Interactive effects between drought and warming in field manipulative experiments across grasslands globally: a systematic review and meta-analysis

James L. King[1*], Pieter A. Arnold[1], Zachary A. Brown[1,2], Peter B. Reich[3,4], Oskar Scherer[1], Susanna E. Venn[5], Adrienne B. Nicotra[1]

[1] Division of Ecology & Evolution, Research School of Biology, The Australian National University, Canberra, ACT 2600, Australia

[2] Commonwealth Scientific and Industrial Research Organisation, Black Mountain Campus, Canberra, ACT 2601, Australia

[3] Institute for Global Change Biology, University of Michigan, Ann Arbor, MI 48130, USA

[4] Department of Forest Resources, University of Minnesota, St. Paul, MN 55108, USA

[5] Centre for Integrative Ecology, School of Life and Environmental Sciences, Deakin University, Burwood, VIC 3125, Australia

Corresponding author: James King (james.king@anu.edu.au)

Abstract

Aim

Interactions between temperature and moisture are possible whereby increases in temperature can result in decreases in soil moisture, exacerbating drought conditions. Both uptake by and emission of carbon from grassland ecosystems are, in large part, governed by temperature and moisture. It is unclear how grasslands will respond to concurrent warming and drought conditions in the future (~2100) or what impact that will have on the terrestrial carbon cycle.

Location

Global.

Major taxa studied

Terrestrial carbon fluxes and pools.

Methods

We performed a systematic review and meta-analysis to synthesise the data from *in situ* field manipulative experiments that investigated the effects of warming and drought factorially, in natural grassland ecosystems around the world. We focussed on experiments that simulated a realistic future climate (>1 $^{\circ}$C warming).

Results

Our review reveals that factorial warming and drought studies are limited to Asia, Europe, and North America. Our analyses indicate that warming and drought will decrease biomass production and soil respiration, and that interactions resulting in greater than additive effects are likely to occur in belowground net primary productivity, soil respiration and net primary productivity. Overall, both inputs and outputs of carbon declined under simulated future conditions, suggesting no change to net ecosystem exchange and carbon stocks.

Main conclusions

This review highlights several knowledge gaps that currently hinder our capacity to fully understand the impacts of temperature and precipitation on grasslands: (1) experimental studies in tropical grassland ecosystems, particularly in the southern hemisphere, (2) experiments that employ belowground warming to realistically simulate future climate change and (3) experiments that collect a range of detailed meta-data including edaphic factors that allows deeper analysis of responses to climate change.

Introduction

Mean temperatures across the globe are increasing and extreme climatic events such as droughts are predicted to become more frequent and severe in the future (IPCC, 2021; Perkins-Kirkpatrick et al., 2024; Vicente-Serrano et al., 2022). The largest fluxes in the terrestrial carbon cycle are photosynthesis and ecosystem respiration (Beer et al., 2010), which are highly sensitive to temperature and moisture, therefore changes in climate are likely to have profound implications for global carbon dynamics (Song et al., 2019). Assessing both uptake and emission of carbon is necessary to understand whole ecosystem processes (Flanagan et al., 2013; Z. Wu et al., 2011). Models suggest future negative effects of warming and drought on carbon sinks (Peñuelas et al., 2017), however field experimentation to validate these predictions is critical.

Soil moisture influences plant ecosystem carbon cycling responses to warming. In cold or wet areas, warming may benefit plant growth by alleviating temperature limitations when there is ample water available (S. Wang et al., 2012). For example, warming often stimulates growth in colder climates (Rustad et al., 2001) but hampers growth in arid regions (León-Sánchez et al., 2020). By stimulating growth and assimilation, warming is also expected to lead to an increase in belowground net primary productivity, root biomass and soil respiration (Rustad et al., 2001; Song et al., 2019; Yan et al., 2022), though there are mechanisms through which acclimation of soil respiration to warming can occur (Sun et al., 2023; J. Zhou et al., 2012). Where warming results in a concomitant decline in soil moisture, however, it may impair plant productivity (De Boeck et al., 2007, Reich et al 2022). Low soil moisture in general causes declines in aboveground biomass production (Song et al., 2019; C. Wang et al., 2021; Wilschut et al., 2022), and can in turn also reduce belowground biomass if the plant is unable to maintain root mass (Arndal et al., 2014). However, drought can also lead to increases in belowground biomass as resources are directed towards roots in response to increased water limitation (Hoeppner & Dukes, 2012; H. Liu et al., 2021). Low soil moisture depresses soil respiration (Canarini et al., 2017; Z. Wu et al., 2011), thereby constraining responses of plants and microbes to warming (G. Liang et al., 2024; Suseela et al., 2012), which can explain conflicting responses of soil respiration to warming observed in field experiments (H. Wang et al., 2023). Therefore, the effects of warming on plants can be highly context-dependent, where climate warming can benefit plant growth during cooler or wetter conditions, but stressful when it is already warm and/or dry within an ecosystem (Reich et al., 2018).

The drying effect of warming on the soil can exacerbate low soil moisture, which may not be clear from experiments analysing single factors. There is a substantial body of work on warming and drought as individual factors on carbon cycling variables, including several meta-analyses (Canarini et al., 2017; Eziz et al., 2017; J. Liang et al., 2013; Lu et al., 2013; Morris et al., 2022; Rustad et al., 2001; C. Wang et al., 2021; Yan et al., 2022). However, the carbon cycle is mediated by multiple factors that rarely occur in isolation. The interactive nature of these factors means that findings from single factor experiments are not suitable for predicting responses to future conditions (Hoeppner & Dukes, 2012; Reich et al., 2020; Sun et al., 2023; Thakur et al., 2019). To address these issues we conducted a comprehensive, targeted systematic review and meta-analysis on field manipulative experiments of warming and drought in factorial combination, for an important ecosystem type: grasslands.

Previous warming and drought meta-analyses have mostly found additive effects (Song et al., 2019; Wilschut et al., 2022; L. Zhou et al., 2016), though occasionally interactive effects have been detected for soil respiration and belowground biomass (Sun et al., 2023; L. Zhou et al., 2020). Due to the paucity of factorial warming and drought experiments, meta-analyses attempting to answer questions related to their interactive effects have previously used studies from across a range of vegetation types (Song et al., 2019; Sun et al., 2023; L. Zhou et al., 2016, 2020) or from across varying experimental conditions (Wilschut et al., 2022), which we argue de-values interpretations of responses when carbon cycle responses to global change factors are strongly dependent on biome (Yan et al., 2022). Further, the often unrealistic growth conditions of a greenhouse or monoculture can result in substantially different findings to a field experiment (Poorter et al., 2016).

It is important to include studies from across the globe to see if there is an overall change in response variables, but it is also important to analyse moderating factors that might explain or be driving differences. For example, some differences in responses can be attributed to the influence of fluctuations in temperature and precipitation (Song et al., 2019; W. Wu et al., 2023). Soil nutrients and characteristics can predict plant growth and distribution patterns (Sun et al., 2022; Zhang et al., 2018). Further, experimental differences such as the magnitude of treatments, duration of experiment and method of implementation can affect responses to warming and drought as well (D. Liu et al., 2021; L. Zhou et al., 2016). For these reasons, we focused on mean annual temperature (MAT), mean annual precipitation (MAP), experiment duration, seasonal precipitation and edaphic factors as moderating factors in our meta-analysis. We targeted grassy ecosystems not only because they are logistically tractable but because they cover ~40 % of Earth's land surface and store ~34% of the world's terrestrial carbon (White et al., 2000). Specifically, we asked: (a) How do factorial field experiments that manipulate warming and drought affect plant biomass, production and soil respiration? (b) Do responses to warming and drought interact, and if so, is that interaction synergistic or antagonistic? (c) How do moderator variables including duration, MAT, MAP, seasonal precipitation and magnitude of treatment, affect responses?

Given patterns described above, we hypothesise that drought will dominate responses in both individual and combined treatments leading to a decrease in plant growth and soil respiration, due to the overriding effect of low soil moisture. Additionally, due to the potential drying effect of warming, we expect that overall, there will be no positive warming effects on plant growth. Further, we predict there will be a negative synergistic interaction (Piggott et al., 2015) for plant biomass, production and soil respiration such that when both warmed and dried, the decreases will be exaggerated. We also predict that there will be no differences in biomass over time as a result of warming and in contrast that the severity of drought effects would increase with increasing duration of treatment, though we would expect there would be an acclimation of soil respiration over time.

Further, we expect that the more severe the drought or heating treatments, the magnitude of treatment, will compound effects. Lastly, we sought to examine whether inherently cool or wet locations would show greater impacts of warming or drying treatments than hotter or drier ones.

