

1 **SUPPLEMENTARY MATERIAL FOR:** “Nitrogen demand, supply, and acquisition strategy  
2 control plant responses to elevated CO<sub>2</sub> at different scales”

3

4 **Methods (cont.)**

5  $\chi$

6 Leaf δ<sup>13</sup>C was used to estimate the time-integrated ratio of leaf intercellular CO<sub>2</sub> concentration to  
7 atmospheric CO<sub>2</sub> concentration ( $\chi$ , unitless) using leaf δ<sup>13</sup>C and chamber air δ<sup>13</sup>C following  
8 Farquhar *et al.* (1989):

9 
$$\chi = \frac{\Delta^{13}C - a}{b - a} \quad (\text{S1})$$

10 where Δ<sup>13</sup>C represents the relative difference between leaf δ<sup>13</sup>C (‰) and air δ<sup>13</sup>C (‰), and is  
11 calculated as:

12 
$$\Delta^{13}C = \frac{\delta^{13}C_{air} - \delta^{13}C_{leaf}}{1 + \delta^{13}C_{leaf}} \quad (\text{S2})$$

13 δ<sup>13</sup>C<sub>air</sub> is the chamber δ<sup>13</sup>C air fractionation, *a* represents the fractionation between <sup>12</sup>C and <sup>13</sup>C  
14 due to diffusion in air, assumed to be 4.4‰, and *b* represents the fractionation caused by Rubisco  
15 carboxylation, assumed to be 27‰ (Farquhar *et al.*, 1989). δ<sup>13</sup>C<sub>air</sub> was quantified in each  
16 chamber by collecting air samples in triplicate for each CO<sub>2</sub> treatment using a 20 mL syringe  
17 (Air-Tite Products Co., Inc., Virginia Beach, VA, USA). Each air sample was plunged into a  
18 manually evacuated 10 mL Exetainer (Labco Ltd., Lampeter, UK) and sent to the University of  
19 California-Davis Stable Isotope Facility, where δ<sup>13</sup>C<sub>air</sub> was determined using a gas inlet system  
20 (GasBenchII; Thermo Fisher Scientific, Waltham, MA, USA) coupled to an isotope ratio mass  
21 spectrometer (Thermo Finnigan Delta Plus XL; Thermo Fisher Scientific, Waltham, MA, USA).  
22 δ<sup>13</sup>C<sub>air</sub> for each CO<sub>2</sub> treatment was estimated by calculating the mean of the triplicate δ<sup>13</sup>C<sub>air</sub>  
23 samples within each chamber, then calculating the mean δ<sup>13</sup>C<sub>air</sub> across all chambers. Specifically,  
24 δ<sup>13</sup>C<sub>air</sub> was -8.81‰ for the ambient CO<sub>2</sub> treatment and -5.95‰ for the elevated CO<sub>2</sub> treatment.

25

26 **Results (cont.)**

27  $\chi$

28 An interaction between CO<sub>2</sub> and nitrogen fertilization (*p*<0.001; Table S3) indicated that the  
29 negative effect of increasing nitrogen fertilization on  $\chi$  (*p*<0.001; Table S3) was stronger under  
30 elevated CO<sub>2</sub> than ambient CO<sub>2</sub> (Tukey test comparing the nitrogen fertilization- $\chi$  slope between

