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UNIVERSITÉ D'OTTAWA
UNIVERSITY OF OTTAWA

To the best in my life, to the memory of my brother Mehdi who dedicated his life for freedom and human rights, to my husband GH. Adel, for his support, understanding, and love and to my little daughter Shiva who had a hard time while mommy was studying.

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ABSTRACT

The management of nitrogen fertilizers is an extremely important aspect of crop production. There is a strong relationship between the rhizosphere biochemistry of winter wheat and nitrogen fertilizers. This study demonstrates the role of different nitrogen fertilizers (NH_4^+, NO_3^-, NH_4^+& NO_3^- and urea) on plant growth and chemical conditions of the rhizosphere such as pH, amino acids, soluble and insoluble sugars of winter wheat var. Fredrick under laboratory conditions.

Wheat growth appeared to be similar with the different N-fertilizers. However, a tendency for more growth was observed under nitrate and a mixed nutrition. The supply of ammonium, a mixture of ammonium and nitrate or urea resulted in an acidic pH in the rhizosphere. Nitrate uptake by plants resulted in a pH increase of the rhizosphere. The amounts and types of amino acids in the rhizosphere were significantly ($P<0.05$) different under the various N-fertilizers. In the wheat rhizosphere supplied with nitrate the total amino acids were less than those of plants supplied with the other N-fertilizers. The greatest total amount was observed under ammonium supply. In the case of nitrate-fed plants there were more basic amino acids in the rhizosphere whereas, plants supplied with other N-fertilizers exuded more acidic amino acids. The amount of amino acids decreased with time.

The different forms of N-fertilizers also produced different effects on the amounts and types of both soluble and insoluble sugars in the rhizosphere. The total soluble sugars were greatest in the rhizosphere of wheat supplied with ammonium and the amounts decreased in the order of nitrate> a mixture> urea. The soluble sugars were also observed to decrease with time. The total insoluble sugar levels were highest in the rhizosphere of plants treated with a mixture of ammonium and nitrate and were lowest in those plants treated with nitrate at 7 days. However, the insoluble sugars of the rhizosphere decreased with time under all treatments except with nitrate treatment. A positive correlation is suggested between the amounts of amino acids and sugars which were exuded and the type of the N-fertilizers. The pH of the rhizosphere is responsible for the exudation of organic compounds, which was greatest at acidic pH.

RESUME

La gestion des fertilisants azotés est un aspect important de la production agricole. Il existe un lien étroit entre la biochimie du rhizosphère pour le blé d'hiver et les fertilisants azotés. Cette étude démontre le rôle des différents fertilisants azotés (NH_4^+, NO_3^-, $NH_4^+ + NO_3^-$, et l'urée) sur la croissance des plantes et les propriétés chimiques du rhizosphère tels que le pH, les acides aminés, les sucres solubles et insolubles. L'étude a porté sur le blé d'hiver de la variété Fredrick, sous des conditions de laboratoire.

La croissance du blé apparaît similaire pour les différents fertilisants. Toutefois, une tendance vers une croissance accrue a été observée sous l'influence du nitrate ou d'une nutrition mixte. L'apport d'ammonium, d'un melange d'ammonium et de nitrate ou d' urée provoque un pH acide dans le rhizosphère. L'assimilation de nitrate par les plantes cause une augmentation de pH du rhizosphère. Les quantités et types d' acides aminés dans le rhizosphère varient ($P<0.05$) selon les différents fertilisants azotés. Dans le rhizosphère du blé alimenté au nitrate, la quantité totale d' acides aminés était moindre que dans les plantes alimenteés avec d' autres fertilisants. La plus grande quantité totale a eté observée sous alimentation d'ammonium. Dans le cas de plantes alimenteés au nitrate, le quantité d'acides aminés basiques dans le rhizosphère étaient plus grande. D'autre part, les plantes

alimenteés avec d'autres fertilisants azotés ont excudé une plus grande quantité d'acides aminés acidiques. Le taux d'acides aminés a diminué avec le temps. Les différentes formes de fertilisants azotés ont aussi provoqué des effets variés sur la quantité et le type de sucres solubles et insolubles dans le rhizosphère. La plus grande quantité totale de sucres solubles se retrouvent dans le rhizosphère du blé alimenté avec de l' ammonium et les quantités ont diminué selon l'ordre nitrate> mélange> urée. On remarque aussi que la quantité de sucres solubles diminuent avec le temps. Les taux de sucres insolubles totaux étaient les plus élevés dans le rhizosphère des plantes traiteés avec un mélange ammonium nitrate, et le plus bas pour des plantes traiteés au nitrate, après 7 jours. Toutefois la quantité de sucres insolubles du rhizosphère a diminué avec le temps pour tous les traitements à l' exception du nitrate. Une relation de proportionnalité existe impliquant les quantités d' acides aminés et de sucres qui ont été exsudés et le type de fertilisants azotés. Le pH du rhizosphère est responsable de l'exsudation des composés organiques la quelle a été le plus élevée sous pH acide.

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CHAPTER 1

INTRODUCTION

1.1 General introduction:

The effective management of nitrogen availability to crops presents a greater challenge to the farm operator than does that of any other fertilizer nutrient. No other nutrient requires as much attention and no other brings greater rewards for wise management (Olson and Kurtz, 1982). Modification of agricultural practices and careful adjustment of fertilizer application to crop needs can minimize the nitrogen cost and loss, and/or nitrate contamination (Nitrogen, No. 162, July - August, 1986) and can provide better crop yields.

Understanding the relation of the root exudate to plant nutrition, especially those rich in nitrogen is essential for the development of better crop production systems. A close relationship between plant nutrition and root exudates exists and it is involved in the modification of the rhizosphere biochemistry (Curl and Truelove, 1986). Since rhizosphere biochemistry holds the answer to many questions relating to the development, function, and diseases of root, intensification of rhizosphere research worldwide could improve crop yields, which are needed to feed an ever-

increasing human population. It would also provide a better understanding of the role that root exudates play in biological and in some cases, chemical control of certain root diseases.

The present study was conducted to determine the effects of various N-fertilizers such as nitrate, ammonium, a 1:1 mixture of ammonium and nitrate and urea on the rhizosphere biochemistry of winter wheat (Triticum aestivum var. Fredrick).

It was hypothesized that the various N-fertilizers change the rhizosphere biochemistry through their effects on the quantity and/or composition of the seed/root exudates. Specific objectives were to study the rhizosphere free amino acids and carbohydrates of winter wheat at different time periods, i.e. 7, 15, and 21 days after germination. Carbohydrate and amino acid studies were chosen because of the essential role of these substances in the rhizosphere effects (Curl and Truelove, 1986) and in plant metabolism. An attempt was made to address the possible role that each nitrogen form has on root exudates, on the basis of their effects on the rhizosphere pH and/or plant growth.

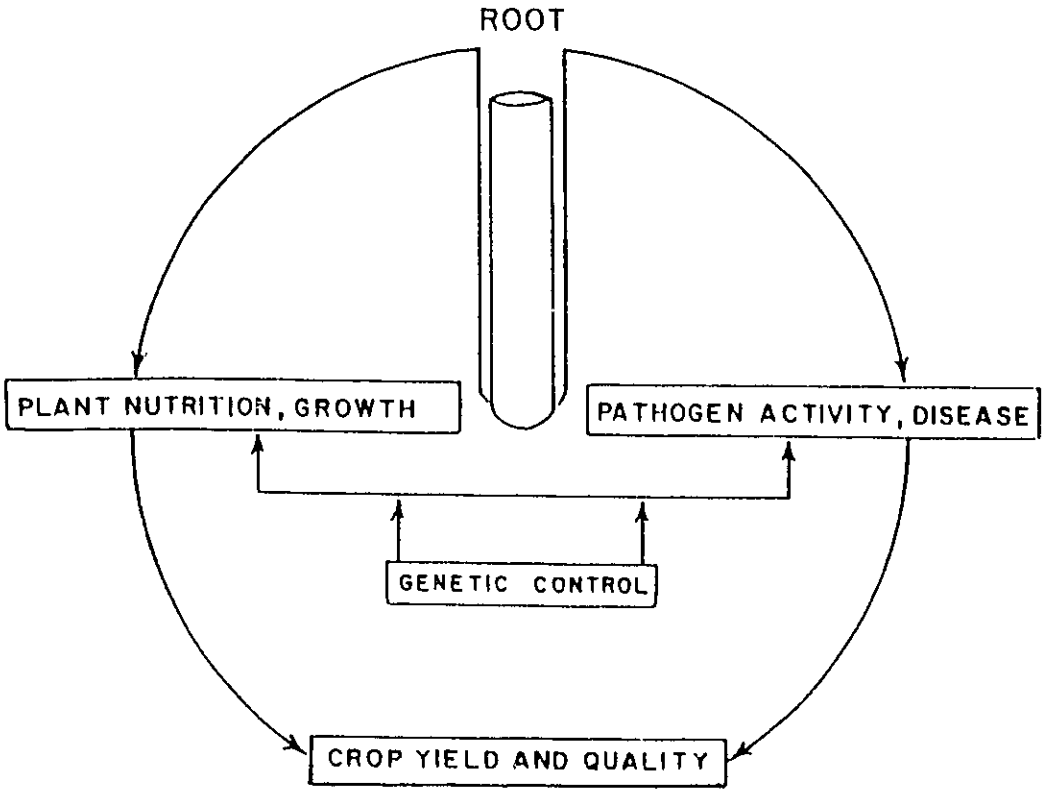
1.2 Literature review:

1.2.1 Rhizosphere:

Plant growth and development are strongly influenced by the rhizosphere, an environment that the plant itself helps to create. This is the zone where microbial activity constitutes a major contribution to plant health and growth (Curl and Truelove, 1986). The critical role of the rhizosphere on plant nutrition and growth and on plant pathogens and pathogenesis has been investigated intensively (Newman, 1978; Smiley, 1978; Deacon and Henry, 1981; Curl and Truelove, 1986; Ming-Muh and Hsieh; 1986; Inskeep and Comfort, 1986; Tari and Anderson, 1988). A schematic diagram of the influence of rhizosphere ecology on crop production and quality is shown in Figure 1 (Curl and Truelove, 1986).

"Rhizo", or "rhiza" is derived from the Greek word for root. Sphere, can mean a ball or zone such as a root system in an environment. Indeed, we are dealing with a unique environment inhabited by a "society" of microorganisms (Curl and Truelove, 1986; Sivasithamparam et al., 1987; Juhnke et al., 1987; Chao et al., 1988). As yet, no natural root system has been discovered without some microflora (Tinker, 1984).

Figure 1. A Schematic Representation of the Influence of rhizosphere on Crop Yield and Quality (Curl and Truelove, 1986).


ROOT
PLANT NUTRITION, GROWTH
PATHOGEN ACTIVITY, DISEASE
GENETIC CONTROL
CROP YIELD AND QUALITY

The existence of the rhizosphere zone, densely populated by microorganisms, is directly due to the release of a wide variety of organic materials by plant roots (Helal and Sauerbeck, 1984; Tari and Anderson, 1988).

Based on extensive studies (Börner, 1960; Vancura, 1964; Vancura and Hovadik, 1965; McDougall, 1970; Vancura and Hanzlikova, 1972; Martin, 1977; Kraffczyk et al., 1984; Curl and Truelove, 1986) many types of materials are known to be released by the root system. They were classified by Hale et al (1981) as follows:

(1) Root exudates: Chemicals released into the rhizosphere by the roots. Root exudation involves several pathways and biochemical mechanisms.

(2) Leakages: Compounds of low molecular weight which diffuse into the apoplast and, via the apoplast, move to the root surface, or leak directly from epidermal or cortical cells. Volatile compounds such as alcohols, fatty acids, alkyl sulfides could be included this category (Curl and Truelove, 1986).

(3) Secretions: Compounds which cross membrane barriers with the aid of metabolic energy.

(4) Mucilage: Compounds originating from root cap cells and root debris.

The amount and/or type of materials release by the roots not only varies with the species of plants and their age, but also the environmental conditions of the plant are important factors (Curl

and Truelove, 1986). For example, Barber and Gunn (1974) and Beck and Gilmour (1983) reported that in crops such as wheat and barley, 3 to 10% of fixed carbon was released from the roots under sterile soil conditions within a few weeks (i.e.8 weeks). Vancura (1964) noted that the loss of up to 7 to 10% of the dry matter of barley and wheat could be accounted for by the release of the root exudates into the rhizosphere. Up to 44% of the photosynthetically fixed carbon by maize plants was transferred to their roots of which 13% was released into the rhizosphere (Helal and Sauerbeck 1984). However, amounts as high as 25% of the root weight have been also suggested to exudate in form of soluble compounds (Newman, 1978). On the other hand, the amounts of insoluble materials lost are thought to range from 1% (Newman, 1978) to 30% (Beck and Gilmour, 1983).

Root exudates constitute the major source of important compounds in the rhizosphere which are readily metabolized by microorganisms. These compounds influence the microbial or fungal activity around the roots in a variety of ways (Kush and Dadarwal, 1981; Polonenko et al., 1984). They support germination of dormant propagules, attract zoospores to the root, direct the growth of germ tubes toward the root, and stimulate infection (Altman and Campbell, 1977; Lockwood, 1985; and Gutterridge et al., 1987). Soybean root exudates have been demonstrated to affect the population of Bradyrhizobium japonicum, an N_2- fixing microorganism

in both artificial media and also in soil (Mahmoud and Angle, 1987). At a low exudate concentration of 0.02%, the growth of B. japonicum was either unaffected or was enhanced, whereas, with a high exudate concentration of 0.04%, growth was inhibited. Joshi (1983) demonstrated the general stimulation of fungi in the rhizosphere by root exudates. Fusarium solaniferum spp. phaseoli, can colonize and produce chlamydospores in the rhizosphere of numerous non-hosts with the aid of root exudates (Schroth and Hendrix, 1982). F. oxysporum invades cortical cells of nonsusceptible weeds and crop plants due to their root exudates (Katan, 1971). Bacterial pathogens of foliage can colonize the rhizosphere of non-hosts by using their root exudates (Kritzman and Zutra, 1983). Also, the infection of winter wheat by Gaeumannomyces graminis was more obvious in the rhizosphere of plants having the greatest amounts of root exudates (Ming Tan and Semenuik, 1970).

Root exudates are mainly comprised of sugars, amino acids, glycosides, organic acids, vitamins, enzymes and a wide variety of miscellaneous compounds derived from plant metabolites (Table 1) (Curl and Truelove, 1986; Mahmoud and Angle, 1987). The nature and role of root exudates were characterized by the work of Vancura (1964); Rovira, (1956) and Bowen (1966). Among the exudates, the carbohydrates and amino acids are considered to be the most important, since they are the simple compounds required to stimulate microbial activity.

Table 1. Substances detected in plant root exudates (from Curl and Truelove, 1986).

Compounds	Exudate
Sugars	Glucose, fructose, sucrose, maltose, ribose, xylose, arabinose, raffinose, oligosaccharide
Amino compounds	Asparagine, alanine, glutamine, aspartic acid, leucine, isoleucine, serine, aminobutyric acid, glycine, cystine, cysteine, methionine, phenylalanine, tyrosine, threonine, lysine, proline, tryptophan, arginine, homoserine, cystathionine
Organic acids	Tartaric, oxalic, citric, malic, acetic, propionic, butyric, succinic, fumaric, glycolic, valeric, malonic
Fatty acids and sterols	Palmitic, stearic, oleic, linoleic, linoleic acids, cholesterol, campesterol, stigmasterol, sitosterol
Growth factors	Biotin, thiamine, niacin, pantothenate, choline, inositol pyridoxine, p-amino benzoic acid, n-methyl nicotinic acid
Nucleotides, flavonones and enzymes	Flavonone, adenine, guanine, uridine, cytidine, phosphatase, invertase, amylase, protease, polygalacturonase
Miscellaneous compounds	Auxin, scopoletine, fluorescent substances, hydrocyanic acid, glycosides, saponin (glucosides), organic phosphorus compounds, nematode cyst or egg-hatching factors, nematode attractants, fungal mycelium growth stimulant mycelium growth inhibitors, zoospore attractants, spore and sclerotium germination stimulants and inhibitors, bacterial stimulants and inhibitors, parasitic weed germination stimulator

It has been reported that sugars and amino acids constitute some 67% of the root exudates of maize (Kraffczyk et al., 1984). Sugars and amino acids are also known to be responsible for the increase in daytime denitrification in the rhizosphere of bean and maize (Scaglia et al., 1985). Amino acids such as serine and aspartic acid play an essential role in soil denitrification processes (Rovira, 1969). Sugars are metabolized in preference to organic acids by microorganisms (Kraffczyk et al., 1984). Kraffczyk et al (1984) found the amount of monosaccharides to be less in nutrient solutions of non-sterile roots than in solutions of sterile roots.

Microorganisms have different capabilities for the utilization of sugars (Krieg and Döbereiner, 1984). For example, in the presence of rhizosphere microorganisms, the amounts of fructose, arabinose and glucose which predominate, decreased by a half, while sucrose was not affected (Kraffczyk et al., 1984). It has been noted that the carbohydrates in an Azotobacter medium, a nitrogen-fixing bacterium able to synthesize large amounts of biologically active substances such as vitamins, amino acids, and auxin, modified the production of these compounds by this organism (Gonzalez-Lopez et al. 1983). Sparling et al. (1981) concluded that glucose increased the microbial biomass, ATP content, and the activities of hydrogenase and amylase.

Rhizobium accumulates along the growing roots of chick pea

plants by chemotaxis toward root exudates (Kush and Dadarwal, 1981). The lowest level of response to chemotaxis for galactose and glucose was found at a 0.1 mM concentration whereas, for aspartate, asparagine, tyrosine and tryptophan, the concentration was 10 µM. The bacterial cells showed chemotaxis towards glutamine at a concentration as low as 1 µM. Finally, Ming Tan and Semeniuk (1970) concluded that part of the exuded carbohydrates could be used by soil-borne root pathogens such as R. solani and Pythium arrhenomanas, for their own development.

The rhizosphere microorganisms also respond differently to amino acids. For example, Hartmann et al. (1988) found that Azospirillum brasiliense, A. lipoferum, and A. amazonense strains showed a species-specific response pattern towards glutamate, histidine, alanine and serine. Azospirillum lipoferum strains grew very well utilizing these amino acids as their sole nitrogen and carbon sources and excreted high amounts of ammonium into the medium, while A. amazonense strains grew best with glutamate and alanine. However, the nitrogen fixation ability of A. amazonense was inhibited at high concentrations of histidine, alanine, serine or glycine. It was also found that A. brasiliense could not grow on histidine and the nitrogen fixation ability of the organism was inhibited by alanine, glycine and leucine (Hartmann et al. 1988).

Root exudates also have a dramatic effect on the concentrations of metals and mineral nutrition of the soil (Inskeep

and Comfort, 1986; Marschner et al. 1987). They are important in increasing the soluble fraction of Fe^{3+}, Cu^{2+}, and Zn^{2+} in the rhizosphere (Inskeep and Comfort, 1986). In the rhizosphere of Pinus radiata, the amount of soluble Fe^{3+} in the presence of exudates was 10^3 to 10^5 times greater than in the absence of exudates (Inskeep and Comfort, 1986). The amount of this ion in the rhizosphere is an important factor in determining Fe uptake; high concentrations of Fe^{3+} providing gradients for diffusion of Fe^{3+} both to and from the root (Lindsay, 1984). In barley, the concentrations of amino acids are important in complexing Cu, especially at pH values greater than 6. The concentration of the Cu/amino acid complex was anywhere from 10 to 10^3 times greater than the free Cu^{2+} plus inorganically complexed Cu. The most important copper/amino acid complexes were found to be Cu-aspartate, Cu-histidine, Cu-glutamine and Cu-serine (Inskeep and Comfort, 1986). Marschner et al. (1987) believed that root exudates might be important for the inactivation of Al^{3+} by complexation and adsorption. In addition, Goda and Reisenauer (1980) suggested that root exudates such as malate were responsible for increasing soil Mn solubility by reducing levels of MnO_2. Reduction of MnO_2 by organic acids present in root exudates also provided a source of other organic acids which further reacted to influence the availability of Fe (Jauregui and Reisenauer, 1982).

1.2.2 Nitrogen fertilizers:

Nitrogen is the most important nutrient for plant growth. Although plants can live in an atmosphere of nitrogen, it is the availability of nitrogen to plants in a biologically useful form that is one of the major factors that limits plant growth (Schubert, 1987). In the future large scale agriculture will depend even more on nitrogen fertilizers. The most rapid expansion in nitrogen fertilizer capacity has taken place within the last five years in Canada. The production of ammonia has grown by 896,000 tonnes since 1983. The construction of two ammonia/urea plants during the 1980s has led also to a rapid build up of urea capacity. Urea production has grown by 46% as compared to 22% for ammonia between 1983 - 1987 (Nitrogen, No. 175, September - October, 1988). The production of N- fertilizers has increased 34% for anhydrous ammonia, 37% for urea, 61% for ammonium nitrate and 21% for ammonium phosphate in Canada in 1988 as compared to 1987 (Statistics Canada PPI).

Nitrogen fertilizers are needed almost universally to maintain the high production levels of nonleguminous crops (Olson and Kurtz, 1982). In legumes, 50 to 75% of the plant nitrogen comes from inorganic nitrogen sources, primarily as nitrate, with only 25 to 50% derived from N_2 - fixation (Hunter et al. 1982).

The major roles of nitrogen in plant growth are as a component of: (a) chlorophyll molecules, (b) amino acids involved in the building of proteins, (c) enzymes. They also involved in carbohydrate utilization, stimulative to root development and activity, and regulation of the uptake of other nutrients (Olson and Kurtz, 1982). The predominant impact of the N- fertilizers on crop quality is to enhance growth and protein content of grain and forage crops (Riedell, 1989). The high protein content is necessary for both processing (e.g. bread baking quality) and for nutrition. It has been shown that N- fertilizers significantly improved wheat growth, with up to fourfold increases in dry matter, shoot number, nitrogen uptake and yield (Hutcheon and Paul, 1966; Bacon et al. 1989). The flour and wheat protein obtained from plants grown with different nitrogen fertilizers vary in their amino acid content. Hence, it is possible to grow wheat with a specific protein content by controlling the nitrogen supply and moisture (Hutcheon and Paul, 1966). Gregory et al. (1981) implied that the continued production of dry matter by cereals during the growth of grain depends on the availability and uptake of sufficient nitrogen.

The major forms of nitrogen available for plant growth are ammonium and nitrate (Gashaw and Mugwira, 1981), although, urea has long been used as a cheap source of nitrogen (Bloom and Finazzo, 1986). Recently, urea consumption increased in Northern Europe because of its availability and low cost. In the five years between 1982 and 1997 urea production worldwide increased by 9.5 million

tonnes or 44%, which includes a growth of 1.5 million tonnes N in the Soviet Union (Nitrogen, No. 180, July - August, 1989).

The question of whether plants should be supplied with nitrate, ammonium or other N- fertilizers is a matter of great practical importance. In some cases, higher growth rates have been obtained with a combination of both ammonium and nitrate (Gashaw and Mugwira, 1981; Trolldenier and Rheinbaben, 1981; Marschner, 1986; Gentry et al. 1989), while in other cases with only ammonium (Sommer and Six, 1982). Gentry et al. (1989) found that wheat plants could not absorb sufficient nitrogen required for maximal growth when the only nitrogen supply available was nitrate. The inclusion of ammonium in the nutrient solution generally resulted in a greater total N uptake, which would minimize N as a growth-limiting factor. However, in other reports, plants showed more growth and greater nitrogen absorption when nitrate rather than ammonium was the source of nitrogen (Haynes and Goh, 1978; Pan et al. 1985; Ohyama et al. 1989). There is also contradictory evidence regarding the presence of ammonium in the rooting zone. It can have either an inhibitory effect on nitrate uptake and assimilation (Dean-Drummond and Glass, 1983; Breteler and Siegerrist, 1984; Ohyama et al. 1989) or a stimulatory effect on nitrate efflux from plants (Minotti et al., 1969). Maize growth was actually reduced by treatment with 100-200 ppm urea (Weiming and Zhiyu, 1987). Finally, in legume plant species, greater growth was found with urea than with nitrate (Andrews et al., 1985).

1.2.3 Nitrogen uptake and assimilation:

The actual process of N uptake by plants requires movement of ionic N species to the root surface for absorption (Olson and Kurtz, 1982). The rate of N uptake by field crops is very rapid during the period of vegetative growth (Olson, 1978). approximately 4 kg / ha daily in corn and wheat, however, large physiological differences in uptake and assimilation patterns exist depending on the N source (Zornoza et al. 1989). The binding between nitrate and soil colloids is negligible and less than for ammonium, therefore, nitrate is mobile and readily transported to the plant roots by mass flow (Olson and Kurtz, 1982). Nitrate is actively (energy dependent) taken up by the root system where it is either reduced and synthesized in the roots to amino acids or transferred into the xylem stream for transport to the shoots (Lee, 1980; Guerrero et al., 1981; Pan et al. 1985; Schubert, 1987). A part of the nitrate may also accumulate in the vacuoles (Granstedt and Huffaker, 1982; Morgan et al., 1985).

Experiments with corn indicated that about 30% of the nitrate taken up by the root system is reduced in the roots, a further 10% is accumulated and 60% translocated to the shoots (Morgan et al., 1985). It has been shown that 58% of the total cellular nitrate accumulated in the leaf vacuoles of barley plants grown in the presence of nitrate (Granstedt and Huffaker, 1982). Root nitrate

reduction is localized in cells at the root periphery (Rufty et al., 1986). An induction phase for nitrate transport into the root has been established for many plant species (Goyal and Huffaker, 1986). The time required for induction of the transporter was found to vary, with periods of 8 to 10 hrs being reported for wheat seedlings (Goyal and Huffaker, 1986). Nitrate reduction in plants is given in the following equation (Guerrero et al., 1981):

$$NO_3^- \xrightarrow{2e^-,\ \text{nitrate reductase}} NO_2^- \xrightarrow{6e^-,\ \text{nitrite reductase}} NH_4^+$$

The proportion of nitrate reduced in the roots and shoots varies among the different plant species (Schubert, 1987) and even among the cultivars of a species such as soybean (Hunter et al. 1982). The enzyme involved in nitrate reduction is nitrate reductase and the amount present in the roots may be the major factor in reduction of nitrate in root system (Olson and Kurtz, 1982). This enzyme requires NADP or NADPH (Oaks and Hirel, 1985) as the reductant. It has been shown that the uptake rate of nitrogen by plants without pretreatment with nitrate is distinctly lower in the first few hours, until the induction of nitrate reductase in the root cell (Jackson et al. 1972) or a nitrate carrier, in the plasma membrane of root cells (Dean-Drummond, 1984). However, it is likely that after reduction, the nitrate absorbed follows the same pathway in the assimilatory processes as does ammonium (Guerrero et al., 1981).

In contrast, ammonium uptake rate is greater than nitrate uptake, since there is no initial lag time, (Goyal and Huffaker, 1986), the majority of ammonium absorbed by plants being assimilated in the roots (Lewis et al. 1982). However, a substantial proportion of ammonium is not taken up in the ionic form i.e. NH_4^+, but as NH_3 that permeates the plasma membrane after deprotonation, leaving H^+ in the external solution (Mengel et al., 1976). Ammonium is assimilated by the enzyme glutamine synthetase and glutamate synthase mainly present in roots (Schubert, 1987).

Urea is known to be taken up by plants in the neutral form (Kirkby and Mengel, 1967; Olszanska, 1968). The actual uptake of urea has not been extensively studied (Criddle et al. 1988), however, both passive (Olszanska, 1968) and active (Hentschel, 1970) uptake have been reported for corn and bean roots, respectively. Olszanska (1968) considered that three phases were involved during the course of urea absorption. The first was adsorption of urea and its diffusion into "free space" which occurs rapidly. The second phase consists in the slow diffusion of urea through the membranes into cells. As the end of this phase, it may be assumed that the urea concentrations in the tissue and external solution are equal. In the third phase, urea decomposition acquires considerable importance. After entering the cells, the urea undergoes enzymatic decomposition by urease into NH_4^+ and CO_2 (Goyal and Huffaker, 1986), and in this manner urea that diffusional

equilibrium, which was achieved during the second phase is disturbed. This causes further amounts of urea to be absorbed.

Ultimately, the processes of uptake, reduction, and translocation result in the incorporation of the various form of nitrogen into amino acids and protein, which are required for the growth and maintenance of plants (Guerrero et al., 1981). Ammonia, which is the end product of nitrate reduction, ammonium uptake and urea hydrolysis, is incorporated into organic compounds by way of one or both of the major routes known to be responsible for the conversion of ammonia-nitrogen into α-amino-nitrogen: the glutamate dehydrogenase and the glutamine synthetase-glutamate synthase pathways (Figure 2) (Guerrero et al., 1981).

1.2.4 Nitrogen fertilizers and the rhizosphere biochemistry:

The nutrient status of the soil can change the anatomy (Newman, 1978; Brown and Hornby, 1987), chemical composition (Newman, 1978) or growth (Turner et al., 1985; Marschner, 1986) of plant root systems. Since these continually discharge organic matter in the form of root exudates into their medium, the chemical and biochemical properties of the rhizosphere will be affected. It has been reported that nitrogen fertilizers can change the rhizosphere pH (Weinberger and Yee, 1984; Marschner et al., 1986; Brown and Hornby, 1987).

Figure 2. Assimilation of Ammonium by 1. Glutamine synthetase; 2. Glutamate synthase; 3. Glutamate dehydrogenase; 4. Asparagine synthetase; red. Ferrodoxin - reduced Ferrodoxin.

(1) (NH_4^+) + (ATP) + (Glutamate)----→ (Glutamine) +(ADP) +(P)

(2) (Glutamine) + (α - Ketoglutarate) ----→ (2 Glutamate)
red. Ferrodoxin

(3) (α - Ketoglutarate) + (NH_4^+) ----→ (Glutamate)
NAD(P)H NAD(P)

(4) (Aspartate) + (NH_4^+) + (ATP)----→ (Asparagine) + (ADP) +(P)
Glutamine Glutamate

Plants that absorb nitrogen as nitrate tend to raise the pH in the rhizosphere while, those absorbing nitrogen as ammonium lower the pH. The rhizosphere pH may be as much as 2 units greater or lesser than the pH of the bulk soil (Marschner, 1986). Weinberger and Yee (1984) have also reported an increase in the rhizosphere pH with a mixture of ammonium and nitrate, but indicated no change in the rhizosphere pH of wheat plant under urea treatment. Other experiments (Kirkby and Mengel, 1967; Miller et al., 1970; Blair et al., 1971; Riley and Barber, 1971; Soon and Miller, 1977) reported a decrease in the rhizosphere pH when ammonium or urea were used as the N-source.

The excretion of hydrogen ions or hydroxyl ions (Weinberger and Yee, 1984; Brown and Hornby, 1987) from root to the solution medium is known to be responsible for pH changes brought about by the various nitrogen fertilizers. The role of organic acids in changing the rhizosphere pH has also been considered (Newman et al., 1987). It was concluded by Nye (1981) that the imbalance between the intake of cations and anions across the root surface is the dominant source of the pH gradient in the rhizosphere.

Biochemical changes in the rhizosphere can occur as a result of the influence of nitrogen fertilizers on the seed/root exudates (Brown and Hornby, 1987). Trolldenier and Rheinbaben (1981) reported that there were higher root exudates in the rhizosphere of wheat plants fed on ammonium fertilizer. Levels of root exudates

were lowest in the rhizosphere of plants treated with nitrate and intermediate in plants supplied with a mixture of ammonium and nitrate. The form of the nitrogen affected both the concentration and composition of the amino acids in wheat root exudates (Ivarson and Sowden, 1970; Brown and Hornby, 1987). Larger numbers of amino acids and amides and in greater concentrations occurred in the root exudates of fertilized plants. Slightly greater concentrations of amino acids were found in the root exudates of plants given ammonium than those of plants given nitrate (Brown and Hornby, 1987). In addition, Ingversen and Ivanko (1971) reported that amino acid exudates of wheat plants supplied with nitrate contained more glutamic and aspartic acids (47 - 58%) than those of plants supplied with a mixture of nitrate and ammonium (38 - 43%).

The effects of various nitrogen fertilizers on the composition and/or quantity of root exudates are also important. Any change in the root exudates of a plant through N-fertilizers could affect or modify plant metabolism and growth (Haynes and Goh, 1978), and plant susceptibility to diseases (Curl and Truelove, 1986; Hartmann et al., 1988). It was suggested that the "take - all" fungus of wheat, Gaeumannomyces graminis grows less well on a medium containing exudates from plants treated with ammonium than with those given nitrate (Huber et al., 1968; Huber and Watson, 1972; Smiley and Cook, 1971; Taylor et al., 1983; Brown and Hornby, 1987). Further, Hornby and Goring (1972) have reported reduced levels of "take - all" disease in wheat when using a mixture of

ammonium and nitrate, intermediate levels with nitrate alone and higher levels of incidence with ammonium alone. Macnish and Speijers (1982) found that in Western Australian conditions, i.e. Mediterranean climate with a hot dry summer and a mild moderately wet winter, nitrate had little or no effect on "take - all" disease while ammonium reduced "take - all" severity and sometimes the incidence. Diseases caused by Phymatotrichum omnivorum, Thielaviopsis basicola, Gaeumannomyces graminis, Verticillium albatrum and Streptomyces scabies were suppressed by ammonium but enhanced by nitrate fertilizers (Curl and Truelove, 1986).

