Elevated CO₂ and temperature increase soil C losses from a soy-maize ecosystem

Running head: Climate change loses soil C

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Abstract

Warming temperatures and increasing CO_2 are likely to have large effects on the amount of carbon stored in soil, but predictions of these effects are poorly constrained. We elevated temperature (canopy: +2.8 °C; soil growing season: +1.8 °C; soil fallow: +2.3 °C) for three years within the 9th-11th years of an elevated CO_2 (+200 ppm) experiment on a maize-soybean agroecosystem, measured respiration by roots and soil microbes, then used a process-based ecosystem model (DayCent) to simulate the decadal effects of warming and CO_2 enrichment on soil C. Both heating and elevated CO_2 increased respiration from soil microbes by ~20%, but heating reduced respiration from roots and rhizosphere by ~25%. The effects were additive, with no heat x CO_2 interactions. Particulate organic matter and total soil C declined over time in all treatments and were lower in elevated CO_2 plots than in ambient plots, but did not differ between heat treatments. We speculate that these declines indicate a priming effect, with increased C inputs under elevated CO_2 fueling a loss of old soil carbon. Model simulations of heated plots agreed with our observations and predicted loss of ~15% of soil organic C after 100 years of heating, but simulations of elevated CO_2 failed to predict the observed C losses and instead predicted a ~4% gain in soil organic C under any heating conditions. Despite model uncertainty, our empirical results suggest that combined, elevated CO_2 and temperature will lead to long term declines in the amount of carbon stored in agricultural soils.

Introduction

Human activity, primarily fossil fuel burning, is increasing atmospheric $[CO_2]$ and raising global mean temperature (Hartmann *et al.*, 2013). These changes are likely to have direct and indirect effects on storage of soil organic carbon (SOC), but estimates of the direction and magnitude of these effects are poorly constrained (Dieleman *et al.*, 2012; Lu *et al.*, 2013). Soils worldwide store over two orders of magnitude more C than annual anthropogenic emissions ($\approx$ 1500 Pg C in the top 1 m; Eswaran *et al.*, 1993), so even small changes in soil C storage in response to climate change could produce large feedbacks to the global C cycle. This may be especially true of the SOC-rich former prairie soils of the agriculturally managed Midwestern United States, where annual tillage, infrequent water limitation, regular fertilization, and frequent pulses of highly labile C from crop residues provide ideal conditions for temperature-controlled microbial activity (Tisdall & Oades, 1980).

Changes in soil C are difficult to detect on short timescales because some pools turn over slowly, with mean residence times of hundreds of years. Although it is conceptually useful to identify the faster-cycling subpools of soil C, we lack experimental methods to measure them directly (Schmidt *et al.*, 2011). Instead, changes in the rate of CO_2 fluxes from soil can be used as a proxy for changes in the soil C cycle by partitioning total CO_2 flux (R_{tot}) into components attributed to "autotrophic" respiration (R_{aut}) from plant roots and rhizosphere organisms, or to "heterotrophic" respiration (R_{het}) from soil microbes in the process of breaking down soil organic matter (SOM). Because R_{het} is the primary avenue for loss of soil C, any change in R_{het} indicates a change in the

rate of soil C loss. R_{het} is strongly controlled by soil temperature and moisture and therefore expected to shift under future climate conditions (Davidson & Janssens, 2006; Subke & Bahn, 2010; Conant *et al.*, 2011; Bradford, 2013). In contrast, changes in R_{aut} are indirectly linked to the rate of C *input* from roots, so a unit change in R_{tot} could indicate either increasing or decreasing soil C. Therefore correct partitioning of fluxes is essential to their use as a proxy for changes in pool size (Kuzyakov & Larionova, 2005).

Previous soil heating experiments have generally shown short-term increases in R_{tot} (Rustad *et al.*, 2001; Wu *et al.*, 2011), except when heating exacerbated soil water limitations (Schindlbacher *et al.*, 2012; Pendall *et al.*, 2013; Suseela & Dukes, 2013; Wall *et al.*, 2013; Wang *et al.*, 2014). This heating effect often diminishes after a few years of treatment. Whether these responses will persist over the long term under climate change depends on whether a particular soil's R_{het} response is modulated by availability of nutrients or C substrates (Luo *et al.*, 2001; Chevallier *et al.*, 2015) or by physiological adaptation of the microbial community (Allison *et al.*, 2010; Bradford, 2013). In addition, few of these studies were able to separate soil respiration into its autotrophic and heterotrophic components. Since R_{het} is strongly controlled by thermal kinetics while R_{aut} responds to a wide variety of non-thermal factors, it has been widely assumed that temperature-associated increases in R_{tot} are driven by increasing R_{het}, but support for this assumption is equivocal (Hartley *et al.*, 2007; Bond-Lamberty & Thomson, 2010; Suseela & Dukes, 2013; Wang *et al.*, 2014).

