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## Supplementary Material

# Effect of N supply on the carbon economy of barley when accounting for plant size

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**Table S1.** Final nutrient concentration in each N treatment.
**Table S2.** Effect of N supply on RGR (relative growth rate) and NAR (net assimilation rate).
**Table S3.** Statistically significant differences among N levels at each time point for several growth parameters.
**Table S4.** Amount of phenotypic plasticity expressed as the coefficient of variation (%) (CV) of growth traits through ontogeny, grouped by levels of N supply.
**Table S5.** Amount of phenotypic plasticity expressed as the coefficient of variation (%) (CV) of growth traits among nitrogen concentrations calculated for several common plant sizes.
**Table S6.** Hierarchical multiple regression assessment of ontogenetic and N-supply dependent changes in leaf thickness (LT) and dry matter content (DMC) while controlling for plant size.
**Table S7.** Plant N concentration (NPL, $g_N\ g_{plant}^{-1}$) and leaf N concentration (NLF, $g_N\ g_{leaf}^{-1}$) in plants grown under 0.5 and 2.5 mM of N.
**Table S8.** Growth response coefficients (GRC) (Poorter and Nagel 2000) calculated for the whole experimental period.

**Figure S1.** Details about the hydroponic system.
**Figure S2.** Experiment display: distribution of nitrogen treatments in the glasshouse.
**Figure S3.** Irradiance during the experiment.
**Figure S4.** Comparison between the three ways of assessing growth parameters in response to N supply: 1) one single harvest at 32 days; 2) overall average across 9 harvests; and 3) at a common plant mass of 400 mg. (A) Leaf dry matter content (Leaf DMC); (B) Stem dry matter content (Stem DMC); (C) Root dry matter content (Root DMC).
**Figure S5.** Leaf dry matter content (LDMC) approximation and leaf thickness $(SLA \times LDMC)^{-1}$ approximation among the 6 N treatments.
**Figure S6.** Effect of nitrogen supply on the root respiration rates of plants subjected to 0.5 and 2.5 mM of N.
**Figure S7.** Carbon concentration (mmol C gplant-1) measured in plants grown under 0.5 and 2.5 mM N.
**Figure S8.** Daily biomass allocation to roots for each nitrogen treatment.

**Table S1.** Final nutrient concentration in each N treatment**.**

| N treatment (mM $NO_3^-$) | Final nutrient concentration (mM) in different N treatments | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **N** | **P** | **K** | **Mg** | **Cl** | **S** | **Mn** | **Zn** | **B** | **Cu** | **Mo** | **Fe** | **Ca** |
| **0.125** | 0.125 | 0.5 | 2.05 | 0.5 | 0.05 | 1.28 | 0.002 | 0.002 | 0.025 | 0.0005 | 0.0005 | 0.1 | 0.06 |
| **0.25** | 0.25 | 0.5 | 2.05 | 0.5 | 0.05 | 1.28 | 0.002 | 0.002 | 0.025 | 0.0005 | 0.0005 | 0.1 | 0.13 |
| **0.5** | 0.50 | 0.5 | 2.05 | 0.5 | 0.05 | 1.25 | 0.002 | 0.002 | 0.025 | 0.0005 | 0.0005 | 0.1 | 0.25 |
| **1.0** | 1.00 | 0.5 | 2.05 | 0.5 | 0.05 | 1.00 | 0.002 | 0.002 | 0.025 | 0.0005 | 0.0005 | 0.1 | 0.25 |
| **1.75** | 1.75 | 0.5 | 2.05 | 0.5 | 0.05 | 0.90 | 0.002 | 0.002 | 0.025 | 0.0005 | 0.0005 | 0.1 | 0.35 |
| **2.5** | 2.50 | 0.5 | 2.05 | 0.5 | 0.05 | 0.50 | 0.002 | 0.002 | 0.025 | 0.0005 | 0.0005 | 0.1 | 0.50 |

**Table S2.** Effect of N supply on RGR (relative growth rate) and NAR (net assimilation rate). The nitrogen concentrations are expressed in mM of nitrate.

| Time (days) | RGR (mg $g^{-1}$ $day^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | **0.125 mM N** | **0.25 mM N** | **0.5 mM N** | **1 mM N** | **1.75 mM N** | **2.5 mM N** |
| 4 | 123 | 121 | 137 | 170 | 138 | 150 |
| 7 | 115 | 118 | 131 | 163 | 136 | 148 |
| 11 | 104 | 115 | 124 | 153 | 134 | 144 |
| 14 | 96 | 112 | 119 | 146 | 132 | 142 |
| 18 | 85 | 109 | 111 | 136 | 130 | 139 |
| 21 | 77 | 106 | 106 | 129 | 128 | 137 |
| 25 | 67 | 103 | 99 | 119 | 126 | 133 |
| 28 | 59 | 100 | 93 | 112 | 124 | 131 |
| 32 | 48 | 97 | 86 | 102 | 122 | 128 |

