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Soil disturbance and invasion magnify CO₂ effects on grassland productivity, reducing diversity

Running title: Disturbance and invasion magnify CO₂ effects

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Abstract

Climate change, disturbance, and plant invasion threaten grassland ecosystems, but their combined and interactive effects are poorly understood. Here, we examine how the combination of disturbance and plant invasion influences the sensitivity of mixedgrass prairie to elevated carbon dioxide (eCO₂) and warming. We established subplots of intact prairie and disturbed/invaded prairie within a free-air CO₂ enrichment (to 600 ppmv) by infrared warming (+1.5° C day, 3° C night) experiment and followed plant and soil responses for five years. Elevated CO₂ initially led to moderate increases in biomass and plant diversity in both intact and disturbed/invaded prairie, but these effects shifted due to strong eCO₂ responses of the invasive forb *Centaurea diffusa*. In the final three years, biomass responses to eCO₂ in disturbed/invaded prairie were 10 times as large as those in intact prairie (+186% vs. +18%), resulting in reduced rather than increased plant diversity (-17% vs. +10%). At the same time, warming interacted with disturbance/invasion and year, reducing the rate of topsoil carbon recovery following disturbance. The strength of these interactions demonstrates the need to incorporate disturbance into predictions of climate change effects. In contrast to expectations from studies in intact ecosystems, eCO₂ may threaten plant diversity in ecosystems subject to soil disturbance and invasion.

Introduction

Elevated CO₂, global warming and disturbances associated with land use threaten biological diversity and ecosystem services globally (Bellard *et al.*, 2012; Dale, 1997; Hoekstra *et al.*, 2005). To date, these processes have been studied largely in isolation, with studies of climate change (here used broadly to include elevated CO₂ [eCO₂]), focusing on relatively undisturbed ecosystems, and disturbance studies considering effects under present-day climate (Perring *et al.*, 2016). However, given that climate and disturbance influence many of the same processes, it is critical to understand their combined and interactive effects (Oliver & Morecroft, 2014; Ostberg *et al.*, 2015; Perring *et al.*, 2016). For example, soil food webs more effectively resist drought, and therefore retain soil carbon and nitrogen, within extensively managed pastures than within intensively managed wheat fields (De Vries *et al.*, 2012). While such interactions are likely to be important, predicting them is complex, requiring data-rich models or multi-factor experiments (Oliver & Morecroft, 2014; Perring *et al.*, 2016).

In grasslands, land use can involve several disturbances that may alter effects of climate change. A few studies have examined particular climate-disturbance combinations. Grazing was found to counteract negative effects of warming on plant species richness on the Tibetan Plateau (Klein *et al.*, 2004), and effects of elevated CO₂ (eCO₂) on legume abundance in New Zealand (Newton *et al.*, 2014). Grassland fires, which can be intentionally or unintentionally associated with land use, are expected to interact with

warming and precipitation change, with significant effects on ecosystem services (Fernandes *et al.*, 2013; Thonicke *et al.*, 2001; Westerling *et al.*, 2006). In California annual grassland, fire led to more positive effects of eCO₂ and nitrogen (N) addition on productivity (Henry *et al.*, 2006).

Soil disturbance is also common in grasslands, resulting from cultivation, energy development, or other intensive forms of land use. Soil disturbances often damage root systems and alter soil structure, with correspondingly large effects on soil properties, resource availability, and plant communities (James *et al.*, 2003; Wilson & Tilman, 1993). These effects of disturbance are likely to influence how plants and soils respond to CO₂ and warming. For example, greater nutrient availability can increase plant productivity responses to eCO₂ (Reich & Hobbie, 2013; Terrer *et al.*, 2019), suggesting the potential for similar patterns with disturbance. Soil resource availability can also interact with eCO₂ and warming to influence plant communities and C cycling, effects that can be mediated by changes in productivity, soil respiration, and the sensitivity of individual species (Pastore *et al.*, 2021; Reich, 2009).

Despite the prevalence of anthropogenic soil disturbance, and its potential for interacting with eCO₂ or warming, there have been few empirical studies of such interactions. Plowing did not interact with warming to influence grassland cover or species richness in Japanese montane grasslands (Suzuki, 2014). In separate studies of intact and disturbed calcareous grassland in Switzerland, eCO₂ had broadly similar

effects on plant productivity and species evenness (Leadley *et al.*, 1999; Niklaus *et al.*, 2001).

Soil disturbance is also frequently associated with plant invasion (Davis *et al.*, 2000; Hobbs & Huenneke, 1992), which itself can interact with climate change (Dukes, 2000; Liu *et al.*, 2017; Sorte *et al.*, 2013). Disturbance may reduce biotic resistance to invasion from native species (D'Antonio & Thomsen, 2004). Furthermore, many invasive plants have traits that allow them to respond positively to changes that involve increases in resource availability, including not only disturbance, but also N deposition, eCO₂, and increases in precipitation (Bradley *et al.*, 2010; Dukes & Mooney, 1999; Seabloom *et al.*, 2015). Across studies, invasive species tend to respond more positively to eCO₂ and warming than do native species (Liu *et al.*, 2017). Consequently, the combination of soil disturbance and invasion has the potential to alter the way grasslands respond to eCO₂ and warming.

Here, we describe an experimental study of how eCO₂ and warming interact with disturbance to influence plant communities and ecosystem properties in mixedgrass prairie. The semiarid mixedgrass prairie is the largest remaining grassland in North America, sustaining both biological diversity and livestock production (Samson *et al.*, 2004). It is threatened by climate change as well as disturbance, particularly energy development and crop production, both of which involve soil disturbance and

associated plant invasion (Heisler-White *et al.*, 2009; Mueller *et al.*, 2016; Ott *et al.*, 2020; Samson *et al.*, 2004).