31 CO<sub>2</sub> treatments:  $p<0.05$ ; Fig. S3), resulting in a stronger downregulation of  $\chi$  under elevated CO<sub>2</sub>  
32 with increasing fertilization. A three-way interaction ( $p<0.001$ ; Table S3) indicated that  
33 interactions between CO<sub>2</sub> and nitrogen fertilization were driven by inoculated plants (Tukey test  
34 comparing the nitrogen fertilization- $\chi$  slope between inoculated plants grown under ambient CO<sub>2</sub>  
35 and inoculated plants grown under elevated CO<sub>2</sub>:  $p<0.001$ ), as there was no difference in the  
36 effect of nitrogen fertilization on  $\chi$  between CO<sub>2</sub> treatments in uninoculated plants (Tukey test  
37 comparing the nitrogen fertilization- $\chi$  slope between uninoculated plants grown under ambient  
38 CO<sub>2</sub> and uninoculated plants grown under elevated CO<sub>2</sub>:  $p>0.05$ ). An interaction between CO<sub>2</sub>  
39 and inoculation ( $p<0.001$ ; Table S3) indicated that elevated CO<sub>2</sub> decreased  $\chi$  in uninoculated  
40 plants (Tukey test of the CO<sub>2</sub> effect in uninoculated plants:  $p<0.001$ ) and increased  $\chi$  in  
41 inoculated plants (Tukey test of the CO<sub>2</sub> effect in inoculated plants:  $p<0.001$ ).  
42

#### 43 *Components of carbon costs to acquire nitrogen*

44 Elevated CO<sub>2</sub> increased  $C_{bg}$  by 100% ( $p<0.001$ ; Table S4), a pattern that was not modified by  
45 nitrogen fertilization (CO<sub>2</sub>-by-nitrogen fertilization interaction:  $p>0.05$ ; Table S4). An  
46 interaction between CO<sub>2</sub> and inoculation ( $p<0.05$ ; Table S4) indicated that the positive effect of  
47 inoculation on  $C_{bg}$  ( $p<0.001$ ; Table S4) was only apparent under ambient CO<sub>2</sub> (Tukey test of the  
48 inoculation effect under ambient CO<sub>2</sub>:  $p<0.001$ ; Fig. S4), as there was no effect of inoculation on  
49  $C_{bg}$  under elevated CO<sub>2</sub> (Tukey test of the inoculation effect under elevated CO<sub>2</sub>:  $p>0.05$ ). An  
50 interaction between nitrogen fertilization and inoculation ( $p<0.001$ ; Table S3) indicated that the  
51 positive effect of increasing nitrogen fertilization on  $C_{bg}$  ( $p<0.001$ ; Table S3) was stronger in  
52 uninoculated plants than inoculated plants (Tukey test comparing the nitrogen fertilization- $C_{bg}$   
53 slope between inoculation treatments:  $p<0.001$ ).

54 Elevated CO<sub>2</sub> increased  $N_{wp}$  by 27% ( $p<0.001$ ; Table S4), a pattern that was enhanced  
55 with increasing nitrogen fertilization (CO<sub>2</sub>-by-nitrogen fertilization interaction:  $p<0.05$ ; Table  
56 S4) but was not modified by inoculation (CO<sub>2</sub>-by-inoculation interaction:  $p>0.05$ ; Table S4). An  
57 interaction between nitrogen fertilization and inoculation ( $p<0.001$ ; Table S4) indicated that the  
58 positive effect of increasing nitrogen fertilization on  $N_{wp}$  ( $p<0.001$ ; Table S4) was stronger in  
59 uninoculated plants than inoculated plants (Tukey test comparing the nitrogen fertilization- $N_{wp}$   
60 slope between inoculation treatments:  $p<0.001$ ).  
61

62 *Nitrogen fixation*

63 There was no effect of CO<sub>2</sub> treatment on root nodule: root biomass ( $p>0.05$ ; Table S5), a pattern  
64 that was not modified by nitrogen fertilization (CO<sub>2</sub>-by-nitrogen fertilization interaction:  $p>0.05$ ;  
65 Table S5). However, an interaction between CO<sub>2</sub> and inoculation ( $p<0.001$ ; Table S5) indicated  
66 that the positive effect of inoculation on root nodule: root biomass ( $p<0.001$ ; Table S5) was  
67 stronger under ambient CO<sub>2</sub> (3129% increase; Tukey test comparing the inoculation effect under  
68 ambient CO<sub>2</sub>:  $p<0.001$ ) than elevated CO<sub>2</sub> (379% increase; Tukey test comparing the inoculation  
69 effect under elevated CO<sub>2</sub>:  $p<0.001$ ). An interaction between nitrogen fertilization and  
70 inoculation ( $p<0.001$ ; Table S5) indicated that the negative effect of increasing nitrogen  
71 fertilization on root nodule: root biomass ( $p<0.001$ ; Table S5) was stronger in inoculated pots  
72 than uninoculated plants (Tukey test comparing the nitrogen fertilization-root nodule: root  
73 biomass slope between inoculation treatments:  $p<0.001$ ; Fig. S5).

