1 Soil carbon responses to past and future CO₂ in three Texas prairie soils

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Abstract

Changes in soil carbon storage could affect and be affected by rising atmospheric CO₂. However, it is unlikely that soils will respond uniformly, as some soils are more sensitive to changes in the amount and chemistry of plant tissue inputs whereas others are less sensitive because of mineralogical, textural, or microbial processes. We studied soil carbon and microbial responses to a preindustrial-to-future CO₂ gradient (250-500 ppm) in a grassland ecosystem in the field. The ecosystem contains three soil types with clay fractions of 15% to 55%: a sandy loam Alfisol, a silty clay Mollisol, and a black clay Vertisol. Soil and microbial responses to atmospheric CO₂ are plant-mediated; and aboveground plant productivity in this ecosystem increased linearly with CO₂ in the sandy loam and silty clay. Although total soil organic carbon (SOC) did not change with CO₂ treatment after four growing seasons, fast-cycling SOC pools increased with CO₂ in the two clay soils. Microbial biomass increased 18% and microbial activity increased 30% across the CO₂ gradient in the black clay (55% clay), but neither factor changed with CO₂ in the sandy loam (15% clay). Similarly, size fractionation of SOC showed that coarse POM-C, the youngest and most labile fraction, increased four-fold across the CO₂ gradient in the black clay, but increased by only 50% across the gradient in the sandy loam. Interestingly, mineral-associated C, the oldest and most recalcitrant fraction, declined 23% across the gradient in the third soil type, a silty clay (45% clay). Our results provide evidence for priming in this soil type, as labile C availability and decomposition rate (measured as soil respiration and soil C mineralization) also increased across the CO₂ gradient in the silty clay soil. In summary, CO₂ enrichment in this grassland increased the fast-cycling SOC pool as in other CO₂ studies, but only in the two high-clay soils. Priming in the silty clay could limit SOC accumulation after prolonged CO₂ exposure. Because soil texture varies geographically,

41 including data on soil types could enhance predictions of soil carbon and microbial responses to
42 future CO₂ levels.

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44 **Keywords:** elevated CO₂, gradient, grassland, soil carbon, decomposition, soil type

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1. Introduction

Globally, soils contain about three times as much carbon (C) as the atmosphere and are the largest terrestrial pool of C (Schlesinger, 1997; Jobbágy and Jackson, 2000). Because C cycles between the atmosphere and soil through photosynthesis and respiration, changes in physiological processes could affect atmospheric CO₂ levels by altering soil C pools. Conversely, changes in atmospheric CO₂ could affect soil C storage through changes in plant and microbial activities. Whether soils will be a CO₂ source or sink under future CO₂ levels remains uncertain (Pepper et al., 2005). Some of this uncertainty surrounds the mechanisms by which soils sequester C and the effects of elevated CO₂ on these mechanisms.

There are several hypothetical outcomes for soil C under elevated CO₂. One is an increase in soil C due to CO₂-induced plant growth. Increased plant growth leads to greater rhizodeposition into soil (Hungate et al., 1997), some of which becomes stabilized as soil organic carbon (SOC) (Fig. 1). Some meta-analyses have found that soil C increases by about 6 % in elevated CO₂ experiments (Jastrow et al., 2005; Luo et al., 2006). Alternatively, soil C could decrease as atmospheric CO₂ rises if decomposition rates also increase. Soil CO₂ efflux (soil respiration), a measure of decomposition, commonly increases at elevated CO₂ (Zak et al., 2000; Jackson et al., 2009). Some of this increase could be due to priming, in which labile C released from roots stimulates microbial growth and the decomposition of older soil organic matter (Fu and Cheng, 2002; Carney et al., 2007; van Groenigen et al., 2014). Because older soil organic matter represents the majority of SOC, priming could limit long-term soil C sequestration. Finally, soil C could remain unchanged at elevated CO₂. Increases in plant C input could be balanced by increases in decomposition, or gains in new SOC pools could be offset by losses in older SOC pools (Sayer et al., 2011). The latter mechanism was found in a grassland CO₂

enrichment experiment after four years of CO₂ treatment; the most labile fractions of SOC increased, whereas the most recalcitrant fractions decreased, leading to a negligible increase in total SOC (Gill et al., 2002; Gill et al., 2006). It is also possible that SOC responses to CO₂ are simply too small to detect, which was the conclusion of a recent meta-analysis (Hungate et al., 2009).

When soil C does respond to elevated CO₂, the response shape is not well known. Most CO₂ experiments include only an elevated and ambient treatment, so it is uncertain whether soil C cycling will change linearly as atmospheric CO₂ rises or will instead show a threshold response. Given the large change in atmospheric CO₂ levels that terrestrial ecosystems have experienced during the past 250 years, and the range predicted for this century, it is useful to study ecosystem responses to CO₂ under a range of CO₂ levels. Ecosystem experiments involving a range of CO₂ levels to date include chaparral (Treseder et al., 2003), wheat (Polley et al., 1993) and grasslands (Ross et al., 2000; Gill et al., 2002; Gill et al., 2006). Many of these studies showed an increase in soil C with CO₂ concentration up to ~500-600 ppm, followed by plateau or decline at higher CO₂ concentrations.

