

Research Article

The response of ecosystem carbon and nitrogen pools to experimental warming in grasslands: a meta-analysis

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Abstract

Carbon (C) and nitrogen (N) coupling processes in terrestrial ecosystems have the potential to modify the sensitivity of the global C cycle to climate change. But the degree to which C–N interactions contribute to the sequestration of terrestrial ecosystem C (C_{seq}), both now and in the future, remains uncertain. In this study, we used a meta-analysis to quantitatively synthesize C and N responses from field experiments on grasslands subjected to simulated warming and assessed the relative importance of three properties (changes in ecosystem N amount, redistribution of N among soil, litter and vegetation, and modifications in the C:N ratio) associated with grassland C_{seq} in response to warming. Warming increased soil, litter and vegetation C:N ratios and approximately 2% of N shifted from the soil to vegetation and litter. Warming-induced grassland C_{seq} was the result of the net balance between increases in vegetation and litter C (111.2 g m^{-2}) and decreases in soil C (30.0 g m^{-2}). Warming-induced accumulation of C stocks in grassland ecosystems indicated that the three processes examined were the main contributors to C_{seq} with the changes in C:N ratios in soil, litter and vegetation as the major contributors, followed by N redistribution, whilst a decrease in total N had a negative effect on C_{seq} . These results indicate that elevated temperatures have a significant influence on grassland C and N stocks and their coupling processes, suggesting that ecological models need to include C–N interactions for more accurate predictions of future terrestrial C storage.

Keywords climate change, carbon sequestration, carbon–nitrogen interactions, grasslands, nitrogen limitation, stoichiometry

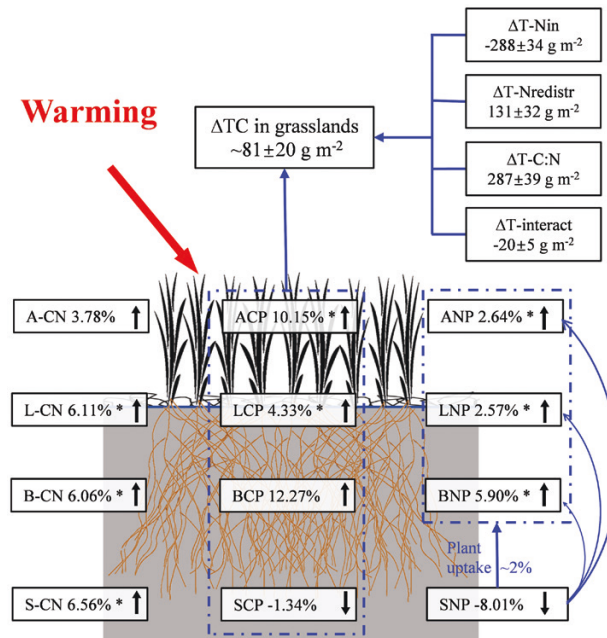
草地生态系统碳氮库对增温响应的整合分析

摘要: 陆地生态系统碳氮耦合过程有可能改变全球碳循环对气候变化的敏感性。然而，碳氮的交互作用对陆地生态系统碳固存的贡献仍不明确。本研究采用Meta分析的方法量化了野外增温条件下草地碳氮储量的变化，并且进一步评估了3个主要的碳氮耦合过程（生态系统氮总量的变化，氮在植被和土壤之间的重新分配，植被与土壤碳氮比的变化）对草地碳固存的相对贡献。增温使得土壤、凋落物和植被的碳氮比增加，并导致约2%的氮从土壤转移到植被和凋落物中。增温提高了植被和凋落物的碳储量(111.2 g m^{-2})，而降低了土壤的碳储量(30.0 g m^{-2})，由此可见，增温提高了整个草地生态系统的

碳储量。碳氮比的变化是温度升高条件下草地碳储量增加的主要贡献者，氮的重新分配次之。相反，氮总量的减少则降低了生态系统的碳储量。这些结果表明，温度升高对草地生态系统碳氮储量的变化及其耦合过程具有显著的影响，建议生态模型考虑碳氮循环的交互作用，以便更准确地预测未来陆地碳储量的变化。

