Amino acids and nitrate as signals for the regulation of nitrogen acquisition

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Abstract
The uptake of nitrogen (N) by roots is known to change with supply in a manner that suggests that the N status of plants is somehow sensed and can feedback to regulate this process. The most abundant source of N in soils for crops is nitrate. Uptake systems for nitrate, ammonium, and amino acids are present in the roots of most plants including crops. As nitrate is assimilated via conversion to nitrite, then ammonium into amino acids, it has been suggested that the internal pools of amino acids within plants may indicate nitrogen status by providing a signal that can regulate nitrate uptake by the plant. In support of this idea, both nitrate and ammonium influx and transporter transcript were shown to decrease in root tissue treated with exogenously applied amino acids. Several different amino acids have been tested for their effects on influx and transcription and glutamine was most effective. The feedback regulation occurs by changing the expression of transporters, but may also involve the post-translational modification of proteins. For example, some of the cytoplasmic enzymes responsible for nitrate assimilation are regulated by phosphorylation and binding of a 14-3-3 protein. The effects of treating plants with glutamine have been examined, first to identify the uptake of the amino acid and then to measure tissue nitrate reductase activity and cellular pools of nitrate. These results are reviewed in terms of feedback regulation and the putative cell sensing systems for N status including a possible specific role for cytosolic nitrate.

Key words: Amino acids, ammonium uptake, feedback regulation, nitrate uptake, nitrogen sensor.

Introduction
It is difficult to find any environment in which nitrogen (N) supply is not a limiting factor for plant growth; this is demonstrated by the fact that the addition of this fertilizer will stimulate the growth of any plant. Soil N is available to plants chiefly as either nitrate (NO\textsubscript{3}\textsuperscript{-}) or ammonium (NH\textsubscript{4}\textsuperscript{+}) and to a lesser extent, amino acids. Nitrogen is a fundamental regulator of plant growth and its supply can so strongly influence plant growth that it once led to the suggestion that NO\textsubscript{3}\textsuperscript{-} was a plant hormone (Trewavas, 1983). The way in which the N status of a plant is sensed is of fundamental importance to agriculture as understanding this mechanism is key to attempts to improve N use efficiency (NUE) by crops. The imbalance between demand and supply for N in crops can result in either sub-optimal yield or the addition of environmentally damaging excesses of fertilizer. The uptake and assimilation of N by roots is known to change with supply in a manner that suggests that the N status of plants is somehow sensed and can feedback to regulate these processes. As NO\textsubscript{3}\textsuperscript{-} is assimilated via conversion to nitrite, NH\textsubscript{4}\textsuperscript{+}, and then into amino acids, it was suggested that the internal pools of downstream N metabolites such as amino acids within plants may indicate N status by providing a signal that can regulate N uptake and assimilation by the plant (Lee and Rudge, 1986; Cooper and Clarkson, 1989).

When whole plant tissues or organs are chemically analysed these measurements show that the N status of a plant is reflected by changes in tissue pools of nitrogenous compounds, including amino acids, NO\textsubscript{3}\textsuperscript{-} and proteins. Many different nitrogen-containing ions and molecules may be signals for N status and could be species specific, but a few favourites have emerged. For example, tissue NO\textsubscript{3}\textsuperscript{-} testing is widely used by farmers as
an indicator for crop fertilizer requirements (Westcott et al., 1993) and NO$_3^-$ has been proposed as a biochemical signal to regulate growth, including lateral roots and shoot:root ratios (Stitt, 1999; Forde, 2002; Schieble et al., 2004). Although, it was recently argued that total shoot protein may actually be a better indicator for shoot:root ratios (Andrews et al., 2006). Laboratory experiments to test feedback regulation have treated plants directly with amino acids and the concentrations applied exogenously to roots are much higher (mM range) than those occurring in the soil (Jones et al., 2002). These high concentrations are used with the specific aim of altering the cellular pools of amino acids but it should be checked that changes in tissue concentrations can actually be measured. Another experimental approach taken has been to use chemical inhibitors to try to block specific steps in the N assimilation pathway. For example, the conversion of NH$_4^+$ to glutamine (Gln) by glutamine synthetase (GS) can be inhibited by methionine sulfoximine (Rawat et al., 1999). In an untreated plant the source for amino acid feedback is likely to reside in the phloem (Gessler et al., 1998).