We have previously documented effects of eCO₂ and warming on intact mixedgrass prairie, including increased plant productivity, reduced nutrient availability and forage quality, altered soil carbon (C), and reduced dominance by common grass species (Augustine *et al.*, 2018; Carrillo *et al.*, 2018; Dijkstra *et al.*, 2010; Morgan *et al.*, 2011; Mueller *et al.*, 2016; Zelikova *et al.*, 2014). We have also found that both eCO₂ and warming can promote plant invasion (Blumenthal *et al.*, 2016; Blumenthal *et al.*, 2013; Reeves *et al.*, 2015). In the present study, we compare effects of eCO₂ and warming on intact mixedgrass prairie to effects on prairie treated with soil disturbance and seeded with five native and five invasive forb species (Fig. 1, Table S1).

Our hypotheses were: **(1)** eCO₂ and warming would increase plant productivity (Mueller *et al.*, 2016) more in disturbed/invaded than in intact prairie due to greater resource availability in combination with species adapted to grow rapidly in disturbed environments; **(2)** eCO₂ would affect plant diversity differently in disturbed/invaded than in intact prairie, potentially increasing diversity (Zelikova *et al.*, 2014) **(a)** more in disturbed/invaded prairie, if disturbance reduced dominance by native grass species, or **(b)** less in disturbed/invaded prairie, if planted native or invasive forbs became dominant; **(3)** Disturbance/invasion would reduce soil C, and eCO₂ and warming effects

on soil C would follow from their effects on plant productivity, increasing C more in disturbed/invaded than in intact prairie.

Materials and Methods

Study Site

The Prairie Heating and CO₂ Enrichment (PHACE) experiment was located in northern mixedgrass prairie near Cheyenne, Wyoming, USA (latitude 41°11'N, longitude 104°54'W, elevation 1930 m). Mean annual temperature was 8 °C and mean annual precipitation was 397 ± 76 mm SD (1984-2013). Precipitation from the onset of growth (DOY 100) to harvest (~ DOY 200) was 281, 246, 204, 120, and 118 mm in 2009-2013, respectively. The native plant community was composed of C₃ graminoids, including *Pascopyrum smithii* (Rydb.) A. Love, *Hesperostipa comata* Trin and Rupr., and *Carex duriuscula* L. Bailey; C₄ grasses, primarily *Bouteloua gracilis* (H.B.K) Lag.; and a variety of forbs and subshrubs, notably *Sphaeralcea coccinea* (Nutt.) Rydb., and *Artemisia frigida* Willd. The site has a history of moderate grazing by cattle and was fenced during the study to keep out both large and small mammals. This was supplemented by trapping of small mammals that got past the fence.

Experimental Design

Climate treatments included: (1) **ct**: ambient CO₂ and temperature, (2) **cT**: infrared warming to increase canopy temperature 1.5 °C during the day and 3 °C at night, (3) **Ct**:

free-air CO₂ enrichment to 600 ppmv, and (4) CT: CO₂ enrichment plus warming. The target CO₂ and temperature levels correspond with late 21st century projections for the region (CMIP3 emissions scenario B1; Kunkel *et al.*, 2013). Global change treatments started in spring 2006 (eCO₂) and spring 2007 (warming) (Fig. 1). Five replications of each treatment were randomly assigned to 20, 3.3-m diameter circular plots (“rings”) within two blocks with slightly different soil types (Morgan *et al.*, 2011). The warming treatment was imposed year-round. Enrichment of CO₂ was limited to daylight hours when green plants were present and the site was not covered with snow. We controlled for potential infrastructure effects by installing dummy FACE tubing and heaters at untreated rings. To minimize dilution of treatment effects through lateral water flow, we buried a 60-cm-deep plastic barrier around each ring. Additional details on the experimental design and system performance are reported in Morgan *et al.* (2011) and LeCain *et al.* (2015).

Each experimental ring was randomly divided into intact prairie and disturbed prairie (including multiple smaller experiments) by a 30-cm deep, below-ground metal barrier oriented parallel to the prevailing northwest winds (Fig. 1).

The disturbed/invaded treatment was designed to mimic key aspects of soil disturbances in mixedgrass prairie – partial plant removal, soil turnover, colonization by invasive species – at a scale small enough for this climate change experiment. Similar changes occur with anthropogenic disturbances such energy development, where road

and well-pad construction can involve removal of plants, storage and subsequent reapplication of topsoil, partial revegetation, and invasion by non-native species (Ott *et al.*, 2020). Within the disturbed prairie section of each ring, we established a 70-cm × 100-cm subplot and partially tilled it (57% of the area) to a depth of 10 cm (Figs. 1, S1). Specifically, we tilled two 20-cm × 100-cm strips, separated by a 15-cm × 100-cm strip of existing prairie vegetation in the center, and bordered by two 7.5-cm × 100-cm strips of prairie vegetation on the sides. Tillage involved turning over, breaking up and homogenizing the soil with mattocks, hoes, and rakes, and was conducted in October 2007. Effects of disturbance alone (not presented here) were monitored for one year.

In November 2008 the entire subplot was seeded with five invasive and five native forb species, six of which successfully established in the study: *Artemisia frigida*, *Centaurea diffusa*, *Chenopodium leptophyllum*, *Grindelia squarrosa*, *Linaria dalmatica*, and *Salsola tragus* (Table S1). We focused on forbs, because many of the most important invasive plants in mixedgrass prairie are forbs, and because this enabled us to locate and track seedlings within the graminoid-dominated ecosystem. Competition between native species and the invasive grass, *Bromus tectorum* was examined in a separate study within the PHACE experiment (Blumenthal *et al.*, 2016). For species selection, we paired each invasive species with a native species by life history, habitat, and where possible phylogeny (Table S1). We selected locally common species to ensure that they were well-adapted to local environmental conditions. All seed was collected from nearby

field sites in Colorado and Wyoming. We sowed $3.43 \text{ g seed}\cdot\text{species}^{-1}\cdot\text{m}^{-2}$ by hand, taking care to distribute seed evenly over both the tilled and vegetated sections of each disturbed/invaded subplot, after which the surface soil was raked and lightly packed to improve seed-soil contact.

