

Inverse analysis of coupled carbon–nitrogen cycles against multiple datasets at ambient and elevated CO₂

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Abstract

Aims

Carbon (C) sequestration in terrestrial ecosystems is strongly regulated by nitrogen (N) processes. However, key parameters that determine the degree of N regulation on terrestrial C sequestration have not been well quantified.

Methods

Here, we used a Bayesian probabilistic inversion approach to estimate 14 target parameters related to ecosystem C and N interactions from 19 datasets obtained from Duke Forests under ambient and elevated carbon dioxide (CO₂).

Important Findings

Our results indicated that 8 of the 14 target parameters, such as C:N ratios in most ecosystem compartments, plant N uptake and external N input, were well constrained by available datasets whereas the others, such as N allocation coefficients, N loss and the initial

value of mineral N pool were poorly constrained. Our analysis showed that elevated CO₂ led to the increases in C:N ratios in foliage, fine roots and litter. Moreover, elevated CO₂ stimulated plant N uptake and increased ecosystem N capital in Duke Forests by 25.2 and 8.5%, respectively. In addition, elevated CO₂ resulted in the decrease of C exit rates (i.e. increases in C residence times) in foliage, woody biomass, structural litter and passive soil organic matter, but the increase of C exit rate in fine roots. Our results demonstrated that CO₂ enrichment substantially altered key parameters in determining terrestrial C and N interactions, which have profound implications for model improvement and predictions of future C sequestration in terrestrial ecosystems in response to global change.

Keywords: Bayesian probabilistic inversion, carbon–nitrogen interactions, carbon-nitrogen coupled model, Duke FACE.

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INTRODUCTION

Rising atmospheric carbon dioxide (CO₂) concentration usually induces carbon (C) sequestration in terrestrial ecosystems (Denman *et al.* 2007). The substantial C accumulation in land ecosystems requires additional nitrogen (N) to match due to their stoichiometric associations in both vegetation and soil (Hungate *et al.* 2003). If ecosystems cannot capture enough N to couple with the increased C storage, terrestrial C sequestration stimulated by elevated CO₂ is likely to be down-regulated

and thus cannot be sustained over time (Hungate *et al.* 2003; Luo *et al.* 2004; Reich *et al.* 2006). A conceptual framework of progressive N limitation has predicted that N limitation on future C sequestration in terrestrial ecosystems in response to rising atmospheric CO₂. The N limitation may become progressively stronger over time unless N fixation is stimulated and/or N losses are reduced, leading to increased N capital (Hungate *et al.* 1999, 2003; Luo *et al.* 2004; Rastetter *et al.* 1992). In addition, the degree of N regulation on terrestrial C sequestration depends on changes in several C–N coupling

parameters, such as the stoichiometric flexibility of C:N ratio, changes in plant N uptake via soil exploration, and N redistribution from soil to vegetation (Luo *et al.* 2004; Rastetter *et al.* 1997). Therefore, it is critical to examine changes in those C–N coupling parameters under elevated CO₂ to fully understand the strength of N regulation on C sequestration in terrestrial ecosystems.

Over the past decade, a number of CO₂ manipulative experiments have been conducted to examine C–N interactions in terrestrial ecosystems (Finzi *et al.* 2006; Gill *et al.* 2006; Hungate *et al.* 2006; Norby and Iversen 2006). These experiments have revealed the potential N regulation of terrestrial C sequestration under elevated CO₂. These experiments have also demonstrated that CO₂ enrichment may alter C–N coupling parameters in terrestrial ecosystems. For instance, C:N ratios in both plant tissues and mineral soil have been observed to exhibit consistent increases in response to rising atmospheric CO₂ (Finzi *et al.* 2006; Gill *et al.* 2006; Hungate *et al.* 2006; Yang *et al.* 2011). Moreover, elevated CO₂ usually enhances plant N uptake from mineral soil (Hungate *et al.* 2006; Norby and Iversen 2006), and results in N redistribution from mineral soil with small C:N ratio to vegetation with large C:N ratio (Gill *et al.* 2006; Hungate *et al.* 2006). In addition, elevated CO₂ may stimulate the increase of ecosystem N capital (Finzi *et al.* 2006; Luo *et al.* 2006). These experimental evidences have greatly advanced our understanding of C–N interactions in terrestrial ecosystems (Luo *et al.* 2006). However, to the best of our knowledge, these experimental results have not yet been synthesized to improve regional and global models, which are used to predict temporal and spatial patterns of terrestrial C dynamics in response to rising atmospheric CO₂.

On the other hand, land surface models have recently been employed to examine terrestrial C–N interactions by incorporating explicit N processes into the original C cycling model (Gerber *et al.* 2010; Jain *et al.* 2009; Sokolov *et al.* 2008; Thornton *et al.* 2007, 2009; Zaehle *et al.* 2010). Those modeling studies consistently revealed that C–N interactions exerted strong effects on terrestrial C dynamics and the consideration of N dynamics substantially reduced the fertilization effects of elevated CO₂ on C sequestration in terrestrial ecosystems. However, few of the models have accounted for variations of the C–N coupling parameters with global change factors. For instance, most of the models usually treated C:N ratios in various ecosystem components as fixed parameters. Without considering the dynamic nature of C–N coupling parameters, available models may lead to unrealistic prediction of future C sequestration in terrestrial ecosystems. Thus, to further improve the model prediction of future C dynamics in terrestrial ecosystems, it is essential to examine whether and how those C–N coupling parameters change with rising atmospheric CO₂.

Data assimilation techniques have been applied to optimize the model representation (Raupach *et al.* 2005) and to estimate model parameters (Wang *et al.* 2009) using actual

observations. The techniques have been successfully applied to estimate key parameters in terrestrial C cycling model, using data either from flux observations (Braswell *et al.* 2005; Knorr and Kattge 2005; Sacks *et al.* 2006; Santaren *et al.* 2007; Tang and Zhuang 2009; Wang *et al.* 2001) or the combination of flux and biometric measurements (Luo *et al.* 2003; Richardson *et al.* 2010; Weng and Luo 2011; Williams *et al.* 2005; Xu *et al.* 2006; Zhang *et al.* 2010). Moreover, data assimilation has also been performed to evaluate the changes in C cycling model parameters under various experimental treatments. For instance, using a deterministic inversion approach, Luo *et al.* (2003) examined changes in C residence time in various ecosystem components under elevated CO₂. Likewise, Zhou *et al.* (2010) investigated changes in C residence time in plant, litter and soil pools in response to experimental warming by using a Bayesian probabilistic inversion approach. However, to the best of our knowledge, the data assimilation approach has not yet been used to examine changes in C–N coupling parameters under CO₂ enrichment.

