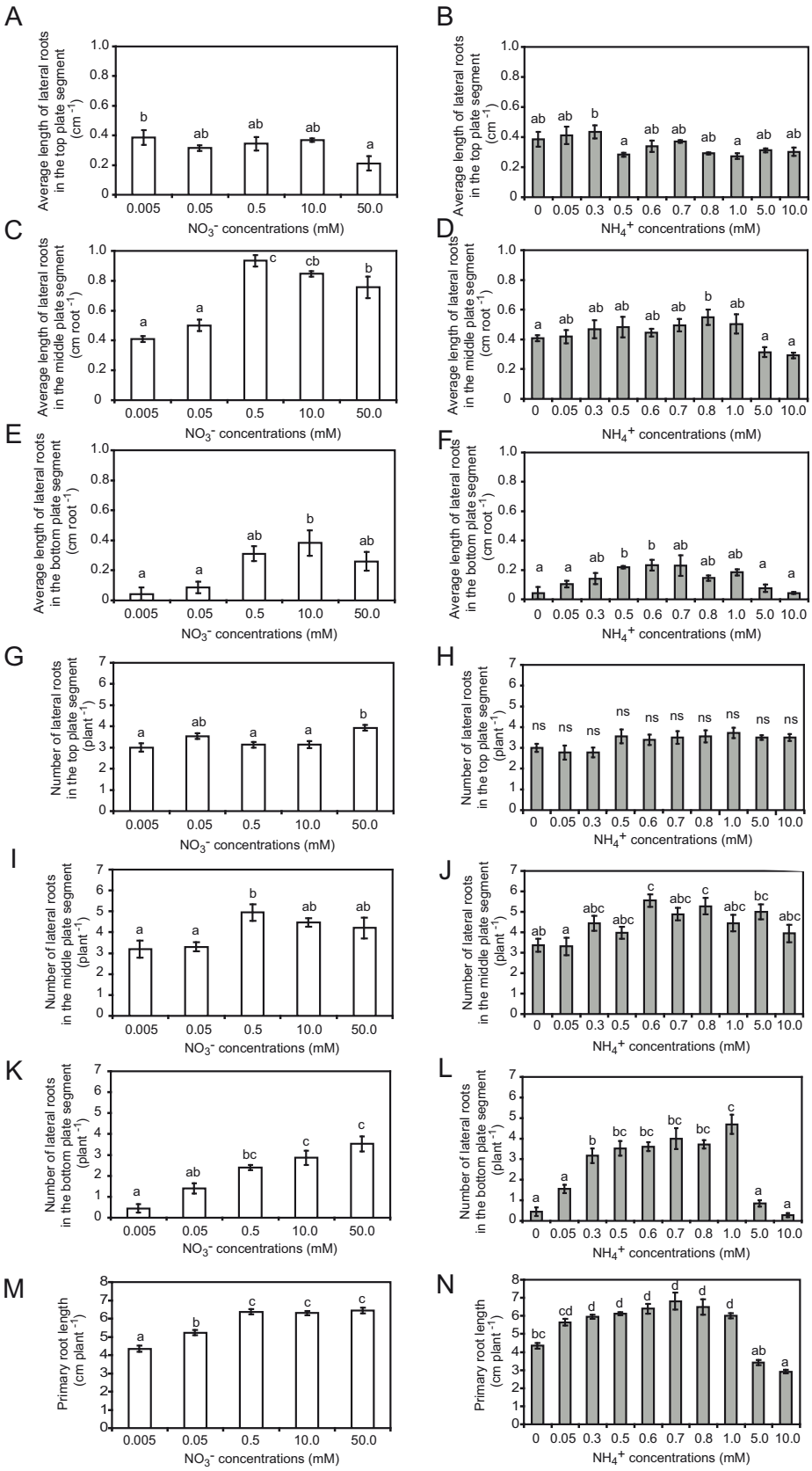
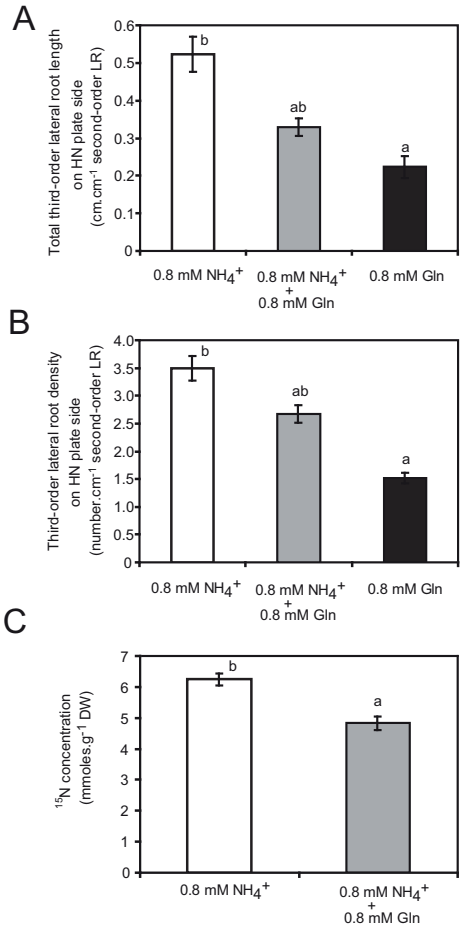


