Nitrogen in physiology. An agronomic perspective and implications for the use of different nitrogen forms

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NITROGEN IN PHYSIOLOGY –

AN AGRONOMIC PERSPECTIVE AND IMPLICATIONS

FOR THE USE OF DIFFERENT NITROGEN FORMS

by

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SUMMARY.

Nitrogen (N) is an essential plant nutrient at the centre of plant metabolism being present in numerous vital organic compounds including amino acids, proteins, nucleic acids and phytohormones. In highly fertile arable soils supplied with N fertilisers, NO_3^- and NH_4^+ ions are usually considered to be the main N forms in soil solution taken up by crop plants, the ratio of NO_3-N to NH_4-N often being in the order of 10-20:1. Soluble organic N is receiving increasing attention. The form of N uptake modifies rhizosphere pH which affects uptake of other soil nutrients. Acquisition of the various N forms is regulated not only by their chemical and spatial availability in the soil, but also by transport systems in the plasma membrane of root cells, root system architecture and mechanisms that regulate the activity of N transport systems and root growth, depending on plant requirements. In most fertile soils well supplied with mineral nutrients, neither N transport from the bulk soil to crop roots nor the efficient processes of NO_3^- and NH_4^+ ion uptake by roots limit N acquisition. Uptake of N is regulated by N demand in which shoot-root interactions play an important role. Nitrate acts as a signal for metabolism and plant development which is associated with cytokinin transport. The biochemistry of N assimilation is well understood but control at molecular level much less so. Nitrogen status of plants can be characterised by the concept of critical N concentration (Nc) in relation to the long-term fall in N concentration in the dry matter as plants age. Nc represents the optimum for growth for a given crop and can be related to actual crop N concentration. Fertilisation is needed when [N] / [Nc], i.e. NNI (nitrogen nutritional index) < 1. By determining N status during crop growth, fertilisation can be carried out more efficiently as exemplified in melon cultivation. Examples are presented to demonstrate practical effects of different N forms including NO_3-, NH_4- and urea-N. These examples include the value of placed stabilised NH_4- N in depressing rhizosphere pH on high-pH soils to increase the availability and uptake of sparingly soluble phosphate and micronutrients iron, manganese, copper, zinc and boron. These latter nutrients play a vital role, protecting plants against both biotic and abiotic stresses. The acidifying effect in the rhizosphere of symbiotically grown legumes can be of similar benefit on high pH soils. The use of NH_4-N fertilisers to depress rhizosphere pH which is also associated with increased Si and Mn uptake is regularly practiced to control some plant diseases such as mildew and take-all in wheat. Water use efficiency (WUE) also relates to the form of N supply. Foliar application of urea is discussed. Signalling effects of stabilised N forms can be used to influence tiller number and yield components in wheat and barley crops. The use of NH_4-N in restricting lateral shoot development in tomato production is discussed.

Keywords: ammonium-N, nitrate-N, urea-N, rhizosphere pH, micronutrient acquisition, crop yield and quality, crop nitrogen status.

CONTENTS

1. INTRODUCTION.

Nitrogen lies at the centre of metabolism of all living organisms. Plants contain about 2-5% N in the dry matter on a dry weight basis, a range of values which may appear low in relation to the C content at about 40%. Nevertheless the bulk of N in plants occurs in organic form with usually only a relatively small amount of inorganic N, which is mainly in the form of nitrate. Organic N constituents include proteins, amino acids, and nucleic acids. All enzymes are proteins so that every enzymatic reaction taking place in plants is dependent on the presence of N. Other nitrogen containing compounds include purines e.g. adenine which form part of the structure of coenzymes such as ATP and NAD+. These together with the pyrimidines (also N compounds) form the organic bases of the nucleic acids (Glass, 1989). Other than the amino acids making up proteins there are in addition about 20 non-protein amino acids. The plant growth regulators, the phytohormones, indole acetic acid (IAA) and the cytokinins which play a crucial role in controlling plant growth and development also contain N. Nitrogen is thus indispensable for the growth and development of crop plants and with the possible exception of potassium (K), is required in much higher amounts than any of the other mineral nutrients.

Despite this high requirement for nitrogen by plants, it is not a constituent of soil parent material and its acquisition during pedogenesis has been almost exclusively the consequence of fixation of molecular nitrogen by micro-organisms either free living or in symbiotic association with plants (Walker and Syers, 1976) As discussed by Bloom (1997) because of this limitation of N supply under natural ecosystems, plants have evolved diverse mechanisms to capture nitrogen including mycorrhizal fungi (Marschner, 1995), insect carnivory (Schulze *et al.,* 1997), root absorption of amino acids from organic soils (Chapin *et al.,* 1993) and leaf absorption from atmospheric deposition (Nicoulaud and Bloom, 1996). Plants have thus evolved highly efficient uptake systems in the acquisition of nitrogen from soil.

The invention of the Haber-Bosch process made almost a hundred years ago, by which molecular nitrogen is converted to ammonia and hence to ammonium fertilisers has had an enormous impact on agriculture. Over the past 50 years global annual use of N fertilisers has increased over twenty fold to reach the present approximate rate of as much as 10^9 million t N/annum (Miller and Cramer, 2005). Nitrogen is no longer the most limiting nutrient in crop production in almost all agricultural countries. In agro-ecosystems the focus of attention is therefore now on how to use nitrogen fertilisers more efficiently and to deal with the environmental problems associated with their use. These include the high energy demand for production, the detrimental effects of nitrate leaching of enhanced eutrophication of surface water bodies, and emissions of N_2O into the atmosphere contributing to global warming.

In this paper we review the acquisition and physiology of nitrogen from an agronomic perspective considering especially the forms of N supplied to crop plants. These forms include nitrate, ammonium N and organic N including urea. We report briefly from the voluminous, burgeoning literature, on the uptake, of these forms of nitrogen and briefly discuss N assimilation. We also take into account the influence of the forms of N supply on the uptake of the other major nutrients taken up as cations and anions and discuss the consequences of the different forms of N nutrition on pH changes in the nutrient medium of solution grown plants and the rhizosphere of soil grown plants. We then consider long distance transport, the distribution of N assimilation between shoot and root and discuss the evidence of short and long distance signalling of N in relation to crop N demand. The concept of plant nitrogen status and its relevance to crop production is then taken into account and we turn to the real world of the soil and the needs of the farmer. Here we attempt by reference to selected examples to show how some of the physiological findings we have discussed in theory can be exploited in the field to improve nitrogen use efficiency especially in relation to different forms of N application.

2. NITROGEN ACQUISITION BY PLANTS.

Acquisition of the various N forms in the soil is regulated not only by their chemical and spatial availability, but also by transport systems in the plasma membrane of root cells, root system architecture and mechanisms that regulate the activity of N transport systems and root growth according to plants' growth requirements (see Jackson *et al.,* 2008).

2.1. Nitrogen forms in the soil and soil root interactions.

The two predominant inorganic sources of N in soils, NH_4- and NO_3-N, differ considerably. Ammonium as a positively charged ion is held on negatively charged sites such as clay and organic matter and is thus relatively immobile. It is also converted readily by nitrification to highly mobile nitrate-N which is not held on soil particles, is soluble in water and can easily be lost from the soil by leaching. Soil aeration, temperature, and pH all have a strong influence on nitrification (Sahawat *et al.,* 2008), so that in most well aerated agricultural soils NO_3^- is the predominant form in which N is absorbed even though the bulk of N fertiliser is applied in the form of urea or ammonium N (Glass, 2009). From 250 soil samples taken at 4 metre intervals over 4 arable fields Miller *et al.,* (2007) showed that nitrate concentrations varied by almost a 100-fold whereas ammonium concentrations which were much lower and less variable than nitrate differed by 10-fold. The ratio of nitrate to ammonium was typically in the range between 10 and 20:1 but on the margins the ratios could be much higher. In many soil analysis data dissolved organic nitrogen which includes amino acids.

Analysis of the average measurements of various forms of N in the soil solutions from the topsoils of four different ecosystems from data collected every two weeks over a thirty six month period are shown in Table 1, overleaf In agreement with the above findings, nitrate is the more dominant inorganic form in all ecosystems. Interestingly though soluble organic N is present in

relatively high concentrations in all soils and the major N form in one of the woodland ecosystems and in pasture even though the pasture had been treated regularly with mineral fertilisers at 80 or 45 kg N/ha. In the arable soil in which applications of mineral N (50-80 kg N/ha) had been applied together with manure, the soluble organic N concentration was more or less equal to that of nitrate N. These finding indicate that even in arable soils the uptake of soluble organic N may be more important than was previously thought as is discussed below.

Table 1: *Concentration of major nitrogen forms in topsoil solution in different ecosystems. (Average measurements from data collected every two weeks over a 36 month period) (Aue, 1993).*

Ecosystem	Depth	NO_3-N	NH_4-N	N_{org}
Dystric Cambisol, (Cambisol-Colluvisol)	cm		mg/L	
Woodland (*Fagus sylvaticus*)	0-12	8.5	1.2	7.8
Woodland (*Alnus glutinosa*)	0-20	2.5	0.2	7.3
Arable land*	0-25	17.5	0.5	17.0
Pasture land**		5.0	0.3	10.5

* Regular fertilisation of maize, oats or sugar beet with manure and KAS (50-80 kg N/ha).

** Regular fertilisation with NPK or KAS (80 or 45 kg N/ha).

In the soil N is transported to plant roots by both mass flow and diffusion. For nitrate, when present at high concentrations in the soil solution, the most important means by which it is moved is by mass flow, the conductive transport in the soil solution from the bulk soil to the root surface driven by transpiration. On the other hand, at lower concentrations of NO_3 and also for NH_4, at the lower concentrations at which is normally present, transport is to a large extent is by diffusion i.e. movement by random thermal motion from a higher to a lower concentration at the root surface induced by N removal during uptake As a rough estimation, mass flow is sufficient to transport NO_3^- to the root surface when the concentration of NO_3^- in the soil solution is above am (assuming that 400 litres of soil solution is transpired by the plant to produce 1 kg of dry matter containing 4% N).(Engels and Marschner, 1995) The contribution of mass flow to total N movement to roots during periods of maximum demand have been estimated at 50% for potato (Asfary *et al.,* 1983), 25% for winter wheat (Barraclough, 1989) and between 15% and 33% in sugar beet, spring wheat, winter barley and winter wheat (Strebel and Duynisveld, 1989). Supply of NO_3 by mass flow to the root surface by mass flow ensures that the nitrate uptake capacity can only limited by the uptake capacity of the roots themselves and not by restricted by transport to the roots. This is not the case with diffusion where the rate of ammonium and nitrate uptake in some poorly supplied soils may be limited by too low a rate of N transport to the roots

In fertile arable soils supporting high yielding crops, however, the concentrations of mineral nutrients in soil solution are often particularly high and especially so for nitrogen which means that supply of N to the root surface is unlikely to be a limiting step in N uptake by the crop (Marschner, 1995). This is so even though the concentrations of nitrate and ammonium in the soil solution may fluctuate enormously (Miller *et al.,* 2007) and be dependent on when the N fertiliser is applied and the time of year the measurements are made particularly in relation to soil temperature which controls nitrification. In the case of the oilseed rape crop, Barraclough (1989) reported a value as low as 87 µM N to ensure adequate supply by diffusion so that uptake was not being impaired
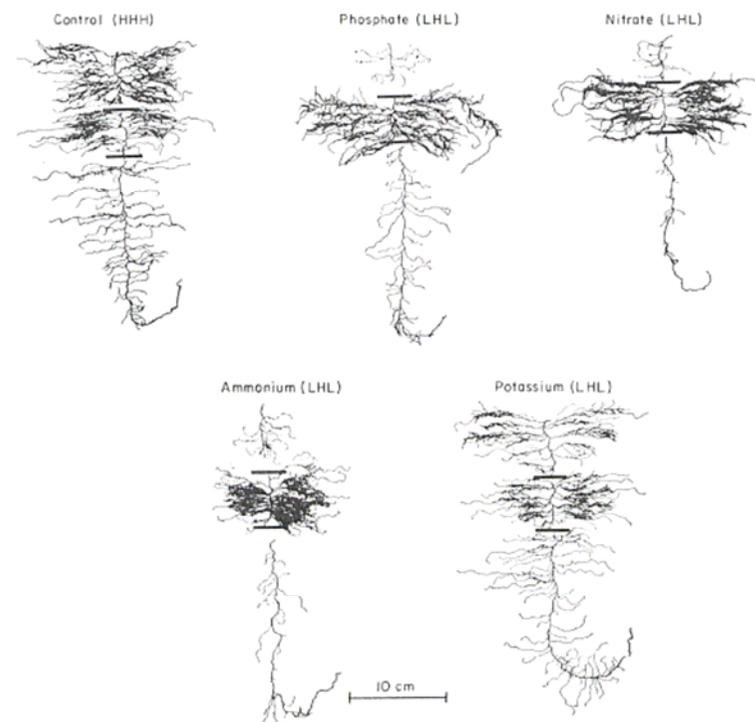
Figure 1: *Root growth response to localised zones of low (L), and high (H), supplies of nitrogen (NO_3 and NH_4), phosphorus and potassium. (after Drew, 1975).*

In addition to nutrient movement towards plant roots, the high plasticity of roots means that roots themselves respond to spatial and temporal nutrient heterogeneity in soils. This is evident in root proliferation in nutrient rich patches in the soil as often observed in sites of fertiliser placement.. The results of the classic experiment of Drew (1975) is shown in Figure 1. in which barley plants grown in sand culture such that different zones of the root system

could be supplied with very low or high concentrations of a *single* inorganic nutrient, with all other nutrients being maintained at high concentration Both with nitrate and ammonium as also with phosphate, exposure of parts of the main seminal roots to high concentrations of each these nutrients caused a localised promotion of the initiation and subsequent extension of both first and second order laterals compared with zones receiving very low concentrations. Interestingly no response was obtained with potassium. As discussed by Forde (2002), it is now recognised that the signal for increased meristematic activity in the lateral roots appears to be a local one coming directly from the nitrate ion. This implies that the lateral root tips are equipped with a nitrate sensor and a signal transduction pathway to convert the nitrate signal into a growth response. Two genes encoding possible components have been identified and an updated model has been presented by Forde (2002). Uncertainties still remain concerning the effect of nitrate on root proliferation in nitrogen rich patches. Robinson (1996) in posing the interesting question 'why do plants bother?' considers that the high mobility of nitrate in soil should limit its contribution to root proliferation for N capture which is in accord with the evidence of many authors of weak relationships between proliferation and nitrate uptake by isolated plants.

