



# Processes regulating progressive nitrogen limitation under elevated carbon dioxide: a meta-analysis

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**Abstract.** The nitrogen (N) cycle has the potential to regulate climate change through its influence on carbon (C) sequestration. Although extensive research has explored whether or not progressive N limitation (PNL) occurs under CO<sub>2</sub> enrichment, a comprehensive assessment of the processes that regulate PNL is still lacking. Here, we quantitatively synthesized the responses of all major processes and pools in the terrestrial N cycle with meta-analysis of CO<sub>2</sub> experimental data available in the literature. The results showed that CO<sub>2</sub> enrichment significantly increased N sequestration in the plant and litter pools but not in the soil pool, partially supporting one of the basic assumptions in the PNL hypothesis that elevated CO<sub>2</sub> results in more N sequestered in organic pools. However, CO<sub>2</sub> enrichment significantly increased the N influx via biological N fixation and the loss via N<sub>2</sub>O emission, but decreased the N efflux via leaching. In addition, no general diminished CO<sub>2</sub> fertilization effect on plant growth was observed over time up to the longest experiment of 13 years. Overall, our analyses suggest that the extra N supply by the increased biological N fixation and decreased leaching may potentially alleviate PNL under elevated CO<sub>2</sub> conditions in spite of the increases in plant N sequestration and N<sub>2</sub>O emission. Moreover, our syntheses indicate that CO<sub>2</sub> enrichment increases soil ammonium (NH<sub>4</sub><sup>+</sup>) to nitrate (NO<sub>3</sub><sup>-</sup>) ratio. The changed NH<sub>4</sub><sup>+</sup>/NO<sub>3</sub><sup>-</sup> ratio and subsequent biological processes may result in changes in soil microenvironments, above-belowground community structures and associated interactions, which could potentially affect the terrestrial biogeochemical cycles. In addition, our data synthesis suggests that more long-term studies, especially in regions other than

temperate ones, are needed for comprehensive assessments of the PNL hypothesis.

## 1 Introduction

Fossil-fuel burning and deforestation have led to substantial increase in atmospheric carbon dioxide (CO<sub>2</sub>) concentrations, which could stimulate plant growth (IPCC, 2013). The plant growth stimulated by CO<sub>2</sub> fertilization and the resulting terrestrial carbon (C) storage could partially mitigate the further increase in CO<sub>2</sub> concentrations and associated climate warming (IPCC, 2013). However, this effect may be constrained by the availability of nitrogen (N), an essential element for molecular compounds of amino acids, proteins, ribonucleic acids (RNAs) and deoxyribonucleic acids (DNAs) in organisms (Rastetter et al., 1997; Oren et al., 2001; Luo et al., 2004; Reich et al., 2006; Norby et al., 2010; Reich and Hobbie, 2013). A popular hypothesis of the N constraint to the CO<sub>2</sub> fertilization effect is progressive N limitation (PNL) (Luo et al., 2004).

Progressive N limitation postulates that the stimulation of plant growth by CO<sub>2</sub> enrichment results in more N sequestered in plant, litter and soil organic matter (SOM) so that the N availability for plant growth progressively declines in soils over time (Luo et al., 2004). The reduced N availability then in turn constrains the further CO<sub>2</sub> fertilization effect on plant growth over longer timescales. However, whether and to what extent PNL occurs depends on the balance of N demand and supply (Luo et al., 2004; Finzi et al., 2006; Walker et al., 2015). If the N supply meets the N de-

mand, PNL may not occur. Otherwise, PNL may lead to a diminished CO<sub>2</sub> fertilization effect on plant growth over time. Some of the site-level studies support (Reich et al., 2006; Norby et al., 2010), while the others refute the PNL hypothesis (Finzi et al., 2006; Moore et al., 2006). To date, no general pattern of PNL across ecosystems has yet been revealed.

Since the key determining PNL occurrence is whether N supply meets N demand (Luo et al., 2004), it is important to understand how N supply changes under elevated CO<sub>2</sub>. The change in the N supply for plant growth under elevated CO<sub>2</sub> is determined by the responses of multiple N cycling processes, including biological N fixation, mineralization, nitrification, denitrification, and leaching (Chapin III et al., 2011). In addition, the responses of these processes to CO<sub>2</sub> enrichment may be influenced by external N addition, such as N deposition and fertilization (Reay et al., 2008). Thus, synthesizing the responses of processes that regulate PNL to CO<sub>2</sub> enrichment may help reveal the general pattern of PNL in terrestrial ecosystems.

In the current study, the main objective was to synthesize data published in the literature on the N limitation to plant growth under enriched CO<sub>2</sub> conditions. Our data synthesis was designed to answer two questions: (i) how do the major processes in the terrestrial N cycle respond to CO<sub>2</sub> enrichment? (ii) Does the CO<sub>2</sub> fertilization effect on plant growth diminish over time? To answer these questions, two sets of data from the literature were collected (Supplement Table S1, Table 1). With the first data set, we quantitatively examined the effects of CO<sub>2</sub> enrichment on all the major processes and pools in the N cycle using meta-analysis. These processes and pools included N sequestered in organic components (i.e., plant tissues, litter and soil organic matter (SOM)), biological N fixation, net mineralization, nitrification, denitrification, leaching, and total inorganic N (TIN), ammonium (NH<sub>4</sub><sup>+</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>) contents in soils. We separated the first data set according to the experimental durations to explore the responses of the N processes to short- vs. long-term CO<sub>2</sub> treatments. In addition, the responses of the N processes to CO<sub>2</sub> enrichment were compared between without and with N addition conditions. The second data set was compiled for the plant growth in decadal free air CO<sub>2</sub> enrichment (FACE) experiments. With the data set, we explored whether the CO<sub>2</sub> fertilization effect on plant growth diminishes or not over time.

