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Nitrogen-economics of root foraging: Transitive closure of the nitrate-cytokinin relay and new systemic signals for N-supply vs. demand

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**Abstract**

As sessile organisms, plants use root plasticity to forage for and acquire nutrients in a fluctuating underground environment. Here, we use genetic and genomic approaches in a “split-root” framework --- in which physically isolated root systems of the same plant are challenged with different nitrogen (N) environments --- to investigate how systemic signaling affects genome-wide reprogramming and root development. The integration of transcriptome and root phenotypes enable us to identify new mechanisms underlying “N-economy” (i.e., N-supply and demand) of plants as a system. Under nitrate-limited conditions, plant roots adopt an “active-foraging strategy”, characterized by Lateral Root (LR) outgrowth and a shared pattern of transcriptome reprogramming, in response to either local or distal nitrate deprivation. By contrast, in nitrate-replete conditions, plant roots adopt a “dormant strategy”, characterized by a repression of LR outgrowth and a shared pattern of transcriptome reprogramming, in response to either local or distal nitrate supply. Sentinel genes responding to systemic N-signaling identified by genome-wide comparisons of heterogeneous vs. homogeneous split-root N-treatments were used to probe systemic N-responses in Arabidopsis mutants impaired in nitrate reduction, hormone synthesis, and also in decapitated plants. This combined analysis identified genetically distinct systemic signals underlying plant N-economy: (i) N-supply: a newly identified long-distance systemic signal triggered by nitrate sensing, and (ii) N-demand: experimental support for the transitive closure of a previously inferred nitrate-cytokinin-shoot-root relay system that reports the nitrate demand of the whole plant, promoting a compensatory root growth in nitrate-rich patches of heterogeneous soil.

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**Introduction**

For all living organisms, the capacity to sense and adapt to environmental change is one of the foremost challenges for survival and propagation. The short-term physiological and morphological responses to fluctuations in the external nutrient environment are even more critical for sessile organisms like plants, giving a particular relevance to the network signaling involved in these adaptive mechanisms. Below ground, plant root plasticity to fluctuating environments is a primary mechanism for optimizing water and nutrient acquisition/use, and depends on the integration of local and systemic signaling. Indeed, plant roots have the ability to sense their environment, enhance their uptake/assimilation systems, and proliferate specifically in nutrient-rich zones (local signaling). This phenomenon is enhanced when the internal nutrient availability is limited (systemic signaling) (1). This dual regulation by local and systemic signaling holds true for nutrients such as nitrate (NO3-), one of the most growth-limiting nutrients. The current model depicting this dual regulation proposes that root growth/development and NO3- transport are; i) regulated locally by NO3- itself, and ii) under a systemic feedback-repression by reduced nitrogen (N)-metabolites (2, 3). One major challenge is to identify the molecular components of these local and systemic N-signaling pathways, and the mechanism for their integration that enables plant roots to properly respond to the varying environmental nutrient scenarios it encounters in the soil.

Recently, it has been demonstrated that root NO3- sensing originates from the functional activity of the NO3- transporter/sensor (transceptor) NRT1.1 (4-6). In particular, root proliferation in NO3- rich zones relies on the dual NO3-/auxin transport activity of this NO3- transceptor (NRT1.1) (7), which illustrates at a mechanistic level the intricate relationship between nutrients, hormones and growth (8). Other key regulatory components of the NO3- perception and signaling pathway have also been identified, such as transcription factors (ANR1, NLP7, SPL9) and kinases (CIPK8, CIPK23). These control root developmental and metabolic activity (*e.g.*, lateral root growth, NO3- transport/assimilation) (5, 9-13). Thus, deciphering the signaling pathways that perceive and integrate external and internal N-status will improve our understanding of how plants coordinate the different N signaling mechanisms to respond and grow in heterogeneous soil habitats.

