



carbon dioxide (CO<sub>2</sub>) (IPCC, 2013), which usually stimulates carbon (C) accumulation in terrestrial ecosystems (Luo et al., 2006). The strength of this sink will almost certainly depend on the interactions of the C cycle with the cycles of nitrogen (N), although in some systems other essential elements like phosphorus (P) may also be important (Rastetter et al., 1992; Cleveland et al., 2013). The accumulation of C in the land ecosystem ultimately depends on stoichiometric amounts of N largely because it is required for the synthesis of the primary CO<sub>2</sub> fixing enzyme, ribulose 1, 5 biphosphate carboxylase/oxygenase and other photosynthetic enzymes (LeBauer and Treseder, 2008; Raven et al., 2004). The efficiency with which N is used by plants and its partitioning between soils and vegetation may also be important in determining how much C is sequestered in response to increased CO<sub>2</sub> concentrations. Interactions between ecosystem C and N cycles have, therefore, generated considerable interest due to their importance in determining the magnitude and sustainability of C sequestration (C<sub>seq</sub>) by terrestrial ecosystems (Jiang et al., 2016; Reich et al., 2006; Shi et al., 2016; Wieder et al., 2015).

Land surface models often ignore the constraints imposed by a limited nutrient-N supply and may overestimate terrestrial C storage (Sokolov et al., 2008; Walker et al., 2015). Some studies have, however, emphasized the importance of C—N interactions and suggest that ecosystem N dynamics can attenuate C responses to global change (Sokolov et al., 2008; Thornton et al., 2009; Thornton et al., 2007; Zaehle et al., 2010). However, C—N interactions are presented differently in different models (Zaehle et al., 2014), making it difficult to quantify C cycle modeling uncertainties and to identify the causes for these uncertainties. This has limited our understanding of the effects of N on the sensitivity of the terrestrial C cycle to global change including increased atmospheric CO<sub>2</sub> concentrations.

The significance of N effects on ecosystem C storage can be assessed by examining what may be the three key mechanisms associated with C—N interactions: changes in N capital, N redistribution between vegetation and soil, and stoichiometric flexibility (Rastetter et al., 1992; Shaver et al., 1992), all of which could be altered by elevated CO<sub>2</sub> (eCO<sub>2</sub>). For example, it has been observed that the C:N ratios of plant tissue and mineral soils increase in response to eCO<sub>2</sub> (Finzi et al., 2006; Hungate et al., 2003; Hungate et al., 2006). Total ecosystem N content may also increase under high CO<sub>2</sub> through alterations in the balance between N inputs and N outputs (Finzi et al., 2006; Sun et al., 2018). In addition, eCO<sub>2</sub> is generally associated with increases in N uptake (Finzi et al., 2007; Hungate et al., 2013; Hungate et al., 2006; Norby and Iversen, 2006) redistributing N from mineral soil to vegetation (Hungate et al., 2013; Hungate et al., 2006; Luo et al., 2006). Published results from other meta-analysis and synthesis work have also indicated significant modifications in the C:N ratios of vegetation and soil, ecosystem N capital, and the redistribution of N between vegetation and soils under eCO<sub>2</sub> (Liang et al., 2016; Luo et al., 2006; Shi et al., 2016; Terrer et al., 2018). However, the relative contributions of these mechanisms to C<sub>seq</sub> in terrestrial ecosystems have rarely been quantified.

Modeling studies have demonstrated that the regulation of ecosystem responses to eCO<sub>2</sub> by N availability varies with different time scales. For instance, Rastetter et al. (1992) predicted that, over a duration of 50 years, the redistribution of N between plant and soil pools and increases in the C:N ratio, dominated C<sub>seq</sub> in response to eCO<sub>2</sub> due to a negligible accumulation of N (less than 1%) from external nutrient sources. Other simulations suggest that (1) over time scale of days to years, ecosystem C<sub>seq</sub> is regulated mainly by flexibility in the C and N stoichiometric relationship, due to instantaneous increases in net primary productivity (NPP) through a stimulation of photosynthesis and enhanced uptake of available N by deeper plant roots (Comins and McMurtrie, 1993; Luo and Reynolds, 1999; McMurtrie and Comins, 1996; Rastetter et al., 1997); (2) over time scales of decades, ecosystem C<sub>seq</sub> is mainly determined by the accumulation of vegetative biomass due to a net shift in N from soils with lower C:N ratios to vegetation with a higher C:N ratio (Luo and Reynolds, 1999; McMurtrie and

Comins, 1996; Rastetter et al., 1997); (3) over time scales of centuries, ecosystem C<sub>seq</sub> depends on the simultaneous accumulation of organic matter in both the vegetation and soils, due to an increased ecosystem N capital, through enhanced N inputs and reduced N losses (Luo and Reynolds, 1999; Rastetter et al., 1997). To our knowledge, few studies have used experimental data to validate the mechanisms underpinning the temporal dynamics of C fixation by terrestrial ecosystems in response to eCO<sub>2</sub>.

The current study was aimed at analyzing published data on the impact of eCO<sub>2</sub> concentrations on ecosystem C and N dynamics in order to answer two main questions: (i) What is the relative contribution of the three mechanisms identified, changes in N capital, changes in C:N ratio and shifts in the partitioning of N between the soil and vegetation pools, to the increment of ecosystem C storage under eCO<sub>2</sub> and (ii) Does the relative contribution change over time?

## 2. Materials and methods

### 2.1. Data collection

We have collected two datasets in order to answer these questions. Using the first dataset, we quantified the effect of eCO<sub>2</sub> on all C pools using a meta-analysis approach and further estimated the relative contributions of the three mechanisms to the change in ecosystem C storage. These C pools and parameters associated with C—N interactions include the C and N sequestered in aboveground shoots, belowground roots, litter and soil pools; the ratios of C and N in shoots, roots, litter and soil pools; and the partitioning coefficients for N for shoots, roots, litter and soil pools. We further extracted data reporting the above variables in forest and grassland ecosystems to investigate whether the relative contributions differed between contrasting ecosystems. The second dataset was compiled from a time series of data from five sites where eCO<sub>2</sub> experiments have been conducted for multiple years. With this dataset, we explored whether the relative contributions of the different mechanisms associated with C<sub>seq</sub> changed over time.

