Simple additive simulation overestimates real influence: altered nitrogen and rainfall modulate the effect of warming on soil carbon fluxes

XIANGYIN NI1,2,3, WANQIN YANG1,4, ZEMIN QI5, SHU LIAO1, ZHENFENG XU1,4, BO TAN1,4, BIN WANG6, QINGGUI WU7, CHANGKUN FU1, CHENGMING YOU1 and FUZHONG WU1,4

1Long-Term Research Station of Alpine Forest Ecosystems, Key Laboratory of Ecological Forestry Engineering, Institute of Ecology and Forestry, Sichuan Agricultural University, Chengdu 611130, China, 2Advanced Science Research Center, The City University of New York, New York, NY 10031, USA, 3Department of Earth and Environmental Sciences, Brooklyn College of The City University of New York, New York, NY 11210, USA, 4Collaborative Innovation Center of Ecological Security in the Upper Reaches of the Yangtze River, Chengdu 611130, China, 5College of Life Science, Neijiang Normal University, Neijiang 641199, China, 6Laboratory of Forestry, Department of Forest and Water Management, Ghent University, Geraardsbergsesteenweg 267, BE-9090 Gontrode (Melle), Belgium, 7Ecological Security and Protection Key Laboratory of Sichuan Province, Mianyang Normal University, Mianyang 621000, China

Abstract

Experiments and models have led to a consensus that there is positive feedback between carbon (C) fluxes and climate warming. However, the effect of warming may be altered by regional and global changes in nitrogen (N) and rainfall levels, but the current understanding is limited. Through synthesizing global data on soil C pool, input and loss from experiments simulating N deposition, drought and increased precipitation, we quantified the responses of soil C fluxes and equilibrium to the three single factors and their interactions with warming. We found that warming slightly increased the soil C input and loss by 5% and 9%, respectively, but had no significant effect on the soil C pool. Nitrogen deposition alone increased the soil C input (+20%), but the interaction of warming and N deposition greatly increased the soil C input by 49%. Drought alone decreased the soil C input by 17%, while the interaction of warming and drought decreased the soil C input to a greater extent (−22%). Increased precipitation stimulated the soil C input by 15%, but the interaction of warming and increased precipitation had no significant effect on the soil C input. However, the soil C loss was not significantly affected by any of the interactions, although it was constrained by drought (−18%). These results implied that the positive C fluxes–climate warming feedback was modulated by the changing N and rainfall regimes. Further, we found that the additive effects of [warming × N deposition] and [warming × increased precipitation] on the soil C loss were greater than their interactions, suggesting that simple additive simulation using single-factor manipulations may overestimate the effects on soil C fluxes in the real world. Therefore, we propose that more multifactorial experiments should be considered in studying Earth systems.

Keywords: additive effect, climate warming, drought, increased precipitation, interaction, multifactorial experiment, nitrogen deposition, soil carbon fluxes

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Introduction

Soil sequesters the largest pool of terrestrial carbon (C) (Lal, 2008), and how soil C fluxes respond to climate warming is a major focus of Earth system research (Davidson & Janssens, 2006; Luo, 2007). In the last two decades, climate warming experiments performed around the world have greatly advanced our understanding of the C fluxes–climate warming feedback (Hobbie & Chapin, 1998; Norby & Luo, 2004; Luo et al., 2009; Natali et al., 2014). A consensus has been that rising temperature stimulates both the soil C influx and efflux (Lu et al., 2013) but has little influence on soil C storage (Sistla et al., 2013). In some global syntheses, a 2 °C increase in temperature has been quantitatively evaluated to stimulate plant biomass production and microbial respiration by 12% (Lin et al., 2010) and 21% (Wang et al., 2014), respectively.

However, the concurrently altered nitrogen level has been another profound forcing driver during the past decades (IPCC, 2013) due to the combustion of fossil
fuels and anthropogenic N fertilization (Schlesinger, 2009; Niu et al., 2016), resulting in the deposition of surplus N on the soil surface and thus alteration of the C cycle (Lu et al., 2011). Numerous syntheses have demonstrated that elevated N considerably increases plant biomass production and the litter input by 54% (Xia & Wan, 2008) and 20% (Liu & Greaver, 2010), respectively, at a global scale because plant growth in most regions remains N-limited (LeBauer & Treseder, 2008). In contrast, Knorr et al. (2005) collected data from 24 publications and found that litter decomposition rate is not significantly affected by N addition at large scale. Experimental observations also indicated that N deposition plays a dual role of accelerating (Adair et al., 2009) or suppressing (Mo et al., 2008) soil C loss through microbial respiration in different ecosystems, whereas Liu & Greaver (2010) suggested that enriched N globally reduces microbial respiration by an average of 8% according to data collected from 410 observations. However, data indicating whether the increased input and decreased loss of soil C induced by N deposition may reduce the climate warming-stimulated release of soil C are not available.