Measurements

We sampled peak aboveground biomass in mid-July. Vegetation was clipped at the ground surface, separated by species, dried at $60 \text{ }^{\circ}\text{C}$, and weighed. The harvest plan was designed to mimic effects of grazing in this typically grazed ecosystem. In the intact prairie, a 1.5 m^2 area was divided into 24 squares, half of which were harvested each year. Individual squares were therefore harvested two or three times during the 5-year study. In the disturbed/invaded subplots, re-harvest was not realistic because dominant species were unpalatable. In addition, some of the short-lived forbs relied on seed set occurring after the time of harvest. Consequently, separate 0.07 m^2 areas (including both tilled and vegetated strips) were harvested each year. In the final year, we harvested all of the plot area not previously harvested (0.28 m^2). To minimize effects of different clipping frequencies among subplots, all areas not harvested during the growing season were clipped to a height of 2 cm following full senescence. To assess the potential effects of different clipping frequencies on biomass production we examined results from a study of grazing intensity at the same site. Methods are described in Derner & Hart (2007).

Sampling for soil C and below-ground biomass was conducted in the week following peak biomass clipping. We collected five soil cores in intact subplots (3-cm diameter, 15-cm depth) and four soil cores in tilled strips within disturbed/invaded subplots (2.54-cm diameter, 15-cm depth). All cores were then split into 0-5 and 5-15 cm samples and composited by depth. Roots were removed by sieving followed by hand picking of sieved soil, then washed, dried, and weighed. A subsample of the composited soil was sieved to 2 mm, inspected to remove visible particulate organic matter, and then treated to remove carbonates (1 M H_3PO_4) (Sherrod *et al.*, 2002). The resulting dried soil was ground to powder and weighed for C analysis on a Finnigan DeltaPlus XP connected to a Carlo Erba NC-2500 elemental analyzer. Pre-treatment soil C was collected in 2005 for each ring, using the same methods, from three cores located around the edge of each ring.

To measure soil NO_3^- availability, we installed resin probes vertically from 2-cm to 7.6-cm depth, between May and October (or late July in the final year of the study). Probes were placed within tilled strips in the disturbed/invaded subplots. Retrieved probes were cleaned with deionized water, extracted with 0.5 N HCl solution, and analyzed colorimetrically for NO_3^- by Western Ag Innovations (Saskatoon, Canada). The NO_3^- values represent a time-integrated proxy for the size of the available NO_3^- pool in surface soil.

Beginning in 2011, once the potential for light limitation became apparent, we quantified light penetration in the disturbed/invaded subplots using a METER LP-80 AccuPAR ceptometer (METER Environment, Pullman, WA, USA). Photosynthetically active radiation was measured above (one measurement per plot) and below (two measurements per plot) the plant canopy between 11:00 and 13:00 hours during cloudless periods, in early August 2011 and 2012 (prior to *C. diffusa* senescence), and late June 2013 (prior to the final harvest).

Analysis

Statistical analyses were conducted in JMP version 15 (SAS Institute, Cary, North Carolina, USA). We analyzed biomass, soil C, and soil N responses with linear mixed models that included random effects of block (to account for spatial covariation) and ring (to account for the split-plot design), and fixed effects of eCO₂, warming, disturbance/invasion and their interactions. For dependent variables measured over time, models also included a random effect of ring x disturbance (to account for repeated measures within subplots), and fixed effects for year and its associated interactions. In analyses of biomass, soil N, and diversity indices, year was a categorical variable, reflecting the expectation that its effects would depend mostly on climatic fluctuations (Mueller *et al.*, 2016). Analyses of soil N also included a random effect to account for the number of days probes were left in the soil each year. In analyses of soil C, year was a continuous variable, to test for differences in the rate of soil C recovery

following disturbance. To account for pre-existing spatial variation in soil C, these models also included pre-treatment soil C as a covariate (Carrillo *et al.*, 2018). To meet model assumptions of homoscedasticity some dependent variables were ln transformed (aboveground biomass, NO_3^-) or squared (light penetration) prior to analysis.

Significant interactions between year, disturbance/invasion, eCO_2 and warming treatments were investigated with post-hoc analyses within disturbance treatments (Table S2).

Shannon's diversity and evenness were calculated from biomass data in R, using the vegan package (Oksanen *et al.*, 2015). Diversity metrics were not quantitatively comparable across subplots because measurement areas differed in size and only disturbed/invaded prairie was seeded with additional species. We therefore analyzed diversity metrics only within disturbance treatments (Table 2). We used bivariate linear regression to test whether species diversity and resin-available NO_3^- were correlated with aboveground biomass, and whether belowground biomass was correlated with diversity and soil C, within disturbance treatment and year.

To assess the relative importance of direct and indirect effects of eCO_2 and warming on diversity, we conducted separate confirmatory path analyses for intact and disturbed/invaded prairie. The models included effects of eCO_2 , warming, and eCO_2 *warming on both aboveground biomass and diversity, and effects of aboveground biomass on diversity. Thus effects of climate change treatments on

diversity could be either direct or indirect, mediated by aboveground biomass.

Analyses were conducted in piecewiseSEM (Lefcheck, 2016) using the following model:

Biomass \sim CO₂ * Temperature + (1|plot) + (1|year); Diversity \sim CO₂ * Temperature + Biomass + (1|plot) + (1|year).

Results

Aboveground biomass - Elevated CO₂ increased aboveground biomass, and the strength of this effect varied with disturbance and year (disturbance \times eCO₂ \times yr; Fig. 2, Table 1).

In disturbed/invaded prairie, eCO₂ interacted with year (Table S2), decreasing aboveground biomass production by 7% and 0.1% in 2009 and 2010, but increasing it by 262%, 169%, and 126% in 2011-2013, respectively. These eCO₂ responses were driven largely by a single invasive forb, *C. diffusa*, most of which was rooted within the tilled portions of the disturbed/invaded subplots, and which first attained high biomass in 2011. Neither warming nor its interaction with year significantly influenced aboveground biomass in disturbed/invaded prairie.

