

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

Results (cont.)

χ

An interaction between CO₂ and nitrogen fertilization ($p<0.001$; Table S3) indicated that the negative effect of increasing nitrogen fertilization on χ ($p<0.001$; Table S3) was stronger under elevated CO₂ than ambient CO₂ (Tukey test comparing the nitrogen fertilization- χ slope between CO₂ treatments: $p<0.05$; Fig. S3), resulting in a stronger downregulation of χ under elevated CO₂ with increasing fertilization. A three-way interaction ($p<0.001$; Table S3) indicated that interactions between CO₂ and nitrogen fertilization were driven by inoculated plants (Tukey test comparing the nitrogen fertilization- χ slope between inoculated plants grown under ambient CO₂ and inoculated plants grown under elevated CO₂: $p<0.001$), as there was no difference in the effect of nitrogen fertilization on χ between CO₂ treatments in uninoculated plants (Tukey test comparing the nitrogen fertilization- χ slope between uninoculated plants grown under ambient CO₂ and uninoculated plants grown under elevated CO₂: $p>0.05$). An interaction between CO₂ and inoculation ($p<0.001$; Table S3) indicated that elevated CO₂ decreased χ in uninoculated plants (Tukey test of the CO₂ effect in uninoculated plants: $p<0.001$) and increased χ in inoculated plants (Tukey test of the CO₂ effect in inoculated plants: $p<0.001$).

Components of carbon costs to acquire nitrogen

Elevated CO₂ increased C_{bg} by 100% ($p<0.001$; Table S4), a pattern that was not modified by nitrogen fertilization (CO₂-by-nitrogen fertilization interaction: $p>0.05$; Table S4). An interaction between CO₂ and inoculation ($p<0.05$; Table S4) indicated that the positive effect of inoculation on C_{bg} ($p<0.001$; Table S4) was only apparent under ambient CO₂ (Tukey test of the inoculation effect under ambient CO₂: $p<0.001$; Fig. S4), as there was no effect of inoculation on C_{bg} under elevated CO₂ (Tukey test of the inoculation effect under elevated CO₂: $p>0.05$). An interaction between nitrogen fertilization and inoculation ($p<0.001$; Table S3) indicated that the positive effect of increasing nitrogen fertilization on C_{bg} ($p<0.001$; Table S3) was stronger in uninoculated plants than inoculated plants (Tukey test comparing the nitrogen fertilization- C_{bg} slope between inoculation treatments: $p<0.001$).

Elevated CO₂ increased N_{wp} by 27% ($p<0.001$; Table S4), a pattern that was enhanced with increasing nitrogen fertilization (CO₂-by-nitrogen fertilization interaction: $p<0.05$; Table S4) but was not modified by inoculation (CO₂-by-inoculation interaction: $p>0.05$; Table S4). An interaction between nitrogen fertilization and inoculation ($p<0.001$; Table S4) indicated that the positive effect of increasing nitrogen fertilization on N_{wp} ($p<0.001$; Table S4) was stronger in uninoculated plants than inoculated plants (Tukey test comparing the nitrogen fertilization- N_{wp} slope between inoculation treatments: $p<0.001$).

Nitrogen fixation

There was no effect of CO₂ treatment on root nodule: root biomass ($p>0.05$; Table S5), a pattern that was not modified by nitrogen fertilization (CO₂-by-nitrogen fertilization interaction: $p>0.05$; Table S5). However, an interaction between CO₂ and inoculation ($p<0.001$; Table S5) indicated that the positive effect of inoculation on root nodule: root biomass ($p<0.001$; Table S5) was stronger under ambient CO₂ (3129% increase; Tukey test comparing the inoculation effect under ambient CO₂: $p<0.001$) than elevated CO₂ (379% increase; Tukey test comparing the inoculation effect under elevated CO₂: $p<0.001$). An interaction between nitrogen fertilization and inoculation ($p<0.001$; Table S5) indicated that the negative effect of increasing nitrogen fertilization on root nodule: root biomass ($p<0.001$; Table S5) was stronger in inoculated pots than uninoculated plants (Tukey test comparing the nitrogen fertilization-root nodule: root biomass slope between inoculation treatments: $p<0.001$; Fig. S5).

