ROOT PHYSIOLOGY: FROM GENE TO FUNCTION

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Aims & Scope:

The Springer Handbook Series of Plant Ecophysiology comprises a series of books that deals with the impact of biotic and abiotic factors on plant functioning and physiological adaptation to the environment. The aim of the Plant Ecophysiology series is to review and integrate the present knowledge on the impact of the environment on plant functioning and adaptation at various levels of integration: from the molecular, biochemical, physiological to a whole plant level. This Handbook series is of interest to scientists who like to be informed of new developments and insights in plant ecophysiology, and can be used as advanced textbooks for biology students.

Root Physiology: from Gene to Function

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Cover Photo: Images of root cross sections taken from the flooding tolerant *Rumex palustris* (whole root system in background). *R. palustris* has been used by groups at Nijmegen University and Utrecht University as a model species for studies of submergence- and flooding-tolerance, at the molecular-to-ecological levels (see Chapter 11). Images of root cross sections from Ankie Ammerlaan (Utrecht University) and whole root system from Eric Visser (Nijmegen University). Cover designed by Ankie Ammerlaan.

Root physiology – from gene to function

Preface

Introduction

In the last decade, enormous progress has been made on the physiology of plant roots, including on a wide range of molecular aspects. Much of that progress has been captured in the following papers, which highlight that the classical boundaries between physiology, biochemistry and molecular biology have vanished. Breakthroughs have been made possible through integration of molecular and whole-plant aspects. There has been a strong focus on a limited number of model species, including *Arabidopsis thaliana*. That focus has allowed greater insight into the significance of specific genes for plant development and functioning. However, many species are very different from *A. thaliana*, in that they are mycorrhizal, develop a symbiosis with N_2-fixing microsymbionts, or have other specialised root structures. Also, some have a much greater capacity to resist extreme environments due to specific adaptations, for example aerenchyma in the case of soil flooding, or are capable of tolerating a wide range of soil chemical constraints, such as acidity, salinity or heavy metal toxicities. Research on species other than *A. thaliana* is therefore pivotal, to develop new knowledge in plant sciences in a comprehensive manner. This fundamental new knowledge can be the basis for important applications in, e.g., agriculture and plant conservation. Although significant progress has been made, much remains to be learnt, especially for many aspects of root physiology. It is envisaged, however, that discoveries made in the recent past will likely lead to major breakthroughs in the next decade.

Resource acquisition and carbon metabolism

The discovery of the role of aquaporins in water transport in both animals and plants has been a major breakthrough for understanding plant water relations, in particular water uptake (Vandeleur et al., 2005). Aquaporins are water-channel proteins (Johansson et al., 2000; Maurel, 1997). Their name is somewhat unfortunate, since the term 'porin' is commonly used for proteins that allow the transport of large molecules in a fairly unspecific manner, whereas we now know that aquaporins, which belong to a class of proteins termed MIPs (membrane-intrinsic proteins), are rather specific. However, some can also transport other small molecules, e.g., glycerol (Zeuthen and Klaerke, 1999) or CO_2 (Uehlein et al., 2003). Knowledge on the regulation of aquaporins contributes to understanding plant responses to some abiotic stresses and might help in the design of new irrigation techniques to improve use of scarce water resources. Aquaporins are involved in the adjustment of the hydraulic conductivity of roots, and therefore in adjustments of the roots' physiology during both phases of wet/dry cycles in natural communities and in horticultural crops with irrigation management using partial root-zone drying (Vandeleur et al., 2005). Wider implications of the regulation of aquaporins for acclimation during abiotic stress include the recent finding that the well-known decrease in root hydraulic conductance during flooding involves gating of a root aquaporin, due to cytosolic acidosis (Tournaire-Roux et al., 2003). Moreover, it can be speculated that aquaporins are important in hydraulic redistribution of water in the root–soil system. Aquaporins would allow a plant control over the exit of water from its roots into a soil with a more negative water potential than that of the roots themselves. This phenomenon, which was first described by Caldwell and Richards (1989) and termed 'hydraulic lift', is quite common in desert species with access to deep water (Yoder and Novak, 1999) and re-hydrates the rhizosphere of surface roots during the night. In contrast to

'hydraulic lift', for trees growing in a soil with a low conductance between the top and deeper layers, water movement *down* the profile might rely on hydraulic redistribution via the taproot (Burgess et al., 1998). So far, there are no data to support any speculations on involvement of aquaporins in hydraulic re-distribution, but it is expected that such information will become available during the next decade.

Major progress has been made on the physiology of uptake and metabolism of nitrogen (Miller and Cramer, 2005) and phosphorus (Raghothama and Karthikeyan, 2005; Smith, 2003a). Genes involved in the transport of these nutrients have been identified, and we are beginning to learn when and where these are expressed. We have also gained a much better understanding of the functioning of specialised roots involved in nutrient acquisition, such as the "proteoid" or "cluster" roots of Proteaceae and *Lupinus albus* (Lambers et al., 2003; Shane and Lambers, 2005). These cluster roots produce and exude vast amounts of carboxylates. Carboxylate release is not restricted to species with cluster roots, but it is the combination of their capacity to release carboxylates in an exudative burst and their structure that allows build-up of high concentrations in the rhizosphere. This ability provides these species with an opportunity to substantially modify their rhizosphere and 'mine' the soil in their immediate vicinity; they are particularly effective in the acquisition of scarcely available phosphorus and micronutrients. Some species belonging to the Cyperaceae have 'dauciform' (=carrot-shaped) roots (Davies et al., 1973; Lamont, 1974; Shane et al., 2005). In many ways, these dauciform roots function in much the same way as cluster roots; their development is suppressed at a high phosphorus supply and when formed they release citrate in an exudative burst (Shane et al., 2005; M.W. Shane and H. Lambers, unpubl.). The combination of biochemical/physiological and anatomical/morphological traits, which allows the build-up of high concentrations of exudates in the rhizosphere might be further exploited considering that the world's phosphorus reserves are dwindling (Vance, 2001).

Major phosphorus reserves are stored in agricultural soils, due to the application of phosphorus fertilisers ('phosphorus bank'). Depending on soil type and agronomic practices in the past,

over 80% of applied phosphorus in fertilisers can be sorbed to soil particles, and hence, largely unavailable for most crop plants. We will need crop species with the root traits of Proteaceae and Cyperaceae to access that phosphorus bank. So far, only a limited number of species with cluster roots have been used in agriculture and horticulture, including *Macadamia integrifolia* (to produce nuts) (Firth et al., 2003), *Lupinus albus* (as a source of protein) (Gardner et al., 1981) and *Aspalanthus linearis* (to produce rooibos tea) (Ratshitaka et al., 2001). Most *Lupinus* species do not make cluster roots of the type produced by *L. albus*, but they do make structures of a similar function (Hocking and Jeffery, 2004). Other *Lupinus* species that are used as crop species, e.g., *L. angustifolius* make sand-binding roots (pers. observation), which may well serve a similar purpose. We are not aware of any species with dauciform roots being intentionally used in managed systems, although, given the relatively wide occurrence of dauciform roots in Cyperaceae (Shane et al., 2005) species with this root type might have been planted in some constructed wetlands. Making greater use of species with cluster roots or similar traits will be of economic benefit, since these plants will be able to access sparingly available phosphorus. In addition, there may be ecological benefits, because an improved capacity for phosphorus acquisition will decrease the need for high rates of phosphorus application, and hence potentially reduce run-off of phosphorus into streams and estuaries, thus limiting eutrophication.

Plants often acquire limiting resources in symbiosis with micro-organisms (Vessey et al., 2005). Our understanding of the legume–rhizobium symbiosis has increased dramatically, not in the least because of the powerful molecular techniques of transcriptomics and metabolomics. These approaches have made it possible to obtain a much improved overview of the metabolic differentiation during nodule development in *Lotus japonicus* (Colebatch et al., 2004). Approximately 860 genes are more highly expressed in nodules than in roots, including one third involved in metabolism and transport. More than 100 of the highly expressed genes encode proteins likely involved in signalling, or regulation of gene expression at the transcriptional or post-transcriptional level. The analysis showed clear signs

of hypoxia in root nodules, as expected; in addition, there were numerous indications that nodule cells also experience phosphorus limitation. Much less is known about other N_2-fixing symbiosis (Vessey et al., 2005).

Mycorrhizal associations can be found in most vascular plant species, and this field of research has developed enormously in the recent past (Graham and Miller, 2005). Molecular tools have revolutionised studies of mycorrhizal diversity and abundance, improving knowledge on host specificity of the symbionts (Graham and Miller, 2005) and highlighting relationships between mycorrhizal fungi diversity as influencing the structure of some communities of vascular plants (e.g., Reynolds et al., 2003). Like the recent discoveries of genes for transport of mineral nutrients in plants (Miller and Cramer, 2005), rapid advances to elucidate genes regulating exchanges of molecules between plant hosts and arbuscular mycorrhizal (AM) fungi are being made. Our views on 'mycorrhizal dependency' need to be revisited, with the discovery that the micro-symbionts can be responsible for most of the phosphorus uptake when there is no, or only a minor, growth response (Smith et al., 2003b).

Carnivory of small animals is a specialised strategy used by some plant species to acquire inorganic nutrients (Adlassnig et al., 2005). Carnivorous species inhabit a range of nutrient-impoverished environments, ranging from fire-prone sand plains to acid peat bogs and aquatic habitats, with, as one might expect, a wide range of root sizes and structures (Adlassnig et al., 2005). In contrast with information available on their traps formed by shoot organs (Juniper et al., 1989), comparatively little is known about the physiology of roots of carnivorous plants; several studies have demonstrated the importance of the roots in water and nutrient uptake for at least some carnivorous species, but not in several others.

Since two books (Day et al., 2004; Lambers and Ribas-Carbo, 2005) dealing with plant respiration have recently been published in Govindjee's series *Advances in Photosynthesis and Respiration*, no review dealing with this aspect of root physiology has been included. Over the last decade or so, we have gained a much better understanding of the respiratory costs of nutrient acquisition in fast- and slow-growing herbaceous species (Poorter et al., 1991; Scheurwater et al., 1998). When plants are grown at a high supply of nitrate, the costs associated with nitrate uptake are considerably greater for inherently slow-growing species, due to a major efflux component (Scheurwater et al., 1999). That passive efflux needs to be compensated by a greater active influx, and thus accounts for greater respiratory costs per unit N acquired. However, greater efflux is not the cause of slower growth; rather, slower growth leads to greater efflux (Ter Steege et al., 1999) and hence greater respiratory costs (Nagel and Lambers, 2002). Considerable information has also become available on the quantitative significance of cyanide-resistant root respiration (Millenaar et al., 2001). Major progress is to be expected on the physiological significance of the alternative path, which has puzzled plant scientists for quite some time. That progress can be expected, because a technique to assess the activity of this path in intact tissues has become more widely available (Ribas-Carbo et al., 2005).

