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

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Abstract

1. In grasslands, interactions between temperature and moisture are possible whereby increases in temperature can result in decreases in soil moisture, exacerbating drought conditions. Both uptake and emission of carbon from grassland ecosystems are, for the most part, governed by climate warming and changing precipitation regimes, yet it remains

unclear how grasslands globally will respond to concurrent warming and drought conditions in the future (~2100) or what impact that will have on the terrestrial carbon cycle.

2. 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 °C warming).
3. 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 net primary productivity (-8.8%) and soil respiration (-18%), and that interactions resulting in greater than additive effects are likely to occur in belowground net primary productivity, soil respiration and net primary productivity. Duration was the most explanatory moderator variable.
4. This meta-analysis highlights several knowledge gaps that hinder our capacity to fully understand the impacts of temperature and precipitation on grasslands: (1) There are no published experimental studies in tropical grassland ecosystems or the southern hemisphere, (2) Experiments that employ belowground warming to realistically simulate future climate change are few and (3) despite a large number of studies from factorial experiments, many of these did not include detailed meta-data including edaphic factors that allow deeper analysis of responses to climate change.
5. *Synthesis*. Overall, emission of carbon fell more than uptake, suggesting that grassland net carbon flux may be more resilient to future climate change than previously thought. Interactive effects between warming and drought are likely, highlighting the risk associated with interpreting results from single factor experiments when making predictions of ecosystem carbon flux responses under future climate scenarios.

Introduction

Mean temperatures across the globe are increasing while extreme climatic events such as droughts and heatwaves 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 from photosynthesis and ecosystem respiration (Beer et al., 2010), which are highly sensitive to both 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; Wu et al., 2011). Individually, climate warming and precipitation both affect carbon cycling. But these factors may also interact, causing responses that are more or less than the sum of their individual effects (Luo et al., 2008; Piggott et al., 2015). Further, as natural phenomena, heat and drought frequently occur together. Meta-analyses so far have found little evidence of compelling interactive effects (but see, Sun et al., 2022; L. Zhou et al., 2020), despite interactive effects being observed in individual studies (Albert et al., 2011; Garten et al., 2009; Hoeppner & Dukes, 2012; Kardol et al., 2010; Ma et al., 2017; Winkler et al., 2016; Zhu et al., 2016). These prior meta-analyses have interrogated single and combined effects well, but it is not clear whether interactive effects detected in experiments are isolated to specific ecosystems or are indicative of broader, global patterns. With an increase in multi-factor experiments, we suggest a focus on realistic, *in situ*, factorial experiments from within a comparable ecosystem type, is necessary to conclusively assess the potential for interactive effects.

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 (Luo et al., 2001; 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; Liu et al., 2021). Low soil moisture depresses soil respiration (Canarini et al., 2017; 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, in particular drought in the presence of heat. The interactive nature of these factors means that the outcomes of single factor experiments cannot be simply compared with those of multi-factor experiments, nor can the findings from single factor experiments be considered suitable for predicting responses to more complex future conditions (Hoeppner & Dukes, 2012; Reich et al., 2020; Sun et al., 2023; Thakur et al., 2019).

Prior meta-analyses have examined overall responses of carbon cycling to combined global change drivers, as well as elucidating some underlying mechanisms, but because there were relatively few

multi-factor experiments at time of compilation, these studies were limited in their capacity to interrogate deeper interactive effects. These studies 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 heat and drought experiments, prior meta-analyses incorporated experiments from across varying experimental conditions and vegetation types. For example, there was large variation with respect to the level of warming implemented, resulting in comparisons of large temperature differences between control and warmed ($\Delta > 9$ °C). This is important because plant and soil responses are often dynamic rather than linear, such that a small increase in temperature may have divergent effects to a large increase in temperature (W. Xu & Yuan, 2017). Further, meta-analyses attempting to answer questions related to interactive climate change 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); however, carbon cycle responses to global change factors are strongly dependent on biome (Yan et al., 2022). Results of a greenhouse or monoculture field trial are not likely to be comparable to those of field manipulations (Poorter et al., 2016). To increase the power to assess interactive effects of drought and heat, our global meta-analysis focused on grasslands and included only field experiments carried out in realistic, natural settings that simulated warming within realistic bounds for the next century (+1-4 °C) in a given site.

Grasslands are manageable and tractable due to short statured vegetation, which are easily measured and can be highly diverse in small areas, allowing a realistic simulation to adequately target a whole community. For this reason, grasslands tend to be studied more than other ecosystems, representing a higher proportion of the literature (Van Sundert et al., 2023). Measures of biomass and gas fluxes are easily taken for the whole ecosystem for short and long duration experiments, making them ideal to test hypotheses. Further, the majority of grassland biomass is near the surface of the soil (± 30 cm), therefore treatments are likely to apply to most of the community (above and belowground biomass), as opposed to woody ecosystems. Simple

manipulations for warming, such as open top chambers (OTC), are more effective for low-stature vegetation as opposed to forests (De Frenne et al., 2010). Finally, grasslands are an important biome the world over, and our meta-analysis encompasses experiments representing a range of climate types that are relevant at the global scale.

In this study, we conducted a systematic review and meta-analysis focusing on mean annual temperature (MAT), mean annual precipitation (MAP), experiment duration, seasonal precipitation and edaphic factors as moderating factors. We limited this study to multi-factor experiments, that realistically simulated climate changes in a single ecosystem type, grasslands. 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 the patterns described above, we hypothesised that drought would 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, despite warming stimulating growth in some ecosystems, due to the potential drying effect, we expected that across ecosystems there would be both positive and negative responses to warming, resulting in no overall effect on plant growth. Further, we predicted that there would 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 would be exaggerated. We also predicted that there would 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 expected there would be an acclimation of soil respiration over time. Further, we expected that the more severe the drought or heating treatments, the magnitude of treatment, would 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.

Materials and 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 (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).

