Plant Soil (2009) 319:85–94
DOI 10.1007/s11104-008-9851-6

REGULAR ARTICLE

Soil moisture surpasses elevated CO_2 and temperature as a control on soil carbon dynamics in a multi-factor climate change experiment

Charles T. Garten Jr. · Aimée T. Classen ·
Richard J. Norby

Received: 17 July 2008 / Accepted: 25 November 2008 / Published online: 16 December 2008

Abstract Some single-factor experiments suggest that elevated CO_2 concentrations can increase soil carbon, but few experiments have examined the effects of interacting environmental factors on soil carbon dynamics. We undertook studies of soil carbon and nitrogen in a multi-factor (CO_2 × temperature × soil moisture) climate change experiment on a constructed old-field ecosystem. After four growing seasons, elevated CO_2 had no measurable effect on carbon and nitrogen concentrations in whole soil, particulate organic matter (POM), and mineral-associated organic matter (MOM). Analysis of stable carbon isotopes, under elevated CO_2, indicated between 14 and 19% new soil carbon under two different watering treatments with as much as 48% new carbon in POM. Despite significant belowground inputs of new organic matter, soil carbon concentrations and stocks in POM declined over four years under soil moisture conditions that corresponded to prevailing precipitation inputs (1,300 mm yr^{-1}). Changes over time in soil carbon and nitrogen under a drought treatment (approximately 20% lower soil water content) were not statistically significant. Reduced soil moisture lowered soil CO_2 efflux and slowed soil carbon cycling in the POM pool. In this experiment, soil moisture (produced by different watering treatments) was more important than elevated CO_2 and temperature as a control on soil carbon dynamics.

Keywords Climate change · Soil moisture · Elevated temperature · Elevated CO_2 · Soil carbon · Soil nitrogen · Old-fields · Particulate organic matter · Mineral-associated organic matter · Soil respiration

Responsible editor: Hans Lambers.

C. T. Garten Jr. (✉) · A. T. Classen · R. J. Norby
Environmental Sciences Division,
Oak Ridge National Laboratory,
P.O. Box 2008, Mail Stop 6036,
Oak Ridge, TN 37831-6036, USA
e-mail: gartenctjr@ornl.gov

Introduction

Recent literature reviews suggest that elevated atmospheric CO_2 is sometimes followed by an increase in soil carbon stocks (Jastrow et al. 2005; Luo et al. 2006). Increased belowground carbon allocation and greater root production can be direct contributors to soil carbon accrual under CO_2-enrichment (Canadell et al. 1996; Luo et al. 2006; De Graaff et al. 2006). Meta-analyses indicate that elevated atmospheric CO_2 can produce small but measurable increases in soil carbon storage, but that contrary results exist (see De Graaff et al. 2006) and that conclusions are not always unequivocal, especially in cases were nutrient limitation appears to constrain plant growth response to elevated CO_2 (Reich et al. 2006; Van Groenigen et al. 2006). Whereas studies of elevated CO_2 alone on terrestrial ecosystems are relatively common, there are few studies of the effects of interactions among CO_2, temperature, and soil moisture on soil carbon dynamics. Moreover, there are relatively few studies of soil carbon dynamics in grasslands or pastures under elevated CO_2, and prior reports sometimes indicate conflicting results. For example, after a decade of exposure, elevated atmospheric CO_2 had no effect on soil carbon stocks in *Lolium perenne* and *Trifolium repens* grasslands at the Swiss FACE experiment (Van Kessel et al. 2006) while an 8-year open-top chamber experiment on native tallgrass prairie in Kansas (USA) indicated increasing soil carbon stocks under elevated CO_2 (Jastrow et al. 2000).

Elevated CO_2 may produce changes not only in belowground carbon allocation that drives soil carbon accumulation but also in root turnover (Fitter et al. 1996) and net soil carbon mineralization (Heath et al. 2005; De Graaff et al. 2006; Finzi et al. 2006). These soil processes are strongly dependent on environmental factors other than CO_2, such as soil temperature and moisture. Therefore, it is reasonable to expect that soil organic matter responses to CO_2-enrichment could be highly site-specific, even species-specific (Torbert et al. 1997), and that increased belowground carbon inputs will translate into higher soil carbon stocks only under circumstances where soil carbon inputs exceed rates of organic matter decomposition (De Graaff et al. 2006). The roles of soil moisture and temperature as modifying factors in the response of soil carbon and nitrogen to elevated CO_2 are not well understood (Tate and Ross 1997), but soil organic matter and nitrogen availability can sometimes be significant limiting factors in the growth response of grassland communities to elevated CO_2 (Zanetti et al. 1997; De Graaff et al. 2006).

We fully expect future increases in atmospheric CO_2 above present day concentrations, but we know little about the interactions among CO_2, temperature, and soil moisture as determinants of ecosystem response. For this reason, we undertook studies of changes in soil carbon and nitrogen in a four-year, multi-factor, CO_2 × temperature × soil moisture experiment near Oak Ridge, Tennessee (USA) to better understand the effects of these factors and their interactions on soil carbon and nitrogen stocks in a constructed old-field community. Prior studies at this site have shown that old-field soil CO_2 efflux increased significantly when atmospheric CO_2 concentrations are elevated in combination with soil moisture (Wan et al. 2007), but the relative contribution of autotrophic and heterotrophic respiration to the observed change is unclear. The primary objective of our study was to test the hypothesis that expected gains in soil carbon and nitrogen under elevated CO_2 could be modified by changes in organic matter decomposition under elevated temperature or decreased soil moisture. A secondary objective was to ascertain, using stable isotope techniques, the rates of transfer of newly fixed carbon to labile and stable soil carbon pools and quantify effects of temperature and moisture on soil carbon stocks under elevated atmospheric CO_2. The latter objective has been identified as a critical research topic for the improved understanding of soil carbon sequestration in grasslands (Tate and Ross 1997).