Perception of the abiotic and biotic root environment

We have gained an appreciation of the fact that roots 'sense' their environment, and that the plant responds in a feed-forward and adaptive manner. The feed-forward response when roots sense adverse conditions in the soil allows acclimation before a major disturbance of the plant's metabolism (Davies et al., 1994). When roots perceive that the soil is flooded, they respond with an enhanced concentration of ethylene in both the roots and above-ground plant parts. Ethylene induces the formation of aerenchyma in roots (Visser and Voesenek, 2005), and also affects adaptive processes in above-ground organs (Voesenek and Blom, 1999). Similarly, roots can sense the availability of water (Davies et al., 1994) as well as nitrogen and phosphorus (De Groot et al., 2003), and signal this information to the shoot, which responds in a feed-forward manner. In the case of water stress, the signalling molecule is ABA (Davies et al., 1994; Schurr et al., 1992). Cytokinins are involved in signalling the plant's N and P status (De Groot et al., 2003; Kuiper et al., 1989; Lambers et a1., 1998). These are some of many examples showing

'signalling' between roots and shoots. More details about signals and signal-transduction pathways are included in the review by Dodd (2005), highlighting that the signals need not always be one of the classical phytohormones, but can, for example, be nitrate or sugars also. Internal signalling in plants, as dependent on plant development and environmental conditions is a rapidly developing field, where much progress can be expected. The challenge will be to integrate the new information into improved crop production systems.

Sensing does not only involve resources, but also other chemical factors in the environment. Aluminium-resistant genotypes must be able to sense the presence of aluminium to respond with the release of aluminium-complexing carboxylates (Kochian et al., 2005). When roots release either mainly citrate or mainly malate, depending on soil pH (Veneklaas et al., 2003), they must be sensing the soil pH or a factor closely associated with it. Sensing environmental conditions is obviously crucial to performance of plants, although we still lack a thorough understanding of the exact mechanisms involved.

Signalling in plants is not only important between different organs of a plant, but also between hosts and parasitic plants (Okubara and Paulitz, 2005). Holoparasitic species are entirely dependent on a host for the completion of their life cycle (Lambers et al., 1998). If their seeds were to germinate in the absence of a suitable host, that would be fatal, especially for those that have extremely small seeds. In fact, both germination (Siame et al., 1993) and the formation of haustoria (Estabrook and Yoder, 1998; Smith et al., 1990), which connect the parasite with its host, depend on chemical signalling between host and parasite. This is an exciting and rapidly developing field. Knowledge of these interactions may appear esoteric, but major applications can be expected, because some parasitic species (e.g., *Striga* and *Orobanche* species) belong to the world's worst weeds (Emechebe et al., 2004; Marley et al., 2004; Rodriguez-Conde et al., 2004). Others (*Cistanche* species) are grown to produce medicine in north-eastern China (Geng et al., 2004). Low-cost and safe signalling molecules that trigger the germination of the seeds of parasitic pest species before crops are sown might be of enormous benefit, especially to farmers in developing countries.

Signalling is also important between hosts and their symbiotic micro-organisms, e.g., rhizobia (Vessey et al., 2005) and mycorrhizal fungi (Graham and Miller, 2005). The intricate interactions that precede the establishment of a functional symbiosis are best understood for the rhizobium–legume symbiosis (Esseling and Emons, 2004). They must also play a role in other symbiotic systems that fix dinitrogen, but the progress in that area has been much slower (Rai et al., 2000; Vessey et al., 2005). Somewhat more is known on signalling between hosts and some mycorrhizal fungi (Graham and Miller, 2005), but much remains to be discovered.

Plants growing in soil with adverse abiotic or biotic conditions

Some species or ecotypes are capable of growing in soils that are naturally enriched with heavy metals, e.g., serpentine or ultramafic soils. The metals may be 'excluded' or absorbed, and stored in compartments where they do not harm the plant's metabolism (Meharg, 2005). Recently, progress has been made on the mechanisms accounting for internal transport and storage of heavy metals as well as metal 'exclusion' (Meharg, 2005). Species or ecotypes that accumulate heavy metals to very high levels are called metallophytes. These are not restricted to soils naturally enriched with heavy metals, but are also found on sites contaminated by heavy metals, e.g., due to mining. Such metallophytes are very important to stabilise contaminated soil, and stop it from spreading over a larger area. Metallophytes have been proposed as a method to clean contaminated soil (phytoremediation) (Meharg, 2005) or extract metals from soil with the intention to mine the metals (phytomining) (Li et al., 2003). To be economically viable options, the metallophytes have to accumulate metals to very high concentrations and produce a lot of biomass in a relatively short time. However, most metallophytes are inherently slow-growing, most likely because they were selected in low-nutrient environments, which are typically inhabited by slow-growing species (Lambers and Poorter, 1992). Genotypes that are both metal resistant and productive need to be selected. Major new discoveries are to be expected in the next

decade, but applications in the context of phyto-remediation and phytomining would appear to be less promising than claimed when first proposed, unless combined with other profit-making operations, e.g., forestry (Robinson et al., 2003).

Mycorrhizas have been claimed to 'protect' higher plants from negative effects of heavy metals in soil (Leyval et al., 1997). It has also been shown that species that belong to a typically non-mycorrhizal family can be mycorrhizal if they are associated with soils with high levels of heavy metals. One example is for California serpentine grassland communities, where *Arenaria douglasii* (Caryophyllaceae) and *Streptanthus glandulosus* (Brassicaceae) were found to be mycorrhizal (Hopkins, 1987). Another example is for *Hakea verrucosa* (Proteaceae), occurring on nickel-containing ultramafic soils in Western Australia (Boulet and Lambers, 2005). These are exciting observations, from an evolutionary perspective as well as because of the possible implications for the rehabilitation of contaminated sites after mining.

Acid soils represent another stress to plant roots. It is not so much the low pH itself that causes the problems, but the fact that the solubility of specific metals strongly depends on pH (Kochian et al., 2005). In particular aluminium is considerably more soluble at low pH. Acid-resistant species typically are aluminium resistant. Resistance is at least partially based on 'exclusion', due to precipitation with citrate, malate or oxalate released from roots, depending on the species (Delhaize et al., 1993; Zheng et al., 1998). Some aluminium-resistant species also accumulate aluminium, and detoxify it internally as aluminium-carboxylate precipitates (Ma et al., 1997; Zheng et al., 1998). As for heavy metal resistance (Meharg, 2005), there is considerable genetic variation for aluminium exclusion (Delhaize et al., 1993; Kochian et al., 2005). This allows important application in agriculture, but care also has to be taken to minimise further soil acidification, which is a common trend in cropping systems (Lambers et al., 1998). Soil amelioration, e.g., using lime, remains important, but does not invariably address sub-soil acidity; therefore, aluminium-resistant genotypes would be important to develop.

Drought, salinity and flooding are abiotic stresses each of which influence plant species composition and productivity in natural and managed systems. Improved knowledge on physiological responses of roots to water stress, including root-to-shoot signalling (Dodd, 2005) should aid design of new irrigation techniques to improve use of scarce water resources (Vandeleur et al., 2005). Since various aspects of resistance to salinity have been reviewed recently (e.g., Munns, 2002; Tester and Davenport, 2003), and in a special issue dealing with dryland salinity in Australia (Lambers, 2003), salinity is not covered in this volume. Flooding regimes shape the composition of natural communities in some areas (Voesenek et al., 2004) and underpin rice production systems; soil waterlogging is also a problem in vast areas of irrigated and rainfed agriculture. Visser and Voesenek (2005) provide a comprehensive review on signals, and signal-transduction pathways, crucial to the perception and acclimation by plant roots to soil flooding. Hormones and signalling pathways that regulate traits for flooding resistance, such as adventitious rooting, aerenchyma formation for gas transport (Colmer and Greenway, 2005; Jackson and Armstrong, 1999) and root metabolism during O_2 deficiency (Gibbs and Greenway, 2003; Jackson and Ricard, 2003) are reviewed by Visser and Voesenek (2005). Substantial gains in knowledge in some areas are highlighted, as are emerging topics that are still poorly understood and will be priority areas for future research.

Roots frequently encounter adverse biotic conditions, due to the presence of microbial pathogens, nematodes, viruses and plant parasites (Okubara and Paulitz, 2005). Our understanding of these interactions has increased enormously, again, in part due to the development of new molecular tools. Developing resistance to root pests and diseases will continue to be important, as chemical protection is not always a desirable option.

Using new genotypes and combinations of crop species based on new ecophysiological information

Allelopathic interactions are very hard to demonstrate in nature, but they are very likely to occur, also in managed ecosystems (Lambers et al., 1998). The interactions may involve micro-organisms (Inderjit, 2005). Allelopathic interactions may account for the invasive nature of some

weeds (Ridenour and Callaway, 2001). There are major possibilities for applications in agriculture. Accessions of wheat (*Triticum aestivun*) differ widely in their potential to inhibit seed germination of ryegrass (*Lolium rigidumi*) (Wu et al., 2000a), a major weed in Australia (Powles and Shaner, 2001). That variation appears to be associated with the release of allelochemicals of a phenolic nature (Wu et al., 2000b), although so far the phenolics have only been assessed in root tissue, not in exudates. Making a crop more competitive, by enhancing its capacity for interference competition, would reduce the need for herbicides.

Facilitation refers to positive effects of one plant on another (Callaway, 1995). It is equally difficult to demonstrate in natural systems as allelopathic interactions are (Hauggaard-Nielsen and Jensen, 2005), but there are numerous examples of increased yields when combinations of crop species are used (Karpenstein-Machan and Stuelpnagel, 2000; Zuo et al., 2000). Such agronomic practices, usually called intercropping, are used in the low-input systems of the tropics, where crops are harvested manually (Willey, 1979), and are also common in China (Zhang et al., 2004) where new intercropping systems continue to be developed (Guixin et al., 2004). If reliable systems can be developed to mechanically harvest intercropped species at the same time, then this would stimulate the development of the practice for broad-area agriculture in other countries.