## 2 Materials and methods

### 2.1 Data collection

For the first data set, a comprehensive literature search with the terms of “CO<sub>2</sub> enrichment (or CO<sub>2</sub> increase)”, “nitrogen” and “terrestrial” was conducted using the online search connection Web of Science in Endnote. Then, papers meeting the following two criteria were selected to do the fur-

ther analyses: (i) including both control and CO<sub>2</sub> enrichment treatments, where the ambient and elevated CO<sub>2</sub> concentrations were around the current and predicted atmospheric CO<sub>2</sub> concentrations by the Intergovernmental Panel on Climate Change (IPCC, 2013), respectively (Fig. S1 in the Supplement); (ii) including or from which we could calculate at least one of the major N pools or processes: soil TIN content, soil NH<sub>4</sub><sup>+</sup> content, soil NO<sub>3</sub><sup>-</sup> content, aboveground plant N pool (APNP), belowground plant N pool (BPNP), total plant N pool (TPNP), litter N pool (LNP), soil N pool (SNP), N fixation, nodule mass and/or number, net mineralization, nitrification, denitrification, and inorganic N leaching. Overall, there were 175 papers included in the first data set (Table S1, References S1). For each paper, means, variations (standard deviation (SD), standard error (SE) or confidence interval (CI)) and sample sizes of the variables in both control and CO<sub>2</sub> enrichment treatments were collected.

For those studies that provided SE or CI, SD was computed by

$$SD = SE\sqrt{n} \quad (1)$$

$$\text{or } SD = (CI_u - CI_l) \sqrt{n}/2u_p, \quad (2)$$

where  $n$  is the sample size,  $CI_u$  and  $CI_l$  are the upper and lower limits of CI, and  $u_p$  is the significant level and equal to 1.96 and 1.645 when  $\alpha = 0.05$  and 0.10, respectively. In some studies, if tissue N concentration and biomass were reported, we multiplied the two parts as N pools. When both APNP and BPNP were provided (or calculated), the two were added together to represent the TPNP. When data from multiple soil layers were provided, they were summed if they were area-based (i.e., m<sup>-2</sup> land), or averaged if they were weight-based (i.e., g<sup>-1</sup> soil). In studies where the respective contents of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> were reported, the TIN was calculated by adding the two together. For all the variables, if more than one result were reported during the experiment period, they were averaged by

$$M = \sum_{i=1}^j \frac{M_i}{j} \quad (3)$$

with standard deviation

$$SD = \sqrt{\frac{\sum_{i=1}^j SD_i^2 (n_i - 1) n_i}{\left(\sum_{i=1}^j n_i - 1\right) \sum_{i=1}^j n_i}}, \quad (4)$$

where  $j$  is the number of results,  $M_i$ ,  $SD_i$  and  $n_i$  are the mean, SD and sample size of the  $i$ th sampling data, respectively (Liang et al., 2013). If additional treatments applied (e.g., N addition), they were treated as independent studies.

Because treatment time and N addition may affect the responses of the N processes to CO<sub>2</sub> enrichment, the data set was divided into different categories: (i) short-term ( $\leq 3$  years) vs. long-term ( $> 3$  years), and (ii) without N addition vs. with N addition. Moreover, the data set was also

**Table 1.** Results on the effect of CO<sub>2</sub> enrichment on ecosystem NPP (or biomass or leaf production) in decadal free air CO<sub>2</sub> enrichment (FACE) experiments over treatment time. The values of the slope, *R*<sup>2</sup> and *P* in the linear regression in Fig. 4 are shown. The lower and upper n (i.e., n and N) in Refs. Schneider et al. (2004), McCarthy et al. (2010) and Reich and Hobbie (2013) mean without and with N addition, respectively. The lower and upper o (i.e., o and O) in Ref. Talhelm et al. (2012) mean without and with O<sub>3</sub> treatment, respectively.

Experiment	Ecosystem type	Treatment years	Variable	Slope	<i>R</i> <sup>2</sup>	<i>P</i>	Reference
Duke_n	Forest	8	NPP	0.50	0.25	0.21	McCarthy et al. (2010)
Duke_N	Forest	8	NPP	−1.39	0.27	0.29	McCarthy et al. (2010)
ORNL	Forest	11	NPP	−1.42	0.38	0.04	Norby et al. (2010)
BioCON_n	Grassland	13	Biomass	0.42	0.05	0.48	Reich and Hobbie (2013)
BioCON_N	Grassland	13	Biomass	0.23	0.01	0.76	Reich and Hobbie (2013)
NZ	Grassland	10	Biomass	0.95	0.05	0.53	Ross et al. (2013)
Swiss_n	Grassland	10	Harvestable biomass	0.30	0.01	0.75	Schneider et al. (2004)
Swiss_N	Grassland	10	Harvestable biomass	1.66	0.47	0.03	Schneider et al. (2004)
NDFF	Desert	9	Standing biomass	−9.54	0.15	0.40	Smith et al. (2014)
Aspen_o	Forest	7	Leaf production	−0.07	0.00	0.97	Talhelm et al. (2012)
Aspen_O	Forest	7	Leaf production	0.09	0.00	0.93	Talhelm et al. (2012)
AspenBirch_o	Forest	7	Leaf production	−5.27	0.77	0.01	Talhelm et al. (2012)
AspenBirch_O	Forest	7	Leaf production	6.48	0.82	0.00	Talhelm et al. (2012)
AspenMaple_o	Forest	7	Leaf production	−9.16	0.40	0.13	Talhelm et al. (2012)
AspenMaple_O	Forest	7	Leaf production	1.11	0.11	0.46	Talhelm et al. (2012)

divided into forest, grassland, and cropland to explore possible differences between ecosystem types.

