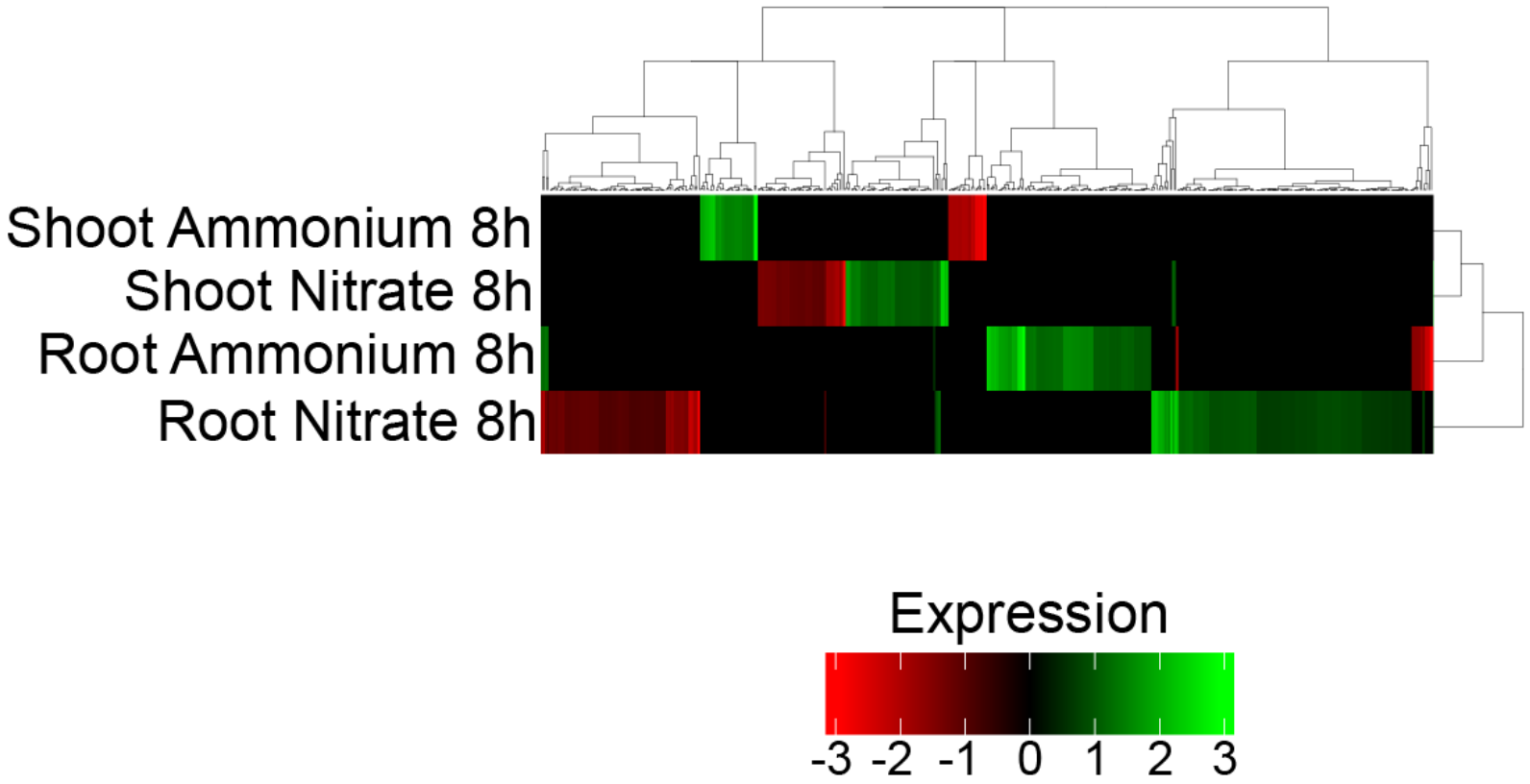
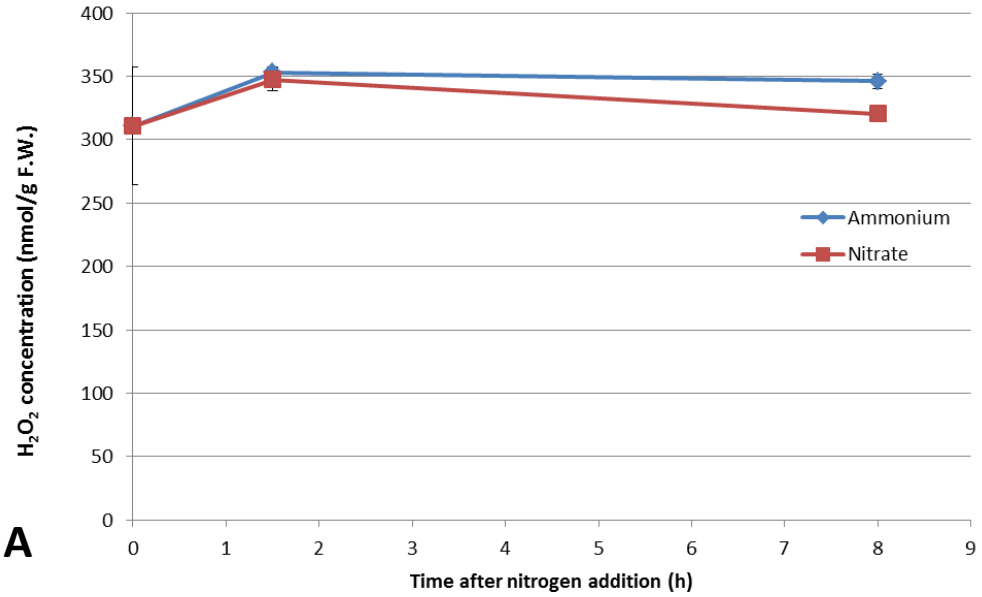


