Regulation of nitrate uptake at the whole-tree level: interaction between nitrogen compounds, cytokinins and carbon metabolism†

ARTHUR GEßLER,1 STANISLAV KOPRIVA1 and HEINZ RENNENBERG1,2

1 Institute of Forest Botany and Tree Physiology, Albert Ludwig University of Freiburg, Georges-Köhler-Allee, Gebäude 053/054, 79110 Freiburg, Germany
2 Corresponding author (heinz.rennenberg@ctp.uni-freiburg.de)

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Summary Pedospheric nitrate uptake is closely integrated with the nitrogen (N) status and demand of the whole tree. Signaling substances communicating the N demand of the shoot to the roots are required in an integrated regulatory system. Beside phloem mobility, such signal compounds must have the potential to repress or increase nitrate uptake either at the transcriptional or post-transcriptional level. Amino compounds cycling within the tree are involved in the regulation of nitrate uptake. In many tree species, inorganic N is generally assimilated in roots, and amino acids—the direct products of N assimilation—are transported in the xylem to the sites of N demand. If the quantity of amino acids transported to the above-ground parts of the tree exceeds shoot N demand, some amino compounds are reallocated to the roots by phloem transport. Particular amino compounds exert transcriptional and post-transcriptional control over nitrate uptake by roots. Induction of nitrate transporters is mediated by nitrate or nitrite, or both, and possibly also by cytokinins, which cycle within the tree and act as both root-to-shoot and shoot-to-root signals. This review focuses on tree-specific requirements for N regulation and signaling, as well as the link between carbon metabolism and nitrate uptake.

Keywords: amino compounds, cycling, glutamine, nitrate assimilation, nitrate transporter.

Introduction

In preindustrial times, nitrogen (N) was thought to be a dominant growth-limiting factor in natural forest ecosystems (Cole and Rapp 1981, Dickson 1989). Consequently, tree metabolism and forest ecosystem functioning were assumed to be adapted to N limitation (Rennenberg et al. 1998). As a result of human activities, N input into terrestrial ecosystems has increased, exposing some forests to a supra-optimal supply of N from wet and dry deposition (Wellburn 1990, Pearson and Stewart 1993, Rennenberg et al. 1998). The atmospheric N load to forests comprises both oxidized N compounds (NO, NO₂, NO₃⁻) originating from fossil fuel combustion by industrial processes and automobiles (Crutzen 1979, Wellburn 1990, Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit 1996, Mosier 2001) as well as reduced N compounds, mainly NH₃ and ammonium, released from intensive agriculture (Fangmeier et al. 1994, Asman et al. 1998). These atmospheric N compounds can be taken up by the above-ground parts of plants (Pearson and Stewart 1993, Burkhardt and Eiden 1994, Muller et al. 1996, Geßler et al. 2000). After removal from the atmosphere by rain events and deposition to the soil, these compounds become available as an N source for roots in addition to the N derived from mineralization processes (Sutton et al. 1995, Rennenberg et al. 1998).

Pedospheric nitrogen uptake is a highly regulated process adapted to the N status and demand of the whole tree (Stulen et al. 1998). Signal substances that communicate the N demand of the shoot to the roots are required in an integrated regulatory system. Experiments in the field and under controlled conditions indicate that the pool of amino compounds (or particular amino compounds within this pool) cycling between the shoot and the roots serve to signal to the roots the internal N status of the whole plant (Cooper and Clarkson 1989, Muller and Touraine 1992, Muller et al. 1996, Kreuzwieser et al. 1997, Geßler et al. 1998a, 1998c). When the N supply of plants exceeds the N demand, the pool of amino acids that are reallocated from the shoot to the roots by way of the phloem increases, thereby providing a signal that leads to the repression of nitrate uptake by fine roots. Whether ammonium uptake is regulated in a similar way remains controversial, because there are contrary results for different plant species (Causin and Barneix 1994, Rennenberg et al. 1998, Rennenberg and Geßler 1999, von Wirén et al. 2000).

