

Supplemental Figures:

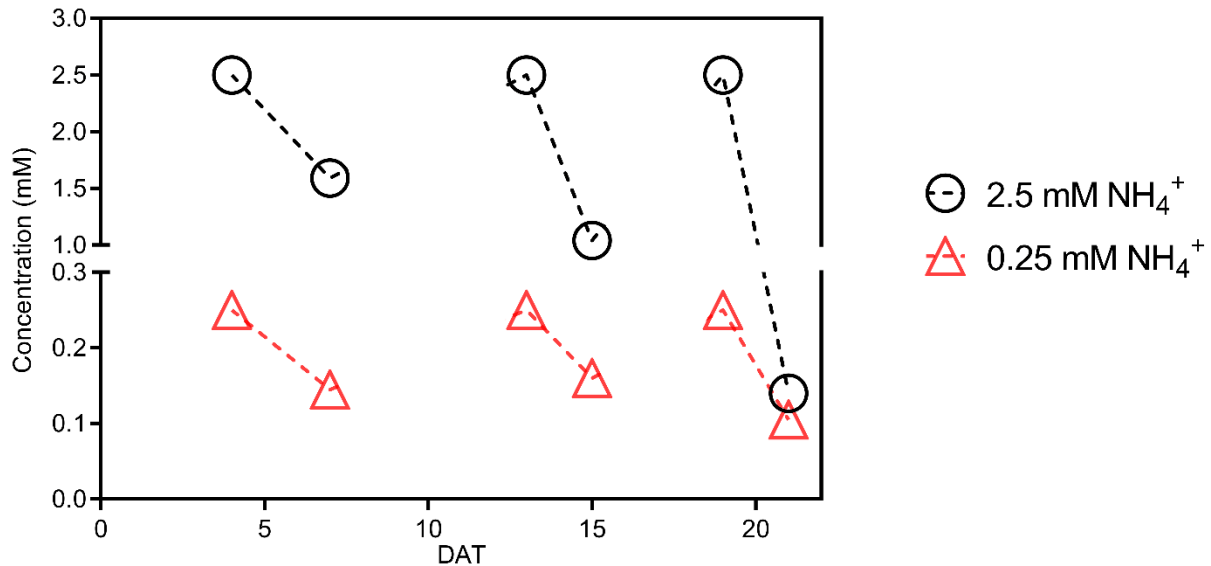


Fig. S1 Pilot study of ammonium depletion in original EcoFABs (Sasse *et al.*, 2019) to establish the size and design of EcoFAB-N used here (see doi: [dx.doi.org/10.17504/protocols.io.b53tq8nn](https://doi.org/10.17504/protocols.io.b53tq8nn)). The EcoFAB had one *B. distachyon* plant growing in revised 0.5 MS medium with 2.5 mM NH₄NO₃ (2.5 mM NH₄) or 0.25 mM NH₄NO₃ (0.25 mM NH₄⁺) for 21 days. Medium were changed at 4, 7 days after transplanting (DAT), followed with changing every 3 days until harvest at 21 DAT. Ammonium concentration in fill medium at 4, 13, 19 DAT and collect medium at 7, 15, 21 DAT was measured by ammonia assay kit (Sigma, AA0100). Data is one replicate.

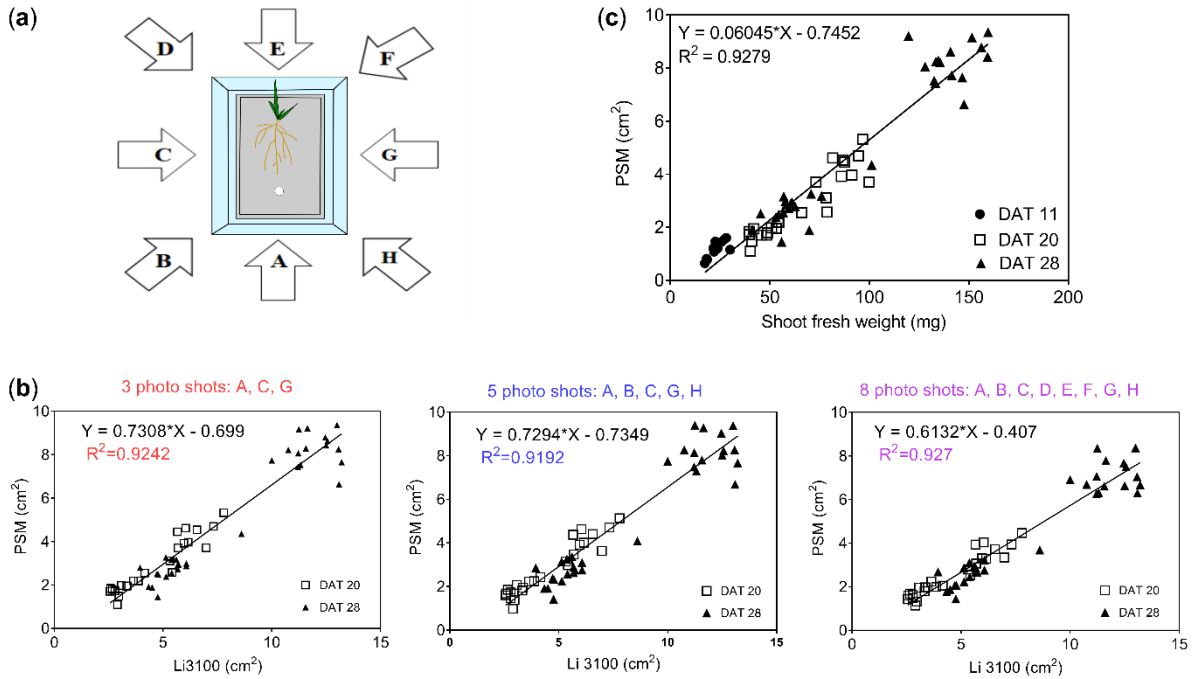


Fig. S2. Non-invasive *B. distachyon* leaf area and invasive leaf area or shoot fresh weight correlations. (a) Imaging scheme of the EcoFAB-N, using a mobile camera positioned at a 45° angle facing downwards. *B. distachyon* images were obtained with the Plant screen mobile software (PSM), and projected leaf area was calculated using the screen segmentation software (see doi: dx.doi.org/10.17504/protocols.io.b53kq8kw). (b) Optimization of number of shots required. Photos from 3, 5, 8 directions were taken at 20, 28 DAT and measured by the method showed in Fig. 1a, b. (c) Correlation between non-invasive leaf area obtained with 3 PSM images as shown in Fig. 1a, b and destructive shoot fresh weight measurements 11, 20, 28 DAT. N plants imaged: 11 DAT (n= 3); 20 DAT (n=6), 28 DAT (n=8).

