**SUPPLEMENTARY MATERIAL FOR:** “Optimal coordination and progressive nitrogen limitation control plant responses to elevated CO2 at difference scales”

**Results (cont.)**

*Components of carbon costs to acquire nitrogen*

Elevated CO2 increased *C*bg (*p*<0.001; Table S3), a pattern that was not modified by fertilization treatment (CO2-by-fertilization interaction: *p*>0.05; Table S3). An interaction between CO2 and inoculation treatment (*p*<0.05; Table S3) indicated that inoculation increased *C*bg under ambient CO2 (Tukey: *p*<0.001), but did not influence *C*bg under elevated CO2 (Tukey: *p*>0.05). An additional interaction between fertilization and inoculation (*p*<0.001; Table S3) indicated that the positive effect of increasing fertilization on *C*bg was stronger in uninoculated plants (Tukey: *p*<0.001).

Elevated CO2 increased *N*wp (*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 treatment (CO2-by-inoculation interaction: *p*>0.05; Table S3). An interaction between fertilization and inoculation (*p*<0.001; Table S3) indicated that the positive effect of increasing fertilization (*p*<0.001; Table S3) was 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 across the fertilization gradient (CO2-by-fertilization interaction: *p*>0.05; Table S4), but not between inoculation treatments (inoculation-by-fertilization interaction: *p*>0.05; Table S4). Specifically, the negative effect of increasing fertilization on nodule biomass (*p*<0.001; Table S4) was stronger under eCO2 than aCO2 (Tukey: *p*<0.001; Fig. S6a), which reduced the increase in nodule biomass under eCO2 with increasing fertilization. A strong interaction between fertilization and inoculation (*p*<0.001; Table S4) was driven by a stronger negative effect of increasing fertilization 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), although an interaction between CO2 and inoculation (*p*<0.001; Table S4) indicated that the general positive effect of inoculation on nodule: root biomass (*p*<0.001; Table S4) was stronger under aCO2 (3129% increase; Tukey: *p*<0.001) than elevated CO2 (379% increase; Tukey: *p*<0.001). The null effect of CO2 on nodule: root biomass was consistently observed across the fertilization gradient (*p*>0.05; Table S4; Fig. S6b). An interaction between fertilization and inoculation (*p*<0.001; Table S4) indicated that the negative effect of increasing fertilization on nodule: root biomass (*p*<0.001; Table S4) was 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 | Coefficient | *χ*2 | *p* | Coefficient | *χ*2 | *p* |
| (Intercept) | - | -1.70\*100 | - | - | 1.24\*10-1 | - | - |
| CO2 | 1 | 9.21\*10-1 | 84.134 | **<0.001** | -3.41\*10-3 | 23.890 | **<0.001** |
| Inoculation (I) | 1 | 1.18\*100 | 41.03 | **<0.001** | 1.68\*10-1 | 134.46 | **<0.001** |
| N fertilization (N) | 1 | 3.38\*10-3 | 152.248 | **<0.001** | 6.69\*10-4 | 529.021 | **<0.001** |
| CO2\*I | 1 | -6.18\*10-1 | 8.965 | **0.003** | 3.68\*10-2 | 1.190 | 0.275 |
| CO2\*N | 1 | -3.66\*10-5 | 1.188 | 0.276 | 1.58\*10-4 | 5.915 | **0.015** |
| I\*N | 1 | -2.22\*10-3 | 22.648 | **<0.001** | -3.20\*10-4 | 55.562 | **<0.001** |
| CO2\*I\*N | 1 | 8.09\*10-4 | 1.109 | 0.292 | -7.54\*10-5 | 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 | Coefficient | *χ*2 | *p* | Coefficient | *χ*2 | *p* |
| (Intercept) | - | 9.41\*10-3 | - | - | 1.32\*10-2 | - | - |
| CO2 | 1 | 1.20\*10-1 | 19.258 | **<0.001** | 9.95\*10-2 | 0.010 | 0.921 |
| Inoculation (I) | 1 | 5.74\*10-1 | 755.02 | **<0.001** | 5.45\*10-1 | 902.063 | **<0.001** |
| N fertilization (N) | 1 | 7.71\*10-6 | 84.376 | **<0.001** | -5.59\*10-6 | 254.741 | **<0.001** |
| CO2\*I | 1 | -4.68\*10-2 | 0.950 | 0.330 | -1.42\*10-1 | 21.632 | **<0.001** |
| CO2\*N | 1 | -1.59\*10-4 | 2.106 | 0.147 | -1.74\*10-4 | 1.590 | 0.207 |
| I\*N | 1 | -5.82\*10-4 | 44.622 | **<0.001** | -7.51\*10-4 | 132.463 | **<0.001** |
| CO2\*I\*N | 1 | 7.26\*10-5 | 0.196 | 0.658 | 1.82\*10-4 | 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 CO2 response 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 of different colored lines

Description automatically generated**

**Figure S2** Effects of CO2, fertilization, and inoculation on the ratio of whole plant biomass to pot volume. Soil nitrogen fertilization is represented on the x-axis. Yellow points and trendlines indicate inoculated individuals grown under ambient CO2, blue points and trendlines indicate uninoculated individuals grown under ambient CO2, red points and trendlines indicate inoculated individuals grown under elevated CO2, and gray points indicate uninoculated individuals grown under elevated CO2. Solid trendlines indicate regression slopes that are different 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 soil fertilization

Description automatically generated**

**Figure S3** 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. Soil nitrogen fertilization is represented on the x-axis. Yellow points and trendlines indicate inoculated individuals grown under aCO2 pooled across inoculation treatments, while blue points and trendlines indicate uninoculated individuals grown under eCO2 pooled across inoculation treatments. Solid trendlines indicate regression slopes that are different from zero (*p*<0.05).

**Figure S4**

**A diagram of different types of soil fertilization

Description automatically generated**

**Figure S4** 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). Soil nitrogen fertilization is represented on the x-axis. Yellow points and trendlines indicate inoculated individuals grown under aCO2 pooled across inoculation treatments, while blue points and trendlines indicate uninoculated individuals grown under eCO2 pooled across inoculation treatments. Solid trendlines indicate regression slopes that are different from zero (*p*<0.05).

**Figure S5**

**A graph of soil fertilization

Description automatically generated**

**Figure S5** Effects of CO2 and fertilization inoculation on the fraction of leaf nitrogen content allocated to structural tissue. Soil nitrogen fertilization is represented on the x-axis. Yellow points and trendlines indicate inoculated individuals grown under aCO2 pooled across inoculation treatments, while blue points and trendlines indicate uninoculated individuals grown under eCO2 pooled across inoculation treatments. Solid trendlines indicate regression slopes that are different from zero (*p*<0.05).

**Figure S6**

A diagram of a root biomass

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). Soil nitrogen fertilization is represented on the x-axis. Yellow points and trendlines indicate inoculated individuals grown under ambient CO2, blue points and trendlines indicate uninoculated individuals grown under ambient CO2, red points and trendlines indicate inoculated individuals grown under elevated CO2, and grey points indicate uninoculated individuals grown under elevated CO2. Solid trendlines indicate slopes that are different from zero (*p*<0.05), while dashed trendlines indicate slopes that are not different from zero (*p*>0.05). Curvilinear trendlines occur as a result of back-transforming models where response variables received either a natural log or square root transformation prior to fitting. Error ribbons represent upper and lower 95% confidence intervals, calculated using the ‘emmeans’ R package (Lenth, 2019).