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, four articles were excluded as they did

not 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. 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

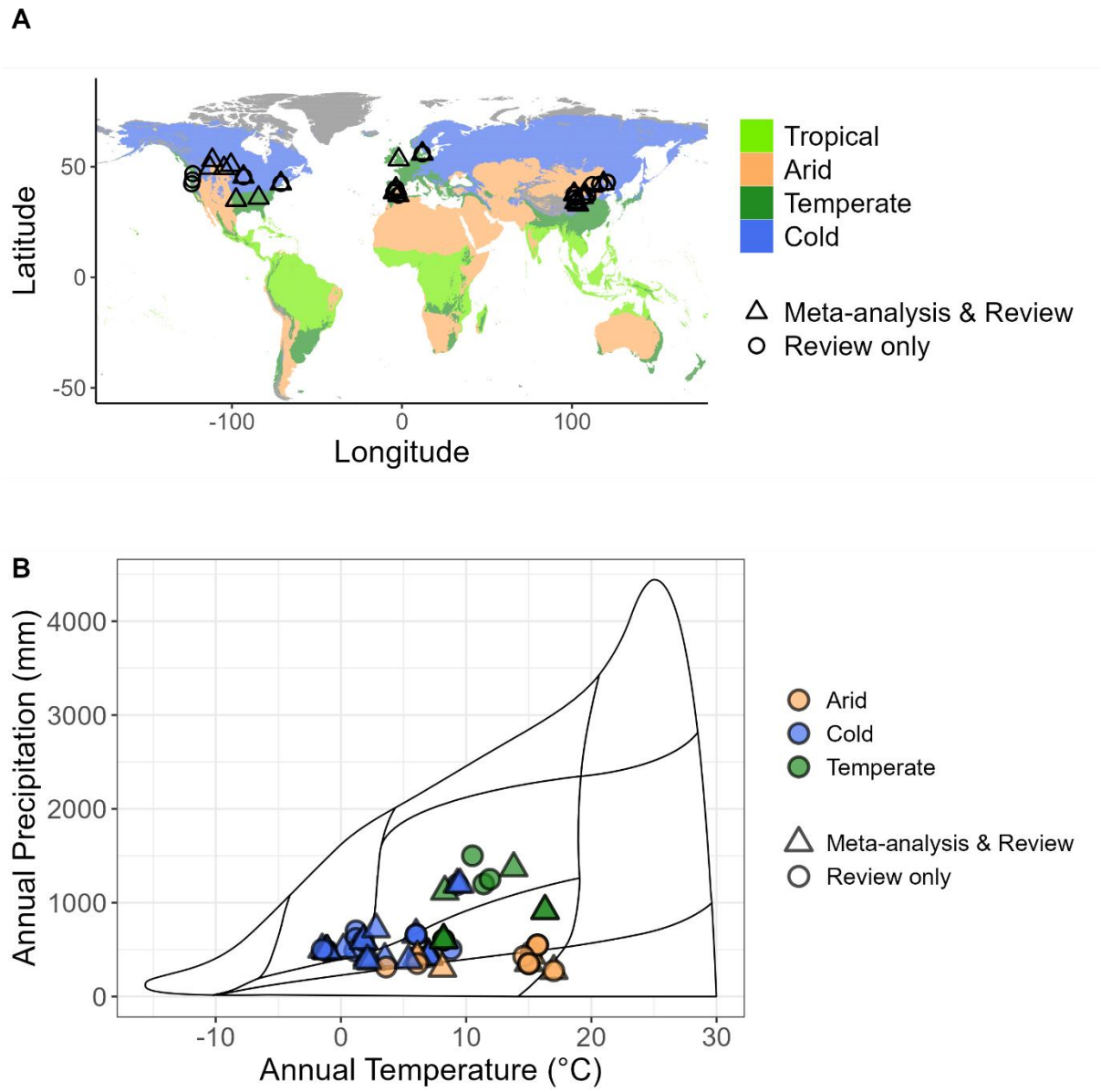
We investigated the main and interactive effects sizes for each response variable for each treatment by calculating Hedges' *d* following (Gurevitch et al., 2000) for conducting factorial meta-analyses. Due to the complex nature of interactions between factors resulting in opposing responses, we used 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 ≤ (+A) < 0 or negative antagonistic (−A), −1 + 1 = 0 < (−A) ≤ 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). Full equations and further details are provided in the Supporting Material.

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.

Results

Systematic review

Our systematic review of *in situ*, field manipulations of drought and warming in global grassland yielded a total of just 65 articles. All 65 articles of the factorial warming and drought manipulative experiments were conducted in the northern hemisphere, in Western Europe (n = 27), China (n = 26) and North America (n = 12) (Fig. 2A). Of the five possible Koppen-Geiger broad climate types that encompass grasslands, the articles were restricted to three (Fig. 2A): Cold (n = 31), Temperate (n = 21) and Arid (n = 20). There were no articles from locations with high average annual precipitation (>2000 mm) or high average annual temperatures (>20 °C) (Fig. 2B).



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

232 **Summary of response variables and included studies**

233 We summarised the response variables reported in the articles and found the most common were
234 aboveground biomass (n = 20) and ecosystem gas fluxes (n = 17) made up of soil respiration (n = 13)
235 as well as methane production (n = 1) and net ecosystem exchange (n = 3). Belowground biomass

and productivity were both reported in 12 studies, where measures of productivity include belowground net primary productivity (n = 7).

Most articles reported results from 2–3 years of manipulation (Fig. 3B), while the duration of the longest experiment was 11 years. In several cases, multiple articles arise 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, all from the same experiment, imposed belowground heating to deep soil (~1 m depth).

