

Chapter 12

Root Strategies for Nitrate Assimilation

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12.1 Introduction

Nitrogen is an essential element in biological molecules, such as nucleotides, amino acids, and proteins, and therefore fundamental for plant growth and development (Marschner 1995). In fact, plant performance, fitness, yield, nutrient efficiency, or susceptibility to biological and environmental stresses is highly dependent on nitrogen mineral nutrition (Epstein and Bloom 2005). Some plants can fix atmospheric nitrogen into organic forms through symbiotic relationships with soil microbes (not covered here), but most plants obtain their nitrogen directly from the soil via absorption of inorganic and organic forms of nitrogen. The concentrations of various forms of N (e.g., organic N, ammonium, nitrate, nitrite, nitrous oxide) in the soil depend on soil type, temperature, and the activities of microorganisms (Nasholm et al. 1998; Jackson et al. 2008).

Nitrate (NO_3^-) is the major N source for higher plants in most agricultural and temperate zone soils (Epstein and Bloom 2005). Distribution and concentration of NO_3^- in soils show substantial spatial and temporal heterogeneity (Jackson and Caldwell 1993), and NO_3^- is the object of intense competition among neighboring plants (Cahill et al. 2010) and between plants and microorganisms (Jackson et al. 1989; Hodge et al. 2000; Miller et al. 2007a). Under conditions of limited NO_3^- availability, the ability of a plant to acquire NO_3^- from its surroundings largely depends on the amount of root area in contact with

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NO_3^- in the soil solution and on the efficiency with which a root can transport NO_3^- from its surroundings into the plant. Under conditions of unlimited soil NO_3^- availability, roots absorb superfluous amounts, and root and shoot NO_3^- concentrations can reach up to 100 mM, most of which is stored within vacuoles (Miller and Smith 1996). Despite considerable variation in the concentration of NO_3^- in the soil solution, root cells keep cytosolic NO_3^- at a controlled level, with values of approximately 3 mM in maize and 4–5 mM in barley (Miller and Smith 1996, 2008; Huang et al. 2012), possibly to minimize oxidative stress (Huang et al. 2012). Nitrate assimilation plays a central role in this homeostasis, along with NO_3^- uptake, efflux, and xylem and vacuolar loading (Crawford and Glass 1998). Nitrate, in addition to being an important nutrient, may serve as an osmoticum for supporting root elongation and may act as signal molecule regulating nitrogen and carbon metabolism and coordinating whole-plant development (Redinbaugh and Campbell 1991; Crawford 1995; Miller et al. 2007b; Krouk et al. 2010a; Dechorgnat et al. 2011; Bloom et al. 2012a). Root strategies for NO_3^- acquisition must cope with soil NO_3^- heterogeneity and cytosolic NO_3^- homeostasis as well as coordinate root growth with NO_3^- sensing, uptake, translocation, and finally, assimilation to organic N.

The relationship between nitrogen acquisition and roots has long been of interest in plant biology and agriculture because of its influence in plant growth and food production (Oaks and Hirel 1985; Oaks 1992). The topic has been reviewed by Miller and Cramer (2005), with an emphasis in the molecular mechanisms that plants use in accessing N in the soil pools; by Jackson et al. (2008), with an emphasis on physiological and ecological functions that contribute to plant–microbe–soil N cycling; by Kraiser et al. (2011), focusing on the integration of nitrogen acquisition strategies from the ecosystem to molecular level; by Forde and Walch-Liu (2009) and Krouk et al. (2010a), with an emphasis in the role of NO_3^- as a signal and its influence on root behavior and NO_3^- regulation, respectively. Here, we review the different strategies that plant roots follow to acquire NO_3^- from the soil.

