Soil-mediated effects of subambient to increased carbon dioxide on grassland productivity

Philip A. Fay[1]*, Virginia L. Jin[2], Danielle A. Way[3,4], Kenneth N. Potter[1], Richard A. Gill[5], Robert B. Jackson[3,4] and H. Wayne Polley[1]

Grasslands are structured by climate and soils[1,2], and are increasingly affected by anthropogenic changes, including rising atmospheric CO_2 concentrations[3,4]. CO_2 enrichment can alter grassland ecosystem function both directly and through indirect, soil-specific effects on moisture, nitrogen availability and plant species composition[5–8], potentially leading to threshold change in ecosystem properties[9]. Here we show that the increase in aboveground net primary productivity (ANPP) with CO_2 enrichment depends strongly on soil type. We found that the ANPP–CO_2 response of grassland was 2.5× greater on two soils with higher plant-available soil moisture and where direct CO_2 effects on ANPP were accompanied by indirect CO_2 effects on ANPP mediated through an increase in soil moisture or increased dominance of a productive C_4 grass. Indirect CO_2 effects on ANPP were absent on a third soil that was less responsive to CO_2 (1.6×). Unexpectedly[6,10], soil N availability changed little with CO_2 and did not seem to drive responses in ANPP. On the more responsive soils, the more productive grass C_4 was favoured with CO_2 enrichment because of greater photosynthetic efficiency. Our results enhance present models of the controls on ecosystem responses to CO_2 (refs 7,8,11) and demonstrate mechanisms by which soils could cause spatial variation in CO_2 effects on ANPP and other ecosystem attributes.

Several mechanisms have been advanced to explain the effects of CO_2 enrichment on ecosystems. CO_2 enrichment may increase plant growth and ecosystem productivity[12–14] by increasing photosynthetic rates and reducing stomatal conductance and transpiration[15–17], thereby increasing water-use efficiency and, potentially, soil-moisture availability[12,18]. CO_2 enrichment may lead to species change when CO_2 effects on photosynthetic capacity or water-use efficiency differ sufficiently among species[8] to change the outcome of species interactions. Species changes based on differential physiological responses to CO_2 may be enhanced on soil types with lower soil moisture and/or N availability (for example, ref. 5), and could reinforce positive effects of CO_2 enrichment on ANPP. However, so far, there have been few direct comparisons of CO_2 responses among soils that differ in properties such as texture that can mediate CO_2 effects on ecosystems.

Here we report that ecosystem response to CO_2 (measured as aboveground productivity response to CO_2) was greatest on soils with higher plant-available soil water (soil water potential, Ψ_S) and where a direct CO_2 effect on ANPP was accompanied by an indirect CO_2 effect on productivity; either an increase in Ψ_S or a change in the dominant grass species. Ecosystem response was reduced on a third soil where only the direct CO_2 effect was present. The study was conducted during 2006–2010 in closed outdoor field chambers that maintain a continuous 500–250 µl l^{-1} CO_2 gradient[19,20] (Supplementary Fig. S1). We studied 60 hydrologically isolated intact soil monoliths (1.5 m^3) in these chambers taken from clay, silty clay and sandy loam soils (Supplementary Table S1) representing three soil orders common in the Blackland prairies of central Texas, USA. The monoliths supported constructed communities of native perennial prairie grasses (C_4) and forbs (Supplementary Table S2). All monoliths were irrigated identically with average growing season rainfall (560 mm).

Aboveground productivity response to CO_2, ΔANPP (the ratio of treatment to 2005 pretreatment ANPP) increased linearly with CO_2 enrichment for silty clay and sandy loam soils ($R^2 = 0.50$–0.59, $P \leq 0.002$), but saturated at near present-day ambient CO_2 on the clay soil ($R^2 = 0.48$, $P = 0.01$, Fig. 1a–c and Supplementary Tables S3, S4). Estimated for the full CO_2 gradient (500–250 µl l^{-1}), ΔANPP increased 2.5× on the sandy loam and silty clay soils, compared with 1.6x on the clay soil (Figs 1a–c and 2). The raw ANPP values (unadjusted for pretreatment ANPP) exhibited similar patterns in CO_2 response ($CO_2 \times$ soil $P < 0.0001$, Supplementary Fig. S2 and Table S3).

