nature plants

ARTICLES

https://doi.org/10.1038/s41477-018-0356-x

Globally consistent influences of seasonal precipitation limit grassland biomass response to elevated CO_2

Mark J. Hovenden[1*], Sebastian Leuzinger[2], Paul C. D. Newton[3], Andrew Fletcher[2], Simone Fatichi[4], Andreas Lüscher[5,6], Peter B. Reich[7,8], Louise C. Andresen[9,10], Claus Beier[11], Dana M. Blumenthal[12], Nona R. Chiariello[13], Jeffrey S. Dukes[14,15], Juliane Kellner[9], Kirsten Hofmockel[16,17], Pascal A. Niklaus[18], Jian Song[19], Shiqiang Wan[19], Aimée T. Classen[20] and J. Adam Langley[21]

Rising atmospheric carbon dioxide concentration should stimulate biomass production directly via biochemical stimulation of carbon assimilation, and indirectly via water savings caused by increased plant water-use efficiency. Because of these water savings, the CO_2 fertilization effect (CFE) should be stronger at drier sites, yet large differences among experiments in grassland biomass response to elevated CO_2 appear to be unrelated to annual precipitation, preventing useful generalizations. Here, we show that, as predicted, the impact of elevated CO_2 on biomass production in 19 globally distributed temperate grassland experiments reduces as mean precipitation in seasons other than spring increases, but that it rises unexpectedly as mean spring precipitation increases. Moreover, because sites with high spring precipitation also tend to have high precipitation at other times, these effects of spring and non-spring precipitation on the CO_2 response offset each other, constraining the response of ecosystem productivity to rising CO_2. This explains why previous analyses were unable to discern a reliable trend between site dryness and the CFE. Thus, the CFE in temperate grasslands worldwide will be constrained by their natural rainfall seasonality such that the stimulation of biomass by rising CO_2 could be substantially less than anticipated.

The capacity of the biosphere to absorb carbon as the atmospheric concentration of CO_2 ($[CO_2]$) increases is a crucial yet uncertain factor in climate science[1]. The fundamental physiology is simple; photosynthesis of most plants is not saturated at current $[CO_2]$, so increasing $[CO_2]$ should stimulate biomass production[2]. Additionally, increasing $[CO_2]$ reduces stomatal aperture, increasing plant water-use efficiency and, by maintaining higher soil moisture storage, increasing productivity in water-limited ecosystems[2]. Together with other minor indirect effects, these two mechanisms produce the CO_2 fertilization effect (CFE) on biomass, defined as the elevated CO_2 (eCO_2)-driven increase in biomass production as a percentage of that in control plots. However, models currently "disagree strongly"[3] on the size of the positive CO_2–productivity feedback, indicating that the processes driving eCO_2 responses are not well characterized, leading to arguments regarding the strength of the CFE[4,5]. The CFE measured in experiments that manipulate $[CO_2]$ varies substantially among studies[6,7] and is considerably lower in open-air experiments than expected from leaf-level and enclosure studies, even for crop plants[8]. Various factors have been proposed to influence the magnitude of the CFE[6–10], but none have explained the large variation observed among experiments. Grasslands occupy over 29% of ice-free land and are consequently important components of the global carbon budget, so the large degree of unexplained variation (~300%[10]) in grassland biomass response to eCO_2 limits our ability to estimate future carbon cycling.

Indirect effects caused by changes in plant water-use efficiency can have a pivotal, and sometimes dominant, influence on the overall biomass response to eCO_2[2,9,11]. These indirect effects probably relate to precipitation patterns and soil moisture conditions[12] and might explain why the CFE responds strongly to precipitation at particular sites and why the mean CFE varies even among similar sites. Despite having a firm theoretical basis, attempts to use water availability to explain the CFE have yielded little success[6,7,10], and individual studies have countered the theory[13,14], suggesting the opposite: that water scarcity can partially limit the CFE. We propose

[1]Biological Sciences, School of Natural Sciences, University of Tasmania, Hobart, Tasmania, Australia. [2]Institute for Applied Ecology New Zealand, Auckland University of Technology, Auckland, New Zealand. [3]Plant Functional Biology, AgResearch, Palmerston North, New Zealand. [4]Institute of Environmental Engineering, ETH Zurich, Zurich, Switzerland. [5]Institute of Agricultural Sciences, ETH Zürich, Zürich, Switzerland. [6]Agroscope, Zürich, Switzerland. [7]Department of Forest Resources, University of Minnesota, St Paul, MN, USA. [8]Hawkesbury Institute for the Environment, Western Sydney University, Sydney, New South Wales, Australia. [9]Department of Plant Ecology, Justus Liebig University of Giessen, Giessen, Germany. [10]Department of Earth Sciences, University of Gothenburg, Gothenburg, Sweden. [11]Department of Geosciences and Natural Resource Management, University of Copenhagen, Copenhagen, Denmark. [12]Rangeland Resources Research Unit, USDA Agricultural Research Service, Fort Collins, CO, USA. [13]Jasper Ridge Biological Preserve, Stanford University, Stanford, CA, USA. [14]Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN, USA. [15]Department of Biological Sciences, Purdue University, West Lafayette, IN, USA. [16]Department of Ecology, Evolution and Organismal Biology, Iowa State University, Ames, IA, USA. [17]Pacific Northwest National Laboratory, Richland, WA, USA. [18]Department of Evolutionary Biology and Environmental Studies, University of Zurich, Zurich, Switzerland. [19]College of Life Science, Hebei University, Baoding, Hebei, China. [20]Rubenstein School of Environment and Natural Resources, University of Vermont, Burlington, VT, USA. [21]Department of Biology, Villanova University, Villanova, PA, USA. *e-mail: Mark.Hovenden@utas.edu.au