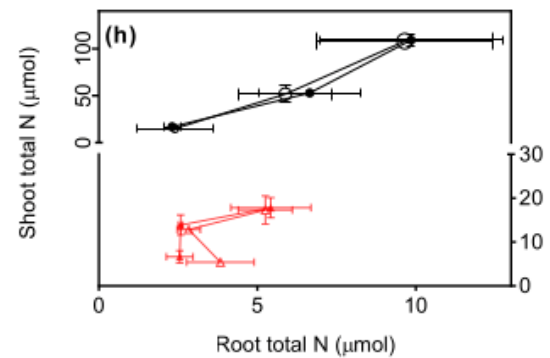
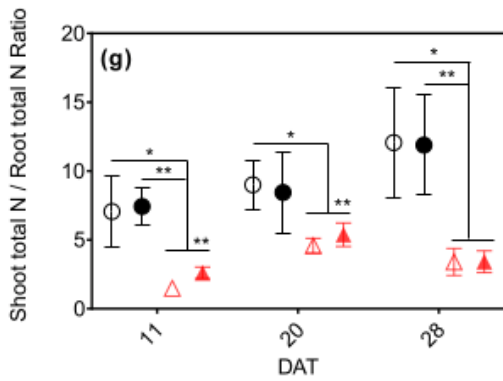
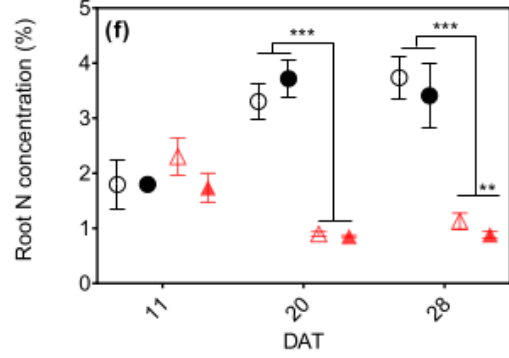
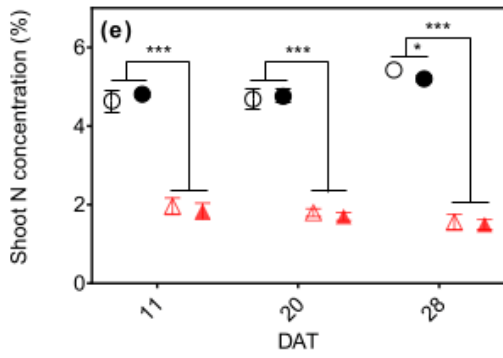
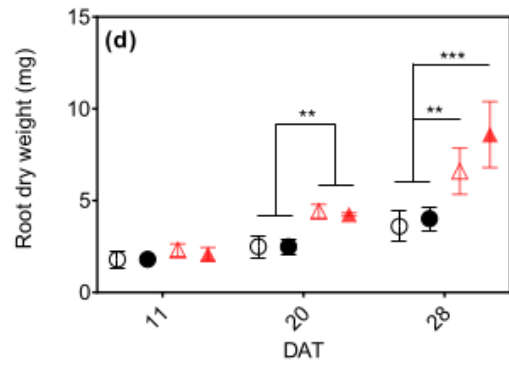
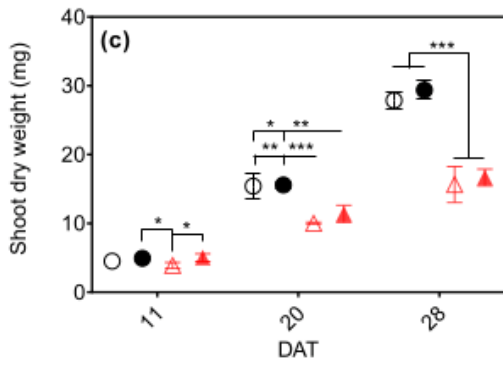
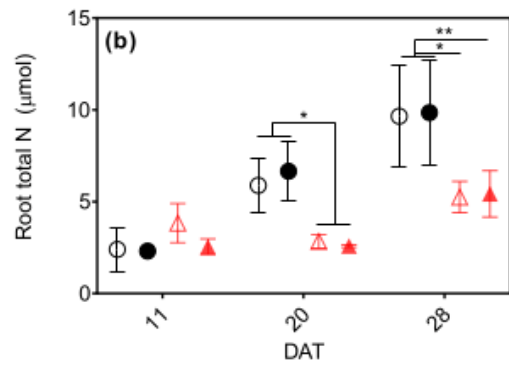
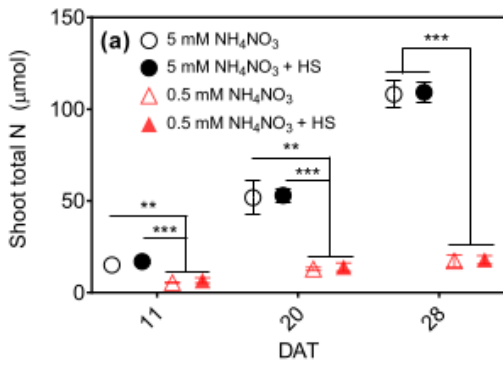


Fig.S3 *B. distachyon* shoot and root total N, dry weight, and N concentration. *B. distachyon* was grown in 5 mM NH₄NO₃ or 0.5 mM NH₄NO₃, with or without *H. seropedicae* (HS), in EcoFAB-N chambers. Data are means ± standard error (n = 3 at 11, 20 DAT and n = 5 at 28 DAT). (a) Shoot total N; (b) Root total N; (c) Shoot dry weight; (d) Root dry weight; (e) Shoot N concentration; (f) Root N concentration; (g) Shoot total N / Root total N ratio. (h) Allocation of total shoot total N (a) and root total N (b) of whole plants over time. Asterisk indicate statistically differences according to unpaired t-test at the 0.05 level. *, p < 0.05; **, p < 0.01; ***, p < 0.001.