Most CO₂ experiments test one soil type, so it is also not clear how soil type influences CO₂ effects on plant productivity, decomposition, and soil C. Soil texture affects productivity and decomposition through factors such as particle surface area and porosity, which influence water holding capacity, cation exchange capacity, and many other factors. Finer-textured (higher silt + clay) soils have greater surface area, allowing greater water and nutrient retention. As a result, they tend to have greater plant productivity in temperate climates (Sala et al., 1988; Reich et al., 1997; Brady and Weil, 2002). Soils with higher silt and clay content also generally sequester more C than sandier soils, with maximum C sequestration at intermediate-to-high

values of silt + clay content (>60% silt + clay) (Burke et al., 1989). This occurs because decomposition is generally slowest in the finest-textured soils, where lower O₂ inhibits aerobic microbial processes, and organic matter is physically protected by clay-humus complexing and soil aggregation (Bosatta and Agren, 1997; Krull et al., 2001; Brady and Weil, 2002). SOC content is positively related to soil clay content both at a global scale (Jobbágy and Jackson, 2000) and a landscape scale (Plante et al., 2006). Soil C models typically use clay content or cation exchange capacity to simulate soil texture effects on physical protection of organic C (Krull et al., 2001; Dungait et al., 2012).

We address how soil type affects the responses of productivity and soil C to CO₂, as well as mechanisms behind soil C dynamics. We studied soil C dynamics in a prairie ecosystem on three soils of contrasting texture (sandy loam, silty clay, clay) that were exposed to a continuous gradient of preindustrial-to-future CO₂ levels (250-500 ppm) in the field. We hypothesized that CO₂-induced plant growth would peak on the intermediate (silty clay) or highest-clay (black clay) soil. We expected that these soil types would provide plants with more water and nutrients, allowing higher growth at elevated CO₂. In contrast, we hypothesized that CO₂ stimulation of decomposition would decrease in finer textured soils. Clays physically protect SOC from decomposition, a dampening effect on substrate-induced decomposition with elevated CO₂. In sum, we expected that the CO₂-induced increase in soil C sequestration would be a positive function of soil clay content, with greatest C sequestration in the silty clay or black clay and the least in the sandy loam.

2. Materials and Methods

2.1. Study system. The research was conducted at the Lysimeter CO₂ gradient (LYCOG) facility operated by the USDA-ARS Grassland Soil and Water Research Laboratory in Temple, TX (Fay et al., 2009; Fay et al., 2012; Polley et al., 2012a). The facility consists of two elongated tunnel-shaped chambers covered in clear plastic. Each chamber is 1.2 m wide and tall and 60 m long. Photosynthesis during daylight and respiration at night were used to create CO₂ gradients. Desired CO₂ concentration gradients were maintained by automatically varying the rate of air flow through chambers in response to changes to photosynthesis (daylight) or respiration rates (night). CO₂ is injected into air entering one tunnel to initiate an elevated to ambient CO₂ gradient (510-380 ppm). Ambient air is introduced into the second tunnel to create an ambient to subambient CO₂ gradient (380-250 ppm). Night-time CO₂ concentrations were regulated at 130-150 ppm above daytime values along each chamber. Cooling coils installed at 5-m intervals along the chambers prevented a temperature gradient from forming. Soil monoliths within chambers were planted with a mixture of perennials--grasses and forbs--representative of the Texas Blackland Prairie located at the site (Table S1). Three soil types were installed along the gradient: a sandy loam Alfisol (Bastil series) with as much as 15% clay and 60-73% sand in the upper 50 cm, a silty clay Mollisol (Austin series) with up to 45% clay in the upper 50 cm and a black clay Vertisol (Houston series) with as much as 55% clay in the upper 50 cm. These three soils span much of the clay content range among elevated CO₂ experiments on soil C (Fig. 2, Tables S2, S3). Bastil soils (fine-loamy, siliceous, active, thermic Udic Paleustalfs) are made from alluvial sediments and commonly occur on stream terraces. Austin soils (fine-silty, carbonatic, thermic Udorthentic Haplustolls) are found in erosional uplands. Houston Black soils (very-fine, smectitic, thermic Udic Haplusterts) are heavy shrink-swell clays found in lowland areas. Soils were excavated in 2002 as intact monoliths (1 m x 1 m

x 1.5 m deep) from sites near the facility, placed into the two chambers, and herbicide was applied to remove existing vegetation. In 2003, a standardized mixture of perennial grasses and forbs was transplanted into the monoliths, representing Texas Blackland Prairie (Table S1). Soils were watered with a metered drip irrigation system using temporal and volume patterns from an average year in central Texas (Fay et al., 2009; Polley et al., 2012a). Mean annual precipitation is 914 mm (1977-2000). The July-August mean maximum temperature is 35°C, and the December mean minimum is 2.9°C. CO₂ treatment began in 2006, and typically extends from April-October, the portion of the growing season during which photosynthesis is sufficiently great to maintain the CO₂ gradient.

2.2. Aboveground Net Primary Production (ANPP). ANPP was measured at the end of each growing season of CO₂ treatment by clipping vegetation to 5 cm height (Polley et al., 2012a). (ANPP data in this paper is from November 2008 and 2009.) Harvested biomass was oven dried for 72 h at 60°C before weighing. Each following January, harvested biomass was returned to its respective monolith along the CO₂ gradient to minimize harvest effects on element cycling. Biomass was shredded with a wood chipper before it was returned to plots to simulate the effect of late-season mowing.

2.3. Soil sampling and C and N measurement. Soils from the CO₂ gradient were sampled at the end of the fourth growing season of CO₂ treatment (Nov 9, 2009). Soil was cored to 15 cm depth, shipped to Duke University (Durham, NC) and air dried. Soils were broken into an aggregate size allowing passage through a 5.6-mm sieve, then aggregates were passed through a 2-mm sieve to remove roots. Soil C was determined by combustion gas chromatography (NC 2100 Soil analyzer, ThermoQuest Italia, S.p.A., Italy). Air-dried, homogenized soil (50-70 mg)

was combusted at 600°C for organic C and at 1050°C for total C and total N. Carbonates make up the difference between organic C and total C.

