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Elevated CO_2 stimulates grassland soil respiration by increasing carbon inputs rather than by enhancing soil moisture

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Abstract

It is not clear whether the consistent positive effect of elevated CO_2 on soil respiration (soil carbon flux, SCF) results from increased plant and microbial activity due to (i) greater C availability through CO_2-induced increases in C inputs or (ii) enhanced soil moisture via CO_2-induced declines in stomatal conductance and plant water use. Global changes such as biodiversity loss or nitrogen (N) deposition may also affect these drivers, interacting with CO_2 to affect SCF. To determine the effects of these factors on SCF and elucidate the mechanism(s) behind the effect of elevated CO_2 on SCF, we measured SCF and soil moisture throughout a growing season in the Biodiversity, CO_2, and N (BioCON) experiment. Increasing diversity and N caused small declines in soil moisture. Diversity had inconsistent small effects on SCF through its effects on abiotic conditions, while N had a small positive effect that was unrelated to soil moisture. Elevated CO_2 had large consistent effects, increasing soil moisture by 26% and SCF by 45%. However, CO_2-induced changes in soil moisture were weak drivers of SCF: CO_2 effects on SCF and soil moisture were uncorrelated, CO_2 effect size did not change with soil moisture, within-day CO_2 effects via soil moisture were neutral or weakly negative, and the estimated effect of increased C availability was 14 times larger than that of increased soil moisture. Combined with previous BioCON results indicating elevated CO_2 increases C availability to plants and microbes, our results suggest that increased SCF is driven by CO_2-induced increases in substrate availability. Our results provide further support for increased rates of belowground C cycling at elevated CO_2 and evidence that, unlike the response of productivity to elevated CO_2 in BioCON, the response of SCF is not strongly N limited. Thus, N limited grasslands are unlikely to act as a N sink under elevated CO_2.

Keywords: BioCON, Cedar Creek LTER, diversity, FACE, nitrogen additions, soil carbon flux

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Introduction

Annually, soil respiration, the combined carbon (C) efflux from belowground autotrophic and heterotrophic respiration, releases roughly 10 times more CO_2 than anthropogenic CO_2 sources (Schlesinger, 1997; Raich *et al*., 2002). That the relatively small anthropogenic flux has had such large effects on atmospheric CO_2 concentrations suggests that even small changes in soil respiration could feed back to significantly exacerbate or mitigate rising CO_2 levels.

Because ecosystems are currently affected by multiple global changes – including rising atmospheric CO_2, increasing N deposition, changes in temperature and precipitation regimes, and changes in plant community composition and diversity – understanding how and why ecosystem processes such as soil respiration (or soil C flux, hereafter SCF) respond to interacting global change variables is vital for predicting and managing ecosystem C fluxes and storage. Enhancing such understanding is the goal of the current study, made in the multifactor Biodiversity, CO_2 and N (BioCON) experiment in eastern Minnesota, USA.

Global changes likely impact SCF by altering one or more of the main drivers of SCF: soil temperature, soil moisture, and C substrate availability (Raich & Schlesinger, 1992; Lloyd & Taylor, 1994; Raich and Tufekcioglu, 2000; Rustad *et al*., 2000; Hogberg *et al*., 2001; Scott-Denton *et al*., 2006). However, these vary on different temporal scales (Reich, 2010) making their effects difficult to characterize. As long as water is not limiting, SCF generally increases with temperature (Lloyd & Taylor, 1994; Wan *et al*., 2007), although its

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sensitivity to temperature varies markedly with time and associated changes in drivers (Mahecha *et al.*, 2010). In water-limited ecosystems (or periods), water availability increases plant and microbial activity and thus SCF directly by alleviating plant and microbial desiccation stress and indirectly by increasing substrate availability (via higher rates of plant growth, photosynthesis, belowground C allocation) and microbial access to substrate (e.g., increased C diffusion through soil water; Wan *et al.*, 2007). In general, respiration increases with C availability; plant respiration is largely dependent on C from current photosynthetic activity (Hogberg *et al.*, 2001) and, under nonlimiting abiotic conditions, microbial respiration increases with labile C availability (Hungate *et al.*, 1997).

Elevated atmospheric CO$_2$ consistently increases SCF (Luo *et al.*, 1996; Zak *et al.*, 2000; Craine *et al.*, 2001a,b; King *et al.*, 2004; Baronti *et al.*, 2008; but see Bader & Körner, 2010), likely by altering at least two of the main drivers of SCF: substrate and water availability. Increasing CO$_2$ may increase plant and microbial C availability by increasing belowground C inputs via increases in photosynthesis (thus photosynthate availability; Hogberg *et al.*, 2001; Lee *et al.*, 2001; Crous *et al.*, 2010; Lee *et al.*, 2011), above- and belowground plant biomass (Reich *et al.*, 2001a, 2006a; Ainsworth & Rogers, 2007; Reich, 2009), and belowground allocation of C to roots, exudates, and mycorrhizae (Matamala & Schlesinger, 2000; Treseder & Allen, 2000; Pendall *et al.*, 2004; Allen *et al.*, 2005; Trueman & Gonzalez-Meler, 2005; Adair *et al.*, 2009). However, elevated CO$_2$ may also increase SCF by increasing soil water availability (a consistent effect of CO$_2$ at the site of this experiment; Appendix S1; Reich *et al.*, 2001b, Reich, 2009). Elevated CO$_2$ decreases plant stomatal conductance (Lee *et al.*, 2001, 2011; Morgan *et al.*, 2004; Niklaus & Korner, 2004; Ainsworth & Rogers, 2007; Leakey *et al.*, 2009), which often results in decreased evapotranspiration (ET; when not offset by increases in leaf area; Nie *et al.*, 1992; Ham *et al.*, 1995; Bremer *et al.*, 1996; Field *et al.*, 1997; Leakey *et al.*, 2009) and plant water loss (increased water use efficiency; Morison, 1993; Owensby *et al.*, 1993; Jackson *et al.*, 1994; Nelson *et al.*, 2004). Declines in ET and plant water use often result in increased soil water availability (Field *et al.*, 1995; Reich *et al.*, 2001b; Hungate *et al.*, 2002; Morgan *et al.*, 2004; Davis *et al.*, 2007; Leuzinger & Korner, 2007). Thus, under elevated CO$_2$, SCF may respond not only to increased C availability, but also to higher soil moisture in elevated relative to ambient CO$_2$ treatments (Craine *et al.*, 2001a,b; Pendall *et al.*, 2003; Wan *et al.*, 2007).

While enhancement of soil water availability has been found to increase plant biomass under elevated CO$_2$ (especially C$_4$ plants; Morgan *et al.*, 2004; Seiler *et al.*, 2009; Owensby *et al.*, 1993; Jackson *et al.*, 1994; Chiariello & Field, 1996; Morgan *et al.*, 2001; Nelson *et al.*, 2004; Leakey *et al.*, 2009), it is not clear if SCF is affected similarly. The higher water use efficiency of plants under elevated vs. ambient CO$_2$ suggests that the response of SCF to elevated CO$_2$ should be greater during dry vs. wet periods (Owensby *et al.*, 1999). However, results thus far have been equivocal (Craine *et al.*, 2001a,b; Pendall *et al.*, 2003; Wan *et al.*, 2007) and there is little direct evidence for or against the hypothesis of CO$_2$-induced increases in soil water content driving higher rates of SCF in elevated CO$_2$ plots (but see Wan *et al.*, 2007).

If elevated CO$_2$ does impact SCF via changes in soil water content, other manifestations of global change, such as elevated atmospheric N inputs or changes in plant diversity, may also affect SCF by altering soil moisture or by interacting with the effects of CO$_2$ on soil moisture. Alternatively, these global change factors may interact to influence SCF through their effects on C availability. We used the BioCON Free Air CO$_2$ Enrichment (FACE) experiment to investigate interacting effects of variation in plant species diversity, N addition, and elevated CO$_2$ on SCF and soil moisture, with a focus on discovering the mechanism(s) behind the effect of elevated CO$_2$ on SCF.

Our aims were (i) to characterize the effects of CO$_2$, N, and diversity on soil moisture and SCF and (ii) to investigate the relationship between treatment effects on soil moisture and on SCF. First, we hypothesized that elevated CO$_2$ would increase soil moisture (Reich, 2009) by decreasing stomatal conductance (Lee *et al.*, 2001, 2011) and therefore likely ET (Fig. 1a). Second, we hypothesized that soil water availability would be lower in enhanced N plots because of increased water demand associated with the larger above- and belowground biomass in enhanced vs. ambient N plots (Fig. 1a; Reich *et al.*, 2001a, 2006a, Reich, 2009). Similarly, we expected that the high biomass and spatial and temporal niche complementarity in diverse vs. monoculture plots would increase plant water use and decrease soil moisture (Fig. 1a). Third, in accordance with previous results, we hypothesized that SCF would be greater in elevated CO$_2$ plots (Appendix S2) because of both the increased availability of C associated with CO$_2$-driven increases in photosynthesis and belowground C inputs, and CO$_2$-induced increases in available soil water (Fig. 1a; Appendix S1). We expected that if elevated CO$_2$ did increase soil water in BioCON, at least a portion of the CO$_2$ effect on SCF would be associated with increased soil water availability (Fig. 1a). Fourth, we hypothesized that elevated N and diversity would increase SCF due to high standing root biomass (Reich *et al.*, 2001a, 2006a), photosynthate production (Lee *et al.*, 2001), and belowground C allocation (Adair *et al.*, 2009), all of which increase C availability for root and microbial respiration relative to ambient N and monoculture plots (Fig. 1a). However, if N and diversity decrease soil moisture, SCF may be slightly reduced by lower levels of soil moisture in high N or diverse plots (Fig. 1a). All of our hypotheses regarding the effects of CO_2, N, and diversity on SCF via their effects on soil moisture assume that increases in soil moisture will positively affect SCF (Fig. 1a). Finally, because BioCON communities are N-limited (Reich *et al.*, 2001a) and adding N increased the positive effect of CO_2 on total biomass over time in BioCON (Reich *et al.*, 2006a,b), we hypothesized that SCF would respond more strongly to elevated CO_2 with added N.

