Letter of Responses

Dear Dr. Rammig,

Thank you very much for handling our manuscript “bg-2015-319”. We appreciate the opportunity to re-submit the manuscript, which has been thoroughly revised based on the reviewers’ comments. In addition, a native English speaker, Dr. Kevin R. Wilcox, who is acknowledged in the revised version, has helped improve the presentation. We hope you will find the revised manuscript acceptable for publication.

Sincerely,
Junyi Liang

The original reviewers’ 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.

Responses to Reviewer #1

I only have one technical remark. On line 114, it should be "between ecosystem types" instead of "among ecosystems".

Response: We have revised the sentence as the reviewer suggested by saying: “Moreover, the dataset was also divided into forest, grassland, and cropland to explore possible differences between ecosystem types” (Lines 123 – 125).

Responses to Reviewer #3

I commend the authors for their detailed consideration of my earlier comments, specifically about the N fixation aspect of the analysis. I understand that the method of meta-analysis is limited in addressing such concerns in all details, but it is important that the results are given in context with these limitations. I think this is now done better, with some appropriate paragraphs in the discussion regarding methodology, experiment durations, and the aspect of zonally varying N-fixer cover. However, it is crucial for these limitations to be acknowledged throughout the manuscript. While a comprehensive analysis was conducted, its general meaningfulness is a bit overstated (e.g. the first sentence of the Summary) in light of what was actually done.

Response: We appreciate the reviewer’s comments. We have carefully revised the manuscript to avoid any overstatement. The first sentence of the *Summary* has been revised to “This study synthesizes data in the literature on the effects of CO_2 enrichment on the terrestrial N cycle to improve our understanding of the N limitation to plant growth under elevated CO_2” (lines 377 – 379). Please find details on other revisions below.

Some (non-exclusive) notes on this:

L203-205 seems to imply that PNL is a natural phenomenon that is now further informed by the N mechanisms that occur under eCO2. Perhaps this should be phrased more carefully, *because the authors point out in the introduction that PNL is a theory that has not consistently been observed in nature.*

Response: The sentence has been re-written to "In this study, we carried out two syntheses on the responses of the terrestrial N cycle and plant growth to CO_2 enrichment to test whether PNL generally occurs across ecosystems" (lines 209 – 211).

L259: "Although a general trend of PNL alleviation has been found in this study...". I disagree with this statement. What is suggested in this study is the general trend of N cycle changes under eCO2 to converge towards increased soil N supply for plant growth, which in theory could alleviate PNL, assuming that this mechanism exists.

Response: This part has been revised to "In this study, it is suggested that the general trend of the N cycle changes under elevated CO_2 converges towards increased soil N supply for plant growth, which in theory could alleviate PNL. However, the PNL alleviation potential may vary across different ecosystems due to asymmetric distributions of biological N fixation" (lines 264 – 268).

L291-294: With the methodological limitations of the analysis (especially the concentration on temperate ecosystems), this statement cannot stand.

Response: The sentence has been deleted. The followed sentence has been revised to "The responses of some N cycling processes that affect N availability are dependent on treatment duration, N addition, and/or ecosystem types" (lines 300 – 304).

In addition to the parts that the reviewer pointed out, we have made some other changes.

Lines 33 – 35: In the end of the *Abstract*, we have added a sentence "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".

Line 68 – 69: the sentence "the main objective was to explore the general pattern of the N limitation to plant growth under enriched CO_2 conditions" has been revised to "the main objective was to synthesize data published in the literature on the N limitation to plant growth under enriched CO_2 conditions".

Although the authors state that they checked the language carefully, there are still a lot of mistakes. Please have the language checked by a native speaker, both for grammar and appropriate scientific writing! For the individual corrections, I only made it to the end of section 4.1, but I think this is enough to illustrate my point:

Throughout: Be consistent with the use of past and present tense, especially in expressions like "our results show" vs "our results showed".

Response: We appreciate the detailed comments. We have checked the language according to the reviewer's comments. In particular, we asked Dr. Kevin R. Wilcox, a native English speaker and a post-doctoral fellow in our laboratory, to help thoroughly check the language of the manuscript. Please find the details below.

L 14, 367, and wherever else the N cycle is mentioned: "Nitrogen cycle" should not be used by itself in a sentence. Either "The nitrogen cycle" or "Nitrogen cycling".

Response: Revised as suggested.

Line 15: “Nitrogen (N) cycle” has been revised to “The nitrogen (N) cycle”.

Lines 19 – 20 and line 71: “in terrestrial N cycle” has been revised to “in the terrestrial N cycle”.

Lines 62 – 63: “the responses of multiple N cycle processes” has been revised to “the responses of multiple N cycling processes”

Line 74 – 75: “all the major processes and pools in N cycle” has been revised to “all the major processes and pools in the N cycle”

Line 209 – 210: “the responses of terrestrial N cycle” has been revised to “the responses of the terrestrial N cycle”

Line 286: “the responses of N cycle processes” has been revised to “the responses of N cycling processes”

Line 303: “the responses of other N cycle processes” has been revised to “the responses of some N cycling processes”

Line 378: “terrestrial N cycle” has been revised to “the terrestrial N cycle”

L15: "...extensive researches have been done..." sounds strange. Maybe just "... extensive research has explored whether..."?

Response: Revised as suggested (lines 16 – 17).

L20: "... but not in soil pool." should be "the soil pool" or "soil pools".

Response: “not in soil pool” has been revised to “no in the soil pool” (lines 21 – 22).

L21: "exist" instead of "exists".

Response: Revised as suggested (line 22).

L27: "...despite of the increases" I think either "in spite of the increases" or "despite the increases" would be correct.

Response: “despite of the increases” has been revised to “in spite of the increases” (line 28).

L28: Check for the used tense, if your "analyses suggest" in L25, then your synthesis should "show" in L28.

Response: “our synthesis showed” has been revised to “our syntheses indicate” (line 29).

L31: Not sure what the "feedback to climate change" means. Shouldn't it be either a feedback between two things or a response to climate change?

Response: The sentence has been revises to “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” (lines 30 – 33).

L35f: I think "stimulated" and "by CO2 fertilization" should go together, so "The plant growth stimulated by CO2 fertilization...".

Response: Revised as suggested (lines 39 – 40).

L38: Unneeded repetition from the previous sentence. Consider "this effect" or similar.

Response: Revised as suggested (line 42).

L38: "constrained by the availability of N" would be more precise.

Response: Revised as suggested (line 43).

L48: I would use "is" instead of "are". Or just "depends" instead of "is dependent".

Response: “are dependent” has been changed to “depends” (line 52).

L66: See comment to L14.

Response: “in terrestrial N cycle” has been revised to “in the terrestrial N cycle” (line 71).

L76f: "the CO2 fertilization effect".

Response: The word “the” has been added (line 84).

L82, 118: I think it reads a bit awkward to have dataset "one" and "two" written out like that.

Response: “for dataset one” has been revised to “for the first dataset” (line 89). “For the dataset two” has been changed to “For the second dataset” (line 126).

L84: "Then, ...".

