


## LETTER

# Elevated CO<sub>2</sub> does not stimulate carbon sink in a semi-arid grassland

Jian Song,<sup>1,2</sup>

Shiqiang Wan,<sup>1,2\*</sup> 

Shilong Piao,<sup>3,4,5</sup> Dafeng Hui,<sup>6</sup>

Mark J. Hovenden,<sup>7</sup> Philippe Ciais,<sup>8</sup>

Yongwen Liu,<sup>9</sup> Yinzhan Liu,<sup>1</sup>

Mingxing Zhong,<sup>1</sup>

Mengmei Zheng,<sup>1</sup> Gaigai Ma,<sup>1</sup>

Zhenxing Zhou<sup>1</sup> and Jingyi Ru<sup>1</sup>

## Abstract

Elevated CO<sub>2</sub> is widely accepted to enhance terrestrial carbon sink, especially in arid and semi-arid regions. However, great uncertainties exist for the CO<sub>2</sub> fertilisation effects, particularly when its interactions with other global change factors are considered. A four-factor (CO<sub>2</sub>, temperature, precipitation and nitrogen) experiment revealed that elevated CO<sub>2</sub> did not affect either gross ecosystem productivity or ecosystem respiration, and consequently resulted in no changes of net ecosystem productivity in a semi-arid grassland despite whether temperature, precipitation and nitrogen were elevated or not. The observations could be primarily attributable to the offset of ecosystem carbon uptake by enhanced soil carbon release under CO<sub>2</sub> enrichment. Our findings indicate that arid and semi-arid ecosystems may not be sensitive to CO<sub>2</sub> enrichment as previously expected and highlight the urgent need to incorporate this mechanism into most IPCC carbon-cycle models for convincing projection of terrestrial carbon sink and its feedback to climate change.

## Keywords

Carbon cycle, climate warming, CO<sub>2</sub> enrichment, forb, grass, increased precipitation, modelling, multi-factor experiment, nitrogen addition, species composition.

Ecology Letters (2019)

## INTRODUCTION

Rising atmospheric CO<sub>2</sub> concentrations can not only stimulate plant growth and productivity directly by enhancing mesophyll CO<sub>2</sub> diffusion and photosynthesis (Sun *et al.* 2014), but also indirectly by reducing leaf stomatal conductance and evapotranspiration (de Boer *et al.* 2011; Lammertsma *et al.* 2011), increasing water-use efficiency (Keenan *et al.* 2013; Fatichi *et al.* 2016) and ameliorating water limitation (Morgan *et al.* 2011; Farrior *et al.* 2015). As a consequence, increasing experimental evidence has unambiguously revealed that CO<sub>2</sub> enrichment can enhance ecosystem carbon (C) uptake and sequestration across various terrestrial biomes – CO<sub>2</sub> fertilisation effects (CFEs; DeLucia *et al.* 1999; Smith *et al.* 2000; Gill *et al.* 2002; Norby *et al.* 2005; Morgan *et al.* 2011; Evans *et al.* 2014; Farrior *et al.* 2015; Roy *et al.* 2016). Based on atmospheric and satellite observations, terrestrial C-cycle models, which are used in IPCC Reports to assess land C budget responses to global change (IPCC 2014), have further attributed increasing global greening and a significant fraction of land C storage to CFEs (Zhu *et al.* 2016a; Huntzinger *et al.* 2017). Moreover, the water-saving mechanisms associated with CO<sub>2</sub> enrichment suggest that CFEs are

more prominent in arid and semi-arid ecosystems (Donohue *et al.* 2013; Fatichi *et al.* 2016), and contribute profoundly to land C sink and global C cycling (Ahlström *et al.* 2015).

As an important biome, grasslands occupy 87 and 54% of the arid and semi-arid regions, respectively (UN 2011). Quantifying the magnitude of grassland responses to rising atmospheric CO<sub>2</sub> is crucial for accurately evaluating future global C budgets. However, large uncertainty of CFEs on grasslands remains due to the confounding impacts by other global change factors including climate warming, changing precipitation regimes and atmospheric nitrogen (N) deposition (Zhu *et al.* 2016b; Carrillo *et al.* 2018). For example, the responses of terrestrial ecosystems to elevated CO<sub>2</sub> (eCO<sub>2</sub>) are dampened by N scarcity (Hungate *et al.* 2003; van Groenigen *et al.* 2006; Reich *et al.* 2006; Reich & Hobbie 2013) and sustained for plants whose association with ectomycorrhizae provides better access to N (Terrer *et al.* 2016, 2018). Changes in climate conditions can also modulate the responses of plant productivity and C allocation to eCO<sub>2</sub>. Several grassland FACE (free-air CO<sub>2</sub> enrichment) experiments have demonstrated that CFEs increase under summer-dominated rainfall (Hovenden *et al.* 2014), but decline in periods of extreme weather conditions (Mueller *et al.* 2016; Roy *et al.* 2016; Obermeier *et al.*

<sup>1</sup>International Joint Research Laboratory for Global Change Ecology, School of Life Sciences, Henan University, Kaifeng, Henan 475004, China

<sup>2</sup>College of Life Sciences, Hebei University, Baoding, Hebei 071002, China

<sup>3</sup>Sino-French Institute for Earth System Science, College of Urban and Environmental Sciences, Peking University, Beijing 100871, China

<sup>4</sup>Key Laboratory of Alpine Ecology and Biodiversity, Institute of Tibetan Plateau Research, Chinese Academy of Sciences, Beijing 100085, China

<sup>5</sup>Centre for Excellence in Tibetan Earth Science, Chinese Academy of Sciences, Beijing 100085, China

<sup>6</sup>Department of Biological Sciences, Tennessee State University, Nashville, TN 37209, USA

<sup>7</sup>Biological Sciences, School of Natural Sciences, University of Tasmania, Private Bag 55, Hobart, Tas 7001, Australia

<sup>8</sup>Laboratoire des Sciences du Climat et de l'Environnement, CEA CNRS UVSQ, Gif-sur-Yvette, France

<sup>9</sup>Key Laboratory of Alpine Ecology and Biodiversity, Institute of Tibetan Plateau Research, Chinese Academy of Sciences, Beijing 100101, China

\*Correspondence: E-mail: swan@ibcas.ac.cn

2017, 2018). In addition, climate warming may enhance CFEs by increasing soil N availability (Hovenden *et al.* 2008, 2017; Dijkstra *et al.* 2010), and/or aggravating water limitation (Morgan *et al.* 2011).

Theoretical analyses further suggest that plant community composition may be a potential mediator of CFEs under multifactorial global change (Kardol *et al.* 2012; Langley & Hungate 2014), partly due to the diverse CO<sub>2</sub> responses of photosynthesis among plant species and related variations in interspecific competition (Poorter & Navas 2003). For example, growth stimulation of C<sub>3</sub> forbs under eCO<sub>2</sub> is higher than that of C<sub>3</sub>/C<sub>4</sub> grasses in two FACE experiments (Lee *et al.* 2011; Newton *et al.* 2014), in contrast to other work (Fay *et al.* 2012; Andresen *et al.* 2017). In addition, a 20-year FACE experiment has revealed that eCO<sub>2</sub> can promote growth of C<sub>3</sub> grasses greater than C<sub>4</sub> grasses during the first 12 years of treatment, but the pattern reverses during the subsequent 8 years (Reich *et al.* 2018). Such differential responses are particularly important because species compositional shifts could dramatically affect community or ecosystem-level responses to eCO<sub>2</sub> (Langley & Megeonigal 2010). Overall, a comprehensive assessment on CFEs should be made together with other global change factors (Reich *et al.* 2014; Zhu *et al.* 2016b) and take plant community composition into consideration (Kardol *et al.* 2012).

Here, we report the second four-factor (eCO<sub>2</sub>, night-time warming, increased precipitation and N addition) experiment in the world, to the best of our knowledge, lasted for 6 years (2012–2017) in a semi-arid temperate steppe on the Mongolian Plateau. As the typical vegetation of the regional grassland biome across the Eurasian continent, this semi-arid temperate steppe is predominately controlled by water availability (Liu *et al.* 2009) and thus expected to respond strongly to eCO<sub>2</sub>. Our previous studies at this experimental site have demonstrated that warming, increased precipitation and N addition consistently enhance gross ecosystem productivity (GEP) to a greater extent than ecosystem respiration (ER), leading to stimulations in net ecosystem productivity (NEP; Wan *et al.* 2009; Niu *et al.* 2010, 2011). Nevertheless, how CO<sub>2</sub> interacts with temperature, precipitation and N manipulations to affect ecosystem C exchange in this semi-arid grassland is still not clear. We hypothesised that (1) warming and N addition would amplify CFEs by increasing soil N availability and/or aggravating water limitation, and (2) on the contrary, increased precipitation would reduce CFEs by suppressing N availability (Hovenden *et al.* 2014) and/or ameliorating water limitation. Finally, although C-cycle models are critical in revealing grassland responses to eCO<sub>2</sub> at large spatiotemporal scales, considerable uncertainty still exists in model projections and needs to be reduced by parameterising with experimental data (Medlyn *et al.* 2015), especially under complex future climate change scenarios. Thus, we also compared our observations with model outputs to evaluate model performance.

