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Experimental warming shifts coupling of carbon and nitrogen cycles in an alpine meadow

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Abstract

Aims Terrestrial ecosystem carbon (C) uptake is remarkably regulated by nitrogen (N) availability in the soil. However, the coupling of C and N cycles, as reflected by C:N ratios in different components, has not been well explored in response to climate change.

Methods Here, we applied a data assimilation approach to assimilate 14 datasets collected from a warming experiment in an alpine meadow in China into a grassland ecosystem model. We attempted to evaluate how experimental warming affects C and N coupling as indicated by constrained parameters under ambient and warming treatments separately.

Important Findings The results showed that warming increased soil N availability with decreased C:N ratio in soil labile C pool, leading to an increase in N uptake by plants. Nonetheless, C input to leaf increased more than N, leading to an increase and a decrease in the C:N ratio in leaf and root, respectively. Litter C:N ratio was decreased due to the increased N immobilization under high soil N availability or warming-accelerated decomposition of litter mass. Warming also increased C:N ratio of slow soil organic matter pool, suggesting a greater soil C sequestration potential. As most models usually use a fixed C:N ratio across different environments, the divergent shifts of C:N ratios under climate warming detected in this study could provide a useful benchmark for model parameterization and benefit models to predict C–N coupled responses to future climate change.

Keywords Bayesian probabilistic inversion, Markov-Chain Monte-Carlo (MCMC), warming, carbon and nitrogen cycles, stoichiometry, alpine meadow

增温对高寒草甸生态系统碳氮循环耦合关系的影响

摘要：陆地生态系统碳吸收受土壤氮素可用性的调节。然而，全球变化背景下的不同生态系统组分的碳氮比及其所反映的碳氮循环耦合关系尚不十分清楚。本文运用数据同化的方法，将一个高寒草甸增温试验的14组数据同化到草地生态系统模型中，从而评估了增温如何影响陆地生态系统的碳氮循环耦合关系。研究结果表明，增温提高了土壤氮素的有效性，降低了土壤活性碳库的碳氮比，导致植物对土壤氮的吸收增加。但是由于植物叶片吸收的碳比吸收的氮增加更多，使得叶片中碳氮比增加，而根部的碳输入增加则低于氮的增加，导致根部的碳氮比减少。同时，增温降低了凋落物碳氮比，可能是在土壤高

氮有效性的条件下，凋落物氮的固定得到增强；而且增温加速了凋落物的分解。同时增温还增加了慢速土壤有机质的碳氮比，使得该土壤碳库的碳固存潜力增大。由于大多数模型在不同的环境中通常使用相对固定的碳氮比，本研究所发现的气候变暖条件下碳氮比的差异变化可为模型参数化提供一个有效的参考，有利于模型对未来气候变化背景下生态系统碳氮耦合关系响应的预测。

关键词：贝叶斯反演，马尔可夫链蒙特卡尔理论(MCMC)，增温，碳氮循环，化学计量，高寒草甸

INTRODUCTION

Global air temperature has sharply increased in recent decades (Cox *et al.* 2000). This increase has been shown to have a great influence on terrestrial ecosystems (Root *et al.* 2003), especially in alpine regions where the ecosystem is highly sensitive to temperature changes (Cheng and Wu 2007; Liu and Chen 2000; Wang *et al.* 2000; Xu and Liu 2007). Many studies have been devoted to understand effects of climate warming on carbon (C) and nitrogen (N) cycles (Beier *et al.* 2008; Dawes *et al.* 2017; Melillo *et al.* 2002). However, most of them specifically focused on how warming affects C or N cycle, separately. We have limited knowledge on the response of C and N interactions to climate warming i.e. essential for fully understanding the C cycling of terrestrial ecosystems and its feedback to climate change.

While the formation of organic matter requires a certain amount of N, the processes of plants absorbing and assimilating C and N are closely related (Raven *et al.* 2004). This relationship is reflected by relatively consistent C:N ratios of various ecosystem components within stoichiometric flexibility (Hessen *et al.* 2004). This proportional relationship controls plant C production and soil organic C decomposition, consequently changing ecosystem C sequestration and its feedback to climate change (Luo *et al.* 2004). If plant C:N ratio keeps relatively stable under climate change, the variation of productivity would be very limited unless the biologically available N is changed (Gruber and Galloway 2008). In contrast, if C:N ratio alters systematically under climate change, ecosystems may undergo large changes in productivity without the need to alter the amount of available N.

In the past two decades, some CO_2 enrichment and N addition experiments have been conducted to test C–N interactions under global change. These previous studies have indicated that elevated CO_2 increased C:N ratios of some ecosystem components while elevated N input decreased C:N ratios (Finzi *et al.* 2006; LeBauer and Treseder 2008; Sullivan *et al.* 2007; Yang and Luo 2011). These results show that the shifts of C:N ratios will adjust or alleviate the effects of CO_2 enrichment or N addition, resulting in the nonlinear effects of different gradient C and N enrichment on ecosystem C storage. In those CO_2 and N addition studies, shifts in C–N coupling parameters under climate warming are seldomly explored (Finzi *et al.* 2011; Williamson *et al.* 2016; Xu and Yuan 2017). Warming influences C cycle by altering the rate of photosynthetic CO_2 uptake and ecosystem respiration (Brooks and Farquhar 1985; Luo 2007; Zhou *et al.* 2007). Moreover, warming usually influences N cycle via promoting soil microbial activity and organic N mineralization (Chapin *et al.* 1995; Ma *et al.* 2015), thus N could be conveyed from mineral soil to plant and changes the C:N ratios of different pools.

Some of the Earth system models have been developed to combine N process and C cycle to more accurately predict future changes in C cycling (Bentsen *et al.* 2013; Jin *et al.* 2020; Kloster *et al.* 2012). However, the key parameters that control C and N coupling were usually set as constant and have large uncertatinties in land models (Luo and Schuur 2020). To minmize these uncertainties, ecologists introduced data assimilation methods into ecological models by using empirical data to constrain model parameters (Luo *et al.* 2011). The constrained parameters, instead of constant ones, have proved to be useful and largely improve the accurancy of model predictions in terrestrial ecosystem C and N cycles under global change (Braswell *et al.* 2005; Shi *et al.* 2016; Wang *et al.* 2001; Weng and Luo 2011). Besides, these constrained parameters can systematically quantify some unmeasurable processes (i.e. interactions between multiple soil C pools) (Yuan *et al.* 2012). But this method requires a high integrity of dataset that includes ecosystem C and N fluxes and pool sizes in different components such as leaf and root, litter, frost floor, mineral soil and microbe (Shi *et al.* 2016). As a result, data assimilation approach has not yet been widely used in terrestiral ecosystem studies (Niu *et al.* 2014). Especially, this approach has not been applied to examine changes in C and N coupling parameters under climate warming.