Previous CO_2 enrichment experiments have generally shown sustained increases in R_{tot} (King *et al.*, 2004; Bernhardt *et al.*, 2006; Pregitzer *et al.*, 2006; Peralta & Wander, 2008; Adair *et al.*, 2011; Carrillo *et al.*, 2011; Drake *et al.*, 2011; Keidel *et al.*, 2015), but there are few reported results from field experiments that manipulate both heat and CO_2 simultaneously. Of those that are reported (Carrillo *et al.*, 2011; Pendall *et al.*, 2011, 2013; Selsted *et al.*, 2012), the observed responses seem to be mostly mediated by water availability, with heat increasing R_{tot} when moisture is available and reducing it when heating produces drier soil. Elevated CO_2 mediates these effects by ameliorating soil water stress through increased plant water use efficiency, but the strength and predictability of this effect seems to vary widely both within and between experiments.

The objective of this study was to measure the root- and SOM-derived components of soil respiration in an intact maize-soybean ecosystem subjected to mid-21st century temperature (+3.5 °C) and CO_2 (585 ppm) conditions under fully open-air conditions at SoyFACE (Urbana IL, USA). We then used a process-based biogeochemical model (DayCent; Parton *et al.*, 1998) to predict the long-term effects of these respiratory responses on soil C storage. We predicted that elevated temperature would increase the activity of soil heterotrophs, leading to increased respiration in root-free soil and long-term losses of C from the most labile pools of SOM. We further predicted that elevated CO_2 would increase plant biomass above and belowground, leading to higher C inputs that would at least partially ameliorate the long-term effect of heat on soil C, and therefore that the long-term fate of soil C at our site would depend on the strength of the interaction between heat and CO_2 effects.

Materials and methods

Site description

Measurements were made at the *Soy*bean *F*ree-*A*ir *C*oncentration *E*nrichment (SoyFACE) experiment (40.04N, 88.23W; elev. 215 m; soyface.igb.illinois.edu), a 32-Ha experimental site near Urbana IL, USA. The site is flat, tile-drained, and has been cultivated for over 100 years. Soils are deep and highly productive, mapped as Flanagan silt loam (fine, smectitic, mesic Aquic Argiudoll) and Drummer silty clay loam (fine-silty, mixed, superactive, mesic Typic Endoaquoll). The mean annual temperature is 11 °C, with monthly mean temperatures ranging from -3 °C in January to 24 °C in July, and annual precipitation is ~1 m, with approximately half falling during the May-September growing season (Angel, 2010a).

The site was managed in a two-year rotation, with maize (*Zea mays* cv 34B43) and soybean (*Glycine max* cv 93B15) alternating between the eastern and western halves of the site. Maize was fertilized with 202 kg N Ha^{-1} yr^{-1} and soybean was not fertilized. The soil was typically chisel plowed each spring before planting, and in the fall after maize harvest but not after soybean. Measurements were taken from 2009-2011 in the west half of the site, where the crop rotation cycle for these years was soybean-maize-soybean. Management of these crops was consistent with previously reported practices at the site (Leakey *et al.*, 2004; Morgan *et al.*, 2005), with the exception that no fall tillage was done after the 2010 maize crop so that the heating equipment could be kept in operation overwinter.

Elevated CO$_2$ and temperature treatments

The field was divided into four experimental blocks, each containing two 20-m octagonal rings spaced 100 m apart to avoid cross-contamination by fumigation gases. One ring in each block was maintained at ambient atmospheric conditions (approximately 390 ppm CO$_2$), while the other was fumigated to a target of 585 ppm CO$_2$ using *F*ree *A*ir *C*oncentration *E*nrichment (FACE) technology (Miglietta *et al.*, 2001). Elevated CO$_2$ was maintained from dawn until dusk throughout the growing season. The high-CO$_2$ rings in the study area have been fumigated every growing season since 2001.

Starting in 2009, each ring was further split by imposing an elevated temperature treatment. One 3-m hexagonal subplot of each ring was equipped with overhead infrared heaters as in Kimball (2005). The heaters were adjusted throughout the growing season to stay 1.4 m above the canopy and were controlled by infrared radiometers to maintain a difference of 3.5 °C between the canopy-top temperature of heated and unheated plots. When rain was falling and when soil temperature was ≤ 5 °C, heating was ineffective and heater output was therefore reduced to a minimum. The heating system operated continuously between June 2009 and September 2011 except during planting when all equipment was removed to allow field tillage, during harvest when power cables were removed to allow harvester traffic, and during a two-week period in January 2010 when the equipment was rebuilt to repair damage from rodents. Further details on the heating treatment are reported in (Ruiz-Vera *et al.*, 2013, 2015; Rosenthal *et al.*, 2014).