For the first dataset, we searched the literature with the terms of “elevated CO<sub>2</sub> (or CO<sub>2</sub> enrichment, or CO<sub>2</sub> fertilization, or CO<sub>2</sub> increase)”, “carbon”, “nitrogen”, “terrestrial” using Web of Science. The resulting database (extracted from research work conducted from 1990 to 2018) contained 12 variables describing C stocks (g C m<sup>-2</sup>) in shoots (ACP), roots (BCP), litter (LCP) and soil (SCP) and the corresponding N stocks (g N m<sup>-2</sup>) in shoots (ANP), roots (BNP), litter (LNP) and soil (SNP) as well as the ratios of C and N in shoots (A-CN), roots (B-CN), litter (L-CN) and soil (S-CN). The following criteria were applied to select the papers to do further analyses: (1) the experiments with ambient and eCO<sub>2</sub> treatments were conducted in the field, where the ambient and eCO<sub>2</sub> were around the current and predicted atmospheric CO<sub>2</sub> concentrations by IPCC (2013); (2) the papers from which we extracted data included actual values or contained information that allowed us to calculate at least one of the 12 variables listed above; (3) experiments lasting for less than one growing season were excluded; (4) Data for C and N pools from the studies examined were expressed in, or could be converted to, g C m<sup>-2</sup> or g N m<sup>-2</sup> ground area. 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). Overall, there were 124 published papers (see References S1) included in the first dataset. The experimental facilities for the eCO<sub>2</sub> field studies used open top chambers (OTC) and Free Air CO<sub>2</sub> Enrichment (FACE). The ecosystem types included forest (mostly from temperate regions), grassland, wetlands, cropland, and desert. All raw data was extracted from the figures and tables in the published papers. For each of the 12 variables, we extracted the mean, standard deviation and sample size. The final database included 1680 rows of observations containing 12 main variables together with additional information (e.g., experimental facilities, ecosystem types, field sites, experimental durations, nitrogen treatments, CO<sub>2</sub> concentrations of treatments) for each site/study. More information on data

extraction and processing are available in Luo et al. (2006) and Liang et al. (2016). The estimations of C gain associated with the three mechanisms were only examined for grasslands and forests due the absence of datasets from the other sites.

For the second data set, five sites with time series data for ecosystem C and N responses to eCO<sub>2</sub> were analyzed (Table 1). The ecosystems consisted of four forests (three deciduous forests and one coniferous forest) and one grassland. These data were used to assess whether the relative contributions of the three mechanisms to ecosystem C gain varies with time. For these five sites, the duration of eCO<sub>2</sub> ranged from 3 to 15 years. For each site, the year, where the C and N responses to eCO<sub>2</sub> in plant and soil pools were reported, was recorded and the relative contributions determined.

### 2.2. Meta-analysis

The natural logarithm transformed response ratio (RR) and its variance (V<sub>RR</sub>) were calculated for each individual observation, see Borenstein et al. (2009), Hedges et al. (1999) and Schulte-Uebbing and de Vries (2018) for more details on the calculations.

$$RR = \ln \left( \frac{\bar{X}_e}{\bar{X}_c} \right) \tag{1}$$

$$V_{RR} = \left( \frac{1}{n_e \times (\bar{X}_e)^2} + \frac{1}{n_c \times (\bar{X}_c)^2} \right) \times \frac{(n_e - 1) \times s_e^2 + (n_c - 1) \times s_c^2}{n_e + n_c - 2} \tag{2}$$

where  $\bar{X}_e$  and  $\bar{X}_c$  are the mean value of each variable for the plots exposed to eCO<sub>2</sub> and ambient conditions, respectively (in g m<sup>-2</sup>); n<sub>e</sub> and n<sub>c</sub> are the corresponding number of replicates for the eCO<sub>2</sub> and ambient treatment, respectively, and s<sub>e</sub> and s<sub>c</sub> are the sample standard deviations.

We calculated the weighted means for the response ratio (RR<sup>++</sup>), as well as the weighted means for the natural logarithm transformed value (lnX<sub>c</sub><sup>++</sup>) for each line of data of the variables under ambient conditions. The random effects model calculates a weighted mean effect size by giving greater weights to observations with lower variances, which are the sum of the within-study variance and the between-study variance (due to sampling errors and variations in the

experimental conditions, respectively) (Borenstein et al., 2009). The weighted mean effect size ±1.96 standard error was used to calculate the 95% confidence interval.

Meta-analysis was performed in MetaWin 2.1 (Rosenberg et al., 2000). Ecosystem type was used as moderators to fit random effects models. The effects of eCO<sub>2</sub> concentrations on the C and N variables were considered significant if the 95% confidence interval for the response ratio did not overlap with 0. The percentage changes were estimated by (exp<sup>RR<sup>++</sup></sup> - 1) × 100%. The mean value ( $\bar{X}_c$ ++) for each variable under ambient conditions was calculated as exp<sup>(lnX<sub>c</sub><sup>++</sup>)</sup>.

### 2.3. Estimation of C gain induced by the three mechanisms and their interactions

Changes in ecosystem C storage (ΔC<sub>Eco</sub>) were attributed to three mechanisms (Eq. (3)): changes in the total ecosystem N content; redistribution of N between vegetation and soils; and flexibility of the C:N ratio in vegetation and soils. Further, we explored the relative contribution of changes in C:N ratio, total N, and N redistribution to ecosystem C accumulation using Eqs. (4), (5), and (6), respectively. The contributions of the interactions of the three mechanisms were assessed using Eqs. (7)–(10).

$$\Delta C_{Eco} = \sum_i^n \Delta C^i = \sum_i^n \left[ N_e \times N f_e^i \times \left( \frac{C}{N} \right)_e^i - N_c \times N f_c^i \times \left( \frac{C}{N} \right)_c^i \right] \tag{3}$$

Assuming the amount of N in vegetation and soils was unchanged and the C:N ratios of vegetation and soils changed due to eCO<sub>2</sub>, then the C increment due to the flexibility of the C:N ratio is:

$$\Delta C_{C:N}^i = \left( N_c \times N f_c^i \right) \times \left[ \left( \frac{C}{N} \right)_e^i - \left( \frac{C}{N} \right)_c^i \right] \tag{4}$$

In the same way, if the C:N ratios of vegetation and soils and the relative distribution of N between vegetation and soils both remain constant, then the C increment due to the change in total ecosystem N is:

$$\Delta C_{Nin}^i = (N_e - N_c) \times N f_c^i \times \left( \frac{C}{N} \right)_c^i \tag{5}$$

**Table 1**

Characteristics of the five elevated CO<sub>2</sub> sites that were analyzed for time dependent changes, along with the sources for the data used in Fig. 3. MAP, mean annual precipitation; MAT, mean annual temperature.