Materials and methods

Design and measurements

Our experiment was conducted within the BioCON FACE experiment. The BioCON experiment is located in an old-field grassland on a nutrient poor, sandy outwash plain in the Long-Term Ecological Research site at the Cedar Creek Ecosystem Science Reserve in Minnesota, USA (Latitude: 45° N, Longitude: 93° W). BioCON Soils (Argic Udipsamments) are homogenous, sandy (93% sand) and poor in soil organic matter with low N content (Dijkstra *et al.*, 2006). Surface soils (0–23 cm) have a field capacity of 11.5% and wilting point of 3.6% [volumetric soil water content (VSWC); Grigal *et al.*, 1974]. Mean annual precipitation (1982–2009) is 800 mm, with ca. 60% falling from May through September (Fig. 2). Mean annual temperature (1982–2009) is 6.7 °C with a mean monthly temperature of −10 °C in January and 22 °C in July. We conducted this study from June to October of 2006, a growing season characterized by higher than average precipitation in April and August and much lower than average precipitation in May, June, and July (Fig. 2). Monthly temperatures were not substantially different than mean monthly temperatures from 1982 to 2005 except in July 2006, which was somewhat warmer than average (Fig. 2). Soil temperatures during July were also substantially higher than documented in previous years (Fig. S2b). Soil moisture was also low during this period, but was not out of the range of measurements taken in previous years (Fig. S1).

The BioCON experiment consists of 354 (2 × 2 m) plots evenly distributed among six 20 m diameter circular areas

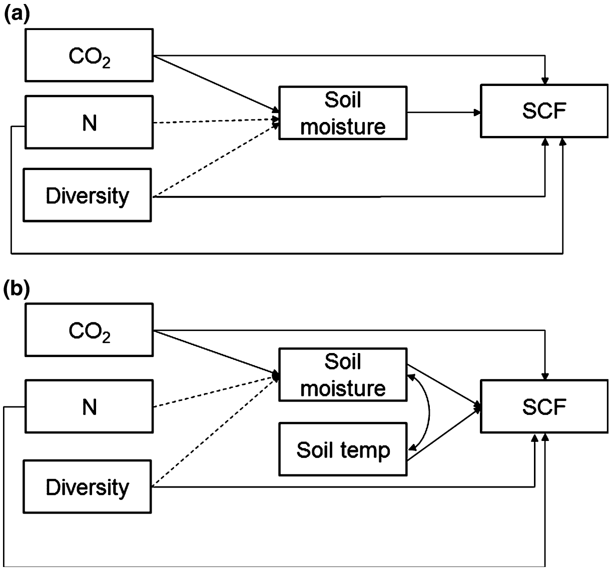

Fig. 1 (a) Conceptual diagram for hypotheses regarding how elevated CO_2, added nitrogen (N), and diversity affect soil carbon flux (SCF) and soil moisture. (b) The model used in the path analysis, which accounts for the effect of soil temperature (Soil temp) on SCF and correlation between soil moisture and soil temperature. Dashed lines indicate hypothesized negative relationships. Solid lines indicate hypothesized positive relationships. Here, we assume that the 'direct' treatment effects (unmediated by soil moisture) on SCF are due to increases in plant- and microbe-available carbon.

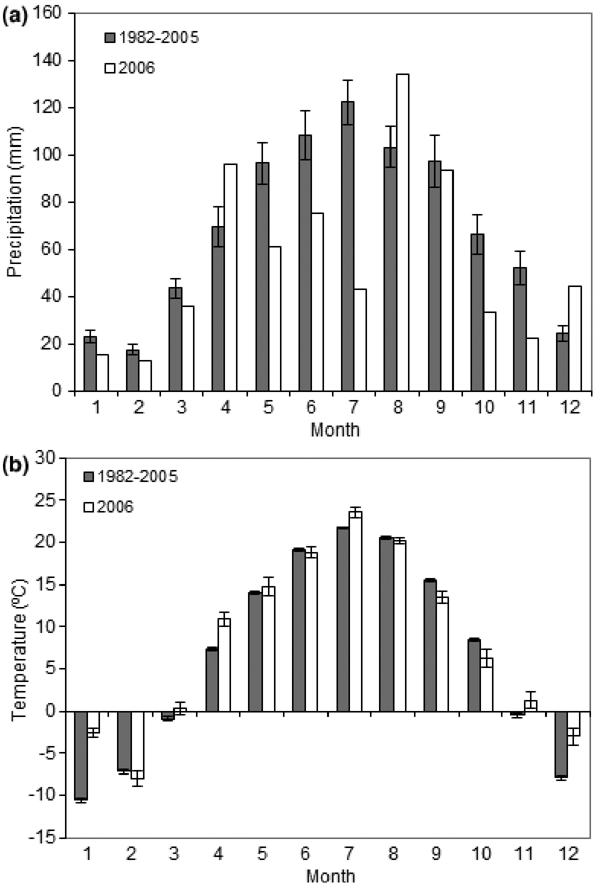

Fig. 2 Average monthly (a) precipitation and (b) temperature for 1982–2005 compared with (a) monthly precipitation and (b) mean monthly temperature in 2006. Error bars are ±1 SE.

(rings). In 1997, these plots were seeded with 1, 4, 9, or 16 species randomly chosen from 16 grassland species in four functional groups (C_3 and C_4 perennial grasses, forbs, and legumes), at a rate of 12 g m^{-2} (divided equally among species in a plot). Since 1998, atmospheric CO_2 concentrations in three rings have been elevated by 180 ppm CO_2 above ambient during each day of the growing season. The remaining three rings have been maintained at ambient CO_2 levels. Half of all plots received 4 g N m^{-2} yr^{-1}, a deposition rate similar to heavily industrialized areas (Vitousek, 1994) and representing roughly a doubling of N availability. BioCON is a split-plot arrangement of treatments in a completely randomized design; CO_2 treatment is the whole-plot factor (three ambient rings and three elevated rings) and the subplot treatments of diversity and N were randomly distributed and replicated in individual plots among the six rings. To mimic presettlement fire frequencies in tall grass prairies, all plots were burned in the early spring in roughly half of the years of this study period (2000, 2002, 2003, and 2005; see Adair *et al.*, 2009 for more experimental detail).

In this study, we used a subset of the BioCON plots: the 16 species plots and the 1 species (monoculture) plots for the perennial C_4 grasses *Andropogon gerardii* and *Bouteloua gracilis*, the perennial C_3 grass *Agropyron repens*, and the nonleguminous forb *Solidago rigida*. These monoculture plots were chosen because they represent a wide range of maximum rooting depths (30–150 g root biomass m^{-2} at a depth of 40–100 cm) and include three functional groups. Across all six rings, we sampled all replicates for each monoculture species (two for each of the four CO_2 by N treatment combinations or 32 plots per sampling) and 9 of the 12 replicates (randomly chosen; three of the four in each ring) per CO_2 by N treatment combination for the 16 species plots (36 plots per sampling), for a total of 68 plots per sampling. We used a subset of plots because measuring SCF and soil moisture in all plots would have required us to measure for multiple days, rather than allowing us to capture both measurements for all plots on a daily basis. We chose to measure the 16 and one species plots to capture the largest possible difference in belowground root biomass.

We measured SCF, soil temperature and soil moisture on 14 days during 2006 (June 1, 26, 30; July 6, 13, 15, 18, 21, 27; August 3, 8, 29; September 25; and October 19). Soil temperature and SCF were measured using a LI-COR 6200 gas exchange system with a LI-COR 6400-09 soil respiration chamber and soil temperature probe (LI-COR, Lincoln, NE, USA; details in Craine *et al.*, 1998, 2001a,b). We used these measurements to estimate daily rates of SCF (where 1 μmol CO_2 m^{-2} s^{-1} is equivalent to 1.04 g C m^{-2} day^{-1}, as in Craine *et al.*, 2001a,b).

Soil moisture was measured at four 17 cm depth increments (0–17, 22–39, 42–59, 83–100 cm) using a Trime FM3 Time Domain Reflectometry system, version P3 with T3 tube-access probe (IMKO Micromodultechnik GmbH, http://www.mesasystemsco.com/product.php?p=8). Use of this probe required the installation of one permanent 5 cm diameter schedule 40 PVC access tube in the soil profile of each plot. Tubes were installed during the summer of 2001. Prior to each measurement, the tube's interior surface was swabbed to remove excess moisture from condensation. The instrument's VSWC output was calibrated to soil-specific VSWC (cm^3 cm^{-3} or %) using VSWC measurements calculated from coincident gravimetric soil moisture and bulk density measurements. The calibrated VSWC values were used in all calculations and data analyses. Total soil water storage (TWS, in cm) from 0 to 100 cm was calculated as the sum of the VSWC for each layer multiplied by the vertical depth increment it represents; the vertical depth increment includes the measured 17 cm and extends to the midpoints between measurements (i.e., % VSWC for 0–17 cm × 19.5 cm + % VSWC for 22–39 × 21 cm + % VSWC for 42–59 cm × 30.5 cm + % VSWC for 83–100 cm × 29 cm).