Over the last decade there has been considerable interest in organic nitrogen uptake by plants especially in the form of amino compounds (Näsholm *et al.,* 2009) and there is now evidence that roots can respond to variations in glutamate concentration in the external environment (Walch-Liu *et al.,* 2006). As glutamate is one of the most abundant amino acids in the soil and a component of dissolved organic nitrogen (DON) its presence in organic rich patches in the soil as in cow pats on pastures could well account for root proliferation at such sites. How external signals from nitrate and glutamate are perceived at the root tip and how the two signalling pathways interact with each other in communicating with internal N status to modulate growth rate has recently been discussed by Forde and Walch-Liu (2009).

2.2 Nitrogen uptake.

2.2.1. Nitrate uptake.

There is an enormous detailed literature on the uptake of nitrate (and ammonium) N and has included reviews and publications by Engels and Marschner (1995), Forde and Clarkson (1999), Glass *et al.* (2002), Miller *et al.* (2007), Over the past 50 years the use of different methodologies has gradually increased our scientific understanding of how nitrate is taken up by plants and we now know the fundamental characteristics of the process. These include: metabolic dependence, enzyme-like kinetics of ion transport, electrophysiological and biochemical aspects of ion transport, the dynamic nature of ion fluxes that change to meet whole plant demand and most recently molecular/genetics characterisation (see Glass, 2009). Over the past decade more and more attention has been given to molecular aspects. Here we provide only an outline of what we see as the salient points of nitrate uptake in relation to agronomy.

There is now very good physiological evidence that nitrate uptake from the soil is dependent on three types of transport system to cope with the variations in nitrate concentrations in cultivated soils (Crawford and Glass, 1998). Two types of high affinity transport systems (HATS), one constitutive (cHATS) and the other inducible (iHATS) are able to take up at low external concentrations for 1 µM to 1 mM. The low affinity transporter (LATS) which displays linear kinetics becomes significant at concentrations above am. Both types of HATS can contribute to nitrate uptake at concentrations above am external concentrations but their transport activity is saturated and expression down-regulated so that their contribution is marginal. These nitrate transporters are encoded by genes from the *NRT1* and *NRT2* families for HATS and LATS respectively. iHATS the induced form of the high affinity system provides a regulatory mechanism for uptake when substrate becomes available. Both nitrate and ammonium uptake are regulated by N status with glutamine acting as a negative feedback signal (Glass *et al.,* 2001) (Figure 2). The high affinity transport systems are highly specific for nitrate and their relatively insensitivity to Cl^- and SO_4^{2-}.which are often present in the soil solution in high concentrations means that there is minimal interference of nitrate uptake at low nitrate concentration (Epstein and Bloom, 2005).
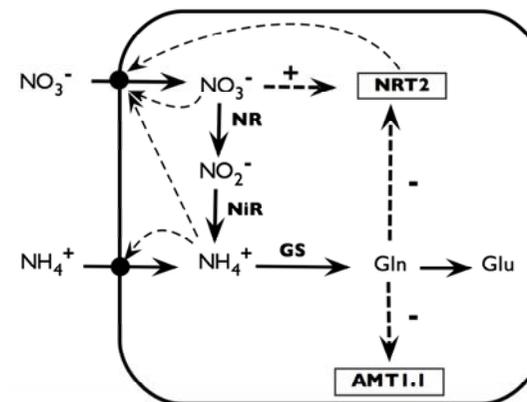
Figure 2: *A model representing feedback processes involved in the regulation of transcript abundances of NRT2 and AMT1 genes by root glutamine concentrations and by direct effects of the transporters by root nitrate and ammonium concentrations (after Glass et al., 2001).*

Nitrate uptake by root cells from the cell wall (the apoplasm) through the plasma membrane to the symplasm is an 'uphill' process requiring energy. Both chemical and electrical potentials oppose nitrate influx i.e. a negatively charged ion has to be transported across a membrane against both a chemical and an electrical gradient (Figure 3, overleaf). The nitrate concentration in the apoplasm (or rhizosphere) can vary widely see section above) but is often be very much lower than the symplasm (5-30 mM). The electrical potential difference across the plasma membrane of 100-200 mV the inside being

negative, is generated by the activity of an H^+ ATPase which pumps H^+ from the cytoplasm into the apoplasm. Short term experiments have revealed that after the supply of nitrate, the pH of the external solution increases and the membrane potential transiently decreases. From this evidence it has been established that nitrate transport across the plasma membrane is coupled to 'downhill' H^+ fluxes via a H^+/NO_3^- symporter with a flux stoichiometry of about $2H^+$ to $1 NO_3^-$, the operation of which stimulates the H^+ ATPase, with subsequent reestablishment of the transmembrane potential. During nitrate uptake the pH of the apoplast (rhizosphere of roots) therefore increases. This increase with nitrate fed plants can be of major agronomic importance

Figure 3: *A model for uptake of NO_3^- and NH_4^+ through the plasma membrane and its coupling with the activity of an electrogenic proton pump (H^+-ATPase) (after Engels and Marschner, 1995).*

Nitrate uptake by roots is sensitive to the availability of cations K^+ Ca^{2+} and Mg^{2+} (see section) but not to NH_4^+. Nitrate uptake is therefore associated with mineral cation uptake. At non toxic levels, ammonium may stimulate or inhibit uptake. It has l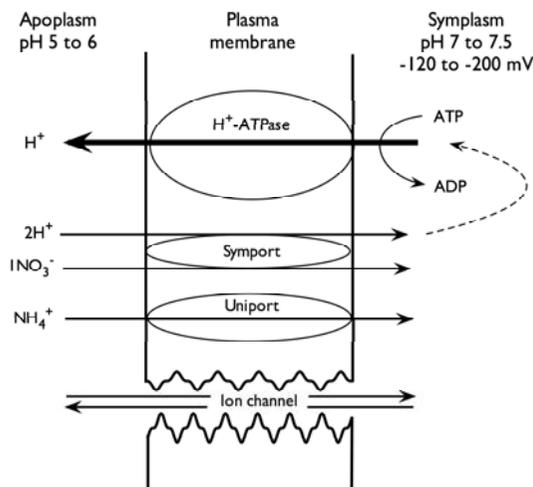ong been recognised that low pH values in the outer medium below 6 stimulate nitrate uptake (Rao and Rains, 1976) presumably because of increased H^+ available for co-transport.

2.2.2. Ammonium uptake.

In well drained aerobic agricultural soils NH_4 is present in much lower concentrations than nitrate as already mentioned. Nevertheless together with nitrate it provides the N supply for crop growth. Some plant species thrive when supplied with NH_4 as the sole N source including the ericaceous species (Pearson and Stewart, 1993) and boreal conifers (Kronzucker *et al.,* 1997). A preference for this form of N nutrition might be expected because of the lower energy demand for uptake. For most agricultural crops, however, with the well known exception of rice grown in paddy soils (Wang *et al.,* 1993), severe toxicity symptoms may ensue when ammonium is supplied in solution as a sole source of nitrogen at concentrations acceptable for nitrate (in the order of >1 mM) (Britto and Kronzucker, 2002).

The high affinity transporter (HATS) for NH_4^+ uptake from 1 μM-1 mM is encoded by genes of the *AMT1* family and expression is also subject to down-regulation by glutamine (see Figure 2). This is the concentration range present in most soils. At higher concentration the low affinity transporters come into play. Comparing NH_4 acquisition by barley which is known to be susceptible to toxicity and rice which is extremely tolerant, Britto *et al.,* (2001), reported that at high external NH_4 concentration $[NH_4^+]O$, barley roots experience a breakdown in the regulation of NH_4 influx leading to excessive amounts of NH_4 in the cytosol. Measurements of NH_4 efflux combined with thermodynamic analysis of the transmembrane electrochemical potential for NH_4 revealed that at elevated $[NH_4^+]O$, barley cells engage a high capacity NH_4^+ efflux system that supports outward fluxes of NH_4^+ against a sizeable gradient. Ammonium efflux was shown to constitute 80% of primary influx providing evidence of futile cycling of N across the plasma membrane of root cells. This futile cycling carries a high energy cost – as much as 40% increase in root respiration – that is independent of N metabolism and causes a reduction in growth. In rice, by contrast, Britto and his colleagues have shown that a defence strategy has evolved an energetically neutral near Nernstian equilibration of NH_4^+ at high $[NH_4^+]O$. These workers suggest that this study characterises the primary events at cellar level which appear highly likely to account for NH_4 toxicity in plants.

Ammonium transport across the plasma membrane at least at high external concentrations (> 1 mM) is believed to occur via an electrogenic uniport i.e. without the requirement of movement of another ion (Ullrich, 1987) (see Figure 3). However, the ATPase driven proton pumping which also occurs across the membrane does in effect operate as an NH_4^+ /H^+ antiport to maintain ionic equilibrium and accounts for the acidifying effect in the rhizosphere of plants supplied with NH_4-N. Ammonium uptake is highly competitive in depressing the uptake of the main mineral cations, K^+, Ca^{2+} and Mg^{2+} (Kirkby and Mengel, 1967; Kirkby, 1968) whereas these ions are largely without effect on the uptake of NH_4^+ (Marschner, 1995).

From an agronomic viewpoint one of the most interesting observations in nitrogen uptake is that ammonium toxicity can be alleviated by co - provision of nitrate (see Britto and Kronzucker, 2002). Additionally by providing both forms of N a synergistic effect on growth can observed by supplying the two forms of nitrogen together. Maximal growth rates can be surpassed by between 40-70% in comparison with either sources alone for plants growing in water culture (Cox and Reisenauer, 1973; Heberer and Below, 1989) with somewhat lower values for plants growing in soil.(Hagin *et al.,* 1990). Various explanations have been proposed many of which suggest that nitrate acts as a signal that stimulates or optimises a multitude of biochemical responses.(Stitt and Krapp, 1999; Tischner, 2000). One suggestion has been that cytokinin synthesis is maximised when NO_3 and NH_4 are supplied together. The possible practical significance of this in improving tomato production is discussed in section 4.12

2.2.3. Urea uptake.

Urea is the major nitrogen form supplied as fertiliser N. In soils urea is hydrolysed rapidly by urease a nickel (Ni) dependent enzyme produced by soil micro-organisms (Watson *et al.*, 1994) into ammonium often with concurrent nitrification into nitrate. Applications of urea to soils may thus expose plant roots to urea as well as ammonium and nitrate for at least for short periods of time. The physiology of the uptake of urea by the roots of higher plant has not been studied in any great detail as discussed in the recent review of Merigout *et al.* (2008). From water culture experiments from the older literature Kirkby and Mengel (1970) demonstrated that urea supplied to roots was for a poor source of N for sunflower as compared to nitrate (Table 2). At 2 mM supply the urea fed plants were less than half the dry weight with just over half the total N content of the nitrate fed plants and showed N deficiency symptoms. Raising the urea concentration in the nutrient solution four fold increased the N concentration in the plants to more or less the same value as in the am nitrate treatment but yield was still lower and huge accumulations of aspartic acid, alanine and asparagine occurred. At the time these experiments were carried out the significance of Ni as a constituent of urease was not appreciated.

