

15 **Abstract:** The nitrogen (N) cycle has the potential to regulate climate change through its
16 influence on carbon (C) sequestration. Although extensive research has explored whether or not
17 progressive N limitation (PNL) occurs under CO₂ enrichment, a comprehensive assessment of
18 the processes that regulate PNL is still lacking. Here, we quantitatively synthesized the responses
19 of all major processes and pools in the terrestrial N cycle with meta-analysis of CO₂
20 experimental data available in the literature. The results showed that CO₂ enrichment
21 significantly increased N sequestration in the plant and litter pools but not in the soil pool. Thus,
22 the mechanisms that drive PNL occurrence partially exist. However, CO₂ enrichment
23 significantly increased the N influx via biological N fixation and the loss via N₂O emission, but
24 decreased the N efflux via leaching. In addition, no general diminished CO₂ fertilization effect
25 on plant growth was observed over time up to the longest experiment of 13 years. Overall, our
26 analyses suggest that the extra N supply by the increased biological N fixation and decreased
27 leaching may potentially alleviate PNL under elevated CO₂ conditions in spite of the increases in
28 plant N sequestration and N₂O emission. Moreover, our syntheses indicate that CO₂ enrichment
29 increases soil ammonium (NH₄⁺) to nitrate (NO₃⁻) ratio. The changed NH₄⁺/NO₃⁻ ratio and
30 subsequent biological processes may result in changes in soil microenvironments, above-
31 belowground community structures and associated interactions, which could potentially affect
32 the terrestrial biogeochemical cycles. In addition, our data synthesis suggests that more long-
33 term studies, especially in regions other than temperate ones, are needed for comprehensive
34 assessments of the PNL hypothesis.

35

36 **1 Introduction**

37 Fossil-fuel burning and deforestation have led to substantial increase in atmospheric carbon
38 dioxide (CO₂) concentrations, which could stimulate plant growth (IPCC, 2013). The plant
39 growth stimulated by CO₂ fertilization and the resulting terrestrial carbon (C) storage could
40 partially mitigate the further increase in CO₂ concentrations and associated climate warming
41 (IPCC, 2013). However, this effect may be constrained by the availability of nitrogen (N), an
42 essential element for molecular compounds of amino acids, proteins, ribonucleic acids (RNAs)
43 and deoxyribonucleic acids (DNAs) in organisms (Rastetter et al., 1997; Oren et al., 2001; Luo et
44 al., 2004; Reich et al., 2006; Norby et al., 2010; Reich and Hobbie, 2013). A popular hypothesis
45 of the N constraint to the CO₂ fertilization effect is progressive N limitation (PNL) (Luo et al.,
46 2004).

47 Progressive N limitation postulates that the stimulation of plant growth by CO₂ enrichment
48 results in more N sequestered in plant, litter and soil organic matter (SOM) so that, the N
49 availability for plant growth progressively declines in soils over time (Luo et al., 2004). The
50 reduced N availability then in turn constrains the further CO₂ fertilization effect on plant growth
51 on long-term scales. However, whether and to what extent PNL occurs depends on the balance of
52 N demand and supply (Luo et al., 2004; Finzi et al., 2006; Walker et al., 2015). If the N supply
53 meets the N demand, PNL may not occur. Otherwise, the CO₂ fertilization effect on plant growth
54 may diminish over time. The PNL hypothesis has been tested in individual ecosystems during
55 the past decade (e.g., Finzi et al., 2006; Moore et al., 2006; Reich et al., 2006; Norby et al., 2010).
56 Some of the site-level studies support (Reich et al., 2006; Norby et al., 2010), while the others
57 refute PNL (Finzi et al., 2006; Moore et al., 2006). To date, no general pattern of PNL across
58 ecosystems has yet been revealed.

59 Since the key determining PNL occurrence is that whether N supply meets N demand (Luo et
60 al., 2004), it is important to understand how N supply changes under elevated CO₂. The change
61 in the N supply for plant growth under elevated CO₂ is determined by the responses of multiple
62 N cycling processes, including biological N fixation, mineralization, nitrification, denitrification,
63 and leaching (Chapin III et al., 2011). In addition, the responses of these processes to CO₂
64 enrichment may be influenced by external N addition, such as N deposition and fertilization
65 (Reay et al., 2008). Thus, synthesizing the responses of processes that regulate PNL to CO₂
66 enrichment may help reveal the general pattern of PNL in terrestrial ecosystems.

67 In the current study, the main objective was to synthesize data published in the literature on the
68 N limitation to plant growth under enriched CO₂ conditions. Our data synthesis was designed to
69 answer two questions: (i) How do the major processes in the terrestrial N cycle respond to CO₂
70 enrichment? (ii) Does the CO₂ fertilization effect on plant growth diminish over time? To answer
71 these questions, two sets of data from the literature were collected (Table S1, Table 1). With the
72 first dataset, we quantitatively examined the effects of CO₂ enrichment on all the major
73 processes and pools in the N cycle using meta-analysis. These processes and pools included N
74 sequestered in organic components (i.e., plant tissues, litter and soil organic matter (SOM)),
75 biological N fixation, net mineralization, nitrification, denitrification, leaching, and total
76 inorganic N (TIN), ammonium (NH₄⁺) and nitrate (NO₃⁻) contents in soils. We separated the first
77 dataset according to the experimental durations to explore the responses of the N processes to
78 short- vs. long-term CO₂ treatments. In addition, the responses of the N processes to CO₂
79 enrichment were compared between without and with N addition conditions. The second dataset
80 was compiled for the plant growth in decadal free air CO₂ enrichment (FACE) experiments.

81 With the dataset, we explored whether the CO₂ fertilization effect on the plant growth diminishes
82 or not over time.

83 2 Materials and Methods

84 2.1 Data collection

85 For the first dataset, a comprehensive literature search with the terms of “CO₂ enrichment (or
86 CO₂ increase)”, “nitrogen” and “terrestrial” was conducted using the online search connection
87 *Web of Science* in Endnote. Then, papers meeting the following two criteria were selected to do
88 the further analyses: (i) including both control and CO₂ enrichment treatments, where the
89 ambient and elevated CO₂ concentrations were around the current and predicted atmospheric
90 CO₂ concentrations by the Intergovernmental Panel on Climate Change (IPCC, 2013),
91 respectively (Fig. S1); (ii) including or from which we could calculate at least one of the major N
92 pools or processes: soil TIN content, soil NH₄⁺ content, soil NO₃⁻ content, aboveground plant N
93 pool (APNP), belowground plant N pool (BPNP), total plant N pool (TPNP), litter N pool (LNP),
94 soil N pool (SNP), N fixation, nodule mass and/or number, net mineralization, nitrification,
95 denitrification, and inorganic N leaching. Overall, there were 175 papers included in the first
96 dataset (Table S1, References S1). For each paper, means, variations (standard deviation (*SD*),
97 standard error (*SE*) or confidence interval (*CI*)) and sample sizes of the variables in both control
98 and CO₂ enrichment treatments were collected.

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

$$100 \quad SD = SE\sqrt{n} \quad \text{Eq. (1)}$$

$$101 \quad \text{or } SD = (CI_u - CI_l)\sqrt{n}/2u_p \quad \text{Eq. (2)}$$

102 where *n* is the sample size, *CI_u* and *CI_l* are the upper and lower limits of *CI*, and *u_p* is the
103 significant level and equal to 1.96 and 1.645 when $\alpha = 0.05$ and 0.10, respectively. In some
104 studies, if tissue N concentration and biomass were reported, we multiplied the two parts as N
105 pools. When both APNP and BPNP were provided (or calculated), the two were added together

106 to represent the TPNP. When data from multiple soil layers were provided, they were summed if
 107 they were area-based (i.e., m² land), or averaged if they were weight-based (i.e., g⁻¹ soil). In
 108 studies where the respective contents of NH₄⁺ and NO₃⁻ were reported, the TIN was calculated
 109 by adding the two together. For all the variables, if more than one result were reported during the
 110 experiment period, they were averaged by

$$111 \quad M = \sum_{i=1}^j \frac{M_i}{j} \quad \text{Eq. (3)}$$

112 with standard deviation

$$113 \quad SD = \sqrt{\frac{\sum_{i=1}^j SD_i^2 (n_i - 1) n_i}{(\sum_{i=1}^j n_i - 1) \sum_{i=1}^j n_i}} \quad \text{Eq. (4)}$$

114 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
 115 sampling data, respectively (Liang et al., 2013). If additional treatments applied (e.g., N addition),
 116 they were treated as independent studies.