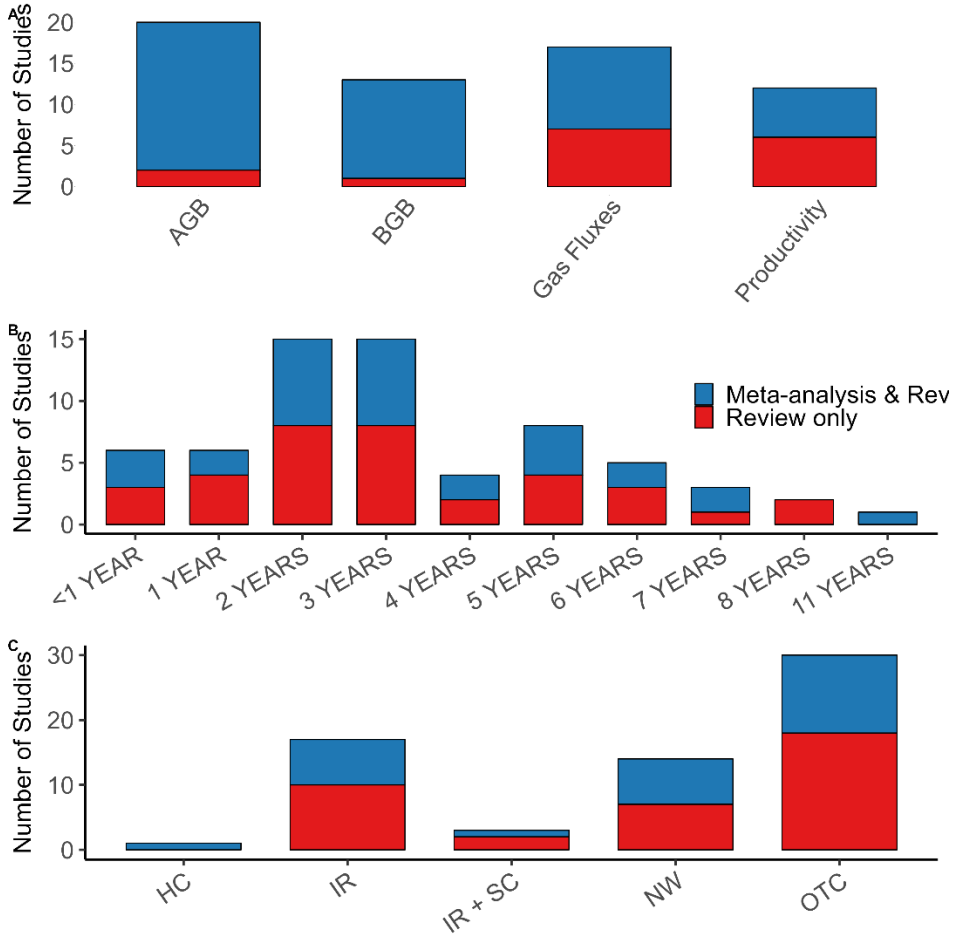

Figure 3. (A) Counts of response variables measured in studies including aboveground biomass (AGB) and belowground biomass (BGB), (B) counts of studies and experimental duration and (C) counts of heating methods using in studies including heating coil (HC), infrared heaters (IR), infrared heaters and 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 could be expected to stimulate growth, we predicted that because it is likely to be associated with drying it would be unlikely to have a positive impact on growth or biomass accumulation, resulting in an overall null effect. We included both 85% and 95% confidence intervals to highlight the high likelihood of effects occurring. This was supported for net primary productivity declining under drought individually (-9.5%, $p = 0.035$) and in combination with warming (-8.8%, $p = 0.055$), while warming alone had no effect (Fig. 4A). Similarly, there was a decrease in aboveground biomass under the combined warming and drought treatment (-9%, $p = 0.054$) and a decline under drought alone (-7.6%, $p = 0.104$), but no change under the warming alone treatment (Fig. 4B).

Belowground, root biomass declined under the combined warming and drought treatment (-18.3%, $p = 0.001$), but there was no change under warming alone nor drought alone (Fig. 4C). Moreover, there were no effects for any of the treatments on belowground net primary productivity (Fig. 4D). We also observed a negative response in soil respiration to drought (-16.9, $p = 0.007$) and the combined warming and drought treatments (-18%, $p = 0.004$) (Fig. 4E), while a positive effect of warming was observed for soil respiration as well (15.3%, $p = 0.039$).

