Rapid changes in root gene expression in response to nitrogen availability: Linking molecular biology, plant physiology, and soil biogeochemical processes

Timothy M Bowles[1,2], Philipp Raab[1], and Louise E Jackson[1]

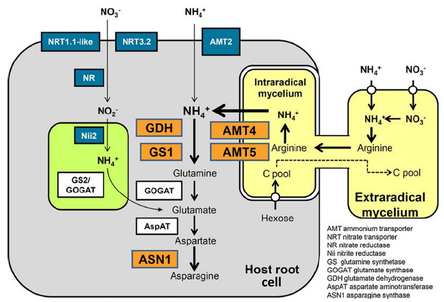
(1) Dept of Land, Air and Water Resources (2) Graduate Group in Ecology; University of California Davis, Davis, CA

I. Why link root gene expression to soil N processes?

- Plants have highly plastic physiological and morphological belowground responses to cope with patchy resources (Hodge et al. 2004, Yang et al. 2008).
- Short-term physiological responses to pulses of N availability include dynamic regulation of root N uptake and assimilation systems at the molecular level (Jackson et al. 2008).
- These changes in root physiology can be monitored through changes in expression levels of specific genes, using molecular biology techniques (Ruzicka et al. 2010/12).
- In systems with rapid soil N cycling, N availability can be difficult to measure or predict. Gene expression can serve as a sensitive "plant's eye view" of the local soil environment and a lens into N availability.

Plants take up soil NH_4^+ and NO_3^- through transporters located in root cortical and epidermal cells. NO_3^- is subsequently reduced to NH_4^+. Resulting NH_4^+ and soil NH_4^+ are incorporated into amino acids via the GS-GOGAT enzymes. These enzymes are the funnel through which soil N is incorporated into the plant N pool. Genes encoding for these enzymes (GS1, GS2, NADH-GOGAT, and Fd-GOGAT) are transcriptionally regulated. Gene expression can be measured by real-time quantitative PCR (RT-qPCR).

Soil N uptake and assimilation in plant roots



from Ruzicka et al. 2012

II. Objectives

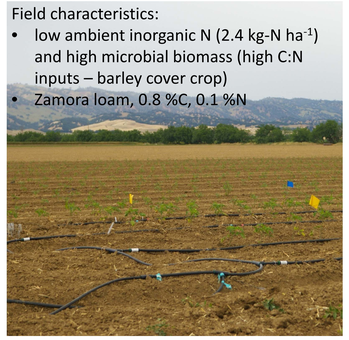
- Determine the short-term molecular physiological response of tomato roots to a pulse of N.
- Measure changes in soil and plant N pools associated with this pulse of N.
- Use root gene expression as an indicator of hard-to-measure soil N dynamics.

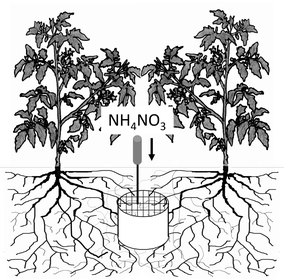
III. Experimental design

- The experiment was conducted on a commercial organic farm during summer 2010 near Esparto, CA.
- Tomatoes (*Solanum lycopersicum* cv. 76R) were transplanted and grown for eight weeks.
- In order to simulate a pulse of N, three levels of NH_4NO_3 were injected into a discrete volume of soil in between plants (see below right):
  - water
  - low-N (9.6 kg NH_4NO_3-N ha^{-1})
  - high-N (62.4 kg NH_4NO_3-N ha^{-1})
- 48, 96 and 120 hours following these injections, soil and roots were sampled from the patch as well as corresponding shoots.
- Evaluations included:

hours post-injection:	48	96	120
root gene expression	•	•	•
soil inorganic N	•	•	•
shoot biomass and N		•	•
root biomass and N	•		•
nitrification potential and potentially mineralizable N	•		•
microbial biomass			•

Field characteristics:
- low ambient inorganic N (2.4 kg-N ha^{-1}) and high microbial biomass (high C:N inputs – barley cover crop)
- Zamora loam, 0.8 %C, 0.1 %N

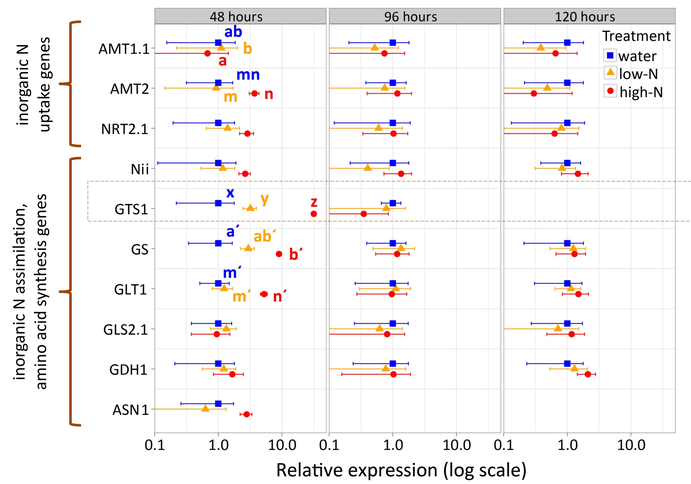




Treatments were designed to create a N patch 10 cm in diameter and 8 cm deep by using a syringe to inject NH_4NO_3 solution at precise locations on a template, centered between plants (no actual ring was used).