Root nodule biomass increased by 30% under elevated CO₂ ($p<0.001$; Table S5), a pattern that was not modified by nitrogen fertilization (CO₂-by-nitrogen fertilization interaction: $p>0.05$; Table S5) or inoculation (CO₂-by-inoculation interaction: $p>0.05$; Table S5; Fig. S5). An interaction between nitrogen fertilization and inoculation ($p<0.001$; Table S5) indicated that the negative effect of increasing nitrogen fertilization on root nodule biomass ($p<0.001$; Table S5) was driven by inoculated plants (Tukey test comparing the nitrogen fertilization-root nodule biomass slope in inoculated plants: $p<0.001$), as there was no effect of nitrogen fertilization on root nodule biomass in uninoculated plants (Tukey test comparing the nitrogen fertilization-root nodule biomass slope in uninoculated plants: $p>0.05$; Fig. S5).

Root biomass increased by 96% under elevated CO₂ ($p<0.001$; Table S5). An interaction between CO₂ concentration and fertilization ($p<0.001$; Table S5) indicated that the positive

effect of increasing nitrogen fertilization on root biomass ($p<0.001$; Table S5) was stronger under ambient CO₂ (Tukey test comparing the nitrogen fertilization-root biomass slope between CO₂ treatments: $p=0.001$). An interaction between CO₂ and inoculation ($p<0.001$; Table S5) indicated that the positive effect of inoculation on root biomass ($p<0.001$; Table S5) was driven by the ambient CO₂ treatment (Tukey test comparing inoculation effect under ambient CO₂: $p<0.001$), as there was no inoculation effect on root biomass under elevated CO₂ (Tukey test comparing inoculation effect under elevated CO₂: $p>0.05$). An interaction between nitrogen fertilization and inoculation ($p<0.001$; Table S5) indicated that the positive effect of increasing nitrogen fertilization on root biomass ($p<0.001$; Table S5) was stronger in uninoculated plants (Tukey test comparing the fertilization-root biomass slope between inoculation treatments: $p=0.001$).

The ratio of total biomass to pot volume

Total biomass: pot volume increased with elevated CO₂, inoculation, and nitrogen fertilization ($p<0.001$ in all cases; Table S6). The positive effect of increasing nitrogen fertilization on biomass: pot volume was stronger in uninoculated plants than inoculated plants (Tukey test comparing the nitrogen fertilization-biomass:pot volume slope between inoculation treatments: $p<0.05$; Fig. S6), and when plants were grown under elevated CO₂ compared to ambient CO₂ (Tukey test comparing the nitrogen fertilization-biomass:pot volume slope between CO₂ treatments: $p<0.001$; Fig. S6).

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 (0 mM N)	35 ppm N (2.5 mM N)	70 ppm N (5 mM N)	105 ppm N (7.5 mM N)	140 ppm N (10 mM 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 (15 mM N)	280 ppm N (20 mM N)	350 ppm N (25 mM N)	630 ppm N (45 mM 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 (°C)	PAR ± SD ($\mu\text{mol m}^{-2} \text{s}^{-1}$)
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 CO₂ concentration, inoculation, and nitrogen fertilization on χ^*

	df	χ^2	p
CO ₂	1	6.809	0.009
Inoculation (I)	1	5.827	0.016
N fertilization (N)	1	109.544	<0.001
CO ₂ *I	1	20.644	<0.001
CO ₂ *N	1	11.839	<0.001
I*N	1	0.013	0.909
CO ₂ *I*N	1	16.901	<0.001

*Significance determined using Type II Wald χ^2 tests ($\alpha=0.05$). P-values less than 0.05 are in bold. Key: df=degrees of freedom, χ^2 =Wald chi-square test statistic, χ =isotope-based ratio of intercellular CO₂ to extracellular CO₂, inversely related to water-use efficiency (unitless)

Table S4 Effects of CO₂ concentration, inoculation, and nitrogen fertilization on components of the carbon cost to acquire nitrogen*

			<i>C_{bg}</i> ^a		<i>N_{wp}</i> ^b	
	df	χ^2	<i>p</i>	χ^2	<i>p</i>	
CO ₂	1	84.134	<0.001	23.890	<0.001	
Inoculation (I)	1	41.030	<0.001	134.460	<0.001	
N fertilization (N)	1	152.248	<0.001	529.021	<0.001	
CO ₂ *I	1	8.965	0.003	1.190	0.275	
CO ₂ *N	1	1.188	0.276	5.915	0.015	
I*N	1	22.648	<0.001	55.562	<0.001	
CO ₂ *I*N	1	1.109	0.292	0.620	0.431	

*Significance determined using Type II Wald χ^2 tests ($\alpha=0.05$). 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. Key: df=degrees of freedom, *C_{bg}*=belowground carbon biomass (gC, numerator of *N_{cost}*), *N_{wp}*=total nitrogen biomass (gN, denominator of *N_{cost}*).

