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

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## Abstract

Nitrogen (N) cycle has the potential to regulate climate change through its influence on carbon (C) sequestration. Although extensive researches have been done to explore whether or not progressive N limitation (PNL) occurs under $CO_2$ 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 terrestrial N cycle with meta-analysis of $CO_2$ experimental data available in the literature. The results showed that $CO_2$ enrichment significantly increased N sequestration in plant and litter pools but not in soil pool. Thus, the basis of PNL occurrence partially exists. However, $CO_2$ enrichment also significantly increased the N influx via biological N fixation, but decreased the N efflux via leaching. In addition, no general diminished $CO_2$ fertilization effect on plant growth over time was observed. Overall, our analyses suggest that the extra N supply by the increased biological N fixation and decreased leaching may potentially alleviate PNL under elevated $CO_2$ conditions. Moreover, our synthesis showed that $CO_2$ enrichment increased soil ammonium ($NH_4^+$) but decreased nitrate ($NO_3^-$). The different responses of $NH_4^+$ and $NO_3^-$, and the consequent biological processes, may result in changes in soil microenvironment, community structures and above-belowground interactions, which could potentially affect the terrestrial biogeochemical cycles and the feedback to climate change.

## 1 Introduction

Fossil-fuel burning and deforestation have led to substantial increase in atmospheric carbon dioxide ($CO_2$) concentrations, which could stimulate plant growth (IPCC, 2013). The stimulated plant growth by $CO_2$ fertilization and the resulting terrestrial carbon (C) storage could partially mitigate the further increase in $CO_2$ concentrations and associated climate warming (IPCC, 2013). However, the stimulated plant growth by $CO_2$ enrichment may be constrained by 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 $CO_2$ fertilization effect is progressive N limitation (PNL) (Luo et al., 2004).

Progressive N limitation postulates that the stimulation of plant growth by $CO_2$ 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 $CO_2$ fertilization effect on plant growth on long-term scales. However, whether and to what extent PNL occurs are dependent 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 demand, PNL may not occur. Otherwise, the $CO_2$ fertilization effect on plant growth may diminish over time. The PNL hypothesis has been tested in individual ecosystems during the past decade (e.g., Finzi et al., 2006; Moore et al., 2006; Reich et al., 2006; Norby et al., 2010). Some of the site-level studies support (Reich et al., 2006; Norby et al., 2010), while the others refute PNL (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 that whether N supply meets N demand (Luo et al., 2004), it is important to understand how N supply changes under elevated $CO_2$. The change in N supply for plant growth under elevated $CO_2$ is determined by the responses of multiple N cycle processes, including biological N fixation, mineralization, nitrification, denitrification, and leaching (Chapin III et al., 2011). In addition, the responses of these processes to $CO_2$ 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 $CO_2$ enrichment may help reveal the general pattern of PNL in terrestrial ecosystems.

In the current study, the main objective was to explore the general pattern of the N limitation to plant growth under enriched $CO_2$ conditions. To do so, two questions were asked: (i) How do the major processes in terrestrial N cycle respond to $CO_2$ enrichment? (ii) Does the $CO_2$ fertilization effect on plant growth diminish over time? To answer these questions, two sets of data from literature were collected (Table S1 in the Supplement, Table 1). With the first dataset, we quantitatively synthesized the effects of $CO_2$ enrichment on all the major processes and pools in N cycle using meta-analysis. These variables 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 ($NH_4^+$) and nitrate ($NO_3^-$) contents in soils. The responses of the N processes to short- vs. long-term $CO_2$ treatment were also explored. In addition, the responses of the N processes to $CO_2$ enrichment under without vs. with N addition conditions were compared. With the second dataset in which the decadal plant growth in free air $CO_2$ enrichment (FACE) experiments were collected, we explored whether $CO_2$ fertilization effect on plant growth diminishes over time.