As a result of our frequent measurements and erratic rainfall (i.e., dry and wet months; Fig. 2), we captured the full range of soil moisture from field capacity to below wilting point (Figs 3 and 4; Fig. S1; averaged across all plots, surface soil VSWC was below wilting point on 4 days and ≥field capacity on 5 days). The dry weather in June and July allowed us to fully capture one postrainfall, soil dry-down event (June 30–July 18) during which rainfall was minimal (ca. 4 mm total) and surface VSWC (0–17 cm) declined from an average of 7.1% on June 26 to 2.8% on July 18 (Figs 3 and 4). We took five SCF-soil water measurements during this dry-down event. All remaining postrainfall measurements were closely

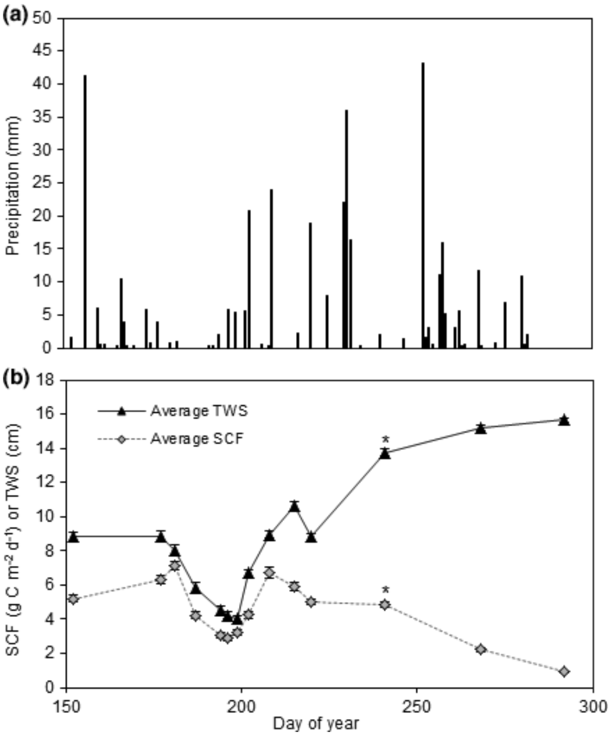

Fig. 3 (a) Daily precipitation, (b) soil carbon flux (SCF) and total water storage (TWS, 0–100 cm) averaged across all treatments from June to October, 2006 (days 152–273). Asterisks indicate the last sampling date of the growing season (29 August 2006). Error bars are ±1 SE.

followed by new rainfall events; from July 18 through the end of August, there were not seven consecutive days with less than 6 mm of rainfall (Fig. 3).

Data analyses

We used two approaches to test our hypotheses regarding the effects of elevated CO_2, diversity and added N on SCF. We note that although our experiment and hypotheses did not specifically address the effect of soil temperature on SCF and soil moisture, we included soil temperature as a covariate in both approaches because it can have large effects on SCF and soil moisture.

The first approach examined our hypotheses for each date separately (within-day time scale; variation in SCF and soil moisture was primarily among plots rather than over time), while the second approach examined our hypotheses at a longer time scale – across the entire growing season (examining variation both among plots and over time). For the first approach, we used path analysis, a subset of structural equation modeling, to fit the model shown in Fig. 1b to the TWS and SCF data for each sampling or time point [Shipley, 2002; R 2.12.0, (R Development Core Team, 2011) laavan package (Rosseel, 2011)]. This model expands our original model (Fig. 1a) by incorporating the influence of soil temperature on soil moisture (via correlation with soil temperature) and SCF (a direct effect; Fig. 1b). Although path analysis allowed us to examine our hypotheses only on a within-day scale, it allowed us to directly test our hypotheses about the direct (via increased C availability) and indirect (via increased soil moisture) effects of

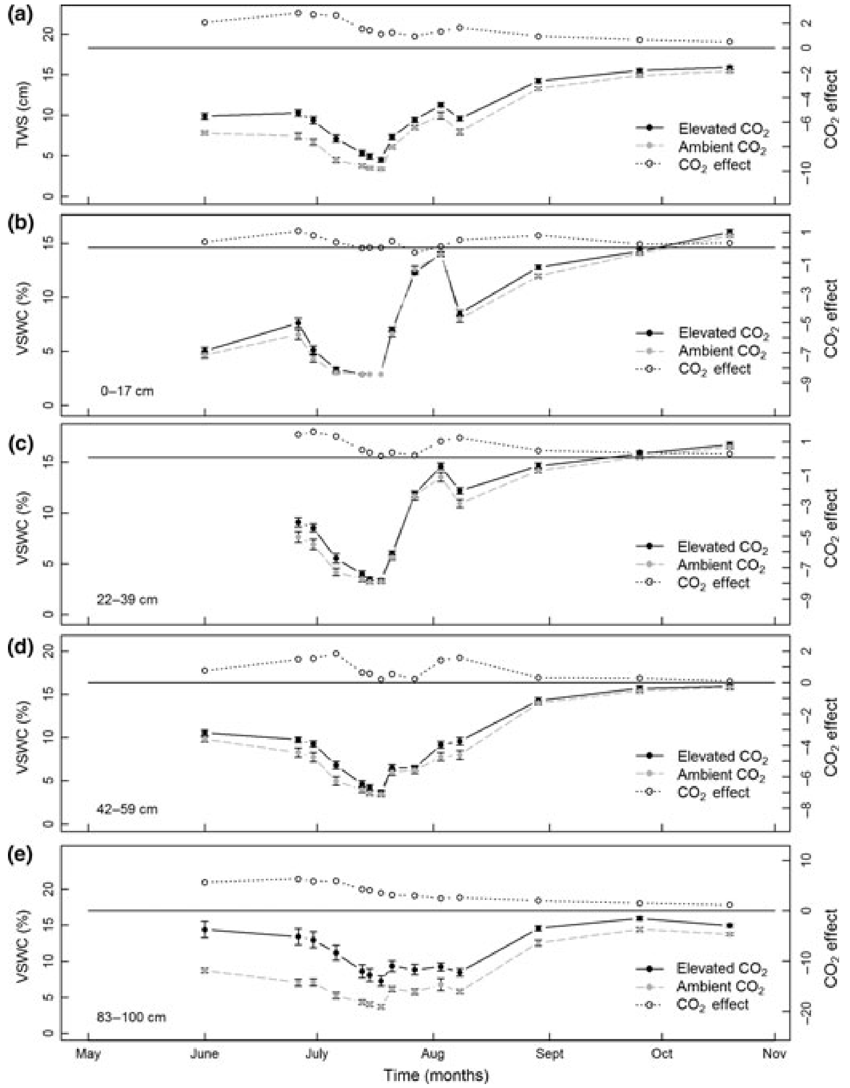

Fig. 4 (a) CO_2 effect on total water storage (TWS, 0–100 cm) and TWS in elevated and ambient CO_2 plots and CO_2 effect on volumetric soil water content (VSWC) and VSWC in elevated and ambient CO_2 plots for (b) 0–17 cm, (c) 22–39 cm, (d) 42–59 cm and (e) 83–100 cm. Absolute CO_2 effects were calculated as the mean TWS or VSWC value of elevated CO_2 plots minus the mean TWS or VSWC value of ambient CO_2 plots. The solid black line without symbols shows where the CO_2 effect is zero. Values less than zero indicate negative effects, whereas values greater than zero indicate positive effects. Error bars are ±1 SE.

Table 1 Results from the repeated-measures (day, D) ANCOVAS for June–October and June–August (growing season) with main effects of CO_2, diversity and nitrogen (N), with ring nested within CO_2 treatment and soil temperature (Soil T) as a covariate on volumetric soil water content (VSWC) at each depth, total water storage in the profile (TWS) and soil carbon flux (SCF)

	June–October						June–August					
Variable	0–17 cm	22–39 cm	42–59 cm	83–100 cm	TWS (0–100 cm)	SCF	0–17 cm	22–39 cm	42–59 cm	83–100 cm	TWS (0–100 cm)	SCF
CO_2	0.584	2.138	3.079	4.017	**15.059**	**18.252**	0.613	2.888	4.768	3.437	**12.0826**	**19.201**
N	1.633	0.990	2.040	0.252	0.509	0.305	1.517	1.287	2.266	0.091	0.639	0.207
CO_2 × N	0.341	1.490	0.018	0.173	0.256	0.541	0.459	1.083	0.006	0.082	0.1555	0.460
D	**784.321**	**680.744**	**285.497**	**56.355**	**368.620**	**146.645**	**823.567**	**707.996**	**270.419**	**60.058**	**440.242**	**137.758**
D × CO_2	**1.975**	**3.332**	**4.677**	**9.721**	**8.626**	**5.968**	**2.156**	**3.263**	**4.163**	**8.987**	**7.8611**	**2.158**
D × N	1.511	**2.000**	0.885	1.005	0.500	1.674	1.650	**2.221**	1.000	1.232	0.7006	1.794
D × CO_2 × N	0.246	0.590	0.686	0.440	0.550	1.618	0.232	0.520	0.660	0.461	0.598	**1.870***
Diversity	0.705	2.462	**7.317**	**4.258**	**3.934**	0.614	0.457	3.081	**8.837**	**5.843**	**5.7**	0.426
D × Diversity	**3.559**	**2.658**	**7.038**	1.576	**4.708**	1.001	**3.741**	**2.416**	**5.901**	1.625	**4.8093**	1.118
CO_2 × Diversity	0.208	0.024	0.093	0.599	0.422	0.178	0.253	0.019	0.099	0.816	0.4907	0.215
D × CO_2 × Diversity	0.318	0.293	1.018	1.459	1.054	0.537	0.328	0.312	1.139	1.251	1.1752	0.632
N × Diversity	0.120	1.530	2.349	1.207	1.731	1.537	0.345	1.848	2.775	1.611	2.1416	1.835
D × N × Diversity	0.641	**2.535**	**1.849** ×	1.241	**2.334**	1.437	0.536	**2.362**	1.425	1.006	**1.9835**	1.235
CO_2 × N × Diversity	0.154	0.174	0.194	1.432	0.338	0.059	0.230	0.212	0.309	1.151	0.2926	0.055
D × CO_2 × N × Diversity	0.318	0.501	0.908	0.573	0.689	0.577	0.257	0.530	0.963	0.757	0.9333	0.647
Soil T	1.560	**10.806**	**16.590**	**28.752**	**25.624**	**83.904**	0.290	**5.975**	**8.648**	**17.222**	**12.101**	**102.794**
r^2	0.9645	0.9645	0.9512	0.8506	0.9586	0.8708	0.9489	0.9517	0.9254	0.8684	0.9670	0.8458

Bold values indicate significant ($P < 0.05$) F ratios. One outlier was excluded from the TWS ANCOVAS.