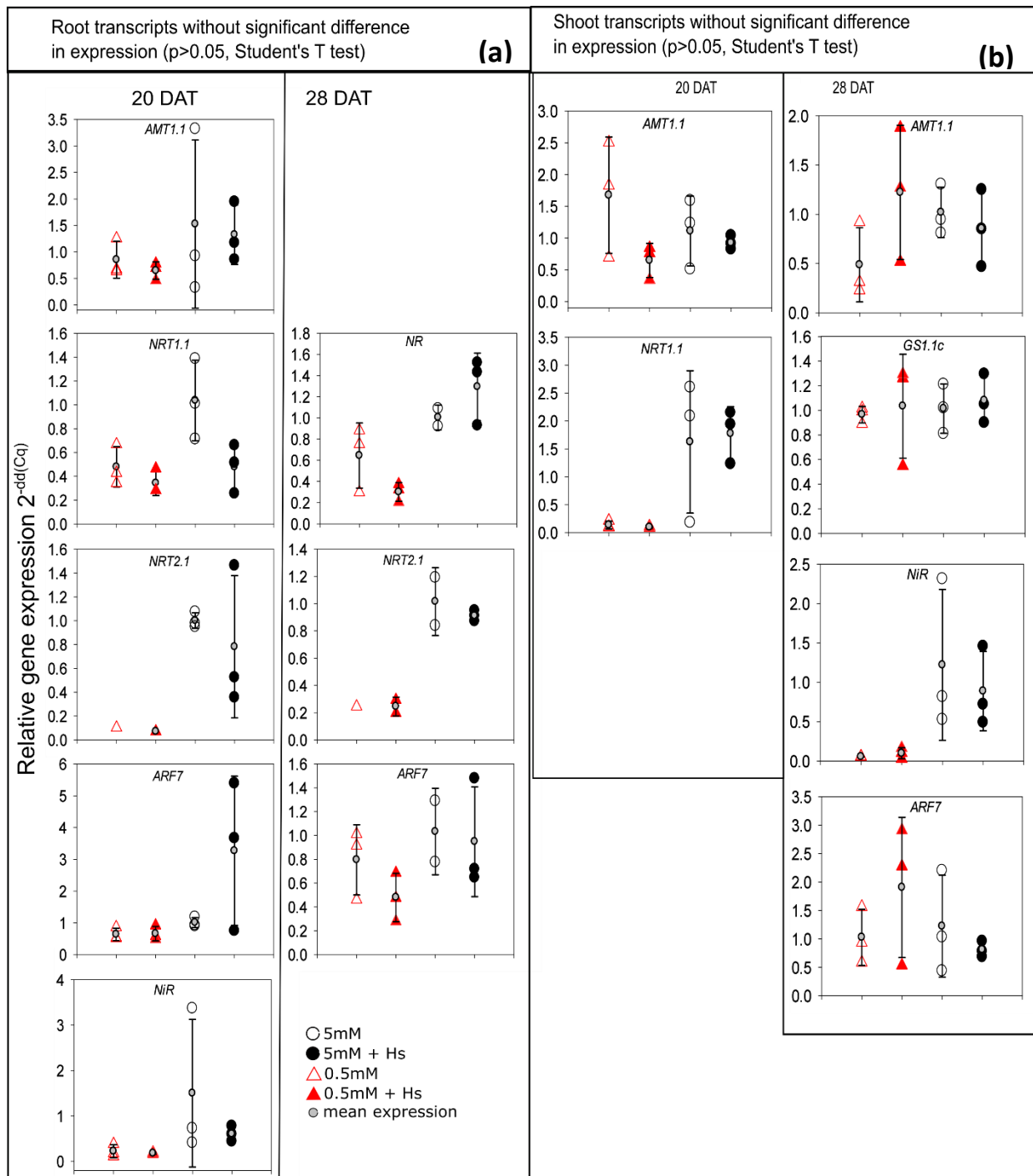


Fig S4: Quantitative Real-Time PCR of selected root (a) and shoot transcripts (b) at 20 and 28 DAT. Relative expression level of AMT1.1, NRT1.1, NRT2.1, NiR, NR, GS1.1c and ARF7 in *B. distachyon* roots, which were grown in 5 mM NH_4NO_3 or 0.5 mM NH_4NO_3 , with or without *H. seropedicae* (HS), in EcoFAB-N chambers for 20, 28 DAT. Expression are normalized to UBQ10, and standardized to the expression in 5 mM NH_4NO_3 at the respective time point. The expression of biological replicates is

depicted using big circles or triangles, while the mean expression is shown on top of each condition using small gray circles and standard deviation error bars. A minimum of 2 biological replicates are shown, except: in NRT 2.1 in Root (20DAT, 0.5 NH₄NO₃ both conditions, and 28 DAT 0.5mM, non-inoculated) where n=1. Asterisks indicate statistical differences according to unpaired t-test for three comparisons: 0.5 vs 5mM NH₄NO₃, and inoculated vs non-inoculated at each respective NH₄NO₃ level. Ammonium transporter 1.1. (AMT1.1), Nitrate transporter 1.1 (NRT1.1), Nitrate transporter 2.1 (NRT2.1), Nitrite reductase (NiR), Nitrate reductase (NR), Cytosolic Glutamine Synthetase (GS1.1c) and Auxin response factor 7 (ARF7)