74 Root nodule biomass increased by 30% under elevated CO<sub>2</sub> ( $p<0.001$ ; Table S5), a  
75 pattern that was not modified by nitrogen fertilization (CO<sub>2</sub>-by-nitrogen fertilization interaction:  
76  $p>0.05$ ; Table S5) or inoculation (CO<sub>2</sub>-by-inoculation interaction:  $p>0.05$ ; Table S5; Fig. S5).  
77 An interaction between nitrogen fertilization and inoculation ( $p<0.001$ ; Table S5) indicated that  
78 the negative effect of increasing nitrogen fertilization on root nodule biomass ( $p<0.001$ ; Table  
79 S5) was driven by inoculated plants (Tukey test comparing the nitrogen fertilization-root nodule  
80 biomass slope in inoculated plants:  $p<0.001$ ), as there was no effect of nitrogen fertilization on  
81 root nodule biomass in uninoculated plants (Tukey test comparing the nitrogen fertilization-root  
82 nodule biomass slope in uninoculated plants:  $p>0.05$ ; Fig. S5).

83 Root biomass increased by 96% under elevated CO<sub>2</sub> ( $p<0.001$ ; Table S5). An interaction  
84 between CO<sub>2</sub> concentration and fertilization ( $p<0.001$ ; Table S5) indicated that the positive  
85 effect of increasing nitrogen fertilization on root biomass ( $p<0.001$ ; Table S5) was stronger  
86 under ambient CO<sub>2</sub> (Tukey test comparing the nitrogen fertilization-root biomass slope between  
87 CO<sub>2</sub> treatments:  $p=0.001$ ). An interaction between CO<sub>2</sub> and inoculation ( $p<0.001$ ; Table S5)  
88 indicated that the positive effect of inoculation on root biomass ( $p<0.001$ ; Table S5) was driven  
89 by the ambient CO<sub>2</sub> treatment (Tukey test comparing inoculation effect under ambient CO<sub>2</sub>:  
90  $p<0.001$ ), as there was no inoculation effect on root biomass under elevated CO<sub>2</sub> (Tukey test  
91 comparing inoculation effect under elevated CO<sub>2</sub>:  $p>0.05$ ). An interaction between nitrogen  
92 fertilization and inoculation ( $p<0.001$ ; Table S5) indicated that the positive effect of increasing

93 nitrogen fertilization on root biomass ( $p<0.001$ ; Table S5) was stronger in uninoculated plants  
94 (Tukey test comparing the fertilization-root biomass slope between inoculation treatments:  
95  $p=0.001$ ).