12.2 Nitrate in the Soil

12.2.1 *Origins and Fates*

In natural ecosystems, NO_3^- is the dominant form of nitrogen available to plants in all but very acidic and anaerobic soils, because of both the ability of particular soil microorganisms (e.g., *Nitrosomonas*, *Nitrosospiras*, and *Nitrobacter* species) to convert ammonium ion (NH_4^+) to NO_3^- and the ubiquitous distribution of these organisms (Oaks 1992; Hiorns et al. 1995). In agricultural systems, the baseline level of NO_3^- is supplemented by the addition of N fertilizers to the soil. Soil NO_3^- concentration averages 1 mM in natural ecosystems (Andrews 1986), whereas it

averages 10 mM, ranging from 0 to 70 mM, in most agricultural systems (Reisenauer 1966). In both natural and agricultural ecosystems, NO_3^- originates (i) through microbial decomposition of soil organic matter via intermediates (nitrification), (ii) through biological fixation, (iii) from atmospheric deposition, and (iv) only in agricultural ecosystems, from the incorporation of fertilizers. The major pathways of NO_3^- losses from soil include (i) leaching to surface and ground water, (ii) microbial conversion to N_2O and N_2 (denitrification), (iii) microbes immobilization, and (iv) soil erosion.

The appearance and disappearance of soil NO_3^- rapidly changes with rainfall and other factors influencing microbial activity such as pH, temperature, and oxygen concentrations, which in turn affect mineralization, nitrification, and denitrification (Haynes 1986). Nitrate with its negative charge is not adsorbed on negatively charged soil particle surfaces, allowing it to move relatively freely through the soil. For these reasons, NO_3^- concentrations in natural and agricultural soils fluctuate greatly both temporally, even diurnally, and spatially even over short distances (Burger and Jackson 2004).

12.2.2 Nitrate in the Rhizosphere

In natural ecosystems, root acquisition of exogenous NO_3^- is a function of its availability, whereas in croplands the addition of fertilizers containing NO_3^- decreases the element of chance (Oaks 1992). NO_3^- enters the rhizosphere because of NO_3^- mobility in the soil (the NO_3^- reaches the root) or activities of the root system itself (the root reaches the NO_3^-). In the first case, NO_3^- mobility in soils results in rapid diffusion to roots and thus easier plant access to the available NO_3^- (Boudsocq et al. 2012). NO_3^- can also reach the rhizosphere by mass flow, linked to transpiration and the depletion of solution near the root surface (Marschner 1995). In the second case, (i) the roots can directly intercept the NO_3^- through their growth or (ii) the roots of some plants can change the rhizosphere's conditions such as releasing oxygen (Kirk and Kronzucker 2005; Li et al. 2008) and exudates (Bais et al. 2006) that greatly influence the density and activity of microbial populations, which in turn can accelerate nitrification of NH_4^+ in the rhizosphere.

12.3 Root Strategies for Nitrate Acquisition

12.3.1 *Ecosystem Level*

12.3.1.1 Associations with Microorganisms

Roots form associations with microorganisms as a strategy to enhance resource capture (Hodge 2009; Kraiser et al. 2011). The majority of plants are capable of associating with arbuscular mycorrhizal fungi, which induce modifications in root system architecture (RSA) (Gutjahr et al. 2009). These modifications can increase plant NO_3^- uptake and improve the nitrogen nutrition of plants, mainly those growing at low levels of nutrients (Cruz et al. 2004). Plant roots also associate with bacteria, which can increase nutrient accessibility, uptake, or both (Bertrand et al. 2000), improving plant growth. These bacteria are referred to as plant growth-promoting bacteria and can produce phytohormones affecting RSA (Persello-Cartieaux et al. 2001) or increase the activity of NO_3^- uptake systems (Bertrand et al. 2000).

12.3.1.2 Competing for Nitrate

Nitrate in the soil is very dynamic and an “object of desire” to individuals of the same species (intraspecific competition) and among different species, regardless of taxonomic affiliation (interspecific competition) (Hodge et al. 2000; Schenk 2006; Cahill and McNickle 2011). The rapid diffusion of NO_3^- through the soil allows different individual plants to be highly efficient at acquiring NO_3^- even when they have restricted or simple RSA (Fitter et al. 2002). Some plants display pronounced proliferation in response to locally applied NO_3^- (Drew 1975; Guo et al. 2002). Because of the mobility of NO_3^- ions in soil, such proliferation increases NO_3^- acquisition by plants when they are in competition with neighbors for a finite, spatially restricted, mixed N source (Robinson et al. 1999).