For the second data set, 15 available time series of plant growth were collected from 7 decadal FACE experiments (Table 1). The ecosystems included nine forests, five grasslands and one desert. Because of the limited data, we included variables that can represent plant growth in one way or another, for example, net primary production (NPP), biomass, and leaf production. These data were collected to reveal whether the effect of CO<sub>2</sub> enrichment on plant growth diminishes over treatment time as proposed by the PNL hypothesis (Luo et al., 2004). In the seven studies, the treatment lasted from 7 to 13 years, and at least 6 years' production measurements were reported. For each data, the percentage change in NPP (or biomass or leaf production) by CO<sub>2</sub> enrichment was calculated. Then, a linear regression between the percentage change and the treatment year was conducted. A significantly negative slope indicates that the effect of CO<sub>2</sub> enrichment on the plant production diminishes over time. A non-significant slope was treated as 0. After deriving all the slopes, the frequency distribution of the slopes were fitted by a Gaussian function:

$$y = y_0 + ae^{-\frac{(x-\mu)^2}{2\sigma^2}}, \tag{5}$$

where *x* is the mean value of each individual interval, and *y* is the frequency of each interval. *y*<sub>0</sub> is the base frequency. *μ* and *σ* are the mean and SD of the distribution.

## 2.2 Meta-analysis

With the first data set, the effect of CO<sub>2</sub> enrichment for each line of data of the N variables was estimated using the natu-

ral logarithm transformed response ratio (RR) (Hedges et al., 1999; Liang et al., 2013):

$$\log_e RR = \log_e (X_E / X_C), \tag{6}$$

where *X*<sub>E</sub> and *X*<sub>C</sub> are the variable values under enriched CO<sub>2</sub> and control conditions, respectively. The variation of the log RR was

$$V = \left( \frac{SD_C^2}{n_C X_C^2} + \frac{SD_E^2}{n_E X_E^2} \right), \tag{7}$$

where *SD*<sub>C</sub> and *SD*<sub>E</sub> are the standard deviation of *X*<sub>C</sub> and *X*<sub>E</sub>, and *n*<sub>C</sub> and *n*<sub>E</sub> are the sample sizes of *X*<sub>C</sub> and *X*<sub>E</sub>.

Then, the random-effects model was used to calculate the weighted mean. In the random-effects model, the weighted mean was calculated as

$$M_{\text{weighted}} = \frac{\sum_{j=1}^k W_j^* M_j}{\sum_{j=1}^k W_j^*} \tag{8}$$

with the variance as

$$V_{\text{weighted}} = \frac{1}{\sum_{j=1}^k W_j^*}, \tag{9}$$

where *k* is the number of studies, *M*<sub>*j*</sub> is the Ln(RR) in study *j*, and *W*<sub>*j*</sub><sup>\*</sup> is the weighting factor which consists of between- and within-study variances (Rosenberg et al., 2000; Liang et al., 2013). The 95 % lower and upper limits (LL<sub>weighted</sub> and UL<sub>weighted</sub>) for the weighted mean were computed as

$$LL_{\text{weighted}} = M_{\text{weighted}} - 1.96 \times \sqrt{V_{\text{weighted}}} \tag{10}$$

and

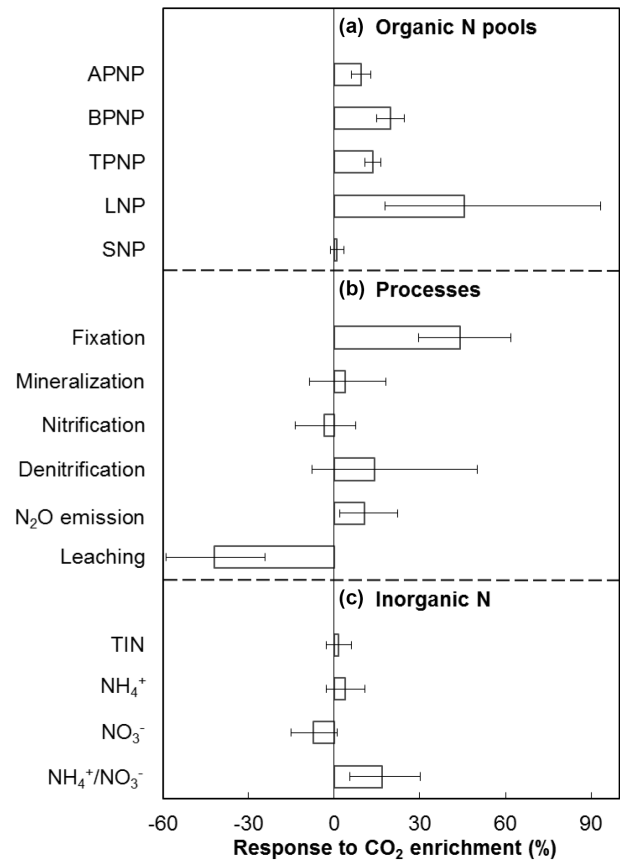
$$UL_{\text{weighted}} = M_{\text{weighted}} + 1.96 \times \sqrt{V_{\text{weighted}}} \quad (11)$$

The weighted mean and corresponding 95 % bootstrapping CI (999 iterations) for each variable and category were calculated in MetaWin 2.1 (details are described in the software handbook by Rosenberg et al., 2000). The results were back-transformed and represented as percentage change by  $(RR - 1) \times 100 \%$ . The response was considered significant if the 95 % CI did not overlap with zero.