This study was designed to examine changes in C–N coupling parameters under CO₂ enrichment using data assimilation approach. Based on measurements of C and N pools in various ecosystem components (i.e. foliage, woody tissues, fine roots, microbe, forest floor soil inorganic N and mineral soil) and fluxes (i.e. litterfall, soil respiration and mineralization and plant N uptake, N input from biological fixation and atmospheric deposition) obtained from Duke Forests during the period of 1996–2005, key parameters in a coupled C and N model (i.e. C:N ratio, N uptake, N allocation coefficient, N input, N loss and the initial value of mineral soil N pool) were estimated through a Bayesian probabilistic inversion for plots with both ambient and elevated CO₂ treatments, respectively. The estimated parameters were then used for a forward analysis to examine ecosystem C and N dynamics in Duke Forests.

METHODS

Data sources

The data used in this study were obtained from the Duke Forest Free-Air CO₂ Enrichment (FACE) experiment at the Blackwood Division, Orange County, North Carolina, USA. The experiment was set on a 15-year-old loblolly pine plantation in 1996 with six plots, each with a size of 30 m in diameter (Hendrey *et al.* 1999). The CO₂ concentration in the three treatment plots has been maintained at 200 ppm above ambient, and the other three control plots have been fumigated with ambient air (Hendrey *et al.* 1999). In this study, eight C datasets from measurements during the period of 1996–2005 were used (Table 1): foliage biomass, woody biomass (Finzi *et al.* 2006), fine root biomass (Pritchard *et al.* 2008), microbial C (Allen *et al.* 2000), litterfall, forest floor C, soil C (Lichter *et al.* 2008) and soil respiration (Jackson *et al.*, 2009). The corresponding N datasets during the same period were also used (Table 1): N pools in leaf, woody tissues (Finzi *et al.* 2002, 2006), fine roots (Finzi *et al.* 2002), microbe (Finzi and

Table 1: observed variables, measurement period and reference sources used in this study

No.	Variable	Measurement period	References
1	Gross primary production	1996–2005	Weng and Luo (2011)
2	Soil moisture	1996–2005	McCarthy et al. (2010)
3	Temperature	1996–2005	McCarthy et al. (2010)
4	Soil respiration	1996–2005	Jackson et al. (2009)
5	Leaf biomass	1996–2005	Finzi et al. (2006)
6	Wood biomass	1996–2005	Finzi et al. (2006)
7	Fine root biomass	1996–2005	Pritchard et al. (2008)
8	Litterfall	1996–2005	Lichter et al. (2008)
9	Microbe biomass	1996, 1998	Allen et al. (2000)
10	Forest floor	1996, 1999, 2002, 2005	Lichter et al. (2008)
11	Mineral SOC	1996, 1999, 2002, 2005	Lichter et al. (2008)
12	Mineralization	1998, 2003	Finzi and Schlesinger (2003)
13	Leaf N	1996–2005	Finzi et al. (2002, 2006)
14	Wood N	1996–2005	Finzi et al. (2002, Finzi et al. 2006)
15	Fine root N	1996–2005	Finzi et al. (2002)
16	Litterfall N	1996–2005	Finzi and Schlesinger (2003)
17	Microbe N	1997–2000, 2002–2004	Finzi and Schlesinger (2003)
18	Forest floor N	1996, 1999, 2002, 2005	Finzi et al. (2006)
19	Mineral soil N	1996, 1999, 2002, 2005	Finzi et al. (2006)
20	Plant N uptake	1996–2005	Finzi et al. (2007)
21	Soil inorganic N	1997–2004	Finzi and Schlesinger (2003)
22	External N input	1996–2002	Finzi et al. (2006)

Schlesinger 2003; Finzi et al. 2006), forest floor and mineral soil (Finzi et al. 2006), litterfall N (Finzi et al. 2002), soil inorganic N pool and soil mineralization (Finzi and Schlesinger 2003; Finzi et al. 2006). In addition, we also used data of plant N uptake (Finzi et al. 2007) and N input from atmospheric deposition and biological fixation (Finzi et al. 2006). Those cited papers described in detail the measurement methods, times and frequencies of these available datasets.

C–N coupled model

The coupled C and N model used in this study was developed from the terrestrial ecosystem (TECO) model (Luo et al. 2003; Weng and Luo 2011; Xu et al. 2006), with eight C and N pools in addition to one mineral N pool. In our coupled C and N (TECO-CN) model, C entered the ecosystem through canopy photosynthesis and was then allocated into foliage (X_1), wood biomass (X_2) and fine roots (X_3) (Fig. 1). Similarly, N was absorbed by plants from mineral soil, and then partitioned among leaf (N_1), woody tissues (N_2) and fine roots (N_3). Plant detritus was transferred to metabolic (X_4) and structure litter (X_5) pools, and was decomposed by microbes (X_6). The structure litter (X_5) was partly respired while partly converted into fast (X_6) and slow soil organic matter (SOM, X_7). During these C cycling processes, N in plant detritus was also transferred among different ecosystem pools (i.e. litter, fast, slow and passive SOM). Mathematically, these C processes could be described by the following first-order ordinary differential equation (Luo et al. 2003):

$$\begin{aligned} \frac{d}{dt} X(t) &= \xi(t)ACX(t) + BU(t) \\ X(0) &= X_0 \end{aligned} \quad (1)$$

where $X = (x_1 \ x_2 \ x_3 \ x_4 \ x_5 \ x_6 \ x_7 \ x_8)^T$, represents C pools in leaf, wood, fine roots, metabolic litter, structure litter, microbe, slow and passive SOM, respectively. $\xi(t)$ is an environmental scalar accounting for temperature and moisture effects on C decomposition (Luo et al. 2003). A describes C transformation among various ecosystem components, given as

$$A = \begin{pmatrix} -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 \\ f_{4,1} & f_{4,2} & f_{4,3} & -1 & 0 & 0 & 0 & 0 \\ 1-f_{4,1} & 1-f_{4,2} & 1-f_{4,3} & 0 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & f_{6,4} & f_{6,5} & -1 & f_{6,7} & f_{6,8} \\ 0 & 0 & 0 & 0 & f_{7,5} & f_{7,6} & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 & f_{8,6} & f_{8,7} & -1 \end{pmatrix}$$

