**SUPPLEMENTARY MATERIAL FOR:** “Nitrogen demand, supply, and acquisition strategy control plant responses to elevated CO2 at different scales”

**Results (cont.)**

*Components of carbon costs to acquire nitrogen*

Elevated CO2 increased *N*wp by 27% (*p*<0.001; Table S3), a pattern that was enhanced with increasing fertilization (CO2-by-fertilization interaction: *p*<0.05; Table S3) but was not modified by inoculation (CO2-by-inoculation interaction: *p*>0.05; Table S3). An interaction between fertilization and inoculation (*p*<0.001; Table S3) indicated that positive effects of increasing fertilization on *N*wp (*p*<0.001; Table S3) were stronger in uninoculated plants (Tukey: *p*<0.001).

Elevated CO2 increased *C*bg by 100% (*p*<0.001; Table S3), a pattern that was not modified by fertilization (CO2-by-fertilization interaction: *p*>0.05; Table S3). An interaction between CO2 and inoculation (*p*<0.05; Table S3) indicated that inoculation increased *C*bg under ambient CO2 (Tukey: *p*<0.001) but did not change *C*bg under eCO2 (Tukey: *p*>0.05). An interaction between fertilization and inoculation (*p*<0.001; Table S3) indicated that positive effects of increasing fertilization on *C*bg were stronger in uninoculated plants (Tukey: *p*<0.001).

*Nitrogen fixation*

Nodule biomass increased by 30% under eCO2 (*p*<0.001; Table S4), a pattern that was modified by fertilization (CO2-by-fertilization interaction: *p*>0.05; Table S4), but not inoculation (inoculation-by-fertilization interaction: *p*>0.05; Table S4). Specifically, negative effects of increasing fertilization on nodule biomass (*p*<0.001; Table S4) were stronger under eCO2 (Tukey: *p*<0.001; Fig. S6a). An interaction between fertilization and inoculation (*p*<0.001; Table S4) indicated that negative effects of increasing fertilization on nodule biomass were stronger in inoculated pots (Tukey: *p*<0.001; Fig. S6a).

There was no effect of CO2 treatment on nodule: root biomass (*p*>0.05; Table S6b), though an interaction between CO2 and inoculation (*p*<0.001; Table S4) indicated that positive effects of inoculation on nodule: root biomass (*p*<0.001; Table S4) were stronger under aCO2 (3129% increase; Tukey: *p*<0.001) than eCO2 (379% increase; Tukey: *p*<0.001). An interaction between fertilization and inoculation (*p*<0.001; Table S4) indicated that negative effects of increasing fertilization on nodule: root biomass (*p*<0.001; Table S4) were stronger in inoculated pots (Tukey: *p*<0.001; Fig. S6b).

**Table S1** Summary table containing volumes of compounds used to create modified Hoagland’s solutions for each soil nitrogen fertilization treatment. All volumes are expressed as milliliters per liter (mL/L)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Compound** | **0 ppm N** | **35 ppm N** | **70 ppm N** | **105 ppm N** | **140 ppm N** |
| **1 M NH4H2PO4** | 0 | 0.165 | 0.33 | 0.5 | 0.67 |
| **2 M KNO3** | 0 | 0.335 | 0.67 | 1 | 1.33 |
| **2 M Ca(NO3)2** | 0 | 0.335 | 0.67 | 1 | 1.33 |
| **1 M NH4NO3** | 0 | 0.165 | 0.33 | 0.5 | 0.67 |
| **8 M NH4NO3** | 0 | 0 | 0 | 0 | 0 |
| **1 M KH2PO4** | 1 | 0.85 | 0.67 | 0.5 | 0.33 |
| **1 M KCl** | 3 | 2.45 | 2 | 1.5 | 1 |
| **1 M CaCO3** | 4 | 3.33 | 2.67 | 2 | 1.33 |
| **2 M MgSO4** | 1 | 1 | 1 | 1 | 1 |
| **10% Fe-EDTA** | 1 | 1 | 1 | 1 | 1 |
| **Trace elements** | 1 | 1 | 1 | 1 | 1 |
|  |  |  |  |  |  |
| **Compound** | **210 ppm N** | **280 ppm N** | **350 ppm N** | **630 ppm N** |  |
| **1 M NH4H2PO4** | 1 | 1 | 1 | 1 |  |
| **2 M KNO3** | 2 | 2 | 2 | 2 |  |
| **2 M Ca(NO3)2** | 2 | 2 | 2 | 2 |  |
| **1 M NH4NO3** | 1 | 3.5 | 0 | 0 |  |
| **8 M NH4NO3** | 0 | 0 | 0.75 | 2 |  |
| **1 M KH2PO4** | 0 | 0 | 0 | 0 |  |
| **1 M KCl** | 0 | 0 | 0 | 0 |  |
| **1 M CaCO3** | 0 | 0 | 0 | 0 |  |
| **2 M MgSO4** | 1 | 1 | 1 | 1 |  |
| **10% Fe-EDTA** | 1 | 1 | 1 | 1 |  |
| **Trace elements** | 1 | 1 | 1 | 1 |  |