Methods

Systematic review of warming and drought experiments in grasslands

We assessed the effects of warming and drought in combination on plant biomass and soil respiration of the world's grasslands. We defined grasslands as non-wetland areas of vegetation making up at least 10% cover, graminoids dominating and trees having less than 10% cover after (Dixon et al., 2014; see supp material). Grassland plants can respond rapidly to changes in temperature relative to longer-lived species (Zhu et al., 2024). Due to their relatively short root structures, grasses and forbs may be more susceptible to the negative effects of drought compared to woody species (Wilschut et al., 2022), making them useful model ecosystems for studying the effects of warming and drought.

We compiled articles from the Manipulation Experiments Synthesis Initiative (MESI) database (Van Sundert et al., 2023) and our own search carried out from 2019 to 13 June 2024 to capture any new factorial climate manipulative experiments that may not have been included MESI (see supp materials for details of search). For articles to be included in the systematic review, there were three main criteria that experiments had to be met. These were (1) it was an *in situ*, field manipulative experiment, (2) it was in a grassland as defined by (Dixon et al., 2014; see supp material), and (3) it contained a warming and a drought treatment in a factorial design. A total of 65 articles were included in our systematic review (see supp material for article exclusion details).

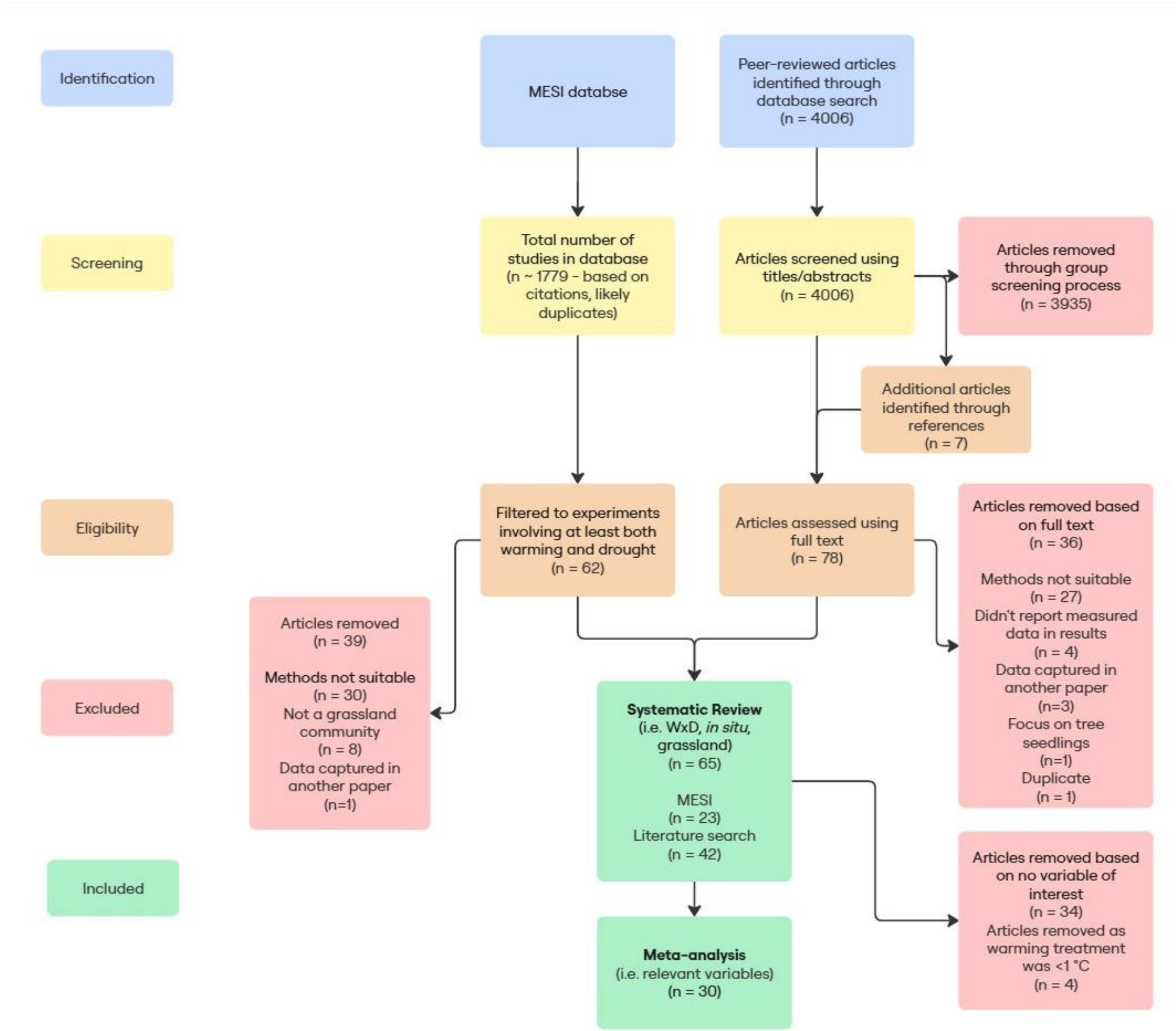

Figure 1. Preferred Reporting Items in Systematic Reviews and Meta-Analyses (PRISMA) flow chart describing articles sourced through literature and database searches and excluded based on criteria.

Meta-analysis

For the meta-analysis, only those articles that met the criteria for the systematic review and had quantitative variables of interest were included. From these, 4 articles were excluded as they did simulate a realistic warming scenario under climate change for the year ~2100 (i.e. imposed warming was <1 °C). Five relevant response variables were identified: net primary productivity (the total of above and belowground net primary productivity), aboveground biomass, root biomass, belowground net primary productivity and soil respiration. Between the literature search and MESI database, we identified 30 articles that were suitable for inclusion for formal meta-analysis, which comprised a total of 467 unique observations.

We recorded the mean, some form of error (e.g. standard deviation or standard error), and number of replicates for each treatment, including the control for each article. Errors were converted to standard deviation. We also assessed a range of moderator variables deemed relevant to interpretation: heating method, heating magnitude (temperature warmed above ambient), drought magnitude (% difference in soil moisture or % precipitation reduced below ambient), experiment duration, mean annual temperature (MAT) and mean annual precipitation (MAP). We use the yearly averages of soil moisture and soil temperature to estimate magnitude of drought and heat relative to control if provided. Otherwise, precipitation reduction (%) or set temperature increase (°C) were used. We also examined whether seasonality of precipitation was an important factor, following the methods of Hovenden et al., (2019); see supp material for details.

Individual and combined effect sizes

We calculated natural log-transformed response ratio (ln*RR*) to assess effect sizes for all response variables to warming, drought and their combination (see supp material for equations).

The effect sizes (ln*RR*) were used in multi-level random effects meta-analytical models, where experiment was nested within article ID as a random effect to account for three important sources of variation: (1) among articles, (2) articles from the same experiment and (3) non-independence of repeated measures from the same article or experiment. Variances were used to calculate overall effect size of each treatment and response variable combination. The 95% confidence intervals (CIs) were extracted from meta-analytical model outputs and used to determine significance, i.e. if 95% CIs overlapped 0 the effect was considered to be non-significant, but if 95% CIs were distinctly positive or distinctly negative, the effect was considered to be significant. A positive effect size indicates a positive impact on the response variable of the treatment and a negative effect size indicates a negative impact on the response variable of the treatment. Heating method was also analysed with this approach.

We then tested for the effects of moderator variables in additional meta-regression models. Specifically, the moderator variables were heating magnitude, drought magnitude, experiment duration, MAP, MAT, and seasonal precipitation, which were assessed using multi-level random effects models with ln*RR* as the response variable. Article ID was nested within experiment as a random effect.

Main and interactive effects of warming and drought

We investigated the main and interactive effects sizes for each response variable for each treatment by calculating Hedges' d as per the methods of (Gurevitch et al., 2000) for conducting factorial meta-analyses. Due to the complex nature of interactions between factors resulting in opposing responses, we used the (Piggott et al., 2015) classification to interpret interaction responses. Briefly, when two factors interact, the result may be additive (equivalent to the sum of the two individual responses, e.g. $-1 +1 = 0$), antagonistic (less than the sum of the individual responses e.g. positive antagonistic (+A), $−1 + 1 = −1 \leq (+A) < 0$ or negative antagonistic (−A), $−1 + 1 = 0 < (−A) \leq 1$) or synergistic (greater than the sum of individual effects e.g. positive synergistic (+S), $−1 + 1 = (+S) > 1$, or negative synergistic (−S), $−1 + 1 = (−S) < −1$). See supp materials for equations.

Main and interactive effects of warming and drought were then implemented in multi-level random effects models, with experiment nested within study ID as random effects and weighted with the inverse of the variance. If the 95% CI of di overlapped 0, the effects were considered as additive (i.e. no different to the sum of the individual effects added together). All analyses were done in the R Environment for Statistical Computing version 4.2.0 (R Core Team, 2024) using the *metafor* (Viechtbauer, 2010) and *orchaRd* (Nakagawa et al., 2023) packages.

