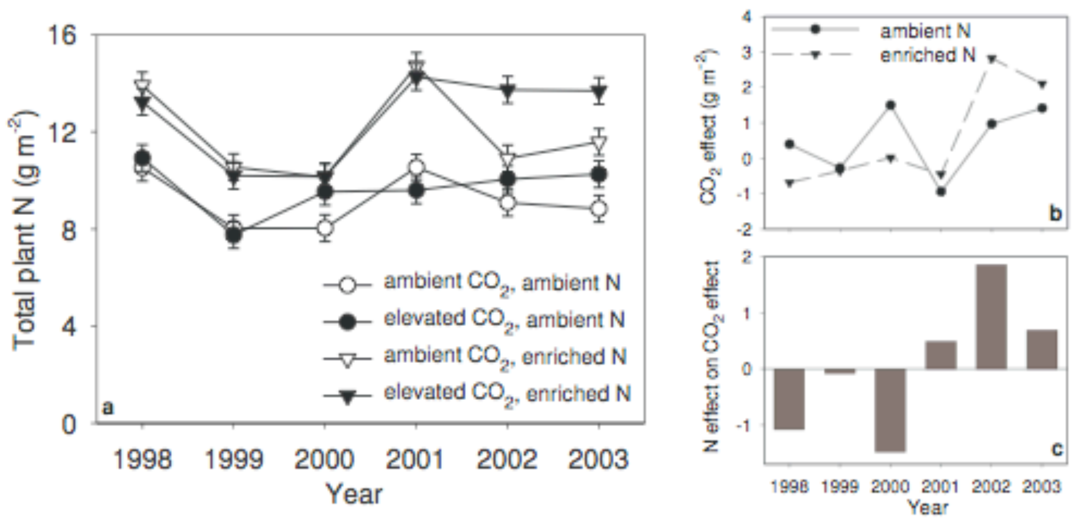
Supplemental Table and Figure Legends

Supplemental Table 1. **Summary of repeated measures analysis of covariance of CO2, N, time, and legume effects on plant biomass.**

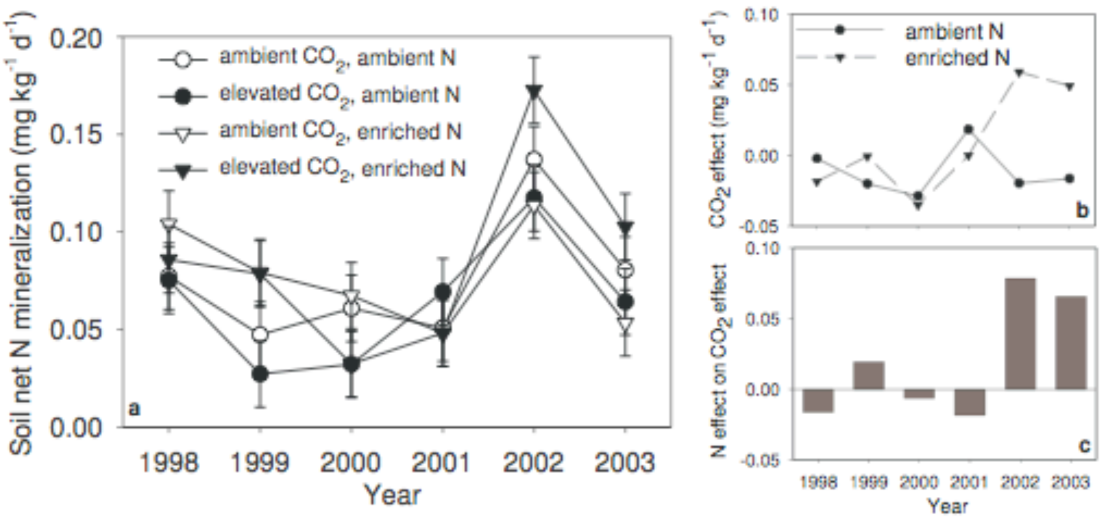
	Total biomass (g m^{-2})	*P*
Whole model R^2	**0.77**	**<0.0001**
Effect	F value	
CO_2	**29.03**	**<0.0001**
N	**7.59**	**0.0059**
Year	**50.43**	**<0.0001**
CO_2*Year	**2.75**	**0.0177**
N*Year	**6.84**	**<0.0001**
CO_2*N	1.33	0.2496
Year*CO_2*N	0.36	0.8746
Total plant N	3934.67	**<0.0001**
CO_2*total plant N	0.09	0.759
N*total plant N	**4.48**	**0.0344**
CO_2*N*total plant N	0.75	0.3859
Year*total plant N	**30.25**	**<0.0001**
CO_2*Year*total plant N	**6.02**	**<0.0001**
N*Year*total plant N	0.59	0.7093
CO_2*N*Year*total plant N	0.73	0.6043

Summary of analyses, using repeated measures analysis of covariance, of the effects of year (1998 to 2003), CO_2 treatment, N treatment, and total plant N contents (g N m^{-2} ground area) and all interactions, on total biomass (g m^{-2}, aboveground plus 0-20 cm belowground). Significant effects are shown in bold.