Methods

Experimental setup

The Old-Field Community, Climate, and Atmosphere Manipulation Experiment (OCCAM) was established on the Oak Ridge National Environmental Research Park (35° 54′ 12″ N, 84° 20′ 22″ W), near Oak Ridge, Tennessee, USA, to study the interactive effects of elevated CO_2, temperature, and soil moisture on the structure and function of a constructed old-field ecosystem. Twelve experimental plots were established at the site on a well-drained, slightly acidic, floodplain alluvium that is classified as Captina silt loam (fine-silty, mesic typic fragiudult) (Norby et al. 1997; Edwards and Norby 1999). Each 4-m diameter plot was trenched through the center and on the outside. Half of each plot was assigned to a "dry" treatment and half to a "wet" treatment. Trenches were lined with insulating foam and a 4-mil PVC film to provide a moisture barrier. A clear plastic canopy (with 92% transmission of PAR) was erected over the top of each chamber to exclude incoming precipitation. Collected rainwater was added weekly to supply the equivalent of 2 mm and 25 mm precipitation to the dry and wet subplots, respectively. Average annual precipitation at the site is 136 cm (26 mm/week). Thus, the total annual rainfall addition to the wet subplots was similar to average annual rainfall and represents "prevailing" conditions. The "dry" subplots had significantly less soil water content (a change from 0.25 to 0.20 cm^3 H_2O cm^{-3} soil; $P \leq 0.001$) than the "wet" subplots (Wan et al. 2007) and can be considered a "drought treatment" because it represents a negative departure from "prevailing soil moisture". A detailed analysis of watering treatment effects on volumetric soil water content has already been published for this experiment (Dermody et al. 2007). In addition, absolute humidity was similar in the chambers, hence chambers with elevated temperatures had lower relative humidity (approximately 16% less) and a higher vapor pressure deficit (1.8 time greater) than ambient temperature chambers (Wan et al. 2007).

In June 2002 existing vegetation at the site was killed using glyphosate and dead biomass was extracted (to a depth of approximately 1 cm) to remove meristems and some of the seed bank. Beyond this, there was a deliberate attempt to minimize surface soil disturbance. Seedlings of seven species (*Plantago lanceolata*, *Andropogon virginicus*, *Festuca pratense*, *Dactylis glomerata*, *Solidago canadensis*, *Trifolium pratense*, and *Lespedeza cuneata*) were planted in the plots in July 2002 and April 2003. Both *Trifolium* and *Lespedeza* are N_2-fixers while the remaining five species are non-N_2-fixing plants. The constructed ecosystems were typical of local old-field communities. Additional details on the site history, experimental setup, changes in species biomass over time, and N_2-fixation rates can be found in Wan et al. (2007), Dermody et al. (2007), or Garten et al. (2008).

In March 2003, open-top chambers (4 m diameter × 2.2 m tall) were installed on the experimental plots. The study was a split-plot design with two levels of CO_2 and temperature and three replicate chambers for each of the following treatment combinations: ambient CO_2—ambient temperature (ACAT), ambient CO_2—elevated temperature (ACET), elevated CO_2—ambient temperature (ECAT), and elevated CO_2—elevated temperature (ECET). In the experimental design, the ACAT chambers were effectively controls for testing effects of elevated atmospheric CO_2 and elevated temperature. Carbon dioxide used for the elevated CO_2 treatment was depleted (−51‰) in ^{13}C relative to ambient air (−8‰). Temperatures were elevated +3°C above ambient and elevated CO_2 was 300 ppmv over ambient air (390 ppmv CO_2). Temperature and CO_2 control was achieved using techniques described by Norby et al. (1997) and Wan et al. (2007). The treatments started in May 2003.

Sampling and analysis

Samples of aboveground biomass were collected for carbon isotope analysis and used in calculations of the fraction of new carbon in soils under elevated CO_2. Plant sampling during the 2003 and 2005 growing seasons and sample handling has been described elsewhere (Garten et al. 2008). Briefly, green leaf samples were collected from multiple individuals of each species in the dry and wet plots within each open-top chamber and the samples were oven dried (70°C) prior to elemental and isotopic analysis. In addition, we obtained samples of plant roots, collected to a 15 cm soil depth in November 2004 for a different purpose, to compare measurements of carbon isotopes in above- and belowground biomass and determine the carbon isotope signature in soil carbon inputs.

Soils were sampled to a 15-cm depth from each open-top chamber in December 2002, prior to the beginning of the experiment, oven dried, sieved (2 mm), and used to measure pre-treatment soil properties. Soil sampling was then repeated in October 2006 (after four growing seasons). Three mineral soil samples (0–15 cm) were collected from the dry and wet side of each chamber (each >300 g). Samples were homogenized by split-plot, oven dried, and sieved (2 mm) so that there was one homogenized sample from the dry and wet side of each chamber. Soil bulk density (1.49 g cm^{-3}) at the site was determined from 15-cm long cores (267 cm^3 per core) collected from six subplots in May 2007.