The non-zero elements ($f_{i,j}$) in matrix A describe C transfer coefficients (i.e. the fractions of the C entering i^{th} pool from j^{th} pool), while the zero elements indicate no C flows between these two pools (Weng and Luo 2011). C is an 8×8 diagonal matrix with diagonal entries given by vector $c = (c_1 \ c_2 \ c_3 \ \dots \ c_8)^T$, representing C exit rates (i.e. the amounts of C per unit mass

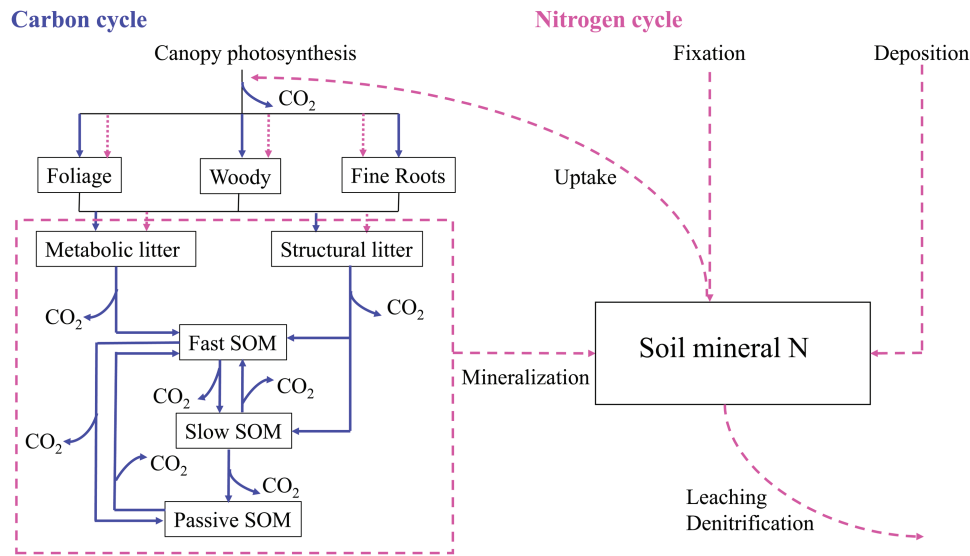


Figure 1: carbon and nitrogen pools and pathways of carbon and nitrogen fluxes in TECO-CN model. Black arrows show carbon cycling processes, while pink arrows indicate nitrogen cycling processes. SOM, soil organic matter.

leaving each pool per day). $B = (b_1 \ b_2 \ b_3 \ 0 \ 0 \ 0 \ 0)^T$ is a vector of allocation coefficients of gross primary production (GPP) partitioned to leaf, wood and fine roots. $U(t)$ represents the C input through GPP, which was simulated using MAESTRA model (Luo *et al.* 2001; Weng and Luo 2011; Xu *et al.* 2006).

The N processes can be described by:

$$\frac{d}{dt} N(t) = \xi A C R^{-1} X(t) + \kappa_u N_{\min}(t) \Pi \quad (2)$$

$$N(0) = N_0$$

where $N = (n_1 \ n_2 \ n_3 \ n_4 \ n_5 \ n_6 \ n_7 \ n_8)^T$, represents N pools in leaf, wood, fine roots, metabolic litter, structure litter, microbe, slow and passive SOM, respectively. R is an 8×8 diagonal matrix with diagonal entries given by vector $R = (r_1 \ r_2 \ r_3 \ \dots \ r_8)^T$, representing C:N ratios in the eight organic N pools. $\Pi = (\pi_1 \ \pi_2 \ 1 - \pi_1 - \pi_2 \ 0 \ 0 \ 0 \ 0 \ 0)^T$, is a vector of allocation coefficients of taken-up N to leaf, wood and fine roots. κ_u is the rate of plant N uptake, and $N_{\min}(t)$ is the amount of available N in soil at time t . The dynamics of the mineral N pool is determined by balance between N input (i.e. N mineralization, biological fixation and atmospheric deposition) and output through plant N uptake and N loss (i.e. leaching and gaseous N fluxes), which can be expressed by

$$\frac{d}{dt} N_{\min}(t) = -(\kappa_u + \kappa_L) N_{\min}(t) + \xi(t) \phi_1^* A C R^{-1} X(t) + F(t) \quad (3)$$

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

where κ_u and κ_L are rates of N uptake and loss, respectively. The second term on the right side of (3) describes the amount of N released during mineralization. $F(t)$ represents N input through biological fixation and atmospheric deposition.

Data assimilation

A Bayesian probabilistic inversion approach was used to optimize the parameters involved in the coupled C and N model. According to the Bayes' theorem, the posterior probability density function (PPDF) $P(p|Z)$ of model parameters p can be estimated from the prior knowledge of parameters p (i.e. a prior probability density function (PDF) $P(p)$) and the information contained in existing observations (i.e. a likelihood function $P(Z|p)$) (4) (Xu *et al.* 2006).

$$P(p|Z) \propto P(Z|p)P(p) \quad (4)$$

The prior PDF $P(p)$ was usually specified by giving a set of limiting intervals for parameters p with uniform distribution (e.g. Luo *et al.* 2003; Weng and Luo 2011; Xu *et al.* 2006). In this study, N-related parameters (i.e. N partitioning coefficient, N uptake, N loss, external N input, the initial mineral N pool and C:N ratio) were the main target parameters to be estimated using the Bayesian probabilistic inversion approach (Table 2). Besides these key parameters, the initial C pools, C exit rates, C allocation coefficients and C transfer coefficients were simultaneously estimated (online supplementary Table 1). The ranges of N partitioning coefficients, N uptake, N loss, external N input and the initial mineral N pool were set according to direct measurements in the Duke forest (Finzi *et al.* 2002; Finzi and Schlesinger 2003; Finzi *et al.* 2006). C:N ratios in various ecosystem components were inverted within ranges based on a global C:N stoichiometric database developed by Yang and Luo (2011). In addition, lower and upper limits of initial C pools, C exit rates, C allocation coefficients and C transfer coefficients were estimated according to a recent modeling analysis conducted in the Duke Forest (Weng and Luo 2011).