Moreover, climate warming may intensify the variability of regional drought (Rahmstorf & Coumou, 2011; Trenberth et al., 2013) and heavy precipitation (Wentz et al., 2007; Allan & Soden, 2008). Water deficits physiologically lead to hydraulic conflict (Ryan, 2011; Rowland et al., 2015) and C starvation (van der Molen et al., 2011; Doughty et al., 2015), resulting in tree mortality (Breshears et al., 2009; Peng et al., 2011). Meanwhile, a decreased water potential triggers a shift in the functional community of soil biota (Bouskill et al., 2016) and slows the decomposition of soil organic matter (SOM) (Yuste et al., 2011). In contrast, increased precipitation provides a sufficient water potential to prevent hydraulic stress and therefore exerts divergent influences on the soil C input and loss (Thomey et al., 2011). Thus, an increase or decrease in rainfall level may amplify or diminish the climate warming-induced increase in soil C fluxes, but quantitative knowledge of these effects is limited.

These single factors or their additive effects have long been used separately to evaluate the influence on terrestrial C cycle (Luo & Weng, 2010). In recent years, increasing awareness has led to the recognition that employing the simple additive effect based on mathematical summation of the effect size of each single factor (Dieleman et al., 2012) may potentially result in bias due to greater variance with a low confidence and thus limit inferences about certain underlying and interacting mechanisms (Leuzinger et al., 2011). However, most of these theoretical deductions were generated under a conceptual framework (Hyvönen et al., 2007; Frank et al., 2015), and extrapolation of the additive effects has not generally been demonstrated in field experiments (Niu et al., 2009). Moreover, the interaction representing the true status of the combined effect between single factors in multifactorial manipulations is poorly understood (Dukes et al., 2005). Dieleman et al. (2012) quantified the interaction of warming and CO2 enrichment and found that the single factor of the CO2 effect is often dominant over the warming effect in stimulating plant production and soil respiration across biomes. However, global N deposition and rainfall regimes are also concurrently altered with climate warming under a global change scenario (IPCC, 2013); whether these forcing drivers modulate the direction and magnitude of the effect of rising temperature on soil C fluxes is unclear.

Here, we hypothesized that N deposition and increased precipitation amplify warming-induced soil C fluxes, whereas drought reduces them. In this meta-analysis, we addressed these gaps by examining the effects of N deposition, drought and increased precipitation on the soil C pool, input and loss or their proxies at 258 experimental sites (see Fig. S1). Thereafter, we performed projections to (i) quantitatively estimate the effect sizes of the three single factors and their interactions with warming on soil C fluxes and equilibrium and then to (ii) compare their interactions and additive effects using multifactorial experiments alone.

Materials and methods

Criteria for data collection

We screened experiments manipulating warming, N deposition, drought and increased precipitation for available data on (i) soil C pools, (ii) inputs and (iii) losses or proxies thereof. A total of 385 peer-reviewed journal articles (Appendix S1, Table S1, Data S1–S3) published before March 2016 that met our criteria were selected via Web of Science (http://apps.webofknowledge.com). The search terms used were ‘warming’, ‘nitrogen (N) deposition’, ‘nitrogen (N) fertilization’, ‘nitrogen (N) addition’ or ‘fertilizer’, ‘drought’, and ‘precipitation’ or ‘rainfall’. The average magnitudes of these manipulations were 2.3 °C, 160 kg N ha−1 yr−1 and a 46% decrease and a 72% increase in the rainfall supply compared with the control for the experiments on warming, N deposition, drought and increased precipitation, respectively.

The assessment of soil C fluxes and equilibrium presents challenges. Thus, we collected proxies of data on the soil C pool, input and loss by following van Groenigen et al. (2014). For the soil C pool, we directly distinguished C storage in the fresh litter layer, soil organic layer and various depths of the soil mineral layer. We used direct measurements of soil C inputs where available. However, in most of the evaluated studies, it was difficult to measure the amount of the true C input to the soil. Therefore, in these cases, we used

belowground litter or biomass production to calculate the C inputs into the soil using the following proxies, in order of availability: coarse (fine) root litter production, coarse (fine) root biomass and total belowground biomass. When belowground biomass was reported for various soil depths in a same article, the data from the uppermost soil layer were used. If data on the belowground C input were not available, we used aboveground litter fall or biomass production (or yield in croplands), in order of availability. For studies in which the biomass of several plant functional types was reported, only the biomass data of trees or vascular vegetation were included in our datasets, and biomass data for moss and lichen species with a low biomass were ignored. In addition, considering that soil respiration via CO₂ emissions constitutes the largest efflux of C from soil to the atmosphere (Schleinger & Andrews, 2000), we did not collect data on the release of methane and dissolved organic carbon in soil. However, the majority of field experiments did not distinguish autotrophic and heterotrophic respiration (four studies vs. 30 studies; 118 studies reporting soil respiration); therefore, we prioritized microbial respiration as a proxy for soil C loss, but soil respiration data were used when microbial respiration data were not available because there is little difference between microbial [(-0.23, -0.009)] for 95% confidence intervals (95% CI) and soil respiration [(-0.039, 0.40)]. Further, several categories were classified to compare the differences of the overall effects of the three global change factors as well as their interactions with warming on soil C pool and fluxes among biomes and ecosystems. For each single factor, facilities (Section 2) and magnitudes of manipulations were also categorized separately (described in detail in Appendix S2).