In addition to amino acids, cytokinins are also involved in N signaling. Cytokinins are thought to communicate changes in the mineral N status of the rhizosphere to the shoot, allowing growth responses to be directly coupled to the N status of the soil. Little is known about shoot-to-root signaling mediated by cytokinins, although there is some evidence that basipetal transport of cytokinins affects N acquisition by roots. Application of exogenous cytokinins to N-deficient plants temporarily reverses deficiency symptoms (Horgan and Wareing 1980) and increases N uptake (Simpson et al. 1982, Trčková and Kamínková 1999) as well as the expression of high-affinity nitrate transporters in roots (Collier et al. 2003).

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The amino acid and cytokinin signaling pathways could form an integrated regulatory system for nitrate uptake in trees with positive and negative effectors: (1) the accumulation of particular amino compounds may serve as a signal for the repression of nitrogen uptake; and (2) the accumulation of cytokinins may increase the expression of genes involved in nitrogen uptake and assimilation. In this review, we discuss possible interactions between cytokinins and amino compounds in signaling the N demand at the whole-tree level and in regulating nitrate uptake. We focus on tree-specific processes such as N storage and remobilization, which may be linked to the regulation of pedospheric N uptake. Because the cytokinin balance in plants is closely linked to carbon metabolism (e.g., Van der Werf and Nagel 1996, Havelange et al. 2000), the regulatory effects of carbohydrates on nitrate uptake are also discussed.

Integrated signaling of tree N status—nitrogen compounds and cytokinins

Nitrate taken up by mycorrhizal and non-mycorrhizal roots is mainly assimilated in amino compounds by belowground tissues (Gojon et al. 1994). As a result, organic nitrogen compounds and only traces of NO$_3^-$ or NH$_4^+$ are transported from the roots to the shoots in the xylem (Dambrine et al. 1995, Geßler et al. 1998b, Schmidt and Stewart 1998). Nitrogen-demanding tissues, e.g., cambial tissues of the stem, leaves, fruits and buds, take N from this xylem-borne pool of amino compounds for the synthesis of proteins and other organic N-containing compounds.

Unused amino compounds in the xylem are thought to be reallocated via the phloem. Experiments with Norway spruce show that various amino compounds are subject to bidirectional exchange between xylem and phloem in twigs, especially when there are no sink tissues requiring N (Geßler et al. 2003). Glutamine (Gln), which is the main form of transported N in many tree species (Sauter and van Cleve 1992, Malaguti et al. 2001, Grassi et al. 2002), is frequently exchanged between xylem and phloem (e.g., Atkins et al. 1980). Assessment of individual amino compounds in the xylem and phloem of trees (e.g., Stoermer et al. 1997, Geßler et al. 1998b) indicates that besides the exchange of amino compounds between xylem and phloem, there is also metabolic interconversion. In Norway spruce trees, mainly Gln and aspartate (Asp) are transported in the xylem from the roots to the shoots, whereas arginine (Arg) as well as Gln accumulate in the phloem in needles and twigs (Stoermer et al. 1997).

Amino compounds loaded into the phloem are generally reallocated in a basipetal direction. When N uptake and transport of soluble N compounds to the shoot exceeds shoot demand, the pool of amino compounds in the phloem expands (Youssefi et al. 2000), and the subsequent increase in amino compound content of the roots inhibits net nitrate uptake. In Norway spruce, phloem-translocated Gln is generally considered to be the compound primarily involved in the down-regulation of root nitrate uptake (Geßler et al. 1998c). However, in several species, other amino compounds such as Arg, alanine (Ala), β-Ala, Asn, glutamate (Glu), methionine (Met) and Asp can also repress nitrate transport (Muller and Touraine 1992). When trees are supplied with an additional N source from atmospheric deposition (NH$_3$ or NO$_x$), net nitrate uptake by fine roots is significantly decreased (Muller et al. 1996, Geßler et al. 1998b).
In beech, the extent of this decrease corresponds to the amount of N taken up as NH₃ by the shoot and is accompanied by a significant increase in basipetal phloem transport of Gln and several other amino compounds. However, in fine roots, only the Gln concentration is increased (Geßler et al. 1998c), indicating a strict control of nitrate uptake by a shoot-to-root Gln signal in this species.