117 Because treatment time and N addition may affect the responses of the N processes to CO₂
 118 enrichment, the dataset was divided into different categories: (i) short-term (≤ 3 years) vs. long-
 119 term (> 3 years), and (ii) without N addition vs. with N addition. Moreover, the dataset was also
 120 divided into forest, grassland, and cropland to explore possible differences between ecosystem
 121 types.

122 For the second dataset, 15 available time series of plant growth were collected from 7 decadal
 123 FACE experiments (Table 1). The ecosystems included 9 forests, 5 grasslands and 1 desert.
 124 Because of the limited data, we included variables that can represent plant growth in one way or
 125 another, for example, net primary production (NPP), biomass, and leaf production. These data
 126 were collected to reveal whether the effect of CO₂ enrichment on plant growth diminishes over
 127 treatment time as proposed by the PNL hypothesis (Luo et al., 2004). In the 7 studies, the

128 treatment lasted from 7 to 13 years, and at least 6 years' production measurements were reported.
 129 For each data, the percentage change in NPP (or biomass or leaf production) by CO₂ enrichment
 130 was calculated. Then, a linear regression between the percentage change and the treatment year
 131 was conducted. A significantly negative slope indicates that the effect of CO₂ enrichment on the
 132 plant production diminishes over time. A non-significant slope was treated as 0. After deriving
 133 all the slopes, the frequency distribution of the slopes were fitted by a Gaussian function:

$$y = y_0 + ae^{-\frac{(x-\mu)^2}{2\sigma^2}} \quad \text{Eq. (5)}$$

134 where x is the mean value of each individual interval, and y is the frequency of each interval. y_0
 135 is the base frequency. μ and σ are the mean and SD of the distribution.

137

138 **2.2 Meta-analysis**

139 With the first dataset, the effect of CO₂ enrichment for each line of data of the N variables was
 140 estimated using the natural logarithm transformed response ratio (RR) (Hedges et al., 1999;
 141 Liang et al., 2013):

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

142 where X_E and X_C are the variable values under enriched CO₂ and control conditions, respectively.

143 The variation of the $\log RR$ was

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

144 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
 145 of X_C and X_E .

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

150
$$M_{weighted} = \frac{\sum_{j=1}^k W_j^* M_j}{\sum_{j=1}^k W_j^*}$$
 Eq. (8)

151 with the variance as

152
$$V_{weighted} = \frac{1}{\sum_{j=1}^k W_j^*}$$
 Eq. (9)

153 where k is the number of studies, M_j is the $Ln(RR)$ in study j , and W_j^* is the weighting factor
154 which consists of between- and within-study variances (Rosenberg et al., 2000; Liang et al.,
155 2013). The 95% lower and upper limits ($LL_{weighted}$ and $UL_{weighted}$) for the weighted mean were
156 computed as

157
$$LL_{weighted} = M_{weighted} - 1.96 \times \sqrt{V_{weighted}}$$
 Eq. (10)

158 and

159
$$UL_{weighted} = M_{weighted} + 1.96 \times \sqrt{V_{weighted}}$$
 Eq. (11)

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

165

166 **3 Results**

167 The meta-analysis from the first dataset showed that CO₂ enrichment significantly increased N
168 sequestered in plants and litter but not in SOM (Figs. 1A, S2). Whereas CO₂ enrichment had
169 little overall effects on N mineralization, nitrification and denitrification, it significantly
170 increased biological N fixation by 44.3% (with 95% CI from 29.5% to 61.8%). The increased
171 biological N fixation was consistent when using various methods except H₂ evolution (Fig. 2A).
172 In legume species, CO₂ enrichment significantly increased nodule mass and number (Fig. 2B). In
173 addition, CO₂ enrichment increased N₂O emission by 10.7% (with 95% CI from 2.0% to 22.3%),
174 but reduced leaching (i.e., -41.8% with 95% CI from -58.9% to -24.3%) (Fig. 1B). Although CO₂
175 enrichment did not change the total inorganic N availability in soils, it increased the soil
176 NH₄⁺/NO₃⁻ ratio by 16.9% (with 95% CI from 5.4% to 30.2%) (Fig. 1C).

177 Treatment time had no effect on most of the variables (overlapped 95% CIs for short- and
178 long-term treatments) except nitrification, which was not changed by short-term treatment, but
179 was significantly reduced (-23.4% with 95% CI from -30.4% to -12.1%) by long-term CO₂
180 enrichment (Fig. 3B). In addition, it seemed that the responses of the NH₄⁺/NO₃⁻ ratio was
181 strengthened over time, representing a neutral response to short-term CO₂ enrichment, but
182 significantly positive and negative responses to long-term CO₂ enrichment (Fig. 3C). The effects
183 of CO₂ enrichment were influenced by N addition (Fig. 3D – F). For example, nitrification was
184 significantly reduced by CO₂ enrichment without N addition by 19.3% (with 95% CI from -40.5%
185 to -0.65%), but was not changed with N addition. Denitrification and N₂O emission responded to
186 CO₂ enrichment neutrally without N addition, but significantly positively with N addition (Fig.
187 3E). Additionally, the responses of some variables to CO₂ enrichment were dependent on
188 ecosystem type (Fig. 3G – I). APNP responded to CO₂ enrichment positively in forests and

189 croplands, but neutrally in grasslands (Fig. 3G). Net mineralization had no response to CO₂
190 enrichment in forests or grasslands, while it was significantly increased in croplands (Fig. 3H).
191 Moreover, the change in the TIN was neutral in forests, grassland, but positive, in croplands,
192 respectively (Fig. 3I). In addition, a positive response of the NH₄⁺/NO₃⁻ ratio was only observed
193 in grasslands (Fig. 3I).

194 The results from the second dataset showed that CO₂ enrichment significantly increased plant
195 growth in most of the decadal FACE experiments (Fig. 4). In addition, the CO₂ fertilization
196 effect on plant growth did not over treatment time change in 11 experiments ($P > 0.05$),
197 decreased in 2 experiments (slope < 0 , $P < 0.05$), and increased in 2 experiments (slope > 0 , $P <$
198 0.05), respectively (Table 1, Fig. 4). Overall, the slope of the response of the plant growth vs.
199 treatment time was not significantly different from 0 (i.e., $-0.37\% \text{ year}^{-1}$ with 95% CI from -1.84%
200 year^{-1} to $1.09\% \text{ year}^{-1}$; Fig. 4).

201

202 **4 Discussion**

203 In this study, we carried out two syntheses on the responses of the terrestrial N cycle and plant
204 growth to CO₂ enrichment to test whether PNL generally occurs across ecosystems.

205

206 **4.1 PNL alleviation**

207 According to the PNL hypothesis, a prerequisite for PNL occurrence is that more N is
208 sequestered in plant, litter and SOM (Luo et al., 2004). Our results showed that elevated CO₂
209 significantly increased N retention in plant tissues and litter, which is consistent with previous
210 meta-analyses (de Graaff et al., 2006; Luo et al., 2006). Thus, the basis of PNL occurrence
211 partially exists. However, the results from the second dataset did not show a general diminished

212 CO₂ fertilization effect on plant growth on the decadal scale, which disagrees with the
213 expectation of the PNL hypothesis, suggesting that N supply under elevated CO₂ may meet the N
214 demand. In this study, we have identified two processes that increase N supply under elevated
215 CO₂, i.e., biological N fixation and leaching.

