**SUPPLEMENTARY MATERIAL FOR:**

**Results (cont.)**

*Nitrogen fixation*

Nodule biomass was stimulated by 30% under elevated CO2 (*p* < 0.001; Table 6), a pattern that was modified across the fertilization gradient (p = 0.479; Table 6), but not between inoculation treatments (*p* = 0.404; Table 6). Specifically, the general negative effect of increasing fertilization on nodule biomass (*p* < 0.001; Table 6) was stronger under elevated CO2 than ambient CO2 (Tukey: *p* < 0.001; Fig. 6A), which reduced the stimulation in nodule biomass under elevated CO2 with increasing fertilization. A strong interaction between fertilization and inoculation (*p* < 0.001; Table 6) was driven by a stronger negative effect of increasing fertilization in inoculated pots (Tukey: *p* < 0.001; Fig. 6B).

There was no effect of CO2 on nodule: root biomass (*p* = 0.767; Table 6), although an interaction between CO2 and inoculation (*p* < 0.001; Table 6) indicated that the general positive effect of inoculation on nodule: root biomass (*p* < 0.001; Table 6) was stronger under ambient CO2 (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* = 0.183; Table 6; Fig. 6C). An interaction between fertilization and inoculation (*p* < 0.001; Table 6) indicated that the general negative effect of increasing fertilization on nodule: root biomass (*p* < 0.001; Table 6) was stronger in inoculated pots (Tukey: *p* < 0.001; Fig. 6D).

[%Ndfa]

**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)** | **Light (%)** |
| 09:00 | 21 | 25 |
| 09:45 | 50 |
| 10:30 | 25 | 75 |
| 11:15 | 100 |
| 22:45 | 21 | 75 |
| 23:30 | 50 |
| 00:15 | 17 | 25 |
| 01:00 | 0 |

**Table 6** 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** | | | **%*N*dfa** | | |
|  | df | Coefficient | *χ*2 | *p* | Coefficient | *χ*2 | *p* | Coefficient | *χ*2 | *p* |
| (Intercept) | - | 9.41E-03 | - | - | 1.33E-02 | - | - |  |  |  |
| CO2 | 1 | 1.20E-01 | 19.258 | **<0.001** | 9.94E-02 | 0.087 | 0.768 |  |  |  |
| Inoculation (I) | 1 | 5.74E-01 | 755.02 | **<0.001** | 5.40E-01 | 903.691 | **<0.001** |  |  |  |
| N fertilization (N) | 1 | 7.71E-06 | 84.376 | **<0.001** | -5.99E-06 | 258.099 | **<0.001** |  |  |  |
| CO2\*I | 1 | -4.68E-02 | 0.950 | 0.330 | -1.38E-01 | 20.614 | **<0.001** |  |  |  |
| CO2\*N | 1 | -1.59E-04 | 2.106 | 0.147 | -1.73E-04 | 1.773 | 0.183 |  |  |  |
| I\*N | 1 | -5.82E-04 | 44.622 | **<0.001** | -7.45E-04 | 133.918 | **<0.001** |  |  |  |
| CO2\*I\*N | 1 | 7.26E-05 | 0.196 | 0.658 | 1.76E-04 | 2.359 | 0.125 |  |  |  |

\*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, %*N*dfa=percent nitrogen fixed from the atmosphere.

**Figure 6**

Chart

Description automatically generated

**Figure 6** Effects of nitrogen fertilization, inoculation treatment, and CO2 treatment on nodule biomass (panel A), nodule: root biomass (panel B), and percent nitrogen fixed from the atmosphere (panel C). Soil nitrogen fertilization is represented continuously 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).