There are some general physiological effects of increasing NO$_3^-$ supply, for example, it can provide an osmoticum, filling vacuoles and driving growth (McIntyre, 1997). Furthermore, changes in supply to the roots may also influence the long-distance flow in the xylem and this effect could result in stimulated transport of plant hormones between the root and shoot. The negative feedback model has become generally accepted by plant biologists, yet the precise nature or mechanism of the signal is not known. Even the exact location of the feedback pool within the plant is not certain, it may be the phloem sap (Gessler et al., 1998; Pal’ove-Balang and Mistrik, 2002), the apoplast or within a subcellular compartment. The link between the changes in plant N status and the molecular sensing mechanisms are difficult to integrate because studying these processes at the cellular level is problematic. These feedback effects on the uptake of each of the three main soil available N forms will be described first and then some possible molecular N sensors, followed by a consideration of the central role of amino acids and NO$_3^-$ in this signalling process.

**Nitrate uptake by roots**

Plant root uptake of NO$_3^-$ can be negatively influenced by the external supply of amino acids or tissue concentrations of amino acids (Lee et al., 1992; Muller et al., 1995). It is a general feature of many different types of plants, including trees (Dluzniewska et al., 2006) and cell cultures (Padgett and Leonard, 1993), that supplying amino acids to roots inhibits NO$_3^-$ uptake. The expression of inducible high affinity NO$_3^-$ transporters can be effectively inhibited by Gln (Quesada et al., 1997; Krapp et al., 1998; Vidmar et al., 2000). Both NO$_3^-$-induced influx and transporter transcript abundance were decreased simultaneously in root tissue treated with exogenously applied amino acids (Vidmar et al., 2000). As amino acids can be inter-converted within plant tissues, chemical inhibitors of the conversion steps were used to identify Gln, rather than glutamate, as being responsible for down-regulating NO$_3^-$ transporter expression (Vidmar et al., 2000). In *Brassica napus*, a negative correlation was found between either shoot N or NO$_3^-$ contents and NO$_3^-$ uptake rates, but pools of free amino acids in roots did not seem to be involved in the control of root NO$_3^-$ uptake (Lainé et al., 1995). More recently for this species, a positive correlation between γ-aminobutyric acid in the phloem and NO$_3^-$ uptake was reported (Beuve et al., 2004). This result is unusual because a negative feedback system is more common for most plants, although even in *Brassica napus* treating the roots with amino acids (Gln, glutamate, and asparagine) decreased NO$_3^-$ uptake and transporter expression (Beuve et al., 2004).

Root NO$_3^-$ uptake systems can be divided into transport systems that operate over different physiological ranges (Crawford and Glass, 1998) and feedback regulation by Gln on these uptake systems appears to be different. For example, *Lolium perenne* plants grown in sterile culture and treated for 24 h with Gln showed decreased activity of the high, but not low affinity NO$_3^-$ uptake system (Thornton, 2004). There are several different routes whereby NO$_3^-$ uptake can be decreased and these include both changes in gene expression and post-translational mechanisms (see review by Miller et al., 2007).

Nitrate uptake can also be influenced by NH$_4^+$ supply and, when a mixed N source is supplied to plants, NO$_3^-$ uptake is usually decreased (Kronzucker et al., 1999). Like amino acids, NH$_4^+$ requires less energy input by the plant because the N is already in a reduced form by-passing this step in assimilation. However, in contrast to amino acids there can be some problems for cellular pH regulation associated with NH$_4^+$ as an N source for the plant (Raven and Smith, 1976) and, at high external concentrations, the energy expended in effluxing this cell toxic ion (Britto et al., 2001).

**Ammonium uptake by roots**

The uptake of NH$_4^+$ by plant roots can be decreased by the external supply of amino acids and NO$_3^-$ (Lee et al., 1992; Rawat et al., 1999). In most agricultural soils plant roots are usually exposed to more NO$_3^-$ than NH$_4^+$ (Miller et al., 2007). Ammonium uptake is mainly mediated by the AMT family of transporters and the regulation of this transport can occur by several different mechanisms. As this topic has been carefully reviewed in detail recently
(Loqué and von Wirén, 2004), only a brief overview of some points that are relevant to transport and N status will be given here. Feeding roots with Gln and the use of a GS inhibitor has led to a model proposing that Gln in the cell altered transcription while cytosolic concentrations of \( \text{NH}_4^+ \) may post-translationally regulate one AMT gene (Rawat et al., 1999). More generally, regulation of uptake can occur at the mRNA level and AMT transcripts are strongly dependent on the N status of the plant, but in \textit{Arabidopsis} the pattern is different for family members. Some AMTs increase expression earlier during N deficiency while others can increase after more prolonged starvation (Loqué and von Wirén, 2004). Split-root experiments have suggested that the local root, rather than whole plant, N status regulates the expression of an \( \text{NH}_4^+ \) transporter (Gansel et al., 2001). This result was different for a \( \text{NO}_3^- \) transporter where the N status of the whole plant was important (Gansel et al., 2001).