216 CO₂ enrichment significantly enhanced the N influx to terrestrial ecosystems through
217 biological N fixation, which reduces dinitrogen (N₂) to NH₄⁺ (Fig. 1B). The enhanced biological
218 N fixation may have resulted from the stimulated activities of symbiotic (Fig. 2B) and free-living
219 heterotrophic N-fixing bacteria (Hoque et al., 2001). In addition, the competition between N₂-
220 fixing and non-N₂-fixing species may have contributed to enhance the biological N fixation on
221 the ecosystem level (Poorter and Navas, 2003; Batterman et al., 2013).

222 In addition, the N efflux via leaching was reduced under elevated CO₂ conditions (Fig. 1B).
223 This could be attributed to the decrease in NO₃⁻, which is the primary N form in leaching,
224 (Chapin III et al., 2011), and the increased root growth which may immobilize more inorganic N
225 in soils (Luo et al., 2006; Iversen, 2010). In contrast, gaseous N loss through N₂O emission
226 increased under elevated CO₂, although this increase was only observed when additional N was
227 applied.

228 The net effect of the responses of N processes to CO₂ enrichment resulted in higher N
229 retention in ecosystems, especially within plant tissues and litter (Fig. S2). Because the product
230 of biological N fixation (i.e., NH₄⁺) and the primary form for N leaching loss (i.e., NO₃⁻) can be
231 directly used by plants, the effects of CO₂ enrichment on the two processes directly increase the
232 N availability for plant growth, potentially alleviating PNL (Fig. 5). The increased N in plant
233 tissues can be re-used by plants via resorption (Norby et al., 2000; Norby et al., 2001), and
234 consequently reduce the N demand from soils. This may be another mechanism that alleviates

235 PNL (Walker et al., 2015). Therefore, the increased N availability from increased N fixation and
236 reduced N leaching could potentially support net accumulation of organic matter in terrestrial
237 ecosystems (Rastetter et al., 1997; Luo and Reynolds, 1999).

238 Since biological N fixation provides at least 30% of the N requirement across natural biomes
239 (Asner et al., 2001; Galloway et al., 2004), our results suggest that the positive response of
240 biological N fixation to CO₂ enrichment plays an important role in alleviating PNL. The PNL
241 hypothesis was proposed to characterize long-term dynamics of C-N coupling in response to
242 rising atmospheric CO₂ concentration. Thus, it is critical to understand the long-term response of
243 biological N fixation to elevated CO₂. In this paper, we have synthesized 12 studies that lasted 4
244 – 7 years and binned them in a long-term category (> 3 years). On average of those long-term
245 studies, CO₂ enrichment increased biological N fixation by 26.2%. The increased biological N
246 fixation is supported by evidence at gene level from long-term experiments. For example, Tu et
247 al. (2015) found that the abundance of *nifH* gene amplicons, which is a widely used marker for
248 analyzing biological N fixation, was significantly enhanced by 12 years of CO₂ enrichment in a
249 grassland (BioCON). However, our synthesis showed a relatively wide 95% confidence interval
250 from 2.54% to 59.8%. The wide range can be partially attributed to the relatively small number
251 of studies. In addition, most studies incorporated in the current synthesis were conducted in
252 temperate regions. Thus, longer-term studies, as well as studies in other regions (e.g., boreal and
253 tropical) are critically needed to reveal more general patterns in the future.

254 In this study, it is suggested that the general trend of the N cycle changes under elevated CO₂
255 converges towards increased soil N supply for plant growth, which in theory could alleviate PNL.
256 However, the PNL alleviation potential may vary across different ecosystems due to asymmetric
257 distributions of biological N fixation (Cleveland et al., 1999). In addition, PNL alleviation may

258 also be influenced by other factors. While most of the long-term experiments did not show a
259 diminished CO₂ fertilization effect, it decreased in two sites (i.e., ORNL and Aspen-Birch) (Fig.
260 4). Plant growth is usually influenced by multiple environmental factors (e.g., nutrients, water,
261 light, ozone). The undiminished CO₂ fertilization effect in most studies indicates that resource
262 limitation (including N) was not aggravated, suggesting that no PNL occurred in these sites.
263 However, in the ORNL and Aspen-Birch (without O₃ treatment) experiments, the diminished
264 CO₂ fertilization effect could be attributed to limitation of N, or other resources, or their
265 combined effect. For example, reduced N availability has been identified as one of the primary
266 factors that lead to the diminished CO₂ fertilization effect on NPP in the ORNL FACE
267 experiment (Norby et al., 2010). In the Aspen-Birch community, however, the deceleration of
268 leaf area increases due to canopy closure was responsible for the diminished CO₂ fertilization
269 effect without O₃ addition (Talhelm et al., 2012). With O₃ addition, O₃ significantly reduced the
270 canopy development, resulting in a relatively open canopy during the experiment period. In
271 addition, the negative effect of O₃ addition increased over time, leading to the apparent increase
272 in the CO₂ fertilization effect (Fig. 4) (Talhelm et al., 2012).

273

274 **4.2 Dependence of the responses of N cycling processes upon methodology, treatment**

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

276 Methodology may potentially influence findings. Cabrerizo et al. (2001) found that CO₂
277 enrichment increased the nitrogenase activity measured by acetylene reduction assay (ARA), but
278 not the specific N fixation measured by the H₂ evolution method. In the studies synthesized here,
279 four methods were used to estimate biological N fixation, including isotope, ARA, H₂ evolution
280 and N accumulation. Among them, ARA and H₂ evolution measure nitrogenase activity (Hunt

281 and Layzell, 1993) whereas isotope and N accumulation methods directly measure biological N
282 fixation. All but the H₂ evolution method showed a significantly positive response to CO₂
283 enrichment (Fig. 2A). The insignificant response shown by the H₂ evolution method was likely
284 because of the small study numbers (i.e., 3). In addition, the biological N fixation measured by
285 ARA, isotope and N accumulation showed similar response magnitudes (Fig. 2A), suggesting
286 consistency among the three methods. However, further assessment on the H₂ evolution method
287 is needed.

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

290 N mineralization, in addition to biological N fixation, is a major source of available N in soils.
291 Our meta-analysis showed no change in the net N mineralization in response to CO₂ enrichment,
292 which is consistent with the results by de Graaff et al. (2006). However, the response of net
293 mineralization was dependent upon ecosystem types, showing no change in forests and
294 grasslands, but significant increases in croplands (Fig. 3H). There may be two reasons for the
295 stimulated net mineralization in croplands. First, N fertilization, which is commonly practiced in
296 croplands, can increase the substrate quantity and quality for mineralization (Barrios et al., 1996;
297 Chapin III et al., 2011; Booth et al., 2005; Lu et al., 2011; Reich and Hobbie, 2013). Second,
298 tillage can alter soil conditions (e.g., increasing O₂ content), which can potentially favor the N
299 mineralization under enriched CO₂ (Wienhold and Halvorson, 1999; Bardgett and Wardle, 2010).
300 These findings suggest that CO₂ enrichment can stimulate the N transfer from organic to
301 inorganic forms in managed croplands.

302 Unlike leaching, the response of nitrification was dependent upon treatment duration (Fig. 3).
303 Nitrification was not changed by short-term treatment, but was significantly reduced by long-

304 term CO₂ enrichment (Fig. 3). One possible reason for the reduced nitrification with long-term
305 CO₂ enrichment is the cumulative effect of hydrological changes. CO₂ enrichment generally
306 reduces stomatal conductance and, consequently, water loss via plant transpiration, leading to an
307 increase in soil water content (Niklaus et al., 1998; Tricker et al., 2009; van Groenigen et al.,
308 2011; Keenan et al., 2013). A synthesis by van Groenigen et al. (2011) shows that CO₂
309 enrichment increases soil water content by 2.6%–10.6%. Increased soil water content may result
310 in less oxygen (O₂) concentration in soils, which could potentially constrain nitrification.

311 In addition, the response of gaseous N loss was dependent on N addition (Fig. 3). The reduced
312 nitrification was only observed under conditions without N addition (Fig. 3E). With N addition,
313 no response of nitrification to CO₂ enrichment was observed (Fig. 3E). Additionally, the
314 response of denitrification to CO₂ enrichment shifted from neutral, without N addition, to
315 significantly positive with N addition (Fig. 3E). One possible reason is that N addition provides
316 more N substrate for nitrifying and denitrifying bacteria (Keller et al., 1988; Stehfest and
317 Bouwman, 2006; Russow et al., 2008). The strengthening trends of both nitrification and
318 denitrification led to a shift of the response of N₂O emission to CO₂ enrichment from neutral
319 without N addition to significantly positive with N addition (Fig. 3E). Our results indicate that
320 CO₂ enrichment significantly increases gaseous N loss when additional N is applied.

