SUPPLEMENTARY MATERIAL FOR: "Nitrogen demand, supply, and acquisition strategy control plant responses to elevated CO_2"

Protocols S1

Leaf nitrogen content

Elevated CO_2 reduced N_{mass} by 51% and increased M_{area} by 44% ($p<0.001$ in both cases; Supplementary Table S5; Supplementary Fig. S1). Increasing nitrogen fertilization increased N_{mass} and M_{area} more strongly under ambient CO_2 than elevated CO_2 (CO_2-by-nitrogen fertilization interaction: $p<0.05$ in both cases; Supplementary Table S5), resulting in a stronger reduction in N_{mass} and a stronger increase in M_{area} under elevated CO_2 with increasing nitrogen fertilization than ambient CO_2 (Supplementary Fig. S2). Increasing nitrogen fertilization increased N_{mass} and M_{area} more strongly in uninoculated plants compared to inoculated plants (inoculation-by-nitrogen fertilization interaction: $p<0.05$ in all cases; Supplementary Table S5; Supplementary Fig. S1).

Dark respiration

CO_2 treatment did not affect R_{d25} ($p>0.05$; Supplementary Table S6). Increasing nitrogen fertilization increased R_{d25} ($p<0.05$; Supplementary Table S6), but this pattern was only observed in uninoculated plants (inoculation-by-nitrogen fertilization interaction: $p<0.001$; Supplementary Table S6; Supplementary Fig. S3A). Inoculated plants exhibited marginally greater R_{d25} than uninoculated plants ($p<0.1$; Supplementary Table S6).

Organ biomasses

Elevated CO_2 increased total leaf, stem, root, and root nodule biomass by 117%, 97%, 96%, and 70%, respectively (p<0.001 in all cases; Supplementary Table S7). Increasing nitrogen fertilization increased total leaf, stem, and root biomasses (p<0.001 in all cases; Supplementary Table S7) more strongly under elevated CO_2 than ambient CO_2 (CO_2-by-nitrogen fertilization interaction: p<0.001 in all cases; Supplementary Table S7). However, increasing nitrogen feritlization decreased root nodule biomass (p<0.001; Supplementary Table S7) similarly between CO_2 treatments (CO_2-by-nitrogen fertilization interaction: p>0.05; Supplementary Table S7). Inoculation increased root biomass (p<0.05; Supplementary Table S7), but this pattern was only observed under ambient CO_2 (CO_2-by-inoculation interaction: p<0.05; Supplementary Table S7). Increasing nitrogen fertilization increased total leaf, stem, and root biomasses (p<0.001 in all cases; Supplementary Table S7) more strongly in uninoculated plants than inoculated plants (inoculation-by-nitrogen fertilization interaction: p<0.001 in all cases; Supplementary Table S7). Increasing nitrogen fertilization decreased root nodule biomass only in inoculated plants (inoculation-by-nitrogen fertilization interaction: p<0.001; Supplementary Table S7)

The ratio of total biomass to pot volume

Total biomass: pot volume increased with elevated CO_2, inoculation, and nitrogen fertilization (p<0.001 in all cases; Supplementary Table S9; Supplementary Fig. S7). Increasing nitrogen fertilization increased biomass: pot volume (p<0.001; Supplementary Table S9) more strongly in uninoculated plants compared to inoculated plants (inoculation-by-nitrogen fertilization interaction: p<0.05; Supplementary Table S9; Supplementary Fig. S7) and more strongly under

47 elevated CO_2 than ambient CO_2 (CO_2-by-nitrogen fertilization interaction: $p<0.001$;

48 Supplementary Table S9; Supplementary Fig. S7).

49

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 $NH_4H_2PO_4$	0	0.165	0.33	0.5	0.67
2 M KNO_3	0	0.335	0.67	1	1.33
2 M $Ca(NO_3)_2$	0	0.335	0.67	1	1.33
1 M NH_4NO_3	0	0.165	0.33	0.5	0.67
8 M NH_4NO_3	0	0	0	0	0
1 M KH_2PO_4	1	0.85	0.67	0.5	0.33
1 M KCl	3	2.45	2	1.5	1
1 M $CaCO_3$	4	3.33	2.67	2	1.33
2 M $MgSO_4$	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 $NH_4H_2PO_4$	1	1	1	1
2 M KNO_3	2	2	2	2
2 M $Ca(NO_3)_2$	2	2	2	2
1 M NH_4NO_3	1	3.5	0	0
8 M NH_4NO_3	0	0	0.75	2
1 M KH_2PO_4	0	0	0	0
1 M KCl	0	0	0	0
1 M $CaCO_3$	0	0	0	0
2 M $MgSO_4$	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 Replication scheme for each unique CO_2-by-inoculation-by-N fertilization combination