*Significant treatment differences between the two ANCOVAS.

treatments on SCF (Fig. 1). For this analysis, we assume that the 'direct' treatment effects on SCF are due to concurrent increases in available C. We used TWS (0–100 cm) as the measure of soil moisture, as it incorporated data from all four soil layers and allowed us to keep the number of analyses manageable (vs. running separate analyses for each of the five soil moisture variables at each time point). Because we had *a priori* hypotheses regarding all pathways in the model and wanted to account for the expected effects of soil temperature on soil moisture and SCF (Fig. 1), we only fit the full (saturated) model to the data from each sampling date. This allowed us to estimate the size of the 'direct' (C effect) and 'indirect' (via soil moisture; the water effect) effects of each treatment on SCF on a within-day or spatial (across plots) basis.

Our second approach tested our hypotheses regarding the effects of elevated CO_2, diversity, and added N on VSWC, TWS, and SCF across the growing season, while accounting for the effects of soil temperature on soil moisture and SCF. We used repeated-measures ANCOVA with ring (the whole-plot) nested within CO_2 treatment (the whole-plot treatment), within-ring plots nested within diversity, CO_2, and N levels as random effects and soil temperature as a covariate (JMP 5.0.1; SAS Institute, Cary, NC, USA). All treatments were considered fixed effects. This ANCOVA was conducted for SCF (g C m^{-2} day^{-1}), TWS (cm; 0–100 cm), and % VSWC at each depth. We expected that the effect of CO_2 on soil moisture would only be present during times of plant activity. Thus, we performed the ANCOVA first for all dates (June–October) and second for only the growing season (June–August). For the June–October ANCOVA, the model failed to converge unless we excluded one outlier (a very high TWS value on August 3). This value was therefore excluded from both ANCOVAS (excluding this value did not change the results of the growing season ANCOVA). We also calculated the CO_2, N, and diversity effects as the mean of elevated CO_2, added N, or 16 species plots minus the mean of ambient CO_2, ambient N, or one species plots, respectively.

This second approach allowed us to examine the direct effects of each treatment on SCF and soil moisture across the growing season, but did not test our hypotheses about indirect treatment effects on SCF via soil moisture. We therefore carried out several analyses to test these hypotheses across the growing season. If a treatment significantly affected soil moisture and SCF in the ANCOVAS, we then examined the relationship between (i) the treatment effect on SCF and soil moisture measurements and (ii) the treatment effect on SCF and the treatment effect on soil moisture. For both relationships, we compared the fit of linear vs. nonlinear (exponential and polynomial) models using Akaike's Information Criterion modified for small sample sizes (AICc; R version 2.8.1). Because the CO_2 effect on VSWC and TWS was negligible after the growing season (when plants were inactive; see Results), we examined these relationships only during the growing season (results for June–October data are presented in Appendix S3).

To estimate the size of the 'indirect' effect of treatments on SCF via soil moisture across the growing season, we used the following procedure. If a treatment significantly affected SCF in the ANCOVA, we fit four models that predicted SCF as a function of VSWC and TWS: linear and polynomial models fit to all data (i.e., one curve for all data) and to the data by treatment (e.g., one curve for elevated CO_2 and one curve for ambient CO_2). We used AICc to choose the model that best fit the data (R version 2.8.1). This allowed us to determine if these treatments altered how SCF responded to changes in soil moisture, that is, if the models fit to the data by treatment significantly improved the model fit to all data. We then used the best model to estimate the portion of the CO_2 effect on SCF that was associated with increased C inputs (the C effect), increased soil moisture (the soil moisture effect) or both. For example, the model that best predicted the relationship between SCF and TWS fit curves to ambient and elevated CO_2 data separately, rather than to all data (see Results). We then estimated effect sizes by combining the elevated or ambient CO_2 model with the ambient or elevated TWS values to generate predictions: the C effect was estimated using the elevated CO_2 model with ambient CO_2 TWS values; the water effect was estimated using the ambient CO_2 model with elevated TWS values; the combined C and water effect was estimated using the elevated CO_2 model with elevated TWS values. We used the predictions from these scenarios to estimate cumulative growing season SCF for each effect by multiplying a plot's predicted daily SCF for two consecutive measurements by the number of intervening days and adding this value to the previously calculated cumulative SCF. We also used this method to estimate growing season SCF in ambient plots (ambient CO_2 equation and ambient TWS values). For these calculations, missing values were replaced with the CO_2 treatment average for the sampling period. Although we fit models to all data (June–October) and growing season data (June–August),

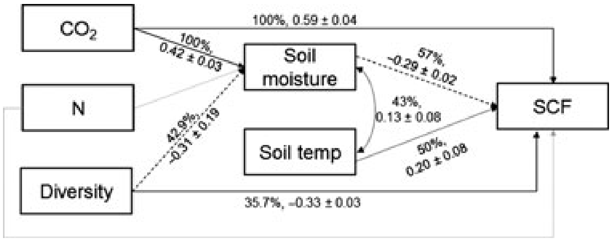

Fig. 5 Percentage of dates each direct effect (pathway) was significant and the average standardized path coefficient ±1 SE for each pathway on those dates. Solid black lines indicate direct positive relationships. Dashed black lines indicate direct negative relationships. Dotted black lines indicate direct relationships that were positive and negative, depending on date. Gray lines indicate direct pathways that were not significant on any date (0% of all dates). Note that while the average effect of soil temperature (Soil temp) on soil carbon flux (SCF) and soil moisture was positive, these effects were both negative on one date. Indirect effects on SCF via soil moisture are not explicitly shown in the figure: elevated CO_2 had a negative indirect effect on SCF on 57% of all dates (average path coefficient = −0.189 ± 0.02); diversity had a positive indirect effect on SCF on 21% of all dates (average path coefficient = −0.082 ± 0.02).

we focus on the June–August results because the CO_2 effect on soil moisture was negligible after the growing season (see Results; model comparison results for all data are presented in Tables S4 and S5, Fig. S6).

Although there was a significant CO_2 by day by N interaction in the growing season ANCOVA (June–August), adding N did not change the overall direction of the CO_2 effect and had only a small impact on the overall size of the CO_2 effect (see Results and Table 1). Additionally, this interaction was not significant in the June–October ANCOVA or in a similar ANCOVA run for all BioCON SCF sampling points (1998–2006; Appendix S2). We therefore examined the indirect effect of CO_2 on SCF via soil moisture across the growing season (as described earlier) averaged across N treatments.

While belowground biomass increases with diversity, elevated CO_2, and added N in the BioCON experiment (Reich *et al.*, 2001a, 2006b; unpublished 1999–2006 data), we found no significant relationships between plotlevel average above- or belowground biomass and average growing season SCF, TWS, or VSWC (at any depth). We therefore do not present these results.

Results

Direct treatment effects on soil moisture

As hypothesized, elevated CO_2 increased VSWC by 23% on average (113% maximum) and TWS by 53% on average (182% maximum; Figs 4 and 5). Across the growing season, elevated CO_2 increased TWS and VSWC at all depths, but the size of the increase varied with day and was related to ambient moisture conditions (repeated-measures ANCOVA CO_2 by Day interaction; Table 1, Fig. 4). In general, the CO_2 effect declined at very low or high VSWC and with declines in plant activity at the end of the growing season (post-August; Fig. 4). During the dry-down period in June through mid-July, the CO_2 effect was larger in the deeper soil layers that retained more soil moisture than in the shallow layers. The CO_2 effect also remained larger in the same deep layers during wet-up in late July while they continued to experience deficits below field capacity (and as shallow layers were rehydrated). For the entire soil profile, the CO_2 effect was largest from June 1 to mid-July when TWS tended to be low but not totally used up. This result was confirmed by the path analysis results, which found larger CO_2 effects on TWS from June through mid-July than later in the growing season (Table 2). As expected, during the post-rainfall event dry-down period from June 30 to July 18 (Julian days 177–199), soils at 0–17 through 42–59 cm depths stayed moist longer in elevated than ambient CO_2 plots (Fig. 4b–d). Both the ANCOVA and path analysis results indicated a consistent positive effect of CO_2 on soil moisture (Figs 4 and 5; Tables 1 and 2).

Diversity (Fig. 6) had variable, but largely negative, effects on growing season soil moisture, decreasing VSWC by a maximum of 42% (mean of 7%; ANCOVA results; Table 1). During the growing season (prior to September), diversity slightly increased VSWC in surface soils when soil moisture was relatively high, but decreased VSWC at all other soil depths (Fig. 6). After the growing season, diversity had little or no effect on VSWC (Day by Diversity interaction; Table 1, Fig. 6). Path analysis results also indicated that, when significant, the within-day effect of diversity on TWS was negative, with no effect of diversity on TWS after the growing season (Fig. 5; Table 2).

In contrast to our expectations, N had no effect on VSWC in shallow and deep soils. However, at intermediate soil depths and for TWS in the soil profile, N reduced or reversed the negative effect of diversity on VSWC, but only during the growing season (N by Diversity by Day interaction; Table 1; Fig. 6c–e). After the growing season, neither N nor diversity substantially altered VSWC or TWS (Table 2; Fig. 6). Path analysis found no significant within-day effects of N on soil moisture, likely due to inability of this method to detect interactions (Fig. 5; Table 2).