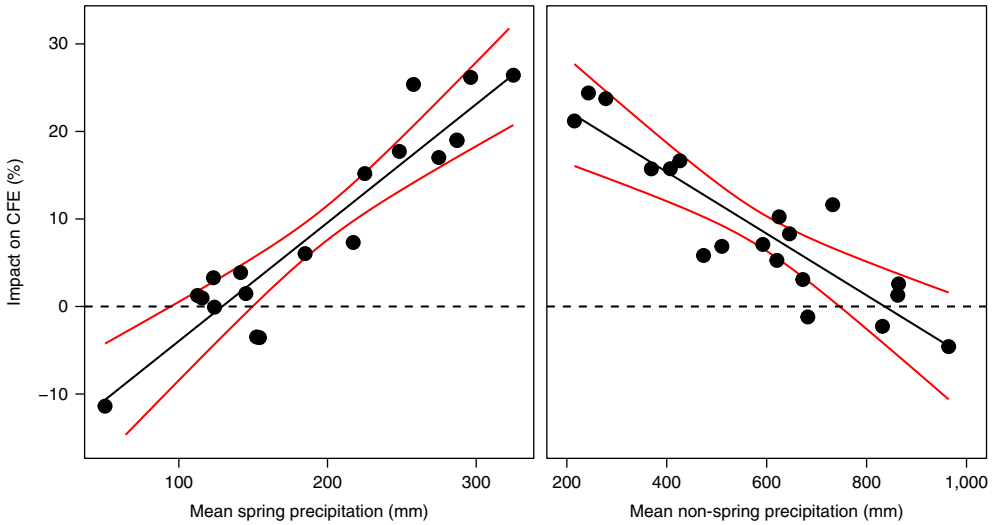

Fig. 1 | Impact of seasonal precipitation on the CFE. Partial regression plots showing the influence on the CFE attributable to spring and non-spring precipitation across 19 grassland eCO_2 experiments. Black lines show the modelled effects, with 95% confidence bands shown in red.

that these apparent contradictions are caused by precipitation having different effects on the CFE at different times of year[15]. Previous work has demonstrated that the seasonal balance of rainfall predicts the CFE at a single site[15], so we suspected that a similar influence might extend across sites. Here, we test the hypothesis that differences in the mean CFE among sites are related to site differences in seasonal precipitation totals.

Experimental results

Using data from 19 grassland CO_2 manipulation experiments and a total of 163 experimental years (Supplementary Table 1), we show that the differences among experiments in the mean CFE are explained extremely well by a stimulatory effect of precipitation in spring and a suppressive effect of precipitation at other times of the year (Fig. 1). The experiments were distributed throughout temperate zones in North America, Europe, Asia and Australasia, covering a wide range of grassland types and environmental factors (Supplementary Table 1). The mean (±s.e.m.) CFE of these experiments was 9.0 ± 1.7% at an average enrichment level of 243 μmolCO_2 mol^{-1} and an average ambient [CO_2] of 375 μmol mol^{-1}, but variation in CFE among the experiments was large, with the site-mean CFE ranging from −7.1% to +20.0% (Supplementary Table 1). We used simple and multiple regression analyses to determine whether variation in the mean CFE among sites was related to climatic and site factors. We tested the impact on the CFE of mean annual, autumn, winter, spring and summer precipitation over the study period at each site, with the seasons defined as being three calendar months in duration with 1 March being the first day of spring in the Northern Hemisphere and autumn in the Southern Hemisphere. We also tested the effects on the mean CFE of mean annual temperature, mean shoot nitrogen content, mean soil carbon-to-nitrogen ratio, mean annual aboveground biomass production, the proportion of C_4 plants at each site, the CO_2 enrichment level and the fumigation technique (chambers versus free-air CO_2 enrichment (FACE) technology). Importantly, variation among experiments in the mean CFE was not explained by any of the tested site variables (Fig. 2), but 74.7% of the variation in the CFE among sites was explained by a two-factor model that incorporated mean spring precipitation and the mean summed precipitation at other times of the year (that is 'non-spring precipitation': $r^2 = 0.747$; $F_{2,16} = 23.6$; $P < 0.00002$; Supplementary Table 2). The site-mean CFE was enhanced by decreasing non-spring precipitation ($P = 0.0002$; Fig. 1), but the effect of low precipitation in spring was negative (that is, the opposite pattern ($P < 0.00001$; Fig. 1)). Thus, the mean CFE for a site was determined by the combination of the stimulatory effect of higher spring precipitation and the stimulatory effect of lower non-spring precipitation (Fig. 1). Considering the range of spring and non-spring precipitation values, the influences of spring and non-spring precipitation on the CFE are relatively evenly balanced, such that their impacts tend to be similar in scale but opposite in influence.

Data from both of the experimental sites and a worldwide precipitation grid covering temperate grassland show that sites that are wetter in spring also tend to be wetter during the rest of the year (Fig. 3 and Supplementary Fig. 1); hence, the contrasting impact of precipitation in spring and non-spring periods constrains the CFE (Fig. 3). This offsetting influence of average spring versus average non-spring precipitation on the CFE explains why mean annual precipitation by itself is a very poor predictor of the CFE (Fig. 2; $r^2 = 0.02$; $P = 0.68$) and why earlier analyses failed to discern any substantial effect of overall site wetness or dryness (usually described by annual metrics) on the degree of stimulation of biomass across sites with markedly different aridity levels. Importantly, none of the other potential predictor variables significantly improved the predictive capacity of the two-factor model (Supplementary Tables 2–4), nor were they strongly correlated with the two predictors (Supplementary Fig. 2), indicating that the observed relationship is unlikely to be mediated by these factors. This offsetting mechanism also explains why the CFE observed in field experiments is mostly lower than anticipated.

Certain site characteristics, such as the proportion of C_4 species in a community[16,17] and nitrogen availability[18,19], can influence the CFE within a site, but our analysis indicates that these ecosystem traits, as well as factors such as mean annual temperature and the degree of CO_2 enrichment, had little influence on differences in the CFE among grassland experiments. Furthermore, the fumigation technique (chambered versus FACE experiments) had no significant impact on the CFE (Supplementary Fig. 3). We suggest that the amount and seasonal distribution of precipitation shape important, relatively stable community and ecosystem properties at a particular site, determining the site's average or 'inherent' CFE. We believe such properties to be the result of long-term (multi-year and evolutionary) processes, and their effects on the biomass CO_2 response differ fundamentally from those of shorter-term physiological mechanisms.