CO_2 treatment	Inoculation treatment	N fertilization	n
Ambient CO_2	Uninoculated	0	2
		35	4
		70	1
		105	2
		140	4
		210	4
		280	3
		350	4
		630	4
	Inoculated	0	4
		35	4
		70	4
		105	4
		140	4
		210	4
		280	4
		350	4
		630	4
Elevated CO_2	Uninoculated	0	2
		35	1
		70	1
		105	4
		140	4
		210	4
		280	4
		350	4
		630	4
	Inoculated	0	4
		35	4
		70	4
		105	4
		140	4
		210	4
		280	4
		350	4
		630	4

Table S4 Replication scheme for each unique CO_2-by-inoculation combination

CO_2 treatment	**Inoculation treatment**	**n**
Ambient CO_2	Uninoculated	28
	Inoculated	36
Elevated CO_2	Uninoculated	28
	Inoculated	36

Table S5 Effects of CO_2 concentration, inoculation, and nitrogen fertilization on leaf nitrogen content and leaf mass per area*

		N_{mass}		M_{area}[a]	
	df	χ^2	*p*	χ^2	*p*
CO_2	1	272.362	**<0.001**	151.319	**<0.001**
Inoculation (I)	1	15.576	**<0.001**	19.158	**<0.001**
N fertilization (N)	1	106.659	**<0.001**	21.440	**<0.001**
CO_2 × I	1	2.025	0.155	0.029	0.866
CO_2 × N	1	22.542	**<0.001**	7.619	**0.006**
I × N	1	11.137	**<0.001**	5.022	**0.025**
CO_2 × I × N	1	0.041	0.839	0.208	0.649

*Significance determined using Type II Wald χ^2 tests (α=0.05). *P*-values less than 0.05 are in bold. Key: [a]=variable was natural log transformed before model fitting, df=degrees of freedom, χ^2=Wald chi-square test statistic, N_{mass}=leaf nitrogen content (gN g^{-1}), M_{area}=leaf mass per unit leaf area (g m^{-2}).

Table S6 Effects of CO_2 concentration, inoculation, and nitrogen fertilization on dark respiration and photosynthetic nitrogen-use efficiency*

		R_{d25}		$PNUE_{gc}$	
	df	χ^2	*p*	χ^2	*p*
CO_2	1	0.256	0.613	300.202	**<0.001**
Inoculation (I)	1	3.094	0.079	9.899	**0.002**
N fertilization (N)	1	5.965	**0.015**	29.696	**<0.001**
CO_2 × I	1	2.563	0.109	0.944	0.331
CO_2 × N	1	2.675	0.102	5.359	**0.021**
I × N	1	12.083	**<0.001**	10.884	**<0.001**
CO_2 × I × N	1	0.244	0.622	0.369	0.544

*Significance determined using Type II Wald χ^2 tests (α=0.05). *P*-values less than 0.05 are in bold. Key: df=degrees of freedom, χ^2=Wald chi-square test statistic, $PNUE_{gc}$=photosynthetic nitrogen-use efficiency at growth CO_2 concentration (μmol CO_2 gN^{-1} s^{-1})

Table S7 Effects of CO_2 concentration, inoculation, and nitrogen fertilization on biomass partitioning*