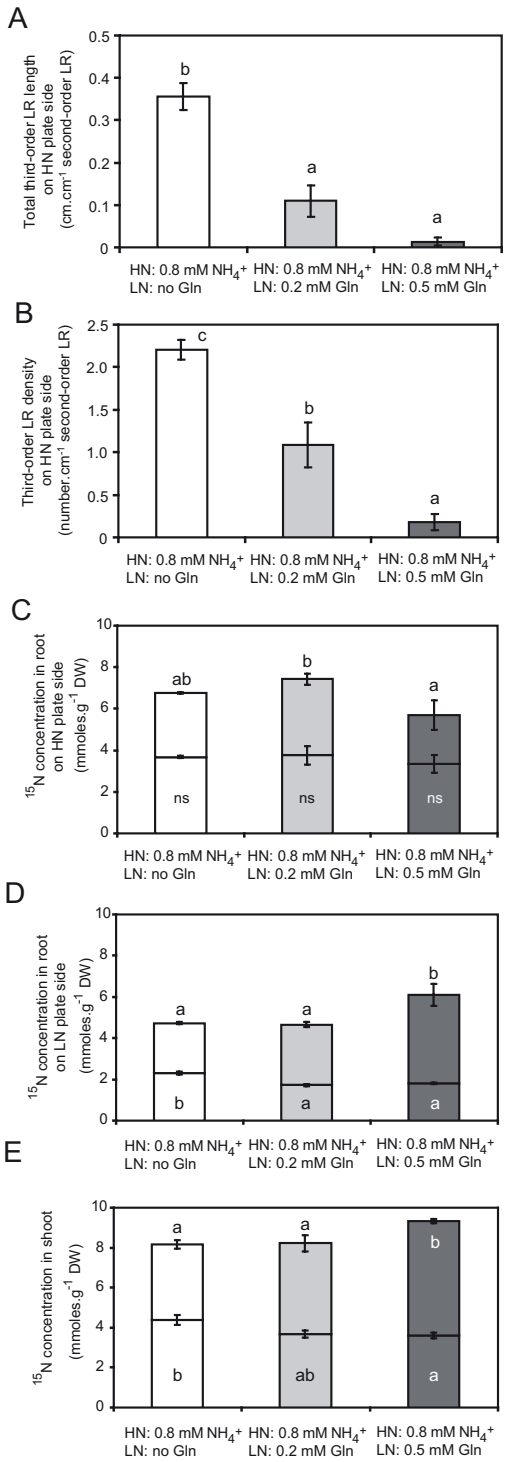
Supplemental Figure 1. Primary and lateral root development under local nitrate and ammonium supply.

(A) to **(F)** Average lateral root length in the top **(A)** and **(B)**, middle **(C)** and **(D)** and bottom **(E)** and **(F)** root compartment. **(G)** to **(L)** Lateral root number in the top **(G)** and **(H)**, middle **(I)** and **(J)** and bottom **(K)** and **(L)** root compartment. **(M)** and **(N)** primary root length. Arabidopsis plants were grown for 15 days on horizontally-split agar plates and supplemented with increasing concentrations of nitrogen in the form of nitrate **(A, C, E, G, I, K, M)** or ammonium **(B, D, F, H, J, L, N)** only in the middle compartment. Bars represent mean values (± SE) of 21 individual plants per treatment and different letters denote significant differences among means at $P < 0.05$ (Tukey's test); ns, not significant.



Supplemental Figure 2. Glutamine represses ammonium-induced third-order lateral root branching.

(A) Third-order lateral root length, **(B)** third-order lateral root density, and **(C)** ^{15}N concentrations in roots from ^{15}N-labeled ammonium supplied to the high N (HN) side. Wildtype plants were grown for 15 days on vertically-split agar plates with the first lateral root growing into a high N compartment (HN) with 0.8 mM ^{15}N-labeled NH_4Cl, 0.8 mM ^{15}N-labeled NH_4Cl together with 0.8 mM glutamine or 0.8 mM glutamine alone, while the remainder of the roots was growing without N supply. Bars represent mean values (± SE) of 10-15 individual plants and different letters denote significant differences among means at $P < 0.05$ (Tukey's test).



Supplemental Figure 3. Systemic repression of ammonium-induced third-order lateral root branching by glutamine.

(A) Third-order lateral root length, **(B)** third-order lateral root density, and **(C to E)** ^{15}N uptake from ^{15}N-labeled ammonium applied to the HN side and its distribution in plants. Concentrations of total nitrogen ($^{14}N+^{15}N$, outer bars) and ^{15}N (inner bars) in **(C)** roots from the high N (HN), **(D)** roots from low N (LN) side of the plate, and **(E)** shoots are indicated. Wild type plantswere grown for 15 days on vertically-split agar plates with the first lateral root growing into a high N (HN) compartment supplied with 0.8 mM ^{15}N-labeled NH_4Cl. Increasing concentrations of glutamine were added to the remainder of the roots growing in a low N compartment (LN). Bars represent mean values of 8-12 individual plants and different letters denote significant differences among means at $P < 0.05$ (Tukey's test); ns, not significant.