On the two soils where ΔANPP increased linearly with CO_2, CO_2 enrichment changed the relative dominance (see Methods, equation (1) for definition) of the C_4 grasses *Sorghastrum nutans* and *Bouteloua curtipendula*. Communities dominated by the mesic tallgrass (*S. nutans*) developed at increased CO_2 whereas communities dominated by the more drought-tolerant midgrass (*B. curtipendula*) developed at subambient CO_2 on the silty clay ($P < 0.0001$) and sandy loam ($P = 0.01$) soils (Fig. 1d–f and Supplementary Tables S3, S4). Similar compositional changes were not observed on the clay soil[21]. Together these two grasses accounted for 61% of ANPP (Supplementary Table S2) and their relative abundance explained ~44% of the variation in ΔANPP on these two soils ($P \leq 0.0001$, Supplementary Fig. S3).

Soil moisture was affected by CO_2 enrichment. As found previously[12,18,22–24], volumetric soil water content (vSWC, Supplementary Fig. S4A) and Ψ_S (Fig. 1g) increased with CO_2 enrichment on all soils. The vSWC–CO_2 response was strongest on the sandy loam (soil × CO_2 $P = 0.0004$, $R^2 = 0.39$, $P < 0.009$, Supplementary Tables S3, S4), where mean vSWC was low compared with the silty clay and clay soils (soil $P < 0.0001$, Fig. 2 and Supplementary Fig. S4A). Conversely, the Ψ_S–CO_2 response was strongest on the clay soil, where mean Ψ_S was more negative compared with the coarser silty clay and sandy loam soils (Figs 1g and 2). These
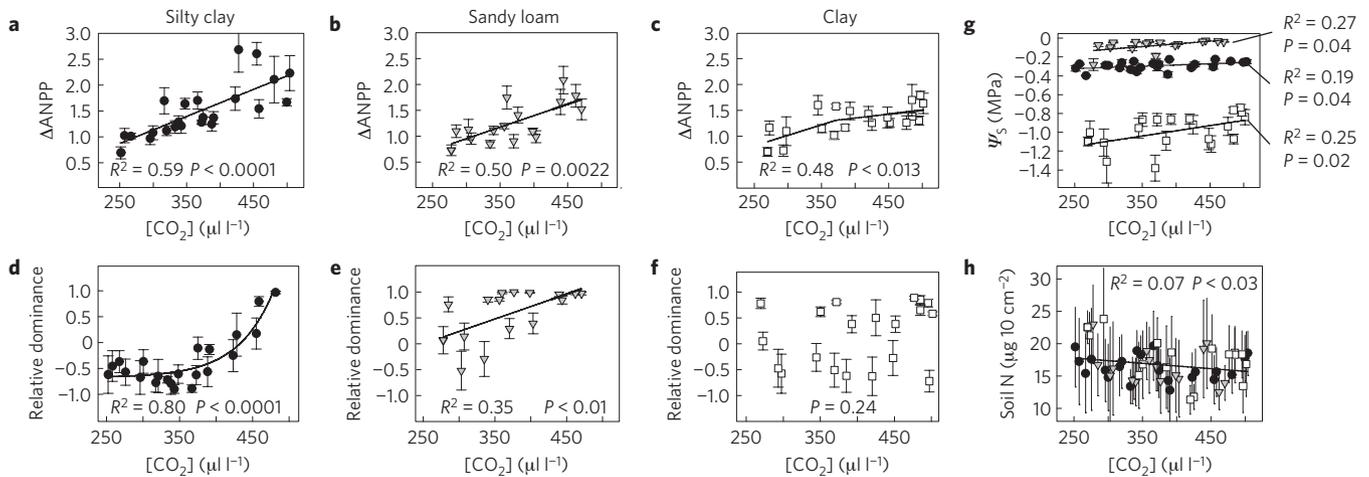
Figure 1 | Responses in aboveground biomass production, dominant grass species and soil moisture and nitrogen on three contrasting soils to a continuous gradient in CO_2. a–c, ΔANPP, the ratio of CO_2-treated ANPP to pretreatment (2005) ANPP. **d–f**, Relative dominance by the tallgrass *S. nutans* (1.0) or midgrass *B. curtipendula* (−1.0). In **d**, two outliers (with low relative dominance at high CO_2) were omitted. If the outliers are included, $R^2 = 0.50$, $P = 0.0007$. **g**, Ψ_S. **h**, Resin-available soil nitrogen (N, $NH_4^+ + NO_3^-$). Symbols in **g,h** corresponded to the soil types in **a–f**. Regression statistics in Supplementary Table S4.
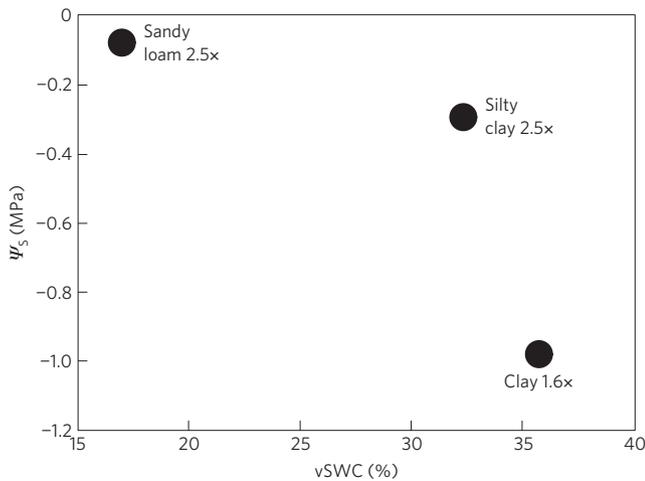