		Leaf biomass[b]		**Stem biomass[b]**		**Root biomass[b]**		**Root nodule biomass[b]**	
	df	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*
CO_2	1	151.836	**<0.001**	98.264	**<0.001**	93.249	**<0.001**	19.258	**<0.001**
Inoculation (I)	1	51.267	**<0.001**	18.744	**<0.001**	6.983	**0.008**	755.020	**<0.001**
N fertilization (N)	1	312.022	**<0.001**	255.837	**<0.001**	195.843	**<0.001**	84.376	**<0.001**
CO_2 × I	1	0.087	0.768	0.046	0.831	3.873	**0.049**	0.950	0.330
CO_2 × N	1	17.727	**<0.001**	18.482	**<0.001**	11.456	**0.001**	2.106	0.147
I × N	1	24.442	**<0.001**	3.743	*0.053*	7.435	**0.006**	44.622	**<0.001**
CO_2 × I × N	1	0.194	0.659	0.483	0.487	0.064	0.800	0.196	0.658

		Leaf mass fraction		**Stem mass fraction**		**Root mass fraction[a]**	
	df	χ^2	*p*	χ^2	*p*	χ^2	*p*
CO_2	1	16.819	**<0.001**	1.695	0.193	2.430	0.119
Inoculation (I)	1	30.317	**<0.001**	24.839	**<0.001**	65.092	**<0.001**
N fertilization (N)	1	38.919	**<0.001**	12.466	**<0.001**	8.127	**0.004**
CO_2 × I	1	6.011	**0.014**	2.717	*0.099*	6.876	**0.009**
CO_2 × N	1	2.361	0.124	2.419	0.120	0.004	0.950
I × N	1	17.762	**<0.001**	32.436	**<0.001**	20.424	**<0.001**
CO_2 × I × N	1	0.553	0.457	0.020	0.888	0.161	0.688

*Significance determined using Type II Wald χ^2 tests (α=0.05). *P*-values less than 0.05 are in bold, while *p*-values where $0.05<p<0.1$ are in italic font. Key: [a]=variable was natural log transformed before model fitting, [b]=variable was square root transformed before model fitting, df=degrees of freedom, χ^2=Wald chi-square test statistic, leaf biomass (g), stem biomass (g), root biomass (g), root nodule biomass (g), leaf mass fraction (unitless), stem mass fraction (unitless), root mass fraction (unitless)

Table S8 Effects of CO_2 concentration, inoculation, and nitrogen fertilization on components of the carbon cost to acquire nitrogen*

		Belowground biomass carbon		**Whole-plant nitrogen biomass**	
	df	χ^2	*p*	χ^2	*p*
CO_2	1	83.286	**<0.001**	27.499	**<0.001**
Inoculation (I)	1	9.237	**0.002**	87.370	**<0.001**
N fertilization (N)	1	139.920	**<0.001**	584.694	**<0.001**
CO_2 × I	1	2.264	0.132	2.758	*0.097*
CO_2 × N	1	25.892	**<0.001**	25.009	**<0.001**
I × N	1	8.388	**0.004**	30.321	**<0.001**
CO_2 × I × N	1	1.169	0.279	2.139	0.144

*Significance determined using Type II Wald χ^2 tests (α=0.05). *P*-values less than 0.05 are in bold, while *p*-values where $0.05<p<0.1$ are in italic font. Key: df=degrees of freedom, χ^2=Wald chi-square test statistic, belowground biomass carbon (gC, numerator of the belowground biomass carbon cost to acquire nitrogen), whole-plant nitrogen biomass (gN, denominator of the belowground biomass carbon cost to acquire nitrogen).

Table S9 Effects of CO_2 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 (α=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