96

97 *The ratio of total biomass to pot volume*

98 Total biomass: pot volume increased with elevated CO<sub>2</sub>, inoculation, and nitrogen fertilization  
99 ( $p<0.001$  in all cases; Table S6). The positive effect of increasing nitrogen fertilization on  
100 biomass: pot volume was stronger in uninoculated plants than inoculated plants (Tukey test  
101 comparing the nitrogen fertilization-biomass:pot volume slope between inoculation treatments:  
102  $p<0.05$ ; Fig. S6), and when plants were grown under elevated CO<sub>2</sub> compared to ambient CO<sub>2</sub>  
103 (Tukey test comparing the nitrogen fertilization-biomass:pot volume slope between CO<sub>2</sub>  
104 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)
<b>1 M NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub></b>	0	0.165	0.33	0.5	0.67
<b>2 M KNO<sub>3</sub></b>	0	0.335	0.67	1	1.33
<b>2 M Ca(NO<sub>3</sub>)<sub>2</sub></b>	0	0.335	0.67	1	1.33
<b>1 M NH<sub>4</sub>NO<sub>3</sub></b>	0	0.165	0.33	0.5	0.67
<b>8 M NH<sub>4</sub>NO<sub>3</sub></b>	0	0	0	0	0
<b>1 M KH<sub>2</sub>PO<sub>4</sub></b>	1	0.85	0.67	0.5	0.33
<b>1 M KCl</b>	3	2.45	2	1.5	1
<b>1 M CaCO<sub>3</sub></b>	4	3.33	2.67	2	1.33
<b>2 M MgSO<sub>4</sub></b>	1	1	1	1	1
<b>10% Fe-EDTA</b>	1	1	1	1	1
<b>Trace elements</b>	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)
<b>1 M NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub></b>	1	1	1	1
<b>2 M KNO<sub>3</sub></b>	2	2	2	2
<b>2 M Ca(NO<sub>3</sub>)<sub>2</sub></b>	2	2	2	2
<b>1 M NH<sub>4</sub>NO<sub>3</sub></b>	1	3.5	0	0
<b>8 M NH<sub>4</sub>NO<sub>3</sub></b>	0	0	0.75	2
<b>1 M KH<sub>2</sub>PO<sub>4</sub></b>	0	0	0	0
<b>1 M KCl</b>	0	0	0	0
<b>1 M CaCO<sub>3</sub></b>	0	0	0	0
<b>2 M MgSO<sub>4</sub></b>	1	1	1	1
<b>10% Fe-EDTA</b>	1	1	1	1
<b>Trace elements</b>	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 CO<sub>2</sub> concentration, inoculation, and nitrogen fertilization on  $\chi^*$ 

	df	$\chi^2$	p
CO <sub>2</sub>	1	6.809	<b>0.009</b>
Inoculation (I)	1	5.827	<b>0.016</b>
N fertilization (N)	1	109.544	<0.001
CO <sub>2</sub> *I	1	20.644	<0.001
CO <sub>2</sub> *N	1	11.839	<0.001
I*N	1	0.013	0.909
CO <sub>2</sub> *I*N	1	16.901	<0.001

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

**Table S4** Effects of CO<sub>2</sub> concentration, inoculation, and nitrogen fertilization on components of the carbon cost to acquire nitrogen\*

			<i>C<sub>bg</sub></i> <sup>a</sup>		<i>N<sub>wp</sub></i> <sup>b</sup>	
	df	$\chi^2$	<i>p</i>	$\chi^2$	<i>p</i>	
CO <sub>2</sub>	1	84.134	<b>&lt;0.001</b>	23.890	<b>&lt;0.001</b>	
Inoculation (I)	1	41.030	<b>&lt;0.001</b>	134.460	<b>&lt;0.001</b>	
N fertilization (N)	1	152.248	<b>&lt;0.001</b>	529.021	<b>&lt;0.001</b>	
CO <sub>2</sub> *I	1	8.965	<b>0.003</b>	1.190	0.275	
CO <sub>2</sub> *N	1	1.188	0.276	5.915	<b>0.015</b>	
I*N	1	22.648	<b>&lt;0.001</b>	55.562	<b>&lt;0.001</b>	
CO <sub>2</sub> *I*N	1	1.109	0.292	0.620	0.431	

\*Significance determined using Type II Wald  $\chi^2$  tests ( $\alpha=0.05$ ). A superscript “<sup>a</sup>” is included after trait labels to indicate if models were fit with natural-log transformed response variables, while a superscript “<sup>b</sup>” 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<sub>bg</sub>*=belowground carbon biomass (gC, numerator of *N<sub>cost</sub>*), *N<sub>wp</sub>*=total nitrogen biomass (gN, denominator of *N<sub>cost</sub>*).