Table 2: *The effect of increasing concentrations of urea as compared with nitrate N supplied in water culture on the growth and nitrogen status of sunflower plants (Kirkby and Mengel, 1970).*

Treatment	Yield	N uptake	Nitrogen	Amino-N
	g DM/10 plants	mg	%	%
2 mM nitrate	10.58	614	5.80	0.23
2 mM urea	4.83	159	3.30	0.04
4 mM urea	6.21	352	5.67	0.14
8 mM urea	7.13	413	5.79	0.67

More recent investigations concerning urea uptake have considered foliar applied urea which is often used in practice. In this respect Nicoulaud and Bloom (1998) demonstrated that a very low concentration of up to 1 µM $NiCl_2$ in the nutrient solution greatly improved the growth of tomato seedlings supplied with N solely by foliar urea.. This finding is in line with the observation that Ni deficient plants showed lower urease activity and that application of urea can be toxic to Ni deficient plants causing leaf tip necrosis. (Krogmeier *et al.,* 1991). The question of whether Ni should be supplied to plants receiving foliar applications of urea is an area of current interest as discussed later (section 4.10).

2.3. Nitrogen assimilation.

2.3.1. Nitrogen metabolism.

After uptake by N transporters, nitrate may be stored in vacuoles or reduced to the -3 valence NH_4^+ state thereby becoming metabolically active for growth.. Depending on plant species and other factors, this can occur in situ in the roots or more usually after transport via the xylem to the leaves.(see Marschner, 1995). The reduction is mediated by two complex enzymes, nitrate reductase NR and nitrite reductase. The first reduction from NO_3^- to NO_2^- involves the Mo containing enzyme nitrate reductase (NR) and NAD(P)H and takes place in the cytoplasm. In leaf cells the nitrite so generated is transported to the chloroplasts where it is further reduced to ammonium by ferredoxin and nitrite reductase NiR). In root cells, reduction takes place in the plastids with energy supplied by the oxidative phosphate pentose pathway from sugars transported from shoot to root. NR and NiR function in series to prevent the accumulation of the highly reactive and potentially toxic nitrite ion.

Ammonium produced by nitrate assimilation as described above, or as supplied as ammonium ions or via nitrogen fixation is then incorporated into amino acids in the chloroplasts. This is achieved by glutamine synthetase (GS) which converts glutamate and NH_4^+ to glutamine, a reaction requiring ATP. Glutamine and α ketoglutarate from mitochondria are then converted to two molecules of glutamate by glutamate synthase (GOGAT). Several amino acids are formed from glutamate and keto acids by transaminases to provide the basis for the synthesis of other amino acids used for building proteins and various N compounds leading to the leading to the synthesis of secondary compounds (Figure 4).

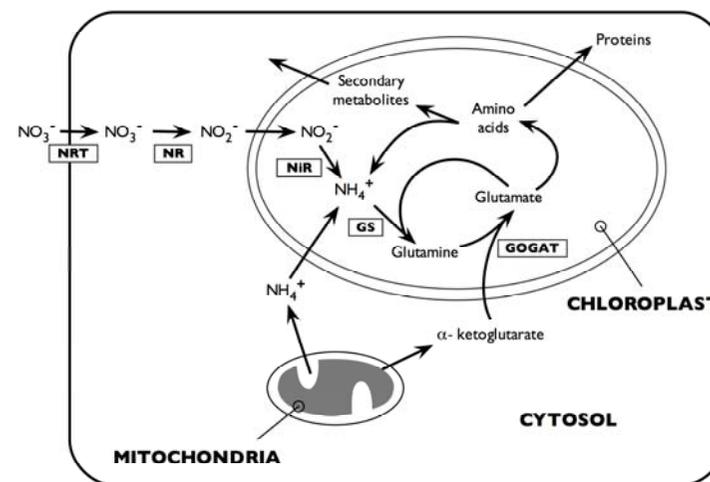**Figure 4**: *Overview of the nitrogen assimilation pathway in green cells (after Lillo, 1994).*

It has been argued that a better understanding of nitrogen metabolism and its regulation at the molecular level might result in improved nitrogen use efficiency particularly in cereals by which supplied nitrogen fertilisers may produce higher yields with greater grain protein contents (Miflin and -Habash,

2002). This work has centred on Glutamine synthetase (GS) and glutamate dehydrogenase (GDH).As described above GS is the major assimilatory enzyme for ammonia produced from nitrate and ammonium nutrition as well as N fixation. Additionally it reassimilates ammonia released as a result of photorespiration and breakdown of proteins and nitrogen transport compounds. The enzyme is widely distributed in plant tissues and organelles and plays a key role in maintaining carbon nitrogen balance. GDH exerts a similar influence in returning carbon in amino acids to reactions of carbon metabolism and tri-carboxylic cycle. Results from transgenic plants containing transferred GS genes suggest that there may be ways in which it might be possible to improve the efficiency by which crops use nitrogen.

2.3.2. *Cation-anion balance during nitrogen uptake and assimilation.*

The form of nitrogen supply (NO_3, NH_4 and N_2 fixation) plays a key role in cation-anion relationships and thus on rhizosphere pH (Kirkby and Mengel, 1967; Römheld, 1986). About 70% of the cations and anions taken up by plants are represented by nitrate or ammonium ions. Nitrate fed plants are characterised by a high anion-cation uptake ratio whereas in ammonium fed plants there is high cation-anion uptake ratio. The difference is dependent mainly on the fate of H^+ released from the roots by the ATPase driven H+ pump in the plasma membrane of root cells. The uptake of nitrate is linked to the influx of about $2H^+$ for NO_3 so that H^+ is removed from the external medium thereby increasing its pH. The uptake of the major cations, K, calcium (Ca), and magnesium (Mg) are favoured by the nitrate ion as balancing cations. During NH_4 uptake which is transported across the membrane by the prevailing electrical gradient independent of the release of protons from H^+ pump in the plasmalemma. NH_4^+ ions strongly compete for uptake with the other major mineral cations whereas anions are favoured to maintain charge balance. The higher uptake of cations than anions from the nutrient medium and acidification of the rhizosphere as occurs with NH_4 nutrition is also characteristic of urea supplied plants or legumes fixing N_2. The practical significance of different N forms modulating rhizosphere pH is discussed in Sections 4.1 to 4.5, 4.7 and 4.8.

2.3.3. *Phytohormones.*

Nitrogen plays a very important role in assuring high yields of crops by the rapid establishment of a large canopy for photosynthesis (high leaf area index), the maintenance of this canopy (leaf area duration) and the establishment of storage organs (a large sink capacity). The way in which N modulates plant growth however, can not totally be accounted for as a direct nutrient effect. For example flower formation in apple trees is affected to a much greater extent by the time or form of N application than by the level of N application. Compared with the control of continuous nitrate supply, even a short term application of ammonium was found to more than double the percentage of buds developing inflorescences (Rohozinski *et al.,* 1986). NH_4-nutrition leads temporarily to increased concentrations of cytokinins (Gao *et al.,* 1992) and arginine which is a precursor of various polyamines with growth regulating functions. Both cytokinins and polyamines appear to regulate flower initiation. Similarly in maize and sorghum grown with NO_3-N and NH_4-N nitrogen, floret and kernel number increased in comparison with NO_3 only nutrition (see Engels and Marschner, 1995). Rapid increases in internal ABA levels may result from lack of nitrogen.(Krauss, 1978). Short term responses to N deprivation such as reduced leaf area, growth or stomatal closure may also be explained by increased leaf ABA concentrations causing reduced cell wall extensibility or stomatal closure (see Engels and Marschner, 1995).

2.4. Cycling and recycling of nitrogen in plants.

The transport of mineral nutrients in plants is often considered solely as a one way process in which nutrients are transported from the root source to the shoot sink. This is not, however, always the case and the reverse can occur for example in foliar application with urea or the absorption ammonia through the leaves when mineral nutrients are transported from the shoots; to the roots via the phloem. The same is true for nitrogen supplied exclusively to the roots where substantial amounts taken up are transported to the leaves is then retranslocated in the phloem to the roots. This cycling process may be described for the completion of one full cycle:

Root → xylem → shoot → phloem → root

This cycling process is of direct agronomic interest because N uptake by crop plants is not only dependent on external factors such as the N concentration at the root surface and temperature but it is also internally regulated by endogenous concentrations of N compounds in the root or particular amino acids transported from shoot to roots. It has also been shown mainly for nitrate fed plants that under conditions where nitrate is not limiting, the uptake of N is closely matched to the growth related demand of the plant. Transport also relates to the forms of nitrogen supply. When nitrate is supplied assimilated can take place both roots and shoots the shoot usually being the more predominant site but for ammonium fed plants assimilation occurs almost exclusively in the root. Nutrient cycling fulfils a number of physiological functions including those discussed below:

2.4.1. *Supplying roots with nitrogen assimilated in shoots.*

A major reason for the cycling of nutrients which are predominantly assimilated in the shoot as is the case for nitrate and sulphate for many plant species, it that is essential that cycling of the assimilation product (i.e. amino acids or glutathione) takes place in order to cover the demand for the roots for these nutrients.

In many cases even when plants have a considerable capacity for NO_3 reduction in the roots, large amounts of reduced N recycles from the shoot far in excess of root demand. Excess reduced N therefore recycling back to the shoot (Jeshke and Pate, 1991). The same observation was made by Simpson *et al.* (1982) in nitrate fed barley plants in which up to 79% of the N translocated in the xylem to the shoot was translocated in the phloem as

reduced N back to the roots. Of this fraction about 21% was incorporated into root tissue and the remainder recycled back to the xylem in the shoot. In young wheat and rye plants Cooper and Clarkson (1989) reported a recycling fraction representing over 60% of the reduced N in the xylem sap. One of the purposes in N cycling is the signalling of shoot demand as discussed below. Another possibility especially for plants growing in the field is to smooth out fluctuations in external nutrient supply from the soil thereby ensuring continuous root growth through soil layers varying in N supply.

For plants supplied with NH_4 N the roots provide are the major site for N assimilation and cover the shoot demand for reduced N. Nevertheless substantial amounts of reduced N also recycle to the roots i.e. the sites of N assimilation (Figure 5). In this experiment with maize comparing ammonium and nitrate nutrition, although the amount of reduced N cycled to the roots was lower in ammonium than nitrate fed plants (0.26/0.81 mmol/d, the amount nevertheless exceeded the amount of total N accumulated in the roots of the ammonium fed plants (0.20 mmol/d). Thus in NH_4-fed plants too some of the reduced N in the xylem sap can represent a recycled fraction (Peuke and Jeschke, 1993). Because of the presence of this variable fraction of reduced N in the xylem, the use the ratio of the proportion of NO_3 to total N in the xylem sap is not a reliable measure of nitrate reduction in the roots because of overestimation (van Beuisichem et al., 1988).
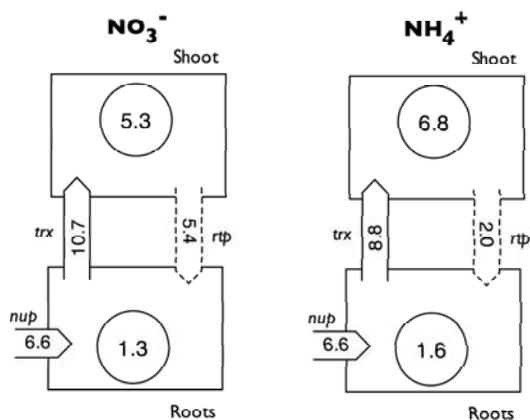

Figure 5: *Nitrogen fluxes between roots and shoots of maize depending on form of N supply (1.0 mM NO_3^- or NH_4^+) All figures are in μ mol/h/g root fresh weight: nup = net uptake, trx = translocation in the xylem, rtp = retranslocation in the phloem. Figures in circles = net accumulation in the root and shoot respectively (Engels and Marschner, 1996).*

Cycling of mineral nutrients in the phloem is closely associated with transport of carbohydrates from source leaves to both shoot sinks (apices, seeds, fruits) and root sinks (root tips). In the main, whereas translocation to shoot sink organs relates closely to nutrient demand, translocation of mineral nutrient to the root is usually in excess of demand the surplus being recycled back to the

shoot in the xylem. One may speculate thus that under nutrient deficiency, a lowered import of nutrients from the phloem to the root can be met by lowering nutrient recycling to the shoot, a means of adjustment which is absent in translocation from the source leaves to the shoot sinks. The increase in root : shoot dry weight ratio associated with N and phosphorus (P) deficiency may therefore be an expression of this type of internal regulation and not only to a higher retention of nutrients taken up by the roots from the soil or nutrient solution. In extreme cases cycling can even lead to a depletion of the amount of nutrient in the shoot in favour of the roots as has been reported for N in soybean (Rufty et al., 1984).