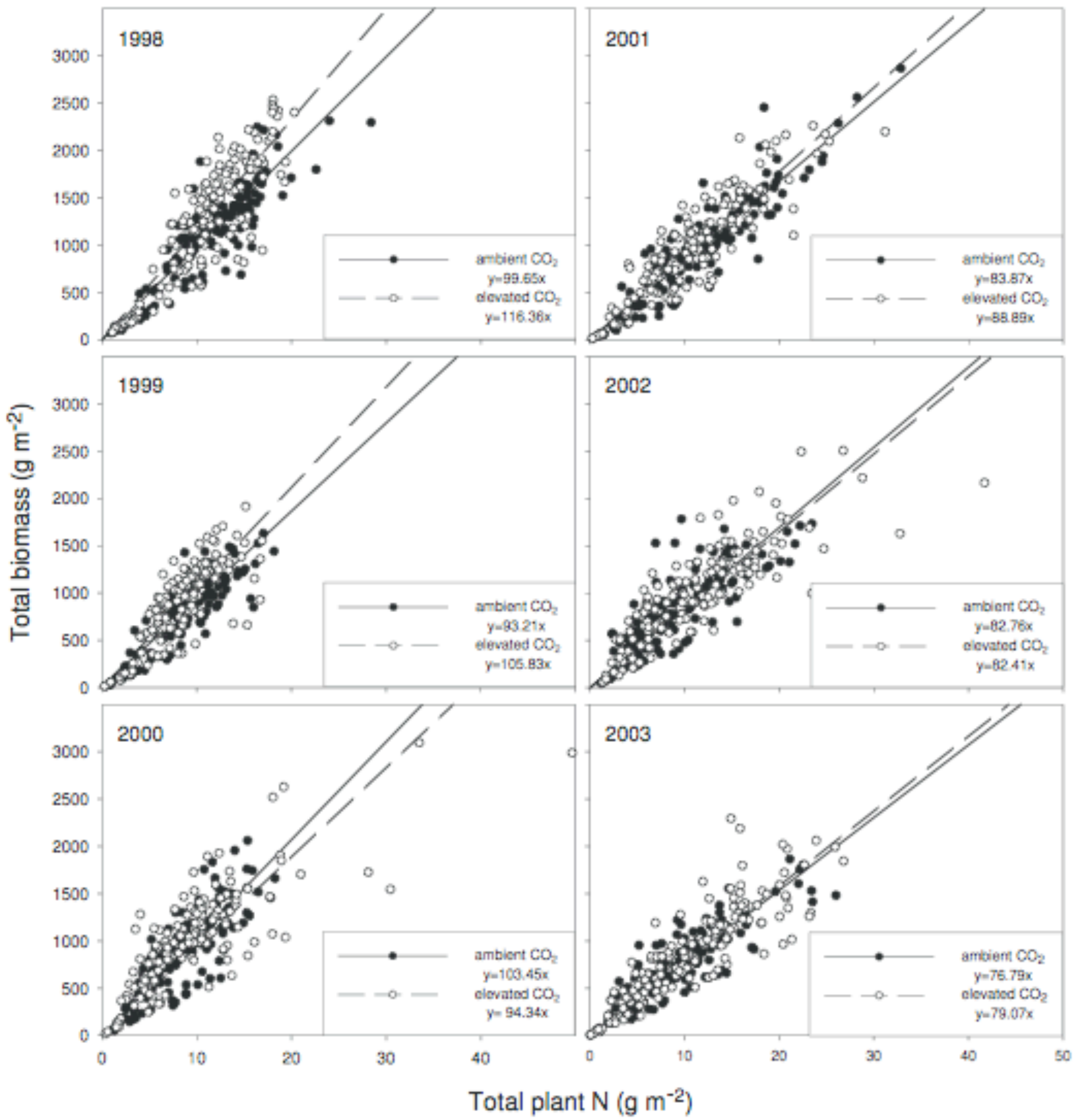
Supplemental Figures



Supplemental Figure 1. Effects of elevated CO_2 and enriched N on total plant N contents per unit ground area over time. A. Total plant N contents per unit ground area (g N m^{-2}) at four CO_2 x N levels (ambient and elevated of each), for each of six years (from 1998 to 2003). Each data point includes plots pooled across diversity treatments and represents the annual mean (plus standard error) of 74 plots sampled once each year. There was a significant interaction (see Table 1) between CO_2, N and year ($P=0.0497$), as well as significant main effect ($P<0.05$) of N. B. The CO_2 effect for total plant N contents per unit ground area, i.e. the CO_2 enhancement (g N m^{-2}) (assessed as E - A; [mean value at elevated CO_2] - [mean value at ambient CO_2]) at ambient vs. enriched N supply over each of the six years. C. The N availability effect on the elevated CO_2 effect for total plant N contents. The N effect is defined as the difference between the CO_2 effect at enriched vs. ambient N ([CO_2 effect at enriched N] – [CO_2 effect at ambient N]).



Supplemental Figure 2. Effects of elevated CO_2 and enriched N on total soil net N mineralization over time. A. Total net soil N mineralization rates (mg kg^{-1} day^{-1}) at four CO_2 x N levels (ambient and elevated of each), for each of six years (from 1998 to 2003). Each data point includes plots pooled across diversity treatments and represents the annual mean (plus standard error) of 74 plots sampled over a one-month field incubation each year. There was a significant interaction (see Table 1) between CO_2 and year ($P=0.0394$), as well as a significant main effect ($P<0.05$) of year. B. The CO_2 effect for net soil N mineralization, i.e. the CO_2 enhancement (mg kg^{-1} day^{-1}) (assessed as E - A; [mean value at elevated CO_2] - [mean value at ambient CO_2]) at ambient vs. enriched N supply over each of the six years. C. The N availability effect on the elevated CO_2 effect for net soil N mineralization. The N effect is defined as the difference between the CO_2 effect at enriched vs. ambient N ([CO_2 effect at enriched N] – [CO_2 effect at ambient N]).