### 3 Results

The meta-analysis of the first data set showed that CO<sub>2</sub> enrichment significantly increased N sequestered in plants and litter but not in SOM (Figs. 1a, S2). Whereas CO<sub>2</sub> enrichment had little overall effects on N mineralization, nitrification and denitrification, it significantly increased biological N fixation by 44.3 % (with 95 % CI from 29.5 to 61.8 %). The increased biological N fixation was consistent when using various methods except H<sub>2</sub> evolution (Fig. 2a). In legume species, CO<sub>2</sub> enrichment significantly increased nodule mass and number (Fig. 2b). In addition, CO<sub>2</sub> enrichment increased N<sub>2</sub>O emission by 10.7 % (with 95 % CI from 2.0 to 22.3 %), but reduced leaching (i.e., -41.8 with 95 % CI from -58.9 to -24.3 %) (Fig. 1b). Although CO<sub>2</sub> enrichment did not change the total inorganic N availability in soils, it increased the soil NH<sub>4</sub><sup>+</sup>/NO<sub>3</sub><sup>-</sup> ratio by 16.9 % (with 95 % CI from 5.4 to 30.2 %) (Fig. 1c).

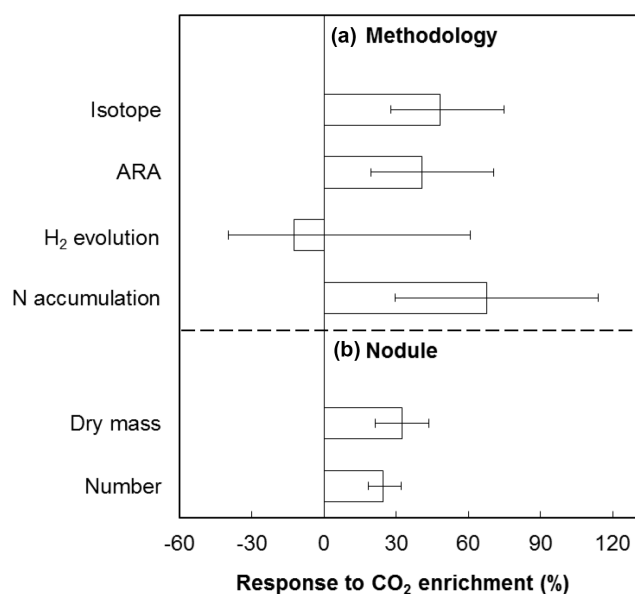
Treatment time had no effect on most of the variables (overlapped 95 % CIs for short- and long-term treatments) except nitrification, which was not changed by short-term treatment, but was significantly reduced (-23.4 with 95 % CI from -30.4 to -12.1 %) by long-term CO<sub>2</sub> enrichment (Fig. 3b). In addition, it seemed that the responses of the NH<sub>4</sub><sup>+</sup>/NO<sub>3</sub><sup>-</sup> ratio was strengthened over time, representing a neutral response to short-term CO<sub>2</sub> enrichment, but significantly positive and negative responses to long-term CO<sub>2</sub> enrichment (Fig. 3c). The effects of CO<sub>2</sub> enrichment were influenced by N addition (Fig. 3d-f). For example, nitrification was significantly reduced by CO<sub>2</sub> enrichment without N addition by 19.3 % (with 95 % CI from -40.5 to -0.65 %), but was not changed with N addition. Denitrification and N<sub>2</sub>O emission responded to CO<sub>2</sub> enrichment neutrally without N addition, but significantly positively with N addition (Fig. 3e). Additionally, the responses of some variables to CO<sub>2</sub> enrichment were dependent on ecosystem type (Fig. 3g-i). APNP responded to CO<sub>2</sub> enrichment positively in forests and croplands, but neutrally in grasslands (Fig. 3g). Net mineralization had no response to CO<sub>2</sub> enrichment in forests or grasslands, while it was significantly increased in croplands (Fig. 3h). Moreover, the change in the TIN was neutral in forests, grassland, but positive, in croplands, re-



**Figure 1.** Results of a meta-analysis on the responses of nitrogen pools and processes to CO<sub>2</sub> enrichment. In (a), APNP, BPNP, TPNP, LNP, and SNP are the abbreviations for aboveground plant nitrogen pool, belowground plant nitrogen pool, total plant nitrogen pool, litter nitrogen pool, and soil nitrogen pool, respectively. In (c), TIN, NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> are total inorganic nitrogen, ammonium, and nitrate in soils, respectively. The error bars represent 95 % confidence intervals.

spectively (Fig. 3i). In addition, a positive response of the NH<sub>4</sub><sup>+</sup>/NO<sub>3</sub><sup>-</sup> ratio was only observed in grasslands (Fig. 3i).

The results from the second data set showed that CO<sub>2</sub> enrichment significantly increased plant growth in most of the decadal FACE experiments (Fig. 4). In addition, the CO<sub>2</sub> fertilization effect on plant growth did not over treatment time change in 11 experiments ( $P > 0.05$ ), decreased in 2 experiments (slope  $< 0$ ,  $P < 0.05$ ), and increased in 2 experiments (slope  $> 0$ ,  $P < 0.05$ ), respectively (Table 1, Fig. 4). Overall, the slope of the response of the plant growth vs. treatment time was not significantly different from 0 (i.e.,  $-0.37 \text{ % year}^{-1}$  with 95 % CI from  $-1.84$  to  $1.09 \text{ % year}^{-1}$ ; Fig. 4).



**Figure 2.** Responses of biological N fixation measured by different methods (a) and nodule dry mass and number in legume species (b). ARA: acetylene reduction assay. Mean  $\pm$  95 % confidence interval.

## 4 Discussion

In this study, we carried out two syntheses on the responses of the terrestrial N cycle and plant growth to CO<sub>2</sub> enrichment to test whether PNL generally occurs across ecosystems.

### 4.1 PNL alleviation

According to the PNL hypothesis, a prerequisite for PNL occurrence is that more N is sequestered in plant, litter and SOM (Luo et al., 2004). Our results showed that elevated CO<sub>2</sub> significantly increased N retention in plant tissues and litter, which is consistent with previous meta-analyses (de Graaff et al., 2006; Luo et al., 2006). Thus, there seems to be evidence for some basic assumptions of the PNL hypothesis. However, the results from the second data set did not show a general diminished CO<sub>2</sub> fertilization effect on plant growth on the decadal scale, which disagrees with the expectation of the PNL hypothesis, suggesting that N supply under elevated CO<sub>2</sub> may meet the N demand. In this study, we have identified two processes that increase N supply under elevated CO<sub>2</sub>, i.e., biological N fixation and leaching.