Given that errors between observed and modeled values follow Gaussian distributions (Luo et al. 2003; Weng and Luo 2011; Xu et al. 2006; Zhang et al. 2010; Zhou et al. 2010), the likelihood function $P(Z|p)$ used in this study can be expressed by

$$P(Z|p) \propto \exp \left\{ -\sum_{i=1}^8 \sum_{t \in Z_i} \frac{[Z_i(t) - \varphi_i X(t)]^2}{2\sigma_i^2(t)} \right\} \quad (5)$$

where $Z(t)$ is measured value, $\varphi X(t)$ is model simulation, and σ is the standard deviation for each measurement. φ is the mapping vector that maps the simulated state variables (C and N content of the eight pools) and fluxes to observational variables (i.e. C and N pools in plant biomass, litterfall and soil and soil respiration and mineralization).

Foliage C and N: $\varphi_1 = (0.75 \ 0 \ 0 \ 0 \ 0 \ 0 \ 0 \ 0 \ 0)$

Woody C and N: $\varphi_2 = (0 \ 1 \ 0 \ 0 \ 0 \ 0 \ 0 \ 0 \ 0)$

Fine root C and N: $\varphi_3 = (0 \ 0 \ 0.75 \ 0 \ 0 \ 0 \ 0 \ 0 \ 0)$

Litterfall C and N: $\varphi_4 = (c_1 \ 0 \ 0 \ 0 \ 0 \ 0 \ 0 \ 0 \ 0)$

Forest floor C and N: $\varphi_5 = (0 \ 0 \ 0 \ 0.75 \ 0.75 \ 0 \ 0 \ 0 \ 0)$

Microbial C and N: $\varphi_6 = (0 \ 0 \ 0 \ 0 \ 0 \ 1 \ 0 \ 0 \ 0)$

Mineral soil C and N: $\varphi_7 = (0 \ 0 \ 0 \ 0 \ 0 \ 1 \ 1 \ 1 \ 1)$

Soil respiration:

Heterotrophic respiration:

$$\varphi_8 = (0 \ 0 \ 0 \ m_4 c_4 \ m_5 c_5 \ m_6 c_6 \ m_7 c_7 \ m_8 c_8)$$

where

$$m_4 = 1 - f_{6,4}$$

$$m_5 = 1 - f_{6,5} - f_{7,5}$$

$$m_6 = 1 - f_{7,6} - f_{8,6}$$

$$m_7 = 1 - f_{6,7} - f_{8,7}$$

$$m_8 = 1 - f_{6,8}$$

Autotrophic respiration (R_a):

$$Ra = (1 - b_1 - b_2 - b_3)U(t)$$

Soil respiration (R_s):

$$Rs(t) = \xi(t)\varphi_8 X(t) + Ra$$

Soil mineralization:

$$\varphi_9 = (0 \ 0 \ 0 \ m_4 c_4 \ m_5 c_5 \ m_6 c_6 \ m_7 c_7 \ m_8 c_8)$$

The posterior PDFs for the parameters of the coupled C and N model were then generated from prior PDFs $P(p)$ with observations Z by a Markov chain Monte Carlo (MCMC) sampling technique, using the Metropolis–Hastings (M–H) algorithm (Hastings 1970; Metropolis et al. 1953) as the MCMC sampler. Specifically, the M–H algorithm was run by repeating

two steps: a proposing step and a moving step. In each proposing step, the algorithm generated a new point p^{new} for a parameter vector p based on the previously accepted point p^{old} with a proposal distribution $P(p^{\text{new}}|p^{\text{old}})$ (6).

$$p^{\text{new}} = p^{\text{old}} + \theta(p_{\text{max}} - p_{\text{min}}) \quad (6)$$

where p_{max} and p_{min} are the maximum and minimum values within the prior range of the given parameter. θ is a random variable between -0.5 and 0.5 with a uniform distribution. In each moving step, point p^{new} was tested to determine whether it should be accepted or not. Whether a new point p^{new} was accepted or not depends on the comparison of $R = \frac{P(p^{\text{new}}|Z)}{P(p^{\text{old}}|Z)}$ with a uniform random number U from 0 to 1. Only if $R \geq U$, then the new point was accepted; otherwise $p^{\text{new}} = p^{\text{old}}$ (Xu et al. 2006). The M–H algorithm were repeated until 300 000 sets of parameter values were accepted, and all the accepted parameter values were then used to construct posterior PDFs (Weng and Luo 2011).

Based on model parameters estimated from the inverse analysis, we examined the effects of elevated CO₂ on C–N coupling parameters for their statistical significance through Student’s t -test (Zhou et al. 2006). The estimated parameters from the inverse analysis were used in the TECO-CN model for a forward analysis to predict C and N dynamics under both ambient and elevated CO₂.

Sensitivity analysis was conducted when parameters were poorly constrained. We tested how sensitive the total ecosystem carbon content to the changes in each of those poorly constrained parameters. We changed each of the parameter by increasing and decreasing their values by 20% and quantified the sensitivity using an index, S . $S = [(R_{\text{ac}} - R_{\text{bc}})/R_{\text{bc}}]$, where R_{ac} is the response after change and R_{bc} is response before change.