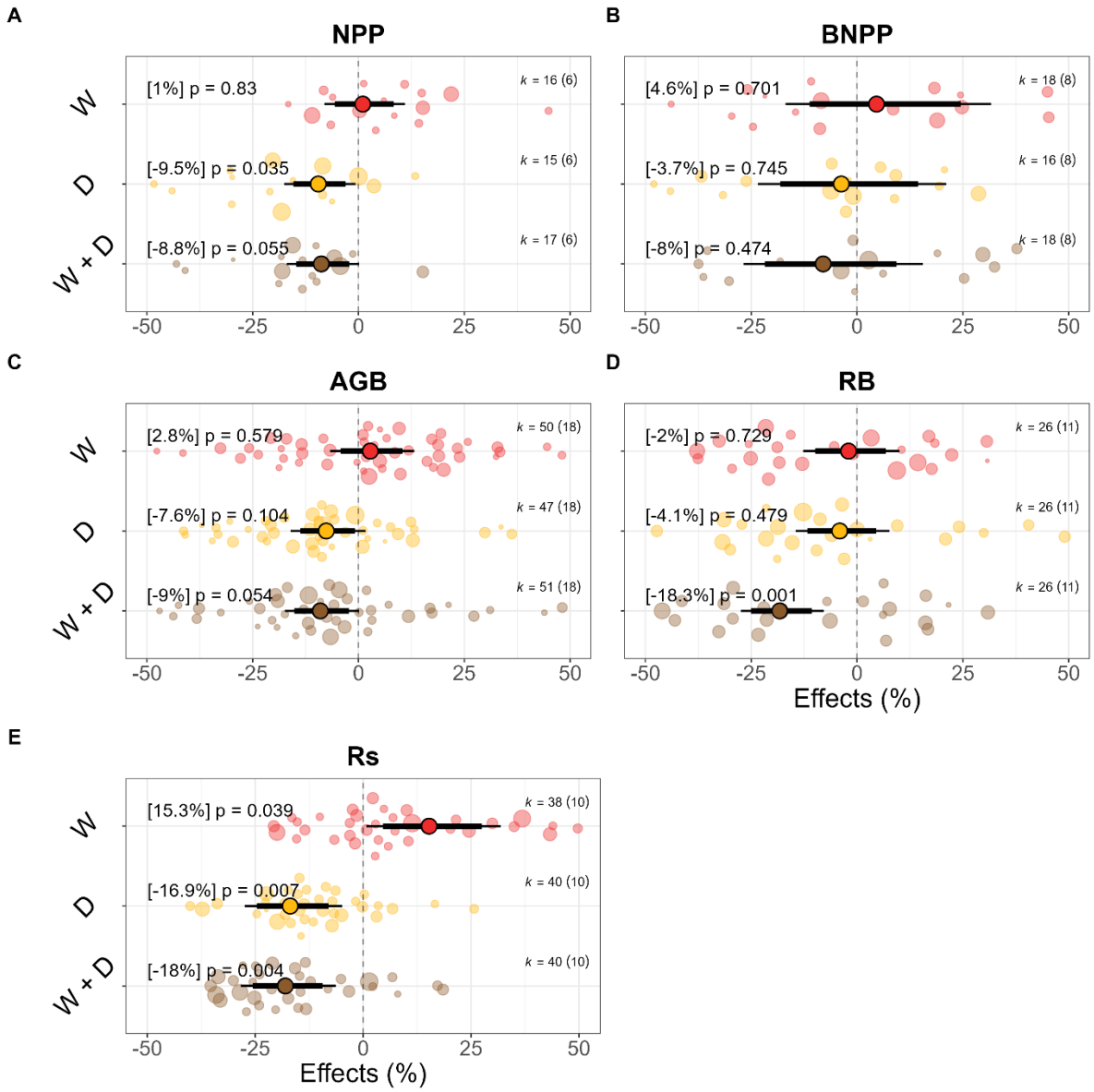

Figure 4. Individual and combined effects of warming and drought on (A) net primary productivity, (B) aboveground biomass, (C) root biomass, (D) belowground net primary productivity and (E) soil respiration. W (warming) treatment, D (drought) treatment and W X D (warming and drought combined) treatment. Effect sizes percent change. Thin error bars indicate 95% confidence intervals (CI) and thick error bars indicate 85% Cis. Percent change is in square brackets. P = p value. k is the number of unique entries contributing to overall effect size, with number of articles in parentheses.

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). Belowground net primary productivity, soil respiration and net primary productivity all had relatively large, negative effect sizes and *p*-values <0.15, indicating that in combination drought and warming result in a negative synergistic interaction (Fig. 5), more than cancelling out any positive effect of warming alone.

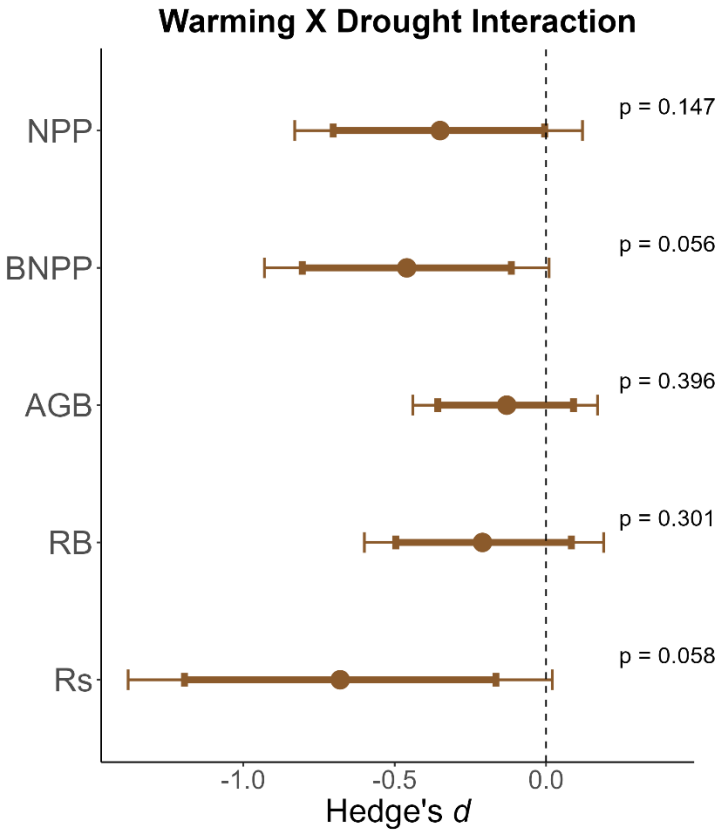

Figure 5. Interactive effects of warming and drought on net primary productivity, aboveground biomass, root biomass, belowground net primary productivity and soil respiration. Effect sizes calculated as Hedges' *d*. Thin error bars indicate 95% confidence intervals (CI) and thick error bars indicate 85% CIs. *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). There were no 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 (using MAT) and dry-to-wet (using MAP) locations, 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 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.