Subsamples of the fresh November 2009 soil cores were also shipped to Brigham Young University (Provo, UT) for physical fractionation, which provides a convenient index of soil C fractions of varying age (Gill et al., 2002; Gill, 2007). First, soils were sieved to 2 mm to remove root biomass. Soil was then dispersed for 18 hours in 0.5 M sodium hexametaphosphate, and the resulting soil slurry was passed through 250 µm and 53 µm sieves. The organic matter collected on these sieves is considered particulate organic matter (POM), and has been shown to have a residence time between a decade and a century, similar to the “slow” or “intermediate” pools in simulation and conceptual models (Six et al., 2002; Gill, 2007; Stewart et al., 2008). Coarse particulate organic matter (POM) C (>250 µm) represents the youngest organic carbon, with residence time shorter than a decade. Fine POM C (53-250 µm) is typically less than a century old. Mineral-associated C (< 53 µm) is the most recalcitrant size fraction, centuries to millennia old (Cambardella and Elliott, 1992; Leavitt et al., 1996; Kelly and Burke, 1997). Mineral-associated C was collected from the soil slurry that had passed through the 53 µm sieve. Each fraction was ground, then acid-treated to remove carbonates. Acid treatment represents an alternative to the combustion method for separating organic from inorganic C; both methods yielded similar total organic C. Soil organic C stocks were estimated using organic carbon concentrations from fractionation and bulk density of each soil type.

2.4. Microbial biomass determination. Active microbial biomass in soils across the CO₂ gradient was determined by substrate-induced respiration (SIR), following the methods of Gill et al. (2006; 2007). SIR is an index of potential microbial activity in the absence of substrate limitation. In preparation for SIR, air-dried soils from the November 2009 cores were re-

moistened to 60% field capacity and pre-incubated for 9 days. Two grams of soil were weighed into 40 mL glass vials with septa. Ten milliliters of 4 g L⁻¹ autolysed yeast solution were added to the soil as a substrate, then vials were capped and headspace CO₂ was measured immediately. Headspace CO₂ was measured again at 2h and 4h using a LI-6200 portable photosynthesis system and LI-6250 CO₂ analyzer (LI-COR, Lincoln, NE). Respiration rate was determined based on the change in headspace CO₂ over these 4h. The rise in headspace CO₂ was approximately linear over this time. Between measurements, vials with the yeast-soil slurry were placed on a table shaker at room temperature (21°C). Microbial biomass was calculated from SIR according to the equation: SMBC = (40.04 x CO₂) + 0.37, where SMBC is soil microbial biomass carbon (µg C g⁻¹ soil) and CO₂ is the respiration rate after substrate addition (µL CO₂ g⁻¹ soil h⁻¹) (Anderson and Domsch, 1978; Bailey et al., 2002).

2.5. Carbon mineralization incubations. The remaining soil (not subjected to SIR) was pre-incubated for three more days (for 12 days total preincubation), then incubated in the lab for one year to assess long-term carbon mineralization (C_{min}) kinetics from along the CO₂ gradient. A one week preincubation before long-term incubation reduces artifacts of previously dried soils (Paul et al., 2001). Soils were maintained in the dark, at ambient (room) CO₂ concentration, with constant moisture and temperature to isolate the effect of native microbial biomass and available carbon on decomposition rates. Soils were incubated at room temperature (21°C) in 1 pint (473 mL) canning jars with septa added to their lids (Paul et al., 2001; Gill, 2007). Each jar contained a plastic cup with 40 g (dry mass) soil moistened to 60% field capacity. Field capacity was determined using soil of the same series (Bastisil, Houston, and Austin), from locations that were the source of soils used in the CO₂ gradient (V Jin, pers. comm.) Ten milliliters of water was added to the bottom of each jar (not contacting the soil) to reduce soil drying. Soils were

weighed monthly to determine evaporation, and an equivalent weight of water was added back. When jars were not being measured for $C_{\min}$, the septa were removed, and the jars were placed uncapped in a dark cabinet. With septa removed, the lids had a small (~0.5 cm diam.) hole for air exchange, to reduce CO_2 buildup in the jars.

$C_{\min}$ rate was calculated based on the rate of CO_2 buildup in jars, measured using a LI-6200 portable photosynthesis system and LI-6250 CO_2 analyzer (LI-COR, Lincoln, NE). Jars were capped, then CO_2 was measured immediately, and again after 2 and 4 hours to determine the average rate of CO_2 concentration buildup. Jar headspace air was sampled using a 1 ml glass syringe. $C_{\min}$ rate was measured initially at 4 day intervals to day 12, then approximately 12 day intervals to day 74, then approximately 24 day intervals to day 233, with final measurements at day 277 and 365. The long term trend in $C_{\min}$ rate was modeled assuming SOC decomposition occurred in two pools (Paul et al., 2001; Gill, 2007):

$$C(t) = C_a * \exp(-k_a t) + C_s * \exp(-k_s t),$$

where $C(t)$ is soil C at time t , C_a is the active C pool at time 0, C_s is the slow-cycling C pool at time 0, k_a and k_s are the decay constants for the active and slow pools, respectively. The derivative of the equation is $C_{\min}$ rate, which was used to fit the data,

$$C_{\min} \text{ rate} = dC/dt = -k_a C_a * \exp(-k_a t) + -k_s C_s * \exp(-k_s t)$$

We made the simplifying assumption that an additional, recalcitrant C pool contributed little to $C_{\min}$ rate. Therefore the model was not constrained to data on total soil C, and the difference between $C_a + C_s$ and experimentally-determined total C represents the recalcitrant C pool. Mean residence time (MRT) for a given pool is the reciprocal ($1/k$) for its respective decay constant.