Sieved soil samples were physically separated into particulate organic matter (POM) and mineral-associated organic matter (MOM) by wet-sieving methods (Cambardella and Elliott 1992). Particulate organic matter is composed of free organic debris from the soil and larger (≥0.053 mm) organic matter fragments released by dispersion of soil aggregates. POM carbon is generally regarded as labile soil carbon when free of charcoal. Mineral-associated organic matter is bound to silt and clay-size particles and also includes smaller (<0.053 mm) organic matter fragments released by dispersion, as well as small amounts of soluble organic carbon. Due to a strong association with silt and clay, MOM carbon is generally regarded as more refractory than POM carbon. The separation was accomplished by shaking a 20 g portion of oven dry soil in a 100 mL solution of sodium hexametaphosphate (5 g L^{-1}) overnight. The mixture was wet sieved through a 0.053 mm sieve. Particulate organic matter (≥0.053 mm) was recovered by back-washing the sieve, filtration, (VWR Grade 315 paper), and oven drying. Mineral-associated organic matter that passed the 0.053 mm sieve (i.e., silt and clay) was also weighed after oven drying.

Plants, whole soil, and the soil fractions (POM and MOM) were milled to a fine, homogenous powder prior to elemental and isotopic analysis. Carbon and nitrogen concentrations were determined using a LECO CN-2000 elemental analyzer (LECO Corporation, St. Joseph, MI). The instrument was calibrated using LECO standards traceable to the National Institute of Standards and Technology (NIST), Gaithersburg, MD. Samples were analyzed for stable carbon isotope ratios ($^{13}C/^{12}C$) using an Integra-CN, continuous flow, isotope ratio mass-spectrometer (SerCon Ltd, Crewe, United Kingdom). Glucose ($\delta^{13}C=-10.2$‰), traceable to NIST, was used as an internal standard for the stable carbon isotope measurements.

Soil respiration was measured during the 2005 and 2006 growing seasons (May–October). At the start of the experiment two PVC soil collars were permanently installed 2 to 3 cm deep in the mineral soil in two locations within each subplot. Collars were cleared of litter and plant material several days prior to measurements. Soil respiration was measured every month during the growing season using a LiCor LI6200 infrared gas analyzer (Li-Cor Inc., Lincoln, NB, USA) with attached soil chamber. A time-integrated growing season rate of soil CO_2 efflux (mol m^{-2} s^{-1}) was calculated from monthly measurements during the 2005 and 2006 growing season.

Isotope calculations

Under elevated CO_2, the fraction (f_N) of new carbon in whole soil, POM, or MOM was calculated from an equation described by Balesdent and Mariotti (1996) and used by others (Hungate et al. 1996; Hagedorn et al. 2003; Hagedorn et al. 2005) in similar studies:

$$f_N = \Delta\delta^{13}C_{sample} / \Delta\delta^{13}C_{plant}$$

where $\Delta\delta^{13}C_{sample}$ is the difference in the carbon isotope signature under elevated and ambient CO_2 for whole soil, POM, or MOM, and $\Delta\delta^{13}C_{plant}$ is the corresponding difference in the plant carbon isotope signature. This equation is applicable to calculations of new carbon at sites initially under mixed C3-C4 vegetation as well as sites with a potentially complex land use history (Balesdent and Mariotti 1996). The fraction of old carbon (f_O) was simply $1-f_N$.

Whereas changes in soil carbon and nitrogen stocks could be evaluated for all treatment combinations, new soil carbon (f_N) was calculated only for the elevated CO_2 chambers. The final plant $\delta^{13}C$ under the dry and wet subplots was, respectively, −42.7 and −43.2‰. The latter values were weighted averages based on $\delta^{13}C$ measurements and the relative contribution of each species to aboveground biomass during the 2003 and 2005 growing seasons (Garten et al. 2008). The plant $\delta^{13}C$ values were similar to $\delta^{13}C$ values measured in roots from dry and wet subplots (−42.8±0.9 and −43.6±0.7‰, respectively) under elevated CO_2. The plant $\delta^{13}C$ under ambient CO_2, averaged in a similar manner, was −28.3‰, and a single value was calculated because the difference between the dry and wet subplots under ambient CO_2 was ≤0.25‰. Roots from the dry and wet subplots under ambient CO_2 (−27.6‰) were less than 1‰ more enriched in ^{13}C than leaves. Soil carbon inputs consist of both above- and belowground detritus. We used aboveground $\delta^{13}C$ values in the calculations because they were similar to $\delta^{13}C$ values in roots and calculations indicated that the difference in f_N using one source or the other was ≤5%. Based on the amount of old carbon ($1-f_N$) remaining after 4 years of exposure to elevated CO_2, turnover times of carbon in whole soil, POM, and MOM were calculated in accordance with methods presented by Balesdent et al. (1987).

Statistical analysis

Data from 2002 were first analyzed using one-way analysis of variance, with four groups (ACAT, ACET, ECAT, ECET) to test for pre-treatment differences in whole soil, POM, and MOM carbon and nitrogen concentrations, C-to-N ratios, and carbon stocks. Second, soils data from 2006 and soil respiration data from 2005 and 2006 were analyzed for treatment differences using a split-plot analysis of variance with a combined random and fixed effects model including the Kenward-Rogers adjustment for degrees of freedom (PROC MIXED, The SAS System, Cary, NC). Last, a combined dataset including measurements of soil carbon and nitrogen from 2002 and 2006 was analyzed using one-way analysis of variance with three groups (pretreatment, post-treatment dry, and post-treatment-wet) to test for changes in soil properties over four years of treatment. Rates of change in soil carbon and nitrogen and the fraction of new soil carbon (f_N) were also calculated but only under conditions of elevated CO_2 based on the pre- and post-treatment measurements from the dry and wet plots in each open-top chamber. A Wilcoxon matched-pairs signed rank test was used to test for effects of watering treatment under elevated CO_2.