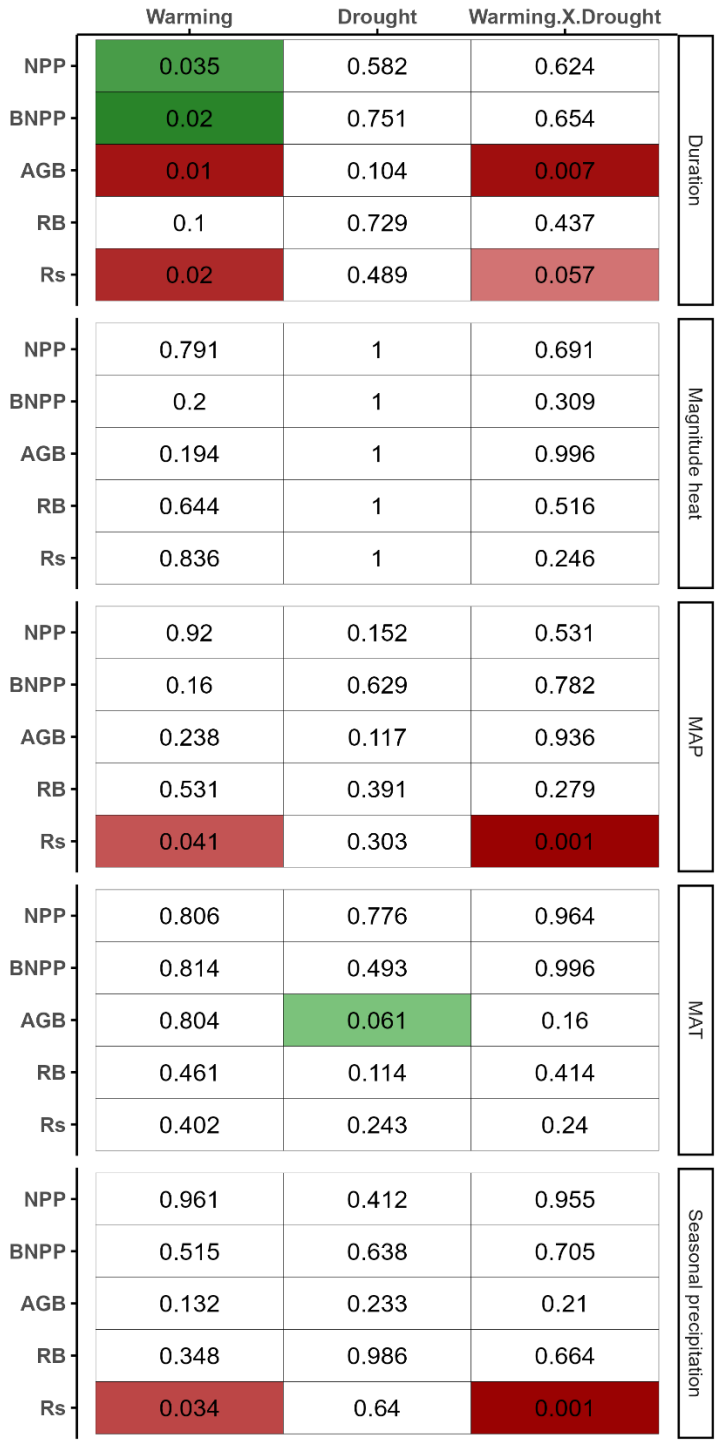

Figure 6. Heat map describing relationships between treatments and moderator variables. The numbers in the cells are p-values, while the colours describe a positive relationship (green) or a negative relationship (red). The strength of the colour represents the smaller the p-value, with p-values above 0.1 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

Summary

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 hypothesised that drought would be a driving factor across responses and that warming would accentuate drought effects, and we did find some evidence to support this. Net primary productivity, belowground net primary productivity and soil respiration all showed a high likelihood of negative synergistic interactions between warming and drought. 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. Warming alone was associated with an increase in soil respiration, yet that was the only response variable to show a strong response to warming across the studies. Other than duration, there was little explanatory power in the moderator variables of treatment magnitude and mean climatic drivers.

Interactive effects are likely

We hypothesised that when both warmed and dried, decreases in responses will be exaggerated, resulting in a negative synergistic interaction (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 a negative synergistic interaction 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). Globally, grasslands are thought to contain ~20% of carbon stocks (Dondini et al., 2023). An increased likelihood of synergistic interactions occurring for net primary productivity and soil respiration has consequences for global carbon cycling, as current models based off single factor experiments are likely to underestimate carbon cycling processes. With multi-factorial warming and drought manipulations becoming more common, the capacity to detect and compare interactive effects increases. Responses in major ecosystem and carbon cycling variables have been found to vary and even switch after longer durations (>10 years), for higher confidence and more robust inference, more long-term manipulations are needed (Melillo et al., 2017; Reich et al., 2020; Sun et al., 2023; L. Zhou et al., 2020).

The importance of examining drought and warming effects in concert

We predicted warming alone would have little overall effect due to heat stimulating growth in some ecosystems but not others, while drought would cause declines in carbon cycling. Drought dominated the responses, resulting in a decline in net primary productivity as well as soil respiration, which together represent the major uptake and emission of carbon from the ecosystem. Warming has been found to either increase or have little effect on plant productivity and soil respiration, thus predictions of future shifts in carbon cycling and effects on the land carbon sink have been difficult to assess (Carey et al., 2016; Dang et al., 2025; Lin et al., 2010; Lu et al., 2013; Luo et al., 2001; Yan et al., 2022). Our results indicate that it is not warming alone that is important, but in combination with drought that represents a realistic future scenario. To date, because many drought experiments have not concurrently imposed a temperature increase, such as would be expected to co-occur with drought (as well as vapor pressure deficit), inference has been limited (Condon et al., 2025). We found that warming increased soil respiration but otherwise had little effect on plant growth. Drought, however, led to declines in most of the response variables, especially when combined with warming. Warming increases the water carrying capacity of the atmosphere, thus, across many parts

of the globe, climate change is predicted to result in less frequent, but more severe precipitation events and therefore longer dry periods between such events (Huntington, 2006). The positive effects of warming may still be detectable in wet years (X. Xu et al., 2012), but our analysis suggests that more often warming will exacerbate the negative effects of drought. Predictions of future carbon cycling based on single factor experiments investigating warming alone are therefore limited in their ability to accurately assess responses. Here, we show that drought tends to override any potential positive effect of warming, as it is well established that low soil moisture is limiting to carbon cycling (Canarini et al., 2017; Song et al., 2019; C. Wang et al., 2021; Wu et al., 2011). It is therefore important to consider both drought and warming when making predictions of future carbon cycling as drought will likely lead to opposite responses than warming alone.

Our meta-analysis indicated warming in combination with drought will have negative impacts for both uptake (net primary productivity) and emission of carbon (soil respiration). The magnitude of the decline in respiration, however, appears to be approximately twice that of net primary productivity (-18 vs -8.8%, respectively), suggesting grassland net carbon flux may be more resilient to future climate conditions than previously thought. Many grasslands are considered to be resilient to drought (Craine et al., 2013; Dass et al., 2018). However, most studies assessing net ecosystem exchange have found net primary productivity to be more sensitive than soil respiration under drought, resulting in switches from a sink to a source under extreme drought (Guo et al., 2025; Shi et al., 2014). Note that net ecosystem exchange was measured in just three of the studies reviewed here, thus was not included in formal analysis. Globally, a growing terrestrial carbon sink in recent decades has been identified to have mitigated the worst effects of climate change so far (Keenan & Williams, 2018). Increases in carbon emission have been predicted to fuel climate change, though here we show there is evidence of little change relative to net primary productivity. With a limited number of experiments, especially long-term, we advocate more work is needed to directly analyse net ecosystem exchange in factorial warming and drought experiments to understand whether there will be persistent effects on carbon source/sink ratios.