Figure S1: Organ specificity in transcriptional responses to nitrate and ammonium. The heat map includes microarray data for genes identified as specifically responsive to nitrate or specifically responsive to ammonium in *Arabidopsis* roots (Patterson et al., 2010) and shoots (this work). In all cases, plants were treated with ammonium or nitrate for eight hours, with a comparison to controls that received no nitrogen. Normalized expression values (fold change 8h/0h) were $\log_2$ transformed, scaled from -3 (repressed) to 3 (induced), and mapped using the R packages "ggplot2" and "Stats" (dendogram distances determined using the Euclidean method). No genes displayed an "ammonium specific" response in both roots and shoots. Seven genes displayed a "nitrate-specific" response in both roots and shoots. The "shared" genes in root and shoot nitrate response are: At1g70780, At2g16660, At2g26980, At3g46880, At3g48100, At4g33960, and At4g37180.

Patterson K, *et al.* (2010) Distinct signalling pathways and transcriptome response signatures differentiate ammonium- and nitrate-supplied plants. *Plant Cell Environ* 33(9):1486-1501.



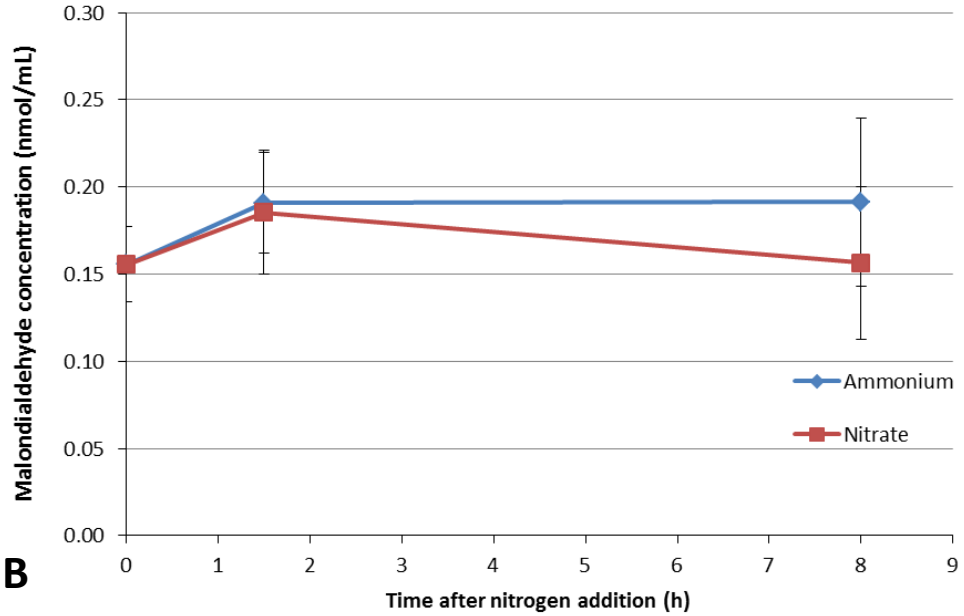


Figure S2: Short-term ammonium and nitrate treatment do not induce oxidative stress in *Arabidopsis* shoots. **A.** Hydrogen peroxide levels in shoots under ammonium and nitrate nutrition. **B.** Quantification of lipid peroxidation in shoots under ammonium and nitrate nutrition using a thiobarbituric acid reactive substances (TBARS) assay. In both experiments, nitrogen-limited plants were supplied with either ammonium or nitrate (1 mM) for the time periods indicated. All data points represent means ± SEM (n = 3). The hydrogen peroxide assay was performed as described in Shin et al. (2005) and the TBARS assay was performed as described in Umbach et al. (2005).

Shin R, Berg RH, & Schachtman DP (2005) Reactive oxygen species and root hairs in Arabidopsis root response to nitrogen, phosphorus, and potassium deficiency. *Plant Cell Physiol* 46: 1350-1357.

Umbach AL, Fiorani F, & Siedow JN (2005) Characterization of transformed *Arabidopsis* with altered alternative oxidase levels and analysis of effects on reactive oxygen species in tissue. *Plant Physiol* 139: 1806-1820.

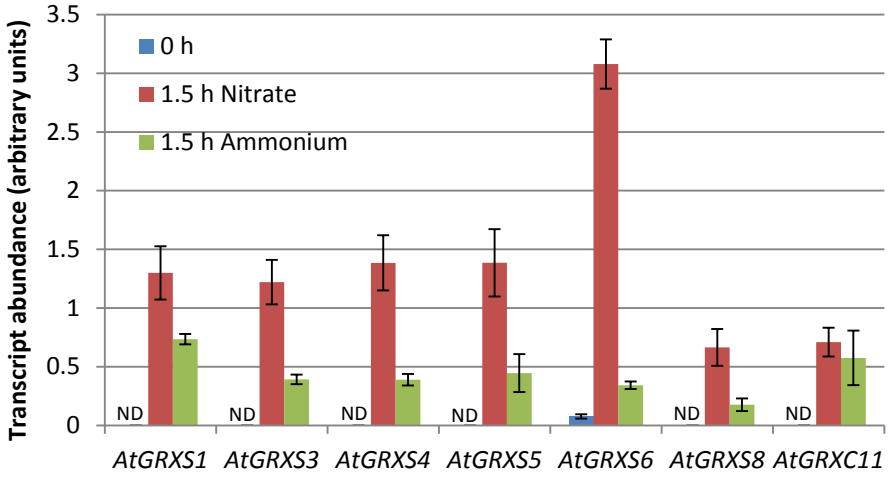


Figure S3: Regulation of glutaredoxin gene expression in *Arabidopsis* roots. Hydroponically-grown plants were nitrogen starved for 26 h and then supplied with either KNO_3 or $(NH_4)_2SO_4$ for 1.5 h. The "0 h" treatment represents nitrogen-starved plants just prior to nitrate or ammonium addition. RNA was isolated from the roots of nitrate-treated and ammonium-treated plants and then glutaredoxin transcript abundance was measured by real-time RT-PCR. Data points represent means ± SEM (n=3). ND = Not detected.