Despite progress in understanding the nature of local nutrient signaling, the signaling mechanisms and the long-distance (systemic) signals through which a plant regulates root activity according to its nutrient status remain largely unknown (3, 14-16). The accumulation of N assimilation products (e.g., amino acids) as a negative feedback signal to mediate root activity (e.g. in particular NO3- uptake), has been proposed (17). Physiological evidence have highlighted their unequivocal role (18) and it has been shown that Glu/Gln-signaling is involved in the N-repression of lateral root outgrowth, involving mir167 and the auxin response factor, ARF8 (19). However, the direct involvement of N assimilation products in systemic N signaling has not been demonstrated (20).

Other putative systemic signals of nutrient status are hormones which have been shown to play an important role in nutrient signaling (8, 20, 21), especially in the case of nitrogen (22). A significant role for cytokinin (CK) as part of systemic N signaling has been proposed because; (*i*) NO3- supply induces an increase in CK content in the xylem of roots and shoots, due to the specific induction of *IPT3*, which encodes an adenosine phosphate-isopentenyltransferase (the first enzyme involved in CK biosynthesis) (23-25), *(ii)* CK regulates the expression of N uptake- and –assimilation related genes (8, 21, 22), as well as root architecture (26-29) and, *(iii)* CK may function as a “root-to-shoot” long-distance signal related to NO3- supply (23, 25). However, an essential experimental validation is missing, since to date, no evidence supports the role of CK as a systemic relay that integrates N-status and the regulation of root activity (N transport and architecture) (22).

In this paper, we establish that CK is a crucial component of a root-shoot-root signaling/relay mechanism involved in conveying the NO3- status of the plant as a “system”, thus enabling a compensatory increase of lateral root growth in NO3- rich zones of a root system foraging for N-resources in a heterogeneous N environment. In addition, our results led us to extend the current model saying that root architecture is under the control of a dual signaling pathway (one local and one systemic) by proposing the existence of at least two genetically independent systemic signals reporting the N-supply and demand of a plant. This has led to our coining of the term and discovery of the systemic signaling mechanisms controlling “Plant Nitrogen Economics”.

**Results and Discussion**

**The split-root system: A framework to study nitrogen-signaling network in Arabidopsis.** To study N-related systemic signaling controlling root development, we utilized the split-root system, in which a single plant is manipulated to create two physically separated root systems that can be supplied with different nutrient media to mimic a heterogeneous soil environment (2, 4, 30-32). Because NO3- is an essential, growth-limiting nutrient and a key signal for gene expression, metabolism, growth and development (3, 33-36), we focused on the different responses of *Arabidopsis* when the NO3- concentration was varied between physically isolated root systems (Fig. 1A). We quantified root architecture in three different NO3- environments: (i) a homogeneous N-replete environment (C.NO3: both compartments have 5 mM KNO3), (ii) a homogeneous N-deprived environment (C.KCl: both compartments have 5 mM KCl), and (iii) a heterogeneous split environment (Sp.NO3/Sp.KCl: one compartment has 5 mM KNO3, and the other 5 mM KCl), from 2 to 4 days after transfer to these conditions. There were no significant differences in the main root (that we considered hereafter as a primary root = PR) length in any of the conditions, showing that root plasticity largely targeted lateral roots (LR) under these growth conditions (Fig. S1).