Across the growing season, TWS and VSWC at all depths but 0–17 cm increased very slightly with soil temperature (Table 1). Path analysis found that soil temperature and TWS were moderately correlated on five dates, but the direction of the relationship varied depending on date (Table 2).

Overall, the results of the June–August and June–October soil moisture ANCOVAs were nearly identical. The sole difference was the loss of one significant effect in the June–August (growing season) ANCOVA (the Day by N by Diversity interaction; Table 1).

Direct treatment effects on SCF

As expected, elevated CO_2 increased SCF by an average of 45% (Table 1, Fig. 7a and c). While elevated CO_2 always increased SCF, the size of the increase was variable depending on day and, during the growing season, N treatment (repeated-measures ANCOVA results; CO_2 by Day and CO_2 by Day by N interactions; Table 1, Fig. 7a and c). During times when water was abundant, adding N increased the size of the CO_2 effect; when soils were drier, adding N slightly reduced or had no effect on the size of the CO_2 effect (Fig. 7c). Overall, the effect of adding N was only slightly positive, increasing the size of the CO_2 effect by only 0.2 g C m^{-2} yr^{-1}. The positive effect of CO_2 on SCF was also seen in the path analysis, where the within-day effect of CO_2 on SCF was consistently positive, but varied in size from small (path coefficient ~ 0.3) to large (path coefficient ~ 0.8; Fig. 5, Table 2).

In contrast to our expectations, increasing diversity from one to 16 species did not significantly affect SCF across the growing season (ANCOVA results; Table 1). However, path analysis indicated that, when analyzed for each date separately, diversity often decreased SCF (Fig. 5, Table 2). Five of the six dates where high diversity reduced SCF were during the dry-down period (July 6–18). The remaining date was during a period of moderate soil moisture (August 8).

The size of the relatively small N effect varied by day (Day by N interaction in June–October ANCOVA), but also varied by CO_2 treatment during the growing season (CO_2 by Day by N interaction; Table 1, Fig. 7a and b). During the growing season, the N effect in the elevated CO_2 plots was largely positive but small, increasing SCF by only 3% on average (Fig. 7b). In ambient CO_2 plots, adding N had little to no effect on SCF (decreasing SCF by 1% on average; Fig. 7b). Averaged across CO_2 treatments, adding N increased SCF by 2%. Analyzing each date separately, path analysis indicated that N had no significant effects on SCF (Fig. 5, Table 2).

As expected, temperature increased SCF across the growing season (Table 1). Path analysis indicated that temperature had variable effects on SCF, depending on day (Table 2, Fig. 5). During periods of high or moderate soil moisture, temperature had either positive or neutral effects on SCF (Table 2). During the dry-down period (July 6–18) increasing soil temperature had either neutral or negative effects on SCF (Table 2).

Direct effect of soil moisture on SCF

Consistent with our hypothesis about the sensitivity of SCF to soil moisture, rates of SCF were clearly influenced

Table 2 Standardized path coefficients from path analyses on each date separately for the direct and/or indirect effects of nitrogen (N), diversity (Div), CO_2 and soil temperature (Soil T) on total water storage in the profile (0–100 cm, TWS) and soil carbon flux (SCF)

	Effects on TWS		Effects on SCF									
Date	Direct CO_2	Direct Div	Direct N	Direct CO_2	Direct Div	Direct N	TWS	Soil T	Indirect CO_2	Indirect Div	Indirect N	Correlation TWS–Soil T
6/1/2006	0.513	0	0	0.629	0	0	−0.306	0	−0.192	0	0	0
6/26/2006	0.589	0	0	0.724	0	0	−0.393	0.315	−0.285	0	0	0.249
6/30/2006	0.544	0	0	0.643	0	0	0	0	0	0	0	0
7/6/2006	0.558	−0.268	0	0.461	−0.432	0	0	−0.345	0	0	0	0.203
7/13/2006	0.446	−0.352	0	0.719	−0.283	0	−0.251	0	−0.180	0.071	0	0.169
7/15/2006	0.453	−0.319	0	0.756	−0.306	0	0	0	0	0	0	0
7/18/2006	0.417	−0.302	0	0.815	−0.378	0	−0.316	0	−0.258	0.119	0	0
7/21/2006	0.385	0	0	0.552	0	0	−0.267	0.264	−0.147	0	0	0.241
7/27/2006	0.304	0	0	0.587	0	0	−0.288	0.249	−0.169	0	0	−0.273
8/3/2006	0.332	−0.256	0	0.483	0	0	−0.288	0.397	−0.139	0	0	0.205
8/8/2006	0.434	−0.376	0	0.684	−0.263	0	−0.209	0	−0.143	0.055	0	0
8/29/2006	0.346	0	0	0.489	0	0	0	0.284	0	0	0	0
9/25/2006	0.278	0	0	0.453	0	0	0	0.242	0	0	0	0
10/19/2006	0.250	0	0	0.289	0	0	0	0	0	0	0	0

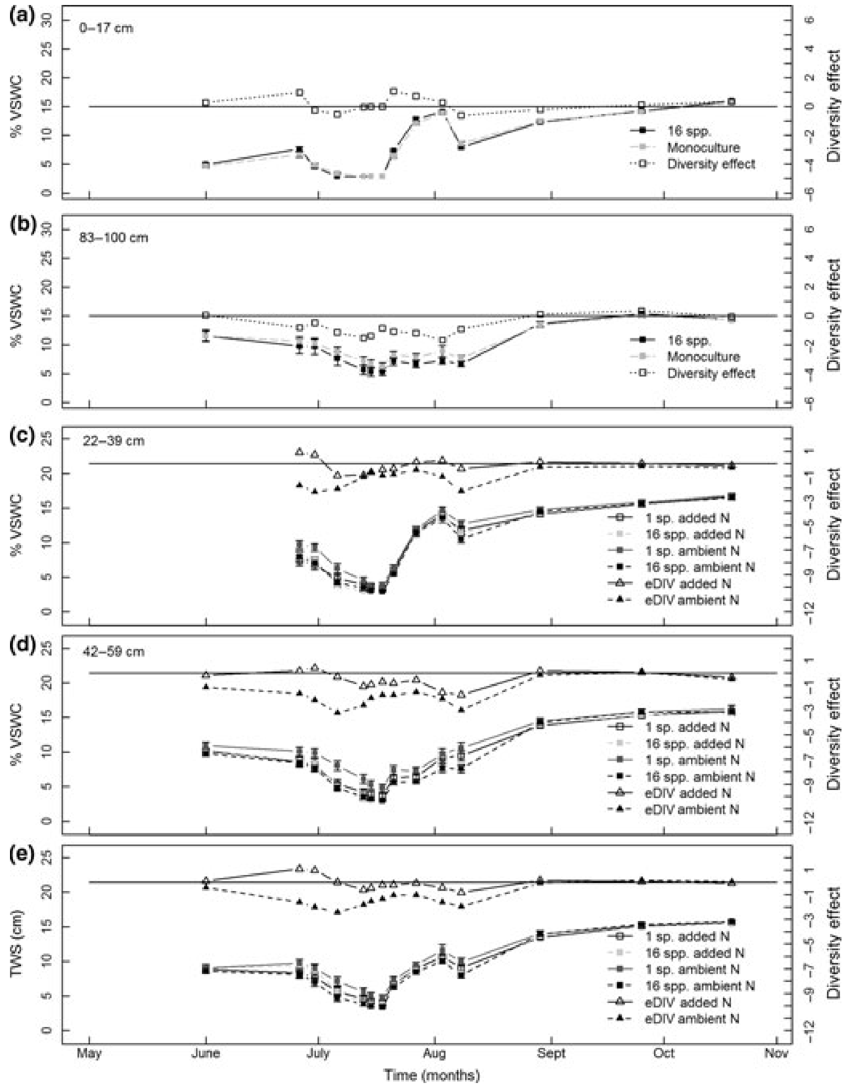

Fig. 6 Absolute diversity (eDIV) effects on volumetric soil water content (VSWC) and %VSWC in one and 16 species plots for (a) 0–17 cm and (b) 83–100 cm. Percent VSWC by diversity and N treatment and eDIV by N treatment on VSWC for (c) 22–39 cm and (d) 42–59 cm soil depths and on (e) total water storage in the soil profile (TWS, 0–100 cm). eDIV was calculated as the mean VSWC or TWS value for 16 species plots minus the mean VSWC or TWS value for one species plots (see Fig. 3 for abbreviations and further detail). The solid black line without symbols shows where eDIV is zero. Error bars are ±1 SE.

by precipitation and soil water content (Fig. 3). SCF in all treatments paralleled TWS from late June to early August (Fig. 3). Across the entire growing season, SCF tended to increase with VSWC and TWS at low to moderate soil moisture levels, but SCF decreased with VSWC and TWS at high soil moisture levels (significant polynomial relationships between SCF and VSWC at all depths except 83–100 cm and TWS; $P < 0.0001$; data not shown).

However, in contrast to our expectations, when each date was analyzed separately, path analysis indicated that plots with high TWS had either slightly lower or the same SCF as plots with low TWS (depending on day; Fig. 5; Table 2). The negative within-day relationship between TWS and SCF occurred at a wide range of soil moisture values (average TWS ranging from 4 to 11 cm; average 0–17 cm VSWC from 2.8% to 14%). Path analysis estimates the relationship between TWS and SCF after accounting for the effects of CO_2, N, and diversity, and soil temperature on both of these factors. Thus, while linear regressions of within-day SCF vs. TWS revealed positive or neutral relationships, linear regressions of the residuals from regressions of TWS and SCF on CO_2, N, diversity, and soil temperature confirmed that, after accounting for treatment effects, the within-day relationships between TWS and SCF were negative or neutral (Table 3).