Table S5 Effects of CO₂ concentration, inoculation, and nitrogen fertilization on investment toward symbiotic nitrogen fixation*

	Root nodule biomass ^b			Root biomass ^b			Root nodule: root biomass ^b	
	df	χ^2	p	χ^2	p	χ^2		p
CO ₂	1	19.258	<0.001	93.249	<0.001	0.010	0.921	
Inoculation (I)	1	755.02	<0.001	6.983	0.008	902.063	<0.001	
N fertilization (N)	1	84.376	<0.001	195.843	<0.001	254.741	<0.001	
CO ₂ *I	1	0.950	0.330	3.873	0.049	21.632	<0.001	
CO ₂ *N	1	2.106	0.147	11.456	<0.001	1.590	0.207	
I*N	1	44.622	<0.001	7.435	0.006	132.463	<0.001	
CO ₂ *I*N	1	0.196	0.658	0.065	0.799	2.481	0.115	

*Significance determined using Type II Wald χ^2 tests ($\alpha=0.05$). 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. Key: df=degrees of freedom, root nodule biomass (g), root biomass (g), root nodule: root biomass (unitless).

Table S6 Effects of CO₂ concentration, inoculation, and nitrogen fertilization on the ratio of total biomass to pot volume (g L⁻¹)^{*}

	df	χ^2	p
CO ₂	1	146.004	<0.001
Inoculation (I)	1	19.320	<0.001
N fertilization (N)	1	279.388	<0.001
CO ₂ *I	1	0.007	0.934
CO ₂ *N	1	49.725	<0.001
I*N	1	9.007	0.003
CO ₂ *I*N	1	0.640	0.434

*Significance determined using Type II Wald χ^2 tests ($\alpha=0.05$). P-values less than 0.05 are in bold. Key: df=degrees of freedom, χ^2 =Wald chi-square test statistic

Figure S1

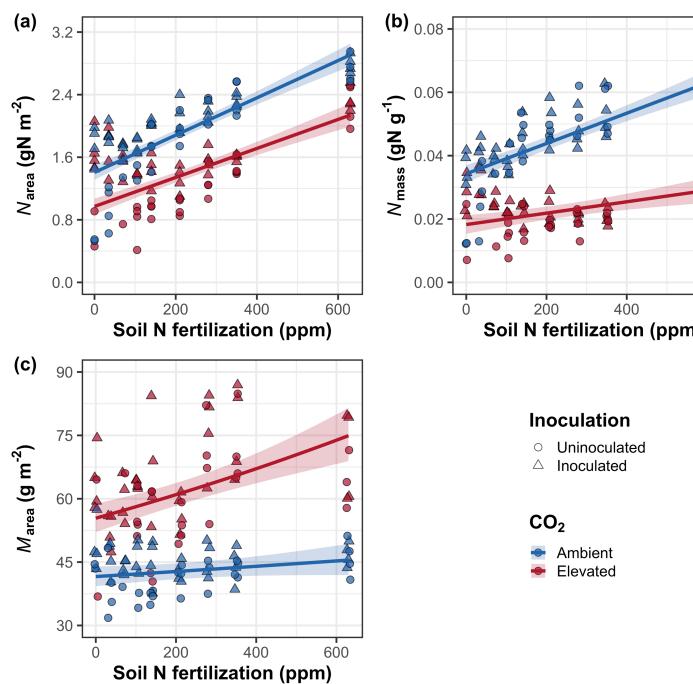


Figure S1 Effects of CO₂ and fertilization inoculation on area-based leaf nitrogen content (a), mass-based leaf nitrogen content (b), and leaf biomass per unit leaf area (c). Fertilization is represented on the x-axis. Red shaded points and trendlines indicate plants grown under elevated CO₂, while blue shaded points and trendlines indicate plants grown under ambient CO₂. Light blue and red circular points and trendlines indicate measurements collected from uninoculated plants, while dark 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 S2