2.6. Soil CO_2 efflux (soil respiration). Soil CO_2 efflux was measured in the field as an index of belowground carbon bioavailability and microbial activity. Soil CO_2 efflux was

measured through soil collars, monthly during each year of CO₂ treatment (May-September, 2006-present), using a LI-COR 6400 infrared gas analyzer (LI-COR, Lincoln, NE). The 10-cm deep PVC soil collars are permanently inserted to 7.5cm depth in the soil (Fay et al., 2009). The collars are near vegetation, but were kept vegetation free to prevent leaf gas exchange from interfering with soil CO₂ efflux measurement. Soil CO₂ efflux represents combined root and microbial respiration.

2.7. Statistical analyses. The relationship between CO₂ concentration and soil variables including organic C, microbial biomass, carbon mineralization, and modeled active and slow C, was analyzed by regression. All statistics were performed in R (R Core Development Team, 2010) with the exception of the SOC physical fraction regressions and the incubation kinetics nonlinear regressions, which were performed in SigmaPlot (Systat Software 2008). The kinetics regression was in the form:

$$C_{\min} \text{ rate} = a \cdot \exp(b \cdot t) + c \cdot \exp(d \cdot t),$$

where $a/b = C_a$ (active C) and $c/d = C_s$ (slow-cycling C).

In this study, sampling was designed to capture the range of CO₂ treatments and soil types. The full CO₂ gradient contains 20 sections, each section containing 4 soil monoliths: two of one soil type and two of another (Table S4). ANPP, soil CO₂ efflux, and SOC physical fractions were measured on 60 monoliths across the CO₂ gradient, consistent with another study (Fay et al., 2012). Regressions on laboratory measurements including microbial biomass, $C_{\min}$ rate, modeled SOC pools, and total SOC concentration, were done using section averages (Table S5). In those regressions, each data point is the average of two soil subsamples within a CO₂ gradient section. Actual sample sizes and planned sample sizes differ slightly due to missing samples (Table S5). ANPP, soil CO₂ efflux, and SOC physical fractions had planned sample

sizes of $n = 16, 24, 20$ (sandy loam, silty clay, black clay) whereas microbial and modeled SOC pools had planned sample sizes of $n = 8, 10, 10$ (sandy loam, silty clay, black clay).

3. Results

3.1. ANPP responses to CO_2 and soil type. ANPP increased linearly with CO_2 concentration in the sandy loam (2008: $R^2 = 0.36$, $p = 0.01$, 2009: $R^2 = 0.32$, $p = 0.02$) and the silty clay (2008: $R^2 = 0.36$, $p = 0.002$, 2009: $R^2 = 0.53$, $p < 0.0001$) soils but not the black clay ($R^2 = 0.07$, $p = 0.27$) during the third and fourth seasons of CO_2 treatment, respectively (2008, 2009, Fig. 3). In both years, average ANPP differed among soil types (ANOVA: $p < 0.05$). Sandy loam and black clay each had higher ANPP than silty clay in 2008 and 2009 (Tukey's HSD: $p \leq 0.05$). Based on regressions of 2008-2009 pooled data, ANPP increased from ~400 to 720 g DW m^{-2} across the CO_2 gradient in the sandy loam soil, or by 1.7 ± 0.5 g m^{-2} ppm^{-1} CO_2 (regression slope $\pm$ SE). In the silty clay soil, ANPP increased from ~250 to 600 g m^{-2} , or by 1.5 ± 0.3 g m^{-2} ppm^{-1} CO_2 . This slope did not differ significantly between sandy loam and silty clay ($p = 0.62$).

3.2. Aboveground and belowground responses compared. In the CO_2 gradient experiment, belowground responses to CO_2 and soil type did not track aboveground responses. The ANPP- CO_2 response was greatest in the sandy loam and silty clay soils (Fig. 3a-b), whereas CO_2 increased active soil microbial biomass most in the black clay (Fig. 4c). Based on regression slope, active microbial biomass increased by 0.42 ± 0.10 μ g C g^{-1} soil ppm^{-1} CO_2 in the black clay. Two measures of decomposition rate— C_{min} rate and soil CO_2 efflux—also increased with CO_2 in the two clay soils but did not respond to CO_2 in the sandy soil. C_{min} rate increased by 0.020 ± 0.006 μ g C g^{-1} soil d^{-1} ppm^{-1} CO_2 across the CO_2 gradient in the silty clay and by

0.018±0.007 $\mu\text{g C g}^{-1} \text{ soil d}^{-1} \text{ ppm}^{-1} \text{ CO}_2$ in the black clay. Soil CO_2 efflux increased by 0.010±0.004 $\mu\text{mol m}^{-2} \text{ s}^{-1} \text{ ppm}^{-1} \text{ CO}_2$ in the silty clay and black clay (Fig. 4c,e,f, Table 1). For $C_{\min}$ rate and soil CO_2 efflux, the slope did not differ significantly between silty clay and black clay ($p > 0.8$)

3.3. Modeled SOC pools from one-year incubation. Active (easily-decomposable) soil organic C increased linearly by 75% across the CO_2 gradient in the black clay, as determined by a two-pool exponential model of $C_{\min}$ rate (Fig. 5b, Table S6). This soil incubation model was significant for all samples ($p < 0.01$, Fig. S1) but better fit the data from the sandy loam ($R^2 > 0.91$ for each CO_2 level) and black clay soils ($R^2 > 0.83$ for each CO_2 level) than silty clay soil (R^2 as low as 0.75; not shown). Modeled slow-cycling C was not affected by CO_2 treatment. On average, black clay soils lost 6%, silty clay soils lost 12%, and sandy loam soils lost 26% of SOC, as determined by combustion of pre- and post-incubation samples (Fig. 6a). The incubation model estimates for SOC loss, based on cumulative C mineralization, were in a similar range: 6-8% loss in black clay and 15-20% loss in sandy loam (data not shown). Although the black clay had higher decomposition rate by soil mass than the silty clay or sandy loam (Tukey's HSD: $p < 0.03$, Fig. 4d-f), the clay-rich soils had less-bioavailable C and lost a smaller proportion of SOC over long-term incubation (Fig. 6). Consistent with this, decomposition rate increased with clay content and SOC concentration, but the modeled active C pool decreased with clay content (Table S6).