2.4.2. Maintenance of cation -anion balance.

As already discussed, nitrogen is quantitatively the most important mineral plant nutrient (2-5% N). In nitrate fed plants in which nitrate assimilation occurs in the shoot, fluxes of the nitrate anion in the xylem must be counterbalanced by equivalent cation fluxes. Quantitatively the most important cation, at least in the monocots is K^+ (2-5% K in the shoot dry matter). In order to reach this same concentration the net translocation of N (atomic weight N 14) has to be 2.8 times higher than K (atomic wt 39) excess K+ must therefore be recycled to the roots.
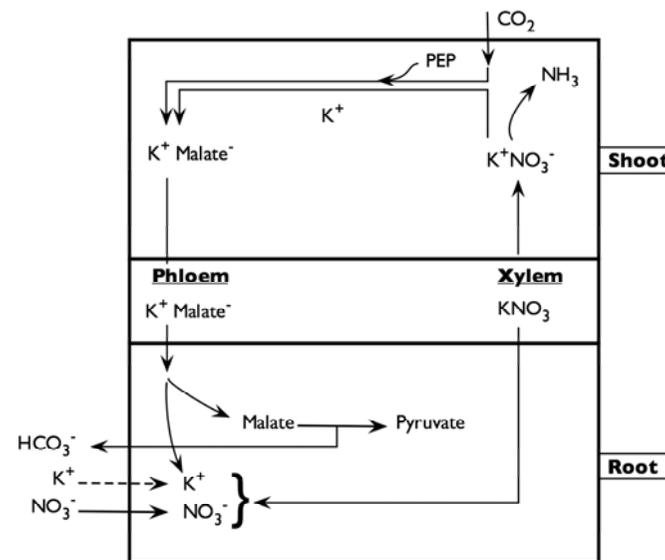
Figure 6: *Model for the circulation of potassium between root and shoot in relation to nitrate and malate transport (PEP, phosphoenolpyruvate). (Based on Ben-Zioni et al., 1971, and Kirkby and Knight, 1977).*

The most well known historical example of cycling is that of K^+ together with malate in the phloem, the so-called malate shuttle (Figure 6). After the decarboxylation of malate in the roots and the release of HCO_3^- in exchange

for the uptake of NO_3^-, the K+ can then recycle as KNO_3 in the xylem. Strong support for the importance of this model has been provided by stem feeding experiments with soybean which reduces about 90% of N in the shoots where stem feeding of K malate induced an increase in bicarbonate release by the roots and net uptake of NO_3^- (Touraine *et al.,* 1992). There are now, however, a number queries relating to this model. Analysis of phloem sap has shown much lower concentrations of malate than K even though ionic balance is maintained by other anions including negatively charged amino acids, sulphate and phosphate (Jeshke *et al.,* 1997; Kirkby, unpublished data). More concerning is the critical argument of Britto and Kronzucker (2005), that nitrate assimilation is a pH neutral process and thus not relevant to subcellular pH homeostasis (i.e. to carboxylate accumulation by the pH stat by means of PEP carboxylase activity). Moreover nitrate uptake by plants is not an exchange process NO_3^-/HCO_3^- as indicate in the model but rather a H^+/NO_3^- symport. Nevertheless ionic balance is maintained in the plant during N and K cycling although the mechanism by which it is achieved is perhaps still elusive.

2.4.3. *Signalling shoot demand for nitrogen.*

Cycling of nitrogen (and other nutrients) between shoots and roots may play an important role in acting as a signal for feedback control in nutrient uptake depending on plant demand for growth. In principle, according to shoot demand, a varied proportion of a nutrient transported in the xylem to the shoot is cycled back to the root. At high shoot demand a lower proportion cycles to the roots acting as a signal for enhanced uptake whereas when shoot demand is low the increase in cycling in the phloem acts to repress root uptake and xylem transport. This is demonstrated in Figure 7 which compares the effect of a high versus a low shoot temperature (low N shoot demand versus high N shoot demand respectively) on the distribution of N uptake between shoot and root. A much higher proportion (51%) of the low N shoot demand was present in the roots as compared with the high N demand treatment.

The mechanisms by which this long distance control is achieved has been, and is continuing to be the subject of much research activity. It has been suggested that recycling of amino acids from shoot to root could provide the means for communicating the N status of the shoot by modification of the N/C ratio of amino acids in the phloem sap to regulate the NO_3 uptake system (Cooper and Clarkson, 1989). However, in a split root system study with the castor oil plant (*Ricinus communis*) it was found that up-regulation of the NO_3 influx system was not correlated with changes in the amino acid composition of the phloem sap nor with the absolute rates of amino acid transport into the root (Tillard *et al.,* 1998). Nevertheless as pointed out by Forde (2002) it cannot be ruled out that subtle short term fluctuations may occur in phloem amino acid composition to which the *NRT2* genes, the genes encoding the LATS nitrate transporters, are sensitive. More recently Forde has suggested that glutamate transport in the phloem could provide a specific signal coordinating the uptake of glutamate from the soil (Forde and Lea, 2007).
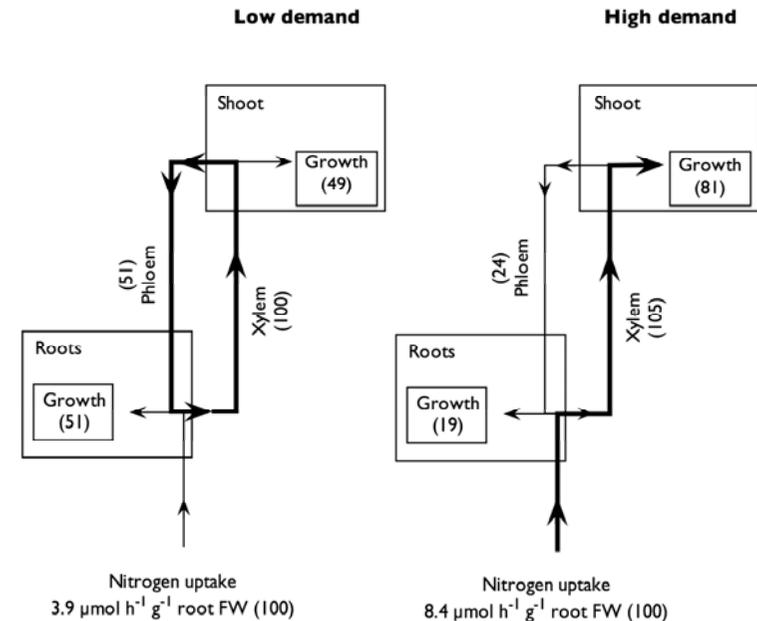

Figure 7: *Model for the regulation of N uptake by shoot demand; the growth related shoot demand of maize was varied by growing the plants with their shoot growing zone (basal 2 cm of the shoot) at either 12°C (low demand) or 24°C (high demand). Plants were supplied with NH_4-N (1 mM). Figures in parenthesis indicate percentage of net N uptake (Engels and Marschner, 1996).*

It has also been suggested that sugars which are present in large concentrations in the phloem sap may also play a role as long distance signals by interacting with NO_3 regulated processes in roots.(see review of Forde, 2002). In accord with the diurnal regulation of NO_3 uptake which occurs in many plant species it seems likely that diurnal fluctuation in the supply of sugars from the shoot act as long range signals to regulate expression of NO_3 transporter genes so helping to coordinate the processes of NO_3 uptake and photosynthesis. A model for the regulation of *AtNRT2.1* and *AtNRT1.1* expressed by local and long range signalling pathways in *Aradopsis* has been proposed by Forde (2002).

The phytohormones including auxins and cytokinins also appear to be involved in signalling. The findings of Rahayu *et al.* (2005) of stimulation of leaf expansion by supply of NO_3 to precultured NH_4-fed tomato plants was associated with a marked increase in concentration of the physiologically active forms of cytokinins in the xylem sap. That the effect could be induced by just 10 µM NO_3 is indicative of cytokinin as a long distance signal mediating the shoot response to NO_3 perception in the roots. Hormonal signals from the roots to the shoots of early transmitters of unfavourable root

zone conditions have also been suggested for various stress conditions including mechanical impedance, water stress and flooding (see Engels and Marschner, 1995).

3. NITROGEN STATUS OF CROP PLANTS.

Soil N is the most important limiting factor for crop production as it strongly modifies plant growth in relation to internal N status. Plant N status is dependent on the amount of total N stored in plants i.e. the plant nitrogen concentration, which is a good indicator of plant growth rates. The term, however, also encompasses the great diversity of N containing compounds in plants. For the agronomist, a knowledge of these forms and their contents is important in order to diagnose N crop status and propose reasoned methods for N fertiliser recommendations. As discussed in the previous section, plants selectively absorb various forms of N from their environment. These for the most part after uptake are rapidly assimilated into a number of N containing pools but some of these N forms may be partly retained in the form in which they were absorbed $e.g.$ NO_3-N. Since growth (i.e. the increase in plant structures) feeds from these internal N pools, it is likely that a knowledge of the pools including their fluxes and the availabilities of the different N contents, is of greater practical importance than that of the overall plant N status. Some indication of this can be seen from the biochemical pathways involved which are summarised in Figure 8. However, only a few pools have shown sufficient variability (see Figure 9) to account for plant response to N, implying that a precise knowledge of these N pools and their assimilation is required in order to manage N fertilisation.
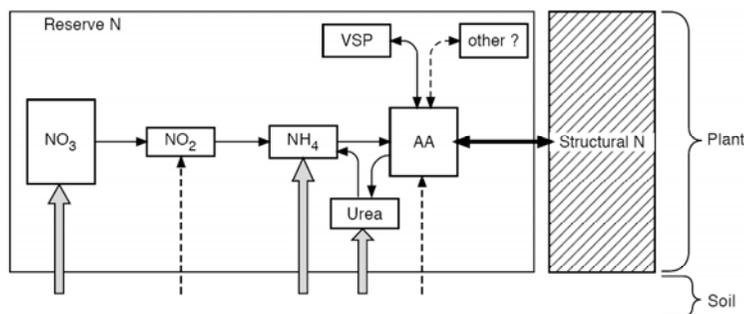

Figure 8: *Plant N pools. The figure differentiates resources, (i.e. N pools used to build structures) from structural N (either metabolic or non-metabolic). The arrows indicate the main fluxes. AA are amino acids and VSP, vegetative storage proteins. The fluxes from the soil indicate uptake, with dashed lines denoting minor processes.*

It is well recognised that these various biochemical forms are not stored in equal concentrations in the plant tissues. Figure 9, a compilation of quantitative data taken from the literature on crops grown under sound agronomic conditions, shows the potential amplitude in shoot and root concentrations of the different forms of N stored in plants as reserve-N. These forms are discussed below.
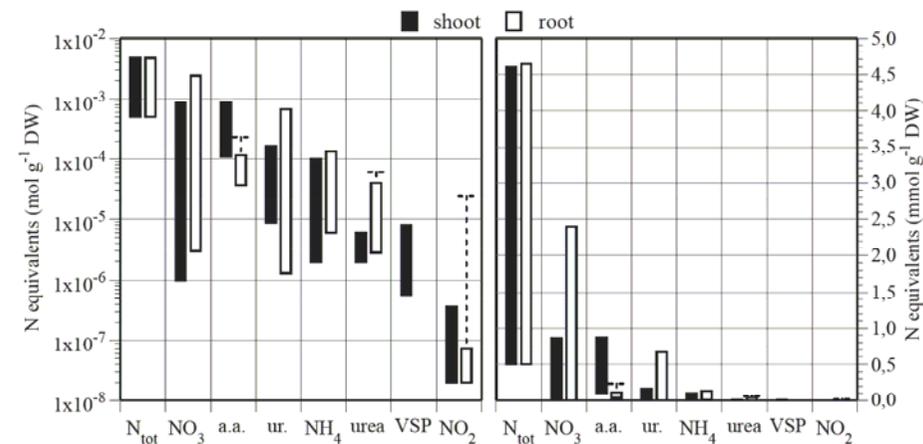

Figure 9. *Amplitude in shoot or root concentrations of total N (N_{tot}) and N-reserves in plants fed with full NO_3 or mixed (NO_3:NH_4) nutrition. Unusual abbreviation for N-reserves are : a.a. amino acids, ur. ureides and VSP vegetative storage proteins. Note that on the left, concentration data (in mol/g DW) are plotted on a log-scale to reveal their large changes with N-forms, while on the right, data (in mmol/g DW) are plotted on a linear scale, to reflect the relative importance of each form in the plant N budget. The dashed lines are maximum values recorded under peculiar nutritional regimes (i.e. full nutrition with the given N-form).*

3.1. Forms of nitrogen in plants.

Plant models usually distinguish between structural and non-structural carbon compounds. As C and N are intrinsically linked in plant growth processes, it seems also appropriate to differentiate N forms based on their use in 'storage' (*in the broad sense*) or in 'growth' (i.e. *increase of structures*). It is emphasised that all non structural N is considered here in the role of a potential resource for the formation of new structures. The term 'structures' is also considered in a broad sense and comprises solid structures (cell walls, organelles …) and metabolic structures (enzymes …). As a basic approach, structural N represents the difference between total N and the N contained in the intermediate metabolites, such as those shown in the Figure 9. Indeed, several forms of N can be absorbed by plants and stored to serve as potential resources for structural growth (see Figure 8). A few of them, however, may accumulate in significant amounts to serve as true intermediate N-reservoirs between soil and structural growth (see Figure 9). These forms have a particularly important role in the plant because they allow the uncoupling of growth from the current uptake processes. For annual crops, only NO_3-N and amino acids are likely to play a prominent part in this uncoupling process. For

trees and perennial crops, the picture is blurred because perennial parts contain most of the DW, implying that any N-compound at a low concentration can build-up a large store and may have a great quantitative importance in the growth of new organs. In particular, this may be the case for the vegetative storage proteins.