CHAPTER 2

MATERIALS AND METHODS

2.1 Chemicals:

Crystalline pure amino acids (99%) were purchased from Sigma Chemical Company[1]. They included the protein amino acids[2]. A mixture of acetylated pure D-[rhamnose, fucose, ribose, arabinose, xylose, allose, mannose, galactose and glucose] (500 ng/ml) was obtained through the courtesy of Dr. I. R. Siddiqui, Agriculture Canada, Ottawa. All other chemicals (reagent grade) used for plant nutrition and analyses are listed in Table 2 along with their suppliers. The ion exchange resin, Amberlite IRA-400 (RN $(CH_3)_3^+$ Cl^-), mesh size (wet) 20 - 50 was from Sigma Chemical Co. Glass distilled, pesticide grade solvents (chloroform and methanol) used for extractions, preparation of solutions and clean - up procedures, were purchased from BDH chemicals[3] and Fisher Scientific Ltd[4].

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[1] Sigma Chemical Co., St. Louis, Missouri, U. S. A.

[2] L[cysteine, tyrosine, alanine, proline, leucine, cystine, phenylalanine, lysine, aspartic acid, hydroxyproline, valine, threonine, tryptophan, isoleucine, histidine, methionine, glutamic acid, arginine, serine, glycine and two amides glutamine and asparagine].

[3] BDH Chemicals, Toronto, Ontario, Canada.

[4] Fisher Scientific LTd., Don Mills, Ontario, Canada.

Table 2 List of chemicals and their suppliers.

Chemical	Formula	Supplier
Acetic acid	CH_3COOH	BDH Chemicals
Acetic anhydride	$(CH_3CO)_2O$	BDH Chemicals
Ammonium hydroxide	NH_4OH	J. T. Baker
Barium hydroxide	$Ba(OH)_2.8H_2O$	Fisher Sci.
Boric acid	H_3BO_3	Sigma Chem.
Calcium chloride	$CaCl_2.2H_2O$	Fisher Sci.
Calcium nitrate	$Ca(NO_3)_2.4H_2O$	BDH Chemicals
Calcium sulphate	$CaSO_4.2H_2O$	BDH Chemicals
Cupric sulphate	$CuSO_4.5H_2O$	Fisher Sci.
di-ammonium hydrogen phosphate	$(NH_4)_2HPO_4$	BDH Chemicals
Ferric citrate	$FeC_6H_5O_7.3H_2O$	J. T. Baker
Hydrochloric acid	HCl	BDH Chemicals
Magnesium sulphate	$MgSO_4.7H_2O$	Fisher Sci.
Manganese sulphate	$MnSO_4.H_2O$	Mallinckrodt Chem. Work, LTd.
2-Mercaptoethanol	$HS.CH_2CH_2OH$	BDH Chemicals
o-Phthaldialdehyde	$C_6H_4(CHO)_2$	Sigma Chem. Co.
Potassium di-hydrogen phosphate	KH_2PO_4	Fisher Sci.
di-Potassium hydrogen phosphate	K_2HPO_4	Fisher Sci.
Pyridine	C_5H_5N	Chromatographic Specialties INC
Silver nitrate	$AgNO_3$	BDH Chemicals
Sodium acetate	$CH_3COONa.3H_2O$	Fisher Sci.
Sodium borohydride	$NaBH_4$	BDH Chemicals
Sodium hydroxide	$NaOH$	Fisher Sci.
Sodium hypochlorite (6% available Chlorine)	$NaOCl$	BDH Chemicals
Sodium molybdate	$Na_2MoO_4.2H_2O$	BDH Chemicals
di-Sodium hydrogen phosphate	$Na_2HPO_4.7H_2O$	Fisher Sci.
Sulphuric acid	H_2SO_4	BHD Chemicals
Tetrahydrofuran	$CH_2(CH_2)_2CH_2O$	BDH Chemicals
Urea	NH_2CONH_2	BDH Chemicals
Zinc sulphate	$ZnSO_4.7H_2O$	Sigma Chem. Co.

High performance liquid chromatography (HPLC) ultra-pure water, generated by a Milli-RO4 coupled to a Milli-Q water purification system (Millipore, Bedford, Mass.) was used in the preparation of all HPLC buffers. Deionized distilled water, used for the preparation of solutions, was prepared by passing tap water through an Illinois Water Treatment Universal Ion Exchanger Model 1 (Sargent - Welch Scientific Co., Toronto, Ont.) before distilling in an all glass still. Pure silica granular sand (60 - 120 mesh) was purchased from Fisher Scientific Ltd. The sand was washed and autoclaved for 20 min. at 250°C and 1.4 Kg cm^{-2} before use.

2.2 Instrumentation:

All glassware was soaked for an hour in a cleaning solution (phosphate free, 20% Extran 300, BDH Chemicals), followed by rinsing with distilled water to ensure they were clean. The glassware was rinsed with acetone and completely dried before use. Glassware that required sterilization was autoclaved for 25 min. at 250°C and 1.4 Kg cm^{-2}. A controlled environment incubator (Conviron, model S10H) was employed for plant growth. A list of general equipment used during the experiments is given in Table 3. A Waters Millipore High Performance Liquid Chromatograph (HPLC) system was employed, it consisted of an automatic pre-column derivatization system (Waters Millipore WISP 710 B), a C-18 guard column, a Water "Resolve" C-18, 5 µm spherical packing (12 cm X 3.9 mm I. D.) column and a fluorescence detector Model 420.

A Hewlett Packard Model 5830A Gas Chromatograph (GC) equipped with a flame ionization detector (FID) and a Hewlett-Packard 18850A GC terminal integrator was used for sugar analyses. Gas chromatography was performed on a DB - 23 Megabore capillary column (30 m X 0.55 mm, I. D., film thickness, 0.5 µm).

2.3 Organisms:

The experimental organisms were the seedlings of winter wheat Triticum aestivum variety Fredrick. The seeds were supplied by Dr. T. Simpson of Agriculture Canada, Ottawa.

2.3.1 Seed sterilization:

Grains of winter wheat were washed for one hour in running tap water followed by sterilization of their surface with a 3% sodium hypochlorite solution for 5 min. The washing was repeated with sufficient sterile distilled water to remove excess sodium hypochlorite. The last washing was checked for traces of chloride ion using 1% silver nitrate.

Table 3 Equipment list and suppliers/manufactures.

Equipment	Model	Suppliers/ Manufacturer
Autoclave	Amsco	Amsco Inc[1].
Balances	Mettler H54AR analytical balance Mettler P163N Mettler PE3600	Fisher Sci. Ltd.
Centrifuge	Sorvall	Ivan Sorvall, Inc[2].
Oven	Isotemperature	Fisher Sci, Ltd.
pH probe and meter	Orion research combination pH probe, Model 91-04 Orion research digital analyzer, Model 501	Canlab Ltd[3].
Rotary evaporator	Buchi	Brinkman[4].
Freeze dryer	Labconco	Fisher Sci. Ltd.
Quantum/Radiometer/ Photometer and spherical quantum sensor	L1-185B	L1 - COR, Inc[5].

[1] Erie, Pennsylvania, U. S. A.

[2] Ivan Sorvall, Inc. U. S. A.

[3] Canlab, Toronto, Ontario, Canada.

[4] Brinkman, Toronto, Canada.

[5] L1 - COR, Inc., U. S. A.

2.3.2 Seed imbibition:

Imbibition was initiated at room temperature (22° ± 1°C) in aerated distilled water. Fifty surface sterilized seeds were aseptically transferred to each of six 500 ml sterile beakers containing 50 ml sterile distilled water. The beakers were plugged and wrapped in aluminium foil to avoid any light exposure of the seeds. The seeds were well aerated with filtered air passed through a Pasteur pipette.

2.3.3 Seed germination:

The growth ability of imbibed wheat seeds was examined by germinating them in sterile distilled water. Fifteen imbibed seeds were placed in each of 100 X 15 mm standard sterilized, disposable plastic Petri dishes (Fisher Scientific Co.) lined with a layer of Whatman No. 4 filter paper, and germinated at 22° ± 1°C in a dark controlled environment with 2/3 of each seed covered with sterile distilled water (about 20 ml). Twenty such Petri dishes were used for each replicate.

2.4 Nitrogen fertilizer solution:

Four nutrient solutions were used, each containing 110 $\mu g\ ml^{-1}$ of total nitrogen. The nitrogen was supplied by modified Hoagland's nutrient solution of Brown and Hornby (1987), as either

nitrate in the form of $Ca(NO_3)_2.4H_2O$ (0.93 gL^{-1}), ammonium as $(NH_4)_2HPO_4$ (0.52 gL^{-1}), or in a mixture (0.465 gL^{-1} of $Ca(NO_3)_2.4H_2O$ and 0.26 gL^{-1} Of $(NH_4)_2HPO_4$), or urea (0.24 gL^{-1}). The other constituents of the plant nutrient solutions are listed in Table 4. The common portion of the nutrient solutions contained 0.12 gL^{-1} of Ca^{2+} and 0.21 gL^{-1} of PO_4^{3-}. The pH of the nutrient solutions was adjusted to 6.0 and they were autoclaved before use at 125°C and 1.4 Kg cm^{-2} for 25 min.

2.5 Plant culture and conditions:

Healthy germinated[1] seedlings showing no contamination were transferred to test tubes (17 X 2.5 cm) containing 10 g of washed sterile silica granular sand (60 - 120 mesh). Each test tube contained only one seedling. Fifteen test tubes were used for each treatment. In parallel experiments the test tubes were treated with 2.1 ml (about 60% of water capacity) of one or another of nutrient solutions at day zero. Each seedling was initially supplied with 231 µg total N. The seedlings were watered with sterile distilled water as needed. For each treatment, test tubes containing sand only (plant excluded) were supplied with the same amount of N - fertilizers and used as controls or non - rhizosphere soils. Also test tubes containing plants were supplied with water only as no nitrogen regime.

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[1] Germination was assessed to have taken place when the root tip pierced the pericarp.

Table 4 Constituents of plant nutrient solutions.

Chemicals	Formula	Amounts ($\mu g\ ml^{-1}$)
Magnesium sulphate	$MgSO_4$	240
Potassium di-hydrogen phosphate	KH_2PO_4	300
di-Potassium hydrogen phosphate	$K_2HPO_4.3H_2O$	350
Calcium sulphate	$CaSO_4.2H_2O$	500
Sodium molybdate	Na_2MoO_4	30
Manganese sulphate	$MnSO_4.H_2O$	1.67
Cupric sulphate	$CuSO_4.5H_2O$	0.24
Zinc sulphate	$ZnSO_4.7H_2O$	0.20
Boric acid	H_3BO_3	1.86
Ferric citrate	$FeC_6H_5O_7.3H_2O$	24

The test tubes were placed in a controlled environment incubator at 22:18°C (day:night) temperature, 5000 Lux light intensity[1] (Dura Test Vita Lite® Westinghouse Agro lite (40 w) and Cool Lite (40 w) fluorescent lamps, (16 h day^{-1}), and 60% relative humidity (RH). The seedlings were harvested after 7, 15 and 21 days of subsequent growth.

[1] Light photosynthetic active radiation of 550 $\mu E\ m^{-2}\ sec^{-1}$ measured by L1 - 185B Quantum/Radiometer/Photometer equipped with L1 - 193SB spherical quantum sensor serial No:SPQA 0536.

2.6 Growth measurement:

2.6.1 Plant height:

At the end of each growth period, the seedlings were removed from the test tubes and separated from the sand particles by vigorously shaking the root - sand mass into the test tube. Plant height was taken as the length from the base of the stem to the tip of the biggest leaf. The height was expressed in mm.

2.6.2 Plant dry weight:

Roots were rinsed clean of adhering sand particles using a continuous flow of distilled water and carefully brushing with a fine hair brush. In this way the root area was cleaned of visible adhering sand particles. The roots were blotted dry and the seedlings divided into roots and shoots. Dry weights were obtained by heating the root and shoot sections in an oven for 24 hours at 80°C. After equilibration with the atmospheric humidity, the weights were taken to an accuracy of ± 0.1 mg on an analytical balance.

2.7 The rhizosphere pH:

At the end of each growth period, the seedlings were gently removed from the sand and the root were shaken into the test tubes.

The pH of each test tube was measured separately using a sand ratio of 2 : 1 with 0.01 M $CaCl_2$. The 0.01 M $CaCl_2$ suspension was shaken for 5 min and the pH of the suspension taken using a combination pH electrode. The pH measurements for 15 test tubes were averaged. The procedure used here for soil pH was recommended jointly by the American Society of Agronomy and the American Society of Testing and Materials (1970). It has been used also by other workers (Smiley and Cook, 1971; Dormmar, 1988) and is reported to minimize variations in soil pH.

2.8 Free amino acids in the rhizosphere:

2.8.1 Extraction of free amino acids:

A review of the different techniques for extracting organic compounds from soil (Paul and Schmidt, 1960; Paul and Tu, 1965; Sowden and Ivarson, 1966; Gilbert and Altman, 1966; Ivarson and Sowden, 1969; Sowden, 1970; Warman and Bishop, 1985; Warman and Bishop, 1987) suggested that 10% ethanol was the most efficient solvent for extraction of amino acids from soil. It is a simple, mild and rapid procedure. Furthermore, it does not require steps involving washing, pH adjustment and chelating, which are necessary with other techniques (Warman and Bishop, 1987). Ethanol, because of its high dielectric constant is known to be a good solvent for most amino acids (Gilbert and Altman, 1966). During the extractions, plants were gently lifted from the sand and the root

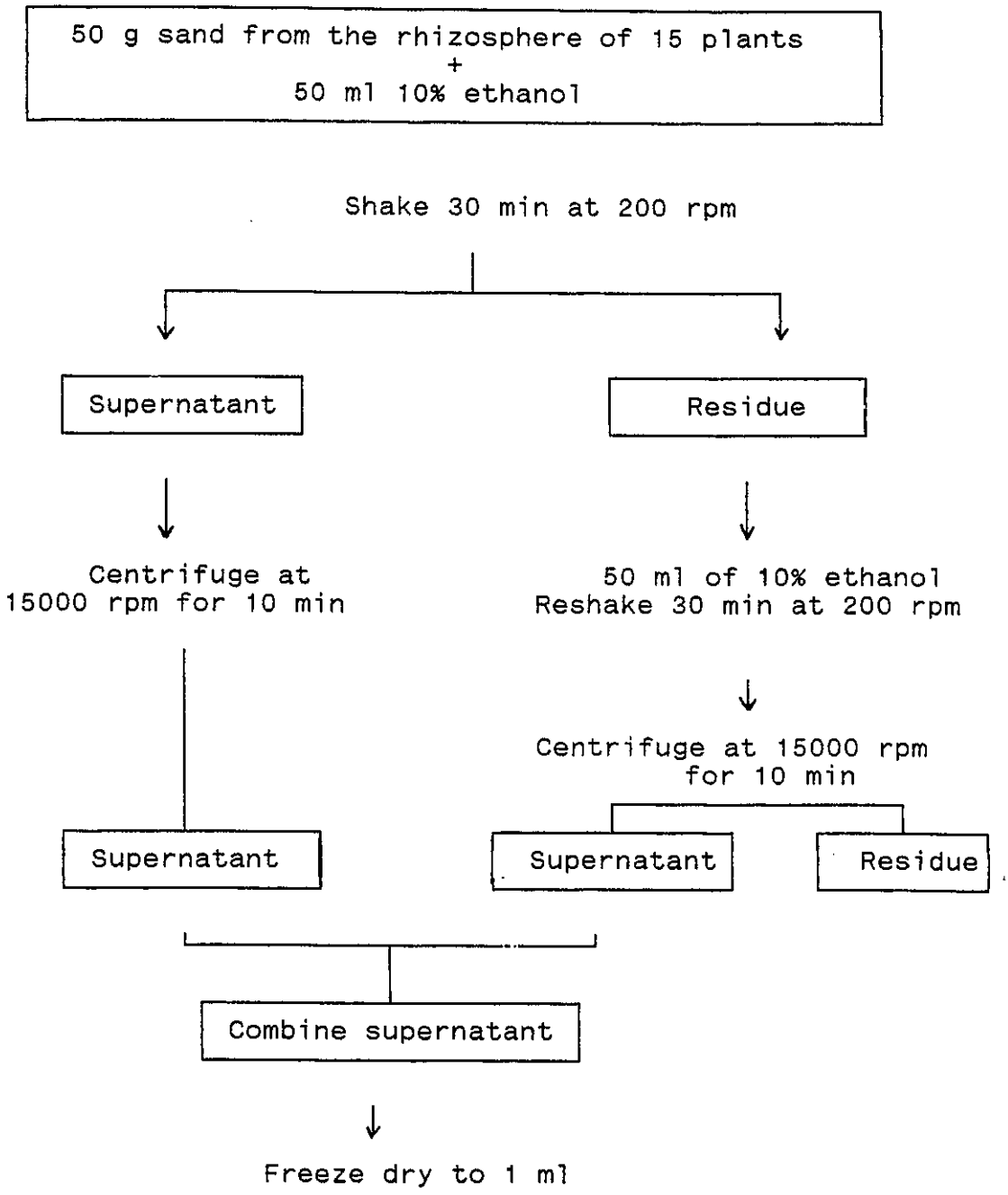
surfaces washed with sterile distilled water. This water was kept for later analysis. An outline of the rhizosphere extraction procedure is shown in Figure 3.

2.8.2 High performance liquid chromatography (HPLC) analysis of amino acids:

The HPLC analysis of soil constituents is relatively new, there being no published report prior to 1985 (Warman and Bishop, 1985). Reversed phase HPLC provides a sensitive, rapid and precise measure of soil amino acids (Warman and Bishop, 1987).

Two solutions were employed in the analysis of amino acids. The first (solution A) was prepared from anhydrous sodium acetate (4.1 g) and di-sodium hydrogen phosphate (7.1 g) dissolved in one liter of water. The pH of the solution was adjusted to 7.0 with concentrated acetic acid. Methanol (20 ml) and 20 ml of tetrahydrofuran were added to the solution. The second solution (B) was 65% aqueous methanol. Both solutions were passed through a Millipore filter (0.4µm) under vacuum and degassed before use. The analysis involved gradient elution.

Figure 3. Flow chart for extracting the free amino acids and sugars in the rhizosphere.


50 g sand from the rhizosphere of 15 plants
+
50 ml 10% ethanol
Shake 30 min at 200 rpm
Supernatant
Residue
Centrifuge at
15000 rpm for 10 min
50 ml of 10% ethanol
Reshake 30 min at 200 rpm
Centrifuge at 15000 rpm
for 10 min
Supernatant
Supernatant
Residue
Combine supernatant
Freeze dry to 1 ml

The elution gradient started with 80/20 mixture of A and B, then the concentration of solution B was increased to 100%, and held there for about 8 min. The total run time was about 24 min.The chromatographic gradient conditions are shown in Table 5. The analysis was carried out at room temperature. Freeze dried samples of amino acids were thawed and centrifuged at 1200 rpm for 2 min. They were filtered through disposable syringe filter (0.4 µm); diluted (100 to 1000 times) and α- aminoadipic acid (0.1 mM) was added as an internal standard. Before injection into the HPLC, the samples were derivatized on a precolumn with o-phthaldialdehyde (OPA) reagent.

Table 5 Chromatographic gradient conditions for HPLC analysis of OPA amino acid derivatives.

Time duration (min)	From (% A/B)	Flow rate (ml min^{-1})
0 (initial)	80 / 20	0
0 - 5	65 / 35	1.5
5 - 10	50 / 50	1.5
10 - 13	35 / 65	1.5
13 - 16	20 / 80	1.5
16 - 20	0 / 100	1.5
20 - 24	0 / 100	1.5

2.8.3 Precolumn derivatization of amino acids:

The following solutions were prepared for the derivatization of the amino acids.

Borate buffer: Boric acid (2.47 g) was dissolved in 90 ml of HPLC distilled water. The pH of the solution was adjusted to 9.5 with 1 N NaOH and the solution allowed to stand for 10 min. The pH was rechecked before making the solution volume up to 100 ml.

o-phthaldialdehyde stock solution (OPA): 50 mg of OPA was dissolved in 1 ml of methanol, to which 6.5 ml of borate buffer was added and the solution was then filtered.

Derivatization: The fluorogenic reaction of primary amines with o-phthaldialdehyde and 2-mercaptoethanol has been used in the separation and derivatization of amino acids by reverse-phase HPLC (Fleury and Ashley, 1983). 2.5 ml of OPA stock solution was added to 20 µl of 2-mercaptoethanol and kept at room temperature overnight. Aliquots (1.5 ml) of the derivatizing solution, a standard and the samples were placed in glass vials, stoppered and loaded on an autosampler. The HPLC autosampler was programmed to make injections, first from a vial containing the derivatizing 2solution and then from a sample vial.

After exactly 2 mins, 10 µl of the mixture (derivatizing reagent and sample) was transferred into the HPLC guard column. This procedure used is that of Fleury and Ashley (1983) and one modified by Joy (1989). The column flow rate was 1.5 ml min^{-1}. The

peak areas of the samples were compared with those of 18 amino acids standards[1] for quantization. Amounts of each amino acid (pmol) were calculated using equation 1.

Equation 1:

$$\text{Amounts of AA (pmole)} = \frac{S \times RF}{(S.\ int) \times (SS.\ int)} \qquad (1)$$

Where: S is the peak area of an amino acid AA in the sample

RF is the response factor of the same AA in the standard mixture

S. int is the peak area of the internal standard in the sample

SS. int is the peak area of the internal standard in the standard mixture

2.9 Rhizosphere soluble sugars:

2.9.1 Extraction of soluble sugars:

The soluble sugars in the rhizosphere were extracted with 10% ethanol. The same extracted sample for the amino acids was used for the soluble sugars (Figure 3).

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[1] The standard amino acid solution prepared by crystalline amino acids dissolved in water.

During preliminary tests for analyzing sugars in sand, no single procedure was found to be fully satisfactory . The methods described here for soluble and insoluble sugars are a modification of procedures outlined by Prehm and Scheil (1978); Dormaar (1984, 1988); and Siddiqi et al. (1986); and I. R. Siddiqui (1989) by personal communication.

2.9.2 Hydrolysis:

50 µl of 12 N HCl was added to a 1 ml of aliquot of the ethanol extract in a 10 X 120 mm test tube. The tubes were sealed and heated overnight at 100°C. The solutions were cooled in an ice bath before the reduction step.

2.9.3 Reduction:

The cooled hydrolysate was made alkaline by addition of 1 ml of 1 N NH_4OH, the pH being determined by the use of pH indicator paper. A solution (25 µg) of 0.2 M $NaBH_4$ in 0.1 N NaOH, was added to the hydrolysate and the mixture was kept at 4°C for 24 hrs. Excess of borohydride was decomposed by adding acetic acid until evolution of gas ceased. The solution was poured into a 50 ml round bottom flask and taken to dryness using a rotary evaporator (35°C). The boric acid was removed by adding 1 ml of methanol and evaporation. This step was repeated three times. The dry residue was subsequently oven dried at 60°C for 0.5 hr.

2.9.4 Acetylation:

200 µl of pyridine and 200 µl of acetic anhydride were added to the dried residue. The mixture was refluxed at 100°C for 20 min. After cooling, the sample was diluted with 2 ml of deionised water and rotary evaporated to remove the extra pyridine. The addition and evaporation steps were repeated five times. The dry residue was dissolved in 4 ml of chloroform and dried under a stream of nitrogen. The residue was redissolved in 100 µl of chloroform and injected into a gas chromatograph.

2.10 Rhizosphere insoluble sugars:

2.10.1 Extraction of insoluble sugars:

The insoluble sugars in the rhizosphere were treated with 72% H_2SO_4 for 1 hour at 5°C. The mixture was shaken occasionally. The hydrolysate was diluted with 70 ml of water and the mixture refluxed at 100°C for 2 hrs. After cooling, the mixture was filtered through Whatman filter paper No. 4 and the volume was adjusted to a 100 ml.

2.10.2 Neutralization:

Five ml of the hydrolysate was neutralized with saturated $Ba(OH)_2$ (about 80 ml) to a pH of 5.5. The mixture was centrifuged

at 500 g for 10 min and filtered with a fine pyrex fritted disc funnel (pore size 4 to 5.5 µm). The volume of the sample was reduced to 15 ml in a rotary evaporator and the pH readjusted to 7.5 with 0.1 N NaOH. The sample was then placed on a column of 5 ml Amberlite IRA-400 (20 to 50 mesh; Cl form) to remove uronides, the resin was eluted with 30 ml water. The eluate was reduced to 15 ml on a rotary evaporator and transferred to a 50 ml round bottom flask with extra washing with 15 ml distilled water.

2.10.3 Reduction:

The sample was treated with 35 mg of $NaBH_4$ in a round bottom flask overnight at room temperature. The extra $NaBH_4$ was decomposed with acetic acid as previously reported. The boric acid was removed by reduction of the sample volume to 1 ml, diluting to 10 ml with methanol and reducing it to dryness. This step was repeated three times. The residue was oven dried before acetylation.

2.10.4 Acetylation:

The reagent consisting of 1 : 2.5 pyridine, acetic anhydride (200 µl : 500 µl) was added to the dried residue and the solutions refluxed at 100°C for 1 hour. The excess pyridine was removed by the addition and evaporation of water five times. The final residue was treated the same as soluble sugars.

2.11 Carbohydrate analysis:

Both the soluble and insoluble sugars were analyzed by gas chromatography (see section 2.2). The column temperature was programmed starting at 100°C for 1 min; then programmed to 220°C at a rate of 10°C min^{-1}. The carrier gas flow was 5 ml min^{-1} of helium and the detector flows were 300 ml min^{-1} of air and 45 ml min^{-1} of hydrogen plus a make up gas of 16 ml min^{-1} of nitrogen. The peak heights of the sample were compared with those of standards. The standard solution consisted of a mixture of acetylated rhamnose, fucose, ribose, arabinose, xylose, allose, mannose, galactose and glucose (500 ng ml^{-1}).

2.12 Data evaluation:

Each experiment was replicated four times for each time period. The results were tested for homogeneity of variance (Bartlett, 1937) and treatments were compared by the use of "Manova" spssx (Statistical packages for the social sciences) at a 0.05 significance level. A VM/370 main frame computer was used for the statistical analyses. Sigmaplot was used to do the regression analyses.

CHAPTER 3

RESULTS

3.1 Growth studies:

3.1.1 Plant height:

After fertilizing the wheat seedlings with different N- fertilizers (NH_4^+, NO_3^-, NH_4^+ & NO_3^- and urea) and growing them for the period of one to three weeks, the plant height of the seedlings was measured at given time periods. The height of the seedlings increased under all fertilizer treatments up to 7 days and was followed by a slower growth rate afterward (Figure 4 , B). No significant difference ($P>0.05$) in seedling growth under various N-fertilizers was noted.

3.1.2 Plant dry weight:

A rapid increase in total dry weight resulted with different N-fertilizers until 7 days (Figure 4 , A). After 7 days, the growth rate decreased. No significant differences ($P>0.05$) were observed in the growth rate (dry weight) of seedlings under different N-fertilizers. However, slightly more shoot dry matter was noted under nitrate or mixed nutrients. Under mixed nutrition, the shoot/root ratio of wheat increased the most (Table 6).

Table 6 Effect of N-fertilizers on dry matter (mg) partitioning and shoot/root ratio of winter wheat after 21 days. Standard deviations shown.

Treatment	Mean Root Dry wt.(mg) (S.D.)	Mean Shoot Dry wt.(mg) (S.D.)	Shoot/root ratio
NH_4^+	32.2±4.9	26.1±2.1	0.81
NO_3^-	33.1±8.4	29.3±1.8	0.89
NH_4^+&NO_3^-	30.0±3.5	30.7±2.0	1.2
$(NH_2)_2.CO$	33.1±0.06	27.2±0.22	0.82

3.2 The rhizosphere pH:

Different N-fertilizers influence the ability of the roots to alter the pH of the rhizosphere (Figure 5). The use of various N-fertilizers did not contribute significantly to any change in the controls (sand without plant). The sand remained at pH 6.0 in all treatments. The rhizosphere pH decreased when the plants were supplied with either ammonium, a mixture of ammonium and nitrate, or urea (Figure 5). However, the pH increased in the rhizosphere of plants which were treated with nitrate. The greatest pH decrease was observed in the rhizosphere of plants which were supplied with

ammonium.

3.3 Free amino acids in the rhizosphere:

All the rhizosphere soils contained most of the amino acids mentioned in the materials and methods section 2.1. Concentrations of amino acids in experimental rhizosphere were corrected where necessary (i.e. concentration of amino acids in controls subtracted from the experimental rhizosphere. Maximum amount of amino acids in controls was 0.01 µmol/ml extract at 7 days). The percent recovery with 10% ethanol extracts for acidic amino acids such as aspartic acid and glutamic acid was 75%, for neutral amino acids such as alanine, glycine and leucine, 80%, and for basic amino acids such as histidine, arginine and lysine, 60%.

Those plants which were treated with water (no nitrogen) showed the sign of stress after 5 days (pale green leaves). Therefore, their results did not considered for comparison. Howevere, their rhizosphere amino acids were measured for 7 days and the results were shown in Appendix A. The quantities of amino acids were significantly ($P<0.05$) different with different N-fertilizers. The total quantity of amino acids extracted from the rhizosphere was greatest in the rhizosphere of plants supplied with ammonium at the three time periods (Figure 6; Table 7). The amino acids of the rhizosphere decreased sharply after 15 days of plant growth. The total amino acids decreased more with the urea

treatment than with those treated with other fertilizers. Table 7 shows the ratio of the amount of total amino acids in the rhizosphere of ammonium-treated plants as compared to the amount of total amino acids for other fertilizers at different time periods. Up to 7 days, almost twice the amounts of amino acids exuded from plants which were supplied with ammonium than from those supplied with nitrate. In contrast, the difference between ammonium and urea is less than other fertilizers. All numerical data are presented in Appendix A.

Table 7 Ratio of total amino acids in the rhizosphere under ammonium treatment vs. other treatments.

Ratio	Days 7	15	21
$NH_4^+:NO_3^-$	1.7	1.1	1.2
$NH_4^+:NH_4^+\&NO_3^-$	1.2	0.71	0.96
$NH_4^+:(NH_2)_2.CO$	1.1	2.3	2.2

More nitrogen exuded from plants under ammonium supply as compared to other nitrogen fertilizers (Table 8). The total nitrogen exuded from plants under ammonium supply was two times the nitrogen exuded from nitrate-fed plants at 7 days. However, the exuded nitrogen under ammonium treatment is 1.5 and 1.3 fold that of those treated with a mixed or urea nutrition respectively (Table 8). With time,

the difference between nitrogen exudate under different nitrogen fertilizers decreased. For example, the exuded nitrogen was 1.3 and 2 fold under ammonium supply in comparison to nitrate or urea treatments at 15 days. At 21 days, the differences between total exudate nitrogen under ammonium supply were 1.4, 1.2 and 1.8 fold that of those under nitrate, a mixture or urea supplies.

The composition of amino acids was also different for each N-fertilizer. The total acidic amin2o acids (aspartic acid and glutamic acid) were significantly different ($P<0.05$) at different time periods (Figure 7, A). At 7 days of plant growth, the total acidic amino acids were 1.6 and 1.5 times more in the rhizosphere of plants supplied with ammonium or urea as compared to those in the rhizosphere of plants treated with nitrate. After 15 days, the rhizosphere of those plants treated with a mixture or with nitrate alone, contained more acidic amino acids (2-7 times) than those which were fed on ammonium or urea. The amounts of acidic amino acids were least (6 times as compared to nitrate) in the rhizosphere of plants which were supplied with urea. The total basic amino acids (histidine, arginine and lysine) was higher in the rhizosphere of plants treated with either nitrate (2 times vs. ammonium) or urea (2.5 times as compared to ammonium) at 7 days (Figure 7, B). However, by 15 days the basic amino acids increased in the rhizosphere of those plants treated with a mixture of ammonium and nitrate and decreased in the rhizosphere of plants treated with nitrate or urea.

Table 8 Effects of various N-fertilizers on nitrogen content (nmol/ml extract/plant) of the rhizosphere at different time periods.
Standard deviations shown.

Treatments	Days 7	15	21
NH_4^+	126±35.8	22.6±1.70	4.28±1.6
NO_3^-	64.8±19.0	17.3±6.97	3.03±1.40
NH_4^+&NO_3^-	84.9±30.7	32.8±5.90	3.46±1.80
$(NH_2)_2.CO$	97.7±20.1	11.4±5.58	2.41±1.61

The total amount of neutral amino acids (alanine, leucine, isoleucine, valine and phenylalanine) was less (1.3 times as compared to ammonium) in the rhizosphere of nitrate-treated plants at 7 days (Figure 8). At 21 days of plant growth, the total acidic, basic and neutral amino acids were lower in the rhizosphere of plants supplied with urea than those of plants supplied with other fertilizers.