First, a site that tends to have wet springs will have communities biologically equipped to take advantage of eCO_2. Repeatedly,

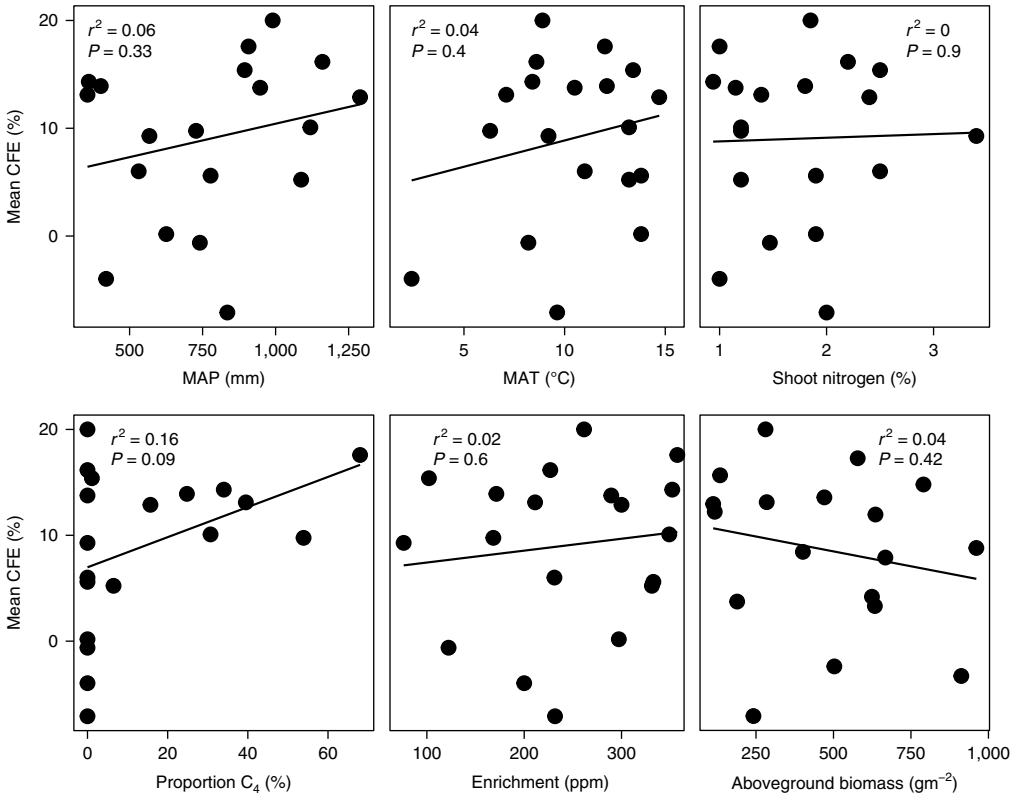

Fig. 2 | The CFE across 19 temperate grassland experiments as a function of different potential drivers. Each point is the mean percentage stimulation of aboveground annual biomass production by eCO_2 (the CFE) for a particular site. Relationships between each driver (mean annual precipitation (MAP); mean annual temperature (MAT); mean nitrogen content of aboveground biomass in control plots (shoot nitrogen); mean proportion of biomass contributed by C_4 species (proportion C_4); mean CO_2 enrichment level (enrichment); and mean aboveground biomass of control plots (aboveground biomass)) and the CFE were analysed by simple regression, with associated r^2 and P values shown in each panel ($n = 19$ independent experiments).

experiments show that grasslands are more responsive to changes in spring precipitation than to changes at other times of the year[20,21], so that spring precipitation is the best predictor of grassland productivity[22] and has a disproportionate influence on community properties key to ecosystem function[23]. Thus, the strong impact of spring precipitation on the CFE is probably mediated via positive relationships with plant species richness[17,24,25], leaf-area index, meristem density[26], microbial community function[27] and ecosystem resource availability, all of which boost the CFE. Additionally, the strong effect of spring precipitation is robust to variation in the definition of spring by about 20 d (Supplementary Fig. 4). The a priori definition of spring we used here (that is, 'calendar spring' 1 March to 31 May in the Northern Hemisphere, and 1 September to 30 November in the Southern Hemisphere) is at the early edge of that range, indicating the importance of including late-spring precipitation to explain variation in the CFE. This agrees with the fact that altering our definition of spring by advancing the commencement date by only 10 d dramatically reduced our ability to explain the variation in CFE among sites, whereas delaying the commencement of spring by up to 20 d had little effect on the predictive power of spring precipitation (Supplementary Fig. 4). Such a strong effect of advancing the definition of spring onset by only 10 d is surprising since the season was defined to span three months, but it indicates the importance of capturing the amount of precipitation that falls within the entirety of the spring period. This suggests that the amount of precipitation that falls while the grassland is in its maximum growth period affects key properties of the community and/or ecosystem, as suggested elsewhere[20–23]. We also tested the effect of site-specific 'growing season' precipitation (Supplementary Table 1) using both broad and narrow definitions of the growing season (see Methods for details), but this analysis explained far less of the variation in the CFE among sites than the spring versus non-spring analysis. This is because definitions of growing seasons often extend far into the summer period, combining periods in which precipitation has opposing effects on the CFE (Fig. 1). In addition, we tested the effect of varying the duration of spring between one month and six months, but again, none of the models approached the ability of the spring versus non-spring model to describe the variation in the CFE. Thus, while the exact timing of the onset of warmer conditions conducive to active growth will vary from site to site and year to year, the traditional definition of the three-month spring period clearly captures the impact of precipitation on important ecosystem properties that have real and measurable effects on productivity.

Second, a considerable proportion of the CFE is obtained from the anti-transpirant effects of eCO_2, which are most pronounced in drier sites[2,9,11]. Therefore, a site that tends to be wet in seasons other than spring has limited opportunities for the benefits of the water-saving effects of eCO_2 to be realized, simply because the soil in such sites will tend to be moist even when not exposed to eCO_2. Thus, the CFE reduces as non-spring precipitation increases, exactly as predicted from theory[2,9,11]. The combination of these two factors determines the site's inherent ability to respond to eCO_2. Importantly, it is a site's mean precipitation in the spring and non-spring periods that determines the mean strength of the CFE. Long-term precipitation averages have a far greater impact on crucial community and ecosystem properties, such as plant community composition, than shorter-term deviations from the average[28], indicating that ecosystem properties link the mean CFE with precipitation, rather than the immediate effects of precipitation on carbon