In this study, we used data assimilation to perform an inverse analysis of coupled C and N cycles, expecting to reveal the changes of C and N coupling parameters under climate warming. The Bayesian probabilistic inversion was used in this study to estimate the C and N coupling parameters (i.e. the C:N ratio of different ecosystem components) and some other key parameters (e.g. N uptake and input, N allocation coefficient, N loss) with the constrains of field observations in a warming expeirment conducted in an apline meadow on Qinghai-Tibet Plateau from 2015 to 2018. The specific questions we addressed in this study included: (i) how does climate warming change the C–N coupling (mostly indicated by C:N ratios) of different ecosystem components? (ii) How will the shifts in C and N coupling influence terrestrial ecosystem C cycle.

MATERIALS AND METHODS

C–N coupled model

The C and N coupled model used in this study was revised from TECO-CN used by Shi *et al.* (2016). The model we used was designed for grassland so we referred it as GECO (Grassland ECOsystem). Different from other full TECO-CN models with canopy module, the C input of our model was from field measurement and eddy covariance tower observation (see Datasets for details), while the other parts of our model are consistent with the full model. There are seven C and N pools and one more mineral nitrogen pool in our model, which include leaf (X1, N1), roots (X2, N2), standing litter (X3, N3), surface litter (X4, N4), fast (X5, N5), slow (X6, N6), passive soil organic matter (SOM, X7, N7) and mineral N pool (Fig. 1). In the GECO model, CO_2 in the atmosphere entered the ecosystem by canopy photosynthesis. Some of the photosynthate was used by plants' respiration, and the remaining was allocated in leaf (X1) and root (X2). The detritus of dead plants then flowed to the litter pool, which contained standing litter (X3) and surface litter (X4). And the underground litter was partly respired by microbes while the rest was converted to fast SOM (X5) and slow SOM (X6). The CO_2 released by the decomposition of soil C eventually returns to the atmosphere. Similarly, plant absorbed N from mineral soil. Then, N was converted to seven N pools which were same as C, and finally returned to soils by microbial mineralization.

GECO model uses matrix-based first-order differential equations to describe the process of carbon transfer between ecosystem carbon pools, which could be represented by:

$$\frac{d}{dt}X(t) = A\xi(t)KX(t) + BU(t) \qquad (1)$$

$$X(0) = X_0$$

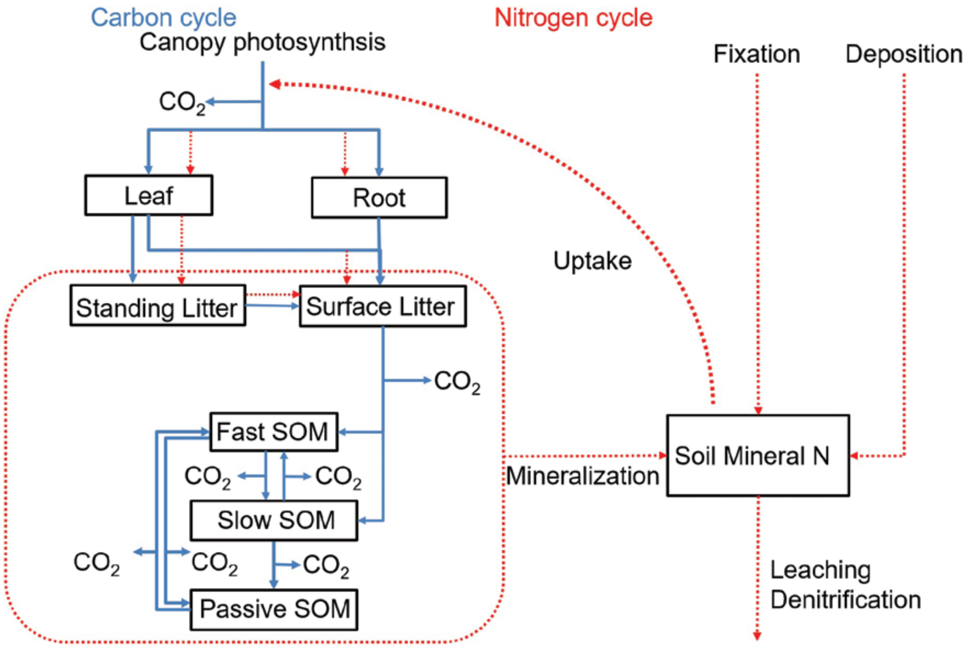

Figure 1: Carbon and nitrogen pools and pathways of carbon and nitrogen cycles in GECO model. Blue arrows show carbon cycling processes, while red arrows indicate nitrogen cycling processes. Abbreviation: SOM = soil organic matter.

where $X = (x_1\, x_2\, x_3\, x_4\, x_5\, x_6\, x_7)^{\mathrm{T}}$, in which x_i represents the C pools in leaves, roots, standing litter, surface litter, fast, slow and passive SOM at time t, respectively. Matrix A represents the proportional relationship of carbon transfer between libraries (Xu *et al.* 2006).

$$A = \begin{bmatrix} -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & -1 & 0 & 0 & 0 & 0 & 0 \\ f_{3,1} & 0 & -1 & 0 & 0 & 0 & 0 \\ 1-f_{3,1} & 1 & f_{4,3} & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & f_{5,4} & -1 & f_{5,6} & f_{5,7} \\ 0 & 0 & 0 & f_{6,4} & f_{6,5} & -1 & 0 \\ 0 & 0 & 0 & 0 & f_{7,5} & f_{7,6} & -1 \end{bmatrix}$$

K is a 7 × 7 diagonal matrix with diagonal entries. The elements on the diagonal indicate the C decay rate from each pool, which is the inverse of the turnover time t_i of C in the pools ($i = 1, 2, \ldots, 7$). U represents the C produced by canopy photosynthesis. B is the proportion of photosynthetic products distributed to leaves and roots, the rest of photosynthetic products are consumed by plant's autotrophic respiration. $\xi(t)$ is an influence function of C decomposition to account for temperature and moisture effects (Luo *et al.* 2003).

