A COMPARISON OF THE INFLUENCE OF DIFFERENT INORGANIC NITROGEN FORMS ON THE PARTITIONING OF CARBON IN C_3 (*TRITICUM AESTIVUM*) AND C_4 (*ZEA MAYS*) PLANTS.

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ABSTRACT

Within cereal plants, NH_4^+ is assimilated in the root while NO_3^- is predominantly assimilated in the shoot. Growth of plants has been found to be affected differently by NO_3^- and NH_4^+ nutrition and it has been considered that these differences originate from the different sites in which the two N forms are assimilated. In particular, it is considered that increased shoot : root ratios observed in C_3 plants grown on NH_4^+ compared to NO_3^- nutrition result from diversion of C from growth into amino compound synthesis within the root. It has been suggested that the lack of influence of the form of supplied N on the shoot : root ratios of maize plants is due to the larger supply of C available from the C_4 photosynthetic mechanism (Lewis *et al.*, 1990).

The biomasses of hydroponically grown wheat (*Triticum aestivum* L. cv. Gamtoos) and maize (*Zea may* L. cv. PNR 394) plants were found to be lower with NH_4^+ as compared to NO_3^- nutrition and the shoot : root ratios of the wheat, but not maize plants, were increased with NH_4^+ nutrition. Smaller biomass accumulation in NH_4^+- compared to NO_3^--fed plants was correlated with lower photosynthetic CO_2 assimilation rates which appeared to be a consequence of reduced stomatal conductances related to reduced shoot moisture contents. Differences in biomass and gas exchange characteristics were evident soon after transfer of the 5 to 7 day old plants into hydroponic culture from nutrient free culture and were compounded during growth. In wheat and maize grown in split-root culture with one root-half in NO_3^-- and the other in NH_4^+-containing nutrient media, the biomass accumulated in the NH_4^+-fed root-half was 40 and 60% respectively of that accumulated by the NO_3^--fed root-half. Thus the roots of both wheat and maize were shown to be intrinsically sensitive to the form of N supplied.

In order to determine whether the changes in biomass accumulation were attributable to altered C partitioning, plants previously supplied with $^{14}CO_2$ through photosynthesis were fractionated into two amino and three carbohydrate fractions. The allocation of N and ^{15}N to inorganic, soluble amino-N and insoluble amino-N fractions was determined to establish what forms of N were present in the shoots and roots. Uptake of N from the root solution was measured using colorimetric techniques. Xylem sap was analyzed to determine the amount of C translocated in the form of amino compounds between the root and shoot and the sites in which the inorganic N was assimilated. Root respiratory O_2 consumption was measured

polargraphically and CO_2 release was monitored using infrared gas analysis or by trapping $^{14}CO_2$ released from plants previously supplied with $^{14}CO_2$ through photosynthesis. Dark HCO_3^- assimilation by roots was determined by measuring $H^{14}CO_3^-$ incorporation from the root solution into 80% ethanol (v/v) soluble acid-stable products.

It was found that there was a greater allocation of ^{14}C to amino compound containing fractions at the expense of allocation of ^{14}C to carbohydrate fractions in NH_4^+- compared to NO_3^--fed plants. The ratios of [^{14}C]carbohydrate : [^{14}C]amino acid were 1.5- and 2.0-fold greater in shoots and roots respectively of 12 mM NO_3^-- compared to 12mM NH_4^+-fed wheat. In 4 and 12 mM N-fed maize the [^{14}C]carbohydrate : [^{14}C]amino ratios were approximately 1.7- and 2.0-fold greater in shoots and roots respectively of NO_3^-- compared to NH_4^+-fed plants. In plants grown in split-root culture with one root-half in 4 mM NO_3^- and the other in 4 mM NH_4^+ containing nutrient media, the ratios of [^{14}C]carbohydrate : [^{14}C]amino acid in the NO_3^--fed root-halves were 2.5- and 2.0-fold higher in wheat and maize respectively than in the root-halves supplied with NH_4^+. This indicates that the allocation of C to the amino compound fractions occurred at the expense of carbohydrate fractions, particularly within the root, and that this change in C allocation could be responsible for reduced root growth.

Allocation of N and ^{15}N within the plant confirmed that NH_4^+-fed plants accumulated more amino compounds than NO_3^- fed plants and it was found that NH_4^+ as compared to NO_3^- nutrition favoured diversion of N into soluble amino compounds, independently of the quantity of N available from either NO_3^- or NH_4^+ nutrition. Wheat roots were found to accumulate large quantities of $^{15}NH_4^+$ (8.5 μg ^{15}N g^{-1} h^{-1}) when supplied with $^{15}NH_4^+$ as a nutrient N source whereas in maize roots very little $^{15}NH_4^+$ accumulated (1.5 μg ^{15}N g^{-1} h^{-1}). It is proposed that this accumulation of $^{15}NH_4^+$ in wheat roots is the result of limited availability of C within the roots of the wheat plants for the detoxification of NH_4^+, in contrast to the situation in maize.

Over 10 h the uptake of NH_4^+ was 1.5- and 1.3-fold greater in wheat and maize respectively than NO_3^- uptake and therefore NH_4^+-fed plants must have diverted more C to the assimilation of NH_4^+ into amino compounds than NO_3^--fed plants, purely on the basis of more N being available in the former. Analysis of xylem sap N contents indicated that the site of nutrient NH_4^+ assimilation was predominantly the root, although some NH_4^+ was translocated

to the shoot, while assimilation of NO_3^- was predominantly shoot based. As a result of root based assimilation of nutrient NH_4^+, wheat and maize translocated larger quantities of amino-C in the xylem sap than did NO_3^--fed plants. In wheat the difference between the rate of C translocation in the xylem of NO_3^-- and NH_4^+-fed plants was 92.2 μmol g^{-1} h^{-1} while in maize the corresponding figure was 806.3 μmol g^{-1} h^{-1}. The larger translocation of C in the xylem sap of NH_4^+- compared to NO_3^--fed plants was confirmed by the fact that, in plants previously supplied with $^{14}CO_2$ through photosynthesis, NH_4^+-fed wheat translocated 1.4-fold and NH_4^+-fed maize 1.2-fold more ^{14}C in the xylem sap than did NO_3^--fed plants. Although cycling of amino-N could have accounted for a substantial proportion of C translocated within the xylem, it is proposed that the loss of C from the roots through translocation of amino compounds from the root to the shoot was the main reason for reduced root extension in wheat plants supplied with NH_4^+ compared to those supplied with NO_3^-.

Respiratory O_2 consumption was 1.4- and 1.6-fold larger in NH_4^+- than in NO_3^--fed wheat and maize plants respectively, possibly reflecting higher energy demands for NH_4^+ uptake and assimilation. It is proposed that the higher O_2 consumption of NH_4^+-fed plants is the consequence of several factors: a) higher rates of NH_4^+ than of NO_3^- uptake, b) demands for ATP and C skeletons for NH_4^+ assimilation, c) lack of large scale NO_3^- reduction within the root and d) the possible role of NO_3^- as an oxidant for respiratory reductant. Apparent respiratory CO_2 release was 1.4- and 1.2-fold larger in NO_3^-- than in NH_4^+-fed wheat and maize plants respectively and was considered to be the consequence of either NO_3^- uptake in exchange for HCO_3^-, as proposed by Ben Zioni *et al.* (1971), or higher dark fixation of HCO_3^- by PEPc in NH_4^+- than in NO_3^--fed plant roots. Changes in respiratory O_2 and CO_2 gas exchange rates resulted in the gas exchange quotient of NH_4^+-fed plants (wheat, 0.5; maize, 0.6) being smaller than those of NO_3^--fed plants (wheat, 1.0; maize, 1.1). Measured rates of dark HCO_3^- assimilation by roots were considerably greater (wheat, 2.6-fold; maize, 8.3-fold) in 4 mM NH_4^+- than in 4 mM NO_3^--fed plants, but differences in respiratory CO_2 release could not be completely ascribed to this mechanism.

The results presented indicate that the differential effects of NH_4^+ and NO_3^- nutrition on the growth of wheat and maize are mediated through differences in photosynthetic rates, C and N partitioning caused by the assimilation of different N sources. The increased shoot : root ratios of NH_4^+- compared to NO_3^--fed wheat may be attributed to enhanced translocation of C from the root to the shoot in the form of amino compounds synthesised in the root from

C which may otherwise have been diverted into root growth. In maize the lack of effect of the form of N on the shoot : root ratios may be ascribed to the greater availability of C from the C_4 photosynthetic mechanism which prevents the accumulation of NH_4^+ within the root and reduces the impact of altered C partitioning on growth.

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ABBREVIATIONS AND SYMBOLS

Γ	CO_2 compensation point
$\Delta\mu_{H+}$	Transmembrane proton electrochemical potential gradient
^{14}C : ^{14}C-N	[^{14}C]carbohydrate : [^{14}C]amino acid ratio
A	Photosynthetic CO_2 assimilation rate
A_{max}	Photosynthetic CO_2 assimilation capacity (under saturating irradiance)
Ba	^{14}C labelled bound protein
Bc	^{14}C labelled bound carbohydrate
CCCP	Carbonylcyanide m-chlorophenylhydrazone
chl	Chlorophyll
Ci	Curie
C_i	Intercellular (sub-stomatal) CO_2 concentration
DCMU	3-(3',4'-dichlorophenyl)-1,1-dimethylurea (diuron)
DHAP	Dihydroxyacetone phosphate
DNP	2,4-dinitrophenol
E	Transpiration rate
EDTA	Ethylenediaminetetra-acetic acid
EtOH	^{14}C labelled water insoluble, ethanol soluble material
G6P	Glucose-6-phosphate
GAP	Glyceraldehyde phosphate
GDH	Glutamate dehydrogenase
GEQ	Gas exchange quotient
GOGAT	Glutamate synthase
Gs	Stomatal conductance to water vapour
GS	Glutamine synthetase
Insol. aN	Insoluble amino-N
IRGA	Infrared gas analyzer
K_m	Michaelis Menten constant
MDH	Malate dehydrogenase
MSO	Methionine sulfoximine
NiR	Nitrite reductase
NR	Nitrate reductase
OAA	Oxaloacetate
OG	2-oxo-glutarate
OPPP	Oxidative pentose phosphate pathway
PEP	Phosphoenolpyruvate
PEPc	Phosphoenolpyruvate carboxylase
PGA	3-Phosphoglycerate
Pi	Inorganic phosphate
PK	Pyruvate kinase
PSI(I)	Photosystem I(I)
Q_{10}	Temperature quotient - the ratio of activity at two temperatures 10 degrees apart
RGR	Relative growth rate
RQ	Respiratory quotient
Rubisco	Ribulose bisphosphate carboxylase and oxygenase
RuBP	Ribulose bisphosphate
RuBPc(o)	Ribulose bisphosphate carboxylase (oxygenase)
S.E.	Standard error

SHAM	Salicylhydroxamate
SLA	Specific leaf area ($cm^2\ g^{-1}$)
Sol. aN	Soluble amino-N
SPS	Sucrose phosphate synthase
Struct.	^{14}C labelled structural material
TCA	Tricarboxylic acid (cycle)
TP	Triose phosphate
Wa	^{14}C labelled water soluble protein and amino acids
Wc	^{14}C labelled water soluble carbohydrate
WUE	Water use efficiency

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1 INTRODUCTION

A requirement exists for N throughout the development of a plant. Quantitatively N represents about 2% of plant dry matter and is a component of proteins, nucleic acids, coenzymes and numerous plant secondary products. Although application of N fertilizer often increases biological and economic yield, N-use efficiency may decrease. Nitrogen may have many other beneficial effects for plants not directly related to yield, e.g. non-specific resistance to various diseases.

The desire to increase economic gain from N application and concern about the pollution potential of excessive N has focused attention on N use by plants. Agricultural fertilizer use is generally inefficient with less than 20 to 25% of N fertilizer added to the soil taken up by plants (Bozzini, 1990). The protein content of crops depends on the ability of the plant to assimilate the N source provided and is important in determining the nutritional and economic value of the crop. While protein quality is genetically determined, protein quantity may to a degree be influenced by the availability of N.

Input of N to cereals such as *Triticum aestivum* (wheat) and *Zea mays* (maize) is from the soil while many other plants, especially the legumes, utilize N fixed from atmospheric N_2 (Schrader, 1984). Utilization of N from the atmosphere (Wellburn, 1990; Raven, 1988), apart from N_2 fixation, and loss of N to the atmosphere (Hocking *et al.*, 1984a) are relatively unimportant for cereals in comparison with utilization of N from the root zone. Soil N is available predominantly as either NO_3^- or NH_4^+ and the responses of plants to these two forms of N vary widely (Haynes and Goh, 1978).

At the extremes some plants cannot tolerate either NO_3^- or NH_4^+ as a sole N source (Bloom, 1988). In many plants NH_4^+ depresses plant growth as compared with NO_3^-, although the opposite has also been reported. The repression of growth by NH_4^+ nutrition may be superficially surprising since NH_4^+ is a more reduced form of N than NO_3^- thus potentially saving the plant energy for reduction. In support of this Chaillou *et al.* (1986) calculated that ATP costs for amino acid and organic acid synthesis *per se* in plants grown on NH_4^+ were half those of plants grown on NO_3^-. It has been suggested that circumstances under which substrate for energy production is limiting would favour the utilization of NH_4^+ over NO_3^- due to the more reduced nature of the former (Turpin *et al.*, 1985). This simplistic estimation

of the energy costs for N reduction and assimilation, however, neglects consideration of the mechanisms of N uptake, site of reduction and assimilation, translocation, charge balance and consequences of the presence of the inorganic N and reduced N for many other aspects of physiology. From evidence derived from root carbohydrates and growth studies it appears that utilization of NH_4^+ as a N source may in fact require more energy expenditure by the plant than the use of NO_3^- (Reisenauer,1978). The wide-ranging ramifications of the effects of the form of N available to the plant are evident from the fact that the form of N may influence the morphology and chemical composition of the plant (Blacquière *et al.*, 1987).

Traditionally NO_3^- has been considered the preferred N source for promoting plant growth. Bloom (1988) in his review of NO_3^- and NH_4^+ as N sources for plant growth suggested that this view stems from experiments conducted with unrealistically high N concentrations or inadequate pH control. In particular, soil NH_4^+ concentrations seldom exceed 1 mM while experimental concentrations are often up to an order of magnitude greater. In addition controlled environment chambers provide generally low light intensities exacerbating N toxicity. Bloom (1988) quaintly suggested that the interaction between plants and NH_4^+ is like that between children and candy - when offered large quantities, they eat more and become sick. While these reservations are appropriate the question still remains as to why, even with adequate pH control and light intensity, growth of NH_4^+-grown wheat and maize plants is diminished in comparison to NO_3^--grown plants (Section 2.2). The physiology behind these differences in response to N form is important to our understanding of the interactions between N metabolism and plant growth.

Four main hypothesis have been offered to explain poor growth on NH_4^+ as opposed to NO_3^- nutrition: 1) acidification of root medium during NH_4^+ assimilation, 2) low carboxylate accumulation in NH_4^+-grown plants, 3) deficiency in inorganic cations as a result of NH_4^+ nutrition and 4) limitation in detoxification of NH_4^+ due to limited C substrate for NH_4^+ assimilation (Allen and Smith, 1986).

It is well known that in plants such as wheat which possess the C_3 photosynthetic pathway, NH_4^+-grown plants are reduced in size relative to NO_3^--grown plants, the roots being particularly responsive (Haynes and Goh, 1978). In C_4 plants such as maize this effect is less apparent with no obviously larger influence of the N form on the roots than on the shoots (Lewis *et al.*, 1990). A possible explanation for these differences is that they arise from the

fact that in both wheat and maize NO_3^- assimilation occurs mainly in the shoot, whereas nutrient NH_4^+ assimilation is restricted to the root (Lewis and Chadwick, 1983; Murphy and Lewis, 1987). It has been suggested that the assimilation of NH_4^+ within the root places demands upon root carbohydrate supply which diverts C away from root extension. The higher photosynthetic capacity of C_4 plants may make available a larger supply of C thus preventing the root of the C_4 plant from being adversely affected (Lewis *et al.*, 1990).

Using wheat and maize as representatives of the C_3 and C_4 photosynthetic pathways respectively, this study examines the effects of NO_3^- and NH_4^+ nitrogen forms on growth, C and N partitioning. The experiments were performed on hydroponically grown plants with strict pH control to eliminate the effects of pH on growth and physiology and avoid problems associated with the complex nature of soils. The specific questions addressed were as follows:

1) Does root based assimilation of NH_4^+ divert too high a proportion of root C away from structural material thus retarding root growth in C_3 plants?
2) Do the energy requirements for root based NH_4^+ assimilation elevate root respiratory C losses above those of NO_3^- assimilating plants?
3) Does the response of C_3 plants to NO_3^- and NH_4^+ differ from that of the C_4 plants?

In order to answer these questions the following aspects of wheat and maize physiology were examined:

1) Growth and gas exchange properties of wheat and maize in response to NO_3^- and NH_4^+ nutrition.
2) Effects of NO_3^- and NH_4^+ nutrition on the partitioning of C and N within wheat and maize.
3) Flux of C and N through the xylem from root to shoot.
4) The role played by root respiration in influencing the allocation of C within the plant.
5) The importance of HCO_3^- assimilation by the roots of NO_3^-- and NH_4^+-fed plants.

2 LITERATURE REVIEW

2.1 NITROGEN IN SOILS

Although this study does not deal with the soil as a source of N, some aspects of N in the soil are briefly reviewed here. Much of the literature on plant-N interactions has been derived from plants grown on soils and some of the effects of N on plant physiology may be related to the physics and chemistry of the soil. In addition, the interest in NH_4^+ as a N source for agricultural crops is sparked at least partially by the behaviour of this ion in the soil.

Nitrogen is a mobile and dynamic element in soils. The amount and form of N in soils depends on the extent of mineralization, N_2 fixation and immobilization of the N by soil particles and microbes. Movement of N through the soil may be through diffusion or mass flow (De Willigen, 1986). Primarily as a result of the biological component of N cycling, the availabilities of NO_3^- and NH_4^+ vary seasonally and the location and form of N within the soil profile varies with factors such as leaching, soil temperature and soil water status (Bloom, 1988).

Although in some soils NH_4^+ is more readily available than NO_3^-, in most agricultural soils the roots of plants absorb N largely as NO_3^-, since this ionic form occurs in higher concentrations than NO_2^- or NH_4^+, and is free to move within the root solution due to the tendency for soils to posses an overall negative charge (Reisenauer, 1978). This has the consequence that NO_3^- is not only readily absorbed by plants but that it is also easily lost from the root zone through leaching which may account for extremely high losses of up to 30% of soil inorganic N per growing season (De Willigen, 1986). Apart from leaching, loss of NO_3^- through denitrification, both biological and chemical, occurs under reducing or anaerobic conditions (Haynes and Goh, 1978) and is especially important in fertilized fields where the loss of N may be enormous (Lewis, 1986).

Some NH_4^+ is usually present in the soil and may affect growth to some extent. In agriculture, application of urea may be used to enhance soil NH_4^+ contents because urea is readily hydrolysed to NH_4^+ in the soil (Harper, 1984) although it is not itself readily utilized by plants (Criddle *et al.*, 1988). Application of nitrification inhibitors has also been used in agriculture to enhance soil NH_4^+ contents (Bock, 1987; Adriaanse and Human, 1991). The

availability of NH_4^+ within the soil may, however, be severely limited because it is tightly held by the micaceous clay minerals of the soil and readily utilized by micro-organisms effectively removing it from the soil solution until mineralization occurs (Lewis, 1986). The problem of limited availability of NH_4^+ may be partially overcome in agriculture through use of K^+ which increases the availability of NH_4^+ by occupying binding sites in the soil (Haynes and Goh, 1978) allowing more effective use of NH_4^+. The availability of NH_4^+ to the plant may also be reduced through conversion of NH_4^+ to NH_3 and subsequent volatilization (Bloom, 1988).

Nitrite uptake by plant roots is not generally considered to be of consequence as a result of the low levels of NO_2^- in the soil and the reported toxicity of this ion. Nitrite may arise in the soil from transformation of N compounds in the soil and rhizosphere, from organic wastes or from NO_3^- containing roots during oxygen stress (Breteler and Luczak, 1982).

The form of N utilized by the plant has important consequences for soil pH. The net acidification which occurs with NH_4^+ uptake and the net alkalinization which occurs with NO_3^- uptake results in differences in solubility, concentration, ionic form, mobility and availability of N in the soil (Marschner, 1991). Uptake of NH_4^+ by many crop plants is increased with increased pH and at high soil pH, NH_4^+ toxicity may result, while at low soil pH N starvation may occur (Findenegg, 1987). Changes in soil pH brought about by uptake of either NO_3^- or NH_4^+ has consequences for the uptake of elements such as K^+ (Findenegg, 1987) and Pi (Sentenac and Grignon, 1985) leading to interactions between N and the availability of other essential nutrients.

The commonly held view that provided root density and soil moisture are high enough, the concentration of NO_3^- in the soil will not limit N uptake unless the NO_3^- concentration is below approximately 0.02 mM has been challenged (Robinson *et al.*, 1991). High NO_3^- concentrations (up to 20 mM) have been found in the root zones of high yielding crops which nevertheless still respond positively to fertilizer applications (Robinson *et al.*, 1991). These authors suggested that the reason for this was related to overestimation by previous workers of the root area effective in NO_3^- uptake. Roots tend to proliferate in localized areas within the soil of high N content (Granato and Raper, 1989) and thus localized portions of the root may be exposed to high N concentrations while other parts of the root system are ineffective in N uptake.

2.2 THE INFLUENCE OF NITROGEN ON GROWTH

The link between N and plant growth is well known and an elevated supply of NO_3^- has been shown to enhance growth of many cereal and pasture grasses (Andrews *et al.*, 1991). This relationship between N and plant growth has been formalized by Hocking *et al.* (1984a) as,

$$W = E \times U \times \left(1-\sqrt{1-\left(\frac{N-T}{M-T}\right)^2}\right)$$

where
- W = daily dry matter production (kg ha^{-1} d^{-1})
- E = evapotranspiration (mm d^{-1})
- U = water use efficiency (kg dry matter ha^{-1} mm^{-1})
- N = tissue N concentration (kg N kg^{-1} dry mass)
- T = threshold N concentration below which growth ceases (0.005 to 0.02 kg N kg^{-1} dry mass)
- M = maximum tissue N concentration (0.01 to 0.08 kg N kg^{-1} dry mass).

This equation depicts production of dry matter by plants to be limited by the availability of N and to be dependent on the growth of the plant and water use. The influence of N on growth has been modelled by other authors more recently (Levin *et al.*, 1989; Hilbert, 1990). Levin *et al.* (1989) provided a model which, in accordance with experimental data, predicted an increased shoot : root ratio with increased N concentration. The shoot component was predicted to increase monotonically in a linear fashion with tissue N concentration (Levin *et al.*, 1989; Hilbert, 1990). Evidence for a relationship between N allocation, shoot : root ratio and relative growth rate has been presented and it has been suggested that shoot : root ratio is controlled by N and that shoot : root ratio is in turn important in determining relative growth rates (Hilbert, 1990). This analysis indicates the importance attached to N in determining growth, but does not discriminate between NO_3^- and NH_4^+ as N sources. A wide variety of responses to NO_3^- and NH_4^+ nutrition have been reported in an assortment of plants grown under differing conditions.

Deleterious effects of NH_4^+ nutrition have been widely reported although some species (e.g. *Oryza sativa*) exhibit a preference for NH_4^+ (Goyal and Huffaker, 1984). Plants growing on acid soils (calcifuges) grow better with NH_4^+ than NO_3^- nutrition while plants with a wide pH tolerance (calcicoles) generally use NO_3^- preferentially (Hageman, 1984). In some cases the reported preference of plants for NH_4^+ may be the consequence of nitrification resulting in the plant receiving a mixture of NO_3^- and NH_4^+ (Spratt and Gasser, 1970). Some of the

variability observed in growth of plants with NO_3^- and NH_4^+ nutrition may result from the role of environmental conditions (e.g. light intensity, shoot and root temperature, soil pH) in determining the response of the plant to the form of the N (Chaillou *et al.*, 1991).

Although the form of N has effects on overall growth of plants, the shoot and the root components respond quite differently. Using *Plantago lanceolata* and *Plantago major* grown on 0.5 mM N, Blacquière *et al.* (1987) found differences in the accumulation of shoot dry matter between NO_3^- and NH_4^+ nutrition but no differences were observed in the roots. Differences in the shoot biomass of these plants were associated with initially higher relative growth rates (RGR) and a high shoot : root ratio in NO_3^--grown plants. Similar results for dry mass accumulation and shoot : root ratios were reported for *Pisum sativum* grown on 1.88 mM N (De Visser and Lambers, 1983). In contrast, shoot : root ratios of *Triticum aestivum* (Cox and Reisenauer, 1973) and *Lolium perenne* (Jarvis, 1987) were higher in plants grown on NH_4^+ than on NO_3^-. These conflicting results are partially the result of the use of different species and also differences in N concentration and other environmental factors. Ammonium nutrition generally depresses plant growth in a concentration dependent fashion and in general NH_4^+ is more deleterious to root growth than shoot growth (Goyal and Huffaker, 1984).

For many plants the best growth and highest protein production rates are achieved with a mixture of NH_4^+ and NO_3^- nutrition (Schrader *et al.*, 1972; Reisenauer, 1978; Gashaw and Mugwira, 1981; Goyal and Huffaker, 1984; Lewis and Chadwick, 1983; Lewis *et al.*, 1986). *Triticum aestivum* plants grown hydroponically on 4 mM NH_4NO_3 had a larger biomass than the equivalent NO_3^--grown plants which were in turn larger than the equivalent NH_4^+-grown plants (Cox and Reisenauer, 1973; Lips *et al.*, 1990). The optimum mixture of NO_3^- and NH_4^+ depends on the species of plant, plant age and the pH of the growth medium (Haynes and Goh, 1978). In *Triticum aestivum* enhanced growth with mixed N sources was attributed to greater tiller development, greater N accumulation and differences induced by N in C partitioning (Herberer and Below, 1989). These authors suggested that the actual ratio of NO_3^-:NH_4^+ was not as important to growth as the mere presence of some NH_4^+ in the growth medium. The importance of the mere presence of NH_4^+, rather than a requirement for a precise ratio of NO_3^-:NH_4^+ to be maintained, is of enormous agricultural significance due to the difficulties in maintaining specific soil NO_3^-:NH_4^+ ratios in agriculture (Adriaanse and Human, 1991).

2.3 THE INFLUENCE OF NITROGEN ON CARBON AND NITROGEN PARTITIONING

The concentration of total N varies between plant organs and also changes during plant development (Hocking *et al.*, 1984a). In young plants N is almost equally partitioned between the shoot and root, but as the plant matures reduced N is accumulated in the shoot. These changes which occur with plant age must be borne in mind when interpreting partitioning data.

A complex set of cause and effect relationships has been described for the effect of NO_3^- on partitioning of N between the shoot and root (Vessey and Layzell, 1987). These authors showed that only N in excess of the requirements of the root was exported to the shoot in *Glycine max*. Roots, therefore, have the highest priority for N in times of N deficiency and this may explain the observation that shoot : root ratios decrease during N stress (Tolley-Henry and Raper, 1986a). Nitrogen deprivation has been shown to cause starch accumulation in leaves and to increase the proportion of photosynthate translocated to the root, resulting in a decline in the shoot : root ratios (Rufty *et al.*, 1988). This enhanced allocation of C to the root was ascribed to a decreased utilization of sucrose in the shoot. Sucrose synthesis in *Triticum aestivum* leaves has been shown to be negatively correlated with NO_3^- reduction; it was suggested that these effects resulted from competition between these processes for photosynthetic energy and C (Van Quy *et al.*, 1991). The function of decreased shoot : root ratios may be to compensate for N deficiency by increasing the N acquisition capacity of the root (Robinson, 1986a; Rufty *et al.*, 1990b; Khamis and Lamaze, 1990).

The site at which the N is assimilated is important in controlling the partitioning of the N between the shoot and root, although extensive cycling of N through the plant has been demonstrated (Section 2.8.1). In many species NO_3^- is predominantly assimilated in the shoot while NH_4^+ is assimilated in the root (Section 2.8.2). In species which reduce NO_3^- predominantly in the leaf, the chloroplasts become a major source of amino acids for protein synthesis. Of leaf protein of grasses and cereals, 65% is made up of Rubisco which is primarily derived from chloroplastic amino acids (Schrader, 1984).

In general, of the total plant N, reduced N in the form of protein accounts for 75 to 90% with free amino compounds accounting for 10 to 25% and NH_4^+ for 0.4 to 4% (Hocking *et al.*,

1984a). Although the partitioning of N between shoot and root may vary with N concentration, the proportion of N diverted into various nitrogenous compounds remains relatively constant. For example, of NO_3^- reduced by the roots of *Zea mays*, a constant proportion, 22 to 27%, was incorporated into protein and insoluble N compounds regardless of NO_3^- concentration in the root medium (Morgan *et al.*, 1985b).

The form of N has an influence on the partitioning of C and N between various nitrogenous components in the plant. Blacquière *et al.* (1987), however, found that levels of reduced N in the shoots of *Plantago lanceolata* and *Plantago major* were not affected by the N source and Lewis *et al.* (1987) found no differences in ^{14}C allocation between N and non-N compounds in *Triticum aestivum* shoots. Blacquière *et al.* (1987) found that the soluble reduced N component of the root was strongly enhanced with NH_4^+ nutrition and Lewis *et al.* (1987) found a significantly reduced ratio of non-N to N compounds in the roots of NH_4^+-compared with NO_3^--grown *Triticum aestivum* plants. The lack of an effect of N form on the reduced N levels in the shoot may be due to the relatively low N concentrations (0.5 to 2 mM) used by these authors. The roots, however, appear to be particularly sensitive to the form of N supplied.

Plants grown on NH_4^+ almost invariably contain higher levels of free NH_4^+ and amide N than those grown on NO_3^- (Goyal and Huffaker, 1984). This observation may be explained by the proposal that NH_4^+ plays a regulatory role in diverting C from carbohydrate biosynthesis into amino acid synthesis (Platt *et al.*, 1977; Robinson and Baysdorfer, 1985). To some extent this C may be derived from carboxylation of PEP for the anaplerotic provision of C for NH_4^+ assimilation (Melzer and O'Leary, 1987).

The influence of N on growth may depend on the role that N plays in determining C partitioning within the plant. In *Triticum aestivum*, allocation of ^{14}C to roots of plants grown on NH_4^+ was higher than in plants grown on NO_3^- (Lewis *et al.*, 1987; Lewis *et al.*, 1990). This change in partitioning with N form was attributed by these authors to the requirement for C within the root for assimilation of NH_4^+. In *Zea mays* Lewis *et al.* (1990) found that allocation of ^{14}C to roots of plants grown on NH_4^+ was lower than in plants grown on NO_3^-. The differences in allocation of ^{14}C to the roots of *Triticum aestivum* and *Zea mays* plants respectively were attributed to differences in the photosynthetic capacities of the C_3 and C_4 pathways of these plants. The proposal was made that the higher photosynthetic capacity of

the C_4 pathway prevented NH_4^+ assimilation from perturbing C allocation significantly in the *Zea mays* plant.

The characteristics of NH_4^+-grown plants which are distinctive from NO_3^--grown plants include lower organic acid contents, higher amino acid contents, and soluble sugar contents that are higher in the shoots and lower in the roots (Chaillou *et al.*, 1991). The concentration of organic acids in the plant is generally enhanced with NO_3^- as compared to NH_4^+ nutrition (Goyal and Huffaker, 1984; Allen and Smith, 1986) and in particular malate appears to accumulate in the former (Chaillou *et al.*, 1991). Accumulation of organic acids has been explained to be a mechanism to counteract OH^- produced during NO_3^- reduction in order to maintain intracellular pH (Section 2.7) although malate has also been implicated in the uptake and translocation of NO_3^- (Section 2.5.1).

Although Blacquière *et al.* (1987) found enhanced malate concentration in NO_3^-- compared with NH_4^+-grown *Plantago lanceolata* and *Plantago major*, these authors found that the concentration of soluble and insoluble carbohydrates in the shoots was not significantly influenced by the N source, in contrast to the findings of Chaillou *et al.* (1991). However, in accord with the results of Chaillou *et al.* (1991), there was a higher soluble and insoluble carbohydrate content in the root fraction of the NO_3^-- compared with NH_4^+-grown plants. Reduced root carbohydrate content in NH_4^+-grown plants was attributed to a measured elevation of root respiratory O_2 consumption caused by NH_4^+ nutrition (Blacquière *et al.*, 1987). Although these authors did not measure CO_2 efflux, they obviously associated enhanced O_2 consumption with greater utilization of root carbohydrate (See Section 2.10.1.2 for further details).

The following conclusions may be drawn concerning the influence of the form of N nutrition on C and N partitioning:

1) Roots are particularly sensitive to the form of N nutrition. In many cases the carbohydrate and reduced N content of the shoots were not influenced by the form of N nutrition while there were large changes in the root.
2) Partitioning of C and N is influenced by the form of N supplied due to the different sites in which the NO_3^- and NH_4^+ are assimilated.
3) The production of amino acids and amides is increased by NH_4^+ nutrition.
4) The production of carboxylates is greater in NO_3^--fed plants.

5) Smaller soluble sugar concentrations in roots of NH_4^+-grown plants may be due to:
   a) elevated root respiratory rates to provide energy for NH_4^+ assimilation and
   b) use of C to provide C skeletons for amino acid synthesis.

2.4 THE INFLUENCE OF NITROGEN ON GAS EXCHANGE CHARACTERISTICS

2.4.1 The influence of nitrogen on photosynthetic carbon dioxide assimilation

Photosynthesis-nitrogen relationships are intrinsically complex, because photosynthesis represents the integrated operation of a series of processes influenced by environmental factors as well as leaf physiology and structure (Field and Mooney, 1983; Kerr *et al.*, 1986). Hunt *et al.* (1985a; b) reported increases in leaf area, specific leaf mass (g m^{-2}), chlorophyll concentration, stomatal density, net photosynthesis, transpiration rate and stomatal conductance with increased NO_3^- nutrition in *Amaranthus powellii*. Thus N influences a large number of the processes governing photosynthesis making the influences of N form and concentration on photosynthesis difficult to interpret. A commonly used technique to gauge photosynthetic CO_2 assimilation is to measure instantaneous photosynthetic rates. Although these techniques have many applications, the instantaneous photosynthetic rates may be poorly correlated with the photosynthetic rates integrated over time due to diurnal variation in photosynthetic rates. Often photosynthetic activity is taken as a measure of the availability of C to the plant for growth and maintenance processes. Photosynthesis is, however, only a part of the C economy of the plant with respiration accounting for the loss of 30 to 50% of the C fixed by the plant (Poorter *et al.*, 1990).

Photosynthetic rates are positively correlated with leaf N concentration (Kerr *et al.*, 1986) and fast growing species have high rates of photosynthesis per unit leaf organic N (Poorter *et al.*, 1990) although, in general, leaf area development is more strongly influenced by N nutrition than photosynthetic rates (Tolley-Henry and Raper, 1986a; Chapin *et al.*, 1988b). A relationship between photosynthetic CO_2 assimilation capacity (A_{max}) and RuBPc activity has been established and Rubisco has been found to form a constant proportion of leaf N, suggesting that A_{max} is limited by N content (Field and Mooney, 1983). Regeneration of RuBP is thought to reflect regeneration of NADPH and photophosphorylation of ATP, although other factors may also limit RuBP regeneration (Farquhar and von Caemmerer, 1982). In *Triticum aestivum* N deficiency has been found to limit photosynthetic reduction capacity and RuBP regeneration, leading to a limitation of A_{max} (Mächler *et al.*, 1988). Thus A_{max} is

limited by leaf nitrogen content through affects on the quantity of Rubisco available and through control of RuBP regeneration.

Evidence for optimal allocation of N to maximize CO_2 assimilation was found by Hunt *et al.* (1985c) suggesting that changes in the photosynthetic rates may be expected to be the result of changes of similar magnitude in all components of CO_2 assimilation, representing a very non-specific effect of N on the photosynthetic machinery (Hunt *et al.*, 1985c). Evans and Terashima (1988) found that changes in the activity of the light reaction and CO_2 assimilation in response to NO_3^- concentrations between 0.5 and 12 mM were accompanied by changes of similar magnitude in chlorophyll contents and many other attributes of the photosynthetic light reaction, confirming the hypothesis of Hunt *et al.* (1985c).

For a given investment of N, C_4 plants have a higher A_{max} than C_3 plants although the photosynthetic rates of the C_3 and C_4 species may be similar due to the accumulation of N in Rubisco in the C_3 but not the C_4 plant (Sage and Pearcy, 1987a). The CO_2 concentrating ability of the C_4 plants leads to CO_2 saturation of the limited RuBPc resulting in maximization of photosynthetic rates for a given N investment in C_4 plants (Sage and Pearcy, 1987b). Although the bundle sheath of the C_4 plant *Digitaria sanguinalis* contained 67% of leaf GOGAT and 80% of leaf GDH, NR and NiR were not detectable in this tissue whereas mesophyll cells of this plant were capable of both NO_3^- and NO_2^- reduction (Moore and Black, 1979). Similar results were reported for *Zea mays* (Neyra and Hageman, 1978). It has been suggested that this compartmentation spatially separates NO_3^- reduction and RuBPc based CO_2 assimilation preventing competition between the two processes (Smirnoff and Stewart, 1985) which may contribute to the higher photosynthetic CO_2 assimilation per unit leaf N of C_4 plants than of C_3 plants (Sage and Pearcy, 1987b). Even within the leaf tissue of C_3 plants there may be localized differences in the abilities of different cell type to undertake N metabolism. Everard *et al.* (1990) found that paraveinal mesophyll protoplasts from leaves of *Glycine max* were better equipped with enzymes for NO_3^- assimilation than mesophyll protoplasts and suggested a division of labour between mesophyll and paraveinal mesophyll cells preventing competition between C and N assimilation.

Although *Triticum aestivum* and *Zea mays* are considered to be C_3 and C_4 plants respectively, some evidence exists that flag leaves, glumes and bracts of *Triticum aestivum* ears may have a C_3-C_4 intermediate physiology (Ziegler-Jöns, 1989a; b) which may be of significance for

grain-filling. Although the division between C_3 *Triticum aestivum* and C_4 *Zea mays* may be to some extent indistinct, the fact remains that the vegetative components of *Triticum aestivum* exhibit typical C_3 characteristics with a lower photosynthetic capacity than *Zea mays*.

Although it is well known that photosynthetic activity is increased with increased N nutrition (Robinson and Baysdorfer, 1985), the role of different forms of N is far from clear. Comparison of the effects of NH_4^+ and NO_3^- on photosynthetic rates has produced results indicating that:

1) Plants grown on NH_4^+ have higher photosynthetic rates (Blacquière *et al.*, 1987 using *Plantago lanceolata* and *Plantago major* grown on 0.5 mM N; Lips *et al.*, 1990 using *Triticum aestivum* grown on 4 mM N).
2) Plants grown on NO_3^- have higher photosynthetic rates (Amory and Cresswell, 1984 using *Zea mays*).
3) The form of N has no affect on photosynthetic rate (Platt *et al.*, 1977 using *Medicago sativa* leaf discs; Lewis *et al.*, 1986 using *Hordeum vulgare* grown on 2 mM N; Lewis *et al.*, 1990 using *Triticum aestivum* grown on 4 mM N).

Thus considerable variation exists in reported effects of NO_3^- and NH_4^+ on photosynthetic capacity. The fact that different results have been found with the same species at the same N concentration (compare Lips *et al.*, 1990 and Lewis *et al.*, 1990) indicates the importance of environmental conditions and experimental techniques in determining the response of photosynthesis to N form.

One of the possible consequences of NH_4^+ nutrition is the uncoupling of photophosphorylation which results in reduced photosynthetic CO_2 assimilation and has been widely reported to occur in isolated chloroplasts at NH_4^+ concentrations as low as 0.6 mM and (Goyal and Huffaker, 1984). In contrast, NH_4^+ stimulation of CO_2 fixation by intact cells and isolated chloroplasts has been reported. This has been attributed to the alteration of stromal pH (alkalinization) and the effects of pH on the activity of RuBPc (Goyal and Huffaker, 1984). Increased pH in the bathing medium of *Spinacea oleracea* chloroplasts led to a lowered stimulation and even an inhibition of photosynthesis by NH_4^+. Thus it appears that the operation of either uncoupling or alkalinization depends on cellular pH and probably requires the transport of the NH_4^+ or NH_3 into the chloroplast for either effect to be manifested.

Net photosynthetic CO_2 uptake is the result of the assimilatory fixation of CO_2 and the release of CO_2 resulting from dark respiration and photorespiration. In general, N addition to N deficient tissue causes an acceleration of respiratory rates but comparison of the effects of NO_3^- and NH_4^+ nutrition on respiration has led to contradictory reports (Goyal and Huffaker, 1984). The effect of N on respiration in both shoots and roots is discussed in more detail later (Sections 2.9.2 and 2.10.1.2 respectively).

2.4.2 The influence of nitrogen on stomatal conductance

A decline of stomatal conductance with N deficiency was observed in *Hordeum vulgare* and *Lycopersicon esculentum* (Chapin *et al.*, 1988b) while elevated NO_3^- nutrition was shown to increase stomatal density and stomatal conductance of *Amaranthus powellii* (Hunt *et al.*, 1985a; b). A general correlation between leaf N and stomatal conductance has been shown (Field and Mooney, 1983). Nitrate has been shown to stimulate stomatal opening in epidermal strips of *Commelina benghalensis* (Raghavendra, 1980) which may suggest a role for N nutrition in controlling stomatal aperture, although the reliability of detached epidermal strips as a test system may be questioned.

It has been suggested that NH_4^+ nutrition results in susceptibility to water stress (Haynes and Goh, 1978). Water uptake rates in *Lycopersicon esculentum* and *Beta vulgaris* were found to be lower in NH_4^+- than in NO_3^--grown plants (Goyal and Huffaker, 1984; Salsac *et al.*, 1987). Lower water use efficiency (g dry mass g^{-1} water) of NH_4^+- as compared with NO_3^--grown plants has been observed for 4 mM N-grown *Triticum aestivum* (Lips *et al.*, 1990). Thus some evidence does exist for differences in water relations between NO_3^-- and NH_4^+-fed plants.

Although NO_3^- and NH_4^+ may affect water relations, the mechanisms for the influence of NO_3^- and NH_4^+ on stomatal conductance have not been explained. Müller *et al.* (1991) found that NH_4^+ stimulated dark CO_2 fixation, predominantly by PEPc, 7-fold in guard cell protoplasts from *Vicia fabia*. The activity of PEPc is known to be inhibited by malate and these authors associated increased vacuolar malate storage with NH_4^+ stimulation of PEPc activity. Increased malate concentration derived from PEPc activity may be expected to stimulate stomatal opening (Müller *et al.*, 1991). Whether NH_4^+ could fulfil this role in intact plants is however dubious because many authors have observed no stimulation of leaf PEPc

activity by NH_4^+ supplied to roots (Schweizer and Erismann, 1985; Arnozis *et al.*, 1988). It is likely that the response of stomatal conductance to N is mediated by the interaction of many biochemical and physiological factors resulting from N metabolism without any direct effects of N on stomatal conductance.

2.4.3 The influence of nitrogen on photorespiration

The photorespiratory pathway is crucial to an understanding of the fixation of CO_2 and the assimilation of N because large amounts of both C and N pass through this pathway. During photorespiration, glycine is converted to serine in the mitochondrion in an ATP liberating step which results in the release of NH_4^+ in stoichiometric quantities to CO_2 evolved from photorespiration (Keys *et al.*, 1978).

$$2\,Glycine + NAD + H_2O \rightarrow Serine + CO_2 + NH_3 + NADH + H^+$$

The major substrate oxidized by plant leaf mitochondria in the light is glycine and plant mitochondria have a glycine/serine transporter, enzymes for glycine metabolism and an OAA transporter that shuttles NADH produced by glycine oxidation out of the mitochondrion (Oliver *et al.*, 1990). The NH_4^+ released in the mitochondrion during the oxidation of glycine to serine by glycine decarboxylase may be reassimilated in the chloroplast or cytoplasm by GS-GOGAT with the participation of NAD(P)H and/or Fd_{rd} (Wallsgrove *et al.*, 1983). Evidence has accumulated for the operation of the chloroplastic GS-GOGAT pathway for photorespiratory NH_4^+ reassimilation, in spite of the presence of GDH in the mitochondrion (Bergmann *et al.*, 1981; Wallsgrove *et al.*, 1983; Martin *et al.*, 1983; Kendall *et al.*, 1986; Wallsgrove *et al.*, 1986; Woo *et al.*, 1987)

With significant photorespiratory activity (over 20% of primary CO_2 fixation), the NH_4^+ produced would have to be assimilated at rates considerably higher than for NH_4^+ derived from NO_3^- reduction since Stulen (1986) calculated that NH_4^+ from NO_3^- reduction was only 10 to 15% of that released in photorespiration. The rates of photorespiratory N cycling have been determined by measuring NH_4^+ accumulation when GS activity was inhibited by MSO and by using [^{15}N]glycine incorporation as an indicator of photorespiration. From such studies Berger and Fock (1983; 1985) found that photorespiratory NH_4^+ release was minimal in *Zea mays* ($\approx$ 1% of photosynthetic CO_2 assimilation) whereas in *Triticum aestivum* NH_4^+ release from photorespiration amounted to 24% of photosynthetic CO_2 assimilation.

The possibility that NO_3^- reduction could be linked to photorespiration for the provision of reductant was raised by Lips (1971). Photorespiration was proposed to function in two capacities:

1) A fast, efficient regulatory system coordinating N and C metabolism.
2) As a source of reducing equivalents from the metabolism of glycolate to glyoxylate for NO_3^- reduction (Lips, 1979). Evidence for the operation of this source of reductant was derived from: a) glycolate stimulation of NR induction, b) apparent localization of NR within the peroxisome together with the enzyme glycolate dehydrogenase for photorespiratory glycolate metabolism (Lips, 1971) and c) correlation between glycolate dehydrogenase activity and NR activity (Roth-Bejerano and Lips, 1973; Kaplan and Lips, 1984). Mann *et al.* (1978) have, however, reported that glycolate was ineffective as a source of reductant for NR and the currently accepted site of NR is in the cytoplasm where sources other than glycolate provide reductant for NO_3^- (Section 2.9.1.1).

Several studies have shown a positive link between N metabolism and photorespiration in C_3 and C_4 plants (Fair *et al.*, 1974; Tew *et al.*, 1974; Cresswell *et al.*, 1979; Amory and Cresswell, 1984) although Marek and Frank (1984) found that N deficiency caused an increase in CO_2 compensation points (Γ) in *Hordeum vulgare*. Increased Γ with NO_3^- as opposed to NH_4^+ nutrition has been found (Fair *et al.*, 1974; Vaklinova *et al.*, 1981). These results were viewed with scepticism by Hall *et al.* (1984) who observed no differences in photorespiratory rates of *Triticum aestivum* and *Hordeum vulgare* grown on NO_3^- or NH_4^+ nutrition or in plants transferred from NO_3^- to NH_4^+ nutrition. Sharma and Sirohi (1988), however, found significantly lower Γ of *Triticum aestivum* grown with NO_3^- compared to NH_4^+ nutrition. These authors linked the smaller Γ to 20% higher PEPc activity in the leaves of *Triticum aestivum* grown with NO_3^- compared to NH_4^+ nutrition. The ratio of oxygenase to carboxylase (RuBPo : PEPc+RuBPc) was thought to be critical in determining the photorespiratory rates of these plants. This latter study highlights the danger of using Γ data as an indicator of photorespiratory rates due to the fact that compensation points are the product of the interactions of complex processes.

2.4.4 The influence of nitrogen on enzyme activities associated with carbon metabolism

The effects of inorganic N nutrition on photosynthesis and photorespiration have been attributed to the influence of N on the enzymes associated with these processes. Nitrogen deficiency was found to limit photosynthetic capacity through limitation of PSII activity (Khamis *et al.*, 1990). Increased NO_3^- nutrition between 1 and 12 mM increased chlorophyll concentration, electron transport rate, photosynthetic reaction centre densities, cytochrome *f* and plastoquinone contents expressed per unit leaf area in *Spinacea oleracea* (Evans and Terashima, 1987). Thus the capacity and activity of the light reaction of photosynthesis is elevated with increased N nutrition.

In general, increased N nutrition elevates soluble protein contents and thus the content of PEPc and Rubisco are also elevated on a fresh mass basis (Avdeeva and Andreeva, 1973; Fair *et al.*, 1974; Tew *et al.*, 1974; Cresswell *et al.*, 1979; Sugiyama *et al.*, 1984; Shieh and Liao, 1985; Robinson and Baysdorfer, 1985; Sugiharto *et al.*, 1990). In some cases the specific activity of photosynthetic enzymes has been reported to change with N nutrition. The specific activity of PEPc from *Oryza sativa* leaves was enhanced by NH_4NO_3 nutrition, although no effect on the specific activity of RuBPc was observed (Shieh and Liao, 1985). Increased specific activity of RuBPc with N deficiency has been found and has been attributed to regulation of RuBPc activity by the amount of assimilatory power available (Mächler *et al.*, 1988).

Ammonium nutrition compared with NO_3^- nutrition enhanced the specific activity of aspartate aminotransferase and reduced the specific activities of NADP-malate dehydrogenase and NADP malic enzyme in *Zea mays* (Bil' *et al.*, 1985). Elevated RuBPc activity in etiolated *Triticum aestivum* grown with NH_4^+ as opposed to NO_3^- was, however, correlated with increased protein content (Golvano *et al.*, 1982). The specific activity of PEPc was enhanced in leaves of plants supplied with NO_3^- compared with NH_4^+ while the roots of NH_4^+-fed plants exhibited increased PEPc activity in comparison to NO_3^--fed plant roots (Schweizer and Erismann, 1985; Arnozis *et al.*, 1988; Garson and Gray, 1991).

Cresswell *et al.* (1979) reported a decrease in the ratio of carboxylase to oxygenase activity of Rubisco for *Themeda triandra* with increased NO_3^- and NH_4^+ nutrition and similar but smaller changes for *Zea mays*. These authors suggested that the changes in the ratios of

carboxylase to oxygenase activity were due to allosteric effects of N on Rubisco. No effects of N content on the ratio of RuBPc : RuBPo or on photorespiration were detected in *Triticum aestivum* (Lawlor *et al.*, 1987a; b) or *Oryza sativa* (Shieh and Liao, 1985).

In summary, increased N nutrition leads to a general elevation in protein synthesis, but differential effects of N concentration and of NO_3^- and NH_4^+ on the carboxylation enzymes point to mechanisms other than a mere elevation of general protein synthesis. Very little consideration of the mechanism of these effects exists in the literature. Although the degree of activation of Rubisco may be controlled by the availability of assimilatory power it does not seem that the activity of the enzyme *per se* is altered by either N form or concentration. On the other hand there appears to be strong evidence for the elevation of PEPc specific activity in the leaves of NO_3^--grown plants in comparison with NH_4^+-grown plants.

2.5 NITROGEN UPTAKE MECHANISMS

Consideration of the uptake systems for NO_3^- and NH_4^+ is applicable to this literature review because of the important implications of mechanisms of these systems for the rates of uptake of different N forms under varying conditions and the interactions of the uptake systems with other aspects of physiology, particularly C metabolism. Nitrite uptake by plant roots is not generally considered to be of consequence as a result of the low levels of NO_2^- in the soil and the reported toxicity of this ion and will not be discussed further.

The systems for NO_3^- and NH_4^+ uptake are separate and are affected differently by pH, temperature and carbohydrate supply (Haynes and Goh, 1978). Root temperature has been indicated to play an important role in determining the specific absorption rates for NH_4^+ and NO_3^-, although overall N intake seems to remain relatively constant with temperature (Clarkson *et al.*, 1986). Ammonium uptake in *Brassica napus* (MacDuff *et al.*, 1987a) and *Hordeum vulgare* (MacDuff and Jackson, 1991) exceeded that of NO_3^- uptake at all temperatures between 3 and 25°C and the uptake of NH_4^+ was less temperature dependent than NO_3^- uptake (MacDuff *et al.*, 1987a; Bowen, 1991; Smart and Bloom, 1991). An effect of temperature on the postulated carriers for N across the plasmalemma has been suggested but White *et al.* (1991) found no evidence for specific effects of temperature on N uptake and concluded that uptake rates were influenced by temperature through plant demand.

The influences of nutrients other than N are important in determining N uptake. Various effects of a deficiency or excess of micro-nutrients have been described (Dhillon *et al.*, 1987) and P deficiency has been shown to limit both NO_3^- and NH_4^+ uptake by *Hordeum vulgare* (Schjørring, 1986) and *Nicotiana tabacum* (Rufty *et al.*, 1990a). Ammonium has also been shown to elevate P uptake and translocation in *Zea mays* (Smith and Jackson, 1987a; b). The interaction between N and the uptake of various inorganic ions has been suggested to account for the effects of the N form on plant growth. For instance, it has been suggested that inhibition of cation uptake by NH_4^+ may be the mechanism of NH_4^+ toxicity (Allen and Smith, 1986).

Uptake of both NO_3^- and NH_4^+ is subject to feedback regulation responding in a complex fashion to plant N status. Uptake of NO_3^- has been found to depend on the amount of bound N in biomass (Larsson and Oscarson, 1990), although at high levels of bound N the size of NO_3^- pools is more important in controlling uptake (Mattsson *et al.*, 1991).

2.5.1 Nitrate uptake mechanism

There is evidence for the existence of at least two distinct systems for NO_3^- uptake in higher plants. One system is constitutive while the activity of the other may be induced 2 to 5 fold above that of the constitutive system (Clarkson, 1986) by NO_3^- concentrations as low as 100 μM (Hole *et al.*, 1990) and exhibits an induction phase lasting between 1 and 3 h (Ashley *et al.*, 1975; Haynes and Goh, 1978). The duration of the induction phase is reduced by higher NO_3^- concentrations (Morgan *et al.*, 1985a) and is maintained by small endogenous accumulations of NO_3^- (MacKown and McClure, 1988). Induction is dependent on *de novo* synthesis of proteins thought to be NO_3^- transporters (MacKown and McClure, 1988; Dhugga *et al.*, 1988a; b; Hole *et al.*, 1990).

Rates of uptake and reduction of NO_3^- are often similar indicating at least some inter-dependence and similar regulation of these two systems (Haynes and Goh, 1978). The suggestion has been made that NR functions for both the reduction and uptake of NO_3^- (Butz and Jackson, 1977; Jones and Morel, 1988; Tischner *et al.*, 1989; 1990) and evidence has been presented that NR is membrane bound (Lips and Avissar, 1972; Ward *et al.*, 1989). Other authors have, however, presented evidence showing that NO_3^- uptake and reduction are

separate (Doddema *et al.*, 1978; Jackson *et al.*, 1986; Ullrich *et al.*, 1990; Marigo *et al.*, 1990; Agüera *et al.*, 1990). This issue is still to be clearly resolved.

Uptake of NO_3^- exhibits an initial high affinity kinetic saturated at about 1 mM NO_3^- with a K_m of between 0.015 and 0.3 mM (Glass, 1988) and a second low affinity system, the rate of which is linear with NO_3^- concentrations above 1 mM (Siddiqi *et al.*, 1990). In *Hordeum vulgare* the low concentration system was identified as the inducible system while the high concentration system was constitutive (Siddiqi *et al.*, 1990). At low concentrations, NO_3^- uptake by *Hordeum vulgare* was shown to be dependent on metabolism, while at higher NO_3^- concentrations (> 20 mM) influx was relatively independent of metabolism (Glass *et al.*, 1990). Considering the relatively large intercellular NO_3^- concentrations (≈ 26 mM) and a nominal external NO_3^- concentration of 10 mM and assuming a representative plasmalemma electrical potential of -150 mV (inside negative), Glass (1988) calculated that NO_3^- uptake alone would require 17 kJ mol^{-1} while at higher exogenous NO_3^- concentrations uptake may be passive (Glass *et al.*, 1990).

It has been suggested that the mechanism of NO_3^- uptake across the plasmalemma is either a 2 H^+:NO_3^- symport (Novacky *et al.*, 1978; Ullrich and Novacky, 1981; McClure *et al.*, 1990a; b) or a NO_3^-:OH^- antiport (Deane-Drummond, 1984c) driven by free energy derived from $\Delta\mu_{H+}$ (Glass, 1988). McClure *et al.* (1990a) indicated that it is not possible to discriminate between NO_3^-:H^+ symports and NO_3^-:OH^- antiports experimentally and that the mechanistic discrimination may be irrelevant due to the dissociation of water. There is evidence for the existence of an H^+ translocating ATPase in the root membrane capable of generating $\Delta\mu_{H+}$ for NO_3^- uptake (O'Neill *et al.*, 1983; Zocchi, 1985; Mengel and Schubert, 1985) and Qiu *et al.* (1985) found evidence for the operation of an electron transport mechanism across the plasmalemma of *Zea mays* root cells coupled to H^+ excretion. Reduction of NO_3^- results in the production of OH^- (Raven and Smith, 1976) which may in turn participate in an antiport for NO_3^- uptake (Thibaud and Grignon, 1981) although NO_3^- uptake is not obligatorily coupled to NO_3^- reduction (Jackson *et al.*, 1986).

In the leaf organic acids may be stored in the vacuoles as salts, or be translocated to the phloem and on down to the roots where they may be decarboxylated and the resulting OH^- excreted, possibly in exchange for further NO_3^- (Ben Zioni *et al.*, 1971). These authors proposed that stoichiometric production of malate with NO_3^- reduction in the shoot allows the

movement of K-malate to the root where the malate may be oxidised to yield $KHCO_3$ which may then exchange for KNO_3. Lack of cycling of K^+ and organic acids (Kirkby and Knight, 1977; Van Beusichem *et al.*, 1985) and excretion of only a portion of the anion charge resulting from NO_3^- uptake (Kirkby and Armstrong, 1980; Van Beusichem *et al.*, 1988) has been cited as evidence against the validity of the Ben Zioni *et al.* (1971) hypothesis. The plants used by these authors, however, reduce NO_3^- in the root to a significant extent and using *Glycine max*, which reduces 90% of NO_3^- in the leaves, convincing evidence has been provided for the validity of the Ben Zioni *et al.* (1971) hypothesis (Touraine *et al.*, 1988). Furthermore extensive cycling of K^+ between root and shoot components of *Triticum aestivum* has been observed (Cooper and Clarkson, 1989). The role for K^+ in NO_3^- translocation has been suggested to be through either K^+ stimulation of ATPase-facilitated xylem loading or K^+-NO_3^- co-transport into the xylem (Rufty *et al.*, 1981). The operation of the Ben Zioni *et al.* (1971) pathway has important implications for the C budget of the plant in terms of the stoichiometric release of CO_2 during NO_3^- uptake.

The uptake of NO_3^- is the net balance between influx and efflux with a half-time for cytoplasmic exchange in the region of 2 minutes in *Zea mays* (MacKlon *et al.*, 1990). Efflux of NO_3^- has been found to be considerable (10 and 80% of influx) (Ashley *et al.*, 1975; Clarkson, 1986; Jackson *et al.*, 1986; Glass, 1988) and to vary with external NO_3^- concentrations (Teyker *et al.*, 1988; Larsson and Oscarson, 1990) and cytoplasmic NO_3^- concentrations (Deane-Drummond, 1984b). The efflux of NO_3^- may represent a fine control mechanism for NO_3^- uptake (Clarkson, 1986).

2.5.1.1 Transport of nitrate into the vacuole

Normal levels of NO_3^- in plant tissue are less than 0.2% of dry weight (Smirnoff and Stewart, 1985) but NO_3^- may accumulate in some circumstances in crop plants up to 10 to 24% of dry weight (Martinoia *et al.*, 1981; Smirnoff and Stewart, 1985). Nitrate exists in two compartments in the cell, an active metabolic cytoplasmic pool and a relatively large vacuolar storage pool (Oaks, 1986). Of the NO_3^- in the tissue, between 0.3 and 30% may be located in the cytoplasmic metabolic pool, the rest being in the vacuolar storage pool (Belton *et al.*, 1985). In times of N deficiency the NO_3^- may be released from the storage pool and metabolized (Barneix *et al.*, 1984a; Tolley-Henry and Raper, 1986b; MacDuff, *et al.*, 1989; Bellaloui and Pilbeam, 1991).

Translocation of NO_3^- into the vacuole (vacuolar NO_3^- < 100 mM) from the cytoplasm (cytoplasmic NO_3^- < 20 mM) is against the concentration gradient and must be active (Glass, 1988) with participation of carrier proteins (Marigo *et al.*, 1990) and an ATPase (Blom-Zandstra *et al.*, 1990; Lew and Spanswick, 1984; Griffith *et al.*, 1986; Clarkson, 1986). Return of NO_3^- from the vacuole to the cytoplasm may represent a passive flux (Glass, 1988) or the operation of a NO_3^-:H^+ symport (Jackson *et al.*, 1986).

It was suggested by MacDuff and Wild (1989) that storage of NO_3^- within the vacuole represents an additional 'demand' on the uptake system. On re-supply of NO_3^- to *Hordeum vulgare* starved of N for 2 days, uptake was higher than in N sufficient plants, but NO_3^- taken up was predominantly stored in the vacuole, rather than being translocated to the xylem (Chapin *et al.*, 1988a). Although one might expect the plasmalemma to play an important role in the regulation of N flux into the cell, control at the tonoplast may be as important (Glass *et al.*, 1985). Large oscillations in NO_3^- uptake by N deprived *Lolium spp.* (Jarvis and MacDuff, 1989) and N sufficient *Glycine max* (Tolley-Henry *et al.*, 1988) have been attributed to alterations in the relative contributions of influx and efflux linked to root carbohydrate resources and the use of vacuolar NO_3^-. Thus the translocation of NO_3^- into the vacuole may represent an additional point of control, not only for cytoplasmic NO_3^- concentration and consequently NO_3^- reduction, but also for NO_3^- uptake and partitioning between shoot and root.

2.5.2 Ammonium uptake mechanism

It has been assumed that biomembranes are permeable to NH_3 without the need for specific transporters and the reported insensitivity of NH_4^+ uptake to temperature ($Q_{10} \approx 1$ between 7 and 17°C) has been taken to imply independence of NH_4^+ uptake from metabolism (Glass, 1988). Glass (1988) criticised this conclusion because, although the NH_3 molecule is neutral and may be readily protonated, at pH < 7 very little is in the unprotonated form. In addition the capacity for acclimation of ion fluxes to temperature may invalidate the Q_{10} measurements and indeed other workers have found Q_{10} ratios for NH_4^+ uptake by *Lycopersicon spp.* (Smart and Bloom, 1991) and *Hordeum vulgare* (MacDuff and Hopper, 1986) to be between 1.5 and 2.4 which is well within the range expected for energy-dependent NH_4^+ uptake. Additional evidence for the metabolic dependence of NH_4^+ uptake comes from the observation that transfer from light to dark and the presence of metabolic inhibitors diminished NH_4^+ uptake

(Kayshap and Singh, 1985; Glass, 1988). Ammonium uptake causes membrane potential depolarization providing evidence for a membrane potential dependent electrogenic uniport for NH_4^+ uptake (Glass, 1988) via a specific ion channel (Goyal and Huffaker, 1986). In *Zea mays* roots, uptake of NH_4^+ had a 1:1 stoichiometry with H^+ efflux representing a possible energy cost for NH_4^+ uptake. The absorption of NH_4^+ by plant roots is rapid but is subject to feedback controls, the most important of which is pH (Reisenauer, 1978) which generally decreases in the growth medium during NH_4^+ uptake (Fuggi *et al.*, 1981). The absorption of NH_4^+ is generally increased with pH which may be partially related to the increased NH_3 concentration at high pH (Reisenauer, 1978). Thus the energy costs of NH_4^+ uptake depend on the exogenous and endogenous NH_4^+ concentrations and the exogenous pH.

Uptake kinetics for NH_4^+ conform to Michaelis-Menten patterns with K_m values ranging between 0.014 and 0.167 mM (Glass, 1988). The uptake of NH_4^+ appears to be the net result of influx and efflux of NH_4^+ in *Triticum aestivum* and *Avena sativa* (Morgan and Jackson, 1988; 1989; MacKlon *et al.*, 1990) possibly controlled by variations in energy supply from the shoots. Evidence for the existence of NH_4^+ in two separate cellular compartments has been presented by Fentem *et al.* (1983a; b). Tissue accumulation of NH_4^+, predominantly in the vacuole, may be as much as 30 μmol g^{-1} fresh mass in *Hordeum vulgare* roots (Glass, 1988).

Absorption of divalent anions (e.g. PO_4^{2-} and SO_4^{2-}) is increased and absorption of divalent cations (e.g. Ca^{2+} and Mg^{2+}) decreased by NH_4^+ nutrition (Cox and Reisenauer, 1973; Reisenauer, 1978; Haynes and Goh, 1978; Chaillou *et al.*, 1986; Salsac *et al.*, 1987; Blacquière *et al.*, 1987) while the influence of NH_4^+ on uptake of monovalent ions is inconsistent (Reisenauer, 1978). Ionic balance may be restored through the synthesis and decarboxylation of organic acids. Differences have been found in the concentration of malate between NO_3^-- and NH_4^+-fed plants with the NO_3^--fed plants having higher concentrations, particularly in the shoots (Blacquière *et al.*, 1987; Morot-Gaudry *et al.*, 1985; Chaillou *et al.*, 1986).

2.5.3 The interaction of nitrate and ammonium uptake

De Visser and Lambers (1983) found that N content of *Pisum sativum* grown on NH_4^+ was lower than in plants utilizing NO_3^-. Blacquière *et al.* (1988) found uptake of N by *Plantago lanceolata* and *Plantago major* from NO_3^- was more rapid than from NH_4^+ unless a mixed N source was provided. In contrast, the rate of absorption of NH_4^+ was shown to be greater than the rate of NO_3^- absorption in *Lolium perenne* (Jarvis, 1987), *Brassica napus* (MacDuff and Wild, 1989), *Hordeum vulgare* (Lewis *et al.*, 1986), *Triticum aestivum* (Cox and Reisenauer, 1973; Lips *et al.*, 1990) and *Zea mays* (Murphy and Lewis, 1987). Thus the relative uptake rates of NO_3^- and NH_4^+ appear to be species specific.

Uptake of NH_4^+ has been reported to be insensitive to NO_3^- supply in some plants (*Phaseolus vulgaris*, Breteler and Siegerist, 1984; *Allium cepa*, MacKlon *et al.*, 1990) while NO_3^- inhibited NH_4^+ uptake in others (*Zea mays*, Murphy and Lewis, 1987; *Triticum aestivum* Deignan and Lewis, 1988). In *Triticum aestivum* NO_3^- inhibited NH_4^+ uptake at high ambient NH_4^+ concentrations but NO_3^- stimulated NH_4^+ uptake at low ambient NH_4^+ concentrations (Criddle *et al.*, 1988). Ota and Yamamoto (1989) found a stimulation of NH_4^+ uptake and assimilation into proteins by small amounts of NO_3^- supplied to *Raphanus sativa*.

Reduced net NO_3^- uptake in the presence of NH_4^+ has been reported for numerous higher plants although little or no effects have been evident in others (Schrader *et al.*, 1972; Jackson *et al.*, 1976; Haynes and Goh, 1978; MacKown *et al.*, 1982; Lewis *et al.*, 1982a; Lewis and Chadwick, 1983). The cultivar of *Hordeum vulgare* used and the type of ions accompanying the NH_4^+ supplied were found to influence the type of response of NO_3^- and K^+ uptake to NH_4^+ (Bloom and Finazzo, 1986). The inhibition of NO_3^- uptake by NH_4^+ has been suggested to result from:

1) Inhibition of NR by NH_4^+ through: a) uncoupling of phosphorylation (Haynes and Goh, 1978) and b) inhibition by assimilation products of NH_4^+ (MacKown *et al.*, 1982).
2) Inhibition of NO_3^- uptake *per se* by NH_4^+ through: a) inhibition by assimilation products of NH_4^+ (Clarkson, 1986; Breteler and Siegerist, 1984; Revilla *et al.*, 1986), b) stimulation of NO_3^- efflux (Deane-Drummond, 1984a; b; 1985), c) inhibition of NO_3^- influx (Lee and Clarkson, 1986; Lee and Drew, 1986; 1989; MacKown *et al.*, 1982; Ingemarsson *et al.*, 1987; Oscarson *et al.*, 1987) and

d) inhibition of K^+ uptake and thus inhibition of translocation of NO_3^- to the shoot leading to reduced NO_3^- influx (Rufty *et al.*, 1982; Pan *et al.*, 1985; Vale *et al.*, 1987; 1988).

The relative contribution of these mechanism to the overall effect of NH_4^+ on NO_3^- uptake varies under different experimental conditions and with different species. Deignan and Lewis (1988) suggested that the interaction of NO_3^- and NH_4^+ suppresses the 'over-rapid' absorption of NH_4^+ by *Triticum aestivum* which may alleviate stress imposed by root-based NH_4^+ assimilation on the supply of C skeletons for root growth, and that this may be the reason for the enhanced growth of plants supplied a mixture of NO_3^- and NH_4^+. It is noteworthy that in a mixed N feed situation there is more N found in the xylem sap of *Zea mays* than in either a sole NO_3^- or a sole NH_4^+ feed situation (Murphy and Lewis, 1987), perhaps contributing to improved growth found with mixed N sources.

2.6 NITRATE, NITRITE AND AMMONIUM ASSIMILATION

The reduction of NO_3^- to NO_2^- is facilitated by nitrate reductase (E.C. 1.6.6.1) in a 2 e^- step. The NR found in higher plants is pyridine nucleotide dependent and the enzyme is soluble (Guerrero *et al.*, 1981) although some authors have suggested that the enzyme is membrane bound (Section 2.5.1).

$$NO_3^- + NAD(P)H + H^+ \rightarrow NO_2^- + NAD(P) + H_2O$$

$$\Delta G^{\circ\prime} = -142.3\ kJ\ mol^{-1}$$

A cytoplasmic location for NR is generally accepted (Oaks, 1979; Campbell, 1988). The reductant for NR is usually NADH although NADPH specific or bispecific forms of the enzyme do exist (Redinbaugh and Campbell, 1981; Dailey *et al.*, 1982; Robin *et al.*, 1985; Campbell, 1988). Both a constitutive and a NO_3^- inducible form of NR exist (Barneix *et al.*, 1984a; Kaplan *et al.*, 1984; Aslam *et al.*, 1987; Oaks *et al.*, 1990) with induction dependent on protein synthesis (Hewitt *et al.*, 1979; Campbell, 1988).

Nitrite must be further reduced to NH_4^+ in a 6 e^- step before the constituent N can enter into organic combinations. Nitrite rarely accumulates and has been reported to be toxic (Hewitt *et al.*, 1976).

$$NO_2^- + 6Fd_{rd} + 7H^+ \rightarrow NH_3 + 6Fd_{ox} + 2H_2O$$

$$\Delta G^{\circ\prime} = -430.9\ kJ\ mol^{-1}$$

The nitrite reductase (E.C. 1.7.7.1) enzyme is thought to occur mainly in the chloroplasts in leaves and in plastids in roots (Magalhães *et al.*, 1974; Neyra and Hageman, 1974; Miflin, 1974; Plaut *et al.*, 1977; Redinbaugh and Campbell, 1991) although other workers have proposed that NiR occurs in the cytoplasm (Grant *et al.*, 1970) or in the peroxisome (Lips and Avissar, 1972). In the chloroplast the reductant for NiR is Fd_{rd} derived from the light reaction (Joy and Hageman, 1966; Neyra and Hageman, 1974; Anderson and Done, 1978). In *Zea mays* roots a non-haeme iron containing protein, similar to Fd, has been identified which is thought to be the *in vivo* reductant for NO_2^- in the root which functions with a pyridine nucleotide reductase similar to Fd-NADP reductase from *Spinacea oleracea* leaves (Suzuki *et al.*, 1985). The NiR enzyme is inducible in the presence of both NO_3^- and NO_2^-, although the former is more effective (Barneix *et al.*, 1984a), and induction is stimulated by light (Gupta and Beevers, 1985).

Two possible routes of NH_4^+ assimilation have been postulated (Lea and Miflin, 1979). Firstly the reductive amination of a keto acid to yield an amino acid directly catalysed by the enzyme L-glutamate:NAD oxidoreductase (E.C. 1.4.1.3) otherwise named glutamate dehydrogenase (GDH).

$$2\text{-}oxo\text{-}glutarate + NH_3 + NAD(P)H + H^+ \rightleftharpoons Glutamate + NAD(P) + H_2O$$

Secondly the initial incorporation of NH_4^+ into glutamine by L-glutamate:ammonia ligase (E.C. 6.3.1.2) otherwise named glutamine synthetase (GS),

$$Glutamate + NH_3 + ATP \rightarrow Glutamine + ADP + Pi + H_2O$$

followed by the transfer of the amine group of glutamine to OG by the enzyme L-glutamine 2-oxo-glutarate aminotransferase (E.C. 2.6.1.53) otherwise named glutamate synthase (GOGAT).

$$Glutamine + 2\text{-}oxo\text{-}glutarate + 2Fd_{rd} + H^+ \rightarrow 2\,Glutamate + 2Fd_{ox}$$

Both NAD(P)H and Fd_{rd} dependent GOGAT have been extracted from several plant tissues but Fd-GOGAT appears to be associated with chloroplasts and root plastids (Oaks and Hirel, 1985; Klaus *et al.*, 1985; Suzuki *et al.*, 1987). The metabolism via both the GDH and GS-

GOGAT routes requires a minimum of two reducing equivalents and 5 C atoms in the form of OG plus an additional ATP in the GS-GOGAT route (Lea and Miflin, 1979).