Figure 2 | Association of vSWC with Ψ_S for the three soils. Each point is the average of all CO_2 concentrations and years, and is annotated with the increase in ΔANPP from 250 to 500 µl l⁻¹ CO_2 estimated from the regression equations fit to Fig. 1a–c (see Supplementary Table S4).

differences in the amount and plant availability of soil moisture were expected from the textural differences among these soils[25]. Resin-available soil N was similar among soils and decreased only weakly with CO_2 enrichment (soil × CO_2 $P = 0.08$, $R^2 = 0.07$, $P = 0.03$, Fig. 1h and Supplementary Tables S3, S4). This contrasts with suggestions that decreased N availability limits productivity gains from increased CO_2 (refs 10,26; but see ref. 6). ΔANPP and relative dominance were more strongly correlated with Ψ_S on the sandy loam ($R^2 = 0.36$–0.41, $P < 0.0001$ and Supplementary Fig. S3) than on the silty clay or clay soils ($R^2 = 0.06$–0.11, $P = 0.004$–0.02). Soil N was not correlated with ΔANPP, Ψ_S, or relative dominance, except for a weak decreasing ΔANPP–soil-N response on the clay soil ($R^2 = 0.08$, $P = 0.01$). These correlations suggest a larger role for vSWC and Ψ_S than for soil N in mediating ANPP and species change on these soils, particularly on the sandy loam soil.