The N processes can be described by this formula:

$$\frac{d}{dt}N(t) = A\xi(t)KR^{-1}N(t) + K_u N_{\min}(t)\Pi \qquad (2)$$

$$N(0) = N_0$$

where $N = (n_1\, n_2\, n_3\, n_4\, n_5\, n_6\, n_7)^{\mathrm{T}}$, in which n_i represents the N pools in leaves, roots, standing litter, surface litter, fast, slow and passive SOM at time t, respectively. R is a 7 × 7 diagonal matrix with diagonal entries, the elements on the diagonal indicate the C:N ratio of each pool. $\Pi = (\pi_1\; 1-\pi_1\, 0\, 0\, 0\, 0\, 0)^{\mathrm{T}}$ is an allocation coefficient vector of N from mineral soil to leaves and roots. K_u is N uptake rate, $N_{\min}(t)$ is the amount of soil available N at time t. The dynamic balance of mineral soil N is determined by the input of mineralization, biological fixation, atmospheric deposition and the output of plants input, leaching and gaseous N fluxes, which can be described by:

$$\begin{aligned} \frac{d}{dt}N_{\min}(t) = & -(K_u + K_L)N_{\min}(t) \\ & + A\xi(t)\varphi_1^{*}KR^{-1}X(t) + F(t) \end{aligned} \qquad (3)$$

$$N_{\min}(0) = N_{\min,0}$$

In formula (3), K_u and K_L represent rates of N uptake and loss, respectively. $A\xi(t)\varphi_1^{*}KR^{-1}X(t)$ represent N mineralization, and $F(t)$ is N input by biological fixation and atmospheric deposition.

In general, parameters used in our model can be divided to three types. They are C decay rates (i.e. c_1, c_2, …), transfer coefficients (i.e. $f_{3,1}$, $f_{4,3}$, …) and C&N coupling parameters (i.e. n_1, n_2, …). C decay rates determine the outflow from corresponding C and N pools, and the transfer coefficients determine the distribution of the outflow and usually measure microbial C or N use efficiencies. C&N coupling parameters link the C cycle and N cycle. These three sets of parameters, combined with the dynamic C pools, make the operation of the entire C–N coupling model (Fig. 1).

Study area

The Hong Yuan field station was located on the eastern of Qinghai-Tibet Plateau (32°84′ N, 102°58′ E), which had a continental plateau frigid temperate monsoon climate. The mean annual precipitation is 747 mm with the mean annual temperature of 1.5 °C, the sunshine duration in a year is about 2000–2400 h, the growing season spans from April to October. The main vegetation type in the study site is alpine meadow, and the soil type is subalpine meadow soil and boggy soil (Song *et al.* 2014). This area is dominated by *Deschampsia caespitosa* (Linn.) Beauv., *Koeleria cristata* (Linn.) Pers., *Gentiana sino-ornata* Balf. f., *Potentilla anserina* L. and *Anemone rivularis* Buch.-Ham (Quan *et al.* 2018).

Experiment and data

Eddy covariance measurements

CO_2 flux was measured by an eddy covariance measurement system installed at a height of 2 m above ground. Meteorological data were measured simultaneously with the eddy covariance system. Soil volumetric water content and soil temperature (T_{soil}) were monitored at a depth of 10 cm. In this study, we used the daily net ecosystem exchange (NEE), gross primary productivity (GPP) and meteorological data from 2015 to 2018. The NEE data quality control, gap-filling and partitioning were according to the method used by Chen *et al.* (2019).

Warming experiments

We used experimental data from a warming experiment near the eddy covariance tower. Block design with two warming treatments (A, ambient temperature; W, warming treatment) (five replications each) was used in this study. In order to minimize the spatial heterogeneity, five replicates per treatment are randomly distributed in experimental plots. The warmed plots were continuously heated by infrared radiators (MSR-2420, Kalglo Electronics Inc., Bethlehem, PA, USA) suspended 1.5 m above the ground since June 2014. The output power was 2000 W, increased soil temperature at 10 cm on average by 2.8 °C (Quan *et al.* 2018). GPP and soil respiration (SR) were measured twice a month by static chambers (LI-6400XT, LI-COR Environmental, Lincoln, NE, USA) in plots with different treatments in growing season from 2015 to 2018. Biometric data were retrieved once a year, including biomass of plant leaves and roots, standing litter, surface litter, and microbes, soil C content, total N content of microbe and soil, and soil inorganic N concentration.

Datasets

The data used for this study included two types: the data to drive the model and the data to be assimilated to constrain parameters of the model. The drive data contain daily climate data of study site from 2015 to 2018, such as soil temperature and soil moisture. In addition, we used daily GPP as C input in the model. The measured GPP at the nearby eddy covariance tower was used as GPP at ambient temperature. Daily GPP at the warming treatment was estimated from correlation analysis between eddy covariance measured GPP and static chamber measured GPP from the ambient plot in the experiment. The response ratio of GPP between the ambient and warming plots was used to interpolate daily GPP values for different warming treatments. The data to be assimilated for parameter constraint include C and N contents in leaf, root, standing litter, surface litter, microbial, mineral soil, and autotrophic and heterotrophic respiration.

Data assimilation

We used Markov-Chain Monte-Carlo (MCMC) method to estimate parameters in the GECO. The method considers the unknown parameters as random variables that conform to a certain prior probability distribution. In this study, N-related parameters (i.e. C:N ratio of different ecosystem components, N uptake, N loss) are targeted parameters of the data assimilation (Supplementary Table S1). Besides these key parameters, the C decay rates, C allocation and transfer coefficients can also be estimated (Supplementary Table S2). According to the Bayesian theorem, the prior knowledge of the parameter and information contained in data are fused together to generate posterior distributions of parameters (Xu *et al.* 2006) as

$$P(p|Z) \propto P(Z|p)P(p) \tag{4}$$

In formula (4), $P(p)$ and $P(p|Z)$ represent the prior probability density function (PDF) and posterior PDF of parameters, respectively. $P(Z|p)$ represent conditional probability density of observation under the prior parameters, which also called the likelihood function of p. We assume that the random error is normally distributed with zero mean, so the likelihood function can be presented by:

$$P(Z|p) \propto \exp\left\{-\sum_{i=1}^{7}\sum_{t\in Z_i}\frac{[Z_i(t)-\varphi_i X(t)]^2}{2\sigma_i^2(t)}\right\} \tag{5}$$