CO<sub>2</sub> enrichment significantly enhanced the N influx to terrestrial ecosystems through biological N fixation, which reduces dinitrogen (N<sub>2</sub>) to NH<sub>4</sub><sup>+</sup> (Fig. 1b). The enhanced biological N fixation may have resulted from the stimulated activities of symbiotic (Fig. 2b) and free-living heterotrophic N-fixing bacteria (Hoque et al., 2001). In addition, the competition between N<sub>2</sub>-fixing and non-N<sub>2</sub>-fixing species may have contributed to enhancing the biological N fixation at the

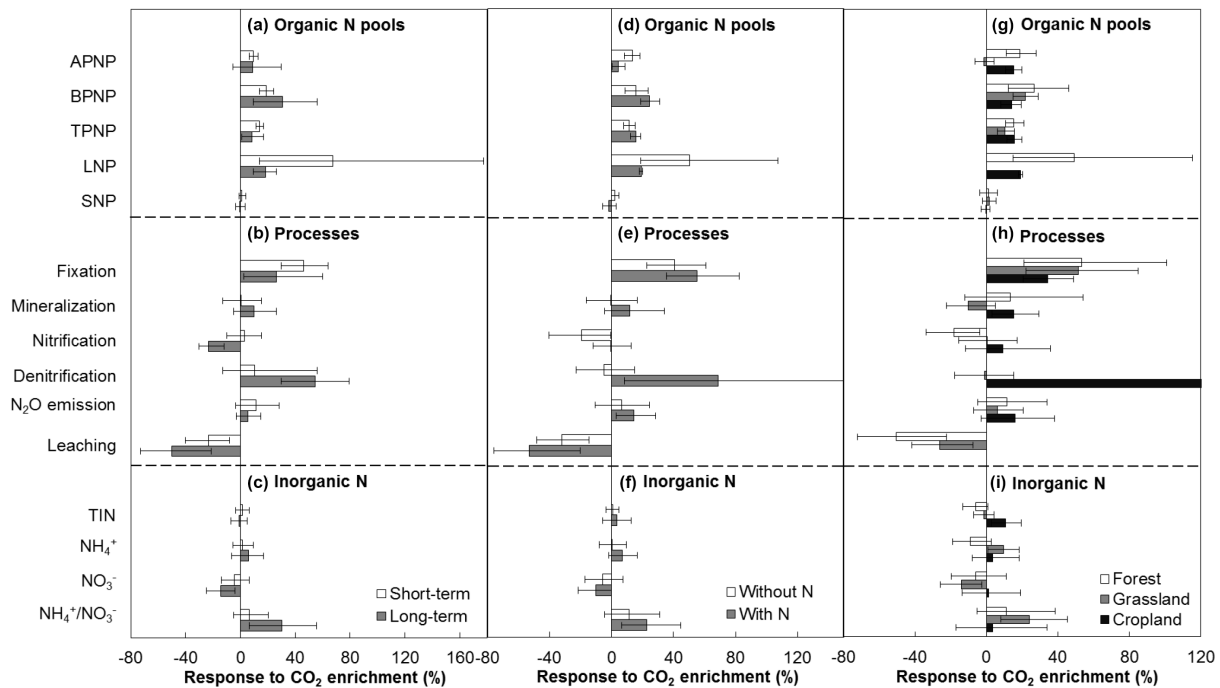
ecosystem level (Poorter and Navas, 2003; Batterman et al., 2013).

In addition, the N efflux via leaching was reduced under elevated CO<sub>2</sub> conditions (Fig. 1b). This could be attributed to the decrease in NO<sub>3</sub><sup>-</sup>, which is the primary N form in leaching. (Chapin III et al., 2011), and the increased root growth which may immobilize more inorganic N in soils (Luo et al., 2006; Iversen, 2010). In contrast, gaseous N loss through N<sub>2</sub>O emission increased under elevated CO<sub>2</sub>, although this increase was only observed when additional N was applied.

The net effect of the responses of N processes to CO<sub>2</sub> enrichment resulted in higher N retention in ecosystems, especially within plant tissues and litter (Fig. S2). Because the product of biological N fixation (i.e., NH<sub>4</sub><sup>+</sup>) and the primary form for N leaching loss (i.e., NO<sub>3</sub><sup>-</sup>) can be directly used by plants, the effects of CO<sub>2</sub> enrichment on the two processes directly increase the N availability for plant growth, potentially alleviating PNL (Fig. 5). The increased N in plant tissues can be re-used by plants via resorption (Norby et al., 2000, 2001), and consequently reduce the N demand from soils. This may be another mechanism that alleviates PNL (Walker et al., 2015). Therefore, the increased N availability from increased N fixation and reduced N leaching could potentially support net accumulation of organic matter in terrestrial ecosystems (Rastetter et al., 1997; Luo and Reynolds, 1999).