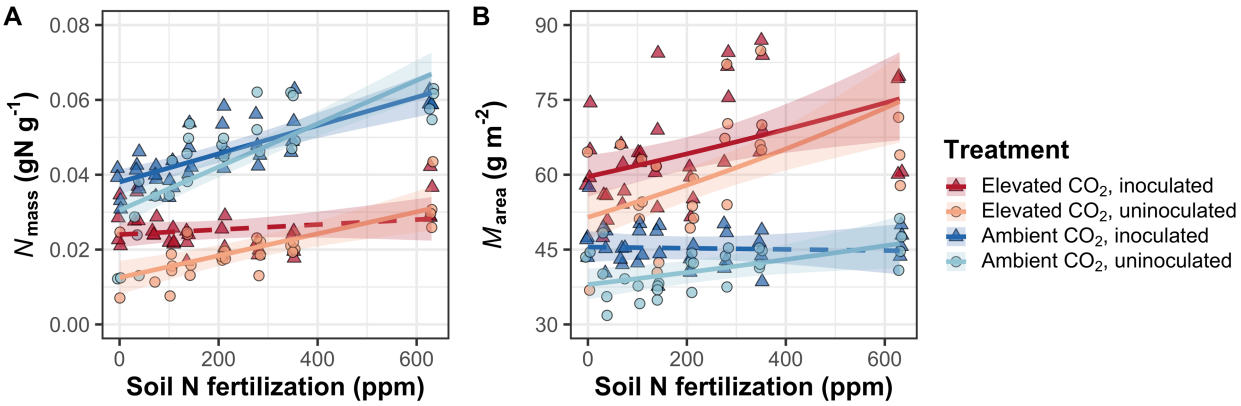

Figure S1 Effects of CO_2 concentration, inoculation, and nitrogen fertilization on mass-based leaf nitrogen content (panel A) and leaf biomass per unit leaf area (panel B). Nitrogen fertilization is on the x-axis in both panels. Red shaded points and trendlines indicate plants grown under elevated CO_2, while blue shaded points and trendlines indicate plants grown under ambient CO_2. Light blue and light red circular points and trendlines indicate measurements collected from uninoculated plants, while dark blue and dark 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). Error ribbons of each trendline represent the upper and lower 95% confidence intervals.