In intact prairie, eCO₂ also increased aboveground biomass in later years of the study (eCO₂ \times yr: Fig. 2, Table S2), but its effects were smaller than in disturbed/invaded prairie: -3%, 1%, 20%, 10% and 24% in 2011-2013, respectively. Warming increased aboveground biomass in intact prairie, and interacted with year, with larger increases in

biomass in 2010, 2012, and 2013, as reported previously (Mueller *et al.*, 2016). Declines in biomass in intact prairie over time were closely associated with similar trends in precipitation (Mueller *et al.*, 2016).

Belowground biomass - We measured belowground biomass in both disturbed/invaded and intact prairie only in 2011, the year with maximum aboveground biomass of *C. diffusa*. At 0-5 cm depth, eCO₂ increased belowground biomass across disturbance treatments by an average of 55% (Fig. S2, Table 1). At 5-15 cm depth, belowground biomass was higher in disturbed/invaded than intact prairie, but was not significantly altered by global change treatments.

Richness, evenness and diversity - As our design did not allow for quantitative comparisons of diversity between disturbance treatments, we analyzed intact and disturbed/invaded prairie diversity indices separately (Table 2). In intact prairie, eCO₂ increased species evenness by an average of 9%, and had similar but marginally significant effects on diversity (Fig. 3). In disturbed/invaded prairie, eCO₂ interacted with year for evenness and diversity, initial increases with eCO₂ giving way to decreases following *C. diffusa* expansion. In 2009-2013, respectively, evenness changed by +5%, +30%, -7%, -38%, -15%, while diversity changed by +8%, +42%, -6%, -30%, -15%. Across rings, diversity declined with increasing aboveground biomass only in disturbed/invaded prairie in 2011-2013 (Fig. S3). Diversity was not significantly correlated with belowground biomass ($p > 0.14$).

We examined the relative strength of direct and indirect predictors of diversity using path analyses. Marginal R^2 values for diversity were 0.12 for intact prairie and 0.35 for disturbed/invaded prairie. Model results support the interpretation that there are strong indirect effects of eCO_2 on diversity in disturbed/invaded prairie, mediated by increases in aboveground biomass (Fig. 4). In disturbed/invaded prairie, eCO_2 increased aboveground biomass ($p = 0.04$), and aboveground biomass was negatively associated with diversity ($p < 0.0001$). In intact prairie, all pathways were non-significant ($p > 0.25$).

Soil C - Trends in shallow (0-5 cm) soil C varied significantly with disturbance and warming (disturbance \times warming \times yr; Fig. 5, Table 1). In intact prairie, soil C declined over the course of the study, a trend that aligned with decreases in precipitation. Soil C was 25% lower in disturbed/invaded than intact prairie in 2010 but recovered substantially by 2013 (disturbance \times yr; Table 1) (Fig. 5). Within disturbed/invaded prairie, warming reduced the rate of soil C recovery (warming \times yr: $F_{1,54} = 5.9$, $p = 0.02$; Table S2). The $eCO_2 \times$ warming interaction previously described for intact prairie (Carrillo *et al.*, 2018), was marginally significant in the subset of years within this study ($F_{1,15} = 4.2$, $p = 0.06$). In 5-15 cm soil C, trends over time were more positive in disturbed/invaded than intact prairie (disturbance \times yr; Table 1), but were not influenced by eCO_2 or warming. Soil C was not significantly associated with belowground biomass in bivariate regressions within disturbance treatments ($p > 0.41$).

Soil NO_3^- - Resin-available NO_3^- decreased with eCO_2 and increased with warming (Mueller *et al.*, 2016), but warming effects were weaker in disturbed/invaded than in intact prairie (disturbance \times warming; Table 1, Fig. S4). NO_3^- was unrelated to aboveground biomass in most years (Fig. S5). Light penetration, measured in disturbed/invaded prairie in 2011-2013, decreased with eCO_2 ($F_{1,15} = 11$, $p = 0.005$; Table S2, Fig. S6).

Discussion

Predicting effects of climate change requires a better understanding of how it will interact with widespread anthropogenic disturbances (Oliver & Morecroft, 2014; Perring *et al.*, 2016). In this study, we found that eCO_2 and warming had very different effects in disturbed/invaded prairie than in intact prairie (Tables 1, S2). Once the invasive forb *C. diffusa* became dominant (2011-2013), eCO_2 increased biomass by an average of 186% in disturbed/invaded prairie, as compared to 18% in intact prairie. Likely as a result of *C. diffusa* effects on aboveground biomass, disturbance/invasion reversed positive effects of eCO_2 on diversity. While eCO_2 increased diversity by 10% in intact prairie, it reduced diversity by 17% in disturbed/invaded prairie. At the same time, disturbance/invasion interacted with warming to influence belowground processes, with disturbance/invasion reducing soil C and warming slowing subsequent

soil C accrual. Together, these patterns indicate that soil disturbance and associated invasion can increase sensitivity of grasslands to climate change.

The increase in aboveground biomass we observed with eCO₂ in disturbed/invaded prairie was much larger than increases observed in other grassland experiments (Hovenden *et al.*, 2019; Terrer *et al.*, 2019). Both disturbance and invasion contributed to this biomass response. Across 2011-2013, 69% of the aboveground biomass in eCO₂ rings was from *C. diffusa* (Fig. 2), a problematic invasive species in western North America. This biennial forb responded particularly strongly to eCO₂ (Reeves *et al.*, 2015), as previously observed for another invasive forb at this site, *Linaria dalmatica* (Blumenthal *et al.*, 2013). The strong eCO₂ response of *C. diffusa* might be due to its rapid growth rate and its C₃ photosynthetic pathway (associated with strong eCO₂ responses, among the native graminoids but not forbs in the PHACE experiment) (Ainsworth & Long, 2005; Mueller *et al.*, 2016). Other seeded species in this study also responded positively to global changes (D. Blumenthal, unpublished data), but they made up much smaller fractions of aboveground biomass, and likely responded to both direct effects of global change treatments and indirect effects mediated by *C. diffusa* dominance.