In formula (5), $Z_i(t)$ and $\varphi_i X(t)$ represent measured value and simulated value of observational variable i at time t, and σ_i is the standard deviation of observational variable i. In this study, i from 1 to 7 represent the C or N contents of leaf, root, annual litter, grass floor, microbe, mineral soil and heterotrophic respiration, respectively. φ_i can transfer the value of X and N (in formulas (1) and (2)) to the variable that i represent. According to the C and N pool structure of the GECO model and the existing empirical knowledge, the mathematical relationship between the size of the carbon pool and each analog quantity is determined, and the observation operator φ is expressed as:

Leaf C and N: $\varphi_1 = (1\,0\,0\,0\,0\,0\,0)$
Root C and N: $\varphi_2 = (0\,1\,0\,0\,0\,0\,0)$
Standing litter C and N: $\varphi_3 = (0\,0\,1\,0\,0\,0\,0)$
Surface litter C and N: $\varphi_4 = (0\,0\,0\,0.5\,0\,0\,0)$
Microbial C and N: $\varphi_5 = (0\,0\,0\,0\,1\,0\,0)$
Mineral soil C and N: $\varphi_6 = (0\,0\,0\,0\,1\,1\,1)$
Heterotrophic respiration:
$\varphi_7 = (0\,0\,0\,m_4c_4\,m_5c_5\,m_6c_6\,m_7c_7)$, in this formula

$m_4 = 1 \text{-} f_{5,4} \text{-} f_{6,4}$
$m_5 = 1 \text{-} f_{6,5} \text{-} f_{7,5}$
$m_6 = 1 \text{-} f_{5,6} \text{-} f_{7,6}$
$m_7 = 1 \text{-} f_{5,7}$

Autotrophic respiration (Ra): $\mathrm{Ra} = (1 - b_1 - b_2)U(t)$
SR (Rs): $\mathrm{Rs}(t) = \xi(t)\varphi_8 X(t) + \mathrm{Ra}$
Soil mineralization:
$\varphi_8 = (0\,0\,m_3c_3\,m_4c_4\,m_5c_5\,m_6c_6\,m_7c_7)$

We used the Metropolis–Hastings (M–H) algorithm as the (Hastings 1970; Metropolis *et al.* 1953) MCMC sampler. This method randomly samples the new parameter value p_{new} according to the recommended posterior distribution, then simulates and introduces it into the model to calculate the new parameter posterior probability p_{new}, and compares it with the previous parameter posterior probability p_{k-1}. We accept the p_{new} only if $R = \frac{P(p^{\text{new}}|Z)}{P(p^{k-1}|Z)}$ > a random number from 0 to 1, or the p_{new} will be rejected and we let $p_k = p_{k-1}$ to start the sampling of next parameter. The M–H algorithm will be repeated until 300 000 sets of parameter values are accepted, and then all accepted parameter values will be used to construct the probability distribution functions (PDFs) (Weng and Luo 2011; Xu *et al.* 2006). When the model is stable, the lower the overall variance is between the simulated and measured values, the better the effect of the model simulation, and it is easier for p_{new} to be accepted.

RESULTS

Model performance as indicated by parameter constraints and observation data fitting

From the shapes of posterior PDFs, we found that parameters like C:N ratios of root, leaf, standing litter, surface litter, fast SOM, slow SOM, plant N uptake (K_u) and external N input ($F(t)$) were well constrained under all three treatments (Fig. 2). While the C:N ratio of passive SOM, the N allocation coefficient to root (π_1), the rate of N loss (K_L) and the initial value of mineral N pool ($N_{\text{min}}(0)$) were poorly constrained (Supplementary Figs S3 and S4). The allocation coefficients of C to root and leaf ($b1$ and $b2$), three decay rates from surface litter, microbe and slow SOM were well constrained. In contrast, the decay rates of root, leaf, standing litter, passive SOM and almost all the transfer coefficients between the different C pools were not well constrained (Supplementary Figs S1 and S2).We chose 100 groups of best fitted parameters which were picked from about 140 000 sets of upgraded parameters to ran GECO model and simulate the C and N dynamics during the period of 2015–2018. The model result showed that the simulation of C pools, as well as N pools, in leaf, standing litter, surface litter and soil matched the observations well both in the ambient and warming condition (Fig. 3). However, the simulations of root C