Results

Pre-treatment differences (2002)

There were no significant differences ($P>0.05$) in soil properties among the four main treatment groups (ACAT, ACET, ECAT, and ECET) prior to the start of the experiment. Means for carbon and nitrogen concentrations, C-to-N ratios, and carbon stocks in whole soil, POM, and MOM were based on pre-treatment measurements from eleven open-top chambers (soils from one chamber could not be found). Summary statistics for the soil properties are presented in Table 1.

Differences in 2006

Considering all the post-treatment 2006 data, there was no difference ($P>0.05$) between ambient and elevated CO_2 treatments for carbon and nitrogen concentrations in whole soil, POM, and MOM. Interactions involving CO_2 × temperature, CO_2 × watering treatment, or CO_2 × temperature × watering treatment were also not statistically significant. Soil moisture, rather than CO_2 or temperature, was the principal factor contributing to differences in the contents of soil carbon and nitrogen. Watering treatment effects were manifested chiefly in POM. Carbon concentrations in POM were significantly ($P\leq0.05$) less under the wet treatment relative to other treatments, and there was no significant interaction between or among watering treatment and temperature or CO_2. Mean carbon stocks in POM were also significantly less ($P\leq0.05$) in the wet treatment, and there was a significant ($P\leq0.05$) watering treatment × temperature interaction. The interaction was caused by greater POM and POM carbon stocks in the dry treatment under elevated temperature, but no difference between watering treatments for POM carbon stocks under ambient temperatures (Fig. 1).

Table 1 Pre- and post-treatment measures of soil carbon and nitrogen in the constructed old-fields

Carbon pool	Measure	Pre-treatment (2002)	Post-treatment (2006)		Probability
			Dry (n=12)	Wet (n=12)	
Whole soil	g N kg^{-1}	1.62±0.08	1.54±0.09	1.49±0.05	ns
	g C kg^{-1}	18.3±0.63	17.7±0.74	17.0±0.31	ns
	C-to-N ratio	11.4±0.2	11.7±0.3	11.5±0.2	ns
	g C m^{-2}	4111±142	3961±165	3821±71	ns
	δ^{13}C (‰)	$-23.28^{a}\pm0.28$	$-26.16^{b}\pm0.24$	$-27.02^{b}\pm0.59$	0.001
POM	g N kg^{-1}	$1.92^{a}\pm0.19$	$1.47^{ab}\pm0.12$	$1.18^{b}\pm0.07$	0.01
	g C kg^{-1}	$29.5^{a}\pm1.9$	$28.3^{ab}\pm1.6$	$23.4^{b}\pm1.4$	0.05
	C-to-N ratio	$16.2^{a}\pm1.0$	$19.9^{b}\pm1.1$	$19.8^{b}\pm0.5$	0.05
	g C m^{-2}	$835^{a}\pm51$	$747^{ab}\pm41$	$641^{b}\pm27$	0.01
	δ^{13}C (‰)	$-24.12^{a}\pm0.37$	$-31.23^{b}\pm0.67$	$-33.29^{c}\pm0.49$	0.001
MOM	g N kg^{-1}	1.58±0.10	1.39±0.09	1.36±0.06	ns
	g C kg^{-1}	15.7±0.5	15.5±0.7	15.4±0.4	ns
	C-to-N ratio	$10.1^{a}\pm0.4$	$11.3^{b}\pm0.3$	$11.4^{b}\pm0.3$	0.05
	g C m^{-2}	3276±105	3213±139	3180±66	ns
	δ^{13}C (‰)	$-23.15^{a}\pm0.31$	$-24.90^{b}\pm0.17$	$-25.54^{b}\pm0.36$	0.001

Post-treatment data are summarized by moisture treatment. Probability values are from analysis of variance comparing the three groups. Means in the same row sharing the same alphabetic superscript are not significantly different (ns = $P>0.05$)

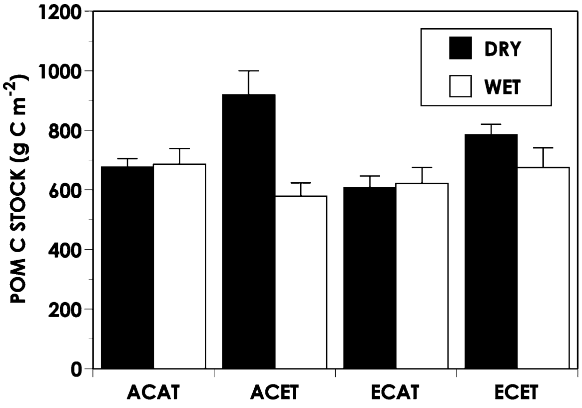

Fig. 1 Comparison of mean (±SE) carbon stocks in particulate organic matter (POM) from surface soils beneath different experimental treatments in 2006 (n=3): AC=ambient CO_2, AT=ambient temperature, EC=elevated CO_2, and ET=elevated temperature

Changes from 2002 to 2006

Nitrogen concentrations, carbon concentrations, and carbon stocks in POM all declined significantly from 2002 to 2006 when soil moisture was maintained at prevailing conditions, i.e. the wet treatment (Table 1). Changes over time in carbon and nitrogen concentrations (or stocks) in POM and MOM were not statistically significant (P>0.05) under the dry treatment. However, there was a significant (P≤0.05) widening of the C-to-N ratio in both POM and MOM from 2002 to 2006 that indicated proportionately greater losses of soil nitrogen relative to soil carbon under both watering treatments (Table 1).