The distribution of amino acids was also different for the various N-fertilizers at a given time and during the course of the experiment. At 7 days of plant growth, the most prominent amino acids were glutamic acid, glutamine and alanine under all treatments (Figure 9). At 15 days of plant growth, glutamine levels

decreased, and serine was not detected (Figure 10). At 21 days other amino acids such as asparagine, glutamine, histidine and arginine were also not detectable (Figure 11). However, gamma amino butyric acid was prominent at 21 days. Comparing the composition of amino acids under different fertilizer regimes at any one of the three time periods showed that in all cases there was a significant difference between treatments ($P<0.05$). At 7 days, plants supplied with ammonium had greater amounts of aspartic acid, glutamine and gamma amino butyric acid compared with those supplied with nitrate, a mixture of ammonium and nitrate or urea. In the case of the nitrate or urea treatments, histidine levels were higher than for the plants treated with ammonium or a mixture of ammonium and nitrate (Figure 9). However, gamma amino butyric acid was not detected in the rhizosphere of plants treated with nitrate. Differences in composition between treatments were more obvious in aspartic acid, threonine, isoleucine and arginine after 15 days (Figure 10). At 21 days of plant growth, the amino acid distributions were more uniform. However, an amino acid tentatively identified as α-amino butyric acid was detected in the rhizosphere of plants which were treated with nitrate, a mixture of ammonium and nitrate or urea.

3.4 Soluble sugars in the rhizosphere:

Standard quantities of the acetylated sugars; glucose, galactose, mannose, rhamnose, fucose, xylose and arabinose were

passed through the gas chromatograph as reference standards. Soluble sugars in the rhizosphere samples were identified and quantified in relation to known amounts of the standards. The percent recovery for soluble sugars was up to 80 to 90%.

All rhizosphere soils (sand) contained most of the sugars mentioned. There were no significant amounts of soluble sugars in the controls (non - rhizosphere soil). The total quantities of soluble sugars were significantly different under various N-fertilizers at a given time and over time. Total soluble sugars increased faster in ammonium fed and nitrate fed plant rhizospheres than those supplied with an equal mixture of ammonium and nitrate or with urea (Figure 12). Under ammonium or nitrate supply, the rhizosphere soluble sugars decreased sharply at 15 days, while, under a mixture of ammonium and nitrate or urea the soluble sugars increased until 15 days. The total soluble sugars were highest in the rhizosphere of ammonium fed plants and lowest in urea fed plants. Table 9 shows the ratio of the amount of soluble sugars in the rhizosphere of ammonium fed plants in relation to the amounts of soluble sugars for other fertilizers at different time periods. The amount of soluble sugars in the rhizosphere under ammonium fertilizer was almost 50 times greater than that of urea fertilizer at 7 days of plant growth. All numerical data are presented in Appendix A.

A closer look at the individual soluble sugars showed that each sugar was also affected differently by the various N-fertilizers. Glucose, the predominant soluble sugar in the rhizosphere under all treatments, showed a fast increase under ammonium or nitrate supply at 7 days, followed by a decrease at 15 and 21 days (Figure 13). In those which were fed on a mixture of ammonium and nitrate or urea, glucose increased slowly until 15 days with a sharp decrease thereafter. The same pattern occurred for arabinose (Figure 14) and xylose (Figure 15). However, the amounts of arabinose and xylose were greater (1.1 times) in nitrate-fed plant rhizospheres than those of ammonium-fed plant at 7 days, and glucose was greater (1.2 times) in ammonium-fed plant rhizospheres. At 15 days of growth, the rhizosphere of plants which were supplied with a mixture of ammonium and nitrate contained higher amounts (1.3 times vs. ammonium) of glucose, arabinose and xylose than those supplied with the other fertilizers.

The pattern of galactose differed from those of glucose, arabinose and xylose (Figure 16). In the rhizosphere of ammonium-fed plants a high amount of galactose was detected at 7 days, followed by a drop at 15 days. However, in the rhizosphere of those plants which were fed on nitrate or a mixture of ammonium and nitrate or urea, galactose increased slowly until 15 days followed by a decrease at 21 days. The pattern of appearance of galactose in the rhizosphere was almost similar in nitrate as the pattern for

a mixture of ammonium and nitrate-fed plants. At 7 days, mannose was not detected in the rhizosphere of ammonium-fed plants.

Table 9 Ratio of total soluble sugars in the rhizosphere under ammonium treatment vs. other treatments at different time periods.

Ratio	Days 7	15	21
NH_4^+ : NO_3^-	1.6	1.3	4.7
NH_4^+ : $NH_4\&NO_3$	22	0.7	5
NH_4^+:$(NH_2)_2$.CO	49.5	1.2	18

However, mannose was detected in those which were fed nitrate, a mixture of ammonium and nitrate or urea (Figure 17). The highest amount of mannose (28 times vs. ammonium) was detected in nitrate-fed plants. No mannose was detected at 21 days under any of the treatments. Unlike other sugars, ribose increased over time in the rhizosphere for the ammonium treatment and a slow development was detected in the rhizosphere of plants supplied with urea (Figure 18). Very small amounts of rhamnose were detected in the rhizosphere of nitrate and ammonium-fed plants. Under ammonium and a mixture of ammonium and nitrate, the main soluble sugars in the rhizosphere were glucose and galactose and fluctuations of these two sugars were more common than those of arabinose and xylose

(Figures 19, A and B). In the rhizosphere of plants which were treated with nitrate the main sugar was glucose and its fluctuation was obvious (Figure 20, A). Intermediate effects have been observed for the rhizosphere of plants which were fed with urea, with the fluctuations of both glucose and galactose over time and appearance of more galactose at 7 days and more glucose at 15 days (Figure 20, B).

The sugar composition was also different under various N-fertilizers. Figures 21, 22 and 23 shows the array of sugars in the rhizosphere of plants under various N-fertilizers at a given time. At 7 days no mannose was detected under urea nutrition (Figure 21). At 15 days mannose and ribose were detected in the rhizosphere of ammonium and urea treated plants respectively and rhamnose was detected only in the rhizosphere of nitrate- fed plants. Therefore the number of sugars increased in the rhizosphere at 15 days (Figure 22). At 21 days mannose was not detected in any treatment, and considerable amounts of glucose were detected only in the rhizosphere of ammonium-fed plants. Rhamnose was also detected in the rhizosphere of both ammonium and nitrate-fed plants (Figure 23).

3.5 Insoluble sugars in the rhizosphere:

Gas chromatography of the reduced and acetylated hydrolyzates of all treatments detected the presence of two deoxy-sugars:

rhamnose and fucose; three pentoses: ribose, arabinose and xylose and three hexoses: mannose, glucose and galactose from the insoluble fraction. There was a small amount of insoluble sugar in the controls which was subtracted from the related sets. The percent recovery for insoluble sugars was 60 to 90%. This recovery corresponds to the recovery of other workers (Dormaar, 1984; 1988).

The quantities of insoluble sugars were significantly different ($P<0.05$) under the different N-fertilizers. The fertilizer mixture of ammonium and nitrate had an greater effect on amounts of the insoluble sugars in the rhizosphere than the nitrate fertilizer did (Figure 24). The ratio of total insoluble sugars exuded into the rhizosphere under ammonium fertilizer as compared to other fertilizers is shown in Table 10. At 7 days of plant growth, more than 4 times the amounts of insoluble sugars was detected in the rhizosphere of ammonium-fed plants in comparison to nitrate-fed plants. The ratio was less (0.39 times and 0.57 times) under mixed or urea treatments as compared to ammonium treatment. However, more insoluble sugars were detected in the rhizosphere of nitrate-fed plants at 15 and 21 days. For example, the total insoluble sugars detected under ammonium treatment was only 0.37 times that of those under nitrate treatment at 21 days (Table 10). All numerical data are presented in Appendix A.

The major components of the insoluble sugars were glucose, galactose and xylose under all treatments. Glucose was highest in the rhizosphere of plants supplied with urea and a mixture of ammonium and nitrate (Figure 25). A slow release of glucose occurred in the rhizosphere of plants which were supplied with nitrate (Figure 25). The rhizosphere of plants which were treated with a mixture of ammonium and nitrate or urea contained more insoluble arabinose and xylose than those which were treated with ammonium or nitrate at 7 days (Figures 26 and 27). Arabinose decreased in all treatments with the exception of nitrate fertilizer at 15 days (Figure 26). A slow increase of arabinose was detected in the rhizosphere of plants treated with nitrate. Insoluble mannose was highest in the rhizosphere of plants supplied with a mixture of ammonium and nitrate at 7 days (Figure 28). However, development of mannose was slow in the rhizosphere of plants supplied with nitrate. Galactose was the major component of the insoluble sugars in the rhizosphere of plants supplied with ammonium (Figure 29, A). However, in those which were treated with nitrate, a mixture of ammonium and nitrate or urea the major components of insoluble sugars were glucose and xylose (Figures 29, B; 30, A and B). Insoluble sugars increased in the rhizosphere of plants supplied with nitrate with time (Figure 30, B). In contrast, in those which were treated with either ammonium, a mixture of ammonium and nitrate, or urea, insoluble sugars decreased over time (Figures 29 and 30).

Table 10 Ratio of total insoluble sugars in the rhizosphere under ammonium treatment vs. other treatments.

Ratio	Days 7	15	21
$NH_4^+:NO_3^-$	4.0	2.3	0.37
$NH_4^+:NH_4^+\&NO_3^-$	0.39	1.6	1.1
$NH_4^+:(NH_2)_2.CO$	0.57	1.7	0.86

The array of the insoluble sugars was also different under various N-fertilizers. At 7 days, fucose was not detected in the rhizosphere of plants treated with a mixture of ammonium and nitrate or urea and xylose , mannose and glucose were not detected in the rhizosphere of plants supplied with nitrate (Figure 31). After 15 days, the kinds of insoluble sugars decreased under all the treatments (Figure 32). Fucose was not detected in the rhizosphere of plants treated with ammonium or nitrate, and galactose was detected only in the rhizosphere of plants fed ammonium. There was not a significant ($P>0.05$) amount of galactose in the rhizosphere of plants treated with urea at 21 days (Figure 33).

Figure 4. Effects of various N-fertilizers on the growth of winter wheat (Triticum aestivum v. Fredrick), n= 4 replicate, 95% confidence intervals shown,

A : Total dry weight, 1st order regression have been done on log dry weight. r NH_4= 0.84; r NO_3= 0.81; r Mix=0.79; r Urea= 0.83.

B : Height, 1st order regression on log height, r NH_4=0.94; r NO_3= 0.95; r Mix= 0.93; r Urea=0.94.

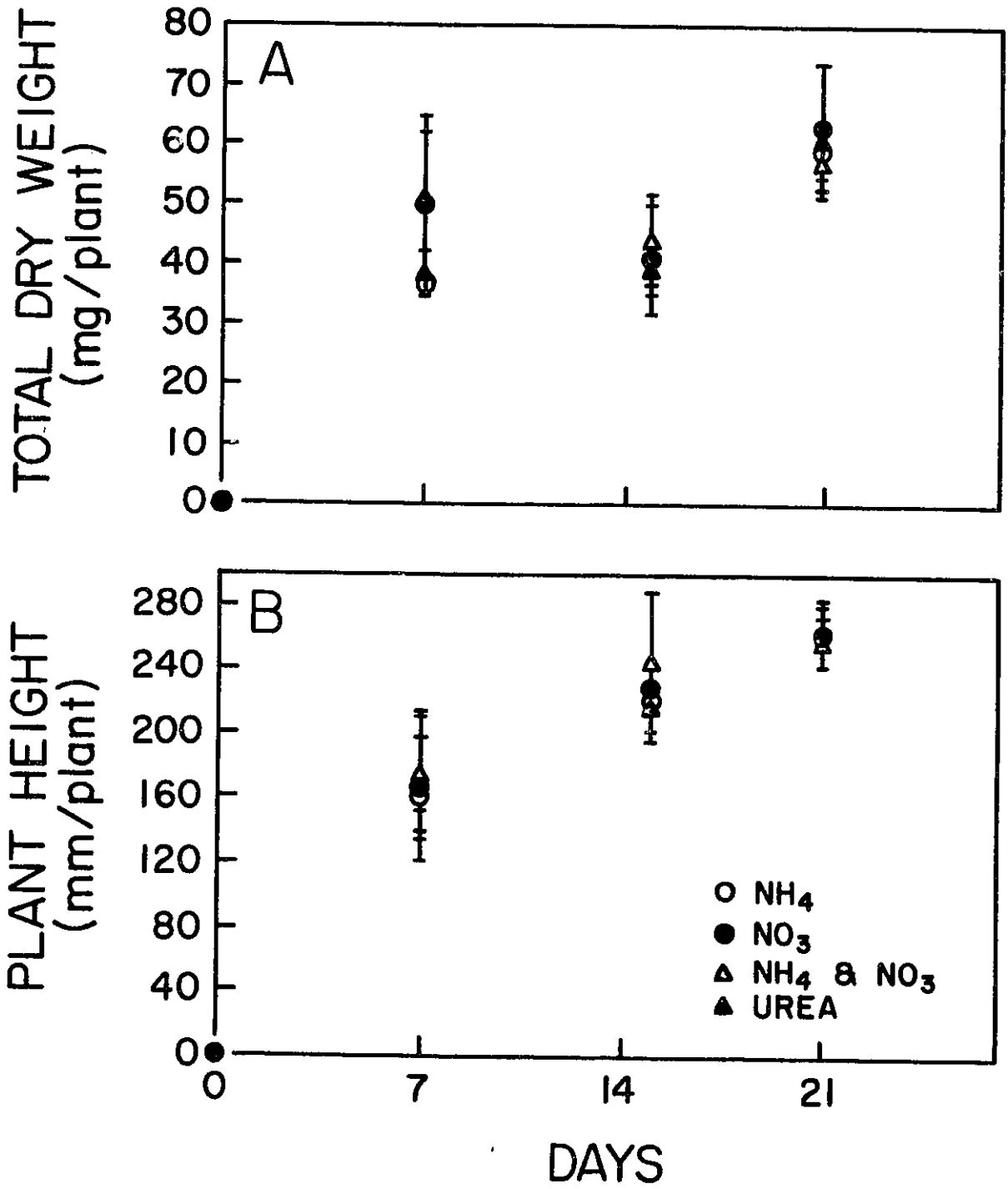

TOTAL DRY WEIGHT (mg/plant)
80
70
60
50
40
30
20
10
0
A
PLANT HEIGHT (mm/plant)
280
240
200
160
120
80
40
0
B
O NH4
● NO3
△ NH4 & NO3
▲ UREA
0
7
14
21
DAYS

Figure 5. Effects of various N-fertilizers on the rhizosphere pH of winter wheat, n= 4 replicates, 95% confidence intervals shown.

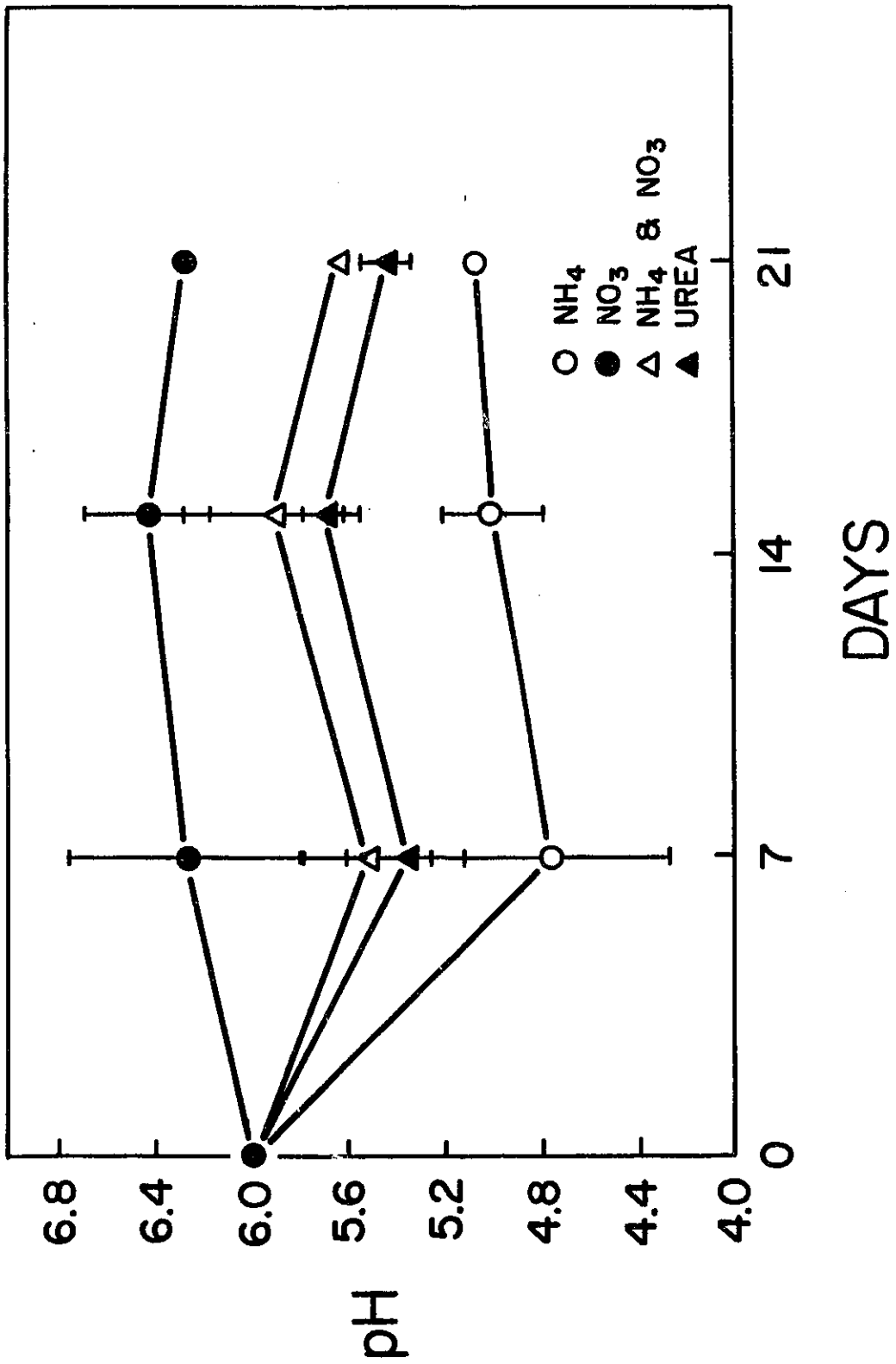

pH
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
0
7
14
21
DAYS
O NH4
● NO3
Δ NH4 & NO3
▲ UREA

Figure 6. Effects of various N-fertilizers on total rhizosphere amino acids at different time periods, n= 4 replicates, 95% confidence intervals shown.

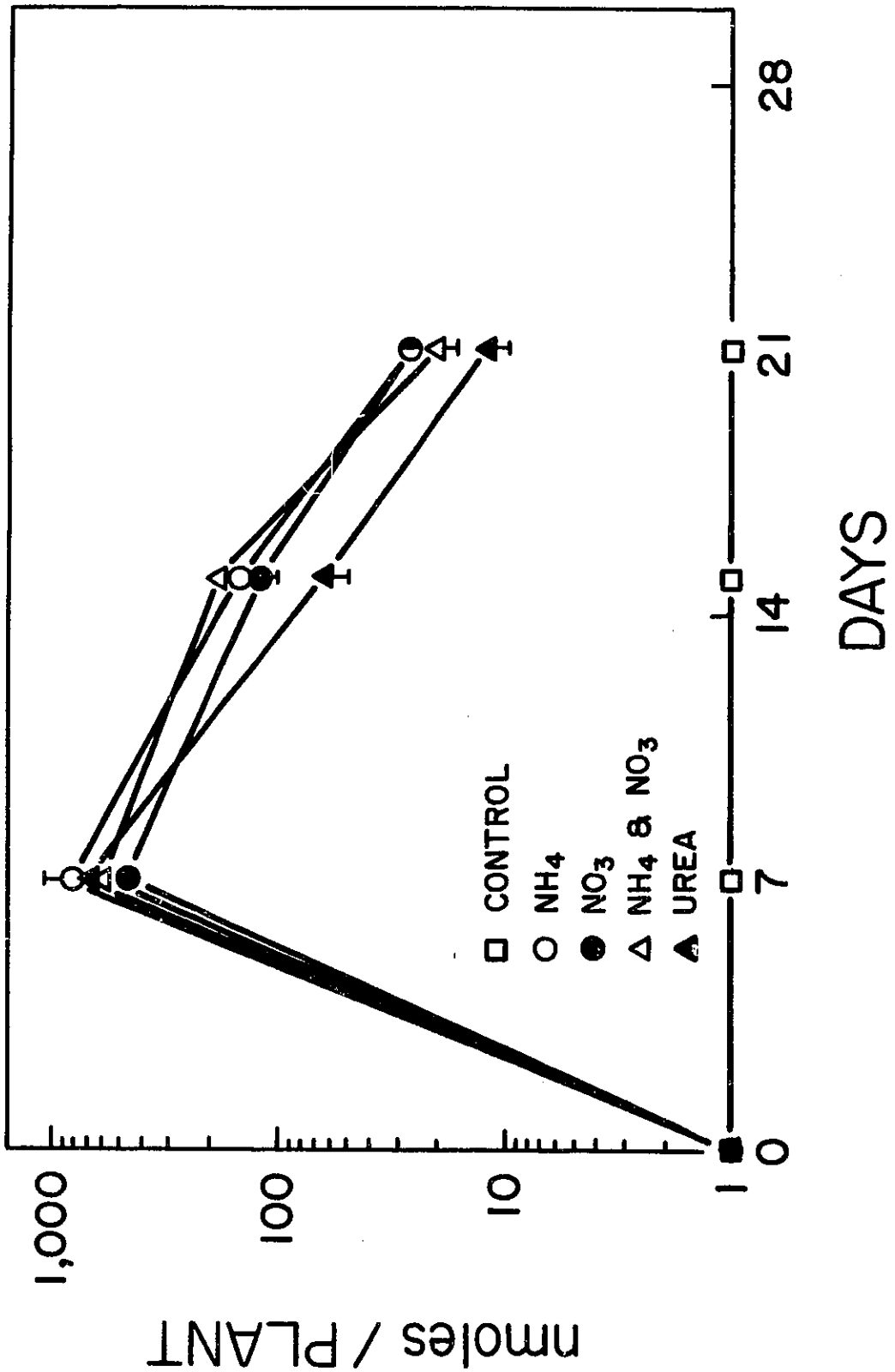

nmoles / PLANT
1,000
100
10
1
0
7
14
21
28
DAYS
CONTROL
NH4
NO3
NH4 & NO3
UREA

Figure 7. Effects of N-fertilizers on (A) acidic and (B) basic rhizosphere amino acids at different time periods, n= 4 replicates, 95% confidence intervals shown.

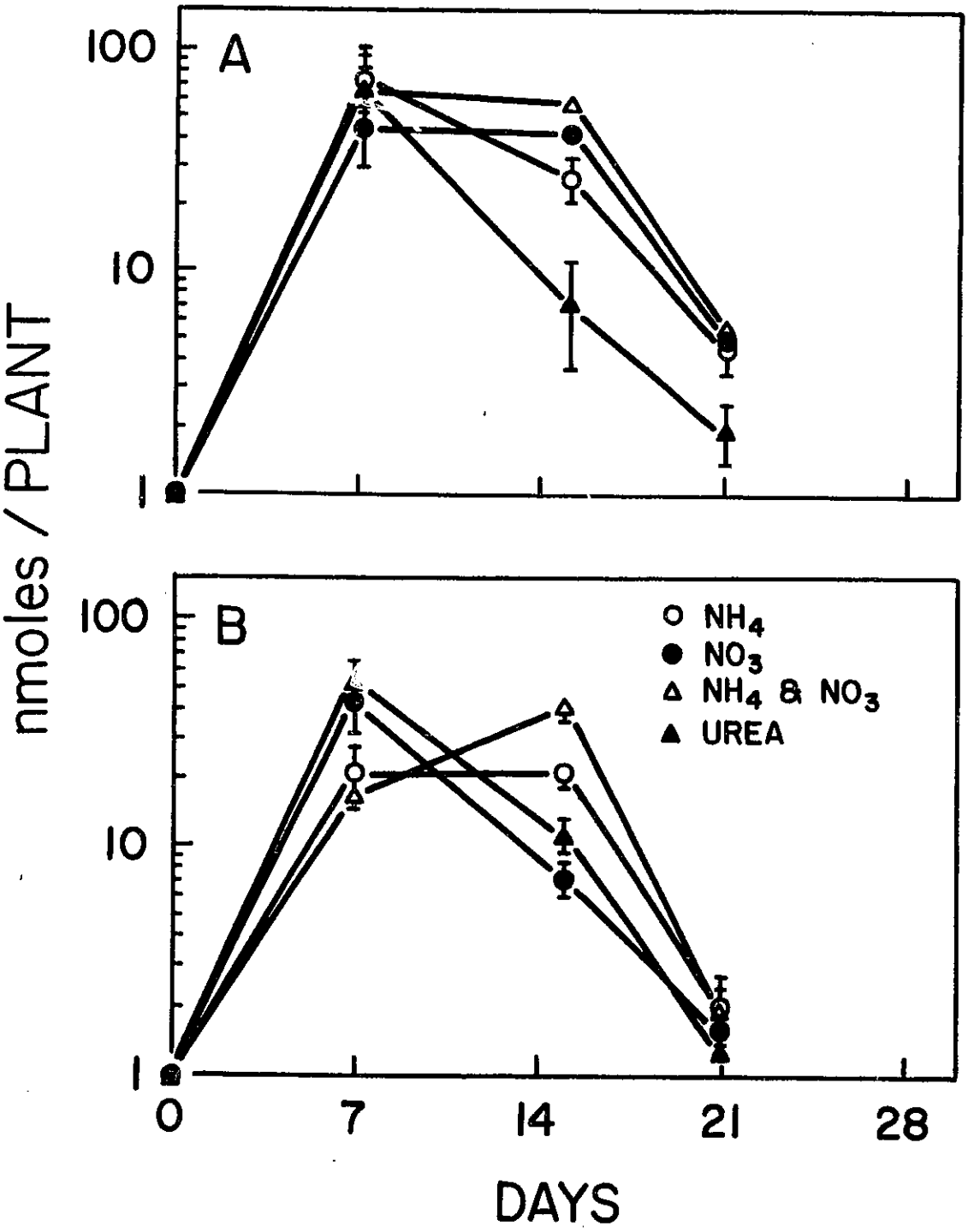

100
A
10
1
nmoles / PLANT
100
B
10
1
NH4
NO3
NH4 & NO3
UREA
0
7
14
21
28
DAYS

Figure 8. Effects of various N-fertilizers on the neutral amino acids in the rhizosphere at different time periods, n= 4 replicates, 95% confidence intervals shown.

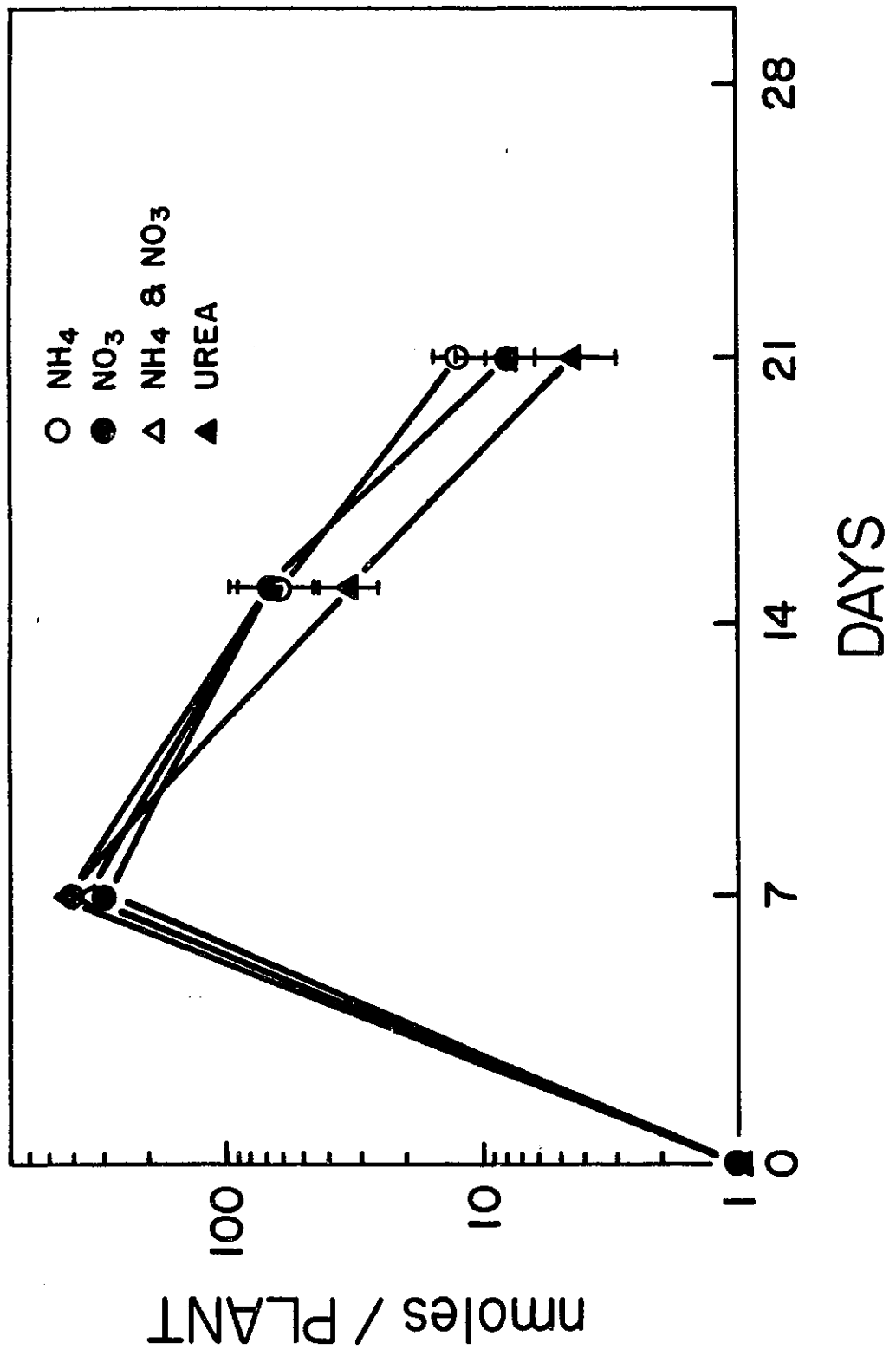

nmoles / PLANT
100
10
1
0
7
14
21
28
DAYS
NH4
NO3
NH4 & NO3
UREA

Figure 9. (part 1) Effects of N-fertilizers on the distribution of the rhizosphere amino acids at 7 days, n= 4 replicates, 95% confidence intervals shown. Histidine was not detected in the rhizosphere of ammonium and mixture treated plants.

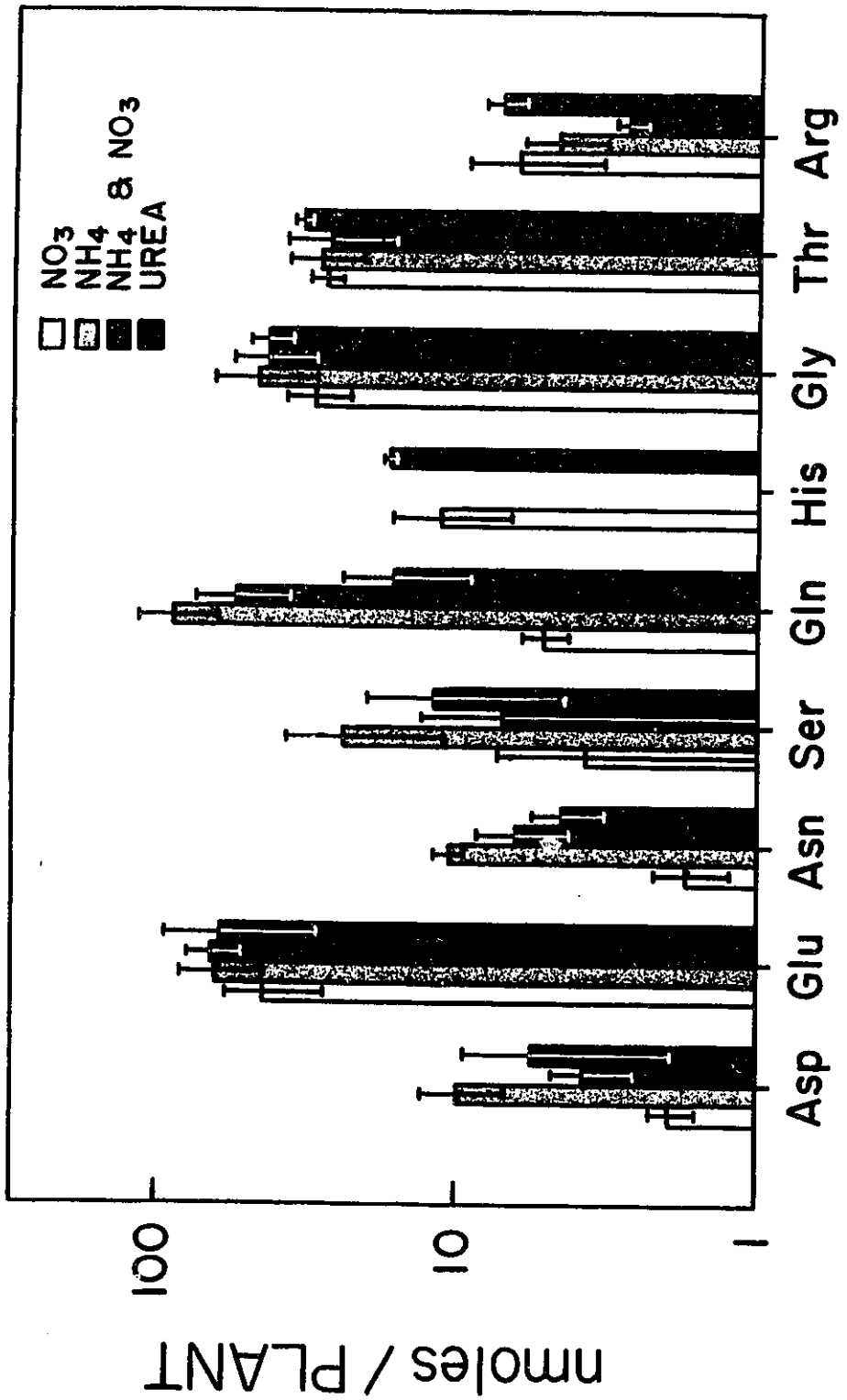

NO3
NH4
NH4 & NO3
UREA
100
10
1
nmoles / PLANT
Asp
Glu
Asn
Ser
Gln
His
Gly
Thr
Arg
AMINO ACIDS

Figure 9 (cont'd) (part 2) Effects of N-fertilizers on the distribution of the rhizosphere amino acids at 7 days, n= 4 replicates, 95% confidence intervals shown.