Name	PopFACE	ORNL FACE	Florida OTC	Duke FACE	BioCON
Location	Tuscany, Italy	Oak Ridge, TN, USA	Cape Canaveral, FL, USA	Durham, NC, USA	Cedar Creek, MN, USA
Latitude	42°22' N	35°54' N	28°38' N	35°58' N	45°24' N
Longitude	11°48' E	84°20' W	80°42' W	79°05' W	93°12' W
MAP (mm)	818	1390	1310	1140	800
MAT (°C)	14.1	14.2	21.8	15.5	6.7
Soil type	Pachic Xerumbrept	Aquic Hapludult	Paolo sand and Pomello sand	Ultic Hapludalf	Argic Udipsamments
Year of treatment initiation	1999	1998	1996	1997	1997
Species	Forest (deciduous): <i>Populus alba</i> , <i>P. nigra</i> & <i>P. euramericana</i>	Forest (deciduous): <i>Liquidambar styraciflua</i>	Forest (deciduous): <i>Quercus myrtifolia</i> , <i>Q. geminata</i> and <i>Q. chapmanii</i>	Forest (conifer): <i>Pinus taeda</i>	Grassland dominated by C3, C4 grasses, legumes and forbs
CO <sub>2</sub> (ppm)	550	550	700 (+350)	550	560
Shoot data	Finzi et al. (2007)	Finzi et al. (2007); Norby et al. (2010)	Seiler et al. (2009); Hungate et al. (2006); Hungate et al. (2013)	Finzi et al. (2006)	Cedar Creek Ecosystem Science Reserve Data <a href="http://www.lter.umn.edu/research/data/">http://www.lter.umn.edu/research/data/</a>
Root data	Finzi et al. (2007)	Finzi et al. (2007); Norby et al. (2010)	Brown et al. (2007); Hungate et al. (2013)	Finzi et al. (2006)	Cedar Creek Ecosystem Science Reserve Data <a href="http://www.lter.umn.edu/research/data/">http://www.lter.umn.edu/research/data/</a>
Litter data	Finzi et al. (2007)	Finzi et al. (2007); Norby et al. (2010)	Hungate et al. (2006); Hungate et al. (2013)	Finzi et al. (2006)	Cedar Creek Ecosystem Science Reserve Data <a href="http://www.lter.umn.edu/research/data/">http://www.lter.umn.edu/research/data/</a>
Soil data	Hoosbeek et al. (2004)	Jastrow et al. (2005)	Brown et al. (2007); Hungate et al. (2013)	Finzi et al. (2006)	Cedar Creek Ecosystem Science Reserve Data <a href="http://www.lter.umn.edu/research/data/">http://www.lter.umn.edu/research/data/</a>

Similarly, if the C:N ratios of vegetation and soils and the amount of N in vegetation and soils remain unchanged, then the C increment due to N redistribution between vegetation and soils is:

$$\Delta C_{shift}^i = N_c \times \left(\frac{C}{N}\right)_c^i \times (Nf_e^i - Nf_c^i) \quad (6)$$

The various contributions to ecosystem C gain induced by the interactions among these three mechanisms are: the interaction of total N change and C:N flexibility Eq. (7); the interaction of N redistribution and C:N flexibility Eq. (8); the interaction of total N change and N redistribution Eq. (9); and the interaction of all the three mechanisms Eq. (10).

$$\Delta C_{Nin+C:N}^i = Nf_c^i \times (N_e - N_c) \times \left[ \left(\frac{C}{N}\right)_e^i - \left(\frac{C}{N}\right)_c^i \right] \quad (7)$$

$$\Delta C_{shift+C:N}^i = N_c \times (Nf_e^i - Nf_c^i) \times \left[ \left(\frac{C}{N}\right)_e^i - \left(\frac{C}{N}\right)_c^i \right] \quad (8)$$

$$\Delta C_{Nin+shift}^i = \left(\frac{C}{N}\right)_c^i \times (N_e - N_c) \times (Nf_e^i - Nf_c^i) \quad (9)$$

$$\Delta C_{Nin+shift+C:N}^i = (N_e - N_c) \times (Nf_e^i - Nf_c^i) \times \left[ \left(\frac{C}{N}\right)_e^i - \left(\frac{C}{N}\right)_c^i \right] \quad (10)$$