Supplemental tables

Table S1 Primers used in this study

| Gene | Phytozome Gene Identifier | Primer | Sequence 5' to 3' | NCBI Reference Sequence |
|----------------------------|---------------------------|--------|----------------------|--|
| Bd_UBQ10 | BdiBd21-3.1G0441800 | Fwd | TGGACTTGCTTCTGTCTGGG | NM_001317880 |
| | | Rev | ACAGGCATAAACTGACGAC | |
| Bd_SamDC | BdiBd21-3.5G0187900 | Fwd | GATGGCGAGAACGTGGAGAA | XM_010241769.2 |
| | | Rev | AAACAAATTGCACGGGGACG | |
| Bd_NRT1.1A | BdiBd21-3.3G0226900 | Fwd | GTGAAATGGGGAAGGGTTTT | XM_003573432.3 |
| | | Rev | ATGCAGCGACATTCAGTCAC | |
| Bd_NRT2.1 | BdiBd21-3.2G0521300 | Fwd | GGCAGCTCGACTTCTTCATC | XM_003572502.2 |
| | | Rev | TAGTCTAGGCGGCTGTGGTT | |
| Bd_GS1.1c | BdiBd21-3.3G0789600 | Fwd | AGCACTCCCAAAGCTCAAA | XM_010237849.3 |
| | | Rev | TGTAAATCGCCCAAATCCTC | |
| Bd_AMT1.1 | BdiBd21-3.5G0198500 | Fwd | AATCCCCAGATTCCCAAAAC | XM_003580067.3 |
| | | Rev | GTGAATCCTTCGTGGTTGCT | |
| Bd_NiR1 | BdiBd21-3.3G0767200 | Fwd | CAGGAGAAGGTGAAGCTGGG | XM_003570520.3 |
| | | Rev | GCAGCTTCAGACGCATCATG | |
| Bd_ARF7 | BdiBd21-3.3G0608800 | Fwd | GGAGGCATGCTGAAGAGTGT | XM_010237246.3 (Zhou <i>et al.</i> , 2018) |
| | | Rev | CAGCCTCCTTACACCGACTC | |
| Bd_NR1 | BdiBd21-3.3G0500200 | Fwd | GTCAAGCGCATCATCGTCAC | XM_003574559.3 |
| | | Rev | GTCGTGAATGCGTTGATGGG | |
| Bd_NRT2.5 | BdiBd21-3.2G0609200 | Fwd | GTCCAAGGCCAAGTTCAGGA | https://doi.org/10.1111/ppl.12716 (Wang, Hüner, & Tian, 2019). |
| | | Rev | CCCAGATTGTCCCGGATGAG | |
| Hs_16s | 1847008 [GenBank] | Fwd | TTCCCGGGTCTTGTACACAC | NR_029329.1 |
| | | Rev | CGTGCGCACTCTAGAAAGGA | |
| Universal bacterial primer | 16s rRNA | 27F | AGAGTTTGATCMTGGCTCAG | https://doi.org/10.1128/AEM.02272-07 (Frank <i>et al.</i> , 2008) |
| | | 1492R | GGTTACCTTGTTACGACTT | |

Table S2. Growth of *B. distachyon* measured non-destructively over time at 4, 11, 17, 23 and 28 days after transplanting (DAT) when roots exposed to 5 mM NH₄NO₃ or 0.5 mM NH₄NO₃, with or without *H. seropedicae* (HS), in EcoFAB-N chambers shown in Fig. 3 (n=10, except 5mM where n=9). All data through time with means \pm standard error (n = 10); Asterisk indicate statistically differences according to unpaired t-test at the 0.05 level. ns, no significant; *, p < 0.05; **, p < 0.01; ***, p < 0.001; ****. (a) Project leaf area results; (b) Total root length; (c) Primary root total length; (d) Lateral root total length; (e) Root hair length.

(a) Project leaf area results

| | Treatments | DAT 4 | DAT 11 | DAT 17 | DAT 23 | DAT 28 |
|---|--------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Project leaf area (cm ²) | 5 mM | 0.49 \pm 0.10 | 1.29 \pm 0.21 | 2.68 \pm 0.49 | 6.20 \pm 0.67 | 8.53 \pm 0.59 |
| | 5 mM + HS | 0.50 \pm 0.12 | 1.35 \pm 0.24 | 3.08 \pm 0.39 | 5.92 \pm 0.98 | 7.57 \pm 1.37 |
| | 0.5 mM | 0.47 \pm 0.13 | 0.97 \pm 0.17 | 1.39 \pm 0.29 | 1.88 \pm 0.36 | 2.39 \pm 0.45 |
| | 0.5 mM + HS | 0.50 \pm 0.09 | 1.09 \pm 0.15 | 1.63 \pm 0.28 | 2.25 \pm 0.16 | 2.97 \pm 0.29 |
| T test | | | | | | |
| T-test to 5 mM | 0.5 mM vs 5 mM | ns | 0.0031 ** | < 0.001*** | < 0.001 *** | < 0.001 *** |
| | 5 mM + HS vs 5 mM | ns | ns | ns | ns | Ns |
| | 0.5 mM + HS vs 5 mM | ns | 0.032 * | < 0.001*** | < 0.001 *** | < 0.001 *** |
| T-test to 0.5 mM | 5 mM + HS vs 0.5 mM | ns | < 0.001 *** | < 0.001 *** | < 0.001 *** | < 0.001 *** |
| | 0.5 mM + HS vs 0.5 mM | ns | ns | ns | 0.011 * | 0.0041** |
| T-test to 0.5 mM +HS | 5 mM + HS vs 0.5 mM + HS | ns | 0.011 * | < 0.001 *** | < 0.001 *** | < 0.001 *** |

(b) Total root length

| | Treatments | DAT 4 | DAT 11 | DAT 17 | DAT 23 | DAT 28 |
|---------------------------|--------------------------|-------------|-------------|--------------|--------------|--------------|
| Total root length (cm) | 5 mM | 1.55 ± 0.15 | 3.98 ± 0.97 | 8.58 ± 1.74 | 13.46 ± 3.48 | 18.80 ± 4.11 |
| | 5 mM + HS | 1.48 ± 0.14 | 4.85 ± 0.79 | 9.74 ± 1.83 | 14.82 ± 2.74 | 25.19 ± 4.85 |
| | 0.5 mM | 1.58 ± 0.18 | 4.37 ± 0.91 | 8.96 ± 2.09 | 13.94 ± 3.90 | 22.12 ± 4.57 |
| | 0.5 mM + HS | 1.53 ± 0.17 | 5.64 ± 1.39 | 11.72 ± 2.73 | 18.77 ± 3.32 | 27.75 ± 4.61 |
| T test | | | | | | |
| T-test to 5 mM | 0.5 mM vs 5 mM | ns | ns | ns | ns | ns |
| | 5 mM + HS vs 5 mM | ns | ns | ns | ns | 0.011 * |
| | 0.5 mM + HS vs 5 mM | ns | 0.012 * | 0.012 * | 0.005 ** | < 0.001 *** |
| T-test to 0.5 mM | 5 mM + HS vs 0.5 mM | ns | ns | ns | ns | ns |
| | 0.5 mM + HS vs 0.5 mM | ns | 0.034 * | 0.027 * | 0.011 * | 0.018 * |
| T-test to 0.5 mM +HS | 5 mM + HS vs 0.5 mM + HS | ns | ns | ns | 0.012 * | ns |