A

	AtGRXS3	AtGRXS4	AtGRXS5	AtGRXS8
AtGRXS3		89.3	91.3	91.6
AtGRXS4	93.1		89.0	88.4
AtGRXS5	95.1	94.1		88.4
AtGRXS8	93.1	92.2	91.2	

B

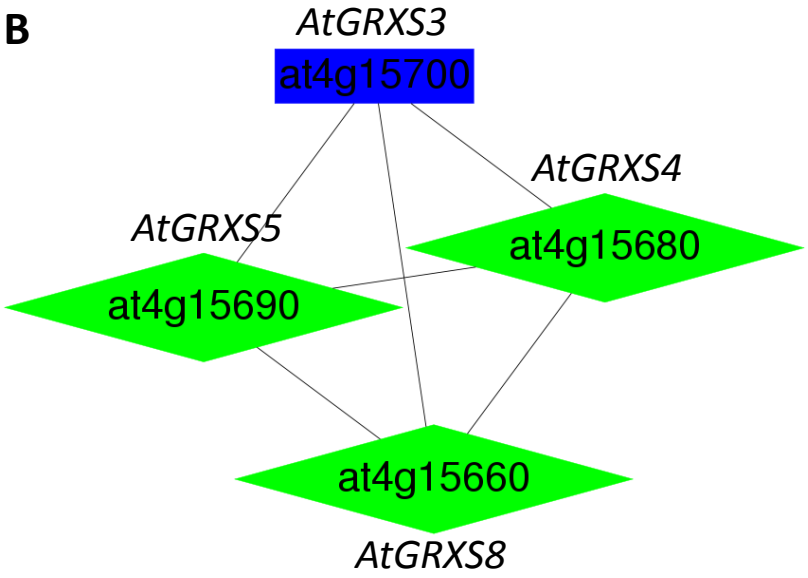


Figure S4: Sequence and regulatory conservation of *AtGRXS3*, *AtGRXS4*, *AtGRXS5*, and *AtGRXS8*. **A.** Nucleotide (yellow) and amino acid (orange) sequence identity of the *AtGRXS3*, *AtGRXS4*, *AtGRXS5*, and *AtGRXS8*. **B.** Co-expression network of *AtGRXS3*. The query gene (*AtGRXS3*/At4g15700) is shown in blue and the co-expressed genes are shown in green. Only genes with Pearson correlation coefficients ≥ 0.75 are shown. Co-expression network was generated using GeneCAT (Mutwil et al., 2008).

Mutwil M, Obro J, Willats WG, & Persson S (2008) GeneCAT--novel webtools that combine BLAST and co-expression analyses. *Nucleic Acids Res* 36(Web Server issue):W320-326.