Since biological N fixation provides at least 30 % of the N requirement across natural biomes (Asner et al., 2001; Galloway et al., 2004), our results suggest that the positive response of biological N fixation to CO<sub>2</sub> enrichment plays an important role in alleviating PNL. The PNL hypothesis was proposed to characterize long-term dynamics of C-N coupling in response to rising atmospheric CO<sub>2</sub> concentration. Thus, it is critical to understand the long-term response of biological N fixation to elevated CO<sub>2</sub>. In this paper, we have synthesized 12 studies that lasted 4–7 years and binned them in a long-term category (> 3 years). On average, in those long-term studies, CO<sub>2</sub> enrichment increased biological N fixation by 26.2 %. The increased biological N fixation is supported by evidence at gene level from long-term experiments. For example, Tu et al. (2016) found that the abundance of *nifH* gene amplicons, which is a widely used marker for analysing biological N fixation, was significantly enhanced by 12 years of CO<sub>2</sub> enrichment in a grassland (BioCON). However, our synthesis showed a relatively wide 95 % confidence interval from 2.54 to 59.8 %. The wide range can be partially attributed to the relatively small number of studies. In addition, most studies incorporated in the current synthesis were conducted in temperate regions. Thus, longer-term studies, as well as studies in other regions (e.g., boreal and tropical) are critically needed to reveal more general patterns in the future.

In this study, it is suggested that the general trend of the N cycle changes under elevated CO<sub>2</sub> converges towards in-



**Figure 3.** Responses of terrestrial nitrogen pools and processes to CO<sub>2</sub> enrichment (Mean ± 95 % confidence interval) as regulated by experimental durations (a–c; short-term: ≤ 3 years vs. long-term: > 3 years), nitrogen addition (d–f), and ecosystem types (g–i). Please see Fig. 1 for abbreviations.

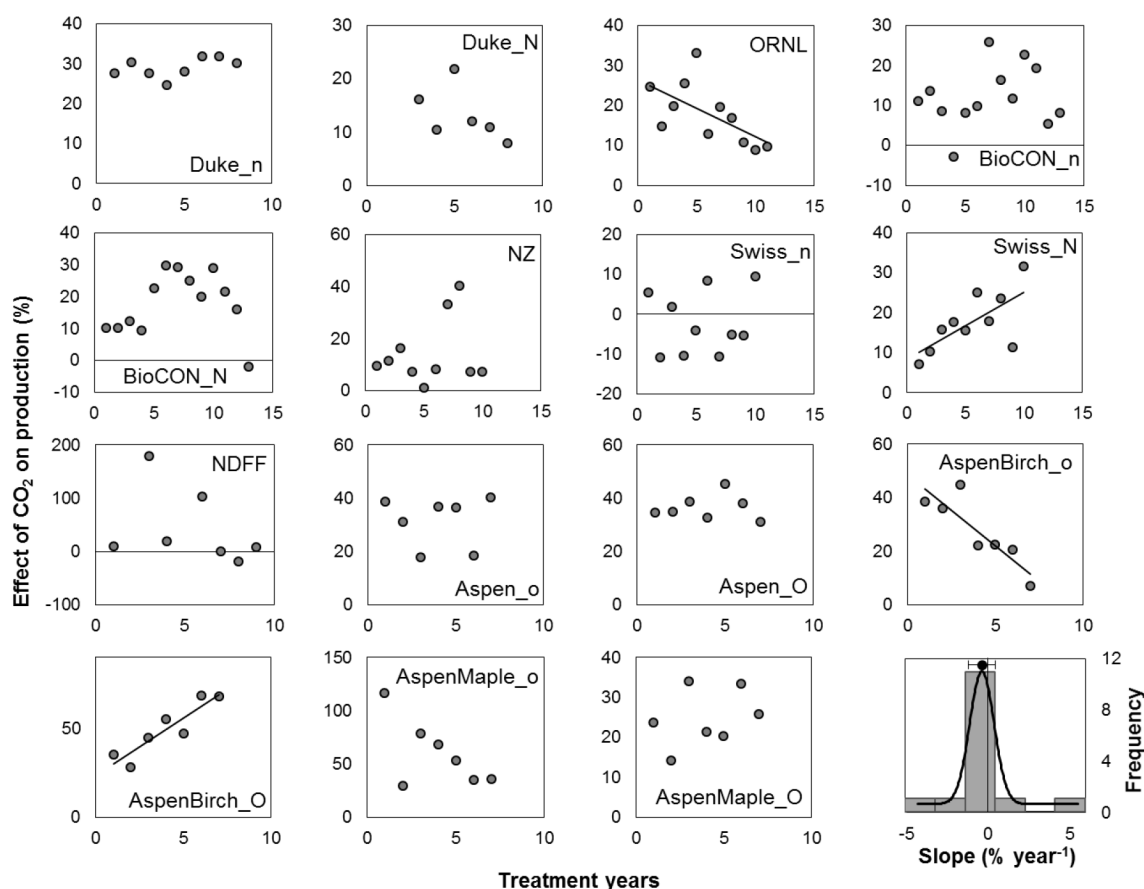
creased soil N supply for plant growth, which in theory could alleviate PNL. However, the PNL alleviation potential may vary across different ecosystems due to asymmetric distributions of biological N fixation (Cleveland et al., 1999). In addition, PNL alleviation may also be influenced by other factors. While a diminished CO<sub>2</sub> fertilization effect on plant growth was not observed in most of the long-term experiments, it occurred in two sites (i.e., ORNL and Aspen-Birch) (Fig. 4). Plant growth is usually influenced by multiple environmental factors (e.g., nutrients, water, light, ozone). The undiminished CO<sub>2</sub> fertilization effect in most studies indicates that resource limitation (including N) was not aggravated, suggesting that no PNL occurred in these sites. However, in the ORNL and Aspen-Birch (without O<sub>3</sub> treatment) experiments, the diminished CO<sub>2</sub> fertilization effect on plant growth was potentially driven by limitation of N, or other resources, or their combined effect. For example, reduced N availability has been identified as one of the primary factors that lead to the diminished CO<sub>2</sub> fertilization effect on NPP in the ORNL FACE experiment (Norby et al., 2010). In the Aspen-Birch community, however, the deceleration of leaf area increases due to canopy closure was responsible for the diminished CO<sub>2</sub> fertilization effect on plant growth without O<sub>3</sub> addition (Talhelm et al., 2012). With O<sub>3</sub> addition, O<sub>3</sub> significantly reduced the canopy development, resulting in a relatively open canopy during the experiment period. In addition, the negative effect of O<sub>3</sub> addition increased over time,

leading to the apparent increase in the CO<sub>2</sub> fertilization effect (Fig. 4) (Talhelm et al., 2012).