Moderating factors explain little variation in impacts of drought and warming

We found that the moderator variables we could test did not effectively explain the observed variation in drought and warming effects, despite the 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. Clearly, as the duration of a manipulation increases, negative drought effects compound (Fig 6; Fig. S2) and initial stimulatory effects of warming diminish or become negative (e.g. aboveground biomass and soil respiration) (Fig 6; Fig. S2). An initial stimulation of growth in response to warming could reflect an increase in nutrient availability through mineralisation (Rustad et al., 2001) that becomes 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 that we observed 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 longer term experiments. While this is somewhat counter-intuitive, given the compounding negative impacts on other response variables discussed above, we propose that if there is a shift in community composition that consists of species that are better able to tolerate

heat or drought stress (Batbaatar et al., 2022) or a composition shift to species with longer-lived aboveground organs, total productivity could increase. These complexities demonstrate that characterising productivity is extremely difficult (Körner, 2021) and emphasise the importance of applying consistent methods to facilitate comparative analyses across studies.

Systematic analysis reveals research gaps and imbalances

We have shown that when data are limited to those from realistic experiments, in natural settings from a single ecosystem type, interactive effects are likely. However, our review also identified substantial holes in the literature that could be addressed in future to further elucidate interactive effects. 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. S1). Despite this substantial increase in multi-factor experiments, there is still relatively little published research in this area, especially in the southern hemisphere. Four continental regions were entirely lacking published studies that met our criteria (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 3000 mm (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, inference from the meta-analysis was also limited by studies focusing on different response variables that cannot be directly compared. Two key variables emerged as most commonly measured, and also most informative. First is net primary productivity, measured through its individual above and belowground components, which is indicative of plant carbon uptake over

438 annual cycles. Second is soil respiration, which is indicative of carbon emission to the atmosphere.
439 Between these two measurements one can gain insight into the total carbon cycling of the
440 ecosystem. However, approximately half of the studies we identified did not measure either of these
441 variables. Further, the dearth of detailed meta-data on experimental sites, such as edaphic factors
442 and soil moisture, meant we were unable to explore other potentially useful explanatory variables.
443 We encourage future publications in this area to include basic carbon cycling variables (net primary
444 productivity and soil respiration) and to report a minimum set of soil measurements that includes
445 soil temperature, soil moisture, pH and organic carbon.

446 Finally, there were only two published experiments in grasslands that employed some form of direct
447 soil warming, one being soil surface (Grime et al., 2008) and the other to a depth of 1 m (Reich et al.,
448 2020) (Fig. 3B). Climate change is predicted to affect temperature both above and belowground
449 (Soong et al., 2020), with belowground warming being a much more constant and continuous impact
450 than manipulations that warm air alone. Experiments that only focus on aboveground warming are
451 missing a key aspect of future climate predictions and could be somewhat unrealistic. We would
452 encourage future researchers to employ manipulations that warm aboveground and the soil directly,
453 to depth, where possible. The DeepSoil 2100 network (Protti Sanchez et al., 2024) aims to address
454 this issue by fostering collaboration across global soil warming experiments.

455 **Conclusion**

456 Our meta-analysis synthesised all available data on field experiments that factorially applied
457 warming and drought treatments in the world's grasslands. These data collectively indicate that
458 drought alone reduces plant biomass, productivity and soil respiration in grasslands and that
459 synergistic negative interactive effects are likely to exacerbate the effects of drought on the major
460 fluxes of carbon – net primary productivity and soil respiration. Our analysis highlights key
461 knowledge gaps and thus we have several recommendations regarding design of experiments.
462 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 (and generally this is larger than the 1–2 °C increases that can be obtained via passive warming) and 3) where possible researchers impose soil warming at depth to realistically simulate future climate conditions.

We advocate that all future experiments measure a core set of carbon cycle response variables to enable better comparison across studies. Net primary productivity (i.e. aboveground net primary productivity and belowground net primary productivity) and soil respiration should be paired measurements in future experiments to quantify uptake and emission of carbon from an ecosystem. By imposing manipulations that share standardised methods, we as a community can generate more effective prediction and modelling capability of the complex interactions between drought and warming impacts of climate change on global grassland carbon dynamics.

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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.