Not all plant species respond to the nutrient-rich zones in the same way (Campbell et al. 1991). Competitively dominant plants exploit nutrient-rich patches to a greater extent simply because they are larger and have higher growth rates rather than because they have greater flexibility within their root system. In contrast, competitively inferior plants, although smaller, allocate more of their new root growth to nutrient-rich areas; that is, they place their new roots with greater precision. Among plants and microorganism, Jackson et al. (1989) showed that in annual grasslands, the NO_3^- pool is consumed as rapidly as it is produced, and microbial uptake is the major factor controlling NO_3^- availability to plants. In the short term (hours), soil microorganisms do compete better than plants for

NO_3^- (Jackson et al. 1989), but after longer periods (days to weeks), plants absorb more NO_3^- than microbes do because of microbial turnover (Inselbacher et al. 2010). Recent work in which substantial microbes turnover was prevented, however, suggested that plants compete directly and more efficiently for NO_3^- than microbes (Inselbacher et al. 2010). Spatial differences in nitrogen availability, distribution of root and microorganisms, relative turnover times of roots and microorganisms, and changes in the soil C:N ratio are the key determinants of “success” in the competition for NO_3^- (Hodge et al. 2000).

12.3.2 *Organism Level*

12.3.2.1 Resource Allocation to Roots

All plants face a basic economic decision: where best to invest their resources (Bloom et al. 1985). The costs associated with getting this wrong may lead to diminished nutrient capture; less resources for reproduction and hence reduced fitness; and at the extreme, competitive exclusion from the particular environment (Hodge 2009). When the roots of some plants encounter a NO_3^- -rich patch, their growth becomes enhanced at the expense of the growth in poorer resource areas (Drew 1975). This means that root development, and especially lateral root initiation, depends on the integrated effects of the local environment and the internal correlative relations between the roots (Gersani and Sachs 1992).

Allocation of resources to above vs. belowground structures or to different parts of the root systems can make budgetary sense. In general, resource allocation to belowground structures relative to aboveground structures increases when N is limiting (Miller and Cramer 2005) (Fig. 12.1). This compensates for N deficiency by increasing the plant’s opportunity to obtain N for sustaining growth (Reynolds and Dantonio 1996; Sims et al. 2012). Such an acclimation response derives from metabolic changes in the shoot and an adjustment of carbohydrate transport to the root (Hermans et al. 2006). NO_3^- in the shoot acts as a long-range signal molecule that regulates root growth (Adgo and Schulze 2002). Among the several changes that result in NO_3^- accumulation in the shoot and could contribute to altered allocation patterns include an inhibition of starch synthesis and turnover in the leaves and a decrease of the transport of sucrose to the roots, resulting in an increase in the root-to-shoot ratio (Rufty et al. 1988). In *Arabidopsis* plants where tissue N is plentiful, NO_3^- can specifically inhibit root system growth while having no effect on shoot system growth (Roycewicz and Malamy 2012). This suggests that plants regulate root-to-shoot ratio not specifically in response to nitrogen starvation but as a general mechanism to tailor their growth to environmental nitrogen supply.



Fig. 12.1 Two months old wheat plants growth in nutrient solution containing 2 mM NO_3^- (left) and 0.2 mM NO_3^- (right) as solely nitrogen source

12.3.2.2 Root Architecture

The root system is fundamentally important for plant growth and survival because of its role in water and nutrient uptake. The architecture of the root system is determined by the pattern of root branching, which in many species displays a high degree of plasticity to enable plant survival under variable environmental conditions (Sultan 2000; Hodge 2009). Soil NO_3^- concentration and overall nutrient status of the plant in concert with the genetic makeup of the plant will determine the pattern of root branching and define the root strategies for NO_3^- acquisition (Hodge 2004; Desnos 2008; Vidal et al. 2010).