SOC loss over one-year incubation was unaffected by the CO_2 gradient. CO_2 had no effect on SOC loss, measured by combustion (Fig. 6a) or estimated by the incubation model (data not shown; $p = 0.07$ for both measured and modeled loss). In contrast to cumulative SOC loss, initial $C_{\min}$ rate $\text{g}^{-1} \text{ SOC}$ increased linearly with CO_2 in the sandy loam and silty clay ($p <$

0.05) implying that CO₂ increased SOC bioavailability (Fig. 6b). Therefore SOC bioavailability increased with elevated CO₂ only for initial decomposition rate, not cumulative decomposition over a year.

3.4. SOC physical fractionation. In the two clay-rich soils, coarse POM-C increased four-fold across the CO₂ gradient (~50 to 240 g C m⁻² in the black clay) but increased by about 50% in the sandy loam (~14 to 21 g C m⁻², Fig. 7a-c). Coarse POM-C represents the youngest, most labile fraction of organic C. Coarse POM-C increased exponentially with CO₂ concentration in the black clay and linearly with CO₂ in the silty clay and sandy loam soils. Fine POM-C is older and less labile than coarse POM-C, and mineral C is the oldest and most recalcitrant fraction. Fine POM-C increased linearly with CO₂ in the sandy loam, but did not respond to CO₂ in the two clay-rich soils (data not shown). Interestingly, mineral C declined 22% across the CO₂ gradient in the silty clay (from ~410 to 320 g C m⁻²), but did not respond linearly to CO₂ in the other two soils.

4. Discussion

Our study addresses two underexplored areas in how soil organic carbon (SOC) responds to rising atmospheric CO₂: the effect of soil type, and the response shape over a CO₂ concentration gradient. As expected, the bioavailability or decomposability of SOC ($C_{\min}$ rate g⁻¹ SOC) decreased with soil clay content (Fig. 6). Although we hypothesized that clay physical protection of SOC would also reduce CO₂-induced decomposition, the two clay soils had the most CO₂-induced decomposition, as measured by $C_{\min}$ rate and soil CO₂ efflux (Fig 4d-i). We found no total SOC sequestration after four growing seasons of CO₂ treatment in any soil (Fig. S2). However, labile SOC components defined by incubation or physical fractionation increased

with elevated CO₂, particularly in the two high-clay soils as hypothesized (Figs. 5, 7). Further, soil C priming may have occurred in the silty clay.

4.1. Investigation of potential C priming. Priming is a process whereby the addition of a substrate to soil either increases or decreases microbial decomposition of existing SOC (Kuzyakov et al., 2000). Under CO₂ enrichment, the increase in labile C input from roots could fuel greater microbial biomass, which decomposes more SOC to meet its metabolic needs (Cheng, 1999; Kelley et al., 2011). Evidence for soil C priming under CO₂ enrichment has been seen in a pine forest (Finzi et al., 2006) and a scrub-oak ecosystem (Carney et al., 2007). In our study, belowground mechanisms consistent with priming occurred in the two clay-rich soils. Labile SOC concentration (Figs. 5, 7a-c) and decomposition rate (Fig. 4d-i) increased linearly with CO₂ in the silty clay and black clay but showed smaller or negligible CO₂ response in the sandy loam. Active microbial biomass increased linearly with CO₂ in the black clay and increased with a linear trend in the silty clay (Fig. 4b-c), suggesting that the increase in labile C at elevated CO₂ stimulated microbial growth. However, we found evidence for a priming response only in the silty clay soil, in which CO₂ enrichment reduced mineral-associated SOC (Fig. 7e). A reduction in mineral C could be caused by factors other than priming, such as leaching; however, the increase in labile C and microbial biomass with CO₂ in the silty clay suggest a priming mechanism. CO₂ had no effect on modeled or measured recalcitrant SOC concentration in the black clay soil, despite its increase in microbial biomass. A priming response could become evident in the black clay in later years, or the soil's high clay content may protect organic matter from decomposition and limit priming.

4.2. Aboveground and belowground responses to CO₂ compared. Aboveground and belowground ecosystem responses to the CO₂ gradient were affected differently by soil type.

Results did not support our hypotheses that CO₂-induced plant growth would be highest and CO₂-stimulated decomposition lowest in the finest-textured soils. Instead, ANPP increased most with CO₂ in the sandy loam soil (Fig. 3a). This pattern has persisted over four growing seasons, likely due to plant growth from increased soil water potential via reduced plant transpiration at elevated CO₂ (Fay et al., 2012). In contrast, belowground CO₂ effects were largest in the black clay soil, including effects on soil CO₂ efflux as well as on fast cycling C pools such as microbial biomass, modeled active C, and coarse POM-C (Table 1; Figs. 4,5,7a-c). ANPP did not respond significantly to CO₂ in the black clay soil, yet soil CO₂ efflux and C_{min} increased. Although we did not measure root growth, we can assume a root:shoot ratio of 4 for grassland (Jackson et al., 1996; IPCC, 2006). Unless root growth and root (autotrophic) respiration increased with elevated CO₂, this implies that heterotrophic respiration drove the efflux response to CO₂ in this soil. Soil CO₂ efflux, an index of microbial and root metabolism in the field, complements the laboratory index of decomposition, C_{min} rate. Despite increased decomposition rate with CO₂ in the black clay, active C accumulated at elevated CO₂, implying either that CO₂ increased root C inputs more than ANPP, or root C inputs were better protected in high-clay soil, making active C responses more apparent in our assays.