**Table S5** Effects of CO<sub>2</sub> concentration, inoculation, and nitrogen fertilization on investment toward symbiotic nitrogen fixation\*

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

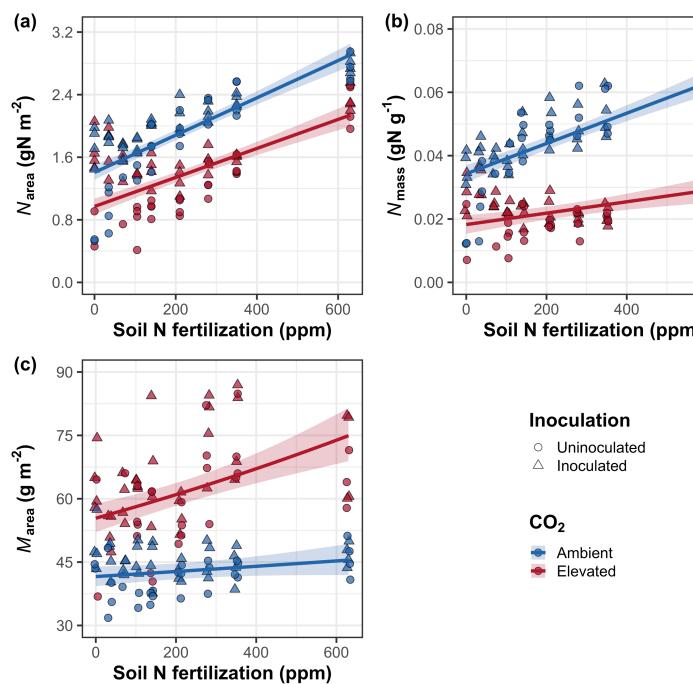
\*Significance determined using Type II Wald  $\chi^2$  tests ( $\alpha=0.05$ ). A superscript “<sup>a</sup>” is included after trait labels to indicate if models were fit with natural log-transformed response variables, while a superscript “<sup>b</sup>” is included if models were fit with square-root 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 CO<sub>2</sub> concentration, inoculation, and nitrogen fertilization on the ratio of total biomass to pot volume (g L<sup>-1</sup>)<sup>\*</sup>

	df	$\chi^2$	p
CO <sub>2</sub>	1	146.004	<b>&lt;0.001</b>
Inoculation (I)	1	19.320	<b>&lt;0.001</b>
N fertilization (N)	1	279.388	<b>&lt;0.001</b>
CO <sub>2</sub> *I	1	0.007	0.934
CO <sub>2</sub> *N	1	49.725	<b>&lt;0.001</b>
I*N	1	9.007	<b>0.003</b>
CO <sub>2</sub> *I*N	1	0.640	0.434

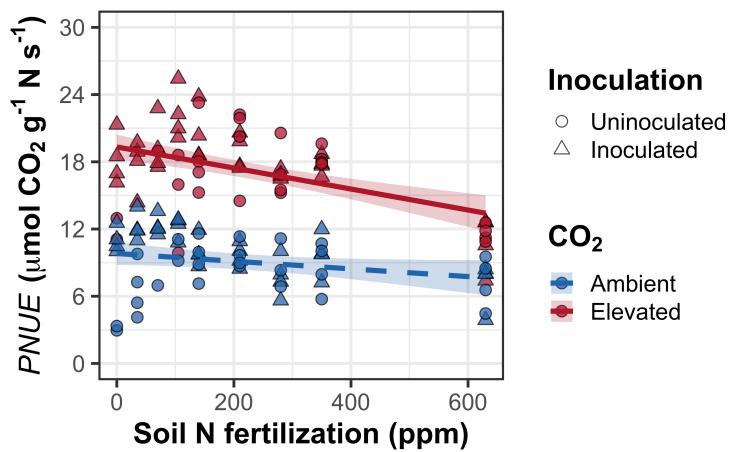
\*Significance determined using Type II Wald  $\chi^2$  tests ( $\alpha=0.05$ ). P-values less than 0.05 are in bold. Key: df=degrees of freedom,  $\chi^2$ =Wald chi-square test statistic