To avoid publication bias, all observations met the following criteria. (i) All of the collected information except for coordinates, altitude and climate data, such as mean annual temperature (MAT) and precipitation (MAP), were obtained directly from the peer-reviewed journal articles. (ii) The treatment and control plots were established under the same ambient natural conditions, which ensure that there was only one variable (one of the three global change factors or their interactions with warming) in this meta-analysis. For example, one study (ref. 7 in Appendix S1) used a randomized complete factorial design with three tillage systems (no-tillage, reduced tillage and conventional tillage) nested by three fertilizer doses. Here, we only collected the data from the no-tillage site without change in tillage type. According to this principle, different sampling sites, tree species and magnitudes of manipulation were considered to be independent and could be included in this meta-analysis. However, invasive species were excluded from our datasets because this goes against the second criterion. (iii) Meta-analysis requires that observations are independent; therefore, only the most recent measurement was used in our datasets if more than one observation for a time series was available from the same study. Furthermore, averaged data from different sampling times were removed to eliminate the temporal effect. When the same results were included in different publications by a same research group, they were only used once. (iv) The sample sizes, means and standard deviations of both the treatment and control were obtained directly or could be calculated from other data presented in the articles. When the data were presented as figures, we used ENGAUGE DIGITIZER 4.1 (Free Software Foundation Inc., Boston, MA, USA) to extract them.

The coordinates, altitude, MAT and MAP of the experimental sites were extracted directly from the studies. When the latitude and coordinates of the experimental sites were not available in a certain article, they could be found in other citations for experiments conducted at the same site. Missing altitude data were obtained using Google Earth (Google Inc., Santa Clara, CA, USA) according to the given coordinates. Some publications did not provide data on MAT and/or MAP; thus, we retrieved these data (2011–2015) from the meteorological station closest to the experimental site using the National Oceanic and Atmospheric Administration website (http://www.noaa.gov; Data S4 and S5).

**Response metrics**

The means (\(\bar{X}_t\) for treatments and \(\bar{X}_c\) for controls), sample sizes (\(n_t\) for treatments and \(n_c\) for controls) and standard deviations (\(S_t\) for treatments and \(S_c\) for controls) of both the treatment and control were provided directly or were calculated using the standard errors or 95% confidence intervals from the studies. These values were employed to calculate the response ratio (\(\ln R\); Eqn 1), variance (\(v\); Eqn 2) and weighting factor (\(w\); Eqn 3) through meta-analysis (Liu & Greaver, 2010; Lu et al., 2011). The frequency distribution of the response ratio was assumed to follow a normal distribution (Fig. S2) and to fit a Gaussian function.

\[
\ln R = \ln(\bar{X}_t/\bar{X}_c) = \ln(\bar{X}_t) - \ln(\bar{X}_c) \quad (1)
\]

\[
v = \frac{S_t^2}{n_t \bar{X}_t} + \frac{S_c^2}{n_c \bar{X}_c} \quad (2)
\]

\[
w = \frac{1}{v} \quad (3)
\]

We calculated the weighted response ratio (\(\ln RR\), Eqn 4) and its 95% bootstrap confidence intervals (Eqn 6 based on Eqn 5) from individual \(\ln R_i\) (\(i = 1, 2, 3, \ldots, n\)) values by assigning a greater weight to the entries with lower variance to improve the precision of our analysis as the mean effect size using a mixed model. Here, \(n\) is the number of entries in a certain category and \(i\) is the \(i\)th entry. If the number of entries used to calculate the mean effect size is lower than 20, the 95% CI will be narrow (Hedges et al., 1999); we conducted a resampling test with 4999 interactions to assess the 95% bootstrap CI and statistical significance. The response of the variable to the manipulation was considered significant if the 95% CI did not overlap with zero. If the mean effect size of a variable in response to manipulation is greater than zero, it is identified as a ‘positive’ effect; otherwise, it is designated ‘negative’. METAWIN 2.0 (Rosenberg et al., 2000) was used to calculate the mean effect sizes and 95% bootstrap CI.
\[
\ln RR = \sum_{i=1}^{n} w_i \ln R_i
\]
\[\frac{1}{\sum_{i=1}^{n} w_i}\]
\[
S_{\ln RR} = \sqrt{\frac{1}{\sum_{i=1}^{n} w_i}}
\]
\[
95\% \text{ CIs} = \ln RR \pm 1.96S_{\ln RR}
\]

We also calculated the percentage change \(P\) (Eqn 7; Lu et al., 2013) of each category based on the weighted response ratio.

\[
P(\%) = (e^{\ln RR} - 1) \times 100
\]

Many studies manipulated only one factor without interactions (Table S1). However, one experiment concurrently considered three single factors (Dukes et al., 2005). In this meta-analysis, we only included the experiments manipulating two single factors to compare their interactions and additive effects (39 studies in total; Table S2). We separated the single factors (named factor 1 and factor 2; i.e., N deposition and increased precipitation, respectively) in these two-factor experiments and then separately examined their differences with the corresponding factor in the single-factor experiments (either N deposition or increased precipitation) using the response ratios. The results indicated that there were no significant differences for a certain factor between the two types of experiments; thus, we included these single-factor experiments in our datasets to evaluate the overall effects of the three single factors (N deposition, drought and increased precipitation) and their interactions with warming on the soil C pool and fluxes.