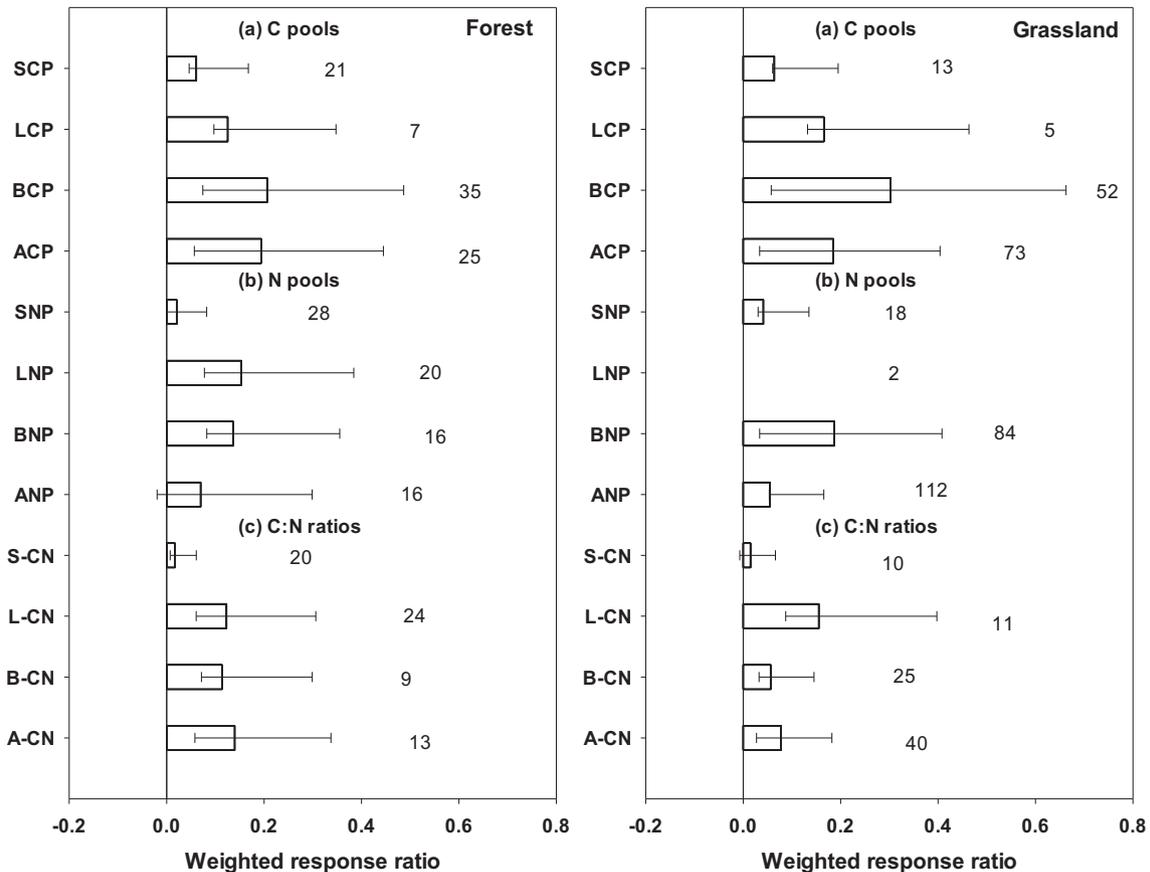
where  $N$ ,  $Nf$ ,  $\frac{C}{N}$  are total ecosystem N content, N partitioning coefficient, and C:N ratio, respectively.  $i$  = aboveground plant, belowground plant, litter, and soil pool.  $e$  = treatment,  $c$  = control.

### 3. Results

#### 3.1. Effects of eCO<sub>2</sub> on C and N accumulation in vegetation and soil

We calculated the relative and absolute change in C and N contents and C:N ratios in plant and soil pools due to eCO<sub>2</sub>. Generally, with eCO<sub>2</sub> the C and N contents increased, as did the C:N ratio in various plant pools (Fig. 1).

Elevated CO<sub>2</sub> increased the C contents of soil, plant litter, plant root, and shoot pools by 6.3%, 13.4%, 22.9%, and 21.4%, respectively in forests and by 6.6%, 18.0%, 35.3%, and 20.3%, respectively in grasslands. The N contents of soil and plant litter, root, and shoot pools were increased by 2.1%, 16.6%, 14.6%, and 7.2%, respectively in the forests and increased by 4.2%, 0%, 20.6% and 5.6%, respectively in the grasslands. However, we were only able to identify two studies that investigated the effects of eCO<sub>2</sub> on the N contents of grassland litter. The relative increase in the C:N ratio in soil and plant litter pools in response to eCO<sub>2</sub> was similar between forest and grassland ecosystems. However, the relative increase in C:N ratio in root and shoot pools in the forests were double the increase found in the grasslands, with eCO<sub>2</sub> increasing C:N ratios in root and shoot pools by approximately 12% and 15%, respectively, in the forest and by 6% and 8%, respectively, in the grasslands (Table S1).



**Fig. 1.** Results of a meta-analysis on the effect of elevated CO<sub>2</sub> concentrations on C and N pools and C:N ratios for forests (left panel) and grasslands (right panel). For (a), S-CN, L-CN, B-CN and A-CN refers to the C:N ratios in soil, litter, belowground plant and aboveground plant pools, respectively and for (b), SNP, LNP, BNP and ANP refers to the N contents in soil, litter, belowground plant and aboveground plant pools, respectively. For (c), SCP, LCP, BCP and ACP refers to the C contents in soil, litter, belowground plant and aboveground plant pools, respectively. The horizontal bars represent the 95% confidence intervals. The sample size for each variable is shown next to the bar.

For the forests, the average increase in C content due to exposure to eCO<sub>2</sub> was approximately 280.3, 52.0, 46.1, and 389.3 g C m<sup>-2</sup> higher in the soil, litter, root, and shoot pools, respectively, compared to the ambient CO<sub>2</sub> treatments. For the grasslands, whilst C accumulation in soil, litter and shoot pools at eCO<sub>2</sub> was lower than that for the forests, there was a similar accumulation of C in root pools. For the grasslands, the net accumulation of C in the soil, litter, root and shoot pools was approximately 181.9, 21.0, 54.2, and 54.4 g C m<sup>-2</sup>, respectively.

Similarly, the N contents of all the pools increased under eCO<sub>2</sub> (Fig. 1 and Table S1). For the forests, the average increase in N content was approximately 4.5, 1.4, 0.8, and 1.9 g N m<sup>-2</sup> for the soil, litter, root, and shoot pools, compared to those under ambient CO<sub>2</sub>. The average N contents of the soil, root and shoot pools under eCO<sub>2</sub> were approximately 13.5, 0.9, and 0.7 g N m<sup>-2</sup> higher, respectively, than those exposed to ambient CO<sub>2</sub>. Grasslands were generally associated with a higher N accumulation in soils, but with a similar or lower N accumulation in roots and shoots, compared with the forests. Again, these results may be biased because of limited data as only two studies on grassland litter were available, both of which showed negligible effects of eCO<sub>2</sub>. After summing the C and N contents in plant and soil pools, the average increase in total ecosystem N content was estimated to be 8.6 and 15.1 g N m<sup>-2</sup> for forests and grasslands, respectively and the corresponding average increase in the total ecosystem C content was 767.6 and 311.4 g C m<sup>-2</sup>.