Tobacco plants with 35S-driven expression of an \textit{Arabidopsis} AMT showed a 30% increase in root uptake of \( \text{NH}_4^+ \) in hydroponics relative to wild-type plants (Yuan et al., 2007). However on soil supplemented with \( \text{NH}_4^+ \) as an N source; these plants showed neither growth nor N acquisition differences from wild-type plants (Yuan et al., 2007). Despite expression being driven by the 35S promoter in these tobacco plants the steady-state transcripts for \textit{AtAMT1;1} were not constitutive, changing with N status of the plants and decreasing after \( \text{NO}_3^- \) or \( \text{NH}_4^+ \) addition to N-deficient roots. This result suggests that the N status of a plant may directly influence mRNA turnover in plants and this may provide another regulatory mechanism for \( \text{NH}_4^+ \) uptake. This result seems to contrast with that for \( \text{NO}_3^- \) transporter transcripts. In \textit{Nicotiana plumbaginifolia} the expression of \( \text{NpNRT2.1} \) driven by \textit{rolD} or the 35S promoters was still high, even after treatment with 10 mM \( \text{NO}_3^- \), when the WT endogenous gene was repressed (Fraiser et al., 2000). Post-translational regulation of \textit{AtAMT1;1} has been shown to occur via the cytosolic C terminus of the protein that results in an alteration of the interaction between the monomer components necessary for transport (Loqué et al., 2007). This mechanism provides a rapid way of regulating \( \text{NH}_4^+ \) transport activity that can be linked to N supply by the phosphorylation of the C terminus.

Amino acid uptake by roots

One underlying assumption for the experiments describing the effects of externally supplied amino acids on uptake of \( \text{NO}_3^- \) and \( \text{NH}_4^+ \) is that these reduced N forms are readily taken up by roots and can directly influence the internal pools of these molecules. Uptake of radiolabelled amino acids by roots has been shown to occur (Watson and Fowden, 1975; Soldal and Nissen, 1978) and this treatment has been shown to increase some tissue pools of amino acids (Lee et al., 1992; Vidmar et al., 2000; Thornton, 2004; Fan et al., 2006). In some environments, such as arctic grassland and salt marsh, soil available amino acids are important sources of N (Henry and Jefferies, 2003; Xu et al., 2006), but in most agricultural situations the importance of this form of N has yet to be demonstrated (Jones et al., 2005). Wild plant roots can acquire soil N through fungi as mycorrhiza (Hodge, 2003) and amino acid transport can be enhanced by this relationship (Sokolovski et al., 2002), but little is known about the regulation of this uptake making it worthy of further investigation.