Thereafter, we calculated the Mahalanobis distances between the interactions and additive effects to compare their differences using the data from only two-factor experiments. A longer Mahalanobis distance means a greater difference between the interaction and additive effect (Sistla et al., 2013). In this meta-analysis, we defined the interaction as the effect size of the combined effect between single factors in field manipulations, and the additive effect was the mathematical summation of the effect sizes of the single factors. These analyses were performed using MATLAB R2012a (MathWorks Inc., Natick, MA, USA).

**Results**

**Warming contributes to the overall effect on soil C equilibrium and fluxes**

When N deposition, drought and increased precipitation as well as their interactions with warming were considered, the size of soil C pool was found to be stimulated by 9\% \((n = 180, P < 0.05)\), but the soil C input (+4\%, \(n = 764\)) and loss (+0.6\%, \(n = 306\)) were not significantly changed \((P > 0.05)\) (Fig. 1). Warming significantly increased both the soil C input and loss (+5\% and +9\%, respectively, both \(P < 0.05\)), but its effect on the size of soil C pool was not significant (+4\%, \(P > 0.05\)).

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**Fig. 1** Percentage changes of the (a) soil carbon pools, (b) inputs and (c) losses in response to nitrogen (N) deposition, drought and increased precipitation as well as their interactions with warming. Error bars represent the standard errors that are calculated by the weighted response ratios. Sample sizes are shown next to the bars, and the vertical lines are drawn at the percentage change values of zero.
Synergetic vs. antagonistic effects between the single factors change the direction and magnitude of the effect of warming on soil C fluxes

Nitrogen deposition significantly ($P < 0.05$) increased the soil C input by 20%, but had no significant effect on soil C loss ($-1\%, \quad P > 0.05$; Fig. 1c). However, the interaction of [warming $\times$ N deposition] considerably increased the soil C input to 49%. Drought significantly (both $P < 0.05$) decreased the soil C input and loss by 17% and 18%, respectively, and the interaction of [warming $\times$ drought] significantly ($P < 0.05$) decreased the soil C input to 22% (Fig. 1b). Increased precipitation significantly ($P < 0.05$) increased the soil C input by 15%, but the effect on soil C loss was not significant ($+14\%, \quad P > 0.05$). However, the interaction of [warming $\times$ increased precipitation] had no significant effect on either the soil C input ($+11\%$) or loss ($+7\%$, both $P > 0.05$).

**Interaction vs. additive effect**

The weighted response ratios of the additive effects of warming interacted with N deposition (Fig. 2c) and drought (Fig. 2d) on soil C inputs, and those of warming interacted with increased precipitation (Fig. 2h) on soil C loss were significantly ($P < 0.05$) greater than that of their interactions, with 42%, 10% and 59% increase in the percentage changes being observed, respectively (Table 1). Greater Mahalanobis distances were observed between the interactions and additive effects for the three two-factor experiments, although significant linear relationships were observed (all $P < 0.01$; Fig. 3c, d, h). However, the greatest Mahalanobis distance was observed between the interaction and the single factor of warming for [warming $\times$ drought] on soil C input (Table 2).

**Discussion**

Warming stimulates soil C influx and efflux

In previous syntheses, researchers have selected certain detailed targets to represent soil C pool and fluxes. For instance, Lu et al. (2013) categorized the soil C influx as gross ecosystem photosynthesis, net primary production, net ecosystem exchange or net photosynthesis rate. In this meta-analysis, the overall input and loss of

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**Fig. 2** Weighted response ratios (ln RR) of the (a, b) soil carbon pools, (c–e) inputs and (f–h) losses in response to the two single factors (factor 1 and factor 2) and their interactions and additive effects using the data from only two-factor experiments. W: warming, N: nitrogen deposition, D: drought, P: increased precipitation. Factor 1 and factor 2 are the first and second single factor, respectively, in each panel. Error bars represent the 95% bootstrap confidence intervals. Sample sizes are shown in each panel, and the vertical dotted lines are drawn at ln RR = 0. Note: ‘$\times$’ represents two-factor experiments.
Table 1 Percentage changes for the interactions and additive effects using the data from only two-factor experiments