One limitation of our analysis is that, with the exception of soil CO₂ efflux, active soil C responses were measured in soil samples taken at the end of the 2009 growing season. Had the soil been sampled earlier in the growing season, active C responses to CO₂ might have been more pronounced, due to higher photosynthesis and belowground C input. Another uncertainty is the large variation in data points above 400 ppm, particularly in the two clay soils. This variation is seen in silty clay ANPP (Fig. 3b) as well as in both clay soils for soil CO₂ efflux and coarse POM fractions (Fig. 4h, 7b-c). Standard errors of prediction (SEP) were higher in the

black clay than the silty loam, indicating greater regression error. In general, the higher variability above 400 ppm in the clay soils may reflect heterogeneity in belowground C inputs or in soil physical characteristics that regulate SOC accumulation, such as aggregation, moisture, or clay content. Additionally, the variability could be a growth effect. Variability could have increased in proportion to plant size at elevated CO₂, as seen in the sandy loam and silty clay, which had the most CO₂-induced plant growth over 2006-2010 (Fay et al., 2012). Variability above 400 ppm reduces our ability to define a CO₂ response shape.

4.3. Other environmental factors in soil C dynamics. Factors such as soil moisture, soil temperature, and plant community composition could explain differences in soil C-CO₂ responses among soil types. Decomposition rates in grassland soil are well known to increase with higher soil moisture and temperature (Mielnick and Dugas, 2000; Epstein et al., 2002). However, the three soils in our experiment experienced similar temperature regimes and the same irrigation regime (Fay et al., 2009). Some difference in decomposition rate may be attributable to water retention differences by soil texture. Contrary to our expectations, soil water potential (plant-available water) was highest in the sandy loam and lowest in the black clay (Fay et al., 2012). In the sandy loam, higher moisture could stimulate decomposition directly through increased microbial activity, or indirectly through higher plant growth and rhizodeposition. Moreover, all three soils experienced increased soil water potential at elevated CO₂ due to reduced plant transpiration (Fay et al., 2012). This water-savings effect was most pronounced in the black clay, and could be a contributing factor as to why microbial biomass and soil CO₂ efflux increased most with elevated CO₂ in the black clay (Table 1). Soil properties such as organic carbon concentration and physical protection of organic matter may limit the role of moisture in decomposition responses, however. Jin et al (2013) varied soil water content

between 25-50% of water-holding capacity in a laboratory incubation with the same three soil types. They found that soil and litter decomposition (per kg soil) differed more by soil type than by moisture, with the highest decomposition occurring in the black clay soil. Another possible factor in soil-specific CO₂ responses is the plant community. On the two clay-rich soils, 30-60% of the CO₂-stimulated increase in ANPP is attributed to an increase in the proportion of a C₄ grass, *Sorghastrum nutans*, within the plant community (Polley et al., 2012b). *Sorghastrum* likely outcompeted the drought-adapted C₄ grass *Bouteloua curtipendula* due to higher photosynthetic rate and greater water use efficiency at elevated CO₂ (Fay et al., 2012). We expect that this community change increased *Sorghastrum*'s contribution to root C that entered clay soils at elevated CO₂. Differences in the chemical quality or simply the availability of C from *Sorghastrum* may have contributed to the more pronounced CO₂ responses of C and microbes in the silty clay and black clay soils.

Another possible factor in the observed SOC dynamics is soil nutrient availability. Because we did not fertilize the soils, any nutrient shift along the CO₂ gradient was likely in response to increased plant growth. Across the three soils, plant-available soil inorganic N declined slightly with elevated CO₂ (Fay et al., 2012). This could indicate increased competition between plants and microbes for soil N. Consistent with this, Kelley et al. (2011) found an increase in recalcitrant N-degrading enzymes with elevated CO₂ in the black clay. In a previous CO₂ gradient experiment with the silty clay, Gill et al. (2006) also found decreased soil N availability with elevated CO₂. However, silty clay in the current experiment showed no change in labile (protein) or recalcitrant (chitin) N-degrading enzyme activity with elevated CO₂ (A. Kelley, unpublished data). Although total soil N increased with clay content (Table S3), plant-available soil inorganic N did not differ among the three soils (Fay et al., 2012), suggesting that

412 silty clay had no more N limitation than the other soils. If N limitation contributed to soil C
413 priming in the silty clay, the effect may be subtle. Although we do not have data on soil P, there
414 was no change in alkaline phosphatase enzyme activity with CO₂ in the silty clay soil (A. Kelley,
415 unpublished data). Therefore, there is no evidence connecting soil C priming to P limitation in
416 the silty clay. Alkaline phosphatase activity did increase with elevated CO₂ in the sandy loam,
417 suggesting P limitation (Kelley et al., 2011). This could partly explain the lack of microbial
418 growth with elevated CO₂ in this soil, compared to increased microbial biomass with elevated
419 CO₂ in the clay soils. We did not measure soil K or micronutrients.