## MATERIALS AND METHODS

### Study site

The experimental site is located in a semi-arid temperate steppe in Duolun County (42°02' N, 116°16' E, 1324 m a.s.l.),

Inner Mongolia, China. Long-term (1954–2016) mean annual temperature is 2.4 °C, and mean annual precipitation is 382 mm with 91% of annual rainfall occurring during the growing season from May to October (China Meteorological Data Sharing Service System). The sandy soil at this study site is classified as Haplic Calcisols (FAO classification). The study site was subjected to livestock grazing prior to 2001 and has been fenced since 2001 to exclude large herbivores with the national policy of ecological restoration in grasslands of China. Ambient N deposition in this area is approximately 20 kg N ha<sup>-1</sup> year<sup>-1</sup> in 2005–2006 (Zhang *et al.* 2008).

### Experimental design

The four-factor experiment was established in May 2011. Each factor had two levels: atmospheric CO<sub>2</sub> concentrations [ambient (aCO<sub>2</sub>) and ambient plus 200 ppm (eCO<sub>2</sub>)], temperature [ambient (aT) and elevated temperature (eT) by night-time (18:00–06:00, local time) warming], precipitation [ambient (aP) and 30% above the ambient precipitation (eP)], and N deposition [ambient (aN) and ambient plus 10 g N m<sup>-2</sup> year<sup>-1</sup> (eN)]. The experiment used a split-plot design, with CO<sub>2</sub>, temperature and precipitation as main treatment factors and N as a subplot factor (Fig. S1). Twenty-four 4-m × 4-m plots were arranged into six rows and four columns, with a 4-m buffer zone between any two adjacent plots. The 24 plots were randomly assigned to the eight combinations of CO<sub>2</sub>, temperature and precipitation, with each treatment having three replicates (blocks). Each of the 24 plots was divided into two subplots, one with N addition and one without. Therefore, this experiment included eight treatments with aN: aCO<sub>2</sub>aTaPaN, eCO<sub>2</sub>aTaPaN, aCO<sub>2</sub>eTaPaN, aCO<sub>2</sub>aTePaN, eCO<sub>2</sub>eTaPaN, eCO<sub>2</sub>aTePaN, aCO<sub>2</sub>eTePaN and eCO<sub>2</sub>eTePaN and eight treatments with eN: aCO<sub>2</sub>aTaPeN, eCO<sub>2</sub>aTaPeN, aCO<sub>2</sub>eTaPeN, aCO<sub>2</sub>aTePeN, eCO<sub>2</sub>eTaPeN, eCO<sub>2</sub>aTePeN, aCO<sub>2</sub>eTePeN and eCO<sub>2</sub>eTePeN.

### Experimental manipulations

Large octagonal open-top chambers (OTCs) were used to manipulate atmospheric CO<sub>2</sub> concentrations (for details see Appendix S1). In each eCO<sub>2</sub> plot, pure CO<sub>2</sub> was released into the OTC via plastic tubes (4 mm in diameter) to achieve a diurnal CO<sub>2</sub> enrichment of 200 ppm over ambient air from June to September of 2011–2017, controlled by LI-820 CO<sub>2</sub> Analyser (LI-COR, Lincoln, NE, USA) and an automatic control system (Luzhai Co., Beijing, China). The instruments were installed and tested in 2011. From 2012 to 2017, mean CO<sub>2</sub> concentrations were 406.7 ± 3.6 ppm and 605.4 ± 8.1 ppm in the aCO<sub>2</sub> and eCO<sub>2</sub> plots, respectively (Fig. S2). The targeted eCO<sub>2</sub> concentration of 600 ppm equals the concentration likely to be reached mid-21st century (IPCC 2014) and is consistent with the treatments of most FACE experiments.

Evidence shows that the global surface temperature is increasing more quickly at night-time than daytime (Easterling *et al.* 1997), leading to a substantial stimulation of C sequestration in the same grassland (Wan *et al.* 2009). Thus, we

warmed plots at night-time using MSR-2420 infrared radiators (Kalglo Electronics Inc., Bethlehem, PA, USA) suspended 2.75 m above the ground, from mid-March to mid-November of 2011–2017. The night-time-warming treatment did increase night-time temperature (by 0.73 °C), but the increased soil temperatures persisted into the day, resulting in a daytime temperature increase of 0.52 °C (Appendix S1), consistent with current climate warming scenarios.

The interannual fluctuations of annual precipitation in local area varied from 35.2% below to 34.1% above the long-term average over the 1954–2010 period (Fig. S3a). Therefore, a 30% increase above ambient precipitation in this project represents a strong but not unrealistic increase in precipitation (Niu *et al.* 2011). After each precipitation event over the 4 months from June to September of 2011–2017, rainfall amount was obtained immediately from a rain gauge adjacent to the experiment (< 200 m). Collected rain water was applied with an automatic sprinkler system right after each natural rain event to avoid changing rainfall frequency. The aP and eP plots received ambient and ambient plus 30% precipitation, respectively (Fig. S3b).

Given the observations of a previous study conducted in this area that ecosystem responses to N addition reached a saturation point at a rate of approximately +10.5 g N m<sup>-2</sup> year<sup>-1</sup> over ambient values (Bai *et al.* 2010), we set the level of N addition as 10 g N m<sup>-2</sup> year<sup>-1</sup>. NH<sub>4</sub>NO<sub>3</sub> was applied in all the eN subplots in mid-June (5 g N m<sup>-2</sup> year<sup>-1</sup>) and mid-July (5 g N m<sup>-2</sup> year<sup>-1</sup>) from 2011 to 2017.

#### Diurnal cycles of ecosystem CO<sub>2</sub> and H<sub>2</sub>O fluxes

Diurnal cycles of ecosystem CO<sub>2</sub> and H<sub>2</sub>O fluxes were measured with a 0.5-m-long × 0.5-m-wide × 0.5-m-high transparent chamber connected to a Li-6400 Photosynthesis System (LI-COR Inc., Lincoln, NE, USA; also see the detail information in Song *et al.* 2015). The chamber was sealed to the surface of an iron frame, which was inserted into the soil at 3-cm depth in each subplot. Steady-state conditions were achieved inside the chamber after 2 min and 9 consecutive logs of CO<sub>2</sub> and H<sub>2</sub>O concentrations were subsequently recorded at 10-s intervals on each frame during a 90-s period. The decreases or increases in the rates of CO<sub>2</sub> and H<sub>2</sub>O concentrations were used to calculate NEP and evapotranspiration (ET; LI-COR Inc. 2003). Following the measurements of NEP, the chamber was vented, covered with an opaque cloth, and put back on the same iron frame. The CO<sub>2</sub> exchange was measured again to calculate ER rate as light was eliminated (and hence photosynthesis). Gross ecosystem productivity was estimated as ER plus NEP. Positive and negative NEP values represent net C uptake and loss, respectively.

Given that soil C loss plays an important role in determining grassland C responses to eCO<sub>2</sub> (Hungate *et al.* 1997), we measured soil respiration (SR) using the same technique (Infra-Red Gas Analyser) with ER measurements. The difference between SR and ER is that ER also includes respiratory C emissions from aboveground plant parts. Thus, measuring both ER and SR allowed us to separate treatment impacts on soil fluxes from those on total ecosystem fluxes. One 5-cm-high PVC collar (10 cm in diameter) was permanently inserted 3 cm into the soil

in each subplot. A CO<sub>2</sub> and H<sub>2</sub>O flux chamber attached to a Li-8100 (LI-COR Inc.) was put on each collar for 1–2 min to measure SR and soil evaporation (E), and then moved to the next collar. The aboveground parts of living plants inside the collars were removed by hand at least 1 day before the measurements. All the ecosystem CO<sub>2</sub> and H<sub>2</sub>O fluxes were measured at 4-h intervals (06:00, 10:00, 14:00, 18:00, 22:00 and 02:00, local time) twice per month on clear and sunny days from May to October in 2012–2017.

Canopy transpiration (T<sub>c</sub>) was estimated as ET minus E. Canopy- and ecosystem-level water-use efficiency (WUE<sub>C</sub> and WUE<sub>E</sub>) were calculated following Niu *et al.* (2011) as:

$$WUE_C = \frac{GEP}{T_c} \quad (1)$$

$$WUE_E = \frac{GEP}{ET} \quad (2)$$

#### NDVI measurements

The Normalized Difference Vegetation Index (NDVI) was used to estimate plant canopy development from 2014 to 2017. Spectral reflectance was measured under cloud-free conditions using an agricultural digital camera (Tetracam Inc., Chatsworth, CA, USA) to monitor NDVI throughout the growing season at 5-day intervals between 11:00 to 13:00 h. Each sample was the mean of four spectra captured with a fibre optic collector (25° field of view) at a height of 50 cm. NDVI was calculated as:

$$NDVI = \frac{\text{Reflectance at 775 nm} - \text{Reflectance at 675 nm}}{\text{Reflectance at 775 nm} + \text{Reflectance at 675 nm}} \quad (3)$$

#### Plant cover estimations

One 1-m × 1-m permanent quadrat was established at the centre of each subplot in June 2011. Plant cover during peak biomass (early August) was assessed in the permanent quadrats using a visual evaluation method once a year from 2012 to 2017. During the measurements, a 1-m × 1-m frame with 100 equally distributed grids (10 cm × 10 cm) was placed above the canopy in each quadrat. The per cent cover of each species was visually estimated in all the grids and summed as species cover. Per cent cover across species was summed to obtain functional group and community cover. Plants were classified into two functional groups: grasses and forbs (Table S1). Over the 6-year period, cover of grasses and forbs accounted for 46 and 54% of community cover, respectively, in the control (aCO<sub>2</sub>-TaPaN) subplots, indicating grass-forb codominance of the temperate steppe.