<table>
<thead>
<tr>
<th>Combined-factor experiment</th>
<th>n</th>
<th>Interaction (%)</th>
<th>Additive effect (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil carbon pool</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Warming × N deposition</td>
<td>1</td>
<td>83.33 ± 11.53</td>
<td>106.25</td>
<td>*</td>
</tr>
<tr>
<td>Warming × Drought</td>
<td>1</td>
<td>34.04 ± 13.80</td>
<td>−16.46</td>
<td>*</td>
</tr>
<tr>
<td>Soil carbon input</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Warming × N deposition</td>
<td>11</td>
<td>18.05 ± 1.54</td>
<td>60.01 ± 3.30</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Warming × drought</td>
<td>25</td>
<td>−34.63 ± 1.05</td>
<td>−24.74 ± 1.32</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Warming × increased precipitation</td>
<td>8</td>
<td>5.15 ± 7.08</td>
<td>−6.98 ± 1.60</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Soil carbon loss</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Warming × N deposition</td>
<td>3</td>
<td>−37.08 ± 5.57</td>
<td>−46.29 ± 0.52</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Warming × drought</td>
<td>5</td>
<td>−31.95 ± 2.53</td>
<td>−11.17 ± 8.31</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Warming × increased precipitation</td>
<td>5</td>
<td>−44.53 ± 2.82</td>
<td>14.14 ± 7.09</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

The percentage changes are calculated by the weighted response ratios and represented as means ± standard errors. P values are assessed by paired t-test at P = 0.05 level and bold values are significant.

*There is only one entry for both the [warming × N deposition] and [warming × drought] experiments reporting soil carbon storages; thus, their additive effects have no standard errors and the P values between their interactions and additive effects are not available.

Soil C were projected to be evaluated within a same framework (van Groenigen et al., 2014) in relation to changes in temperature, N and rainfall regimes, and therefore, this synthesis was intended to generate a general recognition of soil C fluxes that are affected by multiple global change factors.

In accordance with previous syntheses, we found that the soil C loss via microbial and/or soil respiration was stimulated by climate warming by 9% (Fig. 1c; Lu et al., 2013), but the soil C input was lower in our synthesis (+5%) than that assessed by Lin et al. (2010) (+12%). We believe that this discrepancy occurred because our datasets included litter production, which is less sensitive to rising temperature compared with plant biomass production (Sistla et al., 2013). We used direct measurements of soil C stocks as soil C pool and found that soil C storage was slightly increased due to the rising temperature (+4%), but not significantly (P > 0.05; Fig. 1a), suggesting that Earth systems may exhibit resilience to current climate warming to a certain extent (Free & Barton, 2007). Nevertheless, as we hypothesized, elevated N and decreased/increased rainfall regimes exert synergistic, antagonistic or neutral effects on soil C fluxes with climate warming.

**Synergistic: nitrogen deposition amplifies the warming effect in accelerating soil C fluxes**

Approximately 150 Tg yr⁻¹ of anthropogenic N is applied by humans to the Earth’s surface as fertilizer (Schlesinger, 2009) because most plant growth is N-limited worldwide (LeBauer & Treseder, 2008; Treseder, 2008). However, an elevated N level in the soil constrains decomposer activity (Eisenlord et al., 2013) and thus slows SOM decomposition (Janssens et al., 2010; Liu & Greaver, 2010; Frey et al., 2014), which may partly offset the greater loss of soil C triggered by rising temperature (Schindlbacher et al., 2012). Our meta-analysis results showed that the interaction of [warming × N deposition] greatly increased the soil C input (+49%) compared with the single factor of either warming (+5%) or N deposition (+20%) (Fig. 1b). This synergistic effect was clearly related to global progressive nitrogen limitation (LeBauer & Treseder, 2008), but rising temperature may also increase soil N availability (Niu et al., 2016) and thereby promote plant growth. Moreover, most of the fertilizers used in N addition experiments consist of inorganic ammonium or nitrate (Table S1), and N fertilization also stimulates N nitrification of the soil, thus increasing soil inorganic N (Liu et al., 2011), which can be easily assimilated by plants as a nutrient supply.

It is noteworthy that the single factor of N deposition only decreased soil C loss by 1% (Fig. 1c), and the interaction of [warming × N deposition] is also not significant (−24%, P > 0.05). Many studies have demonstrated that climate warming can promote SOM decomposition (Davidson & Janssens, 2006), but enriched N has been shown to decrease the richness of the soil decomposer community (Eisenlord et al., 2013), and this suppression on soil C loss may have been greater than the warming effect in our meta-analysis. These results implied that warming and N deposition (the two large-scale forcing drivers under a global
Fig. 3 Response ratios of the (a, b) soil carbon pools, (c–e) inputs and (f–h) losses for the interactions plotted against the two single factors (factor 1 and factor 2) and their additive effects using the data from only two-factor experiments. W: warming, N: nitrogen deposition, D: drought, P: increased precipitation. Factor 1 and factor 2 are the first and second single factor, respectively, and P values of the regression analyses for factor 1 (●), factor 2 (○) and their additive effects (▲) are shown in each panel. The diagonal dotted lines are 1:1 lines. Note: ‘×’ represents two-factor experiment, and ‘+’ represents additive effect of the two single factors in each panel.