Supplemental Figure 3. Scatterplots demonstrating the CO_2 x year x total plant N interaction shown in Supplemental Table 1. Relationship between total biomass (g m^{-2}) and total plant nitrogen (g N m^{-2}) at ambient CO_2 (closed circles) and elevated CO_2 (open circles) for plots in each year from 1998 through 2003 (≈296 plots per year). The lines were regressed through the origin and the slope is shown in the panel for each year.

Supplemental Materials Table 2**. Summary of repeated measures analysis of covariance of CO2, N, time, and legume effects on plant biomass**

	Total biomass (g m^{-2})	
	F value	*P>F*
Whole model R^2	**0.64**	**<0.0001**
Effect		
CO_2	**5.39**	**0.0809**
N	**38.45**	**<0.0001**
Diversity	**138.65**	**<0.0001**
Year	**668.77**	**<0.0001**
CO_2*Year	0.053	0.4663
N*Year	**36.49**	**<0.0001**
Diversity*Year	**34.46**	**<0.0001**
CO_2*Diversity	0.15	0.7014
N*Diversity	**6.07**	**0.0138**
CO_2*N	0.71	0.4002
CO_2*N*Year	**18.44**	**<0.0001**
Legume biomass	**244.32**	**<0.0001**
CO_2*Legume biomass	0.06	0.8126
N*Legume biomass	3.34	0.0677
Diversity*Legume biomass	**9.09**	**0.0026**
Year*Legume biomass	**28.75**	**<0.0001**
CO_2*Diversity*Year	0.65	0.4215
N*Diversity*Year	0.17	0.6793
CO_2*N*Diversity	0.17	0.6381
CO_2*N*Year	**34.46**	**<0.0001**
CO_2*Year*Legume biomass	0.45	0.5028
N*Year*Legume biomass	**6.58**	**0.0104**
Diversity*Year*Legume biomass	**8.54**	**0.0035**
CO_2*Diversity*Legume biomass	**4.61**	**0.0319**
N*Diversity*Legume biomass	0.30	0.5810
CO_2*N*Legume biomass	**4.3**	**0.0382**
CO_2*Diversity*Year*Legume biomass	1.92	0.1657
N* Diversity*Year*Legume biomass	0.85	0.3573
CO_2*N*Year*Legume biomass	1.52	0.2178
CO_2*N*Diversity*Legume biomass	2.85	0.0917
CO_2*N*Diversity*Year	**8.19**	**0.0042**
CO_2*N*Diversity*Year*Legume biomass	0.71	0.3991

Effects of year, CO_2, N, diversity, year, and total legume aboveground biomass (g m^{-2}) on total biomass (g m^{-2}, aboveground

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Materials and Methods Online