Response: A comma has been added after the word “then” as suggested (line 91).

L85: "..., where the ambient..." instead of "..., and the ambient...".

Response: Revised as suggested (line 92).

L87: "... the Intergovernmental...".

Response: The word “the” has been added (line 94).

L101: It feels like there is a word missing to make this sentence complete.

Response: A word “if” has been added (line 108).

L112: No need to use "nitrogen" when "N" was defined earlier.

Response: The word “nitrogen” has been revised to “N” (line 119).

L118: "For the dataset two,..." Don't use "the" here. Or just write "For the second dataset,...".

Response: “for the dataset two” has been revised to “for the second dataset” (line 126).

L118: Wouldn't "time series" be a more commonly used term?

Response: Revised as suggested (line 126).

L118f: "decadal-long" sounds strange but I might be wrong.

Response: “decadal-long” has been changed to “decadal” (line 127).

L120/121: Did you mean "in one way or another" ?

Response: “on a way or another” has been revised to “in one way or another” (line 129).

L126: "Then, ...".

Response: A comma has been added after the word “then” (line 134).

L135: I recommend using "the first/second dataset" also in the previous section (L82, 118).

Response: Revised as suggested (lines 89, 126).

L140: "logged RR" I am not sure this is a valid expression.

Response: “logged RR” has been changed to “log RR” (line 149).

*L144: "Then, *a or the* random-effects model was used...".*

Response: Revised as suggested (line 153).

L171: "change inorganic N in soils" needs more precision, because you mean abundance, concentration, availability etc. Inorganic N itself is not changed.

Response: The “inorganic N in soils” has been revised to “the total inorganic N availability” (line 180).

L172: "..., it increased the soil NH4+/NO3- ratio...".

Response: A word “the” has been added before “soil NH_4^+/NO_3^- ratio” (line 180).

L178: "the response of the NH4+/NO3- ratio...".

Response: Revised as suggested (line 185).

L192: I would prefer "..., a positive response...".

Response: Revised as suggested (line 197).

L195f: "fertilization effect" and "on plant growth" should stand together, so "...fertilization effect on plant growth did not change over treatment time in 11 experiments...".

Response: Revised as suggested (line 201).

L208: "In PNL hypothesis,..." This should be phrased differently, e.g. "According to the PNL hypothesis,...".

Response: Revised as suggested (line 214).

L210: "retention".

Response: Revised as suggested (line 216).

L213: "PNL hypothesis" needs an article.

Response: A word “the” has been added before “PNL hypothesis” (line 220).

L215: Maybe use "..., i.e. biological N fixation and leaching." Otherwise it looks as if you are listing N supply, biological N fixation and leaching as equals.

Response: Revised as suggested (lines 221 – 222).

L218: "free-living".

Response: Revised as suggested (line 226).

L219-223: Maybe the two sentences should be combined, because you are referencing Poorter and Navas twice. Also, "... when nutrient level is low." is not correct language.

Response: The second sentence has been deleted (lines 229 – 230).

L224: "reduced". Use "decreased" or "was reduced".

Response: The word “reduced” has been revised to “was reduced” (line 231).

L225: "the primary N form in leaching" could be phrased better.

Response: The sentence has been revised to “This could be attributed to the decrease in NO_3^-, which is the primary N form in leaching…” (lines 232 – 233).

L226: "free N" is imprecise.

Response: The “free N” has been revised to “inorganic N” (line 234).

L227-229: Rephrase to something more elegant. E.g. "In contrast, gaseous N loss through N2O emission increased under elevated CO2, although this increase was only observed when additional N was applied.

Response: Revised as suggested (lines 234 – 236).

L237: "plants". Remove "for multiple times".

Response: Revised as suggested (line 242).

L240: "by the increased N fixation". I would prefer "from increased N fixation".

Response: Revised as suggested (line 245).

L247: "the long-term response" or "long-term responses".

Response: A word “the” has been added before “long-term response” (line 251).

L255: "...the relatively small number of studies.".

Response: Revised as suggested (lines 259 – 260).

L262f: "...did not show diminished CO2 fertilization effect,...". Again, this needs an article or a plural.

Response: A word “a” has been added before the word “diminished” (line 269).

L262-264: "CO2 fertilization effect" only needs to be written once in this sentence.

Response: “the CO_2 fertilization effect on plant production” has been revised to “it” (line 270).

L266: "resource limitation (including N)".

Response: Revised as suggested (line 273).

L269: "..., or their combined.". Something missing here.

Response: A word "effect" has been added after the word "combined" (line 276).

L271: Articles (2x).

Response: A word "the" has been added before "ORNL FACE experiment" and "Aspen-Birch community", respectively (lines 278 – 279).

L273: "With O3 addition, O3 significantly reduces...". Write "O3 addition significantly reduced...".

Response: Revised as suggested (line 281).

Other changes for presentation improvement are shown below.

Line number	Previous version	Revised version
9		Dr. Yiqi Luo added one more affiliation.
61 – 62	"The change in N supply: (i)…"	"The change in the N supply…"
70 – 71	"To do so, two questions were asked…"	"Our data synthesis was designed to answer two questions: (i)…"
73	"…data from literature…"	"…data from the literature…"
74	"…we quantitatively synthesized…"	"…we quantitatively examined…"
75	"These variables included…"	"These processes and pools included…"
78 – 80	"The responses of the N processes to short- vs. long-term CO_2 treatment were also explored"	"We separated the first dataset according to the experimental durations to explore the responses of the N processes to short- vs. long-term CO_2 treatments"
80 - 82	"In addition, the responses of the N processes to CO_2 enrichment under without vs. with N addition conditions were compared"	"In addition, the responses of the N processes to CO_2 enrichment were compared between without and with N addition conditions"
82 – 85	"With the second dataset which the decadal plant growth in free air CO_2 enrichment (FACE) experiments were collected, we explored whether CO_2 fertilization effect on plant growth diminishes over time"	"The second dataset was compiled for the plant growth in decadal free air CO_2 enrichment (FACE) experiments. With the dataset, we explored whether the CO_2 fertilization effect on the plant growth diminishes or not over time"
95 – 96	"…the major nitrogen (N) pools or	"…the major N pools or processes…"