Pasture agronomy already uses combinations of species in broad-area agriculture, but further research might enhance productivity of pasture systems if the best combinations of species, and perhaps genotypes within species, can be further refined. For example, when seedlings of mycorrhizal and non-mycorrhizal species are grown together, they tend to have negative effects on each other which are not seen when either seedlings of mycorrhizal species or ones of non-mycorrhizal species grow together (Francis and Read, 1994). The chemical basis of this interference is not known, but the observation may have major implications for plant functioning in natural or managed systems. It may mean that combinations of mycorrhizal and non-mycorrhizal species are less desirable for intercropping, and this will need to be addressed to enhance productivity of intercropping and pasture systems.

Perspectives

Many new discoveries are to be expected in the ecophysiology of roots of native and crop species. One can envisage many applications of the new fundamental knowledge. One area that has not been reviewed in this volume, because it is too new to have generated many publications, is that of signalling in tritrophic below-ground interactions. Similar above-ground interactions are well documented for interactions between plants, their herbivores and 'bodyguards' (Alborn et al., 1997; Kessler and Baldwin, 2001; Sabelis et al., 2001). The first exciting information is now becoming available on interactions between roots of *Thuja occidentalis*, which release chemicals upon attack by weevil larvae (*Otiorhynchus sulcatus*), to attract parasitic nematodes (*Heterorhabditis megidis*), which then prey on the weevil larvae (Van Tol et al., 2001). Similar tritrophic interactions appear to occur in *Zea mays* (T.C.J. Turlings, pers. comm.). It is to be expected that improved knowledge in this area should provide opportunities for applications in plant management systems, similar to those existing for above-ground tritrophic interactions (Turlings and Wäckers, 2004).

Major progress on understanding numerous aspects of root physiology, and under several important environmental constraints, has been made possible by close interactions between ecophysiologists, biochemists and molecular geneticists. These close interactions will be important to achieve new breakthroughs in yield improvement. Such breakthroughs are vitally important, if we are to produce enough food and fibre for the worlds growing population in a sustainable manner.

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Root nitrogen acquisition and assimilation

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Abstract

Nitrogen (N) is the main mineral element in plant tissues and almost all of this nutrient is acquired from the soil by the roots. Nitrogen is available in many different forms in the soil, but the three most abundant forms are nitrate, ammonium and amino acids. The relative importance of these different soil N pools to a plant is difficult to measure and depends on many different environmental factors. Changes in the available amounts and imbalance in the supply of some N forms can even be toxic to plants and in extreme cases can lead to changes in the vegetation. However, the importance of this element for agriculture is reflected in the amounts of N-fertiliser applied to crops and this is a major cost (economic and environmental) for world agriculture. This review covers the molecular mechanisms that the plant uses for accessing these soil N pools and briefly includes consideration of the root N assimilatory pathways that exist in the plant. The soil forms of N that are used by plants depend on many factors, but a series of different transporter and assimilatory genes that can provide access to these pools have been identified. This information can now provide the molecular tools to identify the N sources accessed by a plant and the relative importance of these different pools.

Introduction

Plants require nitrogen (N) throughout their development. This N represents about 2% of total plant dry matter, and is a component of proteins, nucleic acids, coenzymes and numerous plant secondary products. Nitrogen is quantitatively the most abundant of the mineral elements in plant tissues, and enters the food chain mostly as NO_3^- or NH_4^+. The availability of N to plant roots is often an important limitation for plant growth, except where roots develop a symbiosis with N_2-fixing microorganisms (not reviewed). Only a tiny fraction (0.00024%) of planetary N is available to plants in the pedosphere (which includes plants, microbes, fauna, litter and soil). Plants cannot directly access either N_2, which comprises 2% of planetary N, or the 98% of planetary N that is immobilized in the geosphere (Rosswall, 1983). Atmospheric fixation of N_2 due to lightning is thought to account for between

0.5 and 30×10^{12} g N annum^{-1}, and biological N_2 fixation for 45 to 330×10^{12} g N annum^{-1}, 40% of which occurs in the oceans (Rosswall, 1983). The limited bio-availability of N and the dependence of crop growth on this mineral have spawned a massive N-based fertiliser industry worldwide, with annual N-fertiliser consumption currently close to 80×10^{12} g N (Figure 1). An increasingly large proportion of this N is currently applied in 'developing' countries, particularly in Asia, although, the extent of N application in the 'developed' world has declined over the last decade, resulting in a slowing in the rate of worldwide increase of N applications.

Nitrogenous fertilisers and associated contaminants accumulate in some situations to dangerous or even toxic levels, resulting in eutrophication of surface and ground water, and enriching the atmosphere with NH_3 and with N_2O. Considerable leaching of NO_3^- is caused, for example, by excessive application of nitrogenous fertilisers (inorganic and organic) to crops

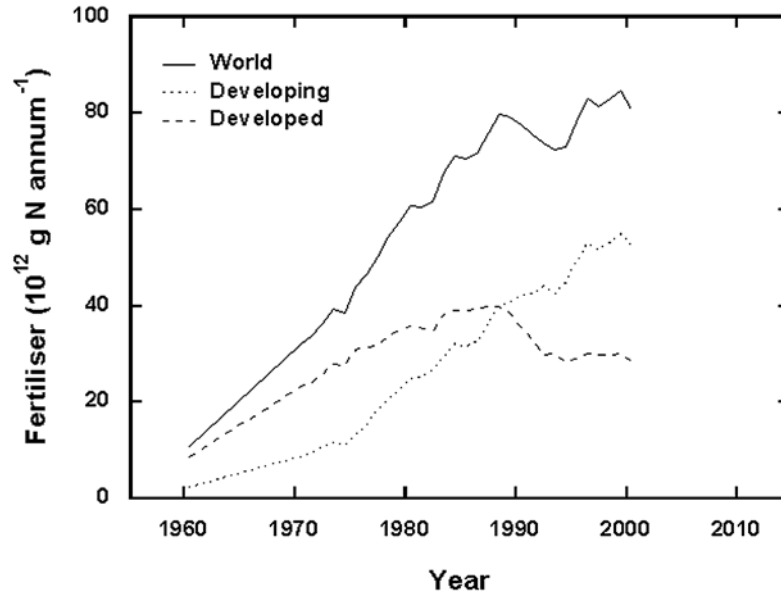*FAX No: +27 21 650 4041. E-mail: mcramer@botzoo.uct.ac.za

Figure 1. The consumption of N-containing fertilisers between 1961/62 and 2000/2001. The developing world was calculated from the data available for Central and South America, Africa (except South Africa), Near East (except Israel), South Asia, East Asia (except Japan), Socialist Asia and Oceania. Nitrogen-containing fertilisers include ammonium sulfate, urea, ammonium nitrate, calcium ammonium nitrate, ammonia direct application, calcium nitrate, sodium nitrate, ammonium chloride, calcium cyanamide, ammonium bicarbonate and combinations including ammonium phosphate, NP, NK and NPK (data plotted from that available from the International Fertiliser Industry Association, www.fertilizer.org/ifa/statistics.asp).

in an attempt to ensure maximum yields. Leaching also depends on soil characteristics, and the amount of water fluxing through the soil. Although less easily leached from soil, NH_4^+ is more toxic to plants than NO_3^- (Dejoux et al., 2000). However, conversion of NH_4^+ to NO_3^- (nitrification) can also contribute to the leaching of N from soils amended with NH_4^+ containing fertilisers. Estimates of total N loss by leaching from NH_4^+-based fertilisers range between 10 and 150 kg N ha^{-1} (International Fertilizer Industry Association, www.fertilizer.org/ifa/statistics.asp). Atmospheric pollution by NH_3 from organic manure, urea and ammonium sulphate might result from NH_3-volatilization. Although estimates of NH_3 volatilization are subject to a great deal of uncertainty, emissions estimates are between 15% and 25% of the applied amount of urea-N for Europe and for the tropics, respectively (Schjørring, 1998). Denitrification losses may be in the range of 5 to 10% of the applied N, of which about 10% is in the form N_2O (International Fertilizer Industry Association, www.fertilizer.org/ifa/statistics.asp), which is a greenhouse gas. Loss of NO_3^- through denitrification, both biological and chemical, occurs under reducing or anaerobic conditions (Haynes and Goh, 1978), and is

especially important in fertilised fields where the loss of N may be enormous (Lewis, 1986). Thus excessive application of N fertilisers has enormous environmental costs, in addition to the economic and ecological costs of the production of the fertilisers. Most farmers are very aware that the excess use of fertiliser can cut their profit margins, but the yield penalties associated with application of too little N are potentially much larger, and it is therefore often economically not worth taking this risk. The balance between these two sides of the equation means that farmers cannot afford to skimp on their N-fertiliser applications, and the excess 'spare' N is deposited into the biosphere. This excess N and other man-made N pollution sources, such as factory and car exhaust, may have major environmental impacts as they supply additional growth potential to native plants. In some extreme cases this release of the growth limitation by N, whatever the source, can result in the invasion of new species, and a change in the landscape. This is the case, for example, with the N_2-fixing Australian *Acacia* spp. which have extensively invaded the 'Fynbos' biome in South Africa. Changes in forest species compositions and vegetation types as a result of agricultural pollution are now widely recognised (Nosengo, 2003), with reports of

changes in forests from the USA (e.g. Kochy and Wilson, 2001), Europe (e.g. Rennenberg et al., 1998) and changes in the UK flora (Pitcairn et al., 2003). This is not acceptable to most people who see this change in the environment as damaging the quality of life. Increasingly, farmers must be paid not just to produce food, but also to protect and maintain the environment.

The N accessed by plants exists in a variety of organic and inorganic forms within the soil. This influences the availability of the N and the uptake of the N by plants. A number of different transporters have been identified as being responsible for the uptake of inorganic (NO_3^- and NH_4^+) and organic N from the soil into roots. These multiple transport systems function under different circumstances, and are subject to complex regulation at the levels of transcription, translation and post-translation. Unlike many other mineral elements, N usually needs to be assimilated in order to participate in the biochemistry of the plant. This introduces a further level of complexity to the system with additional regulatory elements. Nitrate taken up by roots is either reduced *in situ* to NH_4^+ in the root, stored in vacuoles or transported to the shoot. The extent of shoot-based NO_3^- reduction varies between species and environmental circumstances (see below). Reduction of NO_3^- to NH_4^+ is achieved through participation of NO_3^- and NO_2^- reductases, with further assimilation of NH_4^+ into glutamate and glutamine by glutamine synthetase and glutamate synthase (see below).