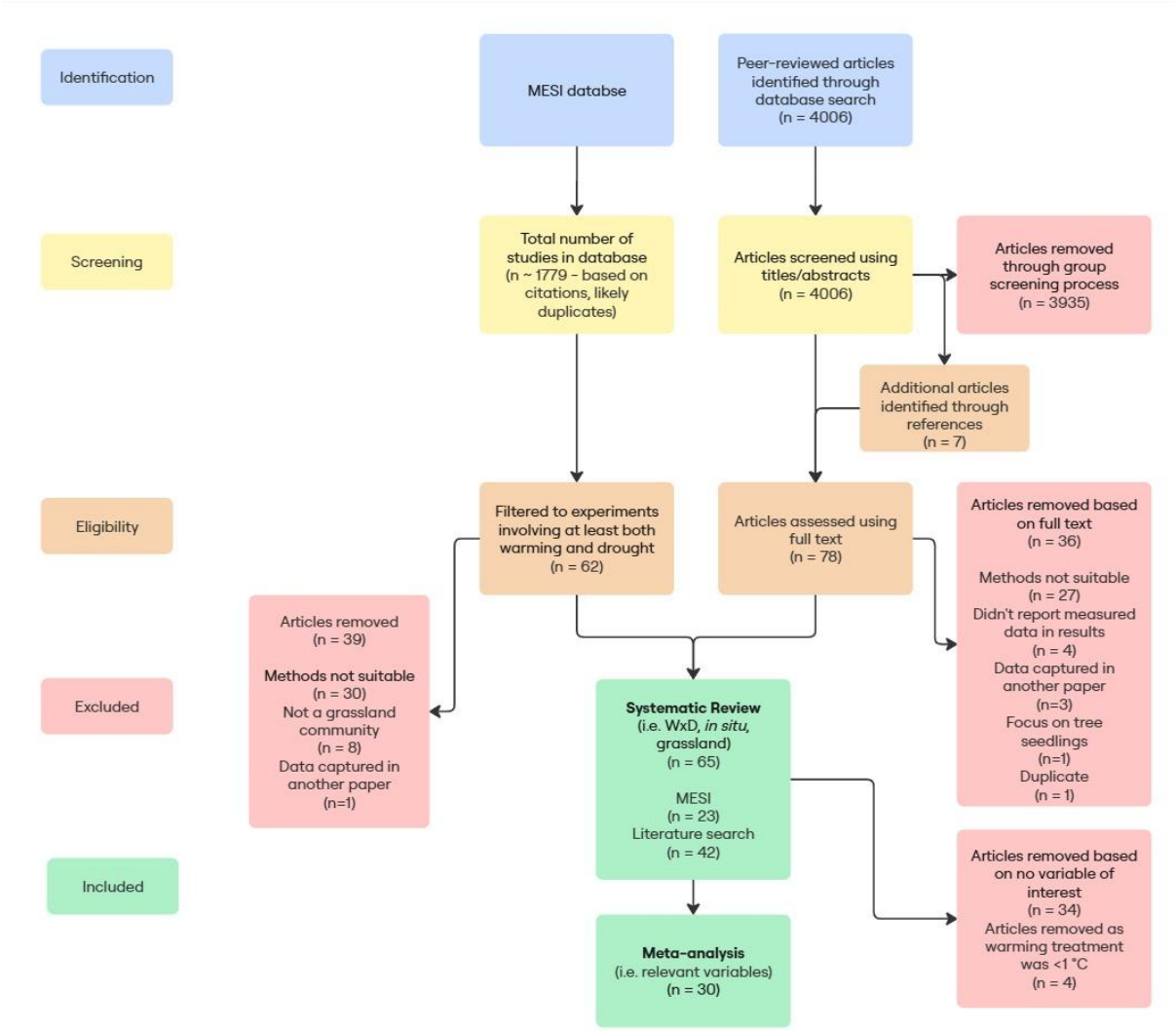


Figure S1. Preferred Reporting Items in Systematic Reviews and Meta-Analyses (PRISMA) flow chart describing articles sourced through literature and database searches and excluded based on criteria.

Meta-analysis 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 km²), 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:

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

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:

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

887 $$dd = \frac{(Xwd + Xd) - (Xw + Xc)}{2S} J(m)$$

888 $$di = \frac{(Xwd - Xw) - (Xd - Xc)}{2S} J(m)$$

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

893 $$v2dd = \frac{\left(\frac{1}{4}\right)\left(\left(\frac{1}{nc}\right) + \left(\frac{1}{nd}\right) + \left(\frac{1}{nw}\right) + \left(\frac{1}{nwd}\right) + dw^2\right)}{2(nc + nd + nw + nwd)}$$

894

895 $$v2dd = \frac{\left(\frac{1}{4}\right)\left(\left(\frac{1}{nc}\right) + \left(\frac{1}{nd}\right) + \left(\frac{1}{nw}\right) + \left(\frac{1}{nwd}\right) + dd^2\right)}{2(nc + nd + nw + nwd)}$$

896

897 $$v2di = \frac{\left(\frac{1}{4}\right)\left(\left(\frac{1}{nc}\right) + \left(\frac{1}{nd}\right) + \left(\frac{1}{nw}\right) + \left(\frac{1}{nwd}\right) + di^2\right)}{2(nc + nd + nw + nwd)}$$