Overall, roots adopted either an “active-foraging strategy”, characterized by outgrowth of LRs in the homogeneous Sp.NO3 and C.KCl conditions, or a “dormant foraging strategy” characterized by a repression of LRs outgrowth in the disparate Sp.KCl and C.NO3 conditions. (Note: A thorough analysis of root development under these diverse conditions is provided in Supplemental Text ‘Root development in Split conditions‘). It is noteworthy that the plant as an integrated system maintained a constant level of root proliferation within the compartments that contained NO3-, as the total LR length in the homogeneous Sp.NO3 compartment was virtually the same as the total LR length in both compartments of the homogeneous C.NO3 roots combined (2.29±0.21 cmLR.PR-1 *vs.* 1.07±0.15 cmLR.PR-1 x 2 root parts=2.15 cmLR.PR-1; Fig. 1B). Altogether, the LR responses in this split-root system seem to display a logical overall adaptive strategy that plants use to optimize nutrient acquisition in different environments. These responses fit with the current model of dual regulation by local NO3- and systemic feedback repression (2, 3); *i.e.*, (i) C.NO3 roots are under a systemic feedback repression in response to high NO3- supply, (ii) In split-root plants that are exposed to NO3- only on one half of their root system, the level of this systemic repression is lower – compared to plants exposed to a homogeneous N-supply. In the split-root case, the combination of a low systemic repression and local NO3- availability leads to LR outgrowth in Sp.NO3 conditions, whereas Sp.KCl roots are only subjected to the systemic repression and, (iii) ??? What comes here??? In C.KCl plants, the systemic repression of LR growth is totally absent, leading to root proliferation. The findings support the hypothesis of the previous model for the control of root development by local and systemic signals (2).

To further our understanding of the molecular underpinnings of these responses to N-supply and demand that enable plants to direct root growth to the more effective N-use area and support plant growth as an integrated “system”, we performed genome-wide analysis of transcriptional changes occurring in short- and long-term exposure to N-supply and -deprivation in the split-root experimental framework.

**Genome-wide reprogramming in response to local *vs.* systemic nitrogen environments**. To understand the molecular basis of the integration of local vs. systemic signals, we undertook a transcriptomic approach across the panel of split-root conditions presented above. RNA from roots of plants exposed to distinct combination of local and distal N-signals (C.NO3, Sp.NO3, Sp.KCl and C.KCl) was extracted at early time points (2 hrs & 8 hrs) and at later time points (2 days), after the beginning of the –N or +N treatment. These early time points were selected in an effort to sample early responses and the dynamics of regulatory change before any significant changes in root morphology. A three-way Analysis of Variance (ANOVA) was performed to statistically analyze these data as a whole (*e.g.*, nitrogen effect, split-root effect, time effect) (Supplemental Text). From this ANOVA analysis, we identified a set of genes whose N-responses were altered by the split-root conditions, *i.e.*, genes that showed a significant interaction between NO3- availability (*i.e.*, presence or absence) and split-root conditions (*i.e.*, homogeneous or heterogeneous): “N-interaction set” of 123 genes (q-val<0.2 and p-val< 0.001; Table S1).

The expression of these 123 genes is significantly affected by a systemic N-signal (*e.g.*, of N supply or demand). The expression values were used to cluster the corresponding split-root experiments on a dendrogram, in order to probe dominant trends in gene expression. At the earliest time-point (2 hrs), this experiment-wise clustering paired the two NO3- treatments together (C.NO3 and Sp.NO3), showing that genes responded at first to the local NO3- environment (Fig. 1C). However, by the later time-points (8 hrs and 2 days), large-scale changes in genome-wide expression among the 123 genes re-arranged the dendrogram of experimental treatment by unexpectedly pairing the Sp.NO3 with the C.KCl treatments, and the C.NO3 with the Sp.KCl treatments (Fig. 1C). This unexpected genome-wide resemblance of disparate conditions closely parallels that observed with LR architecture after 4 days in the same treatments (Compare Fig. 1B and C). Thus, the genes affected by the interaction between NO3- availability and split conditions initially respond to the local root N-environment, but are later controlled by systemic regulatory signals that integrate information about N-status from other parts of the plant. The overall effect is to orchestrate a revised and apparently more effective genome-wide strategy in which a set of molecular changes precedes change in LR architecture in response to system-wide integration of N-systemic signal.