Continued research and improved understanding of the chemistry of N in soils and the biochemistry of N uptake and assimilation may assist in development of improved management practices for natural and crop ecosystems, for example the reduction of N leaching (Spalding et al., 2001). The recognition that there is the possibility to breed improved genotypes capable of more efficient N uptake and utilization has become a new target for research. Most of the crop varieties grown in the developed world have been bred under conditions of high fertiliser input, approaching N saturation. There is an opportunity for the developed world to learn from the more sustainable agricultural systems in the developing world, and their cultivars will be a useful genetic resource in this effort. Improved efficiency of N recovery from soil and improved efficiency of utilization could allow crops to be grown with reduced N-fertiliser applications with contingent environmental and economic advantages. It is now timely and highly pertinent to review our current knowledge of the uptake and assimilation of N by plants. Much of the information on N metabolism is derived from studies on shoots which may or may not be pertinent for roots. This review attempts to provide an overview of N acquisition and assimilation in roots, while focusing on the latest findings relating to the molecular biology and the regulation of these processes.

Nitrogen acquisition

Nitrogen in the soil

Forms and origins of N

Nitrogen in the soil is present as a complex mixture of organic and inorganic forms, and, in addition to seasonal and diurnal changes, is also characterised by an extremely heterogeneous distribution. The transformation of one form into the other comprises what is known as the 'nitrogen cycle' involving the scavenging of organic N by microbial action and re-absorption by plants (Figure 2). Most of the N in soil is present in the form of complex organic molecules, which are converted to NH_4^+ by soil micro-organisms (bacteria and fungi) through mineralisation. Ammonium may then be oxidized via NO_2^- to NO_3^- through a process known as nitrification (*Nitrosomonas spp.*: $NH_3 + 1$ $1/2\ O_2 \rightarrow NO_2^- + H_2O + H^+$, *Nitrobacter spp.*: $NO_2^- + 1/2\ O_2 \rightarrow NO_3^-$). Nitrification is negatively influenced by low soil pH, anaerobic conditions, lack of soil water and temperatures below 5 °C and above 40 °C (Lewis, 1986). Nitrate can, in turn, be converted to nitrogen gases (N_2, N_2O, NO, NO_2) through use of NO_3^- as an electron acceptor in place of O_2 resulting in what is known as 'denitrification'. This occurs when the availability of O_2 is limited, the concentration of NO_3^- high, soil moisture is high, soil carbohydrates are available, and the temperatures are warm (Luo et al., 2000; Strong and Fillery, 2002).

Microbes also utilize inorganic N, and thus immobilize it, sometimes resulting in depletion of N available to plants if adequate carbon (C) is available to support the microbial biomass. The extent of competition between plants and microbes for soil N is complex, due to multiple pathways through which N cycles at variable rates and in varying amounts, and mycorrhizal symbiosis additionally complicates the picture (Hodge et al., 2000a). The availability of N to plants depends on the balance between the rates
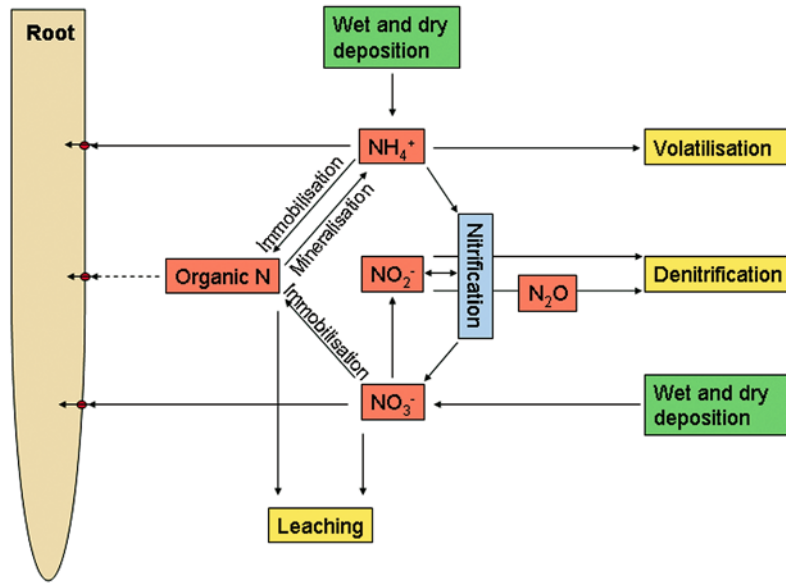
Figure 2. The main pools (boxes) and fluxes between pools (arrows) of N in terrestrial ecosystems, excluding both animals and inputs via N_2 fixation.

of mineralisation, nitrification and denitrification. The rate of mineralisation depends on factors influencing microbial activity such as water content of the soil, aeration of the soil and temperature (Lewis, 1986). If mineralisation is rapid, volatilisation of NH_4^+ to NH_3 can occur. This is favoured by alkaline soil pH and results in acidification (Dejoux et al., 2000). Primarily as a result of the biological component of N cycling, the availabilities of NO_3^- and NH_4^+ vary seasonally and the location and form of N within the soil profile varies with factors such as leaching, soil temperature and soil water status (Bloom, 1988).

The organic N fraction typically comprises 0.1 to 50% of total soil N (Barber, 1984). The current agricultural preference for urea-based fertilisers further contributes to the importance of organic N in the soil (see below). The organic N is in the form of peptides and proteins (ca. 99.5%, e.g., protein–humic complexes and peptides) and the remainder as free amino acids (Jones et al., 2002). Soil micro-organisms secrete proteases into the soil which facilitate the breakdown of proteins and peptides into their constituent amino acid units (Owen and Jones, 2001). The resultant amino acids do not bind strongly to the soil, and therefore do occur as free amino acids in the soil solution. The concentration of free amino acids in the bulk soil solution ranges from 0.1 to 50 mM, with the greatest concentrations in the surface horizons of soils rich in organic matter (Jones et al., 2002). Owen

and Jones (2001) concluded that amino acid concentrations in agricultural soils generally range between ca. 1 and 100 μM. The largest source of amino N in the soil is vegetation, although, fauna, microbes and wet and dry deposition are also sources of varying importance. The concentration of amino acids in plant tissue is typically 1 to 10 mM making this an important source of organic N for the soil. Amino acids may be the dominant form of N in some high-latitude ecosystems. Since mineralisation is temperature dependent, cold anaerobic soils limit N mineralisation and aerobic nitrification, resulting in soils rich in amino compounds (Atkin, 1996). In contrast, many aerobic soils from warmer climes have little amino N since mineralisation proceeds rapidly. Jones et al. (2002) measured the free amino acid concentrations in soils from a range of ecosystem types in Southern Ireland (upland and lowland grasslands, forest, heathland and coastal saltmarsh) using centrifuge-drainage extracts combined with fluorometric assay of the amino acids. These authors found that free amino acids accounted for 24 ± 8 mM, NH_4^+ for 39 ± 14 mM and NO_3^- for 67 ± 42 mM N in the soil solution. Thus amino acids accounted for 10 to 40% of the total soil N in this survey. The possible roles of ecto- and endo-mycorrhizas in facilitating the uptake of organic N are briefly discussed below.

The inorganic N forms utilised by plants are NO_3^- and NH_4^+. Nitrite may arise in the soil from transfor-

mation of N compounds in the soil and rhizosphere, from organic wastes or from NO_3^--containing roots during low oxygen stress (Breteler and Luczak, 1982). However, NO_2^- uptake by plant roots is generally not considered to be of consequence as a result of the low levels of NO_2^- in the soil and the reported toxicity of this ion. Although in some soils NH_4^+ is more readily available than NO_3^-, in most agricultural soils the roots of plants take up N largely as NO_3^-. This is because NO_3^- generally occurs in higher concentrations than either NO_2^- or NH_4^+, and is free to move within the root solution due to the tendency for soils to possess an overall negative charge (Reisenauer, 1978). The high diffusion coefficient of NO_3^- in soil (Table 1) has the consequence that NO_3^- is not only readily available to plant roots, but that it is also easily lost from the root zone through leaching. Leaching may account for extremely high losses of up to 30% of soil inorganic N per growing season (De Willigen, 1986). The concentration of NO_3^- in many agricultural soils is in the millimolar range (1 to 5 mM, Owen and Jones, 2001). As a consequence of the ready use of NO_3^- by plants and micro-organisms and its leachability, concentrations of NO_3^- in the soil solution are usually very variable. In natural systems N is circulated relatively efficiently, with only small losses by denitrification and by leaching of NO_3^-, which is why water draining off natural ecosystems contains very low (e.g. ca. 5 μM) concentrations of NO_3^- (Hagedorn et al., 2001).

Ammonium concentrations in agricultural soils typically range between ca. 20 and 200 μM (Owen and Jones, 2001). However, low pH, low temperature, accumulation of phenolic-based allelopathic compounds in the soil, hydric and anaerobic soils inhibit nitrification and result in NH_4^+ accumulation (Britto and Kronzucker, 2002). Ammonium is relatively immobile in the soil, and less easily lost through leaching. Furthermore, human agricultural and industrial activities (pollution) have resulted in accumulation of NH_4^+ in many agricultural soils (see below). Thus, in some systems, NH_4^+ is the predominant form of N in the soil with concentrations averaging 2 mM in some forest soils up to 20 mM in some agricultural soils (Britto and Kronzucker, 2002). Such high concentrations of NH_4^+ are potentially toxic to some species, possibly due to problems with pH balance (Raven and Smith, 1976), anion/cation imbalance (Chaillou and Lamaze, 2001) and/or the energy drain resulting from the efflux of the ion (Britto and Kronzucker, 2002).

N Mobility in soil

Plants only rely extensively on 'root interception' for the uptake of sparingly soluble nutrients such as P; in contrast, N is mostly delivered to roots through a combination of mass flow and diffusion (De Willigen, 1986). Root interception, although a difficult concept to differentiate from interception combined with diffusion (Marschner, 1995), is thought to account for ca. 1% of N taken up (Barber, 1984). Mass flow relies on transpiration to draw water to the roots. If the rate of N delivery in the transpirational water stream is lower than the root demand for N, then diffusion also plays a role in uptake. Diffusion depends on the concentration gradient and the diffusion coefficient for the particular form of N. Although the diffusion coefficients for NO_3^- and NH_4^+ in water are similar (Table 1), the diffusion coefficients in soil are additionally determined by ion size and charge, viscosity of water, temperature, soil moisture, tortuosity and the soil buffer capacity. For NO_3^- the diffusion coefficient is ca. 1×10^{-10} m^2 s^{-1} (Barber, 1984), while that of NH_4^+ is ca. 10-fold to 100-fold less (Owen and Jones, 2001). This has the consequence that NH_4^+ is less readily leached from the soil than NO_3^-. The corollary of this is that NH_4^+ is also less available in the soil to roots for uptake, although when roots have access to NH_4^+ they take it up more readily than NO_3^- (Lee and Rudge 1986; Colmer and Bloom 1998). This preference for NH_4^+ is, however, modified by environmental factors such as temperature (Clarkson and Warner, 1979). For a maize (*Zea mays*) crop, N supplied by mass flow has been estimated to be ca. 4-fold greater than that supplied by diffusion (Barber, 1984), although this depends on many factors, including the activity of the roots.