关键词：气候变化，碳固存，碳氮耦合，草地，氮限制，化学计量

Graphical Abstract



INTRODUCTION

By the end of this century, global surface temperatures are expected to increase by 1.1–6.4 °C because of increasing concentration of greenhouse gases in the atmosphere (IPCC 2013). The effect of this temperature increase will depend on feedbacks between terrestrial ecosystems and warming-induced changes (Heimann and Reichstein 2008; Luo 2007). Most modeling studies predict that terrestrial ecosystem carbon sequestration (C_{seq}) will be reduced because warming results in more carbon being lost through respiration than is gained by any increases in photosynthesis (Cox *et al.* 2000; Friedlingstein 2015; Heimann and Reichstein 2008; Williams *et al.* 2019). However, experimental investigations have given varied results, with climate warming resulting in increases (Day *et al.* 2008; Oberbauer *et al.* 2007; Sardans *et al.* 2008; Welker *et al.* 2004), decreases (Oberbauer *et al.* 2007) or no change (Luo *et al.* 2009; Marchand *et al.* 2004; Zou *et al.* 2018) in C stocks. These variations can be partly explained by temporal

and spatial variations in how the partitioning of N and its availability regulates ecosystem C cycle processes (Luo 2007; Shaver *et al.* 2000). Uncertainties in the extent to which N regulates the C cycle can lead to significant variations in C-climate feedback predictions (Heimann and Reichstein 2008; Hungate *et al.* 2003).

Understanding C and N coupling is crucial for elucidating how the C cycle responds to climate change because the C and N pathways are closely linked (Hungate *et al.* 2003; McGuire *et al.* 1992; Reich *et al.* 2006). Stoichiometric amounts of N ultimately determine ecosystem C accumulation because it is crucial for the synthesis of the primary CO_2 fixing enzyme, ribulose 1,5 biphosphate carboxylase/oxygenase (RUBISCO) and other photosynthetic enzymes (LeBauer and Treseder 2008; Raven *et al.* 2004). The C:N ratio provides a measure of the relative allocation of C and N in plants and soils and gives information on the efficiency of N use by plants, which is important in determining how much C is sequestered in response to increased CO_2

concentrations (Du *et al.* 2018; Hungate *et al.* 2003; Luo *et al.* 2004; Niu *et al.* 2010; Terrer *et al.* 2018; Thornton *et al.* 2009; Walker *et al.* 2015; Zou *et al.* 2020). The C:N ratio of plant material also impacts on its decomposition by soil microorganisms and whether N is released or immobilized in the soil (Sistla and Schimel 2012). Previous simulations (Cox *et al.* 2000; Friedlingstein 2015; Heimann and Reichstein 2008; Williams *et al.* 2019) have indicated that warming significantly reduced C_{seq} in vegetation and soils by increasing respiration and decomposition, but these did not consider the potential effects of C–N interactions between soils and vegetation. The outcome of any changes will depend on the relative effect of warming on N availability, and its influence on C uptake, as well as on C losses. For instance, C_{seq} could be enhanced if C uptake by the vegetation was promoted by an increase in N availability, due to an increase in warming-related decomposition processes, if the stimulation of C uptake is greater than the associated soil and plant-related C losses (Sokolov *et al.* 2008). Therefore, the effects of warming on C_{seq} could be underestimated if C–N interactions between the vegetation and soil are ignored, resulting in unrealistic predictions of terrestrial feedbacks to climate warming.

In general, there are three key biogeochemical properties associated with changes in ecosystem C_{seq} : (i) alterations in the total ecosystem N amount, through alterations in the balance between N input and N losses, (ii) shifts in N among ecosystem components with different C:N ratios and (iii) changes in the C:N ratio of those components (Luo *et al.* 2004; Rastetter *et al.* 1992; Reich *et al.* 2006; Shaver *et al.* 1992; Walker *et al.* 2015).

Over the past decade, many field-based warming experiments have been conducted to investigate C and N dynamics and C–N coupling (e.g. An *et al.* 2005; Melillo *et al.* 2011; Niu *et al.* 2010). These experiments have demonstrated that all the three biogeochemical properties identified respond to rising temperatures. Increases in plant N use efficiency (NUE) or C:N ratio, or a shift to species with a different NUE at higher temperatures can influence vegetation C fixation and litter decomposition (An *et al.* 2005; Cornelissen *et al.* 2007; Hobbie 1996; Niu *et al.* 2010). Warming was reported to increase the mineralization of N and C in organic forms (Bai *et al.* 2013; Pendall *et al.* 2004; Rustad *et al.* 2001) because of enhanced enzyme activities and increased microbial metabolism (Cookson *et al.* 2007; Koch *et al.* 2007) that might, in turn, increase N immobilization by soil microbes