New soil carbon under elevated CO_2

Under elevated atmospheric CO_2 there was a significant (P≤0.05) depletion in ^{13}C in whole soil (Δ= −2.8‰), POM (Δ=−7.1‰), and MOM (Δ=−1.7‰) from the accrual of new soil organic matter inputs. There was no significant (P>0.05) effect of temperature on the fraction of new soil carbon (f_N) in whole soil, POM, or MOM under elevated CO_2 in 2006. After four growing seasons, between 14 and 19% of the whole soil carbon under the dry and the wet treatment, respectively, was new soil carbon (Table 2). Although a large fraction of POM in the dry treatment was new carbon after four years, approximately equal amounts of the new soil carbon were partitioned between POM (269 g C m^{-2}) and MOM (279 g C m^{-2}). In the wet treatment, approximately 22% more new carbon was partitioned to MOM (388 g C m^{-2}) than POM (317 g C m^{-2}). Based on the ^{13}C analysis of POM in 2006, there was a 49 and 61% decline in old POM carbon in the dry treatment and the wet treatment, respectively. A Wilcoxon matched-pairs signed rank test indicated statistically significant differences between moisture treatments for final stocks of old carbon, the change in carbon stock, and carbon turnover times in POM, but similar effects were not detected in whole soil or MOM (Table 2).

Soil respiration

Watering treatment significantly (P≤0.001) affected soil respiration in 2005, but the rate of soil CO_2 efflux

Table 2 Mean (±SE) fraction of old (f_o) and new (f_N) soil carbon, final old carbon stock (C_{fo}), final new carbon stock (C_{fn}), change in stock, and calculated turnover time of old carbon in whole soil, POM, and MOM following 4 years of exposure of constructed old-fields to elevated CO_2

Carbon pool	Moisture	Old C (f_O)	New C (f_N)	C_{fo} (g C m^{-2})	C_{fn} (g C m^{-2})	Change in stock (g C m^{-2})	Turnover time[c] (years)
Whole soil	Dry	0.851±0.012	0.149±0.012	3330±191	548±46	−871±116	18.6±2.3
	Wet	0.812±0.030	0.188±0.030	3205±96	705±86	−996±156	16.5±2.3
POM	Dry	0.618±0.036	0.382±0.036	428^{a}±34	269±34	-411^{a}±100	9.7^{a}±3.6
	Wet	0.518±0.036	0.482±0.026	332^{b}±10	317±36	-508^{b}±81	4.9^{b}±0.9
MOM	Dry	0.910±0.009	0.090±0.009	2902±182	279±20	−460±43	29.1±4.9
	Wet	0.880±0.018	0.120±0.018	2873±96	388±55	−488±94	31.3±6.7

Means in the same carbon pool with different alphabetic superscripts indicate a significant (P≤0.05) difference between the dry and wet treatments

[c] Assuming exponential decay, turnover time (T) was calculated as: T = t/-ln(C_{fo}/C_i), where t is the duration of the experiment (four years), C_{fo} is the final carbon stock, and C_i is the initial carbon stock (Balesdent et al. 1987)

was unaffected by either elevated CO_2 or temperature. There was a temperature × moisture interaction for soil respiration measurements in 2005 ($P \leq 0.05$) that was caused by a small decline in soil respiration under elevated temperature in the dry treatment. This was contrary to the pattern observed in the wet treatment (Fig. 2). During 2006, the growing season rate of soil CO_2 efflux was significantly affected by both CO_2 ($P \leq 0.05$) and watering treatment ($P \leq 0.001$). Interactions among watering treatment, CO_2, and temperature were not significant. In summary, measurements of growing season soil respiration were consistently reduced by the dry treatment under different treatment combinations of temperature and CO_2 concentration in both 2005 and 2006 (Fig. 2).

Discussion

Short-term (4 years) exposure of the constructed old-fields to elevated CO_2 concentrations produced no detectable change in soil carbon concentrations or stocks. The absence of an effect indicated that soil carbon inputs were unchanged or that decomposition of soil organic matter increased to offset any additional soil carbon input under elevated CO_2. Our data provide evidence for both explanations, but the latter one is more likely. On one hand, elevated CO_2 had no significant effect on aboveground biomass during the first three years of the experiment (Garten et al. 2008) which, consistent with unpublished data collected using mini-rhizotrons, suggests that biomass production and soil carbon inputs were unaffected by the CO_2 treatment. On the other hand, measurements made during the 2006 growing season, as well as data presented by Wan et al. (2007), indicate increased soil respiration under elevated CO_2 that could reflect increased decomposition of existing soil organic matter. Studies in other grasslands indicate that elevated CO_2 can increase organic matter decomposition by altering both microbial biomass and enzyme activity (Drissner et al. 2007).