In previous studies, eCO₂ has often favored invasive over native plants (Liu *et al.*, 2017; Sorte *et al.*, 2013). In this case, however, the effect appears to have been compounded by soil disturbance. Almost all of the *C. diffusa* biomass (on a per-m² basis) was rooted in

the tilled portions of the disturbed/invaded subplots (Fig. 2), suggesting that it required soil disturbance to respond strongly to eCO₂. Disturbances that kill plants tend to increase water and N availability in grasslands (James *et al.*, 2003; Wilson & Tilman, 1993), including within the PHACE study, where plant removal led to six-fold increases in extractable NO₃⁻ (Carrillo *et al.*, 2012). Disturbance may therefore have provided the belowground resources needed for the strong eCO₂ responses of *C. diffusa* and total aboveground biomass. These results suggest that large aboveground biomass responses to eCO₂ may be more common in disturbed landscapes, and may depend on the presence of invasive species with particularly strong responses to eCO₂.

An alternative explanation for the stronger biomass responses in disturbed/invaded prairie than intact prairie could be the lower growing-season harvest frequency in this treatment. However, in a long-term grazing study at the same site and the same years, biomass production was only 19% greater in ungrazed than in moderately grazed (~50% biomass removal) pastures (Fig. S7). This comparison is inexact, but suggests that differences in clipping frequency could account for only a small fraction of the observed 10-fold increase in biomass we observed with disturbance and invasion.

Anthropogenic changes that increase grassland productivity often reduce diversity. For example, nutrient fertilization reduces plant diversity in grasslands around the world, likely by increasing aboveground biomass and reducing light (Borer *et al.*, 2006; Clark *et al.*, 2007; Hautier *et al.*, 2009). Although eCO₂ typically increases grassland productivity

(Hovenden *et al.*, 2019; Terrer *et al.*, 2019), plant diversity responses have been less consistent, and not clearly related to productivity (Komatsu *et al.*, 2019; Morgan *et al.*, 2007; Reich, 2009; Teyssonneyre *et al.*, 2002; Zavaleta *et al.*, 2003; Zelikova *et al.*, 2014). Our results in disturbed/invaded prairie were similar to those seen with nutrient fertilization: large increases in total biomass with eCO₂ were associated with reduced light availability and reduced diversity both over time and among rings (Figs. 3, 4, S3, S6). In contrast to expectations derived from previous studies, primarily conducted in intact grasslands, eCO₂ may threaten plant diversity in disturbed grasslands, particularly when invasive species are present.

An open question is whether the eCO₂ responses of biomass and diversity in disturbed/invaded prairie would be likely to persist over time. The decline in *C. diffusa* biomass in 2013 could indicate a temporary invasion, the effects of dry conditions in 2012 and 2013 (Mueller *et al.*, 2016), or variation associated with *C. diffusa*'s biennial life history. Given the role of disturbance in the eCO₂ response, longer-term responses of biomass and diversity might depend on whether nutrients released by disturbance are immobilized over time (Luo *et al.*, 2004; Perry *et al.*, 2010) or maintained at higher levels, e.g., through positive feedbacks between invasion and nutrient cycling (Ehrenfeld, 2003; Liao *et al.*, 2008). Our results provide little evidence that high biomass production led to net N immobilization by plants or microbes within the time frame of this study.

Although resin-available NO₃⁻ varied widely by year and treatment, it was unrelated or

weakly related (in 2012) to the large increases in biomass we observed with eCO₂ (Figs. S4, S5).

Soil C responses to climate change also differed in intact and disturbed/invaded prairie (Fig. 5). In intact prairie, soil C declined between 2010 and 2013, potentially due to dry conditions during the final two years (Mueller *et al.*, 2016). In contrast, soil C in disturbed/invaded prairie was 25% lower than that in intact prairie in 2010, but recovered by 2013, six years after disturbance. Warming slowed that recovery process in shallow (0-5 cm) soils. Both disturbance and warming effects may have been caused by accelerated decomposition (Carrillo *et al.*, 2018; Walker *et al.*, 2018). Warming effects on decomposition could have been stronger in disturbed/invaded prairie due to C inputs from newly established plants with relatively labile tissue, including *C. diffusa*. This explanation fits with previous work demonstrating greater sensitivity of decomposition to temperature in cultivated than in grassland soils (Conant *et al.*, 2008).

In contrast to our expectations, however, eCO₂ and warming effects on soil C did not follow from their effects on plant biomass. This might be explained by smaller plant biomass responses to eCO₂ and warming belowground than aboveground (Fig. S2) or insufficient time for aboveground plant biomass to be incorporated into the soil. It is also possible, therefore, that increases in plant biomass with eCO₂ would eventually be reflected in soil C. Carbon loss is typical for grassland soils following disturbance, and post-disturbance recovery of soil C is important for mitigating climate change (Conant

et al., 2001; McLauchlan *et al.*, 2006). Our results suggest that warming may slow the short-term recovery of soil C in disturbed environments, even in the presence of high aboveground biomass production.

Disturbed and invaded grasslands are common within the mixedgrass prairie and around the world (Early *et al.*, 2016; Ott *et al.*, 2020; Samson *et al.*, 2004). This study adds to the small set of previous experiments examining how global changes can interact with disturbance or invasion (Bradley *et al.*, 2010; Liu *et al.*, 2017; Perring *et al.*, 2016). Those studies have shown that grazing, clipping, fire and soil disturbance can sometimes alter global change effects (De Vries *et al.*, 2012; Fernandes *et al.*, 2013; Klein *et al.*, 2004; Newton *et al.*, 2014; Teyssonneyre *et al.*, 2002), and that global changes can facilitate invasion (Liu *et al.*, 2017; Sorte *et al.*, 2013). In related work from parallel eCO₂ experiments in intact and disturbed, planted grassland, disturbance initially led to high resource availability and aboveground biomass, but didn't alter eCO₂ effects on plant biomass and species evenness (Leadley *et al.*, 1999; Niklaus *et al.*, 2001). In contrast, we observed marked differences in degree and direction of global change effects with disturbance and invasion. The results demonstrate that disturbance can substantially alter eCO₂ and warming effects, particularly when it favors invasive species (Davis *et al.*, 2000) that respond positively to these global changes (Liu *et al.*, 2017).