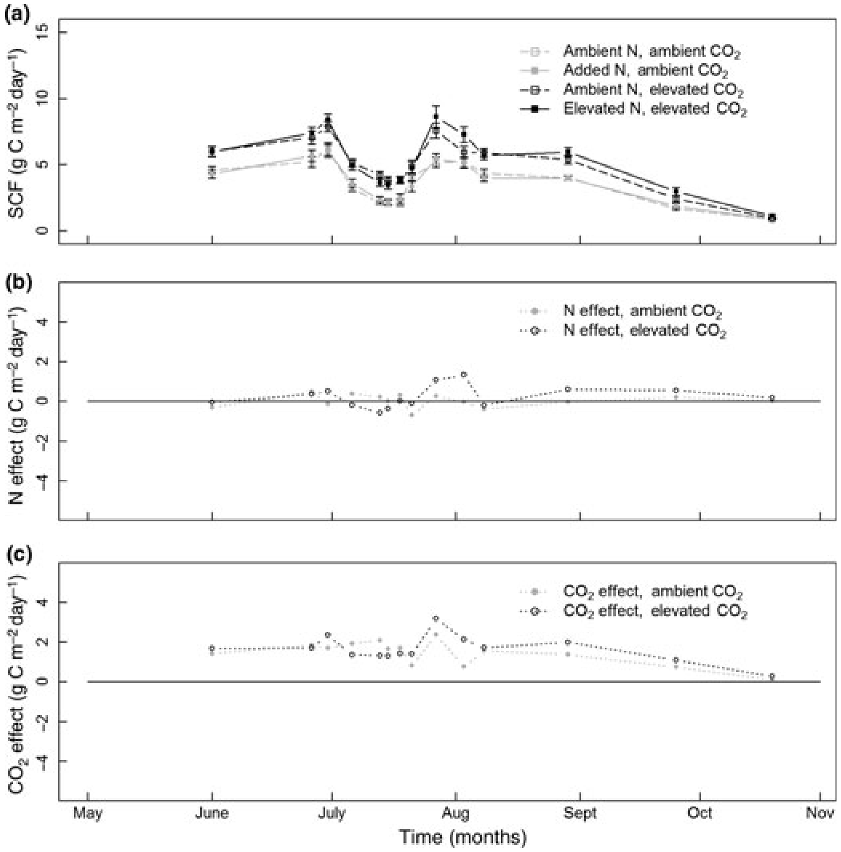

Fig. 7 Average soil carbon flux (SCF) by (a) CO_2 and N treatment and absolute (b) N and (c) CO_2 effects on SCF by N treatment. Absolute CO_2 and N effects were calculated as the mean SCF value for elevated CO_2 or N plots (by N or CO_2 treatment) minus the mean SCF value for ambient CO_2 or N treatments (by N or CO_2 treatment). In panels b and c, the solid black line without symbols indicates where the CO_2 or N effect is zero. Error bars are ±1 SE.

Indirect treatment effects on SCF via soil moisture

Whether examined across the growing season or for each date separately, CO_2-induced increases in soil moisture were unrelated to the positive response of SCF to elevated CO_2. Across the growing season, the CO_2 effect on SCF was relatively consistent and largely unrelated to changes in soil moisture (Fig. 7a and c).

Indeed, across the growing season, there were no significant correlations between the CO_2 effect on SCF and the CO_2 effects on TWS or VSWC at any depth (removing one outlier point from the analysis did not change these results; data not shown). Although the growing season relationships between the CO_2 effect on SCF and the CO_2 effects on all soil moisture measurements were best fit by linear functions (AICc of all other models were ≥ 4 AICc points away from those of the best models), these models explained only 1–10% of the variation in the data and none of the models were statistically significant ($P > 0.05$), indicating that the size of the CO_2 effect on soil moisture was unrelated to the size of the CO_2 effect on SCF.

Elevated CO_2 generally increased SCF at any given soil moisture level (TWS and VSWC at all depths), indicating that CO_2 has substantial effects on SCF apart from increasing soil moisture (Fig. 8). At all values of VSWC, elevated CO_2 increased SCF relative to ambient CO_2 plots (Fig. 8). Regardless of time frame (June–October vs. June–August), the best model for describing the relationship between SCF and any soil moisture variable was a polynomial model with curves fit separately to elevated and ambient CO_2 data (Tables 4 and 5, Fig. 8; see Tables S4 and S5 and Fig. S6 for all data). At all depths except 83–100 cm, elevated CO_2 did not change the shape of the relationship, a convex shape that was driven by reduced SCF at both low and high VSWC. The downward trend at high soil moistures was stronger across all measurements than during the growing season due to the postsenescence coincidence of high soil moistures and low SCF (i.e., September–October; Table S5, Fig. S6). For deep soils (83–100 cm) in the elevated CO_2 plots, the shape of this curve was reversed by the large number of within-growing-season co-occurring high SCF and VSWC values (Fig. 8d).

Table 3 Results from linear regressions of total water storage in the soil profile (TWS, 0–100 cm) vs. soil carbon flux (SCF) and linear regressions of the residuals from the regression of TWS on CO_2, nitrogen, diversity and soil temperature (TWS_{res}) vs. the residuals from the regression of SCF on CO_2, nitrogen, diversity and soil temperature (SCF_{res})

	TWS and SCF			TWS_{res} and SCF_{res}		
Date	*P*-value	R^2	Slope	*P*-value	R^2	Slope
6/1/2006	0.719	0.002	0.034	**0.010**	**0.095**	**−0.235**
6/26/2006	0.455	0.010	0.077	0.008	0.122	−0.299
6/30/2006	0.300	0.020	0.110	0.133	0.041	−0.164
7/6/2006	**0.018**	**0.101**	**0.188**	0.986	0.000	0.002
7/13/2006	0.199	0.028	0.121	**0.026**	**0.083**	**−0.180**
7/15/2006	0.099	0.046	0.165	0.056	0.062	−0.176
7/18/2006	0.411	0.012	0.100	**0.003**	**0.146**	**−0.291**
7/21/2006	0.890	0.000	−0.019	0.058	0.060	−0.277
7/27/2006	0.287	0.020	−0.236	**0.009**	**0.113**	**−0.487**
8/3/2006	0.955	0.000	−0.007	**0.014**	**0.091**	**−0.294**
8/8/2006	0.157	0.031	0.131	0.078	0.048	−0.155
8/29/2006	0.054	0.064	0.260	0.615	0.005	0.055
9/25/2006	0.062	0.059	0.207	0.438	0.010	0.077
10/19/2006	0.671	0.003	0.020	0.832	0.045	−0.009

Significant values are indicated in bold type ($P < 0.05$).

Using the growing season SCF-soil moisture models (Table 5, Fig. 8) to estimate the contributions of increased C inputs and elevated soil moisture to the CO_2 effect on SCF indicated that the majority of the CO_2 effect on SCF was due to increased C inputs rather than increased soil moisture (Fig. 9). Modeled cumulative soil efflux from June to August was 456, 466, 641, and 673 g C m^{-2} for (i) the baseline scenario without either CO_2 effect (ambient CO_2 equation using ambient CO_2 TWS), (ii) the effect of CO_2-induced increased soil moisture alone (ambient CO_2 equation using elevated CO_2 TWS), (iii) the effect of elevated C inputs alone (elevated CO2 equation using ambient CO2 TWS), and (iv) the combined effect of elevated CO_2 via increased C inputs and soil moisture (elevated CO_2 equation using elevated CO_2 TWS), respectively (Fig. 9).

Path analysis indicated that, when each date was considered separately, CO_2 had weak negative effects on SCF via soil moisture (average path coefficient = −0.19; Table 2). On eight sampling dates during the growing season, elevated CO_2 had a small negative effect on SCF by increasing TWS. The direct effect of CO_2 on SCF was three to five times greater than the indirect effect of CO_2 on SCF via soil moisture (Table 2). There was no correlation between the weak negative CO_2 effect on SCF via TWS and average daily TWS (data not shown), indicating that soil moisture status had no effect on the size of this negative CO_2 effect (via soil moisture).

Because diversity did not significantly affect SCF across the growing season, we did not examine relationships between the SCF and soil moisture diversity effects or SCF and soil moisture variables by diversity treatment. However, path analysis indicated that diversity had a very small indirect effect on SCF: on three dry days during the growing season, diversity weakly increased SCF by decreasing TWS (Table 2).

As with the CO_2 effect on SCF, the across-season N effect on SCF was unrelated to the N effect on TWS or VSWC at any soil depth ($r^2 < 0.01$–14.3%; $P > 0.2$; data not shown), indicating that adding N did not affect SCF via effects on soil moisture. The best model for describing the relationship between the N effect on SCF and soil moisture during the growing season was a linear model, but this positive relationship was only significant for the 0–17 cm depth ($P = 0.0496$; $r^2 = 0.33$). Additionally, examination of the relationships between SCF and soil moisture revealed that adding N did not substantially alter rates of SCF at any level (or depth) of VSWC, as the best model for each soil moisture variable used a single equation for all data (Table 4; see Table S4 for all data). Considering each date separately, N had neither direct nor indirect effects on TWS (Table 2).

Discussion

Elevated CO_2 increased SCF by between 21% and 77% on all sampling dates, and by 45% on average. On an absolute basis, the increase in SCF during the growing season was also large and variable (ranging from 1.1 to 2.8 g C m^{-2} day^{-1}). These large effects were likely primarily associated with CO_2-induced increases in C inputs that increased plant and microbial C availability. These increases are consistent with previous BioCON results, which found that elevated CO_2 increased microbial (West *et al.*, 2006), root and soil (Craine *et al.*, 2001b; Appendix S2) respiration. Elevated CO_2 also had a large, although temporally variable, effect on soil moisture (VSWC and TWS), increasing it by 0–113%; however, increased soil moisture could not account for the CO_2-induced stimulation of SCF. The effects of N additions and changes in diversity were much smaller: N and diversity decreased soil moisture by a maximum of 32% and 43%; diversity had either neutral or negative effects on SCF (decreasing SCF 1% on average, 25% at most); and N increased SCF by 3% on average (20% at most).