	processes…”	
131	“…by PNL…”	“…by the PNL hypothesis…”
136	“…the effect… on plant production…”	“…the effect… on the plant production…”
186	“…, representing neutral response to …”	“…, representing a neutral response to …”
197	“… positive response of NH_4^+/NO_3^- …”	“… a positive response of the NH_4^+/NO_3^- ratio …”
218 – 219	“…, the results…showed no general…effect on…”	“…, the results…did not show a general…effect on…”
224 – 226	“The enhanced biological N fixation could result from the stimulated activities of the symbiotic (Fig. 2B) and free-lived heterotrophic N-fixing bacteria”	“The enhanced biological N fixation may have resulted from the stimulated activities of symbiotic (Fig. 2B) and free-living heterotrophic N-fixing bacteria”
227 – 228	“…could also contribute to…”	“…may have contributed to…”
231	“Results showed that…”	“In addition, …”
231 – 232	“…under elevated CO_2 condition”	“…under elevated CO_2 conditions”
237	“…resulted in more N retention…”	“…resulted in higher N retention…”
238	“…, especially in plant tissues and litter”	“…, especially within plant tissues and litter”
247	“…nitrogen requirement…”	“…the N requirement…”
249 – 250	“PNL was proposed to…”	“The PNL hypothesis was proposed to…”
250	“…carbon-nitrogen coupling…”	“…C-N coupling…”
252	“…we synthesize…”	“…we have synthesized…”
256	“For example, Tu et al. (2015) found the abundance…”	“For example, Tu et al. (2015) found that the abundance…”
257	“…enhanced by 12-year CO_2 enrichment…”	“…enhanced by 12 years of CO_2 enrichment…”
268	“In addition, the PNL alleviation may…”	“In addition, PNL alleviation may…”
272	“e.g., nutrients, water, light, ozone, etc.”	“e.g., nutrients, water, light, ozone”
272 – 274	“…resources (including N) limitations are not aggravated, suggesting that no PNL occurs in these sites”	“…resource limitation (including N) was not aggravated, suggesting that no PNL occurred in these sites”
274 – 275	“…in ORNL and Aspen-Birch (without O_3 treatment)…”	“…in the ORNL and Aspen-Birch (without O_3 treatment) experiments…”
279 – 280	“In the Aspen-Birch community, however, deceleration of leaf area increase due to canopy closure is responsible for the diminished CO_2 fertilization effect without O_3 addition”	“In the Aspen-Birch community, however, the deceleration of leaf area increases due to canopy closure was responsible for the diminished CO_2 fertilization effect without O_3 addition”
282	“…resulting in relatively open canopy…”	“…resulting in a relatively open canopy…”
288	“…influence the results”	“…influence findings”

289	"…nitrogenase activity…"	"…the nitrogenase activity…"
290	"…specific N fixation measured by H_2 evolution method"	"…the specific N fixation measured by the H_2 evolution method"
290	"In studies synthesized here…"	"In the studies synthesized here…"
294 – 295	"All but H_2 evolution method showed significantly positive response to CO_2 enrichment"	"All but the H_2 evolution method showed a significantly positive response to CO_2 enrichment"
295 – 296	"The insignificant response by H_2 evolution method was likely because of the small study numbers"	"The insignificant response shown by the H_2 evolution method was likely because of the small study numbers"
296 – 297	"…biological N fixation by…"	"…the biological N fixation measured by…"
297	"…response magnitude"	"…response magnitudes"
298	"…H_2 evolution method…"	"…the H_2 evolution method…"
306	"The meta-analysis…"	"Our meta-analysis…"
309	"…significantly increase in croplands"	"…significant increases in croplands"
311 – 312	"…the substrate quantity and quality for the mineralization"	"…the substrate quantity and quality for mineralization"
313	"Second, tillage can alter the soil conditions…"	"Second, tillage can alter soil conditions…"
314	"…N mineralization…"	"…the N mineralization…"
317	"…is…"	"…was…"
319 – 320	"One possible reason for the reduced nitrification by the long-term CO_2 enrichment is cumulative effect of hydrological change"	"One possible reason for the reduced nitrification with long-term CO_2 enrichment is the cumulative effect of hydrological changes"
320 – 322	"CO_2 enrichment generally reduces the stomatal conductance and the consequent water loss via plant transpiration…"	"CO_2 enrichment generally reduces stomatal conductance and, consequently, water loss via plant transpiration…"
324 – 326	"The increased soil water content may result in less oxygen (O_2) content…"	"Increased soil water content may result in less oxygen (O_2) concentration…"
328	"Reduced nitrification was only observed under without N addition conditions"	"The reduced nitrification was only observed under conditions without N addition"
330 – 331	"Additionally, the response of denitrification to CO_2 enrichment shifted from neutral without N addition to significantly positive with N addition"	"Additionally, the response of denitrification to CO_2 enrichment shifted from neutral, without N addition, to significantly positive with N addition"
332	"...more N substrate to nitrifying and denitrifying bacteria"	"...more N substrate for nitrifying and denitrifying bacteria"
334	"…lead…"	"…led…"
339 – 340	"The increased N_2O emission can partially offset the mitigation of climate change by	"Increased N_2O emissions can partially offset the mitigation of

	stimulated plant CO_2 assimilation as the warming potential by N_2O is as 296 time as that by CO_2"	climate change by the stimulated plant CO_2 assimilation as the warming potential of N_2O is 296 times that of CO_2"
341 – 342	"However, a recent modeling study by Zaehle et al. (2011) has generated an opposite result that CO_2 enrichment reduced radiative forcing of N_2O"	"However, a recent modeling study by Zaehle et al. (2011) found an opposite result showing that CO_2 enrichment reduced radiative forcing of N_2O"
343	"…due to enhanced plant N sequestration…"	"…due to the enhanced plant N sequestration…"
345	"…results in greater N_2O emission"	"…results in a greater N_2O emission"
351	"…respond…"	"…responded…"
352	"…increase NH_4^+ content in soil…"	"…increase the NH_4^+ content in soils…"
353	"…decreased NO_3^- content in soils…"	"…decreased the NO_3^- content in soils…"
353 – 354	"…leading to significant increase in NH_4^+/NO_3^- ratio"	"…leading to a significant increase in the NH_4^+/NO_3^- ratio"
355	"…does not…"	"…did not…"
357	"…C cycle"	"…the C cycle"
360 – 361	"…a significant effect on…"	"…significant effects on…"
364	"…lower turnover rates of …"	"…the lower turnover rates of …"
381	"…, increasing N availability for plant growth"	"…, leading to increased N supply for plant growth"
381 – 383	"The extra N supply by the enhanced biological N fixation and reduced leaching may meet the increased N demand…"	"The additional N supply via the enhanced biological N fixation and the reduced leaching may partially meet the increased N demand…"
383 – 386	"In addition, CO_2 enrichment increased N_2O emission, especially with extra N addition. The increased N_2O emissions can partially offset the mitigation of climate change by stimulated plant CO_2 assimilation"	"In addition, increased N_2O emissions can partially offset the mitigation of climate change by stimulated plant CO_2 assimilation"
386 – 389	"Moreover, the changes in the soil microenvironment, ecosystem communities and above-belowground interactions induced by the different responses of NH_4^+ and NO_3^- to CO_2 enrichment may have long-term effects on terrestrial biogeochemical cycles and climate change, on which further studies are needed"	"Moreover, the changes in the soil microenvironment, ecosystem communities and above-belowground interactions induced by the different responses of NH_4^+ and NO_3^- to CO_2 enrichment may have long-term effects on the terrestrial biogeochemical cycles and climate change"