Structural equation models (SEMs) delineate the simultaneous patterns of causal influence and response among multiple variables[27]. We tested a SEM of likely direct and indirect
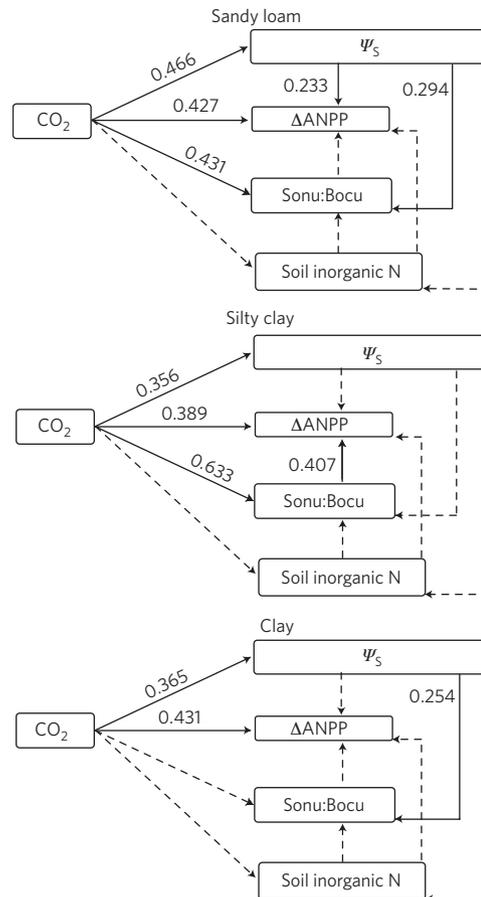

Figure 3 | SEMs for each soil showing hypothesized relationships among CO_2, Ψ_S, relative dominance (Sonu:Bocu) and soil N. Standardized coefficients are given for each path. Significant paths are indicated by solid arrows; non-significant paths were retained, shown with dashed arrows. Sonu, *S. nutans*; Bocu, *B. curtipendula*.

relationships among the independent variable, CO_2, and dependent variables, Ψ_S, soil N, ΔANPP and the relative dominance of *B. curtipendula* and *S. nutans* (Fig. 3). The model adequately fit
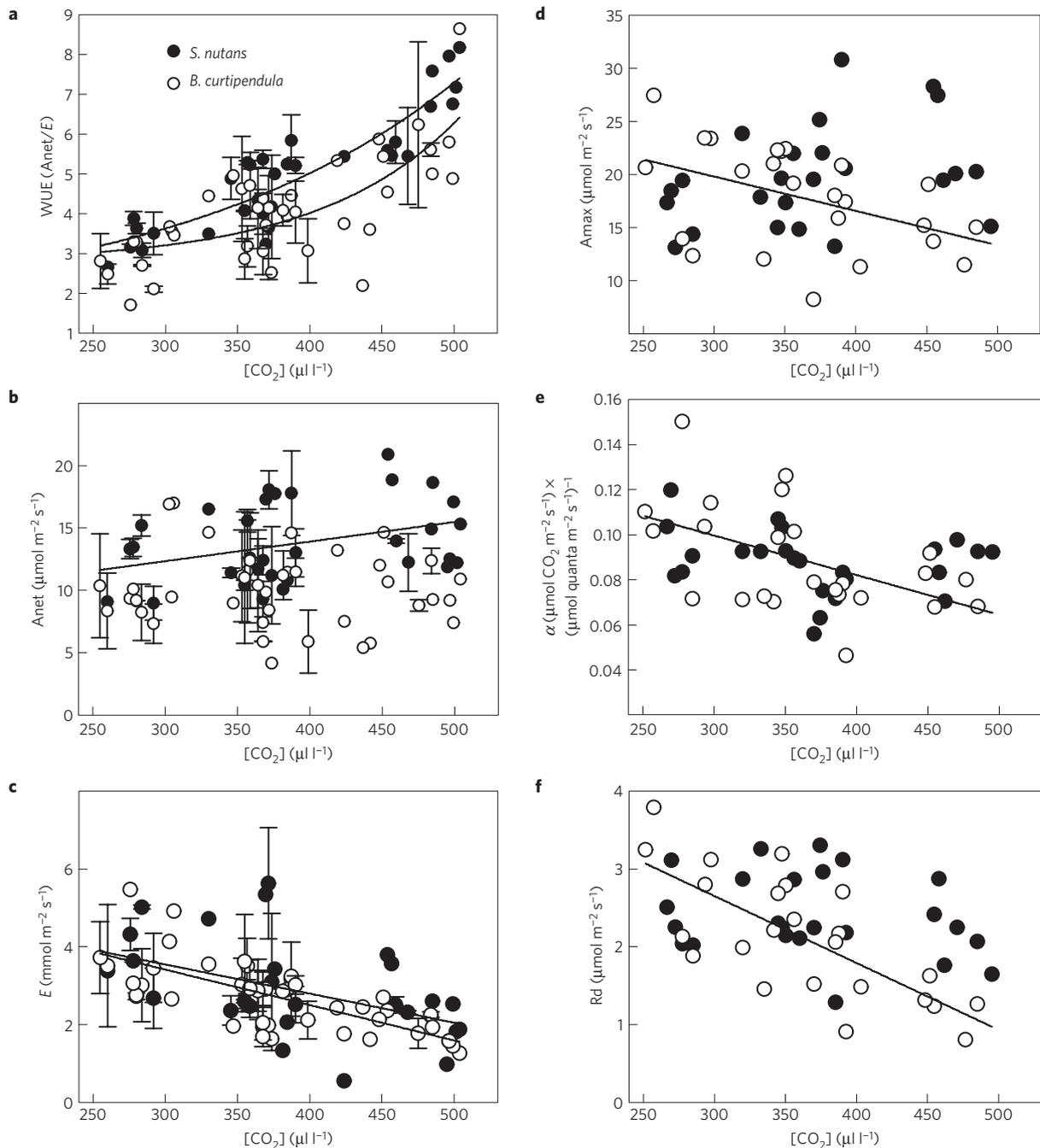
Figure 4 | Physiological responses to CO_2 in the C_4 grasses that assumed dominance at increased (*S. nutans*) and subambient (*B. curtipendula*) CO_2.
a, Leaf photosynthetic WUE computed from **b**, Anet and **c**, transpiration (*E*) measurements. **d**, Amax, **e**, α and **f**, Rd as estimated from light response curves measured on *S. nutans* and *B. curtipendula*. Measurement conditions for **a–c** were: 1,500 $\mu mol\, m^{-2}\, s^{-1}$ photon flux density, leaf temperature 30–35 °C and cuvette CO_2 and H_2O fractions at values corresponding to the gradient. In **d–f**, regression lines are shown for *B. curtipendula*. Regression statistics in Supplementary Table S4.