As the activity of plasma membrane transporters in individual cells can be detected by electrophysiological measurements (Miller et al., 2001) this method has been used to assay for amino acid transport activity in coleoptiles (Krinaide et al., 1984) and roots (Fan et al., 2006). For coleoptiles the shape and duration of the change in membrane potential depended on the net charge of the amino acid (Krinaide et al., 1984). Treating barley roots with Gln gave a change in membrane potential that depended on the concentration applied and could be repeated over several cycles (Fan et al., 2006). However, repeated applications of the amino acid eventually elicited a smaller response perhaps suggesting that the transport system was sensitive to the N status of the cell. The measured affinity of this transport system for Gln was not very high (\( K_m = 1.2 \) mM) when compared with the likely availability of this amino acid in the soil (Jones et al., 2002). To test for root amino acid transporters with a possible role in acquisition from the soil, barley seedlings, grown in the same way as described previously in a full nutrient solution containing 10 mM nitrate (Fan et al., 2006), have been treated with 50 \( \mu \)M lysine. Lysine was tested because uptake of this amino acid by barley roots has been reported (Soldal and Nissen, 1978) and in the root epidermis of \textit{Arabidopsis} the gene encoding the transporter has been identified (Hirner et al., 2006). Epidermal root cells treated with 50 \( \mu \)M lysine usually elicited a significant electrical depolarization (Fig. 1A, B, C), and the shape of the response was similar when reported from either the cytoplasm (Fig. 1A) or vacuole (Fig. 1B). The lysine treatment caused the membrane potential to become less negative (depolarization) and this effect was reversible when the amino acid was removed from the bathing solution (Fig. 1A, B). As parallel membrane potential changes were recorded from both the cytoplasm and the vacuole, this result suggests that the plasma membrane is the source of the electrical event (Miller et al., 2001). Similar electrical responses to lysine could also be obtained from barley cortical root cells (data not shown). This result might suggest that the source of the electrical response was a proton:lysine cotransporter activity at the plasma membrane (Fig. 1A). There is some
evidence for a small acidification after the cell was treated with lysine (Fig. 1A), but this was not observed in all recordings and we cannot explain why this happens when the amino acid supply was removed. However, the time after the first duration of lysine exposure did not appear to significantly alter the magnitude of the transport activity (Fig. 1C). This result shows that the roots of barley seedlings have high affinity amino acid transporters that are able to access soil available N pools and the activity of this uptake system may not depend on the lysine content of the cell or perhaps the plant N status. However, more experiments are needed including molecular studies to check how the activity and expression of this transporter may change with N supply. This lysine transport activity may be analogous to that reported for LHT1 in the Arabidopsis root epidermis and has been identified as a possible target for improving NUE in crops (Hirner et al., 2006). The high affinity of this uptake system is likely to be effective in competing with rhizosphere bacteria for any available lysine.

Clearly the N status of a plant is reflected by changes in tissue pools of nitrogenous compounds. The concentrations of some of these N-containing chemicals in tissues and sap are useful indicators of the N nutritional state of a plant. The missing link between the changes in plant experiments measuring feedback regulation of N uptake are the molecular and cellular mechanisms for the sensing of these changes in plant N status and it is this aspect that will now be reviewed.

Molecular nitrogen sensing systems

Several possible N-sensing systems have been identified that may function through sensing specific amino acids within the plant. Each one of these sensors could be the topic of a review, but for brevity we will only focus on a few key facts for each system.

PII protein

In bacteria, cellular Gln concentrations signal N status through the PII protein and a similar model has been proposed for plants (Moorhead and Smith, 2003). The PII protein is believed to sense both carbon and nitrogen status in bacteria and has been shown to directly regulate the activity of an NH₄⁺ transporter (Coutts et al., 2002). There are three major groups of these proteins and these have been described in detail for microbes (see review by Arcondéguy et al., 2001). Glutamine and 2-oxoglutarate concentrations in bacteria modify phosphorylation status of the PII protein which can then interact with other regulatory elements controlling the expression and activity...
of enzymes involved in either C or N metabolism. In cyanobacteria, 2-oxoglutarate and inorganic N sources, such as NO$_3^-$ and NH$_4^+$, rather than Gln are reckoned to alter the phosphorylation status of PII (see Kobayashi et al., 2005, and references therein). Plant PII homologues have been identified in plants and evidence of a direct interaction between 2-oxoglutarate has been shown (Smith et al., 2003). A protein–protein interaction has been demonstrated between PII-like proteins and secondary components, such as kinases, providing evidence for a similar system in higher plants (Sugiyama et al., 2004). Direct proof of PII as an N sensor in plants remains elusive, although the overexpression of PII in Arabidopsis does impair the ability of plants to sense Gln (Hsieh et al., 1998), knock-out mutants show a phenotype with nitrite or ammonium as an N source (Ferrario-Méry et al., 2005, 2006). A decreased accumulation of ornithine, citrulline, and arginine led to the suggestion that PII interacts directly with N-acetyl glutamate kinase, a key enzyme in arginine biosynthesis (Ferrario-Méry et al., 2006).