321 Our results are consistent with a previous synthesis (van Groenigen et al. 2011). Increased
322 N₂O emissions can partially offset the mitigation of climate change by the stimulated plant CO₂
323 assimilation as the warming potential of N₂O is 296 times that of CO₂. However, a recent
324 modeling study by Zaehle et al. (2011) found an opposite result showing that CO₂ enrichment
325 reduced radiative forcing of N₂O. In their model, less availability of N substrates for nitrification
326 and denitrification due to the enhanced plant N sequestration attributed to the reduced N₂O

327 emission. Our synthesis shows that inorganic N does not decrease. Especially with additional N
328 application, enhanced denitrification by CO₂ enrichment results in a greater N₂O emission.

329

330 **4.3 Changes in soil microenvironment, community structures and above-belowground** 331 **interactions**

332 The meta-analysis showed that the two major forms of soil available N, NH₄⁺ and NO₃⁻,
333 responded to long-term CO₂ enrichment in opposing manners (Fig. 3C). While the enhanced
334 biological N fixation by CO₂ enrichment tended to increase the NH₄⁺ content in soils, the
335 reduced nitrification decreased the NO₃⁻ content in soils, leading to a significant increase in the
336 NH₄⁺/NO₃⁻ ratio (Fig. 3C).

337 Although the total available N did not change under elevated CO₂, the altered proportion of
338 NH₄⁺ over NO₃⁻ in soils may have long-term effects on soil microenvironment and associated
339 aboveground-belowground linkages that control the C cycle (Bardgett and Wardle, 2010). On the
340 one hand, plants would release more hydrogen ion (H⁺) to regulate the charge balance when
341 taking up more NH₄⁺. As a result, the increased NH₄⁺ absorption could acidify the rhizosphere
342 soil (Thomson et al., 1993; Monsanto et al., 2008). The lowered pH could have significant effects
343 on soil microbial communities and their associated ecosystem functions. For example,
344 fungal/bacterial ratio increases with the decrease in pH (de Vries et al., 2006; Rousk et al., 2009).
345 The increased fungal/bacterial ratio may result in lower N mineralization because of the higher
346 C/N ratio of fungi and the lower turnover rates of fungal-feeding fauna (de Vries et al., 2006;
347 Rousk and Bååth, 2007). In other words, the increased fungal/bacterial ratio may slow down the
348 N turnover from organic to inorganic forms. On the other hand, the increased NH₄⁺/NO₃⁻ ratio
349 may increase the N use efficiency because it is more energetically expensive for plants to utilize

350 NO_3^- than NH_4^+ (Chapin III et al., 2011; Odum and Barrett, 2005; Lambers et al., 2008). In
351 addition, since the preferences for plant absorption of different forms of N are different (Chapin
352 III et al., 2011; Odum and Barrett, 2005), the increased $\text{NH}_4^+/\text{NO}_3^-$ ratio may benefit some plant
353 species while depress others, and consequently alter the community structures over time. These
354 diverse changes in soil microenvironment and microbial and plant community compositions
355 could further affect the terrestrial C cycle on long temporal scales, on which more studies are
356 needed.

357

358 **5 Summary**

359 This study synthesizes data in the literature on the effects of CO_2 enrichment on the terrestrial N
360 cycle to improve our understanding of the N limitation to plant growth under elevated CO_2 . Our
361 results indicate that elevated CO_2 stimulates N influx via biological N fixation but reduces N loss
362 via leaching, leading to increased N supply for plant growth. The additional N supply via the
363 enhanced biological N fixation and the reduced leaching may partially meet the increased N
364 demand under elevated CO_2 , potentially alleviating PNL. In addition, increased N_2O emissions
365 can partially offset the mitigation of climate change by stimulated plant CO_2 assimilation.
366 Moreover, changes in soil microenvironments, ecosystem communities and above-belowground
367 interactions induced by the different responses of NH_4^+ and NO_3^- to CO_2 enrichment may have
368 long-term effects on the terrestrial biogeochemical cycles and climate change.

369

370 *Acknowledgements.* We thank two anonymous reviewers for their valuable comments and
371 suggestions, Dr. Kevin R. Wilcox for his help with language checking. This study was
372 financially supported by the US Department of Energy, Terrestrial Ecosystem Sciences grant DE

373 SC0008270 and Biological Systems Research on the Role of Microbial Communities in Carbon
374 Cycling Program grants DE-SC0004601 and DE-SC0010715. Authors declare no conflict of
375 interest.

376

377 **References**

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

379 Physical and biogeochemical controls over terrestrial ecosystem responses to nitrogen
380 deposition, *Biogeochemistry*, 54, 1-39, 2001.

381 Bardgett, R. D. and Wardle, D. A.: Aboveground-belowground linkages: biotic interactions,
382 ecosystem processes, and global change, Oxford University Press, 2010.

383 Barrios, E., Buresh, R. J., and Sprent, J. I.: Nitrogen mineralization in density fractions of soil
384 organic matter from maize and legume cropping systems, *Soil Biology & Biochemistry*, 28,
385 1459-1465, 1996.

386 Batterman, S. A., Hedin, L. O., van Breugel, M., Ransijn, J., Craven, D. J., and Hall, J. S.: Key
387 role of symbiotic dinitrogen fixation in tropical forest secondary succession, *Nature*, 502,
388 224-227, 2013.

389 Booth, M. S., Stark, J. M., and Rastetter, E.: Controls on nitrogen cycling in terrestrial
390 ecosystems: A synthetic analysis of literature data, *Ecological Monographs*, 75, 139-157,
391 2005.

392 Cabrerizo, P. M., González, E. M., Aparicio-Tejo, P. M., and Arrese-Igor, C.: Continuous CO₂
393 enrichment leads to increased nodule biomass, carbon availability to nodules and activity of
394 carbon-metabolising enzymes but does not enhance specific nitrogen fixation in
395 pea, *Physiologia Plantarum*, 113, 33-40, 2001.

396 Chapin III, F. S., Matson, P. A., and Vitousek, P.: Principles of terrestrial ecosystem ecology,
397 Springer, 2011.

398 Cleveland, C. C., Townsend, A. R., Schimel, D. S., Fisher, H., Howarth, R. W., Hedin, L. O.,
399 Perakis, S. S., Latty, E. F., Von Fischer, J. C., Elseroad, A., and Wasson, M. F.: Global
400 patterns of terrestrial biological nitrogen (N₂) fixation in natural ecosystems, *Global*
401 *Biogeochemical Cycles*, 13, 623-645, 1999.

402 de Graaff, M. A., van Groenigen, K. J., Six, J., Hungate, B., and van Kessel, C.: Interactions
403 between plant growth and soil nutrient cycling under elevated CO₂: a meta-analysis, *Global*
404 *Change Biology*, 12, 2077-2091, 2006.

405 de Vries, F. T., Hoffland, E., van Eekeren, N., Brussaard, L., and Bloem, J.: Fungal/bacterial
406 ratios in grasslands with contrasting nitrogen management, *Soil Biology & Biochemistry*, 38,
407 2092-2103, 2006.

408 Finzi, A. C., Moore, D. J. P., Delucia, E. H., Lichter, J., Hofmockel, K. S., Jackson, R. B., Kim,
409 H., Matamala, R., McCarthy, H. R., Oren, R., Phippen, J. S., Schlesinger, W. H.: Progressive
410 nitrogen limitation of ecosystem processes under elevated CO₂ in a warm-temperate forest,
411 *Ecology*, 87, 15-25, 2006.

412 Galloway, J. N., Dentener, F. J., Capone, D. G., Boyer, E. W., Howarth, R. W., Seitzinger, S. P.,
413 Asner, G. P., Cleveland, C. C., Green, P. A., Holland, E. A., and Karl, D. M.: Nitrogen cycles:
414 past, present, and future, *Biogeochemistry*, 70, 53-226, 2004.