Together, the results of this study highlight the need to account for soil disturbance and associated invasion when predicting climate change effects on productivity, diversity

and C cycling (Oliver & Morecroft, 2014; Perring *et al.*, 2016). They also have implications for management of mixedgrass prairie and other semiarid grasslands. It is well known that anthropogenic soil disturbances reduce biological diversity and soil C in grasslands (Davidson & Ackerman, 1993; Hoekstra *et al.*, 2005; Ott *et al.*, 2020; Samson *et al.*, 2004). Our results suggest that eCO₂ and warming will make it more difficult for grasslands to recover from disturbance. In this case, eCO₂ led to dominance by an unpalatable forb and reductions in plant diversity, while warming slowed soil C accumulation. Consequently, limiting soil disturbance and controlling plant invasion, common grassland management objectives, maybe be even more important under future atmospheric and climatic conditions.

Data availability statement: The data that support the findings of this study are openly available in Dryad [DOI still pending].

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Table 1. Mixed-model results for models in which disturbance/invasion was included in the analysis (excluding species richness, evenness, and diversity). Significant *p*-values (<0.05) are in bold.

Treatment	Aboveground Biomass			Root Biomass - 0-5 cm			Root Biomass - 5-15 cm			Resin-available NO ₃ ⁻			Soil C - 0-5 cm			Soil C - 5-15 cm		
	DF	<i>F</i>	<i>p</i>	DF	<i>F</i>	<i>p</i>	DF	<i>F</i>	<i>p</i>	DF	<i>F</i>	<i>p</i>	DF	<i>F</i>	<i>p</i>	DF	<i>F</i>	<i>p</i>
Disturbance	1, 16	86.2	<.0001	1, 32	1.1	0.30	1, 16	6.50	0.02	1, 16	15.8	0.001	1, 15	16.3	0.001	1, 16	0.7	0.40
CO ₂	1, 16	16.7	0.001	1, 32	5.4	0.03	1, 16	0.27	0.61	1, 15	44.2	<.0001	1, 14	1.0	0.34	1, 14	0.5	0.50
Dist.*CO ₂	1, 16	17.4	0.0007	1, 32	1.2	0.28	1, 16	0.67	0.43	1, 16	3.7	0.07	1, 15	0.4	0.54	1, 16	0.0	0.97
Warming	1, 16	4.0	0.07	1, 32	3.1	0.09	1, 16	3.65	0.07	1, 15	17.9	0.0007	1, 13	0.6	0.44	1, 14	1.2	0.29
Dist.*Warm.	1, 16	1.1	0.32	1, 32	1.6	0.22	1, 16	0.61	0.45	1, 16	9.3	0.008	1, 15	0.0	0.83	1, 16	0.8	0.40
CO ₂ *Warm.	1, 16	2.7	0.12	1, 32	2.1	0.16	1, 16	0.01	0.92	1, 15	1.8	0.20	1, 14	1.7	0.21	1, 14	1.4	0.26
Dist.*CO ₂ *Warm.	1, 16	1.0	0.33	1, 32	0.0	0.86	1, 16	0.79	0.39	1, 16	0.0	0.96	1, 15	0.5	0.48	1, 16	1.1	0.31
Yr.	4, 126	11.7	<.0001							4, 127	113.9	<.0001	1, 111	0.0	0.83	1, 112	0.0	0.84
Dist.*Yr.	4, 126	15.3	<.0001							4, 127	13.0	<.0001	1, 111	20.4	<.0001	1, 112	9.1	0.003
CO ₂ *Yr.	4, 126	7.0	<.0001							4, 127	0.5	0.75	1, 111	0.3	0.57	1, 112	0.6	0.46
Dist.*CO ₂ *Yr.	4, 126	3.2	0.01							4, 127	2.1	0.08	1, 111	0.5	0.50	1, 112	0.1	0.82
Warm.*Yr.	4, 126	1.1	0.36							4, 127	5.9	0.0002	1, 111	0.3	0.58	1, 112	1.9	0.17
Dist.*Warm.*Yr.	4, 126	2.1	0.09							4, 127	0.7	0.62	1, 111	4.3	0.04	1, 112	0.2	0.66
CO ₂ *Warm.*Yr.	4, 126	1.1	0.35							4, 127	0.9	0.46	1, 111	0.0	0.89	1, 112	0.4	0.52
Dist.*CO ₂ *Warm.*Yr.	4, 126	0.9	0.49							4, 127	1.0	0.39	1, 111	0.2	0.69	1, 112	0.0	0.98

Table 2. Mixed model results for effects of eCO₂, warming, and year on plant richness, diversity, and evenness in intact and disturbed/invaded mixedgrass prairie. Diversity metrics were only analyzed within disturbance treatments because differences in methods precluded quantitative comparisons across disturbance treatments. Significant *p*-values (<0.05) are in bold.