<u>Results</u>

Systematic review

All 65 articles of factorial warming and drought manipulative experiments were isolated to the northern hemisphere, in North America (n = 12), western Europe (n = 27) and China (n = 26) (Fig. 2A). Of the five possible Koppen-Geiger broad climate types, articles were restricted to three (Fig. 2A): Arid (n = 20), Temperate (n = 21) and Cold (n = 31). There were no articles from locations with high average annual precipitation (>2000 mm) nor high average annual temperatures (>20 °C) (Fig. 2B).

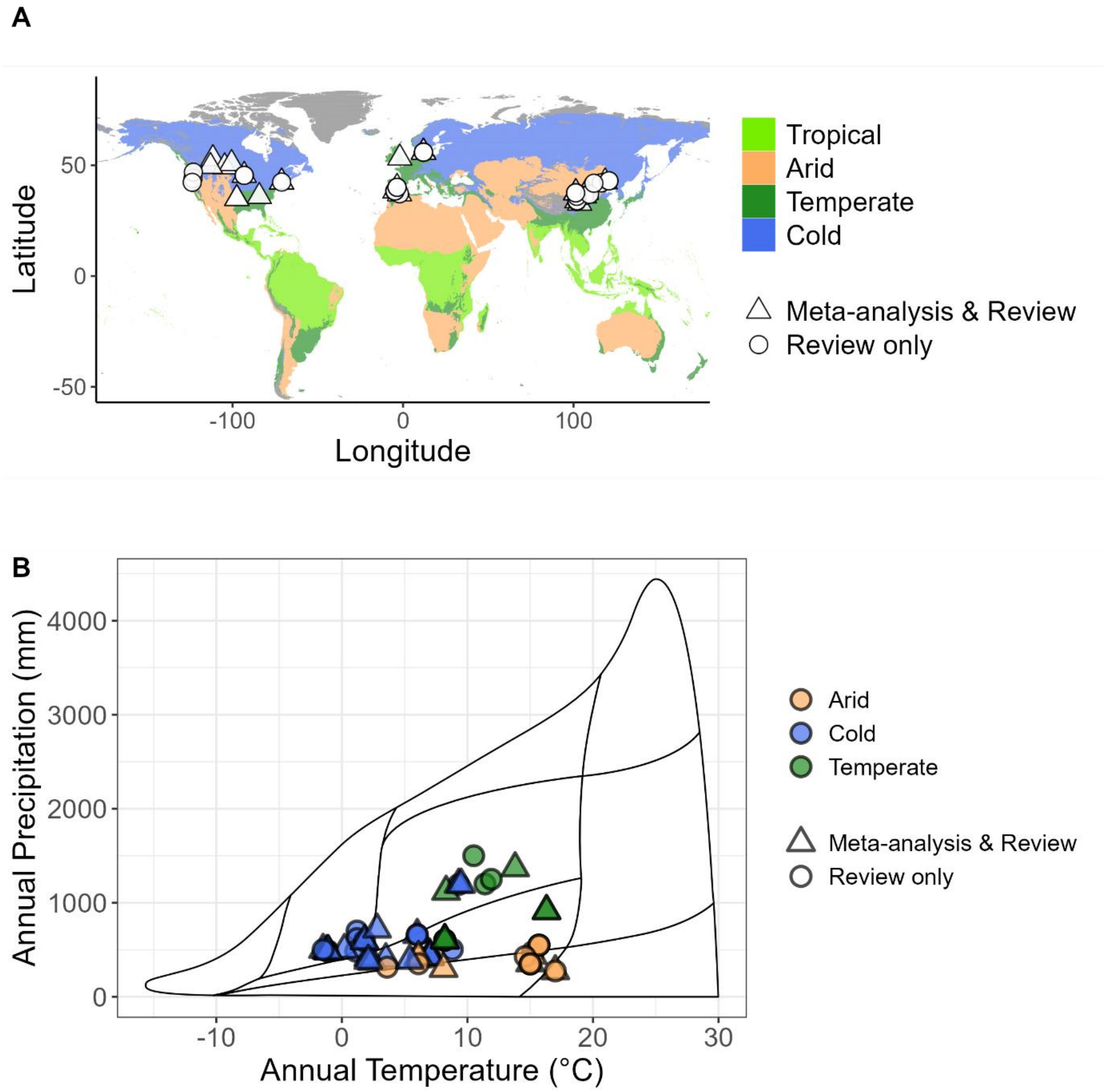

Figure 2. (A) World map displaying locations of experiments with Koppen-Geiger broad climate types and (B) a Whittaker biome plot with blank biomes and points coloured by Koppen-Geiger broad climate types as per (A). Triangles show studies included in both the review and meta-analysis and circles show studies included in the review only.

Summary of response variables and included studies

We summarised the response variables reported in the articles and found the most common were soil traits (n = 36 articles, Fig. 3A), which included physical properties, nutrients, microorganisms, extracellular enzyme activity and environmental DNA measures. Other commonly reported measurements were biomass (aboveground biomass and belowground biomass; n = 20, n = 12 respectively), leaf traits (n = 20; i.e. morphology, physiology, nutrients, epigenetic responses and enzymes), floristics (n = 13) and productivity (n = 12) including belowground net primary productivity (n = 7). Ecosystem gas fluxes (n = 17) were reported as soil respiration (n = 13) as well as methane production and net ecosystem exchange (n = 4). The least studied area was plant reproductive effort (n = 2).

Most articles reported results from 2–3 years of manipulation (Fig. 3B), while the duration of the longest experiment was 11 years, however, note that in several cases multiple articles published from the same long-term experiments. Open-top chambers were the most common form of warming (n = 14), followed by infrared heating (n = 10) and night-warming curtains (n = 8) (Fig. 3C). Only 3 articles (from one experiment) imposed belowground heating, in this case deep soil (to 1 m depth).

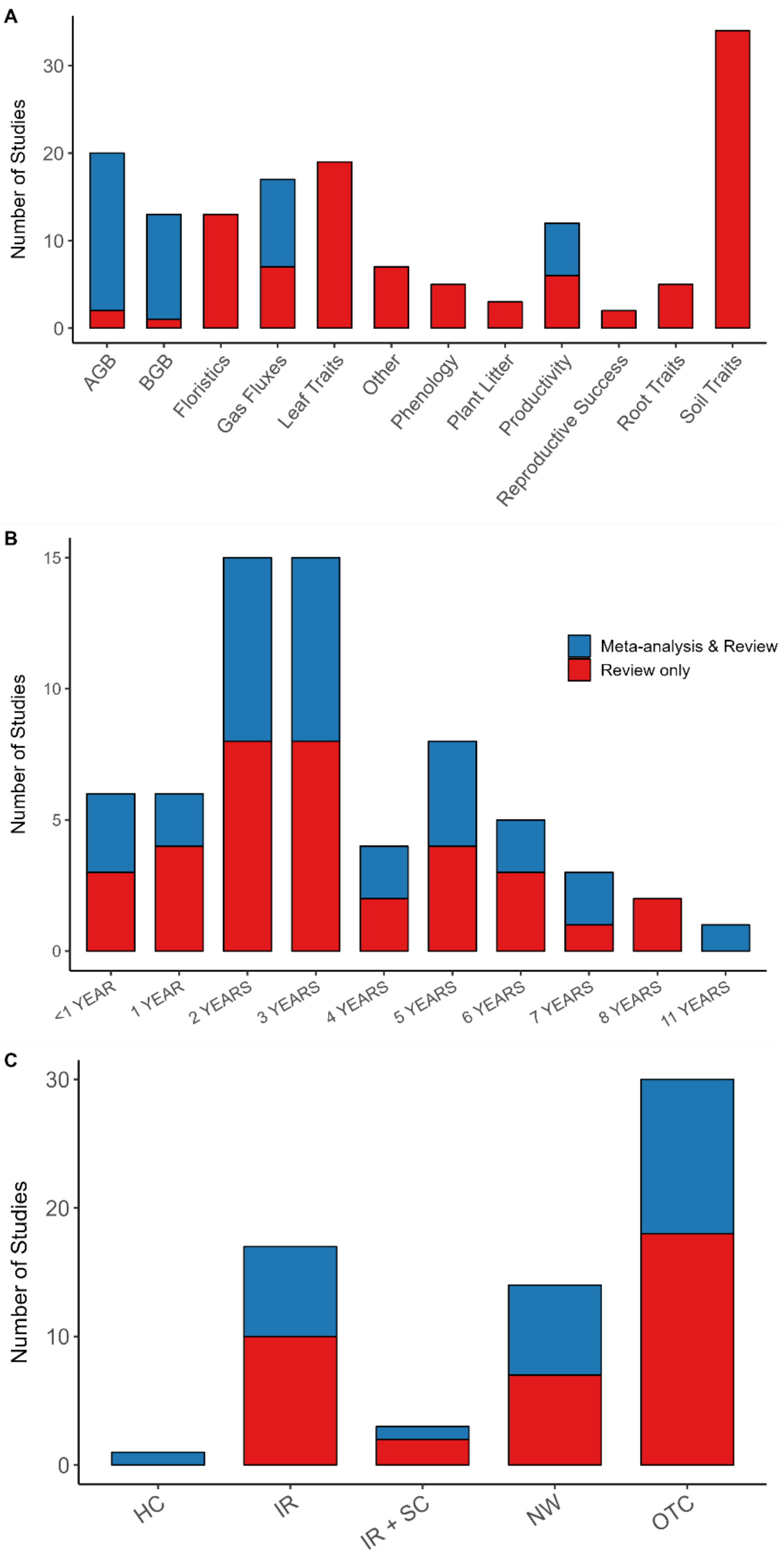

Figure 3. (A) Counts of response variables measured in studies including aboveground biomass (AGB) and belowground biomass (BGB), (B) counts of studies and experimental duration and (C) counts of heating methods using in studies including heating coil (HC), infrared heaters (IR), infrared heaters and soil warming cores (IR + SC), night-warming curtains (NW) and open top chambers (OTC). Blue represents studies that were included in the meta-analysis and review, red represents studies included in the review only.