Measurement of soil properties and CO$_2$ efflux

CO$_2$ efflux from soil was measured at three locations in each plot using 20-cm diameter collars made from PVC pipe. At each location, one collar was inserted 3 cm into the soil to capture total soil respiration (R$_{tot}$), and a second collar was inserted 25 cm to capture respiration by soil heterotrophs (R$_{het}$) by excluding roots and rhizosphere: The top 30 cm of soil contain at least 70% of soybean and 60% of maize root mass (Mayaki *et al.*, 1976; Anderson-Teixeira *et al.*, 2013), so this root-exclusion collar acts as a small trenched plot (Vogel & Valentine, 2005). Collars were installed at crop emergence time each spring and left in place all year, then removed for field tillage just before the next year's planting. This annual reinstallation also eliminated several major limitations that apply to root-exclusion methods in untilled systems: it removed any accumulated difference in C or nutrient availability from previous years of root exclusion, and there was no need to correct for decomposition of roots severed during installation (Hanson *et al.*, 2000) because root biomass at installation was near zero. Respiration by roots and rhizosphere (R$_{aut}$) was calculated for each location as the difference between R$_{tot}$ and R$_{het}$.

CO$_2$ efflux rates were measured using an infrared gas analyzer (LI-8100; Li-Cor, Lincoln NE USA) fitted with a 20-cm static chamber (Li-Cor 8100-103) that rested on top of the soil collar. For each observation, the chamber was closed for two minutes while [CO$_2$] was logged every second. Linear regressions on static-chamber observations underestimate the initial flux rate (Healy *et al.*, 1996), so flux rates were computed in software by the LI-8100, which fit a saturating exponential curve of the form:

$$C_t = C_\infty + (C_0 + C_\infty)e^{-a(t-t0)};$$

where C_0 is [CO_2] at the moment the chamber closed, t is time, and a and C_∞ are fitted parameters representing curvature and [CO_2] at the asymptote, respectively. Evaluating the derivative of C_t at $t = 0$ then gives the instantaneous initial slope $a(C_\infty - C_0)$, which was scaled by the volume of the soil chamber to give CO_2 flux rate at the moment the chamber closed.

Using exponential rather than linear fits is especially important in a FACE setting, because it allows a further correction for initial chamber conditions: Respiration was measured while fumigation was active. Pure CO_2 is released from the upwind side of the ring and mixes to the target concentration as it is blown across the plot (Miglietta *et al.*, 2001), meaning that in elevated-CO_2 plots the flux chamber would sometimes close on a transient high-[CO_2] air mass (up to 2000 ppm). In these cases the CO_2 concentration gradient from soil to chamber air, and thus the rate of diffusion across the soil surface, was small. This meant that for these readings the fitted flux rate 'at the moment the chamber closed' was much smaller than the true equilibrium rate. We corrected this bias using a method recommended by Li-Cor Inc. that re-evaluates the previously fit [CO_2] curve to find the equilibrium flux $a(C_\infty - C_{target})$, where a and C_∞ are taken from the previous curve fit and C_{target} is the daily average [CO_2] in that ring (585 PPM for fumigated rings, 370-400 PPM for unfumigated rings).

Soil temperatures were measured simultaneously with each respiration measurement using a thermocouple probe inserted to 5 cm depth. Soil volumetric water content was measured from 5 to 105 cm depth 2-3 times each week using a capacitance probe and is reported elsewhere (Rosenthal *et al.*, 2014; Ruiz-Vera *et al.*, 2015).

Particulate organic matter (POM), which consists of fragmented but undecomposed plant matter and is used as a proxy for the abundance of labile soil C, was measured using a procedure modified from Marriott & Wander (2006). Briefly, air-dried soil was sieved to 2 mm and a 10-g sample was weighed into a 30-mL plastic bottle. The mouth of the bottle was covered with a 53-µm nylon mesh to retain POM and sand while allowing silt and clay particles to escape. The bottle was submerged in 5% sodium hexametaphosphate (HMP) and shaken for one hour, then the HMP and suspended fines <53 µm were removed, replaced with deionized water, and shaking was repeated until no further fine material was extracted. The remaining POM + sand was transferred to a pouch of 53-µm mesh, rinsed with DI water, dried at 30 °C, and weighed. Samples were then ground in a ball mill (Geno Grinder 2010; BT&C, Lebanon New Jersey, USA) and combusted to determine C content using an elemental analyzer (Costech ECS4010; Costech Analytical Technologies, Valencia, California, USA).

Statistical analysis

Analysis of variance for soil respiration was performed in a complete-block design using CO_2 as a whole-plot fixed effect and heat as a split-plot fixed effect nested within CO_2. Blocks were treated as random, and autocorrelation within plots from

repeated measurement through the season was estimated as a first-order autoregressive function. R_{tot}, R_{het}, and R_{aut} fluxes for each season were analyzed separately as mixed-effects linear models with repeated measures using the nlme and lsmeans packages in R 3.2.4 (Lenth, 2016; Pinheiro *et al.*, 2016; R Core Team, 2016). The date of each survey event was included as a categorical variable to account for within-season changes. Although most of the temporal variation is likely to be driven by weather and crop growth phase, the Day effect was treated as a catchall term and no explicit temperature or moisture covariates were included in the model. Because repeated measurements within the same plot are pseudo-replicates, the three flux measurements from each plot were averaged, giving n=4 observations per treatment in each day. Because experiments with few replicates have low power to detect small differences, we set a significance threshold of $p \leq 0.1$ to minimize the chance of false negative conclusions (Filion *et al.*, 2000). Full statistical output and data-processing scripts are available in the data package for this manuscript (Black *et al.*, 2016).