RESULTS

Inverse analysis of C–N coupling parameters

According to the shape of posterior PDFs, model parameters can usually be grouped into three categories: well constrained, poorly constrained and edge-hitting (Braswell et al. 2005; Luo et al. 2009). Here, parameters of C:N ratios in the foliage (CN_1), woody tissues (CN_2), fine roots (CN_3), metabolic litter (CN_4), fast (CN_6) and slow (CN_7) SOM, plant N uptake (K_u) and external N input ($F(t)$) were well constrained within their prescribed ranges under both ambient and elevated CO₂ (Figs 2 and 3). However, the N allocation coefficient to foliage (π_1), the rate of N loss (K_L) and the initial value of mineral N pool ($N_{\text{min}}(0)$) were poorly constrained (Figs 2 and 3). In addition, C:N ratios in structural litter (CN_5) and passive SOM (CN_8) and N allocation coefficient to woody tissues (π_2) hit edge at the lower limits under both ambient and elevated CO₂ scenarios (Figs 2 and 3). Besides those target parameters, other model parameters were also assimilated in this study. Of them, four initial values for the foliage biomass (X_1),

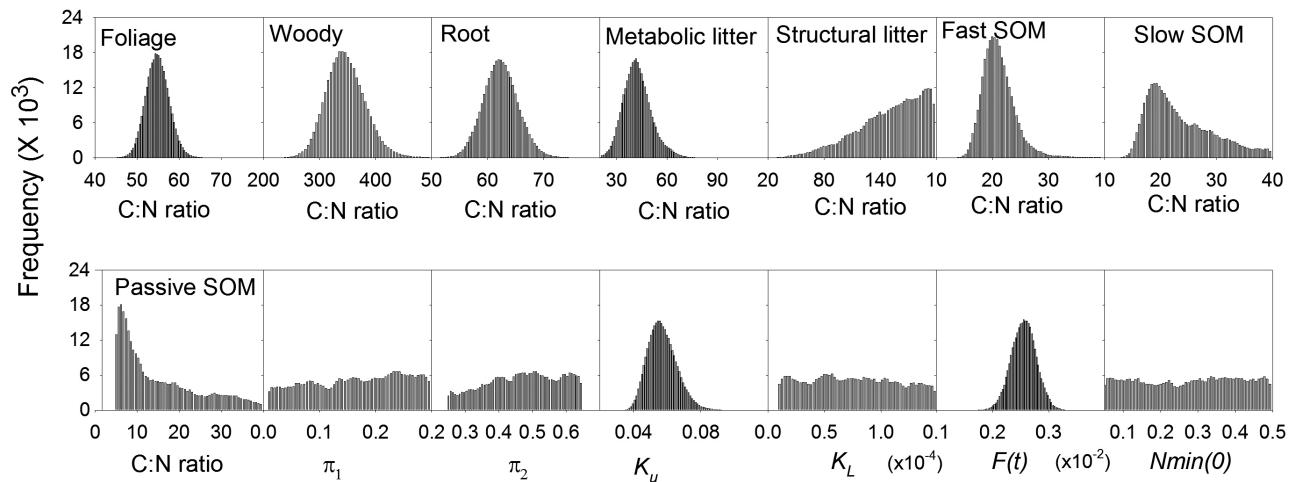


Figure 2: posterior distributions of carbon–nitrogen coupling parameters under ambient CO_2 . SOM, soil organic matter; π_1 , N uptake to leaves; π_2 , N uptake to woody tissues; κ_u , rate of N uptake; κ_r , rate of N loss; $F(t)$, rate of N input; $N_{\text{min}}(0)$, initial value of available N pool.

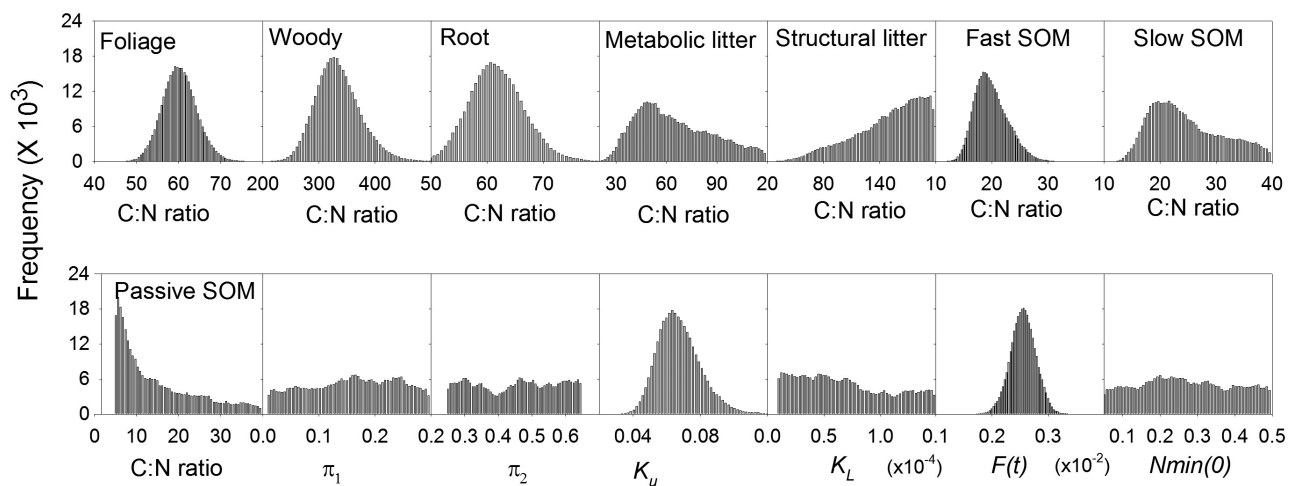


Figure 3: posterior distributions of carbon–nitrogen coupling parameters under elevated CO_2 . SOM, soil organic matter; π_1 , N uptake to leaves; π_2 , N uptake to woody tissues; κ_u , rate of N uptake; κ_r , rate of N loss; $F(t)$, rate of N input; $N_{\text{min}}(0)$, initial value of available N pool.

woody biomass (X_2), fine root biomass (X_3), slow SOM pool (X_7); three exit rates from the foliage and fine root biomass (c_1 and c_3), fast SOM pool (c_6); and three C allocation coefficients for leaf, wood and fine root pools (b_1 , b_2 and b_3) were well constrained (online supplementary Figs 1–2). In contrast, the transfer coefficients among various ecosystem components ($f_{i,j}$) were poorly constrained. By further conducting sensitivity analysis on the poorly constrained parameters, we found that those parameters had little impact on total ecosystem carbon content (online supplementary Table 3).