**Table S2** Summary of the daily growth chamber growing condition program

|  |  |  |
| --- | --- | --- |
| **Time** | **Air temperature (ºC)** | **PAR ± SD (μmol m-2 s-1)** |
| 09:00 | 21 | 278±2 |
| 09:45 | 557±4 |
| 10:30 | 25 | 797±4 |
| 11:15 | 1230±12 |
| 22:45 | 21 | 797±4 |
| 23:30 | 557±4 |
| 00:15 | 17 | 278±2 |
| 01:00 | 0±0 |

**Table S3** Effects of soil nitrogen fertilization, inoculation, and CO2 on components of the carbon cost to acquire nitrogen\*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | **Belowground carbon biomass**a | | **Total nitrogen biomass**b | |
|  | df | *χ*2 | *p* | *χ*2 | *p* |
| (Intercept) | - | - | - | - | - |
| CO2 | 1 | 84.134 | **<0.001** | 23.890 | **<0.001** |
| Inoculation (I) | 1 | 41.03 | **<0.001** | 134.46 | **<0.001** |
| N fertilization (N) | 1 | 152.248 | **<0.001** | 529.021 | **<0.001** |
| CO2\*I | 1 | 8.965 | **0.003** | 1.190 | 0.275 |
| CO2\*N | 1 | 1.188 | 0.276 | 5.915 | **0.015** |
| I\*N | 1 | 22.648 | **<0.001** | 55.562 | **<0.001** |
| CO2\*I\*N | 1 | 1.109 | 0.292 | 0.620 | 0.431 |

\*Significance determined using Type II Wald χ2 tests (α=0.05). A superscript “a” is included after trait labels to indicate if models were fit with natural-log transformed response variables, while a superscript “b” is included if models were fit with square-root transformed response variables. *P*-values less than 0.05 are in bold and p-values where 0.05<*p*<0.1 are italicized. Key: df=degrees of freedom.

**Table S4** Effects of soil nitrogen fertilization, inoculation, and CO2 on the root nodule biomass: root biomass ratio and root nodule biomass\*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | **Root nodule biomassb** | | **Root nodule: root biomassb** | |
|  | df | *χ*2 | *p* | *χ*2 | *p* |
| (Intercept) | - | - | - | - | - |
| CO2 | 1 | 19.258 | **<0.001** | 0.010 | 0.921 |
| Inoculation (I) | 1 | 755.02 | **<0.001** | 902.063 | **<0.001** |
| N fertilization (N) | 1 | 84.376 | **<0.001** | 254.741 | **<0.001** |
| CO2\*I | 1 | 0.950 | 0.330 | 21.632 | **<0.001** |
| CO2\*N | 1 | 2.106 | 0.147 | 1.590 | 0.207 |
| I\*N | 1 | 44.622 | **<0.001** | 132.463 | **<0.001** |
| CO2\*I\*N | 1 | 0.196 | 0.658 | 2.481 | 0.115 |

\*Significance determined using Type II Wald χ2 tests (α=0.05). A superscript “b” is included after trait labels to indicate if models were fit with square-root transformed response variables. *P*-values less than 0.05 are in bold and *p*-values where 0.05<*p*<0.1 are italicized. Key: df=degrees of freedom.