Meta-analysis: Individual and combined effects of warming and drought on biomass and respiration

We predicted that drought would drive declines in plant growth while warming would have little overall effect due to its drying effect. This was supported for net primary productivity, where drought individually and in combination with warming caused a significant decline, while warming alone had no significant effect (Fig. 4A). Similarly, there was a significant decrease in aboveground biomass under the combined warming and drought treatment and a marginally non-significant decline under drought alone, but no change under the warming alone treatment (Fig. 4B).

Belowground, root biomass declined under the combined warming and drought treatment, but there was no change under warming alone nor drought alone (Fig. 4C). Moreover, there were no significant effects for any of the treatments on belowground net primary productivity (Fig. 4D). We also observed a negative response in soil respiration to drought and the combined warming and drought treatments (Fig. 4E). A significant positive effect of warming was observed for soil respiration as well.

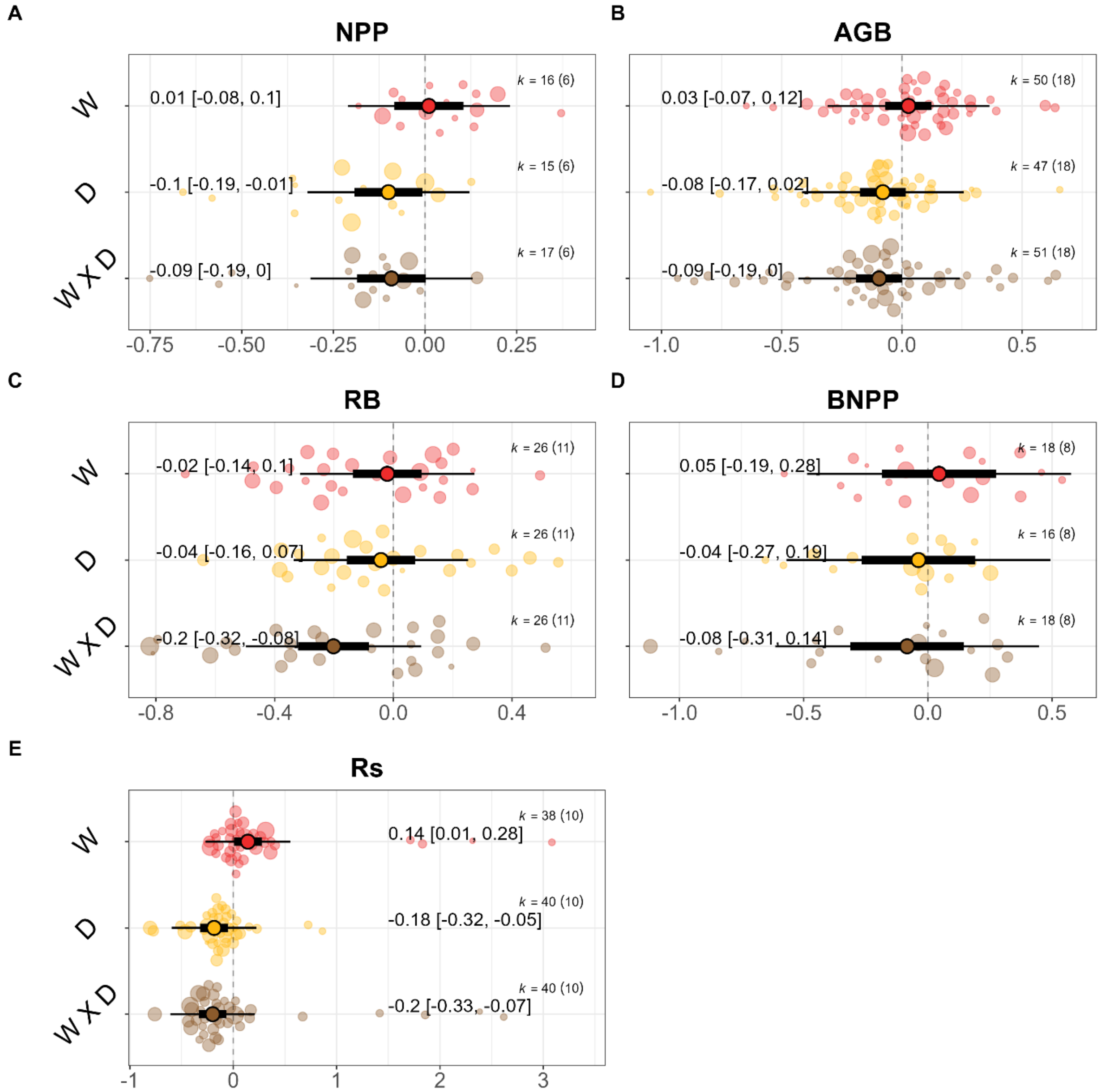

Figure 4. Individual and combined effects of warming and drought on (A) net primary productivity, (B) aboveground biomass, (C) root biomass, (D) belowground net primary productivity and (E) soil respiration. W (warming) treatment, D (drought) treatment and W X D (warming and drought combined) treatment. Effect sizes calculated as natural log response ratio ln*RR*. k is the number of unique entries contributing to overall effect size, with number of articles in parentheses. The confidence intervals are within the square brackets and indicated by the thick bar. The thin line indicates prediction intervals which represent heterogeneity.

Negative synergistic interactive effects of warming and drought

We calculated Hedge's *d* effect size to assess whether interactive effects of drought and warming were synergistic (more than the sum of their individual parts – either positive or negative) or antagonistic (less than the sum of their parts – either positive or negative). While there were no statistically significant interactions between warming and drought detected for any of the response variables at the typical 95% confidence level, all clearly trended towards a negative synergistic interaction, with two that would be significant at a 94% confidence level (Fig. 5). Belowground net primary productivity and soil respiration (and to a lesser extent net primary productivity) had relatively large, negative effect sizes and were only marginally non-significant, indicating a negative synergistic interaction (Fig. 5).

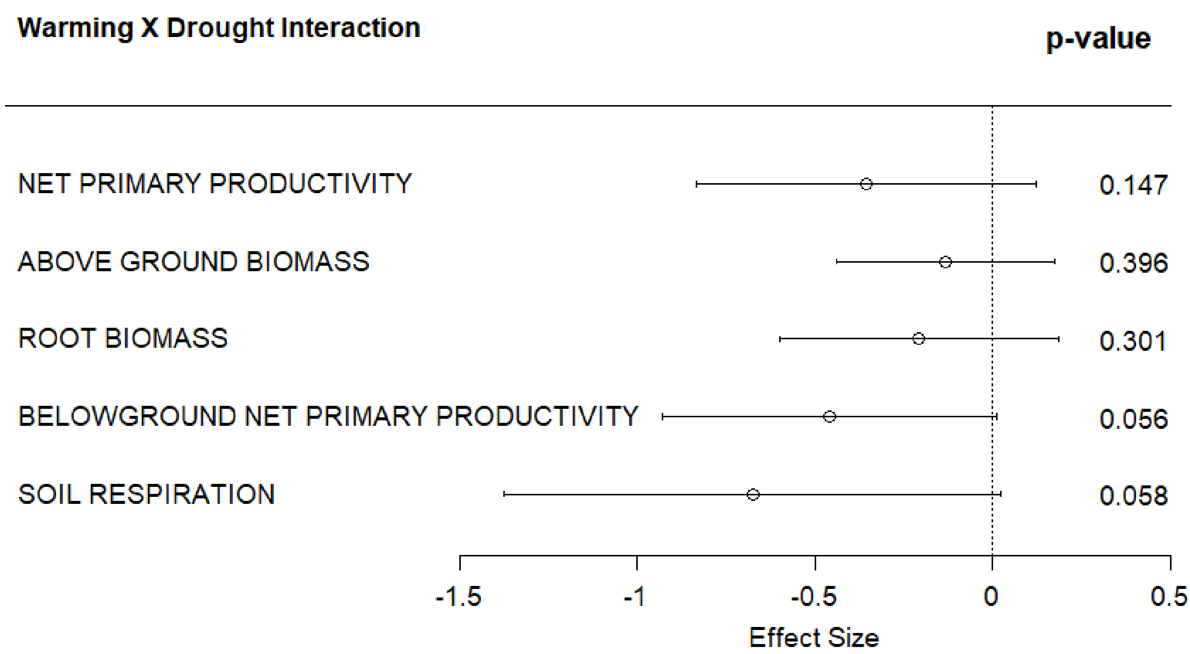

Figure 5. Interactive effects of warming and drought on net primary productivity, aboveground biomass, root biomass, belowground net primary productivity and soil respiration. Effect sizes calculated as Hedges' *d*. Error bars indicate 95% confidence intervals (CI) and *p*-values are displayed in the right-hand column.