Across the growing season, SCF increased with soil temperature, but path analysis revealed that on a within-day scale the effect of temperature varied, becoming positive, negative, or neutral depending on

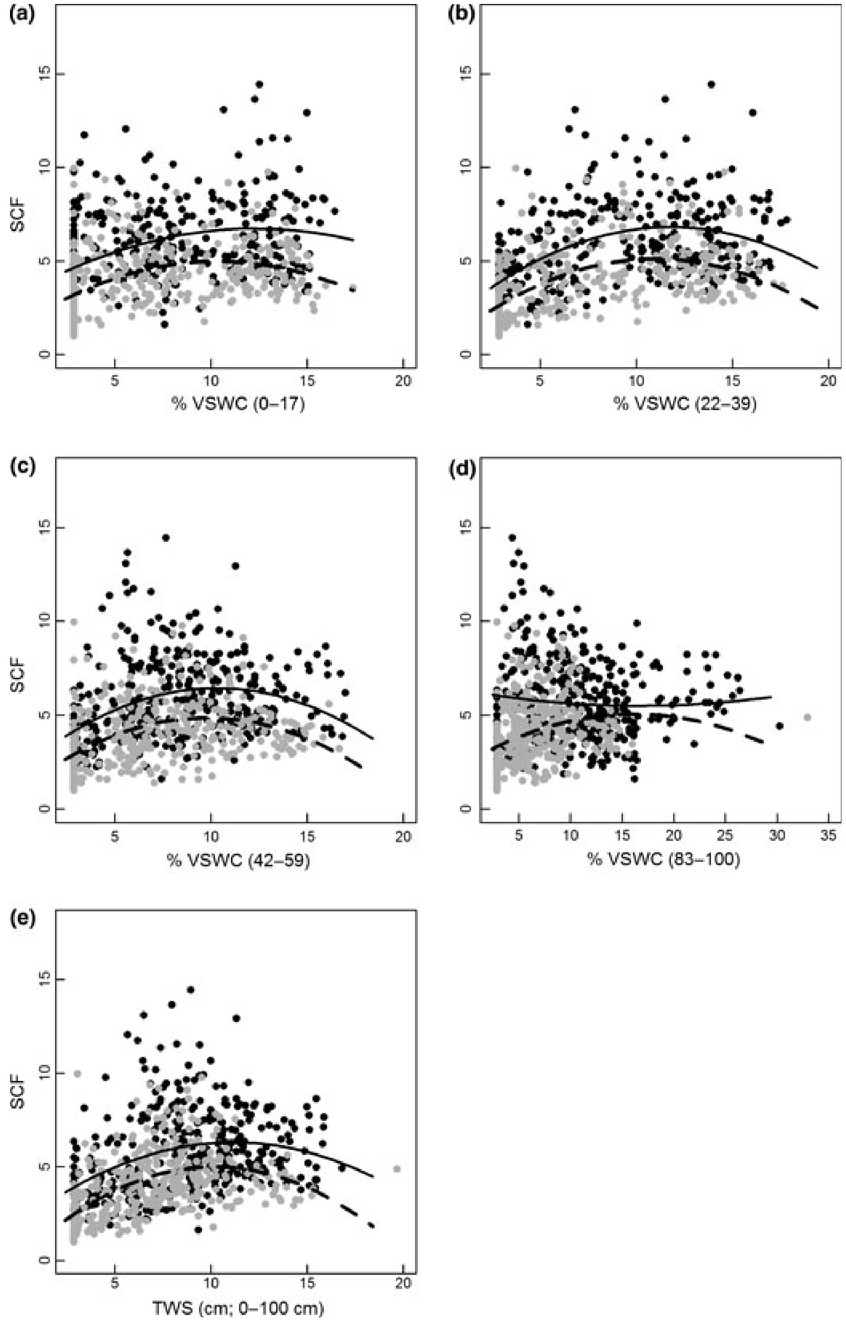

Fig. 8 Relationships between soil C flux (SCF; g C m^{-2} day^{-1}) and % volumetric soil water content (VSWC) for (a) 0–17cm, (b) 22–39 cm, (c) 42–59 cm, (d) 83–100 cm, and for (d) total water storage (TWS, in cm) during the growing season (June – August). Gray dots and dashed lines indicate ambient CO_2. Black dots and solid lines indicate elevated CO_2.

Table 4 Results of model comparison analysis using Akaike's Information Criterion, modified for small sample sizes (AICc) for soil carbon flux as a function of volumetric soil water content (VSWC) or total water storage (TWS) using growing season data (June–August)

Treatment	Soil moisture variable	r^2				AICc				Difference in AICc points			
		Linear	Polynomial	Linear by trt	Polynomial by trt	Linear	Polynomial	Linear by trt	Polynomial by trt	Linear	Polynomial	Linear by trt	Polynomial by trt
CO$_2$	0–17	0.1162	0.1474	0.2594	0.2886	3127.8	3105.4	3001.7	2980.3	147.5	125.1	21.4	0
	22–39	0.1772	0.2463	0.2979	0.3680	2783.4	2729.8	2683.2	2622.3	161.1	107.5	60.9	0
	42–59	0.0789	0.1574	0.2047	0.2746	3158.2	3096.7	3054.1	2994.6	163.6	102.1	59.6	0
	83–100	0.0370	0.0499	0.1690	0.1809	3190.9	3185.1	3086.5	3084.0	106.9	101.0	2.4	0
	TWS	0.1367	0.2032	0.2300	0.2915	3071.7	3017.6	2992.8	2940.7	131.0	76.9	52.1	0
N	0–17	0.1162	0.1474	0.1469	0.1498	3127.8	3105.4	3129.3	3111.4	22.4	0	23.9	6.1
	22–39	0.1772	0.2463	0.2112	0.2500	2783.4	2729.8	2785.2	2734.8	53.6	0	55.4	5.0
	42–59	0.0789	0.1574	0.1119	0.1607	3158.2	3096.7	3159.4	3102.0	61.4	0	62.7	5.3
	83–100	0.0370	0.0499	0.0752	0.0571	3190.9	3185.1	3190.6	3187.6	5.9	0	5.6	2.6
	TWS	0.1367	0.2032	0.1577	0.2049	3071.7	3017.6	3074.4	3024.3	54.1	0	56.8	6.7

Linear and polynomial models were fit to all data or by CO$_2$ and nitrogen (N) treatments (trt). Models with a difference in AICc points of ≥ 4 have considerably less support in the data relative to the best model (Burnham & Anderson, 2002).

day. When soil moisture was low, increasing soil temperature had negative or neutral effects on SCF, suggesting that SCF was limited by the availability of soil moisture. In contrast, during periods of sufficient soil moisture, increasing soil temperature had positive (suggesting that temperature was limiting) or neutral effects (suggesting neither soil moisture nor soil temperature was limiting). These results are consistent with recent work suggesting that the sensitivity of SCF to temperature varies with time and temporal scale and associated changes in drivers (Mahecha *et al.*, 2010).

Elevated CO$_2$

In contrast to our hypothesis, CO$_2$-induced increases in soil moisture contributed little to the positive effect of elevated CO$_2$ on SCF. Across the growing season, we found no relationships between the size of the CO$_2$ effects on SCF and soil moisture; the CO$_2$ effect on SCF did not decline with increasing soil moisture, indicating that elevated CO$_2$ did not affect SCF by increasing plant and microbial water availability during dry periods (Owensby *et al.*, 1999). Finally, we estimated that the across-growing season soil water effect accounted for only 6% of the CO$_2$ effect on SCF. Thus, our results suggest that increased water availability may be a weak driver of the CO$_2$ effect on SCF in this system.

The within-day path analysis results further supported the weak role of soil moisture in driving the CO$_2$ effect on SCF: the within-day effect of CO$_2$ on SCF via soil moisture was actually a small negative effect that was largely obscured by the strong direct positive effect of CO$_2$ on SCF via increased C availability. This negative effect was driven by the unexpected negative within-day relationship between TWS and SCF, a relationship that contrasts with the largely positive response of SCF to TWS across the growing season. This suggests that soil moisture and SCF may be responding to different drivers or processes at these different time scales. Within individual days, this relationship may reflect that both measurements were responding to plot-to-plot variation in total biomass. In other words, plots with high biomass tended to have both low soil moisture and high SCF due to greater rainfall interception and uptake of available soil moisture, which was then used to support plant activity. This relationship would be reinforced by elevated CO$_2$, which in BioCON has increased both above- and belowground biomass (Reich *et al.* 2006a, Reich, 2009). Alternatively, or complementarily, on a within-day scale, SCF measurements may be responding to the physical conditions created by high vs. low soil moisture. As soil moisture increases, the soil pore volume available for vapor transport is reduced, which can

Table 5 Parameters for the best model for describing the relationship between soil carbon flux (SCF) and each soil moisture variable using growing season data (June–August): % volumetric soil water content (VSWC) by depth (0–17, 22–39, 42–59 and 83–100 cm) and total water storage (TWS, 0–100 cm)

Soil moisture variable	Estimated parameters for the best model							
	Elevated CO_2				Ambient CO_2			
	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*
0–17	4.52	0.22	−0.02	−7.55	3.16	0.21	−0.03	−7.07
22–39	4.13	0.27	−0.04	−8.14	2.92	0.23	−0.04	−7.90
42–59	2.94	0.47	−0.04	−4.61	2.42	0.31	−0.04	−5.96
83–100	6.18	−0.06	0.00	−6.99	3.31	0.14	−0.01	−9.03
TWS	2.90	0.42	−0.03	−5.17	1.22	0.50	−0.05	−4.88

For each soil moisture variable (SMV), the best model was a polynomial equation fit to each CO_2 treatment: SCF = $CO_{2elev} \times [(a + b \times \text{SMV} - c \times (\text{SMV} - d)^2] + CO_{2amb} \times [(e + f \times \text{SMV} - g \times (\text{SMV} - h)^2]$, where CO_{2elev} = 1 for elevated CO_2 plots and 0 for ambient CO_2 plots and CO_{2amb} = 1 for ambient CO_2 plots and 0 for elevated CO_2 plots.

reduce the diffusion of CO_2 from soil, assuming similar CO_2 gradients across treatments (Jassal *et al.*, 2005). Thus, within a single day, plots with high TWS could have low SCF measurements (Jassal *et al.*, 2005). Regardless of the source of the negative effect of elevated CO_2 on SCF via TWS, this small effect was overwhelmed by the large, direct positive effect of elevated CO_2 on SCF.