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

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Abstract: ~~Nitrogen~~ The nitrogen (N) cycle has the potential to regulate climate change through its influence on carbon (C) sequestration. Although extensive research has explored ~~es have been done to explore~~ whether or not progressive N limitation (PNL) occurs under CO_2 enrichment, a comprehensive assessment of the processes that regulate PNL is still lacking. Here, we quantitatively synthesized the responses of all major processes and pools in 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. Thus, the mechanisms that drive PNL occurrence partially exist~~s~~. 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 ~~despite~~ in spite of the increases in plant N sequestration and N_2O emission. Moreover, our ~~synthesis~~ syntheses indicate~~showed~~ that CO_2 enrichment ~~increased~~ 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, ~~community structures and~~ above-belowground community structures and associated interactions, which could potentially affect the terrestrial biogeochemical cycles ~~and the feedback to climate change~~. 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 ~~stimulated~~ 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, ~~the stimulated plant growth by CO_2 enrichment~~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. However, whether and to what extent PNL occurs ~~are dependent~~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, the CO_2 fertilization effect on plant growth may diminish over time. The PNL hypothesis has been tested in individual ecosystems during the past decade (e.g., Finzi et al., 2006; Moore et al., 2006; Reich et al., 2006; Norby et al., 2010). Some of the site-level studies support (Reich et al., 2006; Norby et al., 2010), while the others refute PNL (Finzi et al., 2006; Moore et al., 2006). To date, no general pattern of PNL across ecosystems has yet been revealed.

Since the key determining PNL occurrence is that whether N supply meets N demand (Luo et al., 2004), it is important to understand how N supply changes under elevated CO_2. The change in the N supply for plant growth under elevated CO_2 is determined by the responses of multiple N ~~cycle~~ cycling processes, including biological N fixation, mineralization, nitrification, denitrification, and leaching (Chapin III et al., 2011). In addition, the responses of these processes to CO_2 enrichment may be influenced by external N addition, such as N deposition and fertilization (Reay et al., 2008). Thus, synthesizing the responses of processes that regulate PNL to CO_2 enrichment may help reveal the general pattern of PNL in terrestrial ecosystems.

In the current study, the main objective was to ~~explore the general pattern of~~synthesize data published in the literature on the N limitation to plant growth under enriched CO_2 conditions. ~~To do so,~~ Our data synthesis was designed to answer two questions~~ were asked~~: (i) How do the major processes in the terrestrial N cycle respond to CO_2 enrichment? (ii) Does the CO_2 fertilization effect on plant growth diminish over time? To answer these questions, two sets of data from the literature were collected (Table S1, Table 1). With the first dataset, we quantitatively ~~synthesized~~ examined the effects of CO_2 enrichment on all the major processes and pools in the N cycle using meta-analysis. These ~~variables~~ processes and pools included N sequestered in organic components (i.e., plant tissues, litter and soil organic matter (SOM)), biological N fixation, net mineralization, nitrification, denitrification, leaching, and total inorganic N (TIN), ammonium (NH_4^+) and nitrate (NO_3^-) contents in soils. We separated the first dataset according to the experimental durations to explore t~~T~~he responses of the N processes to short- vs. long-term CO_2 treatments~~ were also explored~~. In addition, the responses of the N processes to CO_2 enrichment were compared between ~~under~~ without ~~vs.~~and with N addition conditions~~ were compared~~. ~~With the~~The second dataset was compiled for ~~in which~~ the ~~decadal~~

plant growth in decadal free air CO_2 enrichment (FACE) experiments. With the dataset were collected, we explored whether the CO_2 fertilization effect on the plant growth diminishes or not over time.

2 Materials and Methods

2.1 Data collection

For the first dataset ~~one~~, a comprehensive literature search with the terms of "CO_2 enrichment (or CO_2 increase)", "nitrogen" and "terrestrial" was conducted using the online search connection *Web of Science* in Endnote. Then~~,~~ papers meeting the following two criteria were selected to do the further analyses: (i) including both control and CO_2 enrichment treatments, ~~and~~ 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 ~~nitrogen (N)~~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 α = 0.05 and 0.10, respectively. In some studies, if tissue N concentration and biomass were reported, we multiplied the two parts as N pools. When both APNP and BPNP were provided (or calculated), the two were added together to represent the TPNP. When data from multiple soil layers were provided, they were summed if they were area-based (i.e., m^{-2} land), or averaged if they were weight-based (i.e., g^{-1} soil). In studies where the respective contents of NH_4^+ and NO_3^- were reported, the TIN was calculated by adding the two together. For all the variables, if more than one result were reported during the experiment period, they were averaged by

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

with standard deviation

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

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

Because treatment time and N addition may affect the responses of the N processes to CO_2 enrichment, the dataset was divided into different categories: (i) short-term (≤ 3 years) vs. long-term (> 3 years), and (ii) without N addition vs. with N addition. Moreover, the dataset was also divided into forest, grassland, and cropland to explore possible differences ~~among ecosystems~~between ecosystem types.

For the second dataset ~~two~~, 15 available time ~~courses~~ series of plant growth were collected from 7 decadal ~~long~~ FACE experiments (Table 1). The ecosystems included 9 forests, 5 grasslands and 1 desert. Because of the limited data, we included variables that can represent plant growth ~~on a~~in one way or another, for example, net primary production (NPP), biomass, and leaf production. These data were collected to reveal whether the effect of CO_2 enrichment on plant growth diminishes over treatment time as proposed by the PNL hypothesis (Luo et al., 2004). In the 7 studies, the treatment lasted from 7 to 13 years, and at least 6 years' production measurements were reported. For each data, the percentage change in NPP (or biomass or leaf production) by CO_2 enrichment was calculated. Then, a linear regression between the percentage change and the treatment year was conducted. A significantly negative slope indicates that the effect of CO_2 enrichment on the plant production diminishes over time. A non-significant slope was treated as 0. After deriving all the slopes, the frequency distribution of the slopes were fitted by a Gaussian function:

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

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

2.2 Meta-analysis

With the first dataset, the effect of CO_2 enrichment for each line of data of the N variables was estimated using the natural logarithm transformed response ratio (*RR*) (Hedges et al., 1999; Liang et al., 2013):

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

where X_E and X_C are the variable values under enriched CO_2 and control conditions, respectively. The variation of the log~~ged~~ *RR* was

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

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 of X_C and X_E.

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

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

with the variance as

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

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

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

and

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

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

3 Results

The meta-analysis from the first dataset showed that CO_2 enrichment significantly increased N sequestered in plants and litter but not in SOM (Figs. 1A, 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 croplands, but neutrally in grasslands (Fig. 3G). Net mineralization had no response to CO_2 enrichment in forests or grasslands, while it was significantly increased in croplands (Fig. 3H). Moreover, the change in the TIN was neutral in forests, grassland, but positive, in croplands, respectively (Fig. 3I). In addition, a positive response of the NH_4^+/NO_3^- ratio was only observed in grasslands (Fig. 3I).

The results from the second dataset showed that CO_2 enrichment significantly increased plant growth in most of the decadal FACE experiments (Fig. 4). In addition, the CO_2 fertilization effect over treatment time on plant growth did not over treatment time change in 11 experiments ($P > 0.05$), decreased in 2 experiments (slope < 0, $P < 0.05$), and increased in 2 experiments (slope > 0, $P < 0.05$), respectively (Table 1, Fig. 4). Overall, the slope of the response of the plant growth vs. treatment time was not significantly different from 0 (i.e., -0.37% $year^{-1}$ with 95% CI from -1.84% $year^{-1}$ to 1.09% $year^{-1}$; Fig. 4).