the data for all three soils ($P > 0.55$, Supplementary Table S5) and explained 33–54% of the variation among these variables. On all soils the SEMs contained direct effects of CO_2 enrichment on ΔANPP ($P < 0.0001$). On the sandy loam, there were direct effects of CO_2 enrichment on Ψ_S and relative dominance ($P \le 0.0001$), and Ψ_S effects on ΔANPP and relative dominance ($P \le 0.03$). Thus, on the sandy loam CO_2 enrichment increased ΔANPP and altered C_4 grass dominance directly, and increased ΔANPP indirectly by way of increased Ψ_S ($P \le 0.04$).

The silty clay SEM also contained direct effects of CO_2 enrichment on Ψ_S and relative dominance ($P \le 0.0001$), and

in contrast with the sandy loam, an indirect effect of CO_2 enrichment on ΔANPP by way of the change in relative dominance ($P < 0.0001$). On the clay soil, there was a direct effect of CO_2 enrichment on Ψ_S ($P < 0.0001$) and an indirect effect of CO_2 enrichment on relative dominance by way of Ψ_S, but no effects of Ψ_S, relative dominance, or soil N on ΔANPP. Thus, on the two soils where ΔANPP was most responsive to CO_2, CO_2 enrichment increased ΔANPP directly, as well as indirectly by increasing Ψ_S on the sandy loam, or favouring a productive tallgrass over a midgrass on the silty clay. These indirect CO_2 effects on ΔANPP were absent on the less-responsive clay soil.

We found evidence for physiological mechanisms to explain CO_2 effects on ANPP and soil moisture. CO_2 enrichment increased leaf-level photosynthetic water-use efficiency (WUE = net photosynthesis (Anet) $\times$ transpiration^{-1}; refs 12, 18) in the three most abundant species in these assemblages (*S. nutans*, *B. curtipendula*, (Fig. 4a), and *Solidago canadensis* (data not shown)). Higher WUE was caused by lower transpiration (Fig. 4c) associated with reduced stomatal conductance (data not shown), which lessened soil-moisture depletion at increased CO_2.