Modeling of soil respiration and soil organic carbon

Because a three-year heating experiment is likely too short to detect changes in SOC, we performed an *in silico* experiment using a process-based ecosystem model (DayCent; Parton *et al.*, 1998) to simulate the effects of a 100-year global change manipulation and better understand the long-term effects of elevated CO_2 and temperature on soil carbon dynamics. DayCent has been widely used to model soil C, N, P and S dynamics and trace gas fluxes. It has been particularly well-validated for crop and grassland systems, and is straightforward to modify for predicted future conditions,

making it ideal for simulations of the future ecosystem effects of climate and/or land-use changes (Davis *et al.*, 2010, 2012; Hartman *et al.*, 2011). DayCent model development has been closely tied to previous global change experiments and its input parameters are designed for easy calibration against experimentally measured responses (Parton *et al.*, 2007; Frey *et al.*, 2013).

To predict the medium- and long-term effects of ongoing ecosystem warming and elevated CO_2 on soil carbon cycling, we performed a three-part set of DayCent simulations to simulate the historic development of the SoyFACE site from native prairie into a maize-soy rotation, extended this simulation through the 21st century, then ran the model four times using all factorial combinations of elevated CO_2 and heat.

To calibrate the size and turnover rates of soil C pools, the model was first run to equilibrium by simulating a native tallgrass prairie at pre-industrial $[CO_2]$ of 294 PPM. Each simulation lasted 3867 years and looped over a weather file made by randomly ordering the years of an 1889-2009 temperature and precipitation record for Urbana, Illinois (Angel, 2010b). Vegetation for the spin-up period used prairie grass parameters provided by Hudiburg et al. (2015), with autumn burning every 5th year and low-intensity grazing by bison (10% of foliage removed 3 times per growing season). Soil parameters were based on bulk densities and organic matter contents of undisturbed Illinois prairie remnants (David *et al.*, 2009), and on physical properties of the Flanagan and Drummer soil series (NRCS, 2012). To match the high-moisture conditions predominant in Central Illinois before the introduction of artificial drainage, a standing

water table was simulated from January through May. Soil organic matter turnover times were adjusted to produce steady-state (<1% change per decade in last 100 yr) SOM C and N of 10450 and 760 g m^{-2}, respectively, in the top 20 cm (Figure S1). These totals are comparable to those measured in tallgrass prairie remnants on deep, mesic soils throughout the Midwest (Aref & Wander, 1998; Kucharik *et al.*, 2006; Matamala *et al.*, 2008; Brye & Riley, 2009; David *et al.*, 2009; Jelinski & Kucharik, 2009) and were achieved using turnover rates for the active, intermediate, and slow soil C pools of 11, 0.1, and 0.002 yr^{-1}, giving residence times of 33 days, 10 years and 500 years, respectively.

Annual rows crops were simulated beginning in 1868, the year our site was first recorded as occupied by European settlers. To simulate the change from an untilled, seasonally wet prairie to a tile-drained, annually-tilled crop system, we ceased simulating a standing water table, increased the maximum decomposition rate of intermediate- and slow-turnover organic matter, and reduced leaching rates for N and OM (Table S1). Additionally, we reduced the rate of nonsymbiotic soil N fixation and the fraction of mineralized N lost to nitrification to better match conditions observed in row crop systems (Table S1). Site-specific parameters were based on soil conditions measured at the site (Peralta & Wander, 2008; C. Black, unpublished data; J. Jastrow, unpublished data; Moran & Jastrow, 2010), soil moisture measured at the site (S. B. Gray, unpublished data; Rosenthal *et al.*, 2014; Ruiz-Vera *et al.*, 2015), and historical weather data from the Illinois State Water Survey (Angel, 2010b). Atmospheric [CO_2] was increased linearly to match the rise in industrial fossil fuel burning, from 294 ppm in

1868 to 370 ppm in 2000. Crop-specific parameters for maize and soybeans were developed by Hudiburg et al. (2015) to match the rate and physiological mechanisms of 20th-century crop genetic improvements: maize yield gains have come mostly from increases in planting density and photosynthetic capacity (Duvick, 2005), so we simulated an increase in the maximum daily biomass production rate, with minor adjustments to other parameters (Table S3). In contrast, soybean yield increases have come mostly from improved yield partitioning at constant plant size (Koester *et al.*, 2014), so our soybean parameters were constant except that we increased the maximum harvest index in 1950 and 1980.