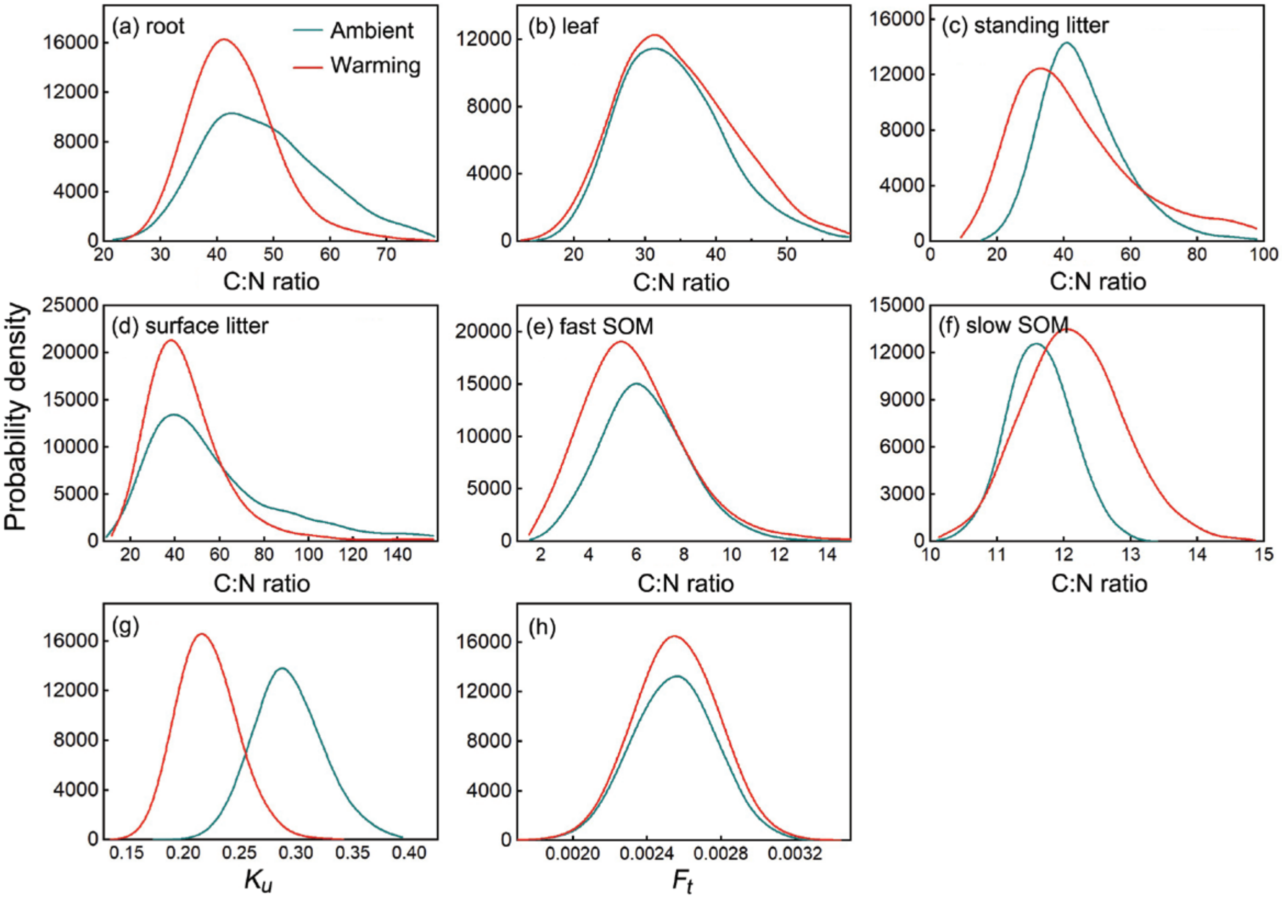

Figure 2: Posterior distributions of carbon–nitrogen coupling parameters under ambient and warming conditions. (**a**) C:N ratios of root, (**b**) leaf, (**c**) standing litter, (**d**) surface litter, (**e**) fast SOM, (**f**) slow SOM, (**g**) rate of N input and (**h**) uptake. F_t = rate of N input, K_u = rate of N uptake, SOM = soil organic matter.

under the ambient condition, and microbe biomass C under either ambient or warming condition (Fig. 3), did not match the observations. We also simulated other factors, including SR, autotrophic respiration (Ra) and heterotrophic respiration (Rh) (Fig. 4). Compared with the observations, most simulations of SR fitted well with the exception of a few lower estimates in 2017.

Shifts in the coupled C and N cycles inferred from estimated parameters

Warming had no significant effects on C:N ratio of passive SOM, N input to ecosystem or the allocation coefficients of N to plants ($P < 0.05$, Fig. 5; Table 1). On the other hand, warming significantly decreased C:N ratio of root, standing litter, surface litter and the microbe but increased C:N ratio of leaf and slow

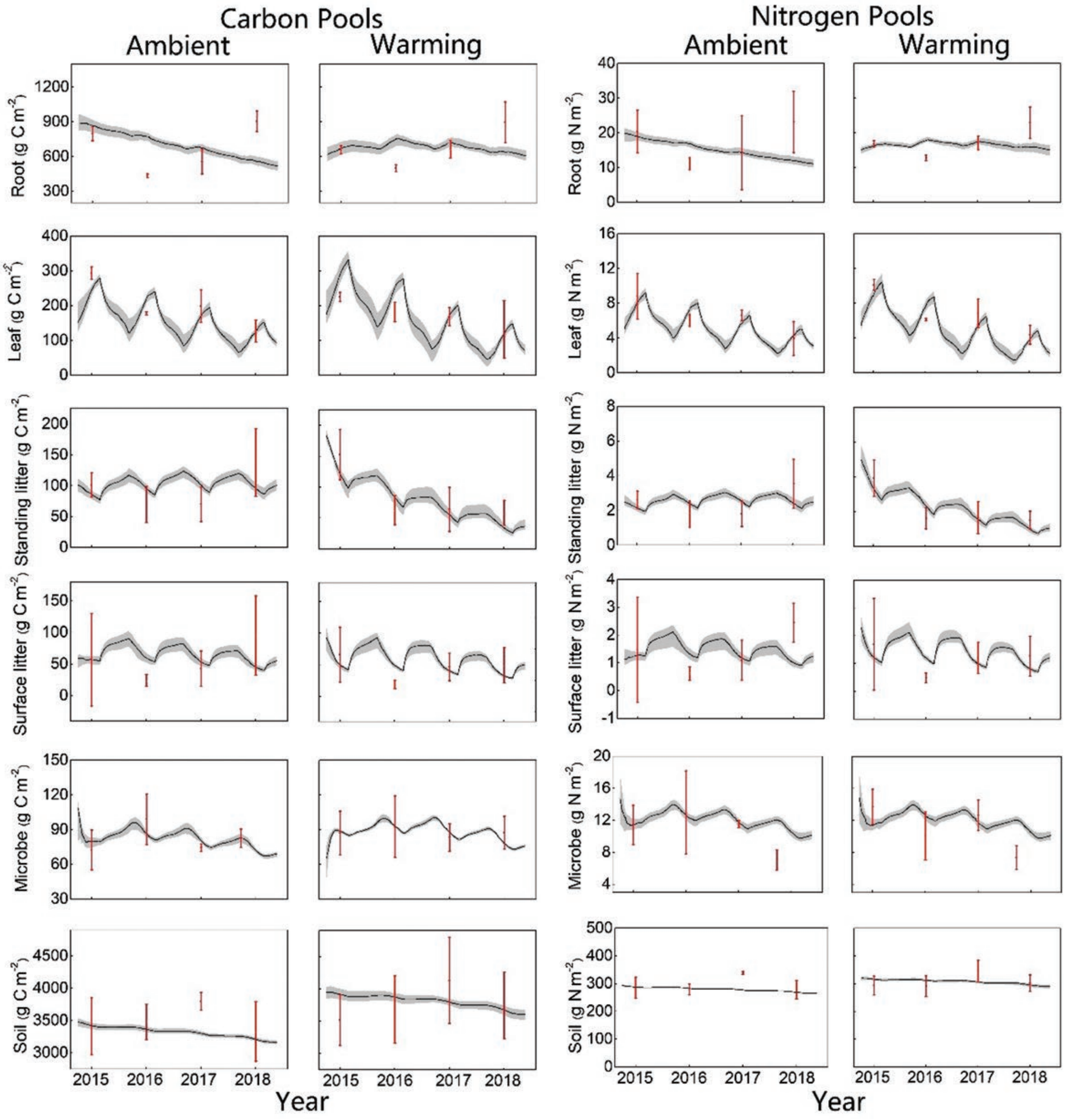

Figure 3: The comparisons of modeled versus measured C and N pools in various ecosystem components under ambient and warming treatments. The red points, black lines and shaded areas represent the observation data, model simulated lines and the range of 25% and 75% quantiles of model simulation, respectively.