	Richness			Diversity (H')			Evenness		
	DF	<i>F</i>	<i>p</i>	DF	<i>F</i>	<i>p</i>	DF	<i>F</i>	<i>p</i>
Intact Prairie									
CO ₂	1, 15	0.1	0.76	1, 15	3.3	0.09	1, 15	8.1	0.01
Warming	1, 15	3.8	0.07	1, 15	1.3	0.28	1, 15	0.0	0.95
CO ₂ *Warm.	1, 15	0.2	0.70	1, 15	0.1	0.73	1, 15	0.1	0.76
Yr.	4, 64	16.1	<0.0001	4, 64	8.6	<0.0001	4, 64	18.8	<0.0001
CO ₂ *Yr.	4, 64	1.6	0.19	4, 64	1.1	0.36	4, 64	1.8	0.13
Warm.*Yr.	4, 64	1.8	0.15	4, 64	0.3	0.88	4, 64	1.6	0.20
CO ₂ *Warm.*Yr.	4, 64	0.2	0.95	4, 64	0.1	0.99	4, 64	0.1	0.98
Disturbed Prairie									
CO ₂	1, 15	1.3	0.28	1, 16	0.1	0.81	1, 15	1.6	0.22
Warm.	1, 15	5.3	0.04	1, 16	2.3	0.15	1, 15	0.0	0.95
CO ₂ *Warm.	1, 15	0.6	0.44	1, 16	0.1	0.80	1, 15	0.6	0.45
Yr.	4, 64	32.7	<0.0001	4, 62	12.8	<0.0001	4, 62	3.7	0.009
CO ₂ *Yr.	4, 64	1.2	0.34	4, 62	6.8	0.0001	4, 62	7.2	<0.0001
Warm.*Yr.	4, 64	0.3	0.89	4, 62	1.7	0.16	4, 62	1.6	0.18
CO ₂ *Warm.*Yr.	4, 64	0.8	0.53	4, 62	2.2	0.08	4, 62	1.4	0.23

Figure Legends

Figure 1. Diagram of study design. Colors indicate the timing and location of treatments and sampling. Soil sampling dots indicate approximate locations of multiple smaller samples. Individual rings (whole plots) were 3.3 m in diameter. Underground barriers separated subplots from one another, and rings from the surrounding prairie.

Figure 2. (A) Responses of aboveground biomass to warming and eCO₂ in intact prairie and disturbed/invaded prairie. Stacked bars for disturbed/invaded prairie show the relative contributions of the dominant invasive forb *Centaurea diffusa*, other seeded invasive forbs, and native species to aboveground biomass. (B) Responses of *C. diffusa* biomass to warming and eCO₂ in tilled and vegetated strips within disturbed/invaded subplots. Treatment codes indicate global change treatments: **ct** = control, **cT** = warmed, **Ct** = eCO₂, **CT** = warmed plus eCO₂. Error bars are +/- 1 SE. See Tables 1 and S2 for statistical results.

Figure 3. Effects of eCO₂ and warming on species richness, evenness, and Shannon's diversity (H') in intact vs. disturbed/invaded prairie. Note that absolute values are not comparable across disturbance treatments and years, due to species addition in disturbed/invaded subplots and variation in sampling effort (0.75 m² in intact prairie, and 0.1 m² in disturbed/invaded prairie in 2009-2012, and 0.28 m² in disturbed/invaded prairie in 2013). Treatment codes indicate global change treatments: **ct** = control, **cT** =

warmed, **Ct** = eCO₂, **CT** = warmed plus eCO₂. Error bars are +/- 1 SE. See Table 2 for statistical results.

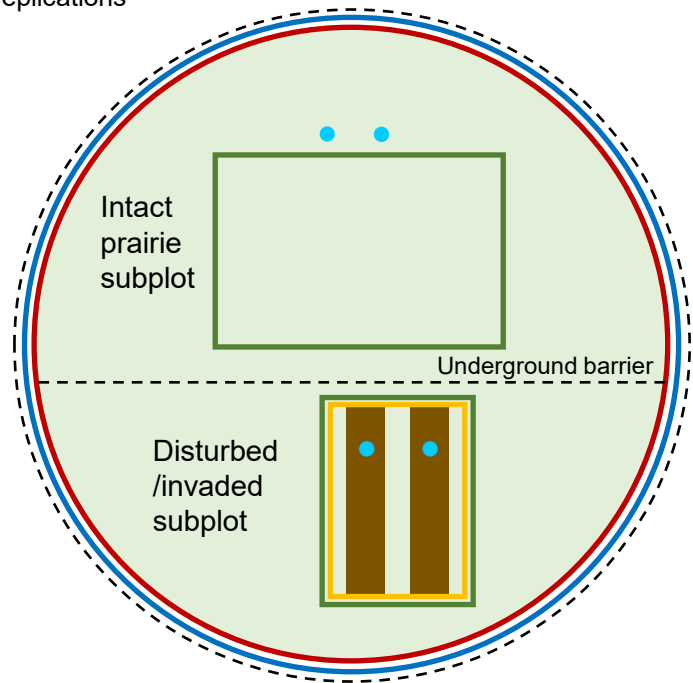
Figure 4. Path analysis assessing the relative strength of direct and indirect effects of eCO₂ and warming on diversity in intact and disturbed/invaded prairie. Blue and red and gray arrows represent significant positive, significant negative and non-significant effects, respectively. Arrow widths are proportional to standardized path coefficients, shown in adjacent numbers. Dashed arrows pass behind aboveground biomass.

Marginal R² values for diversity were 0.12 for intact prairie and 0.35 for disturbed/invaded prairie.