Figure S2

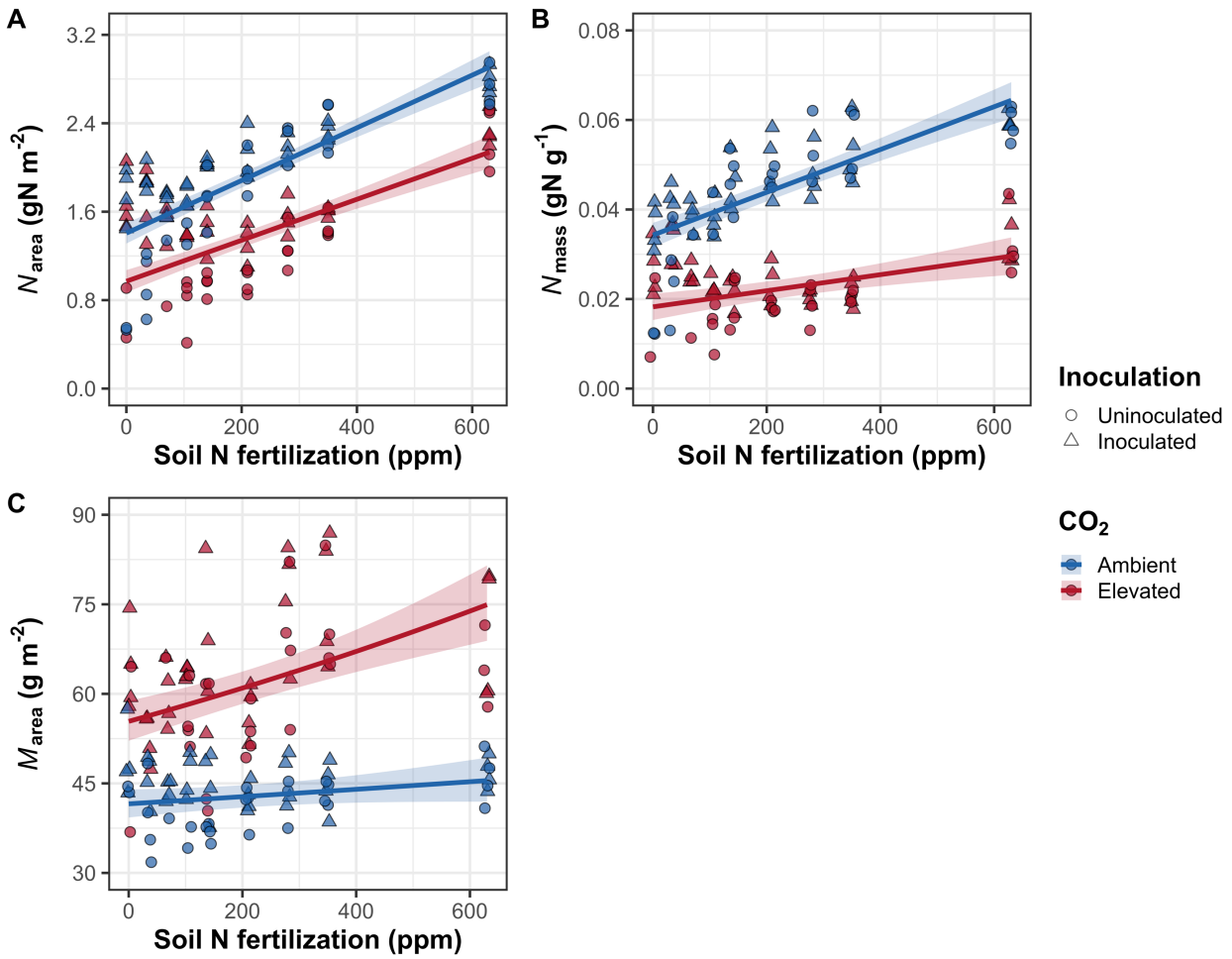

Figure S2 Effects of CO_2 concentration and nitrogen fertilization on area-based leaf nitrogen content (panel A), mass-based leaf nitrogen content (panel B), and leaf biomass per unit leaf area (panel C). Nitrogen fertilization is on the x-axis. Red shaded points and trendlines indicate plants grown under elevated CO_2, while blue shaded points and trendlines indicate plants grown under ambient CO_2. Circular points indicate measurements collected from uninoculated plants, while triangular points indicate measurements collected from inoculated plants. Solid trendlines indicate regression slopes that are different from zero (p<0.05). Error ribbons of each trendline represent the upper and lower 95% confidence intervals.

Figure S3

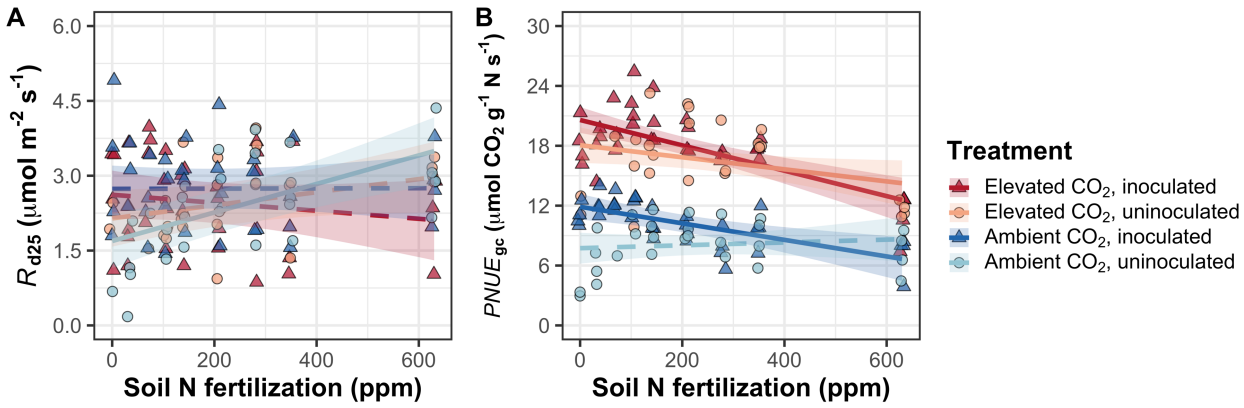

Figure S3 Effects of CO_2 concentration, inoculation, and nitrogen fertilization on dark respiration at 25°C (panel A) and photosynthetic nitrogen-use efficiency at growth CO_2 concentration (panel B). Nitrogen fertilization is on the x-axis in both panels. Red shaded points and trendlines indicate plants grown under elevated CO_2, while blue shaded points and trendlines indicate plants grown under ambient CO_2. Light blue and light red circular points and trendlines indicate measurements collected from uninoculated plants, while dark blue and dark 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). Error ribbons of each trendline represent the upper and lower 95% confidence intervals.