Bidirectional xylem–phloem exchange takes place in the crown and in the trunk or root region. Hence, not all amino compounds transported downward in the phloem originate from the top of the tree. Weber et al. (1998) concluded that Arg cycled only in the belowground parts of Norway spruce trees, because this compound was detected only in the xylem and phloem of roots and was not transported in the xylem of the trunk or in the twigs (Rennenberg et al. 1998).

The movement of amino compounds is even more complex when N remobilization in spring and N storage in autumn is taken into account. For example, during spring remobilization, adult beech trees convert amino compounds in both leaves and stem (Figure 1). In spring, a proportion of the amino compounds originating from storage N is allocated to newly developing leaves by phloem transport. Thus, transiently and locally, the phloem may not contribute to the basipetal circulation of amino compounds. During spring, however, pedospheric N uptake is generally restricted by low soil temperatures (Geßler et al. 1998a); thus, signaling by cycling amino compounds and, hence, plant internal regulation of N uptake seems to play only a minor role at this time. During N storage in autumn, mainly Arg, which is reported to have no signaling function in beech (Geßler et al. 1998a), is transported to storage tissue and may be subject to phloem-to-xylem exchange in twigs and trunks.

Cytokinins, N⁶-substituted adenines, also appear to relay environmental stresses in the rhizosphere to the shoot, particularly changes in mineral N availability around the roots, allowing growth responses to be directly coupled to soil N status. Wagner and Beck (1993) showed that the quantity of cytokinins transported in the xylem was lower in low-N plants compared with controls and was positively correlated with shoot:root ratio. Endogenous cytokinins in several species increase following an increase in N supply (Samuelson et al. 1995, Collier et al. 2000), although how N status is translated into a cytokinin signal is unclear (Mok and Mok 2001). The simplest explanation is that the N concentration in roots is directly proportional to root cytokinin concentration; alternatively, the products of N assimilation may regulate cytokinin biosynthesis or catabolism, or both (Samuelson and Larsson 1993, Beck 1996). The increased transport of cytokinins from roots to shoot (Samuelson and Larson 1993, Wagner and Beck 1993) appears to up-regulate both response regulator genes involved in N signal transduction (Tanguchi et al. 1998, Mok and Mok 2001, Takei et al. 2001) and nitrate reductase expression in leaves (Samuelson et al. 1995). There is also evidence that shoots control cytokinin concentrations in roots, thereby governing cytokinin export to the shoot. Havelange et al. (2000) showed that the accumulation of sucrose in roots stimulated the transport of cytokinins in the xylem, and postulated that sucrose acts as a substrate for metabolism and as a regulatory molecule with a hormone-like function in the root.

Recently, Emery and Atkins (2002) hypothesized that cytokinins, which are transported from roots to shoots in the xylem and from shoots to roots in the phloem (e.g., Baker and Allen 1992, Grayling and Hanke 1992), act as a chemical signal coordinating physiological changes in shoots and roots. It is likely that an expanded or activated pool of cytokinins in the

**Figure 1.** Allocation and cycling of total soluble non-protein nitrogen (TSNN) compounds in beech trees (A) during N mobilization in spring and (B) during N storage at the end of the growing season. The compounds comprised at least 5% of TSNN. Bold printed compounds comprised more than 50% and compounds in italics more than 15% of TSNN (Figure redrawn from Geßler et al. 1998b). Abbreviations: Arg = arginine; Asn = asparagine; Asp = aspartate; Gln = glutamine; and Glu = glutamate.
roots acts in a similar way to the cytokinin pool in the leaves, up-regulating response regulator genes involved in N signal transduction and increasing the expression of enzymes involved in N metabolism (Samuelson et al. 1995, Takei et al. 2001). Trčková and Kamínk (1999) observed that spraying shoots of N-starved wheat plants with cytokinin (N<sup>6</sup>-(meta-hydroxybenzyl)adenosine) increased nitrate net uptake by up to 70%.