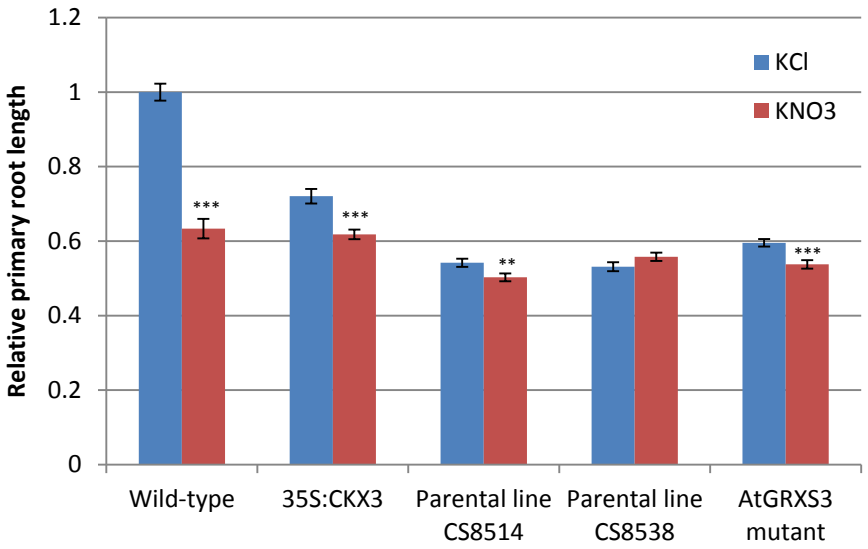


Figure S5: Nitrate-mediated inhibition of root growth in the *AtGRXS3* mutant and the *35S:CKX3* transgenic line. Relative primary root length of 12-day-old wild-type (Columbia-0), *35S:CKX3* (Columbia-0 background), *AtGRXS3* mutant (Nössen background), and *AtGRXS3* mutant parental lines CS8514 and CS8538 (Nössen background) are shown. Plants were grown for nine days in basal ammonium succinate medium and then 5 mM KNO_3 or KCl was added to the medium. After three days, root length was measured. Data was combined from three separate experiments, with raw root length data normalized individually for each experiment by setting the average root length of wild-type plants supplied with KCl equal to 1.0. Data points represent means ± SEM ($n \geq 32$).). Asterisks indicate significant differences (***$P \leq 0.001$; **$P \leq 0.01$) compared to KCl-treated plants of the same genotype.