Amino acids have strongly varying diffusion coefficients in water with lysine, glycine and glutamate having diffusion coefficients of ca. 1×10^{-12}, 1×10^{-11}, 1×10^{-11} m^2 s^{-1}, respectively (Owen and Jones, 2001). These low diffusion coefficients limit the rate of amino acid diffusion in the soil (less than 1 mm day^{-1}, Table 1) making it more likely that they will be consumed by microbes than taken up by roots, since the half-life of amino acids in soils is ca. 4 h. Thus, in practice, many plants may be unable to take up organic N compounds in competition with micro-organisms. This has been demonstrated by a lack of ^{13}C enrichments in the plant tissues supplied with [15]N–[13]C-labelled organic substrates; however, ^{13}C taken up may also have been rapidly lost

Table 1. Calculation of the diffusion rates and sorption behaviour of inorganic N (NH_4^+, NO_3^-) and dissolved organic N (lysine, glycine, glutamate) in soil. The calculations are based upon the addition of 15.5 μM N-solute to the soil (modified from Owen and Jones, 2001)

	Unit	NO_3^-	NH_4^+	Lysine	Glycine	Glutamate
Diffusion coefficient in water	$m^2\ s^{-1}$	1.90×10^{-9}	1.96×10^{-9}	9.03×10^{-10}	1.05×10^{-9}	6.94×10^{-10}
Effective diffusion coefficient in soil	$m^2\ s^{-1}$	3.26×10^{-10}	2.70×10^{-12}	1.12×10^{-12}	9.03×10^{-12}	1.20×10^{-11}
Soil diffusion coefficient in soil relative to NO_3^-		1	8.23×10^{-3}	3.42×10^{-3}	2.76×10^{-2}	3.68×10^{-2}
Diffusion distance in 1 day	m	7.51×10^{-3}	6.80×10^{-4}	4.40×10^{-4}	1.25×10^{-3}	1.44×10^{-3}
Soil solution concentration	μM	77.3	0.62	0.55	3.87	7.73
Amount sorbed to soil	μmol L^{-1} soil	0.00	15.3	15.4	14.7	13.9
Total in soil	μmol L^{-1} soil	15.5	15.5	15.5	15.5	15.5
Percentage of N sorbed of total in soil	%	0	99.2	99.3	95.0	90.0

through respiration (Hodge et al., 2000b). The use of double-label isotopes seems to provide a reliable method for measurements of plant access to soil organic N sources. The low amino acid concentrations in agricultural soils, rapid microbial turnover of organic nitrogen, low diffusion coefficients and low uptake rates suggest that inorganic N will be the dominant N source available to crop plants (Owen and Jones, 2001). There is still some controversy as to the extent to which organic N is accessed by plants. Forest species *in situ* (*Deschampsia flexuosa, Picea abies, Vaccinium myrtillus*) in Sweden were found to take up intact [15]N-^{13}C labelled amino-compounds, which had been added to soils (Persson et al., 2003). In Arctic salt marshes, plant roots were found to take up between 5% and 11% of [15]N–[13]C-labelled glycine supplied, and to contribute to the turnover of organic N in the soil (Hugh et al., 2003). To some extent the controversy in the literature over the degree to which organic N is accessed by plants may result from the use of different techniques and experimental conditions; however, soils also differ widely in their microbial flora. Variation in soil temperature and in microbial flora result in differences of the half-life of organic N in the soil, and thus the access that plants have to this organic N. The importance of factors like soil temperature for microbial activity may reduce the relevance of results obtained from pot experiments in the labora-

tory to the field situation. Furthermore, different plant species may also vary in their ability to intercept and to take up organic N.

Although there is some controversy as to whether plants do access organic N in soil, it is clear that plant roots do in general have the capacity to take up organic N. In a survey of 31 species from boreal communities using a GC-MS to measure [15]N-^{13}C-labelled amino acid uptake, it was found that all the plant species tested, representing a wide variety of plant types, had the ability to take up amino acids from a mixed solution containing 15 amino acids (Persson and Näsholm, 2001). In wheat (*Triticum aestivum*) roots exposed to amino acids at 100 μM, a concentration typical for agricultural soils, rates of net uptake of amino acids ranged between 3 and 33 pmol mm^{-1} root s^{-1}, depending on the amino acid in question (Owen and Jones, 2001). Following uptake, the amino acids enter the root pool of amino compounds, and may be directly incorporated into proteins, deaminated in the root or transported to the shoot.

Fertilisers

The fertilisers used currently include a diverse collection of compounds including organic sources of N, such as animal manures. The major synthetic fertilisers include: (1) ammonium fertilisers (ammonia, 80% N (w/w); ammonium sulphate, 21% N; ammonium

bicarbonate, 17% N); (2) NO_3^- fertilisers (calcium nitrate, 16% N; sodium nitrate, 16% N); (3) ammonium nitrate fertilisers (ammonium nitrate, 34% N; calcium ammonium nitrate, which is a combination of ammonium nitrate and calcium carbonate, 21 to 27% N; ammonium sulphate nitrate, 26 to 30% N); (4) amide fertilisers (urea, 46% N; calcium cyanamide, 20% N); (5) solutions containing more than one form of N (e.g., urea ammonium nitrate solution, 28 to 32% N); (6) slow-release fertilisers (which are either derivatives of urea, granular water-soluble N fertilisers encased in thin plastic film or other means of slow release such as sulphur-coated urea) and (7) multi-nutrient fertilisers containing N (NP, NK and NPK). There has been a dramatic increase in the utilisation of urea-based fertilisers over the last decades, so that urea is currently the predominant form of N fertiliser used (Figure 3).

In agriculture, application of urea may be used to enhance soil NH_4^+ contents because urea is readily hydrolysed to NH_4^+ in the soil (Harper, 1984), but it is not itself readily accessed by plants (Criddle et al., 1988). Urea is a popular form of N fertiliser due to its competitive price and high N concentration (46% of mass) reducing transport and distribution costs. However, N is lost from urea through conversion to NH_4^+ and then NH_3, although, this is less likely to occur from acidic soils with high cation exchange capacities. The enzyme urease converts urea to NH_4^+, and its activity is proportional to the microbial biomass, which in turn depends on the organic matter content of the soil, and on water present in the soil to solubilise the urea. Urease is a ubiquitous enzyme which is produced by micro-organisms in the soil and, because it is highly stable, persists in the soil after decay of the micro-organisms (Watson et al., 1994). Conversion of urea to NH_4^+ consumes H^+ and produces HCO_3^-, resulting in a net pH increase: $CO(NH_2)_2 + H^+ + 2H_2O \rightarrow 2NH_4^+ + HCO_3^-$. The fate of HCO_3^- is pH dependent. Due to the rapid equilibration of H_2CO_3 with CO_2 at acidic pHs it can be described as: $HCO_3^- + H^+ \leftrightarrow H_2CO_3 \leftrightarrow CO_2 + H_2O$ (pKa = 6.4). At more alkaline pHs: $HCO_3^- \leftrightarrow H^+ + CO_3^-$ (pKa = 10.3). Thus at acidic pH, two H^+ are consumed by formation of 2 NH_4^+ from urea, while at extremely alkaline pH there may be no pH implication of urea hydrolysis *per se*. However, at alkaline pH's volatilization of NH_4^+ can reduce soil pH: $NH_4^+ + OH^- \rightarrow NH_4OH \rightarrow NH_3 + H_2O$ (pKa = 9.3). If large amounts of urea are supplied to the soil, then the conversion of this to NH_4^+ can drive the pH up, with consequent promotion of volatilization; this has spurred the use of urease inhibitors to slow the breakdown of urea. However, nitrification of NH_4^+ derived from urea ($2NH_4^+ + 4O_2 \rightarrow 2NO_3^- + 4H^+ + H_2O$) can also cause severe pH decreases in some situations (Nohrstedt et al., 2000). Plant uptake of NH_4^+ derived from urea will further contribute to pH decreases. Thus the effect of urea on soil pH depends on several variables making the pH consequences uncertain.

The most common nitrogenous fertilisers used after urea are compounds containing NH_4^+. The application of NH_4^+-based fertilisers and those containing urea enhances soil NH_4^+ contents and the proportion of N available to the roots in this form. As a result of the high pKa (9.3) for conversion of NH_4^+ to NH_3, NH_4^+ is much more abundant in soil at acidic to neutral pH with only 0.5% of ammoniacal N in the form of NH_3 at pH 7. The utilisation of NH_4^+ has important implications for soil pH, since uptake of this cation results in a strong acidification of the soil. In contrast, uptake of NO_3^- results in net alkalinisation of the soil, albeit, at a much slower rate than that of acidification associated with NH_4^+ uptake. Furthermore, bacterial activity can rapidly convert NH_4^+ to NO_3^-. This nitrification also has an acidification effect, and consequently supply of NH_4^+-N can cause acidification regardless of whether the NH_4^+ is taken up by plant roots. The net acidification that occurs with NH_4^+ uptake and the net alkalinisation that occurs with NO_3^- uptake results in differences in solubility, concentration, ionic form, mobility and availability of N in the soil (Marschner, 1991). Since uptake of NH_4^+ by many crop plants is increased with increased pH, at high soil pH NH_4^+ toxicity may result, while at low soil pH, N starvation may occur (Findenegg, 1987).