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

899 **Moderator variable regressions**

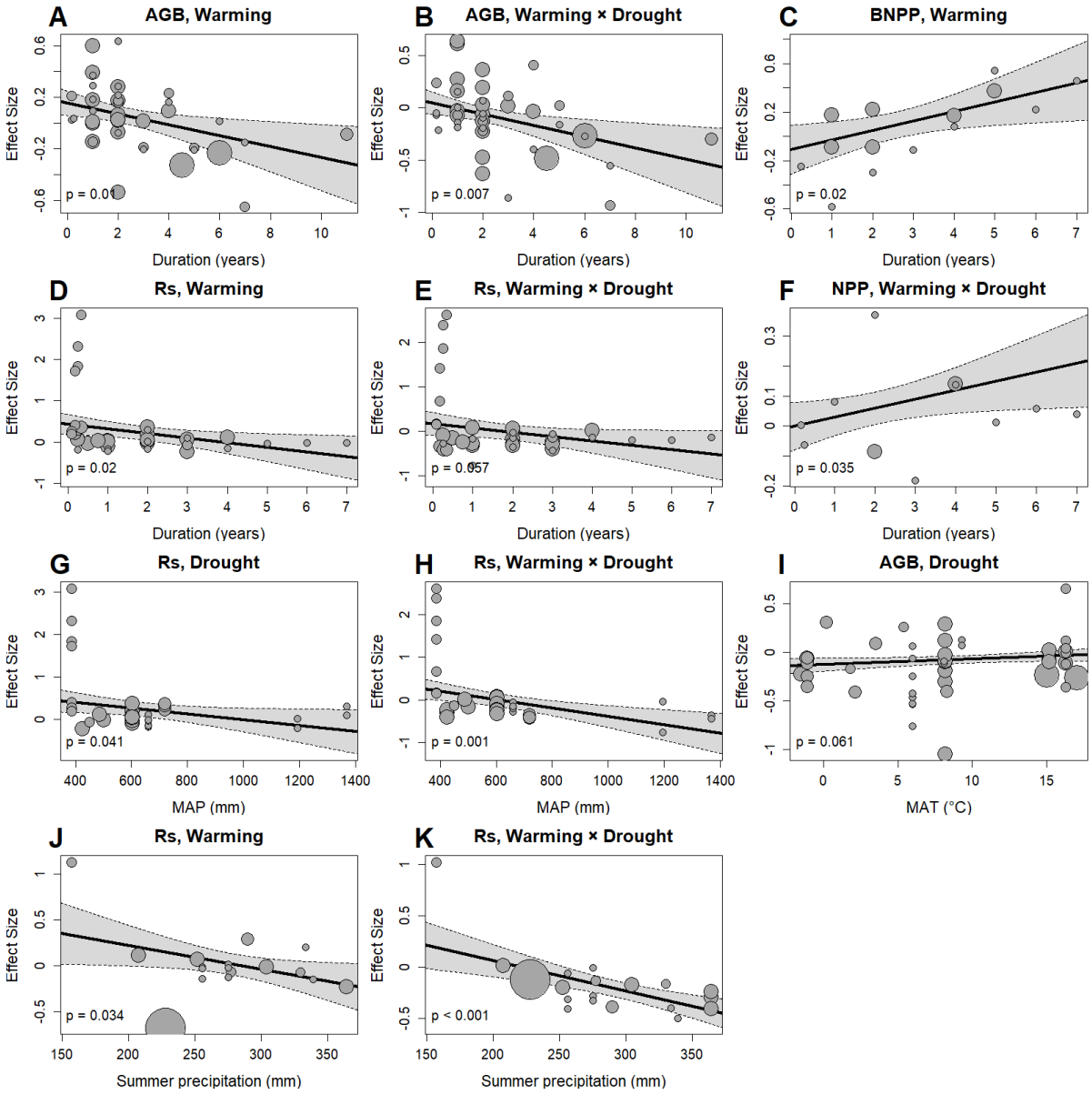
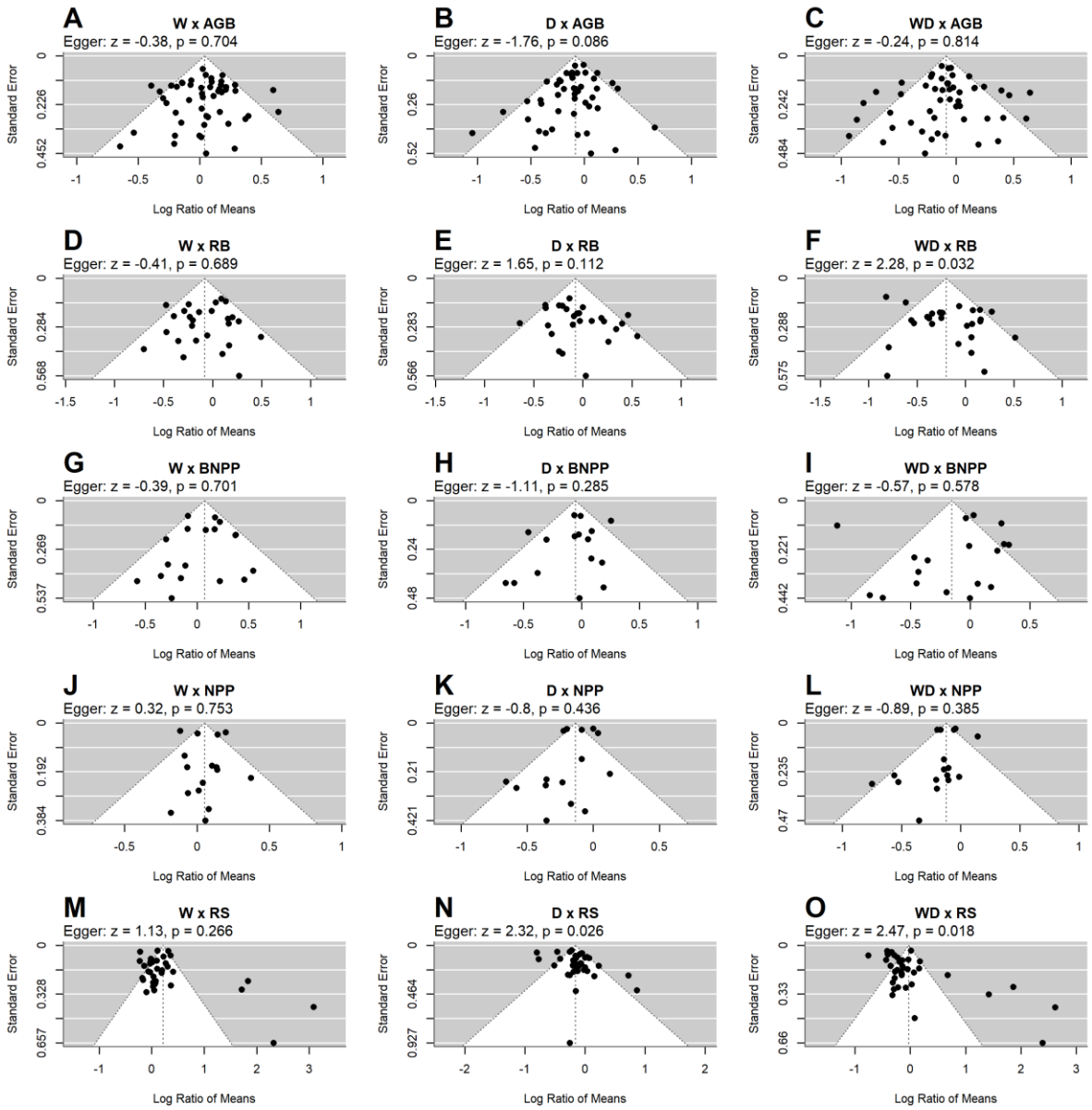


Figure S2. Bubble plots of meta-regressions between the natural log-transformed response ratio (ln*RR*) of experiment duration with (A) AGB (aboveground biomass) under warming (B) and warming drought combination, (C) BNPP (belowground net primary productivity) under warming, (D) Rs (soil respiration) 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



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911 Figure S3. Funnel plots of the natural log-transformed response ratio (ln*RR*) for (A) AGB (aboveground biomass) under
912 warming (B) drought (C) and warming drought combination, (D) RB (root biomass) under warming (E) drought (F) and
913 warming drought combination, (G) BNPP (belowground net primary productivity) under warming (H) drought (I) and
914 warming drought combination, (J) NPP (net primary productivity) under warming (K) drought (L) and warming drought
915 combination, (M) NPP (net primary productivity) under warming (N) drought (O) and warming drought combination.
916 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
917 evidence of publication bias.

918