Ammonium assimilation in higher plants was long thought to begin with the synthesis of glutamate by GDH but the high K_m for NH_4^+ (5.8 mM) of GDH makes it unlikely that this enzyme could function *in vivo* (Lea and Miflin, 1979). It is now believed that the major pathway for NH_4^+ assimilation is the GS-GOGAT pathway (Skokut *et al.*, 1978; Kaiser and Lewis, 1980; Lewis *et al.*, 1983; Baskakova and Izmailov, 1984; Morris *et al.*, 1988). There is little or no evidence in the literature to suggest that GDH plays an important role in NH_4^+ assimilation (Lewis *et al.*, 1982a) although substantial activities of the enzyme are found in plant tissue, particularly roots (Lewis *et al.*, 1982a; Lewis *et al.*, 1983). GS has an extremely low K_m for NH_4^+ of 19 μM which means that cellular NH_4^+ could be kept at very low levels ($\approx$ 100 μM) thus preventing any toxic effects (Lea and Miflin, 1979).

The activities of the GS and GOGAT enzymes are not considered to be inducible although small changes with changes in N nutrition have been reported (León *et al.*, 1990; Lewis *et al.*, 1982a; Murphy and Lewis, 1987). Regulation of NH_4^+ assimilation has often been suggested to involve feedback inhibition by products of NH_4^+ assimilation. Menacho and Vega (1989) suggested that NH_4^+ decreased GS and Fd-GOGAT activity in *Chalamydomonas reinhardtii* while products of NH_4^+ assimilation acted as a negative modulator of NADH-GOGAT.

The energy for the reduction of NO_3^- to NH_4^+,

$$NO_3^- + H_2O + 2H^+ \rightarrow NH_4^+ + 2O_2$$

$$\Delta G^{\circ\prime} = 347\ kJ\ mol^{-1}$$

is derived from 1 NAD(P)H and 6 Fd_{rd} which is approximately equivalent to a total of 4 NAD(P)H. The assimilation of NH_4^+ via the GS-GOGAT pathway requires an additional ATP and 2 Fd_{rd} (equivalent to 1 NAD(P)H). Thus assimilation of nutrient NH_4^+ is considerably energetically cheaper than reduction and assimilation of NO_3^- although this ignores costs for uptake and translocation. There are also small differences between C_3 and C_4 plants in terms of the energy costs for N assimilation. Photosynthetic CO_2 assimilation by C_4 plants is slightly more expensive in terms of energy than assimilation by C_3 plants, but C_4 plants have only minimal rates of photorespiration. From data provided by Edwards and Walker (1983) and

assuming that photorespiratory N cycling in C_4 plants is approximately 1 % of CO_2 assimilation (Berger and Fock, 1983; 1985), the results in Table 2.1 may be derived showing that overall N metabolism in C_3 plants is slightly more energetically expensive than in C_4 plants although nutrient NO_3^- assimilation is cheaper in C_3 plants.

Table 2.1 Theoretical energy requirements for NO_3^- assimilation and photorespiratory N cycling in C_3 and C_4 plants. Percentages in brackets are of total costs for CO_2 assimilation and N metabolism (After Edwards and Walker, 1983).

	Requirements per CO_2 fixed (mol mol^{-1} CO_2)			
	C_3 plants		C_4 plants	
Metabolism	ATP	NAD(P)H	ATP	NAD(P)H
Total costs for CO_2 assimilation and N metabolism	4.73	3.3	5.1	2.3
Photorespiratory N cycling costs	0.17 (3.6%)	0.17 (5.2%)	0.01 (0.2%)	0.01 (0.2%)
Costs for NO_3^- metabolism to glutamate	0.06 (1.3%)	0.3 (9.1%)	0.06 (1.1%)	0.3 (13.0%)
Combined costs of photorespiratory N cycling and NO_3^- metabolism	0.23 (4.9%)	0.47 (14.2%)	0.07 (1.4%)	0.31 (13.5%)

2.7 ACCUMULATION OF IONS ASSOCIATED WITH INORGANIC NITROGEN REDUCTION

Considerable attention has been given to ionic accumulation during the reduction of N and has been accorded much importance by some authors (e.g. Raven and Smith, 1976; Smith and Raven, 1979; Smirnoff and Stewart, 1985). Although the production of these ions undoubtedly occurs during N reduction, the importance of these ions is difficult to evaluate considering the vast number of redox and acid/base reactions which occur in the cell.

Nitrate assimilation generates OH^- ions (1 per NO_3^-) and NH_4^+ assimilation generates H^+ (1 per NH_4^+) which must be excreted or neutralized to maintain a constant cytoplasmic pH (Raven and Smith, 1976; Smith and Raven, 1979; Raven, 1988). Although the cell walls could store some of the H^+ and OH^-, this could only account for a fraction of that produced. For the roots this problem is simply overcome through the excretion of H^+, OH^- or HCO_3^-

into the root medium (Smirnoff and Stewart, 1985) and this excretion may even be linked to the uptake of N.

The distribution of N reductive sites has been proposed to be the result of selective pressures related to the requirement of the plant for the disposal of excess H^+ and OH^- (Raven and Smith, 1976). The site of NH_4^+ assimilation is predominantly the root and N is transported to the shoot as a mixture of amino acids and amides (Section 2.8.3). Ammonium uptake and assimilation by the root involves the acidification of the rooting medium (Raven and Smith, 1976; Allen and Raven, 1987; Allen *et al.*, 1988). If NH_4^+ assimilation does occur in the shoot, sucrose translocated from the shoot may be converted into malate in the root, releasing 2 H^+, which may be exchanged for NH_4^+ ions, and the malate translocated to the shoot where it enters the pH stat and is decarboxylated to yield pyruvate. Raven and Smith (1976) concluded that all the H^+ produced in the reduction of NH_4^+ in the root could not be stored in the vacuole for osmotic and pH reasons and thus the predominant means of detoxification must be through root excretion.

Reduction of NO_3^- actually results in the formation of 2 OH^-, but the production of H^+ by the subsequent assimilation of NH_4^+ results in a net stoichiometry of only 1 OH^- per NO_3^- (Raven and Smith, 1976). This assumes that NO_3^- reduction and NH_4^+ assimilation occurs in the same pH pool, which is probably not the case. Although NO_3^- assimilation can occur in the roots it appears, at least for cereals, that the major site of NO_3^- reduction is the shoot (Section 2.8.2). The reduction of NO_3^- in the root provides none of the problems associated with the leaf in terms of the disposal of OH^- which may be either excreted into the external medium or metabolised via the biochemical pH stat.

Kirkby and Knight (1977) found that with increasing NO_3^- assimilation, the bulk of the anions (OH^-) produced accumulated in the plant as malate with a parallel accumulation of cations, while root efflux of OH^- accounted for only 20% of the anion charge. It has been suggested that the OH^- excretion from the root is the result of NO_3^- reduction in this organ alone (Raven and Smith, 1976). In *Ricinus communis* 70% of all OH^- produced by root NO_3^- and SO_4^{2-} reduction was excreted directly as base (Allen and Raven, 1987) with very little organic anion of root origin being stored in the roots. Of OH^- produced by shoot NO_3^- and SO_4^{2-} reduction 40% was stored in the shoot, the rest being translocated to the roots for excretion. Similar

results were observed in *Brassica napus* (MacDuff *et al.*, 1987b) and in *Phaseolus vulgaris*, although in this plant very little OH^- was excreted (Allen *et al.*, 1988).

PEPc may function as a pH stat and has also been attributed an important anaplerotic role in the TCA cycle. Schweizer and Erismann (1985) found differential effects of NO_3^- and NH_4^+ on PEPc expressed on the basis of both fresh mass and soluble protein which they suggested may be related to a requirement for a pH stat in root and leaf cells supplied with NO_3^- and NH_4^+. The absence of PEPc from the bundle sheath cells of C_4 plants, and thus the lack of a pH stat, has been suggested to account for the lack of NO_3^- reduction in these cells (Neyra and Hageman, 1978; Moore and Black, 1979; Smirnoff and Stewart, 1985).

The use of a biochemical pH stat involves the allocation of large quantities of C to vacuolar storage, unless this C is translocated to the root for excretion. Oxalate is the most efficient storage product because it can neutralize one OH^- for each C. Up to 10% of the organic C may be involved in organic acid formation and the sequestering of organic acid in the vacuole has been estimated to consume about 5-10 ATP/N while transport of C to root probably only requires 2 ATP (Smith and Raven, 1979). Simultaneous supply of NO_3^- and NH_4^+ would alleviate the pH stress imposed by supply of one of the two forms and this may in part account for the synergistic effects of NO_3^- and NH_4^+ supply on growth.

2.8 NITROGEN TRANSLOCATION

Techniques for estimation of translocation in phloem and xylem have been reviewed by Simpson (1986) and it appears that there are considerable grounds for viewing data gathered with these techniques with caution. Phloem sap is particularly difficult to collect. The majority of work in this field has been carried out on legumes which can be induced to bleed freely from both the xylem and the phloem. Suitable techniques for phloem sampling in cereals are, however, absent (Kirkman and Miflin, 1979) and indeed *Triticum aestivum* is known not to bleed readily from the phloem (Simpson *et al.*, 1982). Collection of EDTA enhanced phloem exudate from cut leaves of *Triticum aestivum* was judged prone to error by Simpson and Dalling (1981) after the discovery of increased exudation of phenylalanine and tyrosine with EDTA.

Obtaining sufficient quantities of xylem sap from species with small stems is also often a problem. Utilization of passively exuded xylem sap from cut roots of *Triticum aestivum* is apparently reliable (Kirkman and Miflin, 1979; Simpson *et al.*, 1982) although the possibility exists that apoplastic solution from tissues other than xylem may be collected during collection of xylem sap (Minchin and McNaughton, 1987). Pneumatic extrusion of xylem sap is prone to error as the pressure may force nutrient solution through the root. In addition to the difficulties in obtaining xylem sap, the fact that extensive re-cycling of organic N occurs in the plant means that xylem organic N contents, as an indicator of the site of reduction, over estimate the contribution of the root (Vessey and Layzell, 1987).

2.8.1 Nitrogen redistribution

Both the xylem and the phloem participate in transporting N in plants. The C:N mass ratio for xylem varies from 1.5 to 6 and from 15 to 200 for phloem, the latter being heavily loaded with photosynthate but not inorganic N (Pate, 1980). The xylem is the principal conduit for N transport from root to shoot and different species, depending on nutritional and physiological circumstances, may have a wide range of N forms and concentrations in the xylem sap (Schrader, 1984). Nitrogen translocation from roots in the transpirational stream is not only partitioned in proportion to the transpirational activity of the target organs (Simpson, 1986) but also depends on the sink strength of the tissue which is in turn related to growth rates (Perby and Jensén, 1987). The phloem is the principal conduit for the transport of organic N from shoot to other parts of the shoot or to the root.

Xylem to phloem transfer, which is highly specific and may occur through the transfer cells lining the xylem and phloem of certain tissues, may account for up to 58% of N intake of the shoots of legumes (Simpson, 1986). The transfer of amino compounds from xylem to phloem may have an additional significance due to the fact several amino compounds from *Lupinus albus* (glycine, methionine, aspartate, homoserine, γ-aminobutyric acid and glutamate) are transformed into other substances during transfer, suggesting a symplastic transport into the phloem (Sharkey and Pate, 1975). Phloem to xylem transfer of photoassimilates has also been found in *Lupinus augustifolius* by using ^{11}C labelled photoassimilate (Minchin and McNaughton, 1987). From studies using *Lycopersicon esculentum* Van Bel (1984) concluded that although the apoplastic pathway for transfer between the xylem and phloem was possible a symplastic path was 'more likely' and suggested the involvement of ray cells. In a review

article Van Bel (1990) emphasized the importance of rays in radial transport, their role in C and N cycling and their capacity for selective transfer of metabolites. The function of transfer between xylem and phloem is not clear, but the suggestion has been made that it provides a mechanism for enriching the xylem with photosynthate and the phloem with N. Such a mechanism may be of some consequence to the allocation of N and C to the fruits of plants such as *Lupinus albus* which have low transpirational activity and may thus depend on the phloem for supply (Pate *et al.*, 1979).

In *Triticum aestivum* the proportion of N cycling in the plant represented 18% of the total N in the plant and up to 79% of the N imported by the roots from the shoots was transferred to the xylem for re-export (Simpson *et al.*, 1982). About 50% of N imported daily into the shoots of vegetative *Triticum aestivum* plants was retranslocated to the roots (Lambers *et al.*, 1982). Recycled N represented 50 to 70% of xylem N in *Triticum aestivum* grown on between 1 and 1.5 mM NO_3^- (Cooper *et al.*, 1986a; Cooper and Clarkson, 1989; Larsson *et al.*, 1991). Cooper and Clarkson (1989) suggested that there is only a single amino-N pool in both shoot and roots and that it is this combined pool which regulates N uptake. The proportion of N cycled through the shoots to the roots depends on the physiological status of the plant and is increased by low soil N concentrations and water stress.

The reduction of NO_3^- within the root has been viewed in the context of extensive N recycling within the plant to be for the satisfaction of root demand for reduced N with only excessive reduced N being transported to the shoot (Radin *et al.*, 1978). Oji *et al.* (1989), however, found that root-reduced N did not sustain the N feeding of the roots and that this was largely derived from the shoot. Roots of *Lupinus albus* received on average an amount of N via the phloem in surplus of their growth requirements (Pate *et al.*, 1979). Massive support for the root by shoot based supplies of reduced N has also been demonstrated using split-root culture of *Triticum aestivum* where one half of the root was maintained in an ^{15}N nutrient medium (containing NO_3^-) and the other in a ^{14}N nutrient medium (Lambers *et al.*, 1982; Cooper *et al.*, 1986a). According to the results from these experiments even NO_3^--fed roots could be dependent on the supply of N from the phloem rather than depending on recently absorbed N from the soil.

The cycling of N in a plant is thought to be a consequence of a balance between the capacity of a plant to absorb N, to remobilize protein and to incorporate the N into new structure or

proteins (Simpson *et al.*, 1982). A four compartment model has been proposed to account for the cycling of N through a mature leaf. In the model, N entering the leaf in the xylem first enters a pre-cursor pool from where it may be transferred directly to an efflux pool (phloem) or exchanged with the soluble storage pool (vacuolar) or the pool of insoluble N (leaf protein). The phloem efflux of N from the leaves may be translocated basipetally to the roots (Simpson, 1986). Cytokinins, produced in the roots, have been suggested to play a role in determining the partitioning of N within the plant by influencing the accumulation of N within the leaves (Simpson, 1986).

2.8.2 Nitrate translocation

The advantages of foliar as opposed to root based NO_3^- reduction may be derived from the benefits of NR having access to photosynthetic reductant rather than respiratory reductant (Andrews, 1986a) although ionic balance may also be of consequence (Section 2.7). The site (root or shoot) at which NO_3^- is reduced varies between plant species. In *Oryza sativa* it appeared from ^{15}N studies that nitrate is largely incorporated into glutamine and glutamate in the root (Yoneyama and Kumazawa, 1975). The enzymes associated with NO_3^- reduction in *Hordeum vulgare* and *Triticum aestivum* are predominantly located in the leaves indicating this as the site of NO_3^- assimilation in this plant (Lewis *et al.*. 1982b; Cooper *et al.*, 1986a; Przemeck and Kücke, 1986). In both *Triticum aestivum* (Ashley *et al.*, 1975) and *Zea mays* (Murphy and Lewis, 1987) NO_3^- is predominantly reduced and assimilated in the shoot.

The root is capable of concentrating considerable quantities of NO_3^- in the xylem sap. In *Zea mays* grown on 1 mM NO_3^- xylem NO_3^- concentrations were 10.5 mM (Oaks, 1986), *Ricinus communis* grown on 1 mM NO_3^- accumulated 10 to 15 mM NO_3^- in the xylem (Schobert and Komor, 1990) and *Hordeum vulgare* grown on 2 and 8 mM NO_3^- had 27 and 34 mM NO_3^- respectively in the xylem sap (Lewis *et al.*, 1982a). In *Zea mays* xylem sap analysis has shown that in NO_3^--fed plants 59% of N supplied from the root to the shoot was in the form of NO_3^- with 35% being in the form of amino compounds (Murphy and Lewis, 1987). When these authors examined the content of xylem sap after 4 h supply with $^{15}NO_3^-$ they found that 93% of ^{15}N was in the form of NO_3^- and only 3.5% as amino compounds identifying the shoot as the primary site of NO_3^- assimilation in these plants. Of net influx into *Zea mays* roots during 24 hours of exposure to 1 mM $K^{15}NO_3$, 56% was translocated to the shoots, 30% reduced in the roots and 14% accumulated in the roots (Jackson *et al.*, 1986). In

Hordeum vulgare grown on 1.5 mM NO_3^- 50% of nitrate reduction occurred in the roots (Gojon *et al.*, 1986) and in field grown *Triticum aestivum* 30% of $^{15}NO_3^-$ was reduced within the root (Cooper *et al.*, 1986b).

In *Zea mays* the translocation of NO_3^- via the xylem from root to shoot was found to be concentration dependent (Oaks, 1986). It appears that once the NO_3^- reduction capacity of the shoots is exceeded an accumulation of NO_3^- in the shoot (Oaks, 1986) or root occurs (Schobert and Komor, 1990). Thus the variable proportion of NO_3^- reduced within the root may be a function of the NO_3^- concentration supplied and the limited reduction capacity of the root (Andrews, 1986b). Morgan *et al.* (1985a; b) found that as NO_3^- concentrations were increased more NO_3^- was reduced in the root leading to enhanced reduced N levels in the xylem sap up to a point at which, with increasing NO_3^- in the external medium, translocation of NO_3^- in the xylem was increased. This suggested that at high external NO_3^- concentrations root NR was saturated and excess NO_3^- was allocated to translocation.

The pathway of NO_3^- into the root is likely to be strongly influenced by the position of NR within the root. NR has been reported to occur predominantly in the epidermal cells of the *Zea mays* root (Rufty *et al.*, 1984; Rufty *et al.*, 1986). Lewis *et al.* (1982a) suggested that part of the reason for the relative lack of NO_3^- reduction in the roots of *Hordeum vulgare* may be due to the apoplastic pathway through which this ion may move on its way to the xylem, effectively partitioning the NO_3^- away from the NR enzyme. Entirely apoplastic translocation of NO_3^- across the root is, however, unlikely (Hocking *et al.*, 1984a; Robinson *et al.*, 1991).

MacKown *et al.* (1983) reported little reduction, but appreciable translocation and efflux of NO_3^-, during the influx of this ion while extensive reduction, but little translocation and efflux, occurred in the absence of influx. The uptake of NO_3^- into roots may be thought of as a three compartment system with the cytoplasm functioning both as a destination for NO_3^- from the root solution and the vacuole and as a source of NO_3^- for the vacuole, xylem and root solution (MacKown *et al.*, 1983; Jackson *et al.*, 1986). The NO_3^- in the vacuole does not appear to be reduced when sufficient exogenous NO_3^- is available but is reduced in the absence of exogenous NO_3^- (Jackson *et al.*, 1986) serving as a buffer against changes in xylem NO_3^- flux (Gojon *et al.*, 1991).

The proportion of NO_3^- reduced in the roots may be subject to diurnal variations. Oji *et al.* (1989) were able to show evidence for a strategy of root based NO_3^- reduction which responded to light and dark with root reduction in the dark accounting for 82% of NO_3^- uptake while in the light this figure dropped to 31%. *Glycine max* plants absorbed NO_3^- during both the light and dark portions of the daily cycle at nearly equal rates but the NO_3^- taken up in the dark period was only translocated and reduced, predominantly in the shoot (80%), in the following light period (Rufty *et al.*, 1984). The NO_3^- taken up in the dark by *Glycine max* was accumulated in the root (70%) in the form of NO_3^- (83%) half of which was located in the vacuole (Rufty *et al.*, 1987). In the subsequent light period little exogenous NO_3^- and only 18% of the endogenous root NO_3^- was reduced in the root, the remainder being reduced in the shoot. As the light period progressed uptake from the exogenous source became more important. Rufty *et al.* (1987) concluded that the diurnal fluctuations represented a regulatory mechanism, based on transpiration rates, which coordinated the delivery of NO_3^- to the shoot under conditions favouring photosynthetic NO_3^- reduction.

The degree of root based NO_3^- reduction was suggested by Pate (1980) to depend on the availability of carbohydrate from the shoot. A similar suggestion was made by Pace *et al.* (1990). Raper *et al.* (1991) found large increases in NO_3^- uptake during low light intensity interruption of the dark period and also indicated short term changes in net uptake apparently associated with periods of net NO_3^- efflux and suggested that NO_3^- uptake is related to root carbohydrate content. Radin *et al.* (1978) found evidence to suggest that NO_3^- reduction is a poor competitor for carbohydrate with root growth in *Gossypium hirsutum* although this plant principally reduces NO_3^- in the shoot.

The extent to which NO_3^- is reduced within the root, therefore, depends on: 1) the species concerned, 2) the NO_3^- concentration, 3) the capacity of the shoot for NO_3^- reduction and 4) the capacity of the root for uptake and reduction of NO_3^-, which may be limited by carbohydrate.

2.8.3 Ammonium translocation

Assimilation of NH_4^+ occurs at the site of absorption and thus translocation of NH_4^+ is minimal (Bloom, 1988). Ammonium absorbed by the roots is, in virtually all recorded cases, assimilated via the GS-GOGAT pathway into glutamate and glutamine prior to translocation,

although the transport of other amino compounds may be important in some species (Ta and Joy, 1984). In *Hordeum vulgare* the NH_4^+ concentration found in xylem sap remained small regardless of whether NH_4^+ was supplied alone or with NO_3^- (Lewis *et al.*, 1982a). When Murphy and Lewis (1987) examined xylem sap from *Zea mays* plants grown with NH_4^+ they found that 84% of xylem N was in the organic form with the remainder as NH_4^+. After 4 h supply of $^{15}NH_4^+$, 66% of ^{15}N was found in the organic fraction of N in the xylem with the remainder as NH_4^+. This identifies the root as the principal, but not the only, site of nutrient NH_4^+ assimilation.

2.8.4 Amino compound translocation

In plants which assimilate some NO_3^- in the root, or are supplied with NH_4^+ which is assimilated in the root, the major pathway for amino compound transport is the xylem and amino compounds are an important constituent of xylem sap. The type of organic N translocated in the xylem varies between species but is commonly asparagine and glutamine (Hocking *et al.*, 1984a; Ta and Joy, 1984; Kirkman and Miflin, 1979). The loading of the xylem and phloem with nitrogenous solutes is a selective process (Pate, 1980) probably functioning with proton co-transport (Novacky *et al.*, 1978; Kinraide and Etherton, 1980; Franz and Tattar, 1981; Kinraide *et al.*, 1984). Translocators specific for the net charge on the amino compounds have been proposed (Robinson and Beevers, 1981; Felle, 1981; Wyse and Komor, 1984; Schobert and Komor, 1989) and separate translocators have been proposed for the plasmalemma and the tonoplast (Dietz *et al.*, 1990). It has been suggested that amino compound translocators respond to C/N ratios (Sauer *et al.*, 1983) and are induced by NH_4^+ and NO_3^- (Sauer, 1984).

The form of N supplied to the plant has consequences for the concentration and form of reduced N transported in the plant. Ammonium nutrition compared with NO_3^- nutrition elevated xylem amino compound contents by 300% in *Zea mays* (Murphy and Lewis, 1987) and 500% in *Hordeum vulgare* (Lewis *et al.*, 1982a). The amides, with low C:N ratios, are the major xylem carriers of organic N. In *Zea mays* plants fed with NO_3^-, glutamine is the predominant amino compound in the xylem sap, whereas in NH_4^+-fed plants asparagine levels exceed those of glutamine (Murphy and Lewis, 1987). In *Hordeum vulgare* fed NO_3^- glutamine was the predominant amino compound and its concentration was increased 3-fold by NH_4^+ nutrition (Lewis *et al.*, 1982a). Arginine and γ-aminobutyric acid contents of

Brassica oleracea were enhanced by NH_4^+ compared to NO_3^- nutrition although the bulk of organic N in both phloem and xylem sap was in the form of glutamine (Shelp, 1987). To a large extent the amino compounds translocated in a plant are species specific and the extent to which the form of N influences the amino compound content of the xylem depends on the concentration of N supplied and the site of NO_3^- and NH_4^+ assimilation within the plant.

2.9 INTERACTIONS BETWEEN NITROGEN AND CARBON METABOLISM IN THE SHOOT

2.9.1 Dependence of nitrogen assimilation on photosynthate

The reduction and assimilation of NO_3^-, NO_2^- and NH_4^+ is dependent on C metabolism for provision of C skeletons and energy for synthesis of the enzymes, reducing equivalents for functioning of the enzymes and C skeletons to accept the reduced N (Stulen, 1986). The high energy phosphate and reducing equivalents from the light reaction are available for the reductive assimilation of CO_2 as well as a variety of other processes of which one of the more quantitatively important is the assimilation of N (Anderson, 1981). The source of reductant and C for N assimilation is of considerable importance because of the implications it may have for CO_2 assimilation, C partitioning and productivity.

2.9.1.1 Nitrate reduction

The reducing power for cytoplasmic NO_3^- reduction may be supplied from the chloroplast or from the respiratory processes (Anderson, 1981). It is unlikely that the reductant for NO_3^- is derived from any one source but that the particular source would depend on the physiological status of the leaf.

Much evidence exists for the involvement of the chloroplast in supplying reductant for NO_3^- in the light. The chloroplast envelope is, however, impermeable to NAD(P)H (Walker, 1976) and only slow ATP translocation occurs (Heber and Heldt, 1981). The impermeability of the chloroplast, in particular the inner membrane (Heldt and Sauer, 1971), to these important high energy substances has led to the postulation of shuttles which would involve the movement of intermediates across the membrane.

1) In the dicarboxylate shuttle light generated intermediates in the form of malate may be transported from the chloroplast into the cytoplasm where they may be used

for reduction of NO_3^- (Neyra and Hageman, 1976; 1978; Sawhney *et al.*, 1978a; b; Rathnam, 1978). According to this hypothesis malate produced in the light may be oxidised in the cytoplasm for the formation of NADH, and OAA produced in the process could be recycled into the chloroplast for further reduction. The carriers (Heldt and Rapley, 1970; Heber and Heldt, 1981) and enzymes (Anderson and Done, 1978; Anderson and House, 1979) required for this mechanism have been described. An extension of this cycle including the formation of aspartate from OAA and the translocation of this intermediate across the chloroplast membrane has been proposed (Walker, 1976).

2) In the PGA/DHAP shuttle DHAP, produced in the Calvin cycle, may be exchanged for cytoplasmic Pi or PGA through operation of the phosphate translocator (Heldt and Rapley, 1970; Heldt, 1976; Flugge and Heldt, 1977; 1978; Heber and Heldt, 1981; Anderson, 1981). Within the cytoplasm DHAP may be oxidised by NAD to PGA which may exchange for further DHAP, or be retained in the glycolytic sequences.

The principal difference between the dicarboxylate and the PGA/DHAP shuttles is that the latter involves the loss of reducing potential and phosphorylation potential from the Calvin cycle whereas the former involves only the withdrawal of reducing potential from the light reaction. If DHAP is exchanged for Pi instead of PGA there is also a net export of C from the chloroplast. In addition to these major translocators there also appears to be a specific translocator functioning for the translocation of glucose derived from starch out of the chloroplast (Heber and Heldt, 1981). Glucose in the cytoplasm may enter respiratory metabolism and thus be implicated in cytoplasmic reduction events.

Evidence exists for the *in vivo* operation of both the dicarboxylate shuttle and the PGA/DHAP shuttle in the reduction of NO_3^- (Klepper *et al.*, 1971; Rathnam, 1978; Mann *et al.*, 1978; House and Anderson, 1980). In the absence of added PGA, DHAP or C_4 acids, glyceraldehyde (inhibitor of Calvin cycle activity) inhibits both CO_2 fixation and NO_3^- reduction by *Spinacea oleracea* protoplasts (Rathnam, 1978). This indicates that the physiological mechanism for provision of reductant for NO_3^-, at least in *Spinacea oleracea*, is through the PGA/DHAP shuttle.

The effects of light and dark conditions on NO_3^- metabolism are difficult to interpret because the reduction products of NO_3^- require chloroplastic reductant for further metabolism. Several

reports have indicated that light is essential for foliar NO_3^- reduction (Canvin and Atkins, 1974; Canvin and Woo, 1979) and it has been suggested that light promotes transfer of NO_3^- from the storage to metabolic pools (Ferrari *et al.*, 1973). Evidence for a link between NO_3^- reduction and the photosynthetic light reaction has been provided by the observed stimulation of O_2 evolution from detached *Hordeum vulgare* leaves by NO_3^- (de la Torre *et al.*, 1991). Reduction of NO_3^- in leaves of *Hordeum vulgare* (Soares *et al.*, 1985) and *Zea mays* (Gray and Cresswell, 1983) has, however, been found to occur in both light and dark conditions and dark utilization of NO_3^- by chlorophyll-free mutants of *Oryza sativa* has been reported (Yoneyama, 1984). Apart from the reductant required for NO_3^- there is also evidence that photosynthesis may regulate NR activity through cytosolic ATP/AMP levels (Kaiser and Brendle-Behnisch, 1991; Kaiser and Spill, 1991). Thus NO_3^- reduction may be closely, but not obligatorily, linked to the photosynthetic light reaction which may exert its effect through the supply of reductant or through activation of the NR enzyme.

The postulated involvement of the PGA/DHAP shuttle for supply of reductant for NO_3^- implicates the Calvin cycle in NO_3^- reduction. Inhibition or uncoupling of photophosphorylation resulted in a decreased $^{14}CO_2$ fixation and $^{15}NO_3^-$ reduction, although $^{15}NO_3^-$ reduction was not as strongly inhibited as $^{14}CO_2$ fixation (Atkins and Canvin, 1975). Isolated leaf protoplasts of *Spinacea oleracea* are capable of NO_3^- reduction in the light with a 3 to 4 fold stimulation occurring in the presence of HCO_3^- (Rathnam, 1978). Larsson *et al.* (1985b) reported that NO_3^- uptake and reduction was stimulated by CO_2 at high light intensities while a competitive relationship between NO_3^- and CO_2 existed at low light intensities. This was taken by the authors to imply that photoproduced reductant, transferred across the chloroplast envelope, was involved in NO_3^- reduction and that depletion of this within the chloroplast resulted in the inhibition of CO_2 fixation at low light intensities. Deprivation of CO_2 prevented the assimilation of NO_3^- by *Chlorella vulgaris* (Di Martino Rigano *et al.*, 1985) and *Scenedesmus* (Larsson *et al.*, 1985a). Thus there is strong evidence for the dependence of NO_3^- reduction on the Calvin cycle for provision of intermediates for production of reductant. Transport of photosynthetic intermediates from the chloroplast is dependent on the availability of Pi and it has been shown that P deficiency prevents NO_3^- assimilation in *Scenedesmus* (Larsson *et al.*, 1985a) and results in starch accumulation in *Panicum maximum* (Ariovich and Cresswell, 1983). Leaf extracts of plants supplied with elevated NO_3^- have been reported to have higher SPS activity correlated with reduced starch accumulation (Kerr *et al.*, 1984; 1986; Huber *et al.*, 1985). Similar results were not obtained

with plants dependent on sources of N other than NO_3^- (Kerr *et al.*, 1984). Blackwood and Miflin (1976) found that NO_3^- supplied to *Zea mays* leaves increased the incorporation of ^{14}C into malate and aspartate, but decreased incorporation into sucrose and starch.

2.9.1.2 Nitrite reduction

The chloroplastic location of the NiR enzyme in photosynthetic tissue is strong evidence for the dependence of the enzyme on the photosynthetic provision of reducing equivalents. Nitrite assimilation in chloroplasts is linked to the availability of Fd_{rd} generated through the photosynthetic electron transport reactions (Venkataramana and Das, 1983). The rate at which NO_2^- reduction occurs falls in the range 10 to 25 μmol NO_2^- mg chl^{-1} h^{-1} which would indicate a maximum demand for Fd_{rd} of 200 μmol mg chl^{-1} h^{-1} (Robinson, 1986b). Inhibition of NO_2^- assimilation by DCMU and CCCP indicates that NO_2^- assimilation is closely linked to photosynthetic electron transport (Miflin, 1974; Atkins and Canvin, 1975) although NO_2^- reduction has been shown to occur in the light and dark in leaves of *Hordeum vulgare* (Soares *et al.*, 1985) and *Zea mays* (Gray and Cresswell, 1983). Supply of glyceraldehyde, which inhibits the Calvin cycle, has been found to stimulate NO_2^- reduction (Anderson and Done, 1978). Due to the fact that both photosynthetic CO_2 assimilation and NO_2^- reduction depend on the chloroplastic supply of reductant, competition between the two processes may be expected.

Measurements of Fd indicated that at high light intensity adequate Fd_{rd} would be available to support both CO_2 assimilation and NO_2^- reduction without competition (Buchanan, 1980). No evidence for competition between CO_2 and NO_2^- at high light intensity (1000 μmol m^{-2} s^{-1}) was found by Baysdorfer and Robinson (1985) or Robinson (1986b). Nitrite has, however, been reported to inhibit CO_2 fixation in intact *Spinacea oleracea* chloroplasts in a pH dependent fashion (Purczeldt *et al.*, 1978). This was attributed to stromal acidification which inhibited the activity of fructose and sedoheptulose bisphosphatases which play an important role in the regulation of CO_2 fixation (Purczeldt *et al.*, 1978; Kaiser and Heber, 1983). Similar reasoning was invoked by Larsson *et al.* (1985b) to explain the inhibitory effects of NO_2^- on CO_2 assimilation in *Scenedesmus*. Robinson (1990) has reported evidence for O_2 inhibition of NO_2^- reduction which was attributable to photoreduction of O_2 by ferredoxin. Thus, although NO_2^- reduction is unlikely to compete with CO_2 assimilation at high light

intensities, the effects of NO_2^- on photosynthesis may be mediated by mechanisms other than reductant demand.

2.9.1.3 Ammonium assimilation

In photosynthetic tissue, ammonium derived from NO_2^- is known to be incorporated into amino acids within the chloroplast and is thus reliant on concurrent photosynthesis for the provision of reductant and C skeletons. While a proportion (10%) of immediate photosynthate has been traced into amino acids it is likely that stored C would be required to meet the demand for C during rapid NH_4^+ assimilation (Atkins and Canvin, 1975). Little evidence exists to suggest that chloroplasts can independently synthesize the C skeletons for NH_4^+ assimilation and it is more likely that C leaves the chloroplast as DHAP and is converted to OG in the mitochondrion and cytoplasm before re-entry into the chloroplast for amino acid synthesis (Lea and Miflin, 1979; Elrifi and Turpin, 1986). Ferredoxin is required for assimilation of NH_4^+ in the leaf and considering the flux of NH_4^+, the supply of Fd_{rd} needs to be considerable, especially when the flux of NH_4^+ derived from photorespiration is considered. A component of photosynthetic O_2 evolution has been associated with NH_4^+ assimilation and was linked to the light reaction provision of ATP and Fd_{rd} (Anderson and Walker, 1983).

Inhibition of photophosphorylation by DCMU resulted in a partial inhibition (75%) of NH_4^+ assimilation while low concentrations of the uncoupler CCCP stimulated NH_4^+ assimilation in the leaf (Atkins and Canvin, 1975). This complex result is the consequence of the dependence of NH_4^+ assimilation on both reductant from the light reaction and C directly or indirectly derived from the Calvin cycle. Correlative evidence for the inter-dependence of NH_4^+ and CO_2 assimilation may be derived from the work of Larsson *et al.* (1985b) who showed that NH_4^+ supplied to *Scenedesmus* resulted in the inhibition of CO_2 assimilation independent of light intensity. The inhibitory influence was accompanied by a drop in ATP levels attributed to partial uncoupling by NH_4^+.

The assimilation of NH_4^+ has consequences for both photosynthetic and respiratory C metabolism as evidenced by diversion of assimilate from starch synthesis to TCA cycle intermediates for transamination in *Selenastrum minutum* and *Chlorella pyrenoidosa* (Elrifi *et al.*, 1988). In green algae, supply of NH_4^+ resulted in a repression of CO_2 assimilation and

a stimulation of mitochondrial electron transport. Repression of photosynthetic CO_2 assimilation was attributed to decreased RuBP concentration, possibility as a result of consumption of Calvin cycle intermediates for N assimilation (Elrifi and Turpin, 1986; Elrifi *et al.*, 1988). Amory *et al.* (1991) found that NH_4^+ assimilation in *Selenastrum minutum* had an absolute requirement for CO_2 which was the result of both photosynthetic CO_2 assimilation and dark PEPc fixation of HCO_3^- for the anaplerotic supply of C for NH_4^+ assimilation.

During assimilation of NH_4^+, freshly assimilated CO_2 is withdrawn from organic acids and carbohydrates and is diverted into amino acids (Klaus *et al.*, 1985; Platt *et al.*, 1977). These changes observed in *Medicago sativa* leaf disks were accompanied by an increase in pyruvate and a decrease in PEP in comparison to controls not supplied with NH_4^+ leading to the suggestion that the activity of pyruvate kinase (PK) was increased by NH_4^+ (Platt *et al.*, 1977). Schweizer and Erismann (1985) found no effect of either NO_3^- or NH_4^+ on the activity of PK but found an increase in PEPc activity in leaves of *Phaseolus vulgaris* supplied with NO_3^-, although no effect of NH_4^+ on PEPc activity of leaves was observed. The lack of effect of NH_4^+ on leaf PEPc activity was probably the consequence of the assimilation of NH_4^+ within the root. Stimulation of CO_2 assimilation in oat coleoptiles by NH_4^+ was observed by Engemann and Brown (1980) and was suggested to be due to cytoplasmic acidification, induced by NH_4^+, stimulating light and dark PEPc activity. Oxaloacetate, derived from PEPc or from mitochondrial oxidation of malate, is considered to be metabolised to OG which may be utilized for NH_4^+ assimilation.

2.9.2 Dependence of nitrogen assimilation on glycolysis, the oxidative pentose phosphate pathway and tricarboxylic acid cycle activity

Although light has been implicated in the reduction of NO_3^- it has been suggested that NO_3^- reduction is essentially linked to respiration and that light really only plays a role in controlling the availability of respiratory NADH. In the dark respiration is able to support N assimilation to some extent.

Sawhney *et al.* (1978b) suggested that increased adenylate charge resulting from photophosphorylation inhibits mitochondrial respiration and that the reducing equivalents resulting from glycolysis and the TCA cycle may be diverted into NO_3^- reduction. Plant mitochondrial respiration is, however, apparently relatively insensitive to cytosolic ATP/ADP

ratios (Stitt *et al.*, 1982) but is sensitive to levels of cytoplasmic TP (Hampp, 1985). Whether respiration occurs in the light concurrently with photosynthesis has been the subject of much debate and recent evidence indicates that respiration does continue in the light. McCashin *et al.* (1988) found that the TCA cycle activity of *Triticum aestivum* in the light was 80% of that in the dark. Mitochondrial electron transport in *Selenastrum minutum* has been shown to continue in the light and dark and to be capable of supporting NO_3^- and NO_2^- reduction (Weger and Turpin, 1989) and NH_4^+ assimilation (Weger *et al.*, 1988). Thus the export of reducing equivalents from the chloroplast in the light is sufficient to drive NO_3^- reduction and to maintain relatively high respiration rates, although respiration rates may be reduced to some extent in the light.

The oxidative pentose phosphate pathway (OPPP) could be responsible for the reduction, at least in part, of NO_3^- (Dailey *et al.*, 1982). The operation of this pathway in the cytoplasm normally accounts for a significant proportion of glucose degradation and may be favoured during NO_3^- reduction. NADPH has been shown to act as a physiological reductant of NO_2^- under dark aerobic conditions (Ramarao *et al.*, 1981). The activity of one of the primary enzymes of the OPPP (G6P dehydrogenase) in *Chlamydomonas reinhardtii* has been reported to be increased in NO_3^-- as compared to NH_4^+-fed cells providing evidence for the involvement of the OPPP pathway in NO_3^- assimilation (Hipkin and Cannons, 1985).

Starch and sucrose may play an important role as a source of TP for N assimilation. Weger and Turpin (1989) found that NO_3^- and NO_2^- stimulation of TCA cycle activity in *Selenastrum minutum* was greater than that of NH_4^+. The TCA cycle was also found to continue to operate under anaerobic conditions suggesting that reductant from the mitochondrion was utilized for NO_3^- reduction and assimilation (Weger and Turpin, 1989). Sawhney *et al.* (1978a) found that TCA cycle intermediates stimulated NO_3^- reduction and proposed that NADH generated by the TCA cycle was important for this purpose. Woo and Canvin (1980) proposed that a dicarboxylate shuttle, operating from the mitochondrion, supplied the reductant through malate.

Amino acids were found to be synthesized in the chloroplast from OG derived from TCA cycle intermediates (Weger *et al.* (1988) resulting in increased respiration rates (Turpin *et al.*, 1990b). The measured increases of TCA cycle activity were found to occur mainly between OAA and OG (Guy *et al.*, 1989). The source of OAA was found to be dark PEPc activity

which exceeded RuBPc activity under special circumstances. PEPc has been reported to provide an anaplerotic C source for assimilation of NH_4^+ into amino acids (Guy *et al.*, 1989) and Vanlerberghe *et al.* (1990) have shown a linear relationship between PEPc activity and NH_4^+ assimilation. The activity of PEPc is subject to complex metabolic regulation (Schuller *et al.*, 1990a) and malate has been found to be a potent 'mixed' inhibitor of PEPc, although this inhibition may be overcome by PEP (Wedding *et al.*, 1990).

2.9.3 Dependence of nitrogen assimilation on anaerobic respiration

The use of dark anaerobic conditions for assaying the activity of NR has led to considerable debate as to the importance of anaerobic respiration for NO_3^- and NO_2^- reduction. Although anaerobic conditions are unlikely in plant leaves, several authors have suggested that the NO_3^- may function as an alternative oxidant to O_2 for respiratory reductant (Naik and Nicholas, 1981; Hocking *et al.*, 1984b).

Under dark conditions NO_2^- accumulates in photosynthetic tissue due to a lack of photosynthetic reductant while NO_3^- reduction is favoured by anaerobic conditions due to the lack of competition with O_2 for reductant. In the dark O_2 competes strongly with NO_3^- for NADH, but any factor (anaerobic conditions or respiratory inhibitors) which limits oxidative phosphorylation results in a stimulation of NO_3^- reduction through the removal of the competitive effects of O_2 (Radin, 1973; Hewitt *et al.*, 1979; Mann *et al.*, 1979; Canvin and Woo, 1979; Ben-Shalom *et al.*, 1983; Reed *et al.*, 1983). Very high levels of NO_3^- (100 mM) may competitively overcome the inhibition of NO_3^- reduction by O_2 (Radin, 1973). The view that anaerobic conditions are required for NO_3^- reduction in the dark and that dark conditions prevent NO_2^- reduction is erroneous. It has been clearly shown that NO_3^- reduction may occur under dark aerobic conditions and that NO_2^- reduction continues in the dark (Gray and Cresswell, 1983; Ben-Shalom *et al.*, 1983; Soares *et al.*, 1985).

Evidence for the functioning of NO_3^- as an oxidant for respiratory reductant under anaerobic conditions has been provided (Naik and Nicholas, 1981; Weger and Turpin, 1989) although Lee (1978) and Trought and Drew (1981) found no evidence for this. Vanlerberghe and Turpin (1990) have proposed a model describing the dark anaerobic assimilation of NH_4^+ by *Selenastrum minutum* which was based on evidence of NH_4^+ induced starch metabolism and accumulation of alanine as a fermentation product. Although these results do no indicate a

role for anaerobic respiration in N assimilation under normal physiological conditions, they do indicate the importance of TCA cycle intermediates in provision of reductant and C for N assimilation.

2.9.4 Dependence of nitrogen assimilation on 'chloroplastic respiration'

Metabolism of NO_2^- and NH_4^+ in the leaf in the dark is dependent on reductant available within the chloroplast which may be derived from storage C in the form of starch. 'Chloroplast respiration' describes the operation of the OPPP and glycolytic pathways (starch to PGA) within the chloroplast (Kow *et al.* 1982). Originally it was supposed that the chloroplast derived reducing potential and ATP from the cytoplasm in the dark. Considering the low permeability of the chloroplast membrane to NAD(P)H and ATP it seems unlikely that this would be the case, although the dicarboxylate and PGA/DHAP shuttles could fill this requirement to some extent. Kow *et al.* (1982) reported chloroplastic oxidation of GAP to PGA with the formation of NADP which could be converted to Fd_{rd}. It was suggested that the C source is chloroplastic starch (Chen and Gibbs, 1991). Chen and Gibbs (1991) provided evidence from labelling studies with chloroplasts from *Chlamydomonas reinhardtii* for the involvement of the OPPP, glycolysis and a component of chloroplastic electron transport in 'chloroplast respiration'. Mohanty *et al.* (1991) demonstrated that the reduction of plastoquinone in the dark was enhanced by NH_4^+ and suggested that this was brought about by increased respiratory oxidation of chloroplastic starch for amino acid synthesis leading to increased NAD(P)H production in the chloroplast. The reductant provided by such reactions was suggested to be utilized for a number of reactions including NO_2^- reduction to amino acids (Chen and Gibbs, 1991). This may explain the observation that NO_2^- is reduced in the dark in leaves (Gray and Cresswell, 1983).

2.10 INTERACTIONS BETWEEN NITROGEN AND CARBON METABOLISM IN THE ROOT

The root is dependent on photosynthates translocated from the shoot for supply of C, energy and reductant (Stulen, 1986). Light has been shown to accelerate NO_3^- reduction which has been suggested to result from: 1) light stimulation of NO_3^- uptake, 2) light promotion of transfer of NO_3^- from storage to the metabolic pools, 3) light induced NR synthesis, 4) light activation of pre-existing NR or 5) photosynthetic provision of reductant for NO_3^- assimilation

in the leaves (Naik *et al.*, 1982). From comparison of respiration rates (O_2 consumption) and NR activity Stulen (1986) concluded that NO_3^- reduction in the root is limited by NADH supply.

Because roots are dependent on the supply of sugars from the shoot for growth, and considering the C requirements of N assimilation, it is possible that N assimilation could be an effective competitor for C in the root. Nitrate reduction was found to be more adversely affected than root growth at low endogenous sugar levels suggesting that NO_3^- reduction is a poor competitor for root carbohydrate resources (Radin *et al.*, 1978). Glucose additions to excised or detopped roots enhanced NO_3^- reduction, possibly through facilitation of NO_3^- movement from the storage pool into the metabolic pool (Oaks and Hirel, 1985). The dependence of NO_3^- assimilation in the root of intact plants on the provision of photosynthate is illustrated by the proportion of NO_3^- reduced in dark and light conditions in the root. Rufty *et al.* (1984) showed that uptake of NO_3^- in the dark was only slightly depressed (dark = 7.93 and light = 8.42 μmoles h^{-1}) but total plant NO_3^- reduction was significantly diminished in the dark. Of NO_3^- taken up in the dark by *Glycine max* 70% was accumulated in the root and only reduced, mostly in the shoot, during the subsequent light period (Rufty *et al.*, 1987). The lack of reduction of NO_3^- in the root in the dark has been linked to root carbohydrate content. In roots in the dark over twice as much NO_3^- was reduced as in the light (Aslam and Huffaker, 1982; Oji *et al.*, 1989) indicating the greater capacity of the leaves for NO_3^- reduction.

The sources of NADH in the root for NO_3^- reduction are possibly glycolysis, malate metabolism, the OPPP and mitochondrial dehydrogenases in combination with shuttle systems (Stulen, 1986). Sucrose translocated from the shoot to the root may provide a source of C for operation of glycolysis and the OPPP and malate transported from the shoot to the root may enter the TCA cycle to form NADH (Stulen, 1986). PEPc could also function in an anaplerotic role for the provision of C to the TCA cycle (Arnozis *et al.*, 1988). Naik and Nicholas (1984) provided evidence for the involvement of mitochondrial NADH sources, in the form of the TCA cycle intermediates, in NO_3^- reduction in the root of *Triticum aestivum*. Some participation of non-oxidative decarboxylation of pyruvate in *Triticum aestivum* root NO_3^- reduction was also suggested by these authors.

Nitrite reduction and NH_4^+ assimilation occur in the root plastid. Reductant may be transferred from the cytosol into the plastid via a TP shuttle or possibly be produced in the plastid. Dry *et al.* (1981) have shown that in root plastids, NO_2^- reduction was dependent on the supply of G6P. A negative correlation between NO_2^- accumulation under anaerobic conditions and the level of G6P led to the proposal by these authors that anaerobic metabolism of G6P depleted this metabolite leading to an accumulation of NO_2^-. Glucose-6-P was proposed to be utilized via the OPPP for the provision of NADPH for NO_2^- reduction (Dry *et al.*, 1981; Oji *et al.*, 1985). Bowsher *et al.* (1989) has provided evidence for the utilization of the OPPP for provision of reductant for NO_2^- reduction in *Pisum sativum* root plastids. This was suggested to occur through G6P dehydrogenase reduction of NADP which in turn reduces NO_2^- in the presence of a root electron carrier because NO_2^- reduction was most effectively supported by G6P.

2.10.1 Root respiration

Root respiration is an extremely important component of the C budget of the plant and may account for up to 30% of the daily photosynthate (Blacquière *et al.*, 1987). In *Zea mays* grown on nutrient solutions respiratory CO_2 loss has been reported to account for 39% of photosynthetic CO_2 assimilation and 46% of this respiratory C was lost from the roots (Lambers, 1985). In *Triticum aestivum* it was found that 30% of fixed C was translocated to the roots and 23% of this used in root respiration (Lambers *et al.*, 1982).

Variability in the fraction of carbohydrate translocated to the root and subsequently respired has been explained by Lambers *et al.* (1991) to be partially the result of variation in shoot : root ratios and root growth rates. Higher shoot : root ratios appear to be linearly correlated with root growth respiration. The proportion of photosynthate respired by roots decreases with increasing age possibly as a result of decreases in root growth and ion uptake (Lambers *et al.*, 1991). Root respiration is markedly dependent on the state of the shoot, probably as a result of limitations in substrate supply to the root (Bingham and Farrar, 1988). Root respiration rates have been found to correlate with root carbohydrate content and the turnover of ATP. Limitation of root respiration by carbohydrate has been demonstrated by a correlation between increased sugar content and respiratory activity of *Hordeum vulgare* after pruning of a portion of the roots (Bingham and Farrar, 1988).

Diel fluctuations of respiration rate in *Cucumis sativus* have been reported to correlate with fluctuation in soluble sugar concentrations (Lambers *et al.*, 1991). Root respiration rates are, however, not a simple function of carbohydrate supply and are often better correlated with ion uptake which in turn controls ATP turnover (Lambers *et al.*, 1991). Williams and Farrar (1990) suggested that respiration is under fine control of adenylates, but that the capacity of the respiratory system is determined by the supply of sucrose or the processes requiring metabolic energy. ATP production in root respiration can be formulated as:

$$r_{ATP} = m_{ATP} + \frac{1}{Y_{ATP}^{GR}} RGR + \frac{1}{U_{ATP}^{I}} NIR$$

where

	r_{ATP}	=	ATP production in root respiration
	m_{ATP}	=	ATP consumption in maintenance respiration
	$1/Y_{ATP}^{GR}$	=	ATP requirement for synthesis of cell material
	RGR	=	relative growth rate
	$1/U_{ATP}^{I}$	=	ATP requirement for anion uptake
	NIR	=	net rate of anion uptake.

This equation relates the rate of respiratory ATP production to maintenance, growth and ion uptake requirements (Lambers *et al.*, 1991).

2.10.1.1 'Alternative' respiratory pathway

Mitochondria of higher plants often contain an alternative, CN resistant, salicylhydroxamate (SHAM) sensitive, electron transport pathway which does not support phosphorylation (Lance *et al.*, 1985) and which may account for up to 50% of root respiration (De Visser and Lambers, 1983). In the 'alternative pathway' electrons from ubiquinone by-pass the cyanide sensitive cytochromes and pass instead through an alternative carrier (Lance *et al.*, 1985). High activity of this pathway would reduce the production of ATP (De Visser and Lambers, 1983) since there are no coupling sites on the alternative carriers and thus only one coupling site exists in the 'alternative pathway' instead of the three found in the cytochrome pathway (De Visser *et al.*, 1986).

The extent to which the 'alternative pathway' functions depends on the type of substrate being metabolized and the species involved. The activity of the 'alternative pathway' was found to account for between 0 and 14% of respiration in *Zea mays* and for about 4% of respiration

in *Triticum aestivum* when NADH was utilized as a substrate, although higher activities could be obtained with other substrates (Lance *et al.*, 1985). Under conditions where the activity of the cytochrome pathway is saturated, the 'alternative pathway' is engaged (Blacquière and De Visser, 1984). Weger *et al.* (1990), however, showed that the 'alternative pathway' in *Chlamydomonas reinhardtii*, although present, failed to engage at all when a functional cytochrome oxidase pathway was present.

Under conditions where the cytochrome pathway is saturated (high ATP/ADP ratio, saturating substrate level or uncoupled electron transport) this 'alternative pathway' may function as an 'energy overflow' mechanism for oxidation of carbohydrate in excess of that required by growth, storage and ATP production (Lambers, 1985). Bingham and Farrar (1988), however, found no evidence for the functioning of this pathway as a simple 'energy overflow' mechanism in *Hordeum vulgare* roots. The 'alternative pathway' has also been suggested to play a role as an 'energy overcharge' mechanism whereby additional energy may be produced to overcome limitations imposed by the capacity of the cytochrome pathway (Lambers *et al.*, 1991). Evidence for the functioning of the 'energy overcharge' mechanism has been provided by De Visser *et al.* (1986).

2.10.1.2 Respiration and nitrogen source

One of the important components of root respiration is the provision of ATP for ion uptake which has been termed 'salt respiration'. Lower rates of respiration are found with plants grown on low nutrient concentrations and although this may be related to the rate of ion uptake, it may also be the result of slower growth rate and lower maintenance costs (Lambers *et al.*, 1991). Caution should be exercised when interpreting respiration rates as these may be expressed as biochemical activity, CO_2 release or O_2 consumption and there may not be a correlation between the three measures.

When shoot and root respiration are analyzed (biochemical activity) the root has a lower growth efficiency and higher maintenance coefficient than the shoot. Johnson (1990) has identified ion uptake, N assimilation and ion efflux as being important determinants of respiration in roots. The uptake and reduction of N has been found to represent a major portion of growth respiration while re-uptake of ions to balance leakage was a significant proportion of maintenance respiration (Johnson, 1990). The rate of respiration was, however,

concluded by this author to depend strongly on the site of NO_3^- reduction within the plant, considering that NO_3^- reduction consumes 81% of the energy required for protein synthesis.

Nitrate and NH_4^+ absorption by roots elicited an immediate 160% increase in respiratory O_2 consumption in roots of *Pisum sativum*, 90% of which was attributable to the 'alternative pathway' (De Visser *et al.*, 1986). This was not accompanied by an immediate increase in shoot photosynthesis although this did increase after a 1 hour delay indicating that the respiratory increase was not due to increased photosynthesis, but rather *vice versa*. Granato and Raper (1989) found increased root respiration (CO_2 release) in *Zea mays* in the presence of NO_3^- and that respiration rate was correlated with the amount of reduced N in the root and the proliferation of root lateral branches (Granato *et al.*, 1989).

De Visser and Lambers (1983) showed no clear differences between respiration rates of NO_3^-- and NH_4^+-grown *Pisum sativum* although the activity of the 'alternative pathway' was slightly higher and the cytochrome pathway slightly lower in the NH_4^+-grown plants. Although De Visser *et al.* (1986) do not discuss differences between NO_3^- and NH_4^+ in terms of respiratory O_2 consumption their data show significantly higher rates of respiration, and particularly 'alternative pathway' activity, in NO_3^-- compared with NH_4^+-fed *Pisum sativum*. In contrast, Blacquière (1987) found that NH_4^+-grown *Plantago lanceolata* and *Plantago major* required more respiratory energy than NO_3^--grown plants. These differences in respiration rate, measured as O_2 consumption, were found to be due to a elevation of both the cytochrome and the 'alternative pathway' (Blacquière *et al.*, 1987). In roots of *Triticum aestivum* the 'alternative pathway' has been found to account for 40% of root respiration in NH_4^+-grown plants but NO_3^- grown plants respired almost exclusively via the cytochrome pathway (Barneix *et al.*, 1984b). Differences between the root respiration responses of different species to NO_3^- and NH_4^+ may be due to differences in the extent of NO_3^- reduction within the root.

Yemm and Willis (1956) found with excised *Hordeum vulgare* roots that respiration rates measured as CO_2 release were lower in roots supplied with NH_4^+ than those supplied with NO_3^-. Similar results have been observed in *Triticum aestivum* (Lips *et al.*, 1990). High rates of CO_2 release by roots of NO_3^-- compared with NH_4^+-grown plants may be the result of exchange of HCO_3^- for NO_3^- which may represent up to 20% of respiratory C loss (Barneix *et al.*, 1984b).

Synthesis of amides and amino acids would draw intermediates such as OAA and OG from the TCA cycle. With glucose and NH_4^+ as precursors the synthesis of amides (glutamine and asparagine) would follow the reaction,

$$1.5\,glucose + 4NH_3 + 2NAD \rightarrow Asparagine + Glutamine + 2NADH$$

and the NADH produced may be oxidized via the 'alternative pathway' when ATP and NADH requirements are low (De Visser and Lambers, 1983). Lambers *et al.* (1991), however, commented that the production of NADH in excess of ATP requirements is unlikely under steady state conditions, although it may occur upon introduction of N starved plants to NH_4^+. In roots engaged in reduction of NO_3^- any NADH produced would be completely consumed by NO_3^- reduction (De Visser and Lambers, 1983) and thus a decline in the activity of the 'alternative pathway' may be expected (Barneix *et al.*, 1984b).

Blacquière (1987) has produced a theoretical calculation of the energetic costs of NO_3^- and NH_4^+ respiration with complex dependence on a wide range of physiological processes. On the basis of these calculations Blacquière (1987) concluded that CO_2 production by NO_3^--grown roots would be higher than that of NH_4^+-grown roots, and this, taken together with the effect of N form on root respiratory O_2 consumption results in a higher RQ for the NO_3^--grown plants.

2.10.1.3 Phosphoenolpyruvate carboxylase activity in roots

The activity of PEPc forms an integral part of our understanding of the C economy of the root. The activity of this enzyme has largely been overlooked in literature dealing with root respiration although the activity of PEPc may reduce apparent respiratory CO_2 release from the root. A relationship between PEPc, malate and the capacity to synthesize amino acids in *Triticum aestivum* roots has been established (Oaks, 1986).

Roots of *Phaseolus vulgaris* fed NO_3^- showed no increase in PEPc activity while in NH_4^+-fed plant roots PEPc responded positively (Schweizer and Erismann, 1985). Similar results were obtained with several species including *Triticum aestivum* and *Zea mays* where PEPc activity was up to 380% higher in NH_4^+- than in NO_3^--fed plants (Arnozis *et al.*, 1988). These data strongly support the function of PEPc in providing an anaplerotic source of C for assimilation of NH_4^+ although the role of PEPc in providing a pH stat cannot be dismissed (See Sections

2.9.2 and 2.7 respectively). No quantitative assessment of the importance of PEPc as an anaplerotic source of C for assimilation of N within the roots of higher plants appears to exist as yet in the literature. Arnozis *et al.* (1988) have, however, suggested an alternative function for PEPc in the root. These authors have postulated that the high proportion of organic acids found in the xylem of NH_4^+- compared to NO_3^--fed plants could be the result of the activity of PEPc. It was proposed that the role of the carboxylates derived from PEPc was for ionic balance of xylem sap. Arnozis *et al.* (1988) made no quantitative assessment as to the importance of the two possible functions of PEPc activity in the roots (i.e. providing carboxylates for N assimilation or for xylem sap ionic balance).

3 METHODS AND MATERIALS

3.1 PLANT CULTURE

3.1.1 Wheat

Triticum aestivum L. cv. Gamtoos (donated by SASKO milling, South Africa) was pre-germinated in aerated water for 24 h before planting into vermiculite. After one week in vermiculite the plants were transferred into aerated hydroponic culture where they were grown for a further 3 weeks (unless otherwise specified) and supplied with Long Ashton nutrient medium (Hewitt, 1966) containing 4 or 12 mM KNO_3, NH_4Cl or NH_4NO_3 with iron supplied as Fe-EDTA (Table 3.1).

The stems of the plants were wrapped in foam rubber and wedged in a 2 cm long piece of 1.7 cm internal diameter garden hose wedged into a 2.5 cm hole in an opaque flat plastic lid placed over opaque plastic tanks. Hydroponic solutions (22 l per 8 plants) were replaced once a week for the first two weeks and thereafter twice a week. The pH of the medium (6.5) was monitored, and corrected if necessary, with either NaOH or HCl on a daily basis. Culture took place in a phytotron chamber with an irradiance of 500 μmol m^{-2} s^{-1} supplied by a mixture of 14 x 250 W Wotan (South Africa) metal-halide lamps, 21 x 150 W Wotan sodium vapour and 24 x 150 W Osram (South Africa) incandescent lamps. The photoperiod was 14 h long with 10 h full irradiance, the lamps being switched so as to simulate the diurnal change in light spectral quality and quantity. The day/night temperature was 25/18 °C and the day/night RH was 50/65%.

3.1.2 Maize

Zea mays L. cv. PNR 394 (donated by Agseeds, South Africa) was pre-germinated in aerated water for 24 hours before planting into vermiculite. After 5 days in vermiculite the plants were transferred into aerated hydroponic culture where they were grown for a further 10 days (unless otherwise specified) and supplied with Long Ashton nutrient medium (Hewitt, 1966) modified to contain 4 or 12 mM KNO_3, NH_4Cl or NH_4NO_3 with $FeSO_4$ as an iron source (Table 3.1).

Table 3.1 Nutrient concentrations supplied to hydroponically grown wheat and maize. Fe-citrate was used as an iron source only where specified. Either 4 or 12 mM nitrogen was used.

Salt	Concentration	
	mg l^{-1}	mM
Macronutrients		
$MgSO_4.7H_2O$	368.0	1.5
K_2SO_4	348.0	2.0
$CaCl_2.2H_2O$	588.0	4.0
$NaH_2PO_4.2H_2O$	104.0	0.67
$Na_2HPO_4.12H_2O$	239.0	1.5
Micronutrients		
H_3BO_3	8.58	0.1388
$MnSO_4.4H_2O$	4.64	0.0208
$ZnSO_4.7H_2O$	0.66	0.0023
$CuSO_4.5H_2O$	0.24	0.0033
$Na_2MoO_4.2H_2O$	0.06	0.00025
Iron source		
Fe-EDTA (wheat)	33.00	0.09
Fe-citrate (where specified)	306.10	1.25
$FeSO_4.7H_2O$ (maize)	7.67	1.25
Nitrogen		
KNO_3	0.40	4.0
KNO_3	1.21	12.0
NH_4Cl	0.32	4.0
NH_4Cl	1.28	12.0
NH_4NO_3	0.32	2.0 (4 mM N)
NH_4NO_3	0.96	6.0 (12 mM N)

The stems were wrapped in foam rubber and wedged in a 2.5 cm hole in an opaque plastic lid placed over opaque plastic tanks. Hydroponic solutions (22 l per 8 plants) were replaced once every 5 days for the first ten days and every second day thereafter. The pH of the medium (5.5) was monitored, and corrected if necessary with either NaOH or HCl on a daily basis. Culture took place in a phytotron chamber with an irradiance 1500 μmol m^{-2} s^{-1} supplied by a mixture of 28 x 400 W Wotan metal halide, 14 x 400 W Wotan sodium vapour and 14 x 150 W Osram incandescent lamps. The photoperiod was 14 h long with 10 h full

irradiance, the lamps being switched so as to simulate the diurnal change in light spectral quality and quantity. The day/night temperature was 35/25 °C and the day/night RH was 50/65%.

Experience indicated that maize cultured hydroponically with Fe-EDTA was prone to be chlorotic. For this reason Fe-citrate, as recommended by Hewitt (1966), was tried as an iron source. Although the plants grew superbly with this iron source there was concern over the possibility of heterotrophic citrate utilization and high levels of microbial activity in the nutrient medium. In order to test this possibility plants were grown on 1.25 mM $FeSO_4$, 1.25 mM $FeSO_4$+1.25 mM citrate and 1.25 mM Fe-citrate. The results convincingly show that the supply of citrate to $FeSO_4$ grown plants does affect growth, in particular shoot growth (Figure 3.1). The explanation for this may be that the citrate is acting as a heterotrophic substrate for the root, reducing the dependence of the root on the shoot for carbohydrate supply. The shoot may therefore be able to divert more C into growth. It has been shown that NR is capable of reducing Fe-citrate thereby releasing citrate for metabolic requirements (Campbell and Redinbaugh, 1984). For these reasons it was decided to use inorganic $FeSO_4$ as the iron source. Use was made of Fe-citrate in a ^{14}C-partitioning experiment (Section 3.6), account being taken of the heterotrophic function of this iron source.

In order to determine the optimum level of $FeSO_4$ it was supplied at 0.2, 0.4, 0.7 and 1.4 mM to maize with 4 mM NO_3^- as an N source (Figure 3.2). It was decided to supply a slightly supra-optimal concentration of 1.25 mM initially followed by a boost of 0.9 mM $FeSO_4$ after three days because of the known oxidation of $FeSO_4$ (Hewitt, 1966).

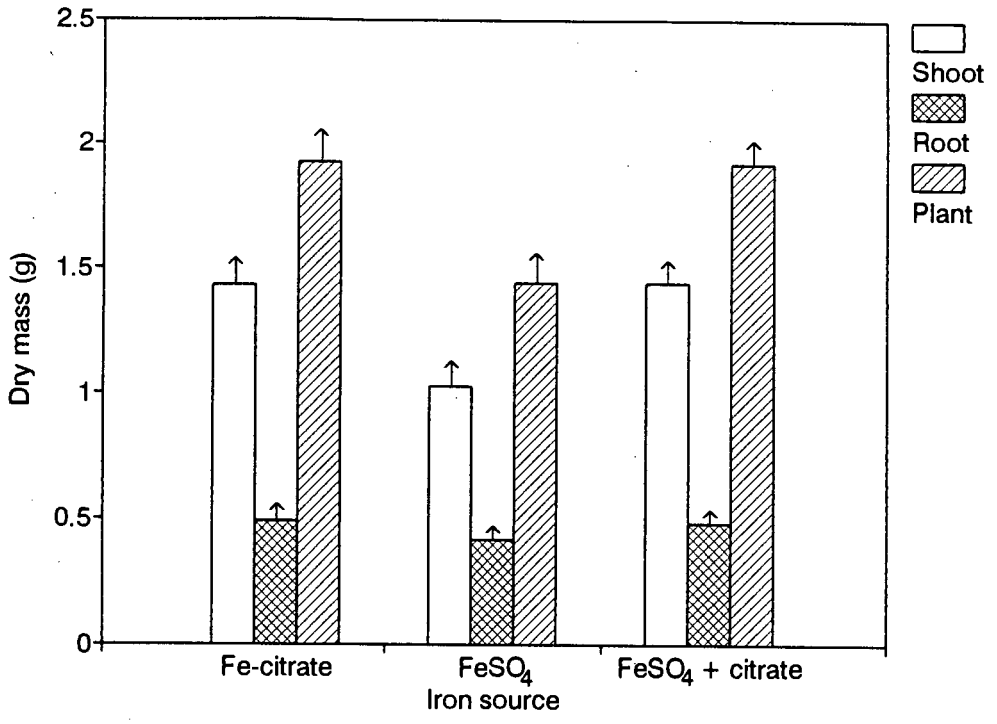

Figure 3.1 Comparison of the effect of 1.25 mM Fe-citrate, 1.25 mM $FeSO_4$ and 1.25 mM $FeSO_4$ + 1.25 mM citrate supplied in the nutrient medium on the dry masses of maize plants grown hydroponically for 10 days with 4 mM NO_3^- as a N source. Bars indicate the S.E.

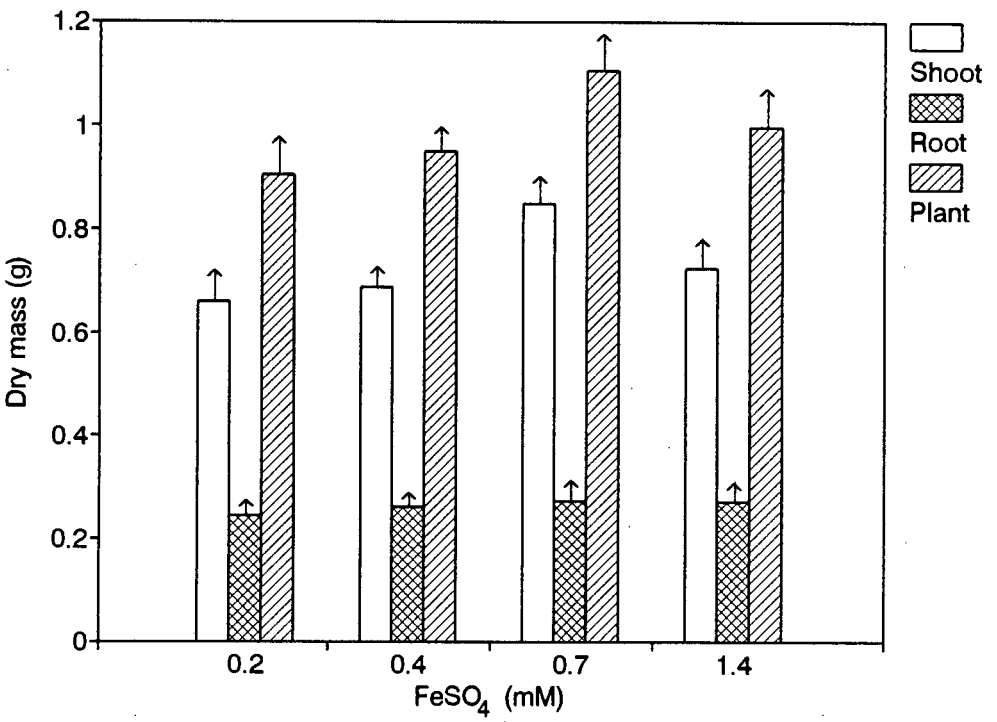

Figure 3.2 Determination of optimum concentration of $FeSO_4$ for maize growth. Plants grown hydroponically for 10 days with 4 mM NO_3^- as an N source. Bars indicate the S.E.

3.1.3 Split-root plant culture

Both wheat and maize plants were cultured with the seminal roots split into two different feeding solutions containing either NO_3^- or NH_4^+ as an N source. Wheat was grown on vermiculite for 1 week prior to transfer into hydroponic culture (as described above) with 4 or 12 mM NH_4NO_3 for 1 week. Maize was grown in vermiculite for 1 week. Thereafter the plants were transferred into split-root culture. The seminal roots were subjectively divided into two, odd roots pruned if necessary, and passed through plastic pipes in the lids of two opaque 1000 ml plastic bottles. At the split the roots were wrapped in foam rubber against injury and desiccation and the plants supported by a plastic frame. One root-half was supplied with Long Ashton nutrient medium containing NO_3^- and the other with NH_4^+. The solutions were aerated and changed every 48 h in the case of wheat (pH=6.5) and every 24 h in the case of maize (pH 5.5).

3.2 COMPARISON OF PLANT MASS AND GAS EXCHANGE CHARACTERISTICS ON VARIED NITROGEN NUTRITION

In order to establish the effect of the form and concentration of N on the plants, both wheat and maize (24 plants per treatment) were grown as indicated in hydroponics with 4 and 12 mM N supplied as NO_3^-, NH_4^+ and NH_4NO_3. The hydroponic tanks containing the plants were randomly arranged within the phytotron chamber.

3.2.1 Fresh and dry mass

The plants were divided into root and shoot components. The roots were washed and carefully blotted dry. The components were then weighed for fresh mass, dried in an oven at 80 °C for 48 hours and re-weighed. The shoot:root ratios and plant moisture contents were calculated.

The phytotron chamber had a characteristic light distribution pattern with lower light intensity toward the back of each bench. To account for this the position of the plants on the bench was incorporated in the analysis of variance. The results were subject to two- (factors: N form and bench position) and three-way (factors: N form, N concentration and bench

position) analysis of variance (Statgraphics Version 4.0, 1989). There was no consistent effect of the position of the plants within the phytotron chamber on the results.

3.2.2 Gas exchange characteristics

Photosynthetic rates of the mid-lamina portion of the youngest fully expanded leaves of 15 plants (wheat - 3 week old, maize 10 day old) from each treatment were measured using a portable ADC (Analytical Development Corporation, Hoddeston, England) LCA3 infrared gas analyzer (IRGA) connected to a Parkinson cereal leaf chamber. The IRGA measurements were performed in the phytotron chamber in which the plants were grown. Air was supplied to the IRGA through a 25 l buffering tank under positive pressure and the excess allowed to escape into the atmosphere. In the case of the wheat plants the photosynthetic area was calculated from measurements of the leaf width at either end of the leaf chamber while the maize leaves filled the chamber window (area = 11 cm^2). The flow rate was set to 300 ml min^{-1} for wheat and 500 ml min^{-1} for maize. The IRGA was set up to pass air at ambient RH over the leaf.

The ADC energy balance equation, used for calculation of the leaf temperature, was modified to reflect the lighting characteristics of the phytotron chamber used according to tables in the ADC LCA3 manual. Calculations of gaseous exchange variables were performed in a spreadsheet using the equations specified in the manual for the ADC LCA3. The following derived values were calculated: assimilation rate (A), stomatal conductance to water vapour (Gs), intercellular CO_2 concentration (C_i), transpiration rate (E) and water use efficiency (WUE, mmol CO_2 per mol H_2O). These derived quantities were used for two- (factors: N form and bench position) and three-way (factors: N form, N concentration and bench position) analysis of variance (Statgraphics Version 4.0, 1989).