First of all, when encountering a NO_3^- -rich patch, a plant maximizes N capture through upregulating inflow and making a more competitive root system (Robinson

et al. 1999). The strategies affecting RSA involve lateral root elongation, lateral root initiation, and primary root growth (Kraiser et al. 2011). A localized area of high NO_3^- stimulates the elongation of lateral roots through a dual-affinity nitrate transporter, *CHL1*, that not only transports NO_3^- but also senses external NO_3^- concentrations (Ho et al. 2009) and activates the *NITRATE REGULATED 1 (ANR1)* (an MADS-box gene) that control changes in root architecture (Guo et al. 2002). *ANR1* initiates a local-range signaling pathway and regulates NO_3^- -stimulated lateral root elongation (Remans et al. 2006a). When plants are grown on high NO_3^- or have high levels of N metabolites, the expression of *ANR1* decreases and lateral root elongation is suppressed (Zhang et al. 1999; Gansel et al. 2001). The net result is that, if roots grown on low NO_3^- are exposed to a localized region of high NO_3^- , then lateral roots proliferate specifically in that region of the roots. The putative high-affinity NO_3^- transporter *NRT2.1*, like *NRT1.1*, serves as a NO_3^- sensor to coordinate the development of the root system. It acts directly on lateral root initiation under NO_3^- -limiting conditions (Lejay et al. 1999; Little et al. 2005; Remans et al. 2006b).

It is interesting to note that root proliferation occurs in localized patches of NO_3^- , which is a relatively mobile nutrient, but not in localized patches of NH_4^+ , which is a relatively immobile nutrient (Leyser and Fitter 1998). A possible explanation for this paradox might be the very mobility of the NO_3^- ion, which makes it an ideal subterranean signal molecule (Forde and Zhang 1998). In unfertilized soils, a major source of nitrogen is from decaying organic matter, which under aerobic conditions releases both NH_4^+ and NO_3^- . The relative immobility of NH_4^+ means that it is the NO_3^- ion that will be the first to reach nearby roots through the soil solution. In this scenario, NO_3^- provides the signal that allows roots to proliferate towards areas where less mobile forms of N are localized within the soil.

Finally, NO_3^- itself stimulates primary root growth, both directly and by antagonizing the inhibitory effect of L-glutamate (Walch-Liu and Forde 2008). This highlights that relative abundance of inorganic nitrogen (e.g., NO_3^-) and organic N (e.g., glutamate) influences RSA and therefore NO_3^- acquisition. In several studies RSA adaptation to external NO_3^- availability was observed whereby NO_3^- was shown to interact with auxin, abscisic acid, and cytokinin signaling pathways (Signora et al. 2001; Garnett et al. 2009; Krouk et al. 2010b; Vidal et al. 2010; Ruffel et al. 2011; Wang et al. 2012).

There are other attributes of a root system in addition to its architecture that dictate its capacity and efficiency for NO_3^- acquisition (Gastal and Lemaire 2002; Miller and Cramer 2005; Volder et al. 2005; Garnett et al. 2009; Hodge 2009). These include the proportion of active roots, root longevity, rooting depth, proportion of fine roots versus thick roots, and number, size, and location of the root hairs.

12.3.3 Molecular Level

12.3.3.1 Root Uptake Systems

To cope with the heterogeneity and dynamic variations of NO_3^- concentrations, plants have evolved at least three NO_3^- transport systems that function according to enzyme kinetics (Crawford and Glass 1998): (i) constitutive (active even when plants have not been previously supplied with NO_3^-) high-affinity transport systems (CHATS) with low values of both K_m (concentration of substrate that gives “half-maximum” absorption) and V_{\max} (maximum rate of absorption), typically 6–20 μM and 0.3–0.82 $\mu\text{mol g}^{-1} \text{h}^{-1}$, respectively; (ii) NO_3^- -inducible (hours to days of exposure to NO_3^-) high-affinity transport systems (IHATS) with higher K_m and V_{\max} values, typically 20–100 μM and 3–8 $\mu\text{mol g}^{-1} \text{h}^{-1}$, respectively; and (iii) constitutive low-affinity transport systems (LATS), which become evident when NO_3^- is plentiful (above 250 μM). Uptake by HATS and LATS is mediated by two families of NO_3^- transporters, NRT1 and NRT2, respectively (Wang et al. 2012). The relative contribution of these transporters to NO_3^- uptake is regulated by negative feedback, linking the expression and activity of NO_3^- uptake to the C:N status of the plant (Lejay et al. 1999; Miller et al. 2007b): under low external NO_3^- concentration, plants upregulate HATS, while under high external NO_3^- concentration or when fertilizers are applied, plants change their dependence from the HATS pathway and root–microbe associations to the LATS pathway. Like RSA, local and long-range signaling pathways regulate the activity of NO_3^- transport system in response to both external NO_3^- and sugars transported from the shoot (Forde 2002).