420 ***4.4. Comparison to other CO₂ experiments: effects of soil type.*** Field soils vary widely
421 in silt and clay content. US soils range from 11% silt + clay in glacially-derived Minnesota and
422 Michigan soils to 90% silt + clay in Kansas tallgrass prairie (Zak et al., 1994). Although soil
423 properties are known to influence organic C accumulation, few elevated CO₂ studies have
424 included more than one soil type. Moreover, results from these experiments are mixed. Soil
425 type did not affect soil C sequestration in an annual grassland under elevated CO₂, perhaps
426 because the two soils tested had similar clay content (Luo et al., 1996; Hungate et al., 1997). In
427 contrast, CO₂ enrichment in a beech-spruce ecosystem stimulated greater net C input and
428 retention in an acidic loam than in calcareous sand (Hagedorn et al., 2001; 2003). Our results,
429 like those of Hagedorn et al. (2001; 2003), imply that clay content influenced SOC accumulation
430 by protecting soil organic matter from decomposition. As in their experiment, the sandiest soil
431 in our experiment had the most CO₂-induced plant growth, but the least labile C accumulation,
432 likely because new C was unprotected from decomposition.

433 There are some caveats in comparing SOC results from our study to others. The physical
434 fractionation method identifies SOC pools based on laboratory procedure, rather than

decomposition kinetics. The coarse POM, fine POM, and mineral-associated C fractions may each contain a heterogeneous mixture of compounds, meaning the fractions may not completely represent the pools in our incubation model or other incubation-based studies (Wander, 2004; Dungait et al., 2012). We present both fraction and incubation results to give a clearer picture of this disconnect, which is an ongoing area of debate in the literature (Wander, 2004). Another caveat is that the modeled C pool sizes in our study are lower than in other incubation-based studies. In our study, modeled active C is $\sim 0.03\text{--}0.07\text{ g kg}^{-1}$ soil in the sandy loam, and $0.03\text{--}0.09\text{ g kg}^{-1}$ soil in the black clay. In comparison, active C was 0.13 g kg^{-1} in forest soil (Haile-Mariam et al., 2000), 4 g kg^{-1} in subalpine meadows (Gill, 2007) and 0.5 g kg^{-1} in agricultural soil (Collins et al., 2000). Modeled slow-cycling C in our study was $\sim 2\text{ g kg}^{-1}$ soil in the sandy loam and $3\text{--}9\text{ g kg}^{-1}$ in the black clay. Other studies measured slow C as $7\text{--}10\text{ g kg}^{-1}$ (Collins et al., 2000; Haile-Mariam et al., 2000; Gill, 2007). Out of simplicity, our model was not constrained to total C, it did not capture a recalcitrant C pool, which may explain these lower estimates. The model ($C_a + C_s$) accounts for only 20% of total SOC, implying that the remaining 80% of SOC is recalcitrant and not influencing decomposition rate.

4.5. Comparison to other CO_2 experiments: shape of CO_2 response. Our study is consistent with others in finding both linear and nonlinear SOC responses to CO_2 . In a chaparral experiment, soil C within water-stable aggregates and total soil C increased linearly over 250–650 ppm CO_2 (Treseder et al., 2003). Total SOC increased exponentially along a natural CO_2 gradient in grassland (CO_2 spring; 368–674 ppm), although a linear model also fit the relationship (Ross et al., 2000). In our study, CO_2 -response shape differed by soil type and response variable. In the black clay, microbial biomass and modeled active SOC increased linearly with CO_2 , whereas coarse POM-C increased exponentially with CO_2 . By contrast, most of the SOC pools

458 did not respond to CO₂ in the sandy loam. The only SOC pool which decreased with CO₂ was
459 mineral-associated C in the silty clay.

460 Our results both support and contrast from those in a previous iteration of the CO₂
461 gradient experiment (Gill et al., 2006). In that experiment, mesic grassland was exposed to a
462 preindustrial-to-future (200-560 ppm) CO₂ gradient for four years. The grassland had formed on
463 the same soil series (Austin) as the silty clay Mollisol in our current experiment; however, it
464 showed nonlinear rather than linear soil C responses to CO₂. Compared to pretreatment values,
465 total SOC in that experiment declined from ambient to subambient CO₂, and had a small but
466 nonsignificant increase from ambient to elevated CO₂. Soil microbial biomass and soil CO₂
467 efflux also had nonlinear responses to CO₂, with curves peaking at about 440 ppm CO₂ (Gill et
468 al., 2002; Gill et al., 2006). Our current study also has evidence for a CO₂ response threshold;
469 coarse POM-C curved upward at about 450 ppm CO₂ in the black clay. In contrast to the
470 previous study, microbial biomass and soil CO₂ efflux increased linearly in the black clay. We
471 hypothesize that the drop in microbial biomass and soil CO₂ efflux above 440 ppm CO₂ in the
472 previous study is due to the drop in plant productivity in the same CO₂ range (Polley et al., 2003);
473 whereas in the present study microbial biomass and soil CO₂ efflux mirror the linear increase in
474 plant productivity and labile SOC with CO₂. One consistent result between the two experiments
475 is that CO₂ enrichment increased labile SOC and decreased mineral-associated SOC in the silty
476 clay soil. Therefore, both experiments provide evidence for a priming response to elevated CO₂
477 in the silty clay soil.

479 **5. Conclusions**

In this prairie ecosystem, soil texture had significant effects on soil organic carbon accumulation and its response to CO₂. Higher-clay soils had greater SOC, and the highest-clay soil had the greatest increase in rapidly cycling SOC pools with CO₂ enrichment. We found evidence of priming; SOC loss from recalcitrant organic matter increased at elevated CO₂ for the intermediate-textured silty clay soil only. SOC may be more physically protected from decomposition in the highest clay soil, preventing priming. Although the sandiest soil had the greatest ANPP increase with CO₂, the lack of soil C responses to CO₂ in this soil suggests that 1) soil properties can override plant-mediated CO₂ effects on soil C cycling or 2) belowground plant responses to CO₂ differed from aboveground responses. Our results are consistent with evidence from other studies that CO₂ enrichment accelerates soil C cycling, but we find that CO₂ effects depend significantly on soil texture. Given the wide range of clay content in elevated CO₂ experiments to date (<10% to >50% clay), a better understanding of soil type effects could improve predictions of soil feedbacks on rising atmospheric CO₂.