The management history of the site before 1980 was inferred from records of crop acreage and fertilizer usage for Champaign County retrieved from the National Agricultural Statistics Service (NASS, 2011). Site management since 1980 is well described (Moran & Jastrow, 2010) and was simulated accordingly (Table S2). Briefly, management progressed from low-yielding mixed maize/oat/pasture in 1869 through increasingly intensive cropping and fertilization to a maize-soybean-oat rotation by 1935 and a maize-soybean rotation by 1950, with fertilization rates and cultivar parameters adjusted each decade to match NASS records. Beginning in 1970 we changed cultivation from moldboard plow every spring and fall to chisel plowing each spring and in fall after maize only, and fertilization rates were held steady at 157 kg N Ha^{-1}. This management schedule was continued though 1999, then concluded in 2000 with one year of winter wheat (Moran & Jastrow, 2010).

To simulate the SoyFACE climate change manipulations, we extended the 20th-century simulation for the years 2001-2109 using the actual 2001-2011 planting and harvest dates of the SoyFACE experimental field. Weather data for 2001-2011 was retrieved from DAYMET (Thornton *et al.*, 2014) and the model was run four times: a control run with actual weather conditions and $[CO_2]$ set to 370 ppm (ambient conditions at the initiation of the SoyFACE experiment), a CO_2-only run with $[CO_2]$ increased by 200 ppm to 570 ppm as a step change in 2001, a heat-only run with daily maximum and minimum temperatures increased 3.5 °C as a step change in 2009, and a heat+CO_2 run with both temperature and CO_2 increased. Note that we did not simulate any further increase in $[CO_2]$ after the step change, so for all model-data comparisons we treated values modeled at 370 or 570 ppm as equivalent to field values observed in 2009-2011 at ~390 or 585 ppm.

Our model calibration strategy was to use the performance of our spin-up and historic hindcast scenarios as indicators of correct parameter calibration, then run the climate change scenarios with no further changes in model tuning. To the extent that model hindcasts do match known conditions, we gain confidence that model predictions for the future are reasonable. To evaluate model performance in more detail, we compared modeled soil temperature, moisture, and respiration rates against our 2009-2011 field observations. We also compared modeled aboveground biomass and grain yields for 2001-2008 against detailed phenological measurements from SoyFACE, using a database compiled by Twine et al (2013). All model parameters and analysis scripts are available online (Appendix 1; Black *et al.*, 2016).

Results

Temperature and CO_2 manipulation

Infrared heating produced a mean temperature increase of approximately 3.1, 2.7, and 2.6 °C at the top of the canopy in 2009, 2010, and 2011, respectively, and CO_2 fumigation consistently maintained ~585 ppm CO_2 during daylight hours (Ruiz-Vera *et al.*, 2013, 2015). Soil temperature at 5-cm depth was increased by 1.8 ± 0.2 °C (mean $\pm$ standard error of daily differences) during the growing seasons (Figure 1). During fallow seasons, heater output was intermittently reduced during extreme cold snaps (less than 16% of total time) but soil temperature for the whole season was still increased by 2.3 ± 0.1 °C (Figure 1). There was no consistent difference in soil temperature between eCO_2 and unfumigated plots given the same heat treatment.

Soil respiration

Overall, heating caused a consistent and large increase in R_{het} but reduced R_{aut} by a similar degree, producing no appreciable net effect of heating on R_{tot}, while eCO_2 increased R_{het} and affected R_{aut} differently each year, with the net effect of a small stimulation in R_{tot} from eCO_2. Averaged across the entire experiment, R_{het} was higher than control by 16, 12, and 48% in the eCO_2, heat, and heat+CO_2 treatments, respectively. R_{aut} was slightly (3%) higher in eCO_2 and lower in heated plots by 21% (heat) and 31% (heat+eCO_2). R_{tot} was higher in eCO_2 treatments by 11% (unheated eCO_2) and 13% (heated eCO_2) but 3% lower in the heated ambient CO_2 treatment (Figure 2).

Separate mixed-model analyses of respiration from each season (Table 1) showed that under soybeans in 2009, R_{tot} was unchanged while R_{het} increased and R_{aut} decreased in both heat and eCO_2 treatments. Under maize in 2010, CO_2 increased R_{tot}, heat increased R_{het}, and there were no differences in R_{aut} between treatments. Under soybeans in 2011, there were no differences in R_{tot} between treatments while R_{het} was higher and R_{aut} was lower in heated plots. R_{het} also showed a three-way interaction between Heat, CO_2, and Day, with higher R_{het} from heated eCO_2 plots on June 24 and July 18 but no statistical difference between treatments on the other days of the season (Figure 3). During the fallow period following soybeans (winter 2009-2010), R_{aut} and R_{tot} did not differ between treatments while R_{het} was higher in eCO_2 plots and had an interactive effect with heat and day, with a trend ($p < 0.12$) for higher R_{het} from heated plots on October 7 and December 31, lower R_{het} from heated plots on December 10, and no statistical difference on the other days. During the fallow period following maize (winter 2010-2011), no component of soil respiration (R_{tot}, R_{het}, R_{aut}) differed between treatments. The main effect of Day was significant in all treatments every season, while the heat by CO_2 and CO_2 by Day interactions were never significant.