12.3.3.2 Vacuolar Loading and Long-Distance Transport

Once inside the root cell cytoplasm, NO_3^- can be translocated across the tonoplast and stored in the vacuoles, be loaded into the xylem vessels and subsequently unloaded in plant aerial tissues, or enter the amino acid biosynthesis pathway (Wang et al. 2012). Nitrate may be an important osmoticum solute in the vacuole of root cells (Zhen et al. 1991). Also in the base of the root growth zone, NO_3^- can be considered a significant component of the osmotic pool supporting its expansion (Bloom et al. 2012a). Long-distance NO_3^- transport (e.g., root-to-shoot NO_3^- transport) is finely tuned in response to various environmental conditions (Smirnoff and Stewart 1985). The influence of the environment on long-distance NO_3^- transport (e.g., transport from root to shoot) is evident in the different shoot and root NO_3^- concentrations found during different times of the day and between plant species (Fig. 12.2). *AtNRT1.5* mediates the first step in loading of NO_3^- into xylem vessels and facilitates root-to-shoot xylem NO_3^- transport (Lin et al. 2008). *AtNRT1.8* and *AtNRT1.9* regulate root-to-shoot NO_3^- translocation; in specific, *AtNRT1.8* mediates NO_3^- removal from xylem and may also diminish root-to-shoot

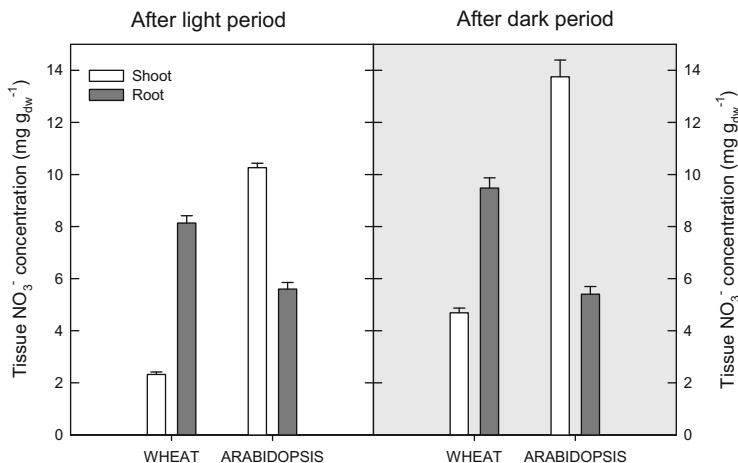


Fig. 12.2 Shoot and root nitrate concentration of wheat (14 days old) and *Arabidopsis* (40 days old) plants after the light and dark period. Wheat and *Arabidopsis* plant growth in nutrient solution containing NO₃⁻ (0.2 mM) as solely N source and in a light/dark cycle of 16/8 and 9/15 h, respectively (Bloom et al. unpublished data)

NO₃⁻ transport (Li et al. 2010), whereas *AtNRT1.9* facilitates the loading of NO₃⁻ into the root phloem and enhances downward NO₃⁻ transport in roots (Wang and Tsay 2011). *AtNRT1.9* prevents excess amounts of NO₃⁻ being transported to the shoot. For example, in different ecotypes of *Arabidopsis*, the capacity to maintain NO₃⁻ reserves under low NO₃⁻ supply confers higher tolerance to low NO₃⁻ environments (North et al. 2009). All together these studies show that roots play a key step in regulating NO₃⁻ distribution in the plant and again highlight that NO₃⁻ is a key component in the regulation of plant development and growth.