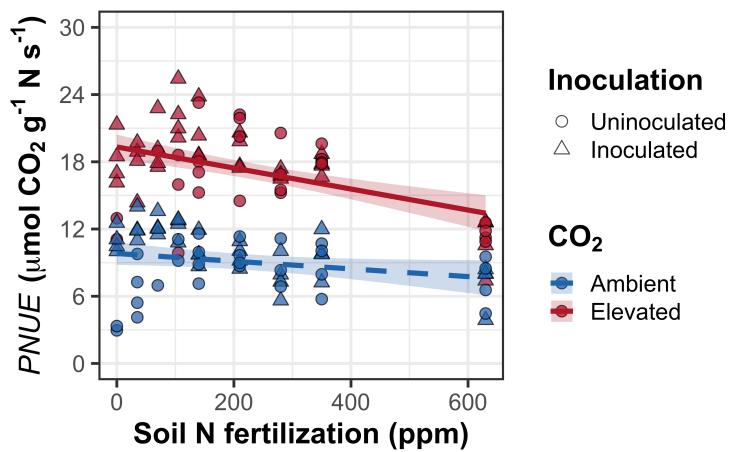


Figure S2 Effects of CO₂ and fertilization inoculation on photosynthetic nitrogen-use efficiency. Fertilization is represented on the x-axis. Red shaded points and trendlines indicate plants grown under elevated CO₂, while blue shaded points and trendlines indicate plants grown under ambient CO₂. Light blue and red circular points and trendlines indicate measurements collected from uninoculated plants, while dark 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 S3

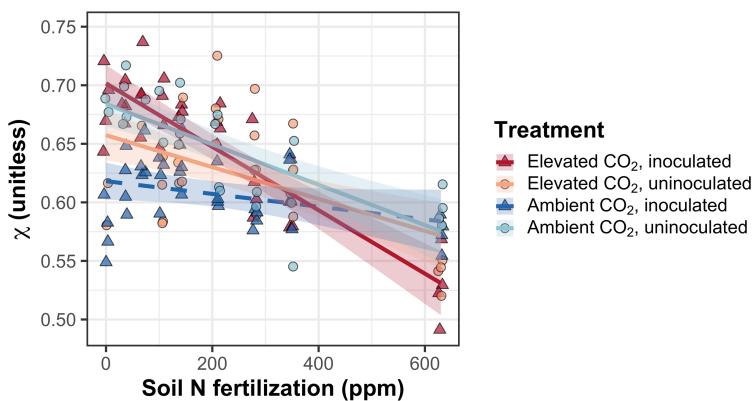


Figure S3 Effects of nitrogen fertilization, inoculation treatment, and CO₂ treatment on χ .

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. Light blue and red circular points and trendlines indicate measurements collected from uninoculated plants, while dark 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