Comparison of parameter distributions showed that plant N uptake, C:N ratios in foliage, fine root, metabolic and structural litter were significantly higher under elevated than ambient CO_2 ($P < 0.05$), whereas CO_2 enrichment did not exert significant effects on C:N ratios in wood tissues and SOM ($P > 0.05$, Fig. 4). Moreover,

elevated CO_2 led to the decrease of C exit rates in foliage, woody biomass, structural litter and passive SOM (online supplementary Table 2), indicating the increase of C residence time in these ecosystem components. By contrast, elevated CO_2 resulted in the increase of C exit rate in fine roots, demonstrating faster fine root turnover under CO_2 enrichment. In addition, C allocation to the foliage became smaller under elevated CO_2 , while C allocation to fine roots tended to be larger under CO_2 enrichment (online supplementary Table 2).

Model prediction of C and N dynamics

Maximum likelihood estimators (MLEs, i.e. the peaks of posterior distributions) were identified for well-constrained parameters, while the sample means were calculated for those poorly constrained parameters

(online supplementary Table 2). Using these MLEs and means in combination with the forward analysis, the TECO-CN model was used to simulate ecosystem C and N dynamics in Duke Forests during the period of 1996–2005. Compared with the model prediction by default parameters, trained TECO-CN model provided reasonably well prediction for ecosystem C dynamics in the Duke Forest under both ambient (online supplementary Fig. 3) and elevated CO₂ (online supplementary Fig. 4). Our results also indicated that modeled N pools in foliage, woody tissues, fine roots and forest floor closely matched with the corresponding measurements

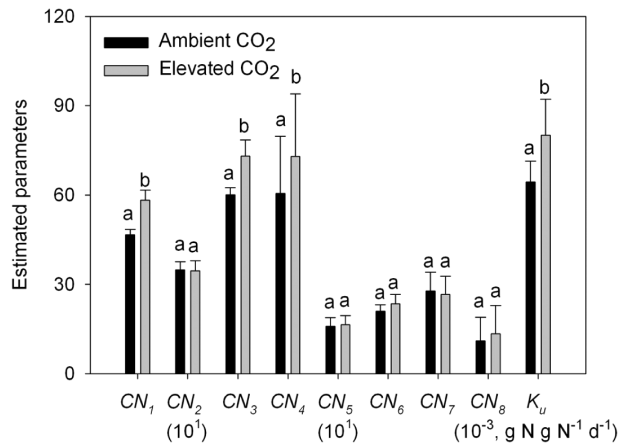


Figure 4: maximum likelihood estimators (MLEs) of carbon–nitrogen coupling parameters under ambient and elevated CO₂. Error bars represent standard deviations of parameters. CN₁, C:N ratio in foliage; CN₂, C:N ratio in woody tissues; CN₃, C:N ratio in fine roots; CN₄, C:N ratio in metabolic litter; CN₅, C:N ratio in structural litter; CN₆, C:N ratio in fast SOM (soil organic matter); CN₇, C:N ratio in slow SOM; CN₈, C:N ratio in passive SOM; K_u, rate of N uptake. Different letters show significant differences at the level of $P < 0.05$.

Table 2: target parameters of TECO-CN model and their prior ranges

Parameters	Description	Units	Lower limit	Upper limit
CN ₁	C:N ratio in foliage	—	5	120
CN ₂	C:N ratio in woody tissues	—	20	800
CN ₃	C:N ratio in fine roots	—	30	100
CN ₄	C:N ratio in metabolic litter	—	20	120
CN ₅	C:N ratio in structural litter	—	0.1	200
CN ₆	C:N ratio in fast SOM	—	5	40
CN ₇	C:N ratio in slow SOM	—	5	40
CN ₈	C:N ratio in passive SOM	—	5	40
π ₁	N uptake to leaves	—	0.01	0.30
π ₂	N uptake to woody tissues	—	0.25	0.65
κ _u	Rate of N uptake	g N g N ⁻¹ day ⁻¹	0.01	0.2
κ _l	Rate of N loss	g N g N ⁻¹ day ⁻¹	1.0 × 10 ⁻⁴	1.5 × 10 ⁻³
F(t)	Rate of N input	g N m ⁻² day ⁻¹	8.0 × 10 ⁻³	4.0 × 10 ⁻²
N ₌₍₀₎	Initial value of available N pool	g N m ⁻²	0.05	0.5

for both ambient (Fig. 5a–c) and elevated CO₂ scenarios (Fig. 6a–c). However, TECO-CN model could not capture the observed declining trend of microbial N content with time (Figs 5d and 6d). In addition, the trained model did not simulate N dynamics in mineral soil well (Figs 5e and f and 6e and f), partly due to the large variations in SOM measurements among different years.

Our TECO-CN model predicted that elevated CO₂ led to C accumulations in foliage, woody tissues, fine roots, forest floor and mineral soil, but did not alter microbial C pool during the period of 1996–2005 (Table 3). In total, elevated CO₂ stimulated the accumulation of ecosystem C pool by 20.4% over the study period. Likewise, our modeling analyses showed that elevated CO₂ increased N pools in all ecosystem components, and stimulated the increase of ecosystem N capital by 8.5% over the study period (Table 3).

DISCUSSION

To the best of our knowledge, this study is the first analysis to estimate C–N coupling parameters using data assimilation approach. Our modeling results showed that 8 of the 14 C–N coupling parameters were well constrained by the available datasets obtained from the Duke Forest, whereas the remaining C–N coupling parameters were poorly constrained by the existing datasets. Our modeling results also revealed that elevated CO₂ induced the increase of C:N ratios in vegetation and litter, the enhanced plant N uptake and the accrual of ecosystem N capital. These results demonstrate that C–N coupling parameters in Duke Forests exhibit significant changes in response to rising atmospheric CO₂. The changes in C–N coupling parameters have important implications for understanding terrestrial C–N interactions and also for improving global coupled C and N models.