Figure S4

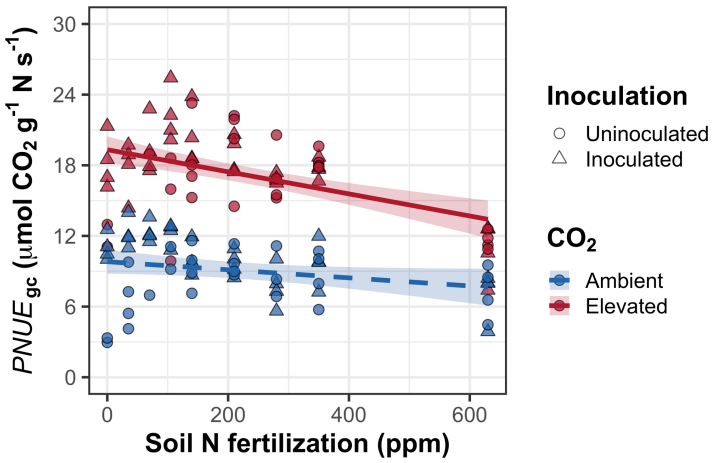

Figure S4 Effects of CO_2 concentration and nitrogen fertilization on photosynthetic nitrogen-use efficiency at growth CO_2 concentration. Nitrogen fertilization is on the x-axis. Red shaded points and trendlines indicate plants grown under elevated CO_2, while blue shaded points and trendlines indicate plants grown under ambient CO_2. Circular points indicate measurements collected from uninoculated plants, while triangular points indicate measurements collected from inoculated plants. Solid trendlines indicate regression slopes that are different from zero (p<0.05). Error ribbons of each trendline represent the upper and lower 95% confidence intervals.

Figure S5

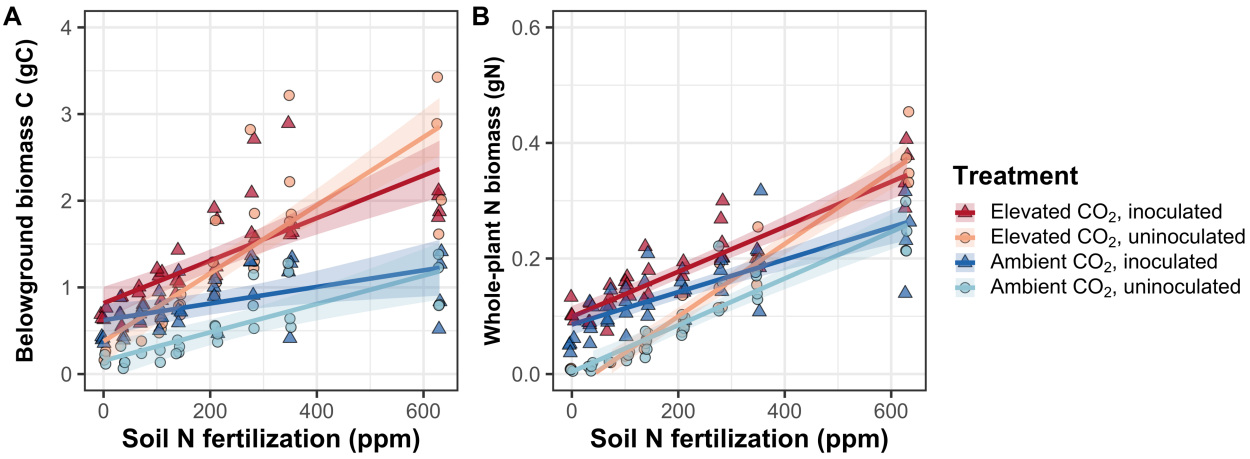

Figure S5 Effects of CO_2 concentration, inoculation, and nitrogen fertilization on belowground biomass carbon (panel A) and total nitrogen biomass (panel B). Belowground biomass carbon is the numerator of N_{cost}, while total nitrogen biomass is the denominator of N_{cost}. Nitrogen fertilization is on the x-axis in both panels. Red shaded points and trendlines indicate plants grown under elevated CO_2, while blue shaded points and trendlines indicate plants grown under ambient CO_2. Light blue and light red circular points and trendlines indicate measurements collected from uninoculated plants, while dark blue and dark 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). Error ribbons of each trendline represent the upper and lower 95% confidence intervals.