Glutamate receptors

Three types of glutamate receptors are known in animals as being important for nerve transmission of signals and, in particular, at the synapse and related genes have been identified in plants (Lam et al., 1998) and Arabidopsis has a family of 20 members (Chiu et al., 2002). These plant proteins can function as calcium channels; they can be opened by both glutamate and glycine and the interaction can be synergistic (Dennison and Spalding, 2000; Dubos et al., 2003). Constitutive expression of one family member increased sensitivity to ionic stress (Kim et al., 2001). There is evidence that one family member has a role in C and N metabolism and the plant hormone, abscisic acid (ABA), is involved in this response (Kang and Turano, 2003). Other family members may play a role in touch and cold sensing (Meyerhoff et al., 2005), growth of hypocotyls (Brenner et al., 2000) and roots (Sivaguru et al., 2003; Walch-Liu et al., 2006). For a role in sensing N status these receptors could detect amino acids in the phloem or apoplast and their cellular localization will be important in identifying this function.

Cytokinins and the His–Asp phosphorelay

There is evidence for the plant cytokinin hormones having a central role in signalling plant N status (Inoue et al., 2001), and these can be listed as follows.

(i) The enzymes for the synthesis of cytokinins are specifically induced by NO$_3^-$ supply and not other nutrients, although treatment with auxin can have a similar effect (Miyawaki et al., 2004).

(ii) The concentrations of active cytokinins in plants changes in parallel with changes in N supply, for example, increasing when additional N is supplied (Takei et al., 2002); these changes can occur by synthesis or mobilization of stored forms.

Cytokinins signals are mediated by a multi-component phosphorylation system, composed of a histidine protein kinase (Kakimoto, 2003). Microarray analysis has identified a battery of over 200 genes that have many different functions that appear to be under the regulation of the cytokinin phosphorelay system (Kiba et al., 2005). The overlap between these genes and those responsive to changes in N status are candidates for a signalling role.

General amino acid control

This system is best understood in yeast, where amino acid biosynthetic pathways can be specifically induced by withholding supply of a specific N source (reviewed by Hinnebusch, 2005). In plants, several different components of a parallel system have been identified and, although the sequence homology is not always strong, some have been shown to complement the yeast mutants (Zhang et al., 2003). It therefore seems very likely that a similar control system for amino acid biosynthetic pathways exists in higher plants and gene knock-out mutants will be important tools for confirming this control system in plants. Yeast utilizes NH$_4^+$ and amino acids directly as N sources so, assuming that the same general control system occurs in plants, it should be integrated with the regulation of NO$_3^-$ uptake and assimilation and this must be a fundamental difference between the two types of organism. Therefore, fungi that can use NO$_3^-$ like Hansenula sp. and Neurospora crassa, may be better model organisms for the situation in plants. In Neurospora, NIT2 a global transcription factor for responses to N has been identified and this protein has a zinc finger DNA-binding GATA domain that regulates the expression of the proteins needed to assimilate NO$_3^-$ (Feng and Marzluf, 1998). Homologues have been found in plants (Daniel-Vedele and Caboche, 1993) and there is some complementation possible between the plant and fungal systems (Rastogi et al., 1997). The Arabidopsis GATA transcription factors are more implicated in light-mediated and circadian-regulated gene expression rather than the regulation of N metabolism (Manfield et al., 2007). A defect in the only nitrate-inducible GATA gene in Arabidopsis led to decreased chlorophyll content and down-regulation of the genes involved in sugar metabolism (Bi et al., 2005).

Cellular mechanisms for feedback regulation of N uptake

At the cellular level the simplest signal is likely to be a change in the compartmental pools of N, in particular
the cytosol, as N influx occurs at the plasma membrane. Although changes in the phloem concentrations of amino acids may indicate a change in N status of the plant, it is actually within root cells where the signal must feedback to elicit changes in N uptake. Here we review what is known about changes in the cellular amino acid and \( \text{NO}_3^- \) pools in relation to N supply.

**Changes in cellular amino acid pools**

There are few values reported for single cell amino acid concentrations and this is due to the technical difficulties associated with these types of measurements. When light and dark spinach leaf tissue was fractionated and compared, the cytosolic concentrations of most amino acids had increased considerably in the light (Winter et al., 1994). For example, Gln concentrations were reported to increase from 4.4 mM to 25.7 mM and yet when diurnal changes in \( \text{NO}_3^- \) influx by roots has been measured there is an increase in uptake during the light period (Macduff and Bakken, 2003). These changes in leaf amino acid cytosolic pools do seem compatible with the negative feedback model for \( \text{NO}_3^- \) uptake, unless the root is behaving differently. The resolution of this type of anomaly depends on obtaining improved methods for measuring cytosolic amino acid pools (e.g. FRET; Okumoto et al., 2005).