4 Discussion

~~The current study~~In this study, we carried out two syntheses on the responses of the terrestrial N cycle and plant growth to CO_2 enrichment to test whether PNL generally occurs across ecosystems.~~reveal the general pattern of PNL and the underlying processes that regulate PNL.~~

4.1 PNL alleviation

~~In~~ According to the PNL hypothesis, a prerequisite for PNL occurrence is that more N is sequestered in plant, litter and SOM (Luo et al., 2004). Our results showed that elevated CO_2 significantly increased N retention~~s~~ in plant tissues and litter, which is consistent with previous meta-analyses (de Graaff et al., 2006; Luo et al., 2006). Thus, the basis of PNL occurrence partially exists. However, the results from the second dataset ~~showed no~~did not show a general diminished CO_2 fertilization effect on plant growth on the decadal scale, which disagrees with the expectation of the PNL hypothesis, suggesting that N supply under elevated CO_2 may meet the N demand. In this study, we have identified two processes that increase N supply under elevated CO_2, i.e., biological N fixation and leaching.

CO_2 enrichment significantly enhanced the N influx to terrestrial ecosystems through biological N fixation, which reduces dinitrogen (N_2) to NH_4^+ (Fig. 1B). The enhanced biological N fixation ~~could result~~may have resulted from the stimulated activities of ~~the~~ symbiotic (Fig. 2B) and ~~free-lived~~free-living heterotrophic N-fixing bacteria (Hoque et al., 2001). In addition, the competition between N_2-fixing and non-N_2-fixing species ~~could also contribute~~may have contributed to enhance the biological N fixation on the ecosystem level (Poorter and Navas, 2003; Batterman et al., 2013).~~A review by Poorter and Navas (2003) suggests that elevated CO_2 could strengthen the competition of N_2-fixing dicots when nutrient level is low.~~

~~Results showed that~~In addition, the N efflux via leaching was reduced under elevated CO_2 conditions (Fig. 1B). This could be attributed to the decrease in NO_3^-, which is the primary N form in leaching, ~~NO_3^-~~ (Chapin III et al., 2011), and the increased root growth which may immobilize more ~~free~~ inorganic N in soils (Luo et al., 2006; Iversen, 2010). In contrast, gaseous N loss through N_2O emission increased under elevated CO_2 ~~in comparison with that under ambient CO_2. But~~, although this ~~the~~ increase was only observed when additional N was applied.

The net effect of the responses of N processes to CO_2 enrichment resulted in ~~more~~ higher N retention in ecosystems, especially ~~in~~ within plant tissues and litter (Fig. S2). Because the product of biological N fixation (i.e., NH_4^+) and the primary form for N leaching loss (i.e., NO_3^-) can be directly used by plants, the effects of CO_2 enrichment on the two processes directly increase the N availability for plant growth, potentially alleviating PNL (Fig. 5). The increased N in plant tissues can be re-used by plants ~~for multiple times~~ via resorption (Norby et al., 2000; Norby et al., 2001), and consequently reduce the N demand from soils. This may be another mechanism that alleviates PNL (Walker et al., 2015). Therefore, the increased N availability ~~by the~~from increased N fixation and reduced N leaching could potentially support net accumulation of organic matter in terrestrial ecosystems (Rastetter et al., 1997; Luo and Reynolds, 1999).

Since biological N fixation provides at least 30% of the N ~~nitrogen~~ requirement across natural biomes (Asner et al., 2001; Galloway et al., 2004), our results suggest that the positive response of biological N fixation to CO_2 enrichment plays an important role in alleviating PNL. The PNL hypothesis was proposed to characterize long-term dynamics of ~~carbon-nitrogen~~C-N coupling in response to rising atmospheric CO_2 concentration. Thus, it is critical to understand the long-term response of biological N fixation to elevated CO_2. In this paper, we have synthesized 12 studies that lasted 4 – 7 years and binned them in a long-term category (> 3 years). On average of those long-term studies, CO_2 enrichment increased biological N fixation by 26.2%. The increased biological N fixation is supported by evidence at gene level from long-term experiments. For example, Tu et al. (2015) found that the abundance of *nif*H gene amplicons, which is a widely used marker for analyzing biological N fixation, was significantly enhanced by 12 ~~-~~years of CO_2 enrichment in a grassland (BioCON). However, our synthesis showed a relatively wide 95% confidence interval from 2.54% to 59.8%. The wide range can be partially attributed to the relatively small number of studies~~study numbers~~. In addition, most studies incorporated in the current synthesis were conducted in temperate regions. Thus, longer-term studies, as well as studies in other regions (e.g., boreal and tropical) are critically needed to reveal more general patterns in the future.

~~Although a general trend of PNL alleviation has been found in this study,~~In this study, it is suggested that the general trend of the N cycle changes under elevated CO_2 converges towards increased soil N supply for plant growth, which in theory could alleviate PNL. However, the PNL alleviation potential may vary across different ecosystems due to asymmetric distributions of biological N fixation (Cleveland et al., 1999). In addition, ~~the~~ PNL alleviation may also be influenced by other factors. While most of the long-term experiments did not show a diminished CO_2 fertilization effect, ~~the CO_2 fertilization effect on plant production~~it decreased in two sites (i.e., ORNL and Aspen-Birch) (Fig. 4). Plant growth is usually influenced by multiple environmental factors (e.g., nutrients, water, light, ozone~~, etc.~~). The undiminished CO_2 fertilization effect in most studies indicates that resource~~s~~ limitation (including N) ~~limitations are~~ was not aggravated, suggesting that no PNL ~~occurs~~ occurred in these sites. However, in the ORNL and Aspen-Birch (without O_3 treatment) experiments, the diminished CO_2 fertilization effect could be attributed to limitation of N, or other resources, or their combined effect. For example, reduced N availability has been identified as one of the primary factors that lead to the diminished CO_2 fertilization effect on NPP in the ORNL FACE experiment (Norby et al., 2010). In the Aspen-Birch community, however, the deceleration of leaf area increases due to canopy closure ~~is~~ was responsible for the diminished CO_2 fertilization effect without O_3 addition (Talhelm et al., 2012). With O_3 addition, O_3 significantly ~~reduces~~ reduced the canopy development, resulting in a relatively open canopy during the experiment period. In addition, the negative effect of O_3 addition ~~increases~~ increased over time, leading to the apparent increase in the CO_2 fertilization effect (Fig. 4) (Talhelm et al., 2012).