| Time (days) | NAR (g $m^{-2}$ $day^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | **0.125 mM N** | **0.25 mM N** | **0.5 mM N** | **1 mM N** | **1.75 mM N** | **2.5 mM N** |
| 4 | 8.3 | 7.4 | 9.3 | 10.3 | 9.7 | 9.2 |
| 7 | 6.2 | 7.7 | 8.8 | 9.5 | 8.3 | 7.9 |
| 11 | 5.9 | 7.1 | 7.6 | 8.5 | 6.5 | 6.7 |
| 14 | 6.0 | 6.7 | 7.7 | 8.3 | 7.6 | 7.0 |
| 18 | 6.1 | 6.9 | 6.6 | 8.4 | 7.7 | 6.8 |
| 21 | 5.0 | 6.6 | 6.5 | 7.4 | 6.9 | 6.6 |
| 25 | 5.5 | 7.3 | 5.9 | 6.7 | 6.1 | 6.4 |
| 28 | 5.0 | 8.9 | 6.2 | 6.6 | 6.9 | 5.9 |
| 32 | 4.5 | 8.6 | 7.1 | 8.2 | 6.8 | 6.9 |

Note: RGR was calculated as the derivative of the 2$^{nd}$ order polynomial was fitted to the natural log of total dry mass plotted against time.

**Table S3.** Statistically significant differences among N levels at each time point for several growth parameters. Data was analysed using one-way analysis of variance (ANOVA). All parameters were natural logarithm transformed. The homogeneity of variances was violated for the following parameters and time points: SLA at days 21 and 32, LMR at day 21 and RMR at day 28. In these cases a Welch ANOVA test was performed. The Welch ANOVA revealed no statistical significant difference between N treatments for SLA at day 21, and statistically significant differences for LMR at day 21, RMR at day 28 and SLA at day 32. *P*-values: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

| | Time points (days) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Parameter** | **4** | **7** | **11** | **14** | **18** | **21** | **25** | **28** | **32** |
| **LAR** | * | n.s. | *** | ** | *** | *** | *** | *** | *** |
| **SLA** | * | ** | *** | ** | n.s. | n.s | * | * | *** |
| **LMR** | n.s. | * | *** | *** | *** | *** | *** | *** | *** |
| **SMR** | n.s. | n.s. | * | n.s. | * | *** | *** | *** | *** |
| **RMR** | * | ** | *** | *** | *** | *** | *** | *** | *** |

**Table S4.** Amount of phenotypic plasticity expressed as the coefficient of variation (%) (CV) of growth traits through ontogeny, grouped by levels of N supply. RGR (relative growth rate, mg $g^{-1}$ $day^{-1}$), NAR (net assimilation rate, g $m^{-2}$ $day^{-1}$), LAR (Leaf area ratio, $m^2$ $kg^{-1}$), SLA (specific leaf area, $m^2$ $kg^{-1}$), LMR (leaf mass ratio, g $g^{-1}$), SMR (stem mass ratio, g $g^{-1}$), RMR (root mass ratio, g $g^{-1}$).

| **Nitrogen concentration (mM $NO_3^-$)** | **RGR** | **NAR** | **LAR** | **SLA** | **LMR** | **SMR** | **RMR** |
|---|---|---|---|---|---|---|---|
| **0.125** | 29.6 | 18.8 | 18.0 | 12.0 | 11.3 | 24.7 | 9.0 |
| **0.250** | 7.5 | 10.6 | 14.4 | 11.7 | 12.1 | 26.5 | 9.9 |
| **0.500** | 15.5 | 15.9 | 9.6 | 12.0 | 14.4 | 24.3 | 3.3 |
| **1.000** | 17.0 | 14.8 | 10.2 | 13.7 | 12.9 | 29.5 | 4.5 |
| **1.750** | 4.3 | 14.6 | 11.1 | 9.9 | 14.1 | 20.2 | 13.1 |
| **2.500** | 5.4 | 13.6 | 9.0 | 9.3 | 14.2 | 23.5 | 14.4 |

**Table S5.** Amount of phenotypic plasticity expressed as the coefficient of variation (%) (CV) of growth traits among nitrogen concentrations calculated for several common plant sizes. RGR (relative growth rate, g $g^{-1}$ $day^{-1}$), NAR (net assimilation rate, g $m^{-2}$ $day^{-1}$), LAR (Leaf area ratio, $m^2$ $kg^{-1}$), SLA (specific leaf area, $m^2$ $kg^{-1}$), LMR (leaf mass ratio, g $g^{-1}$), SMR (stem mass ratio, g $g^{-1}$), RMR (root mass ratio, g $g^{-1}$).