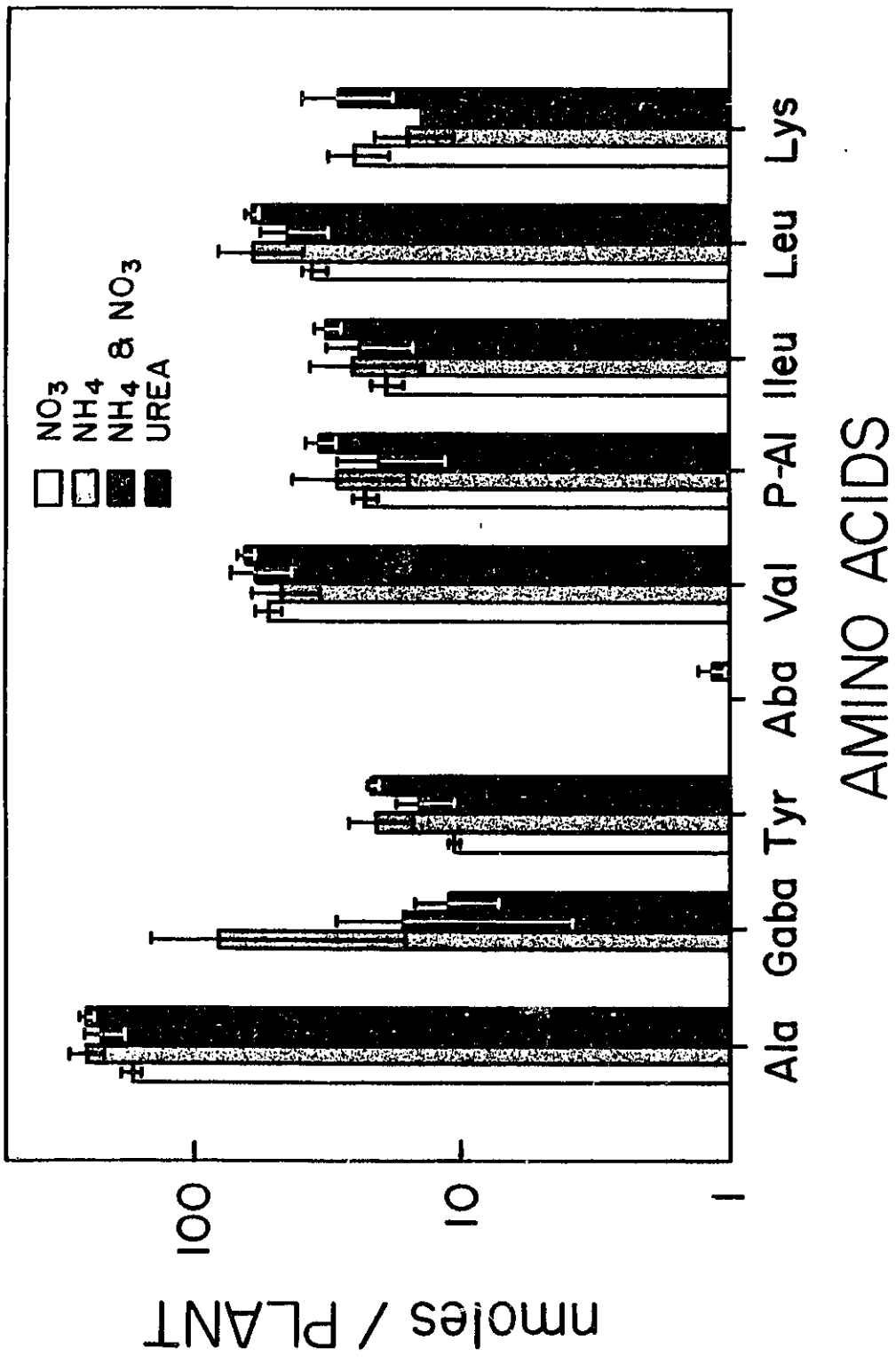

nmoles / PLANT
100
10
1
NO3
NH4
NH4 & NO3
UREA
Ala
Gaba
Tyr
Aba
Val
P-Al
Ileu
Leu
Lys
AMINO ACIDS

Figure 10. (part 1) Effects of N-fertilizers on the distribution of the rhizosphere amino acids at 15 days, n=4 replicates, 95% confidence intervals shown.

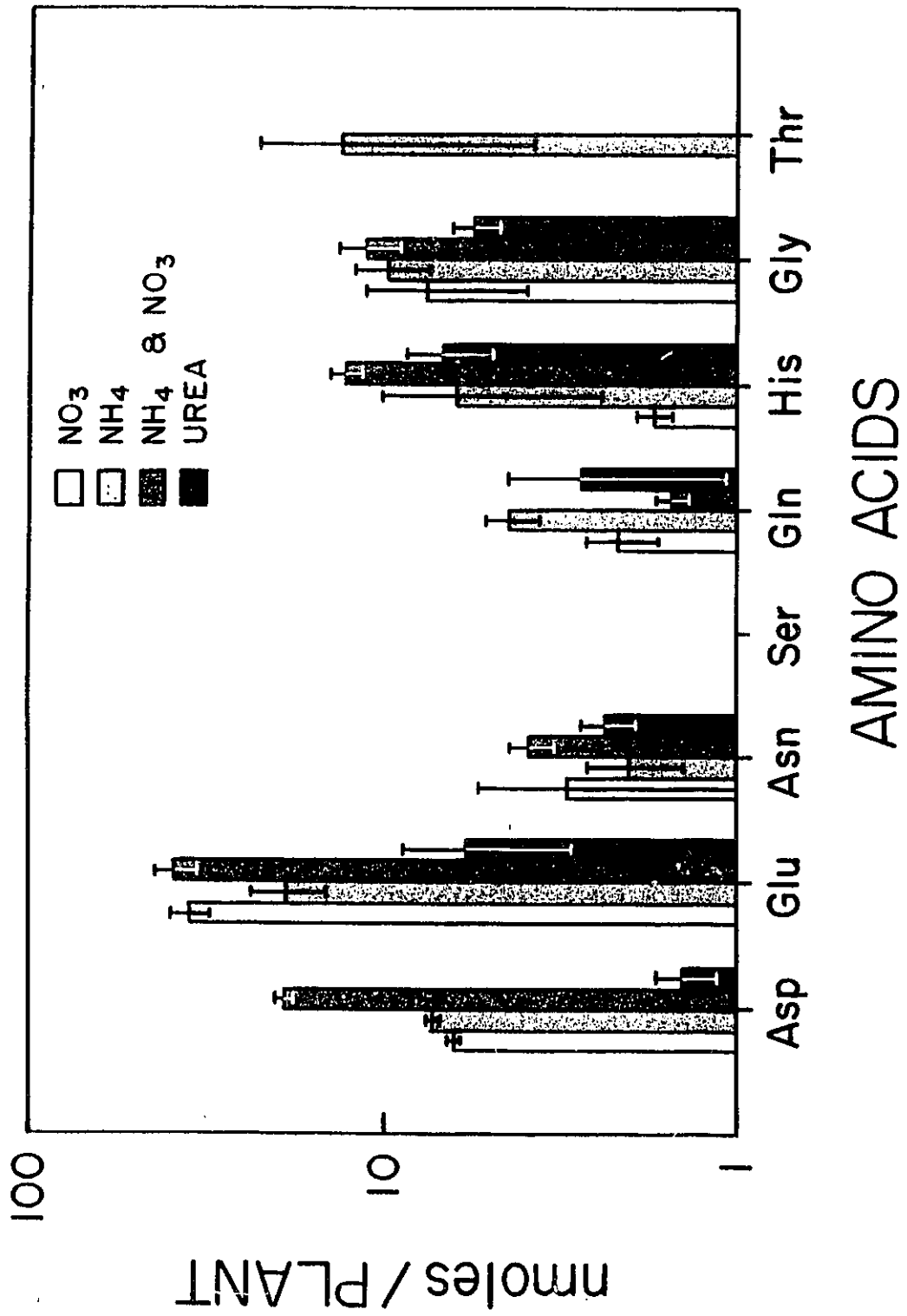

100
10
1
nmoles / PLANT
NO3
NH4
NH4 & NO3
UREA
Asp
Glu
Asn
Ser
Gln
His
Gly
Thr
AMINO ACIDS

Figure 10 (cont'd) (part 2) Effects of N-fertilizers on the distribution of the rhizosphere amino acids at 15 days, n=4 replicates, 95% confidence intervals shown.

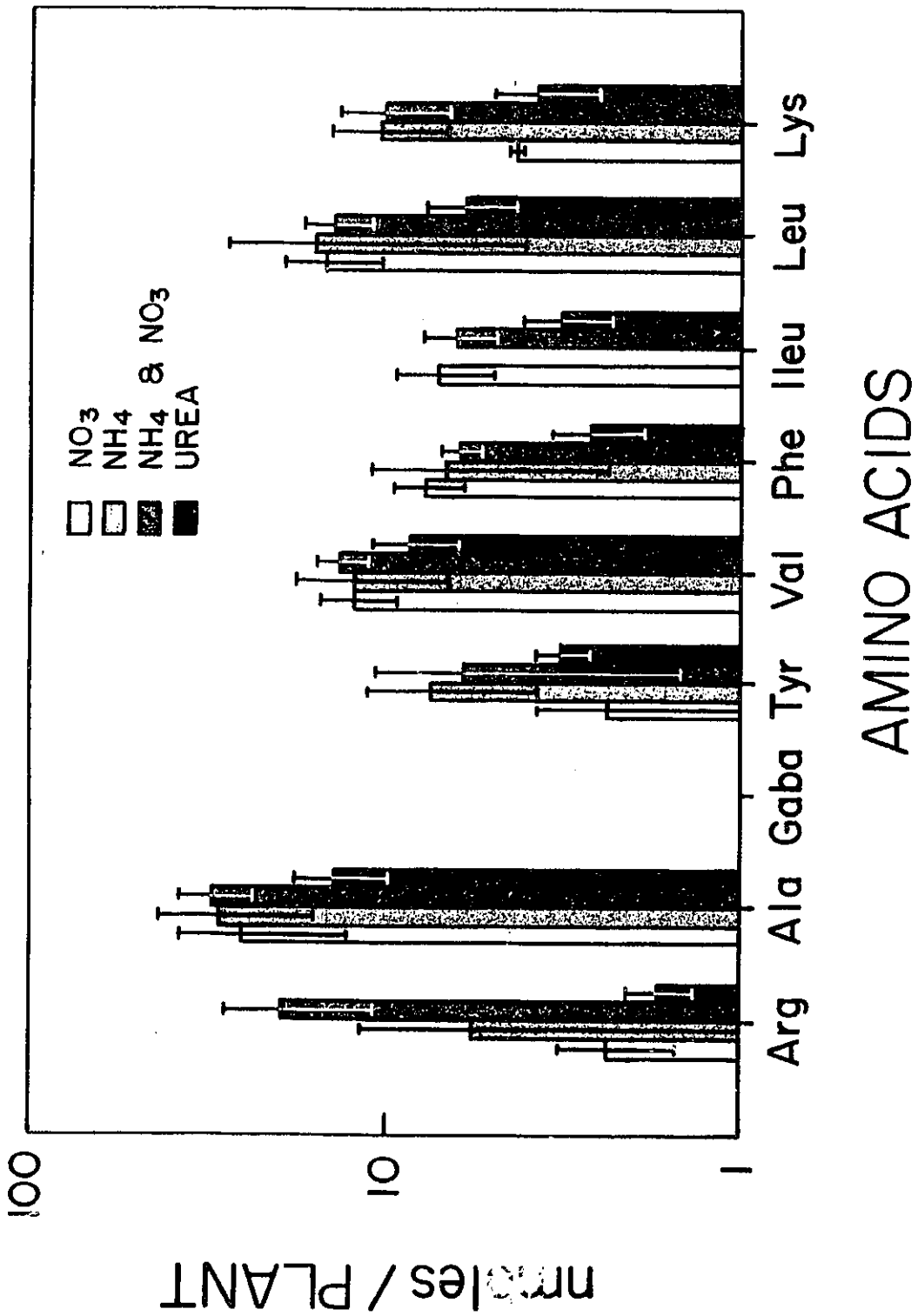

100
10
1
nmoles / PLANT
NO3
NH4
NH4 & NO3
UREA
Arg
Ala
Gaba
Tyr
Val
Phe
Ileu
Leu
Lys
AMINO ACIDS

Figure 11. (part 1) Effects of the various N-fertilizers on the distribution of the rhizosphere amino acids at 21 days, n= 4 replicates, 95% confidence intervals shown.

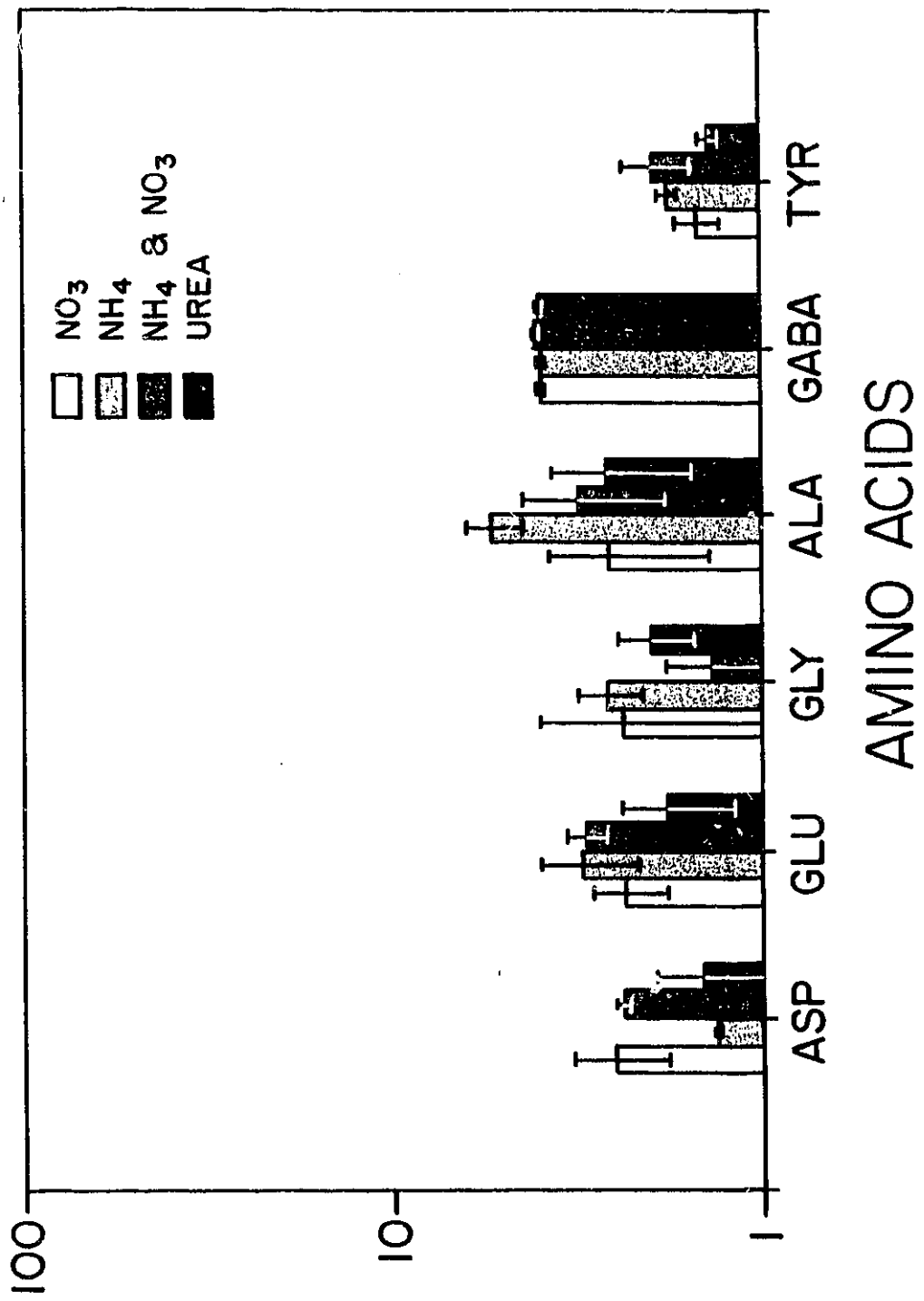

nmoles / PLANT
100
10
1
NO3
NH4
NH4 & NO3
UREA
ASP
GLU
GLY
ALA
GABA
TYR
AMINO ACIDS

Figure 11 (cont'd) (part 2) Effects of the N-fertilizers on the distribution of the rhizosphere amino acids at 21 days, n= 4 replicates, 95% confidence intervals shown.

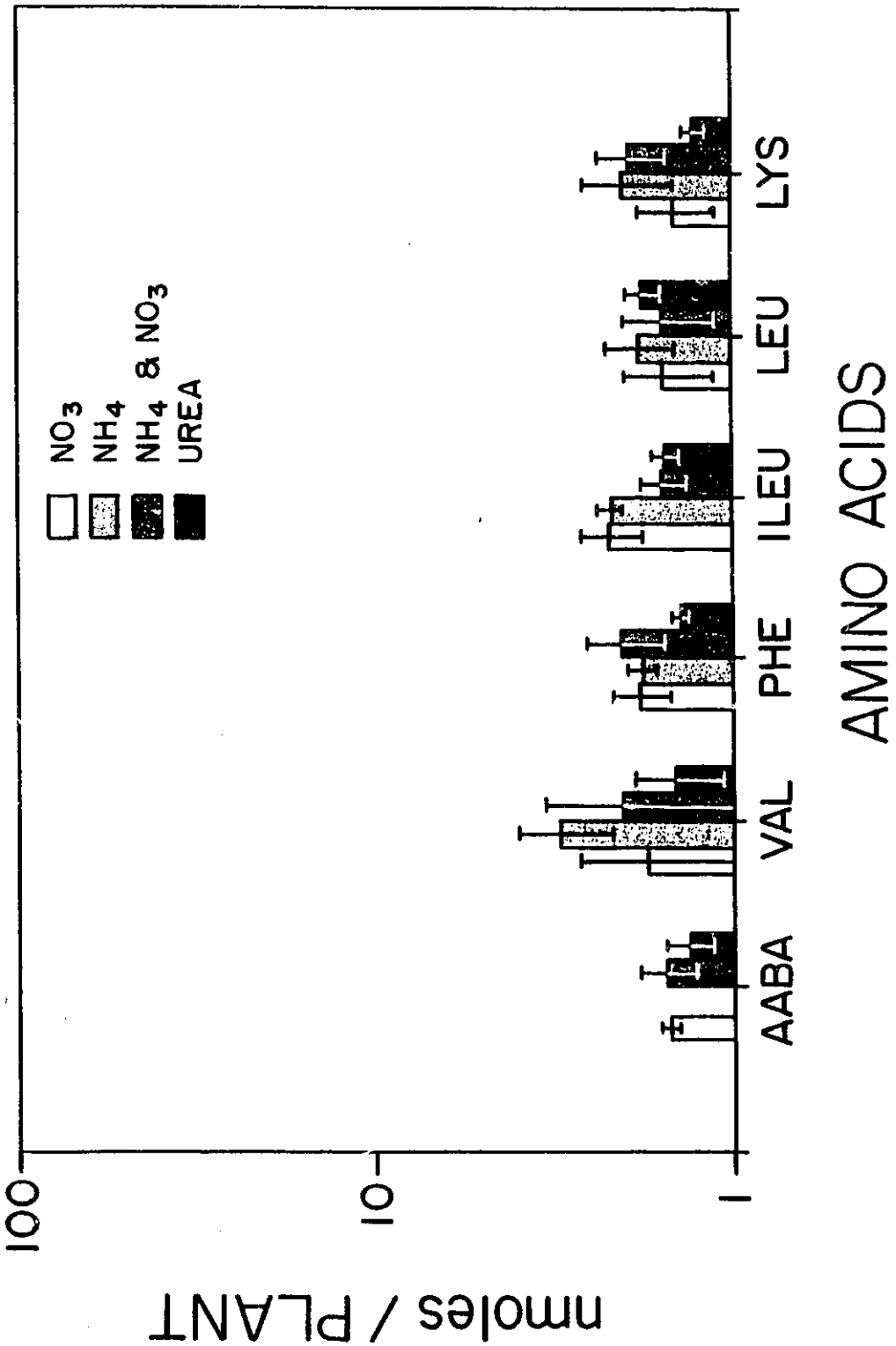

nmoles / PLANT
100
10
1
NO3
NH4
NH4 & NO3
UREA
AABA
VAL
PHE
ILEU
LEU
LYS
AMINO ACIDS

Figure 12. Effects of N-fertilizers on the rhizosphere total soluble sugars of winter wheat over time, n= 4 replicates, 95% confidence intervals shown.

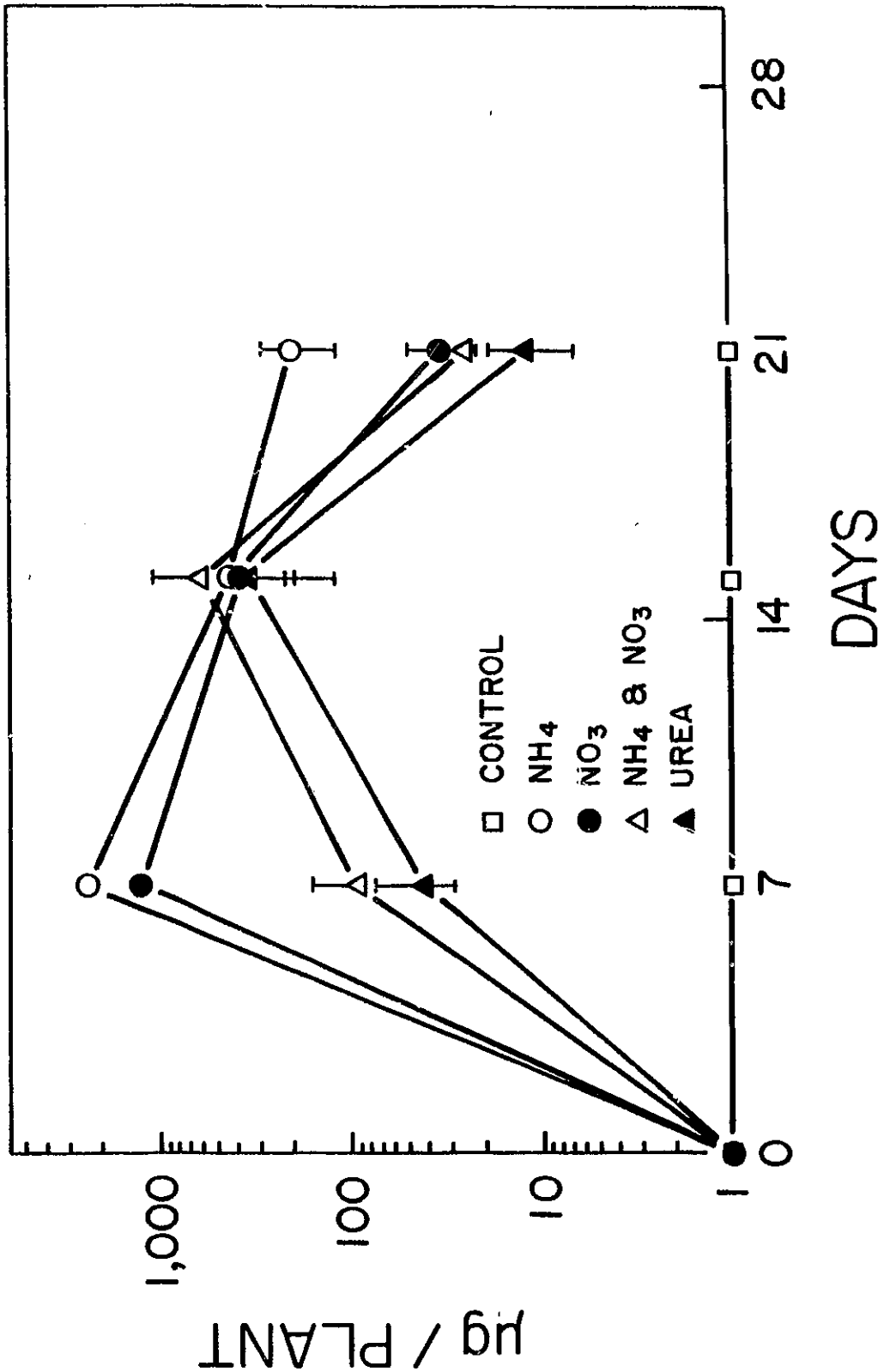

μg / PLANT
1,000
100
10
1
0
7
14
21
28
DAYS
CONTROL
NH4
NO3
NH4 & NO3
UREA

Figure 13. Effects of various N-fertilizers on the rhizosphere glucose of winter wheat over time, n= 4 replicates, 95% confidence intervals shown.

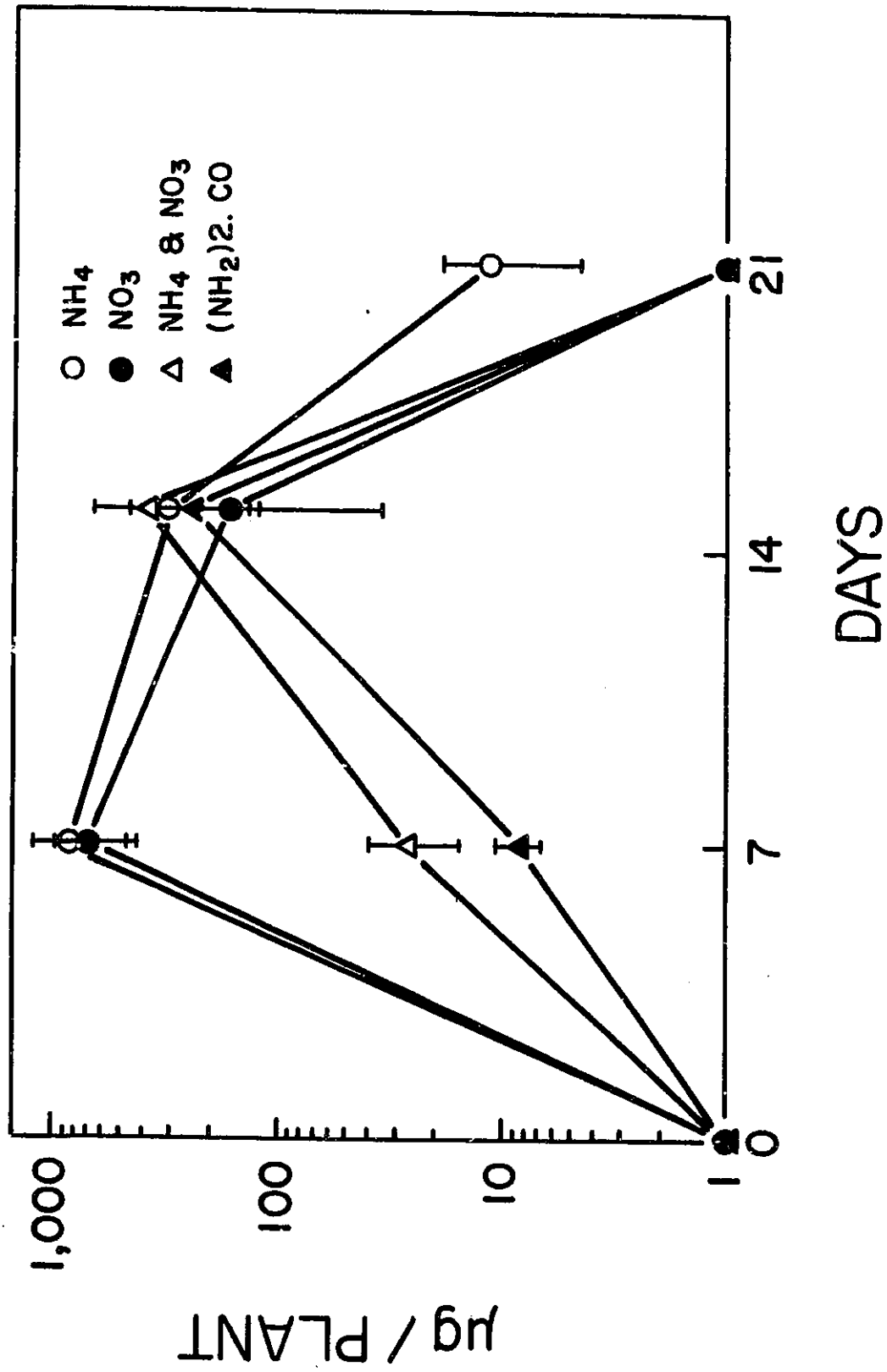

μg / PLANT
1,000
100
10
1
0
7
14
21
DAYS
○ NH4
● NO3
△ NH4 & NO3
▲ (NH2)2. CO

Figure 14. Effects of N-fertilizers on the rhizosphere arabinose of winter wheat over time, n= 4 replicates, 95% confidence intervals shown.

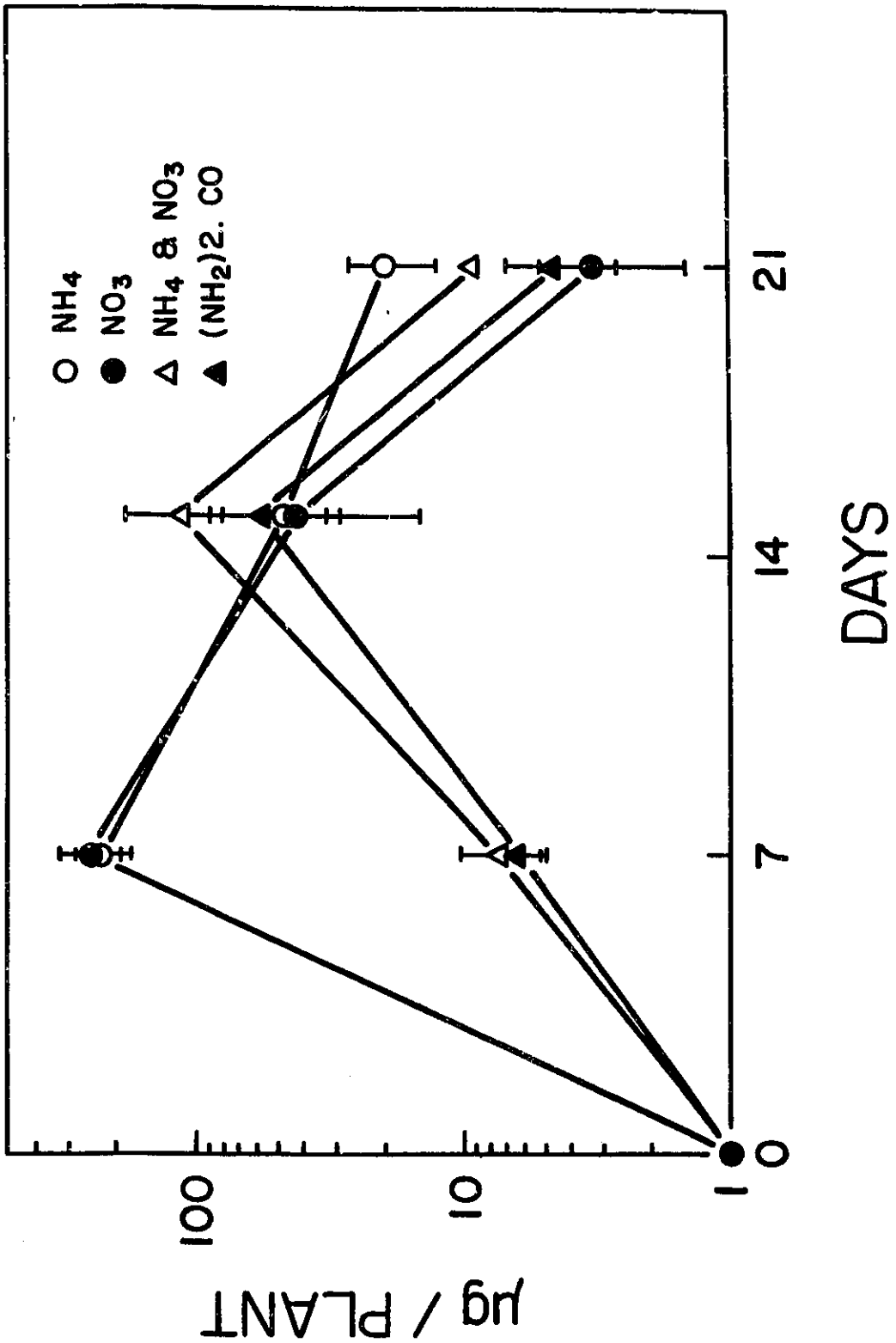

μg / PLANT
100
10
1
0
7
14
21
DAYS
○ NH4
● NO3
△ NH4 & NO3
▲ (NH2)2. CO

Figure 15. Effects of N-fertilizers on the rhizosphere xylose of winter wheat over time, n= 4 replicates, 95% confidence intervals shown.