**Changes in cellular nitrate pools**

\( \text{NO}_3^- \)-selective microelectrodes were used to investigate the effect of treating barley roots with Gln (Fan et al., 2006). Cytosolic \( \text{NO}_3^- \) activities in root cortical and epidermal cells increased to a maximum after about 2 h, but by 6 h the activity was restored to a lower steady-state value (Fig. 2A). Treating roots with Gln for 6 h did not significantly change whole root tissue \( \text{NO}_3^- \), but the pools of this amino acid did increase (Fig. 2B) and there was a decrease in the amount of active \( \text{NO}_3^- \) reductase (NR) in the root tissues (Fig. 2C). These parallel changes in root NR activity can provide an explanation for this change in cytosolic \( \text{NO}_3^- \) as this assimilatory sink for \( \text{NO}_3^- \) was decreased (Fan et al., 2006). The activity of NR is regulated by reversible Mg-dependent phosphorylation and binding of a 14-3-3 protein (Kaiser and Huber, 2001), which has been shown to be responsible for the observed light-regulated fluctuations in its activity in leaf tissue (Tucker et al., 2004). Furthermore, \( \text{NO}_3^- \)-selective microelectrode measurements on Arabidopsis leaf cells (Cookson et al., 2005) have also suggested that there is a strong dependence of cytosolic \( \text{NO}_3^- \) on NR activity. Although this idea does not seem compatible with results obtained using pieces of tobacco leaf which suggested that \( \text{NO}_3^- \) uptake at the plasma membrane can supply an increased rate of \( \text{NO}_3^- \) reduction (Lea et al., 2004). Furthermore, in a recent paper it was shown that there were significant differences in the leaf cytosolic \( \text{NO}_3^- \) pools of two rice cultivars that differed in their NUE (Fan et al., 2007). Changes in the cytosolic \( \text{NO}_3^- \) pool could provide a cellular signal indicating changes in N status and this is worthy of further investigation.

The changes in cytosolic \( \text{NO}_3^- \) activity can act as a signal in plant cells; this does not necessarily imply a second messenger role, but can be a signal regulating gene expression and triggering the mobilization of other N pools (e.g. vacuolar \( \text{NO}_3^- \); van der Leij et al., 1998).

**Future prospects**

An exciting opportunity for unravelling the N signalling process in the future is provided by the use of specific gene mutants. For example, the Gln dumper mutants of Arabidopsis (Pilot et al., 2004), these plants exude excessive amounts of Gln that must be present in the apoplast and may therefore interfere with the usual N signalling systems. Studying the N physiology of these plants may
provide useful information on the N signalling system. The fundamental link between N supply and growth is achieved through altering the transport and amounts of, as well as the sensitivity to, plant hormones as effectors for changing morphology. The role and importance of cytokinins in N status has been described (Inoue et al., 2001). Hormone mutants have been useful for the identification of the role in N signalling for responses such as ABA and lateral root growth (Signora et al., 2001). These tools are likely to continue to be very useful in the future, especially when used with plants that have altered N physiology and transport.

In agriculture and horticulture, N can be applied to the leaves of crops and the effects of these foliar inputs on signalling must be important. The consequences of these applications for N use efficiency by crops may be a significant target for genetic improvement as they do not reflect the usual site for N acquisition. By contrast, for some wild species of plants atmospheric deposition of N can negatively effect their distribution (Aber et al., 2003), but this result may be explained by competition for nutrients rather than a direct effect on signalling. Furthermore, the form of N application can be important in determining if the effect is positive or negative (Pearson and Stewart, 1993).

The N status of plants, and particularly the amino acid pools, must be closely linked to photosynthetic activity and the expression of some transporters depends on the amount of available carbon (Lejay et al., 2003). One interesting aspect of this feedback regulatory mechanism for N status is the change associated with plant development. For example, during senescence and remobilization of stored N pools the amino acids can increase and this will have knock-on effects for assimilatory enzymes such as NR and GS. This situation contrasts with that during early vegetative growth. These changes in plant development do not provide any problems for feedback regulation as the tissue and intracellular signals adjust appropriately. However, the regulatory targets for improving NUE during early vegetative growth will be very different from that at senescence.

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