and N uptake by plants (Bai *et al.* 2013). Warming might also accelerate N depletion through increased leaching losses and gaseous N emissions (Bai *et al.* 2013). Previous meta-analyses and synthesis work have examined warming impacts on C (Lu *et al.* 2013; Wu *et al.* 2011; Yan *et al.* 2019) and N cycles (Bai *et al.* 2013; Rustad *et al.* 2001), but few of them have studied C–N interactions and their role in warming-induced C dynamics. In addition, previous studies investigating N effects on C cycling under warming mainly considered the direct dependence of soil mineralization and N availability on temperature, and did not account for variations in NUE and N uptake by plants in a warmer environment. This could lead to an unrealistic representation of the N cycle and its impact on C feedbacks to climate warming, making it difficult to quantify C cycle modeling uncertainties and to identify the causes for these uncertainties. Therefore, more information is required on the use of field data to examine whether and how those C–N coupling processes change with climate warming.

This study synthesized the available published data on the responses of grassland C and N to increasing temperatures with the main objectives: (i) to investigate the warming impacts on the dynamics of grassland C and N pool sizes (including mineral soil, litter, aboveground vegetation and belowground plant parts), and C–N interactions; and (ii) to examine the primary C–N coupling processes responsible for grassland C_{seq} under warming and their relative importance. To quantitatively assess warming impacts on the dynamics of grassland C and N stocks, we have obtained information from individual research studies that report the effects of elevated temperatures on grassland ecosystems *in situ*, using the approach of Hedges *et al.* (1999).

MATERIALS AND METHODS

Data collection

We searched the literature using the terms ‘warming (or elevated temperature, or temperature increase)’, ‘grassland (or meadow or steppe or savanna or pasture or grass prairie)’, ‘carbon’, ‘nitrogen’ and ‘terrestrial’ using the Web of Science (1997–2018). The compiled database contained 12 variables describing C stocks ($g\ m^{-2}$) in shoots (aboveground vegetation carbon pool, ACP), roots (belowground plant carbon pool, BCP), litter (litter carbon pool, LCP) and soil (soil carbon pool, SCP); N stocks ($g\ m^{-2}$) in shoots (aboveground vegetation nitrogen pool, ANP), roots (belowground

plant nitrogen pool, BNP), litter (litter nitrogen pool, LNP) and soil (soil nitrogen pool, SNP); ratios of C and N in shoots (aboveground vegetation C:N ratio, A-CN), roots (belowground plant C:N ratio, B-CN), litter (litter C:N ratio, L-CN) and soil (soil C:N ratio, S-CN). A further selection of the papers was carried out using the following criteria: (i) experiments with control and warming treatments conducted in the field; (ii) where at least one of our considered variables was reported; (iii) experiments lasted for less than one growing season were excluded. (iv) The means, standard deviations and sample sizes of the chosen variables were directly provided or could be calculated from the published studies. The experimental warming manipulations included open-top chambers, infrared heaters and heating cables, environmental chambers and mesocosm translocations, etc. Overall, there were 52 published papers included in the dataset (Supplementary Table S1 for more information on these 52 studies). All raw information was collected from the published literature from figures and tables. For each of the 12 variables, we extracted the mean, standard deviation and sample size. The C and N content reported in various soil depths were normalized to the same soil depth of 100 cm as described in Yang *et al.* (2011). The final database (Supplementary Table S1) includes 214 rows of observations containing 12 main variables and basic information (e.g. experimental facilities, field sites, experimental durations, temperature increases) related to each study. Grassland types in this study included forbs, grasses, sedges, tundra or shrub–grass mixtures.