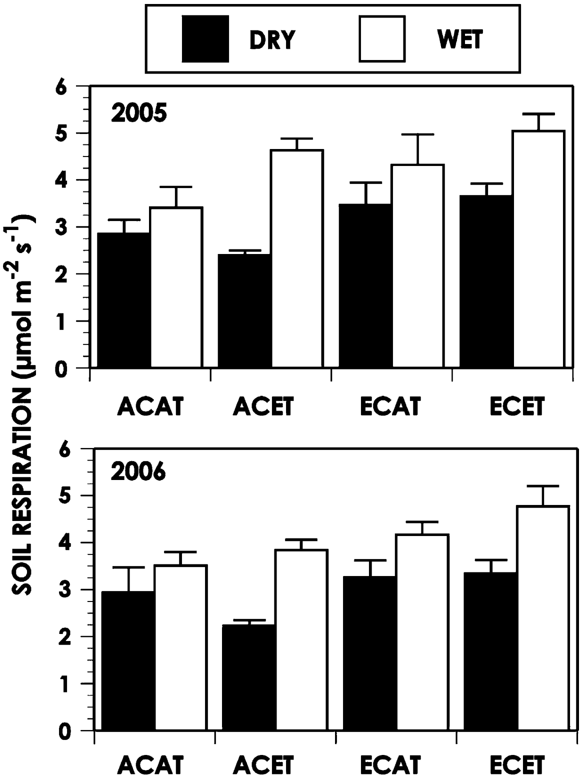

Fig. 2 Mean (±SE) rates of soil CO_2 efflux under different watering treatments during the 2005 and 2006 growing seasons (May–October) (n=12): AC=ambient CO_2, AT=ambient temperature, EC=elevated CO_2, and ET=elevated temperature

Our results are consistent with other studies indicating no measurable change in soil carbon storage under conditions of elevated CO_2 and low soil nitrogen availability (De Graaff et al. 2006), at least over the short-term. Although we do not have experiment-specific measures of nitrogen availability, daily rates of gross soil nitrogen mineralization at a nearby location (same alluvial soil type), averaged over two years, were 0.66 μg N g^{-1} and were the lowest measured mid-season rates among a comparison of three widely distributed, experimental sites (Duke, Rhinelander, and Oak Ridge) (Zak et al. 2003). Moreover, studies in the constructed old-fields indicate high rates of symbiotic N_2-fixation by two legumes with more than 80% of aboveground plant tissue nitrogen derived from the atmosphere (Garten et al. 2008). The absence of an effect of elevated CO_2 on soil carbon storage in the constructed old-fields, even under conditions where symbiotic N_2-fixation contributed 44–51% to aboveground nitrogen stocks, is similar to results from studies in a grass-clover system where, following nine years of exposure to elevated CO_2, symbiotic N_2-fixation did not affect soil carbon storage (Van Groenigen et al. 2003). Although our evidence is indirect, we attribute the absence of an effect of elevated CO_2 on soil carbon storage in the short-term to nitrogen limitations on above- and belowground carbon inputs.

The hypothesis that possible gains in soil carbon under elevated CO_2 would be offset by increased decomposition under elevated temperature was rendered moot by the absence of a significant CO_2 effect on soil carbon and nitrogen under conditions of ambient temperature and the absence of a significant CO_2 × temperature interaction. There was a significant temperature × watering treatment interaction that affected 2006 carbon stocks in POM in the dry and wet treatment plots due to differences in POM mass. Under elevated temperature, the dry subplots contained more POM carbon under ambient CO_2 than under elevated CO_2 (Fig. 1). This interaction appeared to exemplify the following effect where elevated CO_2 can potentially diminish the effect of the reduced watering treatment on soil carbon storage.

Like Wan et al. (2007), we found no significant overall main effect of temperature on mean rates of soil CO_2 efflux in the constructed old-fields but effects of watering treatment that consistently indicated less soil CO_2 efflux in the dry treatment plots. Dermody et al. (2007) have previously addressed the complex, interacting effects of elevated atmospheric CO_2, warming, and reduced precipitation inputs on soil moisture in the OCCAM experiment. Treatment impacts on soil moisture are confounded because warming and less precipitation input both reduce soil moisture. The effect can be magnified under a combination of both warming and drying, but elevated CO_2 can increase soil moisture and potentially offset some of the warming and drying effects via lower stomatal conductance and reduced evapotranspiration. This may explain why the greatest effect of drying on POM carbon was measured under ACET rather than ECET (Fig. 1). Lower precipitation inputs in the dry treatment significantly reduced soil moisture to a 15 cm depth, even in association with elevated atmospheric CO_2 (Dermody et al. 2007). Although a lower relative humidity and a higher vapor pressure deficit have also been reported for OCCAM's elevated temperature chambers (Wan et al. 2007), the latter properties are far less important than soil moisture to belowground processes (like decomposition of organic matter) that affect changes over time in soil carbon and nitrogen stocks. Moisture limitations on rates of soil organic matter decomposition are a probable cause of greater stocks of labile soil carbon under elevated temperature in the dry treatment. However, the latter mechanism could be combined with increased soil carbon inputs under elevated temperature. The elevated temperature treatment increased plant biomass in some old-field species, but temperature effects on particular species were not consistent over time (Garten et al. 2008).

More than elevated CO_2 or temperature, watering treatment (i.e., soil moisture) was the principal driving environmental factor that produced changes in old-field soil carbon and nitrogen concentrations during the four-year experiment. The changes were manifested primarily in particulate organic matter—a pool of fresh, easily decomposable, and rapidly cycling soil organic carbon. As expected, carbon concentrations and stocks in whole soil and MOM were unchanged over four years, because of their longer turnover times (Table 2). The different responses of whole soil, POM, and MOM exemplify the importance of making distinctions between various soil carbon pools in climate change experiments. Although POM carbon and nitrogen concentrations, as well as carbon stocks, declined over time in both watering treatments, the declines from 2002 to 2006 were greatest, and statistically significant, in the wet treatment plots (Table 1). Both aboveground biomass (Garten et al. 2008) and soil CO_2 efflux (Fig. 2) were greater in the wet treatment plots, and losses of soil carbon appeared to outweigh any increase in soil carbon inputs irrespective of elevated CO_2 and temperature.