3.3 GROWTH ANALYSIS

3.3.1 Biomass, leaf areas and moisture contents

Plants (24 to 32 per treatment) were harvested at regular intervals and divided into leaf, stem and root components. The tanks containing the plants were arranged so that equal numbers were at the front and back of each bench to average out any possible 'bench' effects. The fresh mass of each component and the photosynthetic area of leaves was determined using a

Licor LI-3000 (Nebraska, USA) area meter. Fresh mass of root material was determined after careful blotting of roots to dry off excess water. Dry mass was determined after drying of the plants in a ventilated oven for 48 h at 80 °C.

Fresh and dry mass, leaf area, shoot:root ratio, specific leaf area (unit leaf area per unit dry mass) and moisture content values were subject to covariate analysis of variance (covariate: time; factor: N form) using Statgraphics Version 4.0 (1989).

3.3.2 Gas exchange characteristics

Photosynthetic rates of the mid-lamina portion of the youngest fully expanded leaves of 24 to 32 plants per treatment (wheat and maize) were measured at regular intervals using a portable ADC LCA2 IRGA with DL2 data logger (Analytical Development Corporation, Hoddeston, England) connected to a Parkinson cereal leaf chamber. In wheat the measurements were performed on a weekly basis while in maize the measurements were more frequent. The IRGA measurements were performed in the phytotron chamber in which the plants were grown. Air was supplied to the IRGA through a 25 l buffering tank under positive pressure and the excess allowed to escape into the atmosphere. In the case of the wheat plants the photosynthetic area was calculated from measurements of the leaf width at either end of the leaf chamber while the maize leaves filled the chamber window (area = 11 cm^2). The flow rate was set to 300 ml min^{-1} for wheat and 500 ml min^{-1} for maize. The IRGA was set up to pass air at 0% RH over the leaf. Each leaf was retained in the Parkinson leaf chamber for less than one minute.

Data consisting of irradiance, air temperature, RH, CO_2 concentration (reference and analysis) were collected on the data logger and calculations performed in a spreadsheet using the formulae specified in the ADC LCA2 manual to yield assimilation rate, stomatal conductance to water vapour, intercellular CO_2 concentration, transpiration rate and water use efficiency. The ADC energy balance equation, used for calculation of the leaf temperature, was modified to reflect the lighting characteristics of the phytotron chamber used according to tables in the ADC LCA3 manual. All of the derived values were subject to covariate analysis of variance (covariate: time; factor: N form) using Statgraphics Version 4.0 (1989).

3.4 CO_2 RESPONSE CURVES ($A:C_i$)

The response of photosynthetic CO_2 assimilation (A) to varied intercellular CO_2 concentration (C_i) was determined using the mid-lamina portion of the youngest fully expanded leaves of 3 plants from each N treatment using a portable ADC LCA3 IRGA connected to a Parkinson's cereal leaf chamber as described in Section 3.2.2. The IRGA measurements on maize were performed in the phytotron chamber in which the plants were grown. Wheat was transferred to a phytotron chamber delivering 1500 μmol m^{-2} s^{-1} irradiance 48 h prior to the experiment, all other conditions being maintained the same as for the growth conditions of wheat (Section 3.1.1). Ambient air was supplied to the IRGA through a 25 l buffering tank under positive pressure and the excess allowed to escape. The CO_2 concentration of the gas supply to the leaf was varied using the built in facility of the ADC LCA3 for this purpose. Higher than ambient CO_2 concentrations (approximately 1500 ppm) were supplied by mixing industrial grade pure CO_2 with ambient air through precision flow control needle valves (EAS 2000-F01, HyFlo, South Africa) . In the case of the wheat plants the photosynthetic area was calculated from measurements of the leaf width at either end of the leaf chamber. The maize leaves filled the chamber window (area = 11 cm^2). The flow rate was set to 300 ml min^{-1} for wheat and 500 ml min^{-1} for maize. The IRGA was set up to pass air at ambient RH (RH approximately 50%) over the leaf.

The properties of $A:C_i$ curves have been described by Lawlor (1987). The rapid loss of CO_2 at low intercellular CO_2 concentrations is the result of combined photorespiratory activity and dark respiration, with photorespiration enhanced by the low intercellular CO_2 concentrations. The initial slope ($\delta A/\delta C_i$) is controlled by the efficiency of carboxylation and is a reliable predictor of the carboxylation rate ($V_{Rubisco}$). The portion of the curve where $\delta A/\delta C_i$ approaches zero due to limitation in the supply of substrate (RuBP) is termed J_{max} due to the fact that substrate supply is limited by the maximum capacity of the photosynthetic electron transport pathway. The value A_o corresponds to the CO_2 assimilation rate in the absence of stomatal limitations (i.e. intercellular CO_2 concentration = ambient CO_2 concentration). At ambient CO_2, the intercellular CO_2 concentration is less than the ambient CO_2 concentration and the corresponding CO_2 assimilation rate is A_i, which is the CO_2 assimilation rate limited by stomatal conductance.

Least squares first order rate equations with offset were fitted to the $A:C_i$ data using the program Enzfitter Version 1.03 (1987, Elsevier Biosoft, Cambridge, UK). Linear regression was performed on the $A:C_i$ data between 0 ppm and 50 ppm CO_2 to determine the $\delta A/\delta C_i$ where internal CO_2 concentration approaches zero.

3.5 POST-ILLUMINATION GAS EXCHANGE

Using the ADC LCA3 as described above (Section 3.2.2), youngest fully expanded leaves of 6 plants of both wheat and maize from each N treatment (NO_3^- and NH_4^+) were allowed to equilibrate for 5 minutes inside the Parkinson leaf chamber. The chamber window was then covered to completely obscure irradiation. Measurements were recorded at frequent intervals for a 30 minute period. The average values were plotted for each time interval.

3.6 $^{14}CO_2$ PARTITIONING

3.6.1 Supply of $^{14}CO_2$ to plants

Hydroponically grown plants were transplanted from troughs into 1000 ml light tight plastic containers containing fresh Long Ashton nutrient medium (Table 3.1). The plants were enclosed in a perspex canopy (0.7 m x 0.2 m x 0.6 m) sealed with a moat of water. Circulation of the canopy atmosphere (1000 ml min^{-1}) through an ADC Model 225 MK 3 bench IRGA (Analytical Development Corporation, Hoddeston, England) in a closed circuit allowed continuous monitoring of CO_2 concentration within the canopy. The canopy was darkened when the CO_2 concentration reached 340 ppm and $^{14}CO_2$ was then released into the gas circuit from 50 to 100 μCi $NaH^{14}CO_3$ (Amersham International, UK) using 50 ml 10% (v/v) lactic acid as an acidifying agent. Irradiance was restored after 10 minutes had been allowed for equilibration. Plants were retained in the canopy (usually for 45 to 60 minutes) until the CO_2 concentration was reduced and no change in CO_2 concentration could be detected over a 10 minute interval (approximately 60 ppm in the case of wheat and 20 ppm in the case of maize) whereupon the plants were returned to their tanks for either 9 or 24 h prior to harvest. Each treatment was at least in triplicate.

3.6.2 Harvest of $^{14}CO_2$ fed plants and sample analysis

Plants were divided into leaf and root components, weighed and homogenized with an Ika-Ultra-Turrax T25 in 80% (v/v) cold ethanol and extracted for 24 h at 4 °C with occasional agitation. Thereafter the samples were re-homogenized and filtered through Whatman No. 1 paper of known weight.

The extract and residue were separated into the following fractions:

a) ethanol soluble compounds (lipid and lipid soluble compounds),
b) free water soluble neutral and positively charged compounds (mainly carbohydrates)
c) free water soluble negatively charged compounds (mainly amino acids and soluble proteins),
d) bound (HCl-hydrolysed) neutral and positively charged compounds (polysaccharides),
e) bound (HCl-hydrolysed) negatively charged compounds (insoluble proteins),
f) Residue unhydrolysed by HCl (structural material).

3.6.2.1 Ethanol soluble fraction

The 80% ethanol soluble fraction was evaporated to 20 ml and then partitioned twice with petroleum ether (40 to 60 °C BP). After thorough mixing with petroleum ether the samples were allowed to stand for 1 h prior to freezing and decanting the petroleum ether after which the interface was washed with 10 ml fresh petroleum ether. The petroleum ether was evaporated almost to dryness and the residue made up to 100 ml with 96% ethanol. This fraction was taken to represent the ethanol soluble, water insoluble, components of the plants (designated EtOH).

3.6.2.2 Water soluble fraction

The water soluble component, remaining after petroleum ether partitioning, was passed through 6 x 1 cm columns of Dowex 50W-X8 H^+ (100 to 200 mesh particle size) ion exchange resin prepared according to Atkins and Canvin (1971). The water soluble neutral carbohydrate fraction was eluted from the column with 50 ml distilled water and the

remaining negatively charged amino acid and organic acid fraction with 100 ml 2 M HCl. These fractions are henceforth referred to as water soluble carbohydrate (Wc) and water soluble protein and amino acids (Wa).

The Dowex columns were tested for their reproducibility. The same sample was passed through 3 different columns. The standard error as a percentage of the mean for the neutral fraction was 1.76% and for the negatively charged fraction 1.13%. From this test it was concluded that the columns were reliable for separation of the neutral and negatively charged fractions. The fate of soluble protein (Rubisco) on the columns was tested by loading 4 ml of a 1 mg Rubisco ml^{-1} (Sigma) solution onto the column and collecting the eluate in 5 ml fractions. The 5 ml fractions were then reacted with ninhydrin to test for the presence of the protein. Elution with 50 ml H_2O yielded no reaction with ninhydrin. Elution with 100 ml 2 M HCl yielded weak reaction with ninhydrin after 15 ml eluate had passed through the column. Most of the protein was eluted in 20 to 35 ml of 2 M HCl and no further protein was eluted after 40 ml. Thus soluble proteins were eluted in the acid fraction.

3.6.2.3 Bound fraction

The residue was dried at 80 °C for 24 hours before weighing and milling with a Wiley mill using a 0.5 mm mesh size. Samples of 0.2 g milled material were hydrolysed with 10 ml 6 M HCl in hermetically sealed McCartney bottles at 110 °C for 24 h. The hydrolysate was filtered through Whatman No. 1 paper of known weight and the residue, designated structural material (Struct.), dried at 80 °C for 24 h.

The dried residue was weighed and sub-samples of 0.05 g taken in triplicate for oxidation on a Packard Tricarb Model 306 Sample Oxidizer (Illinois, USA). The sub-samples were enclosed in a filter paper envelope which was placed into a platinum wire cage automatically heated electrically in a 100% O_2 gas stream to result in ignition of the sample. Gas released by oxidation of the sample and paper was collected in a column of 8 ml Carbo-sorb (Packard). The Carbo-sorb was then washed from the column into a scintillation vial using 12 ml Insta-gel scintillation fluid (Packard).

The filtrate was twice evaporated at 70 °C to dryness under vacuum (-100 kPa) on a Buchler Instruments Evapo-mix (Fort Lee, USA). The dried sample was resuspended in distilled

water, the pH adjusted to neutrality with 0.1 M NaOH, and volume adjusted to 20 ml. The filtrate was passed through 6 x 1 cm Dowex 50W-X8 columns as described for the water soluble fraction (Section 3.6.2.2) to separate out the neutral carbohydrates (polysaccharide derived) and the negatively charged amino acid (insoluble protein derived) fractions. These fractions are henceforth referred to as the bound carbohydrate (Bc) and bound protein (Ba) fractions respectively.

All samples were counted in a Beckman Instruments LS 5000 TD liquid scintillation counter (California, USA), with Insta-gel scintillation fluid. The scintillation counter was programmed to provide DPM with automatic quench correction through use of the h number technique. Of each sample 0.5 ml was counted in 5 ml scintillation fluid apart from the shoot ethanol soluble fraction where quenching due to chlorophyll was problematic and thus only 0.1 ml sample was counted (volume decided by addition of varying volumes of sample to standards of known DPM).

The results were expressed as Bq mg^{-1} fresh mass and were subjected to Student's T tests (Parker, 1979) for differences between NO_3^-- and NH_4^+-fed plants. As a result of variations in the ability of the plants to absorb CO_2, each plant has a variable amounts of total $^{14}CO_2$ label. In addition, in some experiments not all the plants could be simultaneously supplied with $^{14}CO_2$. The results were therefore expressed on a percentage basis (percentage that any one fraction makes up of the total for a particular plant part). Student's T tests were performed on the arcsine transformed data to attach significance values to data expressed on a percentage basis. The equation used for the arcsine transform was taken from Zar (1984),

$$p' = \frac{1}{2}\left[\arcsin\sqrt{\frac{X}{n+1}} + \arcsin\sqrt{\frac{X+1}{n+1}}\right]$$

where p' = transformed proportion
X = numerator of proportion ($^{14}CO_2$ in one fraction)
n = denominator of proportion (Total $^{14}CO_2$ counts).

The results were also expressed as shoot : root ratios for each fraction and as [^{14}C]carbohydrate : [^{14}C]amino acid ratios (Wc+Bc+Struct. : Wa+Ba) for shoot and root components. These results were subjected to Student's T tests (Parker, 1979) to determine the significance of the differences between NO_3^-- and NH_4^+-fed plants.

3.7 DETERMINATION OF ^{14}C SHOOT : ROOT RATIOS

Hydroponically grown plants (6 replicates per treatment) were supplied with $^{14}CO_2$ as detailed for $^{14}CO_2$ partitioning (Section 3.6.1). After 24 h the plants were separated into shoot and root components and dried at 80 °C for 48 h. The plants were milled in a Wiley mill (mesh 0.5 mm) and 0.05 g material taken for oxidation in a Packard Tricarb Model 306 Sample Oxidizer to determine ^{14}C label present in the root. The results were expressed on the basis of radiocarbon activity per gram root dry mass in units Bq g^{-1} and the shoot : root ratios calculated. These results were subjected to Student's T tests (Parker, 1979).

3.8 DETERMINATION OF INORGANIC N UPTAKE RATES

Plants to be used for uptake measurements were transferred into fresh nutrients 16 h before the experiment was initiated. At the start of the experiment the plants were transferred into fresh nutrients and then sub-samples (4 ml) were withdrawn every 2 h from nutrient solutions (4 replicates per treatment) of both wheat and maize plants (4 and 12 mM N) over a 28 h period and taken for analysis of NO_3^- and NH_4^+ content. During the course of the 28 h period the lights were switched off for a period of 8 h, but temperature was maintained at a constant level (wheat 25 °C, maize 30 °C). The mass of each plant container was determined at each sampling interval to determine the volume of nutrient solution present to allow correction for evaporative and transpirational water loss.

3.8.1 Nitrate

Szechrome NAS reagent (diphenylamine sulfonic acid chromogene) obtained from the Ben Gurion University of the Negev, Applied Research Institute (Israel), was used for NO_3^- determination. Equal volumes of 85 to 86% phosphoric acid (AR) and 95 to 97% sulphuric acid (AR) were mixed and allowed to stand for one week to allow the NO_3^- content of the acid to diminish. The Szechrome reagent was added (5 g l^{-1}) to the mixed acid and the solution shaken until solubilized, gas being released from the container from time to time. To 0.5 ml of unknown, 2.5 to 5 ml reagent was added in a test tube. The tube was sealed, inverted to mix and 5 to 60 minutes after mixing the absorbance at 570 nm determined in a Beckman Model 42 spectrophotometer. The standard curve was prepared using 0.5 ml of KNO_3 solutions giving concentrations of 20, 40, 80, 120, 160, 200 nM.

3.8.2 Ammonium

To 0.5 ml sample 2.5 ml of Nessler's reagent (BDH) was added and mixed. The absorbance at 420 nm was determined 90 s after mixing using a Beckman model 42 spectrophotometer. The standard curve was prepared using 0.5 ml of NH_4Cl solutions giving concentrations of 100, 200, 300, 400, 500 nM.

3.9 ^{15}N ANALYSIS

3.9.1 Sample preparation for analysis of total ^{15}N enrichment

The method employed for sample preparation and analysis was adapted from Deignan and Lewis (1988). Wheat and maize plants (3 replicates per treatment), grown as described (Section 3.1), were transferred to aerated 500 or 1000 ml containers respectively (light tight with respect to roots), containing fresh Long Ashton nutrient medium (Table 3.1) with 4 or 12 mM N enriched to 50% A%E $K^{15}NO_3$ or $^{15}NH_4Cl$ (Amersham International, UK) for 8 daylight hours. Thereafter the plants were divided into shoot and root components, the latter thoroughly washed in deionized water and blotted dry. The material was weighed and killed in liquid N. The plant components were then dried at 80 °C for 48 hours prior to re-weighing, milling with a Wiley mill using a 0.5 mm mesh (Arthur h Thomas, California, USA) and micro-Kjeldahl digestion in 35 cm long tubes with 3 ml 34% (w/v) salicylic acid in concentrated A.R. sulphuric acid (BDH, England) and a 1 g sodium-sulphate-mercuric chloride catalyst tablet (BDH). Standards using 0.01 to 0.1 g Titriplex ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$, Merck, Germany) were prepared for estimation of the efficiency of digestion (typically > 96%). The digest was made up to 15 to 25 ml and 2 to 5 ml aliquots were taken for steam distillation in a Büchi (Switzerland) distillation unit. The samples were alkalinized with 10 ml 50% (w/v) NaOH containing 2.5% (w/v) $Na_2S_2O_5$. The distillate (30 ml) was collected in 2 ml 0.02 M HCl (Titrisol, Merck) and back titrated with 0.005 M NaOH (Titrisol, Merck) using an automatic Schott titrator (TR 85, T80/20, Germany) to pH 5.2. Immediately following back-titration the samples were acidified with 2 ml 0.08 M AR H_2SO_4 (BDH).

3.9.2 Sample preparation for analysis of partitioning of ^{15}N

The method for sample preparation and analysis was adapted from Lewis and Chadwick (1983). Wheat and maize plants (3 replicates of each treatment) grown as described (Section 3.1) were transferred to aerated 500 or 1000 ml light tight containers respectively containing fresh Long Ashton nutrient medium (Table 3.1) with 12 mM N enriched to 50% A%E $K^{15}NO_3$ or $^{15}NH_4Cl$ (Amersham International, UK) for 8 daylight hours. Thereafter the plants were divided into shoot and root components, the latter thoroughly washed in deionized water and blotted dry. The material was weighed and killed in liquid N before homogenization in chilled 80% (v/v) ethanol ($\approx$ 10 ml g^{-1}) with an Ika-Ultra-Turrax T25 (Janke and Kunkel, Germany) dispersion tool. Extraction was allowed to proceed in a cold room (4 °C) for 24 h with occasional shaking prior to re-homogenization. The material was then filtered through Whatman No. 1 filter paper and the residue washed three times with approximately 20 ml 80% ethanol.

The residue, henceforth designated the insoluble amino-N fraction (Insol. aN), contained organic N insoluble in 80% ethanol (insoluble proteins). The 80% ethanol soluble fraction was separated into inorganic N (NO_3^- and NH_4^+) and organic N (amino acids and soluble proteins). The 80% ethanol soluble filtrate was evaporated to 20 ml under an air stream and 4 ml of this passed through 6 x 1 cm columns of Dowex 50W-X8 H^+ (100 to 200 mesh particle size) ion exchange resin (BDH) prepared according to Atkins and Canvin (1971). The water soluble NO_3^- fraction was eluted from the column with 50 ml distilled water and the remaining organic+ammonium fraction with 100 ml 2 M HCl.

3.9.2.1 Insoluble amino-nitrogen fraction (Insol. aN)

The filtered residue was dried in an oven at 80 °C for 24 h and then weighed and milled with a Wiley mill using a 0.5 mm mesh. Duplicate 0.15 g samples were digested and distilled as described for total ^{15}N (Section 3.9.1).

3.9.2.2 Ammonium fraction

Of the soluble fraction duplicate 2 to 5 ml aliquots were distilled with 0.2 g AR MgO_2 (BDH - previously heated to 600 °C in a muffle furnace for 2 h to remove CO_2 which may otherwise interfere with the back-titration) as a base. The reason for the use of MgO_2 as a base is that it is weak in comparison to the NaOH normally used and does not liberate N other than NH_4^+ during limited distillation. The distillate (20 ml) was collected and titrated as described for total ^{15}N analysis (Section 3.9.1).

3.9.2.3 Nitrate fraction

The NO_3^- containing fractions (54 ml) collected from the Dowex columns were reduced in volume under an airstream to 20 ml and duplicate 2 to 5 ml aliquots distilled in a Büchi distillation unit with 0.3 g Devarda's alloy (BDH) as a reductant and 0.2 g MgO_2 as a base. The distillate (30 ml) was collected and titrated as described for total ^{15}N analysis (Section 3.9.1).

3.9.2.4 Ammonium + soluble amino-nitrogen fraction

The 100 ml 2 M HCl eluate from the Dowex columns was evaporated under an air stream to 20 ml. Duplicate 10 ml aliquots were digested and distilled as described for total ^{15}N analysis (Section 3.9.1).

3.9.2.5 Soluble amino-nitrogen fraction (Sol. aN)

The soluble amino-N fraction was calculated from the difference between the ammonium+soluble amino-N fraction and the NH_4^+ fraction.

3.9.3 Determination of ^{15}N enrichment

The distillate of each fraction was heated under an air stream to facilitate evaporation so that N concentration was increased to 25 μg per 0.2 ml sample. The samples were then prepared for ^{15}N analysis using a Jasco molecular emission spectrophotometer (Japan Spectroscopic

Co., LTD., Tokyo, Japan) according to the sodium hypobromite method of Faust (1967). The samples were reacted under vacuum with alkaline hypobromite to release N_2 gas,

$$2\ NH_3 + 3\ NaOBr \rightarrow N_2 + 3\ H_2O + 3\ NaBr.$$

The vacuum system consisted of a high vacuum pump capable of creating a vacuum of 0.1 kPa connected in series with a mercury diffusion pump yielding a final pressure of 1.0 Pa. Two liquid N cold traps were used to reduce water vapour pressure of the system.

The percentage enrichment was calculated as,

$$\%E = \frac{100}{2G\left(\frac{N_{28}}{N_{29}}\right)+1}$$

where $\%E$ = percentage enrichment
N_{28}, N_{29} = peak heights of $^{14}N^{14}N$ and $^{15}N^{14}N$ respectively
G = gain.

This was corrected using a standard curve prepared for the Jasco analyzer with known standards and the natural abundance of ^{15}N subtracted,

$$A\%E = (10.015 \times \%E - 0.192) - 0.3663$$

where $A\%E$ = corrected atom percent enrichment.

The N concentration and the percentage enrichment figures were used to calculate the ^{15}N uptake in units of $\mu g\ ^{15}N\ g^{-1}$ dry mass or fresh mass h^{-1}. The results were subjected to Student's T test (Parker, 1979) and the results shown on the graphs. The results were also expressed on a percentage basis (percentage that any one fraction makes up of the total for a particular plant part). Student's T tests were performed on the arcsine transformed data to attach significance values to data expressed on a percentage basis. The equation used for the arcsine transform is given in Section 3.6.2.3.

3.10 COLLECTION AND ANALYSIS OF XYLEM SAP

3.10.1 Wheat

Hydroponically grown plants (12 replicates per treatment) were supplied with $^{14}CO_2$ as detailed for $^{14}CO_2$ partitioning (Section 3.6.1). After 24 h the shoots were cut from roots leaving a stump of about 1 to 2 cm length. The bleeding sap exuded from the roots was collected with glass capillary tubes for 1 h after cutting as specified by Lewis and Chadwick (1983). Sap was also collected from shoots by pneumatic extrusion using a PMS Instrument Company Schölander pressure chamber (Oregon, USA). Very little bleeding sap was exuded from the roots of wheat plants, especially those grown on NH_4^+, and thus sap extruded under pressure was utilized for analyses.

3.10.2 Maize

Hydroponically grown plants (12 replicates per treatment) were supplied with $^{14}CO_2$ as detailed for $^{14}CO_2$ partitioning (Section 3.6.1). After 24 h the shoots were cut from roots leaving a stump of about 1 to 2 cm length. The bleeding sap extruded from the root was collected with glass capillary tubes for 1 h after cutting as specified by Lewis and Chadwick (1983). Attempts were made to collect sap from shoot and root material placed into a Schölander pressure chamber but this was on the whole unsuccessful due to the compressibility of the tissue. Thus bleeding sap, which was copiously extruded from the cut root, was relied on for further analyses.

3.10.3 ^{14}C content

The xylem sap (10 to 50 μl) was taken for counting in a Beckman LS 5000 TD liquid scintillation counter with 5 ml Insta-gel scintillation fluid. These results were subjected to Student's T tests (Parker, 1979).

3.10.4 Nitrate concentration

The xylem sap was appropriately diluted and the NO_3^- concentration determined using Szechrome NAS reagent as specified previously (Section 3.8.1). These results were subjected to Student's T tests (Parker, 1979).

3.10.5 Separation and quantitative determination of amino acid content

A Beckman Instruments 120C amino acid analyzer was used to separate amino acids contained in approximately 200 μl xylem sap. The sample was loaded onto a 22 cm column of Beckman UR 30 spherical ion-exchange resin. Three lithium citrate buffers, pH 2.83, 3.70 and 3.75 (Kedenburg, 1971) were used. The separated components were automatically mixed with ninhydrin and the mixture heated in a water bath to form the characteristic purple/blue colour. The absorbance of the samples was determined at 540 nm and recorded on a Beckman 125 digital integrator. The sample amino acid concentrations were calculated from the integrator recordings (area under peak) and internal standards,

$$a = \frac{IR_i}{STD_{Ei}} \times 0.5 \times \frac{NL_E}{NL_I} \times \frac{200}{V}$$

where

	a	=	amino compound concentration (μmol ml^{-1})
	IR_i	=	integrator reading for ith sample amino acid
	STD_{Ei}	=	integrator reading for ith external standard amino acid (2 μmol ml^{-1})
	NL_E	=	integrator reading for external standard nor-leucine
	NL_I	=	integrator reading for internal standard nor-leucine (0.5 μmol ml^{-1})
	V	=	sample volume (μl).

The amino compounds separated were: aspartic acid (Asp), threonine (Thr), serine (Ser), asparagine (Asn), glutamic acid (Glu), glutamine (Gln), proline (Pro), glycine (Gly), alanine (Ala), valine (Val), cysteine (Cys), methionine (Met), isoleucine (Ile), leucine (Leu), tyrosine (Tyr), phenylalanine (Pal), γ-amino butyric acid (Gab), lysine (Lys), histidine (His), arginine (Arg) and the concentration of NH_4^+ was also determined. The amino compounds were classed into the categories of: asparagine, glutamine, and 'others' for graphing purposes. Using the number of C and N atoms in each amino acid the C:N ratio for each sample was calculated. The results for each amino compound, NH_4^+ concentration, total amino compound content and overall C:N ratio were subjected to Student's T tests (Parker, 1979).

3.10.6 Transpiration rate determination

The rate of transpiration, an indirect measurement of the flow rate of xylem sap from root to shoot, was gravimetrically determined for each nutrient regime over an 5 h period. The plants (4 replicates) were maintained in plastic containers with a constant air supply. Controls (4 replicates) without plants were similarly treated. Each container was weighed every half hour. The controls were subtracted and the rate of transpiration expressed on the basis of root dry mass. Thus it was possible to calculate:

a) the amount of ^{14}C label leaving the root via the xylem sap

b) The quantity of amino compounds, NO_3^- and NH_4^+ leaving the root in the xylem stream.

These results were subjected to Student's T tests (Parker, 1979).

3.11 ROOT RESPIRATION RATE DETERMINATION

For these experiments the plants were grown in the normal hydroponic tanks, but the hypocotyls were inserted through the plastic lids appropriate for 1000 ml plastic bottles, so as to limit damage to the plants upon later transfer to these bottles. Each lid was fitted with a short 2 cm piece of 1.7 cm internal diameter garden hose through which the foam rubber wrapped plants were inserted. Each lid was equipped with one double sided and one single sided nipple. Ten day old maize and 3 week old wheat plants, supplied with fresh nutrient medium 24 h prior to the experiment, were transferred into 1000 ml opaque plastic bottles containing fresh nutrient medium and the bottles sealed with plastic lids (6 replicates per treatment). The inside of the double sided nipple was connected to a stiff plastic tube reaching to the bottom of the bottle. The outside of the double sided nipple was initially connected to the air supply and the single sided nipple was left unconnected initially.

3.11.1 Respiratory CO_2 release

Respiratory CO_2 release was measured using an ADC Model 225 MK3 bench IRGA set up in differential mode. In this mode the IRGA provided a resolution of $\approx$ 1 ppm. Gas from a cylinder of medical air (Afrox, South Africa) was passed through the root medium and the respiratory CO_2 release rate determined. The gas from the medical air cylinder at relatively high pressure was split into two streams. One stream was passed through a volume control

tap directly into the reference cell of the IRGA. The other stream was passed through a pressure regulator and then into a 12 port manifold. Each port on the manifold was equipped with a high pressure precision needle valve (EAS 2000-F01, HyFlo, South Africa) for precise flow rate control. This assembly was designed to provide accurate control over the flow of gas to each plant root without variation in CO_2 concentration. The pressure regulator ensured that any perturbation in the gas flow through any one of the manifold ports had no effect on the flow through other ports.

Six plants from each treatment were connected to the manifold system set to deliver 500 ml min^{-1} at each port. Each container was sealed using 5% Agar No. 3 (Oxoid, England) applied to the foam rubber wrapped around the plant shoot. The plants were allowed to equilibrate for a period of three hours prior to measurement to overcome problems associated with maintaining equilibrium between the gas and water phases of CO_2 (Browse, 1979). Pumps on the IRGA were used to sample gas from the single sided nipple on each bottle at a flow rate of 100 ml min^{-1}, the remaining gas being allowed to leak away. The gas was passed through the analysis cell of the IRGA and the Δppm recorded. These measurements were repeated three times half hourly. The plants were then harvested, dried at 80 °C for 48 h and weighed. The respiration rate was then expressed on the basis of root dry weight using the equation,

$$R_{CO_2} = \frac{\Delta ppm}{W} \times \frac{1}{22.4} \times \frac{FR}{60000} \times \frac{(T+273)}{273} \times \frac{1.013}{P}$$

where
R_{CO2} = root respiration (nmol g^{-1} s^{-1})
Δppm = analysis - reference CO_2 concentration
W = root mass (g)
FR = flow rate gas over root (ml min^{-1})
T = temperature of nutrient solution (°C)
P = atmospheric pressure (bar).

These results were subjected to Student's T tests (Parker, 1979).

3.11.2 Respiratory O_2 consumption

A Clark type Orion Research Model 97-08 O_2 electrode (Massachusetts, USA) with built in temperature compensation was used to measure root respiratory O_2 consumption. Due to physical constraints it was difficult to introduce the electrode into the bottle in which the plant was maintained. For this reason the electrode was sealed in a 100 ml bottle provided with an

inlet and outlet port arranged to ensure good circulation of the solution passed over the electrode.

Immediately prior to measurement of O_2 consumption the plant was disconnected from the gas supply. Solution from the bottles in which each plant was maintained was withdrawn through the double sided nipple using a peristaltic pump. The solution was pumped over the O_2 electrode and returned to the bottle through the single sided nipple. A 'T' connection with a long narrow diameter tube open to the atmosphere was placed between the pump and electrode to alleviate pressure related variability. The flow rate used was 300 ml min^{-1} ensuring good circulation of the nutrient solution.

The O_2 electrode was connected to an Orion Research Model 701 digital pH meter. The pH meter was in turn connected through a linear instrument amplifier (gain = 10) to a PC-74A analog-to-digital converter card (Eagle Electric Co. (PTY) LTD, South Africa) installed in an IBM compatible computer. Software developed in house was used to read the voltage from the analog-to-digital converter card and to convert, display and store the O_2 concentration (mg l^{-1}) once every 30 s. The instantaneous (recorded every second) value was unreliable due to small variability as a result of pressure fluctuations introduced by the pump. The value stored was thus the average of 30 readings. The electrode was calibrated using the built in electronic zero and a vigorously aerated Long Ashton nutrient solution. The reading from the pH meter (mg l^{-1}) was then monitored over a wide range of O_2 concentrations with the voltage read by the analog-to-digital converter and a least squares linear regression performed on the results to allow real time conversion of voltages to O_2 concentrations.

Oxygen consumption of each plant was measured for 20 to 30 minutes and then the volume of solution measured. The plants were then harvested, dried at 80 °C for 48 h and weighed. The respiration rate was then expressed on the basis of root dry weight using the equation,

$$R_{O_2} = \left(\frac{ppm}{s}\right) \times \frac{1}{W} \times \frac{V}{1000} \times \frac{1}{32}$$

where R_{O2} = root respiration (nmol g^{-1} s^{-1})

ppm/s = rate of O_2 consumption determined from the slope of the plot of $[O_2]$ (ppm = mg l^{-1}) versus time (s)

W = root dry mass (g)

V = volume of nutrient solution.

These results were subjected to Student's T tests (Parker, 1979).

3.11.3 Respiratory $^{14}CO_2$ release

Plants were supplied with $^{14}CO_2$ as specified previously (Section 3.6.1). After 24 h the release of $^{14}CO_2$ from roots into a constant flow of gas was determined. A gas manifold was set up as described for respiratory CO_2 release to deliver a constant flow of gas to each bottle. Each bottle was sealed with 5% agar No.3 and checked for leaks with soapy water. The gas efflux from each bottle was then passed through 50 ml of 10% (v/v) Carbo-sorb (Packard) for 30 minutes. An ADC Model 225 MK 3 bench IRGA was used to monitor flow rates (for leak detection) and CO_2 concentration post CO_2 trap to check the efficacy of the Carbo-sorb. Aliquots of 0.5 ml Carbo-sorb were then counted on a Beckman LS 5000 TD liquid scintillation counter with 5 ml Insta-gel scintillation fluid. The plants were separated into shoot and root components, dried at 80 °C for 48 h and weighed. The plants were milled in a Wiley mill (mesh 0.5 mm) and 0.05 g material taken for oxidation in a Packard Tricarb Model 306 Sample Oxidizer to determine ^{14}C label present in the root. The ^{14}C respiration rate was then expressed on the basis of radiocarbon activity per gram root dry mass in units Bq Bq^{-1} g-1 s^{-1}. These results were subjected to Student's T tests (Parker, 1979).

3.12 ASSIMILATION OF $NaH^{14}CO_2$ BY ROOTS

Plants were grown in hydroponic tanks through plastic lids appropriate for 1000 ml plastic bottles, so as to limit damage to the plants upon later transfer to these bottles. Each lid was fitted with a short 2 cm piece of 1.7 cm internal diameter garden hose through which the foam rubber wrapped plants were inserted. Ten day old maize and 3 week old wheat plants (4 replicates per treatment) were transferred into fresh aerated Long Ashton nutrient medium in 1000 ml darkened plastic bottles 16 h before supplying $^{14}CO_2$ to the plant roots. At the beginning of the 1 h $^{14}CO_2$ feeding period, the plants were transferred into fresh strongly aerated (for 3 h) Long Ashton nutrient medium and 10 μCi $NaH^{14}CO_2$ (0.1 mCi $mmol^{-1}$) was added. After 1 h the plants were removed from the nutrient solution and the roots thoroughly washed under running deionized water. The roots were blotted dry and the plants divided into shoot and root components, weighed and killed in liquid N. The plant components were then transferred to 80% (v/v) ethanol and homogenized with an Ika-Ultra-Turrax homogenizer. The suspension was allowed to extract for 24 h at 4 °C with occasional shaking before re-homogenization. The suspension was acidified with 1 ml of 10 M HCl to release any $^{14}CO_2$ present and then filtered through Whatman No. 1 filter paper and made up to 100 ml with

80% (v/v). A 0.5 ml sub-sample was counted in 5 ml Insta-gel scintillation fluid using a Beckman LS 5000 TD scintillation counter.

The results were expressed as Bq g^{-1} fresh mass h^{-1} and subjected to an analysis of variance with N from and concentration as factors (Statgraphics Version 4.0, 1989). From a knowledge of the specific activity of the $NaH^{14}CO_2$ used, and from the moisture contents of plants harvested for fresh and dry mass determinations simultaneously with those used in this experiment for $H^{14}CO_3^-$ labelling, the rate of HCO_3^- utilization was calculated in units of μmol HCO_3^- g^{-1} dry mass h^{-1}.

3.13 SPLIT-ROOT EXPERIMENTS

Plants cultured on both 4 and 12 mM NO_3^- and NH_4^+ as described in Section 3.1.3 were utilized for:

a) Root respiratory CO_2 release from each root-half (6 replicates per treatment) (Section 3.11.1).

b) Root respiratory O_2 consumption from each root-half (6 replicates per treatment) (Section 3.11.2).

c) Total N content and ^{15}N uptake for each root half; only one root of each plant being supplied with ^{15}N, the other receiving ^{14}N (6 replicates per treatment) (Section 3.9.1).

d) ^{14}C partitioning (5 replicates per treatment) (Section 3.6).

Methods used in these determinations have been described above. Throughout the NO_3^- and the NH_4^+ grown root components were analyzed separately.

4 RESULTS AND DISCUSSION

4.1 GENERAL REMARKS

Leaves of wheat grown on NH_4^+ nutrition were greener in colour and appeared to be more rigid than those of plants grown on NO_3^- nutrition. The NH_4^+-fed wheat plants exhibited a longer inter-node distance and were observed to enter reproductive growth (4 to 5 week stage) earlier than NO_3^--fed plants. From visual assessment, the roots of NO_3^--fed wheat plants were obviously better developed than those of NH_4^+-fed plants. From experience with collecting xylem sap bled from root stumps or through pneumatic extrusion with a Schölander bomb it was obvious that the water potentials of NH_4^+-fed wheat plants were lower than those of NO_3^--fed plants.

Although NH_4^+ nutrition did not appear to reduce maize root growth as noticeably as in the case of wheat plants, the overall growth of the maize plants was considerably reduced by NH_4^+ in comparison with NO_3^- nutrition. Leaf coloration of maize was not noticeably different between NO_3^-- and NH_4^+-fed plants. Of the few maize plants which died during the experiments, all were 12 mM NH_4^+-fed plants. The performance of the maize plants appeared superior when the lower (4 mM) of the two N concentrations was supplied. Results obtained from experiments with maize performed with 12 mM NH_4^+ were generally found to be more variable than those obtained from NO_3^--fed plants, or plants grown on 4 mM N, possibly indicating that at 12 mM NH_4^+ the plants were under NH_4^+ stress. As in the case of wheat, it appeared that the water potentials of NH_4^+-fed maize plants were lower than those of NO_3^--fed plants.

The experimentation reported here was carried out at both 4 and 12 mM N because in some cases the differences in physiology between NO_3^-- and NH_4^+-fed plants were small at the 4 mM N feeding level. Where experimental work was undertaken using 12 mM N, 4 mM N was also used as a comparison.

4.2 BIOMASS CHARACTERISTICS IN RESPONSE TO DIFFERENT NITROGEN SOURCES

The responses of wheat and maize growth to NO_3^-, NH_4NO_3 and NH_4^+ nutrition have been widely reported although the recorded results are often conflicting, possibly as a consequence of variability introduced through nitrification (Spratt and Gasser, 1970), inadequate pH control Bloom (1988) or other environmental factors.

4.2.1 Wheat response

The concentration of N in the nutrient solutions did not have a statistically significant[1] influence on the dry mass, shoot : root ratios or moisture contents of wheat plants, indicating that 4 mM N was sufficient to sustain maximum growth and that 12 mM N was in excess of requirements (Appendix 7.1.1, 3-way analysis of variance). Analysis of variance indicated that the effects of NO_3^- and NH_4^+ nutrition on dry masses, shoot : root ratios and shoot and root moisture contents were significantly different.

With plants grown on 4 mM N, no differences in the dry masses were evident between NO_3^-- and NH_4NO_3-fed plants, while the dry masses of NH_4^+-fed plants were significantly smaller than either NO_3^-- or NH_4NO_3-fed plants (Figure 4.1a). At 12 mM N NH_4NO_3-fed plants had significantly larger dry masses than NO_3^-- and especially NH_4^+-fed plants. Similar enhancement of wheat growth by NH_4NO_3 and reduced growth with NH_4^+ has been observed by other workers (Cox and Reisenauer, 1973; Lips *et al.*, 1990).

Large differences between the shoot : root ratios of NO_3^-- and NH_4^+-fed plants were found, with NH_4NO_3-fed plants forming an intermediate group (Figure 4.1b). Although the shoots of NH_4^+-fed plants were reduced in size compared to those of NO_3^--fed plants, the roots of NH_4^+-fed plants were particularly severely affected. Thus smaller root extension in NH_4^+-fed plants was the main reason for the higher shoot : root ratios of NH_4^+- compared to NO_3^--fed plants. There were no significant differences between 4 and 12 mM N-fed plants with respect to shoot : root ratios.

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[1] Significant is used in the statistical sense to mean rejection of the null hypothesis at either $p < 0.05$, or where specified, at $p < 0.1$.

Shoot moisture contents of NH_4^+-fed plants were significantly lower than those of either NO_3^-- or NH_4NO_3-fed plants whether grown on 4 or 12 mM N (Figure 4.1c). The root moisture contents of the 4 mM N-fed plants appeared to be largely unaffected, while the 12 mM NH_4^+-fed plant roots were intermediate between the 12 mM NO_3^- and NH_4NO_3 treatments. Although the standard errors on the root moisture content data are acceptable, these moisture contents are notoriously difficult to determine due to possible inconsistency, despite rigorous precautions, in the blotting dry of the roots for fresh mass determinations. Statistical analysis showed that although there were no significant effects of N concentration on shoot moisture contents, there were significant interactions between the form and concentration of N, i.e. the effects of NH_4^+ nutrition on shoot moisture contents were exacerbated by higher concentrations of N (Appendix 7.1.1). These findings regarding shoot moisture contents may be of significance in interpreting some of the gas exchange data (Section 4.3.1).

The fact that the form of N does influence the biomass accumulation of wheat plants has been made clear, but the question may still be asked as to whether the influence of the form of N is on the root, the shoot or the whole plant. To overcome the differential effects of NO_3^- and NH_4^+ nutrition on the shoots, the plants were grown in split-root culture with one half of the root in NO_3^-- and the other in NH_4^+-containing nutrient medium allowing direct comparison of NO_3^-- and NH_4^+-fed roots without the problems of different shoot physiology (See Section 3.1.3 for details of methods).

There were significant reductions in the sizes of NH_4^+-fed root-halves in comparison to NO_3^--fed root-halves (Figure 4.2a). The higher concentration of N (12 mM) had a significant deleterious influence on shoot and root growth in comparison to the 4 mM N treatment. The shoot : root ratios of NH_4^+-fed root-halves were higher than those of NO_3^--fed root-halves (Figure 4.2b) indicating that the roots *per se* were sensitive to the form of N supplied.

4.2.2 Maize response

Three-way analysis of variance showed that both the form and concentration of N were highly significant in determining dry masses, shoot : root ratios and shoot moisture contents of maize plants (Appendix 7.1.1). Plants grown on 12 mM N were smaller overall than those grown on 4 mM N, indicating that 12 mM N was in excess of requirements for maximum

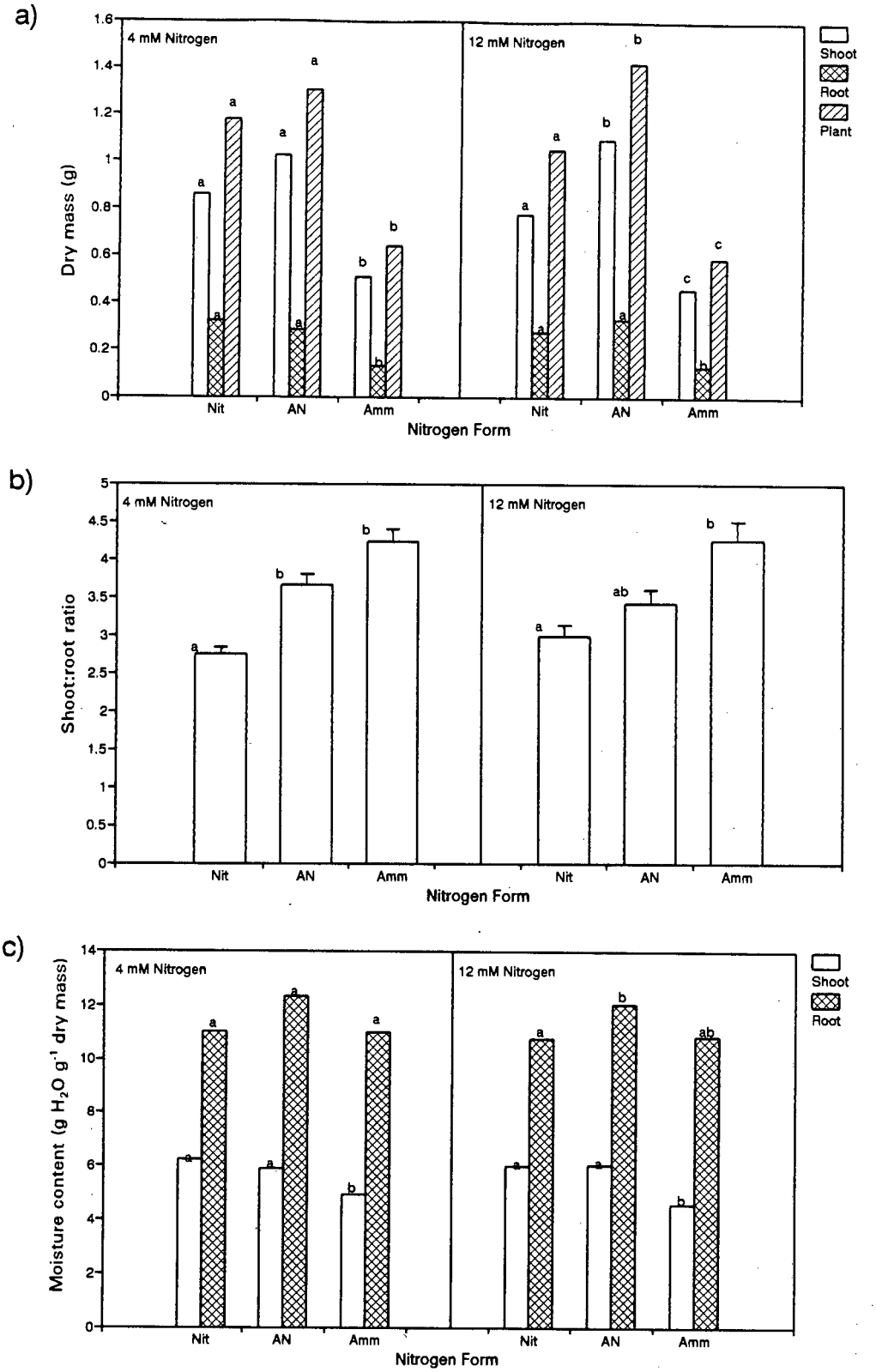


Figure 4.1. a) Shoot, root and plant dry masses; b) shoot : root ratios; c) moisture contents (g H_2O g^{-1} dry mass) of wheat plants grown on 4 or 12 mM NO_3^- (Nit), NH_4NO_3 (AN) or NH_4^+ (Amm). Letters are used to indicate the significance of differences (at 95% confidence interval) between treatments determined using analysis of variance. Where no standard error (S.E.) bar is shown the letters are placed so that the height to the middle of the letter from the data point corresponds with the S.E. Primed and non-primed letters form separate groups indicating significant differences between the groups. Different plant parts or measures were tested separately. Further detail of display of statistical information is provided in Appendix 7.1. (n=24)

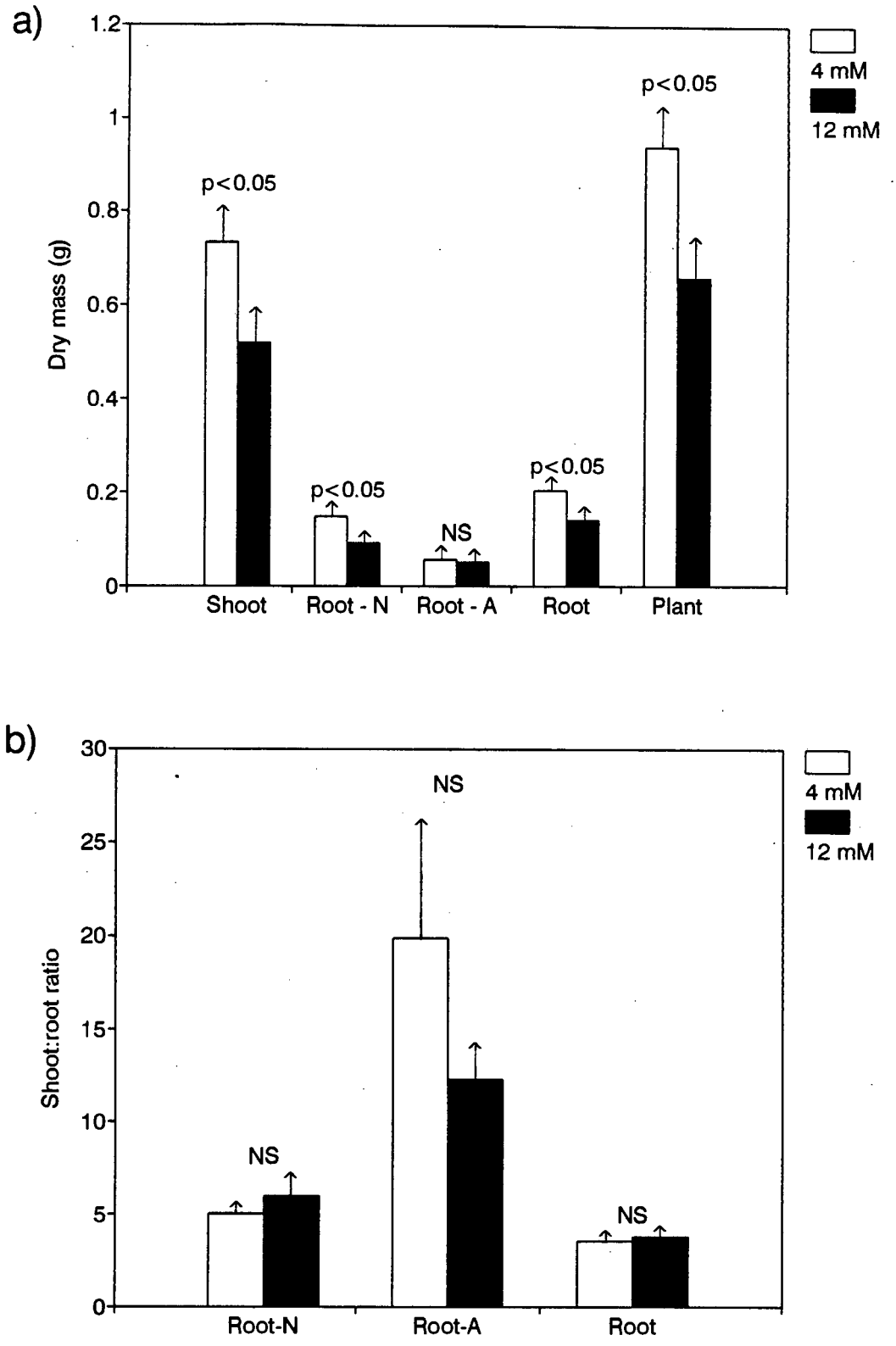


Figure 4.2. a) Dry masses of shoots, NO_3^--fed root halves (Root-N), NH_4^+-fed root halves (Root-A), roots and plants; b) shoot : root ratios of wheat plants grown on 4 or 12 mM N in split-root culture. Results of Student's T test comparisons of 4 and 12 mM N-fed plants are shown above S.E. bars (NS=not significant at 90% confidence interval). Student's T tests of NO_3^-- versus NH_4^+-fed root masses and shoot : root ratios yielded $p<0.05$ for both the 4 and 12 mM N treatments. (n=6)

growth (Figure 4.3a). Strong interaction between the form and concentration of N on shoot dry masses, shoot : root ratios and moisture contents was observed.

The dry masses of both 4 and 12 mM NH_4^+-fed plants were smaller than those of NO_3^--fed plants while no significant differences were found between NO_3^-- and NH_4NO_3-fed plants (Figure 4.3a). In contrast, Schrader *et al.* (1972) reported increased growth of maize with 7 mM NH_4NO_3 compared to NO_3^- and NH_4^+ nutrition, but also found that growth was reduced with NO_3^- compared to NH_4^+ nutrition. Only small differences were observed between the shoot : root ratios of NO_3^--, NH_4NO_3- and NH_4^+-fed plants, although the concentration of N had a small but significant effect of increasing the shoot : root ratios at the higher N concentration (Figure 4.3b). The moisture contents of the maize shoots grown on both 4 and 12 mM NH_4^+ were found to be significantly lower than those of NO_3^-- and NH_4NO_3-fed plants (Figure 4.3c). The situation in the roots with respect to moisture contents was less clear due to the difficulty in accurately determining fresh mass.

As in the case of wheat (Section 4.2.1), split-root experiments were conducted with maize in order to directly compare NO_3^-- and NH_4^+-fed roots without the problems of different shoot physiology. There were significant reductions in the sizes of NH_4^+-fed root-halves in comparison to NO_3^--fed root-halves (Figure 4.4a). The concentration of N had no significant effects on maize shoot or root growth. The shoot : root ratios of NH_4^+-fed root-halves were higher than those of NO_3^--fed root-halves (Figure 4.4b). The alteration of the shoot : root ratio by the form of nutrient N was surprising since no such effect was detected between plants grown entirely on one form of N or the other. This indicates that the roots *per se* were sensitive to the form of N supplied.

4.2.3 Discussion

The absolute sizes of wheat and maize plants were not directly comparable due to the fact that the maize grew much more rapidly and was harvested after only 10 days in hydroponics, while the wheat was harvested after 21 days. However, the form and concentration of N had comparable consequences for both species (Figures 4.1a and 4.3a). The wheat results support the assertion of Goyal and Huffaker (1984) that in general NH_4^+ is more deleterious to root growth than shoot growth. The lack of effect of NH_4^+ nutrition on shoot : root ratios of maize has been reported previously by Lewis *et al.* (1989). Many reasons have been put

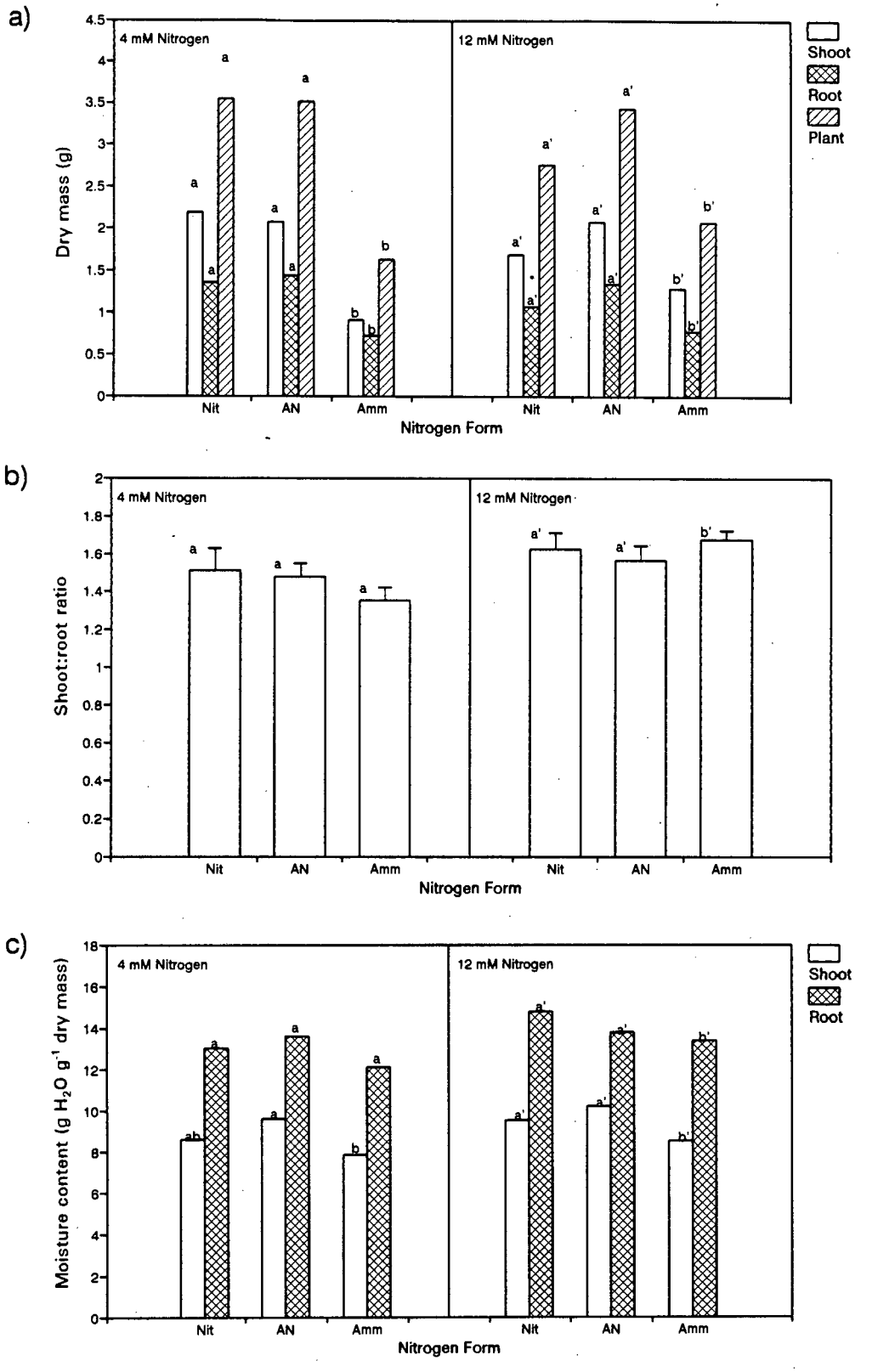


Figure 4.3. a) Shoot, root and plant dry masses; b) shoot : root ratios; c) moisture contents (g H_2O g^{-1} dry mass) of maize plants grown on 4 or 12 mM NO_3^- (Nit), NH_4NO_3 (AN) or NH_4^+ (Amm). Statistics as in Figure 4.1. (n=24)

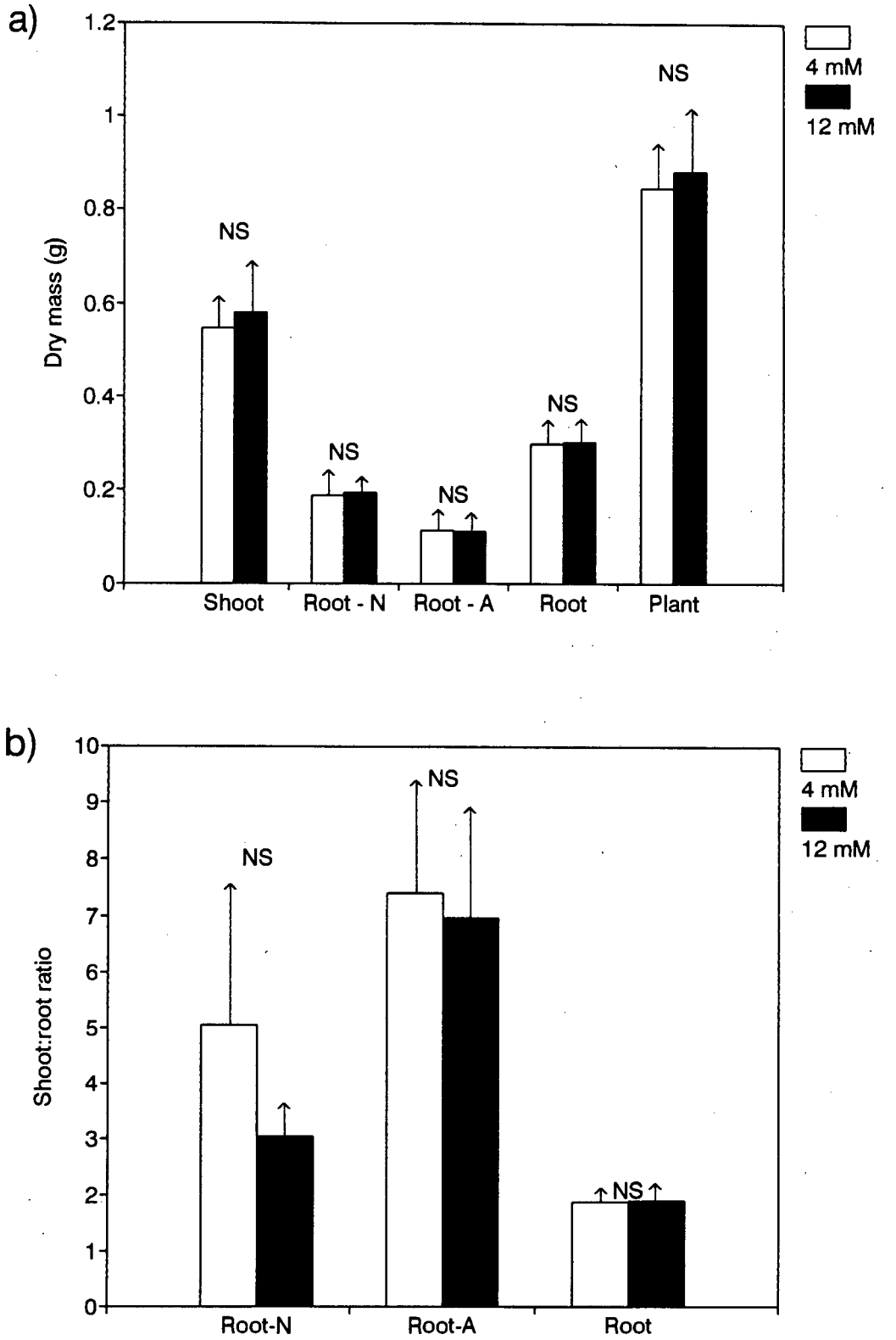


Figure 4.4. a) Dry masses of shoots, NO_3^--fed root halves (Root-N), NH_4^+-fed root halves (Root-A), roots and plants; b) shoot : root ratios of maize plants grown on 4 or 12 mM N in split-root culture. Results of Student's T test comparisons of 4 and 12 mM N-fed plants are shown above S.E. bars (NS=not significant at 90% confidence interval). Student's T tests of NO_3^-- versus NH_4^+-fed root masses and shoot :root ratios yielded $p > 0.1$ for 4 mM N and $p < 0.05$ for 12 mM N treatments. (n=6)

forward to explain reduced plant growth with NH_4^+ compared to NO_3^- nutrition. Three major possibilities are that:

1) Acidification of the root medium by plants utilizing NH_4^+ is deleterious to growth (Allen and Smith, 1986). In the experiments reported here the pH of the root solution was carefully controlled and thus this possibility may be dismissed.
2) Uptake of cations is inhibited by utilization of NH_4^+ (Chaillou *et al.*, 1986) and thus growth is reduced through a limitation in the availability of cations.
3) Ammonium assimilation generates a limitation on C availability for growth. As a result of the assimilation of NH_4^+ within the root, as opposed to the predominantly foliar assimilation of NO_3^-, demands are placed upon root carbohydrate supply which diverts C away from root extension (Lewis and Chadwick, 1983; Murphy and Lewis, 1987). The higher photosynthetic capacity of C_4 plants may make available a larger supply of C thus preventing the root of the C_4 plant from being adversely influenced (Lewis *et al.*, 1990).

On the basis of the results presented thus far it is not possible to exclude the influence of NH_4^+ nutrition upon cation accumulation. It is, however, difficult to understand on the basis of this proposal why maize roots should be less affected by the N source than wheat roots. The proposal that NH_4^+ nutrition limits root growth through competition for root carbohydrate resources is supported by this data. The fact that in the split-root experiments the shoot : root ratios of the root-halves supplied with NH_4^+ were higher in both wheat and maize than those of NO_3^--fed root-halves indicates that NH_4^+ nutrition exerts its influence on the root *per se*. The altered shoot : root ratios in maize may be the result of the inability of the shoot to modify C allocation to the NH_4^+-fed root-half to meet the challenge of NH_4^+ assimilation.

Some evidence does exist in the literature for differences in water relations between NO_3^-- and NH_4^+-fed plants (Haynes and Goh, 1978; Goyal and Huffaker, 1984; Salsac *et al.*, 1987; Lips *et al.*, 1990), but little explanation has been forthcoming for these results. Moisture content is an important physiological variable due to the possible consequences for many aspects of physiology including ion uptake and translocation, cell expansion and gas exchange characteristics.

In summary, the biomass and moisture results showed that:

1) Biomass accumulation was lower with NH_4^+ than with NO_3^- nutrition.

2) The shoot : root ratios of wheat plants were increased with NH_4^+ compared to NO_3^- nutrition.

3) The shoot : root ratios of maize were unaffected by the N source.

4) Shoot moisture contents of both wheat an maize were lower in NH_4^+- than in NO_3^--fed plants.

5) In split-root experiments the shoot : root ratios of the root-halves supplied with NH_4^+ were higher in both wheat and maize than in the root-halves supplied with NO_3^-.

4.3 GAS EXCHANGE CHARACTERISTICS IN RESPONSE TO DIFFERENT NITROGEN SOURCES

4.3.1 Wheat response

Three-way analysis of variance showed that the differences between the CO_2 assimilation rates and stomatal conductances of NO_3^-- and NH_4^+-fed plants were significant (Appendix 7.1.2). The differences in CO_2 assimilation rates, transpiration rates, stomatal conductances and intercellular CO_2 concentrations between 4 and 12 mM N grown plants were also found to be significant. There was also significant interaction between the form and concentration of N in determining the CO_2 assimilation rates, stomatal conductances and intercellular CO_2 concentrations, indicating that the concentration of N was important in determining the magnitude of the differences between NO_3^-- and NH_4^+-fed plants with respect to these physiological variables.

Very little difference was found between 4 mM NO_3^-- and NH_4^+-fed plants with respect to photosynthetic CO_2 assimilation and transpiration rates (Figure 4.5a). In 12 mM N-grown plants, however, there were significant differences in CO_2 assimilation rates, but not transpiration rates (transpiration significant at $p<0.1$ level), between NO_3^-- and NH_4^+-fed plants. At 12 mM N the CO_2 assimilation rates of NH_4^+-fed plants were only 85% of those of NO_3^--fed plants. The lack of differences between the photosynthetic rates of plants grown on 4 mM NO_3^- or NH_4^+ is supported by the results of Lewis *et al.* (1986) using *Hordeum vulgare* grown on 2 mM N and Lewis *et al.* (1990) using wheat grown on 4 mM N, but are contrary to the data of Lips *et al.* (1990) who showed that 4 mM NH_4^+ increased photosynthesis by wheat in comparison with 4 mM NO_3^-.

The estimation of stomatal conductances using the ADC LCA2 or LCA3 IRGA is based on leaf temperature which is calculated from the measured air temperature and light intensity with assumptions concerning the transmission of infrared radiation by the windows of the Parkinson leaf chamber and the quantity of infrared radiation in light. This calculation has been justifiably criticised due to the possibility that the transmission of infrared by the cuvette windows may change with the age of the cuvette and that the quantity of infrared radiation in light varies, particularly when artificial lighting is used. In addition, the optical properties of the leaf may influence the absorption of incident radiation. In this study the calculation of the quantity of infrared radiation was modified when the ADC LCA2 or LCA3 were used to account for the type of artificial lighting used according to tables provided for this purpose with the ADC LCA3 IRGA. The use of the ADC IRGA for estimation of stomatal conductance is thus only reliable for comparative work within a controlled environment on one particular species.

Stomatal conductances were significantly reduced in 12 mM NH_4^+- compared to 12 mM NO_3^--fed plants, with the average stomatal conductance of 12 mM NH_4^+-fed plants being only 64% of that of NO_3^--fed plants (Figure 4.5b). The differences in gas flux (CO_2 and H_2O) between NO_3^- and NH_4^+-fed plants were clearly correlated with the stomatal conductances, implying stomatal limitation of CO_2 assimilation rates. Intercellular CO_2 concentrations were significantly lower in NH_4^+- than in NO_3^--fed plants receiving 12 mM N, although no differences were found in 4 mM N-fed plants (Figure 4.5b). Differences in intercellular CO_2 concentrations are important in determining the rates of photosynthesis. There were no significant differences between the water use efficiencies of any of the treatments (Figure 4.5c).

4.3.2 Maize response

Three-way analysis of variance clearly showed that the form of N nutrition was important in determining the CO_2 assimilation rates, transpiration rates and stomatal conductances of maize (Appendix 7.1.2). The concentration of N was only significant in determining stomatal conductances. Significant interaction between the form and concentration of N was only found with respect to transpiration rates, indicating that the higher N concentration exaggerated the reduction of transpiration rates evident in NH_4^+- compared to NO_3^--fed plants.

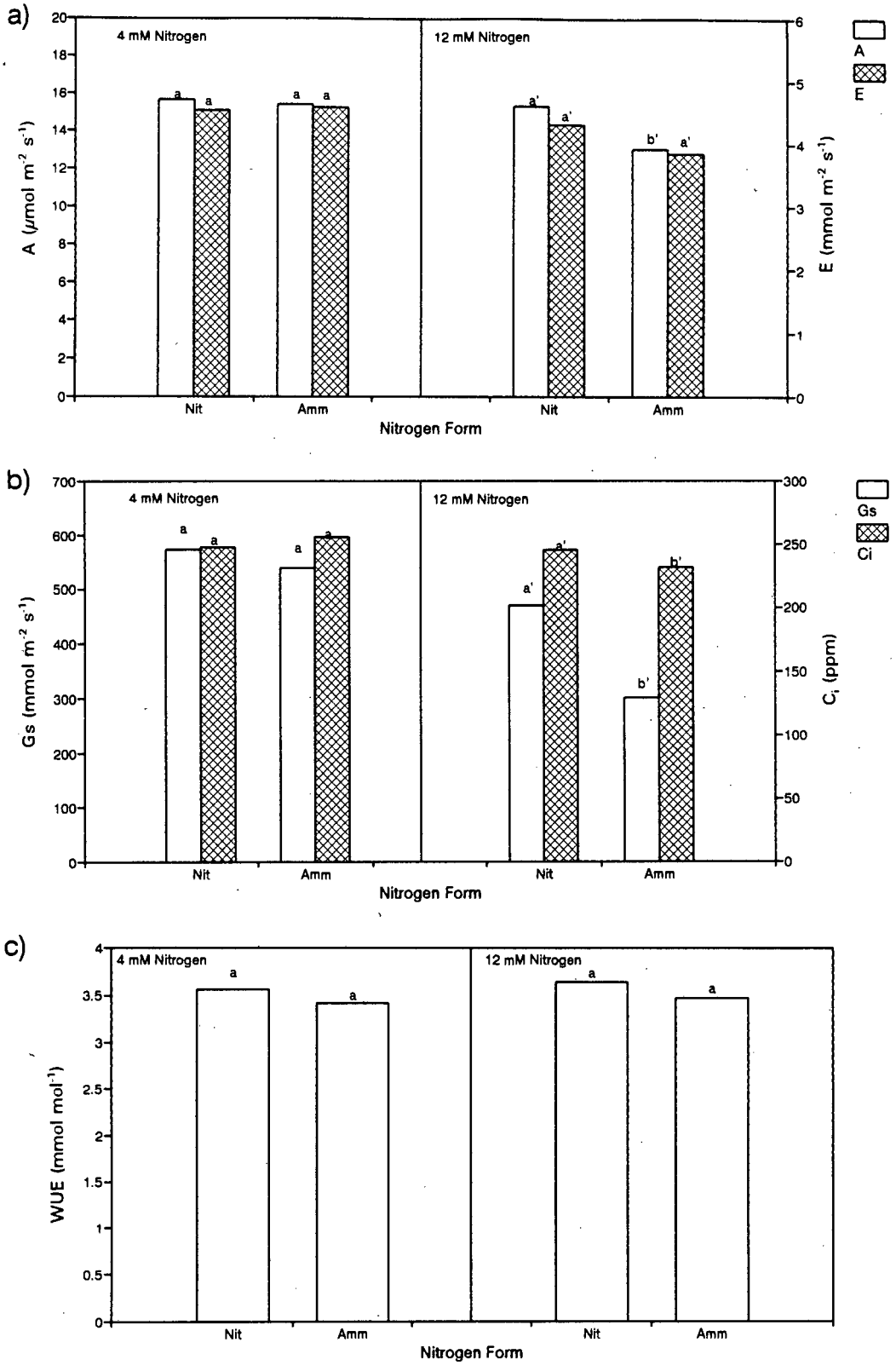


Figure 4.5. a) Carbon dioxide assimilation rates (A) and transpiration rates (E); b) stomatal conductances to H_2O (Gs) and intercellular CO_2 concentrations (C_i); c) water use efficiencies (WUE, mmol CO_2 mol^{-1} H_2O) of wheat plants grown on 4 or 12 mM NO_3^- (Nit) or NH_4^+ (Amm). Statistics as in Figure 4.1. (n=15)

The CO_2 assimilation and transpiration rates of maize grown on 4 mM NH_4^+ showed a significant reduction in comparison to 4 mM NO_3^--fed plants (Figure 4.6a). The average CO_2 assimilation rate of 12 mM NH_4^+-fed plants was only 77% of that of NO_3^--fed plants. At 4 mM N the transpiration rates were significantly lower for NH_4^+- than for NO_3^--fed plants, but these differences were not significant with 12 mM N due to the high variability of NH_4^+-fed plant transpiration rate data. Significantly lower stomatal conductances of NH_4^+-compared to NO_3^--fed plants were observed at both 4 and 12 mM N (Figure 4.6b). The stomatal conductances of 12 mM NO_3^-- and NH_4^+-fed plants were significantly higher than those of 4 mM N plants. Intercellular CO_2 concentrations were decreased in NH_4^+- compared to NO_3^--fed plants at 12 mM N and, to a lesser (not significant) extent, also at 4 mM N. The higher stomatal conductances were associated with higher intercellular CO_2 concentrations in plants grown on 12 mM N compared to 4 mM N. Significantly higher water use efficiencies were found in 4 mM NH_4^+- compared to NO_3^--fed plants, although no significant differences were apparent in the 12 mM N-fed plants (Figure 4.6c).