Meta-analysis

We have calculated response ratios (RRs) for the impacts of warming on grassland C and N dynamics, as is the usual approach in a meta-analysis (Bai *et al.* 2013; Lu *et al.* 2013). The RR is described by the proportion of the mean value of a variable for the whole experimental period of the warming treatment (\bar{X}_t) relative to that in the control treatment (\bar{X}_c). The RR logarithm is used to decrease bias and guarantee a normal sampling distribution (Hedges *et al.* 1999).

$$\ln RR = \ln \left(\frac{\bar{X}_t}{\bar{X}_c} \right) = \ln \bar{X}_t - \ln \bar{X}_c \quad (1)$$

The corresponding variance (v) for each $\ln RR$ was approximated as Hedges *et al.* (1999):

$$v = \frac{s_t^2}{n_t \bar{X}_t^2} + \frac{s_c^2}{n_c \bar{X}_c^2} \quad (2)$$

with s_t and s_c , n_t and n_c representing the standard deviation and sample size in the warming and control treatments, respectively. From this variance, we derived a weighting factor w :

$$w_{ij} = \frac{1}{v} \quad (3)$$

We calculated weighted means for both the RR ($\ln RR_{++}$) and the natural logarithm transformed value ($\ln X_{c++}$) for each line of data of the variables under control conditions.

The random effects model calculates a weighted mean effect size by giving greater weight to observations with lower variances, which are the sum of the within-study variance and between-study variance (due to sampling error and variation in experimental conditions, respectively). Weighted mean effect size ± 1.96 Standard error (Stderr) was used to calculate the 95% confidence interval (CI).

Meta-analysis was performed in MetaWin 2.1 (Rosenberg *et al.* 2000). The effects of warming on the C and N variables were considered significant if the 95% CI for the RR did not overlap with 0. The percentage changes were estimated by $(\exp^{\ln RR_{++}} - 1) \times 100\%$. The mean value (\bar{X}_c) for each variable, under control conditions, was calculated as $\exp^{\ln X_{c++}}$.

Changes in the three key biogeochemical properties associated with C_{seq}

Based on previous studies (Rastetter *et al.* 1992, 1997; Shaver *et al.* 1992), we utilized Equation (4) to link any warming-related changes in grassland C_{seq} (ΔC_{GS}) with the three properties identified: a change in the total ecosystem N content; a redistribution of N among vegetation, litter and soil; and modifications in the C:N ratio in vegetation, litter and soil. Further, we assessed the relationships among changes in C:N ratio, total N and N redistribution and their interaction terms, to ecosystem C accumulation using Equations (5)–(7) and Equations (8)–(11).

$$\Delta C_{GS} = \sum_i^n \Delta C_i = \sum_i^n \left[N_t \times Nf_{ti} \times \left(\frac{C}{N} \right)_{ti} - N_c \times Nf_{ci} \times \left(\frac{C}{N} \right)_{ci} \right] \quad (4)$$

where N , Nf and $\frac{C}{N}$ are total ecosystem N content, N partitioning coefficient and C:N ratio, respectively, and where i refers to the aboveground vegetation, belowground plant parts, litter and soil pools. t = treatment, c = control. Note that the change in ecosystem C associated with changes in the C:N ratio

(N_{in} , shift and interactions) among all components is the sum over all i .

Assuming the amount of N in vegetation, litter and soil was unchanged and the C:N ratios of vegetation, litter and soil was changed by warming, then the changes in C in component i associated with flexibility in the C:N ratio of component i is:

$$\Delta C_{(C:N)i} = (N_c \times Nf_{ci}) \times \left[\left(\frac{C}{N} \right)_{ti} - \left(\frac{C}{N} \right)_{ci} \right] \quad (5)$$

If the C:N ratio of vegetation, litter and soil and the relative distribution of N among those components remain constant, then the changes in C in component i associated with the change in total ecosystem N is given by:

$$\Delta C_{(Nin)i} = (N_t - N_c) \times Nf_{ci} \times \left(\frac{C}{N} \right)_{ci} \quad (6)$$

Assuming also that the C:N ratio of vegetation, litter and soil and the amount of N in those components were unchanged, the change in C in component i associated with a redistribution of N into or out of component i is given by:

$$\Delta C_{(shift)i} = N_c \times \left(\frac{C}{N} \right)_{ci} \times (Nf_{ti} - Nf_{ci}) \quad (7)$$

The changes in C in component i associated with the interactions among these three properties for a specific ecosystem component i , are given by the following: (i) the interaction between the total N change and C:N flexibility (Equation (8)); (ii) the interaction between N redistribution and C:N flexibility (Equation (9)); (iii) the interaction between the total N change and N redistribution (Equation (10)) and (iv) the interaction of all the three mechanisms (Equation (11)).