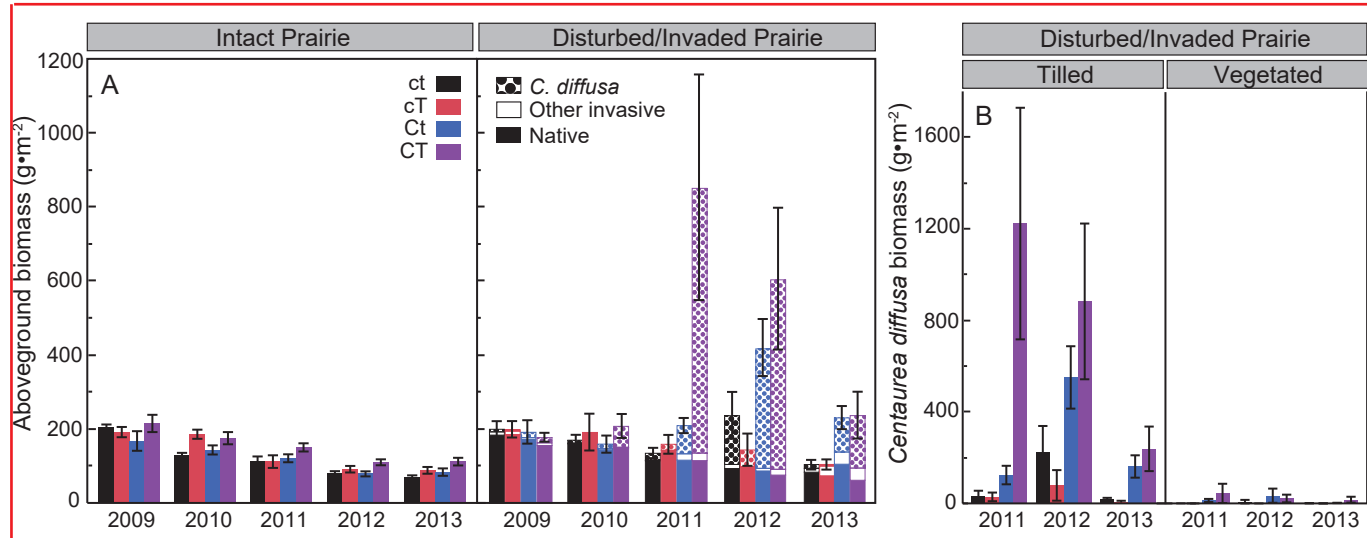
Figure 5. Effects of disturbance/invasion on temporal changes in % soil C at 0-5 cm and 5-15 cm depths by global change treatment. Treatment codes indicate global change treatments: **ct** = control, **cT** = warmed, **Ct** = eCO₂, **CT** = warmed plus eCO₂. Error bars are +/- 1 SE. See Tables 1 and S2 for statistical results.

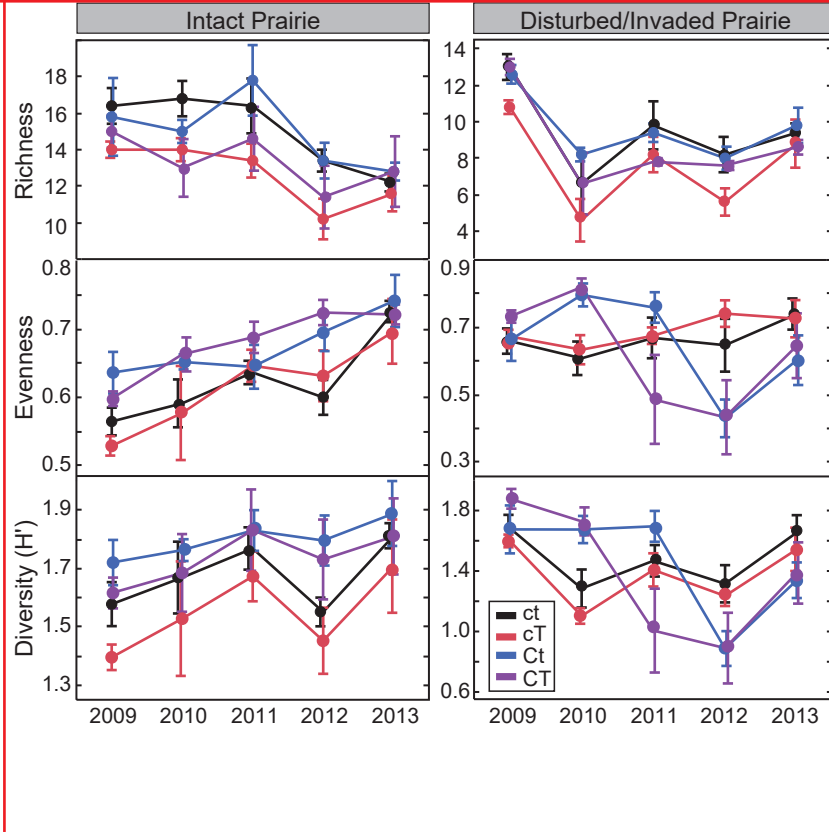
Design:

- 4 whole-plot treatments: control, eCO₂, warming, eCO₂ & warming
- 2 split-plot treatments: intact prairie, disturbed/invaded prairie
- 5 replications



	2006	2007	2008	2009	2010	2011	2012	2013
Elevated CO₂								
Warming								
Disturbance								
Seeding								
Biomass sampling								
Soil C sampling								
Soil N sampling								
Light sampling								





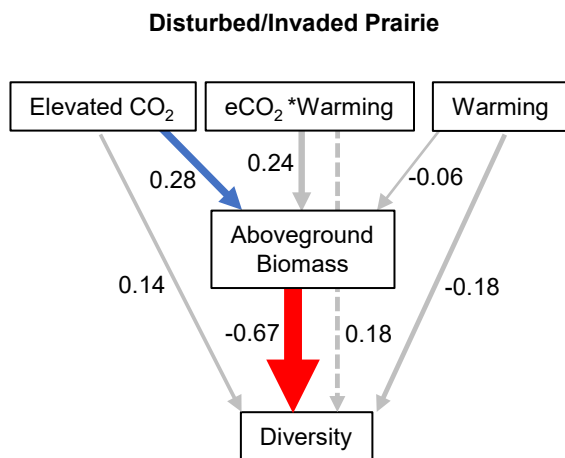
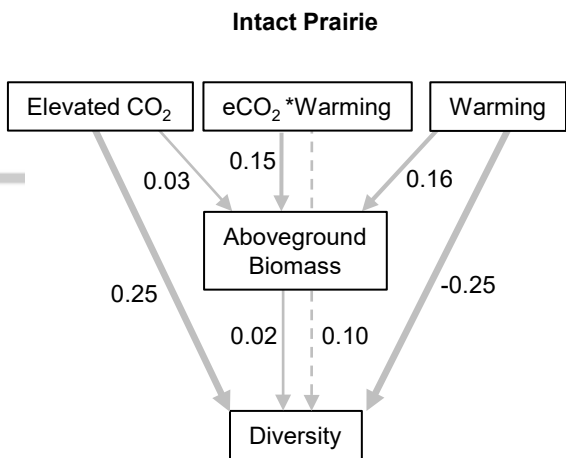


Figure 3

