

# Nitrate and ammonium affect the overall maize response to nitrogen availability by triggering specific and common transcriptional signatures in root

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## Supplementary information

**Figure S1:** Distribution and percentage of differentially expressed genes (DEGs) identified ( $\log_2$  FC  $> |0.58|$ ; FDR  $\leq 0.05$ ) by RNA-Seq analysis from the comparison between  $\text{NO}_3^-$  or  $\text{NH}_4^+$  supplied maize seedlings for 24 h with respect to the control (-N, nitrogen deficient solution). Data are shown as percentage of genes differentially expressed in response to each treatment in  $+\text{NO}_3^-$  treatment (A) and  $+\text{NH}_4^+$  (B) on the total amount of DEGs. DEGs were classified as up-regulated according to their  $\log_2$  fold change values (a  $\log_2$ FC threshold  $> |0.58|$  was set, corresponding to a 1.5-fold change increase or decrease in expression). Among the up- and downregulated DEGs, several ranges of induction or repression are shown as  $\log_2$  of the gene expression fold changes.

**Supplementary dataset 1:** Cuffdiff complete results of pairwise differential expression analyses ( $+\text{NO}_3^-$  vs -N;  $+\text{NH}_4^+$  vs -N).

**Supplementary dataset 2:** Complete results of GO enrichment analysis for the DEGs included in each cluster.

**Table S1:** Summary of reads obtained by RNA-Seq analysis. For each treatment ( $+\text{NO}_3^-$ ,  $+\text{NH}_4^+$ ) or control condition (-N), three biological replicates were processed (R1, R2, R3).

**Table S2:** Expression matrix of differentially expressed genes (DEGs) showing a  $\log_2$  fold change ratio  $> |0.58|$  and a false discovery rate (FDR)-adjusted p-value  $\leq 0.05$  in at least one treatment that were included in the hierarchical clustering analysis. RPKM expression values in  $+\text{NO}_3^-$ , -N and  $+\text{NH}_4^+$  samples and clustering results are reported for each of the 2324 DEGs.

**Table S3:** List of some selected differentially expressed genes (DEGs) showing a  $\log_2$  fold change ratio  $> |0.58|$  and a false discovery rate (FDR)-adjusted p-value  $\leq 0.05$  in at least one treatment that are related to N transport and metabolism. RPKM (Reads Per Kb per Million) expression values in

+NO<sub>3</sub><sup>-</sup>, -N and +NH<sub>4</sub><sup>+</sup> samples, hierarchical clustering results and log<sub>2</sub> fold change ratio are reported.

**Table S4:** List of representative genes from selected GO terms overrepresented among DEGs specifically responsive to NH<sub>4</sub><sup>+</sup>. RPKM: Reads Per Kb per Million.

**Table S5:** List of representative genes from selected GO terms overrepresented among DEGs specifically responsive to NO<sub>3</sub><sup>-</sup>. RPKM: Reads Per Kb per Million.

**Table S6:** List of representative genes from selected GO terms overrepresented among DEGs specifically responsive to both NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>. RPKM: Reads Per Kb per Million.

**Table S7:** Complete quantification for all free amino acids detected as average of µg/g of weighted tissue ± SE and the proportion of total amino acids (%). TOT: calculated sum of total amino acids. Δ% -N: calculated percentage increase/decrease of total free amino acids detected in each treatment (+NO<sub>3</sub><sup>-</sup> or +NH<sub>4</sub><sup>+</sup>) with respect to the N-deficient treatment (-N). The asterisk indicates significant differences with respect to -N treatments (Student's t-test with: \*\* p≤0.01, \* p≤0.05).

**Table S8:** Complete quantification for all hydrolysed amino acids detected as average of µg/g of weighted tissue ± SE and the proportion of total amino acids (%). TOT: calculated sum of total amino acids. Δ% -N: calculated percentage increase/decrease of total hydrolysed amino acids detected in each treatment (+NO<sub>3</sub><sup>-</sup> or +NH<sub>4</sub><sup>+</sup>) with respect to the N-deficient treatment (-N). The asterisk indicates significant differences with respect to -N treatments (Student's t-test with: \*\* p≤0.01, \* p≤0.05).

**Table S9:** Amino acid transitions for HILIC-MS/MS quantification.