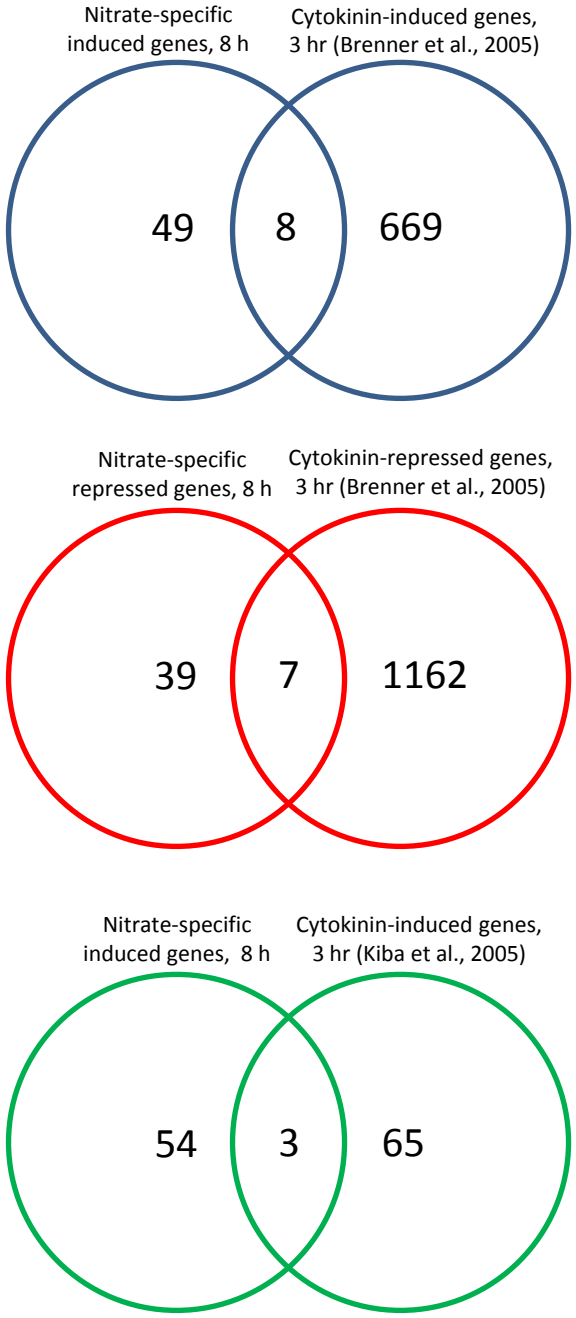


Figure S6: Comparison of global transcriptional responses to nitrate and cytokinin. "Nitrate-specific induced" and "nitrate-specific repressed" gene lists were taken from this work, while "cytokinin-induced" and "cyotkinin repressed" gene lists were taken from Brenner et al. (2005) and Kiba et al. (2005). In the nitrate-induced/cytokinin-induced (Brenner) comparison, three of the eight "shared" genes are glutaredoxins (*AtGRXS4*, *AtGRXS5*, *AtGRXC11*). In the nitrate-induced/cytokinin-induced (Kiba) comparison, two of the three "shared" genes are glutaredoxins (*AtGRXS4*, *AtGRXS6*).

Kiba T, *et al.* (2005) Combinatorial microarray analysis revealing *Arabidopsis* genes implicated in cytokinin responses through the His->Asp phosphorelay circuitry. *Plant Cell Physiol* 46(2):339-355.