Particulate organic matter

POM-C declined from the beginning to the end of the experiment (2009 > all other harvests; Tukey HSD $p < 0.01$; Figure 4) and was approximately 14% lower in eCO_2 plots than in ambient plots (ANOVA F=7.69, $p < 0.01$; Figure 4), but showed no statistically resolvable difference between heated and unheated plots (ANOVA F=0.29, $p > 0.5$). Averaged across all treatments, the top 30 cm of soil contained 588 ± 41 g POM C

m^{-2} (mean ± SE) in Spring 2009, 439 ± 21 in Spring 2010, 444 ± 25 in Spring 2011, and 457 ± 22 in Fall 2011.

DayCent model

DayCent simulations of 20th-century grain yields of maize and soybeans agreed well with historic crop yields from Champaign County and captured about half of the observed year-to-year variation in yield (Figure S2 & Figure S3; root-mean-square error = 82.6 g C m^{-2}, RMSE/mean = 0.53). Modeled total C and N in soil organic matter at the end of the historic agriculture scenario were both very near the values measured at SoyFACE (Figure S1). During the 2001-2008 CO_2 simulation, the temporal dynamics of modeled aboveground biomass within each season matched well with values observed at SoyFACE in those years (Figure S4). Observed soybean grain yields at the site averaged 191 ± 30 g C m^{-2} in ambient plots and 212 ± 39 in eCO_2 plots (Twine *et al.*, 2013); modeled yields for the same years were 194 ± 65 and 254 ± 75 g C m^{-2}, respectively. Observed maize yields averaged 423 ± 15 g C m^{-2} in ambient plots and 412 ± 44 in eCO_2 plots (Leakey *et al.*, 2006; Markelz *et al.*, 2011; Ruiz-Vera *et al.*, 2015); modeled yields for the same years were 432 ± 38 and 460 ± 22 g C m^{-2}, respectively.

Modeled effects on soil temperatures were somewhat higher than the observed differences, with heated model runs 4.1 ± 0.6 °C (mean ± sd) warmer than unheated runs at 5-cm depth during the growing season, while observed differences were less than 2 °C. Additionally, modeled soil temperature differences dropped to 3.5 °C before planting and after harvest, while observed differences were larger then than during the growing season

(~2.5 °C; Figure 1). Modeled temperatures in elevated CO_2 model runs were 0.5 ± 0.4 °C lower than those in ambient runs, but no consistent differences were observed in the field (Figure 1).

Compared to the values observed in the field, DayCent captured the seasonal variation and relative timing in all components of soil respiration, matched its magnitude well for R_{het}, and consistently underpredicted R_{aut}, producing a smaller overall under prediction of R_{tot} (Figure S5).

When the simulation was extended to 100 years of heating and elevated $[CO_2]$, DayCent predicted that CO_2 would increase all soil C pools, producing an increase of about ~4% in total SOM C (Figure 5). In contrast, heating was predicted to produce a rapid drop in all pools that overwhelmed the increased C inputs from eCO_2, producing a loss from heated runs, relative to the control scenario, of 15% of total soil C in the top 20 cm by 2109 (Figure 5).

Discussion

Experimental manipulation of CO_2 and temperature conditions similar to those expected for the mid-21st century increased respiration from soil microbes, likely indicating a drawdown of both labile and protected soil C. This effect would not have been detectable without partitioning respiration into its root and microbial components, and it was not offset by the observed positive effect of CO_2 on plant productivity. Instead, CO_2 increased respiration and reduced POM C, and we saw no evidence of interactions between heat and CO_2 responses, leading us to speculate that eCO_2 primed long-term

losses of SOC. Our model results adequately captured the effects of temperature and the additive effect of the temperature and CO_2 responses, but did not reproduce a CO_2 priming effect, so actual soil C losses may exceed our model-predicted value of 15% in 100 years.

Our results support the prediction that elevated temperature would increase the activity of soil heterotrophs and that this increased respiration would lead to long-term losses of soil C. Three years of heating produced strong and persistent increases in R_{het} (Figure 2; Table 1) and our model results are consistent with these increases leading to losses of C from all soil pools (Figure 5). Because heating reduced R_{aut} simultaneously with increasing R_{het}, there was no change in total soil respiration with heat (Figure 2; Table 1). This result contrasts with many previous studies (reviewed in Rustad *et al.*, 2001; Wang *et al.*, 2014) where R_{tot} increased under heating except under water limitation, but this discrepancy is explained by partitioning fluxes into their autotrophic and heterotrophic components.

Consistent with recent meta-analyses (Dieleman *et al.*, 2012; Wang *et al.*, 2014), we found that R_{het} responded reliably to increased temperature even when opposing changes in R_{aut} masked its effect on R_{tot}. This highlights the importance of separating soil CO_2 fluxes in global change experiments into their root and microbial components. We speculate that this masking may also occur at other sites where R_{tot} was measured without partitioning and found unresponsive to heat, especially those in grasslands and crops where root activity seems less responsive to eCO_2 than in forest systems (Wang *et al.*, 2014).