Table 2 Mahalanobis distances between the interactions and the two single factors (factor 1 and factor 2) and their additive effects

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<tr>
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<tr>
<td>Soil carbon pool</td>
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<tr>
<td>Warming × N deposition</td>
<td>1.69 (1)</td>
<td>0.50 (1)</td>
<td>0.29 (1)</td>
</tr>
<tr>
<td>Warming × drought</td>
<td>2.10 (1)</td>
<td>1.36 (1)</td>
<td>2.14 (1)</td>
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<tr>
<td>Soil carbon input</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Warming × N deposition</td>
<td>0.02 (11)</td>
<td>0.61 (11)</td>
<td>2.13 (11)</td>
</tr>
<tr>
<td>Warming × drought</td>
<td>2.10 (25)</td>
<td>0.01 (25)</td>
<td>0.92 (25)</td>
</tr>
<tr>
<td>Warming × increased precipitation</td>
<td>0.01 (8)</td>
<td>0.39 (8)</td>
<td>1.84 (8)</td>
</tr>
<tr>
<td>Soil carbon loss</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Warming × N deposition</td>
<td>0.84 (3)</td>
<td>1.31 (3)</td>
<td>0.97 (3)</td>
</tr>
<tr>
<td>Warming × drought</td>
<td>1.92 (5)</td>
<td>0.34 (5)</td>
<td>0.77 (5)</td>
</tr>
<tr>
<td>Warming × increased precipitation</td>
<td>1.15 (5)</td>
<td>0.58 (5)</td>
<td>1.58 (5)</td>
</tr>
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</table>

The weighted response ratios are used to calculate the Mahalanobis distances. A longer Mahalanobis distance means a greater difference between the two items of each pair. Sample sizes are shown in the parentheses.

*Factor 1 and factor 2 are the first and second single factor, respectively, in each two-factor experiment.
change scenario) are coupled through a more complex connection impacting ecosystem functioning. However, the currently available studies on this topic are limited, and there is an urgent need for underlying linkages to be built.

**Antagonistic: drought offsets warming-induced increases in soil C fluxes**

Long-standing estimates between terrestrial ecosystems and climate change pay more attention to C fluxes-climate warming feedbacks (Davidson & Janssens, 2006; Luo, 2007), and little insight into the water cycle (which also interacts with C cycle; van der Molen et al., 2011) has been obtained through ecological research (Chapin et al., 2008). Climate warming is projected to generate more frequent drought (Trenberth et al., 2013), which has the potential to trigger tree die-off (Breshears et al., 2009) and therefore decreases plant production and the associated soil C storage. In our meta-analysis, warming increased the soil C input (+15%), while drought reduced it (−17%), and when drought was interacted with warming, the decrease reached 22% (n = 25, P < 0.05; Fig. S1b). One implication of this antagonistic effect is that increased plant growth due to rising temperature requires a greater water supply to sustain physiological metabolism and evapotranspiration, whereas drought deteriorates the water supply to sustain physiological metabolism and growth due to rising temperature requires a greater flux of this antagonistic effect is that increased plant production and the associated soil C storage. In our meta-analysis, warming increased the soil C input (+15%), while drought reduced it (−17%), and when drought was interacted with warming, the decrease reached 22% (n = 25, P < 0.05; Fig. S1b). One implication of this antagonistic effect is that increased plant growth due to rising temperature requires a greater water supply to sustain physiological metabolism and evapotranspiration, whereas drought deteriorates the rainfall-conducting pathway from the soil to the plant, resulting in hydraulic conflict (Breshears et al., 2009; Rowland et al., 2015). Moreover, plants require sufficient available soluble C in warmer environments, but nonstructural carbohydrates may be involved in protecting against water deficits (van der Molen et al., 2011). Hence, it ultimately becomes difficult for plants to fend off ‘C starvation’ (Doughty et al., 2015; Rowland et al., 2015).

However, the interaction of [warming × drought] on soil C loss was not significant (P > 0.05; Fig. S3c), although the single factors of warming and drought both significantly impacted it. This lack of a significant effect probably occurred because drought may lead to a shift in structure of soil microbial community (Yuste et al., 2011) and even cause microbial mortality (Compant et al., 2010), so heterotrophic respiration may also be declined (Rustad et al., 2001). Further, the sample size was too small (n = 5) and therefore presented a broad 95% CI [−0.60, 0.68]. Nevertheless, we have no convincing evidence demonstrating that drought can additively offset the warming-induced stimulation in soil C loss (Schindlbacher et al., 2012). In contrast, a rainfall deficit under a warmer climate may impede plant biomass accumulation and soil C sequestration.