**Figure S1**



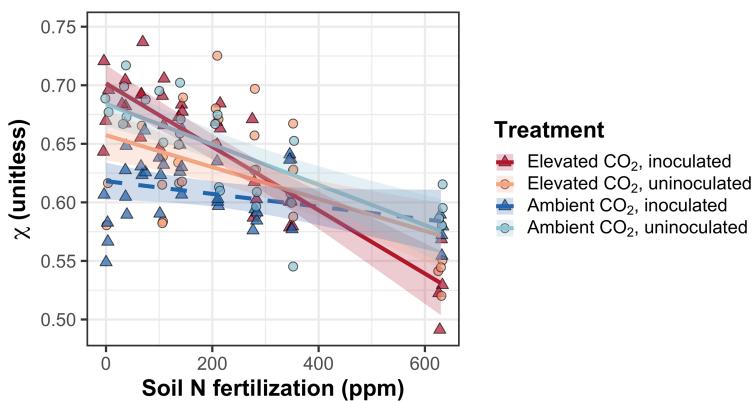
**Figure S1** Effects of CO<sub>2</sub> and fertilization inoculation on area-based leaf nitrogen content (a), mass-based leaf nitrogen content (b), and leaf biomass per unit leaf area (c). Fertilization is represented on the x-axis. Red shaded points and trendlines indicate plants grown under elevated CO<sub>2</sub>, while blue shaded points and trendlines indicate plants grown under ambient CO<sub>2</sub>. 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 CO<sub>2</sub> 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 CO<sub>2</sub>, while blue shaded points and trendlines indicate plants grown under ambient CO<sub>2</sub>. 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 ( $p > 0.05$ ).