#### Statistical analyses

This study focused on the eCO<sub>2</sub> effects under multi-factorial global change. First, four-way repeated-measures analyses of variance (RM-ANOVAS) with a split-plot design were performed to examine the main and interactive influences of CO<sub>2</sub>, temperature, precipitation, and N on NEP, ER, GEP, ET, E, T<sub>c</sub>, WUE<sub>C</sub>, WUE<sub>E</sub>, NDVI, forb cover, and the ratio of forb to community cover across all the 16 treatments. In the mixed effect models (PROC MIXED) used in RM-ANOVAS, between-

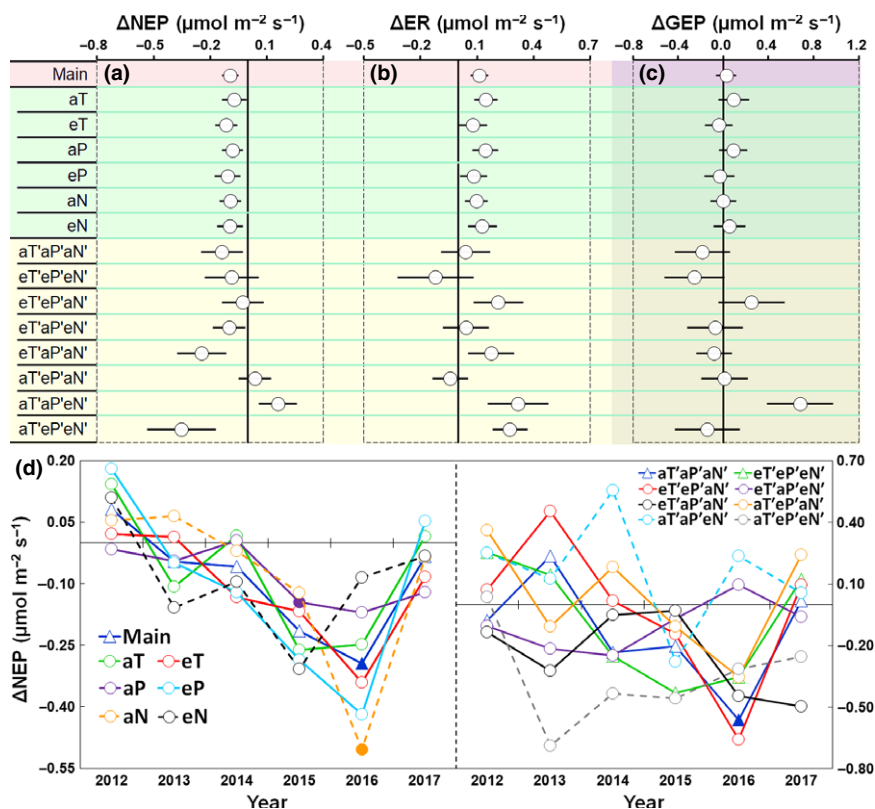
subject effects were assessed as CO<sub>2</sub>, temperature, precipitation, N, and their interactions and within-subject effects were year and its interactions with CO<sub>2</sub>, temperature, precipitation and N (for SAS code see Appendix S2). The N addition treatment was nested within the plot (OTC), and thus the construction of the plot error and the pooled residual error were used to test effects of CO<sub>2</sub>, temperature, precipitation and their interactions as well as influences of N and its interactions with the other three factors, respectively (Hu 2010). The statistical power for the main effects of eCO<sub>2</sub> on NEP, ER, and GEP was 0.92, 0.63 and 0.07, respectively. Second, given the scarcity of four-factor experiments, reporting the eCO<sub>2</sub> effects under different combinations of global change factors is extremely important (Shaw *et al.* 2002). Three-way RM-ANOVAS with a split-plot design were used to examine eCO<sub>2</sub> impacts on these parameters across the eight treatments with aT, eT, aP and eP, respectively. In addition, three-way RM-ANOVAS were performed for these parameters across the eight treatments with aN and eN, respectively. Finally, multiple comparisons using the LSD method were conducted to examine eCO<sub>2</sub> influences on these parameters under each of the eight treatment combinations of temperature, precipitation and N, including aT'aP'aN' (eCO<sub>2</sub>aTaPaN vs. aCO<sub>2</sub>aTaPaN), eT'eP'eN' (eCO<sub>2</sub>eTePeN vs. aCO<sub>2</sub>eTePeN), eT'eP'aN' (eCO<sub>2</sub>eTePaN vs. aCO<sub>2</sub>eTePaN), eT'aP'eN' (eCO<sub>2</sub>eTaPeN vs. aCO<sub>2</sub>eTaPeN), eT'aP'aN' (eCO<sub>2</sub>eTaPaN vs.

aCO<sub>2</sub>eTaPaN), aT'eP'aN' (eCO<sub>2</sub>aTePaN vs. aCO<sub>2</sub>aTePaN), aT'aP'eN' (eCO<sub>2</sub>aTaPeN vs. aCO<sub>2</sub>aTaPeN) and aT'eP'eN' (eCO<sub>2</sub>aTePeN vs. aCO<sub>2</sub>aTePeN).

Over the 6 years, eCO<sub>2</sub>-induced absolute changes were calculated under different treatment combinations. For example, the main effects of eCO<sub>2</sub> were calculated as the difference between all the plots with eCO<sub>2</sub> and aCO<sub>2</sub>. Similarly, the influences of eCO<sub>2</sub> under the aT treatment were examined as the difference between the plots with eCO<sub>2</sub> and aT and the plots with aCO<sub>2</sub> and aT. The significance levels of the eCO<sub>2</sub> effects were determined by RM-ANOVAS and multiple comparisons. In addition, to show potential interannual variations of the eCO<sub>2</sub> impacts, effects in each year were also calculated and provided in Fig. S4.

Simple linear regressions were conducted to examine the spatial relationship of NEP with forb cover and SR and the dependences of NEP changes on the changes in forb cover and SR induced by eCO<sub>2</sub> under different treatment combinations. Stepwise multiple linear regressions were used to investigate which variable has the greatest effect on NEP changes under eCO<sub>2</sub>, with the changes in forb cover and SR as the independent variables. All statistical analyses described above were conducted using SAS 9.4 (SAS Institute, Cary, NC, USA).

To address whether the OTCs changed microclimate and subsequently affected community structure and ecosystem C fluxes, we analysed microclimate changes induced by OTCs



**Figure 1** Means ( $\pm 1$  SE) of eCO<sub>2</sub>-induced absolute changes ( $\Delta$ ) in NEP (a), ER (b) and GEP (c) across the 6 years and NEP changes in each year (d) under different treatment combinations (TCs), including (1) averaged across all the 8 TCs (main effects,  $n = 144$  [3(block)  $\times$  8(TC)  $\times$  6(year)] in panels a–c and  $n = 24$  [3(block)  $\times$  8(TC)] in panel d), (2) averaged across the 4 TCs of aT vs. eT, aP vs. eP, and aN vs. eN ( $n = 72$  [3(block)  $\times$  4(TC)  $\times$  6(year)] in panels a–c and  $n = 12$  [3(block)  $\times$  4(TC)] in panel d), and (3) under each of the 8 TCs including aT'aP'aN', eT'eP'eN', eT'eP'aN', eT'aP'eN', eT'aP'aN', aT'eP'aN', aT'aP'eN' and aT'eP'eN' ( $n = 18$  [3(block)  $\times$  6(year)] in panels a–c and  $n = 3$  [block] in panel d). Dark shadows in panel c highlight GEP = NEP + ER. Filled symbols indicate significant changes at  $P < 0.05$ . See Table 1 for treatment abbreviations.