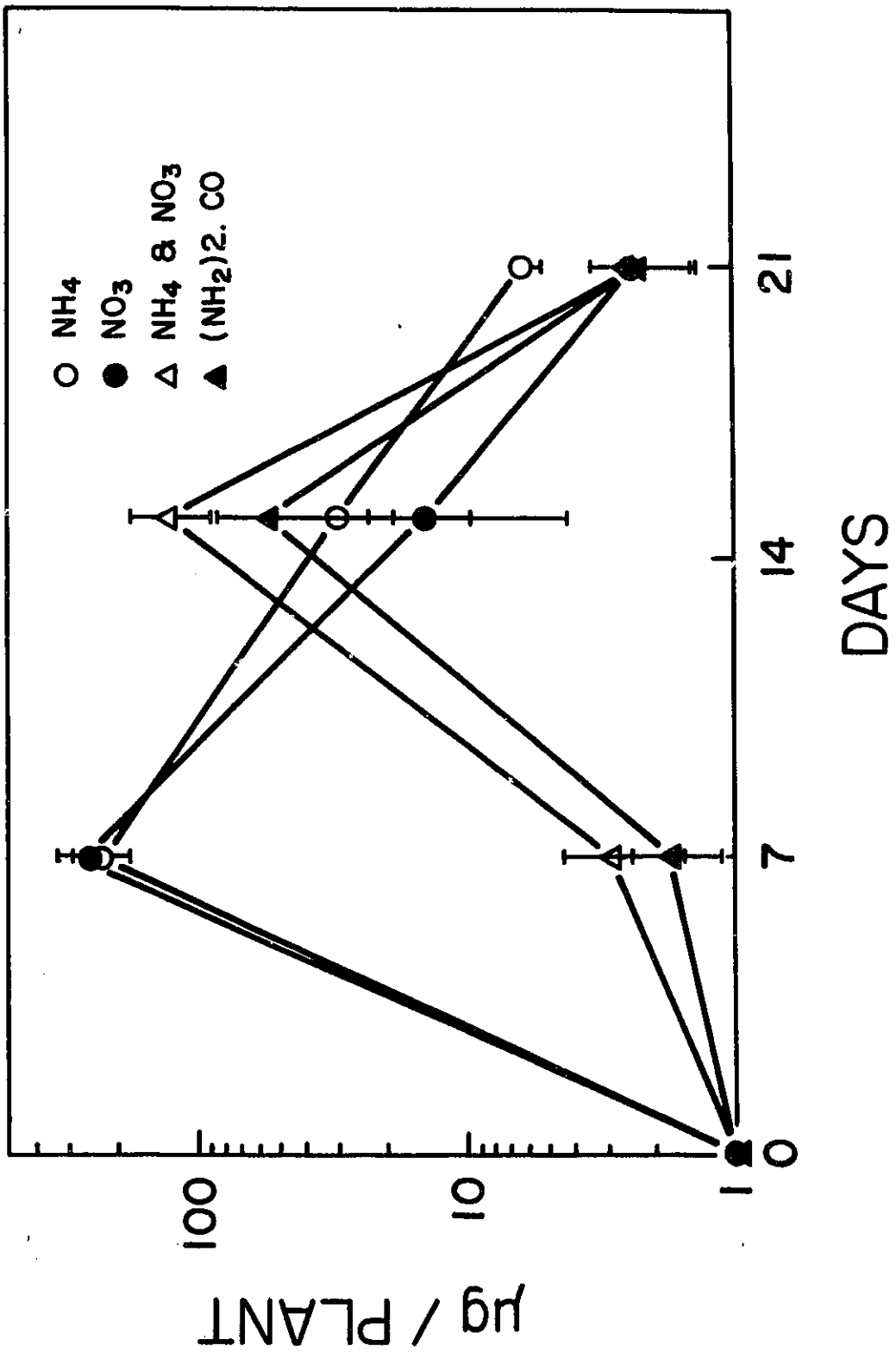

μg / PLANT
100
10
1
0
7
14
21
DAYS
O NH4
● NO3
Δ NH4 & NO3
▲ (NH2)2. CO

Figure 16. Effects of various N-fertilizers on the rhizosphere galactose of winter wheat, n= 4 replicates, 95% confidence intervals shown.

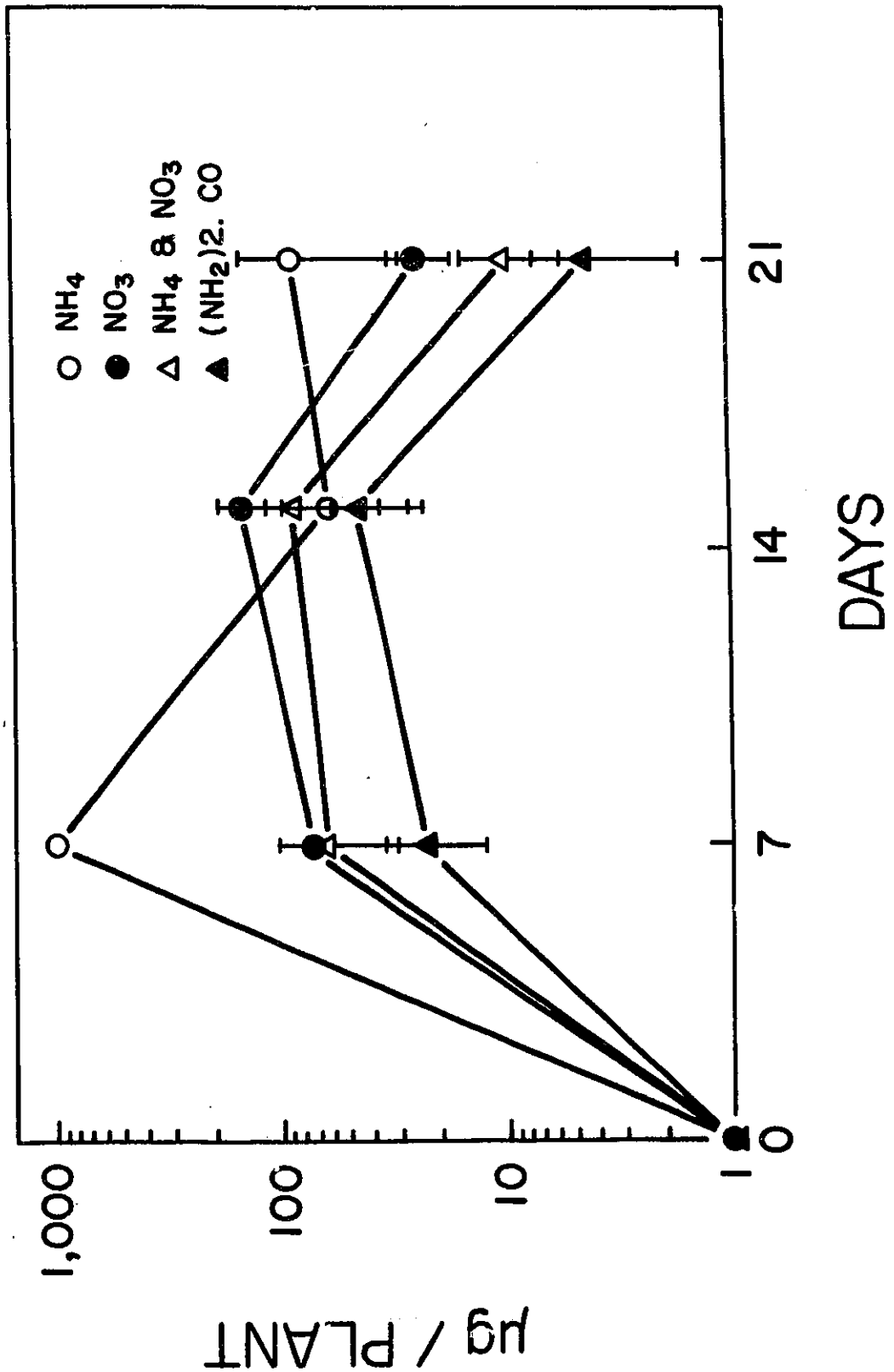

μg / PLANT
1,000
100
10
1
0
7
14
21
DAYS
NH4
NO3
NH4 & NO3
(NH2)2. CO

Figure 17. Effects of N-fertilizers on the rhizosphere mannose over time, n= 4 replicates, 95% confidence intervals shown.

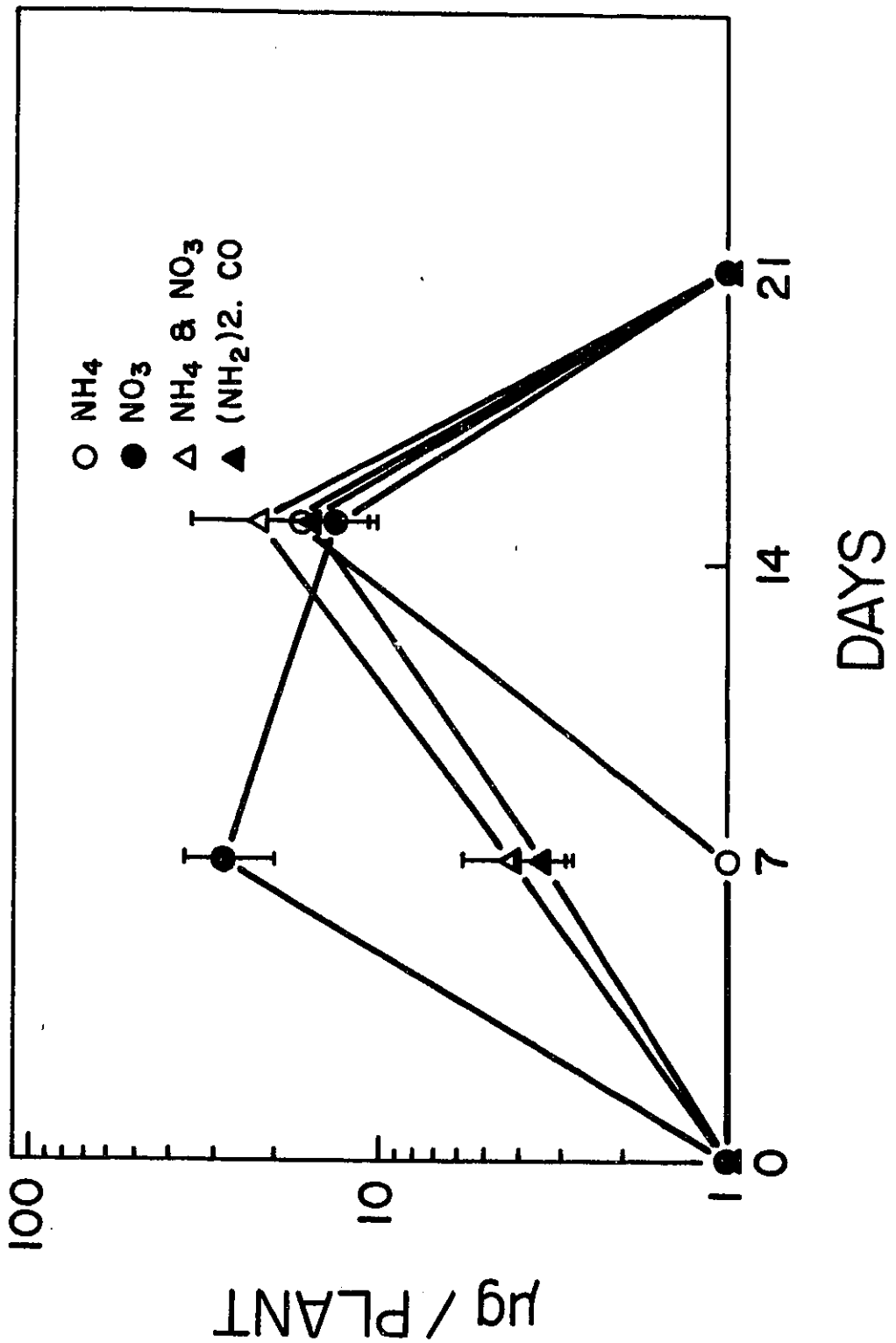

μg / PLANT
100
10
1
0
7
14
21
DAYS
○ NH4
● NO3
△ NH4 & NO3
▲ (NH2)2. CO

Figure 18. Effects of N-fertilizers on the rhizosphere ribose of winter wheat over time, n= 4 replicates, 95% confidence intervals shown.

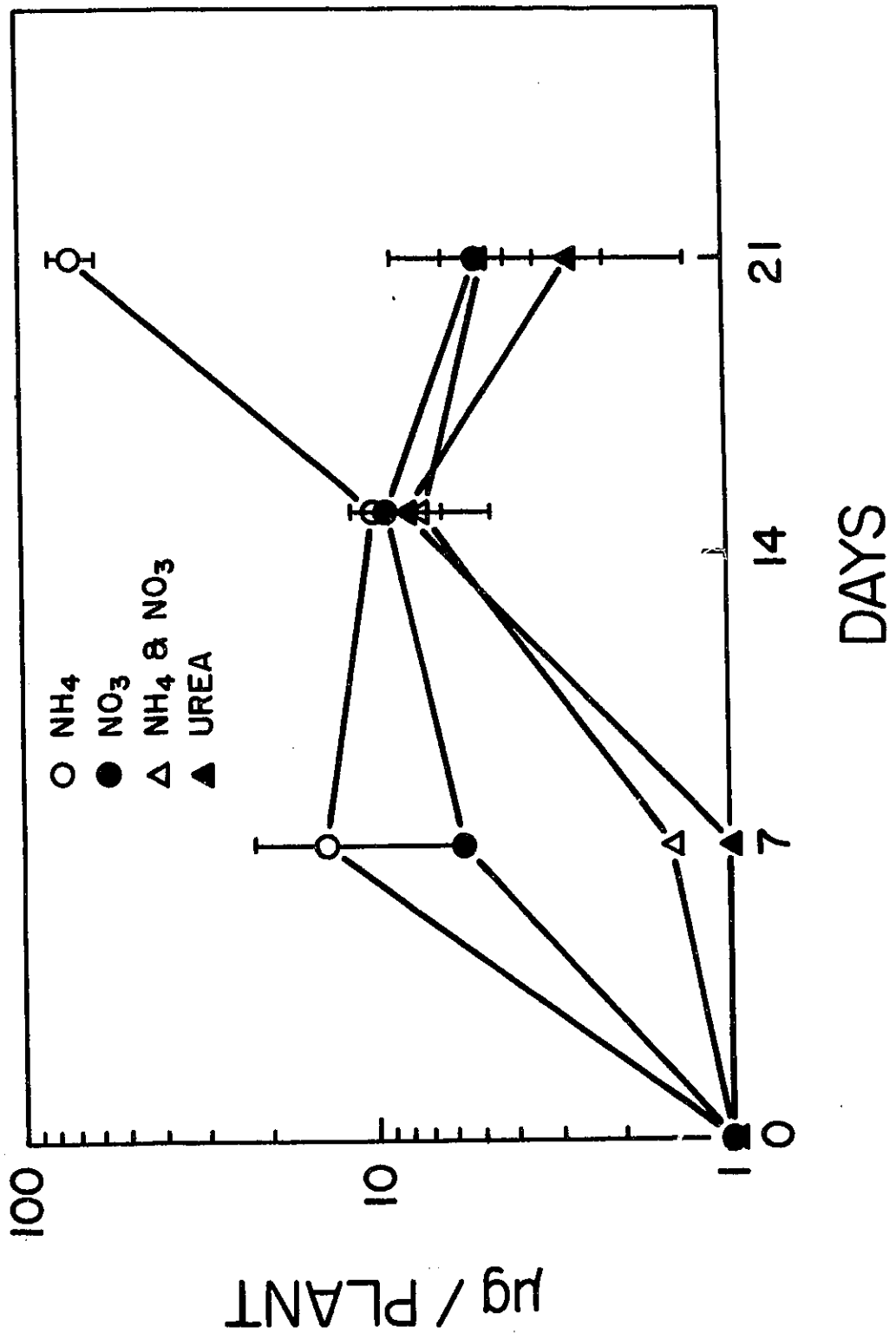

μg / PLANT
100
10
1
0
7
14
21
DAYS
NH4
NO3
NH4 & NO3
UREA

Figure 19. Effects of N-fertilizers on the soluble sugars (A) NH_4^+ and (B) a mixture of NH_4^+ & NO_3^- n= 4 replicates, 95% confidence intervals shown.

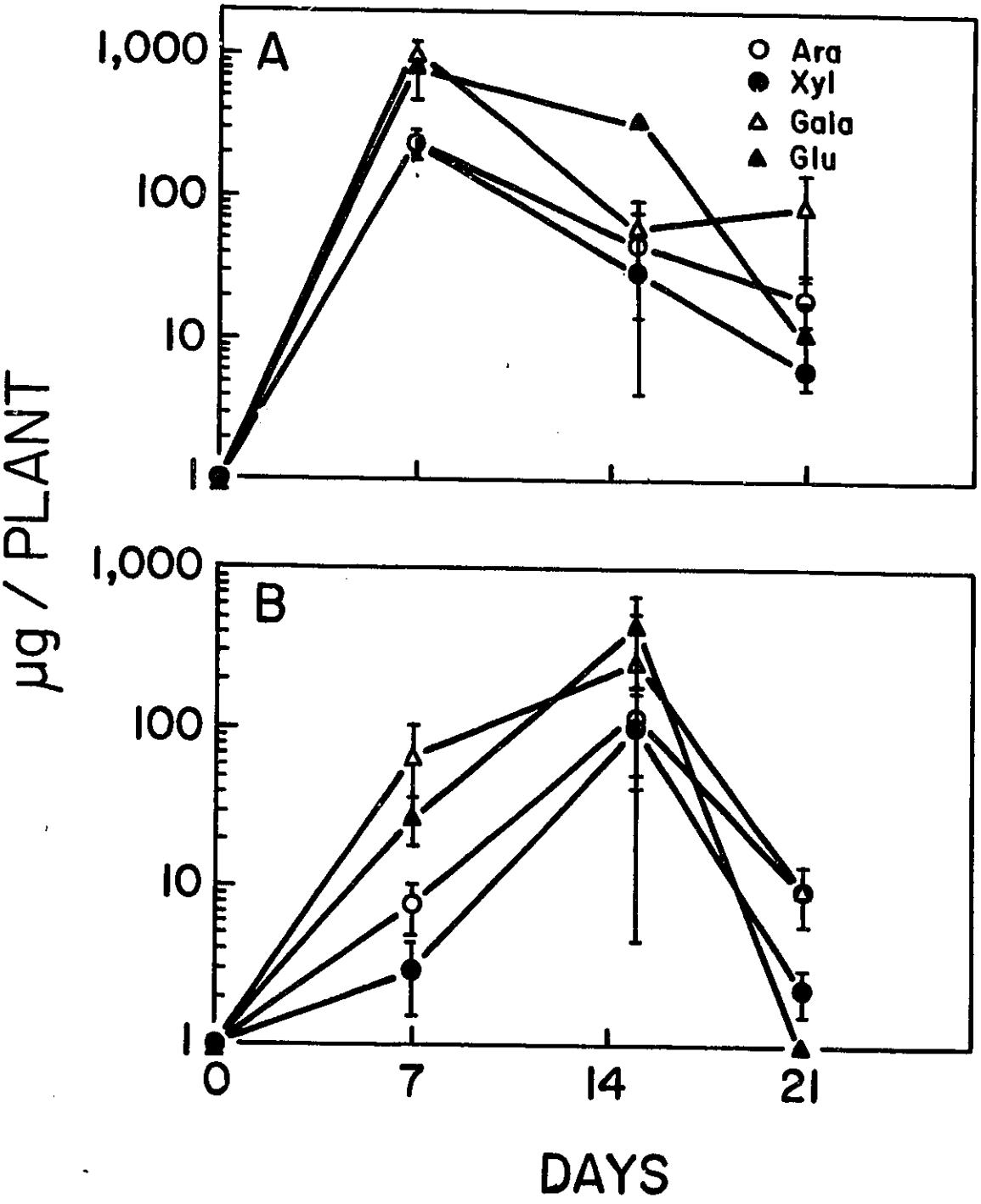

1,000
100
10
1
A
Ara
Xyl
Gala
Glu
µg / PLANT
1,000
100
10
1
B
0
7
14
21
DAYS

Figure 20. Effects of N-fertilizers on the soluble sugars (A) NO_3^- and (B) urea n= 4 replicates, 95% confidence intervals shown.

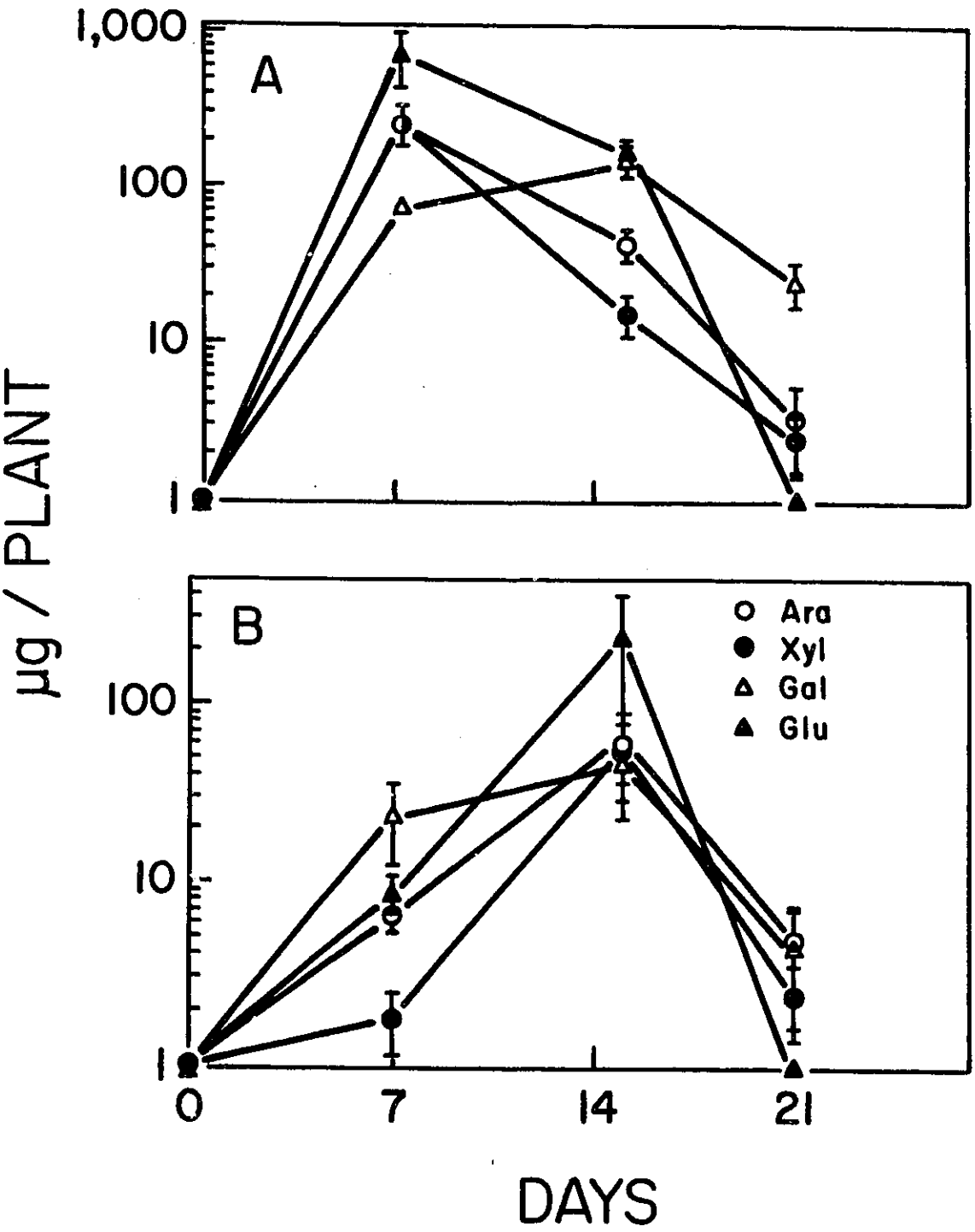

1,000
100
10
1
A
B
100
10
1
μg / PLANT
Ara
Xyl
Gal
Glu
0
7
14
21
DAYS

Figure 21. Effects of various N-fertilizers on several rhizosphere soluble sugars (Ara, Xyl, Man, Gala, Glu, Rib) at 7 days, n= 4 replicates, 95% confidence intervals shown.

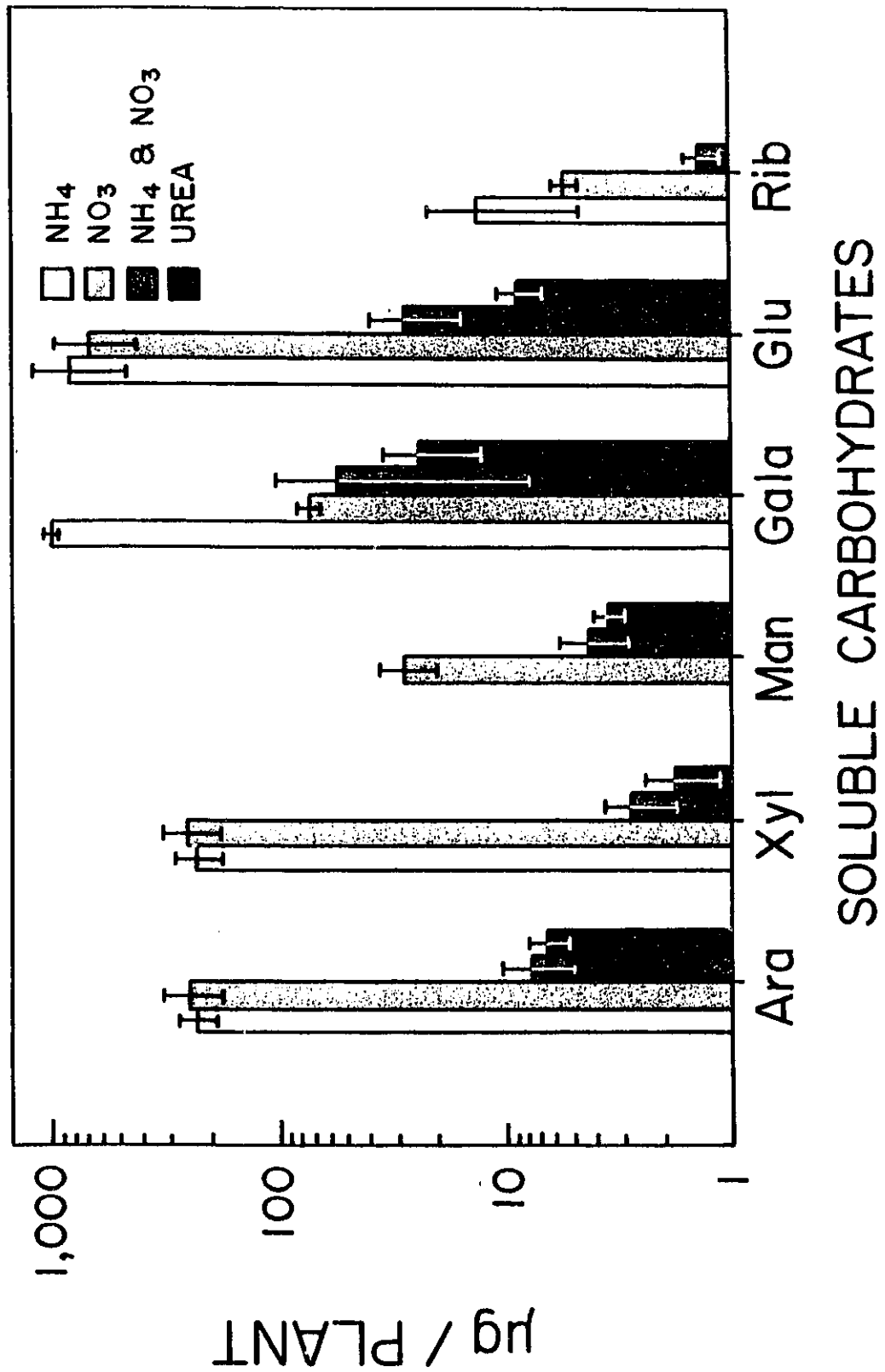

μg / PLANT
1,000
100
10
1
NH4
NO3
NH4 & NO3
UREA
Ara
Xyl
Man
Gala
Glu
Rib
SOLUBLE CARBOHYDRATES

Figure 22. Effects of N-fertilizers on several rhizosphere soluble sugars at 15 days, n= 4 replicates, 95% confidence intervals shown.

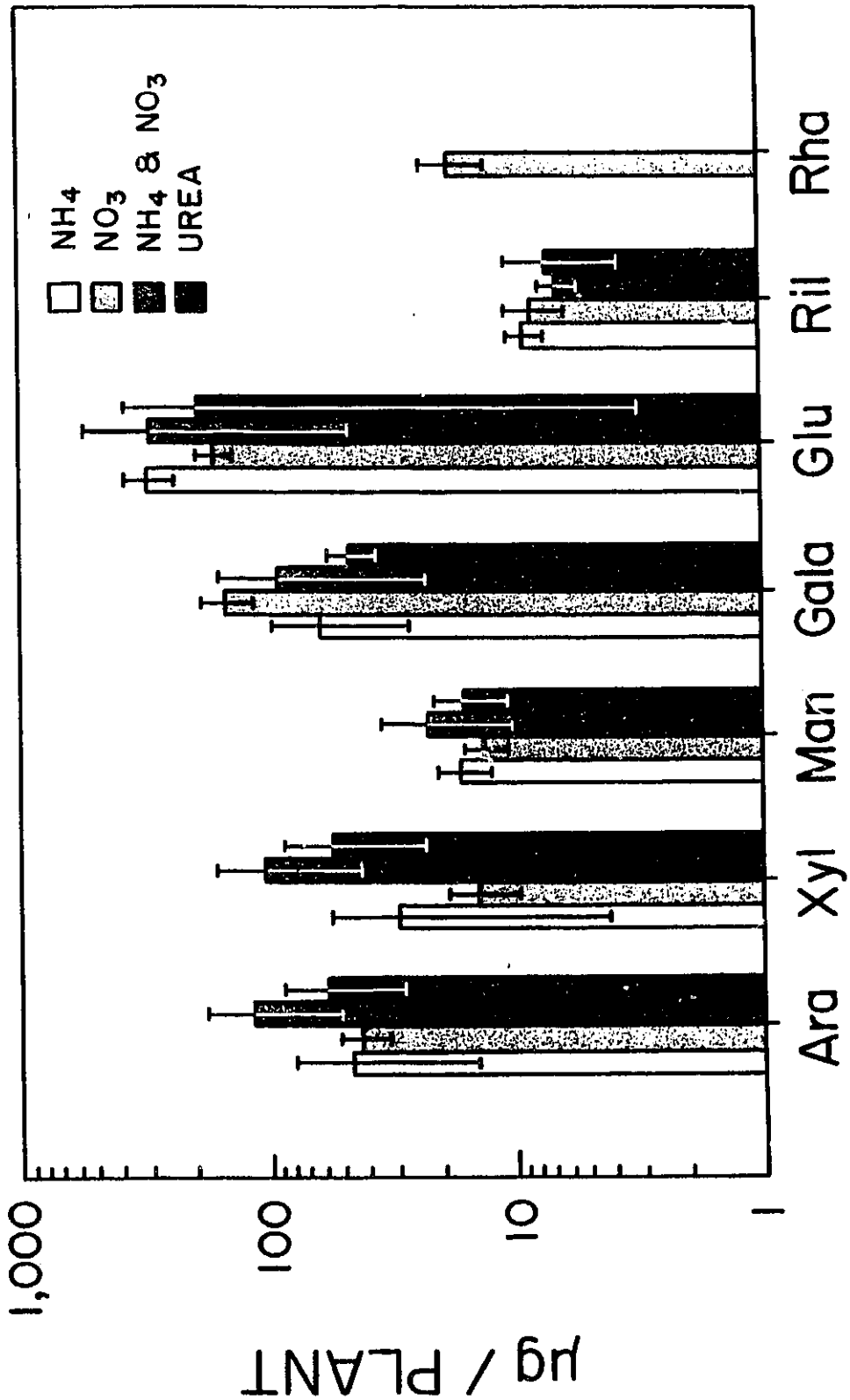

SOLUBLE CARBOHYDRATES
μg / PLANT
1,000
100
10
1
NH4
NO3
NH4 & NO3
UREA
Ara
Xyl
Man
Gala
Glu
Ril
Rha

Figure 23. Effects of various N-fertilizers on several rhizosphere soluble sugars at 21 days, n= 4 replicates, 95% confidence intervals shown.