3.1.1. Storage nitrogen.

Nitrate can be stored as a free ion in vacuoles and retrieved quickly for metabolic use (Blumwald and Poole, 1985). It is a usual form of N transport in the xylem, but not in the phloem which explains why NO_3^- is present both in roots and shoots. Different plant species and organs of plants show contrasting ability to store free NO_3^- (for references, see Cárdenas-Navarro et al., 1999) but nitrate-accumulators are generally plants with low dry matter content implying that plant water plays an important role in relation to NO_3^- storage. It is thus interesting to note that on average, compared to organic farming, conventional farming generates agricultural products of lower dry matter content that also exhibit higher NO_3^- content (Worthington, 2001). Leafy vegetables may contain most of their N as NO_3-N. In contrast, highly N-fertilised cereal crops such as wheat do not seem to accumulate much NO_3^- since this ion accounts for only 10% of N reserves (Justes et al., 1994). It is generally held that trees have low NO_3^- reserves.

Plants take up nitrite although it is a very reactive ion and is generally toxic. It is thus observed that plant NO_2^- content is low, but this is subjected to genetic and environmental variations. Nitrite occurrence in transformed agricultural products is generally associated with poor storage condition (i.e. anoxia) rather than plant nutritional problems.

Ammonium ion (NH_4^+) accumulation in the tissues is toxic to most, if not all plants cultivated with NH_4^+ as sole source of N. A large genotypic variability characterises the threshold at which toxicity manifests itself (Britto and Kronzucker, 2002). The co-provision of nitrate alleviates this toxicity and it is commonly reported to allow for synergistic plant growth responses (Mengel and Kirkby, 1987). The underlying mechanisms are still a matter of debate. Recent papers (Britto et al., 2001; Schjoerring et al., 2002) have suggested that cytosolic NH_4^+ concentration value is in the millimolar range, contradicting the common belief that NH_4^+ tissue and xylem sap concentrations are low. Plant NH_4^+ concentration is strongly influenced by the NO_3^-/NH_4^+ ratios of the rooting medium, but remains usually one order of magnitude lower than that of NO_3^-. Trees may differ from this behaviour.

Amino acids (AA) represent a stable form of N storage in vacuoles and as already discussed in section 2.3 and 2.4, they are transported in the xylem and phloem saps. It is generally considered that in most heterotrophic tissues, AA represent the major form of available N. All studies dealing with plant responses to N nutrition have shown that the pool of free amino acids is positively correlated to N supplies. In young tomato plants, Huanosto Magaña et al. (2009) observed strong daily changes, AA contents being maximum in the afternoon and minimum at dawn. The leaves of these plants stored as much N in the form of AA as they did NO_3^-. In temperate trees, arginine is considered to be an efficient N-transport compound because its concentration is generally high in the xylem sap during autumn and winter periods (Schmidt and Stewart, 1998) and each molecule contains 4 atoms of N (i.e. 32% N). It is also considered, with glutamine, as an important form of N storage for woody species (Nordin et al., 2001).

Urea is a metabolic intermediate produced by N catabolism of arginine, purines or ureides. Urea does not accumulate in the cytoplasm since it is rapidly hydrolysed by urease, giving rise to NH_4^+. Urea may be absorbed and transported through the xylem sap in plant tissues.

In N_2 fixing plants ureides allantoin and allantoic acid are important compounds in the xylem sap. Together with AA (e.g. arginine, glutamine and asparagine), these compounds represent efficient forms for long distance N transport, due to their high N/C ratio (e.g. > 0.4, see Marschner, 1995). This is particularly important in temperate tree species during phases of N remobilisation from - or formation of – vegetative storage proteins (Schmidt and Stewart, 1998), but these remain poorly defined (VSP, see Cooke and Weih, 2005). The common justifications given to assess the storage function of proteins are their low molecular weight, their solubility in water and a marked seasonal variation in their concentration in vegetative organs. In deciduous trees, VSPs are synthesised in autumn and broken down in spring to favour N recycling and investment in early growth. However, other properties such as those conferring resistance to frost and insects are often associated, which poses the question of the true function of VSPs. Moreover, most literature dealing with VSPs gives qualitative rather than quantitative data from which it is difficult to infer their real contribution to the N pool, compared to other N forms. In peach trees, the bark-N undergoes strong annual variation with storage phases in late autumn and depletion in spring (Gomez and Faurobert, 2002). In this study, N from the VSP varied from 2-3% (autumn and winter) to < 1% (summer) of the total N content of the bark. Over the same period, N from the total soluble proteins minus VSPs fraction varied between 20 to 10% of the total. It could be argued, therefore, that the importance of the so-called VSPs relies more on their signalling property rather than on their contribution to the N budget of the plant.

3.1.2. Structural nitrogen.

Growth is usually approximated from the measurement of the dry biomass accumulation. This view is biased, however, because reserves fluctuate and may form a significant part of the total biomass. This may be exemplified in the day-night cycle by comparing total and structural dry biomass accumulations (Huanosto Magaña et al., 2009). Figure 10, overleaf, shows clearly the role of reserves: during the light period, total DW increases mainly due to the photosynthetic C acquisition but it stalls during the dark period. Structural growth, however, proceeds at a constant rate day and night. From these observations it may be inferred that growth becomes uncoupled from resource acquisition owing to fluctuating supplies from internal reserves.

Since N forms important reserves in the DW (see Figure 9) it is logical to apply to N the same principle described for DW, leading to define structural N as the difference between total N and the N contained in the intermediate metabolites, such as those shown in the Figure 8.
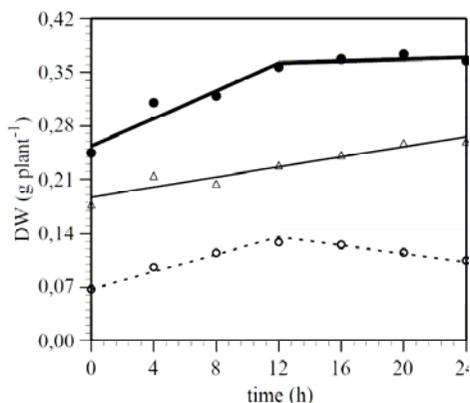

Figure 10. *Changes in the components of the dry biomass (DW) of tomato plantlets in a growth cabinet with constant temperature during day and night (shaded area). The thick line (closed circles) represents total DW, the dotted line (open circles) is the DW of reserves (C-based – sugars, organic acids and N-based – NO_3, NH_4, free AA) and the thin line (open triangles) is the structural DW (i.e. total minus reserve DW). Redrawn from Huanosto Magaña et al. (2009).*

This allows the building of simple trophic growth models based on resource availability. Mirroring the approach used with carbon, the functions of N acquisition from the soil and N use in growth have to be considered separately owing to the existence of plant N reserves which allow the plant to be independent of the soil as a source of N. It is for this reason that growth can continue until plant N reserve exhaustion which allows plant development to take place over the transient periods of shortages in soil N availability. In this case, the concept of 'growth autonomy' sustained by N-reserves replaces the equivocal notion of 'luxury N storage'. The same distinction has also been made in the case of potassium between 'luxury uptake' and 'contingency supply' (Epstein and Bloom, 2005).

This approach is, however, simplistic due to the poor knowledge of the N-source compounds, which is by no means exhaustive, implying that the quantification of the structural-N remains approximate. For instance, it is known that N-containing secondary compounds such as defensive molecules are not structures nor N-resources. For instance, the common cyanogenic compound 'prunasin' has been reported to account for as much as 5% of leaf total N (Graham, 2002). So far, its role has been associated with plant defence against herbivory but neither with growth nor reserves. This suggests that a separate N-pool may be operative other than that required for growth.

The main goal of this modelling scheme is to provide a rationale to interpret plant N concentration changes (i.e. [N], commonly expressed as % in the dry weight). In particular, the aim is to differentiate variations related to internal store filling or depletion (*due to temporal changes in soil N availability*) from those driven by ontogenic changes in the composition of structures. However, as stated above, the approach has clear analytical limitations since calculated structural [N] changes may be real or biased by undetermined N resources. Therefore, it appears also essential to characterise indirectly reserve-N and structural N. This has been done through the concept of critical N and following the long-term ontogenic [N] changes in the dry matter of crops.

3.1.3. Critical nitrogen concentration.

The determination of critical nutrient concentrations in plants has for many years been a subject of interest in agronomy, based on the idea that the '*sufficiency of a nutrient is a function of its percentage content in the plant*' (Macy, 1936). Central to this thinking has been the concept of a level above which plants have '*luxury consumption*', and below which there is '*poverty adjustment*' until a '*minimum percentage*' is reached. Macy (1936) also suggested the probability '*… that the form which the nitrogen takes in the plant is largely a function of its sufficiency, which can be measured in terms of the critical percentages*'. Thus, a direct way to determine N reserves in plants has been to characterise the relationship linking plant biomass to [N] in the dry mass of plants grown over a wide range of N supply. The outcome of such studies has always been a hyperbolic (i.e. saturable) function from which the critical concentration can be determined as the minimum [N] allowing maximum biomass accumulation (Ulrich, 1952). Different experimental methods of determination have been proposed (Adamowicz and Le Bot, 2008; Burns, 1992; Justes *et al.*, 1994) but the general principle is the same as shown in Figure 11.
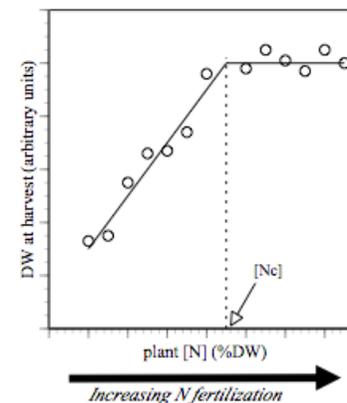

Figure 11. *Mathematical determination of critical N concentration [N_c] as the intercept between two linear regressions. When plant [N] < [N_c] dry weight (DW) increases almost linearly with increasing [N]. For [N] $\geq$ [N_c], DW does not respond to increasing [N].*

It is considered therefore that the excess of N above [N_c], the critical N concentration, gives an estimate of N-reserves. A large variability has been associated to [N_c] determination, therefore, it is important to characterise its ontogenic evolution.

3.2. Changes of nitrogen status during ontogeny.

3.2.1. Progressive nitrogen decrease.

It is well recognised that plant [N] (average N concentration of the entire plant) is not constant but varies enormously (see Figure 9), in particular with plant ageing. Highest [N] may be found in young plants during the exponential growth phase, but [N] decreases progressively thereafter with plant dry biomass accumulation. This is caused by a gradual change in the relative proportions of N-rich *versus* N-poor tissues during the plant's life cycle. Since N is largely involved in C acquisition processes, photosynthetically active leaves are highest in [N] compared to other plant organs but their contribution to total plant biomass (so-called LWR or leaf weight ratio) decreases with the biomass accumulation resulting from stems which are low in [N] (Greenwood, 1990). This phenomenon is particularly magnified for old trees, in which the trunk represents most of the aerial biomass. In closed canopies of annual crops, old and shaded leaves accumulate with time and recycle some of their N to young parts. Thus, their lower N contents also depress the overall plant [N]. A conceptual modelling of this progressive [N] decline during ontogeny was proposed by Hardwick (1987), based on the skin (*high-N*) and core (*low-N*) theory. At the risk of being simplistic, the basic principle of this model considers that plants use most of their N to capture resources in exchange surfaces (the skin, dimension 2, i.e. m^2) and fill the bulk volume (the core, dimension 3, i.e. m^3) with most of the biomass. As these two body parts grow at different rates (i.e. *allometric growth*), the overall surface to volume ratio evolves in time with a dimension 2/3. Consequently, the amount of plant N also evolves with the same dimension, leading to the approximate relation: N $\propto$ DW$^{2/3}$, which can be written in terms of concentration (i.e. N/DW = [N]) as: [N] $\propto$ DW$^{-1/3}$. In other words, [N] declines progressively with DW accumulation.