Moderator variables explain little of experiment variation

We anticipated that the moderator variables would help explain variation in the data by accounting for various contextual differences between individual experiments and location. However, overall, these variables explained surprisingly little (Fig. 6). Net primary productivity under warming and drought combined increased with longer experiment duration while aboveground biomass under warming individually and combined with drought declined (Fig S1A,B,F). Belowground net primary productivity under warming increased though soil respiration under warming and drought in combination with warming decreased with longer experimental length (Fig S1C,D,E, supp). There were no significant responses as result of magnitude heat under any treatment for any response variables. We were unable to test the impact of magnitude for drought due to soil moisture data being inadequately reported.

Finally, we sought to examine whether inherently cool or wet locations would show greater impacts of warming or drying treatments than hotter or drier ones. We therefore examined effect size responses along a gradient of cool-to-warm locations (MAT) and dry-to-wet (MAP), and by exploring the effects of precipitation in more depth through seasonal precipitation for the periods the experiments were carried out. We found that soil respiration declined with increasing MAP under the drought treatment (Fig S1G, supp) and warming combined with drought (Fig S1H, supp), such that soil respiration was more negative in locations with higher MAP. There was a marginally non-significant positive response of aboveground biomass to MAT under drought (Fig S1I, supp), resulting in more negative responses in locations with lower MAT. Soil respiration was negatively correlated with increasing summer precipitation under warming (Fig S1J, supp) and warming combined with drought (Fig S1K, supp) resulting in negative responses to treatments at higher spring precipitation.

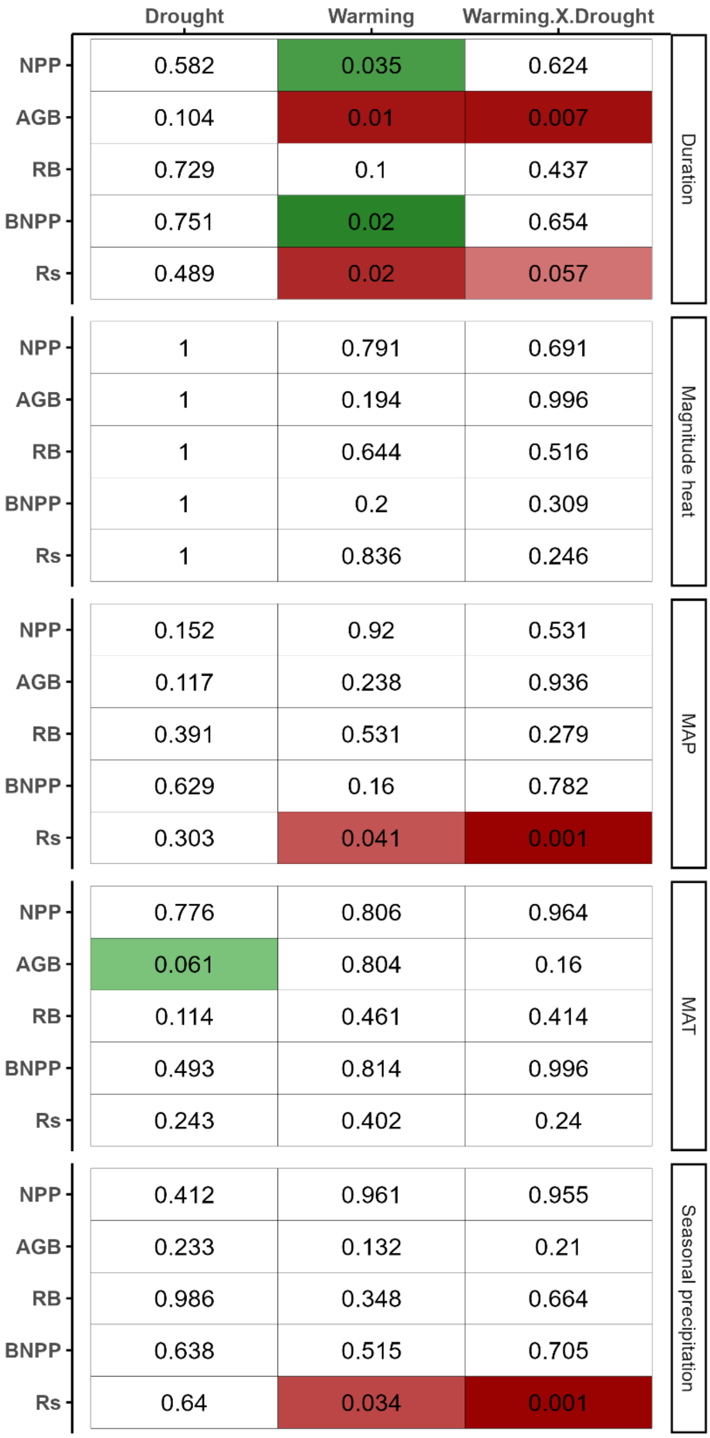

Figure 6. Heat map describing significant relationships between treatments and moderator variables. The numbers in the cells are p-values, while the colours describe a positive relationship (green) or a negative relationship (red). The strength of the colour represents the level of significance, with p-values above 0.1 deemed non-significant and not receiving a colour. Treatments across the top including warming, drought and warming in combination with drought (Warming.X.Drought). Response variables are along the y axis with net primary productivity (NPP), aboveground biomass (AGB), root biomass (RB), belowground net primary productivity (BNPP) and soil respiration (Rs). Moderator variables are on the right-hand side of the plot including experiment duration, magnitude heat, mean annual precipitation (MAP), mean annual temperature (MAT) and seasonal precipitation.

Discussion

Here we synthesised the literature on factorial field experiments that manipulate temperature and water availability in grasslands across the globe. We sought to identify gaps in the literature and assess whether there are consistent patterns of key carbon cycling variables in response to warming, drying and the combination thereof. We predicted that drought would be a driving factor across responses. Our meta-analysis confirmed that drought drove most responses, leading to declines in inputs of carbon into the system including net primary productivity and aboveground biomass and outputs in the form of soil respiration, both individually and in combination with warming (Fig. 4A,B,E). Warming was associated with a significant increase in soil respiration, yet that was the only response variable to respond to warming alone (Fig. 4E). Net primary productivity, belowground net primary productivity and soil respiration all showed high likelihood of negative synergistic interactions between warming and drought (Fig 5). Other than duration, there was little explanatory power in the moderator variables of treatment magnitude and mean climatic drivers. Below we the explore knowledge gaps this work has highlighted, the importance of examining interacting factors, and the insights gained from our exploration of moderator variables.

Systematic analysis reveals research gaps and imbalances

Research effort in this field has expanded substantially in recent years, with 42 more studies added between 2019-2024 (our search) compared to 23 studies from prior to 2019 (MESI database; Fig. 1). Despite this substantial increase in multi-factor experiments, there is still a paucity of published research in this area, especially in the southern hemisphere. Four continental regions were entirely lacking such studies (Africa, South America, South Asia and Oceania; Fig. 2A). No experiments were carried out in 'tropical' Koppen-Geiger climate types (Fig. 2A) nor in regions that receive high annual rainfall (Fig. 2B), despite grasslands existing in regions in excess of 3000mm (Dixon et al., 2014). A large portion of global grasslands are tropical and/or savanna ecosystems, dominated by C_4 grasses, making their omission stark when considering their contribution to global grassland carbon cycling (Dixon et al., 2014; Grace et al., 2006; Scurlock & Hall, 1998). To predict the responses of grasslands to global change more accurately and precisely, geographically and climatically diverse studies are necessary, including tropical and sub-tropical ecosystems.