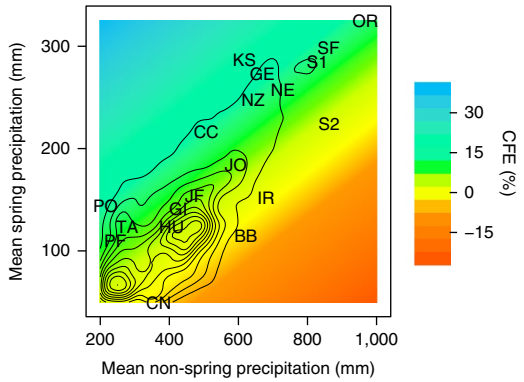

Fig. 3 | Predicted CFE of aboveground biomass for given spring and non-spring precipitation values. Predictions used the formula CFE (%) = 2.94 + 0.135 spring precipitation (mm) − 0.035 non-spring precipitation (mm). The CFE is for a CO_2 enrichment of 243 µmol mol^{-1} above an ambient [CO_2] of 375 µmol mol^{-1}. Suppression of biomass is shown as red, while stimulation of biomass is shown as blue. Contours show the probability density of particular combinations of spring and non-spring precipitation for temperate grasslands worldwide, most of which lie within a range in which the CFE is predicted to be low. The 19 experiments used in this analysis (Brandbjerg, Denmark (BB); Cedar Creek, USA (CC); Duolun, China (CN); Bavaria, Germany (GE); Giessen, Germany (GI); Godollo, Hungary (HU); Oak Park, Ireland (IR); Jasper Ridge FACE, USA (JF); Jasper Ridge open-top chambers, USA (JO); Kansas, USA (KS); Nenzlingen, Switzerland (NE); Bulls, New Zealand (NZ); Oak Ridge, USA (OR); Prairie FACE, USA (PF); Prairie open-top chambers, USA (PO); SERC I, USA (S1); SERCII, USA (S2); Swiss FACE, Switzerland (SF); TasFACE, Australia (TA)) are plotted to show their combination of spring and non-spring precipitation values.

assimilation rates. Thus, increasing spring precipitation increases a site's tendency to possess community traits that boost the response to eCO_2. Unravelling the mechanisms whereby this occurs should now become a key goal of global change ecology and will require concerted, global observational and experimental efforts. The fact that the models with the greatest ability to explain the variation among sites were those that included the entirety of the spring period suggests that processes occurring belowground before shoot emergence and those occurring during the early stages of biomass formation are key to understanding the mean CFE response of a system.

In short, we found that it is the tendency of a site to receive more or less precipitation than another site in spring or in the rest of the year, as indicated by the average values, that influences the site's mean CFE, rather than a direct link between each precipitation event and CO_2-related growth stimulation. This is supported by the fact that interannual variation in the CFE within each site was poorly described by the combination of spring and non-spring precipitation (Supplementary Fig. 5). Within each site, the annual CFE can be affected by a variety of factors, including deviation from the climatic average, as well as lags and legacies of responses to treatments in previous years. For instance, a strong stimulation of biomass production in one year could deplete soil nutrient stocks, leading to suppressed responses in subsequent years[29]. Similarly, conditions that limit growth in one year could lead to the accumulation of nutrients and strong growth responses in subsequent years. In both of these scenarios, the annual CFE values will be divorced from the contemporaneous precipitation since the CFE will be partly dependent on the climatic conditions of antecedent years, such as occurs with other ecosystem processes[30–33]. However, over longer periods, the site average CFE should tend towards the inherent CFE for that location, which is determined by the combination of average values of spring and non-spring precipitation.

Geographical extrapolation

The ability to describe variation in the CFE among grassland sites allows us to project the potential CFE of a site from easily obtained climatic variables (Fig. 3), as is possible for annual net primary productivity[34,35]. By doing this for temperate grasslands worldwide, we found that most grasslands occur in sites in which the combination of spring and non-spring precipitation leads to a low CFE (Fig. 3). Although there is substantial geographic variation in the potential CFE of temperate grasslands, the projected CFE is below 10% in large areas across all continents (Fig. 4), constrained by the seasonality of precipitation in those locations (Fig. 3). The average expected CFE of temperate grasslands from our projections is 6.0 ± 0.03%—one-third lower than that observed in the experiments (Fig. 1) because of the global prevalence of temperate grasslands in sites with low spring precipitation but moderate precipitation at other times of the year (Fig. 3). Thus, predicting eCO_2 effects on grassland biomass production by averaging experimental results without the geographical extrapolation would lead to an overestimation of the CFE.

Conclusions

Clearly, predicting carbon feedbacks to the atmosphere is a global research priority[36], and the CFE is a dominant uncertainty in projecting biosphere feedback effects on the growth of atmospheric [CO_2]. We show consistent, biome-wide interactions of the CFE with precipitation seasonality suggesting that the CFE in grasslands is likely to be less than would be predicted by models that do not accurately represent these counteracting influences of precipitation at different times of the year[3]. Targeted experiments in underrepresented grassland areas—especially the neglected tropical areas and those predicted to have low CFE—would be an efficient way of refining and confirming our capacity to project the impact of eCO_2 on grasslands around the world. Together with a thorough examination of belowground biomass responses to eCO_2 and how biomass responses translate into ecosystem carbon balance, this will be the next important step in improving global predictions of carbon feedbacks from terrestrial ecosystems.

Methods

We collected annual aboveground biomass data from the 19 experiments listed in Supplementary Table 1, all of which were either open-top chamber or FACE experiments located outdoors with plants growing in the soil (that is, not in pots). We used all of the experiments for which annual aboveground biomass data were available either directly from the researchers or from published results. Where experiments included factors other than CO_2 manipulation, such as warming or precipitation removal, we only used the control (ambient) levels of the other factors and therefore examined the CO_2 response independent of other experimental factors, essentially treating each experiment as a single-factor experiment. The Swiss FACE experiment included differing levels of nutrient application as a treatment. We used data from the lower level of nutrient application, which was merely sufficient to replace the nutrients removed during regular biomass harvests. We first calculated the annual CFE as the difference in annual aboveground biomass production between eCO_2 and control plots, expressed as a percentage of the biomass of the control plots. The difference in biomass between elevated and control plots was corrected for any pre-existing difference where these data were available. Most experiments harvested or measured aboveground biomass once per year, but where biomass was harvested more frequently, the individual harvest values were summed at the plot level to obtain the annual aboveground biomass values.