## 2 Materials and Methods

### 2.1 Data collection

For dataset one, a comprehensive literature search with the terms of "$CO_2$ enrichment (or $CO_2$ 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 further analyses: (i) including both control and $CO_2$ enrichment treatments, and the ambient and elevated $CO_2$ concentrations were around the current and predicted atmospheric $CO_2$ concentrations by 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 nitrogen (N) pools or processes: soil TIN content, soil $NH_4^+$ content, soil $NO_3^-$ 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 dataset (Table S1, References S1 in the Supplement). For each paper, means, variations (standard deviation (SD), standard error (SE) or confidence intreval (CI)) and sample sizes of the variables in both control and $CO_2$ enrichment treatments were collected.

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

$$\mathrm{SD} = \mathrm{SE}\sqrt{n} \tag{1}$$

or

$$\mathrm{SD} = (\mathrm{CI_u} - \mathrm{CI_l})\sqrt{n}/2\mathrm{u}_p \tag{2}$$

where $n$ is the sample size, $\mathrm{CI_u}$ and $\mathrm{CI_l}$ are the upper and lower limits of CI, and $\mathrm{u}_p$ is the significant level and equal to 1.96 and 1.645 when $\alpha = 0.05$ and 0.10, respectively. In some studies, 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., $\mathrm{m}^{-2}$ land), or averaged if they were weight-based (i.e., $\mathrm{g}^{-1}$ soil). In studies where the respective contents of $NH_4^+$ and $NO_3^-$ were reported, the TIN was calculated by adding the two together. For all the variables, if more than one result were reported in the same year, they were averaged by

$$M = \sum_{i=1}^{j} \frac{M_i}{j} \tag{3}$$

with standard deviation

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

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).

Because treatment time and N addition may affect the responses of the N processes to $CO_2$ enrichment, the dataset 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 dataset was also divided into forest, grassland, and cropland to explore possible differences among ecosystems.

For the dataset two, 15 available time courses of plant growth were collected from 7 decadal-long FACE experiments (Table 1). The ecosystems included 9 forests, 5 grasslands and 1 desert. Because of the limited data, we included variables that can represent plant growth on a way or another, for example, net primary production (NPP), biomass, and leaf production. These data were collected to reveal whether the effect of $CO_2$ enrichment on plant growth diminishes over treatment time as proposed by PNL (Luo et al., 2004). In the 7 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 $CO_2$ 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 $CO_2$ enrichment on 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:

$$y = y0 + ae^{-\frac{(x-\mu)^2}{2\sigma^2}} \tag{5}$$

where $x$ is the mean value of each individual interval, and $y$ is the frequency of each interval. $y0$ is the base frequency. μ and $\sigma$ are the mean and SD of the distribution.

## 2.2 Meta-analysis

With the first dataset, the effect of $CO_2$ enrichment for each line of data of the N variables was estimated using the natural logarithm transformed response ratio (RR)

(Hedges et al., 1999; Liang et al., 2013):

$$\log_e \mathrm{RR} = \log_e \left( X_\mathrm{E} / X_\mathrm{C} \right) \tag{6}$$

where $X_\mathrm{E}$ and $X_\mathrm{C}$ are the variable values under enriched $CO_2$ and control conditions, respectively. The variation of the logged RR was

$$V = \left( \frac{\mathrm{SD}_\mathrm{C}^2}{n_\mathrm{C} X_\mathrm{C}^2} + \frac{\mathrm{SD}_\mathrm{E}^2}{n_\mathrm{E} X_\mathrm{E}^2} \right) \tag{7}$$

where $\mathrm{SD}_\mathrm{C}$ and $\mathrm{SD}_\mathrm{E}$ are the standard deviation of $X_\mathrm{C}$ and $X_\mathrm{E}$, and $n_\mathrm{C}$ and $n_\mathrm{E}$ are the sample sizes of $X_\mathrm{C}$ and $X_\mathrm{E}$.