Figure S6

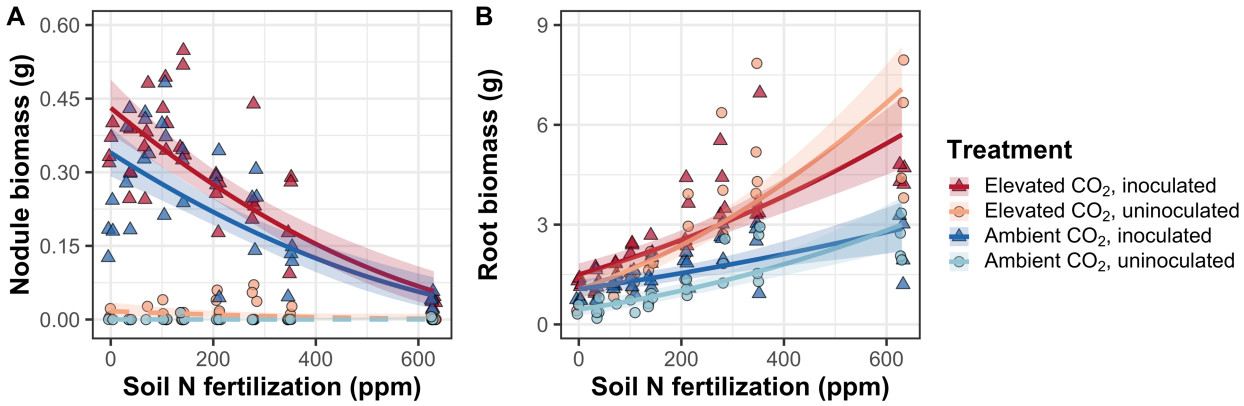

Figure S6 Effects of CO_2 concentration, inoculation, and nitrogen fertilization on root nodule biomass (panel A) and root biomass (panel B). Nitrogen fertilization is on the x-axis in both panels. Red shaded points and trendlines indicate plants grown under elevated CO_2, while blue shaded points and trendlines indicate plants grown under ambient CO_2. Light blue and light red circular points and trendlines indicate measurements collected from uninoculated plants, while dark blue and dark 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). Error ribbons of each trendline represent the upper and lower 95% confidence intervals.

Figure S7

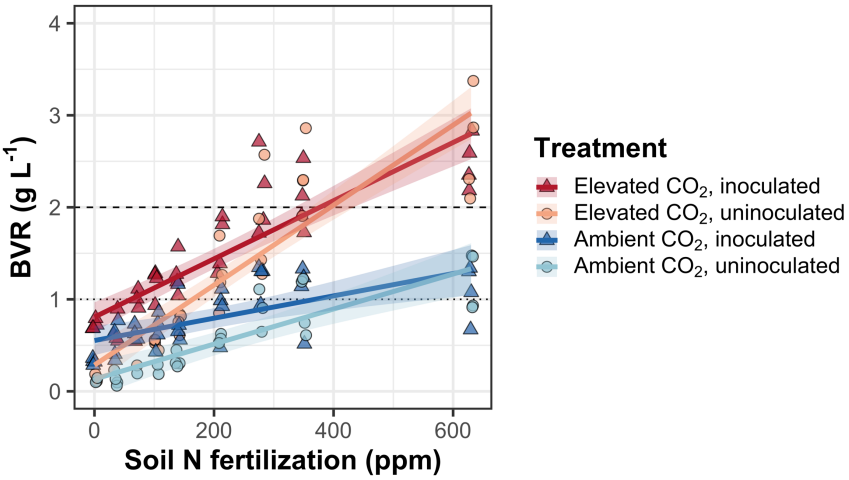

Figure S7 Effects of CO_2 concentration, inoculation, and nitrogen fertilization on the ratio of whole-plant biomass to pot volume. Nitrogen fertilization is on the x-axis in both panels. Red shaded points and trendlines indicate plants grown under elevated CO_2, while blue shaded points and trendlines indicate plants grown under ambient CO_2. Light blue and light red circular points and trendlines indicate measurements collected from uninoculated plants, while dark blue and dark red triangular points indicate measurements collected from inoculated plants. Solid trendlines indicate regression slopes that are different from zero (p<0.05). Error ribbons of each trendline represent the upper and lower 95% confidence intervals. 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}.