**Table 1** Summary (*P*-values) of four-way, split-plot, RM-ANOVAS on the effects of eCO<sub>2</sub>, eT, eP and eN on net ecosystem productivity (NEP), ecosystem respiration (ER), gross ecosystem productivity (GEP), canopy- and ecosystem-level water-use efficiency (WUE<sub>C</sub> and WUE<sub>E</sub>), the Normalized Difference Vegetation Index (NDVI) and forb cover (FO)

Source of variation	NEP	ER	GEP	WUE <sub>C</sub>	WUE <sub>E</sub>	NDVI	FO
eCO <sub>2</sub>	0.095	0.385	0.865	0.901	0.876	0.783	<b>0.010</b>
eT	0.667	0.785	0.993	0.315	0.080	0.535	<b>0.006</b>
eP	0.189	<b>0.022</b>	<b>&lt; 0.001</b>	0.451	0.173	0.145	0.192
eN	0.088	0.055	0.430	0.086	0.057	0.543	<b>&lt; 0.001</b>
eCO <sub>2</sub> × eT	0.706	0.682	0.596	0.825	0.349	0.265	0.419
eCO <sub>2</sub> × eP	0.815	0.713	0.632	0.916	0.988	0.903	0.788
eCO <sub>2</sub> × eN	0.993	0.849	0.824	0.774	0.614	0.903	0.129
eT × eP	0.173	0.099	<b>0.011</b>	0.438	0.462	0.057	0.497
eT × eN	0.191	0.223	0.755	<b>0.034</b>	0.060	0.692	0.143
eP × eN	0.132	0.158	0.698	0.449	0.374	0.951	0.950
eCO <sub>2</sub> × eT × eP	0.201	1.000	0.434	0.219	0.123	0.605	0.078
eCO <sub>2</sub> × eT × eN	0.678	0.130	0.229	0.848	0.344	0.319	0.855
eCO <sub>2</sub> × eP × eN	0.051	0.819	0.129	0.507	0.767	0.430	0.995
eT × eP × eN	0.342	0.667	0.617	0.406	0.964	0.692	0.769
eCO <sub>2</sub> × eT × eP × eN	0.282	0.729	0.611	0.288	0.286	<b>0.020</b>	<b>0.027</b>
year (yr)	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>
eCO <sub>2</sub> × yr	<b>0.012</b>	0.340	0.719	0.603	0.706	0.283	0.069
eT × yr	0.176	0.718	0.281	0.346	0.061	<b>0.040</b>	0.942
eP × yr	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>0.011</b>	<b>0.008</b>	<b>0.001</b>	0.336
eN × yr	0.685	0.053	0.139	0.115	0.299	<b>0.012</b>	0.399
eCO <sub>2</sub> × eT × yr	0.625	0.810	0.925	0.717	0.151	0.423	0.950
eCO <sub>2</sub> × eP × yr	0.141	0.851	0.648	0.797	0.993	<b>0.001</b>	0.160
eCO <sub>2</sub> × eN × yr	0.104	0.375	0.404	0.461	0.137	0.936	0.738
eT × eP × yr	0.629	0.097	0.894	0.193	0.326	0.806	0.259
eT × eN × yr	<b>0.034</b>	0.786	0.514	0.324	0.237	0.580	0.609
eP × eN × yr	0.475	0.601	0.331	0.139	0.344	0.907	0.675
eCO <sub>2</sub> × eT × eP × yr	0.492	0.204	0.085	0.165	0.410	0.630	<b>0.049</b>
eCO <sub>2</sub> × eT × eN × yr	0.604	0.794	0.725	0.533	<b>0.031</b>	0.173	0.559
eCO <sub>2</sub> × eP × eN × yr	0.896	0.644	0.964	0.495	<b>0.012</b>	0.578	0.462
eT × eP × eN × yr	0.776	0.459	0.646	<b>0.023</b>	<b>&lt; 0.001</b>	0.236	0.390
eCO <sub>2</sub> × eT × eP × eN × yr	0.521	0.237	0.555	0.971	0.777	0.765	0.934

Note The bold numerals highlight the significance at  $P < 0.05$ . Key to abbreviations: aCO<sub>2</sub> and eCO<sub>2</sub>: ambient and elevated CO<sub>2</sub>, aT and eT: ambient and elevated temperature, aP and eP: ambient and increased precipitation, aN and eN: ambient and enriched N deposition.

alone, and tried to link them to OTC effects on community structure and C fluxes (Appendix S1). Compared with the unchambered control plots, the OTCs enhanced air temperature (+0.9°C) and humidity (+8.2%), and soil temperature (+1.4°C), but reduced air vapor pressure deficit (−0.15 kPa), leading to decreased community (−15%) and forb cover (−26%). However, the ratio of forb to community cover, GEP, ER, or NEP were not impacted by the OTCs. Nevertheless, given all the other OTCs with different treatments had similar impacts on the above parameters to those of the control treatment with OTCs in comparison with the unchambered control plots, the treatment effects were calculated as the differences between the plots with OTCs. Therefore, the confounding impacts of OTCs could not have influenced the results and conclusions of this work.

### Model simulations

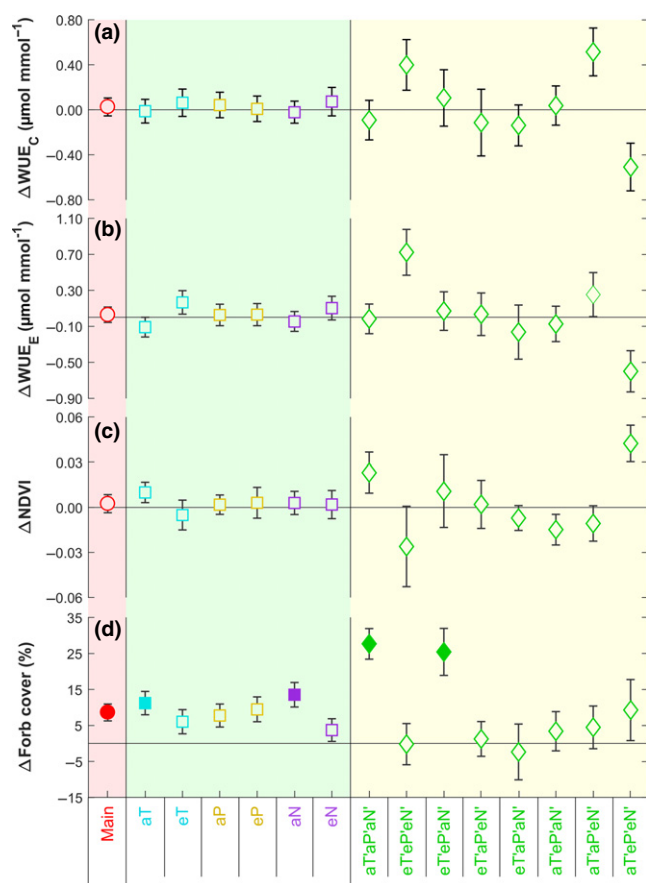
We conducted model simulations from the project ‘Trends and drivers of the regional scale sources and sinks of carbon dioxide’ (TRENDY, <http://dgvn.ceh.ac.uk/node/9>). A total of nine terrestrial C-cycle models (CLM4.5, ISAM, JULES3.2, LPJ, LPJ-GUESS, LPX-Bern, OCN, ORCHIDEE and

VISIT) from TRENDY version 2 were performed in this study. CLM4.5, LPJ-GUESS, LPX-Bern and OCN had considered the impacts of N limitation on the eCO<sub>2</sub> effect, but, the other five models did not couple the interactions between C and N cycling (Le Quéré 2016). We used model results under S1 scenario which was forced by time-varying atmospheric CO<sub>2</sub> concentrations, with climate and land use being constant (Piao *et al.* 2013). Simulated GEP, ER, and NEP at our experimental site were extracted from each model via its latitude and longitude (Duolun County, 42°02′ N, 116°16′ E). Finally, we calculated the eCO<sub>2</sub>-induced changes in GEP, ER, and NEP in Duolun during past three decades as the difference in GEP, ER, and NEP between the time periods of 1983–1987 and 2008–2012. The significance between the changes were tested using *t*-tests.

## RESULTS

### Ecosystem CO<sub>2</sub> exchange responses to eCO<sub>2</sub>

Averaged across all the 6 years and the eight treatment combinations (8 treatments with aCO<sub>2</sub> vs. 8 treatments with eCO<sub>2</sub>), there was no impact of eCO<sub>2</sub> on NEP (4-way RM-



**Figure 2** Means ( $\pm 1$  SE) of  $e\text{CO}_2$ -induced absolute changes in  $\text{WUE}_C$  (a),  $\text{WUE}_E$  (b), NDVI (c) and forb cover (d) under different treatment combinations. Filled symbols indicate significant changes at  $P < 0.05$ . See Table 1 and Figure 1 for abbreviations.

ANOVAS; Fig. 1a, Table 1). However, the  $e\text{CO}_2$  effects on NEP strongly changed with year ( $e\text{CO}_2 \times \text{year}$   $P < 0.05$ ), ranging from an insignificant increase of 157% ( $+ 0.08 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) in 2012 to a significant decrease of 44% in 2016 ( $-0.29 \mu\text{mol m}^{-2} \text{s}^{-1}$ ,  $P < 0.05$ ; Fig. 1d). In addition,  $e\text{CO}_2$  did not affect NEP either when averaged across all the four treatment combinations (aT vs. eT, aP vs. eP, and aN vs. eN; 3-way RM-ANOVAS; Tables S2–S4) or under any of the eight treatment combinations (multiple comparisons; Table S5). Moreover,  $e\text{CO}_2$  had no effect on ER or GEP, the two components of NEP, under any treatment combination (Fig. 1b,c).