These molecular responses observed at the level of the transcriptome, correspond to the morphological responses of the root, and appear to represent a coordinated strategy to anticipate assimilation of newly foraged N. Indeed, despite the different local NO3- conditions, the N-foraging roots (Sp.NO3 and C.KCl) both showed an induction of genes involved in N-uptake and -assimilation, such as *AtNRT3.1* (*NAR2.1*/*WR3*) and *NIR1* (*Nitrite Reductase* *1*) and induction of genes in providing reducing equivalents for N assimilation, such as *G6PD3* (*Glucose-6-phosphate Dehydrogenase 3*) or FNR2 (*Root Ferredoxin:NAPD(H) oxidoreductase 2*) (Table S1). Previously, split-root experiments using *Medicago* (4 days post-treatment) identified these same sentinel genes, among others, as responding to a N-related systemic signaling in addition to a local NO3- signal (31). Interestingly, in the present study, we show that these genes are rapidly regulated by the split-root N-treatment conditions (within 8 hrs), suggesting that their regulation is likely among the first targets of systemic N-signaling, and not a long-term consequence of root adaptation to physiological modifications triggered by the split-root treatment. Overall, these results indicate that systemic signals rapidly (within hours) communicate the NO3- status of the whole root system to alter the expression of a subset of genes mainly involved in N-metabolism and that later changes in genome expression ultimately result in alterations in root architecture.

Surprisingly, very few genes known to be directly involved in LR development or growth were found among this set of systemically-regulated genes (Discussed in Supplemental Text). However, we do not rule out the possibility that genes categorized in “N-metabolism function” have a direct role in LR architecture response. For example, the NO3- transporter *AtNRT2.1* has a role in LR development independently of its NO3- uptake function (37, 38), and has been previously identified as a main target of N-related systemic signaling (18, 39). Indeed, we confirmed the early transcriptional regulation of this gene by the systemic signals using Q-PCR assays (Fig. S2) and revealed an expression pattern of *NRT2.1* that is similar to its functional partner *AtNRT3.1* (40).

**The coordinated molecular and morphological responses triggered by the split-root conditions are driven by NO3- itself and a shoot integrated systemic signal**. A central question is to determine which signals mediate the root growth adaptations to the different levels of NO3- supply in the environment, with respect to gene expression and LR architecture. To efficiently monitor the N x split-root systemic interaction response in a number of different conditions (e.g. mutants and treatments), we identified a set of 8 sentinel genes that responded robustly and showed the same pattern as the dominant trend of the “N-interaction set” of the 123 genes (identified above from the ANOVA analysis), as well as the LR responses in the four types of compartments (*i.e.*, genes up-regulated in Sp.NO3 and C.KCl compared to C.NO3 and Sp.KCl compartments; Supplemental Text). Interestingly, the genes whose expression best correlate with LR architecture largely belong to the NO3- uptake/assimilation functions (Supplemental Text).

Since both NO3- and its downstream assimilates have been implicated in mediating morphological and molecular responses (2, 18, 31, 41, 42), we tested their distinct roles in the split-root responses using an Arabidopsis double mutant in which Nitrate Reductase (*NIA1* and *NIA2* genes) activity is abolished (41). Interestingly, the NR-null mutant still exhibited the usual N-regulated response of the 8 sentinel genes at the 8 hr time-point (Fig. 2A-B). This result shows that NO3- itself, rather than the NO3—assimilates, is sufficient in our conditions to mediate the complete set of early transcriptional N-regulated reprogramming. Therefore, our results demonstrate again that these growth changes are supported by dedicated signaling pathways anticipating (thus independent of) any change in the nutritional status of the plant (8).

To our knowledge, this is the first time that definitive evidence for the role of the shoots themselves in this long-distance root-shoot-root N-signaling has been presented (e.g., direct root-to-root could technically have been invoked in (31, 32) ). We determined that the roots of decapitated plants indeed completely lost the response to the N-systemic signaling, but still responded to local NO3- conditions (Fig. 2A and C). Taken together, these investigations imply that root foraging responses rely on the perception of the system NO3- imbalance/absence of the whole plant and are mediated through a verified root-shoot-root signaling mechanism.