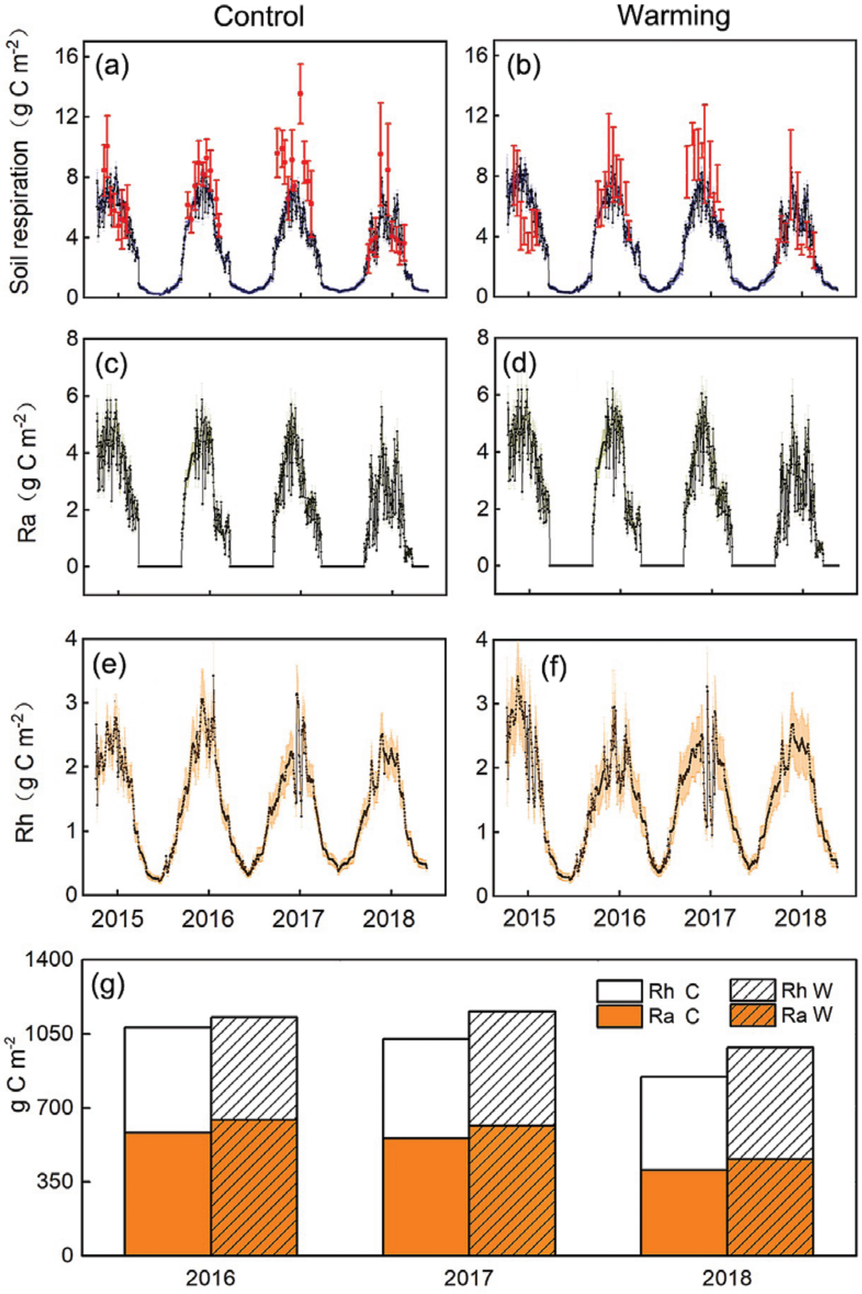

Figure 4: The comparisons of modeled versus measured SR (**a**, **b**). The red points, black lines and purple shaded areas represent the observation data, model simulated lines and the range of 25% and 75% quantiles of model simulation, respectively. Autotrophic respiration (Ra) simulation and the range of 25% and 75% quantiles (**c**, **d**), heterotrophic respiration (Rh) simulation and the range of 25% and 75% quantiles (**e**, **f**) under the ambient (left panels) and warming (right panels); (**g**) the accumulative respiration of Ra and Rh, where white column is Rh, orange column is Ra, and unfilled column represents ambient condition, diagonal stripes represent warming condition.

SOM ($P < 0.05$). At the same time, the N loss and the relative plants N uptake were also significantly decreased by warming. Furthermore, our results showed that most of the decomposition rates of ecosystem components and the distributions of C and N were significantly shifted under warming. Warming led to the increase of C decay rates in standing litter and fast SOM, which mean the decrease of C residence time in these ecosystem components. By contrast, warming resulted in the decrease of C decay rate in fine roots and slow SOM, leading to slower turnover under warming. In contrast, the C decay rates in foliage, surface litter and the allocation coefficients of GPP partitioned