Additional insights on soil carbon dynamics in the constructed old-fields were provided by an analysis of carbon isotopes under elevated CO_2. Even though soil carbon dynamics under elevated CO_2 may not be representative of current-day conditions because of CO_2 induced changes in soil microbial activity (Cardon et al. 2001; Drissner et al. 2007), soil respiration (Heath et al. 2005; Wan et al. 2007), nitrogen availability (De Graaff et al. 2007), and other CO_2-specific soil processes (Niklaus and Falloon 2006), measurements of soil carbon and nitrogen under elevated CO_2 are relevant to soil carbon dynamics in an expected, future, CO_2-rich world. The temperature treatment had no effect on accrual of new soil carbon under elevated CO_2, but the turnover time of old soil carbon in POM was prolonged under reduced soil moisture. A large fraction of POM carbon at the end of the experiment represented newly accrued carbon, but calculations of pool size indicated that amounts of new carbon in MOM equaled or slightly exceeded the partitioning of new soil carbon to POM (Table 2). The partitioning of new soil carbon in the old-field was different than the fate of new carbon in forest soils under elevated CO_2 where the majority of carbon accrual occurs in labile pools (Hagedorn et al. 2003).

In conclusion, the watering treatment surpassed elevated CO_2 and temperature as a determinant of soil carbon dynamics in the constructed old-fields. Reduced precipitation inputs decreased decomposition of soil organic matter and prolonged the turnover time of POM carbon. An imbalance between soil carbon inputs and outputs produced a decline in labile soil carbon stocks under conditions of prevailing soil moisture (the wet treatment). In short-term experiments, like this one, rapidly cycling soil carbon pools (like POM) will be the first indicator of changing dynamics in soil organic matter. Carbon in POM is an important substrate for soil microbial activity and nutrient recycling in the plant-soil system, and declines in POM have been associated with declining soil quality. Long-term declines in soil carbon and nitrogen would tend to promote the kind of community changes in the constructed old-fields that have been previously described, namely increased dominance of an invasive, N_2-fixing forb, *Lespedeza cuneata* (Garten et al. 2008). Our experience with this multi-factor climate change experiment underscores the need for a comprehensive consideration of changes in the soil-plant system that includes the effect of interacting environmental factors (temperature, CO_2, and soil moisture) on individual species responses, plant community composition, and changes in soil carbon and nitrogen that potentially govern long-term changes in ecosystem function.

Acknowledgements The research was sponsored by the U.S. Department of Energy, Office of Science, Biological and Environmental Research Program for Ecosystem Research under contract DE-AC05-00OR22725 with Oak Ridge National Laboratory (ORNL), managed by UT-Battelle, LLC. We wish to thank P. Allen, D.J. Brice, H. Castro, E.C. Engel, E. Felker-Quinn, B. Lu, C. Reilly Sheehan, and S. Wan for their valuable assistance in the laboratory and/or field. We would also like to thank the ORNL/UT Ecosystem Ecology lab group (E. Austin, C. Campany, H. Castro, M. Cregger, C. Iversen, P. Kardol, and M-A. De Graaff) for insightful comments on the draft manuscript.

References

Balesdent J, Mariotti A (1996) Measurement of soil organic matter turnover using ^{13}C natural abundance. In: Boutton TW, Yamasaki S (eds) Mass spectrometry of soils. Marcel Dekker, New York, pp 83–111

Balesdent J, Mariotti A, Guillet B (1987) Natural ^{13}C abundance as a tracer for studies of soil organic matter dynamics. Soil Biol Biochem 19:25–30 doi:10.1016/0038-0717(87)90120-9

Cambardella CA, Elliott ET (1992) Particulate soil organic-matter changes across a grassland cultivation sequence. Soil Sci Soc Am J 56:777–783

Canadell JG, Pitelka LF, Ingram JSI (1996) The effects of elevated $[CO_2]$ on plant-soil carbon below-ground: a summary and synthesis. Plant Soil 187:391–400 doi:10.1007/BF00017102

Cardon ZG, Hungate BH, Cambardella CA, Chapin FSI, Field CB, Holland EA, Mooney HA (2001) Contrasting effects of elevated CO_2 on old and new soil carbon pools. Soil Biol Biochem 33:365–373 doi:10.1016/S0038-0717(00)00151-6

De Graaff MA, Van Groenigen KJ, Six J, Hungate B, Van Kessel C (2006) Interactions between plant growth and soil nutrient cycling under elevated CO_2: a meta-analysis. Glob Change Biol 12:2077–2091 doi:10.1111/j.1365-2486.2006.01240.x

De Graaff MA, Six J, Van Kessel C (2007) Elevated CO_2 increases nitrogen rhizodeposition and microbial immobilization of root-derived nitrogen. New Phytol 173:778–786 doi:10.1111/j.1469-8137.2006.01974.x

Dermody O, Weltzin JF, Engel EC, Allen P, Norby RJ (2007) How do elevated $[CO_2]$, warming, and reduced precipitation interact to affect soil moisture and LAI in an old field ecosystem? Plant Soil 301:255–266 doi:10.1007/s11104-007-9443-x

Drissner D, Blum H, Tscherko D, Kandeler E (2007) Nine years of enriched CO_2 changes the function and structural diversity of soil microorganisms in a grassland. Eur J Soil Sci 58:260–269 doi:10.1111/j.1365-2389.2006.00838.x

Edwards NT, Norby RJ (1999) Below-ground respiratory responses of sugar maple and red maple saplings to atmospheric CO_2 enrichment and air temperature. Plant Soil 206:85–97 doi:10.1023/A:1004319109772

Finzi AC, Sinsabaugh RL, Long TM, Osgood MP (2006) Microbial community responses to atmospheric carbon dioxide enrichment in a warm-temperate forest. Ecosystems (N Y, Print) 9:215–226 doi:10.1007/s10021-005-0078-6