Daily precipitation was obtained from each site individually using data collected on site with automatic weather stations (most sites) or from a nearby meteorological weather station (Kansas and Hungary). In both instances, the weather station was within ~2.5 km of the experimental site. At the Swiss FACE site, the locally collected precipitation data contained short gaps in the record, amounting to ~5% of the total record, so we used data from the nearest meteorological weather station to interpolate the missing values. We used the daily precipitation data to calculate seasonal precipitation totals for each year at each site. The seasons were defined to commence on 1 March (spring in the Northern Hemisphere, and autumn in the Southern Hemisphere), 1 June (summer in the

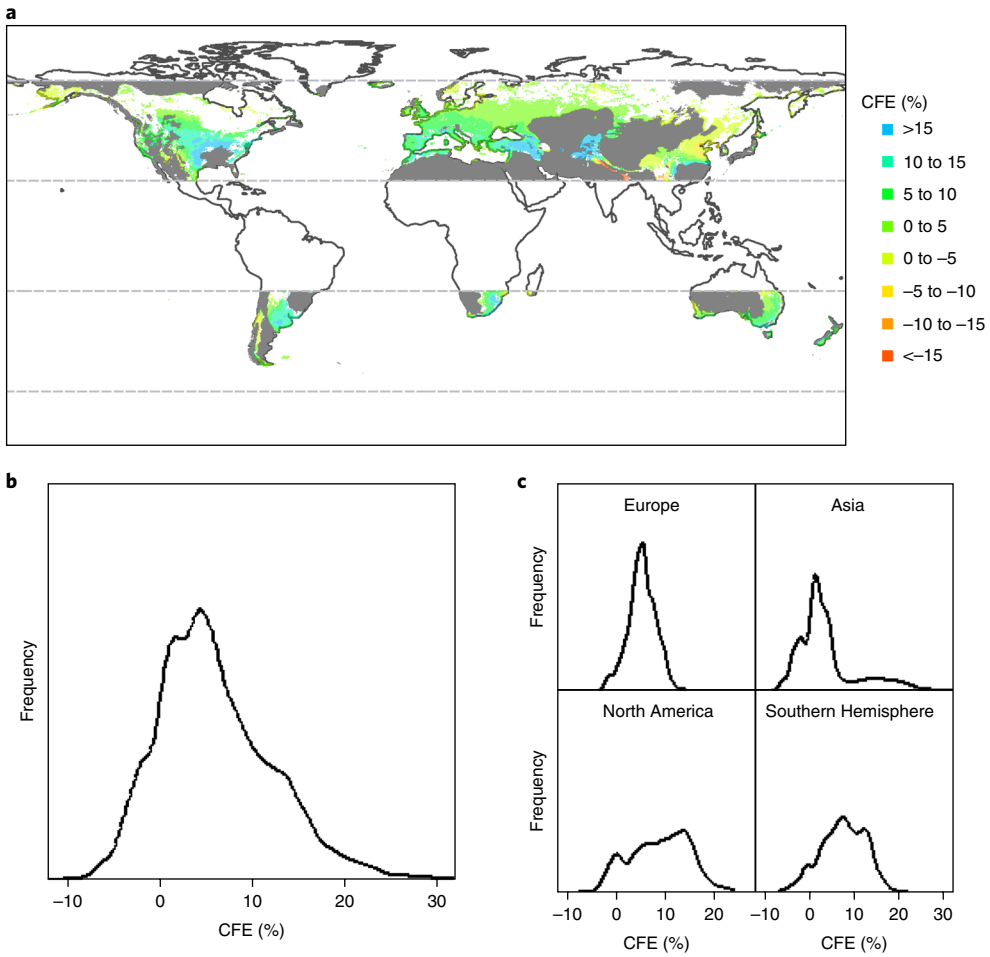

Fig. 4 | Modelled CFE in temperate grasslands. a, Modelled CFE for temperate grasslands, using the formula CFE (%) = 2.94 + 0.135 spring precipitation (mm) − 0.035 non-spring precipitation (mm), for a CO_2 enrichment of 243 μmol mol^{-1} above an ambient [CO_2] of 375 μmol mol^{-1}. The grey areas fall outside the precipitation limits of this analysis. White areas are not temperate zones or not grasslands. The dashed grey lines indicate the Arctic and Antarctic circles as well as the Tropics of Cancer and Capricorn. **b**, Frequency distribution of modelled CFEs in global temperate grassland sites within the precipitation range used to construct the model. **c**, Frequency distributions of modelled CFEs for Europe, Asia, North America and the Southern Hemisphere. The mean CFEs (± s.e.m.) are 5.9 ± 0.03% for Europe (n = 14,604 grid squares), 4.1 ± 0.05 for Asia (n = 24,944 grid squares), 8.9 ± 0.05% for North America (n = 13,764 grid squares) and 7.5 ± 0.05% for the Southern Hemisphere (n = 9,027 grid squares).

Northern Hemisphere, and winter in the Southern Hemisphere), 1 September (autumn in the Northern Hemisphere, and spring in the Southern Hemisphere) and 1 December (winter in the Northern Hemisphere, and summer in the Southern Hemisphere). The seasonal precipitation total was defined as the sum of daily precipitation over the season in each year, and this value was then averaged over all years for which the experiment ran. Annual precipitation was defined as the sum of autumn, winter, spring and summer precipitation totals, with the year commencing on 1 September in the Northern Hemisphere and 1 March in the Southern Hemisphere. The year was defined this way so that it was the year preceding the biomass harvest, which normally occurred in late summer or very early autumn. The seasonal and annual precipitation totals were calculated in the same manner for all experiments.

The characteristics of each experiment to be used as potential drivers of the CFE were supplied by the experimental team from each site or obtained from published values for each experiment. Mean CO_2 enrichment was obtained from annual enrichment values, using annual CO_2 values for elevated and ambient/control plots, then averaged for each site over all years of each experiment. Mean site aboveground biomass production was calculated as the annual aboveground biomass produced in ambient/control plots of each experiment, averaged over all years of the experiment. The proportion of C_4 plants at each site was calculated as the aboveground biomass contribution of C_4 plants as a proportion of the total aboveground biomass in control plots in each experiment, averaged over all years of the experiment. Mean shoot nitrogen was calculated as the mean percentage of nitrogen of aboveground biomass in control plots for each experiment, again averaged over all years for which data were available. Site fertility was also calculated as the total soil nitrogen content and soil carbon-to-nitrogen ratio, but each of these variables had a discontinuous distribution and were thought not to be the most reliable predictors of fertility given that some of the sites were located on organic-rich soils. Nonetheless, each of these fertility indicators was used in turn in the below analyses, with negligible effects on the analysis outcome, thus shoot nitrogen was selected for the final analyses.