WUE and photosynthetic parameters (Fig. 4a–c) also provided a mechanism for the dominance of *B. curtipendula* at subambient CO_2 and of *S. nutans* at increased CO_2. WUE increased more with CO_2 in *S. nutans* than in *B. curtipendula*, because of increased Anet with CO_2 enrichment in *S. nutans* ($P = 0.03$, Fig. 4b, Supplementary Table S4). Furthermore, compared with *S. nutans*, *B. curtipendula* had declining gross photosynthetic capacity (Amax), quantum use efficiency (α), and dark respiration (Rd) with CO_2 enrichment ($0.0001 < P < 0.03$, Fig. 4d–f and Supplementary Table S4). Thus, we posit that at increased CO_2, the taller, more mesic *S. nutans* capitalized photosynthetically on increased soil moisture to outcompete *B. curtipendula*. However, at subambient CO_2, reduced soil moisture combined with increasing Amax, α and Rd gave the advantage to the more drought-adapted midgrass, *B. curtipendula*, in carbon assimilation and growth[28,29]. The change in species composition over subambient to increased CO_2 (250–500 μl l^{-1}) was similar to the change in vegetation from midgrass to tallgrass prairies in the central USA[21].

Our results demonstrate that the ΔANPP–CO_2 response of grassland was greater on soils where soil-moisture availability was high and where direct CO_2 effects on ΔANPP were reinforced by indirect CO_2–ΔANPP effects mediated by an increase in soil-moisture availability on the sandy loam soil or by species change toward a more productive C_4 tallgrass on the silty clay soil. ΔANPP–CO_2 responses were lower on a third soil where the indirect CO_2 effects were absent. Recent models predict that numerous effects will amplify ecosystem responses to CO_2 (refs 7,8). Our results extend these models by showing that soil mediation of the indirect effects of CO_2 can result in both linear and nonlinear ΔANPP responses and threshold-like species change[9], because soil moisture both limited the ΔANPP-CO_2 response and responded to CO_2 (ref. 7). Soil texture is probably the main property of soils mediating these effects, because texture influences both soil-water-holding capacity and plant availability of soil moisture[25]. ANPP of mesic grasslands is more water limited on coarse-textured (compared with fine-textured) soils[11] and hence more likely to benefit from reduced water use at increased CO_2. Understanding soil-mediated controls in ecosystem responses to increased CO_2 is critical for predicting how future ecosystem carbon cycling may differ across the landscape. Soils in the orders studied here occupy approximately 38% of US land area and 20% of global land area, and Mollisols dominate the Great Plains grassland region of North America[25], giving our results potentially broad applicability for understanding past and future responses to altered atmospheric CO_2.

Methods

Study site. The lysimeter CO_2 gradient facility is located in Temple, Texas, USA ($31°05'$ N, $97°20'$ W; ref. 19). Mean annual precipitation is 914 mm (1971–2000), with wetter spring and autumn, and a pronounced summer dry period. Mean maximum temperature is 35 °C and mean minimum is 2.9 °C. The frost-free period is ~250 days.

Soils. Intact soil monoliths (1.5 m³) from a silty clay Mollisol ($n = 24$), a sandy loam Alfisol ($n = 16$) and a heavy clay Vertisol ($n = 20$) were excavated and encased in steel boxes in 2002.

Grassland communities. The monoliths were planted in spring 2003 with seedlings of four perennial C_4 grasses, two C_3 forbs and one legume native to Texas

Blackland prairie (Supplementary Table S2). Other species were regularly removed by hand or selective glyphosate application.

CO_2 chambers. A continuous CO_2 gradient was maintained in two linear chambers (Supplementary Fig. S1A), each composed of ten sections. Each section enclosed a pair of monoliths from two of the three soil types, randomly ordered within each section (Supplementary Fig. S1B). The sandy loam was included in alternating sections.