Neutral: increased precipitation diminishes the warming-induced stimulation of soil C fluxes

Climate warming could amplify high-frequency variability in regional rainfall extremes by approximately 7% per degree Kelvin (Wentz et al., 2007; Allan & Soden, 2008; Bonan, 2008). Given that both climate warming and increased precipitation can increase plant growth (Dukes et al., 2005), it is reasonable to assume a synergistic effect of the two single factors on soil C fluxes. However, our results indicated that although increased precipitation significantly increased the soil C input (+15%; P < 0.05), but when interacted with warming, this effect became marginal (+11%) and was not significant (n = 8, P > 0.05; Fig. S3b). A similar neutral effect was also observed for soil C loss; however, the effect of the single factor of increased precipitation was not significant (P > 0.05), although the 95% CI was much closer to zero [−0.0076, 0.25]; Fig. S3c]. Wetter conditions are favorable for microbial reproduction (Nielsen & Ball, 2015), especially in a warmer environment (Compant et al., 2010), resulting in increased decomposition of SOM (Corrufio et al., 2013, 2015; Lehmann & Kleber, 2015). Furthermore, increased precipitation promotes the release of soluble organic matter from fresh and partly decomposed litter, and this labile C may trigger a ‘priming’ effect that contributes to SOM decomposition (Fontaine et al., 2007; Tamura & Tharayil, 2014). Thus, accelerated loss of soil C may decrease C storage; however, current through-fall collection or exclusion experiments that simulate increased/decreased rainfall regimes do not directly assess the effect on soil C storage (Table S1).

Additionally, the overwhelming majority of these simulations, such as the CLIMATE (Selsted et al., 2012) and EVENT experiments (Jentsch et al., 2007), have been conducted in forests and grasslands (Fig. S1), whereas different ecosystems show great variation in soil C inputs (Qb = 36.9, P = 0.0010; Table S4, Fig. S4). This disequilibrium of the experimental distribution is also observed for different biomes (Qb = 50.5, P = 0.0002 for soil C pool and Qb = 18.4, P = 0.0054 for soil C input; Table S4, Fig. S5). The results of regression analyses indicated that the effect sizes of the soil C pool (n = 182, P = 0.026; Fig. S6a) and input (n = 691, P = 0.0039; Fig. S6e) were positively correlated with an increase in latitude, suggesting that the global alterations in temperature, N and rainfall regimes have a stronger influence in high-latitude regions (Cooper, 2014). In contrast, different facilities (Fig. S7) and magnitudes (Fig. S8) of the manipulations of drought and increased precipitation had no significant (all P > 0.05) effects on the three variables related to the C cycle when all of the entries in this meta-analysis were
considered. However, the magnitude of the treatments may be the prioritized concern when conducting an increased/decreased rainfall experiment at a field site. Thus, more widespread site work, not only involving the magnitudes, should be encouraged to determine how the terrestrial C cycle feeds back to changing rainfall regimes worldwide.

Additive effects overestimate the real influence on soil C fluxes

The coupling of terrestrial C and the climate results from cause–effect connections between factors (Hyvönen et al., 2007), and a single factor has a limited capacity to reflect, and may even erroneously estimate, the real impact. In recent years, increasing concern regarding the potential interactions between these forcing drivers has highlighted the importance of multifactorial experiments for predicting the feedback of Earth systems to climate change (Dijkstra et al., 2005; Field et al., 2007; Mikkelsen et al., 2008; Rustad, 2008; Eisenhauer et al., 2012; García-Palacios et al., 2015). Nevertheless, such multifactorial experiments are still limited, and no general quantitative knowledge has been developed to assess the interactions of these forcing drivers. In our meta-analysis, we compared the interactions and additive effects of the three single factors (N deposition, drought and increased precipitation) and warming. We found that the additive effects of the single factors in some multifactorial experiments, such as (warming × N deposition) and (warming × drought) on the soil C input and (warming × increased precipitation) on the soil C loss, exhibited greater \( P < 0.05 \) Mahalanobis distances with larger differences than the interactions (Table 2) and deviated from 1:1 correlations (Fig. 3). These results suggested that simple additive effects using single-factor manipulations may overestimate the true values under natural field conditions. Here, we demonstrate that multifactorial experiments with interactions for assessing extensive climate change should be more frequently applied in studies on Earth systems.