| Plant size (g) | RGR | NAR | LAR | SLA | LMR | SMR | RMR |
|---|---|---|---|---|---|---|---|
| **0.05** | 12.7 | 11.4 | 7.3 | 6.5 | 10.6 | 5.7 | 9.4 |
| **0.10** | 15.2 | 12.3 | 7.4 | 3.8 | 9.2 | 7.2 | 11.1 |
| **0.15** | 17.8 | 13.2 | 8.2 | 2.1 | 8.5 | 12.1 | 13.0 |
| **0.20** | 20.3 | 13.9 | 9.7 | 1.7 | 8.7 | 15.7 | 15.0 |
| **0.25** | 22.7 | 14.4 | 11.7 | 2.2 | 9.9 | 17.6 | 16.9 |
| **0.30** | 24.9 | 14.7 | 14.2 | 2.7 | 12.0 | 17.9 | 18.7 |
| **0.35** | 26.9 | 14.7 | 16.9 | 3.0 | 14.9 | 16.7 | 20.3 |
| **0.40** | 28.6 | 14.5 | 19.9 | 3.4 | 18.5 | 14.4 | 21.7 |

**Table S6.** Hierarchical multiple regression assessment of ontogenetic and N-supply dependent changes in leaf thickness (LT) and dry matter content (DMC) while controlling for plant size.

| Parameter | Variable | Model 1 | | Model 2 | |
|---|---|---|---|---|---|
| | | B | β | B | β |
| **LT** | Ln Plant Mass | 16.11*** | 0.72 | 15.28*** | 0.69 |
| | N Treatment | | | 6.12*** | 0.20 |
| | $r^2$ | 0.52 | | 0.56 | |
| | $F$ | 415.3*** | | 243.3*** | |
| | $\Delta r^2$ | 0.52 | | 0.04 | |
| | $\Delta F$ | 415.3*** | | 34.4*** | |
| **DMC** | Ln Plant Mass | -0.01* | -0.10 | -0.004 | -0.05 |
| | N Treatment | | | -0.04*** | -0.31 |
| | $r^2$ | 0.01 | | 0.102 | |
| | $F$ | 4.12* | | 21.26*** | |
| | $\Delta r^2$ | 0.01 | | 0.09 | |
| | $\Delta F$ | 4.12* | | 38.00*** | |

Note: The dependent variable DMC and the independent variable plant mass were natural log transformed. The independent variables were entered in two steps - in model 1, ln plant dry mass was entered and held constant. In model 2, N treatment was entered. Therefore the model 1 predictor is ln plant mass, and the model 2 predictors are ln plant mass and N treatment. The change in $r^2$ for model 2 indicates the amount of unique variance accounted for by the independent variables in the second step. B represents the unstandardized coefficient and β represents the standardized coefficient. *p <0.05, ** p < 0.01, *** p < 0.001. Model 1 predictors: ln plant mass, Model 2 predictors: ln plant mass and N treatment. n = 377.

**Table S7.** Plant N concentration ($N_{PL}$, $g_N$ $g_{plant}^{-1}$) and leaf N concentration ($N_{LF}$, $g_N$ $g_{leaf}^{-1}$) in plants grown under 0.5 and 2.5 mM of N.

| Time | $N_{PL}$ | | | | $N_{LF}$ | | | |
|---|---|---|---|---|---|---|---|---|
| (days) | 0.5 mM | se | 2.5 mM | se | 0.5 mM | se | 2.5 mM | se |
| 4 | 0.0271 | 0.0005 | 0.0387 | 0.0014 | 0.0443 | 0.0008 | 0.0569 | 0.0012 |
| 7 | 0.0275 | 0.0006 | 0.0400 | 0.0021 | 0.0471 | 0.0006 | 0.0570 | 0.0010 |
| 11 | 0.0312 | 0.0005 | 0.0447 | 0.0003 | 0.0433 | 0.0014 | 0.0552 | 0.0005 |
| 14 | 0.0321 | 0.0008 | 0.0471 | 0.0007 | 0.0435 | 0.0006 | 0.0561 | 0.0012 |
| 18 | 0.0357 | 0.0005 | 0.0506 | 0.0006 | 0.0485 | 0.0008 | 0.0585 | 0.0008 |
| 21 | 0.0394 | 0.0007 | 0.0539 | 0.0003 | 0.0523 | 0.0013 | 0.0667 | 0.0009 |

**Table S.8** Growth response coefficients (GRC) (Poorter and Nagel 2000) calculated for the whole experimental period (28 days). The GRC's were obtained by using a linear regression approach where the natural log transformed parameters LMR, SLA and NAR were plotted against natural log transformed RGR. The slope of the linear regression is the GRC for each parameter. Each of the GRC values for the different parameters represent their relative contribution to variation of RGR.

| GRCs | | | |
|---|---|---|---|
| ***LMR*** | ***SLA*** | ***NAR*** | **Sum** |
| 0.75 | -0.17 | 0.45 | 1.0 |

**Figure S1.** (A) Ebb-and-flow hydroponic system used in the experiment. The nutrient solutions were stored in the 100 L blue reservoirs located under the bench (one N level treatment per reservoir). (B) Barley plants growing inside PVC tubes on the top of plastic tubs which were filled with nutrient solution. (C) Close up of seedlings growing inside the PVC tubes.