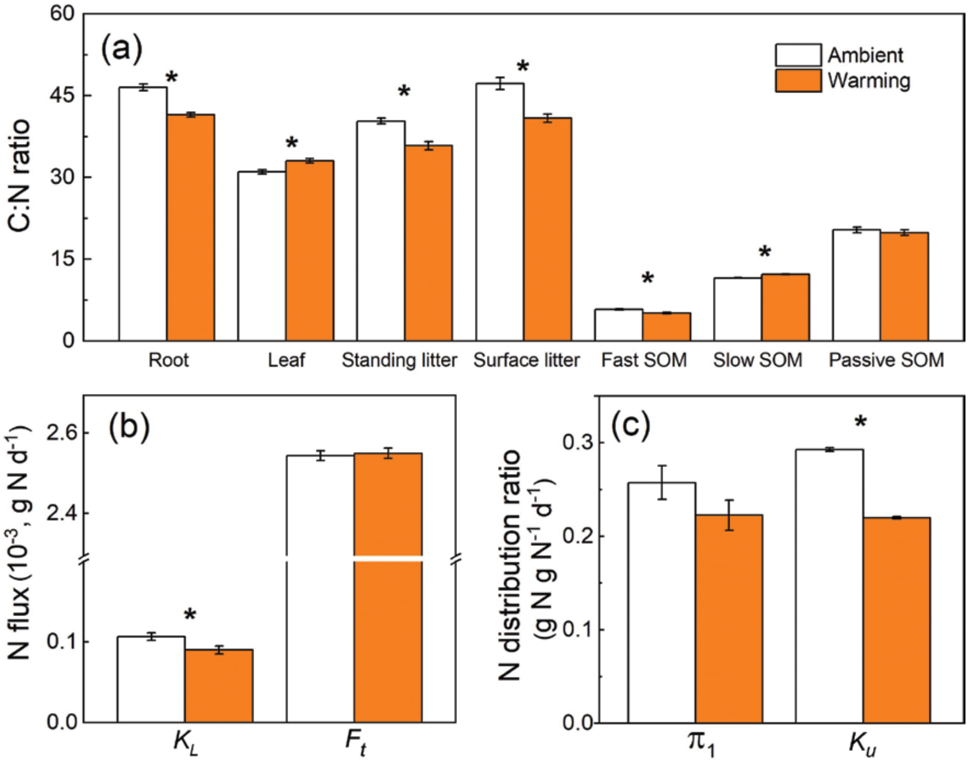

Figure 5: Key parameters of carbon–nitrogen interaction under the ambient and warming treatments. Error bars represent standard deviations of parameters. (**a**) Shifts in C:N ratios of different ecosystem components under warming treatment; (**b**) rate of N loss (K_L) and rate of N input (F_t); (**c**) the proportion of N distributed from plant to leaf (π_1) and relative rate of plant N uptake from soil (K_u). Asterisks show significant differences at the level of $P < 0.05$.

Table 1: The boundaries of priori uniform distributions and the maximum likelihood estimates (MLEs) of the posterior probability functions of some well constrained parameters

		Priori		MLE	
Parameter	**Symbol**	**Minimum**	**Maximum**	**Ambient**	**Warming**
C:N ratio of root	n_1	20	80	41.8	41.4
C:N ratio of leaf	n_2	10	60	30.9	31.4
C:N ratio of standing litter	n_3	1	100	40.2	31.2
C:N ratio of surface litter	n_4	5	160	38.0	36.2
C:N ratio of fast SOM	n_5	1	20	6.1	5.4
C:N ratio of slow SOM	n_6	10	15	11.5	11.9
C:N ratio of passive SOM	n_7	10	30	—	—
N uptake to leaves	π_1	0	0.5	—	—
Rate of N uptake (g N g N^{-1} d^{-1})	K_u	0	0.4	0.29	0.21
Rate of N loss (10^{-5} g N g N^{-1} d^{-1})	K_L	1	20	—	—
Rate of N input (10^{-3}g N m^{-2} d^{-1})	F_t	0.8	4	2.6	2.5
Initial value of available N pool (g N m^{-2})	$N_{\min}(0)$	0.05	0.5	—	—

to root and leaf were not significantly changed under warming (Supplementary Fig. S5). Warming stimulated SR on average by 10.6% in our system, and the stimulation magnitude kept increasing with year. Similarly, Rh and Ra under the warming treatment were 10.4% and 10.9% higher than the ambient treatment, respectively (Fig. 4).

DISCUSSION

This study used the data assimilation approach to reveal the changes of coupled C and N cycles in a grassland ecosystem under climate warming. Although the C:N ratios in some ecosystem components (different soil pools) and the related C and N cycle in our model cannot be measured directly and some of the observations are also incomplete (e.g. N fixation, N input), data assimilation can merge measurements with a model to estimate the states or processes of a system that could not be directly measured. By the means of data assimilation, we can improve the effectiveness of limited measurements by using a model and increase the accuracy of model estimates (Lary 1999; Luo *et al.* 2011). In this study, we used the observations of N pools in different ecosystem components and some prior knowledge about N cycling to infer N fixation, input and loss, which were not directly measured. Based on the constrained parameters, the model simulations indicated that warming significantly shifted some key processes in ecosystem C and N cycle. Those shifts of C and N coupled processes under warming can help us better understand the C–N interaction in grassland ecosystem and improve the reliability of the C and N coupling models.

Warming shifts ecosystem C:N ratios

Our results showed that warming significantly changed most coupled C–N processes. The C:N ratios of different ecosystem components shifted under warming, but with different response direction and magnitude. Meanwhile, some key processes associated with ecosystem N cycle also had a dramatic shift. We found that warming decreased C:N ratio but increased soil N availability in fast SOM pool, which are consistent with some previous reports (Gill *et al.* 2002; Hungate *et al.* 2003; Wang *et al.* 2014; Xu and Yuan 2017). Increased N availability in this ecosystem contributed to an elevated accumulation of soil N, leading to a larger N loss even though the relative proportion of N loss (K_L) had decreased. On the other hand, because plants absorbed larger N amount from soils, and more N was allocated to root under warming, combining with increased C input to both leaf and root from photosynthesis, leading to a higher C:N ratio of leaf and a lower C:N ratio of root under warming compared with those under the ambient. The increased leaf C:N ratio under warming condition indicates that warming might increase the long-term nitrogen use efficiency of plants leaves, which is in line with previous empirical observations taken by Yang and Luo (2011) and Niu *et al.* (2010).