**Figure S1**

**A graph of different types of mass

Description automatically generated**

**Figure S1** Relationships between area-based leaf nitrogen content (a), mass-based leaf nitrogen content (b), and leaf mass per unit leaf area (c) measured on the focal leaf used to generate *A*net/*C*i curves (x-axis) and leaf nitrogen content measured on the leaf used for chlorophyll extractions (y-axis). Blue points refer to leaves grown under ambient CO2 and red points refer leaves grown under elevated CO2. Square points indicate uninoculated pots and circular points indicate inoculated pots. Pearson’s correlation coefficient, associated *p*-values, and the line of the regression line that described each bivariate are included in the top left corner of each plot. The solid black line visualizes the trend given a 1:1 bivariate relationship.

**Figure S2**

**A graph showing different levels of fertilization

Description automatically generated**

**Figure S2** Effects of CO2, fertilization, and inoculation on the ratio of whole plant biomass to pot volume. Fertilization is represented on the x-axis. Red shaded points and trendlines indicate plants grown under elevated CO2, while blue shaded points and trendlines indicate plants grown under ambient CO2. Lighter blue and red circular points and trendlines indicate measurements collected from uninoculated plants, while darker blue and red triangular points indicate measurements collected from inoculated plants. Solid trendlines indicate regression slopes that are different from zero (*p*<0.05), while dashed trendlines indicate slopes that are not distinguishable from zero (*p*>0.05).The dotted horizontal line indicates the point where biomass: pot volume exceeds 1 g L-1, and the dashed line indicates the point where biomass: pot volume exceeds 2 g L-1.

**Figure S3**

**A diagram of different types of soil fertilizers

Description automatically generated**

**Figure S3** Effects of CO2 and fertilization inoculation on area-based leaf nitrogen content (a), mass-based leaf nitrogen content (b), and leaf biomass per unit leaf area (c). Fertilization is represented on the x-axis. Red shaded points and trendlines indicate plants grown under elevated CO2, while blue shaded points and trendlines indicate plants grown under ambient CO2. Lighter blue and red circular points and trendlines indicate measurements collected from uninoculated plants, while darker blue and red triangular points indicate measurements collected from inoculated plants. Solid trendlines indicate regression slopes that are different from zero (*p*<0.05), while dashed trendlines indicate slopes that are not distinguishable from zero (*p*>0.05).

**Figure S4**

**A graph showing the growth of soil fertilization

Description automatically generated**

**Figure S4** Effects of CO2 and fertilization inoculation on photosynthetic nitrogen-use efficiency. Fertilization is represented on the x-axis. Red shaded points and trendlines indicate plants grown under elevated CO2, while blue shaded points and trendlines indicate plants grown under ambient CO2. Lighter blue and red circular points and trendlines indicate measurements collected from uninoculated plants, while darker blue and red triangular points indicate measurements collected from inoculated plants. Solid trendlines indicate regression slopes that are different from zero (*p*<0.05), while dashed trendlines indicate slopes that are not distinguishable from zero (*p*>0.05).

**Figure S5**

**A diagram of soil fertilization

Description automatically generated**

**Figure S5** Effects of CO2 and fertilization inoculation on belowground carbon biomass (a) and total nitrogen biomass (b). Belowground carbon biomass is the numerator of *N*cost, while total nitrogen biomass is the denominator of *N*cost. Fertilization is represented on the x-axis in all panels. Red shaded points and trendlines indicate plants grown under elevated CO2, while blue shaded points and trendlines indicate plants grown under ambient CO2. Lighter blue and red circular points and trendlines indicate measurements collected from uninoculated plants, while darker blue and red triangular points indicate measurements collected from inoculated plants. Solid trendlines indicate regression slopes that are different from zero (*p*<0.05), while dashed trendlines indicate slopes that are not distinguishable from zero (*p*>0.05).

**Figure S6**

A diagram of soil fertilization

Description automatically generated

**Figure S6** Effects of nitrogen fertilization, inoculation treatment, and CO2 treatment on nodule biomass (a) and root nodule biomass: root biomass (b). Fertilization is represented on the x-axis. Red shaded points and trendlines indicate plants grown under elevated CO2, while blue shaded points and trendlines indicate plants grown under ambient CO2. Lighter blue and red circular points and trendlines indicate measurements collected from uninoculated plants, while darker blue and red triangular points indicate measurements collected from inoculated plants. Solid trendlines indicate regression slopes that are different from zero (*p*<0.05), while dashed trendlines indicate slopes that are not distinguishable from zero (*p*>0.05).