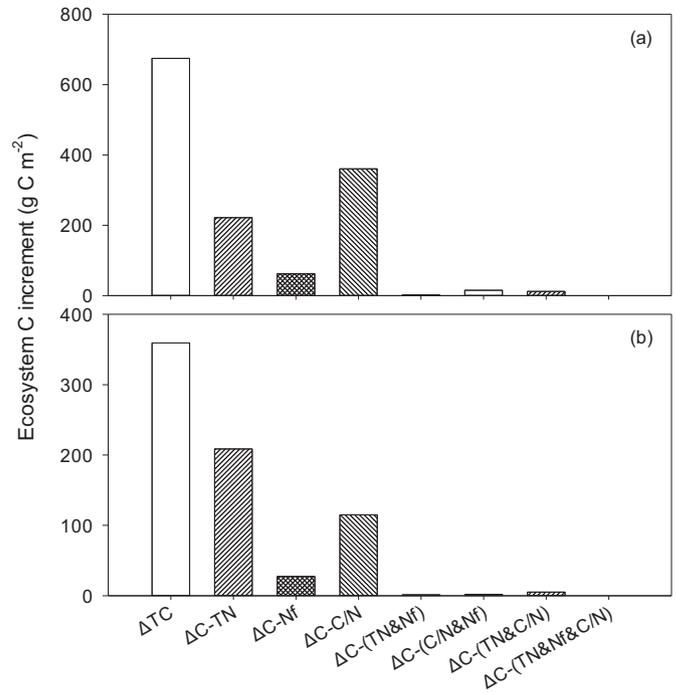
The increases in C content by eCO<sub>2</sub> were greater than the increase in N content in both plant and soil pools, resulting in enhanced C:N ratios in these pools. On average, changes in the C:N ratio in the forest were greater than in the grassland in response to eCO<sub>2</sub> (Fig. 1 and Table S1). In addition, the absolute C increment in vegetation induced by eCO<sub>2</sub> was much higher for the forest (487.3 g m<sup>-2</sup>) than for the grassland (129.6 g m<sup>-2</sup>), largely because the tree woody tissues have higher C:N ratios and a greater C storage capacity. However, the increase in soil C storage was nearly 40% less for grasslands (181.9 g m<sup>-2</sup>) compared to the increase for the forests (280.3 g m<sup>-2</sup>).

### 3.2. Relative contributions of the three mechanisms to the ecosystem C sink

For the forests, the increased C:N ratio contributed 360.5 g C m<sup>-2</sup> (~50% of the total C increment) to the enhanced ecosystem C storage found under eCO<sub>2</sub>, but increases in the total N content and changes in N partitioning between plant and soil only contributed 221.7 and 62.2 g C m<sup>-2</sup>, respectively (Fig. 2a). For the grasslands, the major contributor to the increase in C<sub>seq</sub> was an increase in the total ecosystem N capital (208.5 g C m<sup>-2</sup>, which is approximately 60% of the total C increment), followed by an increase in the C:N ratio of vegetation and soils (115.0 g C m<sup>-2</sup> yr<sup>-1</sup>) and a shift of N from soil to plants (27.3 g C m<sup>-2</sup> yr<sup>-1</sup>) (Fig. 2b). The contribution to ecosystem C storage through the redistribution of N was negligible compared to the other two mechanisms in both ecosystems (Fig. 2). The effects of the interaction terms were of minor significance compared to their individual effects (Fig. 2).

### 3.3. Temporal variation in the relative contributions

In Fig. 3 the responses of five contrasting sites exposed to eCO<sub>2</sub>, with experimental durations ranging from 3 to 15 years, are shown. More information about these sites are presented in Table 1. Our analysis showed that the contributions of the three mechanisms to changes in ecosystem C storage differed over time. Overall, the change in total N content is shown to be an important contributor to the change in ecosystem C storage, however, its contribution to ecosystem C storage had no consistent temporal pattern with increasing contributions to ecosystem C gain at the ORNL FACE site and the Duke FACE site, whilst there were decreasing contributions to ecosystem C gain at the PopFACE site and the BioCON site, over time. Nevertheless, at the Florida OTC site, the increased N loss after 6 years of treatment reduced ecosystem C<sub>seq</sub>, but after 11 years of treatment, a recovery in N availability induced a



**Fig. 2.** The contribution of changes in the total N content ( $\Delta C$ -TN), N redistribution ( $\Delta C$ -Nf), changes in C:N ratio ( $\Delta C$ -C/N) and their interactions, to the ecosystem C storage increment ( $\Delta TC$ ; g C m<sup>-2</sup>) in response to elevated CO<sub>2</sub> ( $\Delta TC$ ), for (a) forests and (b) grasslands.