Due to the C_4 mechanism, maize photosynthetic CO_2 assimilation is largely independent of stomatal conductance when intercellular CO_2 concentrations are maintained above approximately 150 ppm (Section 4.6.2). The intercellular CO_2 concentrations measured here for maize were below 150 ppm and it is, therefore, likely that the reduced stomatal conductance of NH_4^+- compared to NO_3^--fed plants had as a consequence the observed reduced CO_2 assimilation rates.

4.3.3 Discussion

One of the important differences between the maize and wheat plants is the different photosynthetic capacity of the C_4 and C_3 mechanisms respectively. In this investigation the maize plants had higher CO_2 assimilation rates and lower transpiration rates than the wheat plants and thus the water use efficiency of maize was higher. The photosynthetic rates of maize were lower than those reported previously for maize and the results for wheat were higher than those reported previously (NO_3^--fed maize, 25.95; wheat, 8.51 μmol m^{-2} s^{-1}; Lewis *et al.*, 1989). The lower photosynthetic rates of maize found in this investigation may be due to the fact that the phytotron in which the plants were grown was only capable of delivering an irradiance of 1500 μmol m^{-2} s^{-1} whereas the irradiance reported by Lewis *et al.*

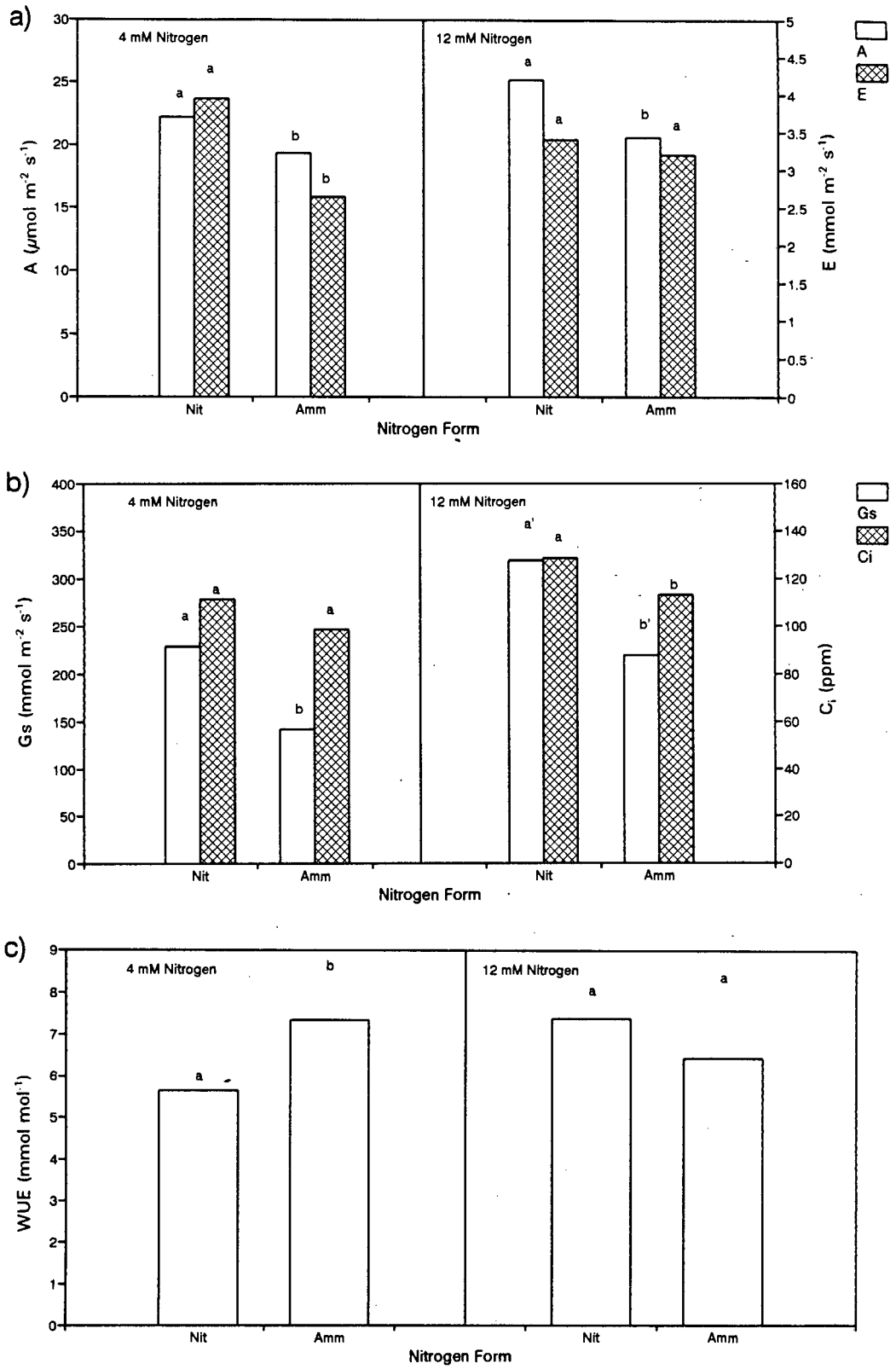


Figure 4.6. a) Carbon dioxide assimilation rates (A) and transpiration rates (E); b) stomatal conductances to H_2O (Gs) and intercellular CO_2 concentrations (C_i); c) water use efficiencies (WUE, mmol CO_2 mol^{-1} H_2O) of maize plants grown on 4 or 12 mM NO_3^- (Nit) or NH_4^+ (Amm). Statistics as in Figure 4.1. (n=15)

(1989) was up to 2500 μmol m^{-2} s^{-1}. In 12 mM N-fed wheat and in 4 mM N-fed maize, NH_4^+ nutrition resulted in decreased CO_2 assimilation and transpiration rates.

Especially in the case of maize (Figure 4.6b), but also in the 12 mM N grown wheat plants (Figure 4.5b), the stomatal conductances of NH_4^+-fed plants were lower than those of NO_3^--fed plants. The stomatal conductances and intercellular CO_2 concentrations were very different between the wheat and maize plants. The average stomatal conductance of 4 mM NO_3^--fed wheat (573.3 mmol m^{-2} s^{-1}) was more than double that of the 4 mM NO_3^--fed maize (228.6 mmol m^{-2} s^{-1}) and the intercellular CO_2 concentration values followed the same pattern. The water use efficiency of the maize plants was considerably higher than that of wheat plants reflecting both the higher CO_2 assimilation rate and the lower transpiration rate of the maize plants. The fact that in both species the moisture contents of NH_4^+-fed plant shoots tended to be lower than those of NO_3^-fed plant shoots (Section 4.2) may be correlated with the differences between stomatal conductances of NO_3^-- and NH_4^+-fed plants.

The lack of large differences between CO_2 assimilation rates of 4 mM NO_3^-- and NH_4^+-fed wheat plants appears to rule out the possibility that reduced photosynthetic C acquisition was responsible for reduced biomass accumulation in these plants. In maize, however, the large differences between the rates of photosynthetic CO_2 assimilation brought about by different N forms may well account, at least partially, for differences in biomass accumulation. The fact that stomatal conductances of NH_4^+-fed wheat (12 mM N) and maize were lower than those of NO_3^--fed plants leads to the conclusion that lower CO_2 assimilation rates were as a consequence of lower stomatal conductances, which were in turn correlated with lower moisture contents of these plants.

The gas exchange data presented are of limited value in determining the amount of photosynthetic C available to the plants. The photosynthetic assimilation of CO_2 may vary with plant age, leaf age, portion of leaf measured and the time of day. The gas exchange characteristics of the mid-lamina portion of the youngest fully expanded leaves of plants of the same age were measured between 2 and 5 hours after the beginning of the photoperiod to standardize the measurements and thus avoid these problems. The results presented above are expressed on the basis of leaf area, as is conventional, but the photosynthetic rates expressed on the basis of leaf mass may provide different information (Section 4.5).

In summary, the gas exchange data has, therefore, shown that:

1) Gas exchange characteristics of wheat were largely unaffected by the N source at 4 mM N, while at 12 mM N the CO_2 assimilation rates and stomatal conductances were lower with NH_4^+ than NO_3^- nutrition.
2) In maize, the photosynthetic CO_2 assimilation rates, transpiration rates, stomatal conductances and intercellular CO_2 concentrations were reduced by NH_4^+ compared to NO_3^- nutrition.

4.4 GROWTH ANALYSIS IN RESPONSE TO DIFFERENT NITROGEN SOURCES

In these experiments the biomass accumulation of the wheat and maize plants was followed over the growing period of these plants. These experiments were performed in order to assess the importance of the form of N on the development of the plants.

4.4.1 Wheat response

The shoot dry masses (Figure 4.7a) and leaf areas (Figure 4.7b) of wheat plants were unchanged by the form of N supplied (Appendix 7.1.3). The roots of NH_4^+-fed plants were reduced in size compared to NO_3^--fed plants (Figure 4.7c). Differences between root dry masses of NO_3^-- and NH_4^+-fed plants were apparent after only 1 week in hydroponic culture, with the average NH_4^+-fed root mass being only 64% of that of NO_3^--fed plants after 4 weeks. Reduced root growth of NH_4^+-fed plants resulted in highly significant differences between the shoot : root ratios of NO_3^-- and NH_4^+-fed plants (Figure 4.8a). The differences between the shoot : root ratios of NO_3^-- and NH_4^+-fed plants increased over time, presumably due to the compound nature of growth. Relative growth rates (slope of the linear regression of the Log_e transformation of dry mass data) of the wheat shoots were unchanged by the form of N supplied while the roots of NH_4^+-fed plants exhibited a reduced relative growth rate in comparison to the roots of NO_3^--fed plants (Figure 4.8b).

Specific leaf areas ($cm^2\ g^{-1}$) were higher in NO_3^-- than in NH_4^+-fed plants indicating that the latter had thicker leaves throughout the growing period (Figure 4.8c). This correlates with the observation that the leaves of NH_4^+-fed plants were more rigid and darker in colour than those of NO_3^--fed plants. The differences between NO_3^-- and NH_4^+-fed plants with respect to specific leaf areas were already present after 1 week in hydroponic culture indicating the

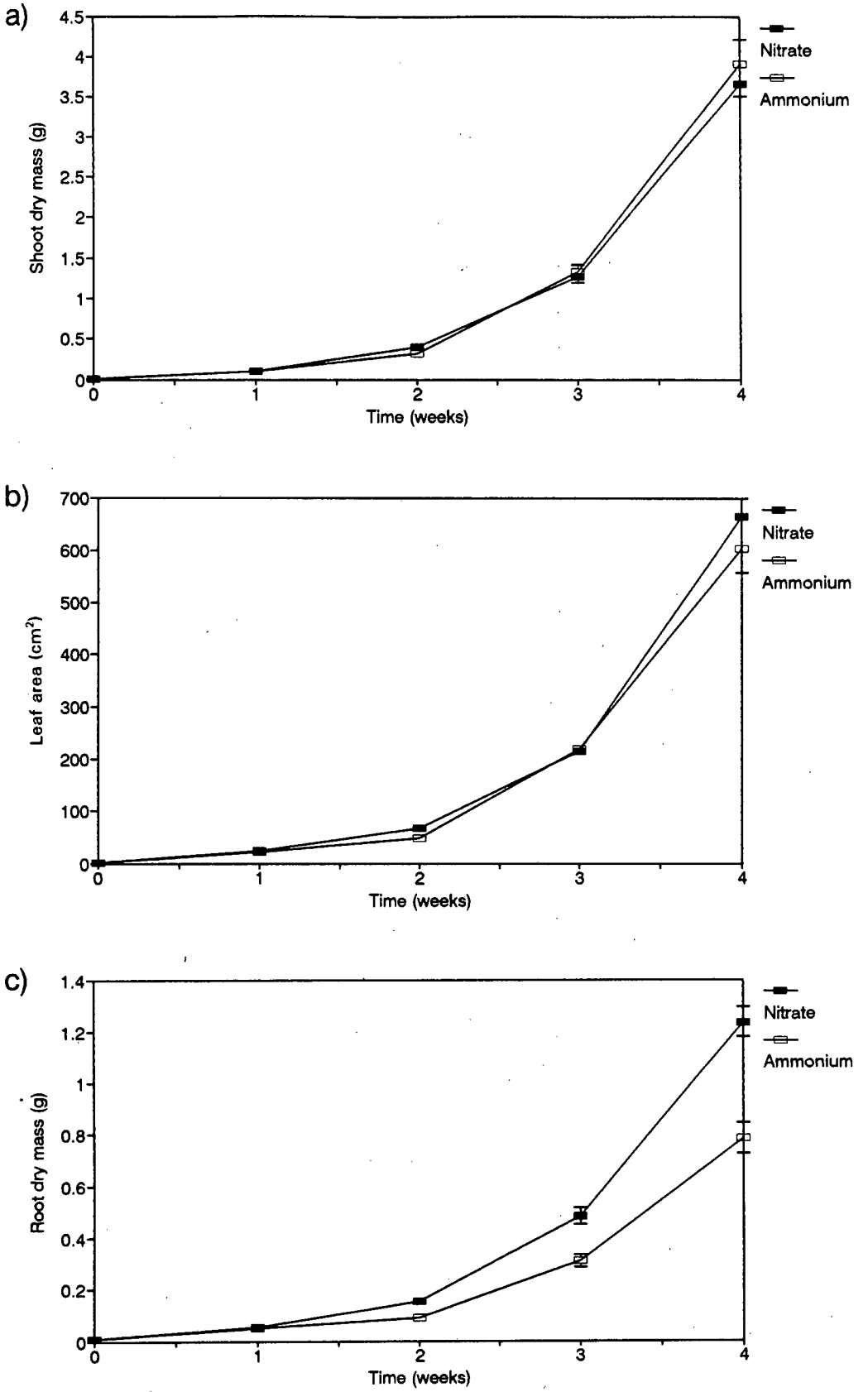


Figure 4.7. a) Shoot dry masses; b) leaf areas; c) root dry masses of wheat plants grown on 4mM NO_3^- or NH_4^+ for 4 weeks. Time=0 is specified as the point at which transfer from nutrient free vermiculite culture to hydroponic culture occurred. Bars show the S.E. where it is larger than the size of the symbols. ($23 < n < 33$)

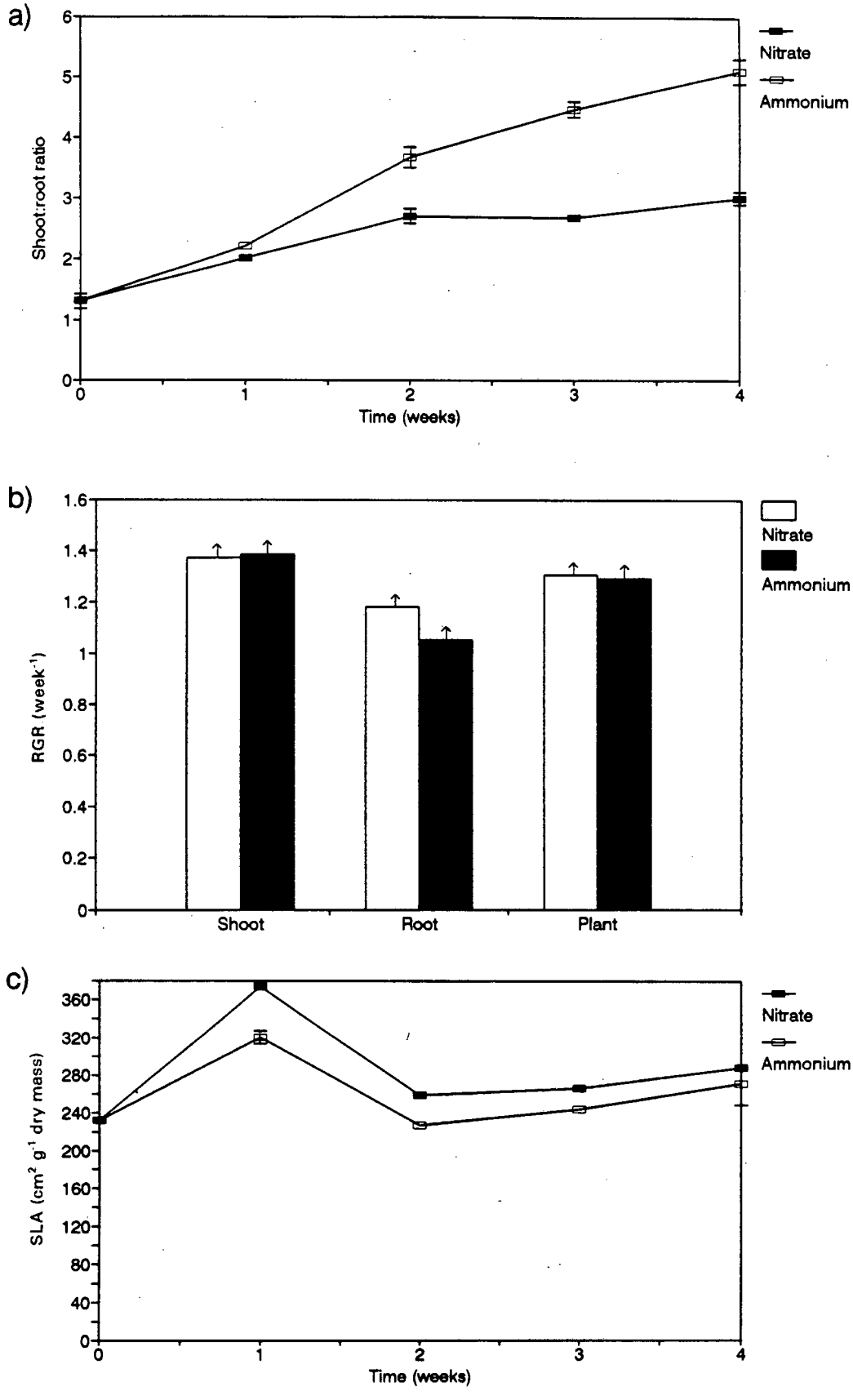


Figure 4.8. a) Shoot : root ratios; b) relative growth rates (RGR, g dry mass produced g^{-1} existing dry mass $week^{-1}$); c) specific leaf areas (SLA, cm^2 leaf area g^{-1} leaf dry mass) of wheat plants grown on 4 mM NO_3^- or NH_4^+ for 4 weeks. Time=0 is specified as the point at which transfer from nutrient free vermiculite culture to hydroponic culture occurred. Bars show the S.E. where it is larger than the size of the symbols. ($23 < n < 33$)

importance of the initial period of exposure of the plant to N. There was a lower shoot moisture content in NH_4^+- compared to NO_3^--fed plants (Figure 4.9), but no differences in the root moisture contents were observed (results not shown). Statistical analysis (Appendix 7.1.3) of the data revealed that there were significant differences between treatments with respect to shoot fresh mass but not dry mass. This is consistent with the significantly smaller moisture contents of NH_4^+- compared to NO_3^--fed shoots.

The biomass, leaf area and moisture content data for wheat, therefore, showed that:

1) Shoot : root ratios were higher in NH_4^+- than in NO_3^--fed plants as a result of reduced root growth in the former and that the shoot : root ratios increased with plant age.
2) Specific leaf areas were higher in NO_3^-- than in NH_4^+-fed plants.
3) The moisture contents of NO_3^--fed wheat plants were higher than those of NH_4^+-fed plants.
4) The influence of the form of N was evident from after only 1 week.

4.4.2 Maize response

Shoot dry masses (Figure 4.10a), leaf areas (Figure 4.10b), root dry masses (Figure 4.10c) and shoot moisture contents were higher in NO_3^-- than in NH_4^+-fed plants 4 days after transfer of the maize plants from nutrient-free vermiculite into hydroponic culture. These differences increased in magnitude with age and statistical analysis illustrated the highly significant nature of these changes (Appendix 7.1.3). The shoot : root ratios (Figure 4.11a) increased for the first nine days and thereafter decreased slightly. There were no significant differences between the shoot : root ratios of NO_3^-- and NH_4^+-fed maize plants, unlike the situation in wheat. Relative growth rates (slope of the linear regression line through the Log_e transformation of dry mass data) of the maize plants were smaller in NH_4^+- than in NO_3^--fed shoots and roots (Figure 4.11b). Although the specific leaf area of NH_4^+-fed plants was significantly higher than that of NO_3^--fed plants, these differences were small (Figure 4.11c). With age the specific leaf areas of both NO_3^-- and NH_4^+-fed plants were reduced. The shoot moisture contents of NO_3^--fed plants were significantly higher than those of NH_4^+-fed plants (Figure 4.12) but the root moisture contents exhibited no consistent trend (data not shown).

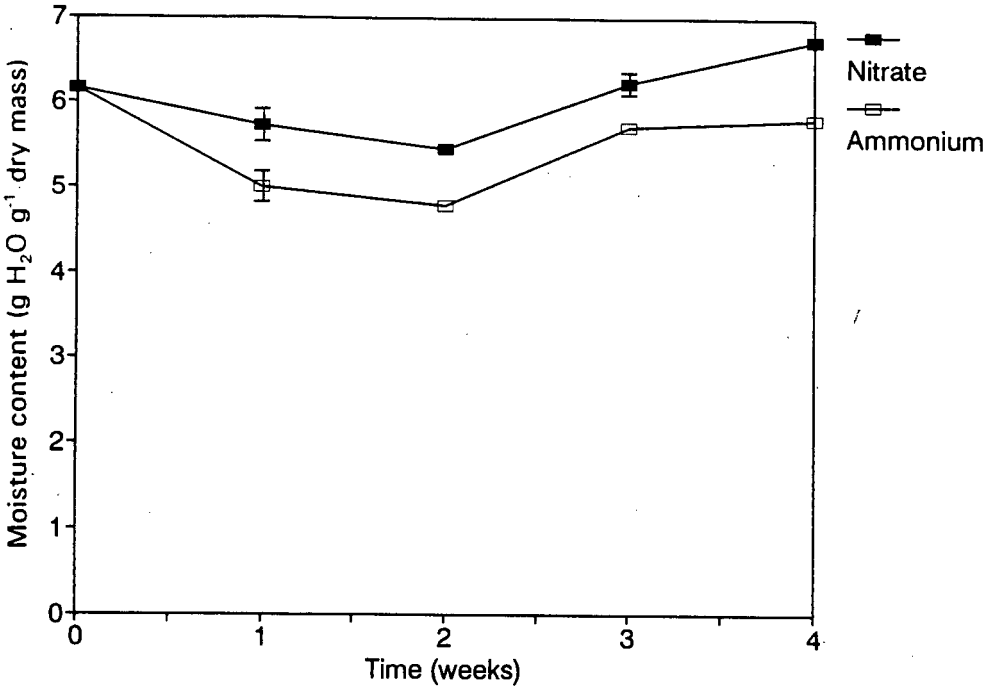


Figure 4.9. Shoot moisture contents (g H_2O g^{-1} dry mass) of wheat plants grown on 4mM NO_3^- or NH_4^+ for 4 weeks. Bars show the S.E. where it is larger than the size of the symbols. ($23 < n < 33$)

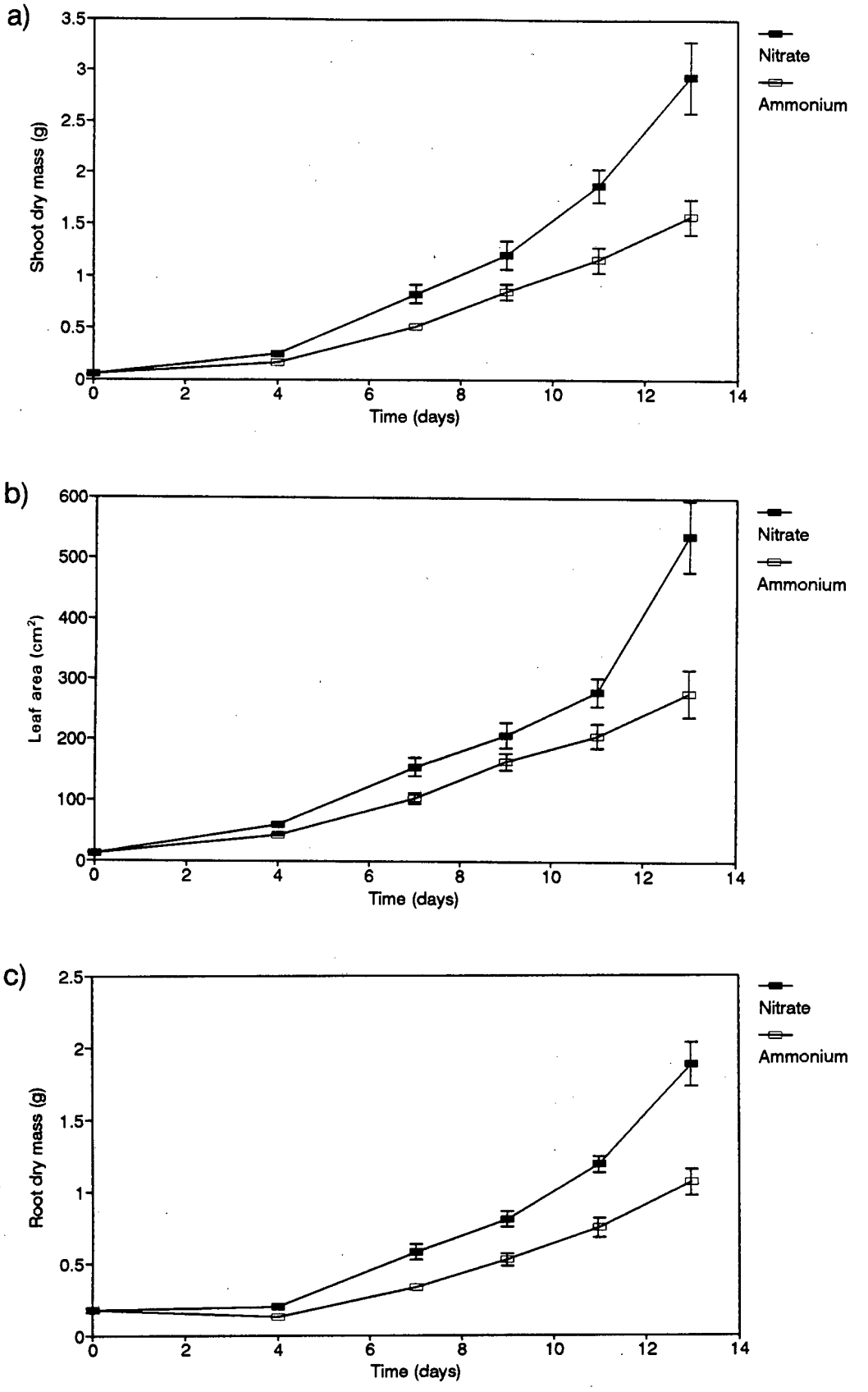


Figure 4.10. a) Shoot dry masses; b) leaf areas; c) root dry masses of maize plants grown on 4 mM NO_3^- or NH_4^+ for 13 days. Time=0 is specified as the point at which transfer from nutrient free vermiculite culture to hydroponic culture occurred. Bars show the S.E. where it is larger than the size of the symbols. ($7 < n < 17$)

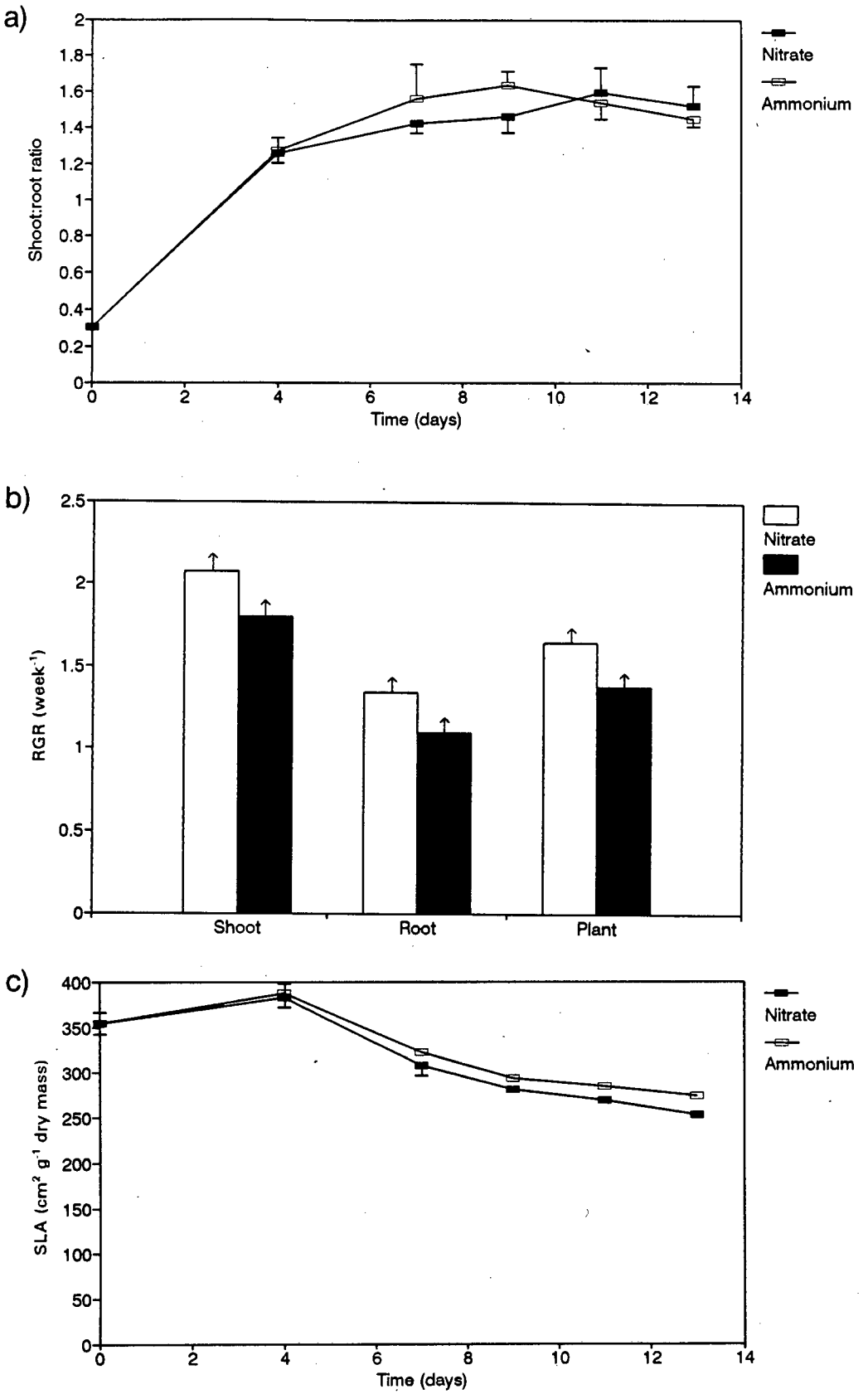


Figure 4.11. a) Shoot : root ratios; b) relative growth rates (RGR, g dry mass produced g^{-1} existing dry mass $week^{-1}$); c) specific leaf areas (SLA, cm^2 leaf area g^{-1} leaf dry mass) of maize plants grown on 4 mM NO_3^- or NH_4^+ for 13 days. Time=0 is specified as the point at which transfer from nutrient free vermiculite culture to hydroponic culture occurred. Bars show the S.E. where it is larger than the size of the symbols. ($7 < n < 17$)

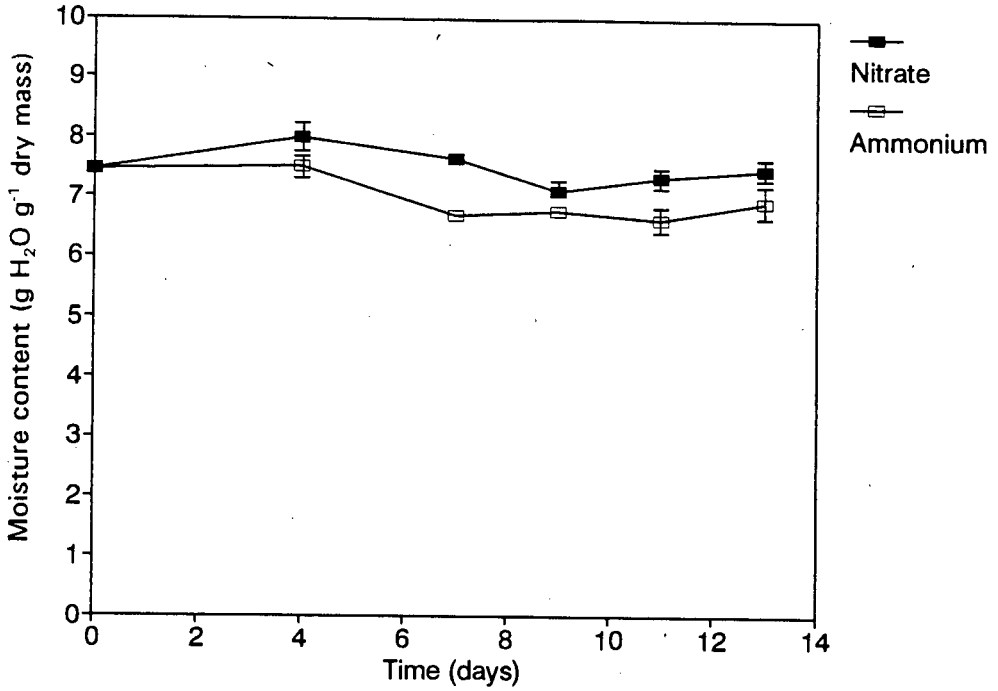


Figure 4.12. Shoot moisture contents (g H_2O g^{-1} dry mass) of maize plants grown on 4 mM NO_3^- or NH_4^+ for 13 days. Bars show the S.E. where it is larger than the size of the symbols. ($7 < n < 17$)

The biomass, leaf area and moisture content data for maize, therefore, showed that:

1) Shoot : root ratios were unaffected by the form of N supplied although the shoot : root ratio did increase with plant age.
2) Specific leaf areas were slightly higher in NH_4^+- than in NO_3^--fed plants.
3) The shoot moisture contents were higher in NO_3^-- than in NH_4^+-fed plants.
4) The influence of the form of N was evident from after only 4 days.

4.4.3 Discussion

Direct comparison of growth-related data between wheat and maize is meaningless due to the very different growth rates exhibited by the two species. Maize plants appeared to be more susceptible to NH_4^+ toxicity than wheat plants with larger differences in growth evident between NO_3^- and NH_4^+ treatments. Biomass accumulation by wheat and maize plants was lower with NH_4^+ than with NO_3^- nutrition, the roots of wheat plants being particularly severely affected. One of the key observations was the fact that the shoot : root ratios of the wheat plants were increased with NH_4^+ nutrition while those of the maize plants were unaffected by the N source. Both wheat and maize exhibited reduced shoot moisture contents in NH_4^+- compared to NO_3^--fed plants.

Changes in the proportion of plant biomass found in the shoot and root components can only be attributed to altered partitioning of C. One of, or a combination of, the following situations are possible:

1) The form of N alters the allocation of photosynthate to the root.
2) The form of N alters the re-translocation of C from the root to the shoot.
3) The form of N alters the loss of C from the root into the root medium by gaseous exchange.

The fact that the roots of maize were not affected by the form of N supplied indicates that either:

1) Maize plants avoid the effect of the different N forms, possibly by having high photosynthetic rates which can support vigorous growth and N assimilation in the roots.
2) Maize plants can respond to the different forms of N by altering: a) the allocation of C to the root, b) the re-translocation of C from root to shoot or c) the loss of C from the root into the root medium by gaseous exchange.

These possibilities will be more fully addressed in subsequent sections. The control of shoot : root ratios, however, has been proposed to be effected through control of carbohydrate partitioning from shoot to root. Nitrogen deprivation enhances allocation of carbohydrate to the roots of plants, thus favouring root growth (Tolley-Henry and Raper, 1986a). Although plants grown on 4 to 12 mM N are certainly not N deficient, the control mechanism of shoot : root ratios through control of carbohydrate allocation from the shoot to the root is possible. In a subsequent sections (4.7.2 and 4.8.4) evidence is, however, presented which shows that the form of N controls the translocation of C from the root to the shoot.

Shoot moisture contents of wheat and maize were lower with NH_4^+ than with NO_3^- nutrition. The literature on the response of plant moisture contents to different forms of N is scanty and devoid of explanations. Ammonium nutrition has been reported to result in susceptibility to water stress (Haynes and Goh, 1978), reduced water uptake rates (Goyal and Huffaker, 1984; Salsac *et al.*, 1987) and lower water use efficiencies (Lips *et al.*, 1990). Two possibilities exist to explain the differences in moisture contents observed between NO_3^-- and NH_4^+-fed plants:

1) NH_4^+ nutrition caused more water to be lost from the shoot. Since the transpiration data indicates that NH_4^+-fed plants had lower transpiration rates on the basis of leaf area (Section 4.3 and 4.5) as a consequence of reduced stomatal conductances, this possibility is negated.
2) NH_4^+ nutrition resulted in reduced water uptake, the mechanism of which is unknown. Transpiration rates measured gravimetrically as water lost per gram of root showed that although NH_4^+-fed plants had slightly lower rates of water loss than NO_3^--fed plants, these results were not significantly different (Figure 4.59).

Detectable differences existed between NO_3^-- and NH_4^+-fed plants with respect to biomass, moisture contents and gas exchange variables 1 week and 4 days after initiation of N feeding to wheat and maize respectively. This indicates that the young plants were extremely sensitive to alterations in N regime. The alteration of the physiology of the young plant by N form is likely to be important in determining the subsequent development of the plant due to the compound nature of growth.

4.5 GAS EXCHANGE CHANGES DURING GROWTH IN RESPONSE TO DIFFERENT NITROGEN SOURCES

In these experiments the gas exchange characteristics of the wheat and maize plants were followed over the growing period. These experiments were performed in order to assess the importance of the form of N on determining the gas exchange characteristics during development of the plants.

4.5.1 Wheat response

The gas exchange variables of wheat changed significantly over the growing period (Appendix 7.1.4). The CO_2 assimilation rates increased (Figure 4.13a), while the transpiration rates (Figure 4.13b) and stomatal conductances (Figure 4.13c) decreased over the 4 week period. The increased CO_2 assimilation rates and reduced stomatal conductances over the growing period resulted in decreased intercellular CO_2 concentrations (Figure 4.14a). Increased CO_2 assimilation rates and decreased transpiration rates resulted in an increase in the water use efficiency (Figure 4.14b) over the 4 week growing period.

No differences were found between the CO_2 assimilation rates, expressed on the basis of leaf area, of NO_3^-- and NH_4^+-fed plants. From the specific leaf areas (Section 4.4.1) the gas exchange variables can be expressed on the basis of leaf mass. Photosynthetic CO_2 assimilation rates expressed on the basis of leaf mass were significantly lower in NH_4^+- than in NO_3^--fed plants throughout the growing period (Figure 13a inset). The differences between the other gas exchange variables (on the basis of leaf area) were significant but small (Appendix 7.1.4). The transpiration rates (Figure 4.13b), stomatal conductances (Figure 4.13c) and intercellular CO_2 concentrations (Figure 4.14a) of NH_4^+-fed plants were found to be lower than those of NO_3^--fed plants, especially during the first week of growth. The water use efficiencies of NH_4^+-fed plants were significantly higher than those of NO_3^--fed plants, although the differences were small (Figure 4.14b). When expressed on the basis of leaf mass, the differences between NO_3^-- and NH_4^+-fed plants with respect to transpiration rates, stomatal conductances and intercellular CO_2 concentrations were magnified (data not shown) because the specific leaf areas of NH_4^+-fed plants were higher than those of NO_3^--fed plants.

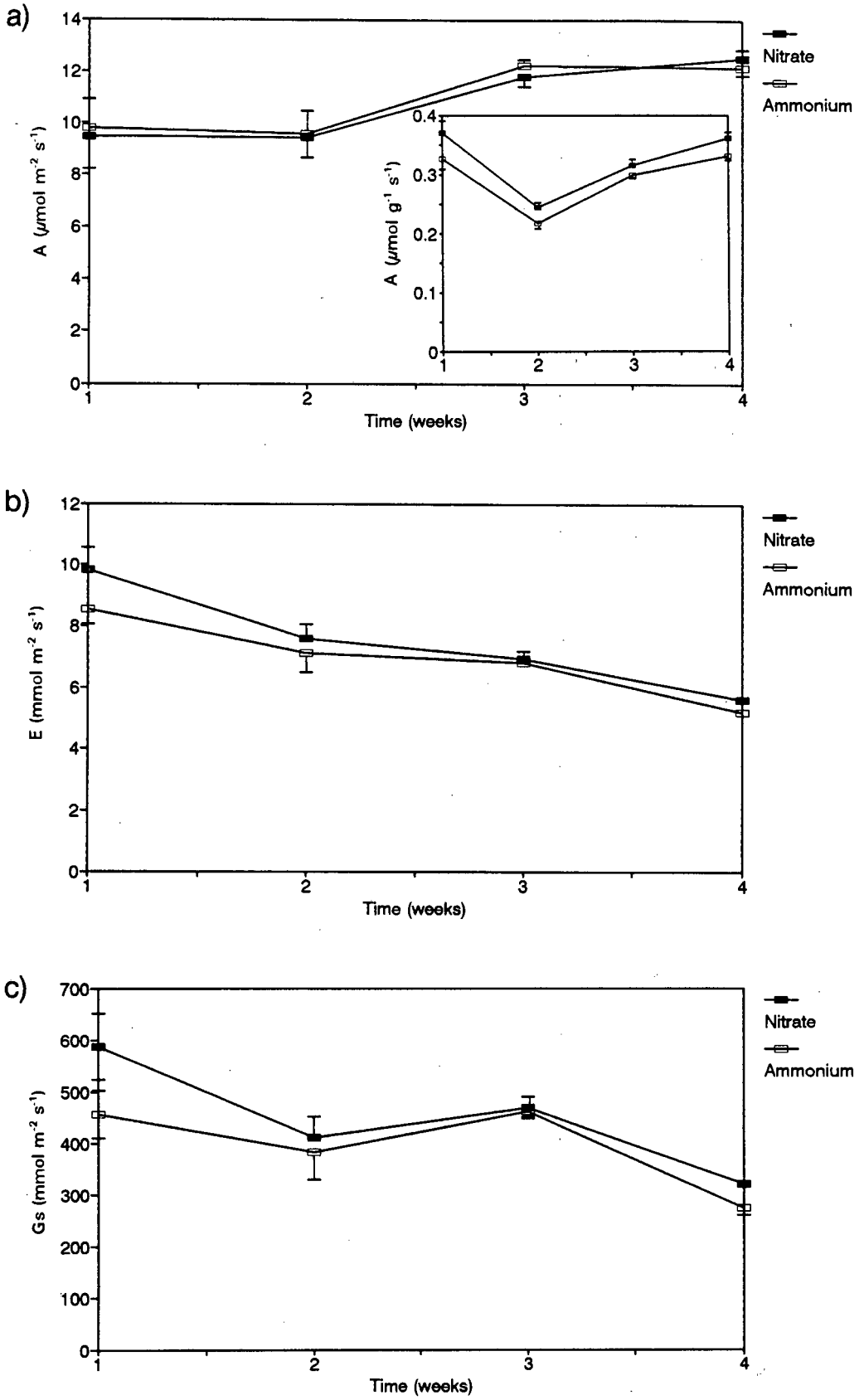


Figure 4.13. a) Carbon dioxide assimilation rates (A, μmol m^{-2} leaf area s^{-1}); b) transpiration rates (E); c) stomatal conductances to H_2O (Gs) of wheat plants grown on 4 mM NO_3^- or NH_4^+ for 4 weeks. Inset shows CO_2 assimilation expressed on the basis of leaf mass (A, μmol g^{-1} leaf dry mass s^{-1}). Time=0 is specified as the point at which transfer from nutrient free vermiculite culture to hydroponic culture occurred. Bars show the S.E. where it is larger than the size of the symbols. (n=24)

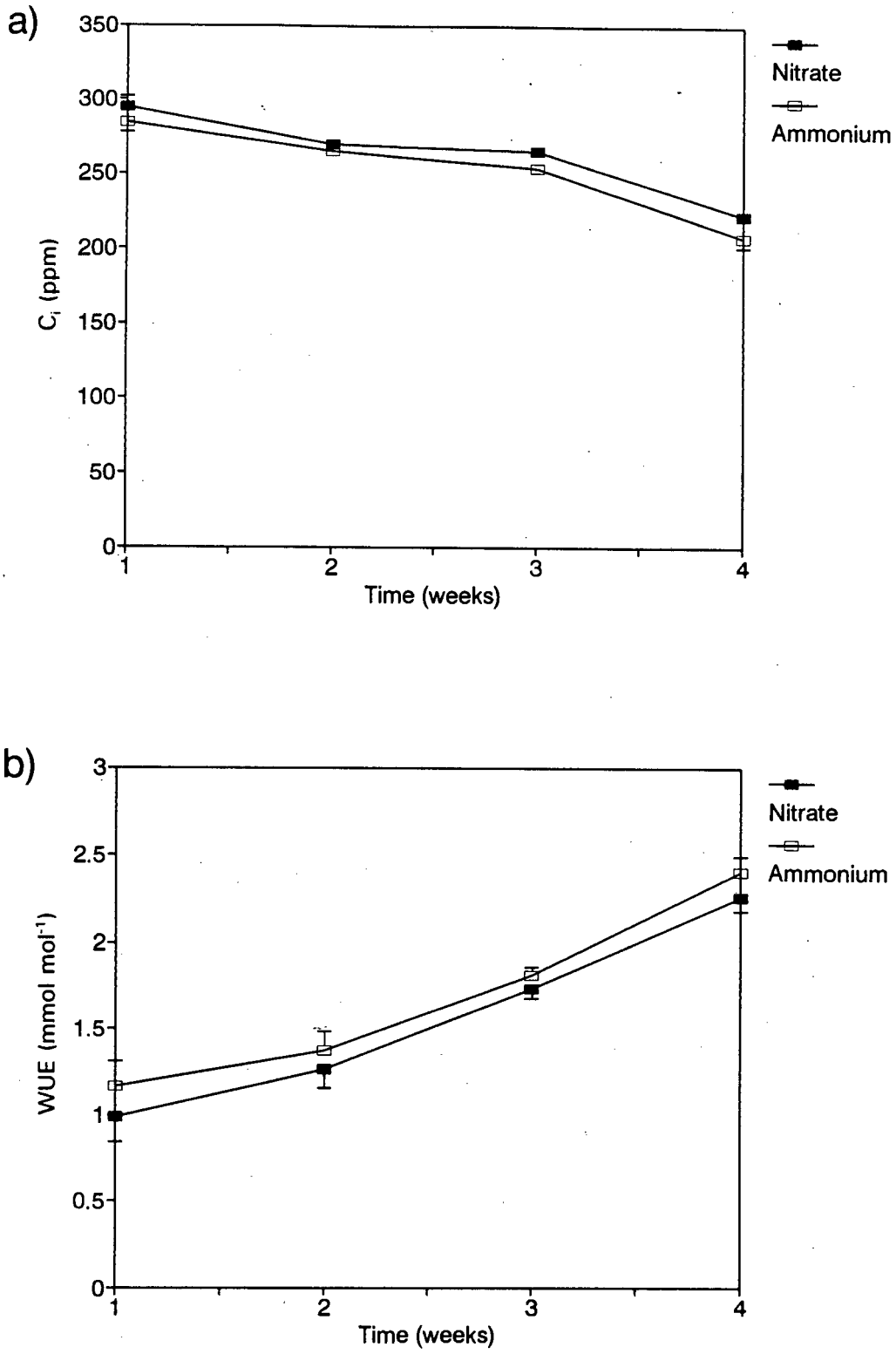


Figure 4.14. a) Intercellular CO_2 concentrations (C_i) and b) water use efficiencies (WUE) of wheat plants grown on 4 mM NO_3^- or NH_4^+ for 4 weeks. Time=0 is specified as the point at which transfer from nutrient free vermiculite culture to hydroponic culture occurred. Bars show the S.E. where it is larger than the size of the symbols. (n=24)

Over the 4 week period the only gas exchange property which increased apart from water use efficiency was the CO_2 assimilation rate. A possibly simplistic interpretation of these results is that the transpiration rates and stomatal conductances were affected by the increase in the shoot : root ratio which occurred during growth. The increased CO_2 assimilation rates are correlated with reduced specific leaf area over the same time period (1 to 4 weeks). Thus the increase in CO_2 assimilation rates may be due to increased assimilatory capacity per unit leaf area.

In summary:

1) Almost no differences were observable between NO_3^-- and NH_4^+-grown plants except for differences in stomatal conductances and transpiration rates evident during the first week of growth.
2) Photosynthetic CO_2 assimilation rates increased while transpiration rates, stomatal conductances and intercellular CO_2 concentrations decreased with plant age.
3) Photosynthetic CO_2 assimilation rates, expressed on the basis of leaf mass, were lower in NH_4^+- than in NO_3^--fed plants.

4.5.2 Maize response

There were no significant changes in photosynthetic CO_2 assimilation rates, transpiration rates, stomatal conductances, intercellular CO_2 concentrations or water use efficiencies over time (Appendix 7.1.4). The CO_2 assimilation rates (Figure 4.15a), transpiration rates (Figure 4.15b) and stomatal conductances (Figure 4.15c) were significantly higher in NO_3^-- than in NH_4^+-fed maize plants (Appendix 7.1.4). No differences were apparent between the intercellular CO_2 concentrations (Figure 4.16a) and water use efficiencies (Figure 4.16b) of NO_3^-- and NH_4^+-fed plants respectively. There appears to be a clear correlation in maize between lower stomatal conductances and lower assimilation rates in plants grown on NH_4^+ compared to NO_3^- nutrition. The slightly higher specific leaf area of NH_4^+- compared to NO_3^--fed maize (Section 4.4.2) was not sufficient to offset the lower CO_2 assimilation rates and transpiration rates of NH_4^+-fed plants (data not shown).

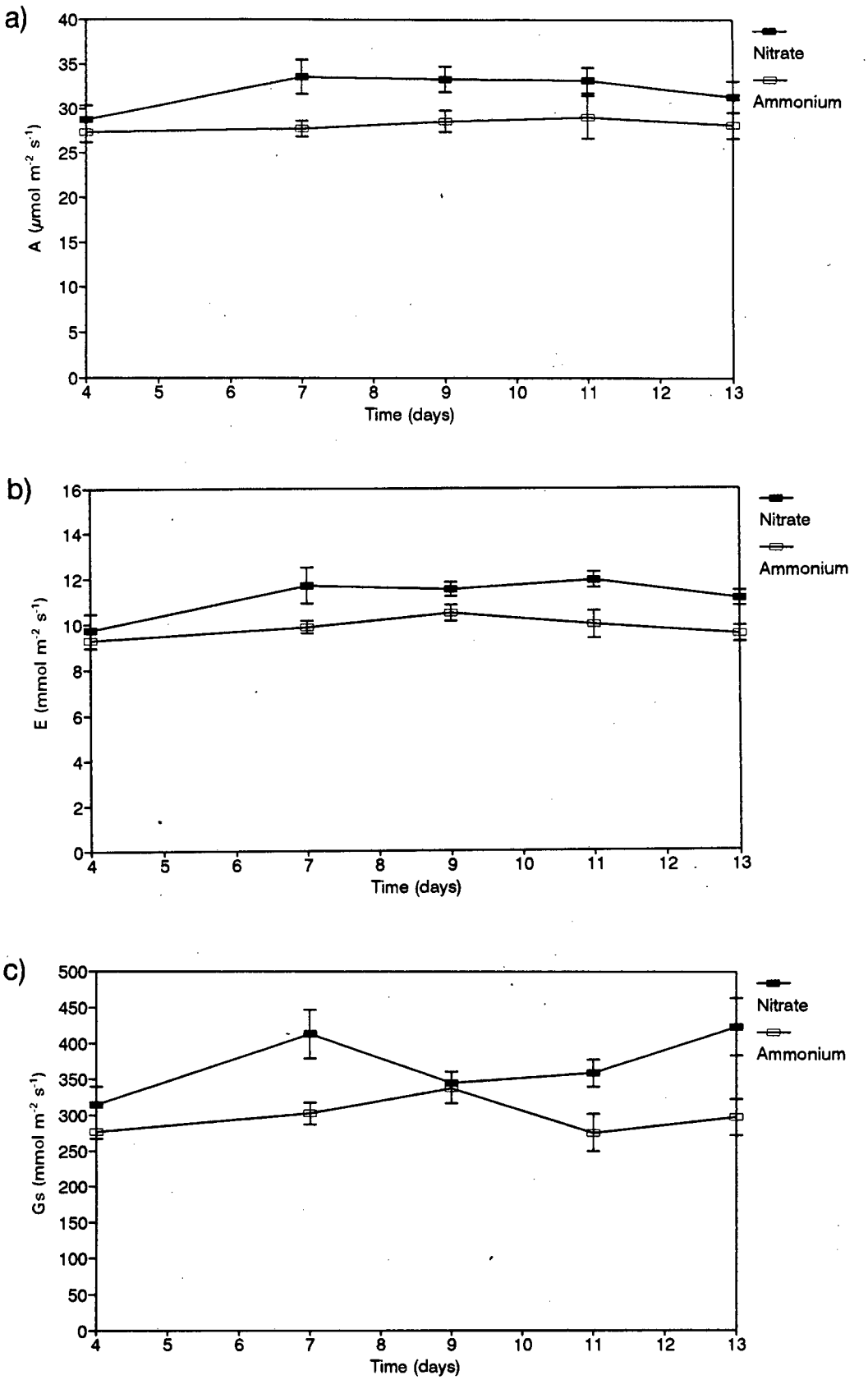


Figure 4.15. a) Carbon dioxide assimilation rates (A); b) transpiration rates (E); c) stomatal conductances to H_2O (Gs) of maize plants grown on 4 mM NO_3^- or NH_4^+ for 13 days. Time=0 is specified as the point at which transfer from nutrient free vermiculite culture to hydroponic culture occurred. Bars show the S.E. where it is larger than the size of the symbols. ($8 < n < 24$)

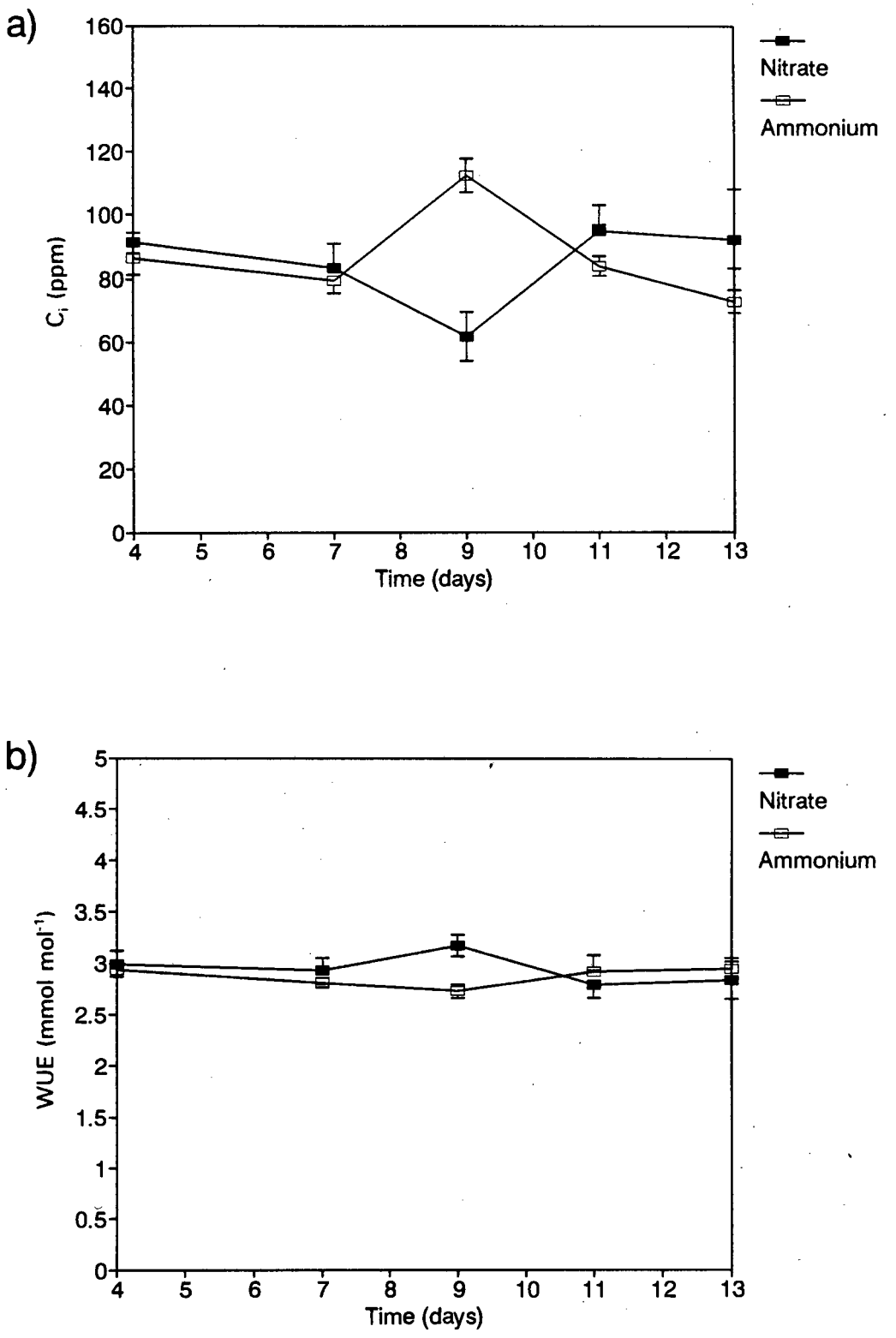


Figure 4.16. a) Intercellular CO_2 concentrations (C_i) and b) water use efficiencies (WUE) of maize plants grown on 4 mM NO_3^- or NH_4^+ for 13 days. Time=0 is specified as the point at which transfer from nutrient free vermiculite culture to hydroponic culture occurred. Bars show the S.E. where it is larger than the size of the symbols. ($8 < n < 24$)

4.5.3 Discussion

Rates of photosynthetic CO_2 assimilation, transpiration and the stomatal conductances of maize plants responded to the form of N supplied whereas those of wheat did not respond or responded only slightly. Comparison of gas exchange characteristics yielded differences between wheat and maize typical for differences between C_3 and C_4 plants.

The smaller biomass of wheat and maize plants grown on NH_4^+ compared to NO_3^- nutrition was correlated with lower photosynthetic rates, although the differences between 4 mM NO_3^-- and NH_4^+-fed wheat plants with respect to photosynthetic rates were only evident when expressed on the basis of leaf dry mass. In maize it is clear that NH_4^+ nutrition resulted in lower photosynthetic rates than NO_3^- nutrition and, therefore, it can be concluded that the reduced C accumulation in NH_4^+-fed plants may have been important in contributing to reduced growth. Although the photosynthetic rates of NH_4^+-fed maize plants were only approximately 10% less than those of NO_3^--fed plants, this difference may be important in determining biomass accumulation. The costs of maintenance respiration would consume a certain minimum proportion of the daily photosynthate, leaving only a proportion of the daily photosynthate for growth. Reduced net photosynthesis would, therefore, have a larger effect on growth than indicated by the magnitude of the drop in photosynthetic rates.

Although increased photosynthetic activity with increased N nutrition is well known (Robinson and Baysdorfer, 1985), considerable variation exists in reported effects of NO_3^- and NH_4^+ nutrition on photosynthetic rates. Some workers have suggested that differences between NO_3^-- and NH_4^+-fed plants with respect to photosynthetic rates may be due to effects of the form of N on the activity of the photosynthetic enzymes (Section 2.4.4). Increased activity of photosynthetic enzymes with NH_4^+ as opposed to NO_3^- nutrition is generally the result of increased protein content of NH_4^+-fed plants compared to NO_3^--fed plants (Golvano *et al.*, 1982) and Rubisco has been found to form a constant proportion of leaf N (Field and Mooney, 1983). Shoot total N has been shown to be higher in NH_4^+- than in NO_3^--fed plants in this investigation (Section 4.8.2), but this was not correlated higher photosynthetic rates in the former. The specific activity of leaf PEPc of both C_3 and C_4 plants has, however, been shown to be enhanced by NO_3^- compared to NH_4^+ nutrition (Schweizer and Erismann, 1985; Arnozis *et al.*, 1988; Garson and Gray, 1991). Altered net photosynthetic rates have been proposed to result from altered rates of photorespiration although data for this proposal are

equivocal. Sharma and Sirohi (1988), however, found evidence in wheat indicating that with NO_3^- nutrition reduced Γ was at least partially the result of enhanced PEPc activity. The possibility that the form of N may have influenced the activity of photosynthetic enzymes cannot be excluded as an explanation for alterations in photosynthetic rates observed in this investigation.

Uncoupling of photophosphorylation by NH_4^+ has frequently been proposed to be responsible for changes in photosynthetic rates of plants supplied with this N source (Goyal and Huffaker, 1984). Supply of NH_4^+ has also been reported to cause stromal acidification with consequences for photosynthetic rates. No unequivocal evidence exists, however, for the operation of these mechanisms in intact tissue. In addition NH_4^+ derived from NH_4^+ nutrition has been shown to be translocated to the shoot to only a limited extent (wheat, Lewis *et al.*, 1987; maize, Murphy and Lewis, 1987).

The photosynthetic rates of both wheat (12 mM N-fed) and maize appear to be closely related to stomatal conductances which may in turn be related to shoot moisture contents. The importance of other mechanisms (direct effects on enzyme activities, uncoupling of photophosphorylation and stromal acidification) in determining the rates of photosynthesis cannot be assessed from the data presented here.

4.6 NON-STEADY STATE GAS EXCHANGE ANALYSIS

Steady state gas exchange analysis provides some information regarding the CO_2 assimilation rates and transpiration rates, but it does not yield much information regarding the physiology responsible for these rates. A more informative approach is to modify some of the environmental variables governing gas exchange and to monitor the gas exchange responses. The physiology of plants is adaptive in that one system may compensate for another and thus underlying differences in physiology may be masked by adaptation to steady state conditions. Modifying controlling variables may disrupt steady state balances built into the system and result in large discrepancies in behaviour. The two factors modified in this investigation were light and CO_2.

4.6.1 Post-illumination burst

Transfer of attached maize and wheat leaves from light to dark conditions resulted in an initial burst (within 1 minute) of CO_2 release which rapidly (within 10 to 15 minutes) declined to the steady state dark respiration rate (Figures 4.17 to 4.20). This typical post-illumination burst is thought to arise from combined photorespiratory and respiratory activity, but primarily the former, continuing for a short period of time (5 minutes) in the dark (Lawlor, 1987). These observations apply to C_3 plants such as wheat, but a C_4 plant such as maize has little photorespiratory activity. There was, however, a small post-illumination burst observed in maize plants, especially those supplied with NH_4^+ nutrition (Figure 4.19). The transfer of plants from light to dark was accompanied by closure of the stomata, indicated by decreasing stomatal conductances, decreased transpiration rates and increased intercellular CO_2 concentrations (Figures 4.17 to 4.20).

There were only small differences in the effects of NO_3^- or NH_4^+ nutrition on post-illumination CO_2 flux in either wheat (Figure 4.17) or maize (Figure 4.19). Stomatal conductances in wheat (Figure 4.17) and maize (Figure 4.19) were, however, initially lower in NH_4^+-fed plants and upon transfer to darkness decreased more rapidly than those of NO_3^--fed plants. Transpiration rates, although initially comparable between NO_3^-- and NH_4^+-fed plants, decreased more rapidly in the latter (wheat, Figure 4.18; maize, Figure 4.20). The much faster decline of transpiration rate upon transfer from light to dark of NH_4^+-fed plants compared to NO_3^--fed plants, and the corresponding change in stomatal conductance, is consistent with the observation that NH_4^+-fed plants have generally lower shoot moisture contents than NO_3^--fed plants (Section 4.2).

4.6.2 A:C_i curves

Although there were only small differences in the relationship between photosynthetic CO_2 assimilation and intercellular CO_2 concentration (A:C_i curves[2]) of NO_3^-- and NH_4^+-fed wheat and maize plants, these curves have been included because of the valuable information they contain for interpreting the gas exchange results from other experiments.

[2] The variables used in characterizing the A:C_i curves are explained in Section 3.4

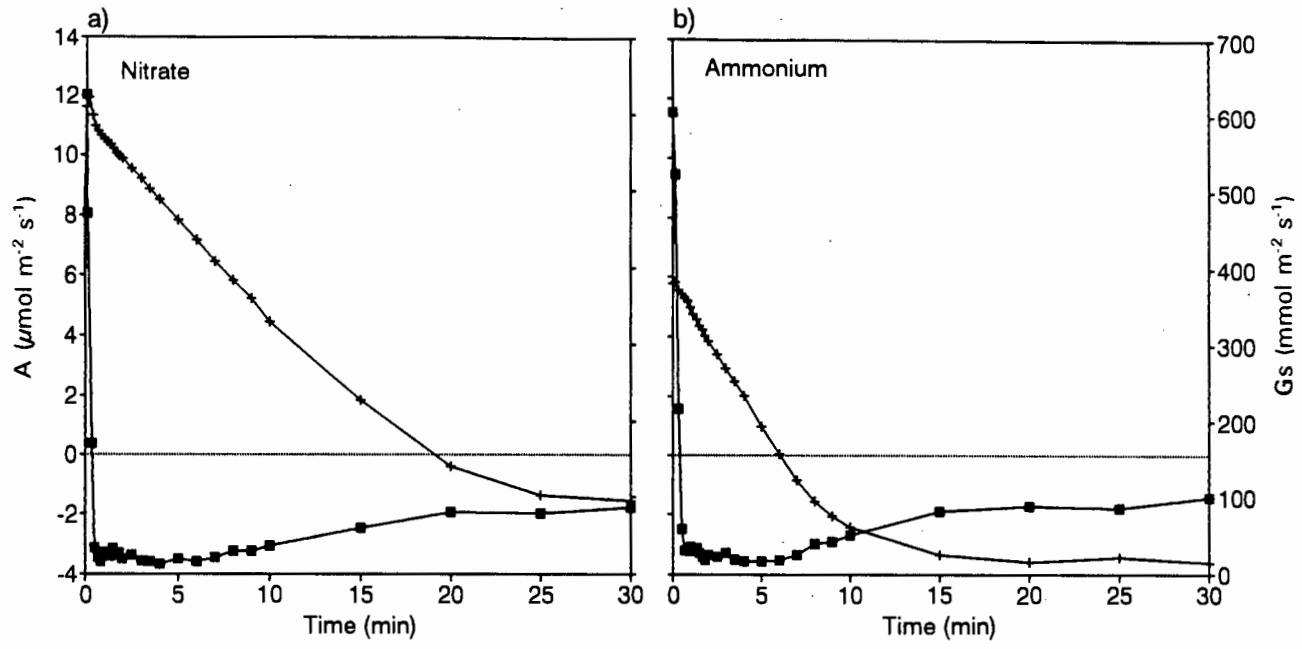


Figure 4.17. Carbon dioxide assimilation rates (A, ■) and stomatal conductances to H_2O (Gs, +) of a) 4 mM NO_3^--fed and b) 4 mM NH_4^+-fed wheat plants after transfer into dark (Time=0). Maximum S.E. of: NO_3^--fed plants, A=1.07 and Gs=63.54; NH_4^+-fed plants, A=0.97 and Gs=45.72. (n=6)

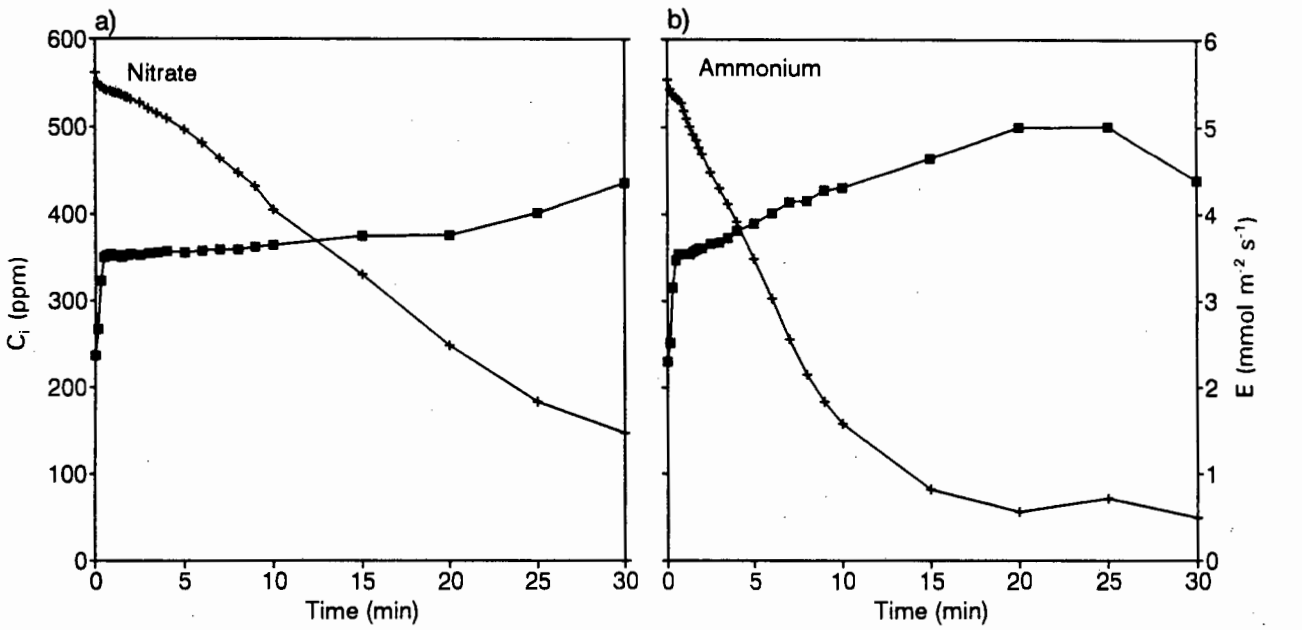


Figure 4.18. Intercellular CO_2 concentrations (C_i, ■) and transpiration rates (E, +) of a) 4 mM NO_3^--fed and b) 4 mM NH_4^+-fed wheat plants after transfer into dark (Time=0). Maximum S.E. of: NO_3^--fed plants, C_i=32.04 and E=0.56; NH_4^+-fed plants, C_i=64.53 and E=0.55. (n=6)

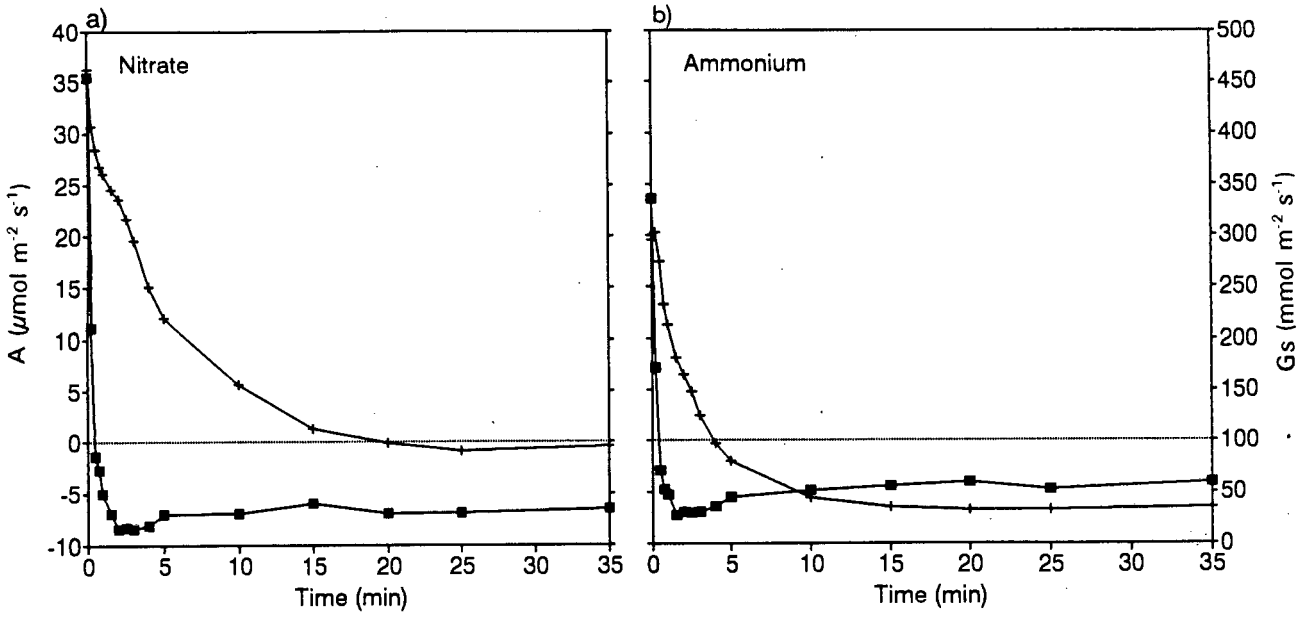


Figure 4.19. Carbon dioxide assimilation rates (A, ■) and stomatal conductances to H_2O (Gs, +) of a) 4 mM NO_3^--fed and b) 4 mM NH_4^+-fed maize plants after transfer into dark (Time=0). Maximum S.E. of: NO_3^--fed plants, A=1.35 and Gs=32.31; NH_4^+-fed plants, A=1.21 and Gs=47.68. (n=6)

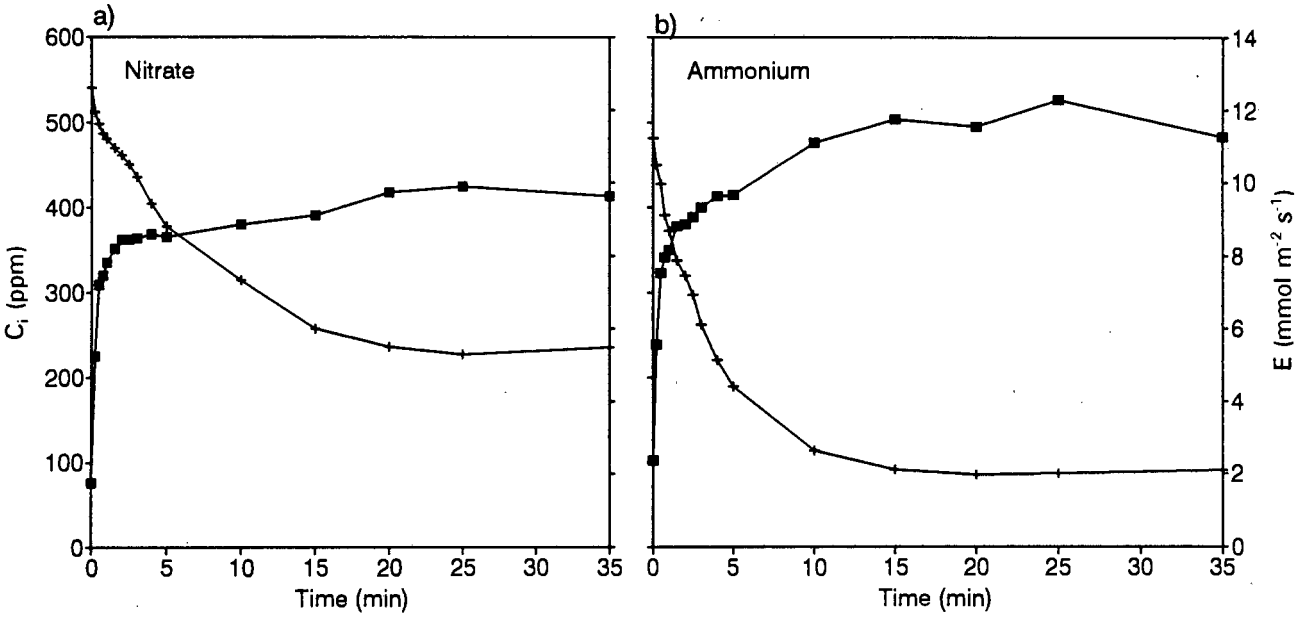


Figure 4.20. Intercellular CO_2 concentrations (C_i, ■) and transpiration rates (E, +)) of a) 4 mM NO_3^--fed and b) 4 mM NH_4^+-fed maize plants after transfer into dark (Time=0). Maximum S.E. of: NO_3^--fed plants, C_i=54.32 and E=0.65; NH_4^+-fed plants, C_i=45.35 and E=0.56. (n=6)

The A:C_i curves of 4 mM N-fed wheat were typical for C_3 plants (Figure 4.21) with a high rate of CO_2 loss at 0 ppm intercellular CO_2 concentration (NO_3^--fed plants 7.34 μmol m^{-2} s^{-1}; NH_4^+-fed plants 10.04 μmol m^{-2} s^{-1}) indicating substantial photorespiratory and respiratory activity, particularly in NH_4^+-fed plants. The differences between NO_3^-- and NH_4^+-fed wheat plants with respect to $V_{Rubisco}$ ($V_{Rubisco} = \delta A/\delta C_i$) were small (Table 4.1) but the higher $V_{Rubisco}$ values of NH_4^+-fed plants may reflect the larger proportion of soluble amino-N found in these plants (Section 4.8.2.1). The link between leaf N and Rubisco content has been demonstrated previously (Field and Mooney, 1983). The maximum rates of RuBP regeneration (J_{max}) and the rates of CO_2 assimilation in the absence of stomatal limitations (A_o) were 1.2-fold greater in NH_4^+- than in NO_3^--fed wheat plants. Photosynthetic rates of NO_3^-- and NH_4^+-fed wheat plants in the presence of stomatal limitation (A_i) were the same.