CO_2 treatments. The CO_2 gradient was controlled at 500–250 μl l^{-1} during May–October beginning in 2006. The plant communities were exposed to ambient conditions during winter. Air enriched to 500 μl l^{-1} CO_2 was introduced to the first linear chamber. Photosynthesis by the enclosed vegetation depleted the air of CO_2 as it transited the chamber. The air flow rate was controlled so that air exited at 380 μl l^{-1} CO_2. Similarly, ambient air was introduced to the second linear chamber and exited at 250 μl l^{-1}. Air temperature was controlled to match the outside ambient temperature. Each monolith received the average growing season rainfall amount for this locale (560 mm). We varied the seasonal pattern of irrigation among years to introduce realistic interannual variability.

Soil N. Soil N was quantified in 2007–2010 by measuring NO_3^- and NH_4^+ in soil solution using ion exchange resins (PRS-probes, Western Ag Innovations). Probes were installed monthly during CO_2 treatment. NO_3^-–N and NH_4^+–N amounts were determined colourimetrically and expressed as μg N 10 cm^{-2}.

Soil moisture. vSWC for 0–40 cm was measured biweekly with a calibrated neutron attenuation probe (503DR Hydroprobe, CPN International). Ψ_S was computed from soil water content using soil water release curves (Supplementary Fig. S4).

ANPP. ANPP was measured each November by clipping all present year growth by species at 5 cm height, drying for 72 h at 60 °C and weighing. The biomass was chipped and returned to the monoliths to minimize nutrient depletion. The relative dominance of two grasses, *S. nutans* and *B. curtipendula*, was estimated from the biomass of the two species:

$$\text{Relative dominance} = \frac{[mass(S.\ nutans) - mass(B.\ curtipendula)]}{[mass(S.\ nutans) + mass(B.\ curtipendula)]} \quad (1)$$

Plant physiology. Anet and stomatal conductance were determined on *S. nutans*, *B. curtipendula* and the forb *S. canadensis* in 12 gradient sections during June or July 2006–2010 with a photosynthesis system (LI-6400 LiCor Biosciences). Newly expanded leaves on two plants per species per monolith were measured. Photosynthetic light response curves were measured during July 2010 at nine light levels from 1,000 to 0 μmol m^{-2} s^{-1}. We estimated the light-saturated rate of Amax, Rd and α from the parameters of rectangular hyperbolae fit to the curves[30].

Data analysis. ANPP values more extreme than 1.5 $\times$ the interquartile range were removed as outliers before analysis. ANPP, ΔANPP, relative dominance and growing season averages of Ψ_S and soil N were fit with an analysis of covariance model using Proc Mixed in SAS 9.2:

$$y = \text{intercept} + \text{soil} + \text{monolith(soil)} + \alpha(CO_2 \times \text{soil}) + \text{year} + \text{soil} \times \text{year} + e$$

Soil was a fixed effect, monoliths within soil type [monolith(soil)] was a random effect, $CO_2 \times$ soil the soil-specific covariate. The simple CO_2 covariate was not fit unless soil $\times CO_2$ was non-significant, because detecting soil-specific CO_2 responses was the analytical objective. Year was fit as a repeated effect using an autoregressive covariance structure (Supplementary Table S3). Year effects will be considered elsewhere.

We fit SEMs to determine the importance of direct CO_2 effects on ΔANPP and of indirect CO_2 effects mediated through Ψ_S, relative dominance and soil N. The hypothesized paths reflected the bivariate relationships among these variables (Supplementary Fig. S3). Models were fit using Proc Calis. We found the same results whether using linearized or untransformed variables; the latter are presented here. Path coefficients were standardized by the variance ratio of the two variables forming the path. Non-significant paths were retained in the final models.

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Author contributions

H.W.P. conceived the experiment and conducted research, P.A.F. conducted research, analysed the data and wrote the manuscript, V.L.J., D.A.W., R.B.J., K.N.P. and R.A.G. conducted research and contributed to the manuscript.