(c) Primary root total length

| | Treatments | DAT 4 | DAT 11 | DAT 17 | DAT 23 | DAT 28 |
|-----------------------------------|--------------------------|-------------|-------------|-------------|-------------|--------------|
| Primary root total length (cm) | 5 mM | 1.55 ± 0.15 | 2.56 ± 0.41 | 4.08 ± 0.88 | 6.04 ± 0.79 | 7.41 ± 0.83 |
| | 5 mM + HS | 1.48 ± 0.14 | 3.01 ± 0.41 | 5.16 ± 0.36 | 8.08 ± 0.85 | 9.64 ± 1.69 |
| | 0.5 mM | 1.58 ± 0.18 | 3.16 ± 0.24 | 4.45 ± 0.64 | 6.70 ± 0.58 | 8.59 ± 1.15 |
| | 0.5 mM + HS | 1.53 ± 0.17 | 3.76 ± 0.27 | 5.98 ± 0.74 | 9.40 ± 1.51 | 11.17 ± 1.23 |
| T test | | | | | | |
| T-test to 5 mM | 0.5 mM vs 5 mM | ns | 0.0017 ** | ns | ns | 0.035 * |
| | 5 mM + HS vs 5 mM | ns | 0.031 * | 0.0032 ** | < 0.001 *** | 0.004 ** |
| | 0.5 mM + HS vs 5 mM | ns | < 0.001 *** | < 0.001 *** | < 0.001 *** | < 0.001 *** |
| T-test to 0.5 mM | 5 mM + HS vs 0.5 mM | ns | ns | 0.0088 ** | < 0.001 *** | ns |
| | 0.5 mM + HS vs 0.5 mM | ns | < 0.001 *** | < 0.001 *** | < 0.001 *** | < 0.001 *** |
| T-test to 0.5 mM +HS | 5 mM + HS vs 0.5 mM + HS | ns | < 0.001 *** | 0.0078 ** | 0.033 * | 0.035 * |

(d) Lateral root total length

| | Treatments | DAT 4 | DAT 11 | DAT 17 | DAT 23 | DAT 28 |
|-----------------------------------|-------------------------|-------|-------------|-------------|-------------|--------------|
| Lateral root total length (cm) | 5 mM | 0 | 1.43 ± 0.95 | 4.50 ± 1.98 | 7.42 ± 3.55 | 11.39 ± 4.34 |
| | 5 mM +HS | 0 | 1.21 ± 0.9 | 4.51 ± 2.38 | 7.25 ± 3.9 | 13.52 ± 4.23 |
| | 0.5 mM | 0 | 1.79 ± 0.86 | 4.58 ± 1.53 | 7.27 ± 2.81 | 15.55 ± 4.82 |
| | 0.5 mM +HS | 0 | 1.88 ± 1.23 | 5.75 ± 2.92 | 9.65 ± 4 | 16.58 ± 5.23 |
| T test | | | | | | |
| T-test to 5 mM | 0.5 mM vs 5 mM | ns | ns | ns | ns | ns |
| | 5 mM +HS vs 5 mM | ns | ns | ns | ns | ns |
| | 0.5 mM +HS vs 5 mM | ns | ns | ns | ns | 0.05 * |
| T-test to 0.5 mM | 5 mM +HS vs 0.5 mM | ns | ns | ns | ns | ns |
| | 0.5 mM +HS vs 0.5 mM | ns | ns | ns | ns | ns |
| T-test to 0.5 mM +HS | 5 mM +HS vs 0.5 mM + HS | ns | ns | ns | ns | ns |

(e) Root hair length

| | Treatments | DAT 4 | DAT 11 | DAT 17 | DAT 23 | DAT 28 |
|--------------------------|-------------------------|-------------|-------------|-------------|-------------|-------------|
| Root hair length (cm) | 5 mM | 0.52 ± 0.09 | 0.49 ± 0.07 | 0.39 ± 0.06 | 0.31 ± 0.04 | 0.26 ± 0.05 |
| | 5 mM +HS | 0.45 ± 0.11 | 0.50 ± 0.09 | 0.33 ± 0.04 | 0.26 ± 0.04 | 0.18 ± 0.06 |
| | 0.5 mM | 0.68 ± 0.04 | 0.74 ± 0.09 | 0.60 ± 0.05 | 0.55 ± 0.02 | 0.54 ± 0.06 |
| | 0.5 mM +HS | 0.74 ± 0.08 | 0.69 ± 0.09 | 0.56 ± 0.09 | 0.42 ± 0.07 | 0.40 ± 0.09 |
| T test | | | | | | |
| T-test to 5 mM | 0.5 mM vs 5 mM | < 0.001 *** | < 0.001 *** | < 0.001 *** | < 0.001 *** | < 0.001 *** |
| | 5 mM +HS vs 5 mM | ns | ns | 0.027 * | 0.015 * | 0.0086 ** |
| | 0.5 mM +HS vs 5 mM | < 0.001 *** | < 0.001 *** | < 0.001 *** | 0.0023 ** | < 0.001 *** |
| T-test to 0.5 mM | 5 mM +HS vs 0.5 mM | < 0.001 *** | < 0.001 *** | < 0.001 *** | < 0.001 *** | < 0.001 *** |
| | 0.5 mM +HS vs 0.5 mM | ns | ns | ns | < 0.001 *** | < 0.001 *** |
| T-test to 0.5 mM +HS | 5 mM +HS vs 0.5 mM + HS | < 0.001 *** | < 0.001 *** | < 0.001 *** | < 0.001 *** | < 0.001 *** |

Table S3. Ammonium and nitrate depletion in the EcoFAB medium, showed in Fig. 4a-b (n=5). Asterisk indicate statistically differences according to unpaired t-test at the 0.05 level. ns, no significant; *, p < 0.05; **, p < 0.01; ***, p < 0.001; ***, p < 0.001; ***, p < 0.001; ***, p < 0.001. (a) ammonium depletion; (b) nitrate depletion.