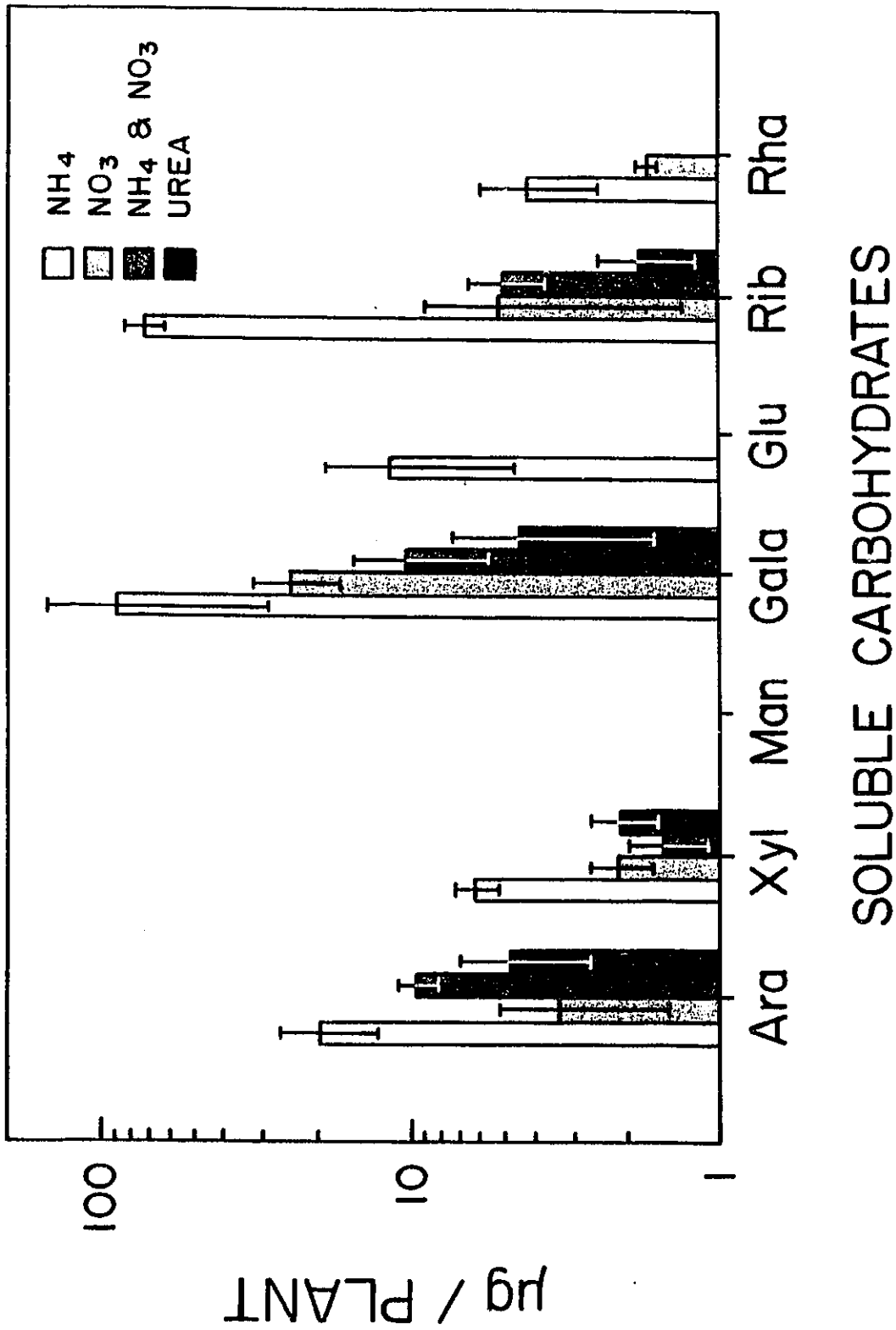

μg / PLANT
100
10
1
NH4
NO3
NH4 & NO3
UREA
Ara
Xyl
Man
Gala
Glu
Rib
Rha
SOLUBLE CARBOHYDRATES

Figure 24. Effects of N-fertilizers on the total insoluble sugars in the rhizosphere of winter wheat over time, n= 4 replicates, standard deviation shown.

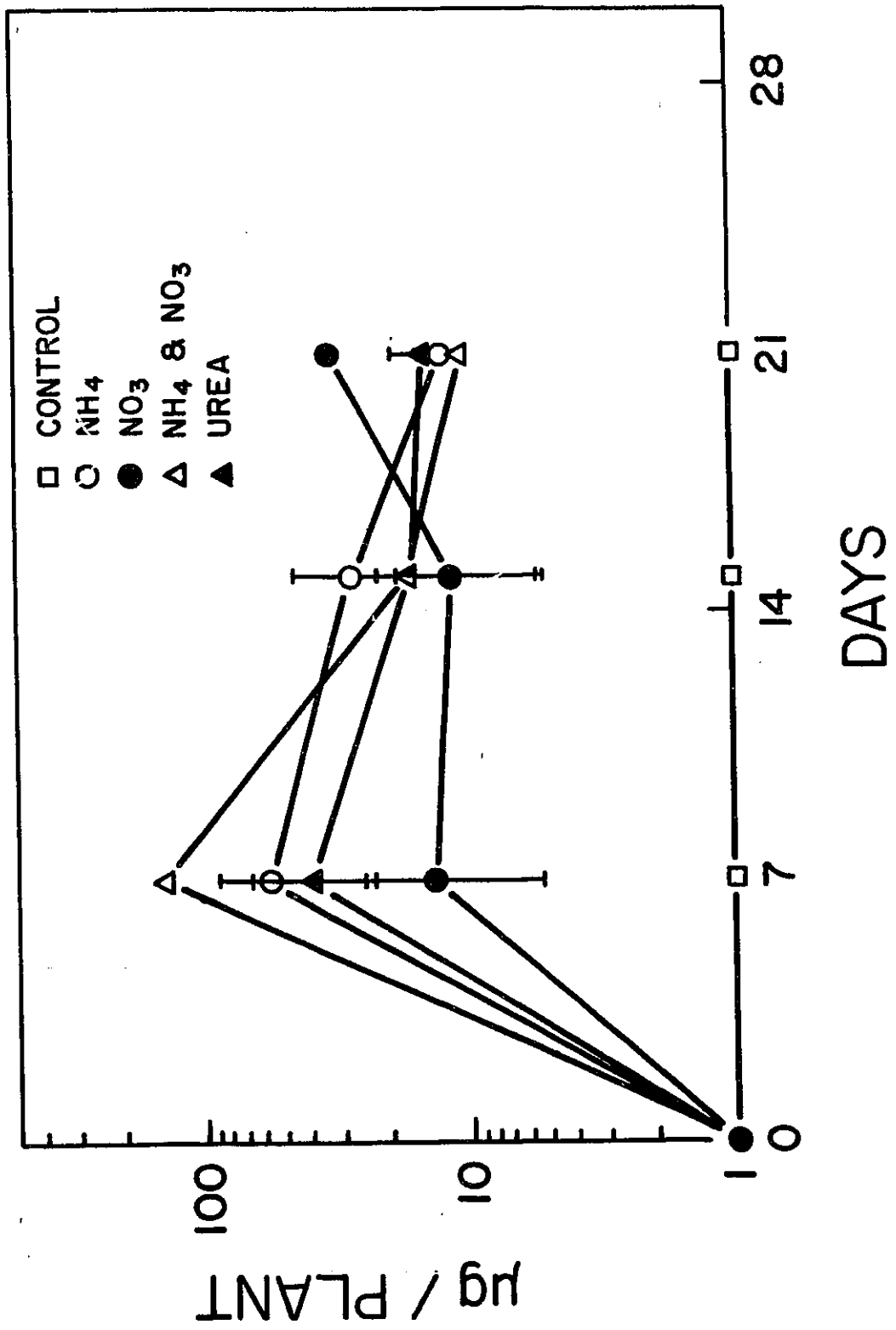

μg / PLANT
100
10
1
0
7
14
21
28
DAYS
CONTROL
NH4
NO3
NH4 & NO3
UREA

Figure 25. Effects of N-fertilizers on the insoluble glucose in the rhizosphere of winter wheat with time, n= 4 replicates, standard deviation shown.

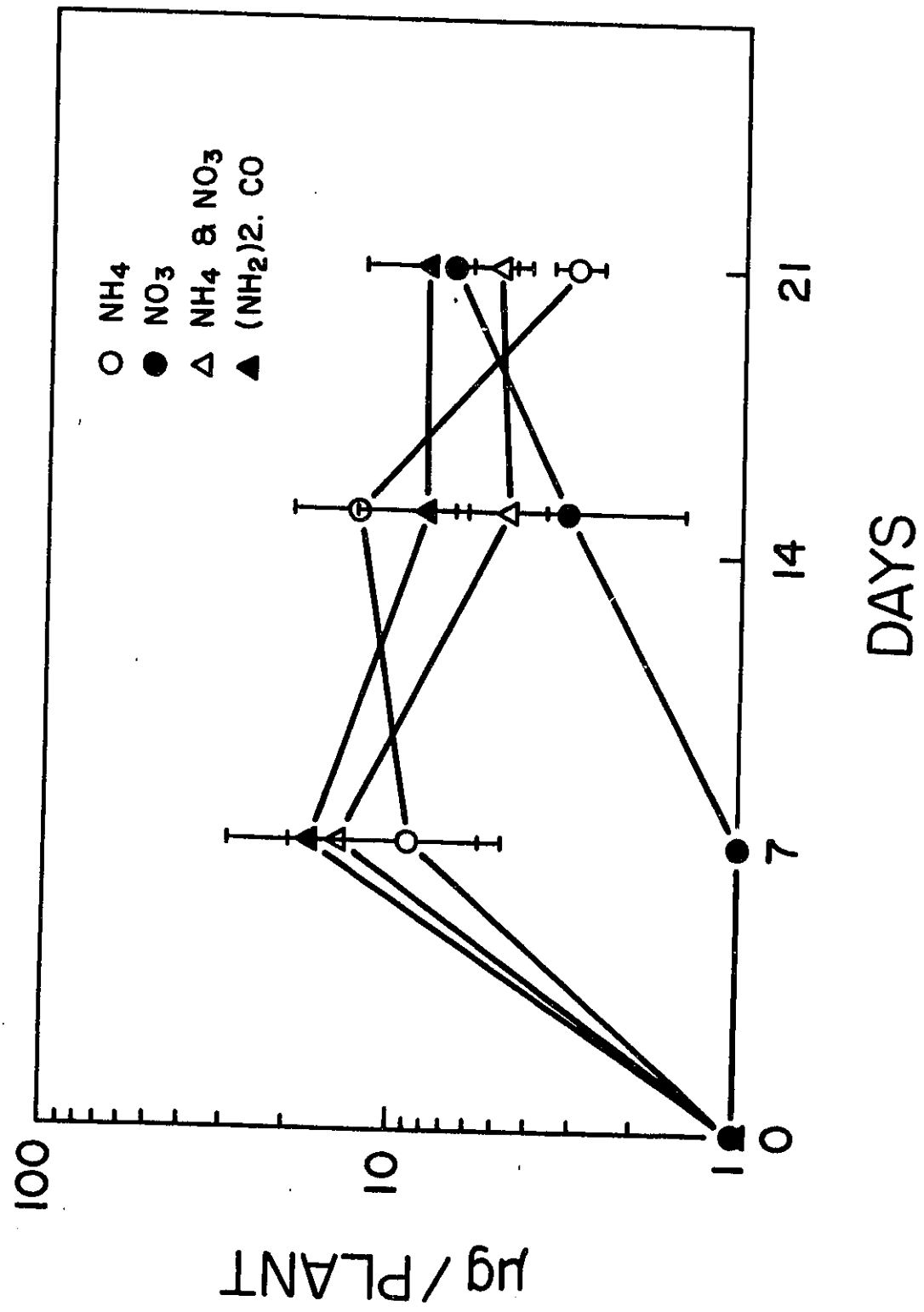

μg / PLANT
100
10
1
0
7
14
21
DAYS
○ NH4
● NO3
△ NH4 & NO3
▲ (NH2)2. CO

Figure 26. Effects of N-fertilizers on the insoluble arabinose in the rhizosphere with time, n= 4 replicates, standard deviation shown.

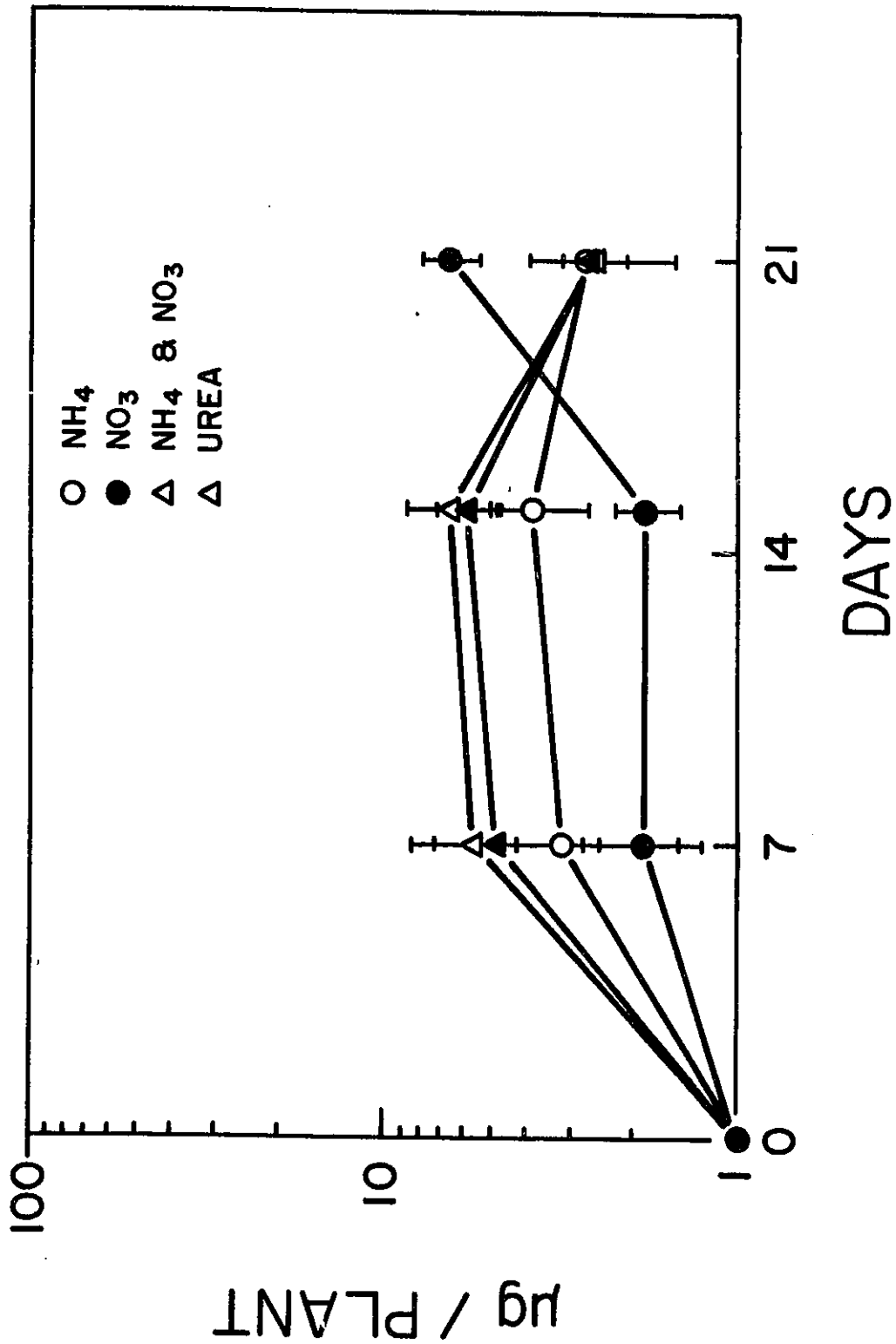

μg / PLANT
100
10
1
0
7
14
21
DAYS
○ NH4
● NO3
△ NH4 & NO3
△ UREA

Figure 27. Effects of N-fertilizers on the insoluble xylose in the rhizosphere with time, n= 4 replicates, standard deviation shown.

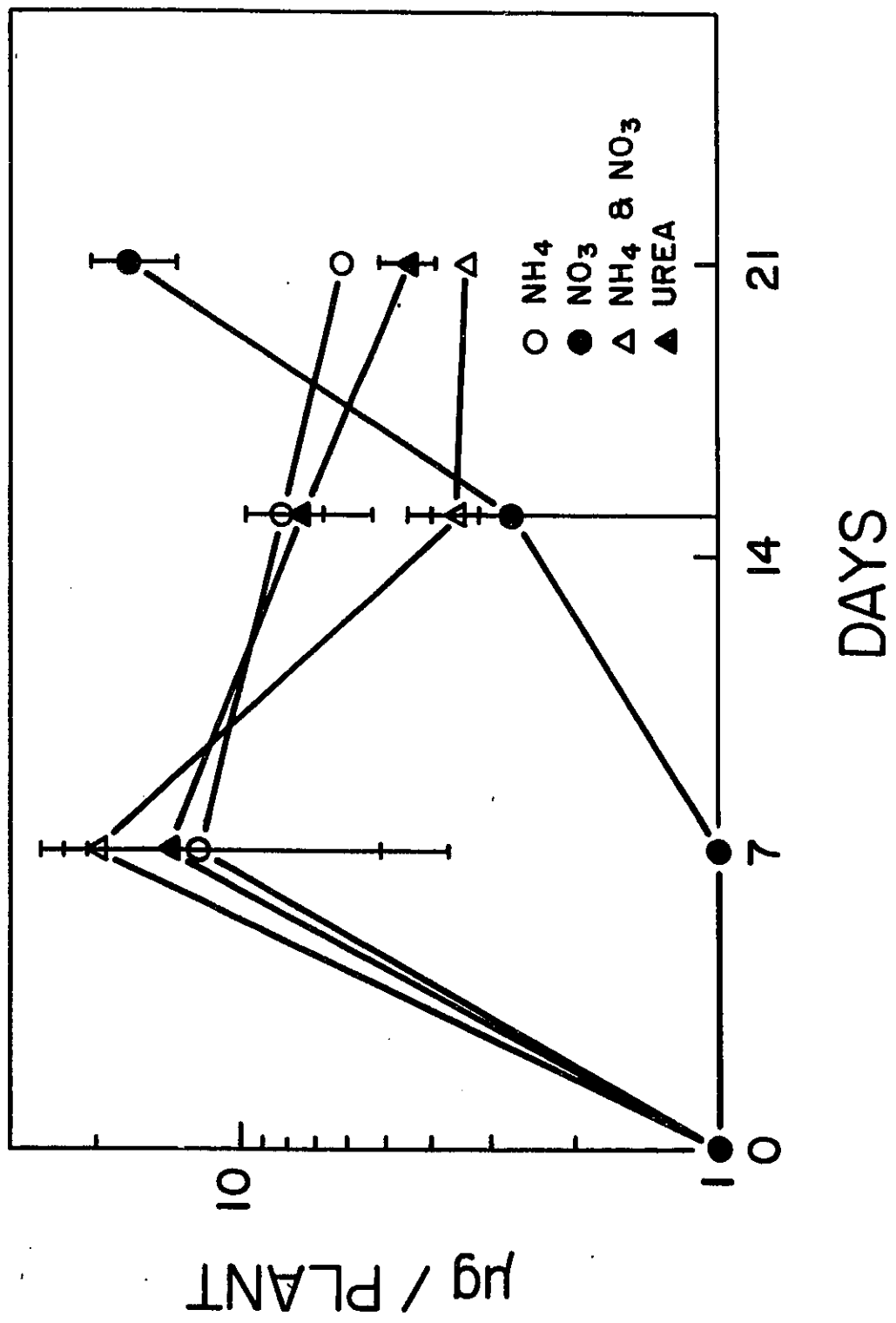

μg / PLANT
0
10
0
7
14
21
DAYS
O NH4
● NO3
Δ NH4 & NO3
▲ UREA

Figure 28. Effects of N-fertilizers on the insoluble mannose in the rhizosphere with time, n= 4 replicates, standard deviation shown.

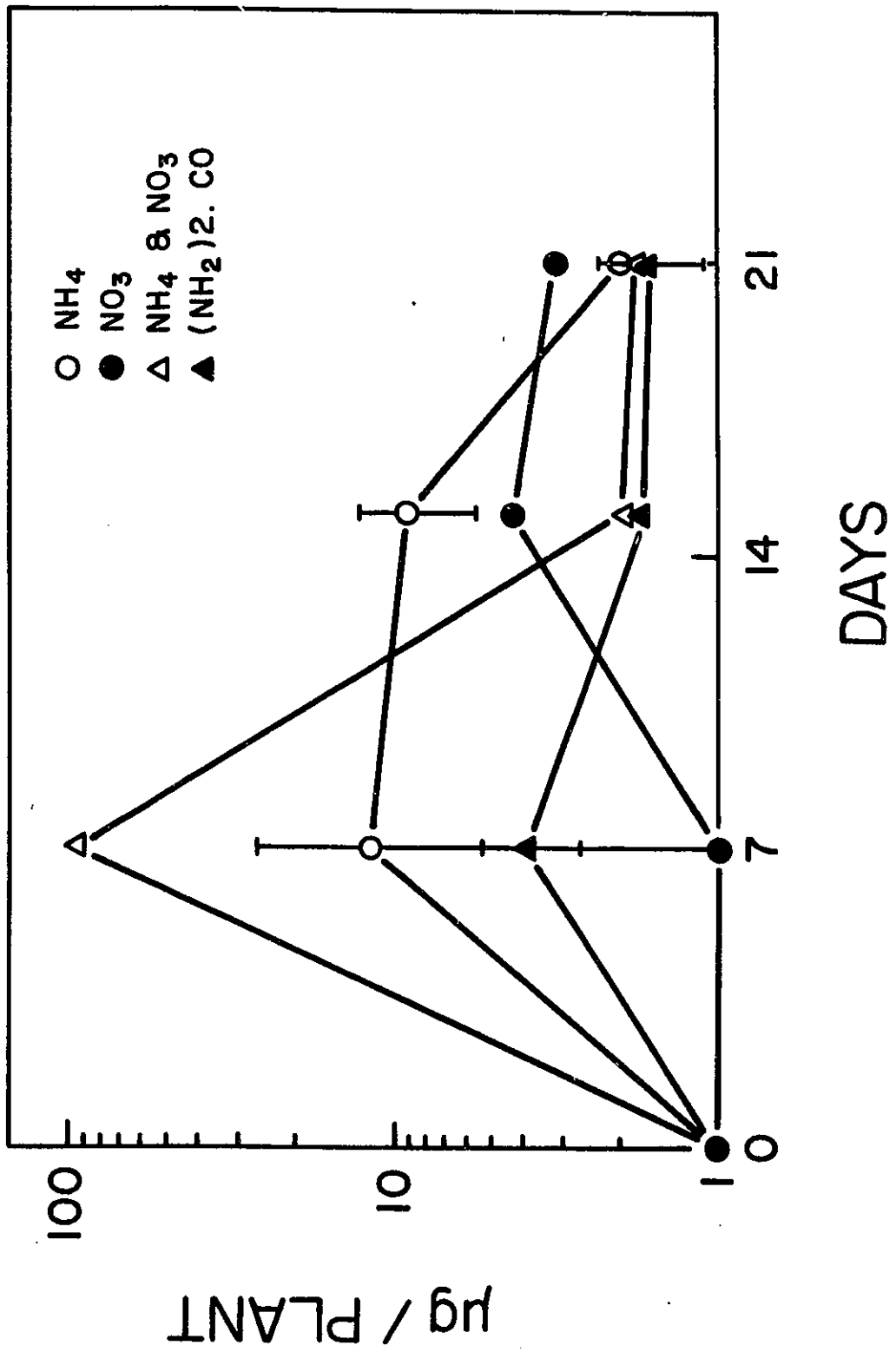

μg / PLANT
100
10
1
0
7
14
21
DAYS
○ NH4
● NO3
△ NH4 & NO3
▲ (NH2)2. CO

Figure 29. Effects of N-fertilizers on the insoluble sugars with time, (A) NH_4^+ and (B) NO_3^-, n= 4 replicates, standard deviation shown.

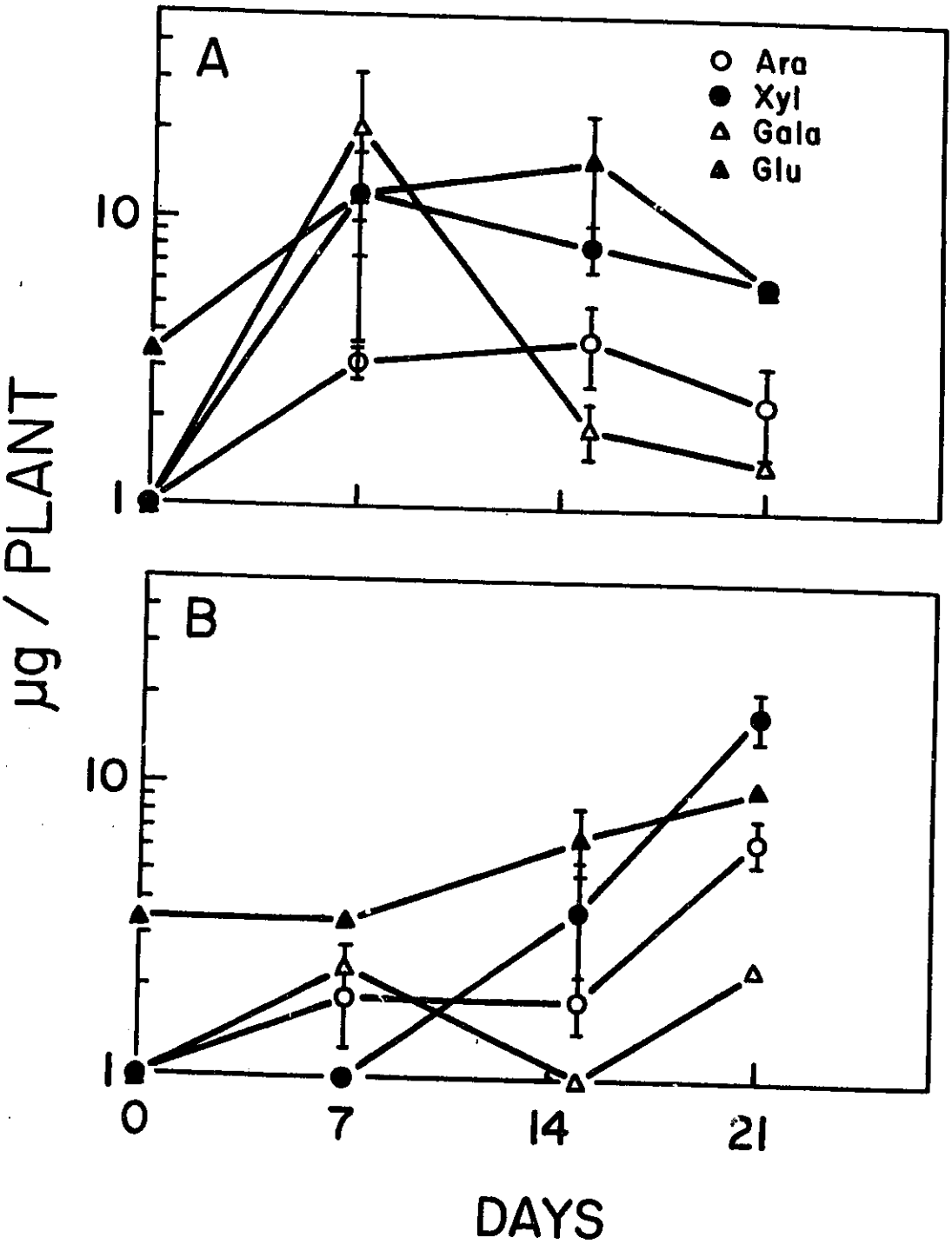

A
B
Ara
Xyl
Gala
Glu
10
1
0
7
14
21
µg / PLANT
DAYS

Figure 30. Effects of (A) a mixture of NH_4^+ & NO_3^- and (B) urea fertilizers on the rhizosphere insoluble sugars with time, n= 4 replicates, standard deviation shown.

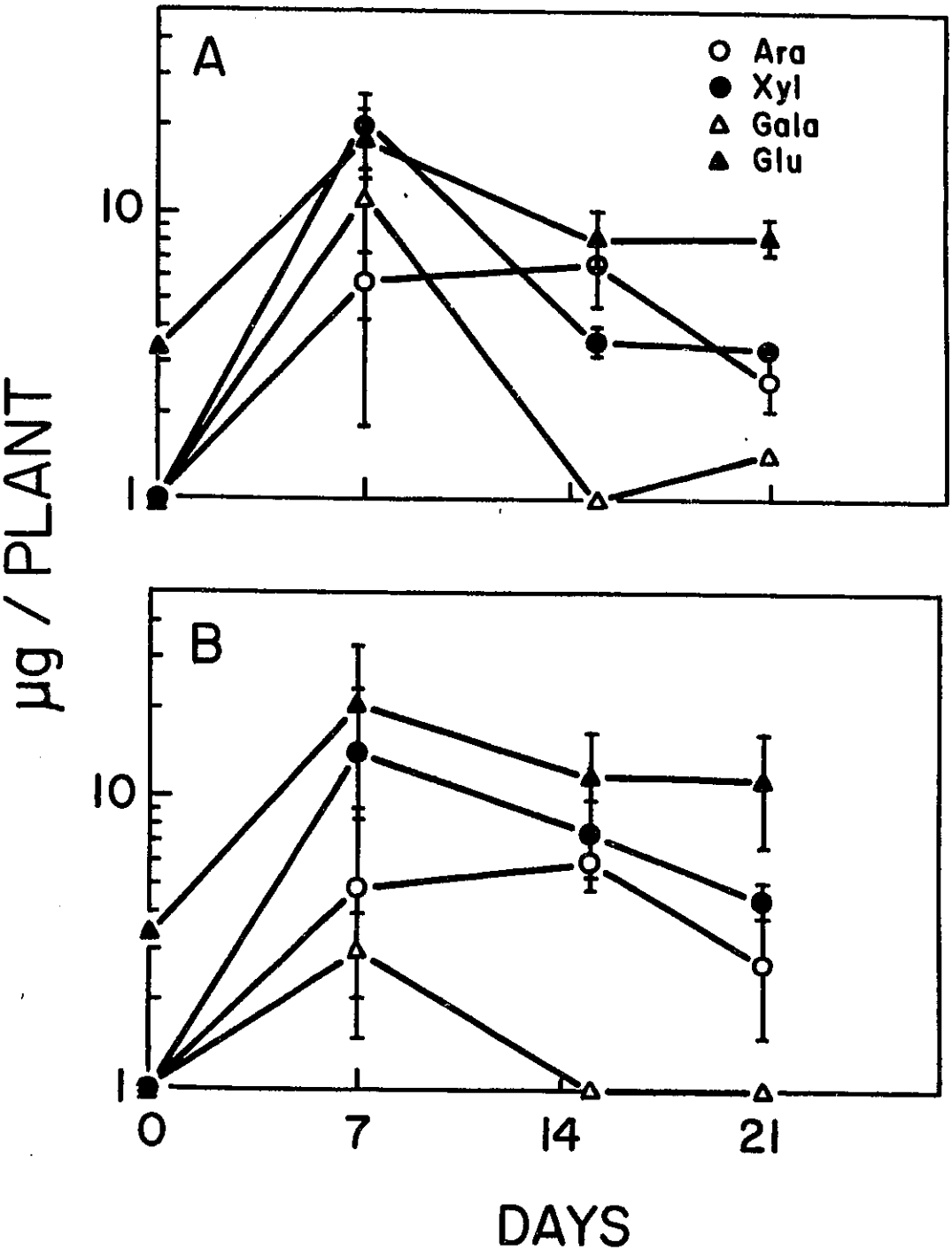

A
B
Ara
Xyl
Gala
Glu
10
1
µg / PLANT
0
7
14
21
DAYS

Figure 31. Effects of different N-fertilizers on various rhizosphere insoluble sugars (Rha, Fuc, Ara, Xyl, man, Gala, Glu, Rib) at 7 days, n= 4 replicates, standard deviation shown.