Use of only one form of N fertiliser can drive soil pH away from the optimum. This can lead to deficiencies of elements such as K^+ (Findenegg, 1987) and P (Sentenac and Grignon, 1985) leading to interactions between N and the availability of other essential nutrients. Nitrogen-related changes in soil pH may also be responsible for the toxicity of certain elements. It may be argued that the extensive problems associated with Al toxicity may be related to the use of NH_4^+-containing fertilisers. On the other hand soil pH can be manipulated simply by modifying the form of N supplied, without the requirement for lime and without the risk associated with acids.
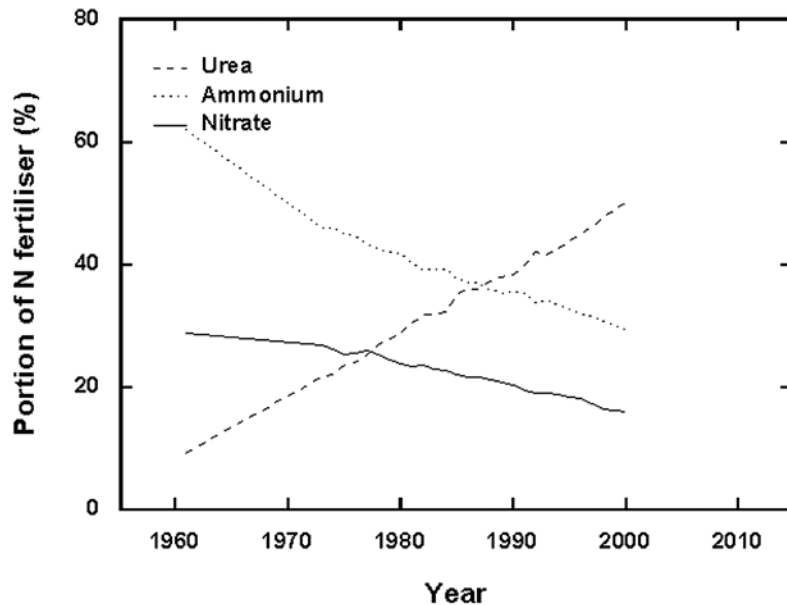
Figure 3. The proportion of total nitrogenous fertiliser applied containing urea, ammonium and nitrate or combinations of these (data plotted from that available from the International Fertiliser Industry Association, www.fertiliser.org/ifa/statistics.asp).

Although high concentrations of NH_4^+ can cause toxicity (see below), it has the benefits of (1) a smaller diffusion coefficient in the soil thus reducing loss of N through leaching, (2) higher specific N content, (3) lower costs, (4) plant incorporation of NH_4^+ avoids the carbon-intensive reduction of NO_3^- to NH_4^+. Thus NH_4^+ may be the N form of choice in some circumstances. However, conversion of NH_4^+ to NO_3^- by nitrification compromises some of these benefits. Nitrification inhibitors have been used in agriculture to enhance soil NH_4^+ contents (Adriaanse and Human, 1991; Bock, 1987). The availability of NH_4^+ within the soil may, however, also be severely limited, because it is tightly held by the micaceous clay minerals of the soil, and readily utilized by micro-organisms effectively removing it from the soil solution until mineralisation occurs (Lewis, 1986). The problem of limited availability of NH_4^+ may be partially overcome in agriculture through additional use of K^+ which increases the availability of NH_4^+ by occupying binding sites in the soil (Haynes and Goh, 1978), allowing more effective use of NH_4^+.

Root structure

The size and architecture of the root system is an important variable for ensuring adequate access to N. The architecture of the root is determined by the pattern of root branching. The species-specific size and architecture of root systems is also strongly determined by a wide range of physical, chemical and biological factors. In general, the size of the root (as measured by total mass, length or area) relative to the rest of the plant (e.g., as expressed by the shoot:root ratio or root mass ratio) increases when N is limiting. Nitrogen deprivation causes starch accumulation in leaves, and an increase in the proportion of photosynthate translocated to the root, resulting in a decline in the shoot:root ratios (Rufty et al., 1988). This enhanced allocation of C to the root was ascribed by these authors to a decreased utilization of sucrose in the shoot. Vessey and Layzell (1987) showed that only N in excess of the requirements of the root was exported to the shoot in *Glycine max*, suggesting that roots have the highest priority for N in times of N deficiency (Tolley-Henry and Raper, 1986), thus promoting root growth. However, there are now indications that root N availability controls developmental cues which in turn determine the demand for growth, thus controlling carbon allocation. Studies with tobacco (*Nicotiana plumbaginifolia*) deficient in NR (Scheible et al., 1997b) and in *Arabidopsis thaliana* (*Arabidopsis*) (Zhang et al., 1999) support the existence of a systemic signal elicited by NO_3^- accumulation that represses root growth. The notion that root growth is favoured by systemic signals under NO_3^- deficiency is also reinforced by the

observation that NO_3^-, but neither glutamine (Tranbarger et al., 2003) nor NH_4^+ (Zhang et al., 1999) supplied to the roots of *Arabidopsis* repressed root growth. Using macro-arrays, Tranbarger et al. (2003) identified transcription factors that were associated with the supply of NO_3^-, but not with glutamine supply. Furthermore, the studies conducted on the effect of N on root architecture suggest that the systemic signal regulating root growth in relation to N status is hormonal; auxin (Zhang and Forde, 2000) or abscisic acid (Signora et al., 2001). The function of decreased shoot:root ratios may be to compensate for N deficiency by increasing the N acquisition capacity of the plant (Brouwer, 1981; Khamis and Lamaze, 1990; Robinson, 1986; Rufty et al., 1990).

Apart from the total size of the root system, there are a large number of other attributes, which dictate its capacity and efficiency for N acquisition. Only a limited proportion of the root may actually be effective in the uptake of N (Robinson, 2001). The acquisition of N also depends on the distribution of the roots active in N uptake within the soil. Rooting depth, which varies greatly between species, determines the ability of a crop to intercept N, particularly NO_3^- during periods of leaching (Gastal and Lemaire, 2002). The construction costs of roots are also an important consideration; fine roots have a higher surface area to volume ratio than thick roots, and thus require less C for construction per unit root length, but may be more expensive for maintenance (per unit root weight). One of the most important attributes is the number, size and location of root hairs, which have an enormous impact on the absorptive surface area of the root.

Nitrogen in the soil is extremely heterogeneous on both a spatial and a temporal scale. Roots tend to proliferate in localized areas within the soil of high N content (Drew and Saker, 1975; Granato and Raper, 1989) and thus specific portions of the root may be exposed to high N concentrations while other parts of the root system are ineffective in N uptake. Plants may sense the soil N concentrations with specific sensors (see below), and also monitor and respond to their own internal N status (Malamy and Ryan, 2001). Many species respond to localised patches of NO_3^- by preferential lateral root proliferation within the nutrient-rich zones (Drew and Saker, 1975). In particular, the availability of NO_3^- affects both the number and location of lateral root initiation sites (Malamy and Ryan, 2001). The stimulatory effect of NO_3^- on root proliferation may seem contradictory to the inhibition of root development at high N concentrations. However, there

seem to be two modes of action: inhibition of root development by a systemic inhibitory signal that results from the accumulation of NO_3^- in the shoot, and a localized stimulatory effect that depends on the local concentration of NO_3^- in the roots (Zhang and Forde, 2000). These authors provided evidence from NR deficient *Arabidopsis* that the localized stimulatory effect is a direct result of NO_3^- (i.e. not amino acids), probably in the leaf, acting on a NO_3^--inducible MADS-box gene (*ANR1*), which encodes a component of the signal transduction pathway linking the external NO_3^- supply to the increased rate of lateral root elongation. The systemic phloem-delivered signal, which is correlated with the N status of the plant, may depend on auxin or an auxin-related pathway for control of lateral root elongation, but not lateral root initiation (Zhang and Forde, 2000). Auxin localization appears to be a key factor in this nutrient-mediated repression of lateral root initiation (Malamy and Ryan, 2001). However, abscisic acid (ABA) applied exogenously inhibits *Arabadopsis* lateral root development through the operation of an auxin-independent pathway (De Smet et al., 2003). These authors showed that a mutation in the *ALF3* gene, which is part of the auxin-dependent regulatory pathway, did not alter the sensitivity of lateral root development to ABA, and that ABA suppresses auxin response in the lateral root primordia. De Smet et al. (2003) proposed a model in which different stages of lateral root initiation and development are regulated by both auxin and ABA.

The question has been posed as to why root proliferation in *Arabidopsis* occurs in localized patches of NO_3^-, which is a relatively mobile nutrient, whereas it does not respond to locally supplied NH_4^+ (Leyser and Fitter, 1998). Zhang and Forde (1998) have argued that this is because roots have evolved to use NO_3^- as a signal molecule, because it is relatively mobile in the soil. This may allow roots to proliferate towards areas where NO_3^-, other forms of N and P are localized within the soil. This ability to proliferate roots in areas with N may also be important in inter-specific competition for N or P (Hodge, 2002).

Plant-rhizosphere interactions

The availability of C in the rhizosphere is a major factor controlling the soil microflora and, consequently, N transformations in the soil (mineralisation, immobilisation, denitrification). A portion of the photosynthetic C is deposited in the soil in the form of root exudates (e.g., humic substances, sugars, organic acids,

amino acids), mucilage and sloughed cells and tissue (Marschner, 1995). Rhizodeposition is a major source of C and N for the soil and its inhabitants (Jensen, 1996). It is therefore of great importance for maintaining the level of microbial activity in the soil. Experiments with disturbed systems have indicated that total C input to agricultural soils can represent 15% to 33% of the C assimilated by plants (Qian et al., 1997). Using C_4 maize (which has a ^{13}C abundance which is distinct from that of C_3 plants), these authors were able to quantify the amount of C contributed to soils previously inhabited by C_3 plants by following changes in ^{13}C abundance. Between 5% (at maturity) and 12% (four-week old maize) of photosynthate was released to the soil as organic carbon. This release of organic C increased denitrification losses from soil by an average of 29% during the early growth stages.

Different portions of the root may exude different organic compounds. Bacterial biosensors were used to asses the exudation of tryptophan and sucrose from roots of *Avena barbata* (Jaeger et al., 1999). Tryptophan exuded from older portions of roots (0.12 to 0.16 m from the tip), while sucrose was most abundant in soil near the root tip. Nutritional circumstances have a significant impact on the type and concentration of exudation that occurs from roots. Al toxicity (Delhaize and Ryan, 1995) and P deficiencies (Shane and Lambers, 2004) strongly influence organic acid exudation. Exudation of carbohydrates and amino acids from roots of plants supplied with NH_4^+ is greater than that from roots supplied with NO_3^- (Cramer and Titus, 2001; Mahmood et al., 2002). This may partially be because plants supplied with NH_4^+ have higher root tissue concentration of amino acids (Cramer and Lewis, 1993), which may be exuded. The notion that carbohydrates simply 'leak' out of the roots has been challenged by work on kallar grass (*Leptochloa fusca*). Mahmood et al. (2002) found that 30-fold differences in sugar exudation between NO_3^-- and NH_4^+-supplied plants were not related to the internal root sugar concentration, or to the different root architecture, or to differential re-absorption of sugars. It was proposed that roots detected soil NH_4^+ concentrations as a signal for diazotrophic bacterial presence, and responded with enhanced sugar exudation. Thus soil exudation is not so much a passive event, but a means of manipulating the C content of the rhizosphere, and thus the soil microbial population.