Our results did not support the prediction that elevated CO_2 would increase plant biomass above and belowground, leading to higher C inputs that would at least partially ameliorate the long-term effect of heat on soil C. Although aboveground plant biomass was higher in eCO_2 plots during soybean years, heating largely negated this difference (Ruiz-Vera *et al.*, 2013) and there was little difference in root mass (S. B. Gray, in prep). R_{het} increased more in heat+eCO_2 plots than in plots given heat alone, indicating increased respiratory losses. Meanwhile R_{aut}, a probable correlate of C inputs from root exudation and turnover, was lower in eCO_2 plots in 2009 and showed no detectable change in other years, and the change in R_{aut} between heat and heat+eCO_2 plots was similar to that between unheated control and eCO_2 plots. One possible explanation for these findings is that the extra C inputs from eCO_2 were priming the breakdown of existing soil C, as seen previously at this and other FACE sites across widely differing ecosystem types (Peralta & Wander, 2008; Moran & Jastrow, 2010; Carrillo *et al.*, 2011; Drake *et al.*, 2011; Hopkins *et al.*, 2014; Fang *et al.*, 2015), rather than offsetting the effect of heat.

The priming hypothesis also is consistent with our observation that POM-C declined from 2009 to 2011 and was lower in eCO_2 plots than in unfumigated plots (Figure 4). The lack of an increase in POM-C with eCO_2 at this site was noted previously and attributed to priming by Peralta *et al.* (2008) after 3 years of fumigation, but it is worth noting that in year 3 the difference in POM-C between treatments was not yet significant. Given that the CO_2 priming effect required most of a decade to become statistically resolvable, it is perhaps not surprising that 3 years of heating did not produce a detectable change in POM-C.

Compared to other experiments that have examined the simultaneous effects of heating and eCO_2 on soil C dynamics, SoyFACE is notable for showing no obvious heat $\times CO_2$ interactions. Although unheated FACE experiments have commonly showed direct effects of eCO_2 on soil respiration (Pendall *et al.*, 2003; Pregitzer *et al.*, 2008; Adair *et al.*, 2011; Drake *et al.*, 2011; Lam *et al.*, 2014), many heat $\times CO_2$ experiments are dominated by indirect effects (Dieleman *et al.*, 2012), which seem to be mediated by the joint effects of CO_2 and heating on soil water availability (Wan *et al.*, 2007; Pendall *et al.*, 2011, 2013; Selsted *et al.*, 2012). Our site, by contrast, showed no significant heat $\times CO_2$ interactions, perhaps because the site is only rarely dry enough to limit respiration. Water content was consistently higher in eCO_2 plots and was lower in heated plots in 2009 and on some days in 2010, but volumetric water content never dropped below 20% and the differences in soil water were not significant in 2011, the driest summer of the study (Rosenthal *et al.*, 2014; Ruiz-Vera *et al.*, 2015). Thus the effects of water availability on R_{het} appear to be additive to the heat effect, not a driving mechanism.

The observed changes in R_{aut} may be caused by differences in root distribution. We have no evidence of changes in total root biomass, however minirhizotron observations from maize in 2010 suggest that elevated CO_2 affected the depth distribution of roots, with greater root length in shallow soils and lower root length in deeper soils, but the effects depended on temperature treatment (S. B. Gray, in prep). In 2009 soybeans in heated plots appeared to use deeper soil water (Rosenthal *et al.*, 2014), possibly indicating a shift of roots toward deeper soil that would have reduced the amount of root-respired C reaching the surface, thus contributing to the reduction in R_{aut} from soybeans we observed that year.

The empirical results from this study are reinforced by forward extrapolations from a process based model which indicates that heat, either singly or combined with increased CO_2, will drive long-term losses in SOC from agricultural soils, adding to losses in SOC caused by aggressive tillage practices. This result is consistent with previous models of $CO_2 \times$ warming experiments in predicting a net loss of soil C under global warming (Parton *et al.*, 2007).

Given the difficulty of inferring SOC changes from short-term direct measurements and the number of known processes that DayCent integrates, we posit that these model results provide our best available prediction of the *direction* of future SOC trajectories in a warming climate, and that that they place a lower bound on the *magnitude* of future losses as CO_2 increases. However, our modelling approach was unable to test the hypothesis of a priming effect of eCO_2 on soil C breakdown, because DayCent's SOC model has an explicitly specified turnover time for each pool. Turnover can be manually increased to simulate priming (Cheng *et al.*, 2013), but this requires a known degree of increase. Our observation that POM-C declined under elevated CO_2 gives an indirect indication that turnover rates have increased, but is not sufficient to constrain the magnitude of the increase, especially in slower-cycling C pools. Instead, increasing model C inputs through CO_2 fertilization lead to an increase in modeled SOM with no decrease in fast C pools that would match our observed drop in POM-C. Therefore our model results probably understate the extent of soil C losses under elevated CO_2. To produce more accurate long-term predictions of SOC dynamics under systems with active priming, models with explicit microbial processes may be needed (Wieder *et al.*, 2013).