#### 4.2 Dependence of the responses of N cycling processes upon methodology, treatment duration, N addition and ecosystem types

Experimental methodology may potentially influence findings. Cabrerizo et al. (2001) found that CO<sub>2</sub> enrichment increased the nitrogenase activity measured by acetylene reduction assay (ARA), but not the specific N fixation measured by the H<sub>2</sub> evolution method. In the studies synthesized here, four methods were used to estimate biological N fixation, including isotope, ARA, H<sub>2</sub> evolution and N accumulation. Among them, ARA and H<sub>2</sub> evolution measure nitrogenase activity (Hunt and Layzell, 1993), whereas isotope and N accumulation methods directly measure biological N fixation. All but the H<sub>2</sub> evolution method showed a significantly positive response to CO<sub>2</sub> enrichment (Fig. 2a). The insignificant response shown by the H<sub>2</sub> evolution method was likely because of the small study numbers (i.e., 3). In addition, the biological N fixation measured by ARA, isotope and N accumulation showed similar response magnitudes (Fig. 2a), suggesting consistency among the three methods. However, further assessment on the H<sub>2</sub> evolution method is needed.

The responses of some N cycling processes that affect N availability are dependent on treatment duration, N addition, and/or ecosystem types (Fig. 3).



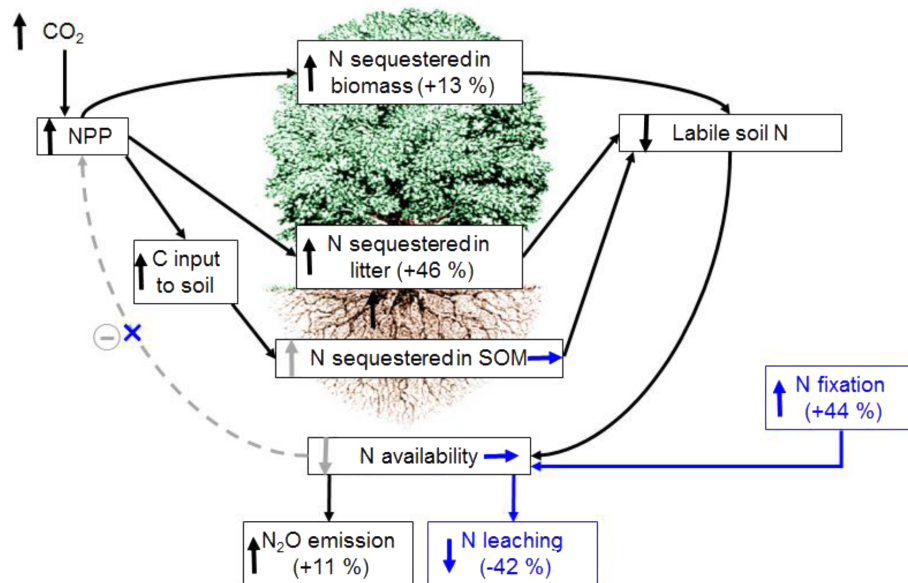
**Figure 4.** Time courses of CO<sub>2</sub> effects on ecosystem NPP (or biomass or leaf production) in decadal-long FACE experiments. Please see Table 1 for details of experiments, references and statistical results. Only statistically significant ( $P < 0.05$ ) regression lines are shown. The panel at the right-low corner shows the distribution of the slopes ( $-0.37\% \text{ year}^{-1}$  with 95 % CI from  $-1.84$  to  $1.09\% \text{ year}^{-1}$ ).

N mineralization, in addition to biological N fixation, is a major source of available N in soils. Our meta-analysis showed no change in the net N mineralization in response to CO<sub>2</sub> enrichment, which is consistent with the results by de Graaff et al. (2006). However, the response of net mineralization was dependent upon ecosystem types, showing no change in forests and grasslands, but significant increases in croplands (Fig. 3h). There may be two reasons for the stimulated net mineralization in croplands. First, N fertilization, which is commonly practiced in croplands, can increase the substrate quantity and quality for mineralization (Barrios et al., 1996; Chapin III et al., 2011; Booth et al., 2005; Lu et al., 2011; Reich and Hobbie, 2013). Second, tillage can alter soil conditions (e.g., increasing O<sub>2</sub> content), which can potentially favour the N mineralization under enriched CO<sub>2</sub> (Wienhold and Halvorson, 1999; Bardgett and Wardle, 2010). These findings suggest that CO<sub>2</sub> enrichment can stimulate the N transfer from organic to inorganic forms in managed croplands.

Unlike leaching, the response of nitrification was dependent upon treatment duration (Fig. 3). Nitrification was not

changed by short-term treatment, but was significantly reduced by long-term CO<sub>2</sub> enrichment (Fig. 3). One possible reason for the reduced nitrification with long-term CO<sub>2</sub> enrichment is the cumulative effect of hydrological changes. CO<sub>2</sub> enrichment is assumed to reduce stomatal conductance and, consequently, water loss via plant transpiration, leading to an increase in soil water content (Niklaus et al., 1998; Tricker et al., 2009; van Groenigen et al., 2011; Keenan et al., 2013). A synthesis by van Groenigen et al. (2011) shows that CO<sub>2</sub> enrichment increases soil water content by 2.6–10.6 %. Increased soil water content may result in less oxygen (O<sub>2</sub>) concentration in soils, which could potentially constrain nitrification.