$$\Delta C_{(Nin*C:N)i} = Nf_{ci} \times (N_t - N_c) \times \left[\left(\frac{C}{N} \right)_{ti} - \left(\frac{C}{N} \right)_{ci} \right] \quad (8)$$

$$\Delta C_{(shift*C:N)i} = N_c \times (Nf_{ti} - Nf_{ci}) \times \left[\left(\frac{C}{N} \right)_{ti} - \left(\frac{C}{N} \right)_{ci} \right] \quad (9)$$

$$\Delta C_{(Nin*shift)i} = \left(\frac{C}{N} \right)_{ci} \times (N_t - N_c) \times (Nf_{ti} - Nf_{ci}) \quad (10)$$

$$\Delta C_{(Nin*shift*C:N)i} = (N_t - N_c) \times (Nf_{ti} - Nf_{ci}) \times \left[\left(\frac{C}{N} \right)_{ti} - \left(\frac{C}{N} \right)_{ci} \right] \quad (11)$$

Calculations of the relative importance of C–N coupling processes for C_{seq} can also be found in Zou *et al.* (2020).

RESULTS

Warming effects on the C and N contents in vegetation, litter and soil

The meta-analysis showed that warming significantly ($P < 0.05$) increased C_{seq} by approximately 10% and 12% in aboveground vegetation and belowground plant parts, respectively (Fig. 1a). Whilst warming also tended to increase C contents in litter but decrease it in soil, neither of these was significant ($P > 0.05$, with 95% CI overlap with 0). The accumulation of N in aboveground vegetation, belowground plant parts and litter pools were increased significantly ($P < 0.05$) by elevated temperature, by approximately 3%, 6% and 3%, respectively. However, soils might lose N ($P > 0.05$, with 95% CI slightly overlap with 0) because of increasing temperature (Fig. 1b). Warming increased the C:N ratio of vegetation, litter and soil, although the impact on the C:N ratio in aboveground vegetation was not significant (Fig. 1c). The proportion of N in soil pools (soil N/total ecosystem N) decreased slightly (2%), which indicates that the proportion of N stored in vegetation and litter pools (1 – soil N/total ecosystem N) increased under warming. Thus, warming resulted in a net N shift from soil to other ecosystem components as plant or litter biomass.

The magnitude of warming had no significant ($P > 0.05$) impact on C and N stocks, or their stoichiometry (Supplementary Table S2). Also, the duration of warming did not influence the changes in most of the variables examined ($P > 0.05$), however, soil N stocks tended to decrease while the soil C:N ratio increased with the length of the warming period ($P < 0.05$, Supplementary Table S2).

Changes in grassland C_{seq} associated with the three key biogeochemical properties

Data synthesis of the responses to elevated temperature indicated that ecosystem C_{seq} increased, on average, by 81.1 ± 19.5 g C m⁻². The increase in C_{seq} was mainly associated with the three key properties identified, given the good relationship between the calculated C_{seq} and the experimental data (Supplementary Fig. S1, $R^2 = 0.85$, $P = 0.024$). The C_{seq} increment associated with an increased C:N ratio under warming was 29.9, 31.3, 36.5 and 189.2 g C m⁻² in aboveground vegetation, belowground plant parts, litter and soil pools, respectively. The change in C_{seq} associated with N redistribution was 72.9, 65.5, 54.5 and –61.5 g C m⁻² in aboveground vegetation, belowground plant parts, litter and