Relationships between the CFE and potential drivers were determined by multiple regression analyses using R[37]. Beginning with all possible combinations of the five precipitation metrics (annual, autumn, winter, spring and summer precipitation totals) and the other six potential drivers (mean annual temperature, mean shoot nitrogen, mean annual aboveground biomass production, proportion of C_4, mean CO_2 enrichment and fumigation technique), we ranked the resultant models using the Akaike information criterion corrected for finite sample size (AIC_c), using the MuMIN package of R[38]. Model competitiveness was determined by observation of the difference in AIC_c between each model and the lowest value of AIC_c obtained (ΔAIC_c). Models were ranked in ascending ΔAIC_c value, and a distinction between competitive and non-competitive models was made by observing any obvious breaks in the sequence of ascending ΔAIC_c. A single two-factor model containing annual and spring precipitation totals was clearly superior to other models and had a 15% probability of being the best model among all possible models, with the next most competitive model only having a 7% probability of being the best model (Supplementary Table 2), so no coefficient averaging was necessary. This model had an r^2 value of 0.75 ($P < 0.00002$), but because spring and annual precipitation were significantly correlated ($r^2 = 0.88$), we replaced the annual precipitation term with non-spring precipitation (that is, the total precipitation in seasons other than spring), which was less strongly correlated with spring precipitation ($r^2 = 0.78$). We also calculated the variance inflation factor (VIF) as an additional test of collinearity.

This was 2.6 for the spring + non-spring precipitation model—approximately half that for the spring + annual precipitation model (VIF = 4.5), indicating that the model incorporating non-spring precipitation had a substantially lower impact from collinearity, and far below 5 (the VIF value generally believed to cause concern[39]). However, collinearity can influence interpretation of a multiple regression relationship and affect predictions using a model containing collinear predictor variables. Therefore, we first tested whether the strength of the regression was influenced by the incorporation of both spring and non-spring precipitation by regressing non-spring precipitation against spring precipitation, calculating the residuals between the non-spring values and the regression line and using these residual values in the model instead, following the method of Harrell[40]. This has the advantage of retaining the information contained in the predictor variable, but removing any collinearity between it (non-spring precipitation) and the remaining term (spring precipitation; $r^2 < 0.01$). This model had an identical r^2 value (0.747; $P < 0.00002$) to that of the original model containing spring and non-spring precipitation, indicating that the original model is robust and, importantly, its interpretation is not subject to error from collinearity. Second, collinearity can inflate the errors involved in making predictions, but only if predictions involve predictor variables that are not similarly correlated[40]. Thus, predictions using the model containing spring and non-spring precipitation would be unreliable if spring and non-spring precipitation were not correlated in the dataset used for predictions. Therefore, we tested the relationship between spring and non-spring precipitation using the entire gridded dataset of mean spring and non-spring precipitation for all temperate grasslands globally (Supplementary Fig. 1). The relationship between spring and non-spring precipitation was almost identical in the global temperate grassland dataset (regression coefficient = 0.27 ± 0.1) as in the dataset used to construct the model (regression coefficient = 0.28 ± 0.05). Since collinearity does not affect predictions made using new data that have the same degree of collinearity as the original data[40], we are confident that the predictions using this model are robust and appropriate.

Therefore, we examined the influence of spring and non-spring precipitation on the CFE by multiple linear regression, also testing for an interaction between spring and non-spring precipitation on the CFE, which was found to be non-significant ($P = 0.24$). Furthermore, we tested the relationship between mean CFE and all combinations between spring and non-spring precipitation and the other six potential, non-precipitation predictors (mean annual temperature, mean shoot nitrogen, proportion of C_4, mean CO_2 enrichment and fumigation technique; Supplementary Table 3) using the same methods as above. Finally, we used a hierarchical approach, adding each of the non-precipitation predictors in turn to the two-factor model and testing whether this led to a significant improvement in model performance using analysis of variance (Supplementary Table 4). We also tested the performance of the seven-term model containing all of these predictors (Supplementary Table 4). None of the resultant three-factor models significantly improved the model performance, nor did the seven-term model (Supplementary Table 4); thus, the most parsimonious model under all of the tests remained the two-factor model. Partial regression analysis was used to determine the effects, with 95% confidence limits, of spring and non-spring precipitation totals on the mean site CFE using the effects package in R[41].

Additionally, we tested the impact of precipitation in and out of the growing season, as opposed to in and out of spring, using a two-factor model and growing season dates estimated for each site individually. We used both broad and narrow definitions of growing season as either the period encompassing non-trivial aboveground growth (broad) or the period of maximum aboveground biomass production (narrow). The variation among sites in the mean CFE was very poorly explained by the combination of growing season and non-growing season precipitation, whether the broad ($r^2 = 0.06$; $F_{2,16} = 0.5$; $P = 0.6$) or narrow ($r^2 = 0.08$; $F_{2,16} = 0.7$; $P = 0.5$) definition of growing season was used. Furthermore, neither growing season (broad definition: $r^2 = 0.04$; $F_{1,17} = 0.7$; $P = 0.4$; narrow definition: $r^2 = 0.05$; $F_{1,17} = 0.8$; $P = 0.4$) nor non-growing season precipitation (broad definition: $r^2 = 0.02$; $F_{1,17} = 0.4$; $P = 0.5$; narrow definition: $r^2 = 0.04$; $F_{1,17} = 0.7$; $P = 0.4$) was correlated with the annual CFE of a site, nor was the proportion of precipitation received during the growing season (broad definition: $r^2 = 0.003$; $F_{1,17} = 0.05$; $P = 0.8$; narrow definition: $r^2 = 0.04$; $F_{1,17} = 0.07$; $P = 0.8$). Hence, variation in the CFE among sites was not related to growing season precipitation.

We tested the impact of varying the definition of spring by either advancing or delaying the commencement date from 1 March/September by 10, 20, 30 or 45 d and testing the impact this alteration had on the performance of the two-factor spring versus non-spring model. The duration of the spring period was maintained at 90 d for all comparisons. Since precipitation data were only available as monthly values for 3 of the 19 experiments, the spring adjustment analysis was done using the remaining 16 sites. Advancing the definition of spring substantially reduced the two-factor model's ability to explain variation among sites in the mean CFE (Supplementary Fig. 4). In contrast, delaying the definition of spring by up to 20 d had little impact on model performance, but longer delays caused it to decline (Supplementary Fig. 4). Therefore, we maintained our definition of spring as commencing on 1 March (Northern Hemisphere) or 1 September (Southern Hemisphere).