While CO_2-induced changes in soil moisture had very little effect on SCF in this old-field grassland, there is some evidence for both positive and negative soil moisture-driven changes in SCF at elevated CO_2 in other ecosystems. Bader & Körner (2010) suggest that, in a forest with inherently high soil moisture, CO_2-induced increases in soil moisture may have resulted in such high soil moistures that SCF was periodically suppressed, leading to no overall change in SCF at elevated CO_2. These results are consistent with the hump-shaped relationship we found between SCF and soil moisture and suggest that this relationship may be extrapolated to predict the suppression of SCF at very high soil moistures. In another old-field grassland, in contrast to our results, Wan *et al.* (2007) found a significant positive relationship between the effects of CO_2 on SCF and soil moisture as well as higher CO_2 effects on SCF at low than at high soil moisture. However, Wan *et al.* (2007) state that their results may be influenced by the inclusion of measurements taken outside of the growing season, when plant activity (and thus SCF) was very low and soil moisture was high. Our results support this idea: while there was no relationship between the CO_2 effect on SCF and soil moisture during the growing season, including the nongrowing season data in our analysis led to several significant relationships between the CO_2 effect and soil moisture, driven by high moisture levels and low SCF rates in the fall when plant activity aboveground had ceased (Figs S3.1 and S3.2). In our view, statistical evidence of interacting CO_2 by soil moisture effects on SCF that confound plant activity with soil moisture is not ecologically or biologically meaningful.

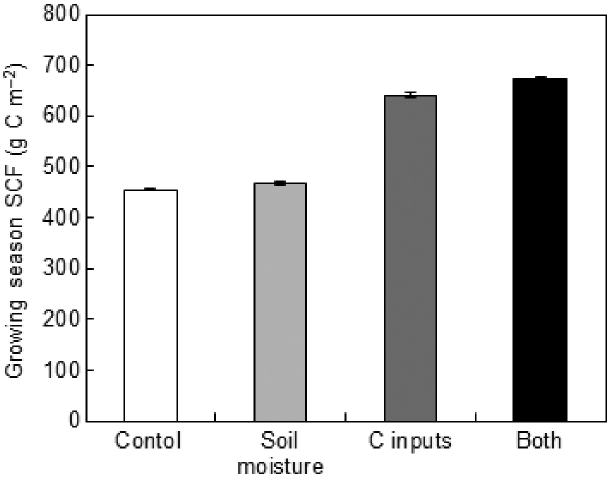

Fig. 9 Cumulative growing season soil C flux (growing season SCF) resulting from ambient CO_2 conditions (control, ambient CO_2), elevated CO_2 (eCO_2) soil moisture conditions (eCO_2 via soil moisture), eCO_2 C inputs (eCO_2 via C inputs), and eCO_2 soil moisture and C inputs (eCO_2 via C inputs + soil moisture). Cumulative growing season SCF was estimated using the predictions from the best model for describing SCF as a function of soil total soil water storage (TWS, 0–100 cm; Table 5; see Materials and methods). Error bars are ±1 SE.

The consistent positive CO_2 effect on SCF was most likely associated with CO_2-driven increases in C inputs and availability. In BioCON, elevated CO_2 increases photosynthesis (Lee *et al.*, 2001, 2011; Crous *et al.*, 2010), labile soil C (Dijkstra *et al.*, 2005), root biomass C (Reich *et al.*, 2001a, 2006b, Reich, 2009), and the total amount of C allocated belowground by plants, much of which is not associated with maintaining root biomass (e.g., root exudation and rhizodeposition; Adair *et al.*, 2009). Thus, by increasing C inputs, elevated CO_2 substantially increases the amount of C available for both plant and microbial respiration. Elevated CO_2 has also increased soil bacterial (but not fungal) biomass and the abundance of genes involved in decomposition of labile C (He *et al.*, 2010), a change that suggests increased microbial biomass (and associated increases in microbial respiration) is linked to increased inputs of labile C in these plots. Additionally, the CO_2 effect on SCF was relatively constant through time and across soil moisture levels, consistent with higher photosynthesis in elevated CO_2 plots across the growing season (T.D. Lee, personal communication). Taken together, past and present BioCON results suggest that the positive effect of CO_2 on SCF, which has been observed in multiple growing seasons of highly varied abiotic conditions (Figs S1 and S2), is associated with CO_2-induced increases in substrate availability rather than with CO_2-induced increases in soil moisture.

Diversity

In contrast to our expectations, increasing diversity did not influence SCF across the growing season, despite substantially greater above- and belowground biomass in the 16 vs. 1 species plots (2006 data not shown). Unexpectedly, our within-day analyses suggested that high diversity slightly decreased SCF (negative direct effect in path analysis), in contrast to previous studies in BioCON that have documented increased in-field SCF and microbial respiration in laboratory incubations with increasing diversity (Craine *et al.*, 2001b; Dijkstra *et al.*, 2005; West *et al.*, 2006). Our within-day path analysis results also indicated that high-biomass diverse plant communities sporadically increased SCF by reducing soil moisture (indirect positive effect in path analysis). As with the negative indirect effect of CO_2 on SCF, this relationship may be driven either by increased interception and uptake of plant available water for plant activity or by reduced vapor-phase soil pore volume and diffusion of CO_2 from the soil.

Across the growing season, SCF and soil moisture were largely positively related, and diversity significantly modified plot-level soil moisture and temperature conditions in ways that may have offset positive effects of diversity on C inputs and thereby SCF: soil moisture was consistently the same or lower in the 16 vs. 1 species plots and growing season soil temperature averaged 1.5 °C less in the 16 vs. 1 species plots (significant diversity effect on soil temperature in repeated-measures ANCOVA, July–October; data not shown). Low soil temperatures in the diverse plots, combined with more days at low soil moistures across this particularly dry growing season, may have resulted in low SCF rates that were limited more by unfavorable abiotic conditions for plants and/or microbes than they were enhanced by the increased C substrate availability in diverse plots (Dijkstra *et al.*, 2005).

Nitrogen

Adding N had small effects on both soil moisture and SCF. In contrast to our hypothesis, adding N reduced the negative effect of diversity on VSWC at intermediate soil depths and had no effect on VSWC in surface or deep soils. As hypothesized, N additions did increase SCF across the growing season, but only very slightly, and N additions had no effect on within-day SCF. On average, N additions increased SCF by 2%, about half of the size of the N effect on SCF in BioCON in 1998 and 1999 (Craine *et al.*, 2001b), suggesting that the positive effect of N on SCF may be declining over time, and/or may be lower in relatively dry summers. The N effect was unrelated to N effects on soil moisture. Combined with the higher above- and belowground biomass in N addition relative to ambient N plots (Reich *et al.*, 2001a, 2006 data not shown), our results suggest that the small N effect on SCF is associated with higher levels of substrate availability. Other studies have similarly found N additions to slightly increase SCF (Brumme & Beese, 1992; Craine *et al.*, 2001b), but still others have found N additions to have neutral (Micks *et al.*, 2004; Ambus & Robertson, 2006) or negative (Mattson, 1995; Butnor *et al.*, 2003; Bowden *et al.*, 2004; Janssens *et al.*, 2010) effects on SCF. The slightly positive N effect on SCF suggests that microbes may not respond to N additions by reducing decomposition of SOM to obtain N and/or that this effect is more than offset by N-induced increases in photosynthesis and photosynthate supply and/or the quality of plant inputs to soils and thus respiration (Reich *et al.*, 2006a).

CO_2 by N interaction

Adding N did affect the size of the CO_2 effect across the growing season, but this effect was variable (increasing or decreasing the size of the CO_2 effect on different days) and was small on average, increasing the size of the CO_2 effect on SCF by 0.2 $g\ C\ m^{-2}\ yr^{-1}$. Furthermore, this interaction was not significant when considered across all sampling points (June–October) and was not significant when considered across all BioCON SCF sampling points (1998–2006; Appendix S2). This suggests that the response of SCF to CO_2 has not been generally or greatly limited (or otherwise affected) by the availability of N.

Conclusions

Our results suggest that the large effect of elevated CO_2 on SCF in terrestrial ecosystems, especially in nonarid systems, may result in large part from CO_2-induced increases in C inputs and availability rather than from CO_2-induced increases in water availability, providing further support for increased rates of belowground C cycling under elevated CO_2 (Hungate *et al*., 1997; Adair *et al*., 2009). Furthermore, unlike the response of biomass to CO_2 (Reich *et al*., 2006a,b), the response of SCF to CO_2 does not appear to be greatly limited by N availability, suggesting that N-limited grasslands are unlikely to sequester C under elevated CO_2.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Effect of elevated CO_2 on soil moisture from 2002 to 2006.
Appendix S2. Effect of elevated CO_2 on soil carbon flux (SCF) from 1998 to 2006.
Appendix S3. Relationships between CO_2 effect on SCF and soil moisture using all data.