415 Hedges, L. V., Gurevitch, J., and Curtis, P. S.: The meta-analysis of response ratios in
416 experimental ecology, *Ecology*, 80, 1150-1156, 1999.

417 Hoque, M. M., Inubushi, K., Miura, S., Kobayashi, K., Kim, H. Y., Okada, M., and Yabashi, S.:
418 Biological dinitrogen fixation and soil microbial biomass carbon as influenced by free-air

419 carbon dioxide enrichment (FACE) at three levels of nitrogen fertilization in a paddy field,
420 *Biology and Fertility of Soils*, 34, 453-459, 2001.

421 Hunt, S. and Layzell, D.B.: Gas exchange of legume nodules and the regulation of nitrogenase
422 activity, *Annual Review of Plant Physiology and Plant Molecular Biology*, 44, 483-511,
423 1993.

424 IPCC: *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to*
425 *the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, Cambridge
426 University Press, Cambridge, United Kingdom and New York, NY, USA, 2013.

427 Iversen, C. M.: Digging deeper: fine-root responses to rising atmospheric CO₂ concentration in
428 forested ecosystems, *New Phytologist*, 186, 346-357, 2010.

429 Keenan, T. F., Hollinger, D. Y., Bohrer, G., Dragoni, D., Munger, J. W., Schmid, H. P., and
430 Richardson, A. D.: Increase in forest water-use efficiency as atmospheric carbon dioxide
431 concentrations rise, *Nature*, 499, 324-+, 2013.

432 Keller, M., Kaplan, W. A., Wofsy, S. C., and Dacosta, J. M.: Emissions of N₂O from Tropical
433 Forest Soils: Response to Fertilization with NH₄⁺, NO₃⁻, and PO₄³⁻, *Journal of Geophysical*
434 *Research-Atmospheres*, 93, 1600-1604, 1988.

435 Lambers, H., Chapin III, F. S., and Pons, T. L.: *Plant Physiological Ecology*, Springer, New
436 York, 2008.

437 Liang, J., Xia, J., Liu, L., and Wan, S.: Global patterns of the responses of leaf-level
438 photosynthesis and respiration in terrestrial plants to experimental warming, *Journal of Plant*
439 *Ecology*, 6, 437-447, 2013.

440 Lu, M., Yang, Y. H., Luo, Y. Q., Fang, C. M., Zhou, X. H., Chen, J. K., Yang, X., and Li, B.:
441 Responses of ecosystem nitrogen cycle to nitrogen addition: a meta-analysis, *New*
442 *Phytologist*, 189, 1040-1050, 2011.

443 Luo, Y. and Reynolds, J. F.: Validity of extrapolating field CO₂ experiments to predict carbon
444 sequestration in natural ecosystems, *Ecology*, 80, 1568-1583, 1999.

445 Luo, Y., Su, B., Currie, W. S., Dukes, J. S., Finzi, A. C., Hartwig, U., Hungate, B., McMurtrie, R.
446 E., Oren, R., Parton, W. J., Pataki, D. E., Shaw, M. R., Zak, D. R., and Field, C. B.:
447 Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide,
448 *Bioscience*, 54, 731-739, 2004.

449 Luo, Y. Q., Hui, D. F., and Zhang, D. Q.: Elevated CO₂ stimulates net accumulations of carbon
450 and nitrogen in land ecosystems: A meta-analysis, *Ecology*, 87, 53-63, 2006.

451 McCarthy, H. R., Oren, R., Johnsen, K. H., Gallet-Budynek, A., Pritchard, S. G., Cook, C. W.,
452 LaDeau, S. L., Jackson, R. B., and Finzi, A. C.: Re-assessment of plant carbon dynamics at
453 the Duke free-air CO₂ enrichment site: interactions of atmospheric [CO₂] with nitrogen and
454 water availability over stand development, *New Phytologist*, 185, 514-528, 2010.

455 Monsant, A. C., Tang, C., and Baker, A. J. M.: The effect of nitrogen form on rhizosphere soil
456 pH and zinc phytoextraction by *Thlaspi caerulescens*, *Chemosphere*, 73, 635-642, 2008.

457 Moore, D. J. P., Aref, S., Ho, R. M., Pippen, J. S., Hamilton, J. G., de Lucia, E. H.: Annual basal
458 area increment and growth duration of *Pinus taeda* in response to eight years of free-air
459 carbon dioxide enrichment, *Global Change Biology*, 12, 1367-1377, 2006.

460 Niklaus, P. A., Spinnler, D., and Korner, C.: Soil moisture dynamics of calcareous grassland
461 under elevated CO₂, *Oecologia*, 117, 201-208, 1998.

462 Norby, R. J., Cotrufo, M. F., Ineson, P., O'Neill, E. G., and Canadell, J. G.: Elevated CO₂, litter
463 chemistry, and decomposition: a synthesis, *Oecologia*, 127, 153-165, 2001.

464 Norby, R. J., Long, T. M., Hartz-Rubin, J. S., and O'Neill, E. G.: Nitrogen resorption in
465 senescing tree leaves in a warmer, CO₂-enriched atmosphere, *Plant and Soil*, 224, 15-29,
466 2000.

467 Norby, R. J., Warren, J. M., Iversen, C. M., Medlyn, B. E., and McMurtrie, R. E.: CO₂
468 enhancement of forest productivity constrained by limited nitrogen availability, *Proceedings*
469 *of the National Academy of Sciences of the United States of America*, 107, 19368-19373,
470 2010.

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

472 Oren, R., Ellsworth, D. S., Johnsen, K. H., Phillips, N., Ewers, B. E., Maier, C., Schafer, K. V.
473 R., McCarthy, H., Hendrey, G., McNulty, S. G., and Katul, G. G.: Soil fertility limits carbon
474 sequestration by forest ecosystems in a CO₂-enriched atmosphere, *Nature*, 411, 469-472,
475 2001.

476 Poorter, H. and Navas, M. L.: Plant growth and competition at elevated CO₂: on winners, losers
477 and functional groups, *New Phytologist*, 157, 175-198, 2003.

478 Rastetter, E. B., Agren, G. I., and Shaver, G. R.: Responses of N-limited ecosystems to increased
479 CO₂: A balanced-nutrition, coupled-element-cycles model, *Ecological Applications*, 7, 444-
480 460, 1997.

481 Reay, D. S., Dentener, F., Smith, P., Grace, J., and Feely, R. A.: Global nitrogen deposition and
482 carbon sinks, *Nature Geoscience*, 1, 430-437, 2008.

483 Reich, P. B. and Hobbie, S. E.: Decade-long soil nitrogen constraint on the CO₂ fertilization of
484 plant biomass, *Nature Climate Change*, 3, 278-282, 2013.

485 Reich, P. B., Hobbie, S. E., Lee, T., Ellsworth, D. S., West, J. B., Tilman, D., Knops, J. M. H.,
486 Naeem, S., and Trost, J.: Nitrogen limitation constrains sustainability of ecosystem response
487 to CO₂, *Nature*, 440, 922-925, 2006.

488 Reich, P. B., Hobbie, S. E., and Lee, T. D.: Plant growth enhancement by elevated CO₂
489 eliminated by joint water and nitrogen limitation, *Nature Geoscience*, 7, 920-924, 2014.

490 Rosenberg, M. S., Adams, D. C., and Gurevitch, J.: *MetaWin: statistical software for meta-*
491 *analysis*, Sinauer Associates Sunderland, Massachusetts, USA, 2000.

492 Ross, D. J., Newton, P. C. D., Tate, K. R., and Luo, D. W.: Impact of a low level of CO₂
493 enrichment on soil carbon and nitrogen pools and mineralization rates over ten years in a
494 seasonally dry, grazed pasture, *Soil Biology & Biochemistry*, 58, 265-274, 2013.

495 Rousk, J. and Bååth, E.: Fungal biomass production and turnover in soil estimated using the
496 acetate-in-ergosterol technique, *Soil Biology & Biochemistry*, 39, 2173-2177, 2007.