(a) Ammonium depletion

| | Treatments | DAT 4 | DAT 8 | DAT 11 | DAT 14 | DAT 17 |
|---|--------------------------|-----------------|-----------------|-----------------|-----------------|------------------|
| Ammonium depletion (μmol) | 5 mM | 4.70 \pm 1.9 | 5.89 \pm 1.01 | 7.48 \pm 0.67 | 7.59 \pm 1.34 | 10.73 \pm 0.78 |
| | 5 mM + HS | 5.26 \pm 1.61 | 3.09 \pm 0.83 | 4.32 \pm 1.61 | 6.61 \pm 1.28 | 9.76 \pm 2.20 |
| | 0.5 mM | 1.24 \pm 0.29 | 1.65 \pm 0.25 | 1.43 \pm 0.31 | 1.28 \pm 0.11 | 1.56 \pm 0.15 |
| | 0.5 mM + HS | 1.52 \pm 0.17 | 1.67 \pm 0.15 | 1.59 \pm 0.15 | 1.45 \pm 0.21 | 1.65 \pm 0.3 |
| t-test | | | | | | |
| t-test to 5 mM | 0.5 mM vs 5 mM | 0.007** | < 0.001*** | < 0.001*** | < 0.001*** | < 0.001*** |
| | 5 mM + HS vs 5 mM | ns | 0.0021** | 0.0042** | ns | ns |
| | 0.5 mM + HS vs 5 mM | 0.01* | < 0.001*** | < 0.001*** | < 0.001*** | < 0.001*** |
| t-test to 0.5 mM | 5 mM + HS vs 0.5 mM | < 0.001*** | 0.0062** | 0.0044380 | < 0.001*** | < 0.001*** |
| | 0.5 mM + HS vs 0.5 mM | ns | ns | ns | ns | ns |
| t-test to 0.5 mM +HS | 5 mM + HS vs 0.5 mM + HS | < 0.001*** | 0.0063** | 0.0044** | < 0.001*** | < 0.001*** |

| | Treatments | DAT 20 | DAT 23 | DAT 26 | DAT 28 |
|---|--------------------------|------------------|-----------------|-----------------|-----------------|
| Ammonium depletion (μmol) | 5 mM | 10.62 \pm 1.77 | 10.74 \pm 2.4 | 9.52 \pm 0.66 | 6.97 \pm 1.99 |
| | 5 mM + HS | 11.34 \pm 1.83 | 9.70 \pm 1.64 | 8.74 \pm 1.73 | 6.70 \pm 2.27 |
| | 0.5 mM | 1.64 \pm 0.11 | 1.73 \pm 0.12 | 1.65 \pm 0.13 | 1.71 \pm 0.22 |
| | 0.5 mM + HS | 1.89 \pm 0.15 | 1.87 \pm 0.13 | 1.89 \pm 0.07 | 1.90 \pm 0.06 |
| t-test | | | | | |
| t-test to 5 mM | 0.5 mM vs 5 mM | < 0.001*** | < 0.001*** | < 0.001*** | < 0.001*** |
| | 5 mM + HS vs 5 mM | ns | ns | ns | ns |
| | 0.5 mM + HS vs 5 mM | < 0.001*** | < 0.001*** | < 0.001*** | 0.001** |
| t-test to 0.5 mM | 5 mM + HS vs 0.5 mM | < 0.001*** | < 0.001*** | < 0.001*** | 0.0012** |
| | 0.5 mM + HS vs 0.5 mM | 0.031* | ns | 0.0122 | ns |
| t-test to 0.5 mM +HS | 5 mM + HS vs 0.5 mM + HS | < 0.001*** | < 0.001*** | < 0.001*** | 0.0015** |

(b) Nitrate depletion

| | Treatments | DAT 4 | DAT 8 | DAT 11 | DAT 14 | DAT 17 |
|--|--------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Nitrate depletion (μmol) | 5 mM | 1.20 ± 1.01 | 2.55 ± 1.53 | 3.91 ± 1.17 | 3.10 ± 2.13 | 8.49 ± 1.19 |
| | 5 mM + HS | 2.19 ± 2.36 | 0.70 ± 1 | 0.12 ± 0.26 | 1.68 ± 1.3 | 8.10 ± 2.92 |
| | 0.5 mM | 0.92 ± 0.27 | 1.44 ± 0.38 | 1.43 ± 0.43 | 1.34 ± 0.22 | 1.57 ± 0.16 |
| | 0.5 mM + HS | 0.93 ± 0.35 | 1.53 ± 0.39 | 1.73 ± 0.15 | 1.56 ± 0.16 | 1.64 ± 0.34 |
| t-test | | | | | | |
| t-test to 5 mM | 0.5 mM vs 5 mM | ns | ns | 0.004** | ns | < 0.001*** |
| | 5 mM + HS vs 5 mM | ns | ns | < 0.001*** | ns | ns |
| | 0.5 mM + HS vs 5 mM | ns | ns | 0.0061** | ns | < 0.001*** |
| t-test to 0.5 mM | 5 mM + HS vs 0.5 mM | ns | ns | < 0.001*** | ns | 0.0011** |
| | 0.5 mM + HS vs 0.5 mM | ns | ns | ns | ns | ns |
| t-test to 0.5 mM +HS | 5 mM + HS vs 0.5 mM + HS | ns | ns | < 0.001*** | ns | 0.0012** |

| | Treatments | DAT 20 | DAT 23 | DAT 26 | DAT 28 |
|--|--------------------------|-----------------|-----------------|-----------------|-----------------|
| Nitrate depletion (μmol) | 5 mM | 6.57 ± 2.78 | 8.64 ± 3.28 | 5.50 ± 0.67 | 3.60 ± 2.3 |
| | 5 mM + HS | 7.75 ± 1.23 | 6.24 ± 3.35 | 3.41 ± 2.8 | 2.33 ± 2.45 |
| | 0.5 mM | 1.66 ± 0.19 | 1.79 ± 0.13 | 1.76 ± 0.13 | 1.85 ± 0.15 |
| | 0.5 mM + HS | 1.88 ± 0.16 | 1.90 ± 0.14 | 1.96 ± 0.09 | 1.94 ± 0.08 |
| t-test | | | | | |
| t-test to 5 mM | 0.5 mM vs 5 mM | 0.0078** | 0.0031** | < 0.001*** | ns |
| | 5 mM + HS vs 5 mM | ns | ns | ns | ns |
| | 0.5 mM + HS vs 5 mM | 0.0097** | 0.0034** | < 0.001*** | ns |
| t-test to 0.5 mM | 5 mM + HS vs 0.5 mM | 0.0011** | < 0.001*** | 0.018* | ns |
| | 0.5 mM + HS vs 0.5 mM | ns | ns | ns | 0.034* |
| t-test to 0.5 mM +HS | 5 mM + HS vs 0.5 mM + HS | 0.0012** | < 0.001*** | 0.02* | ns |

Table S4 Cumulative N depletion in the EcoFAB medium, showed in Fig. 5 (n=5). Asterisk indicate statistically differences according to unpaired t-test at the 0.05 level. ns, no significant; *, p < 0.05; **, p < 0.01; ***, p < 0.001; ***. (a) cumulative N depletion; (b) cumulative ammonium depletion; (c) cumulative nitrate depletion; (d) Delta N , (in cases of different variance unpaired t-test with Welch's correction was taken).