The observation of a 1.4-fold higher rate of CO_2 release from NH_4^+- than from NO_3^--fed wheat plants at 0 ppm intercellular CO_2 is in accord with the data of Sharma and Sirohi (1988) who found that the compensation points of wheat were higher with NH_4^+ than with NO_3^- nutrition. Higher photorespiratory activity of NH_4^+- compared to NO_3^--fed wheat may account for the 1.2-fold higher J_{max} in the former, because CO_2 concentration elevated above ambient levels would have inhibited photorespiration. Considering the small differences between A_i of NO_3^-- and NH_4^+-fed plants which have been confirmed by photosynthetic rates measured previously (Section 4.3.1), the inhibition of photorespiration by high intercellular CO_2 concentrations may thus be expected to result in elevated J_{max} of NH_4^+- compared to NO_3^--fed plants. Lower stomatal conductances in NH_4^+- compared to NO_3^--fed plants may be expected to reduce the intercellular CO_2:O_2 ratio thus facilitating photorespiration. This may partially account for the lower net photosynthetic rates observed in 12 mM NH_4^+- compared to NO_3^--fed wheat.

Maize plants had typical A:C_i curves for a C_4 plant (Figure 4.22) characterized by an initially steep slope and, therefore, a high $V_{Rubisco}$ (Table 4.1). As in the case of wheat, the $V_{Rubisco}$ of NH_4^+-fed maize was slightly higher than that of NO_3^--fed maize, possibly reflecting the larger proportion of soluble amino-N found in these plants (Section 4.8.2.2) although Rubisco has been reported not to accumulate in C_4 plants (Sage and Pearcy, 1987a). When intercellular CO_2 concentration was zero the release of CO_2 was small (NO_3^--fed plants 1.99 μmol m^{-2} s^{-1}; NH_4^+-fed plants 0.56 μmol m^{-2} s^{-1}) indicating little photorespiratory activity. In maize the values for J_{max}, A_o and A_i of NO_3^--fed plants were higher than those of NH_4^+-fed plants.

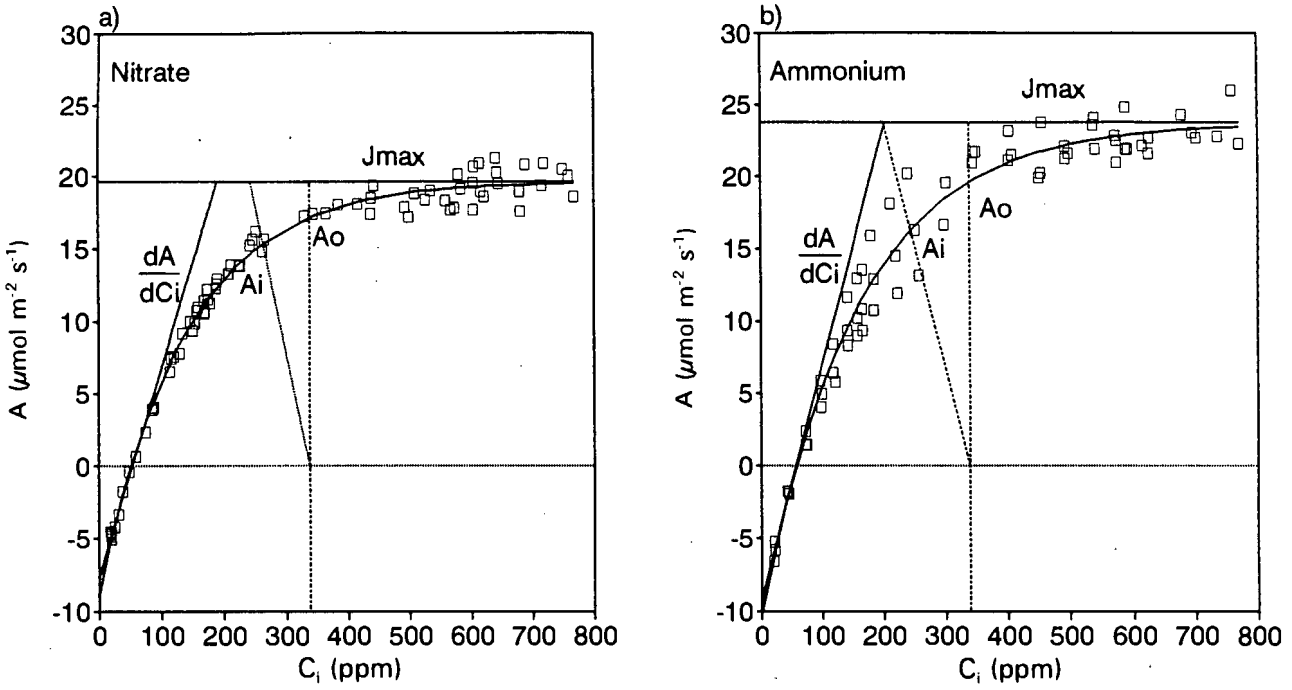


Figure 4.21. A:C_i curves (CO_2 assimilation versus intercellular CO_2 concentration) for a) 4 mM NO_3^--fed and b) 4 mM NH_4^+-fed wheat plants. Symbols on the graph are discussed in the text. (n=3)

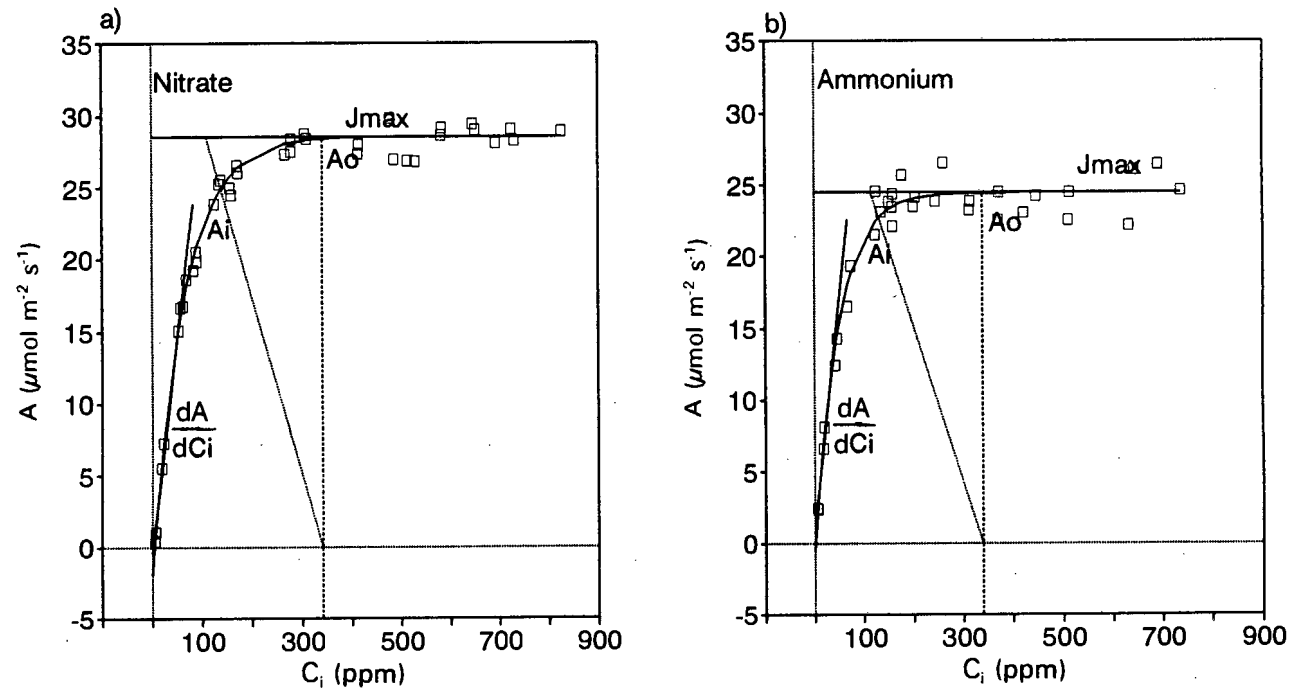


Figure 4.22. A:C_i curves (CO_2 assimilation versus intercellular CO_2 concentrations) for a) 4 mM NO_3^--fed and b) 4 mM NH_4^+-fed maize plants. Symbols on the graph are discussed in the text. (n=3)

This is to be expected from the lower CO_2 assimilation rates observed previously with NH_4^+ nutrition (Section 4.3.2).

Table 4.1 Critical A:Ci curve variables determined from A:Ci curves for 4 mM N-fed wheat and maize (Figures 21 and 22). See text for explanation of symbols.

	Wheat		Maize	
	Nitrate	Ammonium	Nitrate	Ammonium
J_{max}	19.94	24.25	28.61	24.48
$\delta A/\delta Ci$	0.15	0.17	0.29	0.33
A_o	17.09	19.80	28.44	24.46
A_i	15.18	16.79	24.73	22.64

From gas exchange characteristics described previously (Sections 4.3 and 4.5) the differences in photosynthetic CO_2 assimilation between NO_3^-- and NH_4^+-fed plants appear to be the result of reduced stomatal conductances in NH_4^+- compared to NO_3^--fed wheat and maize plants. Photosynthetic rates were invariably associated with reduced stomatal conductances and intercellular CO_2 concentrations (Sections 4.3 and 4.5). To show that the reduced photosynthetic rates were attributable to reduced intercellular CO_2 concentrations, the $A:C_i$ curves may be referred to (Figures 4.21 and 4.22). Wheat typically exhibited intercellular CO_2 concentrations of about 250 ppm (Figures 4.5b and 4.13c). From the $A:C_i$ curves it can be seen that changes in the intercellular CO_2 concentration in the region of 250 ppm would have strong influences on the CO_2 assimilation rate. Maize typically exhibited intercellular CO_2 concentrations of about 100 ppm (Figures 4.6b and 4.16a) which falls in a portion of the $A:C_i$ curves for maize in which small changes in intercellular CO_2 concentration would have a large influence on CO_2 assimilation rates.

4.7 CARBON PARTITIONING AND TRANSLOCATION

The C partitioning experiments were designed to determine whether the changes observed previously (Section 4.2) in root extension in response to different forms of N, particularly in wheat, could be attributed to changes in allocation of C specifically from root resources. In particular, the allocation of C to structural material in the root in comparison to allocation

to other fractions was of interest. The allocation of C between shoot and root and the loading of C onto the xylem were also investigated.

Allocation of C to carbohydrate, amino acid and protein fractions was followed using photosynthetically assimilated $^{14}CO_2$. The ^{14}C containing fractions isolated were 1) water soluble carbohydrate (Wc), 2) amino acids and water soluble protein (Wa), 3) bound carbohydrate (Bc), 4) bound protein (Ba), 5) structural material (Struct.) and 6) water insoluble, ethanol soluble material (EtOH). Although the water insoluble, ethanol soluble fraction was included in all partitioning graphs, this fraction was generally small and difficult to determine experimentally. No systematic changes in this fraction were detected. This fraction contained the leaf chlorophyll and was thus considerably more important in the shoot than the root. The ratios of [^{14}C]carbohydrate : [^{14}C]amino acid (Wc+Bc+Struct : Wa+Ba) and the shoot : root ratios of ^{14}C label in each fraction were calculated for each experiment.

Different quantities of $NaH^{14}CO_3$ were used (50 to 100 μCi) for different experiments and this and other variabilities in ^{14}C supply resulted in the ^{14}C labelling intensity between various experiments not being directly comparable. In addition, not all the plants in any one experiment could be simultaneously supplied with $^{14}CO_2$. Dealing with the data from these experiments was complex due to the fact that it was difficult to experimentally standardize the amount of $^{14}CO_2$ taken up by each replicate of each treatment. To overcome this problem data was expressed on a percentage basis (percentage that any one fraction makes up of the total for a particular plant part) and statistics were calculated on the arcsine transformed data. Unfortunately, use of percentages obscures some of the information potentially available and thus the raw data is presented for reference in Appendix 7.2.

The data in Appendix 7.2 shows that there were considerable variations in the total label present in NO_3^-- and NH_4^+-fed plants. In maize (Figures 7.6 and 7.7) and 12 mM N grown wheat (Figure 7.3) the label present in NH_4^+-fed plants was much lower than that present in NO_3^--fed plants. Differences between NO_3^-- and NH_4^+-fed plants with respect to total ^{14}C label vary with the N concentration and the time between $^{14}CO_2$ supply and harvesting of the plants. The quantity of ^{14}C label found in NO_3^-- and NH_4^+-fed plants does appear to reflect the differences in photosynthetic rates and thus acquisition of $^{14}CO_2$ by the plants to some extent, although the $^{14}CO_2$ present in the plant is the balance between photosynthetic acquisition and respiratory loss. Results presented later show differences in root respiration

rates (Section 4.9.1) and uptake of HCO_3^- by roots (Section 4.10) between NO_3^-- and NH_4^+-fed plants. These factors, together with photosynthetic activity and shoot respiration, must play a role in determining the total ^{14}C label present.

The ^{14}C content of the shoot was generally higher than that of the root (Appendix 7.2) and in general the ^{14}C shoot : root ratios far exceeded the dry mass shoot : root ratios. These experiments provide a non-steady state picture of C allocation and thus the lack of equilibrium between the shoot, which was supplied with the ^{14}C label, and the root is to be expected. The high ^{14}C shoot : root ratios are thus due to translocation limitations and loss of the label from the root, either through respiratory activity or re-translocation to the shoot.

4.7.1 Partitioning of ^{14}C

4.7.1.1 Wheat supplied with 4 mM nitrogen and harvested 24 h after $^{14}CO_2$ supply

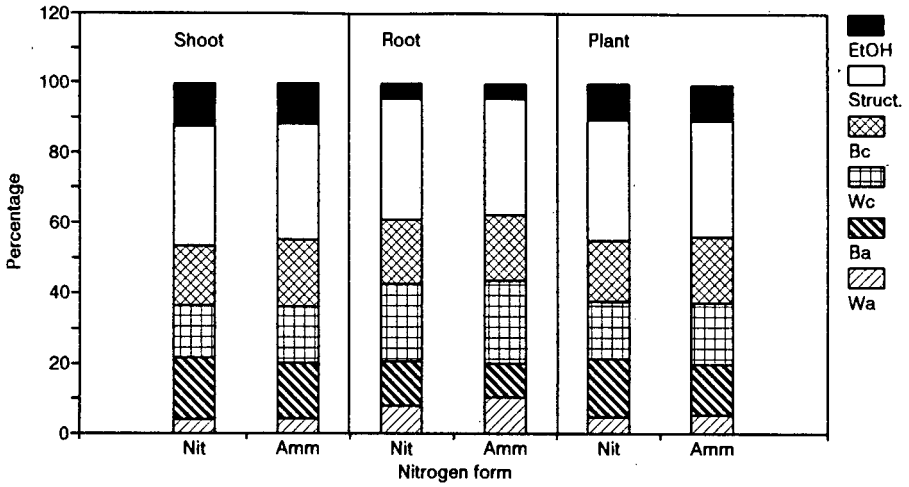
Apart from small significant differences between NO_3^-- and NH_4^+-fed plants with respect to the percentage allocation of ^{14}C to bound protein in the shoots and roots and bound carbohydrates in the shoots, the labelling patterns for NO_3^-- and NH_4^+-fed plants were comparable (Figure 4.23). These results indicate that the large reduction in root growth of NH_4^+- compared to NO_3^- -fed plants was not the result of altered allocation of C within the root. Possible explanations for this result are that either the experimental methodology employed was not sensitive enough to detect the changes in allocation, perhaps due to mobility of ^{14}C between the various fractions, or that the differences in biomass may have been the result of small differences in partitioning throughout the exposure of the plants to the N which were compounded during growth.

No differences were found between the [^{14}C]carbohydrate : [^{14}C]amino (^{14}C : ^{14}C-N) ratios of NO_3^-- and NH_4^+-fed plants (Figure 4.24) although the shoot had a lower ^{14}C : ^{14}C-N ratio than the root. Shoot : root ratios of ^{14}C content of certain fractions (water soluble carbohydrates, amino acids and water soluble protein, structural material) of NO_3^-- and NH_4^+-fed plants were significantly different, but only at the $p<0.1$ level (Figure 4.25). This difference was probably the consequence of a greater total allocation of ^{14}C to the roots of NH_4^+-fed plants. The soluble fractions (water soluble carbohydrates, amino acids and water soluble protein) had lower ^{14}C shoot : root ratios than the insoluble fractions (bound carbohydrates, bound protein, structural material).

4.7.1.2 Wheat supplied with 4 mM nitrogen and harvested 9 h after $^{14}CO_2$ supply

This experiment was conducted to establish the patterns of ^{14}C partitioning without the plants having passed through a dark phase as was normal with a 24 h harvest. It was considered that the lack of differences between NO_3^-- and NH_4^+-fed plants in the 24 h harvest (Section 4.7.1.1) may have been brought about through extensive circulation of C between shoots and roots and the mobility of C between the various fractions. Few differences between NO_3^-- and NH_4^+-fed plants with respect to ^{14}C allocation were, however, found in this experiment (Figure 4.26). There were no differences between the ^{14}C : ^{14}C-N ratios of NO_3^-- and NH_4^+-fed plants (Figure 4.27) and there were only small differences between the ^{14}C shoot : root ratios of NO_3^-- and NH_4^+-fed plants, except that the total ^{14}C shoot : root ratios were lower in NH_4^+- than NO_3^--fed plants (Figure 4.28). Therefore the lack of differences in ^{14}C partitioning after 24 h cannot be solely ascribed to inability of the experimental methodology to detect the differences due to mobility of ^{14}C between various fractions; if this were the case then differences should have been more clearly evident after 9 h than after 24 h. It seems, therefore, that differences between 4 mM NO_3^-- and NH_4^+-fed plants with respect to ^{14}C partitioning were indeed small.

There was a smaller allocation of ^{14}C to structural material and higher ^{14}C : ^{14}C-N ratios in the 9 h experiment than in the 24 h experiment, indicating that the shorter time period limited the flow of ^{14}C into structural material and amino acids (Figures 4.27 and 4.28). The ^{14}C : ^{14}C-N ratios of the root component were much lower than those of the shoot, in contrast to the situation in the 24 h harvest. The low ^{14}C : ^{14}C-N ratios of the roots illustrates the dependence of root based N assimilation on photosynthetically derived C for provision of C skeletons for amino acid synthesis. As in the case of the 24 h harvest (Figure 4.25), the ^{14}C shoot : root ratios in the 9 h harvest were generally higher in the NO_3^-- than in the NH_4^+-fed plants (Figure 4.28). The ^{14}C shoot : root ratios of the water soluble protein and amino acid fraction were particularly small relative to the other fractions indicating root based synthesis of amino acids, particularly in NH_4^+-fed plants.



Fraction	Shoot	Root	Plant
Wa	NS	NS	NS
Ba	$p<0.05$	$p<0.05$	$p<0.05$
Wc	NS	NS	NS
Bc	$p<0.05$	NS	$p<0.05$
Struct.	NS	NS	NS
EtOH	NS	NS	NS

Figure 4.23. Percentage allocation of ^{14}C to water soluble carbohydrates (Wc), amino acids and water soluble proteins (Wa), bound carbohydrates (Bc), bound proteins (Ba), structural material (Struct.) and water insoluble, ethanol soluble material (EtOH) in wheat plants grown on 4 mM NO_3^- (Nit) or NH_4^+ (Amm). Plants were supplied with $^{14}CO_2$ 24 h prior to harvesting. Results of Student's T test comparisons of NO_3^-- and NH_4^+-fed plants performed on arcsine transformed proportions for each fraction are shown in table below graph (NS=not significant at 90% confidence interval). (n=3)

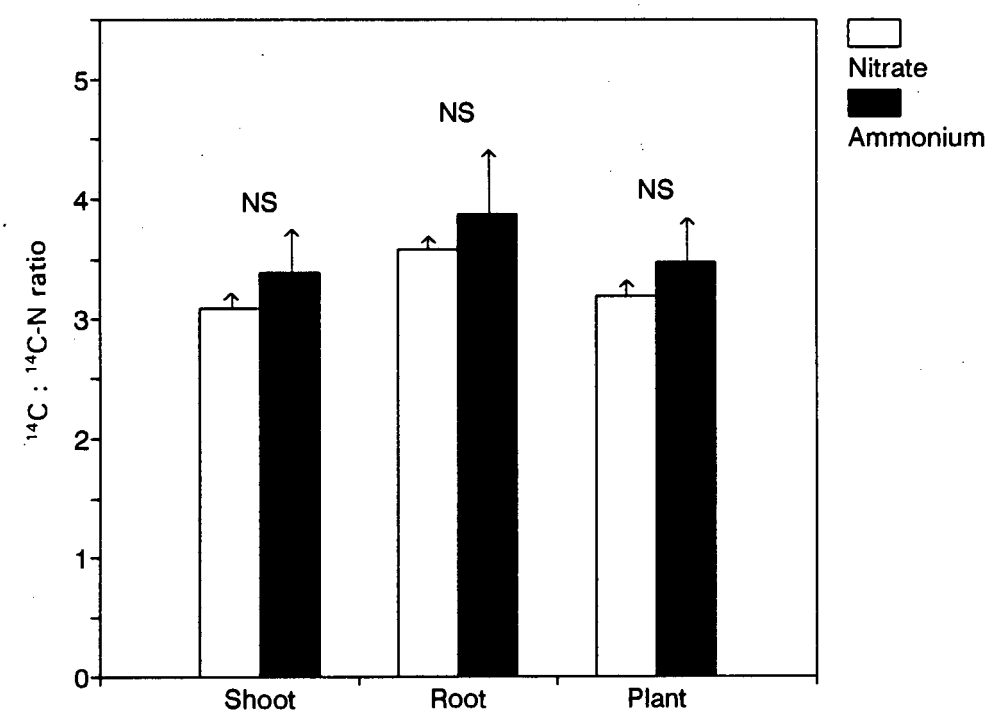


Figure 4.24. Ratios of ^{14}C : ^{14}C-N for wheat plants grown on 4 mM NO_3^- or NH_4^+. Plants were supplied with $^{14}CO_2$ 24 h prior to harvesting. Results of Student's T test comparisons of NO_3^-- and NH_4^+-fed plants are shown above S.E. bars (NS=not significant at 90% confidence interval). (n=3)

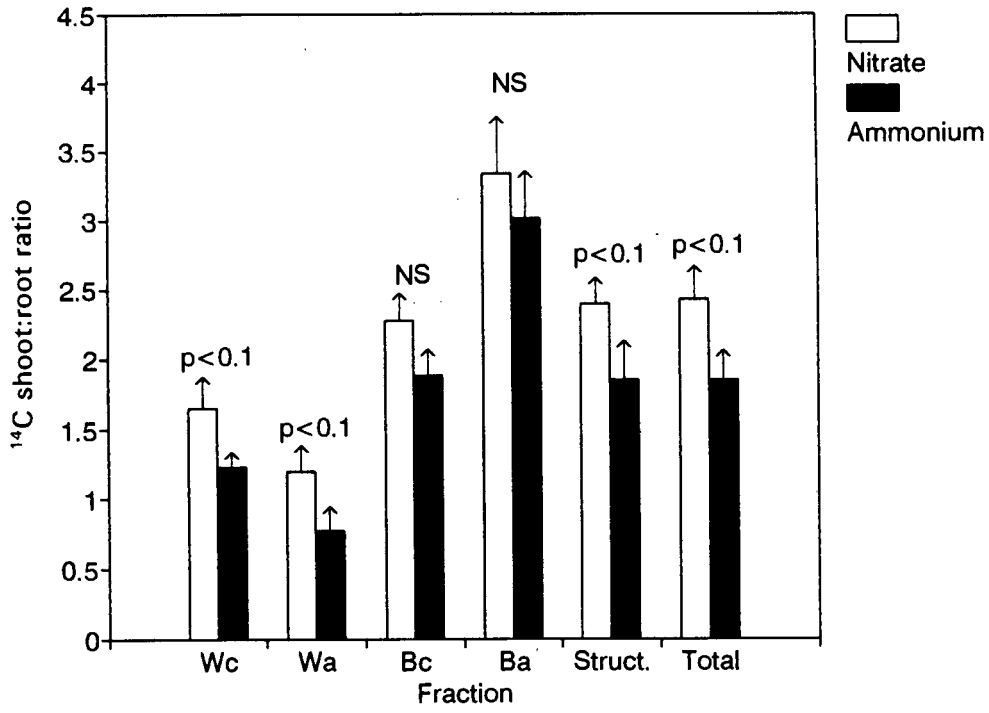
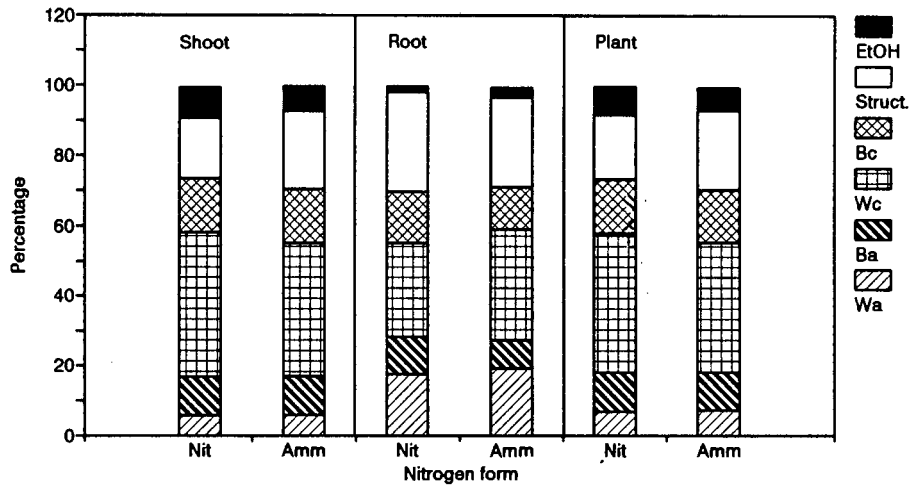


Figure 4.25. ^{14}C Shoot : root ratios of water soluble carbohydrates (Wc), amino acids and water soluble proteins (Wa), bound carbohydrates (Bc), bound proteins (Ba) and structural material (Struct.) in wheat plants grown on 4 mM NO_3^- or NH_4^+. Plants were supplied with $^{14}CO_2$ 24 h prior to harvesting. Results of Student's T test comparisons of NO_3^-- and NH_4^+-fed plants are shown above S.E. bars (NS=not significant at 90% confidence interval). (n=3)



Fraction	Shoot	Root	Plant
Wa	NS	NS	NS
Ba	NS	NS	NS
Wc	NS	NS	NS
Bc	NS	NS	NS
Struct.	p<0.1	NS	NS
EtOH	NS	p<0.05	NS

Figure 4.26. Percentage allocation of ^{14}C to water soluble carbohydrates (Wc), amino acids and water soluble proteins (Wa), bound carbohydrates (Bc), bound proteins (Ba), structural material (Struct.) and water insoluble, ethanol soluble material (EtOH) in wheat plants grown on 4 mM NO_3^- (Nit) or NH_4^+ (Amm). Plants were supplied with $^{14}CO_2$ 9 h prior to harvesting. Other details as for Figure 4.23. (n=3)

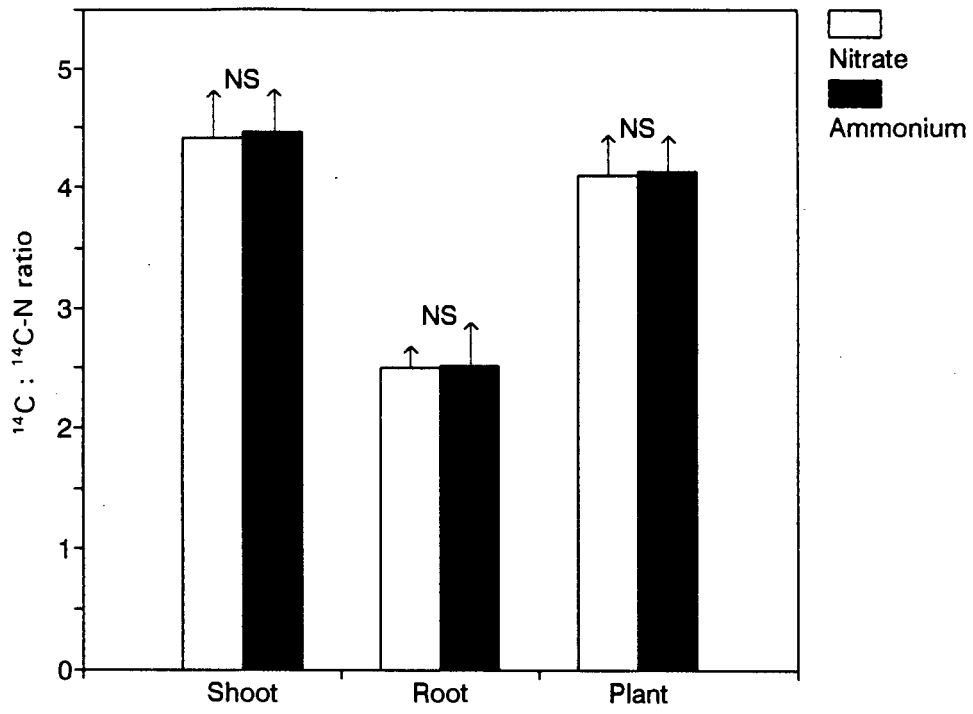


Figure 4.27. Ratios of ^{14}C : ^{14}C-N for wheat plants grown on 4 mM NO_3^- or NH_4^+. Plants were supplied with $^{14}CO_2$ 9 h prior to harvesting. Results of Student's T test comparisons of NO_3^-- and NH_4^+-fed plants are shown above S.E. bars (NS=not significant at 90% confidence interval). (n=3)

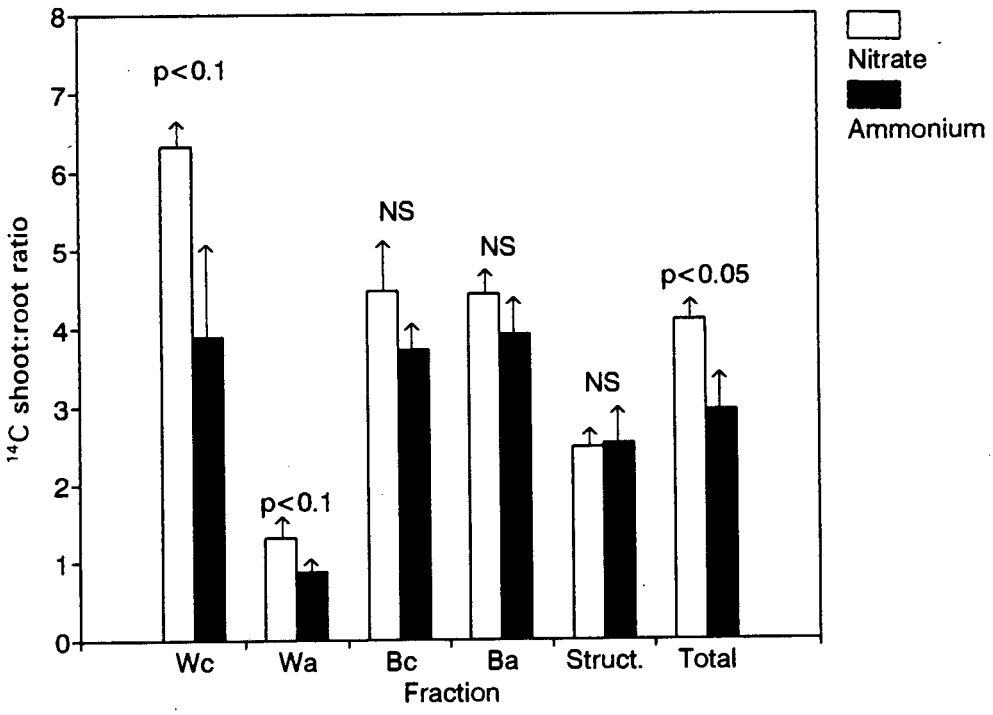


Figure 4.28. ^{14}C Shoot : root ratios of water soluble carbohydrates (Wc), amino acids and water soluble proteins (Wa), bound carbohydrates (Bc), bound proteins (Ba) and structural material (Struct.) in wheat plants grown on 4 mM NO_3^- or NH_4^+. Plants were supplied with $^{14}CO_2$ 9 h prior to harvesting. Results of Student's T test comparisons of NO_3^-- and NH_4^+-fed plants are shown above S.E. bars (NS=not significant at 90% confidence interval). (n=3)

4.7.1.3 Wheat supplied with 12 mM nitrogen and harvested 24 h after $^{14}CO_2$ supply

Despite obvious differences between many other aspects of the physiology of wheat plants grown on either NO_3^- or NH_4^+ nutrition, no major differences could be discerned between ^{14}C allocation patterns in 4 mM N grown plants. In order to magnify the slight differences the plants were grown on 12 mM N. Although this may be an extreme (unnaturally high) N concentration, the objective of the experiment was to trace the physiological causes of reduced root extension, not to replicate natural systems.

The allocation of ^{14}C to structural material was smaller in the 12 mM N experiments than the 4 mM N experiments and with respect to NO_3^--fed plants, allocation of ^{14}C to the structural component of the roots of NH_4^+-fed plants was significantly reduced (Figure 4.29). The ^{14}C : ^{14}C-N ratios of the plants were lower in this experiment (Figure 4.30) than in the 4 mM N experiments (Figures 4.24 and 4.27) and were lower in NH_4^+- than NO_3^--fed plants, principally as a result of increased allocation of ^{14}C to amino acids and water soluble protein and reduced allocation to water soluble carbohydrates and bound carbohydrates. The ^{14}C : ^{14}C-N ratios were higher in the roots than the shoots in the case of the NO_3^--fed plants and the ^{14}C : ^{14}C-N ratios were significantly lower in the NH_4^+- than in the NO_3^--fed plants. The ^{14}C shoot : root ratios of NH_4^+-fed plants were reduced in the soluble fractions (water soluble carbohydrates, amino acids and water soluble protein) and increased in the bound material (bound carbohydrates, bound protein and structural material) in comparison with NO_3^--fed plants (Figure 4.31). Thus, from the 12 mM N-fed wheat it appears that increased amino acid synthesis in the roots of NH_4^+-fed plants was at the expense of root carbohydrates.

As an addendum to this experiment the effect of a heterotrophic supply of C to the roots was investigated by replacing the iron source normally used for wheat culture (Fe-EDTA) with Fe-citrate. There was a significantly larger allocation of ^{14}C to the water soluble protein and amino acid fraction and a smaller allocation to bound carbohydrates in both shoots and roots of NH_4^+- compared to NO_3^--fed plants (Figure 4.32). In addition there was a reduction (only significant at $p < 0.1$) in the allocation of ^{14}C to structural material in NH_4^+-fed plants. The reason for the smaller differences between NO_3^-- and NH_4^+-fed plants in this experiment in contrast to the 12 mM N experiment conducted with Fe-EDTA may lie in the importance of the heterotrophic source of C available from citrate to NH_4^+-fed plants.

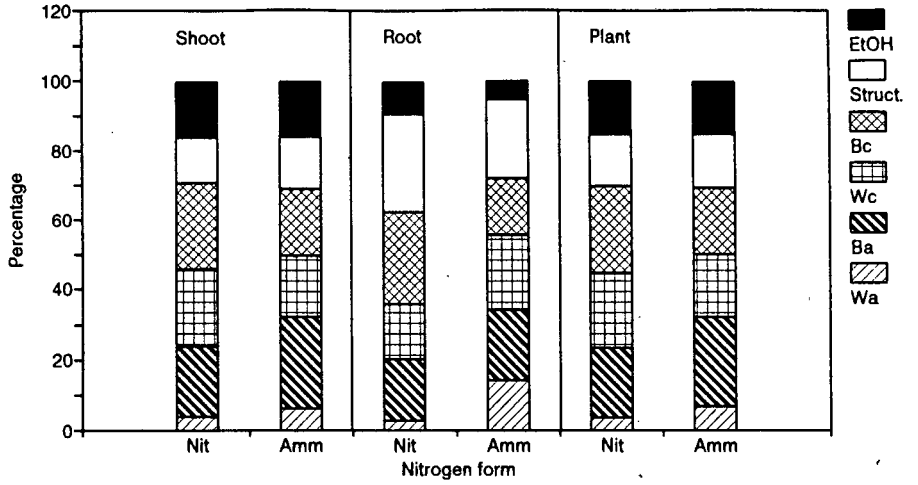
The heterotrophic C source may be expected to reduce the dependence of the root on ^{14}C labelled photosynthate from the shoot, and thus root growth and NH_4^+ assimilation may be expected to compete less for C. As in the 12 mM N experiment conducted with Fe-EDTA, the ^{14}C : ^{14}C-N ratios of NH_4^+-fed plants were lower than those of NO_3^--fed plants, although the differences were small and the ^{14}C : ^{14}C-N ratio, particularly in the root, was lower in the Fe-citrate experiment than the Fe-EDTA experiment (compare Figures 4.30 and 4.33). These results indicate that the citrate was able to replace ^{14}C labelled carbohydrate in the root to some extent. This appeared to enhance the allocation of ^{14}C to amino acids and water soluble protein in the Fe-citrate compared to the Fe-EDTA supplied plants, particularly in the case of NH_4^+-fed plants. The NH_4^+-fed plants had generally lower ^{14}C shoot : root ratios (Figure 4.34) in water soluble fractions (water soluble carbohydrates, amino acids and water soluble protein) and higher ratios in bound material (bound carbohydrates, bound protein and structural material).

From the enhanced incorporation of ^{14}C into amino acids and water soluble protein this experiment, therefore, provides evidence for the root based assimilation of NH_4^+ and NO_3^- into amino acids being limited by the availability of C within the root. This supports the hypothesis that NH_4^+ limits root expansion through competition with root extension for carbohydrate. The role of citrate may, however, be more complex than merely acting as a C source for the root. It has been shown that Fe-citrate is transported through the xylem (Campbell and Redinbaugh, 1984) and it is, therefore, feasible that supplying citrate with Fe enhances the translocation of the Fe to the shoot.

4.7.1.4 Wheat split-root culture: ^{14}C partitioning

Very few differences were evident with respect to ^{14}C partitioning between 4 and 12 mM N-fed wheat grown in split-root culture (Figure 4.35). There were, however, large differences between split-root and normally cultured wheat plants with respect to the ^{14}C partitioning data. In particular there was a smaller allocation of ^{14}C to water soluble carbohydrates and a generally larger allocation to structural material in the split-root experiment.

There was a larger allocation of ^{14}C to the water soluble protein and amino acid fraction in NH_4^+-fed root-halves compared to NO_3^--fed root-halves (Figure 4.35). At 4 mM N, a larger allocation of ^{14}C to bound protein was observed in NH_4^+- compared to NO_3^--fed root-halves.



Fraction	Shoot	Root	Plant
Wa	p<0.05	p<0.05	p<0.05
Ba	p<0.05	NS	p<0.05
Wc	NS	p<0.1	NS
Bc	p<0.1	p<0.05	p<0.05
Struct.	p<0.1	p<0.1	NS
EtOH	NS	p<0.1	NS

Figure 4.29. Percentage allocation of ^{14}C to water soluble carbohydrates (Wc), amino acids and water soluble proteins (Wa), bound carbohydrates (Bc), bound proteins (Ba), structural material (Struct.) and water insoluble, ethanol soluble material (EtOH) in wheat plants grown on 12 mM NO_3^- (Nit) or NH_4^+ (Amm). Plants were supplied with $^{14}CO_2$ 24 h prior to harvesting. Other details as for Figure 4.23. (n=3)

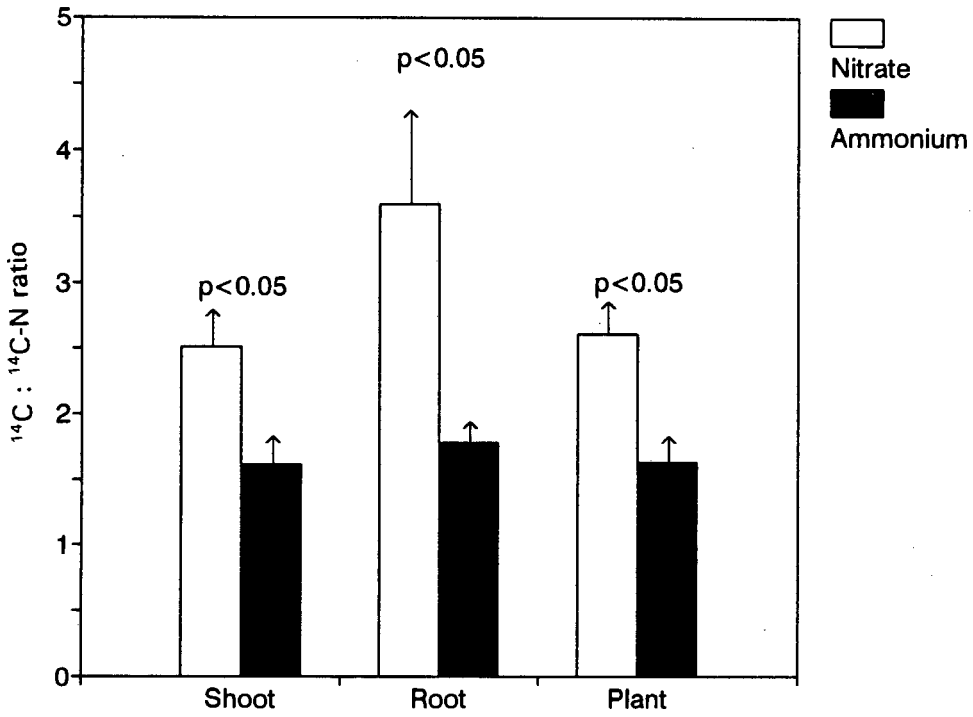


Figure 4.30. Ratios of ^{14}C : ^{14}C-N for wheat plants grown on 12 mM NO_3^- or NH_4^+. Plants were supplied with $^{14}CO_2$ 24 h prior to harvesting. Results of Student's T test comparisons of NO_3^-- and NH_4^+-fed plants are shown above S.E. bars (NS=not significant at 90% confidence interval). (n=3)

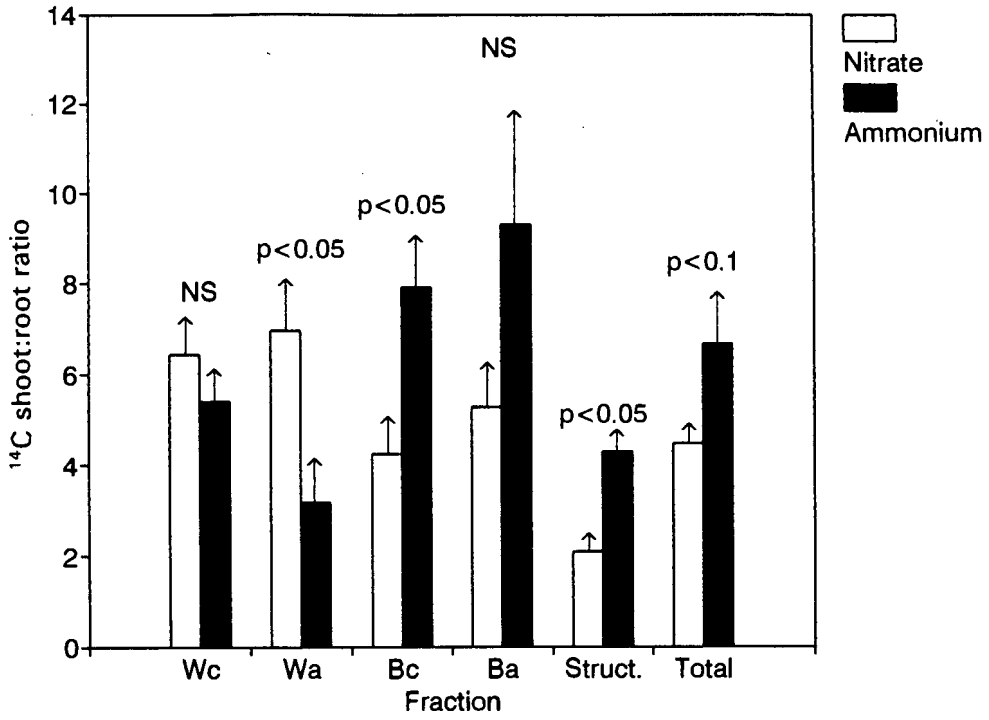
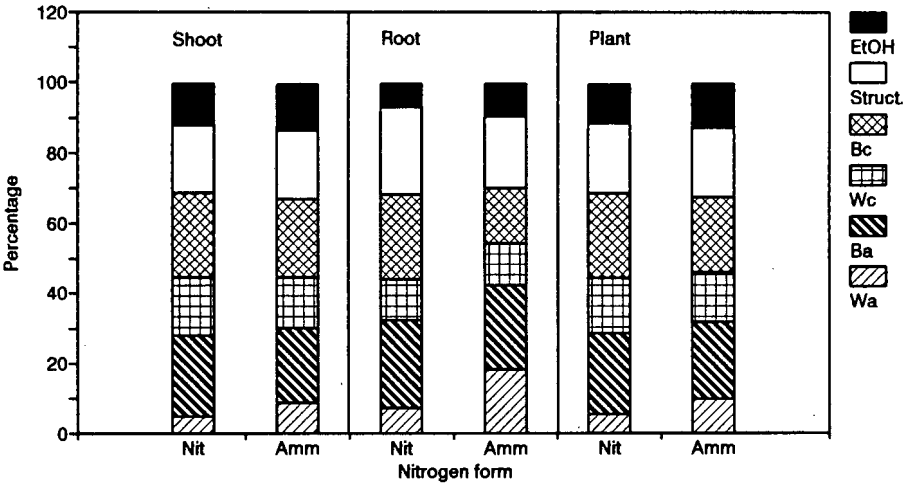


Figure 4.31. ^{14}C Shoot : root ratios of water soluble carbohydrates (Wc), amino acids and water soluble proteins (Wa), bound carbohydrates (Bc), bound proteins (Ba) and structural material (Struct.) in wheat plants grown on 12 mM NO_3^- or NH_4^+. Plants were supplied with $^{14}CO_2$ 24 h prior to harvesting. Results of Student's T test comparisons of NO_3^-- and NH_4^+-fed plants are shown above S.E. bars (NS=not significant at 90% confidence interval). (n=3)



Fraction	Shoot	Root	Plant
Wa	p<0.05	p<0.05	p<0.05
Ba	NS	NS	NS
Wc	p<0.1	NS	NS
Bc	p<0.05	p<0.05	p<0.05
Struct.	NS	p<0.1	NS
EtOH	NS	NS	p<0.1

Figure 4.32. Percentage allocation of ^{14}C to water soluble carbohydrates (Wc), amino acids and water soluble proteins (Wa), bound carbohydrates (Bc), bound proteins (Ba), structural material (Struct.) and water insoluble, ethanol soluble material (EtOH) in wheat plants grown with Fe-citrate as an iron source and with 12 mM NO_3^- (Nit) or NH_4^+ (Amm). Plants were supplied with $^{14}CO_2$ 24 h prior to harvesting. Other details as for Figure 4.23. (n=3)

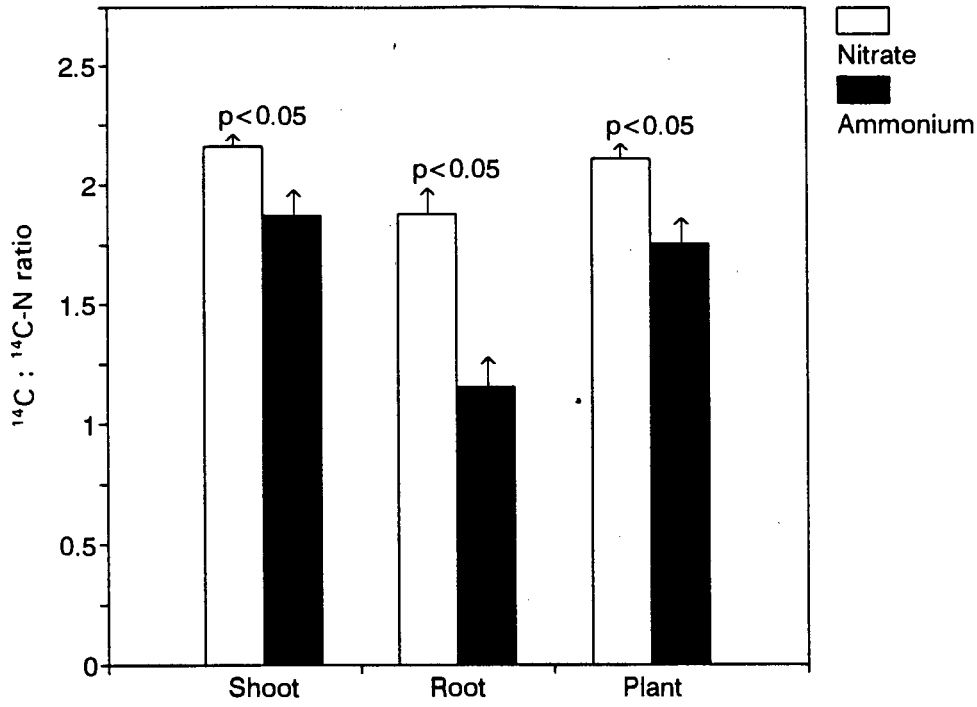


Figure 4.33. Ratios of ^{14}C : ^{14}C-N for wheat plants grown with Fe-citrate as an iron source and with 12 mM NO_3^- or NH_4^+. Plants were supplied with $^{14}CO_2$ 24 h prior to harvesting. Results of Student's T test comparisons of NO_3^-- and NH_4^+-fed plants are shown above S.E. bars (NS=not significant at 90% confidence interval). (n=3)

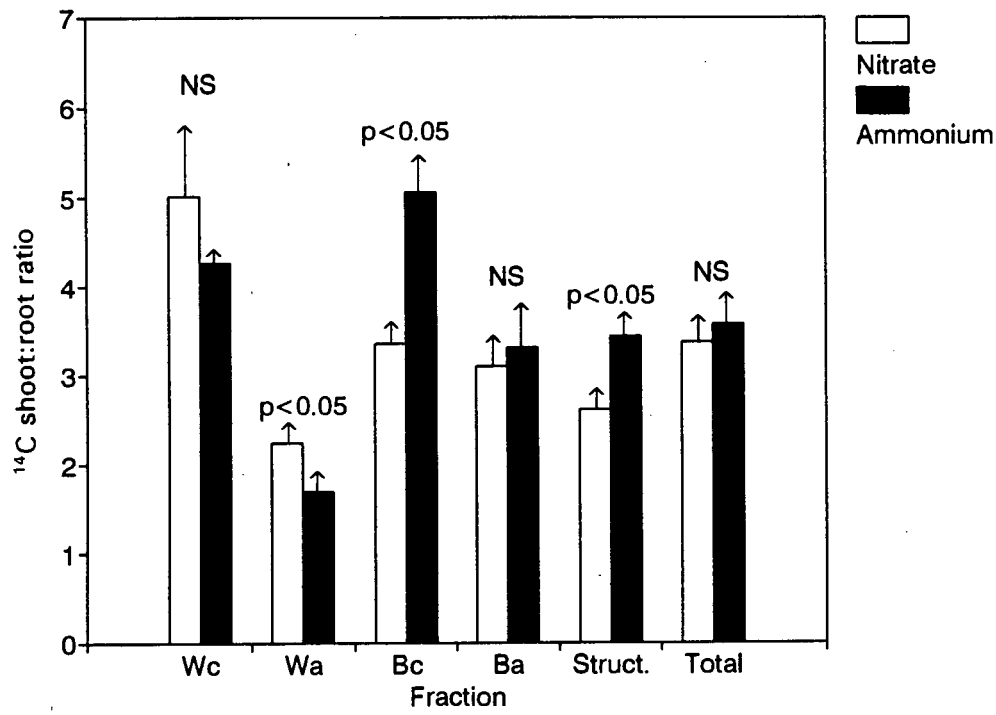


Figure 4.34. ^{14}C Shoot : root ratios of water soluble carbohydrates (Wc), amino acids and water soluble proteins (Wa), bound carbohydrates (Bc), bound proteins (Ba) and structural material (Struct.) in wheat plants grown with Fe-citrate as an iron source and with 12 mM NO_3^- or NH_4^+. Plants were supplied with $^{14}CO_2$ 24 h prior to harvesting. Results of Student's T test comparisons of NO_3^-- and NH_4^+-fed plants are shown above S.E. bars (NS=not significant at 90% confidence interval). (n=3)

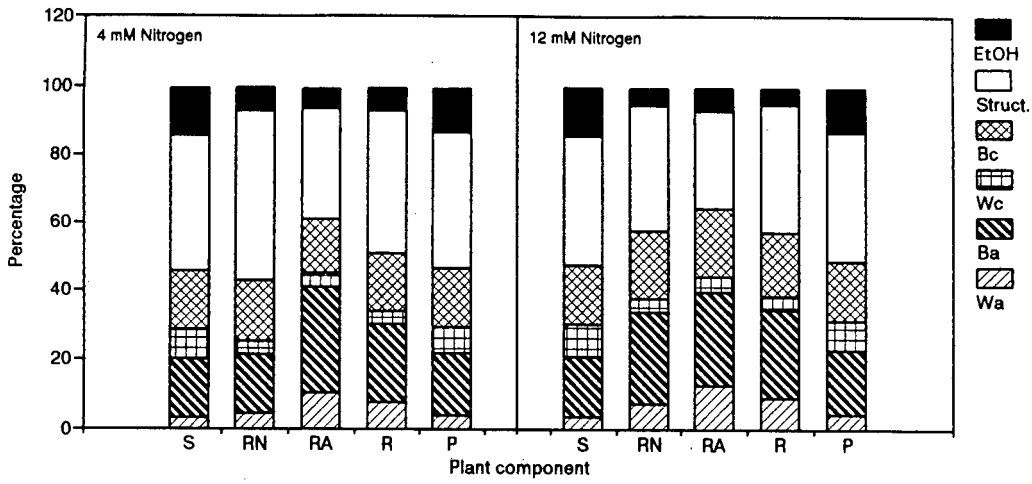
The allocation of ^{14}C to amino acids and water soluble protein and bound protein in 4 mM NH_4^+-fed roots was double that of 4 mM NO_3^--fed roots. These changes were correlated with a smaller allocation of ^{14}C to structural material in the 4 mM NH_4^+- compared to 4 mM NO_3^--fed roots. The roots of plants grown on 4 and 12 mM N had comparable allocation patterns, apart from the fact that the 12 mM NO_3^--fed root-halves had a significantly larger allocation of ^{14}C to bound protein and a smaller allocation to structural material (only significant at $p<0.1$) than the 4 mM NO_3^--fed plants. The ^{14}C : ^{14}C-N ratios of root-halves supplied with NH_4^+ were much lower than those supplied with NO_3^-, indicating the diversion of ^{14}C into amino acids at the expense of carbohydrates (Figure 4.36). There were significantly lower ^{14}C : ^{14}C-N ratios in the 12 mM NO_3^--fed root-halves than in the 4 mM NO_3^--fed root-halves as a consequence of larger allocation of ^{14}C to bound protein in the former.

4.7.1.5 Maize supplied with 4 mM nitrogen and harvested 24 h after $^{14}CO_2$ supply

Allocation of ^{14}C to the water soluble protein and amino acid fraction was increased by NH_4^+ nutrition compared to NO_3^- nutrition in both the shoots and roots, but other fractions were basically unchanged by the form of N supplied (Figure 4.37). Smaller allocation of ^{14}C to structural material in NH_4^+- compared to NO_3^--fed plant roots was only significant at $p<0.1$. As in the case of wheat, there were only small changes in the allocation of ^{14}C with different forms of 4 mM N and therefore 12 mM N was used in a subsequent experiment to magnify the differences.

The ^{14}C : ^{14}C-N ratios were higher in NO_3^-- than NH_4^+-fed plants, principally as a result of the increased allocation of ^{14}C to amino acids and water soluble protein (Figure 4.38). The ^{14}C : ^{14}C-N ratios of the roots were lower than those of the shoots reflecting larger allocation of ^{14}C to water soluble proteins and amino acids and bound proteins, but also smaller allocation to carbohydrates, including structural material, within the roots. The ^{14}C : ^{14}C-N ratios of the NO_3^--fed maize plants were higher than those of the 4 mM N-fed wheat plants, possibly reflecting the larger C supply available from the C_4 photosynthetic mechanism (Section 4.3).

No significant changes in ^{14}C shoot : root ratios between NO_3^-- and NH_4^+-fed plants were observed (Figure 4.39). As in the case of the 4 mM wheat plants, the allocation of ^{14}C to



Fraction	Wa	Ba	Wc	Bc	Struct.	EtOH
N concentration	Nitrate compared with ammonium					
4	p<0.05	p<0.05	NS	NS	p<0.05	NS
12	p<0.05	NS	NS	NS	NS	NS
Component	4 mM compared with 12 mM nitrogen					
Shoot	NS	NS	NS	NS	p<0.1	NS
Root-N	NS	p<0.05	NS	NS	p<0.1	NS
Root-A	NS	NS	NS	p<0.1	NS	NS
Root	NS	NS	NS	NS	NS	NS
Plant	NS	NS	NS	NS	NS	NS

Figure 4.35. Percentage allocation of ^{14}C to water soluble carbohydrates (Wc), amino acids and water soluble proteins (Wa), bound carbohydrates (Bc), bound proteins (Ba), structural material (Struct.) and water insoluble, ethanol soluble material (EtOH) in wheat shoots (S), NO_3^--fed root halves (RN), NH_4^+-fed root halves (RA), roots (R) and plants (P) grown on 4 or 12 mM NO_3^- or NH_4^+ in split-root culture. Plants were supplied with $^{14}CO_2$ 24 h prior to harvesting. Results of Student's T tests performed on arcsine transformed data for differences between NO_3^-- and NH_4^+-fed root halves and between 4 and 12 mM treatments are shown in table below graph. (n=5)

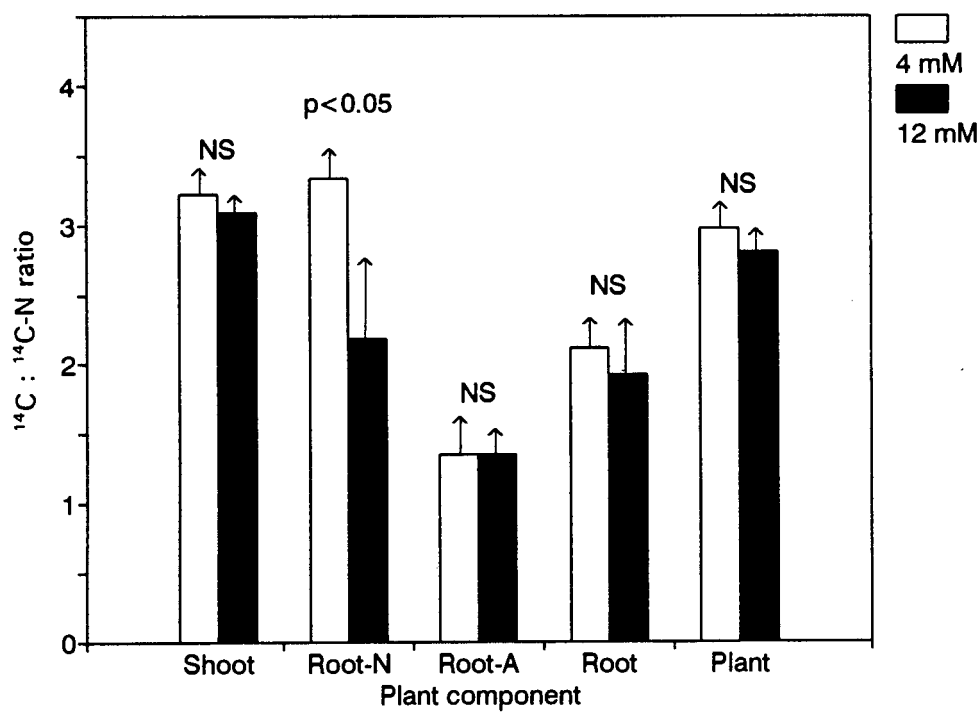


Figure 4.36. Ratios of ^{14}C : ^{14}C-N in wheat plants grown on 4 or 12 mM N in split-root culture. Plants were supplied with $^{14}CO_2$ 24 h prior to harvesting. Results of Student's T test comparisons of 4 and 12 mM N plants are shown above S.E. bars (NS=not significant at 90% confidence interval). Student's T test of NO_3^-- (Root-N) versus NH_4^+-fed root half (Root-A) ^{14}C : ^{14}C-N ratios yielded $p<0.05$ for the 4 mM N and $p>0.1$ for the 12 mM N treatments. (n=5)

amino acids, water soluble protein and bound protein was greater in the roots than in the shoots, leading to a reduced ^{14}C shoot : root ratio of this fraction.

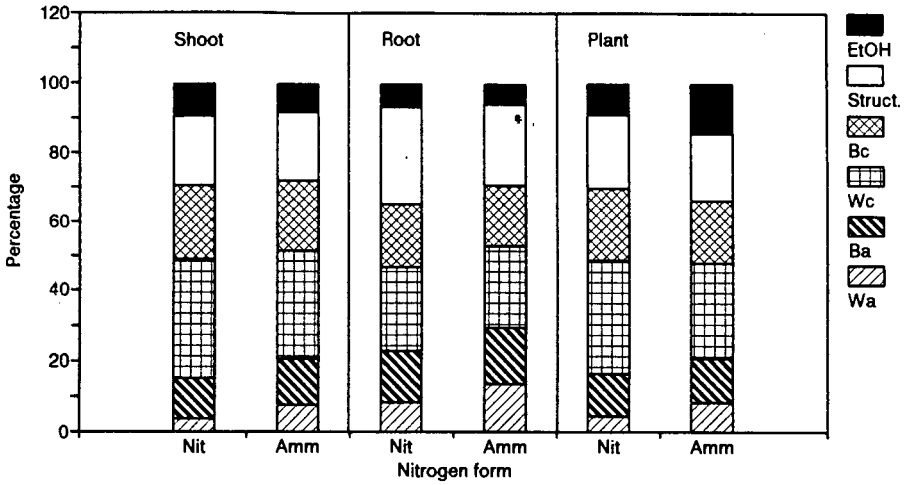
4.7.1.6 Maize supplied with 12 mM nitrogen and harvested 24 h after $^{14}CO_2$ supply

The results for the 12 mM experiment were comparable to those of the 4 mM experiment except that differences between NO_3^-- and NH_4^+-fed plants showed a greater degree of significance (Figure 4.40). There was a significantly greater allocation of ^{14}C to amino acids and water soluble protein and to bound protein and a significantly smaller allocation of ^{14}C to water soluble carbohydrates in both the shoots and the roots of NH_4^+- compared to NO_3^--fed plants. There was also a small, but significant, reduction in the allocation of ^{14}C to the structural material in the roots of NH_4^+- compared to NO_3^--fed plants. There were large differences between NO_3^-- and NH_4^+-fed plants with respect to the ^{14}C : ^{14}C-N ratios (Figure 4.41). As with the 4 mM N experiment (Section 4.7.1.5) the ^{14}C : ^{14}C-N ratios were smaller in the root than in the shoot as a consequence of both enhanced allocation of ^{14}C to amino acids and water soluble protein and bound protein and reduced allocation to water soluble carbohydrates within the roots. There were no significant differences between NO_3^-- and NH_4^+-fed plant ^{14}C shoot : root ratios apart from the slightly higher ratio for NH_4^+-fed plant structural material which was only significant at $p<0.1$ (Figure 4.42).

4.7.1.7 Maize split-root culture: ^{14}C partitioning

Few differences were evident with respect to ^{14}C partitioning between 4 and 12 mM N-fed maize plants grown in split-root culture (Figure 4.43). There were large differences between split-root and normally cultured maize plants with respect to the ^{14}C partitioning data. In particular, there was a lower ^{14}C : ^{14}C-N ratio in the roots of split-root cultured plants compared to normally cultured plants.