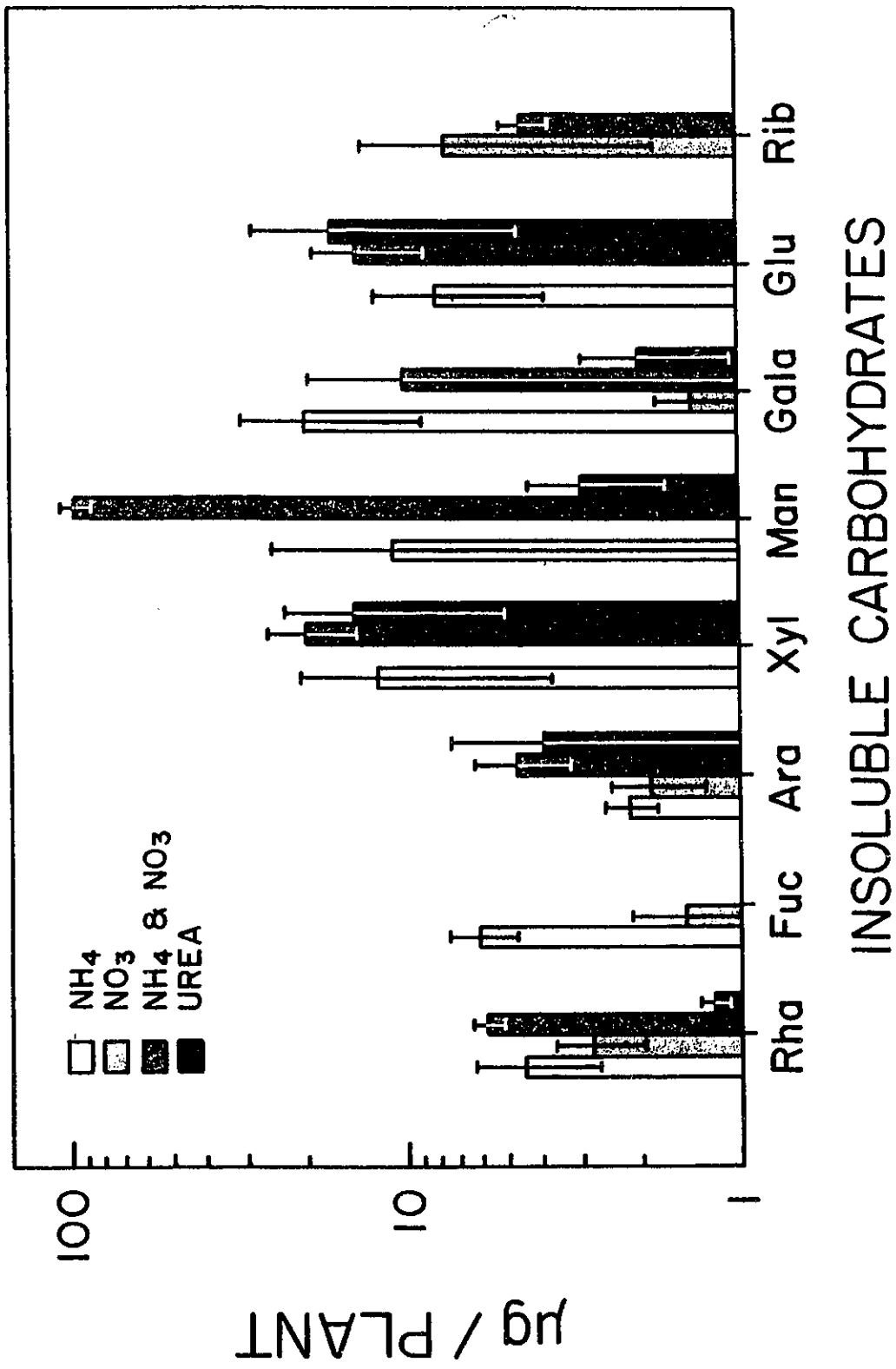

INSOLUBLE CARBOHYDRATES
µg / PLANT
100
10
1
NH4
NO3
NH4 & NO3
UREA
Rha
Fuc
Ara
Xyl
Man
Gala
Glu
Rib

Figure 32. Effects of different N-fertilizers on various rhizosphere insoluble sugars at 15 days, n= 4 replicates, standard deviation shown.

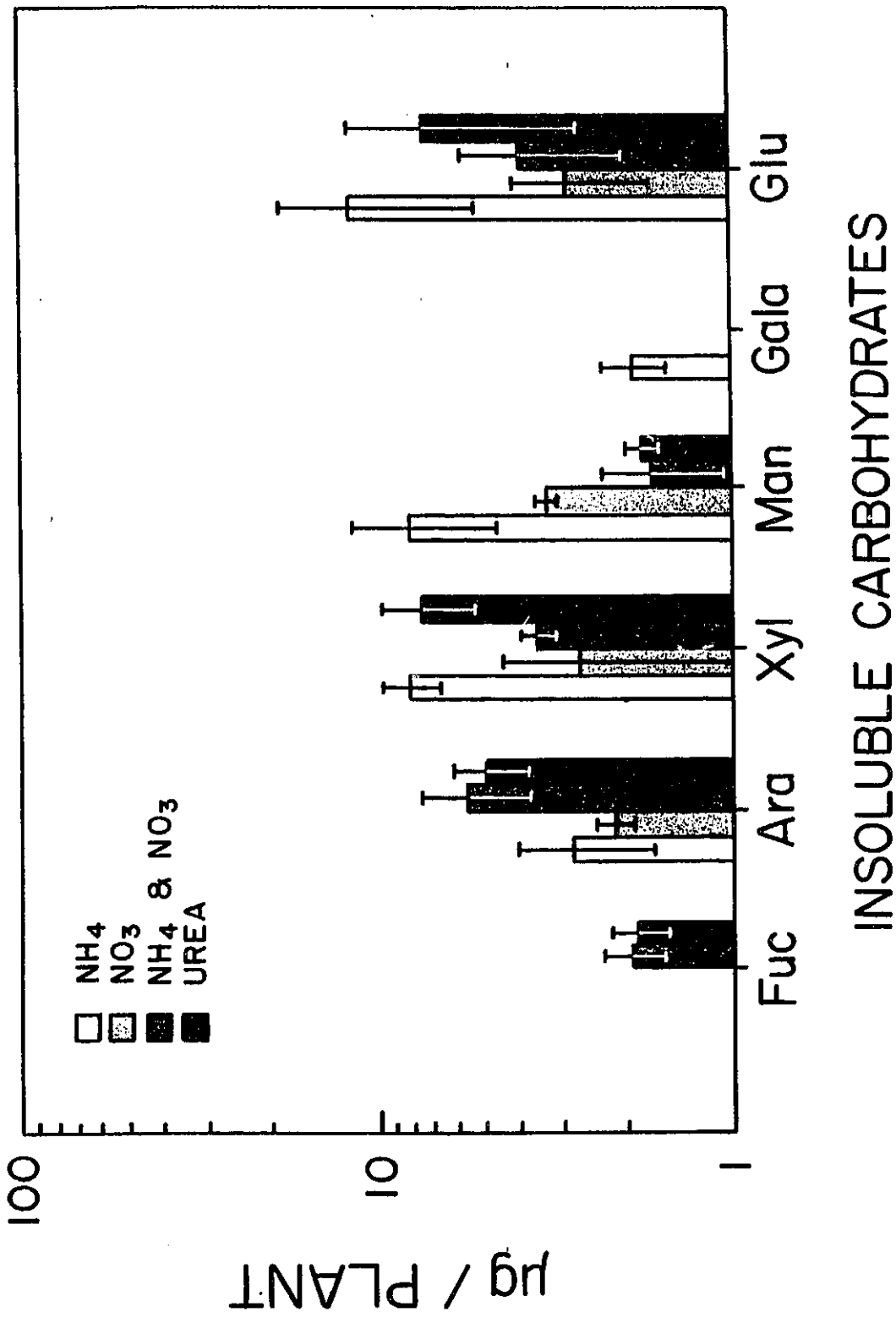
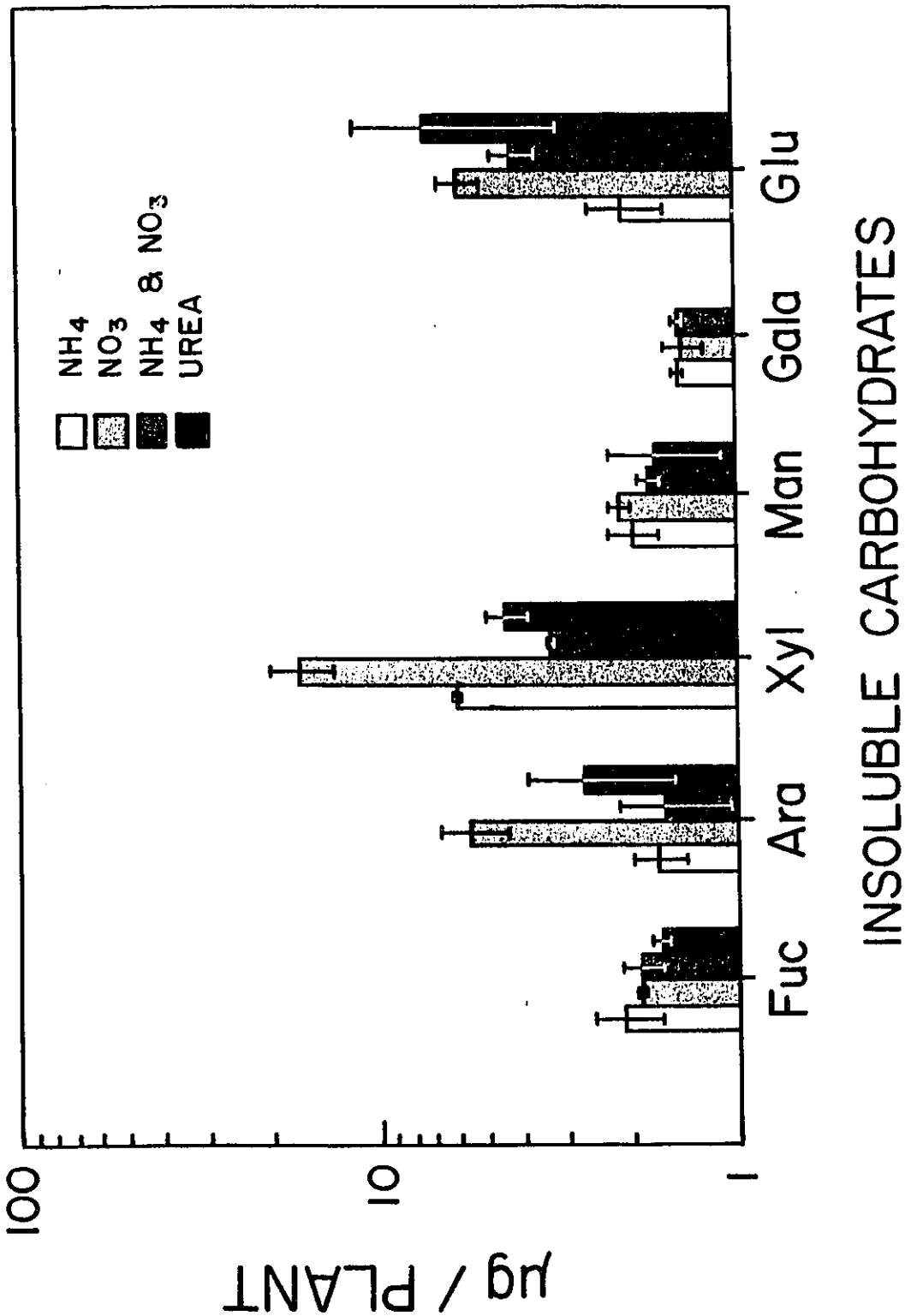

100
10
1
µg / PLANT
NH4
NO3
NH4 & NO3
UREA
Fuc
Ara
Xyl
Man
Gala
Glu
INSOLUBLE CARBOHYDRATES

Figure 33. Effects of different N-fertilizers on various rhizosphere insoluble sugars at 21 days, n= 4 replicates, standard deviation shown.


INSOLUBLE CARBOHYDRATES
µg / PLANT
100
10
1
NH4
NO3
NH4 & NO3
UREA
Fuc
Ara
Xyl
Man
Gala
Glu

CHAPTER 4

Discussion

4.1 Growth studies:

The positive effect of nitrogen fertilizers on crop development is well known, e.g. wheat growth is significantly improved by nitrogen fertilizers at all growth stages (Bacon et al., 1989). However, various studies have reported different responses in plant growth to the form of nitrogen addition (Gashaw and Mugwira, 1981, Sommer and Six, 1986; Marschner, 1986).

In the present study, no significant difference ($P > 0.05$) was observed in the growth (height and total dry weight) of winter wheat variety Fredrick when subjected to different nitrogen fertilizers for up to 21 days. All the plants showed similar growth responses to different fertilizers regardless of nitrogen form. Although statistically not significant, plants which were supplied with nitrate or a mixture of ammonium and nitrate grew slightly more as compared to those treated with either ammonium or urea. Less growth was observed with ammonium alone. These results are in agreement with the findings of Taylor et al. (1983) who found no significant difference in the growth of winter wheat with either ammonium or nitrate. However, our results disagreed with those of Gentry et al. (1989) who showed, with a different cultivar of wheat

(Inbar and Len), that the dry weight increased significantly when plants were supplied with a mixture of ammonium and nitrate as compared to nitrate only for 21 days in hydroponic conditions. They concluded that wheat plants could not absorb sufficient nitrogen for maximal growth when nitrogen was supplied only as nitrate. The current study clearly indicates that wheat can absorb enough nitrogen from a nitrate source and there is no significant difference in growth under nitrate or a mixture of ammonium and nitrate supply. This contradiction could be either due to the use of different cultivars or difference in the experimental conditions. For example, even Gentry et al. (1989) noted a large difference between the two cultivars Inbar and Len. My results also contradict those of Haynes and Goh (1978) who found that ammonium promoted wheat vegetative growth as compared to nitrate. Vegetative growth was not positively influenced by the ammonium supply in the present experiment. In the current study, it was noted that the shoot/root ratio increased in response to the mixed nitrogen nutrient as compared to either ammonium or nitrate. Growth on mixed nitrogen nutrition increased the shoot/root ratio as compared to ammonium or nitrate nutrition respectively. Gentry et al. (1989) noted a 32% increase in shoot/root ratio under a mixed nutrition as compared to nitrate.

In summary, under different forms of nitrogen fertilizers, no significant difference in growth of winter wheat var. Fredrick was noted. It is suggested that this variety can use all forms of

nitrogen fertilizers as nitrogen source, whereas, a mixture of ammonium and nitrate was preferred by Len and Inbar cultivars (Gentry et al., 1989) and cultivar Arthur showed similar response to nitrate and a mixture of ammonium and nitrate (Gashow and Mugwira, 1981). More shoot dry matter was produced under a mixture of ammonium and nitrate. The effects of urea on plant growth were intermediate.

A slight restriction of plant growth due to ammonium and urea nutrition could be related to drastic changes in the rhizosphere pH imposed by ammonium or urea uptake. At low pH values (below 5), ammonium nutrition has detrimental effects on growth (Marschner, 1986) due to induction of H^+ toxicity (Wright et al., 1989) or heavy metal toxicity such as aluminium (Wright et al., 1989). Other than Al, the concentrations of other heavy metals such as Fe, Cu and Zn are also increased in wheat plants under ammonium treatment (Wallace, 1989). However, acidification of the rhizosphere is not the only cause of ammonium toxicity since Magalhaes and Huber (1989) showed that rice growth was not affected by ammonium at low pH (5.7-3.5). The response of a specific plant to different nitrogen forms depends to the activity of its ammonium assimilating enzymes (Magalhaes and Huber, 1989).

Different forms of nitrogen fertilizers may also affect plant growth by altering the cation and anion composition of plants. Ammonium inhibits specific cation uptake in wheat (Wallace, 1989)

and can depress plant growth by inducing a specific cation deficiency. For example, potassium uptake is not only depressed by ammonium (Gashaw and Mugwira, 1981; Gentry et al, 1989) but also, the acidic condition of the rhizosphere under ammonium supply causes an efflux of potassium from the roots to the external medium (Marschner, 1986). Because potassium is required in large amounts by cereal plants (Gentry et al., 1989) and is involved in osmoregulation and cell expansion (Mengel and Arneke, 1982), its deficiency in ammonium-fed plants has adverse effects on plant growth. In addition, ammonium can produce a deficiency of calcium in plants (Pill et al., 1978). Since calcium exerts a crucial role in membrane permeability and maintenance of membrane integrity (Marschner, 1986), its resulting deficiency by ammonium treatment may depress plant growth. In contrast to ammonium, potassium concentration is increased in plants which are treated with nitrate (Gashaw and Mugwira, 1981). On the other hand, ammonium can depress plant growth through free accumulation in green tissues which uncouples photophosphorylation and inhibits photosynthesis (Magalhaes and Huber, 1989). The free accumulation of ammonium caused by carbon deficiency in roots (Magalhaes and Huber, 1989). Therefore, the growth enhancement in nitrate or a mixture of ammonium and nitrate may result from efficiency of cations such as potassium, calcium, magnesium and micronutrient (Wallace, 1989) or from reduced energy requirement for assimilation. As a result, growth responses of plant to different nitrogen sources seems only indirectly attributed to the pH differences.

4.2 pH studies:

Perhaps one of the most important chemical tests of soil as a medium for plant growth is its pH value. The solubility and availability of many nutrients are closely related to soil pH (Shwab et al., 1990). The pH experiments reported here have demonstrated that different nitrogen fertilizers can change the rhizosphere pH of the winter wheat depending on the fertilizer applied. For example, rhizosphere pH decreased when plants were supplied with either ammonium, a mixture of ammonium and nitrate or urea. However, those plants which absorb nitrogen as nitrate alkalinized the rhizosphere. The greatest pH differences were noted in the rhizosphere of ammonium-fed plants. Differences of up to 1.5 pH units between the rhizosphere of plants treated with ammonium and the rhizosphere of plants treated with nitrate were observed. Finally the most extreme rhizosphere pH difference (1.2 units) from the control occurred in the ammonium nitrogen treated plant rhizosphere.

My results seem to contradict those of Weinberger and Yee (1984) who found alkalinity of the pH in the wheat rhizosphere under the influence of a mixture of ammonium and nitrate and no change in the rhizosphere pH under urea treatment. The present results are in agreement with the findings of Nye (1981); Grinsted et al. (1982), Kraffczyk et al. (1984), Schwab et al. (1990) who

observed an acidic rhizosphere pH with a mixture of ammonium and nitrate, ammonium or urea.

Generally, changes in the rhizosphere pH are thought to be brought about predominantly by the net excretion of H^+, OH^- or HCO_3^- from the roots (Marschner, 1986). This excretion is related to the plant cation/anion uptake ratio and is an indication of the need to approach electrochemical balance both in root cells and in the external solution. The form of nitrogen supplied to the plant root has the most important influence on the cation/anion uptake ratio and thus on the rhizosphere pH (Marschner 1986). When ammonium is the nitrogen source, there is a much greater uptake of cations (NH_4^+). Extrusion of an equivalent amount of protons (H^+) into the rhizosphere balances the high uptake of cations vs. anions (Weinberger and Yee, 1984). Proton extrusion into the rhizosphere of plants is promoted by the stimulation effect of ammonium on plasma membrane H^+-ATPase; i.e. the electrogenic pumps, (Marschner, 1986).

As an alternative to H^+ extrusion, influx of HCO_3^+ might be involved in pH regulation under ammonium supply (Smith and Raven, 1979). The acidic condition under ammonium nutrition could be also the result of deprotonation of ammonium to NH_3 in the rhizosphere. Ammonia permeates the plasma membrane leaving H^+ in the external solution (Mengel et al., 1976; Coote et al., 1989). In general, the

ammonium effect on H^+ content of the soil can be summarized as (Coote et al., 1989):

$$HS_a = Q_a [2\alpha + r_a (1 - \alpha) \quad (2)$$

Where:

HS_a = H^+ [in mol ha^{-1}] due to NH_{4+} plant uptake or nitrification,

Q_a = input of ammonium (in form of fertilizer in mol ha^{-1})

α = coefficient representing the portion of ammonium nitrified

$r\alpha$ = coefficient representing the portion removed by plants.

The pH increase following nitrate uptake is a result of both preferential anion (NO_3^-) uptake (Weinberger and Yee, 1984) and nitrate reduction in plants which produces OH^- equivalent (Marschner 1986). These OH^- equivalents can be either effectively neutralized by the H^+ within the cytoplasm or be released into the external solution. In either case, the external pH increases (Marschner, 1986). As well, extrusion of HCO_{3-} from the root cells under nitrate supply has also been reported (Weinberger and Yee, 1984).

Active H^+ influx might also be involved in pH regulation in plants growing under nitrate supply (Schumaker and Sze, 1987). Anions such as NO_{3+} are thought to be accumulated in cells via H^+/anion symport systems by utilizing the proton motive force generated by H^+ extrusion pumps (Schumaker and Sze, 1987). Also, Smith and Raven (1979) observed influx of H^+ for pH regulation in cells growing in very alkaline solution. In general, the effects

of nitrate on soil H^+ (HS_n) were summarized (Coote et al., 1989) as follows:

$$HS_n = (\alpha Q_a + Q_n) [(\gamma -1) l_n - v_n - r_n] \qquad (3)$$

Where:

HS_n = H^+[in mol ha^{-1}] due to NO_3^- plant uptake;

Q_n = nitrate input (as fertilizer in mol (-) ha^{-1});

l_n, v_n and r_n = coefficient representing the portions of nitrate leached, volatilized and removed by the plant

α and Q_a are as in Eq. 2

γ is dependent on soil base saturation and can be estimated experimentally by leaching Ca from soil using 0.002N HCl (Coote et al., 1989).

The acidic rhizosphere pH under a mixture of ammonium and nitrate supply suggested that an excessive cation ($NH4^+$) uptake relative to anion (NO^{3-}) took place in the plants. The pH decrease in the rhizosphere was attributed to the rapid, initial uptake of ammonium ions over nitrate ions. In addition, the presence of ammonium in the medium may have depressed nitrate uptake or reduction (Ullrich, 1983; Dean-Drummond and Glass, 1983). Nitrate uptake or reduction only starts after ammonium has been exhausted or its concentration greatly reduced (Moor and Black, 1979).

Urea would be expected to be physiologically neutral and cause no change in pH (Weinberger and Yee, 1984). However, in the present study, a significant reduction in the rhizosphere pH suggested

higher cation absorption with urea (Kirkby and Mengel, 1967). Therefore, as a result of imbalance in cation-anion uptake, more protons would be released into the rhizosphere of urea-treated plants. This is in agreement with the finding of Bradley et al. (1989) who observed that urea uptake by wheat resulted in acidification of the rhizosphere. However, the pH decrease under urea supply may also be attributed to the original proton extrusion from the H^+-ATPase (Yee, 1984; Marschner, 1986). It seems that the acidic condition under urea treatment was caused by a difference between efflux H^+ to influx of H^+.

In the present study, after 7 days, there was a recovery in the rhizosphere pH of plants treated with ammonium, a mixture of ammonium and nitrate or urea alone. It is possible that at low pH, the efficiency of the H^+-efflux pump at the plasma membrane decreases and influx of H^+ or anion into the cytoplasm increases. For example, it was shown that electropotential increase of root cells from -150 mV at pH 6 to -100 mV at pH 4 inhibited cation uptake (Dunlop and Bowling, 1978). Also, Rufly et al. (1982) showed that a decrease in the pH from 6.1 to 5.1 caused an increase in the ratio of anion to cation uptake from about 1.0 to 1.25 in soybean. The anion uptake is in the form of H^+-anion co-transport across the plasma membrane (Marschner, 1986).

Other ways in which H^+ pumped out of the cell may recycle, have been suggested by Komor et al. (1977) and Novacky et al. (1978). Protons may move into the cell as part of a carrier complex

involving other solutes. Such H^+-linked transport can be either electrogenic or electroneutral. Examples of the former include H^+ symport with sugars (Komor et al., 1977) and amino acids (Novacky et al., 1978). The H^+-anion co-transport system suggests that with a mixture of ammonium and nitrate, the pH decrease from an initial pH of 6.0 to 5.6 could decrease the uptake of ammonium with a concomitant increase in the influx of nitrate. This would explain the pH fluctuation under a mixture of ammonium and nitrate.

In summary, an acidic rhizosphere pH was observed with wheat roots under the treatment of ammonium, mixtures of ammonium and nitrate or urea alone. Nitrate uptake by plants resulted in an increase in the rhizosphere pH. Cation and anion imbalances across the root surface, which influence the proton fluxes have been shown to be the dominant source of pH gradients in the rhizosphere (Nye, 1981; Weinberger and Yee, 1984; Marschner, 1986). The proton flux must be accompanied by other ionic fluxes in order to maintain charge balance and it is probably involved in a number of associations such as various symports, antiports, and possibly current loops (Marschner, 1986). In addition, any calculation of proton fluxes based on changes in the pH can be complicated by the possible occurrance of other processes such as CO_2 evolution from respiration, organic acid effluxes, volatilization, nitrification and denitrification. These could not only provide protons, but also modify the buffering capacity of the soil (Marschner, 1986; Coote et al., 1989).

4.3 Seed/root exudate (Amino acids):

This study confirms that the type of plant nutrition affects the seed/root exudate. Different forms of nitrogen fertilizers affect wheat root amino acid exudate differently, influencing both total quantity as well as the composition of exuded amino acids. The total amounts of the rhizosphere amino acids in wheat seedlings supplied with nitrate was significantly ($P<0.05$) less than those of plants supplied with either ammonium, a mixture of ammonium and nitrate or urea alone. The largest amounts of amino acids were noted to occur in the rhizosphere of plants fed with ammonium. Intermediate amounts of amino acids were detected in the rhizosphere of plants treated with a mixture of ammonium and nitrate or urea alone. These results are in agreement with findings of Brown and Hornby (1987) who showed that the greatest amounts of amino acids were presented in root exudate of wheat seedlings treated with ammonium.

When studying root exudate a certain uptake of released compounds must be taken into account. Bidwell (1981) reported the reabsorption of amino acids by plants, which acted as a additional nitrogen source. Accordingly, the quantities detected represent solely the difference between release and reabsorbed amounts (Kraffczyk et al., 1984). In the present study, the exudate of organic compounds into the rhizosphere was presumably derived from the endosperm as well as from nitrogen uptake and assimilation in

wheat seedlings. A high level of glutamic acid and glutamine in the rhizosphere of wheat seedlings under all treatments suggests that most of the amino acids and amides exuded into the rhizosphere were derived directly from assimilation of the nitrogen source. Other evidence is that the most usual stimulus to glutamine formation is the supply of nitrogen, either as ammonium or nitrate (Bidwell, 1981). In addition, glutamine plays a major role in plants as a detoxifier of ammonium (Lewis and Berry, 1975).

The release of low-molecular weight compounds from roots into the rhizosphere depends on their concentration in the root tissue as well as on the permeability of membranes (Kraffczyk et al., 1984). Therefore, amino acid concentrations are greater in roots of plants supplied with ammonium, a mixture of ammonium and nitrate or urea than those supplied with nitrate. For example, Breteler and Smit (1974) showed that ammonium increased the concentration of free amino acids and amides in the wheat plants. In a comparison of nitrate treatment with a mixture of ammonium and nitrate, Zornoza et al. (1989) demonstrated that in pepper plants a mixture of 80:20 of ammonium and nitrate elevated the concentrations of amino acids in all plant parts, with the roots displaying the greatest increases. However, under nitrate supply, the highest levels of amino acids are found in leaves and the lowest in the roots (Zornoza et al., 1989).

The different effect of nitrogen sources on the amino acids exudated could be linked to the different locations of nitrogen

metabolism as well as different physiological responses by plants. Under ammonium supply, high accumulation of amino acids and amides in roots is not only an immediate response of the plant to detoxify the ammonium (Matsumoto and Tamura, 1980; Marschner, 1986) but it may also be the result of a low rate of protein synthesis or overloading of the reduced nitrogen long distance transport system (Hoff et al., 1974).

In addition, greater accumulation of amino acids and amides under ammonium supply may also result in the retranslocation of amino acids and amides from the shoot to the roots as a feedback control of nitrogen uptake (Simpson et al., 1982). Part of these accumulated amino acids and amides could be exuded into the rhizosphere of plants.

When nitrate is used as the nitrogen source, translocation to the shoots is required since the nitrate reductase system is mostly located in the shoot of wheat plants (Minotti and Jackson, 1970). However, most plants reduce some nitrate in their roots (Minotti and Jackson, 1970; Pan et al., 1985; Schubert, 1987). Less accumulation of amino acids and amides in roots could be responsible for less exudation.

The initial response of plants to the mixture of ammonium and nitrate nutrition is mostly related to the ammonium part of the nutrient. Ammonium apparently is assimilated preferentially in the

roots faster than nitrate because of its toxic effects (Magalhaes and Huber, 1989). In contrast, the nitrate portion of the nutrients can be accumulated or translocated to the shoot (Morgan et al., 1985). It has been shown that seedlings and very young plants preferentially absorb ammonium when supplied with both ammonium and nitrate (Bidwell, 1981). In addition, inhibitory effects of ammonium on nitrate uptake and/or reduction (Dean-Drummond and Glass, 1983; Ohyama et al., 1989) decrease nitrate assimilation. For example, the absorption rate of nitrate decreased to about 33% of the initial rate in soybean after ammonium was added (Ohyama et al., 1989). Therefore, initial root assimilation of ammonium resulted in relatively high amino acid and amide accumulation and hence, greater exudation of these compounds.

Under urea nutrition, the large amount of amino acids exuded in the first week likely indicates a fast linear entry of intact urea molecule into the wheat roots (Bradley et al., 1989). Bradley et al. (1989) noted that assimilation of urea was at least as efficient as that of NH_4NO_3 in wheat. It is likely that after the urea was hydrolysed to ammonia and CO_2, the ammonia is assimilated in the roots to form amino acids and amides. However, Bradley et al. (1989) concluded that urea was utilized differently in wheat plants. They suggested that the NH_3/NH_4 produced via urea hydrolysis is not utilized in the same manner as the NH_3/NH_4 derived from ambient NH_4 and/or from reduction of ambient NO_3. Beuichem and Neeteson (1982) concluded that urea is not assimilated via the

urease and glutamine synthetase/glutamate dehydrogenase systems in maize. In addition, Bidwell (1981) suggested that urea may be incorporated into amino acids directly without hydrolysis. For example, urea condensation with ornithine to form arginine was suggested (Bidwell, 1981). In the present study, the high amounts of arginine in the rhizosphere of urea fed plants could be partly due to direct incorporation of urea into arginine. However, the amounts of exuded amino acids and amides were less under urea supply as compared to ammonium supply. This could be the result of partitioning of urea assimilation into shoot and root (Bradley et al., 1989).

Wheat plants grown on ammonium or urea nutrients lost more nitrogen (Table 8) than those grown on nitrate or a mixture of ammonium and nitrate. For example, during a period of one week, double the amounts of nitrogen were exuded into the rhizosphere under ammonium supply as compared to nitrate supply. The exudation of these pronounced amounts is not economic for plants since most of these materials could be utilized by the rhizosphere microorganisms under non-sterile condition of the field (Marschner, 1986) or could be lost through mineralization (Coote et al., 1989).

Quantitative differences in amino acids exuded from plants given various types of nitrogen fertilizers might be a result of differences in pH of the medium bathing roots. This could affect the permeability of cell membranes resulting in leakage of

nutrients (Hale et al., 1978). Because the acidity of the nutrient medium surrounding the roots caused by uptake of ammonium, a mixture of ammonium and nitrate or urea, increases the permeability of cell membrane and results in greater exudation of amino acids from the roots. More exudation was noted under ammonium or urea supplies. However, because of the large amounts of amino acids and amides accumulated in the roots of plants under ammonium supply, greater exudation was observed. In contrast to ammonium and urea, alkaline pH of the rhizosphere under nitrate supply has less effect on root cell permeability.

The amino acid exudate of wheat seedlings treated with ammonium, a mixture of ammonium and nitrate or urea, contained more acidic amino acids (glutamic acid and aspartic acid) than those of plants supplied with nitrate. Hence, the exudation of acidic amino acids from plant roots into the rhizosphere could be compensated by more cation uptake under these treatments which cause a high internal acidic condition. On the other hand, the more basic amino acids (histidine, arginine and lysine) exude into the rhizosphere of nitrate treated plants could be also compensated by high alkaline internal pH. The basic amino acids were increased in the rhizosphere of plants fed with a mixture of ammonium and nitrate after 15 days, with a concurrent decrease in the acidic amino acids. These results again support the idea of initial assimilation of ammonium over nitrate. Neutral amino acids such as alanine,

leucine, isoleucine, valine and phenylalanine were affected less by the form of nitrogen.