**Cytokinin biosynthesis is essential for root-shoot-root signaling triggering the compensatory root responses to partial NO3- limitation.** How does NO3- as a signal of N-supply (presence) or demand (absence) mediate/amplify a system-wide plant growth response?To date, there have been two types of data linking CK as a second messenger of NO3- signaling. First, CK has been shown to be a root-to-shoot NO3- derived messenger that modulates shoot growth (22-24, 43). Secondly, CK has been shown to control several aspects of N-nutrition, including NO3- -transport and -assimilation steps (thoroughly reviewed and commented in (8, 21, 22)). Thus, it was tempting to speculate - by transitive closure - that NO3- controls CK content that in turns feeds-back on N nutrition (8, 22). However, experimental evidence showing a defect in N-signaling itself in response to a mutation in CK signaling pathway remains to be demonstrated. Since our experimental split-root framework allows us to uncover systemic N-signaling, it represents an ideal experimental design to address the question of the role of CKs in systemic NO3- root-shoot-root signaling.

To test the connection between CK and the N-systemic responses in our experimental system, we repeated the split-root treatments in an Arabidopsis triple mutant for ATP/ADP isopentenyltransferases (*ipt3,5,7*), which has severely reduced CK biosynthesis (28). We first tested the impact of the CK synthesis mutations on the response of the 8 sentinel genes regulated at early time-points in response to systemic signaling triggered by NO3-. Strikingly, we observed that the *ipt3,5,7* mutant was impaired *only* in the differential response between C.NO3 and Sp.NO3, but not in the differential response between C.KCl and Sp.KCl (Fig. 3A-B). Given that this result favored a specific role for CK in the systemic integration of the NO3- status available to the whole root system, our reasoning was that the experimental application of CK specifically to the NO3- compartments (both root compartments of the C.KNO3 plants and only the Sp.NO3 compartment for the Split plants) would mimic the NO3- imbalance in the CK biosynthesis triple mutant. Indeed, the induction of the 8 sentinel genes was restored in Sp.NO3 roots, when CK was supplied to the NO3- -containing compartments (Fig. 3A-C).

Development-wise, we observed that the total LR length in C.NO3, Sp.KCl and C.KCl were unchanged between the wild-type and the *ipt* triple mutant in CK synthesis, ruling out the possibility that the mutant caused a general root growth defect (Fig. 3D). However, as found at the transcriptome level, LR growth stimulation was lost in the Sp.NO3 compartment, compared to C.NO3 (1.46±0.18 cmLR.PR-1 *vs.* 1.28±0.12 cmLR.PR-1, not significant) in the *ipt3,5,7* mutant compared to wild-type, but the stimulation in LR growth in the C.KCl compared to the Sp.KCl, was maintained (2.06±0.17 cmLR.PR-1 *vs* 0.75±0.06 cmLR.PR-1; p-val=4.10-6) (Fig. 3D).

These results led to two conclusions. First, root responses to systemic N-status can no longer be explained by the existence of only one systemic signal as previously proposed (2), but requires the existence of at least two genetically independent systemic signaling pathways. From the evidence above, we propose that the differential response between C.NO3 vs. Sp.NO3 relies on a systemic N-demand signal (-N) whereas the differential response between C.KCl vs. Sp.KCl relies on a systemic N-supply (+N). Second, we identified for the first time an essential component of the systemic N signaling, by demonstrating that the N-demand signal depends on CK biosynthesis. In the N-economics model described below, we develop and discuss the role of –N and +N systemic signals and their interplay in mediating the response of the plant as an integrated system.