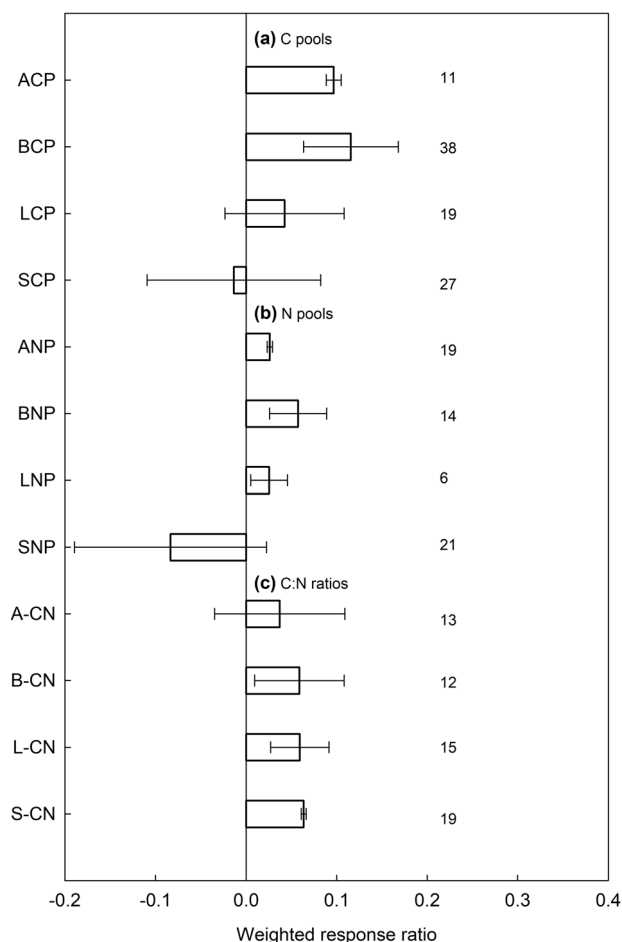


Figure 1: Responses of C:N ratios and C and N pools to warming. In (a), ACP, BCP, LCP and SCP are the C stock in aboveground vegetation, belowground plant parts, litter and soil pools, respectively. In (b), ANP, BNP, LNP and SNP are the N stock in aboveground vegetation, belowground plant parts, litter and soil pools, respectively. In (c), A-CN, B-CN, L-CN and S-CN are the C:N ratios in aboveground vegetation, belowground plant parts, litter and soil pools, respectively. The vertical lines are drawn at $\ln RR = 0$. The number next to each bar is the sample size for each variable. The error bars represent 95% CIs.

soil pools, respectively, whilst the reduction in C_{seq} associated with a loss in total N associated with the same components was 47.6, 31.1, 35.9 and 173.5 g C m⁻². Thus, in total approximately 287 ± 39.2 and 131 ± 31.7 g C m⁻², respectively, of the grassland C increment under warming was associated with an increased C:N ratio and N redistribution. For comparison, the reduction in C associated with a loss in total N was approximately 288 ± 34.0 g C m⁻² (Fig. 2). In contrast, the importance of the interaction terms was minor compared with their major effects and would only have led to a 19.5 ± 5.0 g C m⁻² loss of C. These results were comparable with a field

warming experiment in Oklahoma (Fig. 3), where an increased C:N ratio played a dominant role in ecosystem C storage with increased temperatures (An *et al.* 2005; Niu *et al.* 2010).

DISCUSSION

This study demonstrated that warming increased ecosystem C stocks by ~81 g C m⁻², which fell well within the reported range of 0–150 g C m⁻² in a simulation study examining the responses of a tundra ecosystem to increased temperature (Rastetter *et al.* 1992). The increased C_{seq} was a combination of the net effect of enhanced C accumulation in vegetation and litter and C losses from the soil (Figs 1 and 2). Other work has also reported an increase in C accumulation in vegetation through warming-enhanced plant C fixation (Lu *et al.* 2013; Rustad *et al.* 2001), which might offset or even exceed soil C losses (Zhou *et al.* 2007), leading to an increase in ecosystem C_{seq} (Luo *et al.* 2009). The reasons for the increases in both above- and belowground biomass, as well as litter biomass, and thus the C stocks in these pools, can be attributed to the increase in photosynthesis under warming in grasslands, where plant growth is often limited by temperature. However, our results contrast with model projections of a reduced ecosystem C_{seq} by elevated temperatures

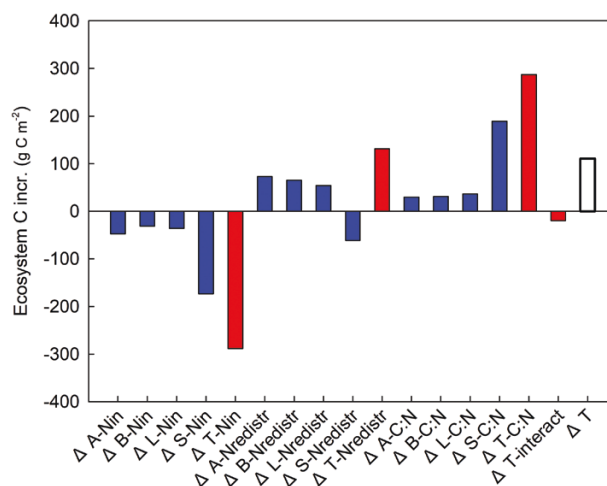


Figure 2: The C_{seq} increment induced by warming and the contributions of variations in three factors, (i) change in total ecosystem N (Nin), (ii) the redistribution of N (Nredistr) and (iii) C:N ratio (C:N). The interactive terms have been aggregated (interact). Blue bars are for the individual ecosystem components (aboveground vegetation (A), belowground plant parts (B), litter (L) and soil (S)). Red bars are the sum over ecosystem components for each of the three factors (T) and for the aggregated interactive terms. The open bar is the total change in ecosystem C.