Ultimately, our meta-analysis was hindered because studies focused on different response variables and thus cannot be directly compared. A few key variables emerge as most commonly measured and also most informative. Net primary productivity, measured through it's individual above and belowground components, is indicative of plant carbon uptake over annual cycles while soil respiration is indicative of carbon loss to the atmosphere. Between these two measurements one can gain an insight into the total carbon cycling of the ecosystem. However, approximately half of the studies we identified did not measure these variables. Further, the dearth of detailed meta-data on experimental sites, such as edaphic factors and soil moisture, prevented analysis of other potentially useful explanatory variables. We encourage future work in this area to include basic carbon cycling variables (net primary productivity and soil respiration) and a minimum set of soil measurements that includes soil temperature, soil moisture, pH and organic carbon.

Finally, there were only two experiments that employed some form of direct soil warming, one being soil surface (Grime et al., 2008) and the other to a depth of 1 m (Reich et al., 2020) (Fig. 3B). Climate change is predicted to affect temperature both above and belowground (Soong et al., 2020). Experiments that only focus on aboveground warming are missing a key aspect of future climate predictions and therefore somewhat unrealistic. We would encourage future researchers to employ manipulations that warm aboveground and the soil directly, to depth, where possible. The DeepSoil 2100 network (Protti Sanchez et al., 2024) aims to address this issue by fostering collaboration between global soil warming experiments.

The importance of examining drought and warming effects in concert

As individual factors, drought and warming had very different impacts. Drought resulted in a decline in net primary productivity as well as soil respiration, which supports our first hypothesis. Drought affects photosynthetic capability and ultimately plant growth (Zlatev & Lidon, 2012). Declines in growth may also reflect shifts in community composition or abundance (Grime et al., 2008) as more drought tolerant plants typically have lower growth rates as part of a conservative strategy (Wright et al., 2004). Such plants are also likely to exhibit a difference in growth strategy, investing relatively more resources belowground in response to drier conditions (Eziz et al., 2017; Z. Wang & Wang, 2023). However, our meta-analysis did not detect significant changes in root biomass nor belowground net primary productivity under drought alone (Fig. 4C,D). Soil respiration declined under drought presumably because low soil moisture supresses plant and microbial activity (Canarini et al., 2017; Z. Wu et al., 2011). Warming however, did not result in any significant effect on plant growth, but did significantly increase soil respiration. Warming can stimulate increases in plant growth (Arndal et al., 2013; D. Liu et al., 2021; Lu et al., 2013; Song et al., 2019), but that response is not always observed (Wilschut et al., 2022). Where no change in growth is detected that may reflect opposing responses of different plant functional types resulting in a net zero effect (Andresen et al., 2009) or may be due to precipitation during the experiment which can mediate responses to warming, with positive responses in wet years but no effect seen in dry years (Xu et al., 2012).

Warming however, stimulates autotrophic and heterotrophic respiration and thus increases overall soil respiration (Rustad et al., 2001; Song et al., 2019; Yan et al., 2022).

We hypothesised that when both warmed and dried, decreases in responses will be exaggerated, resulting in a negative synergistic interactions (a more negative interaction than when individual components are added together; (Piggott et al., 2015)). Belowground net primary productivity, soil respiration and to a lesser extent, net primary productivity, all suggest a high likelihood of an interaction occurring with *p* values < 0.15 (i.e. $> 85\%$ chance of occurring) (Fig. 5). Warming, in the absence of drought, can result in a drought effect through increasing soil drying (Reich et al., 2018; Winkler et al., 2016) and can amplify a drought treatment by further reducing soil moisture (Sheik et al., 2011). Warming and drought can interactively decrease net primary productivity (Hoeppner & Dukes, 2012) while belowground biomass has previously been found to exhibit an antagonistic interaction under warming and drought, (L. Zhou et al., 2020), as has soil respiration (Sun et al., 2023). Synergistic effects have been found to be common in a grassland experiment (Reich et al., 2020), however in a global meta-analysis Song et al., (2019) found no significant responses for these variables highlighting the difficulty of drawing conclusions when comparing studies across diverse ecosystems with opposing responses to treatments. In grasslands, we propose that interactive effects are more likely due to the relatively short root statures and lifespans of the resident plant community. Other woody biomes such as tundra and forest, such as those included in Song et al., (2019), may exhibit greater resilience to these factors, at least within the timeframes that *in situ* experiments tend to operate (predominantly <5 years; Fig. 3B). We believe further work, including more long-term experiments in understudied regions is needed to fully understand the extent of interactive effects from warming and drought as this will have consequences for global carbon stocks.

This meta-analysis indicated warming in combination with drought will have negative impacts for both major inputs of carbon (net primary productivity) and outputs (soil respiration). Taken together, this suggests that there will be little change to net ecosystem exchange, the overall balance of uptake and emission of CO_2. Note that net ecosystem exchange was measured in just 3 of the studies reviewed here. Regardless, the results found in the present meta-analysis do not speak to the magnitude of changes found in net primary productivity and soil respiration and thus consequences for carbon stocks. Globally, in some biomes a growing terrestrial carbon sink has been identified in recent decades that has mitigated the worst effects of climate change so far (Keenan & Williams, 2018). Under severe drought however, net primary productivity is more sensitive than soil respiration, particularly in grassland ecosystems, suggesting a decline in the carbon sink under these conditions (Shi et al., 2014). The likely interactive effects detected in the present meta-analysis suggests the combination of warming and drought decreases net primary productivity and soil respiration more than drought alone. Therefore, we advocate for more work directly analysing net ecosystem exchange in factorial warming and drought experiments to understand whether there will be effects to source/sink ratios.

Moderating factors explain little variation in impacts of drought and warming.

We found that moderator variables did not effectively explain the observed variation in drought and warming effects, particularly considering that we assessed a wide range of comparisons. While this may indicate that climatic factors and experimental decisions are not moderating the warming and drought treatments, there are two other explanations. First, that because not all experiments reported the moderator variables of interest, we did not have the power to detect effects; or second, that the dataset is simply not capturing a broad enough climatic range to detect or quantify these influences. More studies in warmer, wetter climates and more amenable metadata to assess magnitude of manipulation relative to local climatic factors, are needed before such patterns emerge or can be conclusively refuted.

The one exception among moderator variables was the duration of experimental treatments; as the duration of a manipulation increases, negative drought effects compound (Fig 6; supp Fig) and initial stimulatory effects of warming diminish or become negative (e.g. aboveground biomass and soil respiration) (Fig 6; supp Fig). An initial stimulation of growth in response to warming could be reflective of an increase in availability of nutrients through mineralisation (Rustad et al., 2001) that become limiting as they are consumed (Arft et al., 1999). It could also be evidence of compositional shifts to more stress tolerant, less productive species (Grime et al., 2000, 2008; Wright et al., 2004). In contrast to our finding, (L. Zhou et al., 2016) found that soil respiration was positively correlated with increasing duration of manipulation, however we interpret the decline in soil respiration over time in the present study, to suppression of plant growth, resulting in declines in both autotrophic respiration via plant roots and heterotrophic via inputs of carbon into the soil (Selsted et al., 2012). Our meta-analysis also indicated there were increases in productivity under certain treatments in experiments that ran for a longer period of time. While this is somewhat counter-intuitive, given the compounding negative impacts on other response variables discussed above, we argue that if there is a shift in community composition that consists of species better able to tolerate temperature or drought stress (Batbaatar et al., 2022) or to species with longer-lived aboveground organs, total productivity could increase. These complexities demonstrate that characterising productivity is extremely difficult (Körner, 2021) and emphasise how important applying consistent methods to facilitate comparative analyses across studies are.

Conclusion

Our meta-analysis synthesised all available data on field experiments that factorially applied warming and drought treatments in the world's grasslands and indicated that drought alone and in combination with warming reduces plant biomass, productivity and soil respiration in grasslands and interactions are likely to occur for the major fluxes of carbon – net primary productivity and soil respiration. Our analysis further yields several knowledge gaps and recommendations regarding design of experiments. Future work should aim to fill the gaps we identified, namely: 1) understudied regions of the world, including the southern hemisphere and tropical grasslands, need to be considered to better estimate global effects of warming and drought in concert, and 2) experiments need to run for long enough tease out short-term responses of resident plant communities from longer term shifts in composition and carbon cycling. Future experiments should ensure that: 1) detailed meta-data including edaphic factors are collected and reported to allow deeper analysis, 2) the magnitude of warming temperatures reflects projections for particular regions (>1–2 °C generally obtained via passive warming) and 3) where possible researchers impose soil warming at depth to realistically simulate future climate conditions. Finally, we advocate that all future experiments measure a core set of carbon cycle response variables to enable comparison across studies. Net primary productivity (i.e. aboveground net primary productivity and belowground net primary productivity) and soil respiration should be measured in all future experiments to quantify the main inputs and outputs of carbon from an ecosystem. Filling these knowledge gaps and with manipulations that share standardised methods will enable effective prediction and ultimately modelling of the complex interactions of drought and warming effects of climate change on global grassland carbon dynamics.