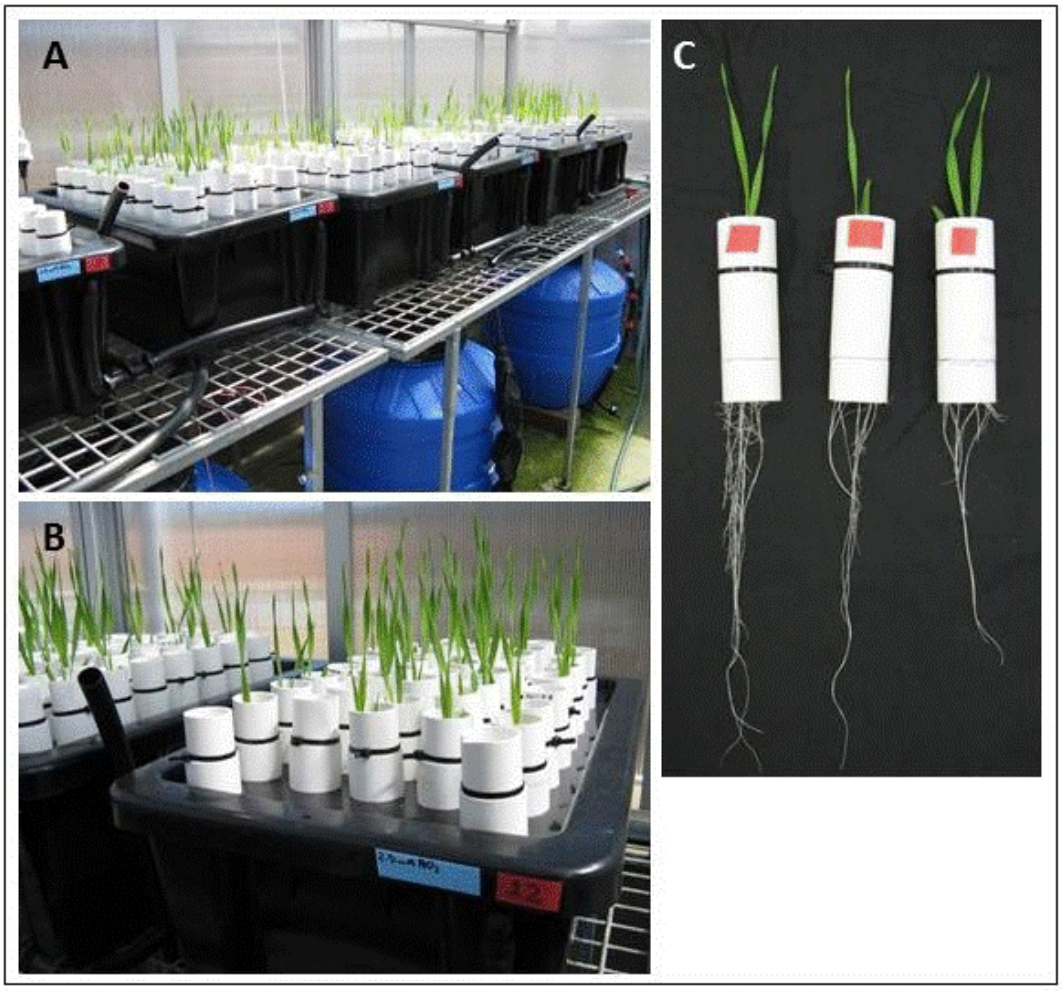

**Figure S2.** Experiment display: distribution of nitrogen treatments in the glasshouse.

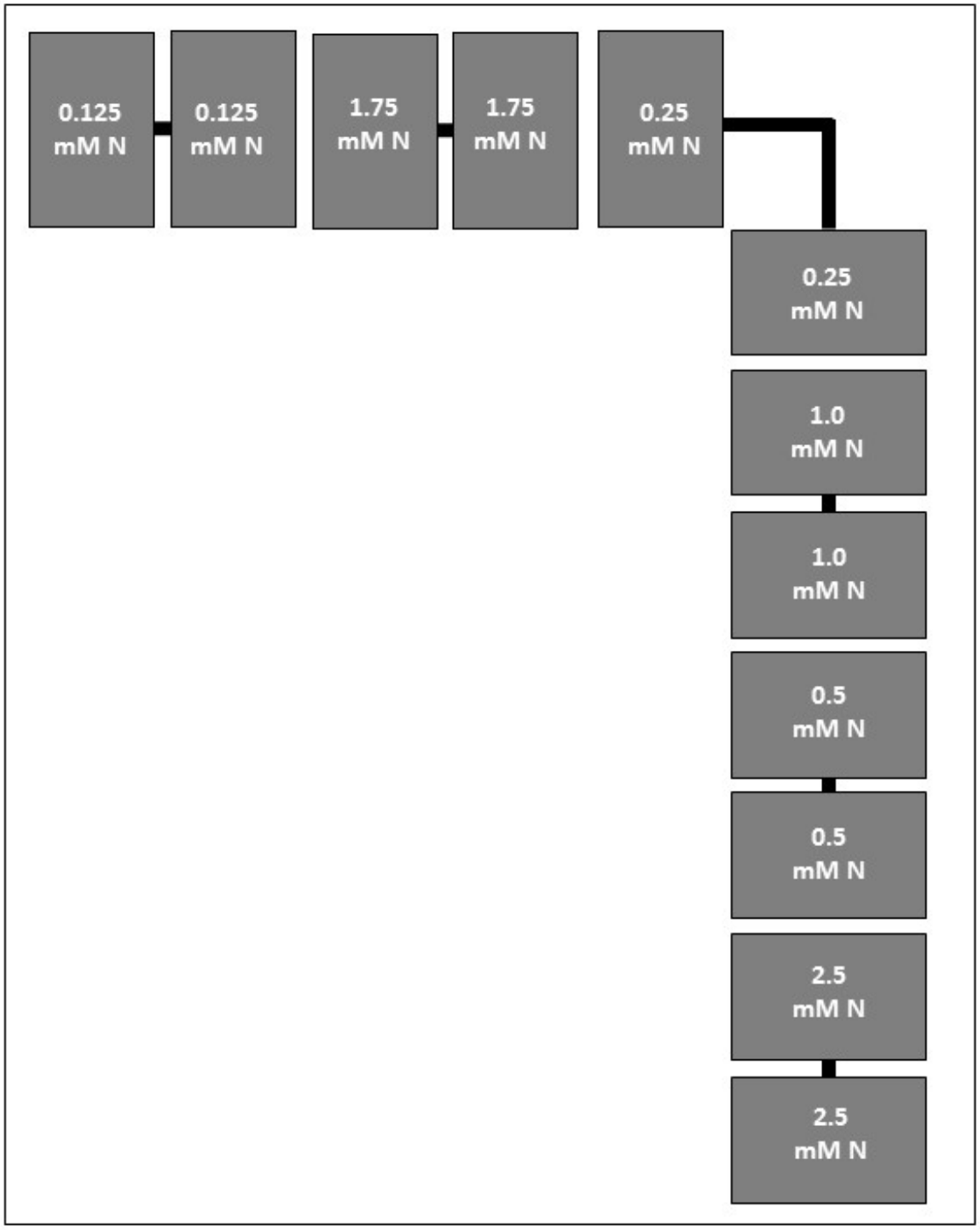

**Figure S3.** Irradiance during the experiment. (A) Photosynthetic active radiation (PAR) at midday; (B) Daily irradiance integral.