497 Rousk, J., Brookes, P. C., and Baath, E.: Contrasting Soil pH Effects on Fungal and Bacterial
498 Growth Suggest Functional Redundancy in Carbon Mineralization, *Applied and*
499 *Environmental Microbiology*, 75, 1589-1596, 2009.

500 Russow, R., Spott, O., and Stange, C. F.: Evaluation of nitrate and ammonium as sources of NO
501 and N₂O emissions from black earth soils (Haplic Chernozem) based on ¹⁵N field
502 experiments, *Soil Biology & Biochemistry*, 40, 380-391, 2008.

503 Schneider, M. K., Luscher, A., Richter, M., Aeschlimann, U., Hartwig, U. A., Blum, H., Frossard,
504 E., and Nosberger, J.: Ten years of free-air CO₂ enrichment altered the mobilization of N
505 from soil in *Lolium perenne* L. swards, *Global Change Biology*, 10, 1377-1388, 2004.

506 Smith, S. D., Charlet, T. N., Zitzer, S. F., Abella, S. R., Vanier, C. H., and Huxman, T. E.: Long-
507 term response of a Mojave Desert winter annual plant community to a whole-ecosystem
508 atmospheric CO₂ manipulation (FACE), *Global Change Biology*, 20, 879-892, 2014.

509 Stehfest, E. and Bouwman, L.: N₂O and NO emission from agricultural fields and soils under
510 natural vegetation: summarizing available measurement data and modeling of global annual
511 emissions, *Nutrient Cycling in Agroecosystems*, 74, 207-228, 2006.

512 Talhelm, A. F., Pregitzer, K. S., and Giardina, C. P.: Long-Term Leaf Production Response to
513 Elevated Atmospheric Carbon Dioxide and Tropospheric Ozone, *Ecosystems*, 15, 71-82,
514 2012.

515 Thomson, C. J., Marschner, H., and Romheld, V.: Effect of Nitrogen-Fertilizer Form on pH of
516 the Bulk Soil and Rhizosphere, and on the Growth, Phosphorus, and Micronutrient Uptake of
517 Bean, *Journal of Plant Nutrition*, 16, 493-506, 1993.

518 Tricker, P. J., Pecchiari, M., Bunn, S. M., Vaccari, F. P., Peressotti, A., Miglietta, F., and Taylor,
519 G.: Water use of a bioenergy plantation increases in a future high CO₂ world, *Biomass &*
520 *Bioenergy*, 33, 200-208, 2009.

521 Tu, Q., Zhou, X., He, Z., Xue, K., Wu, L., Reich, P., Hobbie, S., and Zhou, J.: The Diversity and
522 Co-occurrence Patterns of N₂-Fixing Communities in a CO₂-Enriched Grassland
523 Ecosystem, *Microbial Ecology*, doi:10.1007/s00248-015-0659-7, 2015.

524 van Groenigen, K. J., Osenberg, C. W., and Hungate, B. A.: Increased soil emissions of potent
525 greenhouse gases under increased atmospheric CO₂, *Nature*, 475, 214-U121, 2011.

526 Vitousek, P. M.: *Nutrient cycling and limitation: Hawai'i as a model system*, Princeton
527 University Press, 2004.

528 Walker, A. P., Zaehle, S., Medlyn, B. E., De Kauwe, M. G., Asao, S., Hickler, T., Parton, W.,
529 Ricciuto, D. M., Wang, Y. P., and Wårlind, D.: Predicting long-term carbon sequestration in
530 response to CO₂ enrichment: How and why do current ecosystem models differ?, *Global*
531 *Biogeochemical Cycles*, 29, 476-495, 2015.

532 Wienhold, B. J. and Halvorson, A. D.: Nitrogen mineralization responses to cropping, tillage,
533 and nitrogen rate in the Northern Great Plains, *Soil Science Society of America Journal*, 63,
534 192-196, 1999.

535 Zaehle, S., Ciais, P., Friend, A.D. and Prieur, V.: Carbon benefits of anthropogenic reactive
536 nitrogen offset by nitrous oxide emissions. *Nature Geoscience*, 4, 601-605, 2011.

537

538 **Supporting Information captions**

539 **Figure S1** Distributions of the experimental duration (**A**) and the CO₂ concentrations under
540 ambient (**B**) and elevated (**C**) treatments and their difference (**D**) for the 175 collected studies.
541 Red dashed lines represent the mean values.

542

543 **Figure S2** Summary of the effect of CO₂ enrichment on ecosystem level N budget. Square boxes
544 are nitrogen pools, ovals are nitrogen processes. Red dashed boxes mean the sum of the pools in
545 the boxes. “+”, “-”, and “ns” mean the response to CO₂ enrichment are positive, negative, and
546 not significant, respectively. Please see **Figure 1** for abbreviations.

547

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

549

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

551

552 **Table 1.** Results on the effect of CO₂ enrichment on ecosystem NPP (or biomass or leaf production) in decadal-long free air CO₂
553 enrichment (FACE) experiments over treatment time. The values of the slope, R^2 and P in the linear regression in **Fig. 4** are shown.
554 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
555 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 O₃
556 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
NDFE	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

557

558 **Figure captions**

559 **Figure 1.** Results of a meta-analysis on the responses of nitrogen pools and processes to
560 CO₂ enrichment. In (A), APNP, BPNP, TPNP, LNP, and SNP are the abbreviations for
561 aboveground plant nitrogen pool, belowground plant nitrogen pool, total plant nitrogen
562 pool, litter nitrogen pool, and soil nitrogen pool, respectively. In (C), TIN, NH₄⁺ and
563 NO₃⁻ are total inorganic nitrogen, ammonium, and nitrate in soils, respectively. The error
564 bars represent 95% confidence intervals.

565

566 **Figure 2.** Responses of biological N fixation measured by different methods (A) and
567 nodule dry mass and number in legume species (B). ARA: acetylene reduction assay.
568 Mean ± 95% confidence interval.

569

570 **Figure 3.** Responses of terrestrial nitrogen pools and processes to CO₂ enrichment (Mean
571 ± 95% confidence interval) as regulated by experimental durations (A – C; short-term: ≤
572 3 years vs. long-term: > 3 years), nitrogen addition (D – F), and ecosystem types (G – I).
573 Please see Figure 1 for abbreviations.