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

Methodology may potentially influence ~~the results~~findings. Cabrerizo et al. (2001) found that CO_2 enrichment increased the nitrogenase activity measured by acetylene reduction assay (ARA), but not the specific N fixation measured by the H_2 evolution method. In the studies synthesized here, four methods were used to estimate biological N fixation, including isotope, ARA, H_2 evolution and N accumulation. Among them, ARA and H_2 evolution measure nitrogenase activity (Hunt and Layzell, 1993) whereas isotope and N accumulation methods directly measure biological N fixation. All but the H_2 evolution method showed a significantly positive response to CO_2 enrichment (Fig. 2A). The insignificant response shown by the H_2 evolution method was likely because of the small study numbers (i.e., 3). In addition, the biological N fixation measured by ARA, isotope and N accumulation showed similar response magnitudes (Fig. 2A), suggesting consistency among the three methods. However, further assessment on the H_2 evolution method is needed.

~~The responses of biological N fixation and leaching to CO_2 enrichment are barely influenced by treatment duration, N addition, or ecosystem types (Fig. 3), suggesting that the alleviation of PNL by the increased biological N fixation and decreased leaching generally occurs in terrestrial ecosystems. However, the~~ The responses of ~~other~~ some N ~~cycle~~ cycling processes that affect N availability are dependent on treatment duration, N addition, and/or ecosystem types (Fig. 3).

N mineralization, in addition to biological N fixation, is a major source of available N in soils. ~~The~~ Our meta-analysis showed no change in the net N mineralization in response to CO_2 enrichment, which is consistent with the results by de Graaff et al. (2006). However, the response of net mineralization was dependent upon ecosystem types, showing no change in forests and grasslands, but significant~~ly~~ increases in croplands (Fig. 3H). There may be two reasons for the stimulated net mineralization in croplands. First, N fertilization, which is commonly practiced in croplands, can increase the substrate quantity and quality for ~~the~~ mineralization (Barrios et al., 1996; Chapin III et al., 2011; Booth et al., 2005; Lu et al., 2011; Reich and Hobbie, 2013). Second, tillage can alter ~~the~~ soil conditions (e.g., increasing O_2 content), which can potentially favor the N mineralization under enriched CO_2 (Wienhold and Halvorson, 1999; Bardgett and Wardle, 2010). These findings suggest that CO_2 enrichment can stimulate the N transfer from organic to inorganic forms in managed croplands.

Unlike leaching, the response of nitrification ~~is~~ was dependent upon treatment duration (Fig. 3). Nitrification was not changed by short-term treatment, but was significantly reduced by long-term CO_2 enrichment (Fig. 3). One possible reason for the reduced nitrification ~~by the~~with long-term CO_2 enrichment is the cumulative effect of hydrological changes. CO_2 enrichment generally reduces ~~the~~ stomatal conductance and, consequently, ~~the consequent~~ water loss via plant transpiration, leading to an increase in soil water content (Niklaus et al., 1998; Tricker et al., 2009; van Groenigen et al., 2011; Keenan et al., 2013). A synthesis by van Groenigen et al. (2011) shows that CO_2 enrichment increases soil water content by 2.6% −10.6%. ~~The i~~Increased soil water content may result in less oxygen (O_2) ~~content~~ concentration in soils, which could potentially constrain nitrification.

In addition, the response of gaseous N loss ~~depends~~ was dependent on N addition (Fig. 3). ~~Reduced~~ The reduced nitrification was only observed under conditions without N addition (Fig. 3E). With N addition, no response of nitrification to CO_2 enrichment was observed (Fig. 3E). Additionally, the response of denitrification to CO_2 enrichment shifted from neutral, without N addition, to significantly positive with N addition (Fig. 3E). One possible reason is that N addition provides more N substrate ~~to~~ for nitrifying and denitrifying bacteria (Keller et al., 1988; Stehfest and Bouwman, 2006; Russow et al., 2008). The strengthening trends of both nitrification and denitrification ~~lead~~ led to a shift of the response of N_2O emission to CO_2 enrichment from neutral without N addition to significantly positive with N addition (Fig. 3E). Our results indicate that CO_2 enrichment significantly increases gaseous N loss when additional N is applied.

Our results are consistent with a previous synthesis (van Groenigen et al. 2011). ~~The i~~Increased N_2O emissions can partially offset the mitigation of climate change by the stimulated plant CO_2 assimilation as the warming potential ~~by~~ of N_2O is ~~as~~ 296 times ~~as~~ that ~~by~~ of CO_2. However, a recent modeling study by Zaehle et al. (2011) ~~has generated~~found an opposite result showing that CO_2 enrichment reduced radiative forcing of N_2O. In their model, less availability of N substrates for nitrification and denitrification due to the enhanced plant N sequestration attributed to the reduced N_2O emission. Our synthesis shows that inorganic N does not decrease.

Especially with additional N application, enhanced denitrification by CO_2 enrichment results in a greater N_2O emission.

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

The meta-analysis showed that the two major forms of soil available N, NH_4^+ and NO_3^-, responded to long-term CO_2 enrichment in opposing manners (Fig. 3C). While the enhanced biological N fixation by CO_2 enrichment tended to increase the NH_4^+ content in soils, the reduced nitrification decreased the NO_3^- content in soils, leading to a significant increase in the NH_4^+/NO_3^- ratio (Fig. 3C).

Although the total available N ~~does~~ did not change under elevated CO_2, the altered proportion of NH_4^+ over NO_3^- in soils may have long-term effects on soil microenvironment and associated aboveground-belowground linkages that control the C cycle (Bardgett and Wardle, 2010). On the one hand, plants would release more hydrogen ion (H^+) to regulate the charge balance when taking up more NH_4^+. As a result, the increased NH_4^+ absorption could acidify the rhizosphere soil (Thomson et al., 1993; Monsant et al., 2008). The lowered pH could have ~~a~~ significant effects on soil microbial communities and their associated ecosystem functions. For example, fungal/bacterial ratio increases with the decrease in pH (de Vries et al., 2006; Rousk et al., 2009). The increased fungal/bacterial ratio may result in lower N mineralization because of the higher C/N ratio of fungi and the lower turnover rates of fungal-feeding fauna (de Vries et al., 2006; Rousk and Bååth, 2007). In other words, the increased fungal/bacterial ratio may slow down the N turnover from organic to inorganic forms. On the other hand, the increased NH_4^+/NO_3^- ratio may increase the N use efficiency because it is more energetically expensive for plants to utilize

NO_3^- than NH_4^+ (Chapin III et al., 2011; Odum and Barrett, 2005; Lambers et al., 2008). In addition, since the preferences for plant absorption of different forms of N are different (Chapin III et al., 2011; Odum and Barrett, 2005), the increased NH_4^+/NO_3^- ratio may benefit some plant species while depress others, and consequently alter the community structures over time. These diverse changes in soil microenvironment and microbial and plant community compositions could further affect the terrestrial C cycle on long temporal scales, on which more studies are needed.