**Plant nitrogen economics: A model for systemic signaling of nitrogen-supply and demand**. In this study, we integrated the split-root experimental framework with genomic and genetic approaches, in order to decipher N-related systemic signal controlling root architecture. Overall, the dissection of systemic signaling supports the existence of distinct systemic signals controlling plant N-economics, in which plants balance and respond to N-supply (+N) and N-demand (-N) to efficiently control root growth and the expression of N uptake/assimilation genes, as depicted in Fig.4. Our results build a new model for plant N-economics that proposes the co-existence of systemic signals for both N-supply and N-demand. Our data provided the main following components in support of this “plant nitrogen economics model”: i) *Systemic signals for N-supply and demand*: the LR growth differences observed between root compartments exposed to distinct N-supply/demand environments highlighted the occurrence of several types of N-related systemic signaling, for which the genetic independence has been proven by using the *ipt3,5,7* Arabidopsis mutant in CK synthesis (Fig. 4). Specifically, the differential response between C.NO3 vs. Sp.NO3 highlighted a CK-dependent systemic N-demand (–N signal), whereas the differential response between C.KCl vs. Sp.KCl highlighted the existence of a previously unknown systemic N-supply (+N signal) (Fig. 1B), ii) *NO3- supply is the signal for N-supply and demand*: using an Arabidopsis NR-null mutant (41) in split-root experiments (Fig. 2B), we showed that both local- and distal-NO3- signaling responses are preserved, indicating that NO3- is the signal for both sides of the N economics equation in plants, and iii) *N-supply and demand signals involve a root-shoot-root relay*: decapitation experiments showed that while local NO3- responses are preserved in shootless plants, the systemic signals for distal N-supply or distal N-demand are both lost, invoking a root-shoot-root relay for each (Fig. 2C).

The –N systemic signal for N-demand of our N-economics model (C.NO3 vs. Sp.NO3), has been highlighted in previous studies where root morphological responses were measured (2, 4, 30-32). In our new study, the ability to monitor both root and transcriptome responses, enabled us to identify for the first time a genetically independent +N-systemic signal for N-supply that accounts for the differences between C.KCl and Sp.KCl conditions, the latter of which is exposed to a distal N-supply. Moreover, these differences also imply that a different –N signal (local or systemic) occurs in response to total N-deprivation (in C.KCl), which is distinct from the –N signal perceived in Sp.NO3, since it is not dependent on CK. In the Sp.KCl scenario, this N-deprivation signaling system is repressed by the +N systemic signal (from Sp.NO3) and accounts for the regulation of expression of N-deprivation sentinels such as NRT2.5 (44) (Fig. S3). As such, our study, which combines for the first time split-root conditions, root morphology and genome-wide transcriptome analysis, enabled us to discover new signals for N-supply and demand and to refine previous hypotheses.

Finally, our studies provide the first experimental evidence to support a “transitive closure” for the role of CK in mediating the shoot-root systemic N signal controlling N-uptake/assimilation and LR growth. In previous studies, NO3- (A) was shown to induce (B) CK synthesis, providing evidence for the relationship A->B (22). Previous studies also showed that CK (B) supplied to plants could regulate (C) the expression of genes involved in N-uptake/assimilation and root development, providing experimental evidence for the relationship B→C (22). The transitive closure of A→B and B→C, suggests (by transitive closure) a relationship of A→B→C, but this important relationship in the NO3-/CK relay had not been experimentally validated. The inference of this relationship is noted by the dashed lines in the model in Fig.4 of Kiba et al. which postulates a nitrate-cytokinin relay (22). In our study, by combining the split-root system, NO3- treatment, and a CK biosynthesis mutant, we provide the first experimental evidence that supports the transitive closure of NO3- → CK (systemic signal) → root-shoot-root signaling → activation of root responses, including N-uptake/assimilation and LR development.