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Data and Code Availability Statement

The data and code that support the findings of this study can be found at the following link:

https://figshare.com/s/047771b897dc273c9f78

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Supplementary material

Grassland definition

Dixon et al 2014 definition of a grassland – "In summation, we propose the following definition of grasslands for global application. A natural or semi-natural grassland is defined by the following characteristics: (1) a non-wetland formation; (2) vascular vegetation has at least 10% cover; (3) graminoids have at least 25% cover (but if < 25% cover, graminoids exceed that of other herbaceous and shrub cover); (4) broad-leaved herbs (forbs) may have variable levels of cover and dominance; (5) shrubs have < 25% canopy cover; (6) and trees: (i) in temperate zones, typically have < 10% canopy cover, are < 5 m tall and single-layered, or (ii) in tropical regions, typically have < 40% canopy cover, are < 8 m tall, and are single layered".

Literature search details

Our starting point was the Manipulation Experiments Synthesis Initiative (MESI) database (Van Sundert et al., 2023), which was assembled through the compilation of four sub-databases created from four separate meta-analyses. With a decline in global change studies added to MESI beyond 2018, we decided to supplement the database with our own systematic search from 2019 to 13 June 2024 to capture any new factorial climate manipulative experiments that may not have been included. Our supplementary systematic search of the literature sought to identify articles that presented manipulation of warming and drought under field settings in grassland ecosystems. Here we define the term 'article' as a published paper and 'experiment' as the physical experiment that took place. A single experiment could result in multiple articles through analysing and publishing data on different response variables or data from separate years in the experiment.

The search terms consisted of four categories representing the (1) drought aspect of the experiment, (2) the warming aspect, (3) the nature of the experimental setting (*in situ*) and (4) the ecosystem (grasslands), with a keyword required to be found in each of the four categories (AND). The search string was: (drought* OR dry* OR drier OR precipitation OR "soil moisture" OR "water stress" OR "water deficit" OR "rainfall reduction") AND (warm* OR heat* OR "high temperature*" OR "increas* NEAR/1 temperature*" OR elevated) AND (experiment* OR manipulat* OR field OR "in situ" OR "global change" OR factorial OR "simulate* climat* change*") AND (meadow OR grass* OR steppe* OR plateau OR savanna* OR dryland* OR prairie OR "old-field"). The wildcard symbol * refers to the keyword with any suffix (e.g. warm* could be warming and warms) and the term NEAR/1 requires the keyword to have zero or one word between it and another keyword. Within Web of Science, we ran an advanced search with all databases and used the topic search field for each section. The search was restricted from 2019 – present (13 June 2024), and excluded the preprint citation index and the MEDLINE database. For Scopus we used the same search terms and used the Title-Abstract-Keywords search field, restricted from 2019 - present (13 June 2024) as well. Only primary literature in English were included. Once duplicates, reviews and dissertations were removed, there were a total of 4006 articles.

Systematic review paper screening details

From our literature search, titles and abstracts were screened, resulting in 71 articles. A further 7 articles were identified through references within the relevant 71, resulting in 78 articles total. Of these 78 articles, full texts were read to determine suitability. Of these, 42 met the inclusion criteria with 36 excluded based on unsuitable methods (n = 27), measured data not reported in results (n = 4), data was captured in another paper (n = 3), focus was on tree seedlings (n = 1) or duplication (n = 1).

To find the relevant articles in MESI up to 2019, we first filtered the total number of articles (n ~ 1779) by the treatment column to include only those that included both warming and drought combination experiments (n = 62). From these articles, full texts were then screened to confirm or remove based on ecosystems and methods, with 23 meeting the above criteria and the rest removed based on unsuitable method types (such as greenhouses; n = 27), were not in a grassland ecosystem (n = 8) or contained data that was already captured in another article (re-analysis of same dataset, n = 1). Note that several experiments produced multiple articles and, one dataset in MESI included unpublished data from Dr Shiqiang Wan's lab. The experimental details were published in another article and the dataset included sufficient meta-data to be included in the present study, thus it was deemed suitable to be included.

Meta-analysis variables

We included two aspects of belowground biomass, root biomass and belowground net primary productivity, separately because the former represents a carbon pool and the latter a carbon flux. The studies included here report root biomass taken using a soil core which indicates standing root biomass; belowground net primary productivity was measured through root ingrowth cores giving a measure of fine root growth over the course of a defined period (usually one year). We decided to incorporate measures of aboveground net primary productivity into the aboveground biomass category as these were often considered the same measure between studies. We decided to include all measures of aboveground biomass, even if they were not considered equivalent to aboveground net primary productivity, as their exclusion did not significantly alter the results.

Seasonal precipitation calculation methods

Briefly, monthly precipitation data for each site was downloaded from Worldclim (Harris et al., 2020) using latitude and longitude reported in the studies at a scale of 2.5 ′ (~ 21 km^2), for the period of 2000 to 2021 (the period that the data was available). Monthly precipitation data was summed to get seasonal precipitation totals, such that autumn was September, October and November, winter was December, January and February, spring was March, April and May and summer was June, July and August. Note that northern hemisphere seasons used as there were no southern hemisphere experiments in the dataset. For biomass, the year was defined as the period before sampling was conducted, for example, if aboveground biomass was sampled in June 2020, then the preceding seasonal precipitation totals would be used as the preceding year is important in determining final biomass totals. For soil respiration, we used precipitation data for the season in which the measurements were taken (usually summer) as soil moisture at time of measurement is most important.

Individual and combined effect sizes

$$lnRR = ln\left(\frac{Xt}{Xc}\right)$$

Where *Xt* is the mean of the treatment and *Xc* is the mean of the control.

The variance (*v*) was calculated:

$$v = \frac{St^2}{ntXt^2} + \frac{Sc^2}{ncXc^2}$$

Where *St* and *Sc* are the standard deviations for the treatment and controls, *nt* and *nc* are the sample size of the treatment and controls and *Xt* and *Xc* are as above.

Main and interactive effects of warming and drought

The pooled sample variance, *s*, was calculated for all four treatment groups:

$$s = \sqrt{(nwd} - 1)(Swd){}^\wedge 2 + (nw - 1)(sw){}^\wedge 2 + (nd - 1)(sd){}^\wedge 2 + (nc - 1)(sc){}^\wedge 2)/m$$

Where *nwd*, *nw*, *nd* and *nc* are the sample sizes for warming drought, warming, drought and control and *swd*, *sw*, *sd* and *sc* are the standard deviations for warming drought, warming, drought and control. The degrees of freedom (*m*) for the four treatments are calculated as:

$$nwd + nw + nd + nc - 4$$

And the correction factor *J(m)* was calculated as:

$$J(m) = 1 - \frac{3}{4m - 1}$$

The main effects of each imposed treatment (warming, drought and warming drought) were calculated as:

$$dw = \frac{(Xwd + Xw) - (Xd + Xc)}{2S} J(m)$$

$$dd = \frac{(Xwd + Xd) - (Xw + Xc)}{2S} J(m)$$

$$di = \frac{(Xwd - Xw) - (Xd - Xc)}{2S} J(m)$$

Where *dw* and *dd* are the main effects of warming and drought respectively and *Xwd*, *Xw*, *Xd* and *Xc* are the means of the warming drought, warming, drought and controls respectively. *di* is the interactive effect of warming and drought. Pooled sample variance for each *dw*, *dd* and *di* were calculated as:

$$v2dd = \frac{\left(\frac{1}{4}\right)\left(\left(\frac{1}{nc}\right) + \left(\frac{1}{nd}\right) + \left(\frac{1}{nw}\right) + \left(\frac{1}{nwd}\right) + dw^2\right)}{2(nc + nd + nw + nwd)}$$

$$v2dd = \frac{\left(\frac{1}{4}\right)\left(\left(\frac{1}{nc}\right) + \left(\frac{1}{nd}\right) + \left(\frac{1}{nw}\right) + \left(\frac{1}{nwd}\right) + dd^2\right)}{2(nc + nd + nw + nwd)}$$

$$v2di = \frac{\left(\frac{1}{4}\right)\left(\left(\frac{1}{nc}\right) + \left(\frac{1}{nd}\right) + \left(\frac{1}{nw}\right) + \left(\frac{1}{nwd}\right) + di^2\right)}{2(nc + nd + nw + nwd)}$$

Where *v2dw*, *v2dd* and *v2di* are the pooled sample variance.