Elevated CO_2 and temperature, both singly and in combination, appear to accelerate the loss of soil C from agricultural ecosystems, through probably distinct and potentially additive pathways. Simple measurements of whole-soil respiration were not sufficient to detect these changes, so future experimental work should routinely include partitioning of soil respiration into plant-derived and SOM-derived components. Robust predictions of CO_2 priming effects will require updated ecosystem models that contain explicit microbial dynamics.

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Table 1: Mixed effects model results for rates of soil CO_2 efflux attributed to soil heterotrophs (R_{het}), plant roots and rhizosphere organisms (R_{aut}), and whole soil (R_{tot}). The first six columns show P values for each effect. Boldface values are significant at a preselected threshold of 0.1. The main Day effect was always significant (all $P < 0.02$) and is not shown here to save space. The last two columns show percent change from control for each treatment, presented as the estimated differences ± 1 standard error of whole-season LS means.

Component	Heat	CO_2	Heat × CO_2	Heat × Day	CO_2 × Day	Heat × CO_2 × Day	Heat % change	CO_2 % change
Soy 2009								
R_{aut}	**0.014**	**0.089**	0.552	0.737	0.717	0.988	-53 ± 15	-41 ± 17
R_{het}	**0.035**	**0.068**	0.271	0.790	0.495	0.724	32 ± 11	31 ± 11
R_{tot}	0.475	0.920	0.636	0.859	0.389	0.932	-6 ± 7	0 ± 8
Fallow 2009-2010[1]								
R_{aut}	0.465	0.915	0.845	0.909	0.922	0.899	26 ± 50	-6 ± 43
R_{het}	0.875	**0.097**	0.879	**0.053**	0.775	0.934	5 ± 7	14 ± 7
R_{tot}	0.315	0.424	0.776	0.869	0.915	0.901	12 ± 12	8 ± 12
Maize 2010								
R_{aut}	0.716	0.806	0.917	0.561	0.317	0.277	-8 ± 19	6 ± 20
R_{het}	**0.053**	0.137	0.181	0.704	0.632	0.931	20 ± 8	28 ± 15
R_{tot}	0.263	**0.059**	0.226	0.687	0.642	0.605	8 ± 7	20 ± 7
Fallow 2010-2011								
R_{aut}	0.532	0.796	0.216	0.940	0.339	0.231	-17 ± 30	1 ± 43
R_{het}	0.740	0.800	0.687	0.636	0.310	0.752	6 ± 12	1 ± 12

R_{tot}	0.771	0.953	0.381	0.977	0.204	0.117	-2 ± 11	2 ± 17
Soy 2011								
R_{aut}	**0.018**	0.190	0.864	0.690	0.794	0.381	-48 ± 15	57 ± 33
R_{het}	**0.047**	0.224	0.700	0.801	0.739	**0.091**	27 ± 11	18 ± 12
R_{tot}	0.131	0.252	0.824	0.906	0.822	0.997	-11 ± 6	29 ± 21

[1]Data from March 1, 2010 were excluded from the model because they contained no usable observations from heated plots.

Figure 1: Temperature measured 5 cm below the soil surface at SoyFACE between June 2009 and October 2011. Error bars show treatment means ± 1 standard error for each day.

Figure 2: Seasonal means of CO_2 flux measured from plant roots and rhizosphere (R_{aut}; a), soil heterotrophs (R_{het}; b), and whole soil (R_{tot}; c) at SoyFACE between June 2009 and October 2011. Error bars show treatment LS means ± 1 standard error for each season. Within each season, treatments that share a letter are not statistically different ($P > 0.1$).

Figure 3: CO_2 flux measured from plant roots and rhizosphere (R_{aut}; a), soil heterotrophs (R_{het}; b), and whole soil (R_{tot}; c) at SoyFACE between June 2009 and October 2011. Each season was analyzed separately; vertical grey lines indicate cutoffs between seasons. Error bars show treatment LS means ± 1 standard error for each day.

Figure 4: Particulate organic matter carbon (POM-C) in the top 30 cm of soil at SoyFACE, sampled in spring of 2009 (a), 2010 (b), 2011 (c) and at the end of the experiment in fall 2011 (d). Boxes cover the estimated interquartile range of each group, whiskers extend to the smaller of max/min or 1.5 IQR.

Figure 5: Mean annual values for total soil organic matter (g C m^{-2}) predicted by DayCent in the top 20 cm of SoyFACE soil.

Soil CO$_2$ efflux, µmol m^{-2} sec^{-1}

R_{aut}

R_{het}

R_{tot}

(b)

(a)

(c)

Control   Heat
CO$_2$ Heat+CO$_2$

Soy   Fallow   Maize   Fallow   Soy

2009-07   2010-01   2010-07   2011-01   2011-07

Date