IV. How do roots respond to a soil N pulse: gradual or rapid, many genes or few, sustained or ephemeral?

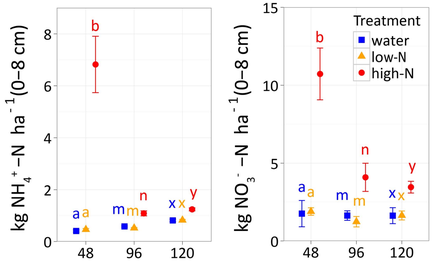
tomato root gene expression and assimilation after a soil N pulse



qRT-PCR analysis of key N uptake and assimilation genes in roots (see table for gene identification). Relative quantity was calculated using the ΔΔCT method with actin (LeACT) as the reference control and the water control group normalized to one. For a given gene, means +/- standard error (n = 5) are shown, with different letters indicating significant differences among treatments at P < 0.05 (ANCOVA with soil gravimetric water content as covariate and Tukey HSD test for mean separation).

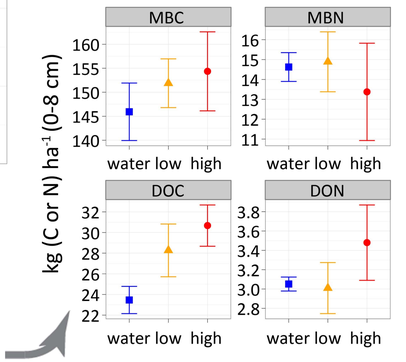
tomato gene	gene name	function
AMT1.1	ammonium transporter 1	NH_4^+ transporter: induced by N deficiency, repressed by N availability
AMT2	ammonium transporter 2	NH_4^+ transporter: induced by NH_4^+ availability
NRT2.1	nitrate transporter	NO_3^- transporter: induced by NO_3^- availability
Nii	nitrite reductase	NO_3^- assimilation: converts NO_2^- into NH_4^+
GTS1 (GS1)	glutamine synthetase	N assimilation, amino acid synthesis (cytosolic)
GS (GS2)	glutamine synthetase	N assimilation, amino acid synthesis (plastids and chloroplasts)
GLT1 (NADH-GOGAT)	glutamate synthase	amino acid synthesis, NADH-dependent (primarily in roots)
GLS2.1 (Fd-GOGAT)	glutamate synthase	amino acid synthesis, ferrodoxin-dependent (primarily in leaves)
GDH1	glutamate dehydrogenase	amino acid synthesis
ASN1	asparagine synthase	amino acid synthesis

soil inorganic nitrogen after a soil N pulse



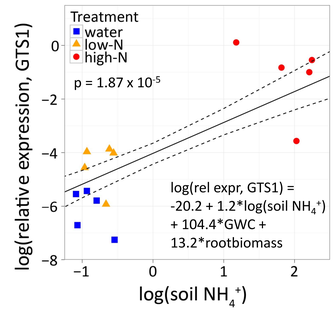
Rapid changes in soil inorganic N concentrations in three treatment groups at three times following NH_4NO_3 injection into soil. Shown are means +/- standard error (n = 5), with different letters indicating significant differences at P < 0.05 within each treatment/time group (one-way ANOVA with Tukey HSD test for mean separation).

soil microbial biomass and dissolved organic matter 120 hours after a soil N pulse



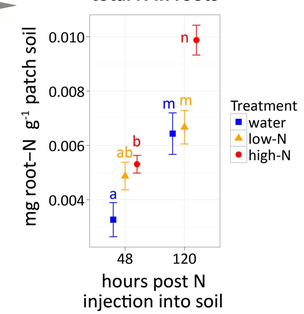
At the end of the experiment, microbial response to N pulse was low. Microbial biomass C and N (MBC, MBN), and dissolved organic C and N (DOC, DON). Shown are means +/- standard error (n = 5). Treatment groups were not significantly different, but treatment effect on DOC was marginal (P = 0.072).

expression of GTS1 at 48 hours after a soil N pulse



Multiple regression model of GTS1 (a key N assimilation gene) expression 48 hours following an N pulse. This model had the best fit (lowest AICc) of all tested. Dashed line: 95% HPDI of the mean.

total N in roots



Rapid N assimilation in roots, especially in the high treatment group. Shown are means +/- standard error (n = 5), with different letters indicating significant differences at P < 0.05 within each treatment/time group (one-way ANOVA with Tukey HSD test for mean separation).

V. Implications for N cycling research

- Plant roots responded rapidly – but transiently – to a N pulse with a suite of changes in expression of N metabolism genes. Changes likely occur even faster than the time scale we measured.
- The low N treatment did not cause a detectable increase in soil inorganic N or other measures of N availability, but root N assimilation genes (especially GTS1 and GS) were significantly upregulated relative to the water control.
  - GTS1 and GS are both glutamine synthetases, part of the GS-GOGAT pathway (ubiquitous in the plant domain) responsible for incorporating soil N into the plant N pool.
  - Expression of these genes in roots may be a highly sensitive physiological indicator of soil N availability.

VI. Agroecological relevance

- Most agronomic tests for N availability measure soil or plant NO_3^- pools. In systems that rely on organic matter transformations to supply plant N, NO_3^- pools may be low despite rapid N dynamics.
- We need better information for nutrient management to deliver multiple ecosystem services: crop productivity, N retention, and C sequestration. With further research, gene expression analysis may become a robust indicator of plant N availability.

VII. Acknowledgments

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