#### Effects of $e\text{CO}_2$ on WUE, NDVI, and plant cover

Over the 6 years and averaged across all the eight treatment combinations,  $e\text{CO}_2$  did not affect either  $\text{WUE}_C$  or  $\text{WUE}_E$  (Fig. 2a,b, Table 1). In addition, no  $e\text{CO}_2$  effect on  $\text{WUE}_C$  or  $\text{WUE}_E$  was detected when averaged across all the four treatment combinations (Tables S2–S4). When analysed separately by each treatment combination,  $e\text{CO}_2$  did not change  $\text{WUE}_C$  or  $\text{WUE}_E$  under any of the eight treatment combinations (Table S5). Similar to WUE, NDVI showed a neutral

response to  $e\text{CO}_2$  under all treatment combinations across the last 4 years from 2014 to 2017 (Fig. 2c).

When averaged across all the eight treatment combinations, the main effects of  $e\text{CO}_2$  substantially enhanced forb cover by 8.6% ( $P < 0.05$ ; Fig. 2d, Table 1). In addition,  $e\text{CO}_2$  also significantly stimulated forb cover by 6.0 and 13.5% when averaged across the four treatment combinations with aT and with aN, respectively (both  $P < 0.05$ ; Tables S2–S4). Moreover,  $e\text{CO}_2$  substantially increased forb cover by 27.7 and 25.4% under the treatment combinations of aT'aP'aN' and eT'eP'aN' (both  $P < 0.01$ ; Table S5).

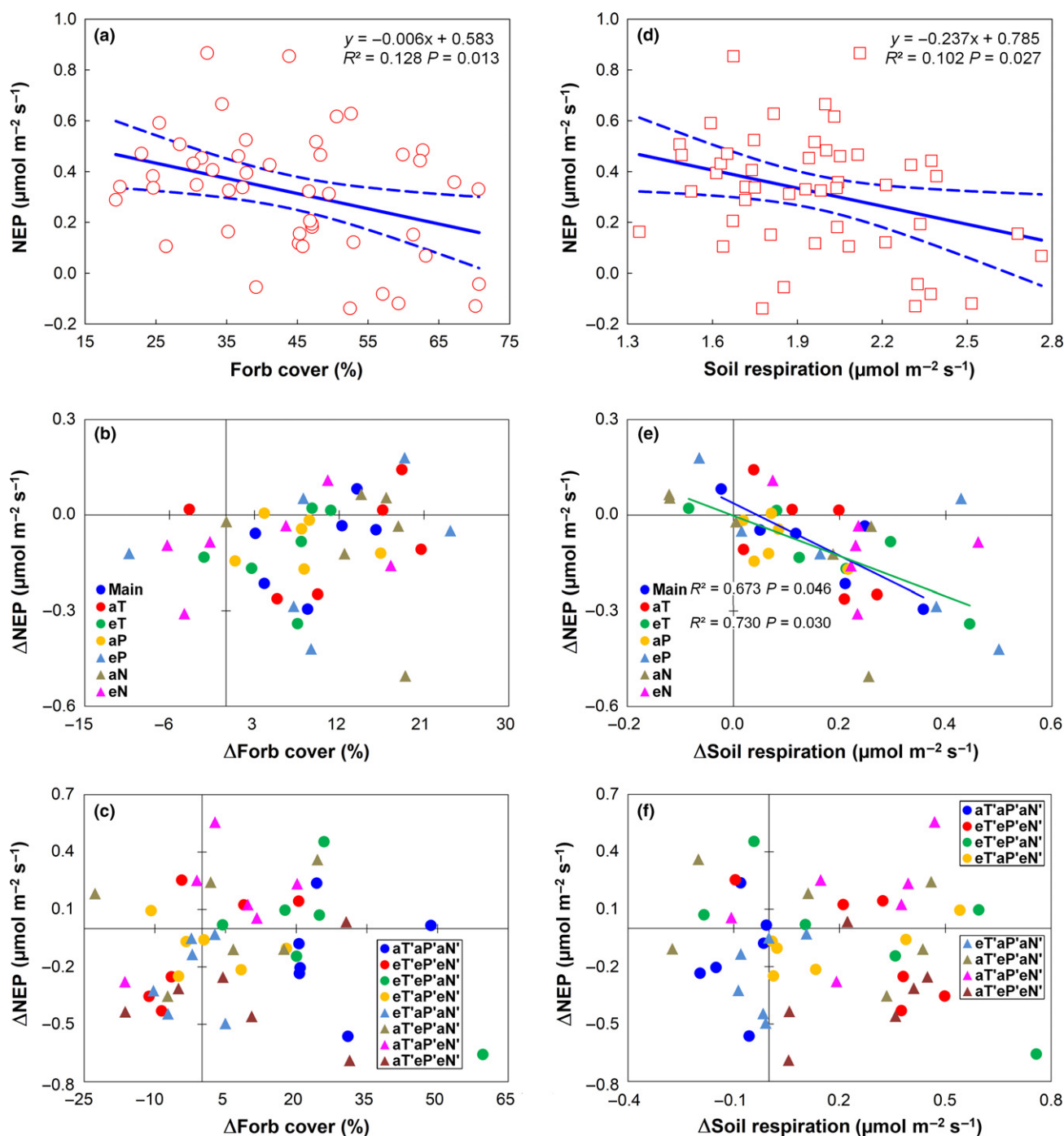
#### NEP responses to $e\text{CO}_2$ affected by community composition and SR

Across all the 48 subplots, NEP showed negatively linear dependences upon both forb cover (Fig. 3a,  $R^2 = 0.128$ ,  $P = 0.013$ ) and SR (Fig. 3d,  $R^2 = 0.102$ ,  $P = 0.027$ ). However, the  $e\text{CO}_2$ -induced changes in NEP had no relationship with the changes in forb cover under  $e\text{CO}_2$  (Fig. 3b,c). By contrast, the NEP responses to  $e\text{CO}_2$  linearly declined from positive to negative with increasing magnitudes of SR changes induced by  $e\text{CO}_2$  when averaged across all the eight treatment combinations (Fig. 3e,  $R^2 = 0.673$ ,  $P = 0.046$ ) and across the four treatment combinations with eT ( $R^2 = 0.730$ ,  $P = 0.030$ ). Nevertheless, NEP changes under  $e\text{CO}_2$  did not correlate with the stimulations of SR under any of the eight treatment combinations (Fig. 3f).

Results of stepwise multiple linear regressions showed that, when averaged across all the eight treatment combinations and across the four treatment combinations with eT,  $e\text{CO}_2$ -induced changes in SR alone explained to 67.3 and 73.0% of the  $e\text{CO}_2$ -induced changes in NEP, respectively (both  $P < 0.05$ ; Table S6). Under the treatment of eT'eP'eN' only, the changes in SR (partial  $R^2 = 0.634$ ,  $P = 0.058$ ) and forb cover (partial  $R^2 = 0.298$ ,  $P = 0.036$ ) together accounted for 93.2% of the changes in NEP under  $e\text{CO}_2$  ( $P < 0.05$ ).

#### DISCUSSION

Elevated  $\text{CO}_2$  alone did not affect NEP in this semiarid temperate steppe, a result which is in contradiction to the widely accepted expectation of a strong  $\text{CO}_2$  fertilisation effect in the water-limited ecosystems as revealed in previous grassland experiments (Bachman *et al.* 2010; Pendall *et al.* 2013; Roy *et al.* 2016). In addition, the  $e\text{CO}_2$  effect on NEP was not changed by any of the other three global change factors or their combinations, leading to no overall effect of  $e\text{CO}_2$  on NEP when averaged across all the eight treatment combinations. These observations are inconsistent with previous findings of nonlinear and non-additive influences of higher-order interaction among global change factors on ecosystem processes by theoretical analyses (Leuzinger *et al.* 2011) or in another four-factor experiment conducted in an annual grassland of North America (Shaw *et al.* 2002; Zhu *et al.* 2016b). The lack of interaction among the four global change factors on ecosystem  $\text{CO}_2$  exchange does not support our hypotheses, even though negative impacts of warming and positive influences of increased precipitation on soil water availability as



**Figure 3** Spatial relationships of NEP with forb cover (a) and soil respiration (d). Linear regression (solid line) with 95% CI (dashed line). Each data point represents a 6-year mean value in each subplot. Dependences of eCO<sub>2</sub>-induced absolute changes in NEP upon the changes in forb cover (b, c) and soil respiration (e, f) under different treatment combinations. Each data point represents eCO<sub>2</sub>-induced absolute changes of NEP, forb cover, and soil respiration in each year. See Table 1 and Figure 1 for abbreviations.

well as marginally negative effects of increased precipitation on soil N availability were detected (Appendix S1). In addition, given that climate conditions can substantially regulate CO<sub>2</sub> fertilisation effects in grasslands (Roy *et al.* 2016; Obermeier *et al.* 2017, 2018), we analysed whether microclimate changes under the warming and increased precipitation treatments mediated NEP responses to eCO<sub>2</sub>. Inconsistent with the widely accepted consensus of stronger CO<sub>2</sub> fertilisation effects

on plants at drier sites (Fatichi *et al.* 2016), NEP responses to eCO<sub>2</sub> declined with warming-induced decreases, but increased with precipitation-induced enhancements in soil moisture in the present study (Fig. S5a,b), providing further support for the predominant role of water availability in controlling semi-arid grassland C cycling (Liu *et al.* 2009), which might have potentially cancelled the positive effects of eCO<sub>2</sub> on ecosystem C sequestration by water-saving mechanisms. More

surprisingly, irrespective of the background N limitation in this semi-arid temperate steppe (Niu *et al.* 2010), eCO<sub>2</sub> did not stimulate C sequestration under the N addition treatment, contradicting our Hypothesis 1 and the conclusions from other grassland experiments (Reich *et al.* 2006; Reich & Hobbie 2013).