Significant moderator variable regressions

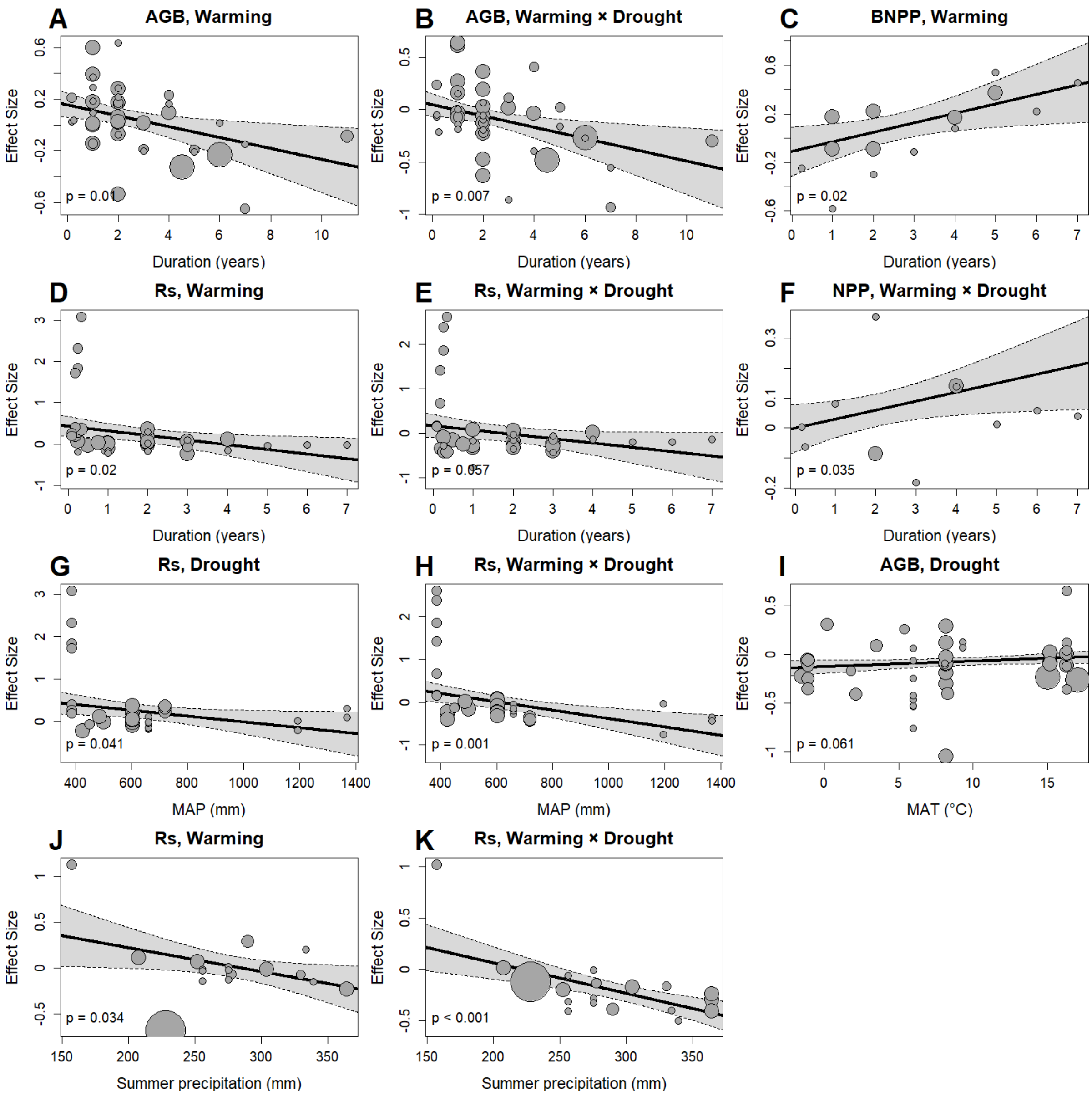

Figure S1. Bubble plots of meta-regressions between the natural log-transformed response ratio (ln*RR*) of experiment duration with (A) AGB (aboveground biomass) under warming (B) and warming drought combination, (C) BNPP (belowground net primary productivity) under warming, (D) Rs (soil respiration) under warming (E) and warming drought combination (F) and NPP (net primary productivity) under warming drought combination. Of MAP (mean annual precipitation) with (G) Rs under drought (H) and warming drought combination. Of MAT (mean annual temperature) with (I) AGB under drought. Of summer precipitation under (J) Rs under warming and (K) warming drought combination. Meta-regression lines are shown as solid black, grey areas indicate 95% confidence intervals. Circle size indicates weight of observation.

Assessment of publication bias

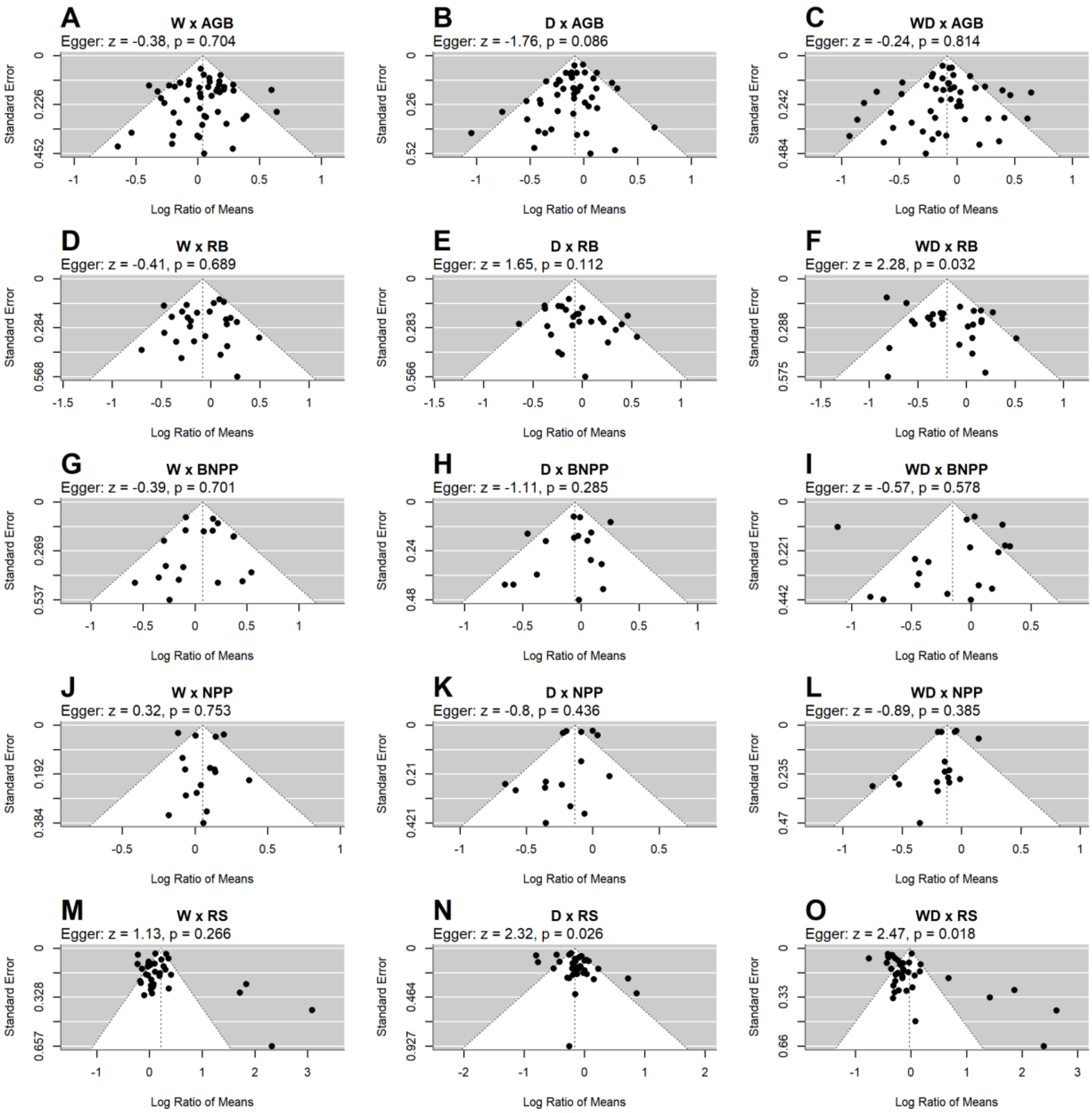

Figure S2. Funnel plots of the natural log-transformed response ratio (ln*RR*) for (A) AGB (aboveground biomass) under warming (B) drought (C) and warming drought combination, (D) RB (root biomass) under warming (E) drought (F) and warming drought combination, (G) BNPP (belowground net primary productivity) under warming (H) drought (I) and warming drought combination, (J) NPP (net primary productivity) under warming (K) drought (L) and warming drought combination, (M) NPP (net primary productivity) under warming (N) drought (O) and warming drought combination. Egger's regression test for publication bias are displayed at the top (z and P values). A P value > 0.05 suggests there is no evidence of publication bias.