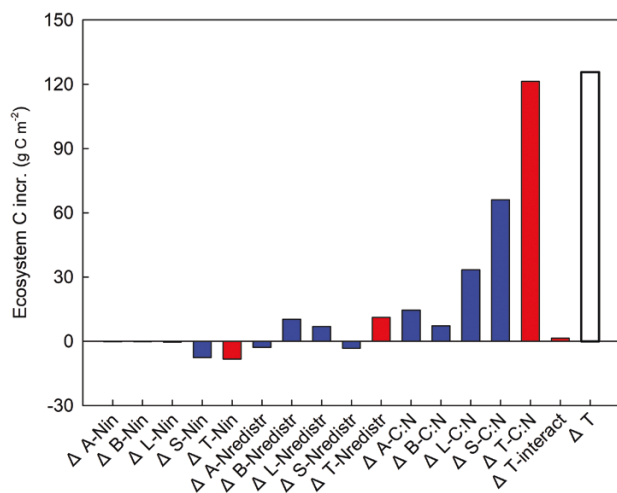


Figure 3: Data from the tallgrass warming experiment site at Oklahoma (Niu *et al.* 2010), showing the C_{seq} increment induced by warming and the contributions of variations in three factors. See Fig. 2 for the abbreviations.

(Cox *et al.* 2000; Friedlingstein *et al.* 2006; Heimann and Reichstein 2008). This discrepancy might be due, in part, to a failure to consider C–N interactions in these model projections (Luo 2007). Including N effects attenuate the sensitivity of the C cycle to climate change (Sokolov *et al.* 2008; Thornton *et al.* 2009). The reported plant growth stimulations and subsequent C fixation by elevated temperatures in the current synthesis are in line with many studies, including other meta-analyses (Lin *et al.* 2010; Rustad *et al.* 2001; Wu *et al.* 2011), and field investigations (An *et al.* 2005; Niu *et al.* 2010). Warming-induced increases in aboveground plant biomass might have enhanced litter production, although warming might also accelerate litter decomposition by stimulating microbial activity (Lu *et al.* 2013; Luo 2007). Enhanced litter decomposition might thus partly offset the higher litter C inputs, leading to little change in C accumulation in litter pools (Fig. 1a). In contrast, C stocks in mineral soils might be depleted (Fig. 1a), which was probably due to warming-enhanced respiratory C losses (Lu *et al.* 2013).

The plant and litter C increments were proportionately larger than the N increments, leading to expanded/widened plant and litter C:N ratios. However, soil C losses were relatively smaller than soil N losses, leading to higher soil C:N ratios as well (Fig. 1a). These results are consistent with other studies showing increased C:N ratios under warming (Day *et al.* 2008; Sardans *et al.* 2008; Welker *et al.* 2004). The enhanced plant biomass and C:N ratios associated with warming produced a lower

quality litter (Fig. 1), which could slow down litter decomposition rates and this might compensate, to some extent, for the increased respiratory C losses under warming (Luo and Zhou 2006; Rustad *et al.* 2001).