5 Summary

~~This synthesis provides a comprehensive assessment of~~This study synthesizes data in the literature on the effects of CO_2 enrichment on the terrestrial N cycle~~, which helps~~ to improve ~~the~~ our understanding of the N limitation to plant growth under elevated CO_2. Our results indicate that elevated CO_2 stimulates N influx via biological N fixation but reduces N loss via leaching, ~~increasing~~ leading to increased N ~~availability~~ supply for plant growth. The ~~extra~~ additional N supply ~~by~~ via the enhanced biological N fixation and the reduced leaching may partially meet the increased N demand under elevated CO_2, potentially alleviating PNL. In addition, ~~CO_2 enrichment increased N_2O emission, especially with extra N addition. The~~ increased N_2O emissions can partially offset the mitigation of climate change by stimulated plant CO_2 assimilation. Moreover, ~~the~~ changes in ~~the~~ soil microenvironments, ecosystem communities and above-belowground interactions induced by the different responses of NH_4^+ and NO_3^- to CO_2 enrichment may have long-term effects on the terrestrial biogeochemical cycles and climate change~~, on which further studies are needed~~.

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Supporting Information captions

Figure S1 Distributions of the experimental duration (**A**) and the CO_2 concentrations under ambient (**B**) and elevated (**C**) treatments and their difference (**D**) for the 175 collected studies. Red dashed lines represent the mean values.

Figure S2 Summary of the effect of CO_2 enrichment on ecosystem level N budget. Square boxes are nitrogen pools, ovals are nitrogen processes. Red dashed boxes mean the sum of the pools in the boxes. "+", "-", and "ns" mean the response to CO_2 enrichment are positive, negative, and not significant, respectively. Please see **Figure 1** for abbreviations.

Database S1 Database extracted from papers listed in References S1.

References S1 Papers from which the first dataset was extracted.

Table 1. Results on the effect of CO_2 enrichment on ecosystem NPP (or biomass or leaf production) in decadal-long free air CO_2 enrichment (FACE) experiments over treatment time. The values of the slope, R^2 and P in the linear regression in **Fig. 4** are shown. The lower and upper n (i.e., n and N) in Refs. Schneider et al., 2004; McCarthy et al., 2010; Reich and Hobbie, 2013 mean without and with N addition, respectively. The lower and upper o (i.e., o and O) in Ref. Talhelm et al., 2012 mean without and with O_3 treatment, respectively.

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

Figure 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% $year^{-1}$).

Figure 5. Mechanisms that alleviate PNL. PNL hypothesis posits that the stimulated plant growth by CO_2 enrichment leads to more N sequestered in long-lived plant tissues, litter and soil organic matter (SOM) so that, the N availability for plant growth progressively declines over time, and plant growth is downregulated (grey symbols). The current synthesis indicates that the basis of PNL occurrence partially exists (i.e., more N sequestered in plant tissues and litter; black symbols). Despite of the increases in plant N sequestration and N_2O emission, stimulated biological N fixation and reduced N leaching can replenish the N availability, potentially alleviating PNL (blue boxes and arrows). Upward, downward, and horizontal arrows mean increase, decrease, and no change, respectively.

Figure 1.

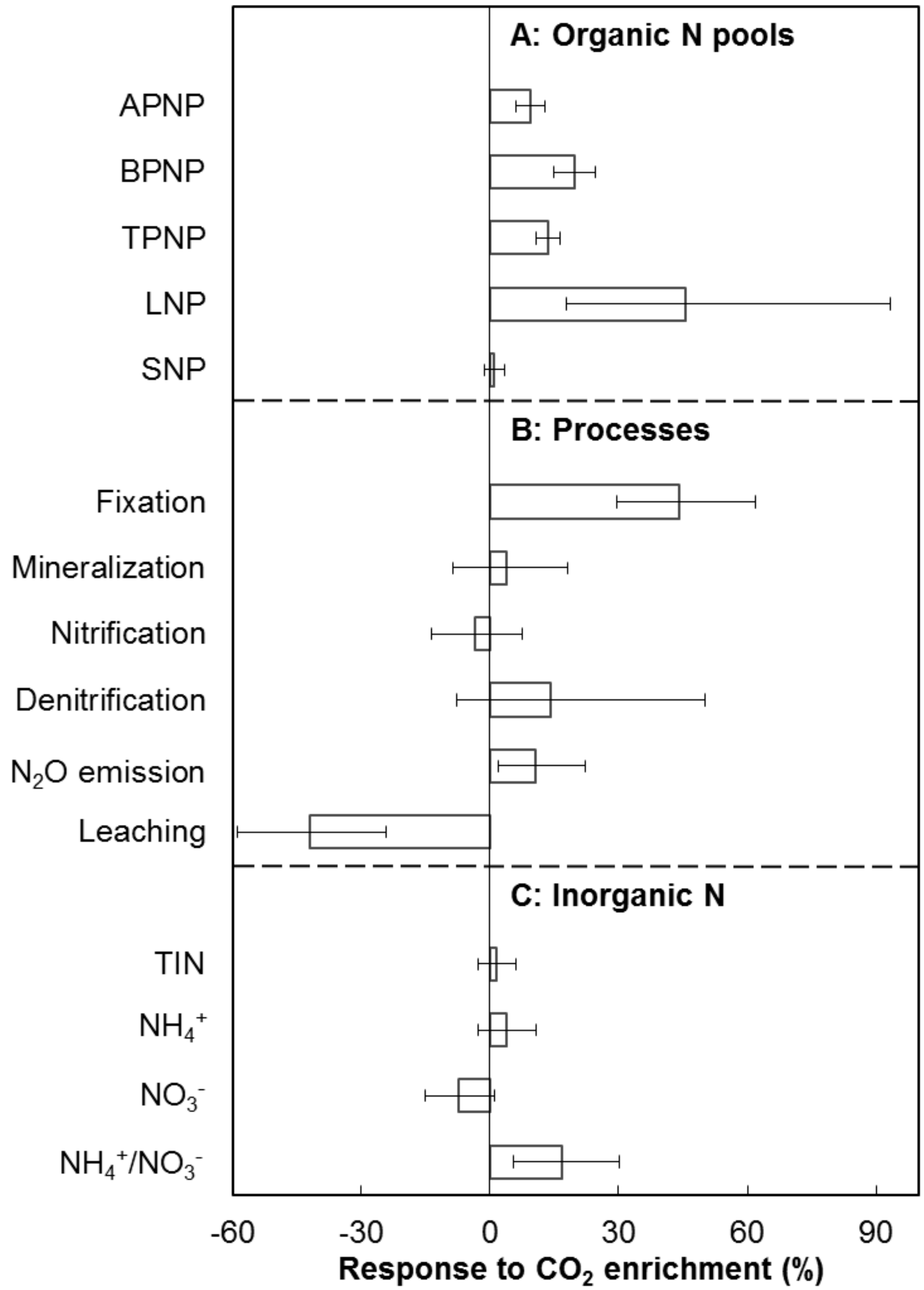

A: Organic N pools
APNP
BPNP
TPNP
LNP
SNP
B: Processes
Fixation
Mineralization
Nitrification
Denitrification
N_2O emission
Leaching
C: Inorganic N
TIN
NH_4^+
NO_3^-
NH_4^+/NO_3^-
-60
-30
0
30
60
90
Response to CO_2 enrichment (%)

Figure 2.