Data conformed to the assumptions of the statistical tests involved, as tested by investigation of residuals, leverage and normality, as well as using Box-Cox plots using the MASS package in R[42]. The only exception was mean annual biomass production of control plots, in which the single data point from the site in Ireland exerted excessive leverage on the relationship with the CFE. Therefore, this single data point was removed from subsequent analyses.

We conducted mapping and spatial analyses in ArcMap 10.3 and ESRI, USA. The 8 km Advanced Very High Resolution Radiometer global land-cover classification[43] product provided moderate oversampling of land-cover classification for wooded grasslands, grasslands and croplands that we determined to be representative of the model target. We added land-cover class, spring and non-spring precipitation to CFE modelled values using the Sample and Spatial Join (nearest geodesic) tools, respectively. Spring and non-spring precipitation values were calculated from a 10 min grid of monthly precipitation values obtained from the Climatic Research Unit at the University of East Anglia CRU CL version 2.0 database, which is available (http://www.cru.uea.ac.uk/data) under the Open Database License. These values were used to calculate the local CFE from the spring + non-spring multiple regression model. We mapped all CFE values for locations meeting model parameters for climate zone and land cover. We visualized the limits of model precipitation parameters by interpolating total precipitation data (ordinary kriging) and classifying the resulting raster with masks applied to tropic and polar zones. Calculations using the geographically projected values of the CFE only included those sites that fell within the range of spring and non-spring precipitation values observed at the experimental sites.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

All data generated or analysed during this study are included in this published article (and its Supplementary Information files) with the exception of the gridded geographic information system data, which are available from https://crudata.uea.ac.uk/cru/data/hrg/tmc/ (precipitation data) and http://glcf.umd.edu/data/landcover/data.shtml (land-cover data).

Received: 29 August 2018; Accepted: 21 December 2018;
Published online: 8 February 2019

References

1. Schimel, D., Stephens, B. B. & Fisher, J. B. Effect of increasing CO_2 on the terrestrial carbon cycle. *Proc. Natl Acad. Sci. USA* **112**, 436–441 (2015).
2. Drake, B. G., GonzalezMeler, M. A. & Long, S. P. More efficient plants: a consequence of rising atmospheric CO_2? *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **48**, 609–639 (1997).
3. Medlyn, B. E. et al. Using ecosystem experiments to improve vegetation models. *Nat. Clim. Change* **5**, 528–534 (2015).
4. Kolby Smith, W. et al. Large divergence of satellite and Earth system model estimates of global terrestrial CO_2 fertilization. *Nat. Clim. Change* **6**, 306–310 (2016).
5. De Kauwe, M. G., Keenan, T. F., Medlyn, B. E., Prentice, I. C. & Terrer, C. Satellite based estimates underestimate the effect of CO_2 fertilization on net primary productivity. *Nat. Clim. Change* **6**, 892–893 (2016).
6. Morgan, J. A. et al. Water relations in grassland and desert ecosystems exposed to elevated atmospheric CO_2. *Oecologia* **140**, 11–25 (2004).
7. Fay, P. A. et al. Dominant plant taxa predict plant productivity responses to CO_2 enrichment across precipitation and soil gradients. *AoB Plants* **7**, plv027 (2015).
8. Ainsworth, E. A. & Long, S. P. What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. *New Phytol.* **165**, 351–372 (2005).
9. Lee, M., Manning, P., Rist, J., Power, S. A. & Marsh, C. A global comparison of grassland biomass responses to CO_2 and nitrogen enrichment. *Phil. Trans. R. Soc. Lond. B* **365**, 2047–2056 (2010).
10. Terrer, C., Vicca, S., Hungate, B. A., Phillips, R. P. & Prentice, I. C. Mycorrhizal association as a primary control of the CO_2 fertilization effect. *Science* **353**, 72–74 (2016).
11. Fatichi, S. et al. Partitioning direct and indirect effects reveals the response of water-limited ecosystems to elevated CO_2. *Proc. Natl Acad. Sci. USA* **113**, 12757–12762 (2016).
12. Leuzinger, S. & Körner, C. Rainfall distribution is the main driver of runoff under future CO_2-concentration in a temperate deciduous forest. *Glob. Change Biol.* **16**, 246–254 (2010).
13. Reich, P. B., Hobbie, S. E. & Lee, T. D. Plant growth enhancement by elevated CO_2 eliminated by joint water and nitrogen limitation. *Nat. Geosci.* **7**, 920–924 (2014).
14. Gray, S. B. et al. Intensifying drought eliminates the expected benefits of elevated carbon dioxide for soybean. *Nat. Plants* **2**, 16132 (2016).
15. Hovenden, M. J., Newton, P. C. D. & Wills, K. E. Seasonal not annual rainfall determines grassland biomass response to carbon dioxide. *Nature* **511**, 583–586 (2014).