We were puzzled by these unexpected results, but at least two other lines of evidence support our results. First, WUE did not respond to eCO<sub>2</sub>. Both theoretical analyses and observations suggest that increased WUE associated with rising atmospheric CO<sub>2</sub> concentration is one of the major mechanisms for the positive eCO<sub>2</sub> effect as eCO<sub>2</sub> promotes stomatal closure and reduces water use (Morgan *et al.* 2011; Fatichi *et al.* 2016). In this study, no positive influence of eCO<sub>2</sub> on WUE was detected at either canopy or ecosystem levels primarily due to little changes in both GEP and water vapour exchange under eCO<sub>2</sub> (Fig. S6, Tables S2–S4, S6). Second, rising CO<sub>2</sub> concentration has contributed to a significant increase in global greenness, based on an indicator of vegetation greening – NDVI (Myneni *et al.* 1997; Zhu *et al.* 2016a). However, plot-level NDVI in this semi-arid temperate steppe did not change after 6 years of CO<sub>2</sub> enrichment, probably accounted for by the trade-offs in plant community given that eCO<sub>2</sub>-induced changes in NDVI increased with increasing forb dominance but declined with increasing grass dominance (Fig. S5c,d).

At least two possible mechanisms may help to explain the neutral responses of NEP to CO<sub>2</sub> enrichment observed in this study. First, community shifts from codominance of grasses and forbs to forb dominance under eCO<sub>2</sub> might contribute to the weak responses of NEP to CO<sub>2</sub> treatment. In this study, eCO<sub>2</sub> promoted forb cover and resulted in a competitive decrease in grass cover, as shown by a significant increase of the proportion of forbs in the whole community from 0.60 averaged across the eight aCO<sub>2</sub> treatments to 0.67 averaged across the eight eCO<sub>2</sub> treatments ( $P < 0.05$ ; Fig. S7, Table S6). The observations imply that eCO<sub>2</sub> stimulates growth of forbs more than that of grasses, which is consistent with the observations from two other grassland CO<sub>2</sub> experiments (Lee *et al.* 2011; Polley *et al.* 2012). However, further analyses on the effects of changing community composition on NEP showed that although NEP significantly decreased with increasing forb cover across all the subplots (Fig. 3a), eCO<sub>2</sub>-stimulated forb cover was not responsible for the reduction in NEP under eCO<sub>2</sub>. Second, accelerated C release from soils in response to eCO<sub>2</sub> might offset the enhancements in C uptake, ultimately leading to increased ecosystem C cycling and turnover rather than C sequestration, which was supported by the findings of several grassland experiments using different CO<sub>2</sub> fumigation techniques (FACE, OTC, and tunnel) and two meta-analyses (Hungate *et al.* 1997; Gill *et al.* 2002; Nie *et al.* 2013; van Groenigen *et al.* 2014; Liu *et al.* 2018). The significantly negative dependence of eCO<sub>2</sub>-induced changes in NEP upon the changes in soil respiration (Fig. 3e) provided strong support for the above arguments. Our findings of the primary controls of soil CO<sub>2</sub> emissions over CO<sub>2</sub> fertilisation effects on NEP are critical for model parameterisation to convincingly assess grassland C sequestration in a high-CO<sub>2</sub> world (Kardol *et al.* 2012; Langley & Hungate 2014).

The unexpected findings of the neutral C-flux responses to CO<sub>2</sub> enrichment from this experiment are not captured by most ecosystem models (Piao *et al.* 2013; Fatichi *et al.* 2016). We compared model outputs of GEP, ER and NEP due to eCO<sub>2</sub> at our experimental site from nine terrestrial C-cycle models used in IPCC Reports with our experimental results to evaluate model performance. Six out of the nine models showed that eCO<sub>2</sub> significantly enhanced both ecosystem photosynthesis and respiration in the semi-arid grassland during 1983–2012, with a significant increment of  $48.5 \pm 14.0 \text{ g C m}^{-2} \text{ year}^{-1}$  for GEP,  $36.4 \pm 10.5 \text{ g C m}^{-2} \text{ year}^{-1}$  for ER and  $12.1 \pm 8.2 \text{ g C m}^{-2} \text{ year}^{-1}$  for NEP when averaged across all the nine models (all  $P < 0.01$ ; Fig. S8). In combination with another study which has revealed negative CO<sub>2</sub> responses of NEP in Mojave Desert (Jasoni *et al.* 2005), it seems that most C-cycle models generally overestimate CO<sub>2</sub> fertilisation effects in arid and semi-arid ecosystems (Donohue *et al.* 2013), even though N limitation is considered in the models (e.g., CLM4.5, LPJ-GUESS and LPX-Bern), suggesting that complex local climate conditions, for example temperature and precipitation, may limit the ability of ecosystem models to make an accurate projection of the eCO<sub>2</sub> effects (Obermeier *et al.* 2017).

## CONCLUSIONS

Using a 6-year field data set of NEP in a water-limited temperate steppe on the Mongolian Plateau, we illustrated that NEP is insensitive to rising atmospheric CO<sub>2</sub> concentrations in a world with multi-factorial global change. Two lines of evidence, including the neutral responses of WUE and NDVI to eCO<sub>2</sub>, supported our conclusion. The neutral effect of eCO<sub>2</sub> on NEP could be mainly attributed to increased soil CO<sub>2</sub> emissions under CO<sub>2</sub> enrichment, which offsetting eCO<sub>2</sub> enhancement on ecosystem C uptake. This surprising result challenges not only the common knowledge of positive CO<sub>2</sub> fertilisation effects on terrestrial C uptake, but also the consensus of stronger CO<sub>2</sub> stimulation on ecosystem C sequestration in arid and semiarid regions (Donohue *et al.* 2013; Fatichi *et al.* 2016). Considering the significance of arid and semiarid ecosystems in land C sequestration and global C cycling (Ahlström *et al.* 2015), and the fact that most terrestrial C-cycle models used in IPCC Reports did not capture the neutral responses of C sequestration to eCO<sub>2</sub> in semi-arid regions, incorporating this mechanism into ecosystem models and parameterising these models using local climate variables should lead to more accurate estimation of terrestrial C budgets.

As a common facility in CO<sub>2</sub> manipulation experiments (Hungate *et al.* 1997; Langley & Megonigal 2010), OTCs used in the present study may create a high temperature and high humidity microenvironment compared with the ambient climate, likely modulating grassland responses to rising atmospheric CO<sub>2</sub> concentrations (Obermeier *et al.* 2017). In addition, although our previous studies at this experimental site have demonstrated that mowing could not change N addition effects on grassland plant community and soil respiration (Yang *et al.* 2012; Du *et al.* 2018), this study conducted in a fenced grassland might fail to investigate possible roles of grazing and mowing, the two common grassland use practices, in regulating grassland responses to CO<sub>2</sub> enrichment,



especially under complex climate change scenarios. Thus, caution should be taken when extrapolating our findings to the regional scale or managed grasslands.

## ACKNOWLEDGEMENTS

The authors thank Juanjuan Han for her constructive suggestions in improving the manuscript, Haijun Yang, Tingjuan Wu, Qian Zhang, Pengshuai Shao, Fanglong Su, Yuan Miao, Hongyan Han, Ang Zhang, Tong Zhang, Jiajia Wang, Jiajia Zhang, and Guoyong Li for their help in the experiment construction and data measurements, and the staff at the Duolun Restoration Ecology Research Station, Institute of Botany, Chinese Academy of Sciences, for their help in maintaining the field facilities. This work was financially supported by the National Natural Science Foundation of China (31430015, 31830012, and 41030104/D0308).

## AUTHOR CONTRIBUTIONS

SQW designed the experiment and built the facility; JS, YZL, MXZ, MMZ, GGM, ZXZ and JYR collected the experimental data; JS, SQW and SLP analysed the experimental data; and YWL and SLP conducted the model simulations. JS, SQW and SLP wrote the first draft of the manuscript, and DFH, MJH and PC contributed substantially to revisions.