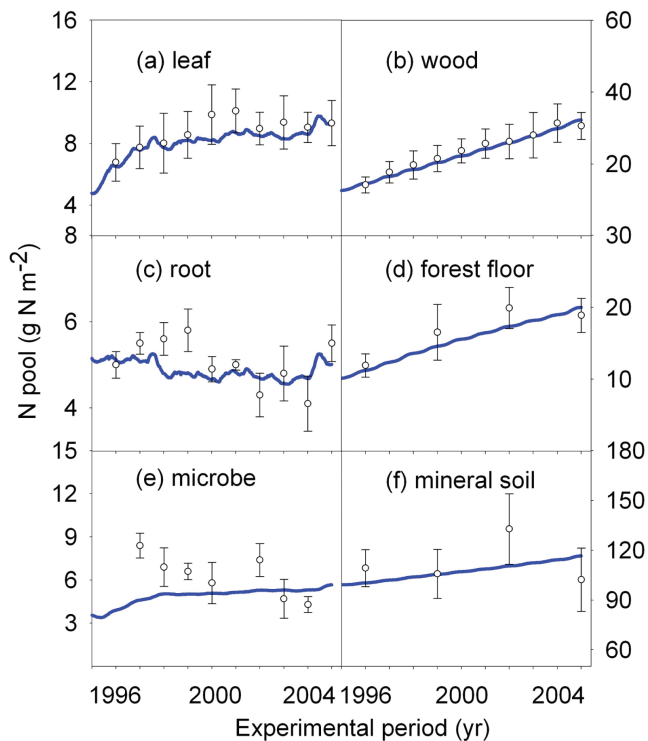


Figure 5: the comparisons of modeled versus measured nitrogen pools in various ecosystem components under ambient CO_2 .

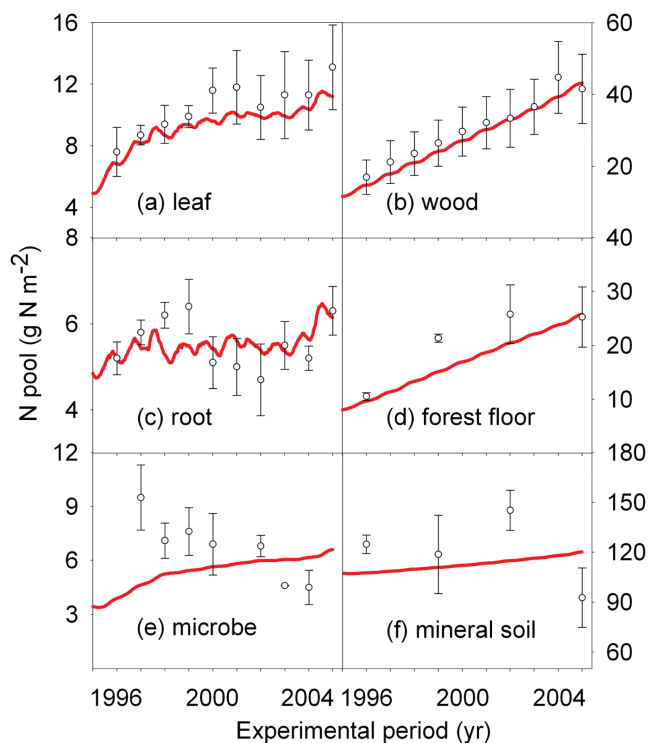


Figure 6: the comparisons of modeled vs. measured nitrogen pools in various ecosystem components under elevated CO_2 .

Constraints of C–N coupling parameters by observations

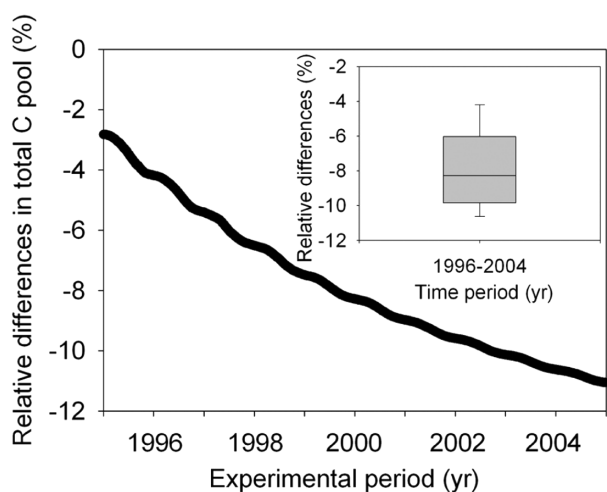
Our inverse analysis indicated that the information contained within the observations from the Duke Forest provided effective constraints on more than one half of the 14 C–N coupling parameters for TECO-CN model. The significant information contained within the observed dataset, which could exert strong effects on model predictions, might be responsible for good constraints of model parameters (Braswell *et al.* 2005; Richardson *et al.* 2010; Xu *et al.* 2006). For instance, C:N ratios in leaf, wood, root, metabolic litter and fast SOM have a large influence on the sizes of model's predicted N pools for these ecosystem components, and these parameters were particularly well-constrained. Similarly, the observed datasets of plant N uptake and external N input from biological fixation and atmospheric deposition provided enough information to constrain the corresponding model parameters (i.e. N uptake and N input). However, the remaining parameters were poorly constrained by the existing datasets. The poorly-constrained model parameters may be due to the inadequate information contained in the available datasets (Richardson *et al.* 2010; Xu *et al.* 2006). For instance, the poor constraints of C:N ratio in metabolic litter observed in this study indicates that differentiating metabolic and structural litter pools in our TECO-CN model would not be of great value unless appropriate measurements were available (Richardson *et al.* 2010; Xu *et al.* 2006). Likewise, short-term observations of C and N pools in mineral soil cannot provide enough information for C:N ratio in the passive SOM with the residence time from centuries to millennia (Xu *et al.* 2006; Zhang *et al.* 2010). Alternatively, isotope data may differentiate SOM with different residence time (Lichter *et al.* 2008), and thus the combination of isotope and biometric data could be expected to provide strong constraints for C:N ratio in the passive SOM.