While plants modify the rhizosphere and the environment for soil micro-organisms, these in turn modify plant physiology. Plant growth enhancement by plant growth-promoting bacteria involves diverse mechanisms including release of indoleacetic acid and cytokinin (Costacurta and Vanderleyden, 1995), reduction in ethylene levels (Wang et al., 2000), stimulation of the ion transport and enhancement of mineral availability (Bertrand et al., 2000). Several plant growth-promoting bacteria have been shown to stimulate root growth (Larcher et al., 2003), probably through hormone release. This modification of root growth has an important impact on N nutrition by increasing NO_3^- uptake capacity and possibly also by directly stimulating NO_3^- transport systems (reviewed by Mantelin and Touraine, 2004). The effects of plant growth-promoting bacteria on plant growth and the acquisition of N are usually greatest in low N fertility environments. Thus inoculation with plant growth-promoting bacteria could potentially have important consequences for enabling plant root growth for increased N acquisition under N deficiency.

Uptake and transport of N

Several recent reviews on the topic of NO_3^- and NH_4^+ transporters have been published (Forde, 2000; Forde and Clarkson, 1999; Touraine et al., 2001; Williams and Miller, 2001), and therefore only an overview of the main topics will be covered in this review. Less is known about uptake systems for other possible soil N sources, although genes encoding transporters for many types of N-containing organic molecules have been identified. The complete genome of *Arabidopsis* was the first to be published for a plant (Bevan et al., 2001), and so at present we have most molecular information for this species. *Arabidopsis* is a wild species and can grow and flower in low-N soils (Miller and Smith, unpublished results).

Nitrate transporters

Nitrate is actively transported across the plasma membranes of epidermal and cortical cells of roots, but net uptake is the balance between active influx and passive efflux. This transport requires energy input from the cell over almost the whole range of concentrations encountered in the soil (Glass et al., 1992; Miller and Smith, 1996; Zhen et al., 1991). It is generally accepted that the uptake of NO_3^- is coupled with the movement of two protons down an electrochemical potential gradient, and is therefore dependent on ATP supply to the H^+-ATPase that maintains the H^+ gradient across the plasma membrane (McClure et al., 1990; Meharg and Blatt, 1995; Miller and

Smith, 1996). Calculations of the energetic requirements for transport suggest that this co-transport is required for a wide range of extracellular NO_3^- concentrations (Miller and Smith, 1996; Siddiqi et al., 1990). For NO_3^- storage in the plant cell, transport at the tonoplast membrane requires a different mechanism and an antiport with H^+ has been suggested (Miller and Smith, 1992). Figure 4 is a schematic diagram that shows NO_3^- uptake and the associated proton-pumping ATPase (H^+-ATPase) that maintains the electrochemical potential gradient to drive the co-transport.

Physiological studies have shown the presence of both high- and low-affinity NO_3^--uptake systems operating at different external NO_3^- concentrations (Aslam et al., 1992; Glass and Siddiqi, 1995). There are believed to be two high-affinity transport systems (HATS) taking up NO_3^- at low concentration (generally below 0.5 mM with low transport capacity) and one low-affinity transport system (LATS) that transports NO_3^- at high concentrations (generally above 0.5 mM with high transport capacity) (Glass and Siddiqi, 1995). Numerous NO_3^- transporters have been cloned from a variety of species, and two distinct gene families, *NRT1* and *NRT2*, have been identified (Crawford and Glass, 1998; Daniel-Vedele et al., 1998; Forde, 2000; Forde and Clarkson, 1999; Williams and Miller, 2001). The Arabidopsis genome contains 52 *NRT1* and 7 *NRT2* family members; it was at first believed that NRT1 mediated the LATS and NRT2 the HATS (Forde and Clarkson, 1999; Zhuo et al., 1999). However, this tidy functional assignment in no longer valid, because in *Arabidopsis* the low-affinity NO_3^- transporter, *AtNRT1.1*, also functions in the high-affinity range (Liu et al., 1999), and these changes in the kinetics of transport are switched by phosphorylation of the protein (Liu and Tsay, 2003). A further complication for the *NRT1* family is that they belong to a much larger family of peptide transporters, the POT, or proton-dependent oligopeptide-transport family which is also known as the PTR or peptide-transport family (Paulsen and Skurray, 1994). Mammalian members of this family can transport peptides of varying sizes (Paulsen and Skurray, 1994). In *Arabidopsis* the pattern of tissue expression for much of the *NRT2* family has been mapped (Orsel et al., 2002; Okamoto et al., 2003). Some of the *NRT2* family require a second gene product for functional activity, but it is not known whether there is an interaction between the gene products (Galván et al., 1996; Zhou et al., 2000).

Some members of both *NRT1* and *NRT2* gene families are NO_3^- inducible and are expressed in the root epidermis, including root hairs, and in the root cortex. Members of both the *NRT1* and *NRT2* families are therefore good candidates for a role in the uptake of NO_3^- from the soil (e.g., Lauter et al., 1996; Lin et al., 2000; Ono et al., 2000; Nazoa et al., 2003). Some family members are constitutively expressed (see Okamoto et al., 2003 for details). For example, in *Arabidopsis AtNRT1.2* is constitutively expressed in the roots, particularly in root hairs and the epidermis (Huang et al., 1999). A detailed description of the tissue expression pattern of *AtNRT1.1* and *AtNRT2.1* has been provided by promoter-reporter gene fusions (Guo et al., 2001; Nazoa et al., 2003). These elegant papers show how much expression changes during development and reveal complicated tissue patterns. For example, *AtNRT1.1* was strongly expressed in the tips of primary and lateral roots but showed weak expression in the root cortex and epidermis (Guo et al., 2001). In contrast, the expression of *AtNRT2.1* was strong in the epidermis, cortex and endodermis of the mature parts of the root (Nazoa et al., 2003). The correlation between $^{13}NO_3^-$ influx and the expression of *AtNRT2.1* and *AtNRT1.1* has led to the suggestion that these two genes may be largely responsible for high and low affinity NO_3^- uptake (Okamoto et al., 2003). It has been suggested that the root cortex is the main site for the uptake of NO_3^- from the soil (Siddiqi et al., 1991), but it is difficult to reconcile this idea with the fact that some NO_3^- transporters are expressed in the epidermis. The expression of both families can be regulated by feedback from N metabolites in many plant species (Touraine et al., 2001). Various amino acids have been tested for their ability to alter the expression and activity of NO_3^- transporters through feedback regulation. Feeding amino acids to roots decreases the expression of NO_3^- transporters (Nazoa et al., 2003; Vidmar et al., 2000). However, identifying which amino acids are responsible for the feedback response is difficult, because they can be assimilated and converted into different amino acids. By using chemical inhibitors to block the conversion of amino acids into other forms, glutamine has been identified as an important regulator (Vidmar et al., 2000). Nitrate transporters are also diurnally regulated, undergoing marked changes in transcript levels and corresponding NO_3^- influx during day/night cycles, with high expression at the end of the light period (e.g., Ono et al., 2000). Sucrose supply in the dark rapidly increases the transcript levels (Lejay et al., 1999), and the diurnal increases in
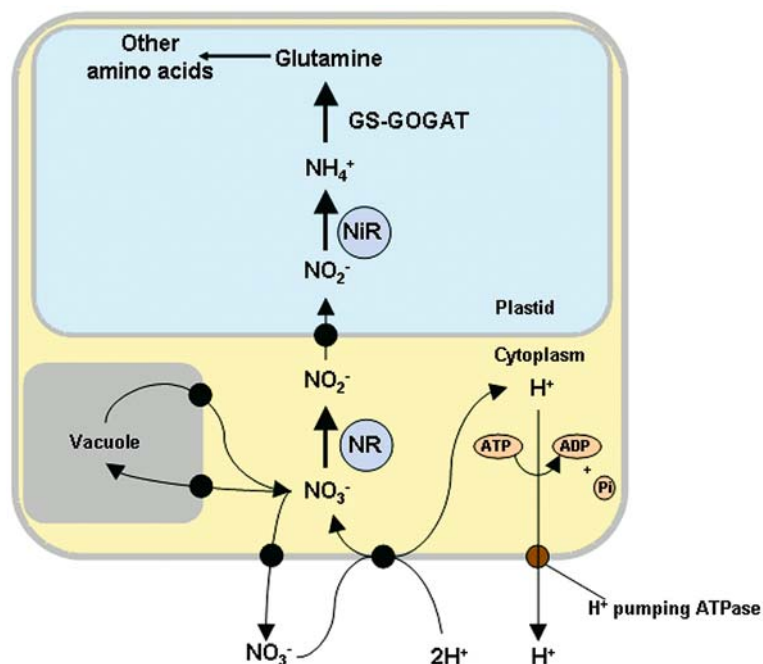
Figure 4. Schematic diagram of NO_3^- uptake and assimilation by plant cells. Key: nitrate reductase, NR; nitrite reductase, NiR; glutamine synthetase, GS; glutamate-2-oxoglutarate aminotransferase, GOGAT (redrawn from Crawford et al., 2000).

expression of root NO_3^-, NH_4^+ and SO_4^{2-} transporters seem to be linked to the changes in sucrose supply to the root which results from photosynthesis during the day (Lejay et al., 2003). These observations indicate the close co-ordination that exists between NO_3^- uptake and C metabolism.

The roles of both *NRT1* and *NRT2* genes in the uptake of NO_3^- from the soil have been demonstrated using mutant plants. A mutant *Arabidopsis* plant deficient in the expression of a *NRT1* gene led to the identification of the first member of this family, although, the original selection of the plant was made using chlorate which is a toxic analogue of NO_3^- (Tsay et al., 1993). Even stronger evidence is available for the *NRT2* family, where double mutant knock-outs of *NRT2* genes in *Arabidopsis* have demonstrated a clear role for these genes in the uptake of NO_3^- from the soil (Filleur et al., 2001). These mutants are deficient in both *AtNRT2.1* and *AtNRT2.2,* and they have lost almost all the NO_3^--inducible HATS, while LATS activity was not altered. Split-root experiments also showed that the double mutant has lost the ability to up-regulate uptake in one part of the root to compensate for N-starvation in another part of the root (Cerezo et al., 2001). In addition, the supply of NH_4^+ to the NO_3^--containing nutrient solution usually inhibits

NO_3^- uptake in the wild-type, but this does not occur in the mutant (Cerezo et al., 2001). These elegant experiments illustrate the powerful use of gene 'knock-out' technology to identify the role of specific transporter genes in N uptake by roots. These results are also important for confirming the function of these genes as NO_3^- transporters, because almost all of the *in planta* expression studies have assumed function on the basis of sequence homology. Sequence similarities may be misleading, especially when a single protein can transport more than one type of ion or molecule, as is the case for both *NRT1* and *NRT2* transporter families. For example, some members of the *NRT1* family can transport amino acids and peptides, and both families can transport NO_2^- when the proteins have been expressed in foreign cells (Miller and Zhou, 2000; Zhou et al., 1998).