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

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

with the variance as

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

where $k$ is the number of studies, $M_j$ is the Ln(RR) in study $j$, and $W_j^*$ 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 ($\mathrm{LL}_\mathrm{weighted}$ and $\mathrm{UL}_\mathrm{weighted}$) for the weighted mean were computed as

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

and

$$UL_{\text{weighted}} = M_{\text{weighted}} + 1.96 \times \sqrt{V_{\text{weighted}}} \tag{11}$$

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) × 100 %. The response was considered significant if the 95 % CI did not overlap with zero.

## 3 Results

The meta-analysis from the first dataset showed that $CO_2$ enrichment significantly increased N sequestered in plants and litter but not in SOM (Figs. 1a and S2 in the Supplement). Whereas $CO_2$ enrichment had little effects on N mineralization, nitrification and denitrification, it significantly increased biological N fixation by 41.02 % (with 95 % CI from 28.68 % to 55.50 %), but reduced leaching (i.e., −25.54 % with 95 % CI from −35.46 % to −13.29 %) (Fig. 1b). In addition, $CO_2$ enrichment increased the content of $NH_4^+$ by 6.46 % (with 95 % CI from 0.55 % to 13.17 %), but decreased that of $NO_3^-$ by 11.09 % (with 95 % CI from −17.65 % to −4.99 %), leading to a neutral change in TIN in soils (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 (−21.77 % with 95 % CI from −29.58 % to −9.93 %) by long-term $CO_2$ enrichment (Fig. 2). In addition, it seemed that the responses of $NH_4^+$ and $NO_3^-$ were strengthened over time, representing neutral response to short-term $CO_2$ enrichment, but significantly positive and negative responses to long-term $CO_2$ enrichment, respectively (Fig. 2c). The effects of $CO_2$ enrichment could be influenced by N addition (Fig. 2). For example, nitrification was significantly reduced by $CO_2$ enrichment without N addition by 19.25 % (with 95 % CI from −39.76 % to −1.43 %), but was not changed with N addition. Denitrification and $N_2O$ emission responded to $CO_2$ enrichment neutrally without N addition, but significantly positively with N addition (30.54 % with 95 % CI from 0.70 % to 113.22 % for denitrification, and 9.33 % with 95 % CI from 0.23 % to 20.49 %; Fig. 2e). Additionally, the responses of some variables to $CO_2$ enrichment were dependent on ecosystem type (Fig. 3). APNP responded to $CO_2$ enrichment positively in forests and croplands, but neutrally in grasslands (Fig. 3a). The net mineralization had no response to $CO_2$ enrichment in forests or grasslands, while it was significantly increased in croplands (Fig. 3b). Moreover, the change in the TIN was negative, neutral, and positive in forests, grassland, and croplands, respectively (Fig. 3c).

The results from the second dataset showed that $CO_2$ enrichment significantly increased plant growth in most of the decadal FACE experiments (Fig. 4). In addition, the $CO_2$ fertilization effect over treatment time on plant growth did not 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 plant growth vs. treatment time was not significantly different from 0 (i.e., −0.37 % $\text{year}^{-1}$ with 95 % CI from −1.84 % $\text{year}^{-1}$ to 1.09 % $\text{year}^{-1}$; Fig. 4).

## 4 Discussion

The current study carried out two syntheses on the responses of terrestrial N cycle and plant growth to $CO_2$ enrichment to reveal the general pattern of PNL and the underlying processes that regulate PNL.

### 4.1 PNL alleviation

In 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 $CO_2$ significantly increased N retentions in plant tissues and litter, which is consistent with previous meta-analyses (de Graaff et al., 2006; Luo et al., 2006). Thus, the basis of PNL occurrence partially exists. However, the results from the second dataset showed no general diminished $CO_2$ fertilization effect on plant growth on the decadal scale, which disagrees with the expectation of PNL hypothesis, suggesting that N supply under elevated $CO_2$ may meet the N demand. In this study, we have identified two processes that increase N supply under elevated $CO_2$, biological N fixation and leaching.