In root-halves fed NH_4^+ the allocation of ^{14}C to water soluble proteins, amino acids and bound proteins was significantly larger while allocation to structural material was smaller than in root-halves supplied with NO_3^- (Figure 4.43). The ^{14}C allocation patterns of roots of NH_4^+-fed maize plants were comparable regardless of the N concentration. Roots of the 12 mM NO_3^--fed plants, however, had a significantly larger allocation of ^{14}C to bound protein and a smaller allocation to bound carbohydrates and structural material (only significant at $p<0.1$)



Fraction	Shoot	Root	Plant
Wa	$p<0.05$	$p<0.05$	$p<0.05$
Ba	NS	NS	NS
Wc	NS	NS	$p<0.1$
Bc	NS	NS	NS
Struct.	NS	$p<0.1$	NS
EtOH	NS	NS	$p<0.05$

Figure 4.37. Percentage allocation of ^{14}C to water soluble carbohydrates (Wc), amino acids and water soluble proteins (Wa), bound carbohydrates (Bc), bound proteins (Ba), structural material (Struct.) and water insoluble, ethanol soluble material (EtOH) in maize plants grown on 4 mM NO_3^- (Nit) or NH_4^+ (Amm). Plants were supplied with $^{14}CO_2$ 24 h prior to harvesting. Other details as for Figure 4.23. (n=4)

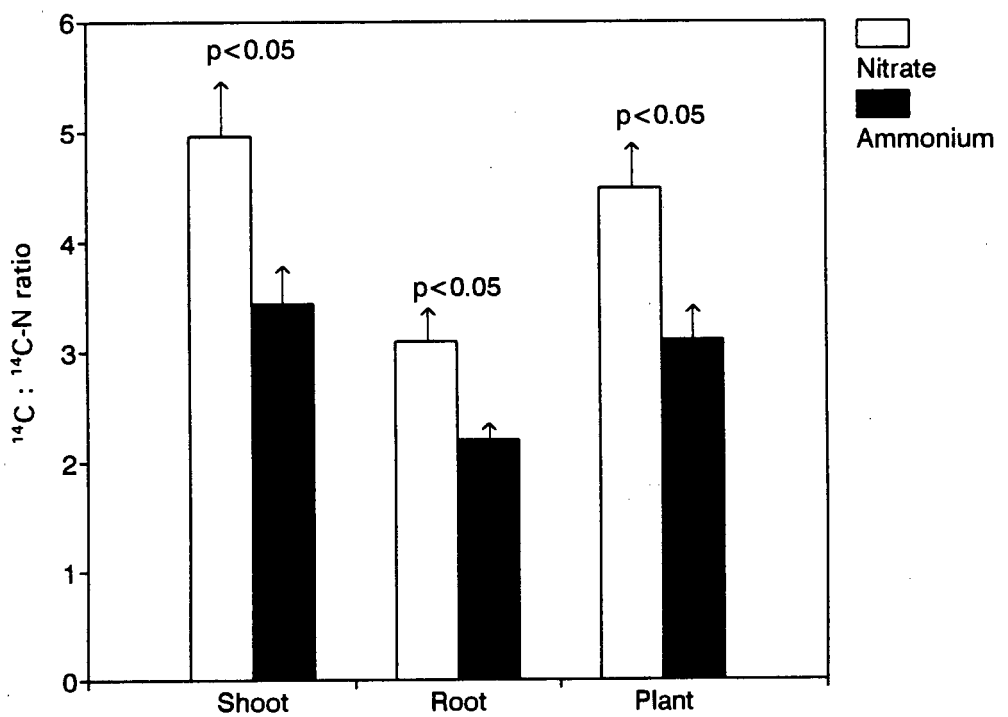


Figure 4.38. Ratios of ^{14}C : ^{14}C-N for maize plants grown on 4 mM NO_3^- or NH_4^+. Plants were supplied with $^{14}CO_2$ 24 h prior to harvesting. Results of Student's T test comparisons of NO_3^-- and NH_4^+-fed plants are shown above S.E. bars (NS=not significant at 90% confidence interval). (n=4)

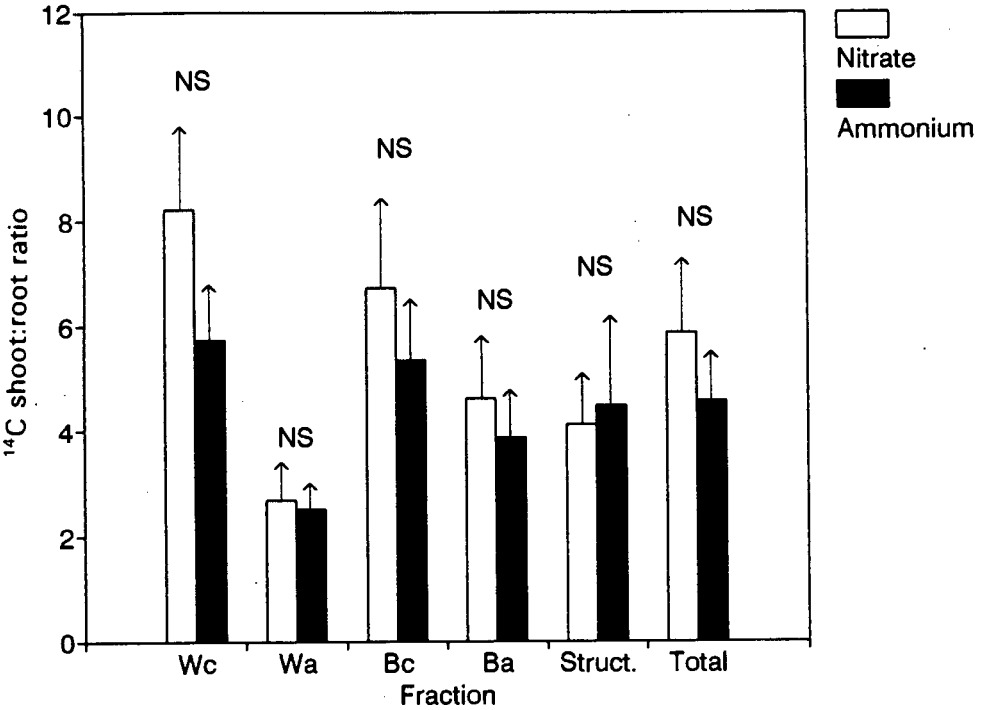
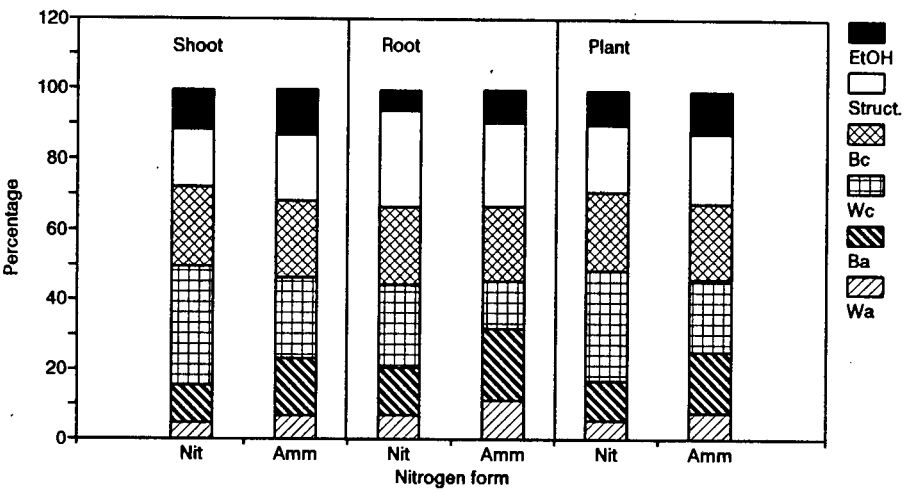


Figure 4.39. ^{14}C Shoot : root ratios of water soluble carbohydrates (Wc), amino acids and water soluble proteins (Wa), bound carbohydrates (Bc), bound proteins (Ba) and structural material (Struct.) in maize plants grown on 4 mM NO_3^- or NH_4^+. Plants were supplied with $^{14}CO_2$ 24 h prior to harvesting. Results of Student's T test comparisons of NO_3^-- and NH_4^+-fed plants are shown above S.E. bars (NS=not significant at 90% confidence interval). (n=4)



Fraction	Shoot	Root	Plant
Wa	p<0.05	p<0.05	p<0.05
Ba	p<0.05	p<0.05	p<0.05
Wc	p<0.05	p<0.05	p<0.05
Bc	NS	NS	NS
Struct.	p<0.1	p<0.05	NS
EtOH	NS	p<0.1	NS

Figure 4.40. Percentage allocation of ^{14}C to water soluble carbohydrates (Wc), amino acids and water soluble proteins (Wa), bound carbohydrates (Bc), bound proteins (Ba) and structural material (Struct.) in maize plants grown on 12 mM NO_3^- (Nit) or NH_4^+ (Amm). Plants were supplied with $^{14}CO_2$ 24 h prior to harvesting. Other details as for Figure 4.23. (n=4)

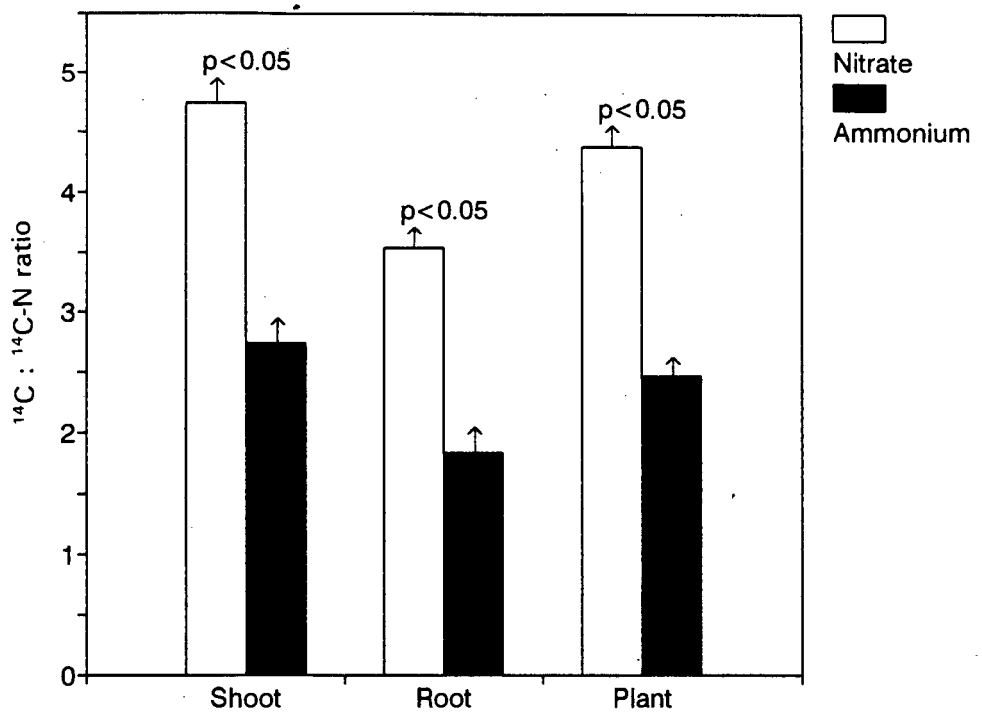


Figure 4.41. Ratios of ^{14}C : ^{14}C-N for maize plants grown on 12 mM NO_3^- or NH_4^+. Plants were supplied with $^{14}CO_2$ 24 h prior to harvesting. Results of Student's T test comparisons of NO_3^-- and NH_4^+-fed plants are shown above S.E. bars (NS=not significant at 90% confidence interval). (n=4)

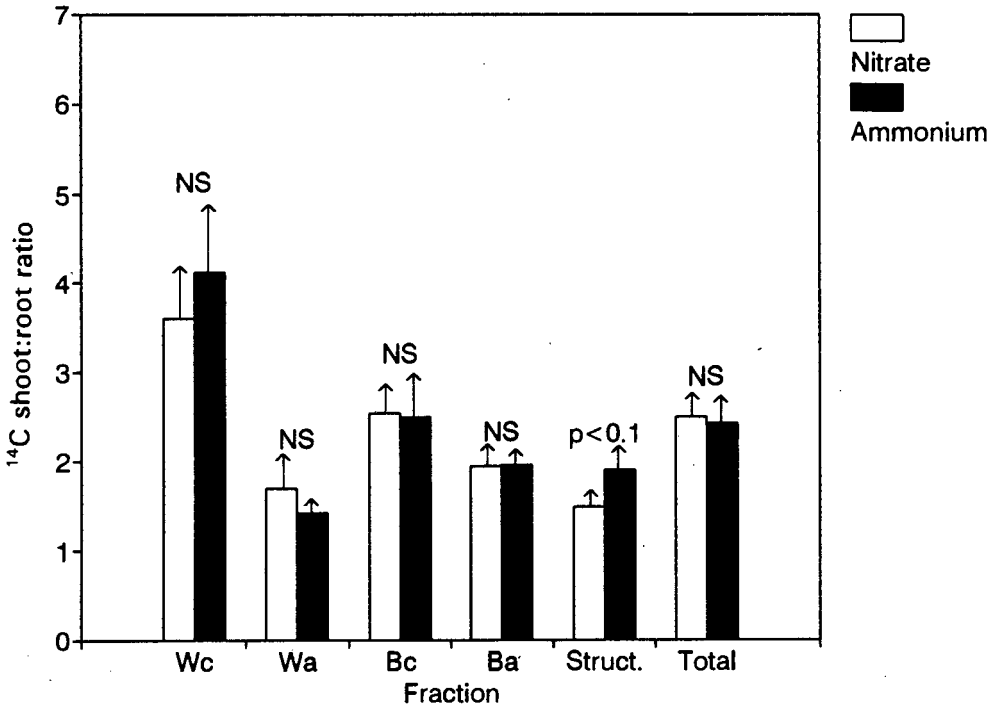


Figure 4.42. ^{14}C Shoot : root ratios of water soluble carbohydrates (Wc), amino acids and water soluble proteins (Wa), bound carbohydrates (Bc), bound proteins (Ba) and structural material (Struct.) in maize plants grown on 12 mM NO_3^- or NH_4^+. Plants were supplied with $^{14}CO_2$ 24 h prior to harvesting. Results of Student's T test comparisons of NO_3^-- and NH_4^+-fed plants are shown above S.E. bars (NS=not significant at 90% confidence interval). (n=4)

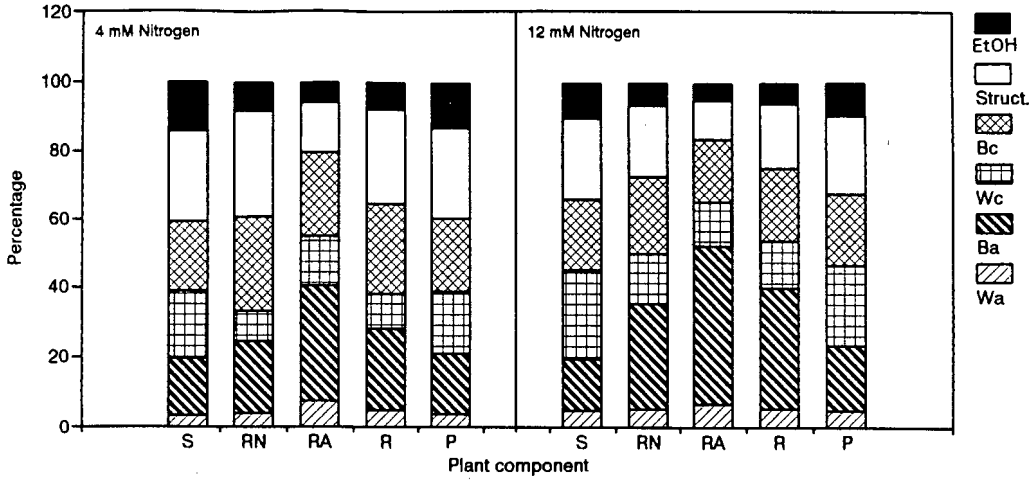
than the 4 mM NO_3^--fed plants. The ^{14}C : ^{14}C-N ratios of the root-halves supplied with NH_4^+ were much lower than those supplied with NO_3^- indicating the diversion of ^{14}C into amino acids at the expense of carbohydrates (Figure 4.44). There were significantly lower ^{14}C : ^{14}C-N ratios in the 12 mM NO_3^--fed root-halves than the 4 mM NO_3^--fed root-halves as a consequence of larger allocation of ^{14}C to bound protein in the former.

4.7.1.8 ^{14}C Shoot:root ratios

Although ^{14}C shoot : root ratios can be obtained from the ^{14}C partitioning experiments these are on a fresh mass basis (Bq mg^{-1} fresh mass). A more reliable method of obtaining these ratios is to use milled dry plant material. The ^{14}C shoot : root ratios were highly variable between experiments, probably because of the large number of physiological processes controlling these ratios. In an experiment designed to determine the ^{14}C shoot : root ratios of maize and wheat (24 h after $^{14}CO_2$ supply to photosynthesizing leaves) it was found that the ^{14}C shoot : root ratios of 4 mM NH_4^+-fed wheat and maize plants were significantly lower than those of the 4 mM NO_3^--fed plants (Figure 4.45). In addition the ratios of the maize plants were smaller than those of the wheat plants. This may indicate that maize is capable of diverting larger amounts of C to the root than wheat to meet the challenge of nutrient NH_4^+ assimilation.

The lower ^{14}C shoot : root ratios of NH_4^+- compared to NO_3^--fed wheat plants are contrary to the results of other authors (Lewis *et al.*, 1987; Lewis *et al.*, 1990). Considering the ^{14}C shoot : root ratios derived from the partitioning experiments it appears that at 4 mM N the ^{14}C shoot : root ratios of wheat were lower in NH_4^+-fed plants than in NO_3^--fed plants while at 12 mM N the opposite was true (Figures 4.25 and 4.31). These results may be the consequence of greater translocation of ^{14}C from the roots to the shoots in NH_4^+-fed plants at the higher N concentration (Section 4.7.2). The lower ^{14}C shoot : root ratios of the 4 mM NH_4^+- compared to 4 mM NO_3^--fed maize plants are supported by the results of Lewis *et al.* (1990). The factors considered to play a role in determining the ^{14}C shoot : root ratios are:

1) Cycling of amino-N between roots and shoots (Simpson *et al.*, 1982).
2) Higher rates of $^{14}CO_2$ loss by NO_3^- roots (Section 4.9.1) possibly linked to exchange of $H^{14}CO_3^-$ for NO_3^- (Ben Zioni *et al.*, 1971).
3) Uptake of CO_2 and re-fixation of $^{14}CO_2$, particularly in NH_4^+-fed plant roots through activity of PEPc (Section 4.10).



Fraction	Wa	Ba	Wc	Bc	Struct.	EtOH
N concentration (mM)	Nitrate compared with ammonium					
4	p<0.05	p<0.05	p<0.05	NS	p<0.05	p<0.05
12	NS	p<0.05	NS	NS	NS	NS
Component	4 mM compared with 12 mM nitrogen					
Shoot	NS	NS	NS	NS	NS	p<0.1
Root-N	NS	p<0.05	NS	p<0.1	p<0.1	p<0.05
Root-A	NS	NS	NS	NS	NS	NS
Root	NS	p<0.05	NS	p<0.05	NS	p<0.05
Plant	NS	NS	NS	NS	NS	p<0.1

Figure 4.43. Percentage allocation of ^{14}C to water soluble carbohydrates (Wc), amino acids and water soluble proteins (Wa), bound carbohydrates (Bc), bound proteins (Ba), structural material (Struct.) and water insoluble, ethanol soluble material (EtOH) in maize shoots (S), NO_3^--fed root halves (RN), NH_4^+-fed root halves (RA), roots (R) and plants (P) grown on 4 or 12 mM NO_3^- or NH_4^+ in split-root culture. Plants were supplied with $^{14}CO_2$ 24 h prior to harvesting. Results of Student's T tests performed on arcsine transformed data for differences between NO_3^-- and NH_4^+-fed root halves and between 4 and 12 mM treatments are shown in table below graph. (n=5)

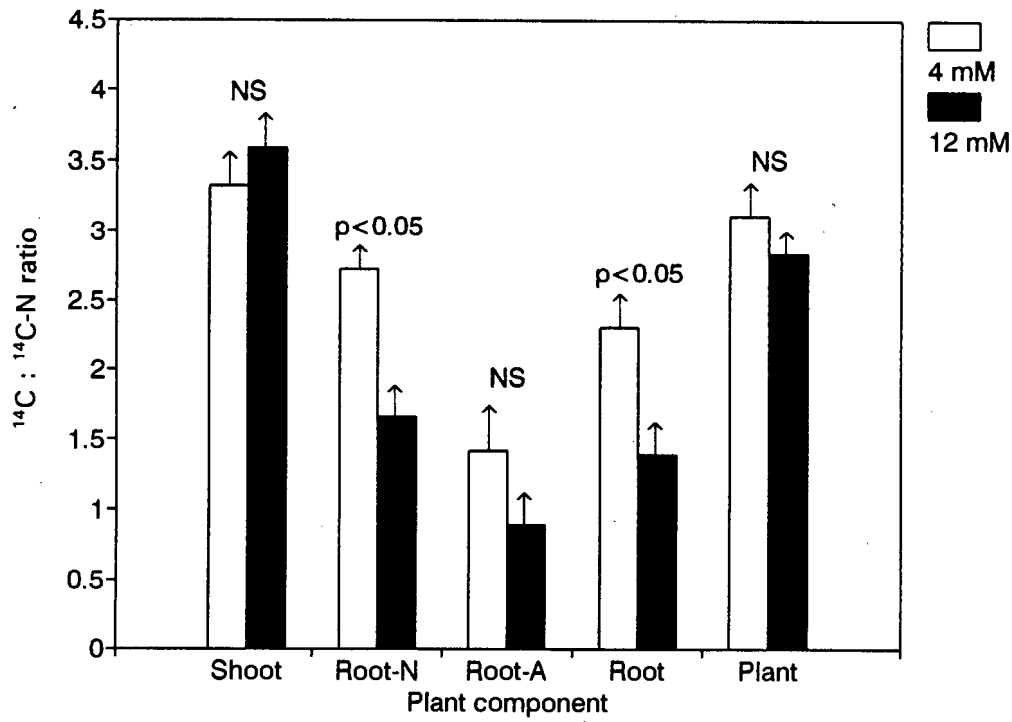


Figure 4.44. Ratios of ^{14}C : ^{14}C-N in maize plants grown on 4 or 12 mM N in split-root culture. Plants were supplied with $^{14}CO_2$ 24 h prior to harvesting. Results of Student's T test comparisons of 4 and 12 mM N plants are shown above S.E. bars (NS=not significant at 90% confidence interval). Student's T-tests of NO_3^-- (Root-N) versus NH_4^+-fed root half (Root-A) ^{14}C : ^{14}C-N ratios yielded $p<0.05$ for both the 4 mM and 12 mM N treatments. (n=5)

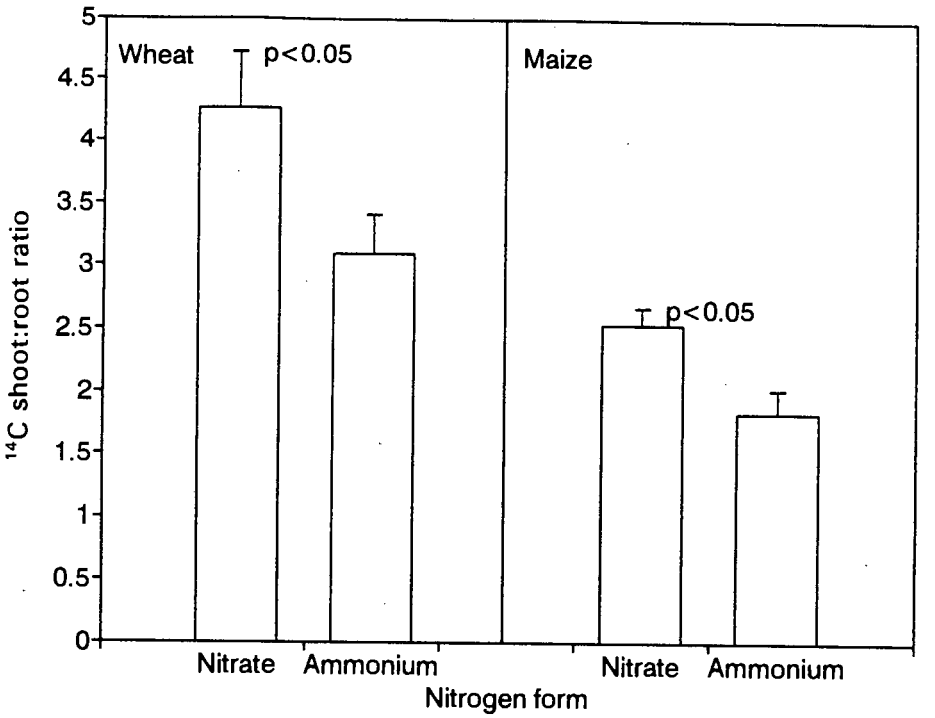


Figure 4.45. Shoot : root ratios of total ^{14}C per g dry mass for both wheat and maize plants grown on 4 mM NO_3^- or NH_4^+. Plant leaves supplied with $^{14}CO_2$ 24 h prior to harvesting. Bars indicate the S.E. Results of Student's T test comparisons of NO_3^-- and NH_4^+-fed plants are shown above S.E. bars. (n=6)

4.7.1.9 Discussion

The influence of N on growth may depend on the role that N plays in determining C partitioning within the plant. Partitioning of ^{14}C has been shown to be dependent on the form and concentration of N supplied in both wheat and maize. The differences between 4 mM NO_3^-- and NH_4^+-fed wheat and maize plants were, however, small. Less ^{14}C was allocated to structural material in both wheat and maize grown with 12 mM N compared to 4 mM N, indicating that increased amino acid synthesis at the higher N concentration was at the expense of allocation of C to structural material. This was also evident from the split-root data. The ^{14}C : ^{14}C-N ratios of maize were higher than those of wheat, particularly in NH_4^+-fed plants, possibly as a consequence of the higher photosynthetic CO_2 assimilation rates of maize. Certain general trends may be noted for 12 mM N-fed wheat and 4 and 12 mM N-fed maize with respect to partitioning of ^{14}C:

1) Relative to NO_3^-, NH_4^+ nutrition increased the allocation of ^{14}C to protein and amino acid components.
2) Allocation of ^{14}C to structural material was reduced, particularly in the root of NH_4^+- compared to NO_3^--fed plants.
3) Higher N concentrations favoured diversion of ^{14}C to the formation of amino acids and proteins over allocation of ^{14}C to carbohydrate and structural components resulting in ^{14}C : ^{14}C-N ratios which were decreased in NH_4^+- compared to NO_3^--fed plants..

Other authors have failed to find differences in ^{14}C partitioning to N and non-N containing fractions of wheat shoots, although a significantly reduced ratio of non-N to N compounds in the roots of NH_4^+- compared to NO_3^--fed plants was observed (Lewis *et al.*, 1987). This may be attributed to the relatively low concentrations of N (2 mM) used by these authors. In the experiments reported here there was a lack of evidence for the effects of N form on the partitioning of ^{14}C within wheat shoots at 4 mM N but at 12 mM there was a significant effect.

The characteristics of NH_4^+-grown plants reported by Chaillou *et al.* (1991) to be distinctive from NO_3^--grown plants include lower organic acid contents, higher amino acid contents and soluble sugar contents that are higher in the shoots and lower in the roots. The results presented here support this assertion to some extent. Certainly the amino acid contents of

NH_4^+-fed plants were higher than those of NO_3^--fed plants, but allocation of ^{14}C to soluble sugars (water soluble carbohydrates) was reduced in both the shoot and root by 12 mM NH_4^+ nutrition.

The data presented here for plants grown entirely on either NO_3^- or NH_4^+ nutrition supports the proposal that the differential effects of NO_3^- and NH_4^+ nutrition on growth are to some extent the result of the sites in the plant at which the respective N forms are assimilated. Larger allocation of ^{14}C label to the amino-N containing fractions (amino acids, water soluble protein and bound protein), particularly in the root, of NH_4^+- compared to NO_3^--fed plants indicates that amino acid synthesis in the root is an important sink for photosynthetic C. The lower ^{14}C : ^{14}C-N ratios of NH_4^+- compared to NO_3^--fed plants confirms that allocation of ^{14}C to the amino-N containing fractions occurs at the expense of the carbohydrate fractions (water soluble carbohydrates, bound carbohydrates and structural material). The fact that allocation of ^{14}C to structural material was generally smaller, particularly in the roots, in NH_4^+-fed plants indicates that the allocation of ^{14}C to the amino-N containing fractions could be responsible for reduced root growth.

The split-root data supports these conclusions and provides a clearer picture than that obtained with the root system grown entirely in one N form. It appears from the split-root data that the limited ^{14}C allocated to the NH_4^+-fed root-halves was insufficient to meet the demands of root growth and N assimilation. The ^{14}C allocated to structural material was considerably reduced, particularly in the case of maize, in NH_4^+-fed root-halves. The deficiency of available C for root functioning is illustrated by the very small allocation of ^{14}C to water soluble carbohydrates.

The maize split-root experiment showed that there were large differences in the allocation of ^{14}C to structural material in the root-halves supplied with NO_3^- and NH_4^+ nutrition. These results may appear surprising since the biomass shoot : root ratios of maize grown without split-root culture were insensitive to the form of N supplied. Biomass data for the maize split-root experiment does, however, show that the root-halves grown in NH_4^+ were much smaller than the halves grown in NO_3^-, thus supporting the partitioning data. Thus the C available to the NH_4^+-fed root-halves from the shoots was probably insufficient to meet the demands of root growth and NH_4^+ assimilation without severe competition between the two. This may result from the inability of the shoot to discriminate between NO_3^-- and NH_4^+-fed root-halves

and inappropriate allocation of C to meet root demands. Small differences between NO_3^-- and NH_4^+-fed maize plants (not split-root plants) with respect to ^{14}C allocation to structural material in the roots were also observed indicating that the same mechanisms determine the effects of NO_3^- and NH_4^+ nutrition in both wheat and maize plants. The lack of response of maize shoot : root ratios to N form may thus be the result of the fact that the differences in ^{14}C partitioning were small and that the larger supply of C available from the C_4 photosynthetic mechanism masked the small changes induced by N form in C partitioning.

The question which still remains is whether the changes in allocation are sufficient to explain the differences observed between NO_3^-- and NH_4^+-fed wheat plants with respect to root biomass accumulation. In particular, ^{14}C partitioning in 4 mM N-fed wheat seemed insensitive to the form of N supplied and yet root extension of plants grown with 4 mM NH_4^+ was only half that of plants grown with 4 mM NO_3^-. Small differences in partitioning throughout the growing period of the plant would be magnified through the compound nature of growth. Thus in spite of the fact that differences in ^{14}C partitioning between NO_3^-- and NH_4^+-fed wheat and maize were small, it is possible that these differences do indeed account for altered growth.

4.7.2 Xylem sap ^{14}C content

If the currently accepted dogma is correct and cereals do reduce and assimilate most NO_3^- in the shoots and most NH_4^+ in the roots (Lewis *et al.*, 1990), then it may be expected that the translocation of amino compounds derived from root based NH_4^+ assimilation in the xylem would be greater in NH_4^+- than in NO_3^--fed plants. Since amino compounds contain C ultimately derived from photosynthate, the ^{14}C labelling of xylem sap in plants previously supplied with $^{14}CO_2$ through photosynthesis should provide a relative indication of the translocation of amino compounds and other organic molecules within the xylem sap. In plants supplied with $^{14}CO_2$ 24 h prior to collection of xylem sap the amount of ^{14}C label in the xylem sap was 1.5- and 1.3-fold greater in NH_4^+- than in NO_3^--fed wheat and maize plants respectively (Figure 4.46a), although the differences between NO_3^- and NH_4^+-fed plants was only significant at $p < 0.1$ in wheat. By utilizing gravimetrically determined transpiration rates expressed on the basis of root dry mass (Figure 4.59), the rates of translocation of ^{14}C from root to shoot were calculated and it was found that in NH_4^+-fed plants 1.4- and 1.2-fold more ^{14}C was translocated from root to shoot than in NO_3^--fed wheat and maize plants

respectively (Figure 46b). These results were, however, highly variable and a large number of replicates were required. The source of the variability lies in the experimental techniques for collection of xylem sap (Simpson, 1986) and the complex relationship which exists between:

1) Control of shoot : root partitioning.
2) Cycling of amino-N between root and shoot (Simpson *et al.*, 1982).
3) Higher rates of CO_2 loss by NO_3^- roots (Section 4.9.1) possibly linked to exchange of $H^{14}CO_3^-$ for NO_3^- (Ben Zioni *et al.*, 1971).
4) Uptake of CO_2 and re-fixation of $^{14}CO_2$ particularly in NH_4^+-fed plant roots through activity of PEPc (Section 4.10).

The results of analysis of xylem sap for ^{14}C content are supported by results presented later showing that the amino-N content of xylem sap is indeed greater in NH_4^+- than in NO_3^--fed plants (Section 4.8.4). Similar results for ^{14}C content of xylem sap have been presented previously by Lewis *et al.* (1990) indicating that the loss of C from the root to the shoot in NH_4^+-fed plants is 1.3- and 1.4-fold greater than that in NO_3^--fed wheat and maize plants respectively.

The amount of ^{14}C translocated per hour in the xylem sap of NO_3^-- and NH_4^+-fed wheat represents 21.8 and 30.0% respectively of the amount of ^{14}C in the root. The corresponding figures for NO_3^-- and NH_4^+-fed maize were 22.3 and 26.8% respectively. This indicates that the translocation of ^{14}C in the xylem sap of wheat and maize was 8.2 and 4.5% higher respectively in NH_4^+- than in NO_3^--fed plants. The equivalent loss of ^{14}C through root respiration in wheat was 0.7 and 0.2% in NO_3^-- and NH_4^+-fed plants respectively (Section 4.9.1). The corresponding figures for NO_3^-- and NH_4^+-fed maize were 1.4 and 1.0% respectively. This illustrates the importance of the losses of ^{14}C from the root via the xylem sap in determining the ^{14}C present in the root. It should not be assumed, however, that the ^{14}C translocated in the xylem is derived solely from root resources. Cycling of N has been demonstrated in many plants and 50 to 70% of the N content of the xylem sap has been found to be recycled N in wheat plants grown on between 1 and 1.5 mM NO_3^- (Cooper *et al.*, 1986a; Cooper and Clarkson, 1989; Larsson *et al.*, 1991). Thus a large proportion of the ^{14}C present in the xylem sap is likely to be derived from recycled amino-N.

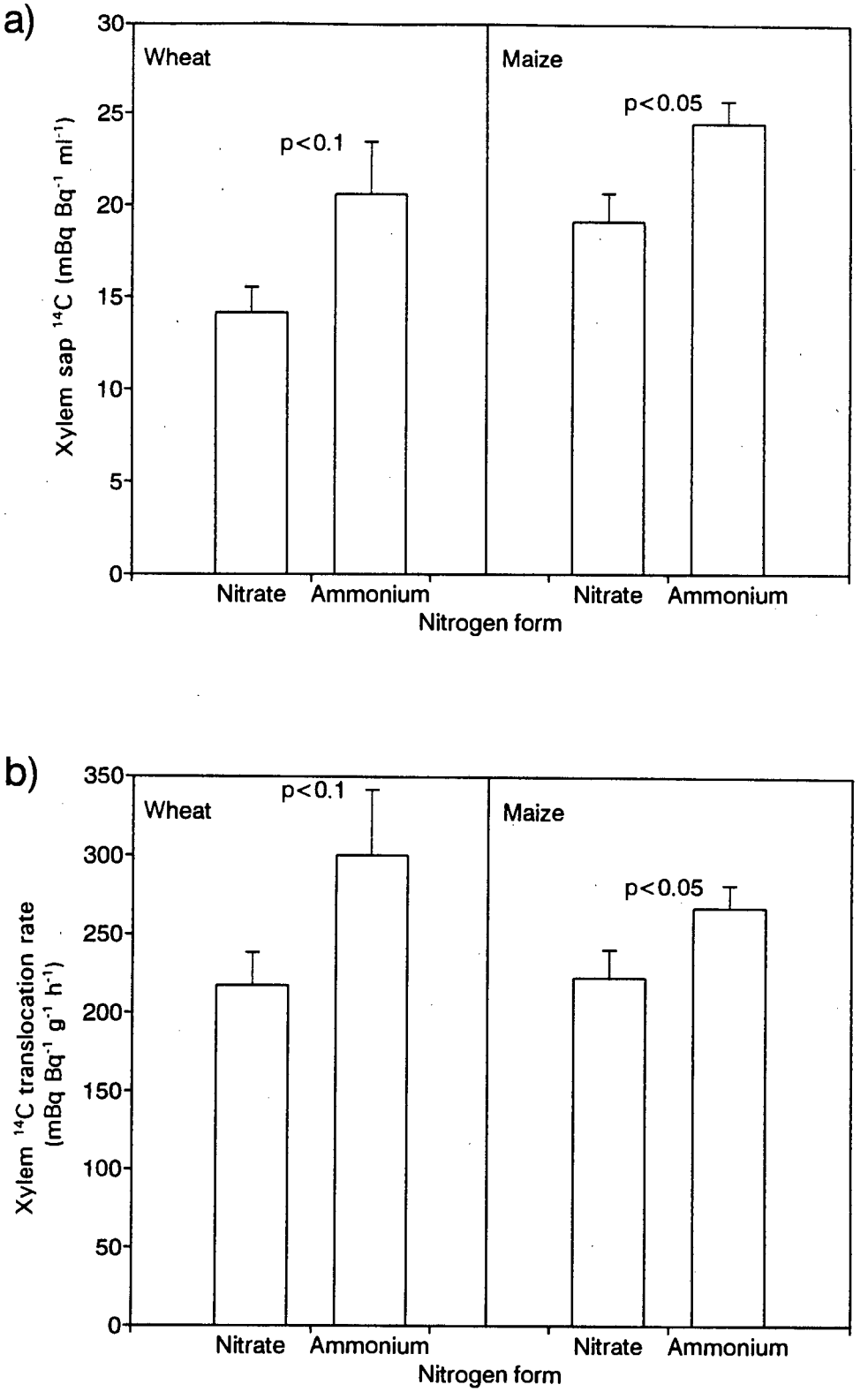


Figure 4.46. a) Xylem sap ^{14}C contents (mBq ^{14}C in sap Bq^{-1} in root ml^{-1} xylem sap; b) Rate of ^{14}C translocation in xylem sap (mBq ^{14}C in sap Bq^{-1} ^{14}C in root g^{-1} root dry mass h^{-1}) for both wheat and maize plants grown on 4 mM NO_3^- or NH_4^+. Bars indicate the S.E. Results of Student's T test comparisons of NO_3^-- and NH_4^+-fed plants are shown above S.E. bars. (n=12)

4.8 NITROGEN UPTAKE, PARTITIONING AND TRANSLOCATION

4.8.1 Nitrogen uptake

Both wheat and maize plants have been shown to exhibit differences in physiology depending on the form of N supplied. An assumption which may erroneously be made when comparing plants grown on the same concentrations of NO_3^- and NH_4^+ is that the plants are exposed to the same level of either ion *in vivo*. The concentration of one inorganic ion may be higher within the plant than the other, merely because the plant can accumulate it more readily. In order to test this possibility the uptake of NO_3^- and NH_4^+ was followed over a 28 h period in both wheat and maize (Figure 4.47).

In both wheat and maize the uptake of N from a 4 mM source was higher in NH_4^+-fed plants than in NO_3^--fed plants. Over 10 h the uptake of NH_4^+ was 1.5- and 1.3-fold greater than NO_3^- uptake in wheat and maize respectively. This experiment was repeated at 12 mM N but the data has not been presented because of variability in the results, mostly due to difficulty in determining small changes in NO_3^- and NH_4^+ concentrations with such high background levels of N. Some of the variability observed in the 4 and 12 mM data may, however, have been the consequence of oscillations in uptake as a result of altered influx and efflux rates as observed in *Lolium spp.* (Jarvis and MacDuff, 1989) and in *Glycine max* (Tolley-Henry *et al.*, 1988).

Transfer of the plants into fresh nutrient medium (Time=0) resulted in a rapid uptake of the inorganic N. Uptake appeared to continue unabated in the dark in maize plants, but in wheat uptake after 4 h in the dark was slow. Upon re-introduction to the light, the uptake of NH_4^+ increased sharply in both wheat and maize. The fact that uptake of NO_3^- occurs in both the light and dark has been demonstrated previously by Oji *et al.* (1989) and Rufty *et al.* (1984). Rufty *et al.* (1987) found that NO_3^- taken up in the dark was accumulated within the root and only reduced during the following light period. The lack of increase in NO_3^- uptake in wheat plants upon re-introduction to the light (Figure 4.47a) may be explained by the observation of Rufty *et al.* (1987) that uptake from exogenous sources becomes important as the light period progresses and as the endogenous NO_3^- from the previous dark period is reduced. The degree of NO_3^- reduction within the root has been proposed to depend on the availability of carbohydrate within the root (Pate, 1980; Pace *et al.*, 1990). The fact that NO_3^- uptake continued unabated in the dark in maize may be related to the availability of C in the

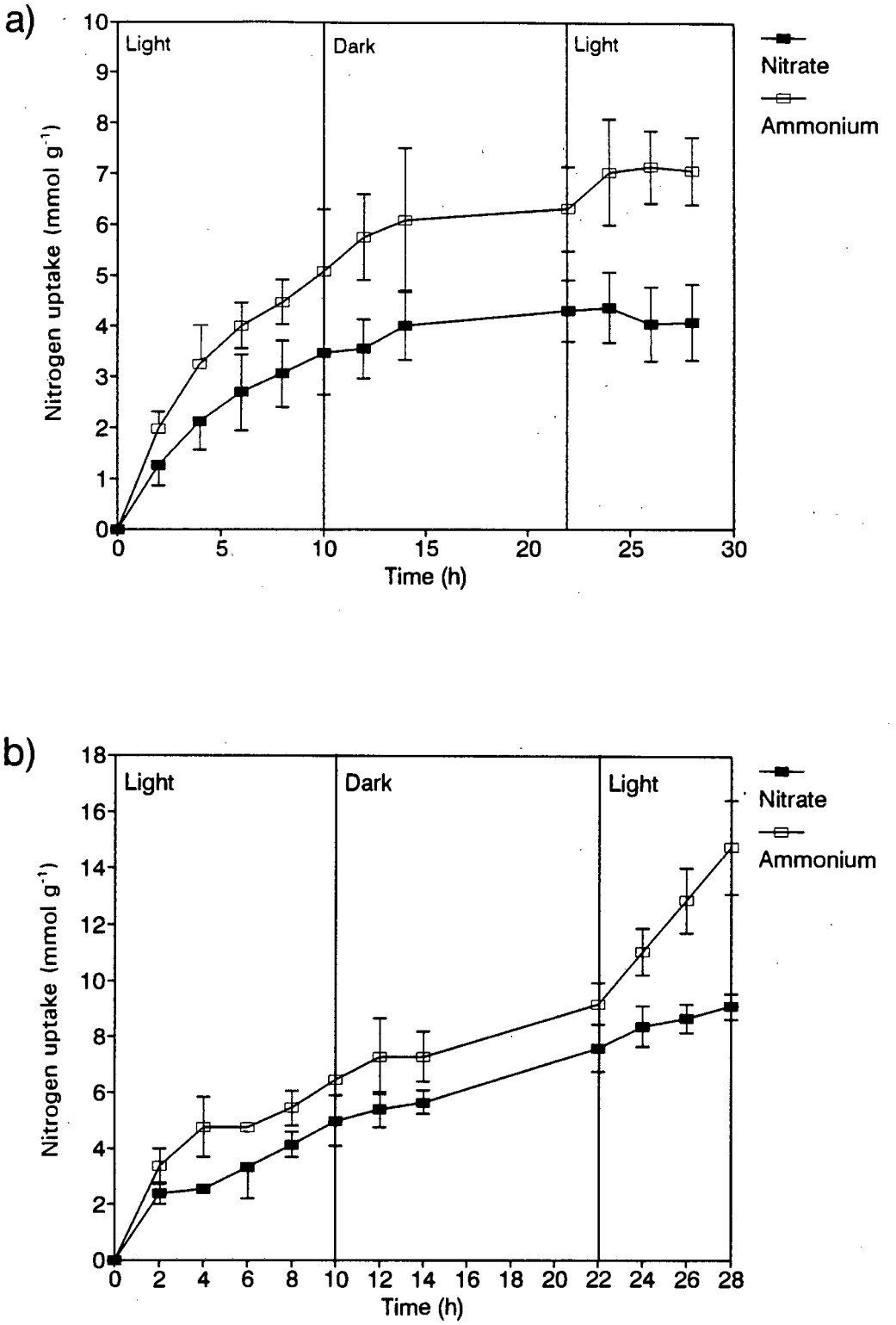


Figure 4.47. NO_3^- and NH_4^+ uptake (mmol g^{-1} root dry mass) by a) wheat and b) maize plants supplied with 4 mM N over a 28 h period including a 12 h period of darkness. Bars indicate the S.E where it is larger than the size of the symbols. (n=4)

roots of this C_4 plant for sustaining NO_3^- uptake and reduction in the dark (Figure 47b). The possible importance of photosynthate for sustained uptake of NH_4^+ is evident in both wheat and maize as a stimulation of NH_4^+ uptake upon exposure to light, although this stimulation may also be attributable to increased translocation of NH_4^+ assimilation products from the root to the shoot in the transpirational stream. Decreased NH_4^+ uptake upon transfer of plants from light to dark has, however, previously been cited as evidence for the metabolic dependence of NH_4^+ uptake (Kashyap and Singh, 1985; Glass, 1988).

This experiment shows that the amount of N received by NO_3^-- and NH_4^+-fed plants is not equivalent. The uptake of NH_4^+ was greater than NO_3^- uptake in both wheat and maize. Similar results have been reported previously for wheat (Cox and Reisenauer, 1973; Lips *et al.*, 1990), barley (Lewis and Chadwick, 1983) and maize (Murphy and Lewis, 1987). Comparisons between NO_3^- and NH_4^+ treatments should be made with the fact that not only is the form of N different, but also the amount that the plant has to assimilate *in vivo*. Thus we might expect that the growth of plants on NH_4^+ as opposed to NO_3^- would place larger demands on plant resources for assimilation of this N source. Evidence for the diversion of ^{14}C to amino-N at the expense of root carbohydrate resources in NH_4^+-fed plants has already been presented (Section 4.7).

4.8.2 Partitioning of nitrogen and ^{15}Nitrogen

In order to determine the effects of NO_3^- as opposed to NH_4^+ nutrition on maize and wheat growth, it was important to establish in what form the N was present within the shoot and root. It was also important to know the destination of recently acquired N, i.e. in which plant part the inorganic N is assimilated into organic nitrogenous molecules. To this end the N contents of four fractions were determined. These were: 1) 80% (v/v) ethanol insoluble particulate material (insoluble amino-N), 2) 80% (v/v) ethanol soluble proteins and amino compounds (soluble amino-N), 3) NO_3^- and 4) NH_4^+. The NO_3^- and NH_4^+ fractions have been collectively termed the inorganic N fraction in the discussion which follows. The ^{15}N contents of these fractions were also determined. Due to the fact that NO_3^- and NH_4^+ are taken up at different rates (Section 4.8.1) comparison of the distribution patterns was confounded. For this reason percentage distribution is presented with statistics calculated on the arcsine transformed data.

4.8.2.1 Wheat response

Total N contents of NH_4^+-fed plants were significantly greater (1.5-fold) than those of NO_3^--fed plants (Figure 4.48a). The quantity of insoluble amino-N in the shoot and root was not affected by the form of N, but the shoot had considerably larger amounts of insoluble amino-N than the root. The quantity of free NO_3^- found in NH_4^+-fed plants was very small and quantities of free NO_3^- found in the shoots and roots of NO_3^--fed plants were comparable. Very little free NH_4^+ was found in NO_3^--fed plants. Smaller amounts of free NH_4^+ were found in the shoots than in the roots of NH_4^+-fed plants. The amount of N in the soluble amino compound fraction was significantly different between NO_3^-- and NH_4^+-fed plants. The larger quantity of N found in this fraction in NH_4^+-fed plants as opposed to NO_3^--fed plants is consistent with the higher ^{14}C : ^{14}C-N ratios in the latter reported previously (Section 4.7).

In the shoots of NH_4^+-fed plants the percentage allocation of N to the inorganic N and insoluble amino-N fractions was small in comparison to NO_3^--fed plants (Figure 4.48b). In the root, however, comparable proportions were found in the inorganic N fractions in both NO_3^-- and NH_4^+-fed plants. The proportion of allocation of N to the soluble amino-N fraction was increased while allocation to the insoluble amino-N fraction was diminished in NH_4^+-fed plant shoots and roots compared to NO_3^--fed plant shoots and roots.

The small amount of NH_4^+ found in the shoots and the large allocation of N to soluble amino-N in the roots of NH_4^+-fed plants may be taken as evidence for the assimilation of NH_4^+ in the roots and the translocation of the resulting amino compounds to the shoot. The greater quantity of total N found in NH_4^+- compared to NO_3^--fed plants supports the observation that the former took up N more rapidly than the latter (Section 4.8.1).

The $^{15}NO_3^-$- and $^{15}NH_4^+$-fed plant shoots had comparable ^{15}N contents although $^{15}NH_4^+$-fed plant roots were considerably ^{15}N enriched in comparison to $^{15}NO_3^-$-fed plant roots (Figure 4.49a). The quantity of free $^{15}NO_3^-$ in the roots was in excess of that in the shoots of $^{15}NO_3^-$-fed plants, but no free $^{15}NO_3^-$ was detected in $^{15}NH_4^+$-fed plants. The quantity of free $^{15}NH_4^+$ in the roots of $^{15}NH_4^+$-fed plants was vastly in excess of that in the shoots. Traces of free $^{15}NH_4^+$ were detected in $^{15}NO_3^-$-fed plants (roots and shoots), presumably as a result of metabolism of $^{15}NO_3^-$ to $^{15}NH_4^+$ or possibly release and re-assimilation of $^{15}NH_4^+$ in the shoot through photorespiratory N cycling (Keys *et al.*, 1978) which has been shown to be

significant in C_3 plants (Berger and Fock, 1983; 1985). The allocation of ^{15}N to soluble amino-^{15}N was considerably enhanced in $^{15}NH_4^+$- compared to $^{15}NO_3^-$-fed plants, especially in the roots.

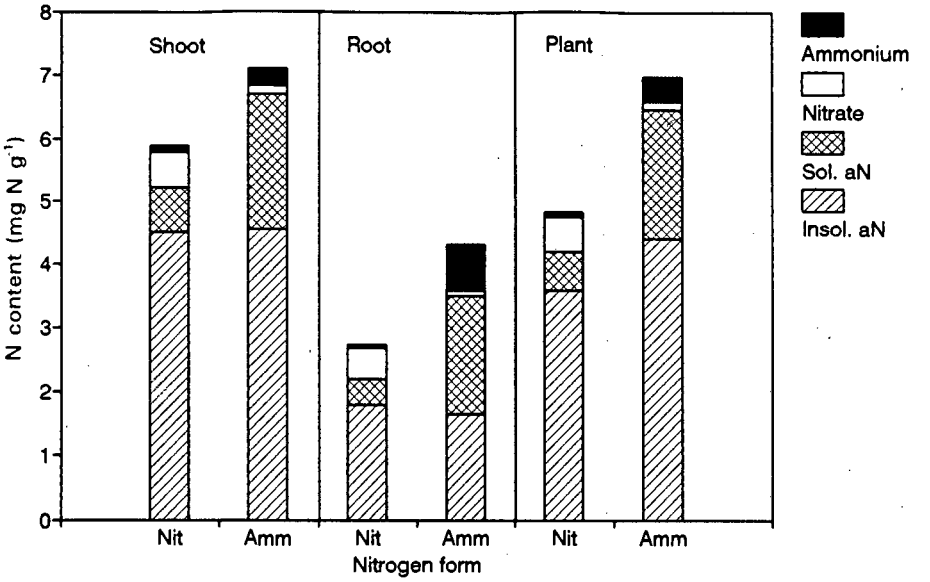
The percentage allocation of ^{15}N to the soluble amino-^{15}N fraction in the shoots was greater in $^{15}NH_4^+$- than in $^{15}NO_3^-$-fed plants, although only small differences were evident in the roots (Figure 4.49b). In spite of the fact that $^{15}NH_4^+$-fed plants accumulated more ^{15}N in the roots, the proportion of ^{15}N in the inorganic ^{15}N fractions of $^{15}NO_3^-$- and $^{15}NH_4^+$-fed plant roots was comparable. The proportion of ^{15}N in the inorganic ^{15}N fractions of the shoots was however significantly smaller in $^{15}NH_4^+$- than in $^{15}NO_3^-$-fed shoots. In $^{15}NH_4^+$-fed plants the large proportion of inorganic ^{15}N in the root, and the large proportion of soluble amino-^{15}N and insoluble amino-^{15}N in the shoot may have been the result of amino compounds, produced by root based assimilation of $^{15}NH_4^+$, being translocated out of the root to the shoot. The large proportion of ^{15}N in the shoot soluble amino-^{15}N fraction may thus reflect the incorporation of ^{15}N into amino acids, proteins and photosynthetic enzymes.

4.8.2.2 Maize response

Total N in NH_4^+-fed plants was significantly higher (1.3-fold) than that in NO_3^--fed plants (Figure 4.50a) in support of the finding that NH_4^+-fed maize plants take up N more rapidly from equimolar sources than do NO_3^--fed plants (Section 4.8.1). The quantity of insoluble amino-N in the root was not significantly affected by the form of N, but was significantly higher in the shoots of NH_4^+- than of NO_3^--fed plants. The quantity of insoluble amino-N (and consequently total N) was considerably greater in the shoots than in the roots. The soluble amino-N content of NH_4^+-fed shoots and roots was significantly increased over that of NO_3^--fed plants. Very little inorganic N was found in the roots of NH_4^+-fed plants while there was a significant amount of this in the shoots of these plants. This must reflect an efficient assimilatory capacity for dealing with free NH_4^+ in the maize root. A significant amount of free NO_3^- was present in the roots of NO_3^--fed plants with only trace amounts of free NH_4^+.

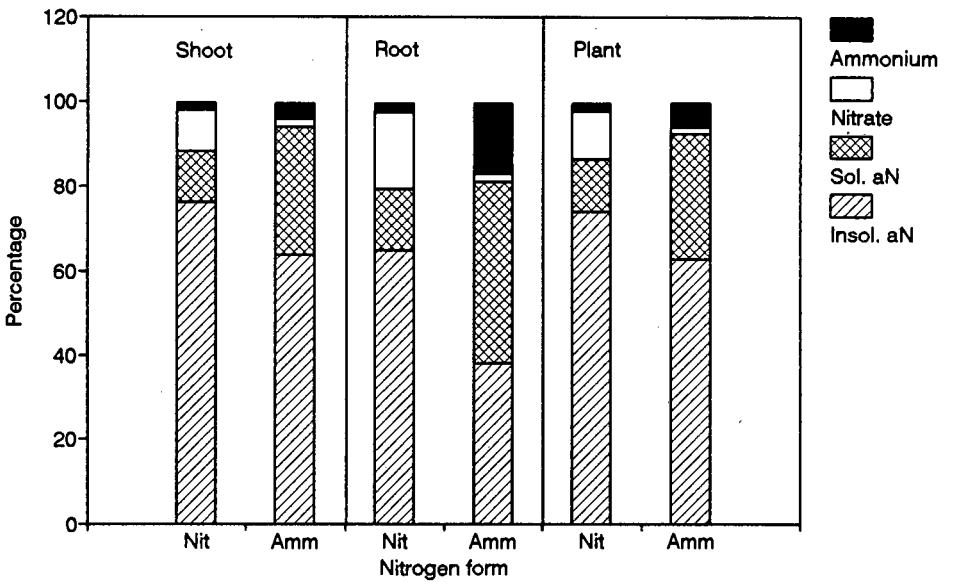
The proportion of soluble amino-N in NO_3^--fed plant roots was clearly smaller than that in NH_4^+-fed plant roots while the reverse was true for the inorganic N components (Figure 4.50b). The proportion of N allocated to soluble amino-N was smaller in the shoots than the roots of NH_4^+-fed plants. There were comparable proportions of inorganic N in the shoots

a)



Fraction	Shoot	Root	Plant
Insol. aN	NS	NS	NS
Sol. aN	p<0.05	p<0.05	p<0.05
Nitrate	p<0.05	p<0.05	p<0.05
Ammonium	p<0.05	p<0.05	p<0.05
Total	p<0.05	p<0.05	p<0.05

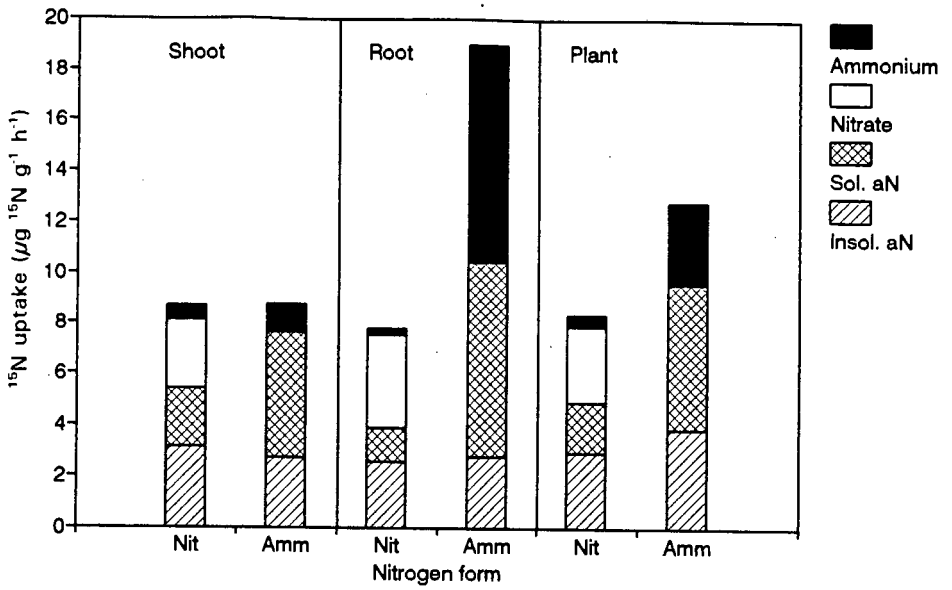
b)



Fraction	Shoot	Root	Plant
Insol. aN	NS	NS	NS
Sol. aN	p<0.05	p<0.05	p<0.05
Nitrate	p<0.05	p<0.05	p<0.05
Ammonium	p<0.1	p<0.05	p<0.05
Total	NS	p<0.05	p<0.05

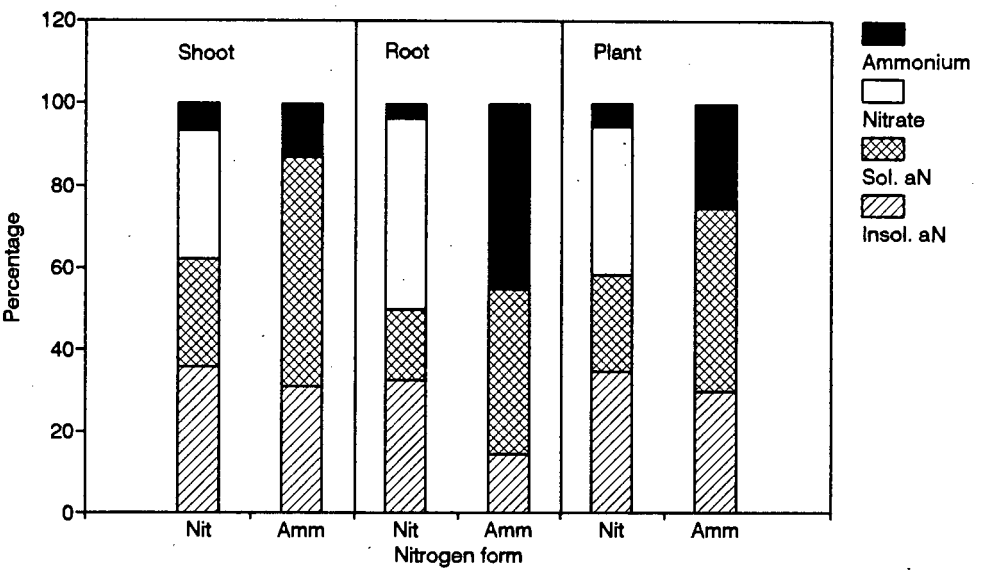
Figure 4.48. a) Partitioning of N (mg N g^{-1} fresh mass) and b) percentage allocation of N to insoluble amino-N (Insol. aN), soluble amino-N (Sol. aN), NO_3^- and NH_4^+ fractions in wheat plants grown on 4 mM NO_3^- (Nit) or NH_4^+ (Amm). Results of Student's T tests on differences in allocation between NO_3^-- and NH_4^+-fed plants are shown in tables below graphs (NS=not significant at 90% confidence interval). Student's T tests on the percentage allocations performed on arcsine transformed data. (n=3)

a)



Fraction	Shoot	Root	Plant
Insol. aN	$p<0.05$	$p<0.05$	$p<0.1$
Sol. aN	$p<0.05$	$p<0.05$	$p<0.05$
Nitrate	$p<0.05$	$p<0.05$	$p<0.05$
Ammonium	NS	$p<0.05$	$p<0.1$
Total	$p<0.05$	$p<0.05$	$p<0.05$

b)



Fraction	Shoot	Root	Plant
Insol. aN	NS	$p<0.05$	NS
Sol. aN	$p<0.05$	$p<0.05$	$p<0.05$
Nitrate	$p<0.05$	$p<0.05$	$p<0.05$
Ammonium	$p<0.1$	$p<0.05$	$p<0.05$
Total	NS	$p<0.05$	$p<0.05$

Figure 4.49. a) Partitioning of ^{15}N (μg ^{15}N g^{-1} fresh mass h^{-1}) and b) percentage allocation of ^{15}N to insoluble amino-^{15}N (Insol. aN), soluble amino-^{15}N (Sol. aN), $^{15}NO_3^-$ and $^{15}NH_4^+$ fractions in wheat plants grown on 4 mM NO_3^- (Nit) or NH_4^+ (Amm). Plants were exposed to ^{15}N for 8 h prior to harvest. Results of Student's T tests on differences in allocation between $^{15}NO_3^-$- and $^{15}NH_4^+$-fed plants are shown in tables below graphs (NS=not significant at 90% confidence interval). Student's T tests for percentage allocations performed on arcsine transformed data. (n=3)

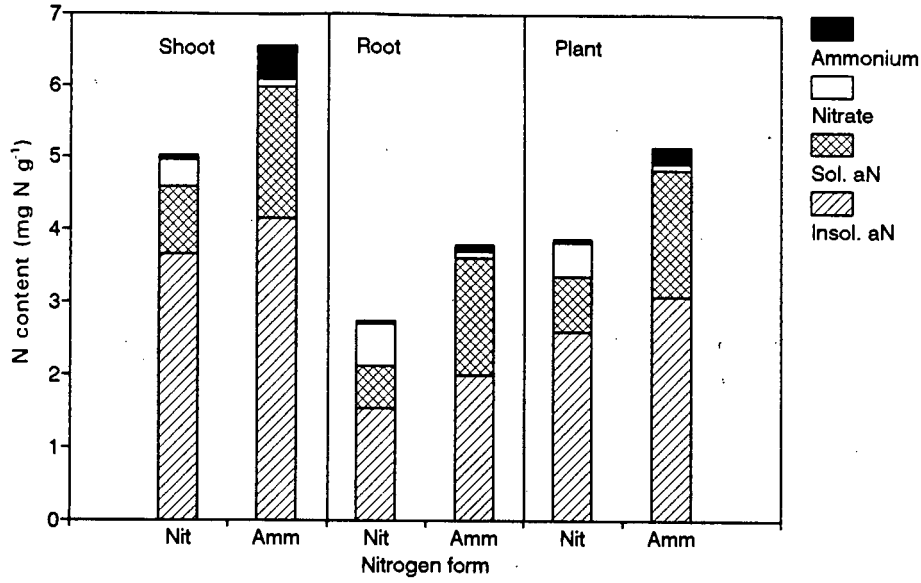
of NO_3^-- and NH_4^+-fed plants, but NO_3^--fed plant roots had a far larger proportion of N in the inorganic N fraction than did NH_4^+-fed plant roots. The small amount of NH_4^+ found in the shoots and the large allocation of N to soluble amino-N in the roots of NH_4^+-fed plants may be the result of efficient NH_4^+ assimilation in the root and translocation of the amino compounds produced to the shoot.

Large amounts of inorganic ^{15}N were found in the roots of both $^{15}NO_3^-$- and $^{15}NH_4^+$-fed plants although the amount of $^{15}NO_3^-$ in $^{15}NO_3^-$-fed roots was far greater than the amount of $^{15}NH_4^+$ in $^{15}NH_4^+$-fed roots (Figure 4.51a), possibly reflecting limited NR capacity in the roots of maize plants. This was in spite of the fact that incorporation of ^{15}N was greater in $^{15}NH_4^+$- than in $^{15}NO_3^-$-fed plants with more total ^{15}N found in roots than shoots. This again reflects a more rapid assimilation of $^{15}NH_4^+$ than of $^{15}NO_3^-$ within the maize root.

The percentage allocation of ^{15}N in shoots was comparable in both $^{15}NO_3^-$ and $^{15}NH_4^+$ treatments, except that the predominant form of the inorganic ^{15}N was different (Figure 4.51b). The shoots had a larger proportion of ^{15}N in the insoluble amino-N fraction than was the case in the roots. A large proportion of ^{15}N-content in $^{15}NH_4^+$-fed roots was in the form of soluble amino-^{15}N with only a small proportion allocated to inorganic ^{15}N. The reverse was true in $^{15}NO_3^-$-fed root.

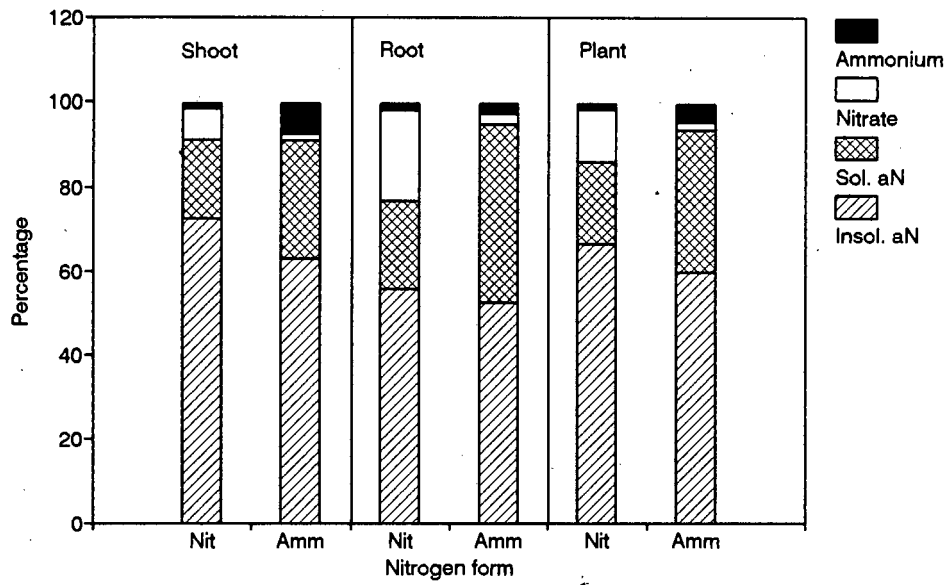
There was a considerable amount of free $^{15}NH_4^+$ in the shoot. This indicates that in the maize plant there was some translocation of free $^{15}NH_4^+$ from root to shoot (Section 4.8.4.2) although free $^{15}NH_4^+$ in the shoot may also be derived from limited photorespiratory N cycling (Berger and Fock, 1983; 1985) which may occur in bundle sheath cells of maize plants. Murphy and Lewis (1987) found that after 4 h supply of $^{15}NH_4^+$ to maize, 66% of ^{15}N was found in the soluble amino-^{15}N fraction in the xylem sap with the remainder as NH_4^+. This identifies the root as the principal, but not the only, site of nutrient NH_4^+ assimilation and agrees well with the 33% of ^{15}N found in the form of $^{15}NH_4^+$ in the maize shoot (Figure 4.51b). There was a small amount of free $^{15}NO_3^-$ in the roots of $^{15}NH_4^+$-fed plants. Whether this resulted from the conversion of $^{15}NH_4^+$ to $^{15}NO_3^-$ within the plant, or by nitrifying bacteria in the growth medium is not known. Measurement of NO_3^- concentrations in $^{15}NH_4^+$ feeding solutions after the feeding period, however, showed that only trace amounts of NO_3^- (0.04 mM $\pm$ 0.04) were present. Only trace amounts of $^{15}NH_4^+$ were found in roots of $^{15}NO_3^-$-fed plants with slightly larger amounts in the shoots of these plants.

a)



Fraction	Shoot	Root	Plant
Insol. aN	$p<0.05$	NS	NS
Sol. aN	$p<0.05$	$p<0.05$	$p<0.05$
Nitrate	$p<0.05$	$p<0.05$	$p<0.05$
Ammonium	$p<0.05$	NS	$p<0.1$
Total	$p<0.05$	NS	$p<0.1$

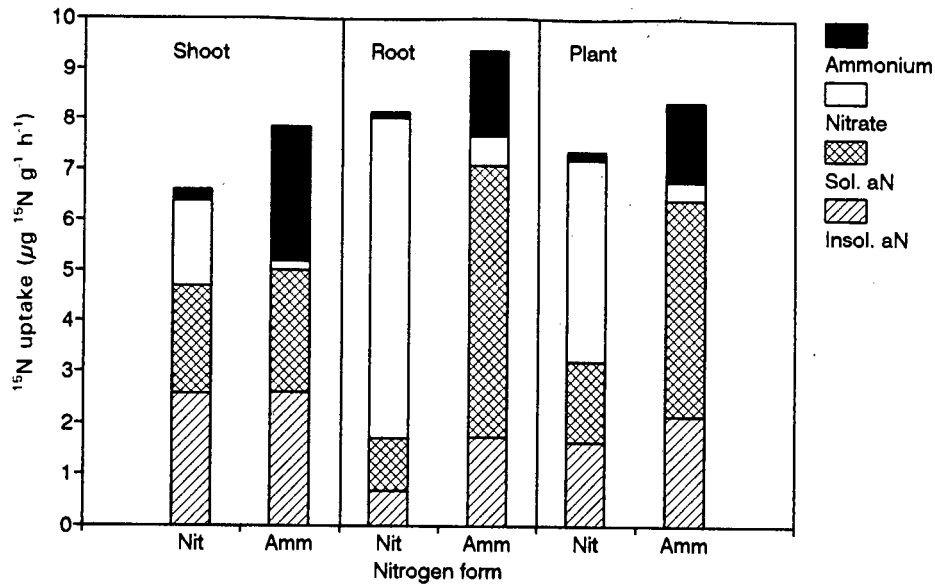
b)



Fraction	Shoot	Root	Plant
Insol. aN	$p<0.1$	NS	NS
Sol. aN	$p<0.05$	$p<0.05$	$p<0.05$
Nitrate	$p<0.05$	$p<0.05$	$p<0.05$
Ammonium	$p<0.05$	NS	NS
Total	$p<0.05$	NS	$p<0.1$

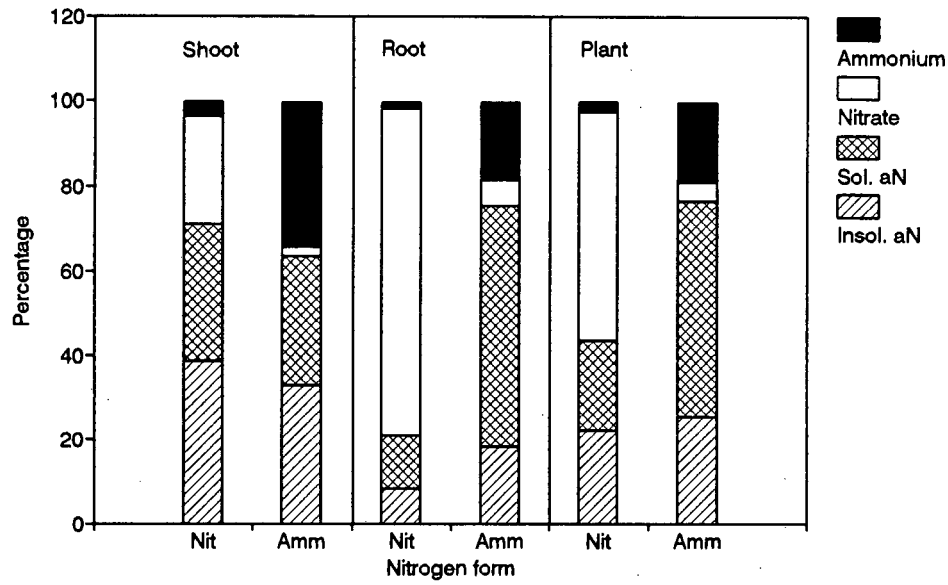
Figure 4.50. a) Partitioning of N (mg N g^{-1} fresh mass) and b) percentage allocation of N to insoluble amino-N (Insol. aN), soluble amino-N (Sol. aN), NO_3^- and NH_4^+ fractions in maize plants grown on 4 mM NO_3^- (Nit) or NH_4^+ (Amm). Results of Student's T tests on differences in allocation between NO_3^-- and NH_4^+-fed plants are shown in tables below graphs (NS=not significant at 90% confidence interval). Student's T tests on the percentage allocations performed on arcsine transformed data. (n=3)

a)



Fraction	Shoot	Root	Plant
Insol. aN	NS	p<0.1	NS
Sol. aN	NS	p<0.05	p<0.05
Nitrate	p<0.05	p<0.05	p<0.05
Ammonium	p<0.05	p<0.05	p<0.1
Total	NS	NS	NS

b)



Fraction	Shoot	Root	Plant
Insol. aN	NS	p<0.1	NS
Sol. aN	NS	p<0.05	p<0.05
Nitrate	p<0.05	p<0.05	p<0.05
Ammonium	p<0.05	p<0.05	NS
Total	NS	NS	NS

Figure 4.51. a) Partitioning of ^{15}N (μg ^{15}N g^{-1} fresh mass h^{-1}) and b) percentage allocation of ^{15}N to insoluble amino-^{15}N (Insol. aN), soluble amino-^{15}N (Sol. aN), $^{15}NO_3^-$ and $^{15}NH_4^+$ fractions in maize plants grown on 4 mM NO_3^- (Nit) or NH_4^+ (Amm). Plants were exposed to ^{15}N for 8 h prior to harvest. Results of Student's T tests on differences in allocation between $^{15}NO_3^-$- and $^{15}NH_4^+$-fed plants are shown in tables below graphs (NS=not significant at 90% confidence interval). Student's T tests on the percentage allocations performed on arcsine transformed data. (n=3)

4.8.2.3 Discussion

The results of the N partitioning experimentation showed that:

1) Uptake of NH_4^+ was more rapid than NO_3^- uptake in both wheat and maize.
2) In both wheat and maize NH_4^+ nutrition resulted in higher soluble amino-N contents than NO_3^- nutrition.
3) Insoluble amino-N contents of wheat were unaffected by the form of N supplied while insoluble amino-N contents of maize were slightly higher in NH_4^+- than in NO_3^--fed plants.
4) Inorganic N contents of plants represented less than 20% of total N and were generally lower in NH_4^+- than in NO_3^--fed plants.
5) Shoots had higher total N and insoluble amino-N contents than roots, although soluble amino-N and inorganic N contents were comparable between roots and shoots.
6) Wheat had higher total N contents (mg N g^{-1} fresh mass) than maize in both NO_3^-- and NH_4^+-fed plants.
7) In wheat and maize the amount of N in the soluble amino-N pool was comparable between shoot and root components, although the root had generally higher soluble amino-N levels, supporting the notion that a single amino acid pool exists in the plant due to extensive N cycling (Simpson, 1986).

The results of the ^{15}N uptake and partitioning experimentation showed that:

1) Soluble amino-^{15}N accumulated in the roots of wheat and maize supplied with $^{15}NH_4^+$.
2) Recently acquired $^{15}NO_3^-$ was found in both the shoot and root components, but assimilation products of NO_3^- were predominantly located in the shoots of both wheat and maize.
3) Accumulation of $^{15}NH_4^+$ occurred in the roots of wheat but not maize.

The total N accumulated by NH_4^+-fed wheat and maize was 1.5- and 1.3-fold greater respectively than that accumulated by NO_3^--fed plants. This is in close accord with the 1.5- and 1.3-fold greater uptake of NH_4^+ by wheat and maize respectively compared to NO_3^- uptake (Section 4.8.1). Thus there was a smaller difference between the uptake of NO_3^- and NH_4^+ in maize plants than in wheat plants. The amount of total N found in NO_3^-- and NH_4^+-

fed wheat plants was 4.8 and 7.0 mg N g^{-1} fresh mass respectively. The corresponding figures for NO_3^-- and NH_4^+-fed maize were 3.9 and 5.2 mg N g^{-1} fresh mass respectively. Thus wheat total N in NO_3^-- and NH_4^+-fed plants was 1.2- and 1.4-fold greater respectively than in maize, indicative of limited C availability in wheat plants and the capacity of maize to assimilate NH_4^+.

The large accumulation of $^{15}NH_4^+$ in $^{15}NH_4^+$-fed roots of wheat was not observed in maize plants. This apparent inability to assimilate the large quantity of $^{15}NH_4^+$ taken up and to translocate the products to the shoot may in part explain the sensitivity of wheat roots to NH_4^+ nutrition and be a consequence of limited resources (carbohydrates) for provision of C skeletons for NH_4^+ assimilation. The percentage of free $^{15}NO_3^-$ in the roots of $^{15}NO_3^-$-fed plants was much higher in maize than in wheat.

Large differences between the rates of ^{15}N uptake into *Hordeum vulgare* from $^{15}NH_4^+$ and $^{15}NO_3^-$ nutrient solutions have been reported previously (uptake of $^{15}NO_3^-$ 20% of $^{15}NH_4^+$ uptake, Lewis and Chadwick, 1983). In this investigation the rate of $^{15}NO_3^-$ uptake by wheat was 60% of the rate of $^{15}NH_4^+$ uptake while the comparable figure for maize was 90%, although the total N content of NO_3^--fed wheat and maize plants was 69 and 75% respectively of that of NH_4^+-fed plants. Lewis and Chadwick (1983) offered no explanation for the differences in the uptake of NO_3^- and NH_4^+ observed between their N and ^{15}N experiments. It has, however, been established that the uptake of both NO_3^- and NH_4^+ is the net result of influx and efflux of these ions (MacKlon *et al.*, 1990; Morgan and Jackson. 1988; 1989) and this may contribute to differences between steady-state N contents and non-steady-state ^{15}N contents.