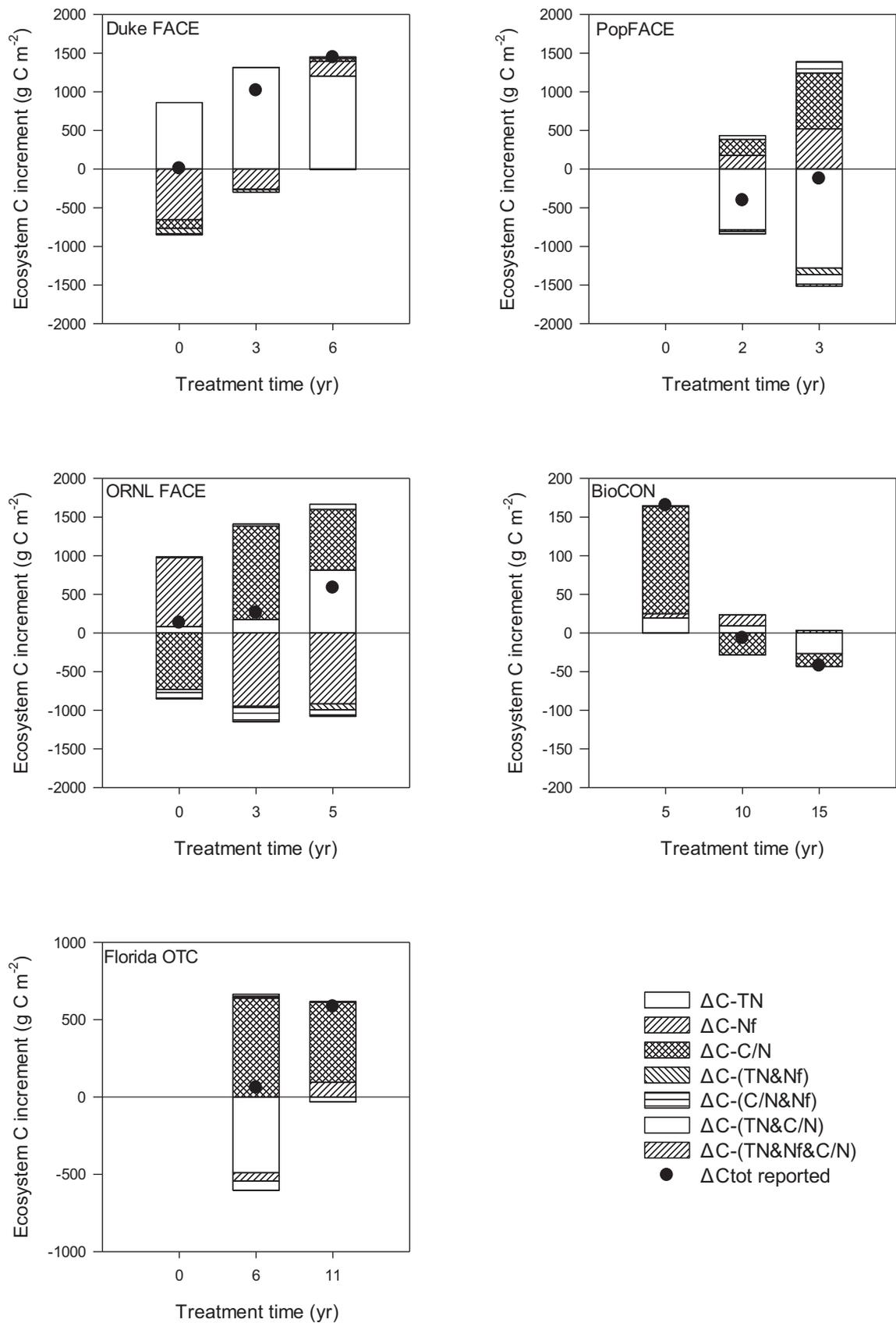
significant increase in C<sub>seq</sub>, though the combined contributions from the other two factors, C:N ratio and N partitioning, showed little variation.

The relative contributions of the flexibility of the C:N ratio to the changes in ecosystem C storage increased dramatically over time at the PopFACE site and at the ORNL FACE site, but at the Duke FACE site its contribution only increased slightly after 6 years of eCO<sub>2</sub>. In contrast, at the Florida OTC, the C:N ratio was the largest contributor, but the relative importance remained unchanged or was slightly reduced after 11 years of treatment. At the BioCON site, the flexibility of the C:N ratio was consistently the largest contributor to ecosystem C<sub>seq</sub> after 5 years of eCO<sub>2</sub>. Surprisingly, 10–15 years of eCO<sub>2</sub> reduced ecosystem C storage, due primarily to the decrease in C:N ratio and N availability, whilst the contribution of N redistribution was quite small and remained largely unchanged over the entire course of the experiment.

The contributions of N redistribution increased over time at the PopFACE, Florida OTC and Duke FACE sites, whereas its contribution decreased over time at the ORNL FACE site. Generally, N redistribution made a small or intermediate contribution to ecosystem C storage except at the ORNL FACE site. This may suggest that at the five experimental sites, at least over a short period of around 5–6 years changes in C:N ratio was a more important contributor to the increment of ecosystem C storage. Again, the interaction terms of the three mechanisms had negligible effects on changes in ecosystem C storage over time.

## 4. Discussion

This study synthesized information from a range of experimental work to investigate the influence of C—N interactions on ecosystem C storage under eCO<sub>2</sub> conditions. The reason for focusing on C—N interactions is because of the central role that N supply has in controlling photosynthesis. Most of the N taken up by plants is used to synthesize the primary carboxylating enzyme, RUBISCO, which largely determines the amount of CO<sub>2</sub> assimilated and, therefore, the amount of carbon



**Fig. 3.** Temporal variations in the C storage increment ( $\text{g C m}^{-2}$ ) induced by elevated  $\text{CO}_2$  at the PopFACE, Duke FACE, ORNL FACE, BioCON, and Florida OTC sites (see Table 1 for more details on these sites) and the contribution of changes in the total ecosystem N content ( $\Delta\text{C-TN}$ ), the C:N ratio ( $\Delta\text{C-C/N}$ ), N partitioning ( $\Delta\text{C-Nf}$ ), and their interactions.

that enters the ecosystem (LeBauer and Treseder, 2008; Raven et al., 2004). Extensive research has shown that there is an almost linear relationship between the rate of photosynthesis, RUBISCO amount and N supply, only tailing off at high N supply (Bekele et al., 2003; Evans, 1989; Evans and Seemann, 1989; Li et al., 2013). Variations in N supply will therefore be reflected in the amount of RUBISCO synthesized and this, in turn, will determine how much carbon enters the system via photosynthesis. Differences in the rate of photosynthesis for the same amount of N supplied will be related to differences in the efficiency of use of N by plants as well as by changes in the specific activity of RUBISCO (Ghannoum et al., 2005). Clearly C input will be critically dependent on N availability/C:N ratio and the ability of plants to modify the C:N ratio without compromising photosynthesis/carbon uptake. Of course, other factors, such as P availability in particular may also have an effect but the evidence indicates that N availability and an ability to modify the C:N ratio has a more significant impact.