Experimental Design. The BioCON (Biodiversity, CO_2 and N) experiment includes 296 2 x 2 m plots arranged in six circular 20-meter diameter rings, located at the Cedar Creek Natural History Area in Minnesota, USA. Plots were established on a secondary successional grassland on a sandy outwash soil after removing the previous vegetation. The experimental treatments were arranged in complete factorial combination of CO_2 (ambient or 560mmol mol^{-1}), N level (ambient and enriched), and species number (1, 4, 9, and 16). Each plot was planted in 1997 with 12 g m^{-2} of seed partitioned equally among all species planted in a plot. The design consisted of a split-plot arrangement of treatments in a completely randomized design. CO_2 treatment is the whole-plot factor and is replicated three times among the six rings. The subplot factors of species number (hereafter called diversity) and N treatment were assigned randomly and replicated in individual plots among the six rings. For each of the four combinations of CO_2 and N levels, pooled across all rings, there were 32 randomly assigned replicates for the plots planted to 1 species, 15 for those planted to 4 species, 15 for 9 species, and 12 for 16 species. Beginning in 1998, the plots assigned to the enriched N treatment were amended with 4 g N m^{-2} yr^{-1}, applied over three dates each year. This N addition is comparable or slightly larger than the average annual net N mineralization rate in similar secondary grasslands on these soils. Beginning in 1998, a free-air CO_2 enrichment system was used during each growing season to maintain the CO_2 concentration at an average of 560 μmol mol^{-1} in elevated treatments during all daylight hours from spring (early April) to fall (late October to mid-November) each year. Three ambient CO_2 rings were treated identically but without additional CO_2.

The 16 species used in this study were all native or naturalized to the Cedar Creek Natural History Area. They include four C4 grasses (*Andropogon gerardii, Bouteloua gracilis, Schizachyrium scoparium, Sorghastrum nutans*), four C3 grasses (*Agropyron repens, Bromus inermis, Koeleria cristata, Poa pratensis*), four N-fixing legumes (*Amorpha canescens, Lespedeza capitata, Lupinus perennis, Petalostemum villosum*) and four non-N-fixing herbaceous species (*Achillea millefolium, Anemone cylindrica, Asclepias tuberosa,* at all CO_2 and N levels. The 4- and 9-species plots were random selections from all species. Plots were regularly weeded to remove unwanted species.

Biomass sampling and biogeochemistry measurements. In each year we assessed above- and below-ground biomass, plant C and N, and soil N in every plot. In June and August of each year, aboveground biomass was harvested in every plot by clipping a 10 x 100 cm strip just above the soil surface. All biomass was collected, sorted to live material and senesced litter, dried and weighed. Live material was considered as current plant biomass. Total belowground biomass (fine roots, coarse roots and crowns) was sampled in every plot within days of aboveground harvests in June and August at 0-20 cm depth using three 5 cm dia. cores in the area used for the aboveground biomass sampling. Roots were thoroughly washed, sorted into fine (<1 mm diameter) and coarse classes and crowns, dried and weighed. Any given area was sampled only once during the six years of this study. All biomass from August harvests was ground and analyzed separately for aboveground and belowground components for C and N concentrations using a CHN analyzer (Carlo-Erba Strumatzione, Milan, Italy). Total plant N pools for the August harvests were estimated by multiplying total live plant biomass by the N concentration. Soil net N mineralization was measured in each plot every year with a semi-open core (2.5 cm diameter) technique using one-month *in situ* incubations at 0-20 cm depth during midsummer. We sieved (2 mm) incubated soil cores, as well as an equal number of soil cores taken at the start of each incubation, and extracted them with 1 M KCl. Extracts were analyzed for NO_3^- and NH_4^+ on an Alpkem auto-analyzer. We measured soil moisture content on a sub-sample by oven drying (48 h, 105°C). We calculated net N mineralization by subtracting initial from final total inorganic N (NO_3^- + NH_4^+).

Statistical analysis. We examined results for the entire 1998-2003 period, using a repeated measures analysis of variance (JMP Statistical Software 5.0.1a) to test for main effects and interactions of the treatments, and whether these changed over time (contrasting across years). Given the nature of both the hypothesized progressive N limitation and the observed patterns over time, year was considered a continuous rather than a of plots over time. To do this, variance among plots nested within CO_2, N and diversity levels was used as a random effect such that measures that co-vary across time (years) were not counted as fully independent. The F statistic for the main effects of N and diversity used the nested effect of plot within CO_2, N and diversity treatments. The F statistic for year and for treatment x year effects used the residual error term. The F statistic for CO_2 used the nested effect of ring within CO_2.