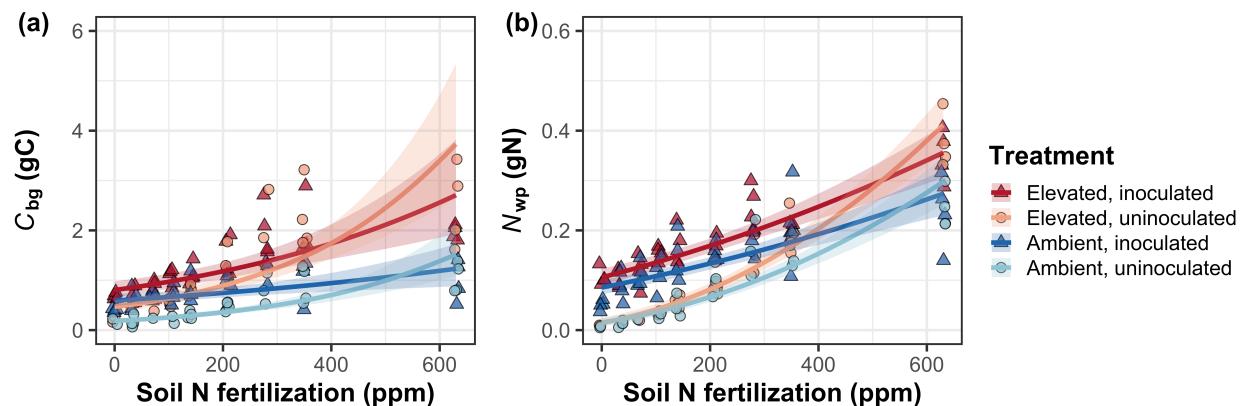
**Figure S3**



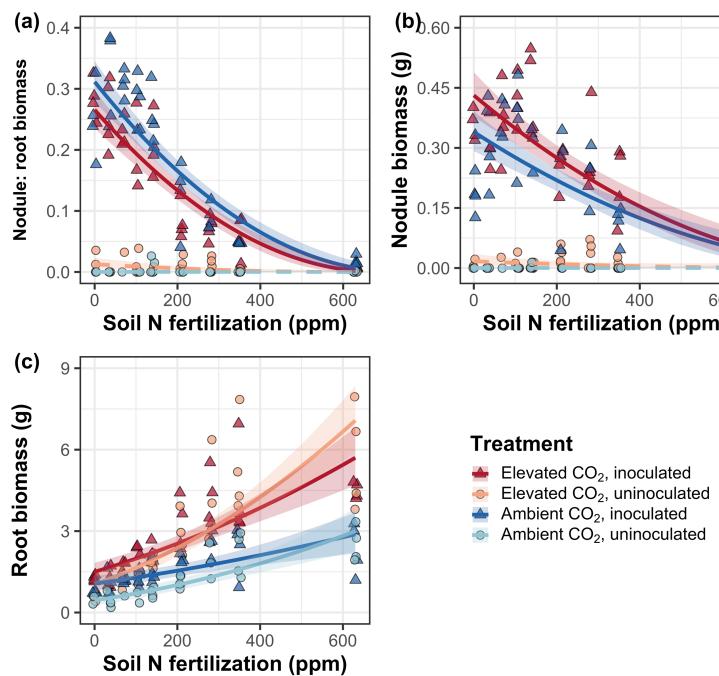
**Figure S3** Effects of nitrogen fertilization, inoculation treatment, and CO<sub>2</sub> treatment on  $\chi$ .

Fertilization is represented on the x-axis. Red shaded points and trendlines indicate plants grown under elevated CO<sub>2</sub>, while blue shaded points and trendlines indicate plants grown under ambient CO<sub>2</sub>. 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 ( $p>0.05$ ).

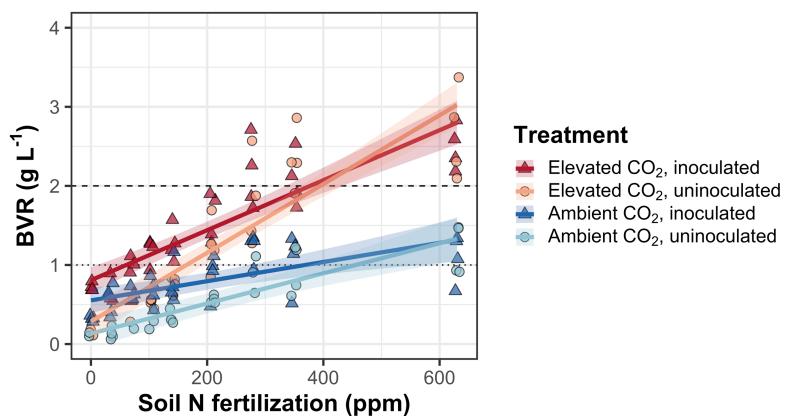
**Figure S4**



**Figure S4** Effects of CO<sub>2</sub> 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 CO<sub>2</sub>, while blue shaded points and trendlines indicate plants grown under ambient CO<sub>2</sub>. 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 S5**

**Figure S5** Effects of nitrogen fertilization, inoculation treatment, and CO<sub>2</sub> 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 CO<sub>2</sub>, while blue shaded points and trendlines indicate plants grown under ambient CO<sub>2</sub>. 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 CO<sub>2</sub>, 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 CO<sub>2</sub>, while blue shaded points and trendlines indicate plants grown under ambient CO<sub>2</sub>. 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<sup>-1</sup>, and the dashed line indicates the point where biomass: pot volume exceeds 2 g L<sup>-1</sup>.