Efflux systems have been studied less than influx systems; however, it is known that efflux is protein-mediated, passive, saturable and selective for NO_3^- (Aslam et al., 1996; Grouzis et al., 1997). Anion channels seem the most obvious route for NO_3^- efflux, because the transport is thermodynamically downhill and genome analysis has identified several gene families that may fulfil this function. The NO_3^--efflux system is under a degree of regulation, induced by

NO_3^- (Aslam et al., 1996), and it is also proportional to whole-tissue NO_3^- concentrations (Teyker et al., 1988). We can therefore predict that the anion channel(s) responsible for NO_3^- efflux must be NO_3^--inducible. Net NO_3^- uptake is regulated by whole-plant demand via shoot-derived signals transported in the phloem to the roots (Imsande and Touraine, 1994; Vidmar et al., 2000). The nature of these feedback signals seems to be amino acid concentrations in the phloem, specifically glutamine (Pal'ove-Balang and Mistrik, 2002; Tillard et al., 1998). Efflux of NO_3^- has been found to be associated with slow growth rates (Nagel and Lambers, 2002). This efflux is, however, a consequence rather than a cause of slow growth. Slow-growing plants from nutrient-poor habitats may simply not be able to exploit high concentrations of NO_3^-, which is then effluxed.

Ammonium transporters
Many plant NH_4^+-transporter (*AMT*) genes have been identified and their function has been confirmed by their ability to complement a yeast mutant deficient in normal NH_4^+ uptake (Ninnemann et al., 1994; von Wirén et al., 2000a). In *Arabidopsis* there are 6 *AMT* genes, while rice (*Oryza sativa*) has 10, and more detailed sequence comparisons have identified two distinct groups within the *AMT* family, denoted *AMT1* and *AMT2* (Shelden et al., 2001; Sohlenkamp et al., 2000). Like the NO_3^- transporters, some *AMT1*-type genes are expressed in root hairs, suggesting that they have a role in uptake of NH_4^+ from the soil (Lauter et al., 1996; Ludewig et al., 2002). Three *AMT1* genes show diurnal changes in expression in roots (Gazzarrini et al., 1999), and the changes in expression during the light period likely result from increases in sucrose availability from photosynthesis during the day (Lejay et al., 2003). More detailed information has been published about the *AMT1*- than about *AMT2*-type transporters, and a correlation between transcript (mRNA) level and NH_4^+ influx has been observed (Kumar et al., 2003), but the role of neither group in uptake from the soil has been clearly established. Although, *Arabidopsis* plants deficient in one of the root-expressed *AMT1* genes showed altered leaf morphology and a 30% decrease in NH_4^+ influx, there were no effects on growth when compared with wild-type plants in a range of conditions (Kaiser et al., 2002). Based on these observations it was suggested that redundancy within the *AMT*-family may compensate for the loss of this transporter.

Similarly, inhibiting the mRNA transcript level of the single *AMT2* in *Arabidopsis* failed to significantly alter growth of the plant, although the actual uptake of NH_4^+ was not measured (Sohlenkamp et al., 2002). One of the *AMT2* transporters is constitutively expressed in the plasma membrane of most tissues including the nodules of a N_2-fixing species, suggesting that it may have a general role in the recovery of NH_4^+ effluxed from all tissues, not only the nodule (Simon-Rosin et al., 2003). Some *AMTs* are constitutively expressed (Suenaga et al., 2003), but for most the expression depends on the availability of NH_4^+ (von Wirén et al., 2000b). The expression of one tomato (*Lycopersicon esculentum*) *AMT1* gene was induced by the presence of N_2-fixing bacteria in the rhizosphere (Becker et al., 2002). In species like paddy rice that chiefly make use of NH_4^+ as a soil N source more of the *AMT1* genes show NH_4^+-induced expression when compared with *Arabidopsis* and tomato that chiefly use NO_3^- as an N source (Sonoda et al., 2003). However, in contrast to most situations for NO_3^-, the expression of some *AMTs* is repressed by the presence of NH_4^+, with the mRNA increasing when less NH_4^+ is available. As described for the NO_3^- transporters (Nazoa et al., 2003; Vidmar et al., 2000), the expression of an *AMT1* gene and NH_4^+ influx were suppressed when plants were supplied with glutamine, suggesting feedback regulation from downstream N metabolites (Rawat et al., 1999).

As for NO_3^-, NH_4^+ transport in plant cells can also be demonstrated by electrophysiology (Ayling, 1993; Wang et al., 1994). Electrophysiology can be used to determine the NH_4^+-transporter kinetics which suggested that NH_4^+ entry into cells may be mediated by cotransport with protons (Ayling, 1993; Wang et al., 1994). However, the energy requirements for uptake of a cation (e.g., NH_4^+) compared to an anion (e.g., NO_3^-) are different. The uptake of NH_4^+, like the uptake of K^+, could be through a channel, and chiefly driven by the negative membrane potential of the plant cell. Several examples of K^+ channels expressed in the root epidermis have been identified (e.g., Downey et al., 2000; Hartje et al., 2000) and gene knock-out studies could identify whether these have a role in NH_4^+ uptake. There is evidence from patch-clamp studies that NH_4^+ ions can enter cells through K^+ channels (White, 1996), and it may be that this is an important route for the entry of NH_4^+ into root cells. This topic is worth investigation using plants that have disrupted plasma-membrane K^+-channel activity, especially given the

lack of direct evidence for the role of *AMTs* in NH_4^+ uptake by root cells. More detailed functional analysis of the *AMT* genes, using heterologous expression, suggests that they may have a channel-type structure that can be composed of several different multiples of *AMT* protein units (Ludewig et al., 2003). The functional activity of the whole protein complex may be modified by altering the *AMT* component units. Electroneutral uptake of N as ammonia (NH_3) may occur by entry through membrane channels and aquaporins may provide a molecular route for this transport (Niemietz and Tyerman, 2000; Howitt and Udvardi, 2000). Aquaporins may also provide a route for efflux across the plasma membrane and for accumulation in the vacuole. The relatively alkaline pH of the cytosol will favour NH_3 flux both into the vacuole and into the apoplast.

The energetic requirements for pumping NH_4^+ out of cells has been identified as a possible cause for the toxic effect of the ion on some types of plants (Britto et al., 2001a, see below). The gene(s) responsible for this NH_4^+ efflux process have not yet been identified, but the thermodynamic mechanism for such a process requires an ATPase or an anti-port somehow exchanging H^+ and NH_4^+. It is not clear why K^+ entry and cytosolic concentration should be regulated while those of NH_4^+ are poorly regulated, but like Na^+ entry during salt stress, perhaps the plant cannot avoid this problem when exposed to high concentrations of these cations. Therefore accurate measurements of the soil concentrations of NH_4^+ may be important for answering these questions for plants growing in soil. The toxic effects of NH_4^+ depend on there being high external concentrations of the cation, perhaps greater than 20 mM (Britto and Kronzucker, 2002). As mentioned above, since the cytosolic pH is usually more alkaline than that of the vacuole and the apoplast, the chemical gradient for NH_3 favours passive exit of this molecule from the compartment. The plant *AMT* gene family function as high-affinity NH_4^+-uptake systems when they are expressed in yeast (von Wirén et al., 2000a). The requirement for an active efflux mechanism at high external NH_4^+ concentrations does not easily fit with the constitutive expression of some of these genes, so more expression analysis is needed to clarify this point.

N fluxes along the length of roots

Net uptake of NO_3^- and NH_4^+ along roots has been mapped using ^{15}N labelling of root segments (Lazof et al., 1992) and ion-selective microelectrode techniques (Henriksen et al., 1990; Taylor and Bloom, 1998). These measurements generally show that the site of most NO_3^- and NH_4^+ uptake is just behind the root meristem. In maize, NO_3^- elicited net H^+ uptake only at the root tip (0–1 mm), but H^+ extrusion in all regions (Taylor and Bloom, 1998). This correlates with symport of $H^+:NO_3^-$ into the root tip. Rapid NO_3^- net uptake was found between 0 and 40 mm behind the root tip, decreasing between 40 and 60 mm. Ammonium-elicited H^+ extrusion was detected in all regions, except for the region 6 to 11 mm from the apex (Taylor and Bloom, 1998). In the region 11 mm from the apex there is hardly any elongation in maize primary roots (Sharp et al., 1988); it is possible that H^+ extrusion is already maximal, that NH_4^+ is stored rather than assimilated, or that NH_4^+ is translocated away from this region. Net uptake of NH_4^+ increased steadily with distance behind the root tip (measured up to 60 mm). When both NH_4^+ and NO_3^- were supplied, NO_3^- net uptake was suppressed at all locations along the root (Colmer and Bloom, 1998; Taylor and Bloom, 1998). Although there is a peak of N uptake just behind the root tip, it is sometimes overlooked that this represents only a 2- to 3-fold increase over that found in the older parts of the root further from the apex. Transporter gene expression studies suggest that mature parts of the root are also significant sites of uptake (Nazoa et al., 2003).

Organic N uptake

Gene families have been identified that are responsible for transporting amino acids (reviewed in Ortiz-Lopez et al., 2000), urea (Liu et al., 2003), oligopeptides (Koh et al., 2002; Steiner et al., 1994), purines (Gillissen et al., 2000), nucleosides (Li et al., 2003) and N-containing heterocyclic compounds (Desimone et al., 2002), but their role in uptake from the soil is still uncertain. This oligopeptide transporter (*OPT*) family is not related to the NTR1 (PTR) family described previously, but both that are able to transport peptides.

Arabidopsis has a large family of at least 46 putative amino acid/auxin transporters which can be sub-divided into some smaller groups based on sequence comparisons, but the functions of the family members are not well characterised. There is a smaller group of 9 related general amino-acid transporters, and some others specifically for auxin and amino acids such as lysine, histidine and proline. An amino-acid transporter, possibly for both histidine and proline, from *Mesembryanthemum crystallinum* is specifically