Brenner WG, Romanov GA, Kollmer I, Burkle L, & Schmulling T (2005) Immediate-early and delayed cytokinin response genes of *Arabidopsis thaliana* identified by genome-wide expression profiling reveal novel cytokinin-sensitive processes and suggest cytokinin action through transcriptional cascades. *Plant J* 44(2):314-333.

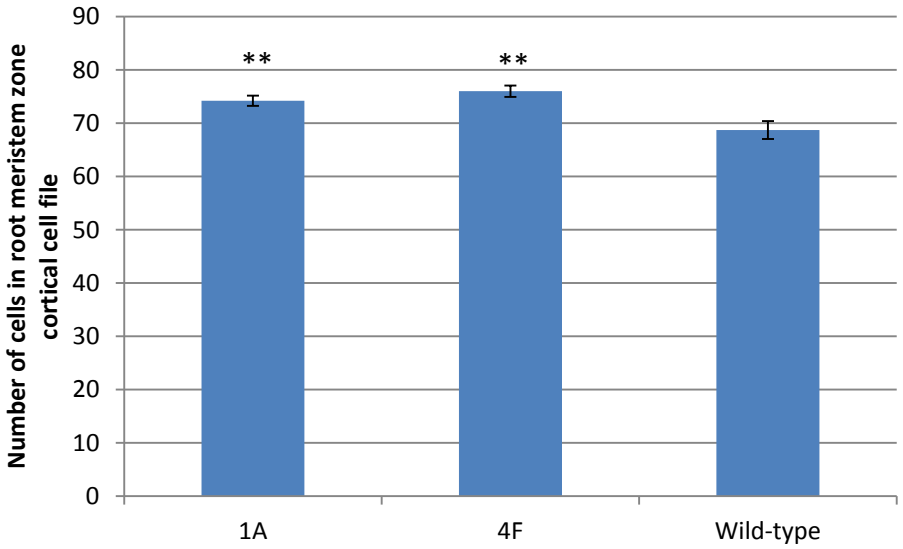


Figure S7: Increase in the number of root meristem cortical cells in *AtGRX3* RNAi lines (1A, 4F). Data points represent means ± SEM (n ≥ 19). All plants were ten-days-old and were grown on vertically-oriented plates. ** = P ≤ 0.01 compared to wild type. Whole roots were stained for three minutes in 10 µg/ml propidium iodide, rinsed twice in distilled water, and then mounted in 5% (v/v) glycerol for confocal microscopy. Confocal microscopy analysis was performed as described by Dello Ioio et al. (2007).

Dello Ioio R, *et al.* (2007) Cytokinins determine *Arabidopsis* root-meristem size by controlling cell differentiation. *Curr Biol* 17(8):678-682.