High N availability in soil could decrease C:N ratio of surface litter by increasing N immobilization (An *et al.* 2005). Previous studies showed that microbes imported N into decomposing litter during initial decay (Gosz *et al.* 1973; Staaf and Berg 1982), indicating that higher N content in microbe under warming also contributes to the decrease of surface litter C:N ratio. The microbe C and N turnover rate increased under warming, which might reduce the size of microbe C and N pools, as well as the transfer coefficient from microbe to slow SOM. Those in combination contributed to the reduced flux from fast SOM pool (lower C:N ratio) to slow SOM pool (higher C:N ratio), and then the increased C:N ratio of slow SOM. In contrast, C:N ratio of passive SOM was relatively stable, warming have limited influence on it, which may be due to the fact that passive SOM is chemically stable and has a protracted turnover time, which meant that it would take longer time to change stoichiometry (Parton *et al.* 1993; Trumbore 1997).

Those shifts of C:N ratio revealed the mechanism underlying warming effects on ecosystem C–N interaction. In contrast to many N deficiency ecosystem, which have to raise their C:N ratio to increase N use efficiency and thereby alleviate N limitation under warming (Niu *et al.* 2010), alpine meadow ecosystem has sufficient soil nitrogen content (Zhao and Zhou 1999). Warming alleviates the temperature limitation on SOM decomposition (Chapin *et al.* 1995; Dalias *et al.* 2002), which increases soil N availability and then influences the whole ecosystem N cycle (Thornton *et al.* 2009). This increase of N released to ecosystem from soil organic pool reduces C:N ratios of most ecosystem components, thus relieving N limitation in this ecosystem.

Constraints of parameters by observation

In this study, 8 out of 12 C–N coupled parameters were well constrained by the observed data from the warming experiment. Parameters like C:N ratio of root, leaf, standing litter, surface litter, microbe and slow SOM were well constrained, which is similar to the study reported about the effect of elevated CO_2 on terrestrial ecosystem carbon cycling (Shi *et al.* 2016). Whether model parameters could be well constrained depends on the critical information contained in the observation data (Richardson *et al.* 2010; Xu *et al.* 2006). C:N ratio of leaf, root, surface litter, standing litter, fast SOM and slow SOM can be constrained well mainly because those parameters are critical for C and N predictions in GECO model, and they have high sensitivity to model performance and thus provide constraints on key processes. In addition, the poorly constrained parameters may result from the lack of information in the existing datasets (Richardson *et al.* 2010; Shi *et al.* 2016; Xu *et al.* 2006). Therefore, developing more specific or useful datasets are very essential and promising for better understanding ecosystem biogeochemical cycle. At the same time, some other factors may also impact the constraining of parameters, such as the length of observations, as short-term data may not able to provide enough information for some ecosystem components that need longer term to turn over (Lichter *et al.* 2008). For example, in this study, the passive SOM has a long turnover time, short-term experiment cannot capture C cycle processes related to this pool. Therefore, long-term experiments are invaluable in establishing models and to detect more subtle changes that cannot be captured by short-term experiments. Moreover, some information from other sources like isotope labeling can also provide more accurate and precise data to comprehend some biogeochemical cycle processes (Kuzyakov *et al.* 2000), especially those related to soil and microbial mechanisms which are not easily to be detected by traditional methods.

Implications for ecosystem carbon cycle and model development

This study reveals that alpine meadow ecosystem responds to warming by both changing C:N ratios of ecosystem components and increasing soil available N content. Changing C:N ratios of different ecosystem components reflects different adjustment strategies of ecosystem processes and functions in response to warming. Previous studies on N regulation of C cycling under climate warming mainly considered the direct dependence of mineralization and soil N availability on temperature, but neglected changes in C:N ratios in a warmer environment (Sokolov *et al.* 2008; Thornton *et al.* 2009). This may lead to an incongruous representation of the N cycle and its impact on C feedback to climate warming. Warming-induced changes in C:N ratio will also strongly affect C balance, even transfer the ecosystem from C source to C sink. For instance, a slight but a significant increase in C:N ratio of slow SOM under warming indicates that soil may have more carbon storage potential. Meanwhile, we found that C loss rate of the slow SOM pool slowed down under warming treatment with a decrease of slow SOM decomposition rate, which may reduce C emission from the soil. This shift in soil C cycle may benefit more C accumulation in slow SOM instead of being emitted as a greenhouse gas.

Changes of C and N coupled parameters under warming revealed in this study also have important implications for the establishment of ecosystem models to simulate C–N coupled processes. Because these shifts of C–N coupling parameters with warming have hardly seen to be incorporated into global-coupled C and N models used to predict C–N interactions in terrestrial ecosystems (Finzi *et al.* 2011; Liang *et al.* 2018; Luo and Schuur 2020; Sistla *et al.* 2014; Williamson *et al.* 2016; Xu and Yuan 2017). If those changes were ignored, land surface models may overestimate the N limitation on terrestrial ecosystems C dynamics and underestimate the C storage in slow SOM pool (Hungate *et al.* 2003). Biogeochemical models need to use dynamic C:N ratios under climate warming, instead of constant ones. Especially in some N limited areas, the modified C:N ratios may benefit ecosystem C sequestration in slow SOM under climate warming, which cannot be captured by models if they use a constant C:N ratio. We thus expect that the uncertainty of predictions will be largely reduced with the constrained model parameters under different climate scenarios. Furthermore, if the parameters under continuous environmental changes are constrained by further data assimilation based on the manipulative experiments, models will perform better by using those dynamic and correct parameters to simulate the ongoing and growing environmental change in the future, and it will become more feasible to adjust parameters for different sites with heterogeneity to carry out global-scale assessment.

CONCLUSIONS

Based on a 4-year field warming experiment and the data assimilation method, this study carried out an inverse analysis of coupled C and N cycles and revealed the changes of C and N coupling parameters under climate warming. We found most of those C–N coupled parameters in the model could be well constrained by the observations. Warming not only increased soil N availability but also shifted C:N ratios in the majority of ecosystem components. These changes would benefit a large C sequestration potential due to the increases of C:N ratio in slow SOM. The findings could provide a useful benchmark for model parameterization and improve terrestrial ecosystem models to predict responses of C–N coupled dynamics to future climate warming from a systematic perspective.

Supplementary Material

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

Figure S1: Posterior distributions of model parameters under ambient.

Figure S2: Posterior distributions of model parameters under warming.

Figure S3: Posterior distributions of carbon–nitrogen coupling parameters under ambient conditions.

Figure S4: Posterior distributions of carbon–nitrogen coupling parameters under warming conditions.

Figure S5: Some key parameters under different treatments.

Table S1: The N related of model and their prior ranges.

Table S2: The free parameters of model and their prior ranges.

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