12.3.3.3 Assimilation: Where and When?

NO₃⁻ that is not transported to the shoot or vacuoles is reduced to nitrite (NO₂⁻) in the cytosol *via* NO₃⁻ reductase and then further to NH₄⁺ by NO₂⁻ reductase in the plastids (Crawford et al. 2000). NH₄⁺ is then added to C skeletons to produce glutamine and glutamate through the sequential actions of glutamine synthetase and glutamate synthase, which are located in the root plastids (Lam et al. 1996). In the root, carbohydrate oxidation provides the approximate 10 mol ATP and reductants needed for NO₃⁻ assimilation (Neuhaus and Emes 2000). The biochemical reactions responsible for root NO₃⁻ uptake and assimilation are so energy intensive that this process largely determines the carbon balance of a plant as well as its nitrogen budget (Bloom et al. 1992). Despite considerable research effort, the relative proportion of NO₃⁻ that is reduced in the shoot and root is still a matter of

considerable debate (Nunes-Nesi et al. 2010). It is known that NO_3^- assimilation may vary between the root and the shoot tissue depending on the species and the growth conditions (Miller and Cramer 2005). Generally, species native to temperate regions rely more heavily on root NO_3^- assimilation than do species of tropical or subtropical origins (Andrews 1986). Roots also appear to be the predominant site of NO_3^- assimilation when plants are grown under low external NO_3^- availability (Gojon et al. 1991; Scheurwater et al. 2002).

Timing, as well as location, of NO_3^- assimilation will have significant implications for the plant energy budget and therefore for plant performance and adaptation (Nunes-Nesi et al. 2010). During the day, shoots can use surplus light to assimilate NO_3^- and divert relatively little energy away from photosynthetic carbon assimilation and thus detract little from plant growth (McDermitt and Loomis 1981; Bloom et al. 1989). Under these conditions, NO_3^- assimilation will have lower energy cost in the leaf than in the root, because the reducing equivalents and ATP for NO_3^- assimilation are obtained without any decrease in the rate of CO_2 fixation. When light limits photosynthesis or during the night, however, no advantage will be gained in assimilating NO_3^- in the shoot, because NO_3^- assimilation and CO_2 fixation will directly compete for ATP and reductant generated by photosynthetic electron transport (Canvin and Atkins 1974), leading to a decrease in CO_2 fixation. Another disadvantage of leaf NO_3^- assimilation, independent of the light level, is that hydroxyl ions generated in the leaf during this process must be neutralized by the synthesis of organic acids (in the root, the pH balance may be maintained via decreased proton excretion or increased bicarbonate excretion) (Smirnoff and Stewart 1985). *In vitro* studies showed that day and night cycles do not influence NO_3^- reductase activities in roots, suggesting that rates of NO_3^- assimilation in the root are similar day and night (Stohr and Mack 2001). Still, the *in vivo* rates of root NO_3^- assimilation in comparison with shoot NO_3^- assimilation and the influence of light and dark cycle away its clarification. In addition, root NO_3^- assimilation will gain in both physiological and ecological importance because the elevated atmospheric CO_2 concentrations anticipated during the next few decades strongly inhibit shoot NO_3^- assimilation in C_3 plants (Bloom et al. 2010, 2012b).

Because leaf NR is a highly regulated enzyme (Lillo et al. 2004), NO_3^- assimilation is central for achieving cytosolic NO_3^- homeostasis (Cookson et al. 2005; Huang et al. 2012). Roots also maintain cytosolic NO_3^- homeostasis under deprivation and resupply of NO_3^- (Zhen et al. 1991; van der Leij et al. 1998), but the role of NO_3^- assimilation in keeping cytosolic NO_3^- homeostasis in the root has received less attention probably because the physiological and/or environmental events that lead to changes in root NR activity are less obvious.