Supplemental Table 1. The presence of ammonium and nitrate triggers lateral root development. Macroscopic measurements of total length and total numbers of first-, second- or third-order lateral roots as well as primary root length of Arabidopsis wildtype plants grown on vertically-split agar plates for 15 days on either local nitrogen supply (0.8 mM NO_3^- or NH_4^+ on HN side with 5 µM NO_3^- on the LN side; LN vs. NO_3^- and LN vs. NH_4^+) or homogenous nitrogen supply (0.8 mM NO_3^- or NH_4^+ on both sides; NO_3^- vs. NO_3^- and NH_4^+ vs. NH_4^+). Values represent means (± SE) of 12-20 individual plants and different letters denote significant differences among means at $P < 0.05$ (Tukey's test); nd, not determined.

	HN plate side					LN plate side					
	Lateral root length (cm.plant $^{-1}$)			Lateral root number (plant $^{-1}$)		Lateral root length (cm.plant $^{-1}$)			Lateral root number (plant $^{-1}$)		Primary root length (cm.plant $^{-1}$)
	First-order	Second-order	Third-order	Second-order	Third-order	First-order	Second-order	Third-order	First-order	Second-order	
LN $_{VS}$ NO_3^-	10.60 ±0.45b	35.84 ±3.92b	0.79 ±0.19a	25.64 ±1.56c	4.00 ±0.82a	46.60±3.35b	1.97±1.02a	nd	32.45±2.09n.s	4.36±1.70a	13.96±0.59b
NO_3^- $_{VS}$ NO_3^-	8.54 ± 0.95b	11.24 ±3.52a	0.12 ±0.10a	13.60±1.89ab	2.41 ±0.24a	58.81±4.51b	27.58±4.87c	nd	38.25±4.36n.s	59.25±11.5b	12.79±0.44a
LN $_{VS}$ NH_4^+	4.81 ±0.34a	7.45 ±0.58a	2.57 ±0.24b	22.06±0.40bc	18.88 ±0.41c	28.88±1.68a	1.85±0.24a	0.03±0.01a	28.06±1.70n.s	8.24±1.09a	11.05±0.52a
NH_4^+ $_{VS}$ NH_4^+	5.09 ±0.43a	4.46 ±1.35a	0.66 ±0.22a	11.23 ±3.80a	9.65 ±3.21b	20.29±2.97a	7.80±2.37b	0.57±0.05b	37.00±3.46n.s	59.33±5.48b	11.17±0.82a

Supplemental Table 2. Reconstituted expression of *AMT1;3* restores third-order lateral development in *qko* also when primary roots are pruned.
Macroscopic analysis of total second- and third-order lateral root length and density on the ammonium-supplied side (HN, high N) and on the N-deficient side (LN, low N). Wildtype (Col-0), *qko* (*amt1;1, amt1;2, amt1;3, amt2;1*), *qko+11* (*amt1;2, amt1;3, amt2;1*) or *qko+13* plants (*amt1;1, amt1;2, amt2;1*; Yuan et al., 2007) were grown for 15 days on vertically-split agar plates with a localized supply of 0.8 mM ammonium. Values represent means (± SE) of 15-20 individual plants and different letters denote significant differences among means at $P < 0.05$ (Tukey's test); ns, not significant.

	HN plate side				LN plate side			
	Total lateral root length (cm.cm^{-1} LR)		Lateral root density (number.cm^{-1} LR)		Total lateral root length (cm.cm^{-1} LR)		Lateral root density (number.cm^{-1} LR)	
	Second-order	Third-order	Second-order	Third-order	Second-order	Third-order	Second-order	Third-order
wt	2.06 ±0.13 ns	0.47 ±0.03 b	5.02 ±0.22 a	2.83 ±0.36 c	1.84 ±0.18 ns	0.11 ±0.05 ns	2.39 ±0.17 ns	0.35 ±0.07 ns
qko+11	2.12 ±0.12 ns	0.26 ±0.02 a	6.21 ±0.49 b	1.76 ±0.16 ab	1.51 ±0.12 ns	0.09 ±0.03 ns	2.33 ±0.12 ns	0.28 ±0.08 ns
qko+13	1.84 ±0.07 ns	0.44 ±0.05 b	5.33 ±0.30 ab	2.50 ±0.19 bc	1.43 ±0.10 ns	0.05 ±0.02 ns	2.32 ±0.10 ns	0.20 ±0.05 ns
qko	2.21 ±0.34 ns	0.15 ±0.02 a	4.24 ±0.38 a	1.02 ±0.11 a	1.55 ±0.24 ns	0.02 ±0.01 ns	2.81 ±0.15 ns	0.17 ±0.02 ns