$CO_2$ enrichment significantly enhanced the N influx to terrestrial ecosystems through biological N fixation, which reduces dinitrogen ($N_2$) to $NH_4^+$ (Fig. 1b). The enhanced biological N fixation could result from the stimulated activities of the symbiotic (Fig. S3 in the Supplement) and free-lived heterotrophic N-fixing bacteria (Hoque et al., 2001). In addition, the competition between $N_2$-fixing and non-$N_2$-fixing species could also contribute to enhance the biological N fixation on the ecosystem level (Poorter and Navas, 2003; Batterman et al., 2013). A review by Poorter and Navas (2003) suggests that elevated $CO_2$ could strengthen the competition of $N_2$-fixing dicots when nutrient level is low.

The N efflux via leaching reduced under elevated $CO_2$ condition (Fig. 1b). This could be attributed to the decrease in the primary N form in leaching, $NO_3^-$ (Chapin III et al., 2011), and the increased root growth which may immobilize more free N in soils (Luo et al., 2006; Iversen, 2010).

The increased N influx via biological N fixation and the reduced N loss through leaching result in more N retention in the biosphere (Fig. S2). Because the product of biological N fixation (i.e., $NH_4^+$) and the primary form for N leaching loss (i.e., $NO_3^-$) can be directly used by plants, the effects of $CO_2$ enrichment on the two processes directly increase the N availability for plant growth, potentially alleviating PNL (Fig. 5). In addition, the increased N retention by $CO_2$ enrichment is primarily in plant tissues and litter but not in SOM (Figs. 1 and S2). The increased N in plant tissues could be re-used by plant for multiple times 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 by the 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).

Although general PNL alleviation has been found in this study, the alleviation potential varies among sites and may be influenced by other factors. While most of the long-term experiments did not show diminished $CO_2$ fertilization effect, the $CO_2$ fertilization effect on plant production decreased 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, etc.). The undiminished $CO_2$ fertilization effect in most studies indicates that resources (including N) limitations are not aggravated, suggesting that no PNL occurs in these sites. However, in ORNL and Aspen-Birth (without $O_3$ treatment), the diminished $CO_2$ fertilization effect could be attributed to limitation of N, or other resources, or their combined. For example, reduced N availability has been identified as one of the primary factors that lead to the diminished $CO_2$ fertilization effect on NPP in ORNL FACE experiment (Norby et al., 2010). In Aspen-Birch community, however, deceleration of leaf area increase due to canopy closure is responsible for the diminished $CO_2$ fertilization effect without $O_3$ addition (Talhelm et al., 2012). With $O_3$ addition, $O_3$ significantly reduces the canopy development, resulting in relatively open canopy during the experiment period. In addition, the negative effect of $O_3$ addition increases over time, leading to the apparent increase in the $CO_2$ fertilization effect (Fig. 4) (Talhelm et al., 2012).

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

The responses of biological N fixation and leaching to $CO_2$ enrichment are barely influenced by treatment duration, N addition, or ecosystem types (Figs. 2 and 3), suggesting that the alleviation of PNL by the increased biological N fixation and decreased leaching generally occurs in terrestrial ecosystems. However, the responses of other

N cycle processes that affect N availability are dependent on treatment duration, N addition, and/or ecosystem types (Figs. 2 and 3).

N mineralization, in addition to biological N fixation, is a major source of available N in soils. The meta-analysis showed no change in the net N mineralization in response to $CO_2$ 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 significantly increase in croplands (Fig. 3b). 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 the mineralization (Barrios et al., 1996; Chapin III et al., 2011; Booth et al., 2005; Lu et al., 2011; Reich and Hobbie, 2013). Second, tillage could improve the soil conditions (e.g., $O_2$ content) and stimulate the turnover rate of soil fauna, increasing N mineralization (Wienhold and Halvorson, 1999; Bardgett and Wardle, 2010). These findings suggest that managements (in croplands) may stimulate the N transfer from organic to inorganic forms.