12.4 Nitrate Use Efficiency

To achieve the doubling in global food production anticipated during the next 50 years will require a threefold increase in nitrogen fertilization rate (Frink et al. 1999). Nitrogen fertilizer will play a key role in this expansion and intensification of agriculture. Unfortunately, excess N compounds released from agricultural systems are detrimental to the environment, threatening the quality of air, water, and soil (Canfield et al. 2010). In addition, such releases are a waste of valuable resources and may cause human health problems. Today, intensification of agriculture must be done through nitrogen management strategies that do not compromise the environment (Matson et al. 1997; Godfray et al. 2010). To this end, improving the efficiency with which plants obtain nitrogen from the environment is of critical importance (Xu et al. 2012), and nitrogen use efficiency (NUE) of individual plants plays a key role. NUE has two components, nitrogen uptake efficiency (NUpE) and nitrogen utilization efficiency (NUtE) (Epstein and Bloom 2005). Roots are the key plant organ for improvement of both NUpE and NUtE (Xu et al. 2012) because they determined NO_3^- uptake and have a critical role in NO_3^- assimilation and transport to other parts of the plant. Root traits can be selected using traditional breeding and marker-assisted selection (Good et al. 2004). Natural variation of NUE in genetic resources can help to select root traits (Chardon et al. 2010). Some important root traits seem to be controlled by a single dominant gene (Werner et al. 2010); for example, overexpressing cytokinin-degrading cytokinin oxidase/dehydrogenase (*CKX*) genes resulted in an elongation of primary root and an increase in root branching and root biomass. NUpE can be improved by targeting root morphology, root-to-shoot ratios, and root NO_3^- transporters (Garnett et al. 2009; Werner et al. 2010). NUtE can be improved by targeting NO_3^- assimilation enzymes (Andrews et al. 2004) and mitochondria metabolism (Foyer et al. 2011). Nevertheless, a combination of traditional breeding and transgenic approaches will be needed to make significant improvements in NUE because of the multiple interacting genetic and environmental factors that govern NUE.

12.5 Conclusions

Nitrogen (N) is a major constituent of plant macromolecules, and nitrate (NO_3^-) is the predominant form of inorganic N available to higher plants in aerobic soils. The concentration of NO_3^- in soil solutions ranges from lower than 100 μM in natural ecosystems to higher than 10 mM in agricultural ecosystems. Nitrate, both from indigenous soil resources and from N inputs contributing to the plant-available soil N pool, varies greatly temporally and spatially. This N pool becomes the object of intense competition among plants and, in some environments, between plants and microorganisms. To cope with the heterogeneity of NO_3^- concentration in the soil,

to meet the energy requirements for its assimilation, and to secure a favorable carbon/nitrogen balance within the plant, plant roots have evolved diverse strategies for NO_3^- acquisition. These strategies extend from (i) the ecosystem level, where there are associations with some microorganisms to acquire NO_3^- and competition with others for NO_3^- ; (ii) the organism level, where plants optimize the allocation of resources between roots and shoots and the pattern of root system branching; and (iii) the molecular level, where the location (root vs. shoot) and time (diurnal cycles) of NO_3^- uptake, transport, and assimilation must adapt to the prevailing environmental conditions. Understanding the diverse strategies that plant roots employ to convert external NO_3^- to organic N will provide valuable information that can be used to improve plant N utilization efficiency and thereby enhance the sustainability of agricultural production under changing climates.

As sessile organisms, plants have evolved developmental and metabolic patterns that acclimate to the prevailing environmental conditions. In particular, plant roots adopt different strategies to acquire NO_3^- from the soil:

- Some plant roots establish associations with diverse microorganisms to ensure NO_3^- accessibility and uptake.
- Flexibility, precision, and relative turnover times of roots are important characteristics in the competition with other plants and microbes for NO_3^- .
- Plant roots sense NO_3^- as a signal. Local (external NO_3^-) and long-range (internal N status) signaling pathways adjust root system architecture and resource allocation, respectively, to the physiological state of the plant and the distribution of NO_3^- in the environment.
- Plant roots can modify their capacity to acquire NO_3^- in a range of concentrations by modulating the expression and function of genes in different NO_3^- uptake systems.
- The transport of NO_3^- between the root and shoot will determine the partition of NO_3^- assimilation between root and shoot.
- Location and timing of NO_3^- assimilation will be adjusted according to the energy budget and C metabolism.
- Recent progress in the understanding of the molecular basis of the root responses to external supply of NO_3^- suggests that root responses are largely regulated by hormone homeostasis and signaling pathways.

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