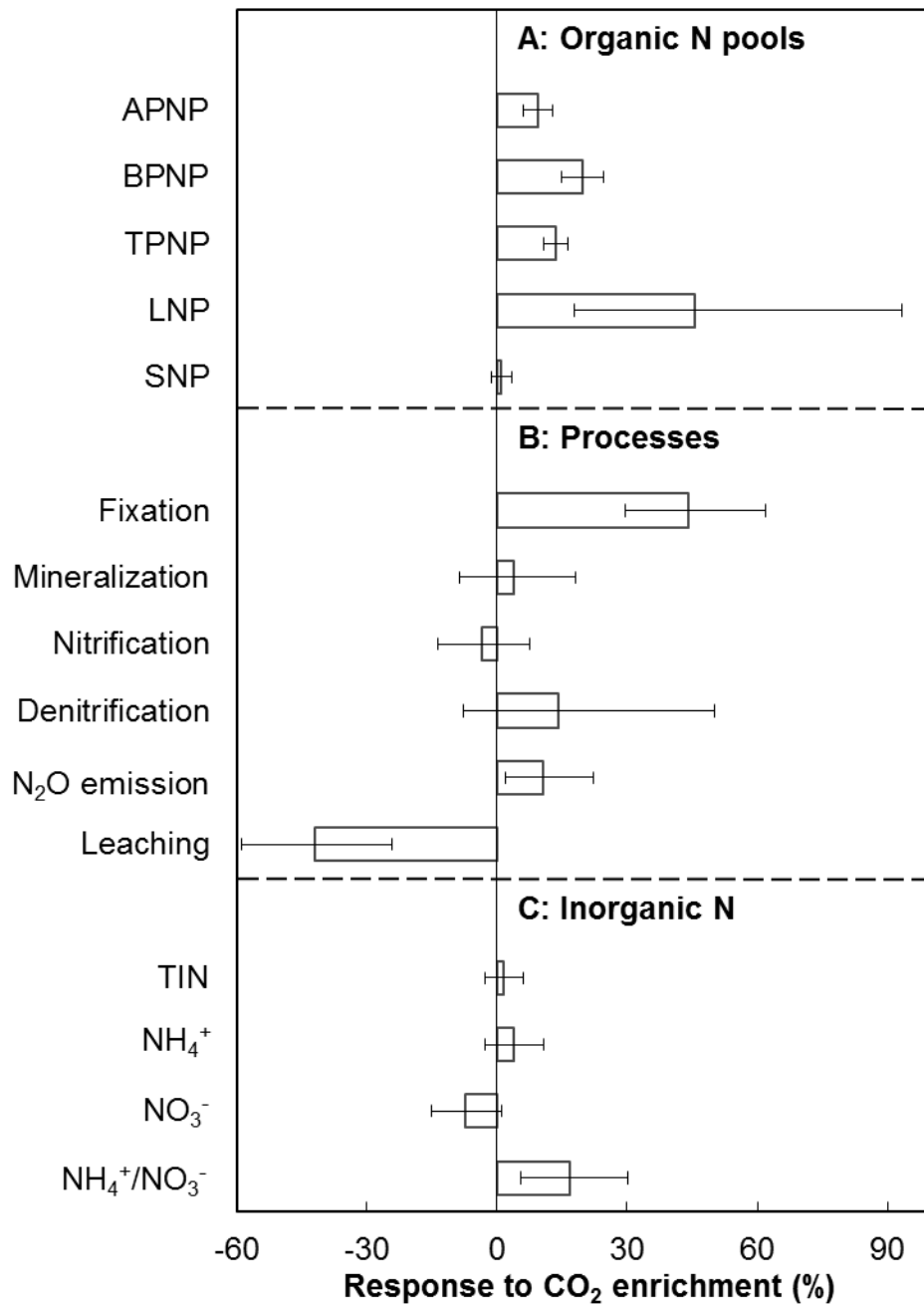
574

575 **Figure 4.** Time courses of CO₂ effects on ecosystem NPP (or biomass or leaf production)
576 in decadal-long FACE experiments. Please see **Table 1** for details of experiments,
577 references and statistical results. Only statistically significant ($P < 0.05$) regression lines
578 are shown. The panel at the right-low corner shows the distribution of the slopes (-0.37%
579 year⁻¹ with 95% CI from -1.84% year⁻¹ to 1.09% year⁻¹).

580

581 **Figure 5.** Mechanisms that alleviate PNL. PNL hypothesis posits that the stimulated
582 plant growth by CO₂ enrichment leads to more N sequestered in long-lived plant tissues,
583 litter and soil organic matter (SOM) so that, the N availability for plant growth
584 progressively declines over time, and plant growth is downregulated (grey symbols). The
585 current synthesis indicates that the basis of PNL occurrence partially exists (i.e., more N
586 sequestered in plant tissues and litter; black symbols). Despite of the increases in plant N
587 sequestration and N₂O emission, stimulated biological N fixation and reduced N leaching
588 can replenish the N availability, potentially alleviating PNL (blue boxes and arrows).
589 Upward, downward, and horizontal arrows mean increase, decrease, and no change,
590 respectively.
591