Results from studies with beech (Collier et al. 2003) support the hypothesis that physiologically active cytokinins are transported from shoots to roots and signal changes in shoot N status. The significant increase in cytokinin content (especially Z-types) in the phloem in response to NH<sub>3</sub> exposure is thought to reflect an increase in the flux of cytokinins from shoot to root.

**Molecular characteristics of nitrate uptake systems**

At the molecular level, there is influx and efflux of nitrate between soil and roots (Aslam et al. 1994, Chaillou et al. 1994). It is unclear whether efflux is a consequence of membrane leakiness or mediated by particular transport system(s). In non-mycorrhizal beech roots and in Fagus–Laccaria mycorrhizae, efflux increases with increasing nitrate supply, amounting to 80–90% of influx at an external nitrate concentration of 1 mM (Kreuzwieser et al. 1997, 2000). The assumed regulators of net nitrate uptake (i.e., ammonium and Glu) seem to reduce nitrate by reducing influx rather than by increasing efflux.

Nitrate uptake by plant roots is complex and involves different active systems. Low-affinity transport systems (LATS) are responsible for uptake at external nitrate concentrations greater than 1 mM (Siddiqi et al. 1990). At lower nitrate concentrations (between 1 μM and 0.25 mM)—which is the range typically found in the soil solution of natural forest ecosystems—high-affinity transport systems (HATS) dominate (Behl et al. 1988). Both LATS and HATS possess distinct constitutive and inducible components (Glass et al. 2001), with LATS having a greater capacity for nitrate uptake than HATS. Thus, HATS seem to be important for N acquisition at low external nitrate concentrations, whereas LATS are responsible for mass nitrate uptake when external concentrations are high (Touraine and Glass 1997). The first low-affinity nitrate transporter (AtNRT1.1) was cloned from Arabidopsis thaliana (L.) Heynh. as a herbicide sensitivity gene CHL1 (Tsay et al. 1993). Subsequently, another three members of the LATS gene family were identified in Arabidopsis (Hatzfeld and Saito 1999, Huang et al. 1999). Because nitrate uptake in AtNRT1.1 mutant plants was also reduced in the HATS range of nitrate concentrations, it was suggested that this transporter functions as a dual-affinity transporter (Wang et al. 1998, Liu et al. 1999).

In the model plant A. thaliana, four genes for low-affinity nitrate transporters (AtNRT1.1–AtNRT1.4) have been identified, and the NRT2 gene family encoding the high-affinity transporters comprises seven members. The sequence identity among the four low-affinity transporter genes is relatively low, whereas the primary structures of the genes from the NRT2 family are highly conserved (Okamoto et al. 2003). The NRT1 gene family belongs to a superfamily of peptide transporters (PTR) comprising about 50 genes. The best characterized members of this gene family are AtNRT1.1 and AtNRT1.2. The mRNA of AtNRT1.1 accumulates predominantly in the epidermis near the root tips of young roots, but also in the endodermis and the cortex of mature roots, whereas the AtNRT1.2 transcript is mainly found in the epidermis and in root hairs (Huang et al. 1996, 1999). Analysis by RT-PCR revealed that mRNAs for the four AtNRT1 genes are present at similar or higher frequencies in leaves as in roots (Okamoto et al. 2003). Steady-state transcript levels of AtNRT1.2 and AtNRT1.3 were higher than those of AtNRT1.1 and AtNRT1.4.