Although frequently reported in modeling studies (Rastetter et al., 1992; Shi et al., 2016; Walker et al., 2015; Zaehle et al., 2014) changes in C and N dynamics in response to eCO<sub>2</sub> have not, to the best of our knowledge, been compared with experimental findings. Our study showed consistent results with the previous model simulations with eCO<sub>2</sub> resulting in higher C:N ratios in vegetation and litter, increased plant N uptake, and an increase in total ecosystem N content (Fig. 1). In terms of the relative importance of the three mechanisms to ecosystem C storage under eCO<sub>2</sub>, changes in the C:N ratio contributed over 50% of the forest C increment, whilst increased N accumulation and N redistribution contributing ~30% and ~9%, respectively. For the grassland, increased N accumulation contributed as much as 60% of the increase in ecosystem C storage, with changes in the C:N ratio and N redistribution contributing ~30% and ~8%, respectively (Fig. 2).

However, there are some limitations in the approach used for this study. Firstly, the elevated CO<sub>2</sub> experiments differed in the extent of CO<sub>2</sub> enrichment. The difference among studies varied from 500 ppm to more than 800 ppm. The ecosystem responses to eCO<sub>2</sub> are known to be dependent on CO<sub>2</sub> concentrations (Gill et al., 2002; Zhou et al., 2008). Therefore, comparisons might have been made by considering the difference in CO<sub>2</sub> concentrations between control plots and experimental plots, but this information was missing for many eCO<sub>2</sub> experiments especially those using OTC's. In addition, the observations collected for forest ecosystems in our database are mostly from temperate regions, thus caution is required in extrapolating our results to forests in general.

#### 4.1. Changes in the key mechanisms due to eCO<sub>2</sub>

Exposure of plants to eCO<sub>2</sub> usually stimulates leaf photosynthesis (Ainsworth and Rogers, 2007; Dusenge et al., 2018) with consequent increases in net primary productivity (De Kauwe et al., 2016; Finzi et al., 2007), which could increase the C:N ratio due to the accumulation of non-structural and structural carbohydrates (Güsewell, 2004). Any litter would, therefore, have a higher C:N ratio and fix more inorganic N, which may, in turn, result in an N limitation, thereby placing a particular emphasis on the importance of changes in C:N ratio (Rastetter et al., 1992). Changes in litter quality will affect the decomposition rate and affect soil C accumulation (Rastetter et al., 1992). Given that the relative input of N into soils was smaller than that of C, the soil C:N ratio also increased (Fig. 1).

Biological fixation and atmospheric deposition are two major external sources for the accrual of N capital in terrestrial ecosystems. N input from these two processes has been demonstrated to be significant by Liang et al. (2016) in a meta-analysis on the alleviation of progressive N limitation, and they suggest that the extra N was supplied by increased biological N fixation and decreased leaching. This may sustain ecosystem C<sub>seq</sub> under high CO<sub>2</sub> concentrations despite increases in plant N sequestration and losses of N through N<sub>2</sub>O emission. In addition, an increase in soil N availability stimulated by interactions between

plant roots and microorganisms in the rhizosphere may be important in releasing N to support a greater N uptake under high CO<sub>2</sub> (Finzi et al., 2007; Liang et al., 2016; Luo et al., 2006; Phillips et al., 2011; Shi et al., 2016). Iversen (2010) found that eCO<sub>2</sub> could stimulate fine root production and deeper root penetration (Iversen, 2010), which often resulted in more plant uptake of N from deeper soil depths, compared to plants exposed to ambient CO<sub>2</sub> (Finzi et al., 2007; Phillips et al., 2011). An increased uptake of N by enhanced root exploration of deeper parts of the soil profile has also been observed in the Duke FACE site (Finzi et al., 2006; Pritchard et al., 2008), which induced a shift in N from the soil to the above ground vegetation under eCO<sub>2</sub>.

#### 4.2. The relative importance of the three mechanisms

Simulation studies (e.g., Rastetter et al., 1992; Walker et al., 2015) demonstrate that eCO<sub>2</sub> stimulation of C cycling is mainly regulated by the three mechanisms mentioned earlier, which has also been confirmed in this study, because the amount of C<sub>seq</sub> predicted via these processes showed a good relationship with experimental data (Fig. S1). To assess the contribution of each of these mechanisms to C storage in response to eCO<sub>2</sub> a mass balance approach was used. To do this the ecosystem C increments (Hungate et al., 2006) of the different pools (aboveground shoot, belowground root, litter, and soil pools) were partitioned according to whether they were due to an increased C:N ratio, an increased shift of N from soil to vegetation, an increased N capital, or the interactive combination of these three mechanisms (Eqs. (3)–(10)).

Through an increased N input and/or reduced N losses C storage can be increased. Mechanistically this is because N availability plays a critical role in photosynthesis and there is often a stoichiometric relationship between these elements in both vegetation and soil (Hungate et al., 2003; Luo et al., 2004; Rastetter et al., 1992). Previously published work showed that leaching losses of N from terrestrial ecosystems was reduced under eCO<sub>2</sub>, though gaseous losses of N are promoted (Liang et al., 2016); in addition, inputs of N through atmospheric deposition and biological fixation are significantly higher under eCO<sub>2</sub> (Liang et al., 2016). In our synthesis, we found a net increase in ecosystem N content of approximately 8.6 and 15.1 g N m<sup>-2</sup> for forest and grassland, respectively. Based on these results, we can predict that the ecosystem C<sub>seq</sub> can be sustained by the net accrual of N capital because inputs of N are large enough to compensate for the reduction in soil N availability by N immobilization in plant biomass and SOM.

The redistribution of N between vegetation and soil under eCO<sub>2</sub> could be important, in the absence of any change in C:N ratio or total ecosystem N content. A shift of N from soil, with a C:N ratio of ~15, into long-lived plant biomass (i.e., wood) with a C:N ratio of ~200, would significantly increase N use efficiency (NUE, C fixation per unit of N) and thus increase ecosystem C storage (Hungate et al., 2003; Luo et al., 2006; Rastetter et al., 1992). However, due to the small net shift of N from soil to vegetation, its contribution to ecosystem C storage, for both forest and grassland, was relatively small (Fig. 2). The contribution of redistributed N to ecosystem C storage was higher in forests than in grasslands because the C:N ratio of woody forest vegetation (~200:1) is much higher than the C:N ratio of non-woody grassland vegetation (~50:1) (Luo et al., 2006; Rastetter et al., 1992). Therefore, the capacity of an ecosystem to increase its C storage by this mechanism will vary with the C:N ratio of the aboveground vegetation.