Unlike leaching, the response of nitrification is dependent upon treatment duration (Fig. 2). Nitrification was not changed by short-term treatment, but was significantly reduced by long-term $CO_2$ enrichment (Fig. 2). One possible reason for the reduced nitrification by the long-term $CO_2$ enrichment is cumulative effect of hydrological change. $CO_2$ enrichment generally reduces the stomatal conductance and the consequent 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 $CO_2$ enrichment increases soil water content by 2.6–10.6 %. The increased soil water content may result in less oxygen ($O_2$) content in soils, which could potentially constrain nitrification.

In addition, the response of gaseous N loss depends on N addition (Fig. 2). Reduced nitrification was only observed under without N addition (Fig. 2e). With N addition, no response of nitrification to $CO_2$ enrichment was observed (Fig. 2e). Additionally, the response of denitrification to $CO_2$ enrichment shifted from neutral without N addition to significantly positive with N addition (Fig. 2e). One possible reason is that N addition provides more N substrate to nitrifying and denitrifying bacteria (Keller et al., 1988; Stehfest and Bouwman, 2006; Russow et al., 2008). The strengthening trends of both nitrification and denitrification lead to a shift of the response of $N_2O$ emission to $CO_2$ enrichment from neutral without N addition to significantly positive with N addition (Fig. 2e). Our results indicate that $CO_2$ enrichment significantly increases gaseous N loss when additional N is applied.

### 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, $NH_4^+$ and $NO_3^-$, respond to long-term $CO_2$ enrichment in opposing manners (Fig. 2c). On the one hand, the enhanced biological N fixation by $CO_2$ enrichment significantly increased $NH_4^+$ content in soil. On the other hand, the reduced nitrification decreased $NO_3^-$ content in soils (Fig. 2b and c).

Although the total available N does not change under elevated $CO_2$, the altered proportion of $NH_4^+$ over $NO_3^-$ in soils may have long-term effects on soil microenvironment and associated aboveground-belowground linkages that control C cycle (Bardgett and Wardle, 2010). On the one hand, plants would release more hydrogen ion ($H^+$) to regulate the charge balance when taking up more $NH_4^+$. As a result, the increased $NH_4^+$ absorption can acidify the rhizosphere soil (Thomson et al., 1993; Monsant et al., 2008). The lowered pH could have significant effect on soil microbial community composition. For example, the 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 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 $NH_4^+/NO_3^-$ ratio may increase the N use efficiency because it is more energetically expensive for plants to utilize $NO_3^-$ than $NH_4^+$

(Chapin III et al., 2011; Odum and Barrett, 2005; Lambers et al., 2008). In addition, since the preferences for plant absorption of different forms of N are different (Chapin III et al., 2011; Odum and Barrett, 2005), the increased $NH_4^+/NO_3^-$ 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 sequestration on long temporal scales, on which more studies are needed.

## 5 Summary

This synthesis provides a comprehensive assessment of the effects of $CO_2$ enrichment on terrestrial N cycle, which helps improve the understanding of the N limitation to plant growth under elevated $CO_2$. Our results indicate that elevated $CO_2$ stimulates N influx via biological N fixation but reduces N loss via leaching, increasing N availability for plant growth. The extra N supply by the enhanced biological N fixation and reduced leaching may meet the increased N demand under elevated $CO_2$, potentially alleviating PNL. Moreover, the changes in the soil microenvironment, ecosystem communities and above-belowground interactions induced by the different responses of $NH_4^+$ and $NO_3^-$ to $CO_2$ enrichment may have long-term effects on terrestrial biogeochemical cycles and climate change, on which further studies are needed.

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**Table 1.** Results on the effect of $CO_2$ enrichment on ecosystem NPP (or biomass or leaf production) in decadal-long free air $CO_2$ enrichment (FACE) experiments over treatment time. The values of the slope, $R^2$ 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; 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 $O_3$ treatment, respectively.

| Experiment | Ecosystem type | Treatment years | Variable | Slope | $R^2$ | $P$ | 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) |

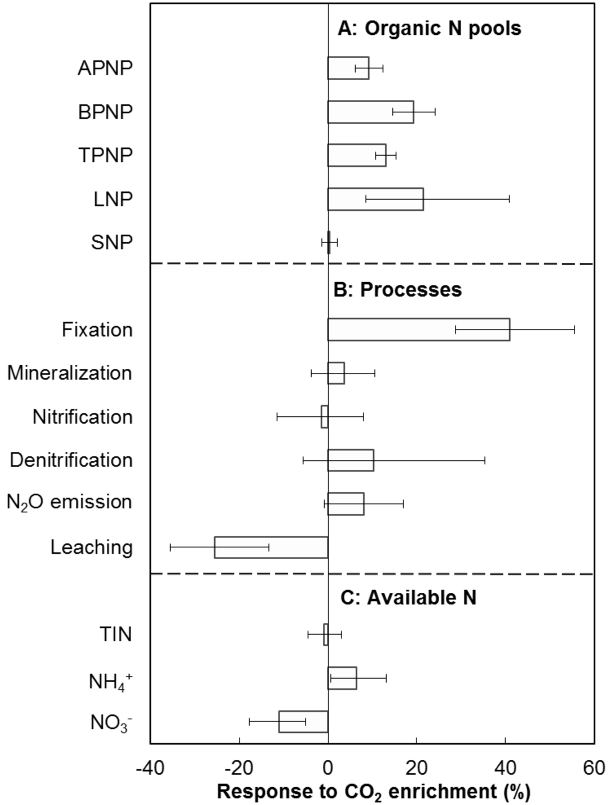

**Figure 1.** Results of a meta-analysis on the responses of nitrogen pools and processes to $CO_2$ enrichment. In **(a)**, APNP, BPNP, TPNP, LNP, and SNP are the abbreviations for aboveground plant nitrogen pool, belowground plant nitrogen pool, total plant nitrogen pool, litter nitrogen pool, and soil nitrogen pool, respectively. In **(c)**, TIN, $NH_4^+$ and $NO_3^-$ are total inorganic nitrogen, ammonium, and nitrate in soils, respectively. The error bars represent 95 % confidence intervals.

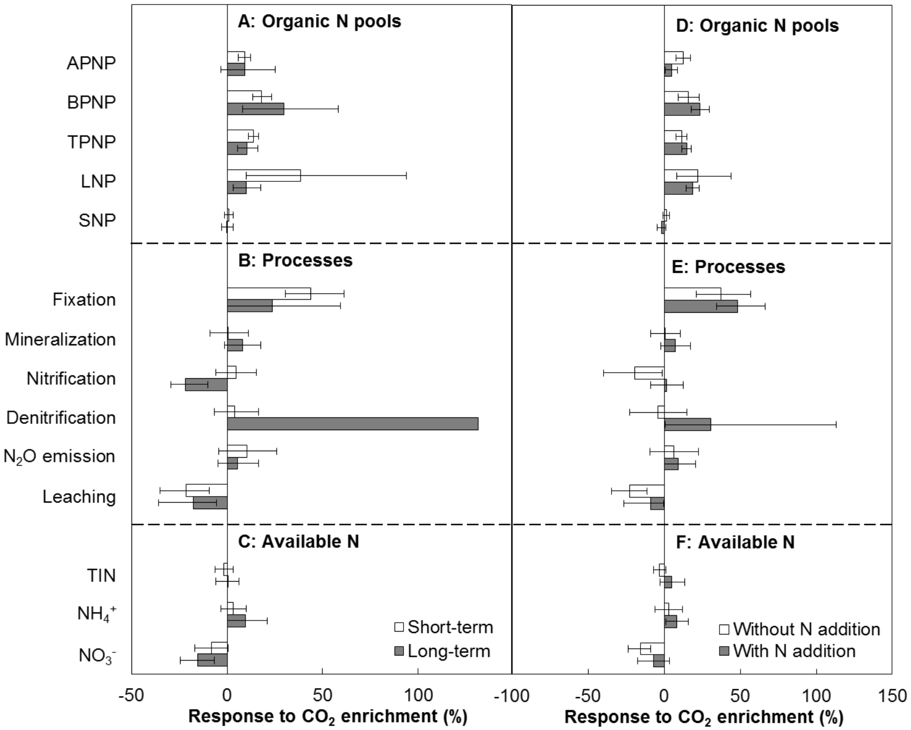

**Figure 2.** Responses of terrestrial nitrogen pools and processes to $CO_2$ enrichment (Mean ±95 % confidence interval) as regulated by experimental durations (**a** – **c**; short-term: ≤ 3 years vs. long-term: > 3 years) and nitrogen addition (**d** – **f**). Please see Fig. 1 for abbreviations.

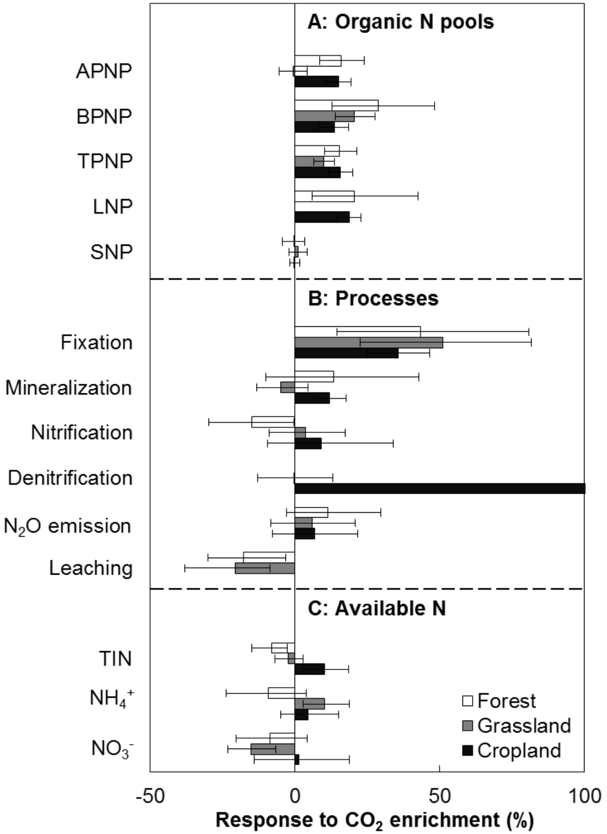

**Figure 3.** Responses of terrestrial nitrogen pools and processes to $CO_2$ enrichment in three types of ecosystems (Mean ±95 % confidence interval). Please see Fig. 1 for abbreviations.

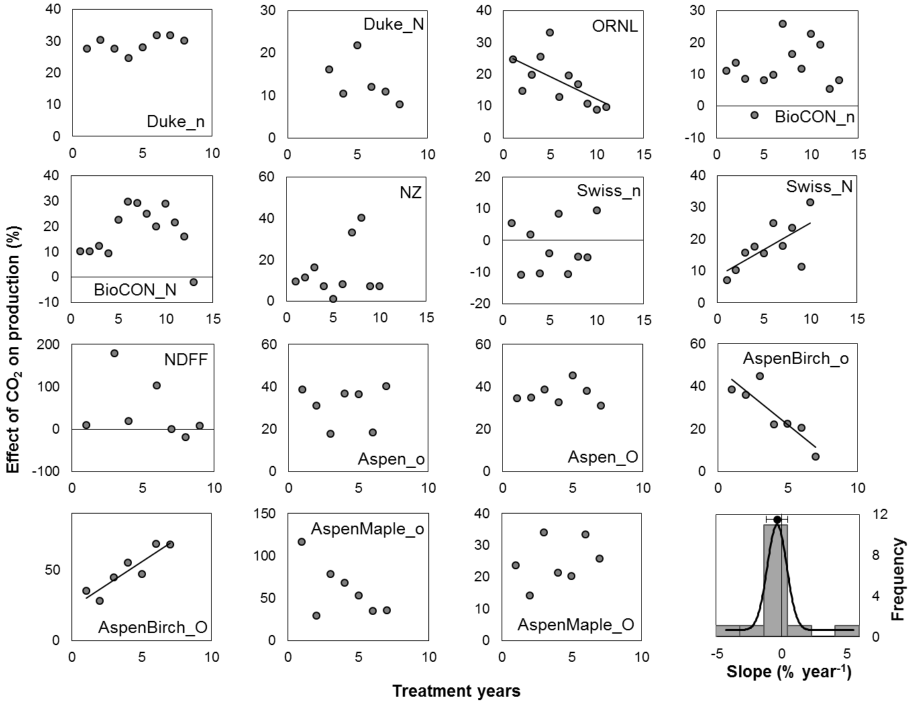

**Figure 4.** Time courses of $CO_2$ effects on ecosystem NPP (or biomass or leaf production) in decadal-long FACE experiments. Please see Table 1 for details of experiments, references and statistical results. Only statistically significant ($P < 0.05$) regression lines are shown. The panel at the right-low corner shows the distribution of the slopes ($-0.37\,\%\ \text{year}^{-1}$ with 95 % CI from $-1.84\,\%\ \text{year}^{-1}$ to $1.09\,\%\ \text{year}^{-1}$).

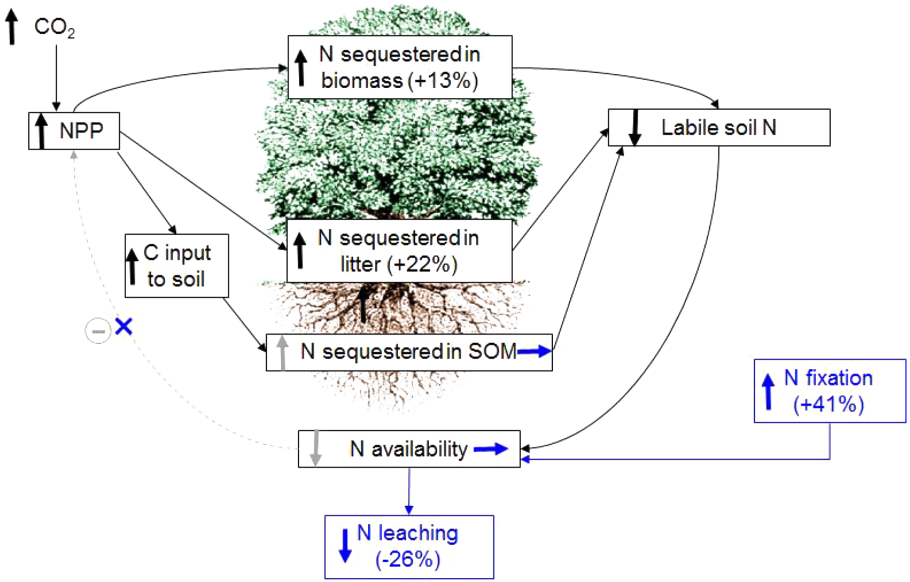

**Figure 5.** Mechanisms that alleviate PNL. PNL hypothesis posits that the stimulated plant growth by CO$_2$ enrichment leads to more N sequestered in long-lived plant tissues, litter and soil organic matter (SOM) so that, the N availability for plant growth progressively declines over time, and plant 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). However, 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.