Differences in the composition of amino acids and amides are associated with different effects of nitrogen forms on different specific pathways of amino acid metabolism (Bidwell, 1981; Magalhaes and Huber, 1989). It is now widely accepted that the assimilation of nitrogen in most higher plants takes place mainly via glutamine synthetase (GS) (Magalhaes and Huber, 1989). However, at high NH_4 concentrations, glutamate dehydrogenase (GDH) and asparagine synthetase (AS) pathways can react directly with ammonium as nitrogen assimilation enzymes (Magalhaes and Huber, 1989). In addition, in order to minimize the carbon loss caused by nitrogen transport, nitrogen-rich compounds such as the amides glutamine, asparagine and amino acid arginine translocate the bulk of the assimilated nitrogen to the shoot under ammonium supply (Streeter, 1979). In the present study, the high levels of glutamine, arginine, glutamic acid and aspartic acid in the rhizosphere of plants treated with ammonium are an indication of an effective glutamine synthetase pathway in wheat roots. On the other hand, low glutamine in the rhizosphere of plants treated with nitrate is likely related to less active glutamine synthetase pathway in the roots. An intermediate amount of these amine and amides in the rhizosphere of plants treated with a mixture of ammonium and nitrate or urea suggest a more active pathway in the roots of these plants. These suggestions can be supported by the

findings of Magalhaes and Huber (1989) who showed that glutamine synthetase activity in the roots of tomato, corn and rice increased with ammonium compared to nitrate. In addition, large amounts of alanine in the rhizosphere of ammonium, a mixture of ammonium and nitrate or urea is representative of the more active glutamic acid-pyruvate tranaminases pathway in the roots of these plants than those of nitrate treated plants (Bidwell, 1981). High levels of serine in the rhizosphere of ammonium treated plants suggests that the metabolism of this amino acid is also increased with ammonium supply. High levels of serine were also detected in tomato plants under ammonium treatment (Ikeda et al., 1974).

The proportion of root exudate decreased with the plants age. The amount of amino acids detected in the rhizosphere decreased 30 fold in three weeks under ammonium treatment. The decrease of root exudate with plant age has been also reported in maize by Haller and Stolp (1985).

In summary, greater exudation of amino acids and amides under ammonium supply is a result of the following combination of effects; high accumulation of amino acids and amides, poor protein synthesis, overloading long distance translocation of nitrogen and severe acidic pH. Those plants treated with either a mixture of ammonium and nitrate or urea responded more like ammonium treated plants than like nitrate treated plants. The results of this study

suggest prior assimilation of ammonium over nitrate and mostly assimilation of urea into the roots.

Increased exudation of acidic amino acids in the rhizosphere of plants treated with ammonium, a mixture of ammonium and nitrate or urea, and greater exudation of basic amino acids under nitrate supply is a compensatory response of plants to internal pH. The neutral amino acids were less affected by the form of nitrogen. The synthesis of more basic amino acids with low C/N ratio is more economical for plants due to less carbon utilization. Therefore, nitrate appears to be more economical for plants with regard to synthesis of more basic amino acids and less nitrogen loss. Eighteen different amino acids and amides were found in the rhizosphere of wheat. Among them, a higher exudation of the amides glutamine, and asparagine and the amino acid alanine obviously accounted for greater synthesis of these compounds in the roots under ammonium treatment. Higher amounts of aspartic acid, glutamic acid and serine were also observed. It is possible that differences in the composition of amino acids and amides are linked to the different location of nitrogen metabolism as well as activity of the ammonium assimilating enzymes in those locations. Whereas ammonium is rapidly incorporated into the roots as amino acids and amides via a highly active GS/GDH/AS pathways (Magalhaes and Huber, 1989), partitioning of nitrate or urea into the root and shoot and less active enzyme systems in roots decreases the accumulation of these materials in the roots. Rapid uptake of ammonium stimulates

the glutamine synthetase pathway as compared to other pathways (Magalhaes and Huber, 1989). Hence, more glutamic acid, aspartic acid, glutamine and asparagine are produced serving as detoxifiers as well as a more economic long distance donors. In contrast, under nitrate supply, plants have the choice of using other pathways as well as glutamine synthetase by having more control on nitrate assimilation through its accumulation, efflux and translocation.

4.4 Root exudate (Carbohydrates):

Quantitatively, sugars are the predominant compounds in the rhizosphere (Curl and Truelove, 1986). The different forms of nitrogen fertilizers have different effects on wheat root soluble and insoluble sugars. The total soluble sugars were greatest in the rhizosphere of wheat seedlings supplied with ammonium, and decreased in the order of nitrate> a mixture of ammonium and nitrate> urea. The total carbon released into the rhizosphere in the form of soluble sugars was 50 times greater under ammonium fertilizer than that under urea fertilizer. Therefore, high exudation of carbon under ammonium may cause a carbon deficiency in roots and accelerate the toxicity of ammonium in plants through accumulation of free ammonium in green tissues (Magalhaes and Huber, 1989). This deficiency could affect plant growth especially at the seedling stage when plants do not have strong photosynthetic processes.

The relationship between plant carbohydrates and nitrogen sources has been documented for more than 20 years (Kirkby, 1968; Michael et al., 1970). The amounts of soluble sugars increase in wheat or other plants more under ammonium nutrition than with other nitrogen sources (Kirkby, 1968; Breteler and Smit, 1974; Haynes and Goh, 1978). Ammonium assimilation has a large carbohydrate requirement as carbon skeleton for the synthesis of amino acids and amides. It has been shown that uptake of ammonium by young potato plants is a result of the carbohydrate supply from the parent tuber (Michael et al., 1970). The sugars exuded into the rhizosphere are derived from seed and shoot carbohydrates which are translocated into the roots. The translocation of soluble sugars from shoot to the roots has already been documented (Oaks and Hirel, 1985; Pace et al., 1990). Results from the present study also suggest that fast uptake and assimilation of ammonium in wheat roots increases the rate of translocation of soluble sugars from seed and/or shoot toward the roots. Therefore, more soluble sugars accumulate in the root and because of the acidic pH of the rhizosphere more sugars exudate into the rhizosphere under ammonium supply. Thus ammonium may tax a plant's carbohydrate reserve and its uptake in large quantities may put a severe strain on the carbohydrate metabolism of the plant (Bidwell, 1981).

Under nitrate nutrition, reduction and assimilation of nitrate in roots also requires some sugars (Ohyama et al., 1989; Pace et al., 1990). The synthesis of [^{14}C] glutamine, [^{14}C] asparagine, [^{14}C]

glutamic acid and [^{14}C] aspartic acid at the expense of ^{14}C-saccharides in the roots is evidence of mobilization of the accumulated carbohydrates when wheat seedlings receive nitrate (Talouizte et al., 1984). If shoots are removed, there is a rapid loss of soluble sugars from the root and a corresponding reduction in the capacity of the roots to reduce exogenous nitrate (Oaks and Hirel, 1985; Pace et al., 1990). Pace et al. (1990) demonstrated that nitrate reduction is more sensitive to sugar than nitrate uptake. When carbohydrate levels are high as a result of light treatment or the addition of glucose or sucrose to the medium, the nitrate reductase and the reduction of nitrate were also relatively high (Aslam and Oaks, 1975; Aslam and Huffaker, 1982; Pace et al., 1990). However, fewer sugars are translocated into the roots because of nitrate partitioning. In addition, the alkaline pH of the rhizosphere under nitrate nutrition has less effect on cell permeability. In a mixture of ammonium and nitrate, preferential assimilation of ammonium in roots requires carbohydrates. However, due to the inhibitory effect of ammonium on nitrate, less nitrogen assimilation occurs in a given time. Therefore, fewer carbohydrates will be translocated into the root. Due to stepwise assimilation of ammonium and nitrate, translocation of sugars into the roots is expected to increase with time. This could be the reason for the gradual increase of soluble sugars into the rhizosphere up to 15 days under a mixture of ammonium and nitrate supply. Under urea treatment, probably fewer carbohydrates are necessary for urea assimilation due to its more direct incorporation into amino acids

without hydrolysis to ammonia. However, partial hydrolysis of urea could be slow and gradual. Therefore, slow release of soluble sugars in the rhizosphere of urea treated plants occurs.

The dominant soluble sugars in the rhizosphere of wheat were glucose, arabinose, xylose and galactose under all nitrogen treatments. However, their proportions and amounts differed under different nitrogen fertilizers. Greatest amounts of these sugars occurred in the rhizosphere of plants which were treated with ammonium and the least in the rhizosphere of nitrate treated plants. The dominance of both glucose and galactose in the rhizosphere of plants treated with ammonium suggest that these two sugars are probably important for ammonium assimilation. On the other hand, the high amounts of glucose in the rhizosphere of nitrate treated plants is an indication of high accumulation of this sugar in the roots and the importance of glucose in nitrate reduction and assimilation.

Glucose stimulates nitrate reduction in the root cells (Minotti and Jackson, 1970) and increases the size of the metabolic pool of nitrate within the root. Therefore, glucose increases the induction potential of the nitrate reductase enzyme (Aslam and Oaks, 1975; Dean-Drummond et al., 1980). Addition of glucose to excised or detopped roots resulted in enhanced levels of nitrate reduction (Dean-Drummond and Clarkson, 1979; Dean-Drummond et al., 1980; Pace et al., 1990). In the absence of glucose, the wheat

roots not only failed to reduce nitrate but also exude more nitrate to external solutions (Minotti and Jackson, 1970).

The amounts of soluble sugars decreased with time in the rhizosphere of plants which were treated with ammonium or nitrate In contrast, soluble sugars increased up to 15 days in those plants which were treated with a mixture of ammonium and nitrate or urea. Presumably the slow release of these sugars under a mixture of ammonium and nitrate or urea is based on stepwise utilization of ammonium and nitrate in the former and gradual assimilation of urea under urea nutrition. The dominance of both glucose and galactose in the case of the mixture nutrition again supports preferential ammonium metabolism. Under urea nutrition, more galactose and more glucose at 7 and 15 days could indicate either the intermediate effects of urea fertilizer relative to ammonium and nitrate or a different utilization pathway. The amounts of minor sugars such as mannose and ribose were also different under various nitrogen fertilizers. However, slow release of some individual sugars such as galactose and ribose was detected under ammonium treatment.

In summary, differences in the amount and number of soluble sugars in the rhizosphere of winter wheat under various nitrogen fertilizers could be the direct effect of the different forms of nitrogen on the plant's amino acid metabolism (Bradley et al., 1989; Magalhaes and Huber, 1989), on cation/anion balance, the rhizosphere pH (Marschner, 1986), and different locations of

nitrogen metabolism (Trolldenier and Rheinbaben, 1981). Incorporation of ammonium into amino acids and amides in the roots occurs at the expense of carbohydrates (Talouizte et al., 1984). Therefore, carbohydrate flux rate toward the roots increased. The acidic pH of the rhizosphere increases the exudation of accumulated soluble sugars into the rhizosphere.

Physiological differences in uptake and assimilation between ammonium and nitrate (Zornoza et al., 1989; Magalhaes and Huber, 1989) are responsible for differences in the amount and number of sugars in the rhizosphere. Although a large amount of carbohydrates are required for nitrate reduction and assimilation, because of partition of nitrate reduction and assimilation into root and shoot, less carbohydrate flux toward the root is expected. As a result of the latter as well as the alkaline rhizosphere pH, fewer soluble sugars are exuded into the rhizosphere. In a mixture of ammonium and nitrate, because of the inhibitory effect of ammonium on nitrate reductase activity (Breteler and Smit, 1974; Gentry et al., 1989) less nitrogen is available for plants at a given time. Therefore, less sugars are needed for nitrogen assimilation. However, after the ammonium is utilized, stored nitrate could be used. As a result, low but gradual flux of carbohydrates toward the roots could occur. These could be the cause of a slow, gradual release of soluble sugars. Partitioning of urea assimilation between roots and shoots or direct incorporation decreases the

influx of soluble sugars to the roots and causes a low, gradual exudation of sugars into the rhizosphere.

Different nitrogen fertilizers have also different effects on the rhizosphere insoluble sugars. Although in very small amounts, the total insoluble sugars were highest in the rhizosphere of plants treated with a mixture of ammonium and nitrate and lowest in those treated with nitrate. Glucose, galactose and xylose were the major component of insoluble sugars. The amounts of each individual insoluble sugars were also different under different nitrogen fertilizers. For example, glucose was highest in the rhizosphere of plants supplied with urea whereas xylose and mannose increased under a mixture of ammonium and nitrate. All insoluble sugars increased with time under nitrate treatment. Insoluble sugars in the rhizosphere are usually derived from mucilage (Angers and Mehuys, 1989) which consists mainly of polysaccharides and polygalctoronic acids. These materials are secreted by root cap cells and are also released by epidermal cells (Dormaar, 1988). The production of the mucilage is positively correlated with plant growth (Trolldenier and Hecht-Buchholz, 1984). In the present study, high amounts of insoluble sugars in the rhizosphere of plants fed with a mixture of ammonium and nitrate and its increase with time in those fed with nitrate are positively related to plant growth. This is correlated with the growth study results.

The lowest concentration of insoluble sugars occurred at 7 days, coincidentely with high nitrate concentration. Nitrate attenuated a drop in the root insoluble sugar content without affecting the corresponding shoot content and being deprived of nitrate for 7 days, increased the synthesis of structural materials in both shoots and roots (Talouizte et al., 1984). When the nitrate concentrations decreased over time the inhibitory effects on insoluble sugars decreased and more were synthesized. The observation of greatest concentration of insoluble sugars in the rhizosphere of plants treated with ammonium, a mixture of ammonium and nitrate or urea suggests that a protective mechanism for acidic pH is present in the root. The insoluble sugars of plant origin, such as xylose, glucose, mannose and galactose are likely to be involved in the structural stability of soil aggregates (Benzing-Purdie and Nikiforuk, 1989; Angers and Mehuys, 1989). Aggregate stability has considerable influence on plant growth, through effects on such features as aeration, infiltration, drainage characteristics and seedling emergence (Cheshire et al., 1989; Angers and Mehuys,1989). Therefore, more rhizosphere insoluble sugars under the effects of nitrate or a mixture of ammonium and nitrate will benefit plant growth and root function.

In summary, the highest levels of insoluble sugars in the rhizosphere of plants supplied with a mixture of ammonium and nitrate could be caused by both higher growth and acidic rhizosphere pH. Nitrate has an inhibitory effect on insoluble sugar

metabolism. However, after 7 days, when the inhibitory effect of nitrate decreased due to its lower concentration in the medium, the amount of insoluble sugars increased. Ammonium and urea both have an intermediate effect on the rhizosphere insoluble sugars.

CONCLUSIONS

As a result of nitrogen's critical roles in plant growth and it's cost, the management of nitrogen resources is an extremely important aspect of crop production. Nitrogen fertilizers exert pronounced effects on the biochemistry of the winter wheat rhizosphere. The rhizosphere pH will change due to an imbalance in cation/anion uptake by plants under the influence of different nitrogen fertilizers. The quantity and composition of seed/root exudate varies under the different nitrogen fertilizers. The total amounts of amino acids, amides and soluble sugars in wheat rhizosphere are higher under ammonium supply than those of other nitrogen fertilizers. However, the total amounts of insoluble sugars in the rhizosphere of plants fed with a mixture of ammonium and nitrate is greater than those treated with ammonium, nitrate or urea.

Under ammonium supply, the rapid uptake of ammonium increases the risk of toxicity in plants. Plants react by immediately converting ammonium to amino acids mainly glutamic acid, aspartic acid, glutamine and asparagine in the roots. Ammonium assimilation needs a large amount of soluble sugars as the carbon source. This nutrient increases the flux of carbohydrates from shoot or seed toward the roots. As a result, accumulation of these compounds into the roots could result in increased amounts being exuded into the rhizosphere due to the effect of acidic pH on root cell

permeability. Therefore, a positive correlation between the amount of materials which exude and their concentration in the roots is suggested. However, since plant growth responses are similar to nitrogen fertilizers regardless of the nitrogen forms, more root exudate is unrelated to plant vigour and growth. In contrast, the insoluble sugars in the rhizosphere could be related to plant growth as well as pH.

Nitrate nutrition by plants has some advantage for plants in comparison to ammonium alone. Ammonium could deplete the carbohydrate reserves of the plant due to fast utilization. The accumulation of amino acids and amides in the roots and the acidic pH of the root zone increases the amounts of exudation. As a result, not only high amounts of nitrogen and carbon are lost through exudation under ammonium nutrition but also these exuded materials can increase other processes such as denitrification in the rhizosphere which has adverse effect on nitrogen content (Scaglia et al., 1985). For example, high amounts of amino acids especially serine and aspartic acid in the rhizosphere play an essential role in soil denitrification (Rovira, 1969).

Although, there is uptake of exuded materials back to the root, because of competition between plant and microorganisms, most of these materials might be removed from the plant medium (Scaglia et al., 1985). Carbohydrate and amino acid deficiency could have an adverse effects on plant growth especially at the seedling stage

when photosynthesis is not strong enough. In addition, competition between ammonium and other cations such as potassium, calcium and micronutrient impair the synthesis of starch, cellulose, protein and increase plant cell permeability (Marschner, 1986; Wright et al., 1989). It is suggested that the plant response to ammonium supply is complex and it is not only related to ammonium itself but also to other factors such as cation concentration in plants, photosynthesis etc.

Nitrate uptake and assimilation are more controlled by plants through efflux control (Criddle et al., 1988) and accumulation in vacuoles. Besides, the partitioning of nitrate reduction and assimilation into root and shoot decreases the energy needs. In addition, there is no competition between nitrate and other cations. It is not as toxic as ammonium. Also the pH change is not acidic and harmful as ammonium on root cell permeability. Less amino acids and amides exudate under nitrate nutrition. In this variety the supply of a mixture of ammonium and nitrate is also recommended. This mixture could give plants the chance of easy available nitrogen in the form of ammonium when nitrate reductase is not active yet at the seedling stage. The inhibitory effects of ammonium on nitrate assimilation has some advantage for plants. Plants could accumulate nitrate and release it for further use. Therefore, it can benefit long term availability of nitrogen. Mixed nutrients have less effect on cation or micronutrient uptake by the plant. In addition, the mild acidic pH caused by the mixture

could be considered under alkaline soil conditions. Less soluble sugars and more insoluble sugars exude under a mixed nutrition and this benefits plant growth.

Urea has a similar effect on growth of this variety, but it is not recommended due to its high acidic effects on rhizosphere pH and increased exudation of amino acids. However, this fertilizer is known mostly as a foliar fertilizer (Bidwell, 1981) and it also goes under fast hydrolysis in nonsterile soils. Further studies should be carried out to analyze these aspects in more detail.

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Appendix A: 1) Numerical data of the rhizosphere amino acids.

Amounts[1] of amino acids in the rhizosphere of winter wheat after 7 days.

Amino acid	Treatments				
	water	NH_4	NO_3	mix	urea
Asp	7.72	9.94	1.97	3.73	5.65
Glu	36.1	63.1	42.9	65.0	61.0
Asn	0.00	10.7	1.75	6.38	4.44
Ser	4.45	24.0	3.73	7.07	12.0
Gln	2.12	87.1	5.16	54.4	16.3
His	2.75	0.00	11.4	0.00	16.8
Gly	11.7	46.3	29.9	42.7	42.8
Thr	0.00	28.6	27.5	26.6	33.1
Arg	1.28	4.70	6.34	2.72	7.15
Ala	22.2	256	173	222	254
Gaba	0.00	80.5	0.00	16.5	11.2
Tyr	8.41	20.8	10.7	14.2	21.5
Aba?	0.00	0.00	0.00	0.00	.156
Val	12.9	46.9	53.0	58.1	63.7
Phe-Ala	3.40	6.70	7.76	6.17	2.63
Ileu	5.69	25.3	19.3	23.8	32.1
Leu	11.2	60.5	36.2	44.7	61.1
Lys	6.21	16.2	25.4	14.2	29.2

[1] Each amount is the average of four replicates. This amount is for one plant.

Amounts of amino acids in the rhizosphere of winter wheat after 15 days.

Amino acid	Treatments			
	NH_4	NO_3	mix	urea
Asp	7.27	6.33	19.1	1.42
Glu	19.0	35.4	39.0	5.89
Asn	3.86	2.03	3.86	2.35
Ser	N.D.	N.D.	N.D.	N.D.
Gln	4.37	2.15	1.53	2.76
His	6.22	1.72	12.77	6.79
Gly	9.70	7.52	11.1	5.51
Thr	13.1	N.D.	N.D.	N.D.
Arg	4.67	1.40	19.1	0.731
Ala	29.6	25.3	31.0	13.9
Gaba	N.D	N.D.	N.D.	N.D.
Tyr	6.49	1.36	5.07	2.25
Aba?	N.D.	N.D.	N.D.	N.D.
Val	12.3	12.3	13.6	8.53
Phe-Ala	6.70	7.76	6.18	2.63
Ileu	N.D.	7.17	6.33	3.22
Leu	16.0	14.8	14.1	6.00
Lys	10.5	4.34	10.1	3.77

N.D. Not detected.

Amounts of amino acids in the rhizosphere of winter wheat after 21 days.

Amino acid	Treatments			
	NH_4	NO_3	mix	urea
Asp	1.33	2.54	2.41	1.46
Glu	3.11	2.36	3.03	1.80
Asn	N.D.	N.D.	N.D.	N.D.
Ser	N.D.	N.D.	N.D.	N.D.
Gln	N.D.	N.D.	N.D.	N.D.
His	N.D.	N.D.	N.D.	N.D.
Gly	2.64	2.39	1.37	2.00
Thr	N.D.	N.D.	N.D.	N.D.
Arg	N.D.	N.D.	N.D.	N.D.
Ala	5.45	2.61	3.17	2.65
Gaba	4.02	4.02	4.15	4.06
Tyr	1.80	1.50	1.96	1.39
Aba?	N.D.	0.51	0.55	0.34
Val	3.07	1.74	2.04	1.15
Phe-Ala	0.80	0.83	1.06	0.40
Ileu	1.20	1.22	0.91	0.55
Leu	1.84	1.56	1.26	0.79
Lys	2.04	1.47	0.96	0.30

N.D. Not detected.

Amounts[2] of soluble sugars in the rhizosphere of winter wheat after 7 days.

Sugars	Treatments			
	NH_4	NO_3	Mix	Urea
Ara	234	248	7.61	6.55
Xyl	233	254	2.70	1.77
Man	N.D.	28.1	4.29	3.5
Gala	1008	74.1	55.8	8.68
Glu	847	697	27.5	8.68
Rib	13.2	5.40	1.36	N.D.

Amounts of soluble sugars in the rhizosphere of winter wheat after 15 days.

Sugars	Treatments			
	NH_4	NO_3	Mix	Urea
Ara	46.9	42.7	116	58.5
Xyl	30.4	14.2	104	55.1
Man	16.7	13.3	22.5	16.0
Gala	61.5	150	90.2	46.8
Glu	311	167	303	191
Rib	9.05	8.40	6.60	7.25
Rha	N.D.	18.1	N.D.	N.D.

[2] Each amount is the average of four replicates. The amount is for one plant.

Amounts of soluble sugars in the rhizosphere of winter wheat after 21 days.

Sugars	Treatments			
	NH_4	NO_3	Mix	Urea
Ara	19.7	3.30	9.52	4.79
Xyl	6.19	2.14	1.53	2.12
Man	N.D.	N.D.	N.D.	N.D.
Gala	87.8	24.3	10.4	4.46
Glu	11.6	N.D.	N.D.	N.D.
Rib	71.6	5.08	5.00	1.83
Rha	4.18	1.72	N.D.	N.D.

Amounts of insoluble sugars in the rhizosphere of winter wheat after 7 days.

Sugars	Treatments			
	NH_4	NO_3	Mix	Urea
Ara	2.17	1.86	4.73	3.93
Xyl	12.2	N.D.	20.1	14.2
Man	11.0	N.D.	96.6	2.99
Gala	19.8	1.38	10.2	2.01
Glu	8.08	N.D.	13.8	10.6
Rib	N.D.	7.59	4.44	N.D.
Rha	4.49	2.81	5.84	1.21
Fuc	6.10	1.46	N.D.	N.D.

Amounts of insoluble sugars in the rhizosphere of winter wheat after 15 days.

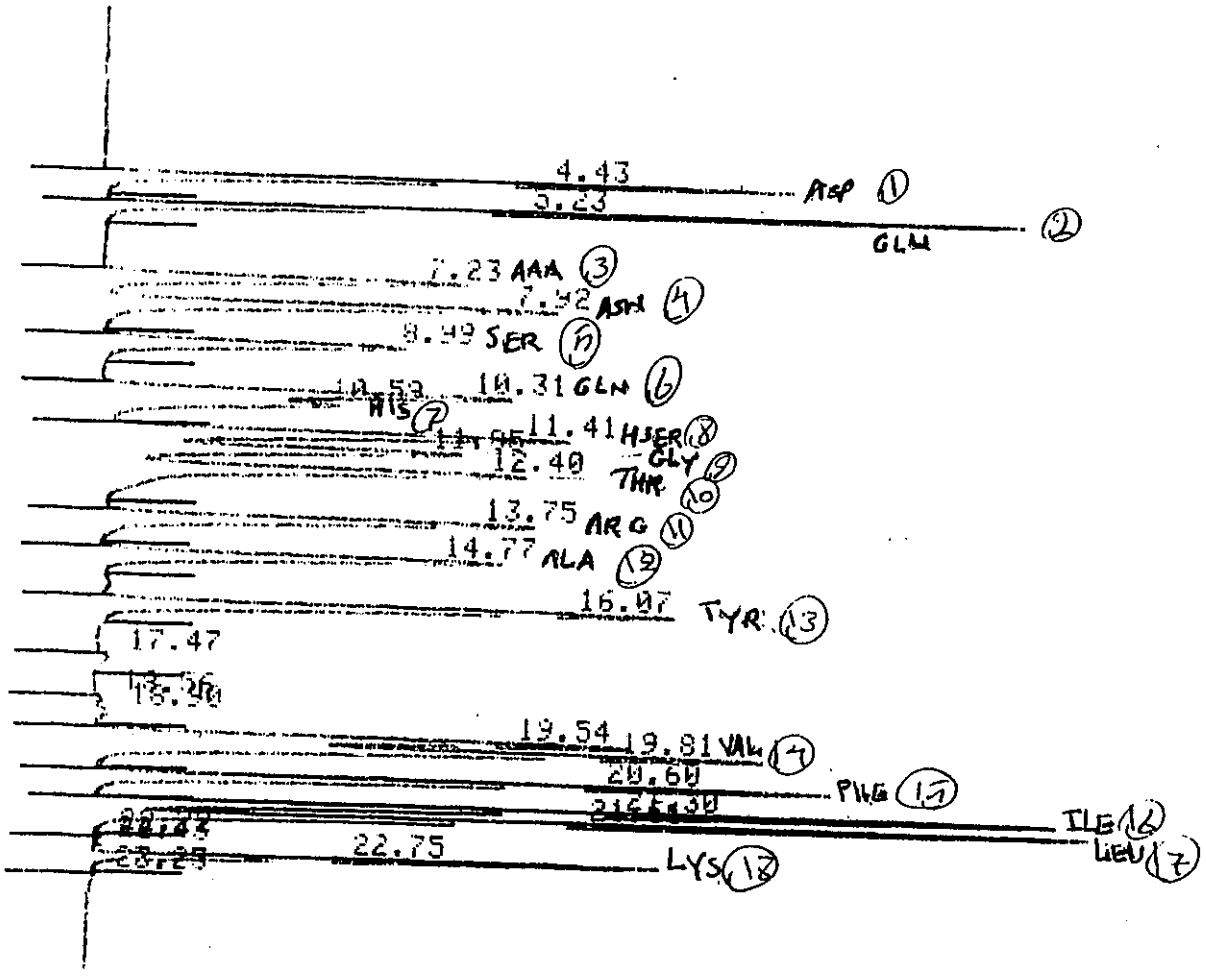
Sugars	Treatments			
	NH_4	NO_3	Mix	Urea
Ara	2.85	2.17	5.66	4.98
Xyl	8.16	2.72	3.58	7.55
Man	8.20	3.33	1.67	1.80
Gala	1.91	N.D.	N.D.	N.D.
Glu	11.9	2.89	3.89	7.36
Rib	N.D.	N.D.	N.D.	N.D.
Rha	N.D.	N.D.	N.D.	N.D.
Fuc	N.D.	N.D.	1.94	1.87

Amounts of insoluble sugars in the rhizosphere of winter wheat after 21 days.

Sugars	Treatments			
	NH_4	NO_3	Mix	Urea
Ara	1.70	5.59	1.61	2.71
Xyl	6.10	17.0	3.38	4.48
Man	1.99	2.16	1.79	1.70
Gala	1.47	1.42	1.47	N.D.
Glu	2.09	5.98	4.22	7.41
Rib	N.D.	N.D.	N.D.	N.D.
Rha	N.D.	N.D.	N.D.	N.D.
Fuc	2.11	1.89	1.89	1.68

APPENDIX B. Sample of the amino acid chromatogram.

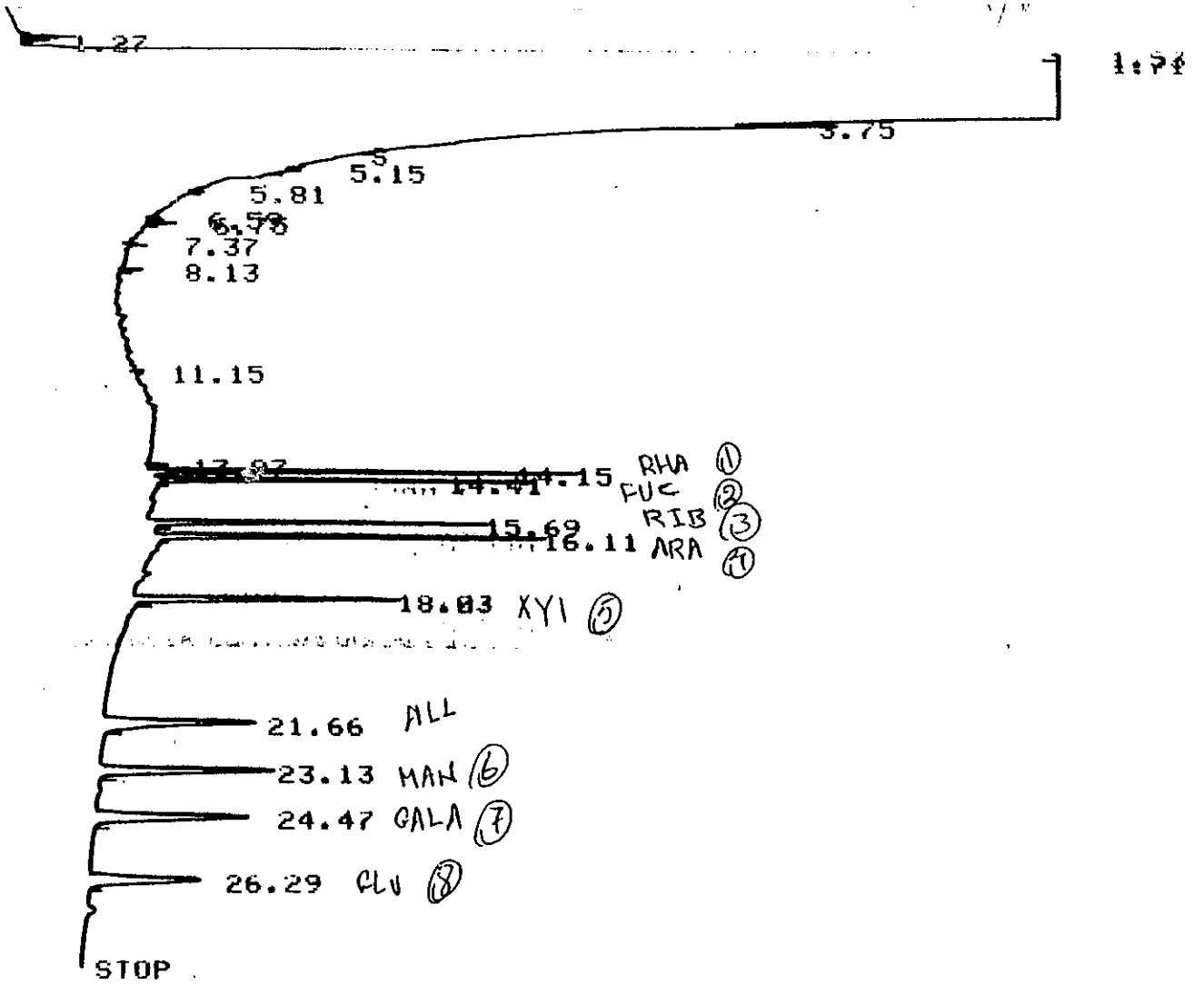
Abbreviation: Asp, aspartic acid; Glu, glutamic acid; AAA, alpha amino adipic acid (internal standard); Asn, asparagine; Ser, serine; Gln, glutamine; His, histidine; Gly, glycine; Thr, threonine; Arg, arginine; Ala, alanine; Tyr, tyrosine; Val, valine; Phe, phenylalanine; Ile, isoleucine; Leu, Leucine; Lys, lysine.



Sample of the sugar chromatogram. 1. Standard chromatogram; 2. sample chromatogram.

Abbreviation: Rha, rhamnose; Fuc, fucose; Rib, ribose; Ara, arabinose; Xyl, xylose; All, allose (internal standard); Man, mannose, Gala, galactose; Glu, glucose.

1. Standard chromatogram



2. Sample chromatogram