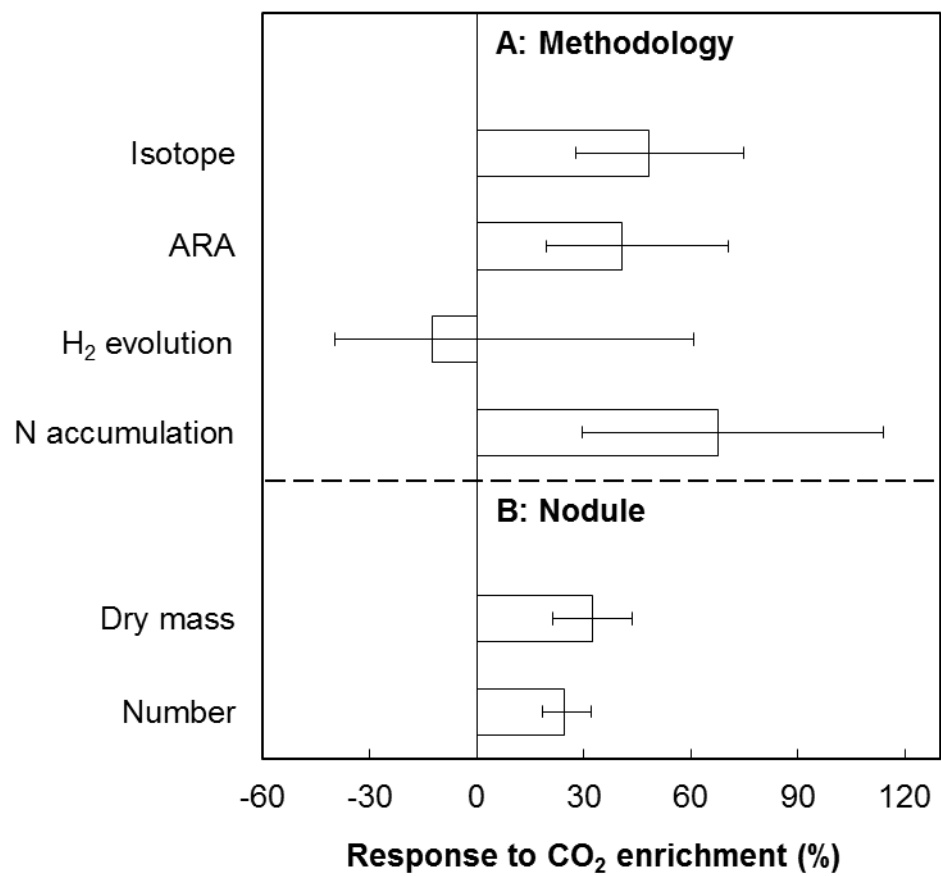
592 **Figure 1.**



593

594

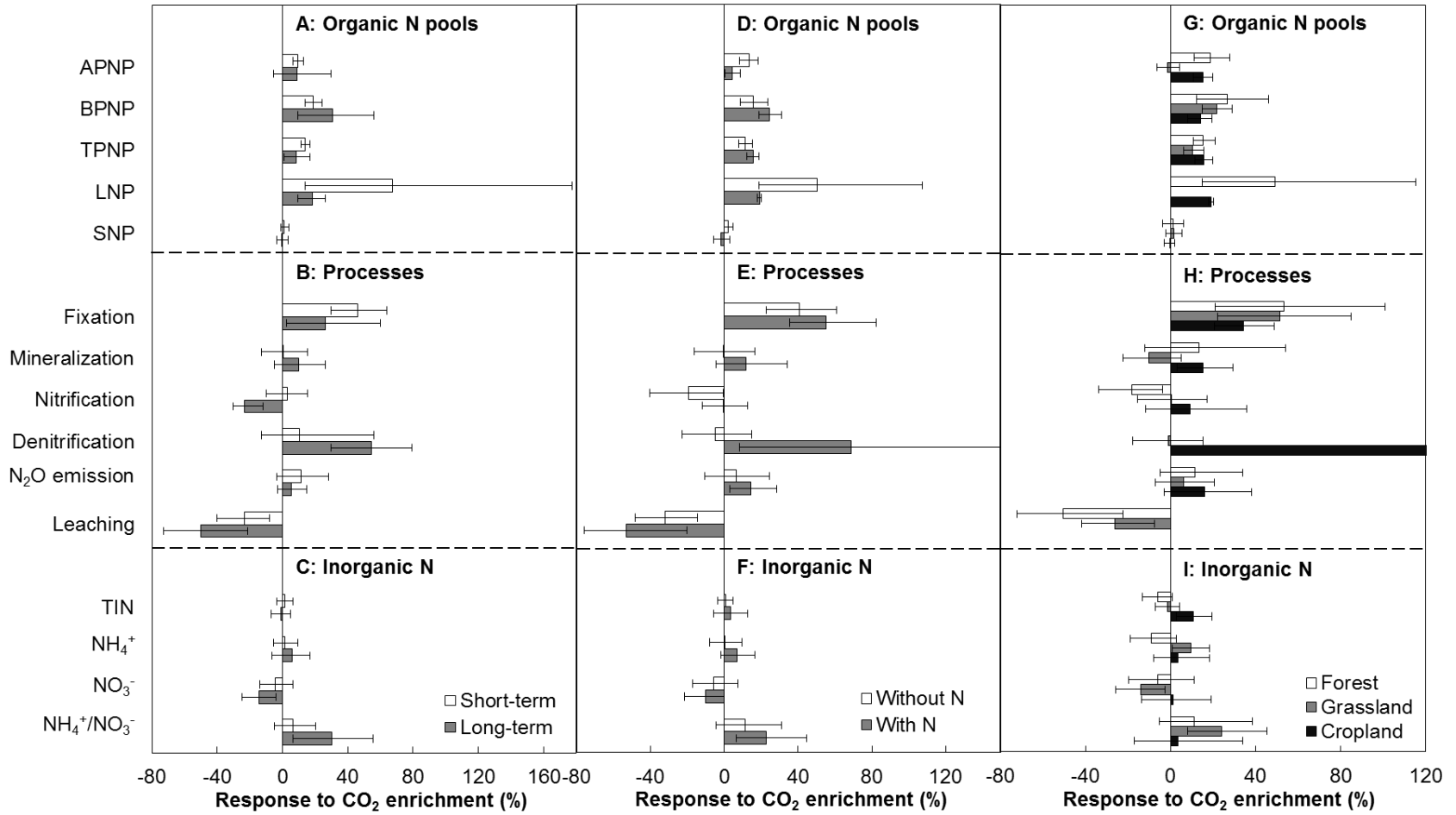
595 **Figure 2.**



596

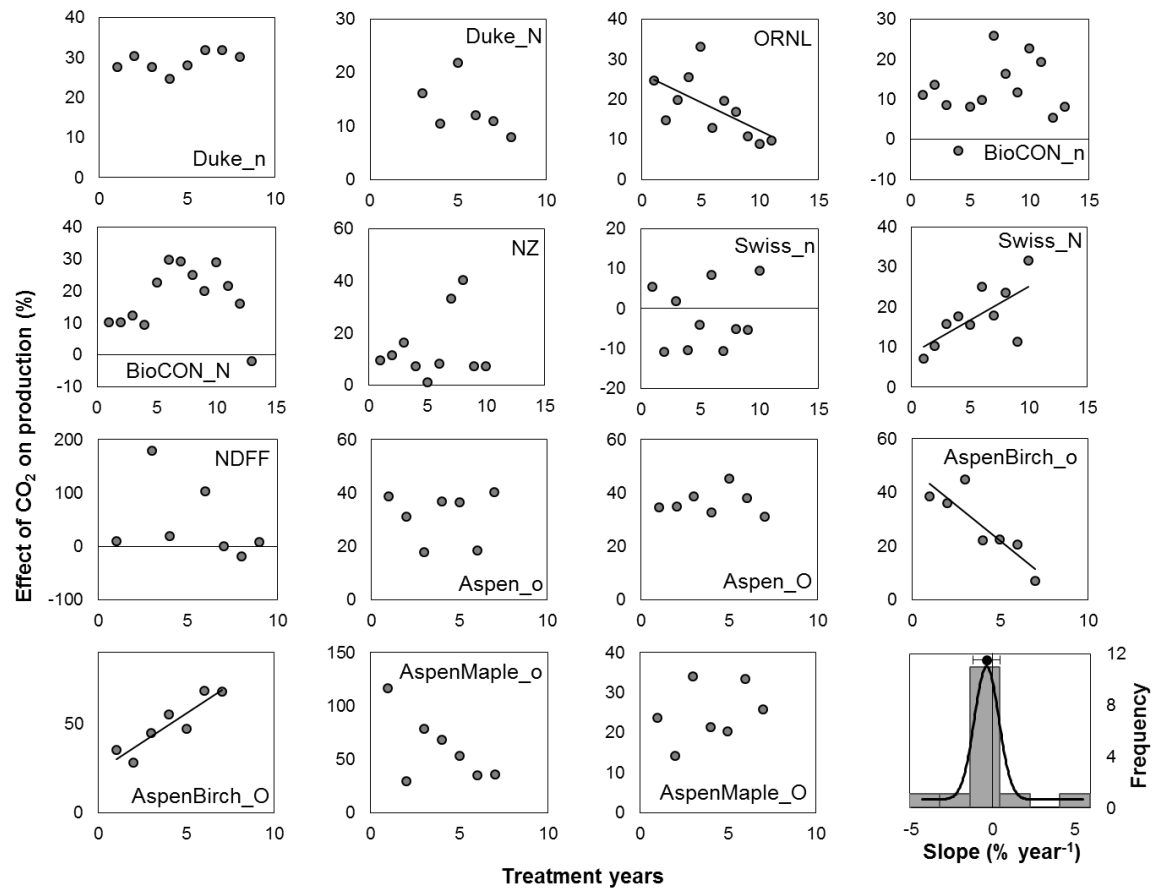
597

598 **Figure 3.**



599

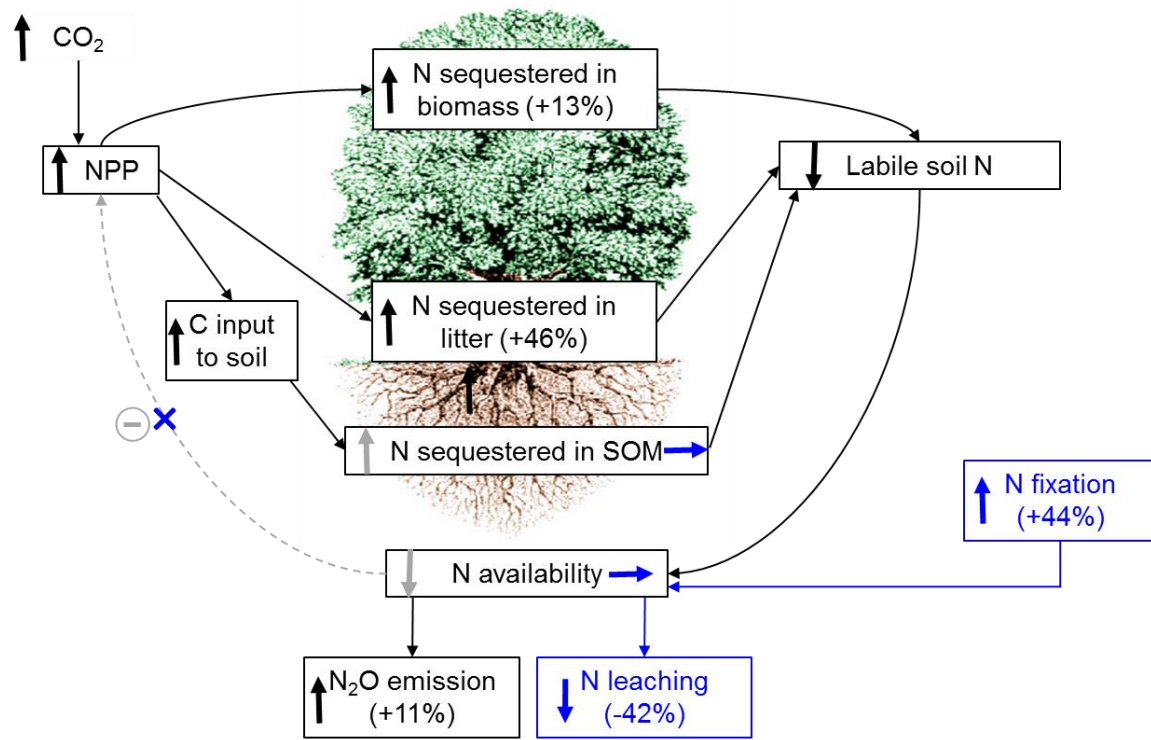
600 **Figure 4.**



601

602

603 **Figure 5.**



604

605