CKs and its antagonistic partner, auxin, are well known to act in concert to tune plant development (45). Thus, it will be of interest to integrate the recent findings on the role of auxin signaling in the control of LR development by local NO3- availability, with the work on CK and systemic N signaling presented herein. On one hand, NO3- promotes (through the action of NRT1.1) auxin accumulation in lateral roots, which promotes its elongation (7). On the other hand, CK synthesis is necessary to induce LR development in response to a systemic –N signal (Fig. 3). According to our results, the role of CKs in a root-shoot-root communication is clear, but several scenarios can be hypothesized for the exact location of their actions. Since we show that systemic N-demand signal is lost in decapitated plants, it is very likely that CKs act in shoots. Given that NO3- provision induces CK production and translocation towards the shoots (23-25), we believe that CKs play an important role as an integrator of the NO3- status in shoots. However, whether the CKs themselves function in the shoot-to-root relay –or as yet unknown upstream and downstream signals of CK- function there remains to be experimentally explored. Preliminary results tend to indicate that it is likely that another signal downstream of CK plays a role in this shoot-root N-demand signal. Indeed, by examining the type-A ARR genes, which constitute a family of primary CK-response genes (46), we observed that these genes are globally regulated by the local NO3- presence in roots (up-regulation in C.NO3-Sp.NO3 vs. Sp.KCl-C.KCl; microarray data), whereas ARR expression is up-regulated in proportion to global NO3- levels in shoots (up-regulation in Control.NO3 vs. Split vs Control.KCl; Q-PCR assays) (Fig. S4). As auxin transport from shoot-to-root is believed to be a reporter of N-status of the plant (20), and that CK may control auxin transport and synthesis (47-49), it is tempting to hypothesize that auxin may be part of the long-distance signal informing the roots of the integrated N-status of the shoot. Furthermore, since auxin is taken up by NRT1.1 locally to stimulate root development, according to the local NO3- environment, the combination between the auxin and CK models would provide a large panel of developmental programs of N-economics of root development described herein.

**Materials and Methods**

**Plant materials.** All *Arabidopsis thaliana* plants were in Columbia background, the wild-type used in this study. The *CycB1::GUS* line used to measure the lateral roots initiation events, the *NR-null* mutant in nitrate reductase (*chl3-5/nia1-2*) and the *ipt3,5,7* triple mutant in CK biosynthesis were respectively obtained from Philippe Nacry (Biochemistry and Plant Molecular Physiology at Montpellier, France), Nigel Crawford (University of California at San Diego, USA) and Sabrina Sabatini (University ‘La Sapienza’ at Rome, Italy).

**Split-root system and treatments.** Split-root conditions applied to Arabidopsis have been adapted from (4). Details of the procedures used are given in Supplemental text.

**Analysis of root growth.** Two, three, and four days after the transfer of the split-root plants to selected treatment media, a minimum of 10 plates for each condition was scanned at 400 dpi (Epson Perfection V350 Photo). Root growth was analyzed as previously described (4). Statistical comparisons of means between treatments and/or genotype were performed using the student’s t-test. Each experiment including wild-type and/or mutants was performed twice, displayed the same result and one experiment was shown.

**Analysis of genome-wide expression.** Genome-wide expression was performed using Affymetrix (ATH1) and for selected examples by (Q-PCR). Statistical analysis has been performed according to (13). Details of the procedures are given in Supplemental text.

The Affymetrix Microarrays data have been deposited in NCBI’s Gene Expression Omnibus in compliance with MIAME standards (http://www.ncbi.nlm.nih.gov/geo/) and are accessible through Gene Expression Omnibus Series accession number GSE22966.