(a) Cumulative N depletion

| | Treatments | DAT 4 | DAT 8 | DAT 11 | DAT 14 | DAT 17 |
|---|--------------------------|------------------|------------------|------------------|------------------|------------------|
| Cumulative N depletion (μmol) | 5 mM | 13.37 \pm 2.55 | 21.81 \pm 3.92 | 33.20 \pm 3.3 | 43.88 \pm 4.96 | 63.10 \pm 6.59 |
| | 5 mM + HS | 14.91 \pm 3.92 | 18.70 \pm 4.05 | 23.14 \pm 3.27 | 31.43 \pm 3.69 | 49.28 \pm 4.37 |
| | 0.5 mM | 9.62 \pm 0.55 | 12.71 \pm 0.66 | 15.57 \pm 1.28 | 18.19 \pm 1.48 | 21.32 \pm 1.62 |
| | 0.5 mM + HS | 9.91 \pm 0.41 | 13.12 \pm 0.84 | 16.44 \pm 0.98 | 19.44 \pm 1.14 | 22.73 \pm 1.71 |
| t-test | | | | | | |
| t-test to 5 mM | 0.5 mM vs 5 mM | 0.021* | 0.0018** | < 0.001*** | < 0.001*** | < 0.001*** |
| | 5 mM + HS vs 5 mM | ns | ns | 0.0018** | 0.0031** | 0.0069** |
| | 0.5 mM + HS vs 5 mM | 0.028* | 0.0025** | < 0.001*** | < 0.001*** | < 0.001*** |
| t-test to 0.5 mM | 5 mM + HS vs 0.5 mM | 0.018* | 0.012* | 0.0015** | < 0.001*** | 0.018* |
| | 0.5 mM + HS vs 0.5 mM | ns | ns | ns | ns | ns |
| t-test to 0.5 mM +HS | 5 mM + HS vs 0.5 mM + HS | 0.022* | 0.017* | 0.0025** | < 0.001*** | 0.022* |

| | Treatments | DAT 20 | DAT 23 | DAT 26 | DAT 28 |
|---|--------------------------|------------------|-------------------|-------------------|--------------------|
| Cumulative N depletion (μmol) | 5 mM | 80.29 \pm 8.53 | 99.68 \pm 11.43 | 114.70 \pm 11.9 | 125.27 \pm 15.01 |
| | 5 mM + HS | 68.38 \pm 6.13 | 84.33 \pm 3.93 | 96.48 \pm 5.13 | 105.51 \pm 8.62 |
| | 0.5 mM | 24.63 \pm 1.72 | 28.14 \pm 1.87 | 31.55 \pm 1.93 | 35.11 \pm 1.86 |
| | 0.5 mM + HS | 26.49 \pm 1.94 | 30.27 \pm 2.01 | 34.12 \pm 2.03 | 37.96 \pm 2.14 |
| t-test | | | | | |
| t-test to 5 mM | 0.5 mM vs 5 mM | < 0.001*** | < 0.001*** | < 0.001*** | < 0.001*** |
| | 5 mM + HS vs 5 mM | 0.047* | 0.033* | 0.021* | 0.047* |
| | 0.5 mM + HS vs 5 mM | < 0.001*** | < 0.001*** | < 0.001*** | < 0.001*** |
| t-test to 0.5 mM | 5 mM + HS vs 0.5 mM | < 0.001*** | < 0.001*** | < 0.001*** | < 0.001*** |
| | 0.5 mM + HS vs 0.5 mM | ns | ns | ns | ns |
| t-test to 0.5 mM +HS | 5 mM + HS vs 0.5 mM + HS | < 0.001*** | < 0.001*** | < 0.001*** | < 0.001*** |

(b) Cumulative ammonium depletion

| | Treatments | DAT 4 | DAT 8 | DAT 11 | DAT 14 | DAT 17 |
|---|--------------------------|-----------------|------------------|------------------|------------------|------------------|
| Cumulative ammonium depletion (μmol) | 5 mM | 4.70 ± 1.9 | 10.59 ± 2.33 | 18.07 ± 2.03 | 25.66 ± 2.49 | 36.39 ± 3.12 |
| | 5 mM + HS | 5.26 ± 1.61 | 8.34 ± 1.06 | 12.67 ± 1.18 | 19.28 ± 1.85 | 29.03 ± 1.05 |
| | 0.5 mM | 1.24 ± 0.29 | 2.89 ± 0.29 | 4.32 ± 0.56 | 5.60 ± 0.59 | 7.16 ± 0.64 |
| | 0.5 mM + HS | 1.52 ± 0.17 | 3.19 ± 0.23 | 4.78 ± 0.36 | 6.23 ± 0.56 | 7.88 ± 0.72 |
| t-test | | | | | | |
| t-test to 5 mM | 0.5 mM vs 5 mM | 0.007** | < 0.001*** | < 0.001*** | < 0.001*** | < 0.001*** |
| | 5 mM + HS vs 5 mM | ns | ns | 0.0015** | 0.0027** | 0.0019** |
| | 0.5 mM + HS vs 5 mM | 0.01* | < 0.001*** | < 0.001*** | < 0.001*** | < 0.001*** |
| t-test to 0.5 mM | 5 mM + HS vs 0.5 mM | < 0.001*** | < 0.001*** | < 0.001*** | < 0.001*** | < 0.001*** |
| | 0.5 mM + HS vs 0.5 mM | ns | ns | ns | ns | ns |
| t-test to 0.5 mM +HS | 5 mM + HS vs 0.5 mM + HS | < 0.001*** | < 0.001*** | < 0.001*** | < 0.001*** | < 0.001*** |