Under eCO<sub>2</sub> conditions, the most important contributor to C<sub>seq</sub> in both forests and grasslands was the changes in C:N ratio. Our synthesis indicated substantial increases in C:N ratio in vegetation and soil pools of ecosystems exposed to eCO<sub>2</sub> (Fig. 1). An increase in the C:N ratio can enhance ecosystem C<sub>seq</sub>, which is a direct result of an increase in NUE in the ecosystem (Luo et al., 2004). The relative contributions of an increase in C:N ratio to the ecosystem C storage increment were approximately 53% and 32% for forest and grassland, respectively (Fig. 2). Despite the increase in N content in all ecosystem compartments, eCO<sub>2</sub>

still leads to an increase in C:N ratio because C accumulates faster than N; for each incremental increase in N quantitatively more C is assimilated (Fig. 1). However, It remains unclear how long the increase in C:N ratio of compartment pools in terrestrial ecosystems will last, even if N availability declines, to sustain ecosystem  $C_{seq}$ .

#### 4.3. Temporal dependence

Generally in the early period of  $CO_2$  enrichment (< 5–6 years) changes in C:N ratio is the dominant mechanism contributing to  $C_{seq}$  (Fig. 3). During longer periods of  $eCO_2$  the redistribution of N between vegetation and soil started to play a more important role, though its contribution to ecosystem C storage was rather small. In contrast the contribution of total N change to ecosystem C storage showed an inconsistent or even an opposite trend over time, depending on the ability of the system to gain or lose N.

Modeling studies predict a peak increase in  $C_{seq}$  during the first 1–3 years due to increases in the C:N ratio in plant tissues caused by stimulated photosynthetic C fixation under  $eCO_2$ . For instance, Luo and Reynolds (1999) predicted that when  $CO_2$  concentration rose from 350 ppm to 700 ppm, the  $C_{seq}$  rate reached  $210 \text{ g C m}^{-2} \text{ yr}^{-1}$  in the first 1–3 years and declined thereafter unless enough N was supplied by either increased N mineralization or soil exploration to maintain C uptake. Rastetter et al. (1997) also found that NPP was increased by approximately  $300 \text{ g C m}^{-2} \text{ yr}^{-1}$  under high  $CO_2$  conditions during the first year and then rapidly declined within 10 years. For the PopFACE, ORNL FACE, BioCON, and Florida OTC sites the early period (2–6 years) of  $eCO_2$  resulted in increased C fixation but as the magnitude of ecosystem  $C_{seq}$  is also regulated by N supply this may explain why C storage did not increase any further at the PopFACE site or only increased marginally at the Florida OTC site (Fig. 3).

For time scales of 50–80 years, as N accumulates in vegetation to support C assimilation, there is a net shift of inorganic N from soil to vegetation (Luo et al., 2004; Rastetter et al., 1997; Reich et al., 2006). If the rate of N uptake exceeds the rate of N supply to inorganic pools, progressive N limitation may occur (Luo et al., 2004). Over 100 years, further C accumulation requires an increase in the total ecosystem N content, which will be determined by the net balance of N replenishment and N loss from the ecosystem (Luo et al., 2004; Rastetter et al., 1997; Reich et al., 2006). However, even the longest  $eCO_2$  experiments have been running for less than 20 years (Table 1), which makes it difficult to quantify the long-term contributions of these factors. At the BioCON site, however, N availability was insufficient to sustain ecosystem  $C_{seq}$ , indicating that PNL may occur in some sites over relatively shorter periods of  $eCO_2$  than might be expected (Fig. 3). Nevertheless, our results provide evidence that over the short to intermediate term, a shift of N between vegetation and soil, as well as changes in the C:N ratio are the more likely mechanisms supporting high rates of ecosystem C storage under  $eCO_2$ .

## 5. Conclusions

This study evaluated the contribution of three principal mechanisms underlying ecosystem C—N coupling and  $C_{seq}$  under  $eCO_2$ : changes in ecosystem N capital, redistribution of N between plant and soil, and changes in the C:N ratio. The predictions of their net effects are more difficult to generalize because these factors sometimes have the opposite effect and the relative magnitude of the impact will vary depending on the  $CO_2$  concentration, vegetation type/composition, and time scale. In addition, other ecosystem attributes, such as moisture availability and temperature, growing season length and soil cation exchange, may also show considerable variation. In some ecosystems, other elements, especially P, may be important in limiting C storage. Our analysis did not address the impact of these additional factors. Therefore, future work needs to be carried out in other ecosystems and a greater range of forest sites, including tropical forests and comparable sites differing in N

deposition where a direct comparison could be made of the impact of increased N inputs. Our analysis showed that  $eCO_2$  increased C and N accumulation in vegetation, litter, and soil pools, and C accumulated faster than N in these pools leading to increased C:N ratios. The relative contribution of the three mechanisms varied temporally and differed across ecosystem types. The flexibility of the C:N ratio was the dominant contributor to  $C_{seq}$  (contributed 50% of the C increment induced by  $eCO_2$ ) in forest ecosystems, with changes in N capital being the dominant contributor to  $C_{seq}$  (contributing 60% of the C increment induced by  $eCO_2$ ) in grasslands. In addition, changes in the C:N ratio made a more significant contribution to ecosystem  $C_{seq}$  during the early period of  $CO_2$  enrichment. These results suggest that ecological models may have to consider the effect of vegetation type and time scale on C—N interactions for improved predictions of the impact of global change on the terrestrial C cycle.

#### CRedit authorship contribution statement

**Junliang Zou:** Methodology, Visualization, Investigation, Writing - original draft. **Qianyu Li:** Investigation. **Bruce Osborne:** Writing - review & editing. **Yiqi Luo:** Conceptualization, Supervision.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Appendix A. Supplementary data

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