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**Figure legends**

Fig. 1. *Arabidopsis* roots display a coordinated morphological and molecular strategy in response to a heterogeneous NO3- environment. (A) Diagram shows the physical split-root experimental set up used to detect N-related systemic signaling. Such roots are subjected to three different treatments: ‘Control KNO3’ plants received KNO3 on both sides of the root system (C.NO3), ‘Control KCl’ plants received KCl on both sides (C.KCl), and ‘Split’ plants received KNO3 (Sp.NO3) on one side and KCl (Sp.KCl) on the other. The gray line in each set-up represents a physical gap between the media in the two compartments that keeps conditions on the two sides isolated. (B) Lateral root (LR) responses in the split-root treatments showing the total LR proliferation in each of the four distinct conditions. At the top, the bar graph depicts the total LR length (cm) normalized by the length of the primary root (PR) (cm) as cmLR.PR-1. For C.NO3 and C.KCl, measurements on both 1/2s of the root systems were pooled and averaged. Each bar graph represents the mean of at least 10 roots. The different letters on top of the bars indicate statistically significant differences (p≤0.05; t-test). Error bars=standard error. At the bottom, one representative set of LRs illustrating the trends in LR length in the different treatments is shown. (C) Genes whose NO3- response was altered in the split-root experiments showed a similar pattern of change as LRs. The heat map depicts the expression pattern of 123 genes that showed an interaction between NO3- availability and split conditions in ANOVA. The same set of genes was used to generate dendrograms to cluster experiments at the different time-points. The numbers at each node in the dendrogram represent bootstrap values from permutation tests.

Fig. 2. The coordinated response of roots in these disparate N-environments requires sensing of NO3- itself and is mediated through the shoot. For each of the 3 panels, the bar graph represents the relative mRNA levels of the *Glucose-6-Phosphate Dehydrogenase 3* (*G6PDH3*) gene, and the line graph represents the relative mRNA accumulation of the 8 genes used to monitor N and split root interaction effects (as described in text). The asterisks in between the two bars indicate significant differences between the corresponding two compartments. The numbers on the line graph are the average percentage of relative mRNA accumulation increase for the 8 genes, either between systemic N-signaling (Sp.NO3 vs. C.NO3) or (C.KCl vs. Sp.KCl\_, or between total NO3-  (C.NO3 + Sp.NO3) vs total KCl (C.KCl + Sp.KCl). Trends are shown for (**A**) the wild-type background (plants were grown in the conditions used for the NR-null mutant), (**B**) the *NR-null* mutant (41) in which Nitrate Reductase activity is abolished and, (**C**) wild-type roots of plants decapitated at the time they were transferred to the split or homogeneous treatments. All roots were harvested for RNA expression analysis 8 h after treatment.

Fig. 3. CK mediates coordination of root responses in a heterogeneous environment by allowing a compensatory LR growth in the NO3- rich area. Expression of *G6PDH3* and the 8 sentinelgenes were assayed by Q-PCR in the standard set of treatments used in (**A**) the wild-type, (**B**) the CK synthesis mutant *ipt3,5,7* background (28), and (**C**) the *ipt3,5,7 mutant* in which CK was added back to the roots in the NO3- compartments. The asterisks indicate the significant differences between two compartments. The numbers on the line graphs are the average percentage of relative mRNA accumulation increase for the 8 sentinel genes, either between Sp.NO3 and C.NO3, C.KCl and Sp.KCl. N.A.=Non Applicable. (**D**) Total LR length (cm LR.PR-1) is shown in WT compared to the *ipt3,5,7* mutant. The different letters on top of the bars indicate statistically significant differences (p≤0.05; t-test). Error bars=standard error

**Fig. 4.** A model of systemic signals involved in plant nitrogen economics. Systemic signals for N-supply and N-demand that control NO3- metabolism genes and LR development in plants exposed to heterogeneous nitrogen environments. We propose the existence of several systemic signals to account for a systems wide integration of nitrogen economics coordinating the root responses in heterogeneous N-environments: 1. An inductor CK dependent N-demand (-N) signal (in pink), 2. A repressive N-supply (+N) signal in Split-root plants (in black) and, 3. A N- starvation signal that is either local or systemic signal in C.KCl conditions and that is CK independent (in blue). These systemic signals of N-supply and N-demand, act likely in combination with NO3- local signal (in red), to control root molecular and developmental phenotypes and coordinate a plant systemic wide response to its perceived nitrogen economics.