| | Treatments | DAT 20 | DAT 23 | DAT 26 | DAT 28 |
|---|--------------------------|------------------|------------------|------------------|------------------|
| Cumulative ammonium depletion (μmol) | 5 mM | 47.01 ± 4.11 | 57.76 ± 5.58 | 67.28 ± 5.57 | 74.24 ± 7.12 |
| | 5 mM + HS | 40.38 ± 1.82 | 50.08 ± 2.1 | 58.82 ± 2.4 | 65.52 ± 4.43 |
| | 0.5 mM | 8.80 ± 0.68 | 10.53 ± 0.75 | 12.18 ± 0.74 | 13.89 ± 0.74 |
| | 0.5 mM + HS | 9.77 ± 0.8 | 11.63 ± 0.83 | 13.53 ± 0.85 | 15.43 ± 0.9 |
| t-test | | | | | |
| t-test to 5 mM | 0.5 mM vs 5 mM | < 0.001*** | < 0.001*** | < 0.001*** | < 0.001*** |
| | 5 mM + HS vs 5 mM | 0.017* | 0.031* | 0.022* | ns |
| | 0.5 mM + HS vs 5 mM | < 0.001*** | < 0.001*** | < 0.001*** | < 0.001*** |
| t-test to 0.5 mM | 5 mM + HS vs 0.5 mM | < 0.001*** | < 0.001*** | < 0.001*** | < 0.001*** |
| | 0.5 mM + HS vs 0.5 mM | ns | ns | 0.045* | 0.031* |
| t-test to 0.5 mM +HS | 5 mM + HS vs 0.5 mM + HS | < 0.001*** | < 0.001*** | < 0.001*** | < 0.001*** |

(c) Cumulative nitrate depletion

| | Treatments | DAT 4 | DAT 8 | DAT 11 | DAT 14 | DAT 17 |
|--|--------------------------|-----------------|-----------------|-----------------|------------------|------------------|
| Cumulative nitrate depletion (μmol) | 5 mM | 1.20 ± 1.01 | 3.75 ± 1.85 | 7.66 ± 1.71 | 10.76 ± 3.07 | 19.25 ± 3.89 |
| | 5 mM + HS | 2.19 ± 2.36 | 2.89 ± 3.28 | 3.01 ± 3.18 | 4.68 ± 3.23 | 12.78 ± 4.13 |
| | 0.5 mM | 0.92 ± 0.27 | 2.36 ± 0.41 | 3.78 ± 0.77 | 5.12 ± 0.92 | 6.70 ± 1.02 |
| | 0.5 mM + HS | 0.93 ± 0.35 | 2.46 ± 0.67 | 4.19 ± 0.71 | 5.75 ± 0.76 | 7.38 ± 1.1 |
| t-test | | | | | | |
| t-test to 5 mM | 0.5 mM vs 5 mM | ns | ns | 0.0032** | 0.0079** | < 0.001*** |
| | 5 mM + HS vs 5 mM | ns | ns | 0.023* | 0.02* | 0.043* |
| | 0.5 mM + HS vs 5 mM | ns | ns | 0.0056** | 0.013* | < 0.001*** |
| t-test to 0.5 mM | 5 mM + HS vs 0.5 mM | ns | ns | ns | ns | 0.013* |
| | 0.5 mM + HS vs 0.5 mM | ns | ns | ns | ns | ns |
| t-test to 0.5 mM +HS | 5 mM + HS vs 0.5 mM + HS | ns | ns | ns | ns | 0.023* |

| | Treatments | DAT 20 | DAT 23 | DAT 26 | DAT 28 |
|--|--------------------------|------------------|------------------|------------------|------------------|
| Cumulative nitrate depletion (μmol) | 5 mM | 25.82 ± 4.79 | 34.46 ± 6.10 | 39.96 ± 6.54 | 43.56 ± 8.06 |
| | 5 mM + HS | 20.54 ± 5.07 | 26.78 ± 2.70 | 30.19 ± 3.86 | 32.52 ± 4.97 |
| | 0.5 mM | 8.35 ± 1.07 | 10.14 ± 1.15 | 11.90 ± 1.21 | 13.75 ± 1.15 |
| | 0.5 mM + HS | 9.26 ± 1.22 | 11.17 ± 1.26 | 13.12 ± 1.26 | 15.06 ± 1.31 |
| t-test | | | | | |
| t-test to 5 mM | 0.5 mM vs 5 mM | < 0.001*** | < 0.001*** | < 0.001*** | < 0.001*** |
| | 5 mM + HS vs 5 mM | ns | ns | 0.03* | 0.043* |
| | 0.5 mM + HS vs 5 mM | < 0.001*** | < 0.001*** | < 0.001*** | < 0.001*** |
| t-test to 0.5 mM | 5 mM + HS vs 0.5 mM | < 0.001*** | < 0.001*** | < 0.001*** | < 0.001*** |
| | 0.5 mM + HS vs 0.5 mM | ns | ns | ns | ns |
| t-test to 0.5 mM +HS | 5 mM + HS vs 0.5 mM + HS | 0.0014** | < 0.001*** | < 0.001*** | < 0.001*** |

(d) Delta N, W indicates t-test using Welch's correction

| | Treatments | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Mean \pm SD |
|------------------------|------------------------------|----------|----------|----------|----------|----------|--------------------|
| Delt N (μ mol) | 5 mM | 8.98 | -3.21 | 10.38 | 29.20 | -7.75 | 7.521 \pm 14.39 |
| | 5 mM + HS | -6.93 | -5.59 | -30.47 | -14.74 | -9.71 | -13.49 \pm 10.12 |
| | 0.5 mM | 16.34 | 8.89 | 11.49 | 9.39 | 16.64 | 12.55 \pm 3.731 |
| | 0.5 mM + HS | 16.81 | 12.67 | 16.13 | 18.36 | 9.60 | 14.71 \pm 3.536 |
| t-test | | | | | | | |
| t-test to 5 mM | 0.5 mM vs 5 mM (W) | | | | | | ns |
| | 5 mM + HS vs 5 mM | | | | | | 0.028* |
| | 0.5 mM + HS vs 5 mM (W) | | | | | | ns |
| t-test to 0.5 mM | 5 mM + HS vs 0.5 mM (W) | | | | | | 0.0028** |
| | 0.5 mM + HS vs 0.5 mM | | | | | | ns |
| t-test to 0.5 mM +HS | 5 mM + HS vs 0.5 mM + HS (W) | | | | | | 0.0021** |

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