Results presented here support the assertion of Goyal and Huffaker (1984) that plants grown on NH_4^+ almost invariably contain higher levels of free NH_4^+ and amino-N than those grown on NO_3^-. This observation may be explained by the proposal that NH_4^+ plays a regulatory role in diverting C from carbohydrate biosynthesis into amino acid synthesis (Platt *et al.*, 1977; Robinson and Baysdorfer, 1985). Unlike the findings of Blacquière *et al.* (1987) for shoots of *Plantago lanceolata* and *Plantago major*, this study demonstrated significant differences in amino-N contents of the shoots of NO_3^-- and NH_4^+-fed plants. The finding of Blacquière *et al.* (1987) that soluble amino-N contents in the roots of NO_3^-- and NH_4^+-fed plants were significantly different is supported by this investigation.

Although the roots of NH_4^+-fed plants take up more N than those of NO_3^--fed plants, the additional N is not diverted into insoluble amino-N within the root. The fact that the insoluble amino-N contents of the roots of both wheat and maize remained constant regardless of the N source supports the proposal of Vessey and Layzell (1987) that only N in excess of the requirements of the root was exported to the shoot in *Glycine max*. Much of the partitioning evidence presented here may be interpreted with respect to the fact that wheat and maize predominantly assimilate NO_3^- in the shoot while NH_4^+ is predominantly assimilated in the root (Section 2.8.2). Experimental evidence for this division of labour between root and shoot is provided in Section 4.8.4. In species which reduce NO_3^- predominantly in the leaf, the chloroplasts are likely to become a major source of amino acids for protein synthesis (Schrader, 1984). This is supported by the observation that assimilation products of $^{15}NO_3^-$ are predominantly located in the shoots.

The percentage distribution of total N fits approximately with the generalized distribution of the total plant N presented by Hocking *et al.* (1984a), in which amino-N in the form of protein accounts for 75 to 90% of total N with free amino compounds accounting for 10 to 25% and NH_4^+ for 0.4 to 4%. However, in this investigation the NH_4^+ and NO_3^- contents of roots contributed up to 20% of total N.

4.8.3 Split-root culture: Total nitrogen and ^{15}Nitrogen contents

In split-root culture experiments, there were no major differences between the total N contents of 4 and 12 mM N-fed wheat respectively (Figure 4.52a) or of 4 and 12 mM N-fed maize respectively (Figure 4.52b) and differences between NO_3^-- and NH_4^+-fed root-halves were small. The total N contents of 4 and 12 mM wheat were 1.7- and 1.6-fold higher respectively than those of maize.

The allocation patterns of ^{15}N between plant parts did not vary much with the concentration (4 or 12 mM) of ^{15}N in either wheat or maize (Figure 4.53). Experimentally only one half of any particular plant root was supplied with ^{15}N. The ^{15}N contents of 4 mM $^{15}NH_4^+$-enriched wheat plants were smaller than those of $^{15}NO_3^-$-enriched wheat plants, while at 12 mM N there were no differences between the two (Figure 4.53a). The efficacy of a root in taking up N depends not only on the ability of the plant to take up the particular form of N, but also on the physical size of the root. Normally NH_4^+ is taken up at rates exceeding

NO_3^- uptake (Section 4.8.1). The lower ^{15}N content of $^{15}NH_4^+$-fed plants is thus probably due to the physically small NH_4^+-root (See biomass data, Sections 4.2.1 and 4.2.2) which was limited by the lower (4 mM) ^{15}N concentration in its ability to take up the N. ^{15}Nitrate- and $^{15}NH_4^+$-enriched maize plant ^{15}N contents were comparable at 4 mM ^{15}N. When fed 12 mM ^{15}N the $^{15}NH_4^+$-enriched plants contained much less ^{15}N than $^{15}NO_3^-$-enriched plants indicating impaired operation of this root-half at high NH_4^+ concentrations.

The proportion of ^{15}N located in the component parts (shoots, root-halves supplied with NO_3^- and root-halves supplied with NH_4^+) was calculated as a percentage of the total ^{15}N content for both wheat and maize (Figure 4.54). The percentage of the total plant ^{15}N found in the roots of the wheat and maize, whether grown on 4 or 12 mM N, remained fairly constant. In maize there was, however, a much larger transfer of ^{15}N from the root-halves exposed to the ^{15}N to the halves not exposed. Transfer of ^{15}N from one root-half to the other in wheat and maize accounted for 4 to 8% and 11 to 32% respectively of N in the recipient root-half, indicating that cycling of N does occur within these plants. The amount of ^{15}N moved from the ^{15}N supplied to the ^{14}N supplied root was of similar magnitude to that reported by others for split-root experiments where one root-half was maintained on $^{15}NO_3^-$ culture and one in $^{14}NO_3^-$ culture (Lambers *et al.*, 1982; Cooper *et al.*, 1986a). There was a larger transfer of ^{15}N from the root supplied with $^{15}NH_4^+$ to the root supplied with $^{14}NO_3^-$ than from the $^{15}NO_3^-$- to the $^{14}NH_4^+$-supplied root, possibly resulting from the more rapid uptake of NH_4^+ than of NO_3^-.

In summary, the results for the split-root N and ^{15}N partitioning experiments showed that:

1) Nitrogen concentration did not affect the total N contents of wheat or maize significantly.
2) Wheat plants had higher total N contents than maize plants.
3) The smaller size of NH_4^+-fed root-halves may have limited ^{15}N uptake.
4) In wheat, transfer of ^{15}N from one root-half to the other accounts for between 4 and 8% of N in the recipient root-half while in maize transfer from the enriched root accounts for between 11 and 32% of N in the recipient root in 4 and 12 mM N-fed plants respectively.
5) The shoot was enriched with ^{15}N to a constant proportion of total ^{15}N regardless of whether $^{15}NO_3^-$ or $^{15}NH_4^+$ was supplied.

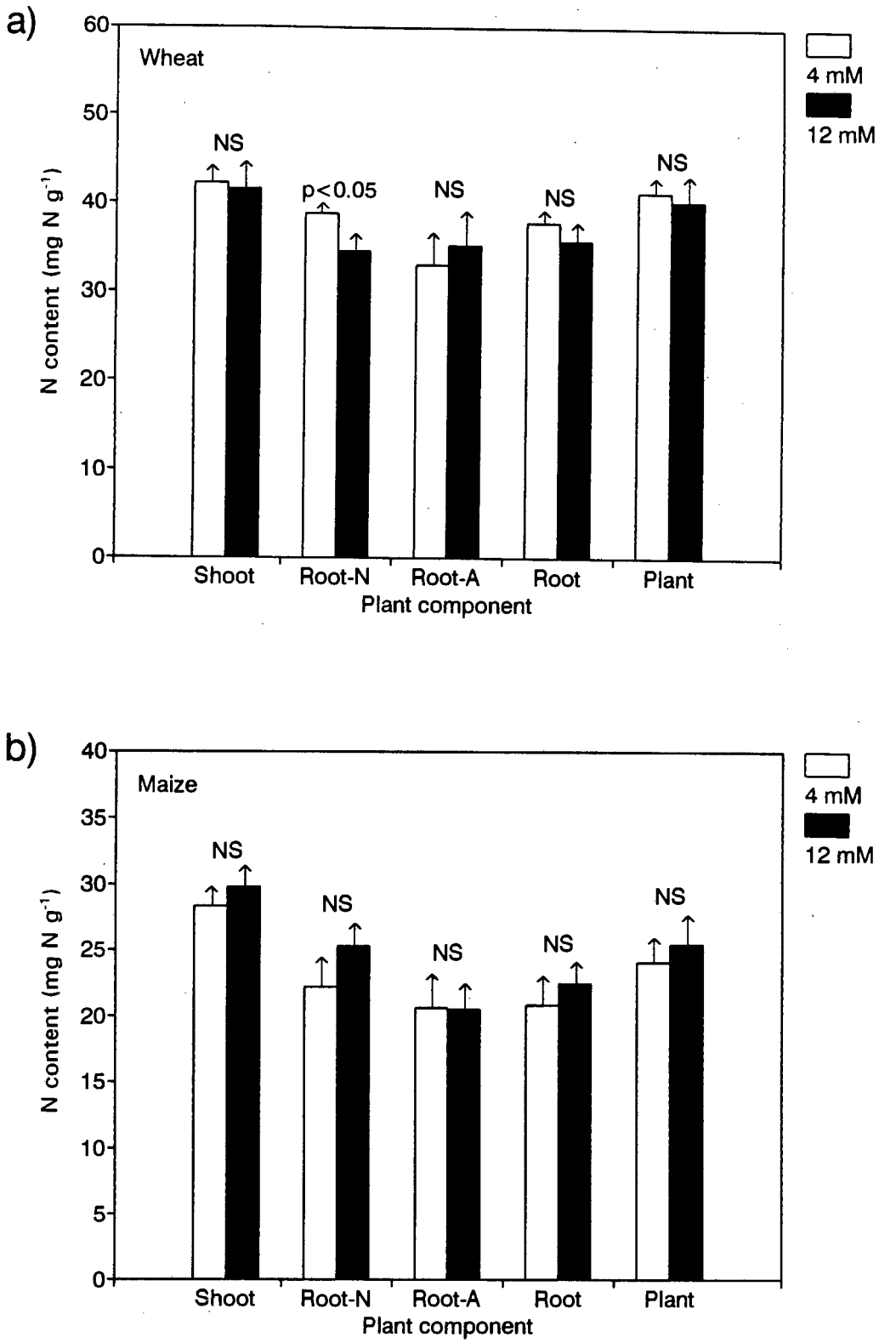
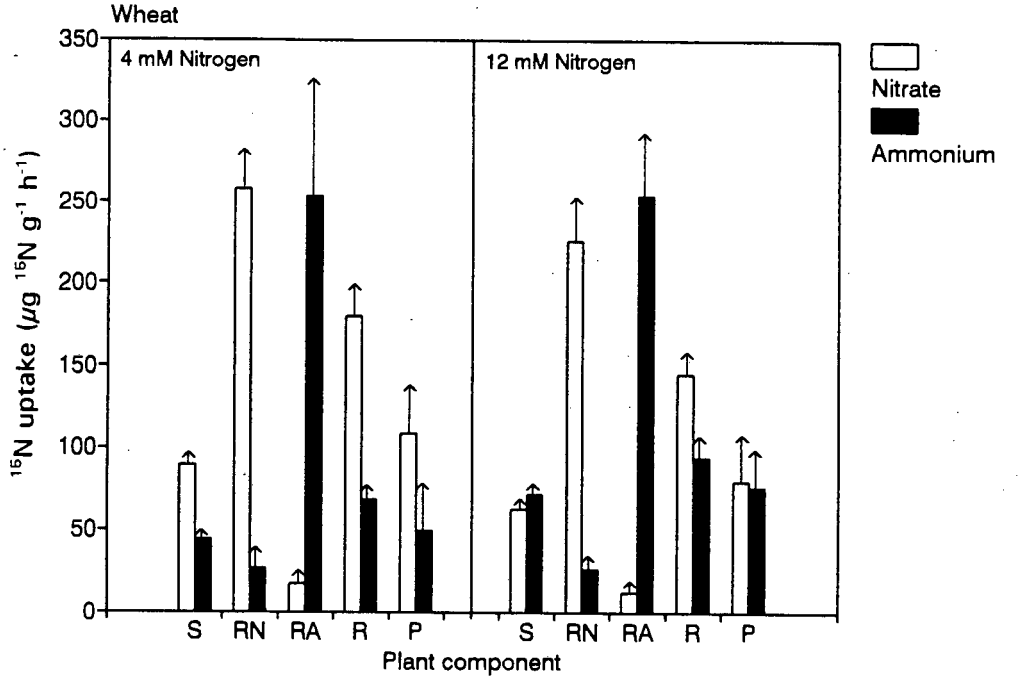


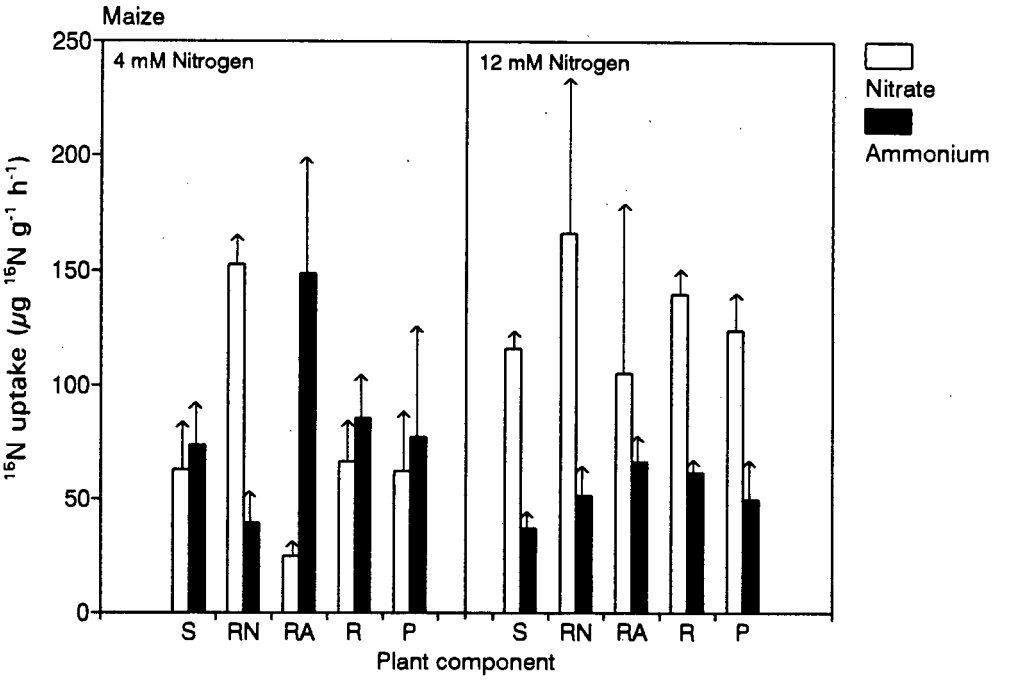
Figure 4.52. a) Wheat and b) maize total N contents (mg N g^{-1} dry mass) of shoots, NO_3^--fed root halves (Root-N), NH_4^+-fed root halves (Root-A), roots and plants grown on 4 or 12 mM N in split-root culture. Bars indicate the S.E. Student's T tests of NO_3^-- (Root-N) versus NH_4^+-fed root halves (Root-A) yielded: wheat, $p<0.1$ for 4 mM N and $p>0.1$ for 12 mM N treatments; maize, $p>0.1$ for 4 mM N and $p<0.05$ for 12 mM N treatments. (n=6)

a)



Wheat	S	RN	RA	R	P
4 mM	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$	NS
12 mM	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$	NS

b)



Maize	S	RN	RA	R	P
4 mM	NS	$p<0.05$	$p<0.05$	NS	NS
12 mM	$p<0.05$	NS	NS	$p<0.05$	$p<0.05$

Figure 4.53. a) Wheat and b) maize ^{15}N uptake (mg N g^{-1} dry mass h^{-1}) and allocation to shoots (S), NO_3^--fed root halves (RN), NH_4^+-fed root halves (RA), roots (R) and plants (P) grown on 4 or 12 mM N in split-root culture. Bars indicate the S.E. Results of Student's T tests on differences for each component between NO_3^-- and NH_4^+-fed plant roots are shown in table below graphs (NS=not significant at 90% confidence interval). (n=6)

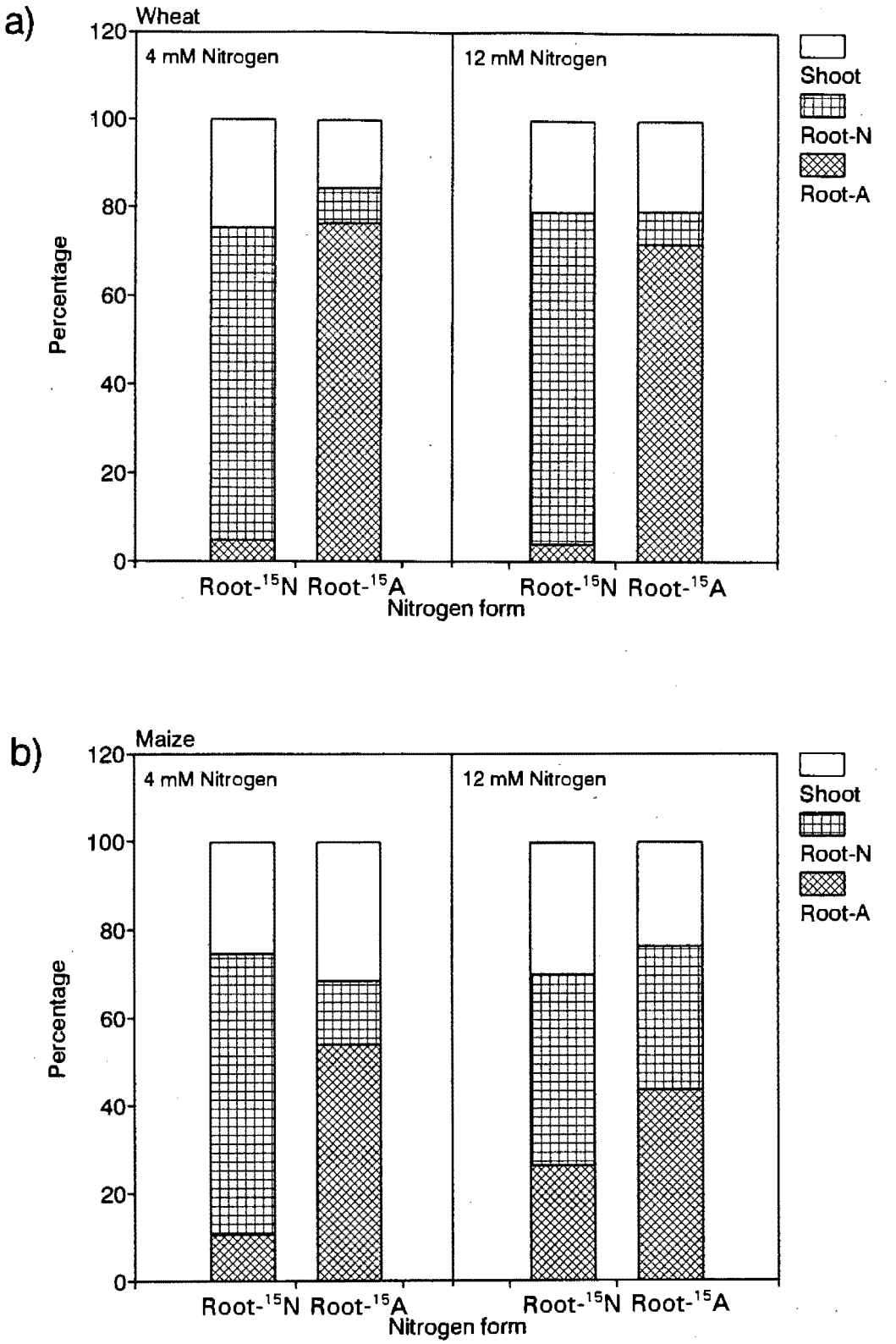


Figure 4.54. Percentage ^{15}N of total ^{15}N in a) wheat and b) maize shoots, NO_3^--fed root halves (Root-N) and NH_4^+-fed root halves (Root-A). The plants were grown on 4 or 12 mM N. The roots of the plants were supplied with NO_3^- or NH_4^+ with one root half of a plant being supplied with ^{15}N and the other with ^{14}N. The root which received the ^{15}N is indicated below the bars.(n=6)

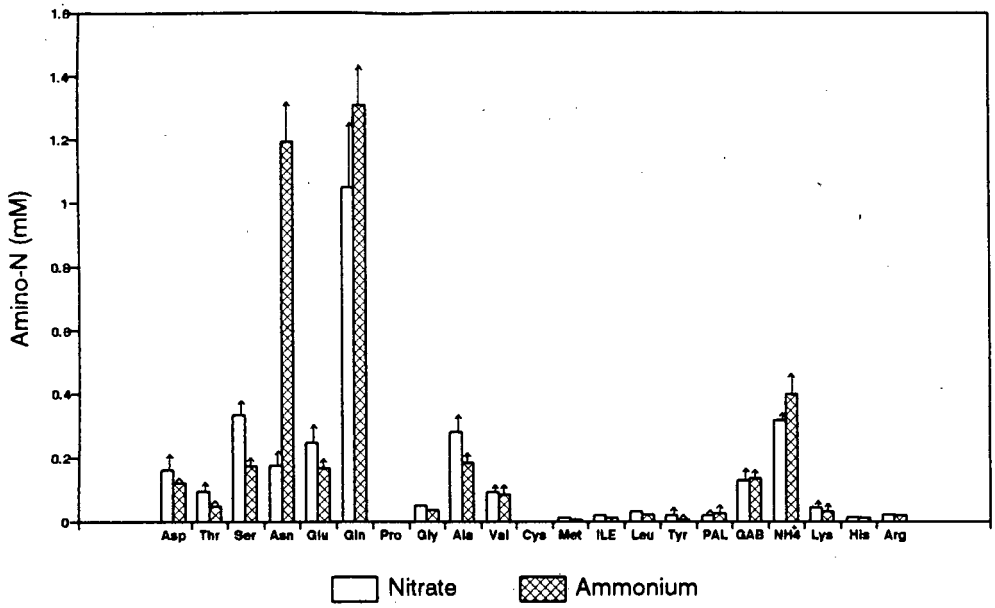
4.8.4 Xylem sap nitrogen content

From ^{14}C (Section 4.7), N and ^{15}N partitioning (Section 4.8.2) it appears that production of amino compounds is favoured by NH_4^+ nutrition over NO_3^- nutrition. The amount of insoluble amino-N in the roots has been shown to be comparable between NO_3^-- and NH_4^+-fed plants. The large scale production of amino compounds in the roots of NH_4^+-fed plants may thus require transport of these amino compounds from the root to the shoot. One may, therefore, expect an increase in the amino-N content of the xylem sap. Apart from changes in the quantity of amino compounds in the xylem sap, there may also be changes in the forms of amino compounds translocated. The transport of ^{14}C through the xylem of plants supplied with $^{14}CO_2$ through photosynthesis has previously been described (Section 4.7.2). Analysis of xylem sap amino-N contents provides a quantitative estimation of the amount of C translocated in the form of amino-C.

4.8.4.1 Wheat response

On a qualitative basis the forms of amino-N found in the xylem sap were comparable in NO_3^-- and NH_4^+-fed wheat plants. However, the quantity of certain amino compounds was considerably dependent on the form of N supplied (Figure 4.55). In particular there were large differences between the asparagine and glutamine contents of NO_3^- and NH_4^+-fed plants. Lewis *et al.* (1982) observed 3.5- and 7.7-fold higher levels of glutamine and glutamate respectively but only slightly higher asparagine contents in the xylem sap of 2 mM NH_4^+- compared to 2 mM NO_3^--fed *Hordeum vulgare* plants. In wheat Lewis *et al.* (1987) found 3.7- and 1.4-fold larger asparagine and glutamine levels respectively in NH_4^+- compared to NO_3^--fed plants. The results presented here showed 6.0- and 1.3-fold larger asparagine and glutamine levels respectively in 4 mM NH_4^+- compared to 4 mM NO_3^--fed plants. The larger differences between NO_3^-- and NH_4^+-fed plants with respect to asparagine concentration in this investigation compared to that of Lewis *et al.* (1987) may result from the different levels of N nutrition employed.

There was 1.6-fold more amino-N translocated in the xylem sap of NH_4^+- than of NO_3^--fed plants (Figure 4.56). Larger differences between NO_3^-- and NH_4^+-fed plants with respect to amino-N contents have been reported previously (Lewis *et al.*, 1982; Lewis *et al.*, 1983). The fact that the content and type amino compounds varied with N source resulted in not only



Asp	Thr	Ser	Asn	Glu	Gln	Pro	Gly
NS	$p<0.05$	$p<0.05$	$p<0.05$	NS	NS	NS	$p<0.1$
Ala	Val	Cys	Met	ILE	Leu	Tyr	PAL
$p<0.1$	NS	NS	$p<0.05$	$p<0.05$	$p<0.05$	NS	NS
GAB	NH_4^+	Lys	His	Arg	Total	C:N ratio	
NS	NS	NS	NS	NS	NS	$p<0.05$	

Figure 4.55. Amino compound composition of xylem sap of wheat plants grown on 4 mM NO_3^- or NH_4^+. Bars indicate the S.E. Results of Student's T tests for differences between NO_3^-- and NH_4^+-fed plants with respect to the concentrations of each amino compound, NH_4^+, total amino compound concentrations and C:N ratios are shown in table below graph. (n=12)

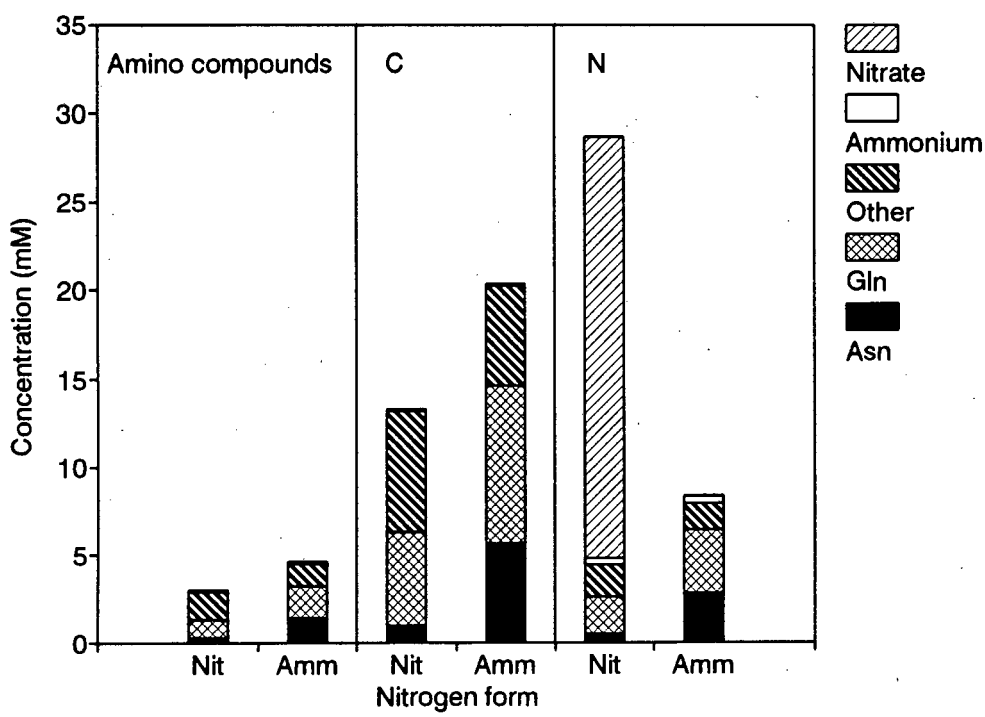


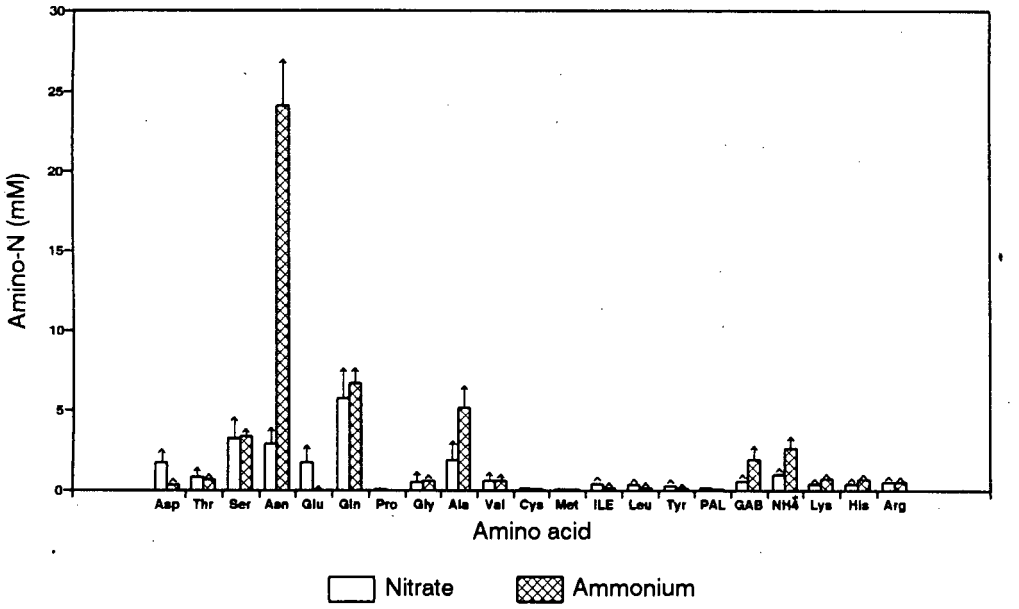
Figure 4.56. Comparison of the concentrations of glutamine (Gln), asparagine (Asn), all other amino compounds (Other), NO_3^- and NH_4^+ between wheat plants grown on 4 mM NO_3^- (Nit) or NH_4^+ (Amm). Results are shown as amino compound, carbon (C) and nitrogen (N) concentrations. (n=12)

greater translocation of amino compounds, but also significant changes in the C:N ratios of the xylem sap. The overall C:N ratio for the amino compounds translocated in the xylem sap of NH_4^+-fed plants averaged 2.06 $\pm$ 0.03 while that of NO_3^--fed plants averaged 3.02 $\pm$ 0.02. The reason for this change of C:N ratio was that the amino compounds produced by NH_4^+-fed plant roots in excess of those from NO_3^--fed plant roots (i.e. asparagine and glutamine) were N rich. The quantity of NH_4^+ in the xylem sap of NO_3^--fed plants was less than in NH_4^+-fed plants, but not significantly so. Thus it appeared that NH_4^+ compared to NO_3^- nutrition altered the quantity and form of amino compounds, particularly the amides, in the xylem sap resulting in improved C efficiency for N translocation from root to shoot.

The NH_4^+ content of NO_3^-- and NH_4^+-fed plants was comparable (approximately 0.3 mM) and did not make a significant contribution to the total N in the xylem sap. The NO_3^- concentration in the xylem sap of 4 mM NO_3^--fed wheat was 23.9 $\pm$ 2.4 mM which is considerably less than the 34.6 mM reported for 8 mM NO_3^--fed *Hordeum vulgare* (Lewis *et al.*, 1982a). The fact that the xylem sap NO_3^- concentrations were high and amino-N contents were low in NO_3^--fed plants provides strong evidence for the assimilation of NO_3^- within the leaves of these plants. The extremely low NH_4^+ and high amino-N contents in the xylem sap of NH_4^+-fed plants provides strong evidence for the assimilation of NH_4^+ within the roots of these plants.

4.8.4.2 Maize response

Comparable changes in amino compound composition of maize xylem sap to those of wheat were observed. Large increases in asparagine concentrations were observed along with significant increases in alanine concentrations with NH_4^+ compared to NO_3^- nutrition (Figure 4.57). The magnitude of this increase in asparagine contents is comparable to the 8-fold increase reported by Murphy and Lewis (1987) for 2 mM N-fed maize. These authors, however, found a doubling of glutamine contents in NH_4^+- compared to NO_3^--fed plants which was not observed in this investigation. There was also a significant increase in the NH_4^+ content of NH_4^+-fed plant xylem sap over that of NO_3^--fed plants, although this contributed only a slightly to the total N in the xylem sap. This may, however, explain the observation that $^{15}NH_4^+$ did accumulate in the shoots of $^{15}NH_4^+$-fed maize plants (Section 4.8.2.2). Overall in NH_4^+- compared to NO_3^--fed plants, there were 1.9-fold higher levels of amino compounds and the C:N ratios of the amino compounds in the xylem sap of



Asp	Thr	Ser	Asn	Glu	Gln	Pro	Gly
p<0.05	NS	NS	p<0.05	p<0.05	NS	NS	NS
Ala	Val	Cys	Met	ILE	Leu	Tyr	PAL
p<0.01	NS	NS	p<0.01	NS	NS	NS	NS
GAB	NH_4^+	Lys	His	Arg	Total	C:N ratio	
p<0.01	p<0.05	p<0.05	NS	NS	p<0.05	NS	

Figure 4.57. Amino compound composition of xylem sap of maize plants grown on 4mM NO_3^- or NH_4^+. Bars indicate the S.E. Results of Student's T tests for differences between NO_3^-- and NH_4^+-fed plants with respect to the concentrations of each amino compound, NH_4^+, total amino compound concentrations and C:N ratios are shown in table below graph. (n=12)

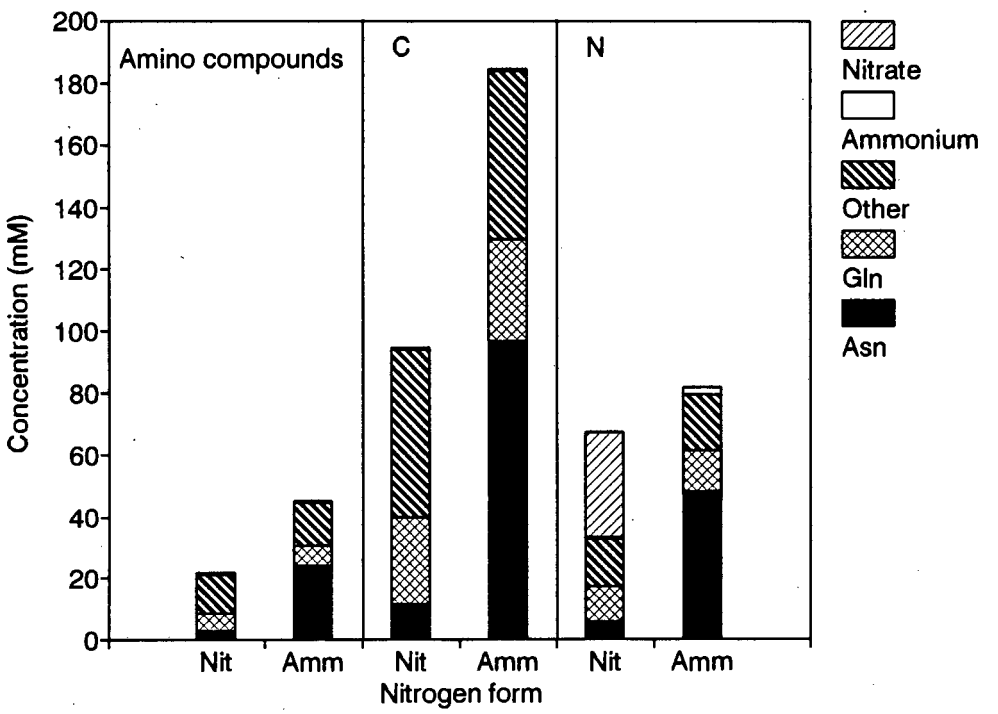


Figure 4.58. Comparison of the concentrations of glutamine (Gln), asparagine (Asn), all other amino compounds (Other), NO_3^- and NH_4^+ between maize plants grown on 4 mM NO_3^- (Nit) or NH_4^+ (Amm). Results are shown as amino compound, carbon (C) and nitrogen (N) concentrations. (n=12)

NH_4^+-fed plants were 2.32 ± 0.05 while those of NO_3^--fed plants were 2.83 ± 0.06 (Figure 4.58). The amount of NO_3^- in the xylem sap was 34.1 ± 3.3 mM which was considerably more than the 15.2 mM reported by Murphy and Lewis (1987) for 2 mM NO_3^--fed maize.

4.8.4.3 Discussion

Direct comparison of wheat and maize xylem sap N contents should be subject to caution due to differences in experimental methodology. The xylem sap from wheat was collected by placing the shoots under pressure in a Schölander bomb while that from maize was collected as bleeding sap from root stumps. The reason for this difference was due to technical problems experienced in inserting maize plants into the Schölander bomb due to the compressibility of the tissue. Although bleeding sap was collected for a short time interval to ensure that only sap already in the vessels was collected, bleeding sap may be more concentrated than sap exuded under pressure (see Section 2.8 for discussion on these techniques).

The N concentration in the xylem is the principal determinant of the amount of N translocated from root to shoot. The rate at which this translocation occurs is, however, at least partially dependent on the transpiration rate, although the importance of transpiration for ion translocation in maize has been questioned by Tanner and Beevers (1990). The transpiration rate was measured gravimetrically for both wheat and maize (Figure 4.59) and was expressed as the loss of water per gram root so as to relate N translocation to the size of the root. Although in both wheat and maize the transpiration rates of NO_3^--fed plants were slightly higher than those of NH_4^+-fed plants, these differences were not significant. Lower transpiration rates in NH_4^+- compared to NO_3^--fed plants may have been expected since other authors have reported reduced H_2O uptake by NH_4^+-fed plants (Goyal and Huffaker, 1984; Salsac *et al.*, 1987). The transpiration rate of the wheat plants was higher than that of the maize plants, probably as a result of the higher shoot : root ratios of the wheat plants. The small differences which exist between NO_3^-- and NH_4^+-fed plants with respect to transpiration rates do not alter the calculated translocation of C in either plant significantly (Table 4.2). The amount of C translocated in wheat and maize plants was 1.5- and 1.7-fold greater respectively in NH_4^+- than in NO_3^--fed plants. The importance of the quantity of C translocated in the xylem sap may be appreciated from the fact that the amount of C translocated in the xylem sap in amino compounds was considerably greater than the amounts

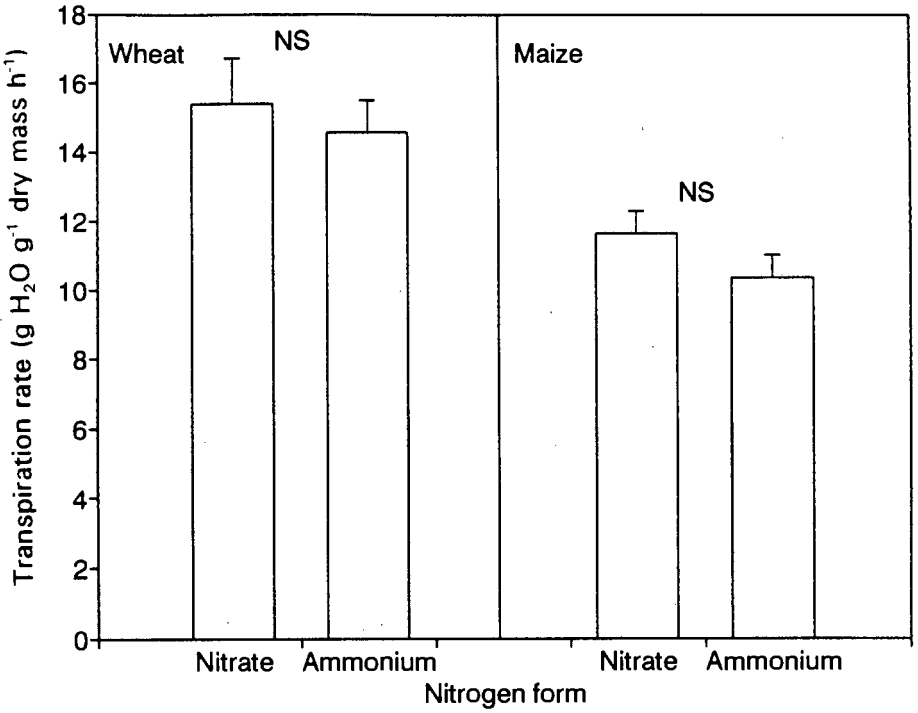


Figure 4.59. Transpiration rates (g water g^{-1} root dry mass h^{-1}) of wheat and maize plants grown on 4 mM NO_3^- or NH_4^+. Bars indicate the S.E. Results of Student's T test comparisons of NO_3^-- and NH_4^+-fed plants are shown above S.E. bars (NS=not significant at 90% confidence interval). (n=4)

of CO_2 lost from the root through root respiration (Section 4.9.1). The fact that the translocation of amino-C in the xylem sap of maize was much greater than in wheat may reflect the greater availability of C in the former from photosynthesis for the assimilation of inorganic N.

Table 4.2 Comparison of the rates of translocation of C in xylem sap of wheat and maize plants supplied with 4 mM NO_3^- or NH_4^+.

Plant species	Xylem sap C concentration (μmol g^{-1} dry mass h^{-1})	
	NO_3^--fed plants	NH_4^+-fed plants
Wheat	205.02	297.26
Maize	1105.68	1911.96

The fact that extensive re-cycling of organic N and exchange of organic N between xylem and phloem occurs in plants means that xylem organic N contents, as an indicator of the site of reduction, over estimate the contribution of the root (Simpson, 1986; Vessey and Layzell, 1987). The extent of N cycling is known to be considerable in wheat (Simpson *et al.*, 1982; Lambers *et al.*, 1982; Cooper *et al.*, 1986a; Cooper and Clarkson, 1989; Larsson *et al.*, 1991). This has led to the hypothesis that there is only a single amino-N pool in both shoot and roots and that it is this combined pool which regulates N uptake (Cooper and Clarkson, 1989). In this investigation the quantity of amino-N in the xylem sap was 15 and 48% of the total N in the xylem sap of wheat and maize respectively. The actual proportion of amino-N in the xylem sap appears to be variable (barley 13%, Lewis *et al.*, 1982a; wheat 26%, Lewis *et al.*, 1987; maize 35%, Murphy and Lewis 1987; all plants supplied 2 mM NO_3^-). Extensive re-cycling of amino-N implies that the translocation of large quantities of amino-C in the xylem does not necessarily indicate that this C is lost from the root exclusively. The fact that NH_4^+ is predominantly assimilated within the root while NO_3^- is predominantly assimilated within the shoot implies that most of the amino compounds in the xylem sap of NH_4^+-fed plants must, however, be derived from the root while most of the amino compounds in the xylem sap of NO_3^--fed plants must be derived from the shoot.

The xylem sap amino-N data supports the finding that ^{14}C transport via the xylem sap was enhanced in NH_4^+- compared to NO_3^--fed plants (Section 4.7.2). These data indicate that the

enhanced translocation of amino compounds from the root to shoot may be responsible for reduced C availability within the roots of NH_4^+-fed plants and thus for reduced root extension in NH_4^+- compared to NO_3^--fed wheat plants.

In summary, it has been shown that:

1) NO_3^- assimilation is predominantly shoot based and that NH_4^+ assimilation occurs in the root of both wheat and maize.
2) More amino-C is translocated in the xylem of NH_4^+- compared to NO_3^--fed wheat and maize plants.
3) In spite of the more rapid absorption of NH_4^+ than of NO_3^- by wheat, the NO_3^--fed plant translocates more N in the xylem than the NH_4^+-fed plant. Similar observations were made by Lewis *et al.* (1982).
4) In spite of the more rapid absorption of NH_4^+ than of NO_3^- by maize, the NH_4^+-fed plant translocates only slightly more N in the xylem than the NO_3^--fed plant. Similar observations were made by Murphy and Lewis (1987).

4.9 ROOT-GAS EXCHANGE CHARACTERISTICS

It has already been shown that plants grown on NH_4^+ nutrition translocate more C in the form of amino-C from the root to the shoot. The possibility exists, however, that in addition to the differences in C translocation from root to shoot, there is a larger loss of C from the roots of plants supplied with either form of N into the root environment in the form of CO_2. Loss of C from the roots as CO_2 may be derived from root respiratory activities or other metabolic processes. Since no experimental distinction between CO_2 from respiratory or other sources was made, the loss of CO_2 from the root is dealt with as respiration which is the common practice in the literature.

4.9.1 Root oxygen consumption and carbon dioxide release

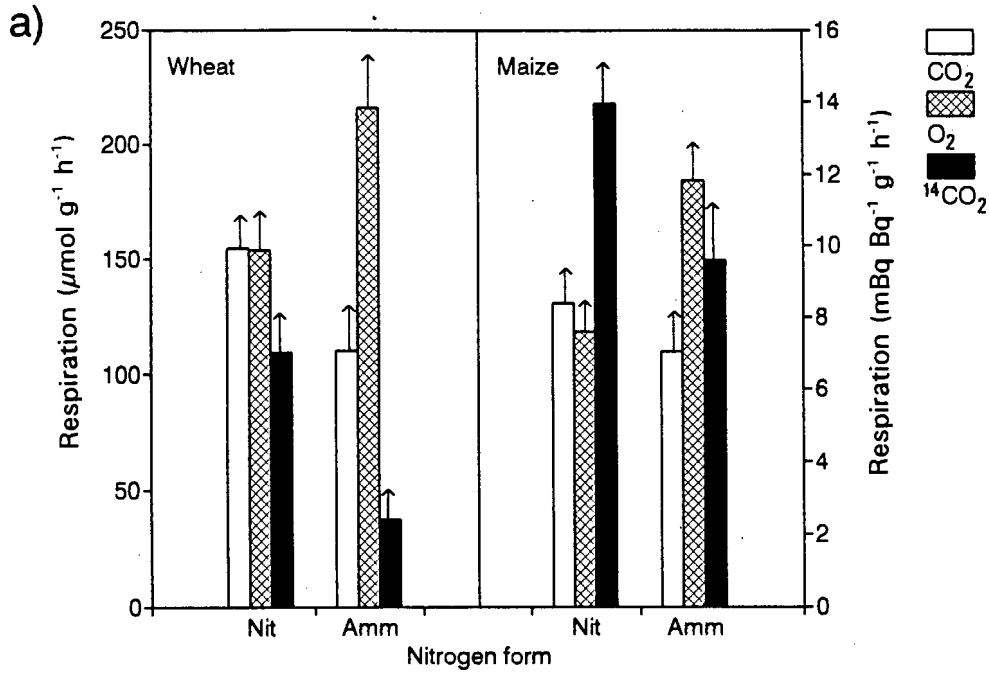
Increased N nutrition has been reported to result in increased respiratory activity of the roots (De Visser *et al.*, 1986). Assimilation of NH_4^+ within the root may require the expenditure of respiratory energy and C for the assimilation of this N into amino acids via the GS-GOGAT pathway. This energy and C expenditure may be hypothesized to be larger for root based NH_4^+ assimilation than NO_3^- reduction and assimilation which is primarily foliar in

wheat and maize. An additional factor which needs to be considered here is the larger flux of NH_4^+ than of NO_3^- through the root due to the higher uptake rates of the former.

Root respiration may be measured as either CO_2 efflux or O_2 influx. Use of CO_2, or $^{14}CO_2$ efflux from plants supplied with $^{14}CO_2$ 24 h prior to measurement, provides an indication of the amount of C released from respiratory processes, predominantly the TCA cycle. The use of CO_2 as a measure of root respiration is, however, problematical because there is a possibility that the root may release HCO_3^- in exchange for NO_3^- (Ben Zioni *et al.*, 1971), thereby obscuring the true respiration rate. An additional problem with the use of CO_2 as an indicator of root respiration is that there is a possibility that PEPc in the root may re-fix a proportion of the respired CO_2 (Section 4.10). The loss of CO_2 is, however, important because it represents a drain on the carbohydrate resources of the root and may thus compete with root extension. Use of respiratory O_2 consumption as a measure of respiration avoids these problems and reflects the activity of the respiratory electron transport pathway through both the cyanide sensitive and cyanide insensitive ('alternative pathway') pathways (Lance *et al.*, 1985). For our purposes it was not important to discriminate between these two pathways.

Wheat grown with NO_3^- (Figure 4.60a) showed a 1.4-fold higher CO_2 efflux rate than equivalent NH_4^+-fed plants. The rates of $^{14}CO_2$ efflux from NO_3^--fed wheat plants were 2.9-fold greater than those from NH_4^+-fed wheat plants. Oxygen consumption was, however, 1.4-fold higher in NH_4^+- than in NO_3^--fed wheat roots, indicating that the rate of respiratory electron transport of NH_4^+-fed plants was higher than that of NO_3^--fed plants. Release rates of CO_2 in NO_3^-- and NH_4^+-fed maize roots were comparable while $^{14}CO_2$ release rates were significantly higher (1.5-fold) in NO_3^-- than in NH_4^+-fed plants (Figure 4.60a). Oxygen consumption, however, was significantly greater (1.6-fold) in NH_4^+- than in NO_3^--fed maize roots.

The changes in O_2 and CO_2 flux have as a consequence changes in the gas exchange quotient (GEQ) which is a similar expression to the more usual RQ, but emphasizes the measurement of net CO_2 efflux and net O_2 influx using gas exchange methods. The GEQ's of NO_3^--fed wheat and maize plants were comparable, as were the GEQ's of NH_4^+-fed wheat and maize (Figure 4.60b) and thus the differences between NO_3^-- and NH_4^+-fed plant GEQ's were consistent in both wheat and maize.



	Wheat	Maize
CO_2	p<0.05	NS
O_2	p<0.05	p<0.05
$^{14}CO_2$	p<0.05	p<0.05

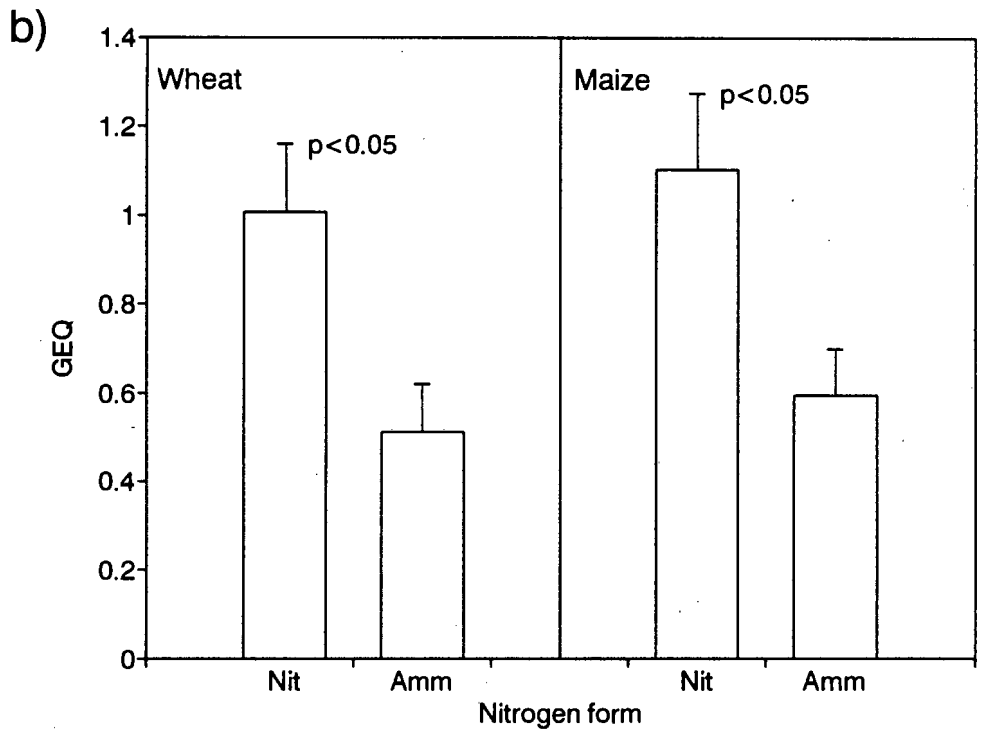


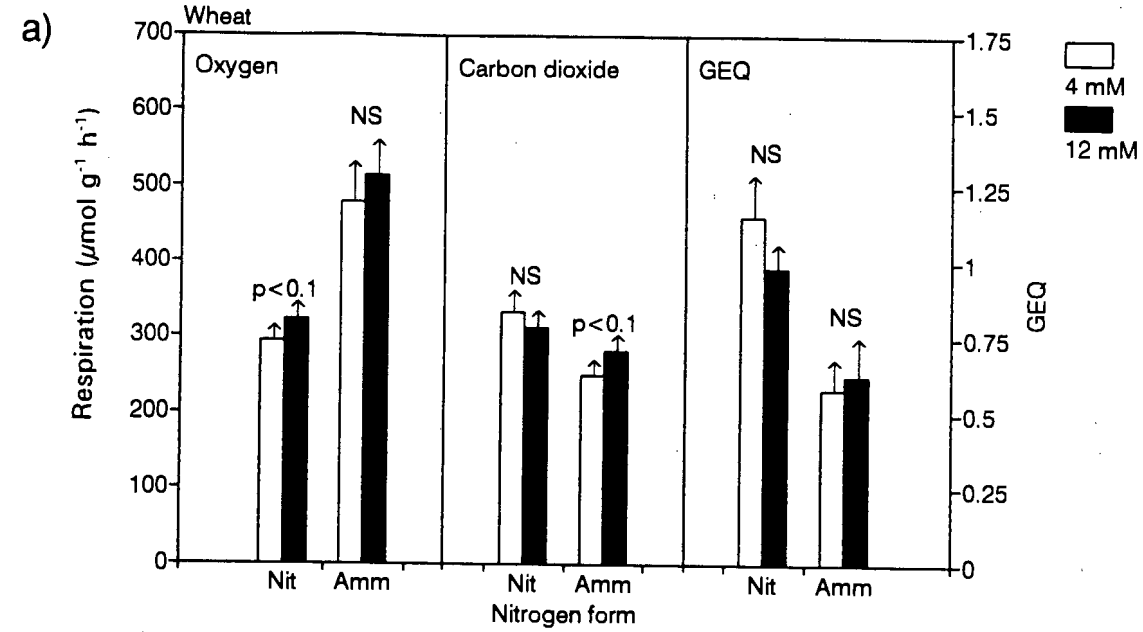
Figure 4.60. a) Gaseous exchange of roots with respect to CO_2 (μmol g^{-1} dry mass h^{-1}) and $^{14}CO_2$ release (Bq Bq^{-1} g^{-1} h^{-1}) and O_2 consumption (μmol g^{-1} dry mass h^{-1}); b) gas exchange quotient (GEQ, mol CO_2 mol^{-1} O_2) of 4 mM NO_3^- (Nit) or NH_4^+-grown (Amm) wheat and maize plants. Bars indicate the S.E. Results of Student's T tests for differences between NO_3^- and NH_4^+-fed plants are shown in table below graph. (CO_2 and $^{14}CO_2$, n=6; O_2, n=8)

The release of $^{14}CO_2$ is expressed on the basis of the amount of ^{14}C measured per unit mass of root material and, therefore, is independent of the absolute amount of ^{14}C within the root. Root $^{14}CO_2$ release is derived from $^{14}CO_2$ assimilated in the leaves by photosynthesis. This $^{14}CO_2$ is likely to be more extensively located in the mobile pools of carbohydrate than steady state C and thus serves as an indicator of the dependence of root respiration on recently acquired C. This explains the larger differences observed between NO_3^-- and NH_4^+-fed plants with respect to $^{14}CO_2$ release than with respect to CO_2 release. The 2.0- and 4.0-fold higher rates of $^{14}CO_2$ release from NO_3^-- and NH_4^+-fed plants respectively in maize compared to wheat are consistent with the observation that the ^{14}C shoot : root ratios of wheat were 1.7-fold higher than those of maize in both NO_3^-- and NH_4^+-fed plants (Section 4.7.1.8).

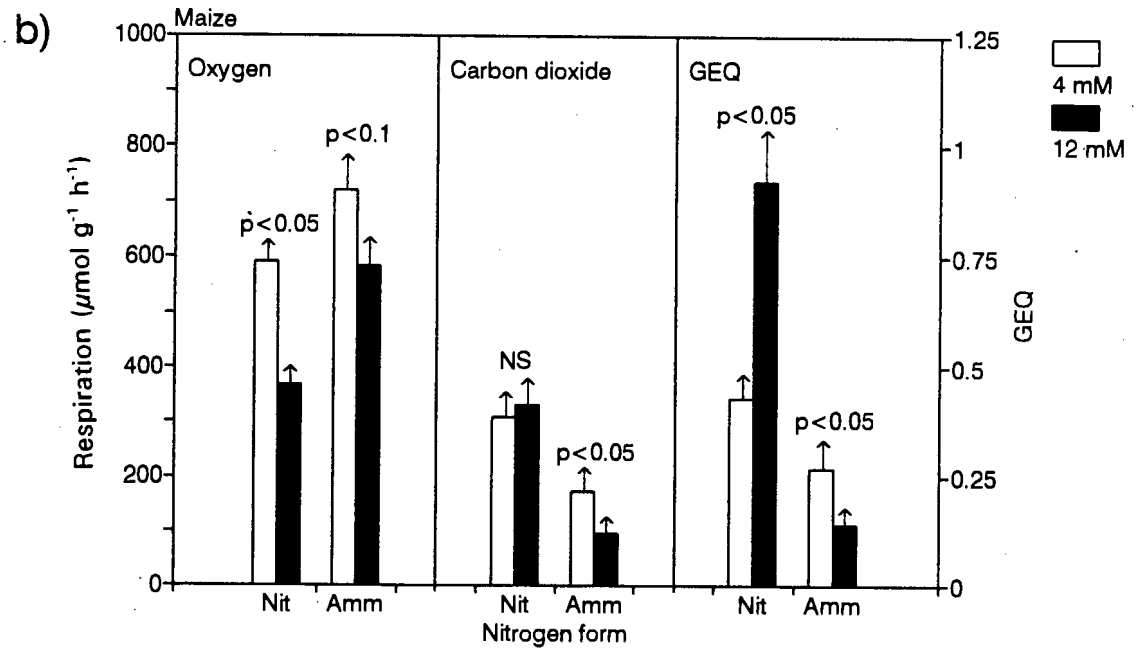
4.9.2 Split-root oxygen consumption and carbon dioxide release

Oxygen uptake by NO_3^-- and NH_4^+-fed wheat root-halves did not change significantly between 4 and 12 mM N (Figure 4.61a). There was, however, a significantly higher O_2 consumption by NH_4^+- compared to NO_3^--fed root-halves. Although differences in CO_2 release between the 12 mM NO_3^-- and NH_4^+-fed root-halves were significant, the differences at both 4 and 12 mM N were relatively small. The GEQ's of NH_4^+-fed root-halves were considerably lower than those of NO_3^--fed root-halves. From this data it appears that the respiration rate, measured as O_2 consumption, of NH_4^+-fed root-halves was higher than that of NO_3^--fed root-halves. The lack of a large difference between CO_2 release rates of NO_3^-- and NH_4^+-fed root-halves should be viewed with the differences in O_2 consumption in mind. The consumption of O_2 by respiration is mechanistically linked to the respiratory production of CO_2. Thus, unless there is an alteration in the substrate utilized for respiration, there must be some process apart from respiration (glycolysis, TCA cycle and respiratory electron transport) responsible for the differences between O_2 and CO_2 flux.

In maize plants (Figure 4.61b) the differences between 4 and 12 mM N feeding were clearer than in wheat with a reduced rate of O_2 evolution at the higher N concentration. As in the case of wheat, there were significantly higher O_2 consumption rates in NH_4^+- compared to NO_3^--fed root-halves. The CO_2 release rates were significantly reduced in NH_4^+- compared to NO_3^--fed root-halves. These results have as a consequence significantly lower GEQ's in NH_4^+-fed root-halves.



N concentration (mM)	Oxygen	Carbon dioxide	GEQ
4	p<0.05	p<0.05	p<0.05
12	p<0.05	p<0.1	p<0.05



N concentration (mM)	Oxygen	Carbon dioxide	GEQ
4	p<0.05	p<0.05	p<0.05
12	p<0.05	p<0.05	p<0.05

Figure 4.61. Split-root a) wheat and b) maize O_2 uptake (μmol g^{-1} dry mass h^{-1}), CO_2 (μmol g^{-1} dry mass h^{-1}) release and gas exchange quotients (GEQ, mol CO_2 mol^{-1} O_2) of 4 or 12 mM NO_3^- (Nit) or NH_4^+-fed (Amm) root halves. Results of Student's T test comparisons of 4 and 12 mM N plants are shown above S.E. bars (NS=not significant at 90% confidence interval). Results of Student's T tests for differences between NO_3^- and NH_4^+-fed root halves (both 4 and 12 mM) are shown in tables below graphs. (n=6)

4.9.3 Discussion

In wheat 52 to 67% of carbohydrates translocated to the root are respired while in maize the corresponding figures are 44 to 57% (Lambers *et al.*, 1991). Thus root respiration may be appreciated to be an important component of the C budget of the plant. A positive correlation has been shown to exist between shoot : root ratios and root respiratory activity (g O_2 g^{-1} dry mass) and it has been proposed that this may result from the availability of carbohydrate to the root and demands on the root for ion uptake (Lambers *et al.*, 1991). The fact that the shoot : root ratios of NH_4^+-fed wheat plants were greater than those of NO_3^--fed plants may, therefore, be expected to result in a higher respiratory rate in NH_4^+-fed plants. This is consistent with the observed respiration rates for wheat, but in maize, where the shoot : root ratios were not altered by the different N forms, comparable patterns of root respiration were observed to those of wheat.

One of the important components of root respiration is the provision of ATP for ion uptake which has been termed salt respiration. It has been shown that in both wheat and maize the rates of NH_4^+ uptake exceed those of NO_3^- uptake (Section 4.8.1). Ignoring the events after entry of the ions into the plant, the costs of NH_4^+ uptake may, therefore, be expected to be higher. The evidence for the existence of 2 H^+:1 NO_3^- symporter for NO_3^- uptake and a 1 H^+: 1 NH_4^+ antiporter for NH_4^+ uptake has been reviewed (Section 2.5). If the H^+ used in these mechanisms is pumped out across the plasmalemma through the functioning of an H^+ translocating ATPase then the costs of NO_3^- absorption should be higher than those of NH_4^+ absorption per mole N taken up.

It is, however, by no means certain that an H^+ translocating ATPase is obligatorily involved in NO_3^- uptake (Deane-Drummond, 1984c) and it may be that NO_3^- is exchanged for OH^- derived from decarboxylation of organic acids (Ben Zioni *et al.*, 1971) or that NO_3^- is exchanged for OH^- derived from the reduction of NO_3^- within the root (Raven and Smith, 1976). The uptake of NO_3^- has, however, been found not to be obligatorily linked to NO_3^- reduction (Jackson *et al.*, 1986) and NO_3^- reduction has been shown to occur only to a limited extent in the roots of wheat and maize (Section 4.8.4). Furthermore the energy required for NO_3^- uptake depends on the exogenous and endogenous concentrations of NO_3^- (Glass, 1988) and influx/efflux of NO_3^- from the root has been indicated to vary with exogenous and endogenous NO_3^- concentrations (Deane-Drummond, 1984b; Teyker *et al.*, 1988; Larsson and

Oscarson, 1990). It is thus difficult to assess the importance of NO_3^- uptake *per se* as an energy cost to the root and thus the importance of this process in determining root respiration.

The H^+ exchanged for NH_4^+ across the root plasmalemma may be derived from the assimilation of NH_4^+ within the root (Raven and Smith, 1976). The fact that NH_4^+ uptake is at least partially dependent on metabolism (Glass, 1988; Kashyap and Singh, 1985) is not contrary to this proposal. The uptake of NH_4^+ appears to be the net result of influx and efflux (Morgan and Jackson, 1988; 1989; MacKlon *et al.*, 1990) possibly controlled by variations in energy supply from the shoots. The absorption of NH_4^+ is generally increased with pH which may be partially related to the increased NH_3 concentration at high pH (Reisenauer, 1978), the uptake of which is likely to be passive (Glass, 1988). Thus the energy costs of NH_4^+ uptake depend on the exogenous and endogenous NH_4^+ concentrations and the exogenous pH.

One mechanism favoured by many authors to explain the effects of NH_4^+ on metabolism is the uncoupling phosphorylation. Uncoupling of respiratory phosphorylation would result in more rapid oxidation of respiratory substrates and consequently more rapid O_2 consumption. The evidence for the role of uncoupling by NH_4^+ in intact tissues is, however, weak. Although uncoupling could explain the enhanced O_2 consumption of NH_4^+- compared to NO_3^--fed plants, it would not explain the lower CO_2 release from NH_4^+-fed plants.

The rate of root respiration (O_2 consumption) has been found to depend strongly on the site of NO_3^- reduction within the plant, which is not surprising considering that NO_3^- reduction consumes 81% of the energy required for protein synthesis (Johnson, 1990). Evidence has been presented for the assimilation of NO_3^- predominantly in the leaves and the assimilation of NH_4^+ in the roots (Section 4.8.4). The fact that NH_4^+ assimilation occurs within the root means that the requirements for ATP, reductant and C must be met by root resources. It is by no means clear that these requirements would necessarily increase root respiration with respect to respiratory electron transport because the requirements for reductant and C may be seen as processes competing for respiratory substrate. The assimilation of NH_4^+ utilizes OG produced within the TCA cycle resulting in increased activity of the TCA cycle, especially between OAA and OG (Guy *et al.*, 1989). Increased TCA cycle activity may increase the levels of reductant which may account for enhanced O_2 consumption by NH_4^+-fed roots.

With glucose and NH_4^+ as precursors the synthesis of amides (glutamine and asparagine) would produce NADH which may be oxidized via either the cytochrome or 'alternative pathway' when ATP and NADH requirements are low (De Visser and Lambers, 1983). This may explain the increased rate of O_2 consumption found in this study with plants grown on NH_4^+ as compared to NO_3^- nutrition. Several authors have found that NH_4^+ compared to NO_3^- stimulates the rate of root respiratory O_2 consumption (Barneix *et al.*, 1984b; Blacquière, 1987) and have attributed this to stimulation of 'alternative pathway' activity which has been found to account for 40% of root respiration in NH_4^+-grown plants (Barneix *et al.*, 1984b).

Although NO_3^- is predominantly assimilated in the shoots, assimilation of NO_3^- within the roots does occur to some extent. Results presented here show that 15% and 48% of N in the xylem sap of wheat and maize respectively was in the reduced form possibly indicating that root reduction does occur (Section 4.8.4). The actual extent of root reduction is, however, difficult to assess because of the significant recycling of N which is known to occur. The reduction of NO_3^- to NH_4^+ is an extremely energetic process requiring the utilization of the equivalent of 4 NAD(P)H. The further incorporation of NH_4^+ produced by NO_3^- reduction would require 1 ATP and 2 Fd_{rd} (equivalent to 1 NAD(P)H.) The utilization of reductant may imply that NO_3^- acts as an alternative oxidant for respiratory reductant thereby decreasing respiratory O_2 consumption as has been proposed by other authors (Naik and Nicholas, 1981; Hocking *et al.*, 1984b; Weger and Turpin, 1989). Naik and Nicholas (1984) provided evidence for the involvement of mitochondrial NADH sources, in the form of the TCA cycle intermediates, and anaerobic pyruvate metabolism in NO_3^- reduction in the root of wheat. The reduction of NO_2^- derived from NO_3^- also depends on the carbohydrate resources of the root for reductant through operation of the OPPP (Dry *et al.*, 1981; Oji *et al.*, 1985; Bowsher *et al.*, 1989). Respiratory O_2 consumption in plants supplied with NO_3^- nutrition has been shown to be almost exclusively via the cytochrome pathway (Barneix *et al.*, 1984b). One of the proposed functions of the 'alternative pathway' is to provide an 'energy overflow' mechanism oxidising reductant in excess of the capacity of the cytochrome pathway. With NO_3^- nutrition NADH is not likely to be in excess of the capacity of the cytochrome pathway (Barneix *et al.*, 1984b).

The GEQ results presented in this investigation show that the release of CO_2 relative to O_2 consumption was consistently higher in NO_3^-- than NH_4^+-fed plants. On the basis of theoretical calculations Blacquière (1987) predicted that the RQ of NO_3^--fed plants would be

higher than that of NH_4^+-fed plants. Yemm and Willis (1956) found with excised *Hordeum vulgare* roots that respiration rates measured as CO_2 release were lower in roots supplied with NH_4^+ than those supplied with NO_3^- and similar results have been observed in wheat (Lips *et al.*, 1990). The linkage between respiratory CO_2 and O_2 flux is usually close with the RQ being fairly typical for tissue dependent on particular respiratory substrates. The differences between NO_3^-- and NH_4^+-fed plants with respect to GEQ may be partially the result of NO_3^- functioning as an oxidant for respiratory reductant, thereby reducing the consumption of O_2. The fact that NH_4^+ assimilation actually enhances O_2 consumption as indicated by De Visser and Lambers (1983) without the requirement for respiratory CO_2 release may also contribute to lower GEQ values in NH_4^+-fed plants.

The proposed uptake of NO_3^- in exchange OH^-, possibly derived from malate decarboxylation (Ben Zioni *et al.*, 1971), also has implications for the respiratory GEQ values. High rates of CO_2 release by roots of NO_3^-- compared to NH_4^+-fed plants have been shown to be the result of exchange of HCO_3^- for NO_3^- which may represent up to 20% of respiratory C loss (Barneix *et al.*, 1984b). Malate translocated from the shoot to the root for participation in NO_3^- uptake as proposed by Ben Zioni *et al.* (1971) may represent an additional source of C to NO_3^--fed plant roots. Thus the effect of loss of HCO_3^- in exchange for NO_3^- uptake on root carbohydrate resources may be minimized through dependence of this process on C derived from the shoot. Another possibility is that respiratory CO_2 loss is reduced within NH_4^+- compared to NO_3^--fed root as a result of the dark assimilation of HCO_3^- by PEPc (Section 4.10).

The greater loss CO_2 from NO_3^-- than from NH_4^+-fed plant roots does not support the proposal that reduced root extension in NH_4^+-fed plants, particularly in wheat, is the consequence of reduced availability of C within the root for root growth. The differences between CO_2 loss from NO_3^-- and NH_4^+- fed plant roots were, however, small relative to the differences between NO_3^-- and NH_4^+-fed plants with respect to the amount of C translocated in the xylem. In wheat the difference between the rates of respiratory CO_2 losses in NO_3^-- and NH_4^+-fed plants was 44.4 μmol g^{-1} h^{-1} while in maize the corresponding figure was 21.0 μmol g^{-1} h^{-1}. In wheat the difference between the rate of C translocation in the xylem of NO_3^-- and NH_4^+-fed plants was 92.2 μmol g^{-1} h^{-1} while in maize the corresponding figure was 806.3 μmol g^{-1} h^{-1}. The rates of respiratory CO_2 loss and xylem C translocation are, however, not

directly comparable because of the existence of extensive recycling of amino-N within the plants.

In summary:

1) Root respiratory O_2 consumption was enhanced in NH_4^+- compared to NO_3^--fed plants as a result of the fact that NH_4^+ is assimilated predominantly within the root. The mechanism of NH_4^+ enhancement was possibly through: a) demands for ATP and C skeletons, b) lack of NO_3^- reduction within the root or c) the possible role of NO_3^- as an oxidant for respiratory reductant.
2) Root respiratory CO_2 release was higher in NO_3^-- compared to NH_4^+-fed plants, possibly as the result of: a) NO_3^- uptake in exchange for HCO_3^- and/or b) higher dark fixation of HCO_3^- in NH_4^+- than in NO_3^--fed plants.

4.10 ROOT $H^{14}CO_3^-$ UPTAKE

Dark fixation of HCO_3^- by roots may occur as a consequence of the presence of the enzyme PEPc in root tissue. In order to determine whether the activity of this enzyme could be responsible for the changes measured in GEQ (Section 4.9.1), roots were exposed to nutrient solutions containing $H^{14}CO_3^-$ and the amount of ^{14}C label present in the acidified 80% (v/v) ethanol soluble fraction determined after a one hour labelling period. Measurement of accumulated label does not necessarily reflect the absolute activity of the PEPc enzyme, because it is likely that a proportion of the ^{14}C label would have been respired.

4.10.1 Wheat response

Analysis of variance (Appendix 7.1.5) showed that the uptake of $H^{14}CO_3^-$ was significantly enhanced (up to 4-fold) in NH_4^+- compared to NO_3^--fed plants (Figure 4.62). Elevated $H^{14}CO_3^-$ uptake in NH_4^+- compared to NO_3^--fed plants was accompanied by increased ^{14}C shoot : root ratios of NH_4^+-fed plants over those of NO_3^--fed plants (Figure 4.63). Thus NH_4^+-fed plants accomplished a much greater transfer of ^{14}C labelled assimilate to the shoot.