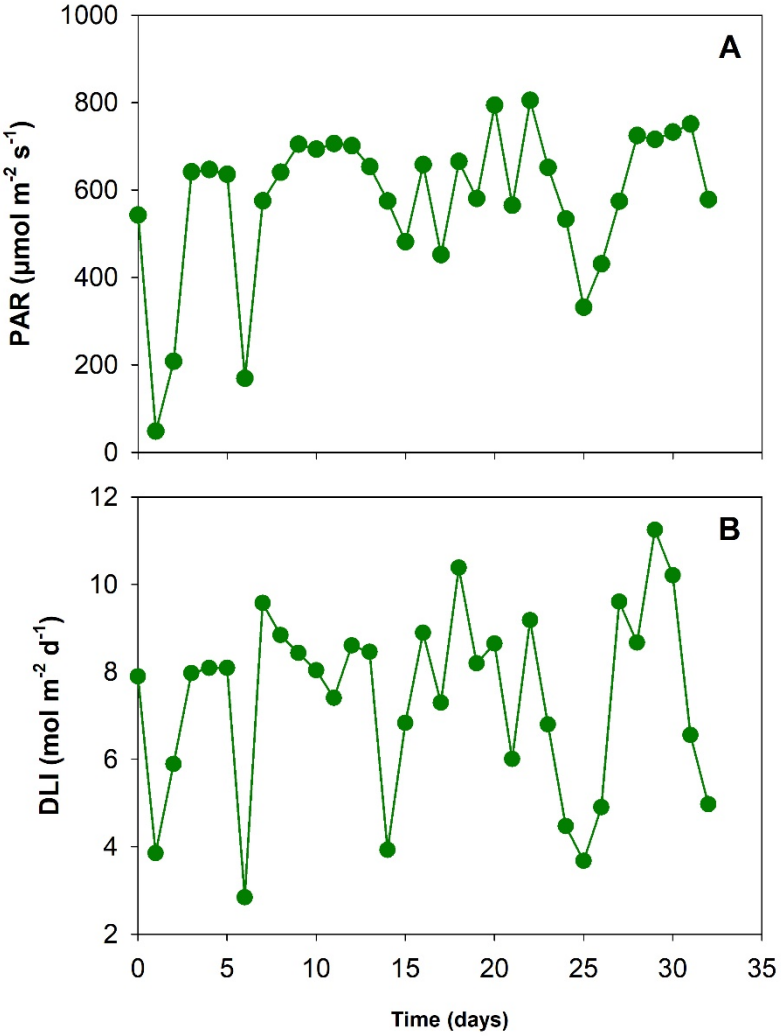

**Figure S4.** Comparison between the three ways of assessing growth parameters in response to N supply: 1) one single harvest at 32 days; 2) overall average across 9 harvests; and 3) at a common plant mass of 400 mg. (A) Leaf dry matter content (Leaf DMC); (B) Stem dry matter content (Stem DMC); (C) Root dry matter content (Root DMC). The error bars represent the standard error of the means (n=7 for day 32 and common mass of 400 mg, and N=63 for the overall average).

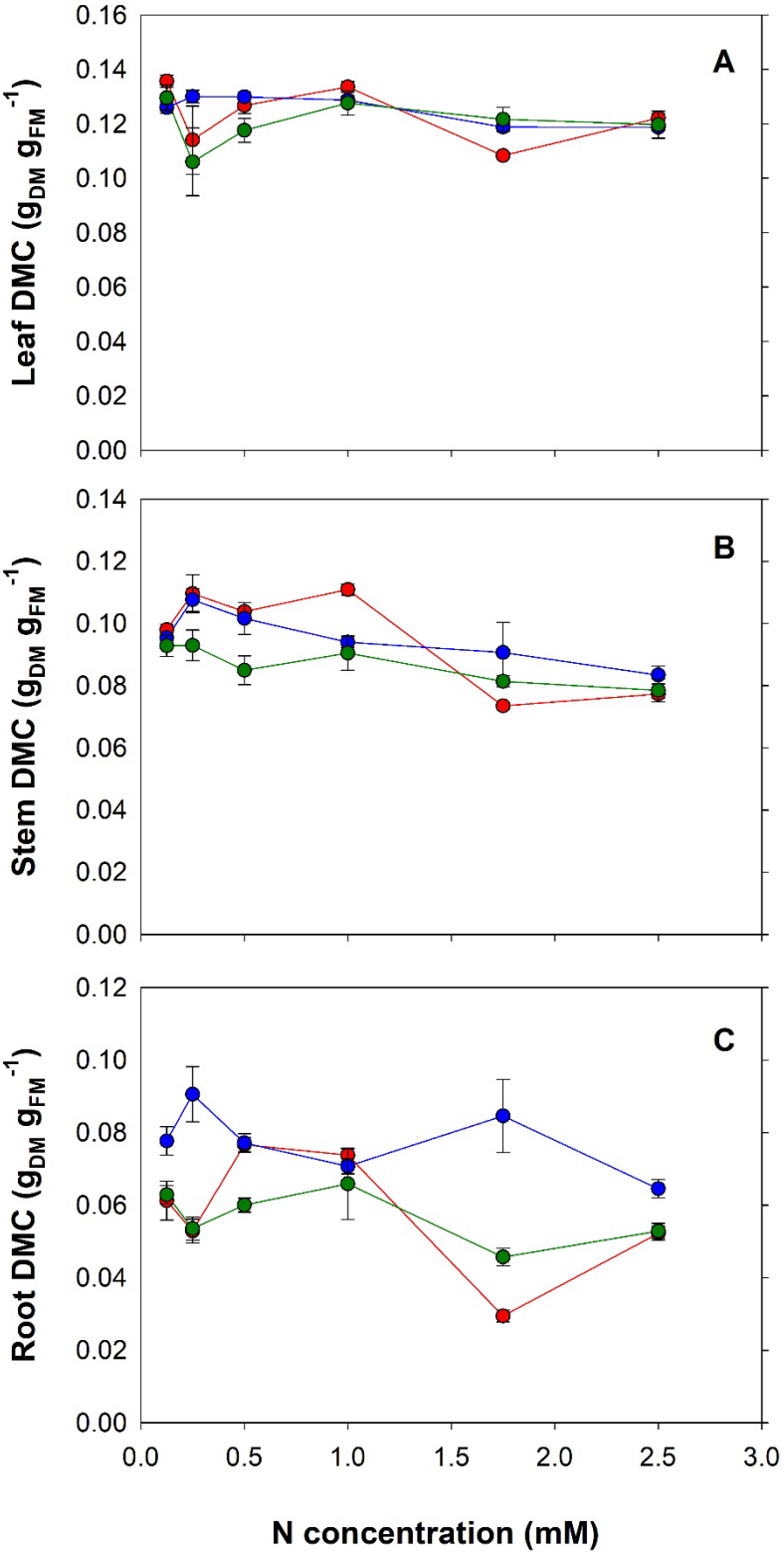

**Figure S5.** Leaf dry matter content (LDMC) approximation and leaf thickness (SLA x LDMC)$^{-1}$ approximation among the 6 N treatments.