This progressive decline has been studied empirically on canopies using fertilisation trials designed to determine [N_c] (see Figure 1) during plant ontogeny (*i.e.* at different harvest dates). The outcome of such studies (see for example Justes *et al.*, 1994) is the finding that from canopy closure onwards, [N_c] also declines with DW accumulation, following a simple allometry having two parameters 'a and b' (Greenwood *et al.*, 1991):

$$[N_c] = a \times DW^{-b} \qquad (Eqn\ 1)$$

where parameter 'a' is the initial [N_c] of exponentially growing plants and parameter 'b', the slope of the progressive decline.

The Figure 12 shows that [N_c] clearly separates N-limited plants from those of adequate N-status in the whole data set. It also indicates that maximum and

minimum plant [N] decrease during ontogeny. The difference between maximum and critical [N] represents N-reserves capable of sustaining growth during periods of N deficiency. By contrast, the growth of plants below [N_c] depends solely on ambient soil N availability.
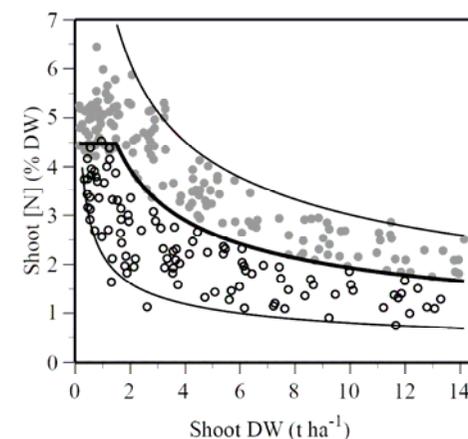
Figure 12. *Changes in shoot [N] of wheat with dry biomass accumulation. The data are taken from fertilisation trials at multiple locations, varieties, densities and years. The thick solid line ([N_c] = 5.35 DW$^{-0.44}$) has been determined specifically on independent data sets, and separates N-limited plants (open circles) from N-sufficient plants (closed circles). Thin lines are envelope curves for maximum and minimum plant [N] changes during ontogeny. Redrawn from Justes et al. (1994).*

3.2.2. Towards a universal concept?

So far, all studies of the kind described above have reached the same conclusion (see the book of Lemaire, 1997). A generalisation of the concept of progressive [N_c] decline with DW accumulation was proposed (Greenwood *et al.*, 1990) in a study which highlighted the difference between C$_3$ and C$_4$ crops, the latter having a lower [N_c] for the same DW (Figure 13).
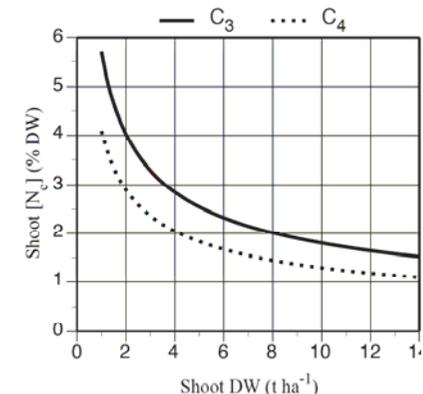
Figure 13. *General [N_c] changes with DW accumulation for C$_3$ (solid line, [N_c]=5.7 x DW$^{-0.5}$) and C$_4$ (dashed line, [N_c]=4.1 x DW$^{-0.5}$) crops, as proposed by Greenwood et al. (1990).*

Table 3: *Parameters of the critical N decline (Equations 2 and 4) for six crops. RUE data are from Gosse et al. (1986) and Muchow and Coates (1986). Parameters a and b are taken from several sources (Lemaire and Salette, 1984; Justes et al., 1994; Ney et al., 1997; Plénet and Cruz, 1997; Colnenne et al., 1998).*

	C_3 crops				C_4 crops		c.v. (%)
	wheat	rape	fescue	pea	maize	sorghum	
a	5.35	4.48	4.80	5.08	3.40	3.90	16.4
b	0.44	0.25	0.32	0.32	0.37	0.39	19.0
RUE	2.0	1.8	1.9	1.7	2.5	2.4	16.0
ε	0.060	0.061	0.061	0.059	0.054	0.057	4.7

Table 3 combines the values determined for parameters 'a and b' in Eqn. 1, from a literature survey on multiple plant species. Indeed, C_4 have lower 'a' values than C_3 species, which is consistent with their lower [N] shown in Figure 13. Parameter 'b', which assesses the slant of [N] progressive decline, is more variable but does not discriminate C_4 from C_3 crops. Thus it needs specific calibration. The data suggest that 'b' varies mostly in relation to light penetration in the foliage. Young plants grow exponentially as long as their leaves are not self-shading. They have also constant $[N_c]$ inferring b = 0 for all genotypes. However, upon canopy closure, 'b' values are reported to raise, from 0.25 in rapeseed (Colnenne *et al.*, 1998) to 0.53 in linseed (Flénet *et al.*, 2006). This variation is attributed to light extinction along the stand's profile that is altered by the canopy structure. Indeed, Lemaire and Gastal (1997) determined low 'b' (0.11) when sorghum was grown as isolated plant with respect to light interception, but high 'b' (0.34) when it was grown in dense stands. Thus, it is likely that the $[N_c]$ progressive decline is highly influenced by agricultural practices.

Because C_3 and C_4 plants do not share the same photosynthetic pathway, Greenwood *et al.* (1990) proposed to formulate N accumulation from absorbed radiation in a way similar to that used for DW accumulation during RUE determination (radiation use efficiency, g/MJ). Indeed, crop DW (g) is known to increase linearly with absorbed photosynthetically active radiation (R_a, MJ), the slope of the relation being RUE:

$$dDW = RUE \times dR_a \qquad (Eqn\ 2)$$

Following this idea, Adamowicz and Le Bot (2008) introduced an equivalent term that they called NRUE (Nitrogen Radiation Use Efficiency, g N/MJ) in order to write :

$$dN_c = NRUE \times dR_a \qquad (Eqn\ 3)$$

Considering the reported allometry between $[N_c]$ and DW (Eqn 1), they calculated that:

$$NRUE = RUE \times a \times \frac{(1-b)}{100} \times DW^{-b} \ or \ NRUE = \varepsilon \times DW^{-b} \qquad (Eqn\ 4)$$

Table 3 gives examples of this 'ε' parameter calculated from literature data. Compared to above parameters (a and b), 'ε' appears remarkably stable, and only slightly lower in C_4 crops. Indeed, it is tempting to approximate NRUE with a unique mean 'ε' value:

$$NRUE = 0.059 \times DW^{-b} \qquad (Eqn\ 5)$$

Figure 14 is a graphical output of Eqn. 5, which highlights the similarity of C_3 and C_4 plants to capture N_c in relation to absorbed radiation, and the difference between the same species in the capture of C (i.e. dry weight). It also shows that opposite in contrast to RUE, NRUE depends on crop biomass
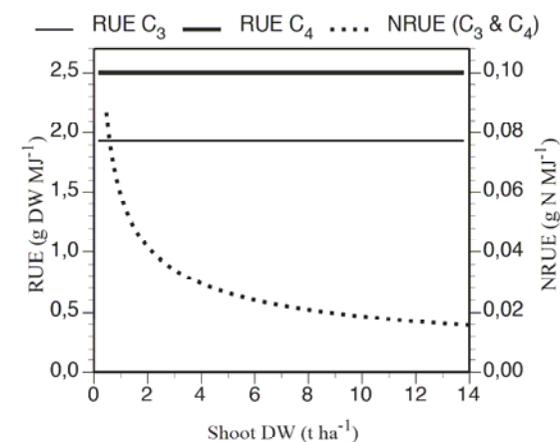
Figure 14. *Comparison of the mean radiation use efficiencies of C_3 and C_4 crops for dry weight (RUE, g DW/MJ, left axis) and for nitrogen (NRUE, g N/MJ, right axis) along the crops cycles.*

From this viewpoint, the critical N accumulated in the crops with light interception depends solely on ontogeny (parameter 'b') but not on a particular photosynthetic metabolism. In fact, the lower $[N_c]$ in C_4 crops would arise from their higher C acquisition rate resulting in greater dilution of endogenous N. The independence of NRUE from C metabolism has also been checked in experiments with tomato (C_3 plants), grown under ambient or CO_2 enriched atmosphere (Adamowicz and Le Bot, 2008). Although, air enrichment increased DW by *c.a.* 20% and decreased the plant [N], NRUE remained unchanged.

This observation is valuable because light absorption is yet one of the main inputs in crop models serving to predict biomass production (through RUE)

and, thus, it could also be used to predict crop N requirements through NRUE, based on the single parameter 'b' that would eventually need specific calibration.

3.2.3. Conceptual usage of [N$_c$] for N fertilisation.

Since [N$_c$] represents the ideal status for the growth of a given crop, it can be considered at any time of the season that fertilisation is only required when actual [N] < [N$_c$]. The ratio between these two concentrations may be expressed as a dimensionless index which has been called the NNI (nitrogen nutrition index, Lemaire and Gastal, 1997) :

$$NNI = \frac{[N]}{[N_c]}$$ (Eqn. 6)

NNI is an expression of plant N status, which can be used to distinguish between limiting (NNI < 1) or sufficient (NNI ≥ 1) conditions of soil N availability for growth. Nitrogen fertilisation should thus be restricted to the crop where NNI < 1. When crops are growing in the field, however, determination of NNI is not a possible option as [N] measurements rely on long-term laboratory procedures, including phases of drying and grinding plant material prior to total N determination. Alternative 'quick-tests' have been proposed and these methods based on the principles developed above are now being used commercially in the field. The methods used measure particular forms of reserve-N stored in plant tissues, especially NO_3-N, which is known to fluctuate widely in concentration. This is of advantage in so far that the changes in NO_3-N concentration in the leaves and stems of plants reflect excess N storage in the plant (see Section 2.3, Figure 8 and Section 3.1.1. above) In addition, however, NO_3-N accumulation can reflect environmental factors and this has to be taken into account, based on the principles developed above. The Jubil® method using sap NO_3-N analysis of stems is one such method developed in France to estimate plant NNI throughout the growing season to adjust N fertilisation of winter wheat (Justes *et al.*, 1997). The method is used as a guide for final N fertilisation decision making (40 to 80 kg N/ha) during stem elongation between Feekes stage 6 (first node) and Feekes stages 8 to 9 (booting).

A similar test has been developed on melon (Dumoulin and Le Bot, 2001) and other vegetable crops (method PILazo®, Raynal-Lacroix *et al.*, 2005), and it is used widely in the French melon fields to control N fertilisation. The authors established the referential linking petiole sap NO_3-N concentration to NNI and devised an operational grid serving as a decision making tool to help growers in their fertilisation strategy. In practice, split-fertilisation is applied during the crop growth, with part of the N-fertiliser applied at planting, based on soil N analysis, the rest being applied between planting and fruit setting if sap NO_3 concentration declines below a threshold value of 3,500 mg NO_3/L.

Table 4 gives an example of the saving in N fertiliser that may be expected from the method. In this particular example it was 120 kg N/ha, highlighting the particular situation of vegetable crops which are often excessively fertilised with N (Le Bot and Adamowicz, 2005). The benefit of the method both in preventing NO_3-N pollution and in improving crop quality by controlling NO_3-N accumulation has also to be considered.

Table 4: *Comparison of two fertilising strategies in a melon field managed by a professional grower. The control strategy was the usual N application according to a precise timetable. Instead, in the PILazo® procedure fertilisation was applied only when sap NO_3 fell below 3500 ppm.*

	Agronomic performance			Fertilisation
	Yield kg/m^2	Mean weight g per fruit	° Brix	kg/ha
Control	2.46	1060	13.4	192
PILazo®	2.37	1104	13.4	72

Apart from the direct methods described above indirect methods, not discussed in this paper, such as those based on optical leaf properties may also be used in the assessment of NNI.

4. PRACTICAL IMPLICATIONS FOR USE OF DIFFERENT NITROGEN FORMS.

As recognised in the previous sections more and more information is still being published on nitrogen physiology in plants particularly on the regulation of uptake and assimilation of N, mainly using modern molecular biological approaches. This is of scientific interest and increases the potential for future development

of GM crops. However, management of N fertiliser application is currently of much more practical importance in achieving higher yields, better food quality and less environmental pollution, than is new knowledge of gene regulation of N uptake and assimilation. Bridging the gap between the huge body of existing knowledge on the physiology of nitrogen and its application to agricultural practice is becoming more and more important. There is a great need therefore for all scientists involved with N physiology to be in regular contact and discussion with farmers so that they may understand agronomic problems in the field at first hand and may carry out their research accordingly. In this view we endorse the remark of the great French microbiologist Louis Pasteur that 'there is no applied science but only the application of scientific knowledge thinking and technique to practical problems'.

The following selected examples below demonstrate practical implications for the use of different N forms in relation to the role of N in physiology. Some of these examples have already been utilised in practice by progressive farmers. The application of others still needs to be assessed under field conditions for efficiency and economic value.