This corresponds with the measured increase in amino-N concentration and ^{14}C concentration in xylem sap of NH_4^+- compared to NO_3^--fed plants (Section 4.7.2 and 4.8.4). Thus it appears that PEPc may be responsible for the assimilation of some HCO_3^- in the root which

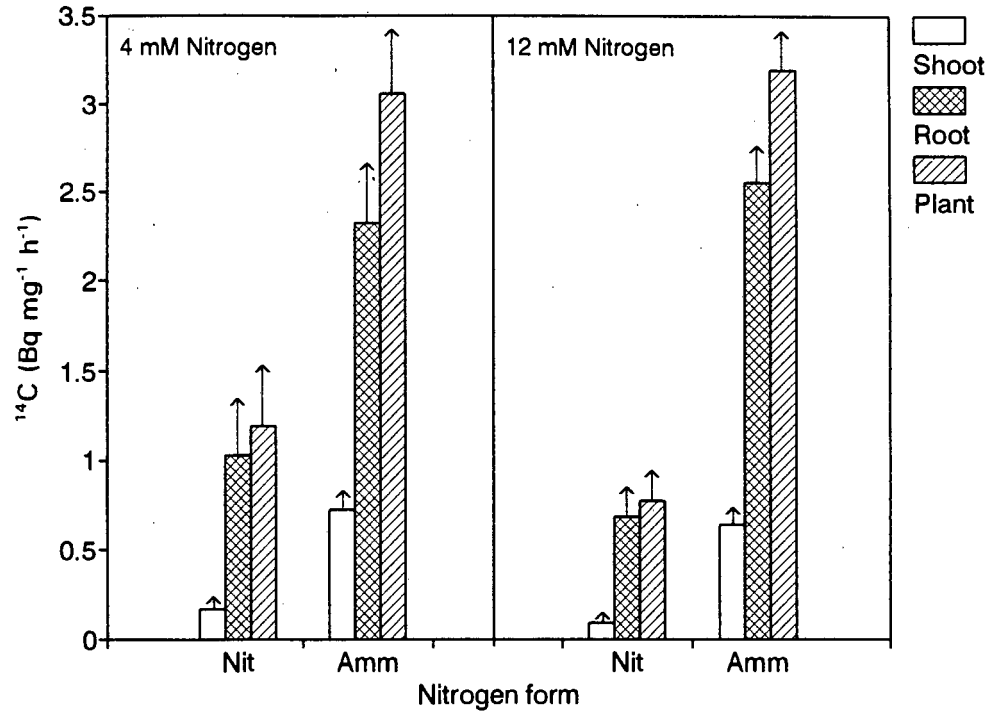


Figure 4.62. Root uptake and subsequent partitioning of $NaH^{14}CO_3$ (Bq mg^{-1} fresh mass h^{-1}) by 4 or 12 mM NO_3^- (Nit) or NH_4^+-grown (Amm) wheat plants. Bars indicate the S.E. The calculated rates of HCO_3^- uptake by the plants in units of μmol HCO_3^- g^{-1} root dry mass h^{-1} were: 4 mM N, NO_3^--fed plants 4.83 ± 1.18, NH_4^+-fed plants 12.39 ± 1.28; 12 mM N, NO_3^--fed plants 3.09 ± 0.48, NH_4^+-fed plants 12.81 ± 0.70. (n=4)

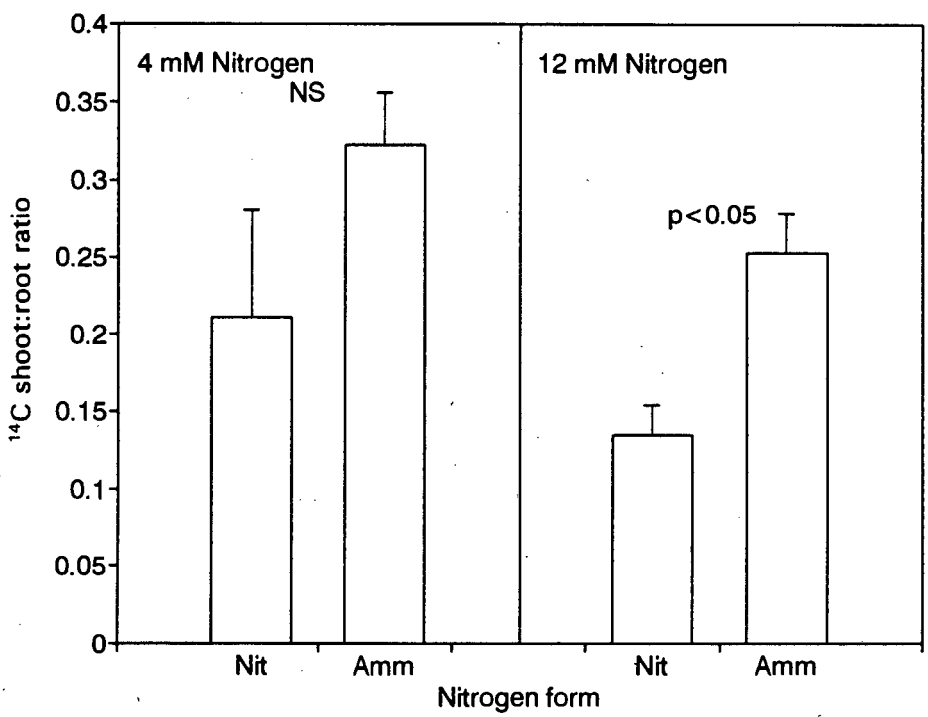


Figure 4.63. ^{14}C Shoot : root ratios of wheat plants resulting from $NaH^{14}CO_3$ uptake by roots grown on 4 or 12 mM NO_3^- (Nit) or NH_4^+ (Amm). Results of Student's T test comparisons of NO_3^-- and NH_4^+-fed plants are shown above S.E. bars (NS=not significant at 90% confidence interval). (n=4)

may provide an anaplerotic source of C for the synthesis of amino acids by the root in addition to C supplied from the shoot. The absolute amount of HCO_3^- calculated to be involved is small in comparison with overall root respiration rates (Section 4.9.1). This may be partially the result of the underestimation of $H^{14}CO_3^-$ assimilation by PEPc, because of the possibility of respiratory release of a proportion of the assimilated $^{14}CO_2$.

4.10.2 Maize response

The results for maize were comparable to those for wheat plants (Figure 4.64). There was a highly significant (approximately 10-fold) larger accumulation of $H^{14}CO_3^-$ by NH_4^+- compared to NO_3^--fed plants in maize (Appendix 7.1.5). This was accompanied by higher ^{14}C shoot : root ratios in NH_4^+- compared to NO_3^--fed plants (Figure 4.65). The ^{14}C shoot : root ratios of the maize plants were, however, almost double those of the wheat plants. This indicates the high capacity of the maize plant for translocating the assimilated label from the roots to the shoots as demonstrated by the high amino compound concentrations in the xylem sap of the maize plants (Section 4.8.4).

4.10.3 Discussion

The enzyme responsible for dark fixation of HCO_3^- in the root is probably PEPc and the higher uptake of HCO_3^- by NH_4^+-fed roots compared to NO_3^--fed roots may be attributed to the enhanced functioning of PEPc in NH_4^+-fed roots. Roots of *Phaseolus vulgaris* fed NO_3^- showed no increase in PEPc activity while PEPc activity responded positively to NH_4^+ nutrition (Schweizer and Erismann, 1985). Similar results were obtained with several species including wheat and maize where PEPc activity of the roots was up to 380% higher in NH_4^+- than in NO_3^--fed plants (Arnozis *et al.*, 1988). PEPc could function in an anaplerotic role for the provision of C to the TCA cycle (Arnozis *et al.*, 1988). No quantitative assessment of the importance of PEPc as an anaplerotic source of C in the roots seems to exist as yet in the literature.

PEPc, in combination with malic enzyme, may function as a pH stat although this is unlikely to be effective in countering the production of H^+ from NH_4^+ assimilation in the long term (Smith and Raven, 1979). It has also been suggested that PEPc in the root has a role in supplying malate and other organic acids for translocation in the xylem sap to the shoot

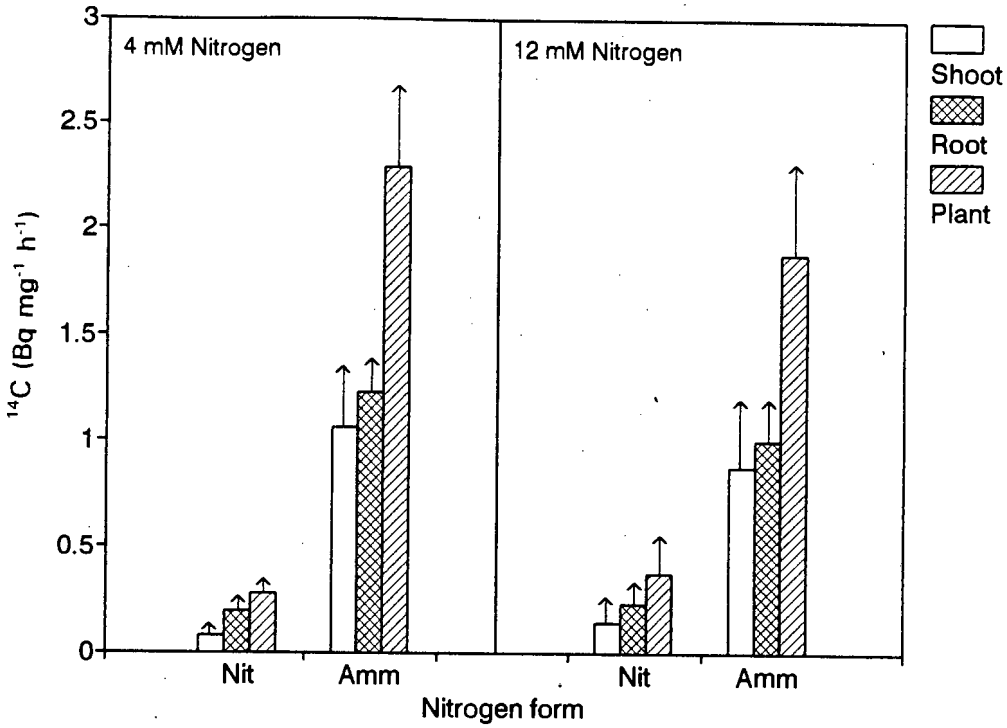


Figure 4.64. Root uptake and subsequent partitioning of $NaH^{14}CO_3$ (Bq mg^{-1} fresh mass h^{-1}) by 4 or 12 mM NO_3^- (Nit) or NH_4^+-grown (Amm) maize plants. Bars indicate the S.E. The calculated rates of HCO_3^- uptake by the plants in units of μmol HCO_3^- g^{-1} root dry mass h^{-1} were: 4 mM N, NO_3^--fed plants 1.33 ± 0.16, NH_4^+-fed plants 10.41 ± 1.6; 12 mM N, NO_3^--fed plants 2.04 ± 0.77, NH_4^+-fed plants 9.4 ± 1.99. (n=4)

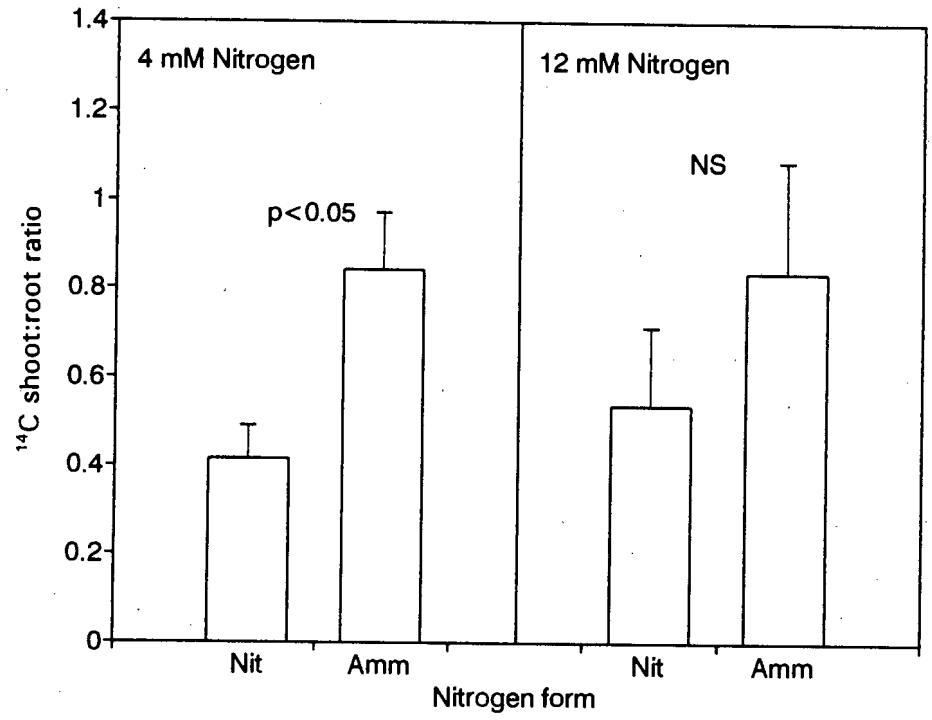


Figure 4.65. ^{14}C Shoot : root ratios in maize plants resulting from $NaH^{14}CO_3$ uptake by roots grown on 4 or 12 mM NO_3^- (Nit) or NH_4^+ (Amm). Results of Student's T test comparisons of NO_3^-- and NH_4^+-fed plants are shown above S.E. bars (NS=not significant at 90% confidence interval). (n=4)

(Arnozis *et al.*, 1988). The anaplerotic role of PEPc is postulated to occur through the OAA derived from PEPc being metabolised to OG which may be utilized for NH_4^+ assimilation. In photosynthetic cells there is a considerable amount of evidence for the role of PEPc in anaplerotic provision of C for amino acid synthesis (Guy *et al.*, 1989; Vanlerberghe *et al.*, 1990; Amory *et al.*, 1991).

In the experiments reported here the net incorporation of ^{14}C into 80% ethanol soluble acid stable products was measured. The net incorporation may be expected to be lower than the rate of PEP carboxylation with $H^{14}CO_3^-$ by PEPc due to release of $^{14}CO_2$ through respiration. Incorporation of $H^{14}CO_3^-$ via PEPc results in OAA specifically labelled in the ß carboxyl group, i.e. the fourth C atom (Edwards and Walker, 1983). Oxaloacetate processed through the TCA cycle would result in ^{14}C labelled OG since CO_2 loss from citrate during formation of OG occurs from C originally derived from PEP. Thus only if all the OG derived from anaplerotic $H^{14}CO_3^-$ assimilation by PEPc is transaminated by GS-GOGAT will the amount of ^{14}C label in the plant be representative of ^{14}C diverted into amino acid synthesis alone. Since it is unlikely that all the OG will be transaminated, release of $^{14}CO_2$ from the roots will occur.

The rates of net HCO_3^- (μmol g^{-1} dry mass h^{-1}) uptake by wheat roots were 3.1 and 5.7% of respiratory O_2 consumption in NO_3^-- and NH_4^+-fed wheat respectively. The corresponding figures for maize were 1.1 and 5.6% in NO_3^- and NH_4^+-fed plants respectively. Thus dark HCO_3^- fixation by PEPc accounts for a small but significant proportion of root respiration. In wheat differences between NO_3^-- and NH_4^+-fed plants with respect to rates of HCO_3^- assimilation could only account for about 17.0% of the differences in respiratory CO_2 evolution while in maize HCO_3^- assimilation could account for 43.3% of the differences. This does not, however, take into account the fact that the rate of respiratory O_2 consumption was higher in NH_4^+- than in NO_3^--fed plants, and thus overestimates the importance of HCO_3^- assimilation. Thus the differences between NO_3^-- and NH_4^+-fed plants with respect to root HCO_3^- uptake may partially explain the differences with respect to respiratory CO_2 release from roots, but other mechanisms must also be functioning. According to Barneix *et al.* (1984b), 20% of respiratory CO_2 release of wheat is utilized for exchange with NO_3^- and this is sufficient, taking into account the activity of root PEPc, to account for the differences observed with respect to respiratory CO_2 release between NO_3^-- and NH_4^+-fed plants.

The ^{14}C shoot : root ratios of wheat and maize supplied with $H^{14}CO_3^-$ to the root were significantly higher in NH_4^+- than NO_3^--fed plants. This indicates that the products of dark fixation of $H^{14}CO_3^-$ were translocated to the shoot with higher efficiency in NH_4^+- than in NO_3^--fed plants. The reason for this is probably related to the root based assimilation of NH_4^+ in comparison to the shoot based assimilation of NO_3^-. Anaplerotic C derived from PEPc would be incorporated into amino compounds within the roots of NH_4^+-fed plants and translocated to the shoots. In plants using NO_3^- as a N source the smaller degree of amino compound synthesis within the roots would limit the amount of ^{14}C translocated to the shoots.

5 CONCLUSIONS

The main hypotheses examined in this investigation were that:

1) Differences between the shoot : root ratios of NO_3^-- and NH_4^+-fed wheat plants were due to the root based assimilation of NH_4^+ and the shoot based assimilation of NO_3^-.
2) Assimilation of NH_4^+ within the root diverts carbohydrate from root extension into the production of amino compounds, thereby competing with root growth.
3) The lack of influence of the form of nutrient N on the shoot : root ratios of maize plants was due to the larger supply of C available from the C_4 photosynthetic mechanism.

This investigation has shown that:

1) Biomass accumulation of wheat and maize plants was lower with NH_4^+ than with NO_3^- nutrition.
2) The shoot : root ratios of wheat plants were increased with NH_4^+ compared to NO_3^- nutrition, while the shoot : root ratios of maize were unaffected by the N source.

Under the experimental conditions employed, it was thus confirmed that there were differences in the response of wheat and maize to the form of nutrient N supplied and that the shoot : root ratios of wheat were sensitive to the form of N supplied. In split-root experiments, however, the shoot : root ratios of the root-half supplied with NH_4^+ were higher in both wheat and maize than in the root-halves supplied with NO_3^-. This indicates that maize roots are sensitive to the form of N supplied and it may be suggested that limited allocation of C to the NH_4^+-fed root-half resulted in competition between NH_4^+ assimilation and root growth.

The following conclusions have been reached regarding the results presented:

5.1) Shoot moisture contents of wheat and maize were significantly lower in NH_4^+- than in NO_3^--fed plants. This was not a consequence of faster transpirational water loss (μmol m^{-2} s^{-1}) from NH_4^+- than from NO_3^--fed plants. The differences must therefore have resulted from reduced water uptake by the roots. Although transpirational water

loss, measured gravimetrically and expressed on the basis of root mass, was lower in NH_4^+- than in NO_3^--fed plants, this difference was not statistically significant.

5.2) Differences between NO_3^-- and NH_4^+-fed plant biomasses were apparent soon after introduction of the N into the root medium of both wheat and maize, and these differences may be expected to be compounded during growth.

5.3) Photosynthetic rates expressed on the basis of leaf area of 4 mM N-fed wheat were unaffected by the form of N supplied. However, the photosynthetic rates of 12 mM NH_4^+-fed wheat plants were only 85% of those of 12 mM NO_3^--fed wheat plants. In maize supplied with 4 and 12 mM NH_4^+ the photosynthetic rates were 87 and 82% respectively of those of NO_3^--fed plants. The reduced photosynthetic rates could partially account for reduced biomass accumulation in the NH_4^+-fed plants.

5.4) Although differences in photosynthetic rates with different N forms may be attributable to factors related to metabolism, there was a correlation between photosynthetic rates, stomatal conductances and transpiration rates suggesting that the mechanism responsible for reduced photosynthesis was reduced stomatal conductance. The lower stomatal conductances of NH_4^+- compared to NO_3^--fed plants may be the consequence of reduced shoot moisture contents in the NH_4^+-fed plants. The role of different N forms in controlling plant moisture contents and consequently stomatal conductance has received scant attention in the literature.

5.5) In wheat the specific leaf areas ($cm^2\ g^{-1}$) of 4 mM NH_4^+-fed plants were lower (approximately 10%) than those of 4 mM NO_3^--fed plants. As a consequence the photosynthetic rates of wheat expressed on a dry mass basis, were significantly lower in 4 mM NH_4^+- than in 4 mM NO_3^--fed plants. In maize the specific leaf areas were only slightly greater in 4 mM NH_4^+- than in 4 mM NO_3^--fed plants.

5.6) The rates of uptake of NH_4^+ were higher than the rates of NO_3^- uptake in both wheat (1.5-fold) and maize (1.3-fold). This implies that the NH_4^+-fed plants would have to divert more C resources into the provision of C skeletons for the assimilation of N into amino compounds than in the case of the NO_3^--fed plants (See paragraph 5.8).

5.7) Changes in ^{14}C partitioning induced by the different forms of N were generally small. Changes observed in ^{14}C partitioning represent differences current at the time of harvesting. It is likely that the large differences in biomass accumulation are the consequence of small differences in partitioning which exist throughout the growing period of the plant and which are compounded by growth.

5.8) Relative to NO_3^-, NH_4^+ nutrition in both wheat and maize increased the allocation of ^{14}C to protein and amino acid components, particularly in the roots. In 12 mM N-fed wheat the allocation of ^{14}C to protein and amino acid fractions was 8.4 and 14% higher in shoots and roots respectively of NH_4^+- compared to NO_3^--fed plants. The corresponding figures for 4 mM N-fed maize were 5.5 and 6.7% for shoots and roots respectively and for 12 mM N-fed maize were 7.7 and 11.2% for shoots and roots respectively. The diversion of ^{14}C into protein and amino acid fractions may have two causes: a) NH_4^+ taken up is detoxified through assimilation to prevent accumulation of NH_4^+ thus favouring synthesis of amino compounds, b) NH_4^+ is taken up more rapidly than NO_3^- and therefore more amino-N is formed.

5.9) Allocation of ^{14}C to structural material was 6.0% lower in wheat and 3.6% lower in maize roots supplied with 12 mM NH_4^+ compared to 12 mM NO_3^--fed roots. The allocation of ^{14}C to structural material within the shoots of wheat and maize plants was little changed by the form of N supplied, although there were changes in the allocation to other carbohydrate fractions. The changes in allocation of ^{14}C were associated with ^{14}C : ^{14}C-N ratios which were decreased in NH_4^+- compared to NO_3^--fed plants. The ^{14}C : ^{14}C-N ratios were 1.5- and 2.0-fold greater in shoots and roots respectively of 12 mM NO_3^-- compared to 12 mM NH_4^+-fed wheat. In both 4 and 12 mM NO_3^--fed maize the ^{14}C : ^{14}C-N ratios were approximately 1.7- and 2.0-fold greater in shoots and roots respectively than in plants supplied with 4 or 12 mM NH_4^+. The allocation of ^{14}C to the amino compound containing fractions at the expense of the carbohydrate fractions in the root is proposed to be responsible for reduced root growth in wheat plants. The lack of response of maize shoot : root ratios to N form may be the result of smaller differences in the allocation of C to the structural fraction.

5.10) The maize split-root experiment showed that the C available to the NH_4^+-fed root-halves from the shoots was probably insufficient to meet the demands of root growth

and NH_4^+ assimilation without severe competition between the two processes. There were large differences in the allocation of ^{14}C to structural material between the root-halves supplied with either NO_3^- or NH_4^+. This was correlated with a smaller dry masses of the root-halves supplied with NH_4^+ than the halves supplied with NO_3^- nutrition.

5.11) In both wheat and maize, NH_4^+ nutrition resulted in the allocation of a higher proportion of N to the soluble amino-N fraction than did NO_3^- nutrition. Insoluble amino-N contents were only slightly higher in NH_4^+- than in NO_3^--fed plants. In wheat supplied with $^{15}NH_4^+$, up to 40% of the total ^{15}N in the roots was in the form of $^{15}NH_4^+$ while in maize only 18% was in the form of $^{15}NH_4^+$. This may reflect the inability of wheat to detoxify large quantities of NH_4^+ taken up.

5.12) The xylem sap of NH_4^+-fed plants was enriched with amino compounds in comparison to that of NO_3^--fed plants. In particular the concentrations of glutamine and asparagine in wheat and asparagine and alanine in maize were increased by NH_4^+ nutrition. The quantity of NH_4^+ translocated in the xylem sap was small, but the amount of NO_3^- translocated by NO_3^--fed plants was large (wheat, 23.9 $\pm$ 2.4 mM; maize, 34.1 $\pm$ 3.3 mM). These data indicate that the site of nutrient NH_4^+ assimilation is the root, while the predominant site of NO_3^- assimilation is the shoot. The larger quantities of amino compounds in the xylem sap of NH_4^+- compared to NO_3^--fed plants resulted in faster rates of translocation of C in NH_4^+- compared to NO_3^--fed wheat and maize plants. In wheat the difference between the rate of C translocation in the xylem of NO_3^-- and NH_4^+-fed plants was 92.2 μmol g^{-1} h^{-1} while in maize the corresponding figure was 806.3 μmol g^{-1} h^{-1}. Although cycling of amino-N could have accounted for a substantial proportion of carbon translocated within the xylem, it is proposed that the loss of carbon from the roots through translocation of amino compounds from the root to the shoot was the main reason for reduced root extension in wheat plants supplied with NH_4^+ compared to those supplied with NO_3^- nutrition.

5.13) The xylem sap of NH_4^+-fed plants contained more ^{14}C derived from photosynthetically assimilated $^{14}CO_2$ than the xylem sap of NO_3^--fed plants. In wheat and maize respectively 1.4- and 1.2-fold more ^{14}C was translocated in the xylem sap from the root to the shoot of NH_4^+- compared to NO_3^--fed plants. This confirms that the

translocation of amino compounds from the root to shoot in NH_4^+-fed plants results in a larger loss of C from the roots than is the case in NO_3^--fed plants (See paragraph 5.12).

5.14) The shoot : root ratios of ^{14}C found in wheat and maize 24 h after supply of $^{14}CO_2$ to photosynthesizing leaves were lower in NH_4^+- than in NO_3^--fed plants. This may be attributable to many factors and probably contributed to the higher ^{14}C contents of xylem sap from plants supplied with NH_4^+ compared to NO_3^- nutrition.

5.15) Root respiratory O_2 consumption was enhanced in NH_4^+- as compared to NO_3^--fed wheat and maize plants 1.4- and 1.6-fold respectively. The factors contributing to NH_4^+ enhancement of root respiratory O_2 consumption may be: a) demands for ATP and C skeletons for NH_4^+ assimilation, b) lack of large scale NO_3^- reduction within the root, c) the possible role of NO_3^- as an oxidant for respiratory reductant and d) higher rates of NH_4^+ than of NO_3^- uptake. Thus NH_4^+ uptake and assimilation is more expensive than NO_3^- uptake and assimilation in terms of energy costs to the root.

5.16) Root respiratory CO_2 release was 1.4-fold higher in NO_3^-- than in NH_4^+-fed wheat plants, and 1.2-fold higher in NO_3^-- than in NH_4^+-fed maize plants. This negates the possibility that increased respiratory activity of NH_4^+-fed plants in comparison to NO_3^--fed plants could result in reduced biomass accumulation in the roots of the former. The factors possibly contributing to lower CO_2 release from NH_4^+- than from NO_3^--fed plants are: a) NO_3^- uptake in exchange for HCO_3^-, b) higher dark fixation of HCO_3^- in NH_4^+- than in NO_3^--fed plants. From these results NO_3^- uptake and assimilation is more expensive than NH_4^+ uptake and assimilation in terms of C costs to the root. In wheat the difference between the rates of respiratory CO_2 losses in NO_3^-- and NH_4^+-fed plants was 44.4 μmol g^{-1} h^{-1} while in maize the corresponding figure was 21.0 μmol g^{-1} h^{-1}. These differences are small in comparison to the differences between NO_3^-- and NH_4^+-fed plants with respect to C translocation in the xylem sap (See paragraph 5.12).

5.17) The GEQ's of wheat and maize supplied with NH_4^+ nutrition were approximately half those of NO_3^--fed plants. Although root respiration has received much attention in the literature, few authors have measured respiratory CO_2 and O_2 flux simultaneously.

5.18) Dark fixation of $H^{14}CO_3^-$ by roots of wheat and maize was 4- and 10-fold greater respectively in NH_4^+- than in NO_3^--fed plants. This may partially, but not completely, explain the differences in terms of respiratory CO_2 release from roots. In wheat differences between NO_3^-- and NH_4^+-fed plants with respect to rates of HCO_3^- assimilation could only account for 17.0% of the differences in respiratory CO_2 evolution while in maize HCO_3^- assimilation could account for 43.3% of the differences. The fact that NH_4^+-fed roots do assimilate HCO_3^- more rapidly than NO_3^--fed roots would result in reduced enrichment of ^{14}C labelled xylem sap of NH_4^+-fed plants and the underestimation of the amount of C allocated to amino compound fractions using ^{14}C techniques. The pathways thought to be responsible for mediating the gas-exchange responses of the root to NO_3^- and NH_4^+ nutrition are presented in Figure 5.1.

The results presented here have shown that the reason for reduced biomass accumulation in NH_4^+- compared to NO_3^--fed wheat and maize plants is partially the result of lower photosynthetic rates of the NH_4^+-fed plants and appeared to be related to the water status of the plant. The large differences between NO_3^- and NH_4^+-fed of wheat and maize plants after 21 and 10 days respectively appeared to be the result of small differences in physiology compounded during growth. The more rapid uptake of NH_4^+ compared to NO_3^-, and the root based assimilation of nutrient NH_4^+, diverts C from root growth into amino compound synthesis which results in increased levels of amino compounds in the xylem sap. The reason that the roots are more sensitive to the form of N than the shoots of wheat is that the translocation of amino compounds to the shoot represents a major loss of C from the root. This has the effect of relocating root carbon to the shoot, thereby limiting root growth and maintaining shoot growth.

The following major physiological differences between wheat and maize may be of importance in determining the response of these plants to the form of N supplied:

1) Photosynthetic rates of maize were higher than those of wheat.
2) Translocation of NH_4^+ and amino compounds to the shoot was more extensive in maize than in wheat.
3) Accumulation of $^{15}NH_4^+$ was observed to occur in wheat roots, but not maize roots.

4) Wheat plants have higher total N contents than maize plants and the differences between the rates of NH_4^+ and NO_3^- uptake were larger in wheat than in maize.

These observations may be interpreted as implying that in maize compared to wheat the larger supply of photosynthetic C may have masked the effects of NH_4^+ nutrition on root growth. The larger availability of carbon may have prevented the accumulation of NH_4^+ in the roots of maize and account for the much larger rates of translocation of amino compounds in the xylem sap of maize compared to wheat plants.

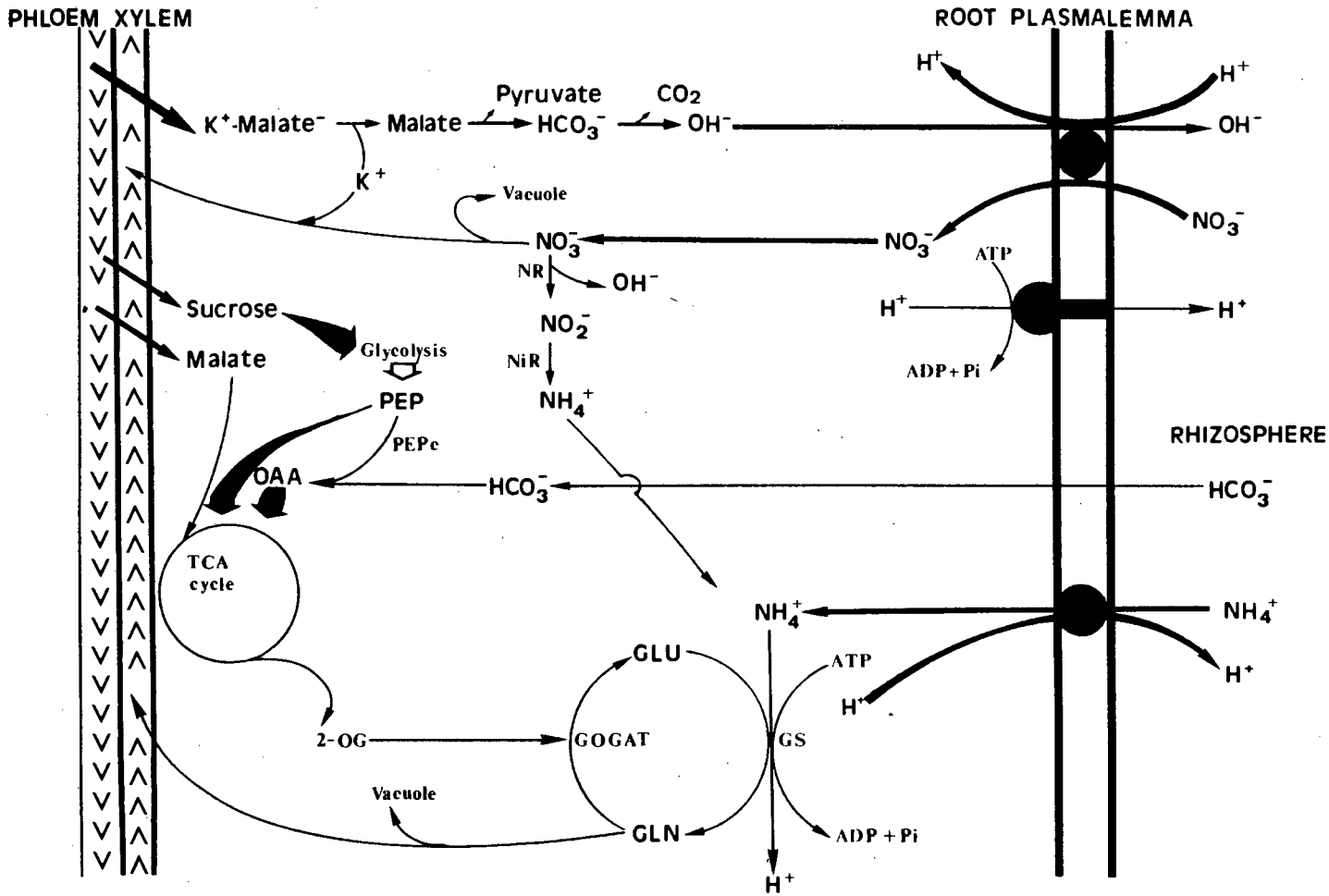


Figure 5.1 The interaction of NO_3^- and NH_4^+ uptake and assimilation with aspects of root metabolism responsible for determining the gas exchange characteristics. Cycling of K^+ between the shoot and root and decarboxylation of malate for production of OH^- exchanged for NO_3^- across the plasmalemma after Ben Zioni *et al.* (1971); anaplerotic functioning of PEPc for provision of C for NH_4^+ assimilation after Turpin *et al.* (1990). NO_3^- uptake is shown as occurring through a transporter functioning as an antiport with OH^- or as symport with H^+; NH_4^+ uptake is shown as occurring through a transporter functioning as an antiport for H^+. Possibility that ATPase functions for the generation of $\Delta\mu_{H+}$ across plasmalemma for facilitation of NO_3^- and NH_4^+ uptake after Glass (1988).

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7 APPENDICES

7.1 STATISTICAL ANALYSES

In the following tables results from statistical tests performed as described in the Methods and Materials section are presented. For each test the F ratio, probability value that the null hypothesis may be rejected (p-value) and degrees of freedom (d.f.) are presented. In cases where the F ratio >1000 this is given as 1000. Where $p<0.0001$ this is specified as $p=0.000$.

Where appropriate the results of multiple range tests using Tukey's test at the 95% confidence interval are shown. The results of the multiple range tests are shown by the positioning of the symbol "*". If the symbols are above one another, there was no significant difference between the corresponding treatments listed below the symbols. If the symbols are displaced relative to one another there was a significant difference between the corresponding treatments.

The following principles were adhered to for the display of the results of analysis of variance on graphs:

1) Significant differences between N concentrations (4 and 12 mM) indicated by the presence of primed letters in the 12 mM results.
2) Different N concentrations were tested separately and thus use of the same letters on both 4 and 12 mM graphs does not imply any statistical information.
3) Different plant components (shoot, root, plant) and different measures (e.g. CO_2 assimilation and transpiration rates) were tested separately. Use of the same letters for these separate tests therefore does not imply any statistical information.

7.1.1 Analysis of variance of wheat and maize dry mass, shoot : root ratio and moisture content data

Both 3-way and 2-way analysis of variance was performed on the dry masses (shoot, root and plant), shoot : root ratios (S:R) and moisture contents of wheat (Table 7.1) and maize (Table 7.2) plants. The plants were grown with either 4 or 12 mM NO_3^-, NH_4NO_3 or NH_4^+. The

factors in the 3-way analysis of variance were the form and concentration of N and the effect of the position of the plants on the bench within the phytotron. The 3-way analysis of variance was performed to test the significance of the N concentration as a treatment. Tukey multiple range tests were performed after the 3-way analysis of variance. Results of the multiple range tests indicate the presence or absence of significant differences between N treatments. Results of these tests are displayed on the corresponding graphs.

The factors in the 2-way analysis of variance were N form and bench position. The 2-way analysis of variance tested the effects of NO_3^- (N), NH_4NO_3 (AN) and NH_4^+ (A) on the biomass and moisture content data. Tukey multiple range tests were performed after the 2-way analysis of variance and indicate the presence or absence of significant differences between N treatments. Results of these tests are displayed on the corresponding graphs.

7.1.2 Analysis of variance of wheat and maize gas exchange data

Both 3-way and 2-way analysis of variance was performed on the gas exchange data of wheat (Table 7.3) and maize (Table 7.4) plants. The plants were grown with either 4 or 12 mM NO_3^-, NH_4NO_3 or NH_4^+. The data tested were the CO_2 assimilation rates (A), transpiration rates (E), stomatal conductances to H_2O (Gs), intercellular CO_2 concentrations (C_i) and water use efficiencies (WUE). The factors in the 3-way analysis of variance were the form and concentration of N and the effect of the position of the plants on the bench within the phytotron. The 3-way analysis of variance was performed to test the significance of the N concentration as a treatment.

The factors in the 2-way analysis of variance were N form and bench position. The 2-way analysis of variance tested the effects NO_3^- (N), NH_4NO_3 (AN) and NH_4^+ (A) on the gas exchange data. Results of these tests are displayed on the corresponding graphs.

7.1.3 Covariance analysis of wheat and maize fresh and dry mass, leaf area, shoot : root ratio, specific leaf area and moisture content data

Covariance analysis of the data for fresh masses, dry masses, leaf areas (L. area), shoot : root ratios (S:R), specific leaf areas (SLA) and moisture contents of wheat plants grown with 4 mM NO_3^- or 4 mM NH_4^+ for a 4 week period (Table 7.5) and of maize plants

grown with 4 mM NO_3^- or 4 mM NH_4^+ for period of 13 days (Table 7.6). The covariate was time and the factor the form of N used.

7.1.4 Covariance analysis of wheat and maize gas exchange data

Covariance analysis of gas exchange data of wheat plants grown with 4 mM NO_3^- or 4 mM NH_4^+ for a 4 week period (Table 7.7) and of maize plants grown with 4 mM NO_3^- or 4 mM NH_4^+ for period of 13 days (Table 7.8). The data tested were the CO_2 assimilation rates (maize, A expressed on the basis of leaf area; wheat, A (area) expressed on the basis of leaf area or A (mass) expressed on the basis of leaf mass), transpiration rates (E), stomatal conductances to H_2O (Gs), intercellular CO_2 concentrations (C_i) and water use efficiencies (WUE). The covariate was time and the factor the form of N used.

7.1.5 Analysis of variance for data concerning $NaH^{14}CO_3$ uptake by wheat and maize roots

Data for the shoot, root and whole plant contents of $NaH^{14}CO_3$ and ^{14}C shoot : root ratios resulting from the root uptake of $NaH^{14}CO_3$ were subjected to 2-way analysis of variance. The plants (both wheat and maize) were grown with either 4 or 12 mM NO_3^- or NH_4^+ (Table 7.9). The factors in the analysis of variance were the concentrations and form of N.

Table 7.1 Analysis of variance of wheat dry mass, shoot : root ratio and moisture content data.

	Dry mass				Moisture content	
	Shoot	Root	Plant	S:R	Shoot	Root
3 - Way ANOVA						
F ratio						
MAIN EFFECTS	18.374	20.175	18.816	17.488	19.635	3.544
N form	36.153	40.151	37.325	31.356	34.507	5.557
N concentration	0.360	0.216	0.330	0.060	1.050	0.549
Bench	0.005	0.074	0.019	0.086	3.294	0.059
2-FACTOR INTERACTIONS	0.935	1.459	1.086	1.660	2.048	0.657
N form X N concentration	0.037	0.158	0.011	1.218	2.389	0.449
N form X Bench	1.578	1.909	1.762	1.567	2.687	0.106
N concentration X Bench	0.476	0.106	0.370	2.833	0.383	2.464
p - value						
MAIN EFFECTS	0.000	0.000	0.000	0.000	0.000	0.009
N form	0.000	0.000	0.000	0.000	0.000	0.005
N concentration	0.556	0.648	0.573	0.809	0.308	0.468
Bench	0.942	0.789	0.891	0.772	0.072	0.812
2-FACTOR INTERACTIONS	0.461	0.209	0.372	0.150	0.077	0.657
N form X N concentration	0.963	0.854	0.989	0.299	0.096	0.639
N form X Bench	0.211	0.153	0.176	0.213	0.072	0.899
N concentration X Bench	0.499	0.749	0.550	0.095	0.544	0.119
d.f.	127	127	127	127	127	127
Multiple range test	*	*	*	*	*	*
	*	*	*	*	*	*
	*	*	*	*	*	*
N form	A	A	A	N	A	N
	N	N	N	AN	AN	A
	AN	AN	AN	A	N	AN
2 - Way ANOVA						
4 mM Nitrogen						
F ratio						
MAIN EFFECTS	9.865	14.778	10.773	22.799	8.627	2.134
N form	12.646	16.589	13.191	26.693	5.313	3.135
Bench	0.168	0.037	0.063	1.920	2.639	0.769
Interaction	2.405	3.156	2.750	1.381	0.413	0.075
p - value						
MAIN EFFECTS	0.000	0.000	0.000	0.000	0.000	0.106
N form	0.000	0.000	0.000	0.000	0.008	0.051
Bench	0.688	0.851	0.806	0.171	0.110	0.393
Interaction	0.126	0.081	0.103	0.245	0.530	0.788
d.f.	63	63	63	63	63	63
Multiple range test	*	*	*	*	*	*
	*	*	*	*	*	*
	*	*	*	*	*	*
N form	A	A	A	N	A	A
	N	AN	N	AN	AN	N
	AN	N	AN	A	N	AN
12 mM Nitrogen						
F ratio						
MAIN EFFECTS	16.297	14.745	16.135	8.695	23.427	3.144
N form	24.360	21.598	24.026	12.418	33.559	4.512
Bench	1.599	2.548	1.924	3.865	5.845	0.002
Interaction	0.698	1.035	0.826	0.311	4.996	5.746
p - value						
MAIN EFFECTS	0.000	0.000	0.000	0.000	0.000	0.032
N form	0.000	0.000	0.000	0.000	0.000	0.015
Bench	0.211	0.116	0.171	0.054	0.019	0.966
Interaction	0.416	0.313	0.377	0.585	0.029	0.020
d.f.	63	63	63	63	63	63
Multiple range test	*	*	*	*	*	*
	*	*	*	**	*	**
	*	*	*	*	*	*
N form	A	A	A	N	A	N
	N	N	N	AN	N	A
	AN	AN	AN	A	AN	AN

Table 7.2 Analysis of variance of maize dry mass, shoot : root ratio and moisture content data.

	Dry mass				Moisture content	
	Shoot	Root	Plant	S:R	Shoot	Root
3 - Way ANOVA						
F ratio						
MAIN EFFECTS	20.422	22.760	22.499	9.436	25.899	19.671
N form	35.133	38.369	38.603	15.644	45.681	32.489
N concentration	5.628	12.135	8.254	6.098	11.808	13.567
Bench	8.577	3.540	6.895	1.038	0.084	0.038
2-FACTOR INTERACTIONS	7.481	4.073	6.337	5.890	9.621	9.134
N form X N concentration	2.359	1.712	2.071	7.985	22.342	19.145
N form X Bench	12.525	7.867	11.175	1.391	2.568	2.633
N concentration X Bench	10.265	3.017	7.564	11.138	0.702	2.177
p - value						
MAIN EFFECTS	0.000	0.000	0.000	0.000	0.000	0.000
N form	0.000	0.000	0.000	0.000	0.000	0.000
N concentration	0.019	0.001	0.005	0.015	0.001	0.000
Bench	0.004	0.062	0.010	0.310	0.775	0.847
2-FACTOR INTERACTIONS	0.000	0.002	0.000	0.000	0.000	0.000
N form X N concentration	0.098	0.184	0.130	0.001	0.000	0.000
N form X Bench	0.000	0.001	0.000	0.253	0.081	0.076
N concentration X Bench	0.002	0.085	0.007	0.001	0.413	0.142
d.f.	143	143	143	143	143	143
Multiple range test	*	*	*	*	*	*
	*	*	*	*	*	*
	*	*	*	*	*	*
N form	A	A	A	A	A	A
	N	N	N	N	N	N
	AN	AN	AN	AN	AN	AN
2 - Way ANOVA						
4 mM Nitrogen						
F ratio						
MAIN EFFECTS	19.964	10.841	17.088	4.632	7.359	2.534
N form	26.868	16.125	24.052	3.217	8.063	3.643
Bench	21.773	5.214	15.047	11.474	1.790	0.067
Interaction	29.872	11.327	22.828	10.353	0.504	1.827
p - value						
MAIN EFFECTS	0.000	0.000	0.000	0.005	0.000	0.064
N form	0.000	0.000	0.000	0.046	0.001	0.032
Bench	0.000	0.026	0.000	0.001	0.186	0.799
Interaction	0.000	0.000	0.000	0.000	0.606	0.169
d.f.	71	71	71	71	71	71
Multiple range test	*	*	*	*	*	*
	*	*	*	*	**	*
	*	*	*	*	*	*
N form	A	A	A	A	A	A
	AN	N	AN	AN	N	N
	N	AN	N	N	AN	AN
12 mM Nitrogen						
F ratio						
MAIN EFFECTS	17.989	15.572	18.823	17.473	19.418	25.488
N form	25.761	22.548	26.884	25.171	29.075	38.163
Bench	0.033	0.146	0.031	0.097	3.286	2.295
Interaction	0.636	0.692	0.657	0.680	2.129	2.070
p - value						
MAIN EFFECTS	0.000	0.000	0.000	0.000	0.000	0.000
N form	0.000	0.000	0.000	0.000	0.000	0.000
Bench	0.857	0.708	0.863	0.760	0.074	0.135
Interaction	0.532	0.504	0.522	0.510	0.127	0.134
d.f.	71	71	71	71	71	71
Multiple range test	*	*	*	*	*	*
	*	*	*	*	*	*
	*	*	*	*	*	*
N form	A	A	A	A	A	A
	N	N	N	N	N	N
	AN	AN	AN	AN	AN	AN

Table 7.3 Analysis of variance of wheat gas exchange data.

	A	E	Gs	Ci	WUE
			3 - Way ANOVA		
F ratio					
MAIN EFFECTS	6.279	2.787	14.718	3.305	1.074
N form	6.780	1.311	10.139	0.491	1.518
N concentration	7.819	6.917	27.531	9.297	0.250
Bench	4.387	0.209	6.674	0.192	1.393
2-FACTOR INTERACTIONS	2.213	1.687	5.465	3.831	0.069
N form X N concentration	4.547	1.851	4.499	5.956	0.006
N form X Bench	0.700	0.662	5.550	3.762	0.002
N concentration X Bench	1.401	2.559	6.502	1.880	0.199
p - value					
MAIN EFFECTS	0.001	0.045	0.000	0.024	0.364
N form	0.011	0.255	0.002	0.493	0.221
N concentration	0.006	0.010	0.000	0.003	0.623
Bench	0.039	0.654	0.011	0.667	0.241
2-FACTOR INTERACTIONS	0.092	0.175	0.002	0.012	0.976
N form X N concentration	0.036	0.177	0.037	0.017	0.942
N form X Bench	0.414	0.427	0.021	0.055	0.967
N concentration X Bench	0.240	0.113	0.012	0.174	0.661
d.f.	100	100	100	100	100
			2 - Way ANOVA		
			4 mM Nitrogen		
F ratio					
MAIN EFFECTS	0.272	0.382	5.660	0.864	0.768
N form	0.112	0.030	0.444	1.336	0.518
Bench	0.433	0.733	10.876	0.392	1.018
Interaction	4.417	0.673	0.004	0.401	0.200
p - value					
MAIN EFFECTS	0.763	0.685	0.006	0.428	0.470
N form	0.743	0.864	0.516	0.254	0.483
Bench	0.521	0.406	0.002	0.541	0.318
Interaction	0.041	0.425	0.953	0.537	0.662
d.f.	49	49	49	49	49
			12 mM Nitrogen		
F ratio					
MAIN EFFECTS	9.737	2.562	9.683	3.746	0.806
N form	12.917	3.005	19.367	5.568	1.197
Bench	6.129	2.002	0.011	1.774	0.382
Interaction	11.331	3.613	14.766	4.917	0.433
p - value					
MAIN EFFECTS	0.000	0.088	0.000	0.031	0.453
N form	0.001	0.090	0.000	0.023	0.280
Bench	0.017	0.164	0.917	0.189	0.546
Interaction	0.002	0.064	0.000	0.032	0.521
d.f.	50	50	50	50	50

Table 7.4 Analysis of variance of maize gas exchange data.

	A	E	Gs	Ci	WUE
			3 - Way ANOVA		
F ratio					
MAIN EFFECTS	4.891	3.992	7.012	2.663	1.172
N form	12.230	6.546	13.780	3.393	1.423
N concentration	1.577	0.270	5.468	2.256	0.837
Bench	2.732	1.575	0.593	0.536	0.496
2-FACTOR INTERACTIONS	2.893	1.731	0.928	0.497	0.956
N form X N concentration	0.942	3.554	0.060	0.453	0.528
N form X Bench	8.178	0.641	2.409	0.024	0.850
N concentration X Bench	4.868	3.905	1.401	0.271	0.186
p - value					
MAIN EFFECTS	0.005	0.013	0.001	0.061	0.330
N form	0.001	0.014	0.001	0.073	0.239
N concentration	0.215	0.611	0.024	0.141	0.374
Bench	0.105	0.216	0.453	0.476	0.492
2-FACTOR INTERACTIONS	0.045	0.173	0.434	0.686	0.421
N form X N concentration	0.347	0.065	0.811	0.512	0.479
N form X Bench	0.006	0.436	0.127	0.878	0.371
N concentration X Bench	0.032	0.054	0.242	0.611	0.673
d.f.	55	55	55	55	55
			2 - Way ANOVA		
			4 mM Nitrogen		
F ratio					
MAIN EFFECTS	2.657	7.796	4.877	0.673	2.195
N form	5.314	14.104	8.299	0.481	4.101
Bench	2.116	1.914	0.721	0.116	0.705
Interaction	0.135	0.027	0.000	0.009	0.035
p - value					
MAIN EFFECTS	0.089	0.002	0.016	0.521	0.132
N form	0.029	0.001	0.008	0.504	0.053
Bench	0.158	0.178	0.413	0.741	0.418
Interaction	0.720	0.872	0.997	0.928	0.854
d.f.	29	29	29	29	29
			12 mM Nitrogen		
F ratio					
MAIN EFFECTS	3.391	1.693	2.956	2.749	0.496
N form	6.173	1.201	5.817	4.449	0.153
Bench	0.382	2.390	0.023	1.486	0.882
Interaction	6.396	0.518	1.496	0.024	0.689
p - value					
MAIN EFFECTS	0.052	0.207	0.073	0.091	0.616
N form	0.021	0.285	0.025	0.049	0.704
Bench	0.550	0.136	0.883	0.239	0.368
Interaction	0.019	0.487	0.234	0.879	0.424
d.f.	25	25	25	25	25

Table 7.5 Covariance analysis of wheat fresh and dry mass, leaf area, shoot : root ratio, specific leaf area and moisture content data.

	Fresh mass			Dry mass		
	Shoot	Root	Plant	Shoot	Root	Plant
F ratio						
Covariate - Time	489.817	395.13	502.114	502.897	493.754	529.685
Factor - N form	0.373	32.07	6.918	0.158	21.97	0.49
p - value						
Covariate - Time	0.000	0.000	0.000	0.000	0.000	0.000
Factor - N form	0.548	0.000	0.009	0.696	0.000	0.492
d.f.	237	237	237	237	237	237
		Dry mass		Moisture content		
	L. area	S:R	SLA	Shoot	Root	Plant
F ratio						
Covariate - Time	483.663	278.486	1.543	13.074	47.267	3.734
Factor - N form	0.634	88.369	6.607	33.325	9.325	74.083
p - value						
Covariate - Time	0.000	0.000	0.215	0.000	0.000	0.055
Factor - N form	0.435	0.000	0.011	0.000	0.003	0.000
d.f.	237	237	237	237	237	237

Table 7.6 Covariance analysis of maize fresh and dry mass, leaf area, shoot:root ratio, specific leaf area and moisture content data.

	Fresh mass			Dry mass		
	Shoot	Root	Plant	Shoot	Root	Plant
F ratio						
Covariate - Time	NA	NA	NA	NA	NA	NA
Factor - N form	NA	NA	NA	NA	NA	NA
p - value						
Covariate - Time	NA	NA	NA	NA	NA	NA
Factor - N form	NA	NA	NA	NA	NA	NA
d.f.	118	118	118	118	118	118
		Dry mass		Moisture content		
	L. area	S:R	SLA	Shoot	Root	Plant
F ratio						
Covariate - Time	NA	NA	NA	NA	NA	NA
Factor - N form	NA	NA	NA	NA	NA	NA
p - value						
Covariate - Time	NA	NA	NA	NA	NA	NA
Factor - N form	NA	NA	NA	NA	NA	NA
d.f.	118	118	118	118	118	118

Table 7.7 Covariance analysis of wheat gas exchange data.

	A (area)	A (mass)	E	Gs	Ci	WUE
F ratio						
Covariate - Time	53.379	3.83	91.091	3.805	180.164	198.963
Factor - N form	0.166	8.474	10.054	7.762	12.211	6.321
p - value						
Covariate - Time	0.000	0.068	0.000	0.053	0.000	0.000
Factor - N form	0.688	0.004	0.002	0.006	0.001	0.013
d.f.	190	190	190	190	190	190

Table 7.8 Covariance analysis of maize gas exchange data.

	A	E	Gs	Ci	WUE
F ratio					
Covariate - Time	1.245	3.121	1.962	0.082	0.386
Factor - N form	6.238	10.644	8.902	0.086	0.004
p - value					
Covariate - Time	0.267	0.080	0.164	0.779	0.543
Factor - N form	0.014	0.002	0.004	0.773	0.953
d.f.	104	104	104	104	104

Table 7.9 Analysis of variance for data concerning $NaH^{14}CO_3$ uptake by wheat and maize roots.

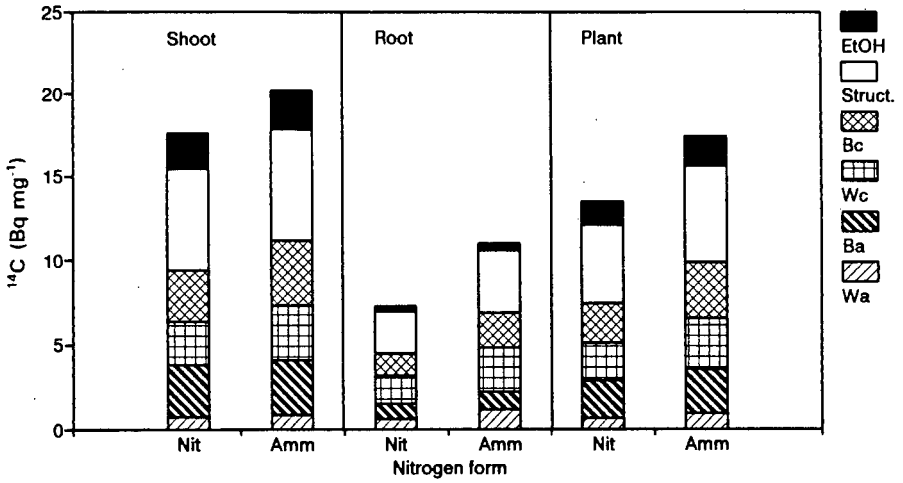
Wheat

	Shoot	Root	Total	S:R
F - value				
MAIN EFFECTS	91.903	25.796	40.304	5.248
N concentration	3.807	0.067	0.335	3.018
N form	179.998	51.524	80.274	7.478
Interaction	0.019	1.617	1.322	0.008
p - value				
MAIN EFFECTS	0.000	0.000	0.000	0.023
N concentration	0.075	0.803	0.580	0.108
N form	0.000	0.000	0.000	0.018
Interaction	0.893	0.228	0.273	0.933
d.f.	15	15	15	15

Maize

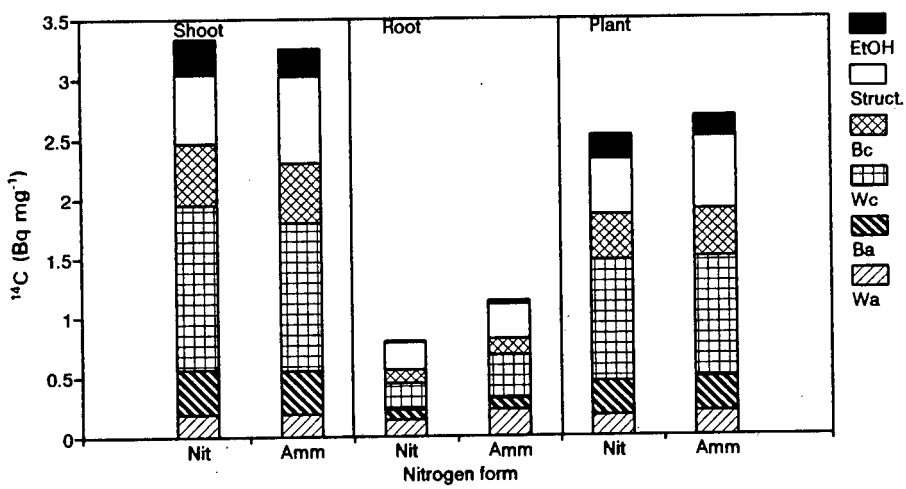
	Shoot	Root	Total	S:R
F - value				
MAIN EFFECTS	10.309	37.894	20.701	2.295
N concentration	0.094	0.857	0.317	0.110
N form	20.525	74.930	41.085	4.480
Interaction	0.416	1.617	0.860	0.144
p - value				
MAIN EFFECTS	0.003	0.000	0.000	0.143
N concentration	0.767	0.383	0.590	0.749
N form	0.001	0.000	0.000	0.056
Interaction	0.538	0.228	0.382	0.715
d.f.	15	15	15	15

7.2 ^{14}C PARTITIONING - UNPROCESSED DATA



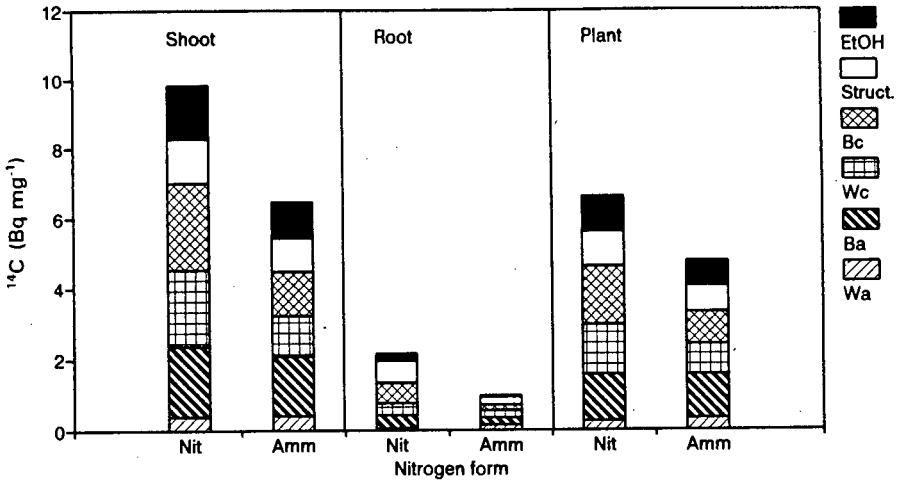
Fraction	Shoot	Root	Plant
Wa	NS	p<0.1	NS
Ba	NS	NS	p<0.05
Wc	p<0.05	p<0.05	p<0.05
Bc	p<0.05	p<0.05	p<0.05
Struct.	NS	p<0.05	p<0.05
EtOH	NS	p<0.05	p<0.05

Figure 7.1. Allocation of ^{14}C (Bq mg^{-1} fresh mass) to water soluble carbohydrates (Wc), amino acids and water soluble proteins (Wa), bound carbohydrates (Bc), bound proteins (Ba), structural material (Struct.) and water insoluble, ethanol soluble material (EtOH) in wheat plants grown with 4 mM NO_3^- (Nit) or NH_4^+ (Amm). Plants were supplied with $^{14}CO_2$ 24 h prior to harvesting. Significance of differences between NO_3^-- and NH_4^+-fed plants calculated from Student's T tests indicated in table below graph (NS=not significant at 90% confidence interval). (n=3)



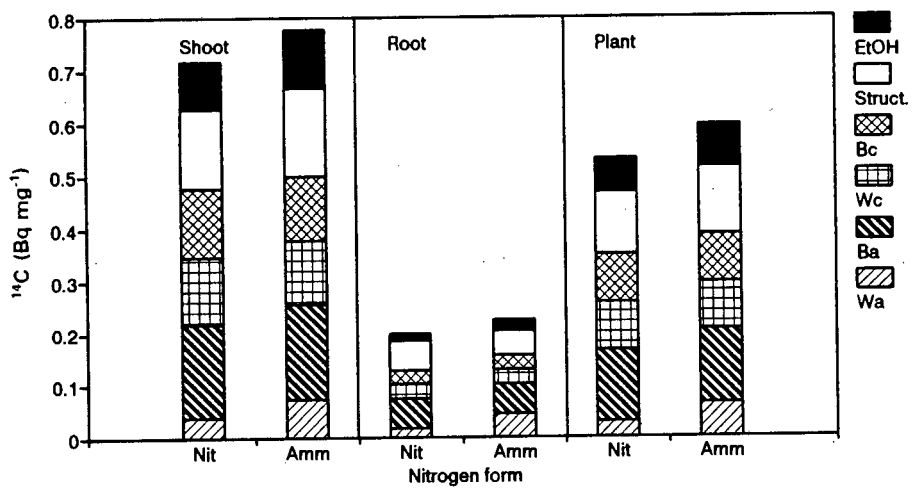
Fraction	Shoot	Root	Plant
Wa	NS	p<0.05	NS
Ba	NS	NS	NS
Wc	NS	NS	NS
Bc	NS	NS	NS
Struct.	NS	NS	p<0.1
EtOH	NS	p<0.05	NS

Figure 7.2. Allocation of ^{14}C (Bq mg^{-1} fresh mass) to water soluble carbohydrates (Wc), amino acids and water soluble proteins (Wa), bound carbohydrates (Bc), bound proteins (Ba), structural material (Struct.) and water insoluble, ethanol soluble material (EtOH) in wheat plants grown with 4 mM NO_3^- (Nit) or NH_4^+ (Amm). Plants were supplied with $^{14}CO_2$ 9 h prior to harvesting. Statistics as in Figure 7.1. (n=3)



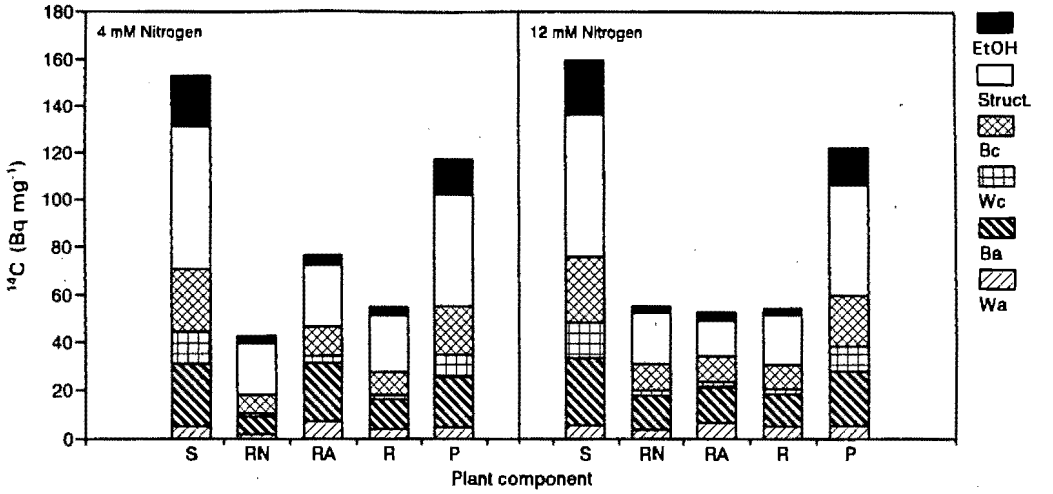
Fraction	Shoot	Root	Plant
Wa	NS	p<0.05	p<0.05
Ba	NS	p<0.05	NS
Wc	p<0.05	p<0.1	p<0.05
Bc	p<0.05	p<0.05	p<0.05
Struct.	p<0.05	p<0.05	p<0.05
EtOH	p<0.05	p<0.05	p<0.05

Figure 7.3. Allocation of ^{14}C (Bq mg^{-1} fresh mass) to water soluble carbohydrates (Wc), amino acids and water soluble proteins (Wa), bound carbohydrates (Bc), bound proteins (Ba), structural material (Struct.) and water insoluble, ethanol soluble material (EtOH) in wheat plants grown with 12 mM NO_3^- (Nit) or NH_4^+ (Amm). Plants were supplied with $^{14}CO_2$ 24 h prior to harvesting. Statistics as in Figure 7.1. (n=3)



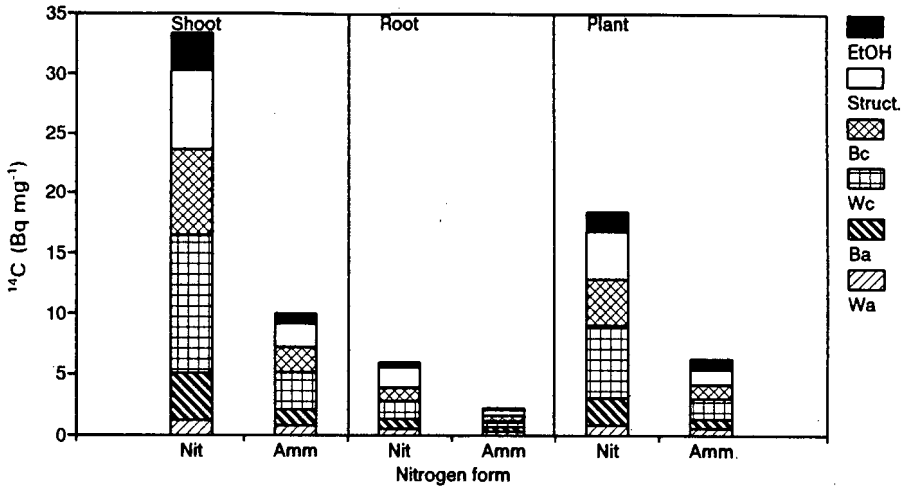
Fraction	Shoot	Root	Plant
Wa	p<0.05	p<0.05	p<0.05
Ba	NS	NS	NS
Wc	NS	NS	NS
Bc	NS	p<0.05	NS
Struct.	NS	p<0.1	p<0.1
EtOH	NS	NS	p<0.05

Figure 7.4. Allocation of ^{14}C (Bq mg^{-1} fresh mass) to water soluble carbohydrates (Wc), amino acids and water soluble proteins (Wa), bound carbohydrates (Bc), bound proteins (Ba), structural material (Struct.) and water insoluble, ethanol soluble material (EtOH) in wheat plants grown with Fe-citrate as an iron source and with 12 mM NO_3^- (Nit) or NH_4^+ (Amm). Plants were supplied with $^{14}CO_2$ 24 h prior to harvesting. Statistics as in Figure 7.1. (n=3)



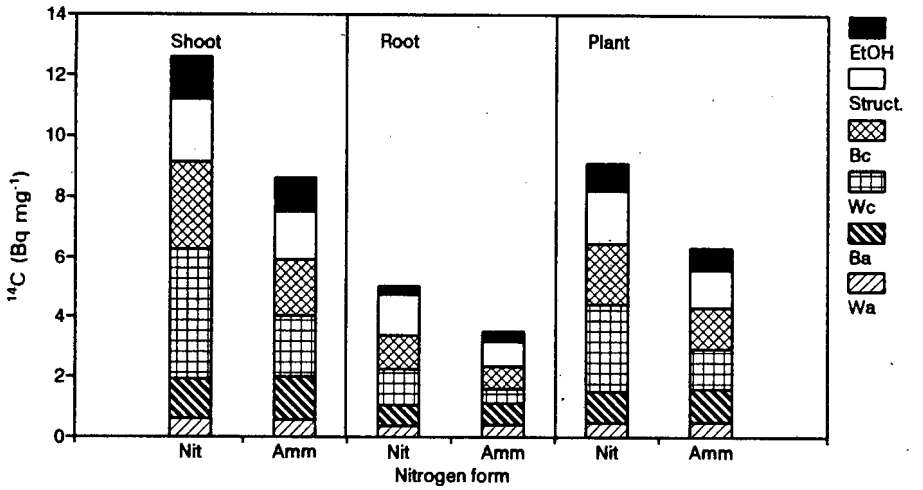
Fraction	Wa	Ba	Wc	Bc	Struct.	EtOH	Total
N concentration (mM)	Nitrate compared with ammonium						
4	p<0.05	p<0.05	p<0.05	p<0.1	NS	NS	p<0.05
12	p<0.05	NS	NS	NS	NS	NS	NS
Component	4 mM compared with 12 mM nitrogen						
Shoot	NS	NS	NS	NS	NS	NS	NS
Root-N	p<0.05	p<0.05	p<0.05	p<0.1	NS	NS	p<0.1
Root-A	NS	NS	p<0.1	NS	p<0.1	NS	p<0.05
Root	NS	NS	NS	NS	NS	NS	NS
Plant	NS	p<0.05	NS	p<0.1	NS	NS	p<0.05

Figure 7.5. Allocation of ^{14}C (Bq mg^{-1} fresh mass) to water soluble carbohydrates (Wc), amino acids and water soluble proteins (Wa), bound carbohydrates (Bc), bound proteins (Ba), structural material (Struct.) and water insoluble, ethanol soluble material (EtOH) in wheat shoots (S), NO_3^--fed root halves (RN), NH_4^+-fed root halves (RA), roots (R) and plants (P) grown with 4 or 12 mM NO_3^- or NH_4^+ in split-root culture. Plants were supplied with $^{14}CO_2$ 24 h prior to harvesting. Results of Student's T tests on differences between NO_3^-- and NH_4^+-fed plants and between 4 and 12 mM treatments are shown in tables below graph. (n=5)



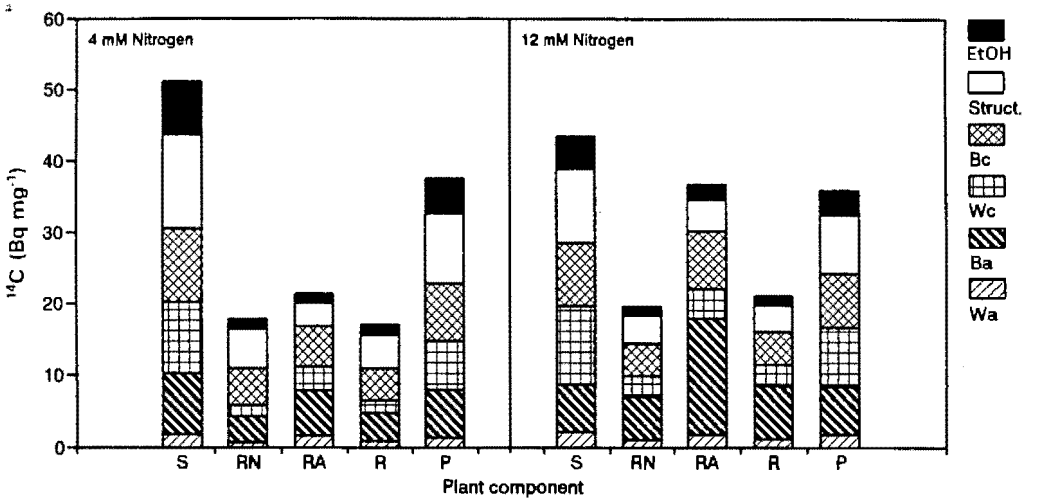
Fraction	Shoot	Root	Plant
Wa	p<0.05	NS	p<0.05
Ba	p<0.05	p<0.05	p<0.05
Wc	p<0.05	p<0.05	p<0.05
Bc	p<0.05	p<0.05	p<0.05
Struct.	p<0.05	p<0.05	p<0.05
EtOH	p<0.05	NS	p<0.05

Figure 7.6. Allocation of ^{14}C (Bq mg^{-1} fresh mass) to water soluble carbohydrates (Wc), amino acids and water soluble proteins (Wa), bound carbohydrates (Bc), bound proteins (Ba), structural material (Struct.) and water insoluble, ethanol soluble material (EtOH) in maize plants grown with 4 mM NO_3^- (Nit) or NH_4^+ (Amm). Plants were supplied with $^{14}CO_2$ 24 h prior to harvesting. Statistics as in Figure 7.1. (n=4)



Fraction	Shoot	Root	Plant
Wa	NS	NS	NS
Ba	NS	NS	NS
Wc	p<0.05	p<0.05	p<0.05
Bc	p<0.05	p<0.05	p<0.05
Struct.	p<0.05	p<0.05	p<0.05
EtOH	NS	NS	NS

Figure 7.7. Allocation of ^{14}C (Bq mg^{-1} fresh mass) to water soluble carbohydrates (Wc), amino acids and water soluble proteins (Wa), bound carbohydrates (Bc), bound proteins (Ba), structural material (Struct.) and water insoluble, ethanol soluble material (EtOH) in maize plants grown with 12 mM NO_3^- (Nit) or NH_4^+ (Amm). Plants were supplied with $^{14}CO_2$ 24 h prior to harvesting. Statistics as in Figure 7.1. (n=4)



Fraction	Wa	Ba	Wc	Bc	Struct.	EtOH	Total
N concentration (mM)	Nitrate compared with ammonium						
4	NS	NS	NS	NS	NS	NS	NS
12	p<0.1	p<0.1	NS	NS	NS	NS	NS
Component	4 mM compared with 12 mM nitrogen						
Shoot	NS	p<0.1	NS	NS	p<0.1	p<0.05	p<0.05
Root-N	NS	p<0.05	NS	NS	NS	NS	NS
Root-A	NS	NS	NS	NS	NS	NS	NS
Root	NS	p<0.05	NS	NS	NS	NS	NS
Plant	NS	NS	NS	NS	NS	p<0.1	NS

Figure 7.8. Allocation of ^{14}C (Bq mg^{-1} fresh mass) to water soluble carbohydrates (Wc), amino acids and water soluble proteins (Wa), bound carbohydrates (Bc), bound proteins (Ba), structural material (Struct.) and water insoluble, ethanol soluble material (EtOH) in maize shoots (S), NO_3^--fed root halves (RN), NH_4^+-fed root halves (RA), roots (R) and plants (P) grown with 4 or 12 mM NO_3^- or NH_4^+ in split-root culture. Plants were supplied with $^{14}CO_2$ 24 h prior to harvesting. Results of Student's T tests on differences between NO_3^-- and NH_4^+-fed plants and between 4 and 12 mM treatments are shown in tables below graph. (n=5)