**Regulation of nitrate uptake and assimilation by carbohydrates**


Nitrate uptake is regulated by atmospheric CO<sub>2</sub> concentration through the availability of carbohydrates (Rufty et al. 1989, Delhon et al. 1996, Constable et al. 2001). Furthermore, nitrate uptake is under diurnal regulation with maximum activity during the day and minimum activity at night (Le Bot and Kirkby 1992, Lejay et al. 1999). The decrease in N uptake at night is reversed by feeding with sucrose, which affects both HATS and LATS at the transcriptional level (Lejay et al. 1999). The regulation of nitrate uptake and nitrate transporter mRNA accumulation by light and sucrose are strongly correlated (Lejay et al. 2003). Although the molecular mechanism underlying regulation by sucrose remains to be elucidated, it appears to differ from the general sugar-sensing mechanisms involving sucrose signaling and hexose transport (Lejay et al. 2003). It is not known if cytokinins in roots, which may be linked to basipetal sugar transport (Havelange et al. 2000), are involved in this regulation.

**Regulation of nitrate transport by N compounds and cytokinins at the molecular level**

Exposure of N-deprived plants to 1 mM nitrate strongly induces AtNRT1.1 mRNA in roots, but only transiently in leaves. AtNRT1.2 is constitutively expressed in both organs, whereas AtNRT1.4 is unaffected by nitrate in the roots but is induced in the leaves. The expression pattern of AtNRT1.3 indicates that this mRNA accumulates in leaves following nitrate exposure, but is repressed in roots (Okamoto et al. 2003). These results are consistent with previous observations indicating that AtNRT1.1 and AtNRT1.2 are responsible for the inducible and constitutive components of LATS, respectively (Huang et al. 1996, 1999). Expression of AtNRT1.1 undergoes a diurnal
rhythm with a maximum during the light period. The \textit{AtNRT1.1} transcript level is increased by sucrose at night, resulting in increased nitrate uptake (Lejay et al. 1999). It is not subject to feedback inhibition by NH\textsubscript{3} or Gln, but is repressed by nitrite (Loque et al. 2003). Nitrate starvation also represses \textit{AtNRT1.1} mRNA accumulation (Lejay et al. 1999).

The first plant component of HATS (\textit{NRT2.1}) was identified in barley (Trueman et al. 1996) and later shown to be part of the inducible system (Zhuo et al. 1999). The \textit{NRT2} family in \textit{Arabidopsis} consists of seven genes. All of these genes, except \textit{AtNRT2.7}, are mainly expressed in roots (Orsel et al. 2002, Okamoto et al. 2003). However, the expression patterns of the genes differ significantly after feeding nitrate to N-starved plants (Okamoto et al. 2003). Both \textit{AtNRT2.1} and \textit{AtNRT2.2} are strongly induced by nitrate in roots, but their mRNA levels decline in response to nitrate feeding in leaves. In contrast, \textit{AtNRT2.3} mRNA is unaffected by nitrate concentration in roots but is induced by nitrate in leaves. In response to nitrate feeding, \textit{AtNRT2.4} and \textit{AtNRT2.5} transcripts are reduced, whereas \textit{AtNRT2.6} and \textit{AtNRT2.7} exhibit constitutive expression (Okamoto et al. 2003). Vidmar et al. (2000) observed a similar time course of induction and down-regulation of the \textit{NRT2.1} transcript and nitrate influx. These data are consistent with the conclusion that \textit{AtNRT2.1} is responsible for the inducible HATS (Lejay et al. 1999, Filleur et al. 2001, Nazoa et al. 2003). In contrast to the low-affinity transporters, \textit{AtNRT2.1} mRNA levels are increased by N starvation (Lejay et al. 1999). The mRNA levels of six \textit{AtNRT2} transporters (\textit{AtNRT2.2} was not analyzed) are substantially higher in \textit{A. thaliana} grown under N-limiting conditions compared with N-sufficient conditions (Orsel et al. 2002).