Acknowledgements

We thank John Kim and Sean Berthrong for their constructive criticism on earlier drafts of this manuscript. Virginia Jin determined field capacity of the soils used in the CO₂ gradient, and provided soil samples for testing. Alexia Kelley helped with the incubation experiment and provided enzyme data. Chris Kolodziejczyk, Kyle Tiner, and Katherine Jones operated CO₂ chambers. This project was supported by funding from the US Department of Energy (Program in Ecosystem Research no. ER64242), the National Science Foundation, Sigma Xi, and the US Department of Agriculture. Mention of trade names or commercial products in this publication does not imply recommendation or endorsement by the US Department of Agriculture. USDA is an equal opportunity provider and employer.

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642

Table 1. Summary of soil C cycle responses to the CO₂ gradient

Part of C cycle		Sandy loam	Silty clay	Black clay
		15% clay	45% clay	55% clay
C Inputs	ANPP	● +60%, linear*	● +40%, linear	no change
C pools (suggests mechanism)	Total SOC	no change	no change	no change
	active microbial biomass	no change	linear trend (p = 0.1)	● +18%, linear
	model: active C	no change	poor model fit	● +75%, linear
	Coarse POM-C	● +50%, linear	● +400%, linear	● +400%, exponential
	Mineral-C	no change	● -23%, linear	no change
	model: slow C	no change	poor model fit	no change
C losses	C _{min} rate	no change	● +56%, linear	● +31%, linear
	Soil CO ₂ efflux	no change	● +29%, linear	● +30%, linear

*● indicates variables with statistically significant responses to CO₂ treatment.

1 **Figure Legends**

2 Fig. 1. Conceptual diagram for prairie soil C cycle responses to elevated CO₂. Positive
3 effects are (+) signs, negative effects are (-) signs. Rising CO₂ (1) stimulates plant
4 growth (2), resulting in greater plant C inputs to soil (3) and reduced CO₂ (1). SOC
5 accumulates (4) if plant C inputs exceed decomposition losses (5). SOC decreases if
6 decomposition exceeds plant inputs, possibly as the result of “priming.” A priming
7 response involves change in quality or quantity of plant inputs to soil (3), change in
8 microbial biomass (6), and change in decomposition of SOC (5).

9

10 Fig. 2. Soil textures in this study, in the context of eight representative published studies
11 of soil C responses to elevated CO₂. The duration of CO₂ treatment is also shown.
12 Details of each study are listed in Table S2.

13

14 Fig. 3. Aboveground net primary productivity in 2008 and 2009, the third and fourth
15 growing seasons of CO₂ treatment. Linear regressions are significant in the sandy loam
16 (a) and silty clay (b) for both years ($p < 0.05$), but not in the black clay (c). SEP is
17 standard error of prediction.

18

19 Fig. 4. (a-c) Active microbial biomass measured by substrate-induced respiration (SIR).
20 Microbial biomass had a linear trend with CO₂ in the silty clay (b) and a significant linear
21 increase with CO₂ in the black clay (c). (d-f) Carbon mineralization rate at the start of a
22 one-year soil incubation. (g-i) Soil CO₂ efflux (soil respiration) in the field averaged

over the 2009 growing season (May-September, $n = 5$ for each data point, error bars are standard error).

Fig. 5. Active SOC pools in sandy loam (a) and black clay (b) as calculated by the incubation kinetics model. The dotted lines in (b) are the 95% confidence interval for the regression of active SOC vs. CO_2 . The modeled slow SOC pools (not shown) had no significant response to CO_2 treatment. Modeled SOC pools for the silty clay are omitted due to poor model fit (R^2 as low as 0.75 compared to $R^2 > 0.83$ for sandy loam and black clay).

Fig. 6. (a) SOC lost over the one-year incubation (cumulative carbon mineralization), expressed as a percentage of initial SOC. On average, black clay soils lost 6%, silty clay soils lost 12%, and sandy loam soils lost 26% of SOC. SOC loss was determined by combustion of pre- and post-incubation samples. (b) SOC lability based on $C_{\min}$ rate g^{-1} SOC at the start of incubation.

Fig. 7. Organic C in physical fractions of the sandy loam (a, d), silty clay (b, e) and black clay (c, f). Coarse POM C (a-c) represents the youngest, fastest-cycling SOC. The y-scale of (a) and (b) is enlarged to show variation. Mineral C (d-f) represents the oldest and slowest-cycling SOC.

46 **Figures**

47

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Fig. 6. (a) SOC lost over the one-year incubation (cumulative carbon mineralization), expressed as a percentage of initial SOC. On average, black clay soils lost 6%, silty clay soils lost 12%, and sandy loam soils lost 26% of SOC. SOC loss was determined by combustion of pre- and post-incubation samples. (b) SOC lability based on C_{min} rate g⁻¹ SOC at the start of incubation.

Fig. 7. Organic C in physical fractions of the sandy loam (a, d), silty clay (b, e) and black clay (c, f). Coarse POM C (a-c) represents the youngest, fastest-cycling SOC. The y-scale of (a) and (b) is enlarged to show variation. Mineral C (d-f) represents the oldest and slowest-cycling SOC.