The external N supplied to an ecosystem can enhance C_{seq} without any redistribution of resources or alteration in the stoichiometry of its components (Luo *et al.* 2004; Rastetter *et al.* 1992). Since the rate of supply of N is often quite slow, substantial N accumulation and the associated accumulation of C might take a very long time (Luo *et al.* 2004, 2006). To assimilate more atmospheric CO_2 , plants need to take up more N from soils, potentially resulting in a decrease in soil N pools (Fig. 1). In addition, warming can also lead to the faster decomposition of soil organic matter so that there is the increased possibility for the leaching of inorganic or dissolved organic N. In addition, warming can potentially increase N_2O emissions, although this is not always the case (Zou and Osborne 2020). All of these three possibilities would lead to a decrease in soil N pools under warming (Bai *et al.* 2013). A depletion of ecosystem N of approximately $10.6 \pm 1.7 \text{ g N m}^{-2}$ was also found in this study, which was associated with an approximately $288 \pm 34.0 \text{ g C m}^{-2}$ loss of C in grasslands. Nevertheless, net nitrification and N mineralization were increased by 32.2% and 52.2%, respectively, leading to a 20% increase in soil mineral N availability with elevated temperatures (Bai *et al.* 2013). Increased soil N availability might stimulate N uptake and assimilation by vegetation that, in turn, promotes the growth of both roots and shoots (Beier *et al.* 2008; Lu *et al.* 2013). Consequently, elevated temperatures stimulated N accumulation in vegetation (Bai *et al.* 2013), and was accompanied by increased C accumulation in both aboveground vegetation and belowground plant parts through stimulated plant growth (Fig. 1a). Therefore, a climate warming-induced stimulation of soil respiratory losses could be compensated by warming-induced increases in net primary production resulting in the net accumulation of C.

Climate models generally incorporate increases in plant N uptake and N mineralization as the main responses to warming (Sokolov *et al.* 2008; Thornton *et al.* 2009), which results in an increase in plant growth and C accumulation (Rustad *et al.* 2001; Wan *et al.* 2005; Welker *et al.* 2004). However, our results showed that warming-induced C_{seq} in grasslands is associated with not just a total N change, but

also the redistribution of N among ecosystem components, and modifications in the C:N ratio (Fig. 2). An increased C:N ratio might be a physiological adjustment to reduced N availability caused by increasing temperature (Luo 2007). In addition, more N was allocated from soil, with a lower C:N ratio, into other ecosystem components with a higher C:N ratio (Fig. 1). Importantly, these modifications can result in an increase in C_{seq} without additional external N inputs. However, an external input of N will be important in the long term as the ecosystem becomes depleted in N because N is an essential nutrient for plant and microbial growth and its supply can ultimately limit grassland C_{seq} capacity (Hungate *et al.* 2003; Luo *et al.* 2004; Rastetter *et al.* 1992; Terrer *et al.* 2018). Therefore, models need to incorporate more information on the range of warming effects on N inputs, remobilization and utilization for more realistic projections of C cycling under future climate change scenarios.

CONCLUSIONS

Our synthesis examined the responses of grassland N and C stocks and their interactions to simulated climate warming based on three key biogeochemical properties: changes in the total amount of N, redistribution of N among soil, litter and vegetation, and modifications in the C:N ratio, all of which were found to make a significant contribution to C_{seq} . Increases in grassland C_{seq} in response to warming was due to the contrasting effects of increasing temperatures on vegetation and soil C pools. However, warming-induced increases in grassland C_{seq} were larger than C losses, leading to an increased ecosystem C_{seq} . As a consequence, more N is taken up from soils to support the increase in primary productivity, resulting in a decrease in soil N stocks. The accumulation of C was faster in vegetation and litter but declined slower in soil than N, leading to higher C:N ratios. Of the major coupling processes examined changes in the C:N ratio contributed the most to warming-induced C_{seq} in grassland ecosystems, followed by N redistribution. In contrast, the reductions in total ecosystem N amount led to C losses in grasslands subject to experimental warming. However, increases in grassland C_{seq} associated with a higher C:N ratio and N redistribution was larger than C losses because of a reduced N capital, leading to an increase in ecosystem C_{seq} . This suggests that the impact of C–N interactions on future C dynamics in a warmer world need to be accounted for in climate models.

Supplementary Material

Supplementary material is available at *Journal of Plant Ecology* online.

Table S1: The list of papers with detailed information from which data were extracted for this study.

Table S2: Regression analysis of $\ln RR$ of ACP, BCP, LCP, SCP, ANP, BNP, LNP, SNP, A-CN, B-CN, L-CN and S-CN in relation to warming magnitude and duration.

Figure S1: Calculated and reported changes in the C stocks for the aboveground vegetation pool (ACP), the belowground plant pool (BCP), the litter pool (LCP), the soil pool (SCP) and the total ecosystem pool, as well as the 1:1 relationship.

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Authors' Contributions

Junliang Zou: methodology, visualization, investigation and writing—original draft preparation. Juying Wu: funding acquisition. Bruce Osborne: writing—reviewing and editing. Yiqi Luo: conceptualization and supervision.

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