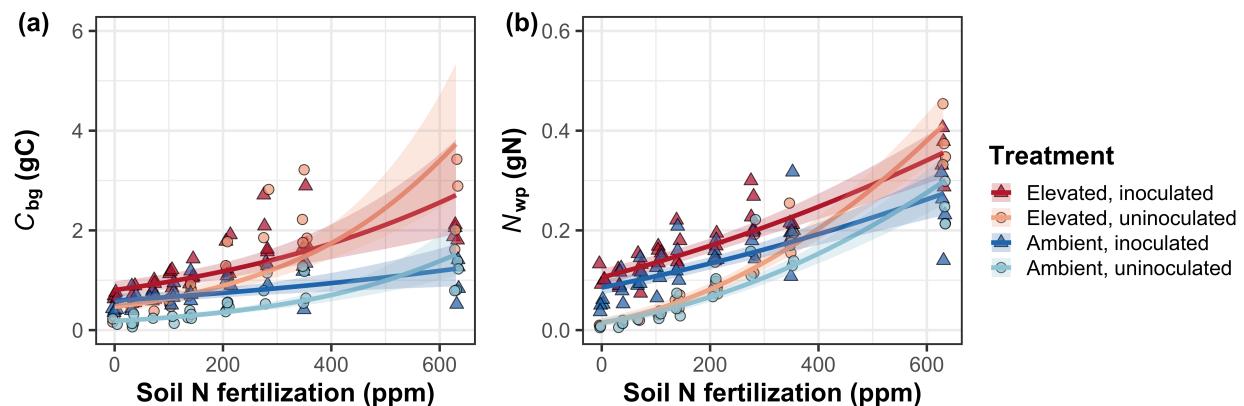


Figure S4 Effects of CO₂ and fertilization inoculation on belowground carbon biomass (a) and total nitrogen biomass (b). Belowground carbon biomass is the numerator of N_{cost} , while total nitrogen biomass is the denominator of N_{cost} . Fertilization is represented on the x-axis in all panels. Red shaded points and trendlines indicate plants grown under elevated CO₂, while blue shaded points and trendlines indicate plants grown under ambient CO₂. Light blue and red circular points and trendlines indicate measurements collected from uninoculated plants, while dark blue and red triangular points and trendlines 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 ($p > 0.05$).

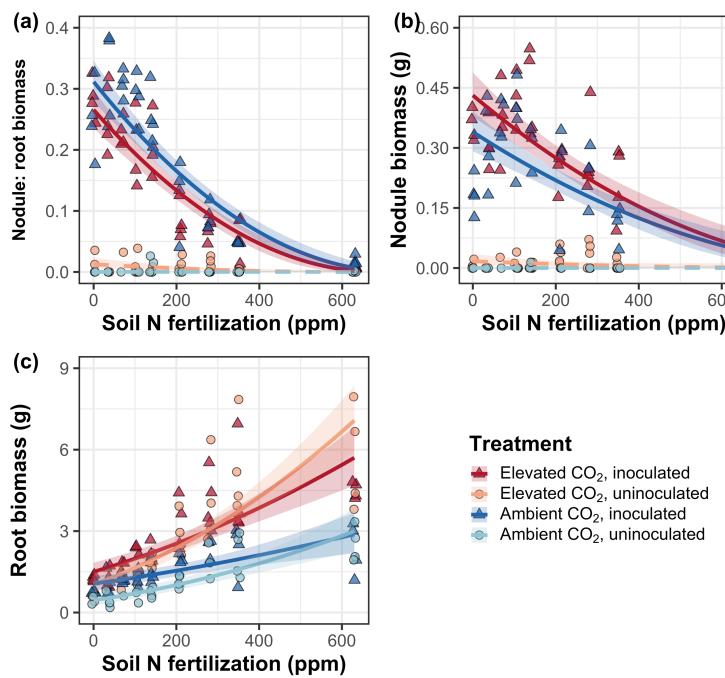
Figure S5

Figure S5 Effects of nitrogen fertilization, inoculation treatment, and CO₂ treatment on root nodule biomass: root biomass (a), root nodule biomass (b), and root biomass (c). Nitrogen 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. Light blue and red circular points and trendlines indicate measurements collected from uninoculated plants, while dark 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 ($p > 0.05$).

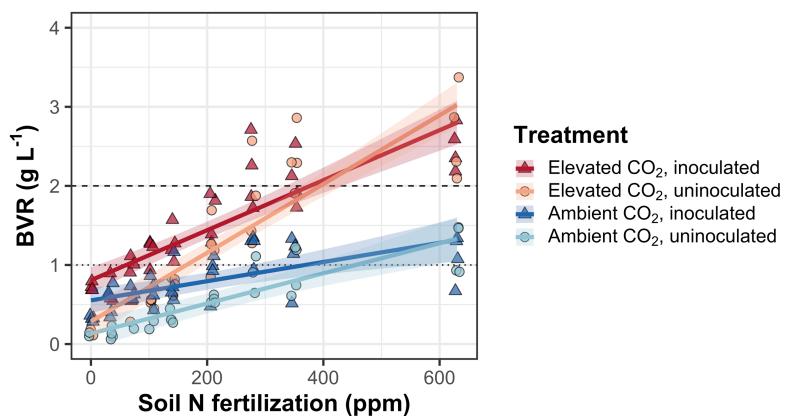
Figure S6

Figure S6 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. Light blue and red circular points and trendlines indicate measurements collected from uninoculated plants, while dark blue and red triangular points indicate measurements collected from inoculated plants. 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.