4.1. Rhizosphere management by NH_4-N / NO_3-N supply to improve crop nutrition.

It is well established that the form in which mineral N fertilisers are taken up by crop plants (NO_3 - or NH_4-N) modulate rhizosphere pH (Römheld, 1986) (see section 2.3.2). These pH changes regulate nutrient availability and uptake from the soil and can be exploited in agricultural practice. The influence of NH_4-N application in depressing rhizosphere pH can be of major importance in increasing the availability of sparingly soluble mineral nutrients such as phosphate and various micronutrients particularly on soils of neutral and high pH (Thomson *et al.,* 1993) (Table 5).

Table 5: *Effect of nitrogen form on nutrient uptake by Phaseolus vulgaris on a sandy loam (pH 6.8, phosphorus supply rock phosphate) (Thomson et al., 1993).*

Nitrogen form	pH		Uptake (μ/m root length)					
	bulk	rhizosphere	P	K	Fe	Mn	Zn	Cu
Ca(NO_3)$_2$	6.6	6.6	123	903	55	8	7	1.4
(NH_4)$_2SO_4$ – N-serve®	5.7	5.6	342	1127	71	20	13	2.0
(NH_4)$_2SO_4$ + N-serve®	6.6	4.5	586	1080	166	35	19	4.6

® N-serve is a registered trade mark of Dow Agro Sciences LLC.

4.2. Rhizosphere pH management to improve disease resistance.

Increasing disease resistance of crops can also be achieved by enhancing the availability of silicon (Si) and micronutrients by lowering rhizosphere pH by $NH_4 – N$ nutrition. For example this treatment can increase the resistance of wheat to mildew as indicated in Figure 15 (Römheld, 1990). Presumably the increase in Si resulting from the higher NH_4-N supply increases resistance of
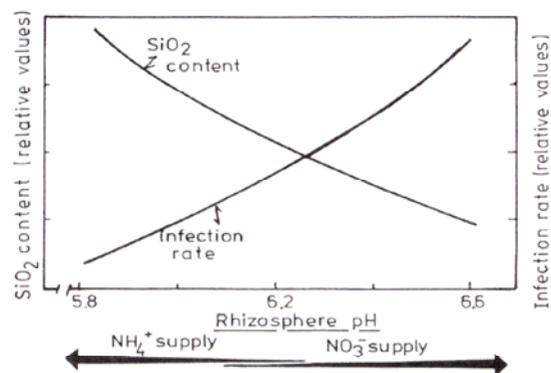

Figure 15: *Schematic representation showing the effect of NH_4-N in reducing rhizosphere pH thus increasing silicon (Si) uptake with its possible effect of lowering disease infection rate in plants as exemplified by increased resistance of wheat to mildew (Römheld 1990).*

epidermal cell walls to fungal attack. It is common practice in the USA to use ammonium N stabilised by the nitrification inhibitor N serve to depress take all (*Gaemannomyces graminis*) in wheat (see Huber and Thompson, 2007). The mechanism behind this ammonium effect is twofold: a) a direct effect of the lower pH at the root surface inhibiting the growth of the pathogen hyphae and b) an indirect effect of pH lowering in the rhizosphere in promoting Mn mobilisation and plant uptake. The influence of NH_4-N in depressing rhizosphere pH and increasing micronutrient uptake by dwarf French bean (*Phaseolus vulgaris* L.) is shown in Figure 16 (Sarkar and Wyn Jones, 1982). It is now becoming increasingly obvious that micronutrients play a major role in protecting plants against both adverse biotic and abiotic factors.
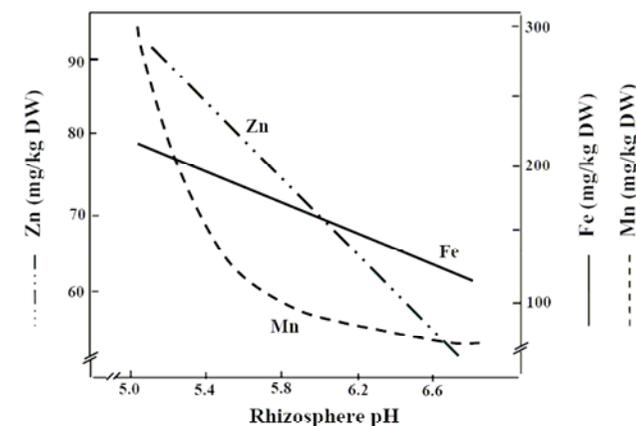

Figure 16: *The influence of NH_4-nutrition in depressing rhizosphere pH of dwarf French bean (Phaseolus vulgaris L.) with related increase in uptake of the micronutrients Zn, Mn and Fe (Sarkar and Wyn Jones, 1982).*

4.3. Use of natural nitrification inhibitors in agroecosystems to improve disease control.

Subbarao *et al.,* (2009) have shown that the common grass *Brachiaria humidicola* releases biological nitrification inhibitors from its roots as measured by biological nitrification inhibition (BNI). These plants therefore take up NH_4 –N from the soil which lowers rhizosphere pH thereby also enhancing manganese (Mn) mobilisation and uptake. We suggest that this may be of direct relevance to the incidence of the disease citrus variegated chlorosis (CVC) also known as citrus dieback syndrome which for the past twenty years has been devastating the citrus industry in Brazil. The causal agent of CVC is vectored by sharpshooter leafhoppers which feed on xylem thereby transferring a xylem inhibiting bacterium. It has been observed that orchards which are managed conventionally with intensive herbicide application and clean soil under the trees, are much more prone to CVC than biologically managed systems in which *Brachiaria* is used as a cover crop under the citrus trees. From this evidence it has been hypothesised that CVC

may be controlled in a similar way to that of take all in wheat (i.e. by a beneficial effect of micronutrients and particularly Mn) (Figure 17). Although this hypothesis has yet to be proved, it is strongly supported by field observations. Trees from biologically managed citrus orchards with *Brachiaria* grass as a cover crop have leaves which are three fold higher in both Mn and zinc (Zn) and such trees show much lower incidence of CVC than those from conventionally managed orchards (Römheld, unpublished).
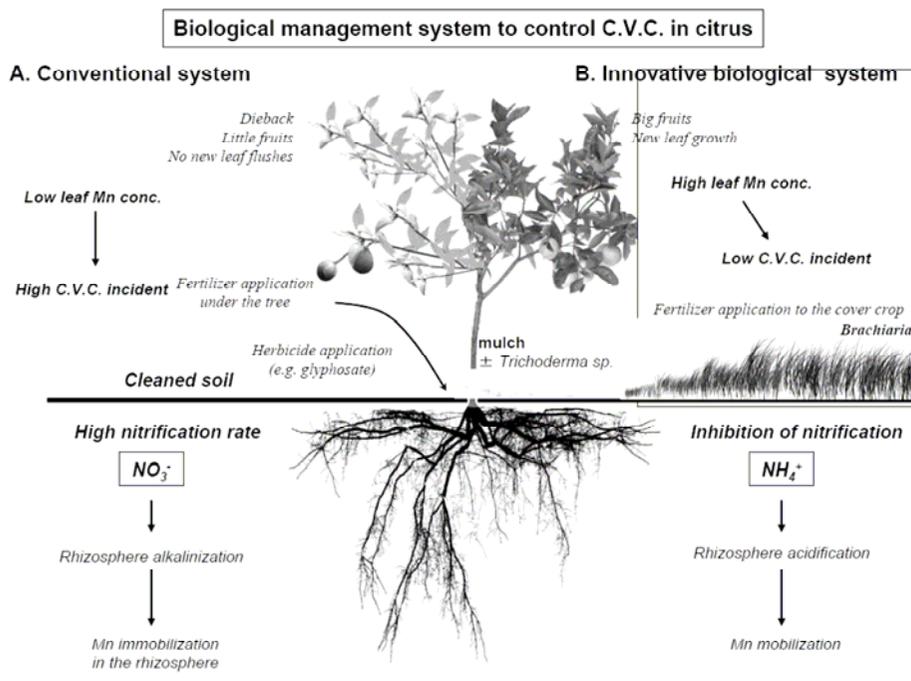

Figure 17: *Biological management system in the control of citrus variegated chlorosis (CVC) (Römheld, unpublished).*

It is of interest that Subbarao *et al.*, (2007) have also detected natural nitrification inhibitors in the roots of other crop plants including sorghum (*Sorghum bicolor* L.) and ground nut (*Arachis hypogaea* L.) and have suggested that BNI capacity might be managed or might be introduced into pastures and crops by means of expression of this phenomenon via genetic improvement. The exciting possibility of introducing BNI genes from the perennial wild relative of wheat *Leymus racemosus* (*Triticeae*) to combat nitrification in wheat farming has been discussed by Subbarao *et al.*, (2007a).

4.4. Placement of NH$_4$ –N to improve exploitation of rhizosphere acidification by induction of 'cluster roots'.

In as yet unpublished research work of Jianbo Shen from the Department of Plant Nutrition at the Chinese Agricultural University (CAU) at Beijing it has been shown in a field study on the calcareous soils of North China Plain (NCP), that placed application of NH$_4$- N can induce intense cluster root formation in maize at the site of placement. This root proliferation is comparable to what has been observed in the Proteaceae (Lambers *et al.*, 2006) and white lupin (Dinkelaker *et al.*, 1989) and is associated with a pH acidification from 7.8 to 5.0 in the placement zone giving rise to enhanced P nutritional status of the maize plants.

In the near future it is envisaged to exploit these artificial cluster roots in non proteaceous species in improving the acquisition of P and micronutrients by intercropping maize cultivated in this way with various other crop species. Such placement of NH$_4$ -N together with supplements of other possible limiting nutrients (e.g. Zn, Mn, Ni, boron (B)) has also the potential to improve resistance to stress factors such as drought, diseases, high temperatures and frost.

4.5. Improved frost resistance by use of granulated ammonium sulphate.

Improving frost resistance in early spring and late autumn is vital in many agricultural countries such as in northern China, northern India, and in eastern Europe. It is particularly relevant in Alberta, West Canada which has a vegetation period of less than 100 days. Here frost resistance has become of greater relevance as a consequence of change of cultivation practice from ploughing to no till or conservation till (R. Davage 2009, pers. comm.). Young plants growing under no till conditions are more at risk of frost attack because the soil is covered by a vegetative mat and the plants are therefore unable to benefit from the sun's radiation in raising the temperature of the darker coloured soil. Further there are field observations that foliar sprays containing mineral nutrients (K, Zn, B, Ca, P) as pre or post frost applications as well as seed dressings can prevent or alleviate frost damage in farmers' fields (R. Davage 2009, pers. comm.).

In relation to the use of different N forms, there are field observations in East Germany and the Ukraine that application of granulated as compared to non granulated ammonium sulphate or ammonium nitrate can alleviate frost damage of winter rape in a similar way to that obtained by foliar application of copper sulphate (Cu SO$_4$) solution at 200 g/ha (B. Bauer 2009, pers. comm.). These findings may again be interpreted as a consequence of rhizosphere acidification caused by the granulated ammonium sulphate treatment increasing the mobilisation of Cu particularly on sites of limited Cu availability. The role of Cu in alleviating frost resistance might be explained by the influence of the enzyme Zn Cu super oxide dismutase (SOD) which enhances the detoxification of oxygen radicals formed in plants under frost conditions.

4.6. Influence of nitrogen forms on paddy and upland rice.

It is generally believed that rice (*Oryza sativa* L.) has a special need for ammonium –N (Mengel and Kirkby, 2001). However, there are observations by (Lin, pers. comm. 2009), that paddy rice and upland rice differ in preference for N form for optimal growth (Figure 18). This difference between

both rice groups appears to correspond to the different predominant N forms of NH_4- and NO_3-N likely to be present in paddy and upland field soils. However, this finding of Lin needs further investigation using different rice cultivars and physiological explanations to account for the observed differences between both rice groups. In practical terms this is an interesting area of research because of the move away from paddy rice to upland (or aerobic rice) is likely to continue as a consequence of increasing water scarcity.
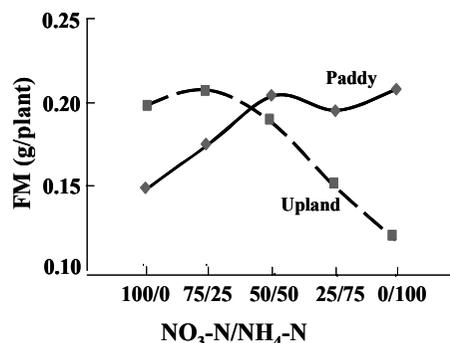

Figure 18: *Influence of the NO_3-N / NH_4-N ratio supplied in solution culture at a total concentration of 1 mM nitrogen on the growth of upland and paddy rice (Lin, pers. comm. 2009).*

4.7. Influence of nitrogen form on water use efficiency.

In a number of experiments comparing NH_4- and NO_3-N - at concentrations below those causing NH_4 toxicity - it has been noted that NH_4-fed plants are often higher in dry matter content than NO_3 – fed plants suggesting a difference between the two N forms in water utilisation (Kirkby unpublished data).This difference in behaviour was confirmed in a water culture experiment of Souri *et al.,* 2009 in which tomato plants pretreated with 2 mM NO_3-N for three weeks were transferred into either NO_3 or NH_4 nutrient solutions, the NH_4 solutions being buffered using $CaCO_3$ to a pH of about 6.5 (Table 6).