## REFERENCES

- Ahlström, A., Raupach, M.R., Schurgers, G., Smith, B., Arneth, A., Jung, M. *et al.* (2015). The dominant role of semi-arid ecosystems in the trend and variability of the land CO<sub>2</sub> sink. *Science*, 348, 895–899.
- Andresen, L.C., Yuan, N., Seibert, R., Moser, G., Kammann, C.I., Luterbacher, J. *et al.* (2017). Biomass responses in a temperate European grassland through 17 years of elevated CO<sub>2</sub>. *Global Change Biol.*, 24, 3875–3885.
- Bachman, S., Heisler-White, J.L., Pendall, E., Williams, D.G., Morgan, J.A. & Newcomb, J. (2010). Elevated carbon dioxide alters impacts of precipitation pulses on ecosystem photosynthesis and respiration in a semi-arid grassland. *Oecologia*, 162, 791–802.
- Bai, Y., Wu, J., Clark, C.M., Naeem, S., Pan, Q., Huang, J. *et al.* (2010). Tradeoffs and thresholds in the effects of nitrogen addition on biodiversity and ecosystem functioning: evidence from inner Mongolia Grasslands. *Global Change Biol.*, 16, 358–372.
- de Boer, H.J., Lammertsma, E.I., Wagner-Cremer, F., Dilcher, D.L., Wassen, M.J. & Dekker, S.C. (2011). Climate forcing due to optimization of maximal leaf conductance in subtropical vegetation under rising CO<sub>2</sub>. *Proc. Natl Acad. Sci. USA*, 108, 4041–4046.
- Carrillo, Y., Dijkstra, F., LeCain, D., Blumenthal, D. & Pendall, E. (2018). Elevated CO<sub>2</sub> and warming cause interactive effects on soil carbon and shifts in carbon use by bacteria. *Ecol. Lett.*, 21, 1639–1648.
- DeLucia, E.H., Hamilton, J.G., Naidu, S.L., Thomas, R.B., Andrews, J.A., Finzi, A. *et al.* (1999). Net primary production of a forest ecosystem with experimental CO<sub>2</sub> enrichment. *Science*, 284, 1177–1179.
- Dijkstra, F.A., Blumenthal, D., Morgan, J.A., Pendall, E., Carrillo, Y. & Follett, R.F. (2010). Contrasting effects of elevated CO<sub>2</sub> and warming on nitrogen cycling in a semiarid grassland. *New Phytol.*, 187, 426–437.
- Donohue, R.J., Roderick, M.L., McVicar, T.R. & Farquhar, G.D. (2013). Impact of CO<sub>2</sub> fertilisation on maximum foliage cover across the globe's warm, arid environments. *Geophys. Res. Lett.*, 40, 3031–3035.
- Du, Y., Han, H., Wang, Y., Zhong, M., Hui, D., Niu, S. *et al.* (2018). Plant functional groups regulate soil respiration responses to nitrogen addition and mowing over a decade. *Funct. Ecol.*, 32, 1117–1127.
- Easterling, D.R., Horton, B., Jones, P.D., Peterson, T.C., Karl, T.R., Parker, D.E. *et al.* (1997). Maximum and minimum temperature trends for the globe. *Science*, 277, 364–367.
- Evans, R.D., Koyama, A., Sonderegger, D.L., Charlet, T.N., Newingham, B.A., Fenstermaker, L.F. *et al.* (2014). Greater ecosystem carbon in the Mojave Desert after ten years exposure to elevated CO<sub>2</sub>. *Nat. Clim. Change*, 4, 394–397.
- Farrior, C.E., Rodriguez-Iturbe, I., Dybzinski, R., Levin, S.A. & Pacala, S.W. (2015). Decreased water limitation under elevated CO<sub>2</sub> amplifies potential for forest carbon sinks. *Proc. Natl Acad. Sci. USA*, 112, 7213–7218.
- Fatichi, S., Leuzinger, S., Paschalis, A., Langley, J.A., Barraclough, A.D. & Hovenden, M.J. (2016). Partitioning direct and indirect effects reveals the response of water-limited ecosystems to elevated CO<sub>2</sub>. *Proc. Natl Acad. Sci. USA*, 113, 12757–12762.
- Fay, P.A., Jin, V.L., Way, D.A., Potter, K.N., Gill, R.A., Jackson, R.B. *et al.* (2012). Soil-mediated effects of subambient to increased carbon dioxide on grassland productivity. *Nat. Clim. Change*, 2, 742–746.
- Gill, R.A., Polley, H.W., Johnson, H.B., Anderson, L.J., Maherali, H. & Jackson, R.B. (2002). Nonlinear grassland responses to past and future atmospheric CO<sub>2</sub>. *Nature*, 417, 279–282.
- van Groenigen, K.J., Six, J., Hungate, B.A., de Graaff, M.A., van Breemen, N. & van Kessel, C. (2006). Element interactions limit soil carbon storage. *Proc. Natl Acad. Sci. USA*, 103, 6571–6574.
- van Groenigen, K.J., Qi, X., Osenberg, C.W., Luo, Y. & Hungate, B.A. (2014). Faster decomposition under increased atmospheric CO<sub>2</sub> limits soil carbon storage. *Science*, 344, 508–509.
- Hovenden, M.J., Newton, P.C.D., Carran, R.A., Theobald, P., Wills, K.E., Vander Schoor, J.K. *et al.* (2008). Warming prevents the elevated CO<sub>2</sub>-induced reduction in available soil nitrogen in a temperate, perennial grassland. *Global Change Biol.*, 14, 1018–1024.
- Hovenden, M.J., Newton, P.C.D. & Wills, K.E. (2014). Seasonal not annual rainfall determines grassland biomass response to carbon dioxide. *Nature*, 511, 583–586.
- Hovenden, M.J., Newton, P.C. & Osanai, Y. (2017). Warming has a larger and more persistent effect than elevated CO<sub>2</sub> on growing season soil nitrogen availability in a species-rich grassland. *Plant Soil*, 421, 417–428.
- Hu, L. (2010). *SAS® Statistical Analysis Tutorial*. Publishing House of Electronics Industry, Beijing, China.
- Hungate, B.A., Holland, E.A., Jackson, R.B., Chapin, F.S. III, Mooney, H.A. & Field, C.B. (1997). The fate of carbon in grasslands under carbon dioxide enrichment. *Nature*, 388, 576–579.
- Hungate, B.A., Dukes, J.S., Shaw, M.R., Luo, Y. & Field, C.B. (2003). Nitrogen and climate change. *Science*, 302, 1512–1513.
- Huntzinger, D.N., Michalak, A.M., Schwalm, C., Ciais, P., King, A.W., Fang, Y. *et al.* (2017). Uncertainty in the response of terrestrial carbon sink to environmental drivers undermines carbon-climate feedback predictions. *Sci. Rep.*, 7, 4765. <https://doi.org/10.1038/s41598-017-03818-2>.
- IPCC. (2014). Climate Change 2014: Synthesis Report. In: *Contribution of Working Groups I, II, and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (eds Core Writing Team, Pachauri, R.K. & Meyer, L.A.). IPCC, Geneva, Switzerland, pp. 1–9.
- Jasoni, R.L., Smith, S.D. & Arnone, J.A. (2005). Net ecosystem CO<sub>2</sub> exchange in Mojave Desert shrublands during the eighth year of exposure to elevated CO<sub>2</sub>. *Global Change Biol.*, 11, 749–756.
- Kardol, P., de Long, J.R. & Sundqvist, M.K. (2012). Crossing the threshold: the power of multi-level experiments in identifying global change responses. *New Phytol.*, 196, 323–326.
- Keenan, T.F., Hollinger, D.Y., Bohrer, G., Dragoni, D., Munger, J.W., Schmid, H.P. *et al.* (2013). Increase in forest water-use efficiency as atmospheric carbon dioxide concentrations rise. *Nature*, 499, 324–327.