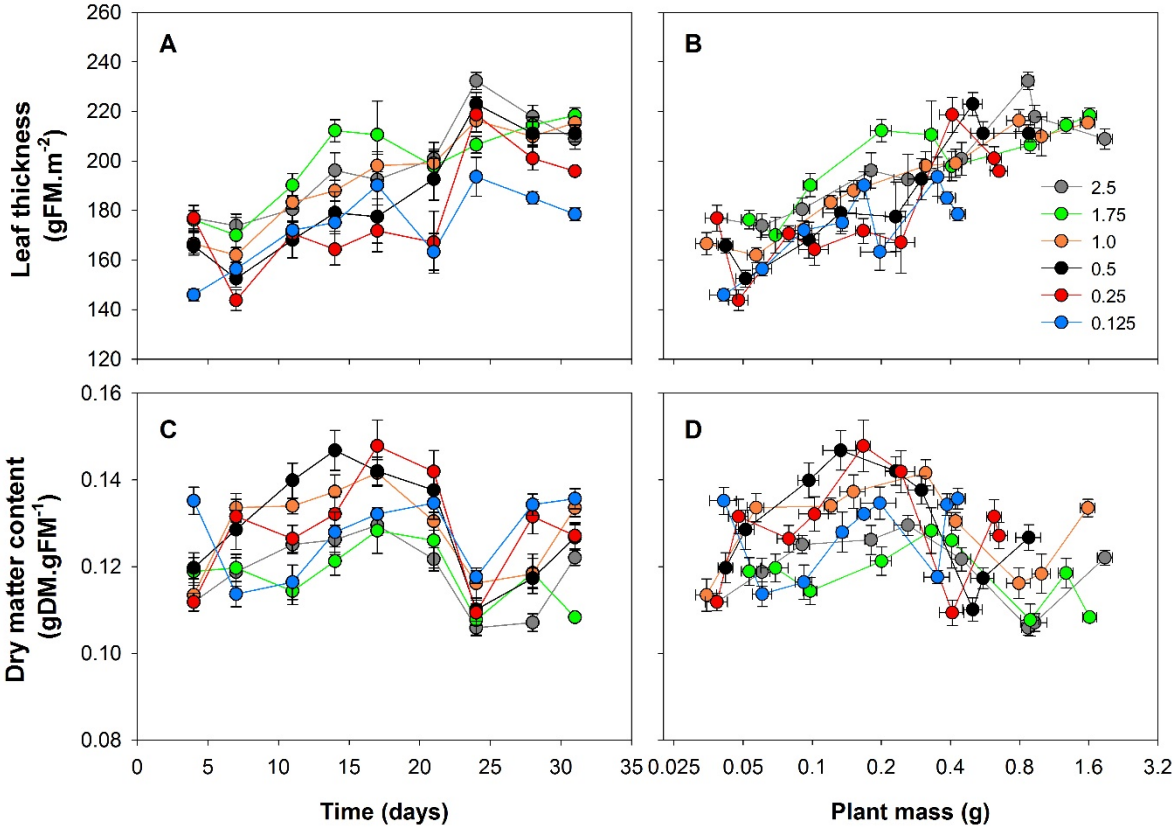

**Figure S6.** Effect of nitrogen supply on the root respiration rates of plants subjected to 0.5 and 2.5 mM of N. Root respiration rate on (A) a leaf area basis (µmol $O_2$ $m^{-2}$ $s^{-1}$) vs time (days); (B) Root respiration rate on a leaf area basis (µmol $O_2$ $m^{-2}$ $s^{-1}$) vs plant dry mass (g).