Table 6: *Effect of N form (NH_4 - versus NO_3-N) in water use efficiency (WUE), by tomato plants growing in a nutrient solution buffered at pH 6.5. All plants were precultured with 2 mM NO_3-N for three weeks before the eight day period for water use measurement (Souri et al., 2009).*

Nitrogen supply		Water consumption
Form	mM	ml / plant / 24h
Nitrate-N (NO_3 –N)	2	66.3
	4	67.5
Ammonium-N (NH_4 –N)	2	37.5
	4	42.5

Water use at the lower level of N supply by the NH_4-plants was only just over a half that of that of the comparative NO_3-fed plants, this value increasing only to about two thirds at the higher N level. The authors observed similar effects in soil grown tomato plants especially in the presence of a nitrification inhibitor in the NH_4-treatment.

In contrast to these general observations of a superior effect of NH_4- N in water use efficiency, Singh and Kothari (pers comm. 2005) observed that both growth and transpiration coefficient in Japanese mint (*Menthe arvensis* L.) was highly dependent on soil pH (Table 7) Higher water use efficiency with NH_4- N supply was only observed on the alkaline soil. Presumably this arose as a consequence of NH_4 – N supply acidifying the rhizosphere thereby enhancing both the mobilisation of P and limiting micronutrients such as Zn, Mn and B to improve growth. On the acid soil by contrast, growth and water use efficiency was slightly higher with NO_3 – N supply possibly because of the alkalising effect in the rhizosphere alleviating Al toxicity.

Table 7: *Form of N – nutrition on the dry weight and transpiration coefficient of Japanese mint (Menthe arvensis L.) growing on an acid and an alkaline soil (Singh and Kothari, pers. Comm. 2005).*

Treatment	DW g shoot/plant	Transpiration coefficient
Alkaline soil		
NO_3 –N	0.88	1087
NH_4 -N	2.59	608
Acid soil		
NO_3 –N	1.13	750
NH_4 -N	0.98	780

In reviewing the literature on the effects of N form on WUE in plants, Lu *et al.,* 2005 conclude that effects appear to be much dependent on plant species, experimental conditions and in particular on nutritional regimes. In view of the increasing importance of the need for efficient water usage by crops especially in relation to the usage of N, the nutrient which is often applied in highest amounts to crops, there is an urgent need for further research in this area.

4.8. Use of legumes in rotations to improve phosphorus and micronutrient use efficiency.

It is well known that legumes fixing molecular nitrogen (biological nitrogen fixation, BNF) have a lower rhizosphere pH than legumes supplied with nitrate N (Römheld, 1986) (see section 2.3.2.). Similarly to plants supplied with NH_4-N, they are better able to access P and micronutrients from sparingly soluble sources in neutral and high pH soils (Table 8). This means of

improving P acquisition by legumes with BNF can be exploited in their production by placing some water soluble P (e.g. superphosphate) as a starter together with broadcast and much less available rock phosphate, the availability of which progressively increases as the effect of rhizosphere acidification comes into play as shown in Figure 19 from the findings of De Swart and van Diest (1987).

Table 8: *Influence of nitrogen fertilisation (NO$_3^-$ / symbiotic N$_2$ fixation) on rhizosphere pH and phosphorus uptake by soybeans (Aguilar and van Diest, 1981).*

Form of N supply	Yield g/pot	Rhizosphere pH	P uptake mg/pot
Nitrate-N (NO$_3$ –N)	7.1	8.0	5
N$_2$ fixation (*Rhizobia*)	11.5	5.3	38

The importance of legume crops in their ability to supply nitrogen to agriculture, and in this case also P, still seems to be very much underrated. The significance of biological nitrogen fixation was certainly appreciated by Fritz Haber who in his Nobel Prize acceptance speech of 1920 for his work on ammonia synthesis concluded 'It may be that this solution is not a final one. Nitrogen fixation bacteria teach us that nature with her sophisticated forms of chemistry still understands and utilises methods which we do not know how to imitate'.
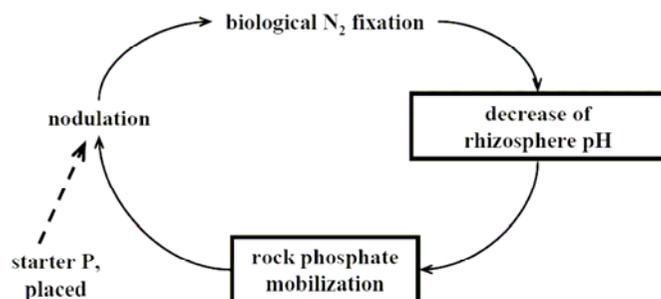

Figure 19: *Schematic representation of the mobilisation of rock phosphate by legumes on a low P soil (pH 6, Koubou bean, Pueraria japonica) De Swart and van Diest 1987).*

4.9. Inhibition of germination by ammonia toxicity after placed urea application.

The use of urea as a mineral nitrogen fertiliser is becoming of increasing importance as a consequence of its lower price on the market Besides the problem of inefficient use of urea on calcareous soils as a consequence of loss of released ammonia, urea toxicity on seed germination and particularly for placement of urea has also to be considered (Bremner and Krogmeier, 1988, 1989). These authors found inhibition of wheat germination only at high levels of urea supply but not with ammonium sulphate, suggestive of NH$_3$ rather than ammonium toxicity. In a subsequent experiment in which a small amount of the urease inhibitor phenylphosphodiamidate was added to the urea fertiliser the absence of any detrimental effect on germination confirmed that the cause of the toxicity was ammonia rather than urea. This finding is of particular relevance in relation to the possible beneficial use of the inhibitor in seed dressing and placement of urea.

4.10. Urea toxicity following foliar application of urea to plants of low nickel status.

Urea is often made as a late foliar application to improve grain yields and quality. In soybean and other ureide - type legumes, regardless of form of N nutrition, large amounts of urea can accumulate in leaves to give the necrotic symptoms of leaf tip burn particularly under Ni deficient conditions as observed by Eskew *et al.,* (1983) In a later study Krogmeier *et al.,* 1991) investigated the effect of the addition of a urease inhibitor together with foliar applied urea to plants supplied with increasing amounts of Ni, an essential component of urease. These workers were able to demonstrate the causal effect between Ni deficiency and leaf tip necrosis following foliar application of urea as a consequence of accumulation of toxic amounts of urea because of the lower activities of urease, essential for urea assimilation. A similar beneficial effect of Ni was reported for foliar applied urea on tomato seedlings (Nicoulaud and Bloom, 1998). Urea is a metabolite in all plants and must be maintained at a low level in order to prevent toxicity (Marschner, 1995). In cowpeas (*Vigna unguiculata* L.Walp.), Walker *et al.,* (1985) reported that even plants not receiving urea (i.e. NO$_3$- or NH$_4$-N fed plants), require Ni during the period after flowering during protein catabolism and the retranslocation of N from older leaves to seeds. The requirement for Ni by other crops is therefore certainly a possibility in the need to prevent the accumulation of urea. This particularly may be the case when late foliar applications of urea are given for the benefit of grain filling in cereals, a period which also coincides with remobilisation and translocation of large amounts of nitrogen compounds from senescing flag leaves.

4.11. Regulation of tillering of wheat to improve grain yields.

In fields in East Germany and in the Ukraine farmers have made the observation that that placed urea depresses tillering of wheat and barley seedlings (Figure 20, overleaf) (Bauer, 2004). This reduction in tillering allows the formation of larger ears with larger grains, farmers reporting higher grain yields of about 15-20% when slightly higher seed rates were also used. The potential benefit of urea placement still needs to be confirmed in practice with the addition of a urease inhibitor. Some indication of the physiological background of the effect has been established from a water culture experiment with varying supply of nitrate and urea supplied together with a urease

inhibitor. The results showed that increasing the proportion of urea inhibited cytokinin concentration in the xylem sap, the lower concentration of xylem transport of cytokinin correlating well with the decrease in tillering (Bauer *et al.,* 2009). Further field and laboratory studies are underway to verify these observations and their relevance to farming practice.
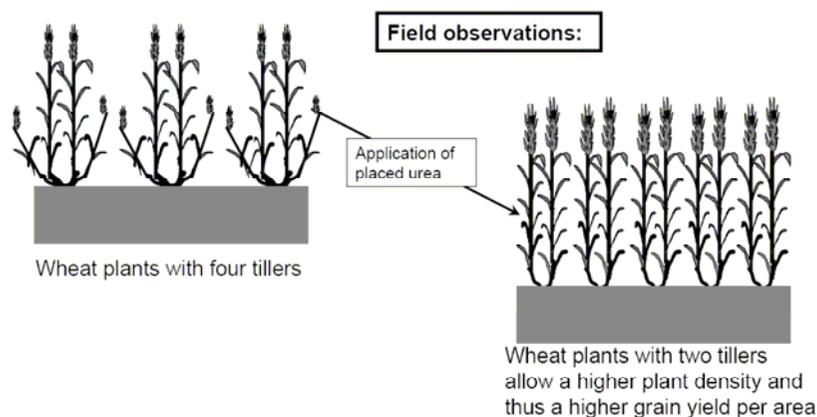

Figure 20: *Schematic representation of the effect of placed application of urea in increasing tillering in wheat (Bauer et al., 2009).*

4.12. Reduction of axillary shoots in tomato production using NH_4-N as a sole nitrogen source.

Nitrate at very low concentrations of supply (5-10 µM) has a signalling function as discussed in Section 2.3.4 and results in a higher division and expansion of leaf cells. This modulation of shoot growth by nitrate observed in various plant species is regulated by long distance cytokinin signalling (Rahayu, *et al.,* 2005). In tomato supplied solely with NH_4 –N and without the nitrate signalling effect, axillary shoots were inhibited without affecting fruit yield (Rahayu, 2003).

This finding is of extreme interest in horticultural practice because the repeated removal of axillary (lateral) shoots is extremely labour intensive. However , continuous supply of N in the form of NH_4 as a sole N source under modern nutrient film technique of tomato production in greenhouses, fruit quality is lower due to the promotion of blossom end rot (BER) symptoms. This disorder is caused by inhibited Ca uptake and translocation into fruits of plants supplied with NH_4-N and is well known in different crop species (Kirkby and Pilbeam, 1984). It would thus seem appropriate that for tomato production grown hydroponically that NH_4-N should be provided during the vegetative period but replaced by NO_3-N during reproductive growth to guarantee good fruiting quality and control of BER symptoms. This approach needs to be evaluated further in horticultural systems before general recommendations can be made in practice.

4.13. Promotion of flower induction by NH_4-N.

It has been known for many years that NH_4 nutrition can promote flower initiation in various plant species including apple trees (Marschner, 1995). Detailed studies with apple seedlings have shown that this promoted flower initiation is regulated by an interaction of cytokinins, arginine and polyamines (Gao *et al.,* 1992; Rohozinski *et al.,* 1986) (Table 9). This ammonium effect on flowering has been confirmed in practice in apple orchards of North Germany (Lüdders and Bünemann, pers. comm.) It would be of interest to test whether NH_4 supply to apple trees may be used to counteract the well known observed alternative bearing syndrome.

Table 9: *Shoot growth and flower induction of apple as affected by the form of nitrogen supply (based on Gao et al., 1992).*

Form of N supply	Shoot length	Flowering buds
	cm	% emerged
Nitrate-N (NO_3 –N)	328	7.4
Ammonium nitrate (NH_4NO_3)	268	8.2
Ammonium-N (NH_4 -N)	209	20.7

5. CONCLUSIONS.

Despite the huge and increasing body of information concerning the physiology of nitrogen in plants there is a wide gap between this accumulated knowledge and its application to improving yield production and quality of crops. Extensive studies made in molecular biology have so far been disappointing in this respect. On a global scale mankind is still dependent on the Haber Bosch process for the production of nitrogen fertilisers. Their use has increased twenty fold over the past fifty years to keep pace with the food demands of the rising world population. This trend also looks set to continue in the future as the world population continues to expand. Nowadays nitrogen is no longer a limiting plant nutrient in most agricultural countries. Attention is currently focused on how to use these fertilisers most efficiently to increase crop yields and quality and how best to avoid any detrimental environmental problems associated with their application. Improving nitrogen management of crops is an important way forward to achieve these aims as indicated by the selected examples cited above which demonstrate that the use of different forms of nitrogen can be highly effective. In order to bridge the gap between physiological knowledge and the practical problems faced by farmers, closer links need to be established between agricultural scientists, extension workers and the farming community.

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