- Lammertsma, E.I., de Boer, H.J., Dekker, S.C., Dilcher, D.L., Lotter, A.F. & Wagner-Cremer, F. (2011). Global CO<sub>2</sub> rise leads to reduced maximum stomatal conductance in Florida vegetation. *Proc. Natl Acad. Sci. USA*, 108, 4035–4040.
- Langley, J.A. & Hungate, B.A. (2014). Plant community feedbacks and long-term ecosystem responses to multi-factored global change. *AoB Plants*, 6, plu035. <https://doi.org/10.1093/aobpla/plu035>
- Langley, J.A. & Magonigal, J.P. (2010). Ecosystem response to elevated CO<sub>2</sub> levels limited by nitrogen-induced plant species shift. *Nature*, 466, 96–99.
- Le Quéré, C. (2016). Global carbon budget 2016. *Earth Syst. Sci. Data*, 8, 605–649.
- Lee, T.D., Barrott, S.H. & Reich, P.B. (2011). Photosynthetic responses of 13 grassland species across 11 years of free-air CO<sub>2</sub> enrichment is modest, consistent and independent of N supply. *Global Change Biol.*, 17, 2893–2904.
- Leuzinger, S., Luo, Y., Beier, C., Dieleman, W., Vicca, S. & Körner, C. (2011). Do global change experiments overestimate impacts on terrestrial ecosystems?. *Trends Ecol. Evol.*, 26, 236–241.
- Li-COR Inc. (2003). *6400 Photosynthesis System, Instruction Manual*. LI-COR Biosciences, Lincoln, NE.
- Liu, W., Zhang, Z. & Wan, S. (2009). Predominant role of water in regulating soil and microbial respiration and their responses to climate change in a semiarid grassland. *Global Change Biol.*, 15, 184–195.
- Liu, S., Ji, C., Wang, C., Chen, J., Jin, Y., Zou, Z. *et al.* (2018). Climatic role of terrestrial ecosystem under elevated CO<sub>2</sub>: a bottom-up greenhouse gases budget. *Ecol. Lett.*, 21, 1108–1118.
- Medlyn, B.E., Zaehle, S., de Kauwe, M.G., Walker, A.P., Dietze, M.C., Hanson, P.J. *et al.* (2015). Using ecosystem experiments to improve vegetation models. *Nat. Clim. Change*, 5, 528–534.
- Morgan, J.A., LeCain, D.R., Pendall, E., Blumenthal, D.M., Kimball, B.A., Carrillo, Y. *et al.* (2011). C<sub>4</sub> grasses prosper as carbon dioxide eliminates desiccation in warmed semi-arid grassland. *Nature*, 476, 202–205.
- Mueller, K.E., Blumenthal, D.M., Pendall, E., Carrillo, Y., Dijkstra, F.A., Williams, D.G. *et al.* (2016). Impacts of warming and elevated CO<sub>2</sub> on a semi-arid grassland are non-additive, shift with precipitation, and reverse over time. *Ecol. Lett.*, 19, 956–966.
- Myneni, R.B., Keeling, C.D., Tucker, C.J., Asrar, G. & Nemani, R.R. (1997). Increased plant growth in the northern high latitudes from 1981 to 1991. *Nature*, 386, 698–702.
- Newton, P.C., Loeffering, M., Parsons, A.J., Brock, S.C., Theobald, P.W., Hunt, C.L. *et al.* (2014). Selective grazing modifies previously anticipated responses of plant community composition to elevated CO<sub>2</sub> in a temperate grassland. *Global Change Biol.*, 20, 158–169.
- Nie, M., Pendall, E., Bell, C., Gasch, C.K., Raut, S., Tamang, S. *et al.* (2013). Positive climate feedbacks of soil microbial communities in a semi-arid grassland. *Ecol. Lett.*, 16, 234–241.
- Niu, S., Wu, M., Han, Y., Xia, J., Zhang, Z., Yang, H. *et al.* (2010). Nitrogen effects on net ecosystem carbon exchange in a temperate steppe. *Global Change Biol.*, 16, 144–155.
- Niu, S., Xing, X., Zhang, Z., Xia, J., Zhou, X., Song, B. *et al.* (2011). Water-use efficiency in response to climate change: from leaf to ecosystem in a temperate steppe. *Global Change Biol.*, 17, 1073–1082.
- Norby, R.J., DeLucia, E.H., Gielen, B., Calfapietra, C., Giardina, C.P., King, J.S. *et al.* (2005). Forest response to elevated CO<sub>2</sub> is conserved across a broad range of productivity. *Proc. Natl Acad. Sci. USA*, 102, 18052–18056.
- Obermeier, W.A., Lehnert, L.W., Kammann, C.I., Müller, C., Grünhage, L., Luterbacher, J. *et al.* (2017). Reduced CO<sub>2</sub> fertilisation effect in temperate C<sub>3</sub> grasslands under more extreme weather conditions. *Nat. Clim. Change*, 7, 137–141.
- Obermeier, W.A., Lehnert, L.W., Ivanov, M.A., Luterbacher, J. & Bendix, J. (2018). Reduced summer aboveground productivity in temperate C<sub>3</sub> grasslands under future climate regimes. *Earth's Future*, 6, 716–729.
- Pendall, E., Heisler-White, J.L., Williams, D.G., Dijkstra, F.A., Carrillo, Y., Morgan, J.A. *et al.* (2013). Warming reduces carbon losses from grassland exposed to elevated atmospheric carbon dioxide. *PLoS ONE*, 8, e71921. <https://doi.org/10.1371/journal.pone.0071921>.
- Piao, S., Sitch, S., Ciais, P., Friedlingstein, P., Peylin, P., Wang, X. *et al.* (2013). Evaluation of terrestrial carbon cycle models for their response to climate variability and to CO<sub>2</sub> trends. *Global Change Biol.*, 19, 2117–2132.
- Polley, H.W., Jin, V. & Fay, P.A. (2012). CO<sub>2</sub>-caused change in plant species composition rivals the shift in vegetation between mid-grass and tallgrass prairies. *Global Change Biol.*, 18, 700–710.
- Poorter, H. & Navas, M.-L. (2003). Plant growth and competition at elevated CO<sub>2</sub>: on winners, losers and functional groups. *New Phytol.*, 157, 175–198.
- Reich, P.B. & Hobbie, S.E. (2013). Decade-long soil nitrogen constraint on the CO<sub>2</sub> fertilisation of plant biomass. *Nat. Clim. Change*, 3, 278–282.
- Reich, P.B., Hobbie, S.E., Lee, T., Ellsworth, D.S., West, J.B., Tilman, D. *et al.* (2006). Nitrogen limitation constrains sustainability of ecosystem response to CO<sub>2</sub>. *Nature*, 440, 922–925.
- Reich, P.B., Hobbie, S.E. & Lee, T.D. (2014). Plant growth enhancement by elevated CO<sub>2</sub> eliminated by joint water and nitrogen limitation. *Nat. Geosci.*, 7, 920–924.
- Reich, P.B., Hobbie, S.E., Lee, T.D. & Pastore, M.A. (2018). Unexpected reversal of C<sub>3</sub> versus C<sub>4</sub> grass response to elevated CO<sub>2</sub> during a 20-year field experiment. *Science*, 360, 317–320.
- Roy, J., Picon-Cochard, C., Augusti, A., Benot, M.L., Thiery, L., Darsonville, O. *et al.* (2016). Elevated CO<sub>2</sub> maintains grassland net carbon uptake under a future heat and drought extreme. *Proc. Natl Acad. Sci. USA*, 113, 6224–6229.
- Shaw, M.R., Zavaleta, E.S., Chiariello, N.R., Cleland, E.E., Mooney, H.A. & Field, C.B. (2002). Grassland responses to global environmental changes suppressed by elevated CO<sub>2</sub>. *Science*, 298, 1987–1990.
- Smith, S.D., Huxman, T.E., Zitzer, S.F., Charlet, T.N., Housman, D.C., Coleman, J.S. *et al.* (2000). Elevated CO<sub>2</sub> increases productivity and invasive species success in an arid ecosystem. *Nature*, 408, 79–82.
- Song, J., Wu, D., Shao, P., Hui, D. & Wan, S. (2015). Ecosystem carbon exchange in response to locust outbreaks in a temperate steppe. *Oecologia*, 178, 579–590.
- Sun, Y., Gu, L., Dickinson, R.E., Norby, R.J., Pallardy, S.G. & Hoffman, F.M. (2014). Impact of mesophyll diffusion on estimated global land CO<sub>2</sub> fertilisation. *Proc. Natl Acad. Sci. USA*, 111, 15774–15779.
- Terrer, C., Vicca, S., Hungate, B.A., Phillips, R.P. & Prentice, I.C. (2016). Mycorrhizal association as a primary control of the CO<sub>2</sub> fertilisation effect. *Science*, 353, 72–74.
- Terrer, C., Vicca, S., Stocker, B.D., Hungate, B.A., Phillips, R.P., Reich, P.B. *et al.* (2018). Ecosystem responses to elevated CO<sub>2</sub> governed by plant-soil interactions and the cost of nitrogen acquisition. *New Phytol.*, 217, 507–522.
- UN. (2011). *Global Drylands: A UN System-Wide Response*. First published in October 2011 by the United Nations Environment Management Group. (eds) Ban, K., Achim, S. & Luc, G., pp. 22–32. Available at <https://www.unep-wcmc.org/resources-and-data/global-drylands-a-un-system-wide-response>. Last accessed 17 August 2018.
- Wan, S., Xia, J., Liu, W. & Niu, S. (2009). Photosynthetic overcompensation under nocturnal warming enhances grassland carbon sequestration. *Ecology*, 90, 2700–2710.
- Yang, H., Jiang, L., Li, L., Li, A., Wu, M. & Wan, S. (2012). Diversity-dependent stability under mowing and nutrient addition: evidence from a 7-year grassland experiment. *Ecol. Lett.*, 15, 619–626.
- Zhang, Y., Zheng, L., Liu, X., Jickells, T., Cape, J.N., Goulding, K. *et al.* (2008). Evidence for organic N deposition and its anthropogenic sources in China. *Atmos. Environ.*, 42, 1035–1041.
- Zhu, Z., Piao, S., Myneni, R.B., Huang, M., Zeng, Z., Canadell, J.G. *et al.* (2016a). Greening of the Earth and its drivers. *Nat. Clim. Change*, 6, 791–795.

Zhu, K., Chiariello, N.R., Tobeck, T., Fukami, T. & Field, C.B. (2016b). Nonlinear, interacting responses to climate limit grassland production under global change. *Proc. Natl Acad. Sci. USA*, 113, 10589–10594.

#### **SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Editor, Johannes Knops

Manuscript received 11 June 2018

First decision made 17 July 2018

Second decision made 3 October 2018

Manuscript accepted 23 October 2018