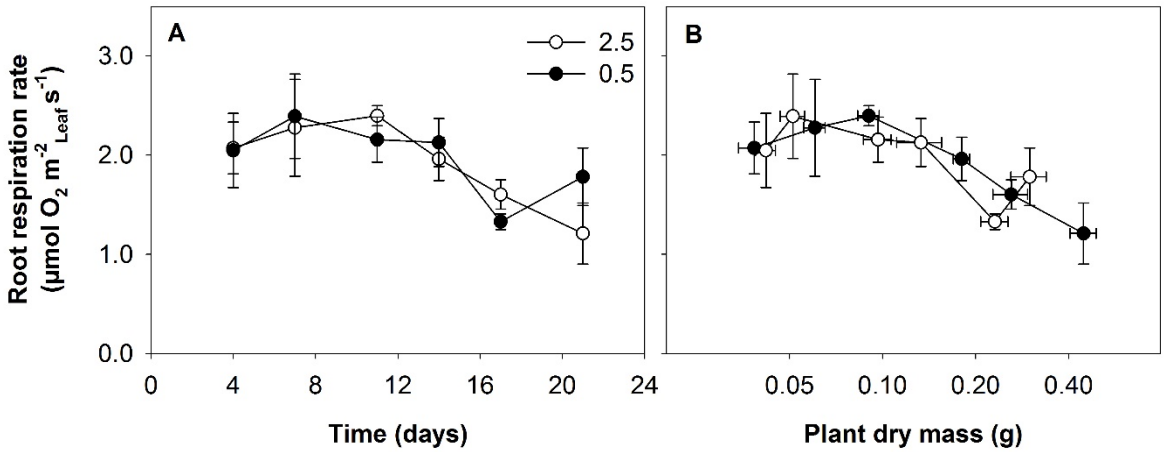

**Figure S7.** Carbon concentration (mmol C $g_{plant}^{-1}$) measured in plants grown under 0.5 and 2.5 mM N. The values are the means ± SE of 3 replicates of whole plants from time point 6 (day 21). The independent samples T-test revealed a statistically significant difference between N treatments at $p < 0.05$.

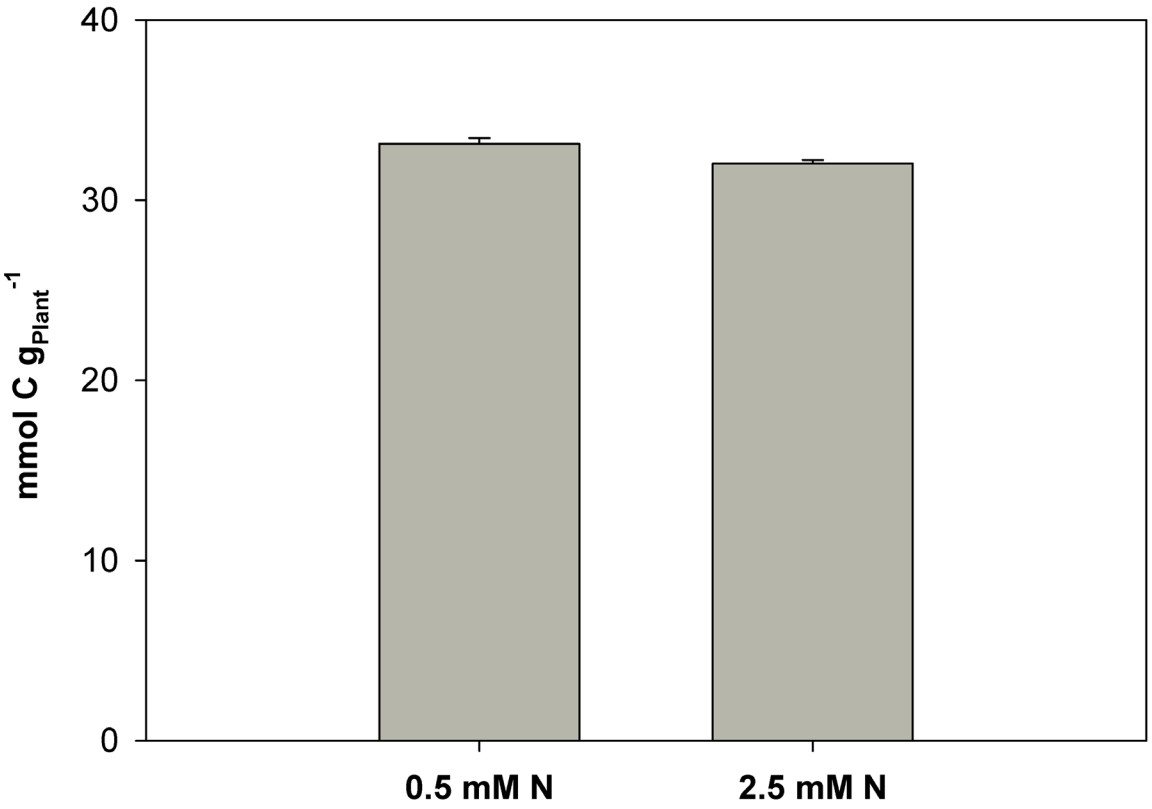

**Figure S8.** Daily biomass allocation to roots for each nitrogen treatment. (A) Root allocation across time; (B) root allocation as a function of plant mass (g). The line patterns in panel B represent the part of the data without common plant masses for all N treatments. See the calculations section of the supplementary information for details on how the calculations of root mass allocation were performed. The nitrogen concentrations in mM of nitrate are represented by the different colours according to the legend in Figure 3B. The y-axis is logarithmic in figure B.