Uncertainty analysis

Meta-analysis provides a statistical method for calculating the mean effect size across different categories, but the data used in this meta-analysis may also present uncertainties because of the limitations of currently available experimental results. First, the collected data mainly came from temperate forests and grasslands in the Northern Hemisphere, particularly for the experiments of drought and increased precipitation, while the data collected from other critical regions were unrepresentative because few studies have been conducted in these regions (Fig. S1). Therefore, the lack of true ‘global’ data may influence the confidence achieved. Second, some targets (i.e., soil C storage) and factorial experiments (i.e., drought and increased precipitation) have received less attention because they are difficult to analyze and expensive to conduct. Thus, there were only 182 entries for the soil C pool, and few observations related to interactions have been collected. Third, some methods employed for analytical syntheses and field experiments limit our understanding of ecological linkages. In this meta-analysis, we expected to obtain a quantitative estimation about the soil C influx and efflux; however, the actual amount of the C input to the soil is difficult to be evaluated. Thus, we used litter and plant biomass production, in order of availability, as proxies for evaluating the soil C input. Moreover, autotrophic and heterotrophic respiration is difficult to distinguish in the field; therefore, we used microbial respiration and soil respiration (in order of availability) for assessing soil C loss (van Groenigen et al., 2014), although using soil respiration \([\text{ln RR} = −0.11 \text{ with } (−0.22, −0.0092) \text{ in } 95\% \text{ CI}]\) may underestimate the effect size of microbial respiration \([\text{ln RR} = 0.0048 \text{ with } (−0.035, 0.045) \text{ in } 95\% \text{ CI}]\). However, this meta-analysis was also projected to enrich our current understanding of how soil C fluxes and equilibrium respond to the interactive elevated N, altered rainfall and rising temperature.

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Conflict of interests

All authors declare no conflict of interests.

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Selsted MB, van der Linden L, Iliom A et al. (2012) Soil respiration is stimulated by elevated CO₂ and reduced by summer drought: three years of measurements in a multifactor ecosystem manipulation experiment in a temperate heathland (CLIM)ATE. *Global Change Biology*, 18, 1216–1230.


### Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Global distribution of the experimental sites included in this meta-analysis.

**Figure S2.** Frequency distribution of the response ratios (ln R) for (a–e) soil carbon pools, (f–j) inputs and (k–o) losses in response to nitrogen deposition, drought and increased precipitation as well as their interactions with warming.

**Figure S3.** Weighted response ratios (ln RR) of the (a) soil carbon pools, (b) inputs and (c) losses in response to nitrogen deposition, drought and increased precipitation as well as their interactions with warming.

**Figure S4.** Weighted response ratios (ln RR) of the (a) soil carbon pools, (b) inputs and (c) losses in response to nitrogen deposition, drought and increased precipitation as well as their interactions with warming across different ecosystems.

**Figure S5.** Weighted response ratios (ln RR) of the (a) soil carbon pools, (b) inputs and (c) losses in response to nitrogen deposition, drought and increased precipitation as well as their interactions with warming across different biomes.

**Figure S6.** Relationships between the response ratios of the (a–d) soil carbon pools, (e–h) inputs and (i–l) losses in response to nitrogen deposition, drought and increased precipitation as well as their interactions with warming and the corresponding latitudes, mean annual temperature (MAT) and precipitation (MAP) at the experimental sites.

**Figure S7.** Weighted response ratios (ln RR) of the (a) soil carbon pools, (b) inputs and (c) losses in response to warming, nitrogen deposition, drought and increased precipitation using different facilities (Methods).

**Figure S8.** Weighted response ratios (ln RR) of the (a) soil carbon pools, (b) inputs and (c) losses in response to warming, nitrogen deposition, drought and increased precipitation using different magnitudes.

**Table S1.** Overview of the experiments involving warming, nitrogen deposition, drought and increased precipitation as well as their interactions included in this meta-analysis.

**Table S2.** Overview of the two-factor experiments included in this meta-analysis.

**Table S3.** The sample sizes (n) and P values of independent t-tests for assessing the effects of a certain factor between the single-(sin) and two-factor (tvo) experiments.

**Table S4.** Between-group heterogeneity (Qb) and the probability (P) of the effect of nitrogen deposition, drought and increased precipitation as well as their interactions with warming on soil carbon pools, inputs and losses across different factors, ecosystems, biomes, facilities (Section 2) and magnitudes.

**Appendix S1.** The peer-reviewed journal articles used in this meta-analysis.

**Appendix S2.** Categories for data assimilation in this meta-analysis.

**Data S1.** Overview of the studies on warming, nitrogen deposition, drought and increased precipitation reporting soil carbon storage included in this meta-analysis.

**Data S2.** Overview of the studies on warming, nitrogen deposition, drought and increased precipitation reporting soil carbon inputs or proxies included in this meta-analysis.

**Data S3.** Overview of the studies on warming, nitrogen deposition, drought and increased precipitation reporting soil carbon losses or proxies included in this meta-analysis.

**Data S4.** Mean monthly temperature during 2011–2015 at the experimental sites for which temperature data were not available in the articles.

**Data S5.** Mean monthly precipitation during 2011–2015 at the experimental sites for which precipitation data were not available in the articles.
To investigate how altered nitrogen and rainfall modulate the effect of warming on soil carbon fluxes, we synthesized global data on soil carbon pool, input and loss from experiments simulating nitrogen deposition, drought and increased precipitation and quantified the responses of soil carbon fluxes and equilibrium to the three single factors and their interactions with warming. We found that the positive carbon–warming feedback was modulated by the changing nitrogen and rainfall regimes. Further, we found that simple additive simulation overestimated the ‘real’ effects on soil carbon fluxes, suggesting that more multifactorial experiments should be considered in studying Earth systems.