Fitter AH, Self GK, Wolfenden J, Van Vuuren MMI, Brown TK, Williamson L, Graves JD, Robinson D (1996) Root production and mortality under elevated atmospheric carbon dioxide. Plant Soil 187:299–306 doi:10.1007/BF00017095

Garten CT Jr, Classen AT, Norby RJ, Brice DJ, Weltzin JF, Souza L (2008) Role of N_2-fixation in constructed old-field communities under different regimes of $[CO_2]$, temperature, and water availability. Ecosystems (N Y, Print) 11:125–137 doi:10.1007/s10021-007-9112-1

Hagedorn F, Spinnler D, Bundt M, Blaser P, Siegwolf R (2003) The input and fate of new C in two forest soils under elevated CO_2. Glob Change Biol 9:862–872 doi:10.1046/j.1365-2486.2003.00638.x

Hagedorn F, Maurer S, Bucher JB, Siegwolf RTW (2005) Immobilization, stabilization and remobilization of nitrogen in forest soils at elevated CO_2: a ^{15}N and ^{13}C tracer study. Glob Change Biol 11:1816–1827 doi:10.1111/j.1365-2486.2005.01041.x

Heath J, Ayres E, Possell M, Bardgett RD, Black HIJ, Grant H, Ineson P, Kerstiens G (2005) Rising atmospheric CO_2 reduces sequestration of root-derived soil carbon. Science 309:1711–1713 doi:10.1126/science.1110700

Hungate BA, Jackson RB, Field CB, Chapin FS (1996) Detecting changes in soil carbon in CO_2 enrichment experiments. Plant Soil 187:135–145 doi:10.1007/BF00017086

Jastrow JD, Miller RM, Owensby CE (2000) Long-term effects of elevated atmospheric CO_2 on below-ground biomass and transformations to soil organic matter in grassland. Plant Soil 224:85–97 doi:10.1023/A:1004771805022

Jastrow JD, Miller RM, Matamala R, Norby RJ, Boutton TW, Rice CW, Owensby CE (2005) Elevated atmospheric carbon dioxide increases soil carbon. Glob Change Biol 11:2057–2064 doi:10.1111/j.1365-2486.2005.01077.x

Luo Y, Hui D, Zhang D (2006) Elevated CO_2 stimulates net accumulations of carbon and nitrogen in land ecosystems: a meta-analysis. Ecology 87:53–63 doi:10.1890/04-1724

Niklaus PA, Falloon P (2006) Estimating soil carbon sequestration under elevated CO_2 by combining carbon isotope labeling with soil carbon cycle modeling. Glob Change Biol 12:1909–1921 doi:10.1111/j.1365-2486.2006.01215.x

Norby RJ, Edwards NT, Riggs JS, Abner CH, Wullschleger SD, Gunderson CA (1997) Temperature-controlled open-top chambers for global change research. Glob Change Biol 3:259–267 doi:10.1046/j.1365-2486.1997.00072.x

Reich PB, Hungate BA, Luo Y (2006) Carbon-nitrogen interactions in terrestrial ecosystems in response to rising atmospheric carbon dioxide. Annu Rev Ecol Evol Syst 37:611–636 doi:10.1146/annurev.ecolsys.37.091305.110039

Tate KR, Ross DJ (1997) Elevated CO_2 and moisture effects on soil carbon storage and cycling in temperate grasslands. Glob Change Biol 3:225–235 doi:10.1046/j.1365-2486.1997.00094.x

Torbert HA, Rogers HH, Prior SA, Schlesinger WH, Runion GB (1997) Effects of elevated atmospheric CO_2 in agro-ecosystems on soil carbon storage. Glob Change Biol 3:513–521 doi:10.1046/j.1365-2486.1997.d01-173.x

Van Groenigen KJ, Six J, Harris D, Blum H, Van Kessel C (2003) Soil ^{13}C-^{15}N dynamics in an N_2-fixing clover system under long-term exposure to elevated atmospheric CO_2. Glob Change Biol 9:1751–1762 doi:10.1111/j.1365-2486.2003.00706.x

Van Groenigen KJ, Six J, Hungate BA, De Graaff MA, Van Breemen N, Van Kessel C (2006) Element interactions limit soil carbon storage. Proc Natl Acad Sci USA 103:6571–6574 doi:10.1073/pnas.0509038103

Van Kessel C, Boots B, De Graaff M-A, Harris D, Blum H, Six J (2006) Total soil C and N sequestration in a grassland following 10 years of free air CO_2 enrichment. Glob Change Biol 12:2187–2199 doi:10.1111/j.1365-2486.2006.01172.x

Wan S, Norby RJ, Ledford J, Weltzin JF (2007) Responses of soil respiration to elevated CO_2, air warming, and changing soil water availability in a model old-field grassland. Glob Change Biol 13:1–14 doi:10.1111/j.1365-2486.2007.01433.x

Zak DR, Holmes WE, Finzi AC, Norby RJ, Schlesinger WH (2003) Soil nitrogen cycling under elevated CO_2: a synthesis of forest FACE experiments. Ecol Appl 13:1508–1514 doi:10.1890/03-5055

Zanetti S, Hartwig UA, Van Kessel C, Luscher A, Hebeisen T, Frehner M, Fischer BU, Hendrey GR, Blum H, Nosberger J (1997) Does nitrogen nutrition restrict the CO_2 response of fertile grassland lacking legumes? Oecologia 112:17–25 doi:10.1007/s004420050278