16. Morgan, J. A. et al. C_4 grasses prosper as carbon dioxide eliminates desiccation in warmed semi-arid grassland. *Nature* **476**, 202–205 (2011).
17. Langley, J. A. & Megonigal, J. P. Ecosystem response to elevated CO_2 levels limited by nitrogen-induced plant species shift. *Nature* **466**, 96–99 (2010).
18. Reich, P. B. & Hobbie, S. E. Decade-long soil nitrogen constraint on the CO_2 fertilization of plant biomass. *Nat. Clim. Change* **3**, 278–282 (2013).
19. Lüscher, A., Daepp, M., Blum, H., Hartwig, U. A. & Nösberger, J. Fertile temperate grassland under elevated atmospheric CO_2—role of feed-back mechanisms and availability of growth resources. *Eur. J. Agronomy* **21**, 379–398 (2004).
20. Darenova, E., Holub, P., Krupkova, L. & Pavelka, M. Effect of repeated spring drought and summer heavy rain on managed grassland biomass production and CO_2 efflux. *J. Plant Ecol.* **10**, 476–485 (2017).
21. Bates, J. D., Svejcar, T., Miller, R. F. & Angell, R. A. The effects of precipitation timing on sagebrush steppe vegetation. *J. Arid Environ.* **64**, 670–697 (2006).
22. Smart, A. J., Dunn, B. H., Johnson, P. S., Xu, L. & Gates, R. N. Using weather data to explain herbage yield on three great plains plant communities. *Rangeland Ecol. Manage.* **60**, 146–153 (2007).
23. Epstein, H. E., Burke, I. C. & Lauenroth, W. K. Response of the shortgrass steppe to changes in rainfall seasonality. *Ecosystems* **2**, 139–150 (1999).
24. Reich, P. B. et al. Species and functional group diversity independently influence biomass accumulation and its response to CO_2 and N. *Proc. Natl Acad. Sci. USA* **101**, 10101–10106 (2004).
25. Lüscher, A., Hendrey, G. R. & Nösberger, J. Long-term responsiveness to free air CO_2 enrichment of functional types, species and genotypes of plants from fertile permanent grassland. *Oecologia* **113**, 37–45 (1998).
26. Wilcox, K. R., Blair, J. M., Smith, M. D. & Knapp, A. K. Does ecosystem sensitivity to precipitation at the site-level conform to regional-scale predictions? *Ecology* **97**, 561–568 (2016).
27. Averill, C., Waring, B. G. & Hawkes, C. V. Historical precipitation predictably alters the shape and magnitude of microbial functional response to soil moisture. *Glob. Change Biol.* **22**, 1957–1964 (2016).
28. Tredennick, A. T., Kleinhesselink, A. R., Taylor, J. B. & Adler, P. B. Ecosystem functional response across precipitation extremes in a sagebrush steppe. *PeerJ* **6**, e4485 (2018).
29. Luo, Y. et al. Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. *Bioscience* **54**, 731–739 (2004).
30. Cable, J. M. et al. Antecedent conditions influence soil respiration differences in shrub and grass patches. *Ecosystems* **16**, 1230–1247 (2013).
31. Ogle, K. et al. Quantifying ecological memory in plant and ecosystem processes. *Ecol. Lett.* **18**, 221–235 (2015).
32. Peltier, D. M. P., Fell, M. & Ogle, K. Legacy effects of drought in the southwestern United States: a multi-species synthesis. *Ecol. Monogr.* **86**, 312–326 (2016).
33. Reynolds, J. F., Kemp, P. R., Ogle, K. & Fernandez, R. J. Modifying the 'pulse-reserve' paradigm for deserts of North America: precipitation pulses, soil water, and plant responses. *Oecologia* **141**, 194–210 (2004).
34. Knapp, A. K. & Smith, M. D. Variation among biomes in temporal dynamics of aboveground primary production. *Science* **291**, 481–484 (2001).
35. Sala, O. E., Parton, W. J., Joyce, L. A. & Lauenroth, W. K. Primary production of the central grassland region of the United States. *Ecology* **69**, 40–45 (1988).
36. IPCC *Climate Change 2014: Synthesis Report* (eds Core Writing Team, Pachauri, R. K. & Meyer, L. A.) (IPCC, 2014).
37. R Development Core Team *R: A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing, 2018).
38. MuMIn: Multi-Model Inference. R package version 1.15.6 (2016).
39. Logan, M. *Biostatistical Design and Analysis Using R: A Practical Guide* (Wiley-Blackwell, Chichester, 2010).
40. Harrell, F. E. *Regression Modeling Strategies: With Applications to Linear Models, Logistic Regression and Survival Analysis* (Springer, New York, 2001).
41. Fox, J. Effect displays in R for generalised linear models. *J. Stat. Softw.* **8**, 1–27 (2003).
42. Venables, W. N. & Ripley, B. D. *Modern Applied Statistics with S* 4th edn (Springer, New York, 2002).
43. Hansen, M., DeFries, R., Townshend, J. R. G. & Sohlberg, R. Global land cover classification at 1 km resolution using a decision tree classifier. *Int. J. Remote Sens.* **21**, 1331–1365 (2000).

Acknowledgements

We thank R. Brinkhoff for assistance with collating the data for this analysis. This research was initiated at the workshop 'Using results from global change experiments to inform land model development and calibration', which was co-sponsored by the US-based INTERFACE Research Coordination Network and Research Group of Global Change Ecology at Henan University (funded by MOST2013CB956300 and NSFC41030104/ D0308).

Author contributions

S.L., J.A.L., M.J.H. and S.F. conceived the research idea and designed the study, with assistance from P.C.D.N. and K.H. M.J.H., S.L., P.C.D.N., J.A.L. and S.F. performed the analysis and, together with A.L. and P.B.R., led the writing of the manuscript. A.F. performed the mapping and all geographical analyses. P.C.D.N., M.J.H., J.A.L., L.C.A., D.M.B., N.R.C., J.S.D., J.K., A.L., P.A.N., C.B., P.B.R., S.W. and J.S. contributed unpublished data. All authors contributed to the final version of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41477-018-0356-x.

Reprints and permissions information is available at www.nature.com/reprints.

Correspondence and requests for materials should be addressed to M.J.H.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s), under exclusive licence to Springer Nature Limited 2019

nature research

Corresponding author(s): Mark Hovenden

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Fully described in methods section for the GIS analyses (too long to place here). No code used in data collection.
Data analysis	Fully described in methods section. Standard multiple regression analyses only used in the widely used analysis platform R. Packages used also listed in methods. As the statistical methods were extremely routine, we have not provided code.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All data generated or analysed during this study are included in this published article (and its supplementary information files) with the exception of the gridded GIS data, which are available from https://crudata.uea.ac.uk/cru/data/hrg/tmc/ for the precipitation data and from http://glcf.umd.edu/data/landcover/data.shtml for

the landcover data. Raw data fog Figures 1, 2 and 3 are provided in the supplementary tables. Raw data for figure 4 are available in the GIS links provided above and described in the methods section and data availability statement.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	This study is a synthesis of the results from nineteen separate experiments.
Research sample	An individual experiment.
Sampling strategy	All possible field elevated CO2 experiments on grassland included (i.e. NO sampling).
Data collection	Data collected from the literature (i.e. published results) or directly from researchers involved in the experiments.
Timing and spatial scale	The 19 experiments varied in timing and spatial scale. Duration of each experiment is included in Table S1. ALL years of data used for each experiment.
Data exclusions	None. All possible data included.
Reproducibility	The synthesis is precisely an attempt to determine how universal the responses are across various experiments, and how the results are affected by climatic variables.
Randomization	N/A.
Blinding	N/A.

Did the study involve field work? ☐ Yes ☒ No

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging