<div align="center">**Letter of Responses**</div>

Dear Dr. Rammig,

Thank you very much for your comments. The manuscript has been thoroughly revised based on your comments. Please find detailed responses below. We hope you will find the revised manuscript acceptable for publication.

Sincerely,
Junyi Liang

The original comments are in italic and colored blue, and our responses follow. All line numbers indicated in the responses are those in the marked-up revision.

"Thus, the mechanisms that drive PNL occurrence partially exist". Sentence not clear.

Response: This sentence and the previous one have been merged and re-written to "The results showed that CO_2 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_2 results in more N sequestered in organic pools." (Lines 20 – 23)

Line 52: "over longer time scales"?

Response: Revised as suggested. The revised sentence reads, "The reduced N availability then in turn constrains the further CO_2 fertilization effect on plant growth over longer time scales" (Lines 50 – 52)

Line 54: Not clear. "Otherwise PNL may lead to…"?

Response: Revised as suggested. The revised sentence reads, "Otherwise, PNL may lead to a diminished CO_2 fertilization effect on plant growth over time." (Lines 54 – 55)

Line 55: remove sentence.

Response: The sentence has been removed (Lines 56 – 58).

Line 58: insert: the PNL hypothesis

Response: Revised as suggested (Line 59).

Line 84: remove "the"

Response: The word "the" has been removed (Line 83).

Line 172: "of"

Response: The word "from" has been changed to "of" (Line 169).

Line 217: "Thus, the basis of PNL occurrence partially exists". Sentence not clear. Do you mean: "Thus, there seems to be evidence for some basic assumptions of the PNL hypothesis"?

Response: The sentence has been revised to "Thus, there seems to be evidence for some basic assumptions of the PNL hypothesis." (Lines 212 – 213)

Line 228: "at"

Response: The word "on" has been changed to "at" (Line 223).

Line 253: "On average, in those long-term studies, CO2 enrichment…"

Response: Revised as suggested. The revised sentence reads, "On average, in those long-term studies, CO_2 enrichment increased biological N fixation by 26.2%" (Lines 247 – 248)

Line 269: Sentence not clear. A diminished CO2 fertilization effect on what? And what decreased in the two sites?

Response: The sentence has been revised to "While a diminished CO_2 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)" (Lines 261 – 263)

Line 276: Is this really an attribution? "…the diminished CO2 fertilization effect on plant growth (?) was potentially driven by limitation of N, or other resources…"

Response: Revised as suggested. The revised sentence reads, "…the diminished CO_2 fertilization effect on plant growth was potentially driven by limitation of N, or other resources, or their combined effect" (Lines 267 – 269)

Line 280: on plant growth?

Response: Yes. The sentence has been revised to "…the deceleration of leaf area increases due to canopy closure was responsible for the diminished CO_2 fertilization effect on plant growth without O_3 addition" (Lines 271 – 273)

Line 288: Methodology of your approach or of the experimental approach?

Response: The sentence has been revised to "Experimental methodology may potentially influence findings" (Line 281)

Line 320: There is no general agreement that this is true in all ecosystems. I would suggest to remove "generally" and replace it by "is assumed"

Response: Revised as suggested. The revised sentence reads, "CO_2 enrichment is assumed to reduce stomatal conductance…" (Lines 310 – 311)

Line 338: "our results…" which results?

Response: We have merged this paragraph with the previous one for better connection. This part after the revision reads, "Our results indicate that CO_2 enrichment significantly increases gaseous N loss when additional N is applied. This is consistent with a previous synthesis (van Groenigen et al. 2011)." (Lines 325 – 327)

Line 342: change "radiative forcing" to "emissions"

Response: Revised as suggested (Line 311).

Line 342 – 344: sentence not clear, please revise.

Response: The sentence has been revised to "In their model, elevated CO_2 enhanced plant N sequestration and consequently, decreased the N availability for nitrification and denitrification in soils, which led to the reduced N_2O emissions" (Lines 331 – 334). A word "however" has been added in the followed sentence (Line 334).

Line 384: "In addition, our analysis indicates that increased…"
Line 385: "may"

Response: Revised as suggested. The revised sentence reads, "In addition, our analysis indicates that increased N_2O emissions may partially offset the mitigation of climate change by stimulated plant CO_2 assimilation" (Line 372 – 374).

Processes regulating progressive nitrogen limitation under elevated carbon dioxide: A

meta-analysis

J. Liang[1,*], X. Qi[1], L. Souza[1,2], Y. Luo[1, 3, *]

[1]Department of Microbiology and Plant Biology, University of Oklahoma, Norman, Oklahoma

73019, USA

[2]Oklahoma Biological Survey, University of Oklahoma, Norman, Oklahoma 73019, USA

[3]Center for Earth System Science, Tsinghua University, Beijing 100084, China

[*]**Corresponding authors:** Junyi Liang (jliang@ou.edu) and Yiqi Luo (yluo@ou.edu). 101

David L. Boren Blvd., Norman, Oklahoma 73019, USA. Fax: +1 405 325 7619. Tel: +1 405 325

6519.

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_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 the 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 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_2 results in more N sequestered in organic pools. ~~Thus, the mechanisms that drive PNL occurrence partially exist.~~ However, CO_2 enrichment significantly increased the N influx via biological N fixation and the loss via N_2O emission, but decreased the N efflux via leaching. In addition, no general diminished CO_2 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_2 conditions in spite of the increases in plant N sequestration and N_2O emission. Moreover, our syntheses indicate that CO_2 enrichment increases soil ammonium (NH_4^+) to nitrate (NO_3^-) ratio. The changed NH_4^+/NO_3^- 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_2) concentrations, which could stimulate plant growth (IPCC, 2013). The plant growth stimulated 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, 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_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~~over longer time scales. 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 demand, PNL may not occur. Otherwise, PNL may lead to a diminished CO_2 fertilization effect on plant growth over time~~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),

59 while the others refute the PNL hypothesis (Finzi et al., 2006; Moore et al., 2006). To date, no

60 general pattern of PNL across ecosystems has yet been revealed.

61 Since the key determining PNL occurrence is that whether N supply meets N demand (Luo et

62 al., 2004), it is important to understand how N supply changes under elevated CO_2. The change

63 in the N supply for plant growth under elevated CO_2 is determined by the responses of multiple

64 N cycling processes, including biological N fixation, mineralization, nitrification, denitrification,

65 and leaching (Chapin III et al., 2011). In addition, the responses of these processes to CO_2

66 enrichment may be influenced by external N addition, such as N deposition and fertilization

67 (Reay et al., 2008). Thus, synthesizing the responses of processes that regulate PNL to CO_2

68 enrichment may help reveal the general pattern of PNL in terrestrial ecosystems.

69 In the current study, the main objective was to synthesize data published in the literature on the

70 N limitation to plant growth under enriched CO_2 conditions. Our data synthesis was designed to

71 answer two questions: (i) How do the major processes in the terrestrial N cycle respond to CO_2

72 enrichment? (ii) Does the CO_2 fertilization effect on plant growth diminish over time? To answer

73 these questions, two sets of data from the literature were collected (Table S1, Table 1). With the

74 first dataset, we quantitatively examined the effects of CO_2 enrichment on all the major

75 processes and pools in the N cycle using meta-analysis. These processes and pools included N

76 sequestered in organic components (i.e., plant tissues, litter and soil organic matter (SOM)),

77 biological N fixation, net mineralization, nitrification, denitrification, leaching, and total

78 inorganic N (TIN), ammonium (NH_4^+) and nitrate (NO_3^-) contents in soils. We separated the first

79 dataset according to the experimental durations to explore the responses of the N processes to

80 short- vs. long-term CO_2 treatments. In addition, the responses of the N processes to CO_2

81 enrichment were compared between without and with N addition conditions. The second dataset

82   was compiled for the plant growth in decadal free air CO_2 enrichment (FACE) experiments.

83   With the dataset, we explored whether the CO_2 fertilization effect on ~~the~~ plant growth diminishes

84   or not over time.

2 Materials and Methods

2.1 Data collection

For the first dataset, 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, where the ambient and elevated CO_2 concentrations were around the current and predicted atmospheric CO_2 concentrations by the Intergovernmental Panel on Climate Change (IPCC, 2013), respectively (Fig. S1); (ii) including or from which we could calculate at least one of the major 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). 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

$$SD = SE\sqrt{n} \qquad \text{Eq. (1)}$$

$$\text{or } SD = (CI_u - CI_l)\sqrt{n}/2u_P \qquad \text{Eq. (2)}$$

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

108   to represent the TPNP. When data from multiple soil layers were provided, they were summed if

109   they were area-based (i.e., m^{-2} land), or averaged if they were weight-based (i.e., g^{-1} soil). In

110   studies where the respective contents of NH_4^+ and NO_3^- were reported, the TIN was calculated

111   by adding the two together. For all the variables, if more than one result were reported during the

112   experiment period, they were averaged by

113
$$M = \sum_{i=1}^{j} \frac{M_i}{j} \qquad \text{Eq. (3)}$$

114   with standard deviation

115
$$SD = \sqrt{\frac{\sum_{i=1}^{j} SD_i^2 (n_i - 1) n_i}{\left(\sum_{i=1}^{j} n_i - 1\right) \sum_{i=1}^{j} n_i}} \qquad \text{Eq. (4)}$$

116   where j is the number of results, M_i, SD_i and n_i are the mean, SD and sample size of the ith

117   sampling data, respectively (Liang et al., 2013). If additional treatments applied (e.g., N addition),

118   they were treated as independent studies.

119   Because treatment time and N addition may affect the responses of the N processes to CO_2

120   enrichment, the dataset was divided into different categories: (i) short-term (≤ 3 years) vs. long-

121   term (> 3 years), and (ii) without N addition vs. with N addition. Moreover, the dataset was also

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

123   types.

124   For the second dataset, 15 available time series of plant growth were collected from 7 decadal

125   FACE experiments (Table 1). The ecosystems included 9 forests, 5 grasslands and 1 desert.

126   Because of the limited data, we included variables that can represent plant growth in one way or

127   another, for example, net primary production (NPP), biomass, and leaf production. These data

128   were collected to reveal whether the effect of CO_2 enrichment on plant growth diminishes over

129   treatment time as proposed by the PNL hypothesis (Luo et al., 2004). In the 7 studies, the

130 treatment lasted from 7 to 13 years, and at least 6 years' production measurements were reported.

131 For each data, the percentage change in NPP (or biomass or leaf production) by CO_2 enrichment

132 was calculated. Then, a linear regression between the percentage change and the treatment year

133 was conducted. A significantly negative slope indicates that the effect of CO_2 enrichment on the

134 plant production diminishes over time. A non-significant slope was treated as 0. After deriving

135 all the slopes, the frequency distribution of the slopes were fitted by a Gaussian function:

136
$$y = y0 + ae^{-\frac{(x-\mu)^2}{2\sigma^2}} \qquad \text{Eq. (5)}$$

137 where x is the mean value of each individual interval, and y is the frequency of each interval. $y0$

138 is the base frequency. μ and σ are the mean and SD of the distribution.

139

140 **2.2 Meta-analysis**

141 With the first dataset, the effect of CO_2 enrichment for each line of data of the N variables was

142 estimated using the natural logarithm transformed response ratio (RR) (Hedges et al., 1999;

143 Liang et al., 2013):

144
$$\log_e RR = \log_e(X_E/X_C) \qquad \text{Eq. (6)}$$

145 where X_E and X_C are the variable values under enriched CO_2 and control conditions, respectively.

146 The variation of the log RR was

147
$$V = \left(\frac{SD_C^2}{n_C X_C^2} + \frac{SD_E^2}{n_E X_E^2}\right) \qquad \text{Eq. (7)}$$

148 where SD_C and SD_E are the standard deviation of X_C and X_E, and n_C and n_E are the sample sizes

149 of X_C and X_E.

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

151 effects model, the weighted mean was calculated as

152
$$M_{weighted} = \frac{\sum_{j=1}^{k} W_j^* M_j}{\sum_{j=1}^{k} W_j^*} \qquad \text{Eq. (8)}$$

153   with the variance as

154
$$V_{weighted} = \frac{1}{\sum_{j=1}^{k} W_j^*} \qquad \text{Eq. (9)}$$

155   where k is the number of studies, M_j is the $Ln(RR)$ in study j, and W_j^* is the weighting factor

156   which consists of between- and within-study variances (Rosenberg et al., 2000; Liang et al.,

157   2013). The 95% lower and upper limits ($LL_{weighted}$ and $UL_{weighted}$) for the weighted mean were

158   computed as

159
$$LL_{weighted} = M_{weighted} - 1.96 \times \sqrt{V_{weighted}} \qquad \text{Eq. (10)}$$

160   and

161
$$UL_{weighted} = M_{weighted} + 1.96 \times \sqrt{V_{weighted}} \qquad \text{Eq. (11)}$$

162   The weighted mean and corresponding 95% bootstrapping CI (999 iterations) for each

163   variable and category were calculated in MetaWin 2.1 (details are described in the software

164   handbook by Rosenberg et al., 2000). The results were back-transformed and represented as

165   percentage change by $(RR - 1) \times 100\%$. The response was considered significant if the 95% CI

166   did not overlap with zero.

167

3 Results

The meta-analysis ~~from~~ of the first dataset showed that CO_2 enrichment significantly increased N
sequestered in plants and litter but not in SOM (Figs. 1A, S2). Whereas CO_2 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_2 evolution (Fig. 2A).
In legume species, CO_2 enrichment significantly increased nodule mass and number (Fig. 2B). In
addition, CO_2 enrichment increased N_2O 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_2
enrichment did not change the total inorganic N availability in soils, it increased the soil
NH_4^+/NO_3^- 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_2
enrichment (Fig. 3B). In addition, it seemed that the responses of the NH_4^+/NO_3^- ratio was
strengthened over time, representing a neutral response to short-term CO_2 enrichment, but
significantly positive and negative responses to long-term CO_2 enrichment (Fig. 3C). The effects
of CO_2 enrichment were influenced by N addition (Fig. 3D – F). For example, nitrification was
significantly reduced by CO_2 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_2O emission responded to
CO_2 enrichment neutrally without N addition, but significantly positively with N addition (Fig.
3E). Additionally, the responses of some variables to CO_2 enrichment were dependent on
ecosystem type (Fig. 3G – I). APNP responded to CO_2 enrichment positively in forests and

191   croplands, but neutrally in grasslands (Fig. 3G). Net mineralization had no response to CO_2

192   enrichment in forests or grasslands, while it was significantly increased in croplands (Fig. 3H).

193   Moreover, the change in the TIN was neutral in forests, grassland, but positive, in croplands,

194   respectively (Fig. 3I). In addition, a positive response of the NH_4^+/NO_3^- ratio was only observed

195   in grasslands (Fig. 3I).

196   The results from the second dataset showed that CO_2 enrichment significantly increased plant

197   growth in most of the decadal FACE experiments (Fig. 4). In addition, the CO_2 fertilization

198   effect on plant growth did not over treatment time change in 11 experiments ($P > 0.05$),

199   decreased in 2 experiments (slope < 0, $P < 0.05$), and increased in 2 experiments (slope > 0, $P <$

200   0.05), respectively (Table 1, Fig. 4). Overall, the slope of the response of the plant growth vs.

201   treatment time was not significantly different from 0 (i.e., -0.37% year^{-1} with 95% CI from -1.84%

202   year^{-1} to 1.09% year^{-1}; Fig. 4).

203

204   **4 Discussion**

205   In this study, we carried out two syntheses on the responses of the terrestrial N cycle and plant

206   growth to CO_2 enrichment to test whether PNL generally occurs across ecosystems.

207

208   **4.1 PNL alleviation**

209   According to the PNL hypothesis, a prerequisite for PNL occurrence is that more N is

210   sequestered in plant, litter and SOM (Luo et al., 2004). Our results showed that elevated CO_2

211   significantly increased N retention in plant tissues and litter, which is consistent with previous

212   meta-analyses (de Graaff et al., 2006; Luo et al., 2006). Thus, ~~the basis of PNL occurrence~~

213   ~~partially exists~~there seems to be evidence for some basic assumptions of the PNL hypothesis.

11

214   However, the results from the second dataset did not show a general diminished CO_2 fertilization

215   effect on plant growth on the decadal scale, which disagrees with the expectation of the PNL

216   hypothesis, suggesting that N supply under elevated CO_2 may meet the N demand. In this study,

217   we have identified two processes that increase N supply under elevated CO_2, i.e., biological N

218   fixation and leaching.

219   CO_2 enrichment significantly enhanced the N influx to terrestrial ecosystems through

220   biological N fixation, which reduces dinitrogen (N_2) to NH_4^+ (Fig. 1B). The enhanced biological

221   N fixation may have resulted from the stimulated activities of symbiotic (Fig. 2B) and free-living

222   heterotrophic N-fixing bacteria (Hoque et al., 2001). In addition, the competition between N_2-

223   fixing and non-N_2-fixing species may have contributed to enhance the biological N fixation ~~on~~ at

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

225   In addition, the N efflux via leaching was reduced under elevated CO_2 conditions (Fig. 1B).

226   This could be attributed to the decrease in NO_3^-, which is the primary N form in leaching,

227   (Chapin III et al., 2011), and the increased root growth which may immobilize more inorganic N

228   in soils (Luo et al., 2006; Iversen, 2010). In contrast, gaseous N loss through N_2O emission

229   increased under elevated CO_2, although this increase was only observed when additional N was

230   applied.

231   The net effect of the responses of N processes to CO_2 enrichment resulted in higher N

232   retention in ecosystems, especially within plant tissues and litter (Fig. S2). Because the product

233   of biological N fixation (i.e., NH_4^+) and the primary form for N leaching loss (i.e., NO_3^-) can be

234   directly used by plants, the effects of CO_2 enrichment on the two processes directly increase the

235   N availability for plant growth, potentially alleviating PNL (Fig. 5). The increased N in plant

236   tissues can be re-used by plants via resorption (Norby et al., 2000; Norby et al., 2001), and

237   consequently reduce the N demand from soils. This may be another mechanism that alleviates

238   PNL (Walker et al., 2015). Therefore, the increased N availability from increased N fixation and

239   reduced N leaching could potentially support net accumulation of organic matter in terrestrial

240   ecosystems (Rastetter et al., 1997; Luo and Reynolds, 1999).

241   Since biological N fixation provides at least 30% of the N requirement across natural biomes

242   (Asner et al., 2001; Galloway et al., 2004), our results suggest that the positive response of

243   biological N fixation to CO_2 enrichment plays an important role in alleviating PNL. The PNL

244   hypothesis was proposed to characterize long-term dynamics of C-N coupling in response to

245   rising atmospheric CO_2 concentration. Thus, it is critical to understand the long-term response of

246   biological N fixation to elevated CO_2. In this paper, we have synthesized 12 studies that lasted 4

247   – 7 years and binned them in a long-term category (> 3 years). On average, ~~of~~ in those long-term

248   studies, CO_2 enrichment increased biological N fixation by 26.2%. The increased biological N

249   fixation is supported by evidence at gene level from long-term experiments. For example, Tu et

250   al. (2015) found that the abundance of *nif*H gene amplicons, which is a widely used marker for

251   analyzing biological N fixation, was significantly enhanced by 12 years of CO_2 enrichment in a

252   grassland (BioCON). However, our synthesis showed a relatively wide 95% confidence interval

253   from 2.54% to 59.8%. The wide range can be partially attributed to the relatively small number

254   of studies. In addition, most studies incorporated in the current synthesis were conducted in

255   temperate regions. Thus, longer-term studies, as well as studies in other regions (e.g., boreal and

256   tropical) are critically needed to reveal more general patterns in the future.

257   In this study, it is suggested that the general trend of the N cycle changes under elevated CO_2

258   converges towards increased soil N supply for plant growth, which in theory could alleviate PNL.

259   However, the PNL alleviation potential may vary across different ecosystems due to asymmetric

260   distributions of biological N fixation (Cleveland et al., 1999). In addition, PNL alleviation may

261   also be influenced by other factors. While a diminished CO_2 fertilization effect on plant growth

262   was not observed in most of the long-term experiments did not show a diminished CO_2

263   fertilization effect, it decreased occurred in two sites (i.e., ORNL and Aspen-Birch) (Fig. 4).

264   Plant growth is usually influenced by multiple environmental factors (e.g., nutrients, water, light,

265   ozone). The undiminished CO_2 fertilization effect in most studies indicates that resource

266   limitation (including N) was not aggravated, suggesting that no PNL occurred in these sites.

267   However, in the ORNL and Aspen-Birch (without O_3 treatment) experiments, the diminished

268   CO_2 fertilization effect on plant growth could be attributed towas potentially driven by limitation

269   of N, or other resources, or their combined effect. For example, reduced N availability has been

270   identified as one of the primary factors that lead to the diminished CO_2 fertilization effect on

271   NPP in the ORNL FACE experiment (Norby et al., 2010). In the Aspen-Birch community,

272   however, the deceleration of leaf area increases due to canopy closure was responsible for the

273   diminished CO_2 fertilization effect on plant growth without O_3 addition (Talhelm et al., 2012).

274   With O_3 addition, O_3 significantly reduced the canopy development, resulting in a relatively

275   open canopy during the experiment period. In addition, the negative effect of O_3 addition

276   increased over time, leading to the apparent increase in the CO_2 fertilization effect (Fig. 4)

277   (Talhelm et al., 2012).

278

279   **4.2 Dependence of the responses of N cycling processes upon methodology, treatment**

280   **duration, N addition and ecosystem types**

281   Methodology Experimental methodology may potentially influence findings. Cabrerizo et al.

282   (2001) found that CO_2 enrichment increased the nitrogenase activity measured by acetylene

283   reduction assay (ARA), but not the specific N fixation measured by the H_2 evolution method. In

284   the studies synthesized here, four methods were used to estimate biological N fixation, including

285   isotope, ARA, H_2 evolution and N accumulation. Among them, ARA and H_2 evolution measure

286   nitrogenase activity (Hunt and Layzell, 1993) whereas isotope and N accumulation methods

287   directly measure biological N fixation. All but the H_2 evolution method showed a significantly

288   positive response to CO_2 enrichment (Fig. 2A). The insignificant response shown by the H_2

289   evolution method was likely because of the small study numbers (i.e., 3). In addition, the

290   biological N fixation measured by ARA, isotope and N accumulation showed similar response

291   magnitudes (Fig. 2A), suggesting consistency among the three methods. However, further

292   assessment on the H_2 evolution method is needed.

293   The responses of some N cycling processes that affect N availability are dependent on

294   treatment duration, N addition, and/or ecosystem types (Fig. 3).

295   N mineralization, in addition to biological N fixation, is a major source of available N in soils.

296   Our meta-analysis showed no change in the net N mineralization in response to CO_2 enrichment,

297   which is consistent with the results by de Graaff et al. (2006). However, the response of net

298   mineralization was dependent upon ecosystem types, showing no change in forests and

299   grasslands, but significant increases in croplands (Fig. 3H). There may be two reasons for the

300   stimulated net mineralization in croplands. First, N fertilization, which is commonly practiced in

301   croplands, can increase the substrate quantity and quality for mineralization (Barrios et al., 1996;

302   Chapin III et al., 2011; Booth et al., 2005; Lu et al., 2011; Reich and Hobbie, 2013). Second,

303   tillage can alter soil conditions (e.g., increasing O_2 content), which can potentially favor the N

304   mineralization under enriched CO_2 (Wienhold and Halvorson, 1999; Bardgett and Wardle, 2010).

305   These findings suggest that CO_2 enrichment can stimulate the N transfer from organic to

306   inorganic forms in managed croplands.

307   Unlike leaching, the response of nitrification was dependent upon treatment duration (Fig. 3).

308   Nitrification was not changed by short-term treatment, but was significantly reduced by long-

309   term CO_2 enrichment (Fig. 3). One possible reason for the reduced nitrification with long-term

310   CO_2 enrichment is the cumulative effect of hydrological changes. CO_2 enrichment ~~generally~~ is

311   assumed to reduce~~s~~ stomatal conductance and, consequently, water loss via plant transpiration,

312   leading to an increase in soil water content (Niklaus et al., 1998; Tricker et al., 2009; van

313   Groenigen et al., 2011; Keenan et al., 2013). A synthesis by van Groenigen et al. (2011) shows

314   that CO_2 enrichment increases soil water content by $2.6\% - 10.6\%$. Increased soil water content

315   may result in less oxygen (O_2) concentration in soils, which could potentially constrain

316   nitrification.

317   In addition, the response of gaseous N loss was dependent on N addition (Fig. 3). The reduced

318   nitrification was only observed under conditions without N addition (Fig. 3E). With N addition,

319   no response of nitrification to CO_2 enrichment was observed (Fig. 3E). Additionally, the

320   response of denitrification to CO_2 enrichment shifted from neutral, without N addition, to

321   significantly positive with N addition (Fig. 3E). One possible reason is that N addition provides

322   more N substrate for nitrifying and denitrifying bacteria (Keller et al., 1988; Stehfest and

323   Bouwman, 2006; Russow et al., 2008). The strengthening trends of both nitrification and

324   denitrification led to a shift of the response of N_2O emission to CO_2 enrichment from neutral

325   without N addition to significantly positive with N addition (Fig. 3E). Our results indicate that

326   CO_2 enrichment significantly increases gaseous N loss when additional N is applied.

327   ~~Our results are~~This is consistent with a previous synthesis (van Groenigen et al. 2011).

328   Increased N_2O emissions can partially offset the mitigation of climate change by the stimulated

329   plant CO_2 assimilation as the warming potential of N_2O is 296 times that of CO_2. However, a

330   recent modeling study by Zaehle et al. (2011) found an opposite result showing that CO_2

331   enrichment reduced ~~radiative forcing~~emissions of N_2O. In their model, elevated CO_2 enhanced

332   plant N sequestration and consequently, decreased ~~less availability of N substrates~~the N

333   availability for nitrification and denitrification in soils, which led to ~~due to the enhanced plant N~~

334   ~~sequestration attributed to~~ the reduced N_2O emissions. ~~Our~~ However, our synthesis shows that

335   inorganic N does not decrease. Especially with additional N application, enhanced denitrification

336   by CO_2 enrichment results in a greater N_2O emission.

337

338   **4.3 Changes in soil microenvironment, community structures and above-belowground**

339   **interactions**

340   The meta-analysis showed that the two major forms of soil available N, NH_4^+ and NO_3^-,

341   responded to long-term CO_2 enrichment in opposing manners (Fig. 3C). While the enhanced

342   biological N fixation by CO_2 enrichment tended to increase the NH_4^+ content in soils, the

343   reduced nitrification decreased the NO_3^- content in soils, leading to a significant increase in the

344   NH_4^+/NO_3^- ratio (Fig. 3C).

345   Although the total available N did not change under elevated CO_2, the altered proportion of

346   NH_4^+ over NO_3^- in soils may have long-term effects on soil microenvironment and associated

347   aboveground-belowground linkages that control the C cycle (Bardgett and Wardle, 2010). On the

348   one hand, plants would release more hydrogen ion (H^+) to regulate the charge balance when

349   taking up more NH_4^+. As a result, the increased NH_4^+ absorption could acidify the rhizosphere

17

350   soil (Thomson et al., 1993; Monsant et al., 2008). The lowered pH could have significant effects

351   on soil microbial communities and their associated ecosystem functions. For example,

352   fungal/bacterial ratio increases with the decrease in pH (de Vries et al., 2006; Rousk et al., 2009).

353   The increased fungal/bacterial ratio may result in lower N mineralization because of the higher

354   C/N ratio of fungi and the lower turnover rates of fungal-feeding fauna (de Vries et al., 2006;

355   Rousk and Bååth, 2007). In other words, the increased fungal/bacterial ratio may slow down the

356   N turnover from organic to inorganic forms. On the other hand, the increased NH_4^+/NO_3^- ratio

357   may increase the N use efficiency because it is more energetically expensive for plants to utilize

358   NO_3^- than NH_4^+ (Chapin III et al., 2011; Odum and Barrett, 2005; Lambers et al., 2008). In

359   addition, since the preferences for plant absorption of different forms of N are different (Chapin

360   III et al., 2011; Odum and Barrett, 2005), the increased NH_4^+/NO_3^- ratio may benefit some plant

361   species while depress others, and consequently alter the community structures over time. These

362   diverse changes in soil microenvironment and microbial and plant community compositions

363   could further affect the terrestrial C cycle on long temporal scales, on which more studies are

364   needed.

365

366   **5 Summary**

367   This study synthesizes data in the literature on the effects of CO_2 enrichment on the terrestrial N

368   cycle to improve our understanding of the N limitation to plant growth under elevated CO_2. Our

369   results indicate that elevated CO_2 stimulates N influx via biological N fixation but reduces N loss

370   via leaching, leading to increased N supply for plant growth. The additional N supply via the

371   enhanced biological N fixation and the reduced leaching may partially meet the increased N

372   demand under elevated CO_2, potentially alleviating PNL. In addition, our analysis indicates that

373   increased N_2O emissions ~~can~~ may partially offset the mitigation of climate change by stimulated

374   plant CO_2 assimilation. Moreover, changes in soil microenvironments, ecosystem communities

375   and above-belowground interactions induced by the different responses of NH_4^+ and NO_3^- to

376   CO_2 enrichment may have long-term effects on the terrestrial biogeochemical cycles and climate

377   change.

378

385

386   **References**

387   Asner, G.P., Townsend, A.R., Riley, W.J., Matson, P.A., Neff, J.C., and Cleveland, C.C.:

388   Physical and biogeochemical controls over terrestrial ecosystem responses to nitrogen

389   deposition, Biogeochemistry, 54, 1-39, 2001.

390   Bardgett, R. D. and Wardle, D. A.: Aboveground-belowground linkages: biotic interactions,

391   ecosystem processes, and global change, Oxford University Press, 2010.

392   Barrios, E., Buresh, R. J., and Sprent, J. I.: Nitrogen mineralization in density fractions of soil

393   organic matter from maize and legume cropping systems, Soil Biology & Biochemistry, 28,

394   1459-1465, 1996.

395   Batterman, S. A., Hedin, L. O., van Breugel, M., Ransijn, J., Craven, D. J., and Hall, J. S.: Key

396   role of symbiotic dinitrogen fixation in tropical forest secondary succession, Nature, 502,

397   224-227, 2013.

398   Booth, M. S., Stark, J. M., and Rastetter, E.: Controls on nitrogen cycling in terrestrial

399   ecosystems: A synthetic analysis of literature data, Ecological Monographs, 75, 139-157,

400   2005.

401   Cabrerizo, P. M., González, E. M., Aparicio-Tejo, P. M., and Arrese-Igor, C.: Continuous CO_2

402   enrichment leads to increased nodule biomass, carbon availability to nodules and activity of

403   carbon-metabolising enzymes but does not enhance specific nitrogen fixation in

404   pea, Physiologia Plantarum, 113, 33-40, 2001.

405   Chapin III, F. S., Matson, P. A., and Vitousek, P.: Principles of terrestrial ecosystem ecology,

406   Springer, 2011.

407   Cleveland, C. C., Townsend, A. R., Schimel, D. S., Fisher, H., Howarth, R. W., Hedin, L. O.,

408   Perakis, S. S., Latty, E. F., Von Fischer, J. C., Elseroad, A., and Wasson, M. F.: Global

409   patterns of terrestrial biological nitrogen (N_2) fixation in natural ecosystems, Global

410   Biogeochemical Cycles, 13, 623-645, 1999.

411   de Graaff, M. A., van Groenigen, K. J., Six, J., Hungate, B., and van Kessel, C.: Interactions

412   between plant growth and soil nutrient cycling under elevated CO_2: a meta-analysis, Global

413   Change Biology, 12, 2077-2091, 2006.

414   de Vries, F. T., Hoffland, E., van Eekeren, N., Brussaard, L., and Bloem, J.: Fungal/bacterial

415   ratios in grasslands with contrasting nitrogen management, Soil Biology & Biochemistry, 38,

416   2092-2103, 2006.

417 Finzi, A. C., Moore, D. J. P., Delucia, E. H., Lichter, J., Hofmockel, K. S., Jackson, R. B., Kim,

418 H., Matamala, R., McCarthy, H. R., Oren, R., Pippen, J. S., Schlesinger, W. H.: Progressive

419 nitrogen limitation of ecosystem processes under elevated CO_2 in a warm-temperate forest,

420 Ecology, 87, 15-25, 2006.

421 Galloway, J. N., Dentener, F. J., Capone, D. G., Boyer, E. W., Howarth, R. W., Seitzinger, S. P.,

422 Asner, G. P., Cleveland, C. C., Green, P. A., Holland, E. A., and Karl, D. M.: Nitrogen cycles:

423 past, present, and future, Biogeochemistry, 70, 53-226, 2004.

424 Hedges, L. V., Gurevitch, J., and Curtis, P. S.: The meta-analysis of response ratios in

425 experimental ecology, Ecology, 80, 1150-1156, 1999.

426 Hoque, M. M., Inubushi, K., Miura, S., Kobayashi, K., Kim, H. Y., Okada, M., and Yabashi, S.:

427 Biological dinitrogen fixation and soil microbial biomass carbon as influenced by free-air

428 carbon dioxide enrichment (FACE) at three levels of nitrogen fertilization in a paddy field,

429 Biology and Fertility of Soils, 34, 453-459, 2001.

430 Hunt, S. and Layzell, D.B.: Gas exchange of legume nodules and the regulation of nitrogenase

431 activity, Annual Review of Plant Physiology and Plant Molecular Biology, 44, 483-511,

432 1993.

433 IPCC: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to

434 the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, Cambridge

435 University Press, Cambridge, United Kingdom and New York, NY, USA, 2013.

436 Iversen, C. M.: Digging deeper: fine-root responses to rising atmospheric CO_2 concentration in

437 forested ecosystems, New Phytologist, 186, 346-357, 2010.

438   Keenan, T. F., Hollinger, D. Y., Bohrer, G., Dragoni, D., Munger, J. W., Schmid, H. P., and

439   Richardson, A. D.: Increase in forest water-use efficiency as atmospheric carbon dioxide

440   concentrations rise, Nature, 499, 324-+, 2013.

441   Keller, M., Kaplan, W. A., Wofsy, S. C., and Dacosta, J. M.: Emissions of N_2O from Tropical

442   Forest Soils: Response to Fertilization with NH_4^+, $NO3^-$, and PO_4^{3-}, Journal of Geophysical

443   Research-Atmospheres, 93, 1600-1604, 1988.

444   Lambers, H., Chapin III, F. S., and Pons, T. L.: Plant Physiological Ecology, Springer, New

445   York, 2008.

446   Liang, J., Xia, J., Liu, L., and Wan, S.: Global patterns of the responses of leaf-level

447   photosynthesis and respiration in terrestrial plants to experimental warming, Journal of Plant

448   Ecology, 6, 437-447, 2013.

449   Lu, M., Yang, Y. H., Luo, Y. Q., Fang, C. M., Zhou, X. H., Chen, J. K., Yang, X., and Li, B.:

450   Responses of ecosystem nitrogen cycle to nitrogen addition: a meta-analysis, New

451   Phytologist, 189, 1040-1050, 2011.

452   Luo, Y. and Reynolds, J. F.: Validity of extrapolating field CO_2 experiments to predict carbon

453   sequestration in natural ecosystems, Ecology, 80, 1568-1583, 1999.

454   Luo, Y., Su, B., Currie, W. S., Dukes, J. S., Finzi, A. C., Hartwig, U., Hungate, B., McMurtrie, R.

455   E., Oren, R., Parton, W. J., Pataki, D. E., Shaw, M. R., Zak, D. R., and Field, C. B.:

456   Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide,

457   Bioscience, 54, 731-739, 2004.

458   Luo, Y. Q., Hui, D. F., and Zhang, D. Q.: Elevated CO_2 stimulates net accumulations of carbon

459   and nitrogen in land ecosystems: A meta-analysis, Ecology, 87, 53-63, 2006.

460 McCarthy, H. R., Oren, R., Johnsen, K. H., Gallet-Budynek, A., Pritchard, S. G., Cook, C. W.,

461   LaDeau, S. L., Jackson, R. B., and Finzi, A. C.: Re-assessment of plant carbon dynamics at

462   the Duke free-air CO_2 enrichment site: interactions of atmospheric [CO_2] with nitrogen and

463   water availability over stand development, New Phytologist, 185, 514-528, 2010.

464 Monsant, A. C., Tang, C., and Baker, A. J. M.: The effect of nitrogen form on rhizosphere soil

465   pH and zinc phytoextraction by Thlaspi caerulescens, Chemosphere, 73, 635-642, 2008.

466 Moore, D. J. P., Aref, S., Ho, R. M., Pippen, J. S., Hamilton, J. G., de Lucia, E. H.: Annual basal

467   area increment and growth duration of *Pinus taeda* in response to eight years of free-air

468   carbon dioxide enrichment, Global Change Biology, 12, 1367-1377, 2006.

469 Niklaus, P. A., Spinnler, D., and Korner, C.: Soil moisture dynamics of calcareous grassland

470   under elevated CO_2, Oecologia, 117, 201-208, 1998.

471 Norby, R. J., Cotrufo, M. F., Ineson, P., O'Neill, E. G., and Canadell, J. G.: Elevated CO_2, litter

472   chemistry, and decomposition: a synthesis, Oecologia, 127, 153-165, 2001.

473 Norby, R. J., Long, T. M., Hartz-Rubin, J. S., and O'Neill, E. G.: Nitrogen resorption in

474   senescing tree leaves in a warmer, CO_2-enriched atmosephere, Plant and Soil, 224, 15-29,

475   2000.

476 Norby, R. J., Warren, J. M., Iversen, C. M., Medlyn, B. E., and McMurtrie, R. E.: CO_2

477   enhancement of forest productivity constrained by limited nitrogen availability, Proceedings

478   of the National Academy of Sciences of the United States of America, 107, 19368-19373,

479   2010.

480 Odum, E. P. and Barrett, G. W.: Fundamentals of Ecology, Thomson Brooks/Cole, 2005.

481 Oren, R., Ellsworth, D. S., Johnsen, K. H., Phillips, N., Ewers, B. E., Maier, C., Schafer, K. V.

482   R., McCarthy, H., Hendrey, G., McNulty, S. G., and Katul, G. G.: Soil fertility limits carbon

483   sequestration by forest ecosystems in a CO_2-enriched atmosphere, Nature, 411, 469-472,

484   2001.

485   Poorter, H. and Navas, M. L.: Plant growth and competition at elevated CO_2: on winners, losers

486   and functional groups, New Phytologist, 157, 175-198, 2003.

487   Rastetter, E. B., Agren, G. I., and Shaver, G. R.: Responses of N-limited ecosystems to increased

488   CO_2: A balanced-nutrition, coupled-element-cycles model, Ecological Applications, 7, 444-

489   460, 1997.

490   Reay, D. S., Dentener, F., Smith, P., Grace, J., and Feely, R. A.: Global nitrogen deposition and

491   carbon sinks, Nature Geoscience, 1, 430-437, 2008.

492   Reich, P. B. and Hobbie, S. E.: Decade-long soil nitrogen constraint on the CO_2 fertilization of

493   plant biomass, Nature Climate Change, 3, 278-282, 2013.

494   Reich, P. B., Hobbie, S. E., Lee, T., Ellsworth, D. S., West, J. B., Tilman, D., Knops, J. M. H.,

495   Naeem, S., and Trost, J.: Nitrogen limitation constrains sustainability of ecosystem response

496   to CO_2, Nature, 440, 922-925, 2006.

497   Reich, P. B., Hobbie, S. E., and Lee, T. D.: Plant growth enhancement by elevated CO_2

498   eliminated by joint water and nitrogen limitation, Nature Geoscience, 7, 920-924, 2014.

499   Rosenberg, M. S., Adams, D. C., and Gurevitch, J.: MetaWin: statistical software for meta-

500   analysis, Sinauer Associates Sunderland, Massachusetts, USA, 2000.

501   Ross, D. J., Newton, P. C. D., Tate, K. R., and Luo, D. W.: Impact of a low level of CO_2

502   enrichment on soil carbon and nitrogen pools and mineralization rates over ten years in a

503   seasonally dry, grazed pasture, Soil Biology & Biochemistry, 58, 265-274, 2013.

504   Rousk, J. and Bååth, E.: Fungal biomass production and turnover in soil estimated using the

505   acetate-in-ergosterol technique, Soil Biology & Biochemistry, 39, 2173-2177, 2007.

506   Rousk, J., Brookes, P. C., and Baath, E.: Contrasting Soil pH Effects on Fungal and Bacterial

507   Growth Suggest Functional Redundancy in Carbon Mineralization, Applied and

508   Environmental Microbiology, 75, 1589-1596, 2009.

509   Russow, R., Spott, O., and Stange, C. F.: Evaluation of nitrate and ammonium as sources of NO

510   and N_2O emissions from black earth soils (Haplic Chernozem) based on ^{15}N field

511   experiments, Soil Biology & Biochemistry, 40, 380-391, 2008.

512   Schneider, M. K., Luscher, A., Richter, M., Aeschlimann, U., Hartwig, U. A., Blum, H., Frossard,

513   E., and Nosberger, J.: Ten years of free-air CO_2 enrichment altered the mobilization of N

514   from soil in *Lolium perenne* L. swards, Global Change Biology, 10, 1377-1388, 2004.

515   Smith, S. D., Charlet, T. N., Zitzer, S. F., Abella, S. R., Vanier, C. H., and Huxman, T. E.: Long-

516   term response of a Mojave Desert winter annual plant community to a whole-ecosystem

517   atmospheric CO_2 manipulation (FACE), Global Change Biology, 20, 879-892, 2014.

518   Stehfest, E. and Bouwman, L.: N_2O and NO emission from agricultural fields and soils under

519   natural vegetation: summarizing available measurement data and modeling of global annual

520   emissions, Nutrient Cycling in Agroecosystems, 74, 207-228, 2006.

521   Talhelm, A. F., Pregitzer, K. S., and Giardina, C. P.: Long-Term Leaf Production Response to

522   Elevated Atmospheric Carbon Dioxide and Tropospheric Ozone, Ecosystems, 15, 71-82,

523   2012.

524   Thomson, C. J., Marschner, H., and Romheld, V.: Effect of Nitrogen-Fertilizer Form on pH of

525   the Bulk Soil and Rhizosphere, and on the Growth, Phosphorus, and Micronutrient Uptake of

526   Bean, Journal of Plant Nutrition, 16, 493-506, 1993.

527 Tricker, P. J., Pecchiari, M., Bunn, S. M., Vaccari, F. P., Peressotti, A., Miglietta, F., and Taylor,

528 G.: Water use of a bioenergy plantation increases in a future high CO_2 world, Biomass &

529 Bioenergy, 33, 200-208, 2009.

530 Tu, Q., Zhou, X., He, Z., Xue, K., Wu, L., Reich, P., Hobbie, S., and Zhou, J.: The Diversity and

531 Co-occurrence Patterns of N_2-Fixing Communities in a CO_2-Enriched Grassland

532 Ecosystem, Microbial Ecology, doi:10.1007/s00248-015-0659-7, 2015.

533 van Groenigen, K. J., Osenberg, C. W., and Hungate, B. A.: Increased soil emissions of potent

534 greenhouse gases under increased atmospheric CO2, Nature, 475, 214-U121, 2011.

535 Vitousek, P. M.: Nutrient cycling and limitation: Hawai'i as a model system, Princeton

536 University Press, 2004.

537 Walker, A. P., Zaehle, S., Medlyn, B. E., De Kauwe, M. G., Asao, S., Hickler, T., Parton, W.,

538 Ricciuto, D. M., Wang, Y. P., and Wårlind, D.: Predicting long-term carbon sequestration in

539 response to CO_2 enrichment: How and why do current ecosystem models differ?, Global

540 Biogeochemical Cycles, 29, 476-495, 2015.

541 Wienhold, B. J. and Halvorson, A. D.: Nitrogen mineralization responses to cropping, tillage,

542 and nitrogen rate in the Northern Great Plains, Soil Science Society of America Journal, 63,

543 192-196, 1999.

544 Zaehle, S., Ciais, P., Friend, A.D. and Prieur, V.: Carbon benefits of anthropogenic reactive

545 nitrogen offset by nitrous oxide emissions. Nature Geoscience, 4, 601-605, 2011.

546

547 **Supporting Information captions**

548 **Figure S1** Distributions of the experimental duration (**A**) and the CO_2 concentrations under

549 ambient (**B**) and elevated (**C**) treatments and their difference (**D**) for the 175 collected studies.

550 Red dashed lines represent the mean values.

551

552 **Figure S2** Summary of the effect of CO_2 enrichment on ecosystem level N budget. Square boxes

553 are nitrogen pools, ovals are nitrogen processes. Red dashed boxes mean the sum of the pools in

554 the boxes. "+", "-", and "ns" mean the response to CO_2 enrichment are positive, negative, and

555 not significant, respectively. Please see **Figure 1** for abbreviations.

556

557 **Database S1** Database extracted from papers listed in References S1.

558

559 **References S1** Papers from which the first dataset was extracted.

560

561 **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

562 enrichment (FACE) experiments over treatment time. The values of the slope, R^2 and P in the linear regression in **Fig. 4** are shown.

563 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

564 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

565 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

566

Figure captions

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 biological N fixation measured by different methods (A) and

nodule dry mass and number in legume species (B). ARA: acetylene reduction assay.

Mean ± 95% confidence interval.

Figure 3. 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), nitrogen addition (D – F), and ecosystem types (G – I).
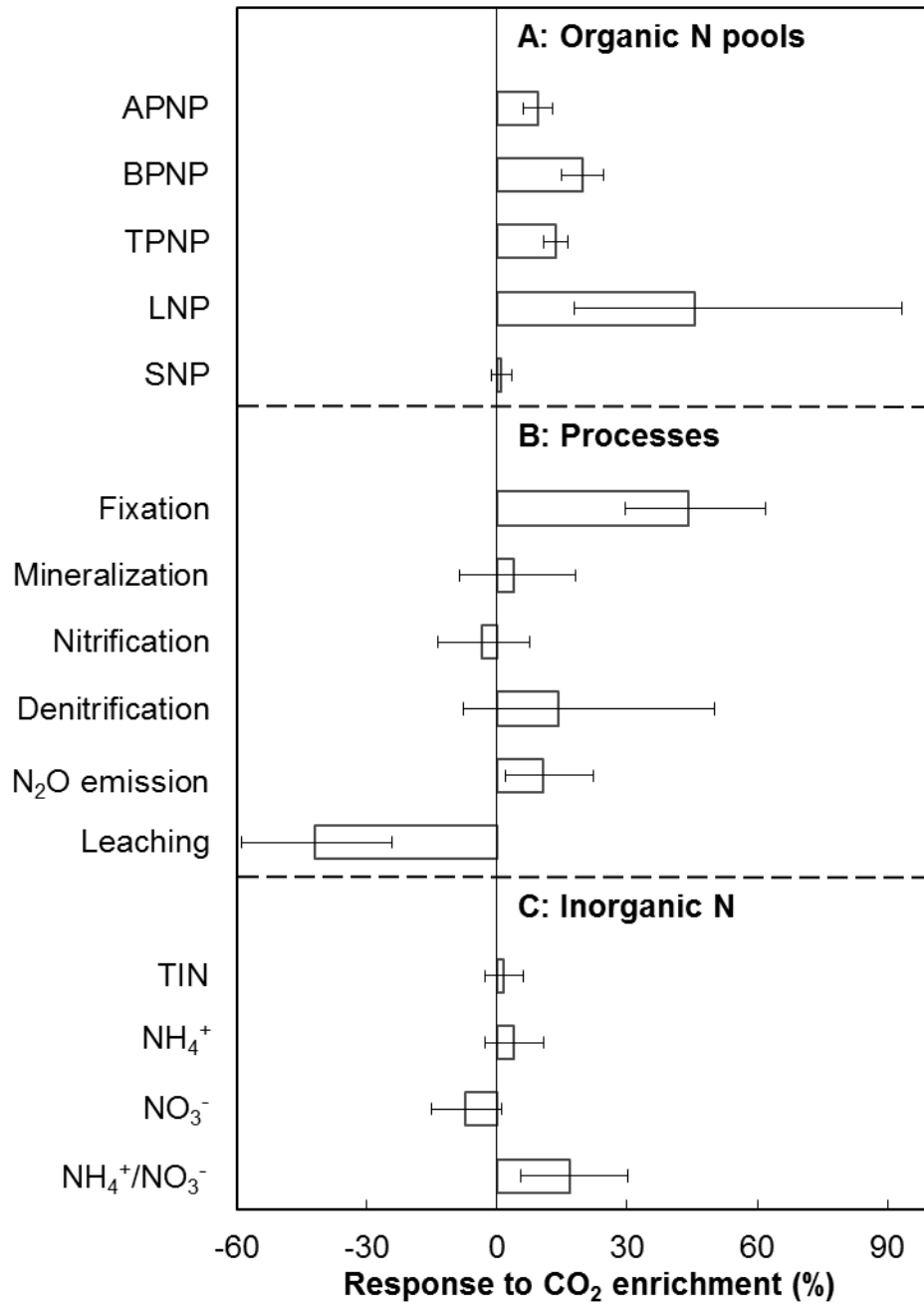
Please see Figure 1 for abbreviations.

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%

$year^{-1}$ with 95% CI from -1.84% $year^{-1}$ to 1.09% $year^{-1}$).

590 **Figure 5.** Mechanisms that alleviate PNL. PNL hypothesis posits that the stimulated

591 plant growth by CO_2 enrichment leads to more N sequestered in long-lived plant tissues,

592 litter and soil organic matter (SOM) so that, the N availability for plant growth

593 progressively declines over time, and plant growth is downregulated (grey symbols). The

594 current synthesis indicates that the basis of PNL occurrence partially exists (i.e., more N

595 sequestered in plant tissues and litter; black symbols). Despite of the increases in plant N

596 sequestration and N_2O emission, stimulated biological N fixation and reduced N leaching

597 can replenish the N availability, potentially alleviating PNL (blue boxes and arrows).

598 Upward, downward, and horizontal arrows mean increase, decrease, and no change,
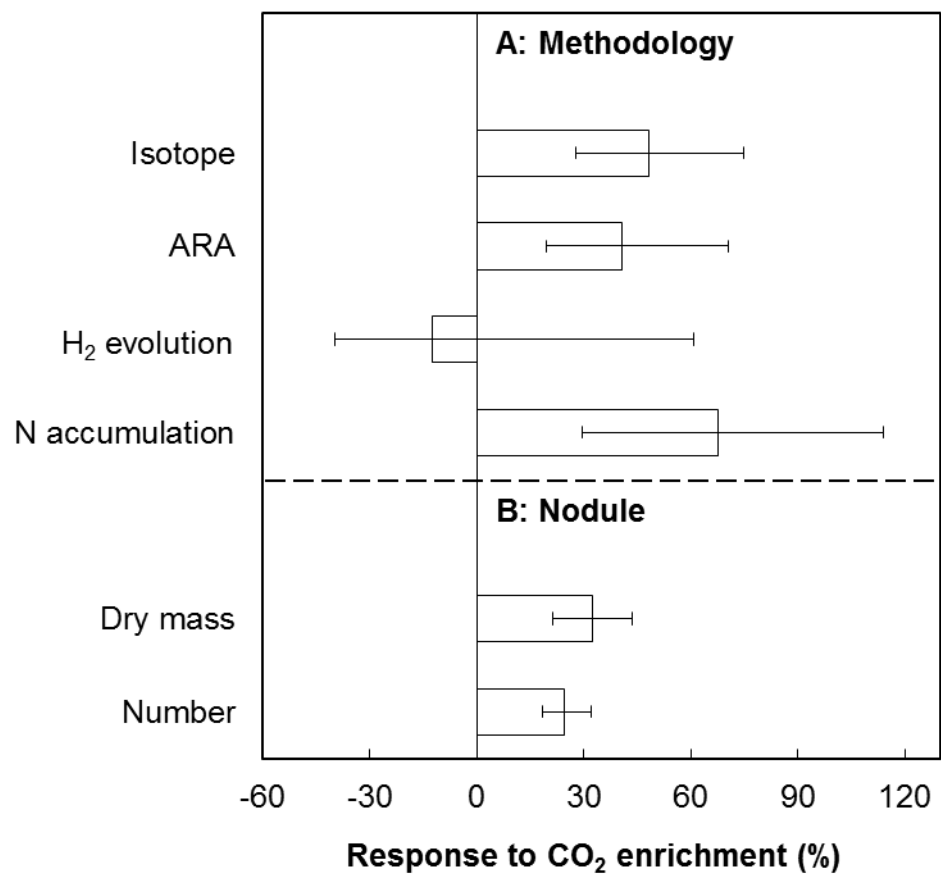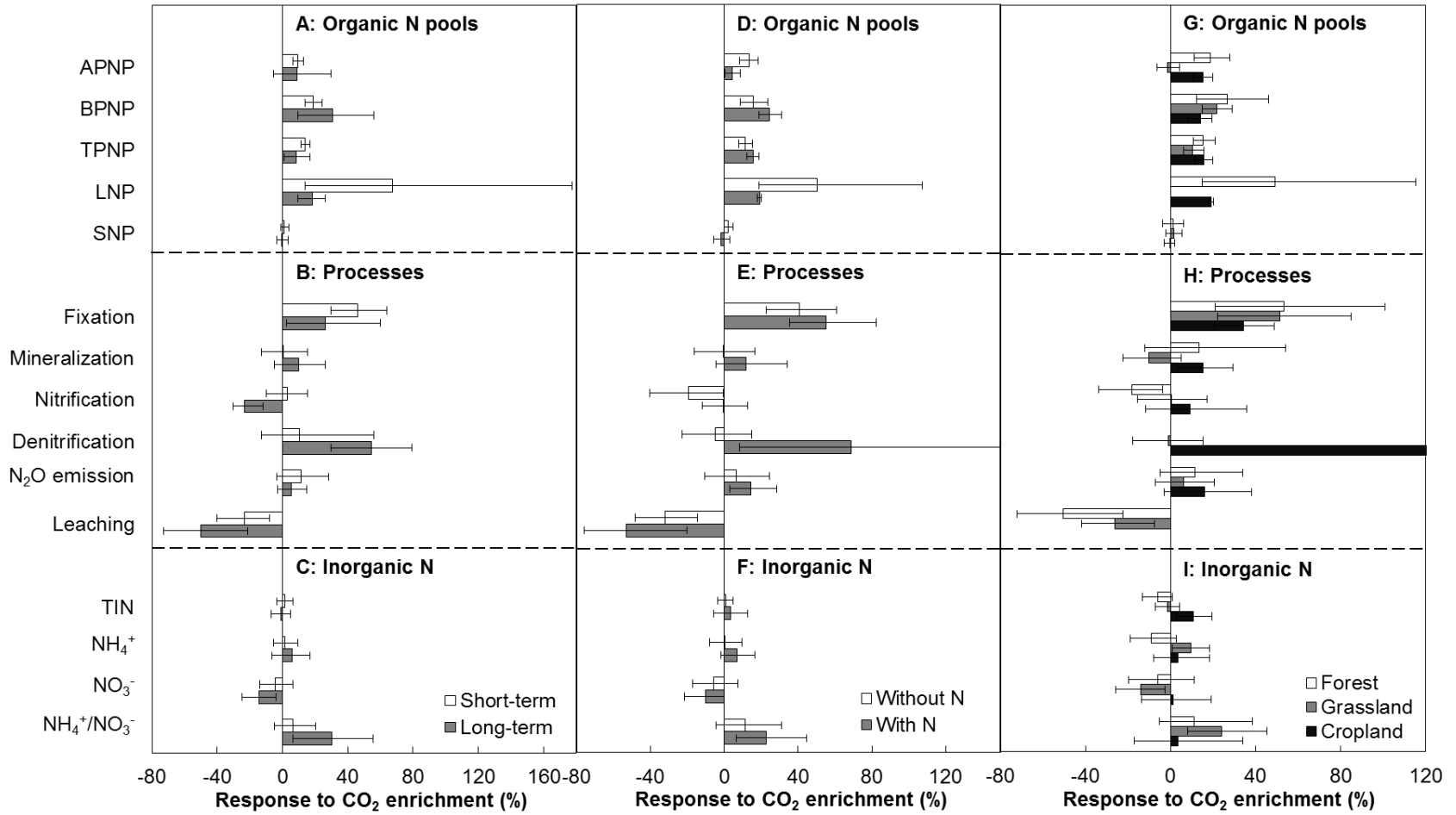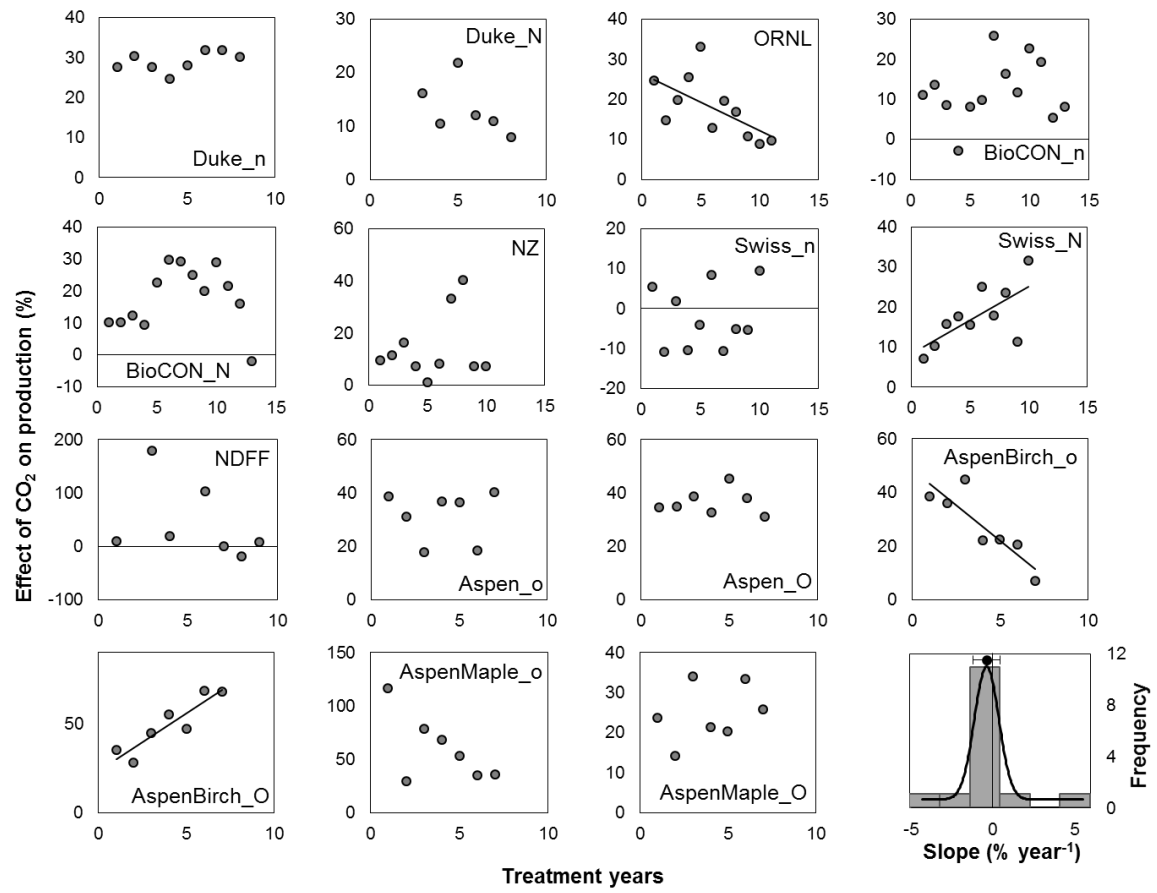
599 respectively.

600

601   **Figure 1.**

A: Organic N pools

APNP
BPNP
TPNP
LNP
SNP

B: Processes

Fixation
Mineralization
Nitrification
Denitrification
N_2O emission
Leaching

C: Inorganic N

TIN
NH_4^+
NO_3^-
NH_4^+/NO_3^-

-60   -30   0   30   60   90

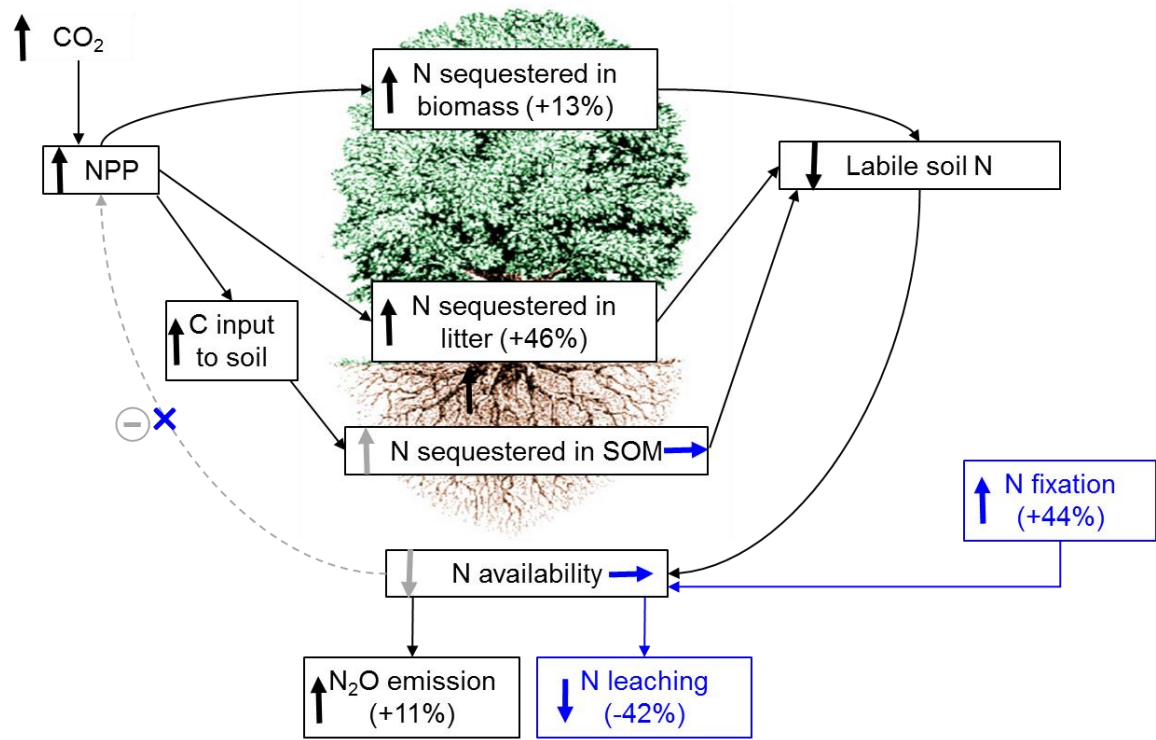**Response to CO_2 enrichment (%)**

602

603

604   **Figure 2.**

605

606

Figure 3.

Figure 4.

612   **Figure 5.**

613

614