Changes in C–N coupling parameters due to elevated CO_2

Our modeling results showed that C–N coupling parameters exhibited substantial flexibility under CO_2 enrichment. Of them, C:N ratios in foliage, fine roots and litter exhibited consistent increases in response to rising atmospheric CO_2 . The increases of C:N ratios in both foliage and fine roots may be due to the dilution of the N concentration by the accumulation of non-structural carbohydrates (Güsewell 2004) or the metabolic down-regulation of enzymes involved in photosynthesis under elevated CO_2 (Crous *et al.* 2010; Luo *et al.* 1994). The increase of litter C:N ratio could be driven by the increases of C:N ratio in plant tissues since litter in terrestrial ecosystems is constituted of plant detritus. In contrast, C:N ratios in wood tissues and SOM did not exhibit significant changes under CO_2 enrichment. The relatively stable C:N ratios may be related to the C pool characteristics in these two components. It is well known that C pools in these two

Table 3: the effects of CO₂ enrichment on carbon and nitrogen pools in various ecosystem components, calculated as percentage changes in carbon and nitrogen pools under elevated CO₂ compared with those under ambient CO₂

Ecosystem component	Carbon pool (g C m ⁻²)			Nitrogen pool (g N m ⁻²)		
	Ambient	Elevated	Relative changes (%)	Ambient	Elevated	Relative changes (%)
Leaf	425.1	552.7	30.0	8.03	9.17	14.2
Wood	7792.1	9538.0	22.4	22.25	27.11	21.8
Fine roots	292.5	338.9	15.9	4.87	5.49	12.7
Microbe	111.4	109.8	-1.5	4.90	5.36	9.5
Forest floor	737.9	954.2	29.3	15.31	16.80	9.7
Mineral soil	2108.1	2317.0	9.9	107.17	112.38	4.9
Total	11467.2	13810.5	20.4	162.54	176.32	8.5

**Figure 7:** relative difference in modeled total ecosystem carbon pool in the Duke Forest using two sets of parameters. First, those C–N coupling parameters derived from observations under ambient CO₂ are used to predict carbon dynamics under CO₂ enrichment. For comparison, C–N coupling parameters estimated from observations under elevated CO₂ are also used to predict carbon dynamics under CO₂ enrichment. Then, the relative difference in modeled total ecosystem carbon pool is calculated to illustrate the effects of the changes in C–N coupling parameters on model prediction. The insert is a Box and Whisker plot, showing the relative difference in modeled total ecosystem carbon pool using two sets of parameters.

components are very large and have slow turnover rate. Thus, it is less possible to detect significant differences in C:N ratios in these components during short periods.

Our modeling results also indicated that elevated CO₂ induced the increases of plant N uptake and ecosystem N capital in the Duke Forest. Theoretically, the accrual of ecosystem N capital could result from the enhanced N inputs from biological fixation and atmospheric deposition, the reduced N losses through leaching and gaseous N losses or the greater root exploration and acquisition of N in formerly root-free areas (Luo et al. 2004, 2006). The external N input from both biological fixation and atmospheric deposition may contribute to the accrual of N capital in terrestrial ecosystems, but its effect has been demonstrated to be negligible in Duke

FACE experiments (Finzi et al. 2006). Likewise, there is little evidence that the decreases in N losses through leaching or gaseous fluxes are significant under elevated CO₂ (McKinley et al. 2009). By contrast, it has been frequently reported that CO₂ enrichment may lead to greater root production and deeper root distributions (Iversen 2009), resulting in larger acquisition of N from the deep soil that are not accessed by plants under ambient CO₂ (Finzi et al. 2007; Iversen 2009). The enhanced root exploration observed in Duke Forest (Pritchard et al. 2008) could enable vegetation to absorb available N from the deep soil, and induce greater N uptake under elevated CO₂. In addition, the soil N availability stimulated by the rhizospheric interactions between plants and microbes could be an important source of N supporting the larger plant N uptake under elevated CO₂ (Finzi et al. 2007; Phillips et al. 2010). Interestingly, a recent analysis by Drake et al. (2011) has supported this point, and demonstrated that the increases in the belowground C flux under CO₂ enrichment stimulated microbial activity, accelerated the rate of SOM decomposition, stimulated plant uptake of N bound to the SOM, and thus contributed to the sustainable C sequestration in Duke Forest during the period of 1996–2005.

Implications for global-coupled C and N model development

Our results have important implications for development of coupled C and N models to improve prediction of terrestrial C sequestration as regulated by N dynamics. Our results demonstrate that C–N coupling parameters exhibit significant shifts under CO₂ enrichment. The changes in C–N coupling parameters may alleviate the degree of N limitation on terrestrial C sequestration in response to rising atmospheric CO₂ in three ways: (i) the increase in C:N ratio (i.e. N-use efficiency) can enable terrestrial ecosystems to sequester C without additional N inputs, but only in the short-term period; (ii) enhanced N uptake by plants as a result of increases in root production that stimulate SOM decomposition and N cycling alleviates N constraints to C sequestration over years to possible decades; (iii) the increase in ecosystem N capital may contribute to the long-term sustainable stimulation of terrestrial C sequestration by rising atmospheric CO₂ (Luo et al. 2004; Rastetter et al.

1997). However, these variations of the C–N coupling parameters with rising atmospheric CO₂ have not yet been incorporated into current global-coupled C and N models used to predict C–N interactions in terrestrial ecosystems. As a consequence, land surface models may overestimate the limitation of N on terrestrial C dynamics and thus result in unrealistic prediction for future C sequestration in terrestrial ecosystems (Hungate *et al.* 2003). For instance, total ecosystem C pool in the Duke Forest under CO₂ enrichment was underestimated by ~7.8% if TECO-CN model parameters at ambient conditions were used to predict C dynamics at treatment scenarios (Fig. 7). It further showed the cumulative nature of the relative difference in total C pool, indicating that the underestimation would become much greater especially for typical long-term predictions. Thus, to improve the accuracy of model prediction, the dynamic C–N coupling parameters should be incorporated into land surface models when projecting future C dynamics in terrestrial ecosystems. However, caution should be paid when extrapolating our findings to other ecosystem given our study site was a fast-growing young plantation during the study period. Responses to elevated CO₂ could be different in mature forest ecosystems.

SUPPLEMENTARY MATERIAL

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

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Conflict of interest statement. None declared.

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