It has been postulated that, among the potential products of N assimilation, only nitrate or nitrite are capable of inducing increased HATS expression, and hence, increasing nitrate influx (Glass et al. 2002, Nazoa et al. 2003). Neither nitrate nor nitrite cycles within the tree (Gebler et al. 1998b), and hence, although they can adapt nitrate uptake to pedospheric availability, they are unable to serve as a plant integrating signal. However, there is evidence for the induction of a high-affinity nitrate transporter by cytokinins in beech (Collier et al. 2003). The accumulation of Z-type cytokinins in beech roots as a consequence of increased [9R]Z transport in the phloem resulted in an increase in the frequency of a mRNA encoding a high-affinity nitrate transporter. The authors speculate that, in a first step, the accumulation of Z-type cytokinins in response to [9R]Z transport results in a transient increase in nitrate net uptake owing to the intensified expression of high-affinity nitrate transporters. As a consequence, nitrate assimilation, and thus synthesis of amino compounds, increases as previously observed when HATS are induced (Siddiqi et al. 1989, Glass and Siddiqi 1995, Kronzucker et al. 1995). The accumulation of newly assimilated amino acids in the roots may exert a feedback inhibition on nitrate uptake (Aslam et al. 2001). Because the transcript level of the high-affinity nitrate transporter remained high, albeit net uptake decreased, amino acids may control nitrate uptake at the post-transcriptional level (Figure 2). Hence, Collier et al. (2003) concluded that a signal mediated by amino compounds can override the cytokinin signal. It has been demonstrated that the accumulation of amino acids in roots controls nitrate uptake at the transcriptional level. Feeding \textit{Arabidopsis} roots with amino acids resulted in an increase in \textit{NRT2} transcript frequency (Zhuo et al. 1999, Vidmar et al. 2000) and Gln is thought to act as the signal. Vidmar et al. (2000) postulated that nitrate and ammonium act as signals at the post-transcriptional level, resulting in decreased influx at constant mRNA levels.

**Conclusions**

Nitrate uptake is subjected to up- and down-regulation depending on plant N status and external factors (Figure 3). Nitrate transporters, which are induced by nitrate or nitrite, or both, adapt nitrate uptake capacity to pedospheric nitrate availability. Amino compounds derived from N assimilation are transported to the shoot to satisfy the demand for reduced N. Particular amino compounds such as Gln are re-allocated basipetally to the roots and can act as signals to repress nitrate uptake both at the transcriptional and post-transcriptional levels. In addition, products of N assimilation in roots induce pro-

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**Figure 2.** Model of the regulation of nitrate uptake by cytokinins and the interaction with amino acid in roots.
A grant (RE 515/20) is gratefully acknowledged. DFGR research fellowship to A.G. (Contract No. GE 1990/3-1) and by financial support by the Deutsche Forschungsgemeinschaft by a... leaf N status. Phloem-transported cytokinins appear to up-regulate regulator genes involved in N signal transduction and root-to-shoot transport of cytokinins. In the shoot, cytokinins up-regulate regulator genes involved in N signal transduction. Possibly, some of the cytokinins are reallocated back to the roots. In addition, iP-type cytokinins appear to be synthesized in stems, meristems and leaves (Morris 1997, Emery et al. 2000) and may be directly converted by transhydroxylation into fully active forms following a change in leaf N status. Phloem-transported cytokinins appear to up-regulate nitrate uptake by roots at the transcriptional level.

Two regulatory pathways appear to be involved in the regulation of N uptake by roots to meet the N demand of the tree and may interact as follows: (1) The accumulation of particular amino compounds (especially Gln) in roots serves as signal for the repression of N uptake at the transcriptional and post-transcriptional levels. (2) The accumulation of cytokinins up-regulates the expression of genes involved in N uptake when not repressed by amino compounds. The proposed regulatory system has positive and negative effectors allowing fine tuning of N uptake by signaling substances that are able to integrate N status at the whole-tree level.

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Crutzen, P.J. 1979. The role of NO and NO2 in the chemistry of the troposphere and stratosphere. Annu. Rev. Earth Planet. Sci. 7: 443–472.


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