In addition, the response of gaseous N loss was dependent on N addition (Fig. 3). The reduced nitrification was only observed under conditions without N addition (Fig. 3e). With N addition, no response of nitrification to CO<sub>2</sub> enrichment was observed (Fig. 3e). Additionally, the response of denitrification to CO<sub>2</sub> enrichment shifted from neutral, without N addition, to significantly positive with N addition (Fig. 3e). One possible reason is that N addition provides more N sub-



**Figure 5.** Mechanisms that alleviate PNL. PNL hypothesis posits that the stimulated plant growth by CO<sub>2</sub> enrichment leads to more N sequestered in long-lived plant tissues, litter and soil organic matter (SOM) so that, the N availability for plant growth progressively declines over time, and plant growth is downregulated (grey symbols). The current synthesis indicates that the basis of PNL occurrence partially exists (i.e., more N sequestered in plant tissues and litter; black symbols). Despite of the increases in plant N sequestration and N<sub>2</sub>O emission, stimulated biological N fixation and reduced N leaching can replenish the N availability, potentially alleviating PNL (blue boxes and arrows). Upward, downward, and horizontal arrows mean increase, decrease, and no change, respectively.

strate for nitrifying and denitrifying bacteria (Keller et al., 1988; Stehfest and Bouwman, 2006; Russow et al., 2008). The strengthening trends of both nitrification and denitrification led to a shift of the response of N<sub>2</sub>O emission to CO<sub>2</sub> enrichment from neutral without N addition to significantly positive with N addition (Fig. 3e). Our results indicate that CO<sub>2</sub> enrichment significantly increases gaseous N loss when additional N is applied. This is consistent with a previous synthesis (van Groenigen et al., 2011). Increased N<sub>2</sub>O emissions can partially offset the mitigation of climate change by the stimulated plant CO<sub>2</sub> assimilation as the warming potential of N<sub>2</sub>O is 296 times that of CO<sub>2</sub>. However, a recent modelling study by Zaehle et al. (2011) found an opposite result showing that CO<sub>2</sub> enrichment reduced emissions of N<sub>2</sub>O. In their model, elevated CO<sub>2</sub> enhanced plant N sequestration and consequently, decreased the N availability for nitrification and denitrification in soils, which led to the reduced N<sub>2</sub>O emissions. However, our synthesis shows that inorganic N does not decrease. Especially with additional N application, enhanced denitrification by CO<sub>2</sub> enrichment results in a greater N<sub>2</sub>O emission.

#### 4.3 Changes in soil microenvironment, community structures and above-belowground interactions

The meta-analysis showed that the two major forms of soil available N, NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>, responded to long-term CO<sub>2</sub> enrichment in opposing manners (Fig. 3c). While the enhanced

biological N fixation by CO<sub>2</sub> enrichment tended to increase the NH<sub>4</sub><sup>+</sup> content in soils, the reduced nitrification decreased the NO<sub>3</sub><sup>-</sup> content in soils, leading to a significant increase in the NH<sub>4</sub><sup>+</sup> / NO<sub>3</sub><sup>-</sup> ratio (Fig. 3c).

Although the total available N did not change under elevated CO<sub>2</sub>, the altered proportion of NH<sub>4</sub><sup>+</sup> over NO<sub>3</sub><sup>-</sup> in soils may have long-term effects on soil microenvironment and associated aboveground-belowground linkages that control the C cycle (Bardgett and Wardle, 2010). On the one hand, plants would release more hydrogen ion (H<sup>+</sup>) to regulate the charge balance when taking up more NH<sub>4</sub><sup>+</sup>. As a result, the increased NH<sub>4</sub><sup>+</sup> absorption could acidify the rhizosphere soil (Thomson et al., 1993; Monsanto et al., 2008). The lowered pH could have significant effects on soil microbial communities and their associated ecosystem functions. For example, fungal/bacterial ratio increases with the decrease in pH (de Vries et al., 2006; Rousk et al., 2009). The increased fungal/bacterial ratio may result in lower N mineralization because of the higher C / N ratio of fungi and the lower turnover rates of fungal-feeding fauna (de Vries et al., 2006; Rousk and Bååth, 2007). In other words, the increased fungal/bacterial ratio may slow down the N turnover from organic to inorganic forms. On the other hand, the increased NH<sub>4</sub><sup>+</sup> / NO<sub>3</sub><sup>-</sup> ratio may increase the N use efficiency because it is more energetically expensive for plants to utilize NO<sub>3</sub><sup>-</sup> than NH<sub>4</sub><sup>+</sup> (Chapin III et al., 2011; Odum and Barrett, 2005; Lambers et al., 2008). In addition, since the prefer-



ences for plant absorption of different forms of N are different (Chapin III et al., 2011; Odum and Barrett, 2005), the increased  $\text{NH}_4^+ / \text{NO}_3^-$  ratio may benefit some plant species while depress others, and consequently alter the community structures over time. These diverse changes in soil microenvironment and microbial and plant community compositions could further affect the terrestrial C cycle on long temporal scales, on which more studies are needed.

## 5 Summary

This study synthesizes data in the literature on the effects of  $\text{CO}_2$  enrichment on the terrestrial N cycle to improve our understanding of the N limitation to plant growth under elevated  $\text{CO}_2$ . Our results indicate that elevated  $\text{CO}_2$  stimulates N influx via biological N fixation but reduces N loss via leaching, leading to increased N supply for plant growth. The additional N supply via the enhanced biological N fixation and the reduced leaching may partially meet the increased N demand under elevated  $\text{CO}_2$ , potentially alleviating PNL. In addition, our analysis indicates that increased  $\text{N}_2\text{O}$  emissions may partially offset the mitigation of climate change by stimulated plant  $\text{CO}_2$  assimilation. Moreover, changes in soil microenvironments, ecosystem communities and above-belowground interactions induced by the different responses of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  to  $\text{CO}_2$  enrichment may have long-term effects on the terrestrial biogeochemical cycles and climate change.

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