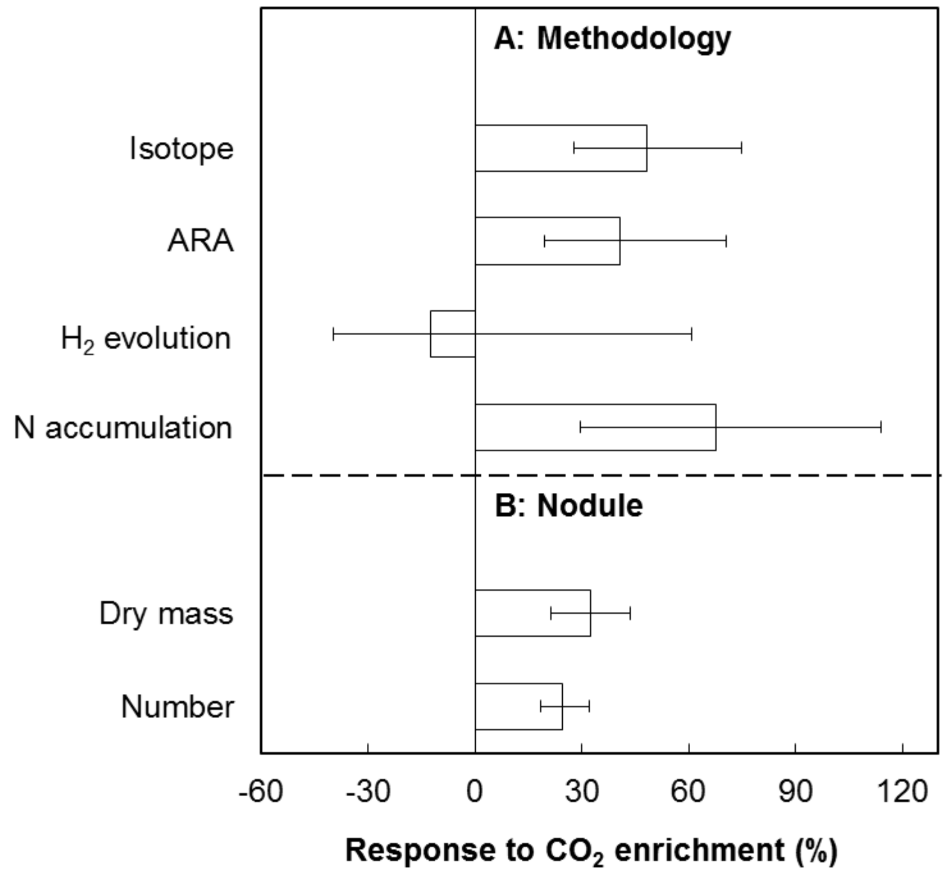

A: Methodology
Isotope
ARA
H_2 evolution
N accumulation
B: Nodule
Dry mass
Number
-60
-30
0
30
60
90
120
Response to CO_2 enrichment (%)

Figure 3.

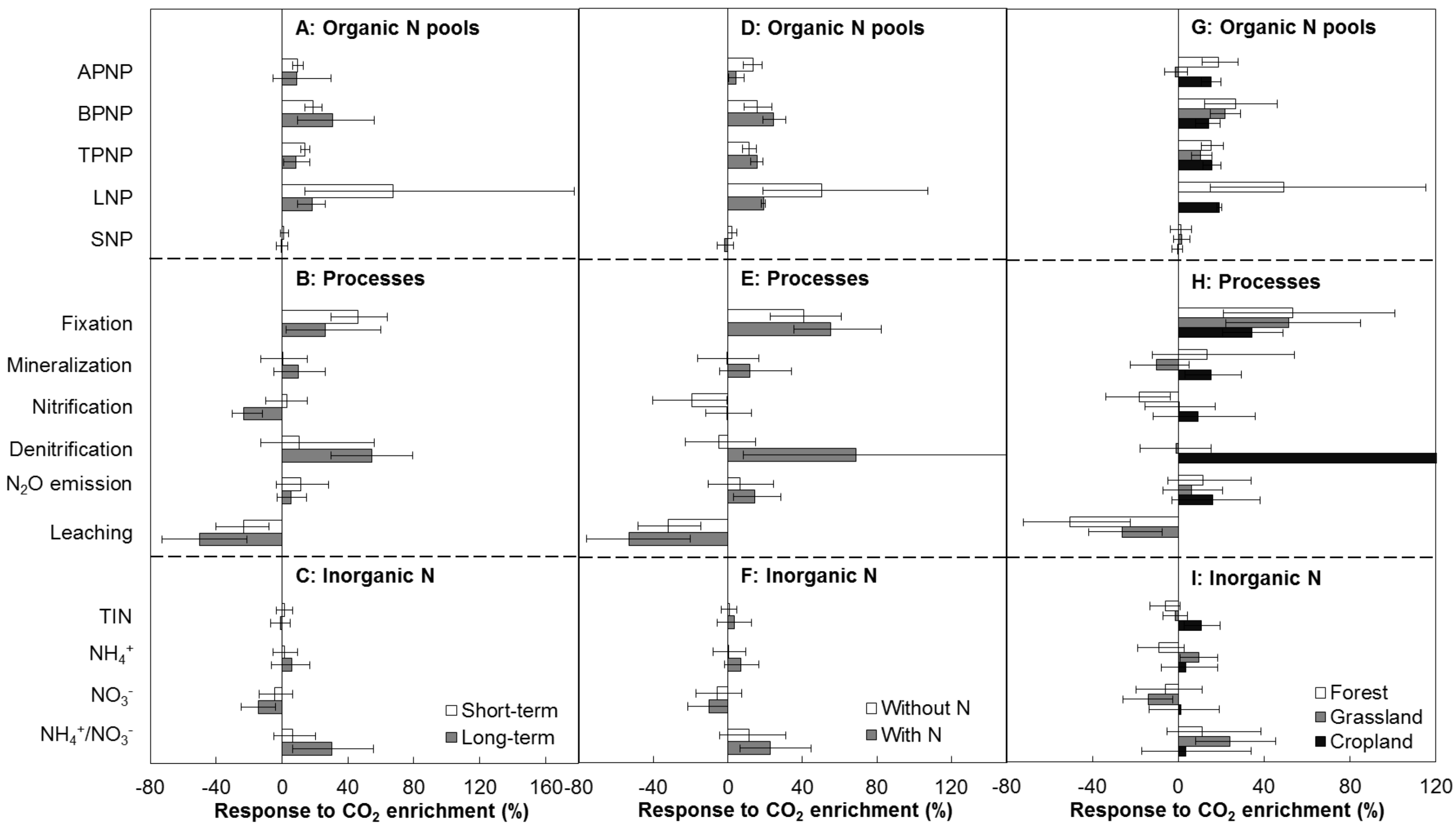

A: Organic N pools
B: Processes
C: Inorganic N
D: Organic N pools
E: Processes
F: Inorganic N
G: Organic N pools
H: Processes
I: Inorganic N
APNP
BPNP
TPNP
LNP
SNP
Fixation
Mineralization
Nitrification
Denitrification
N_2O emission
Leaching
TIN
NH_4^+
NO_3^-
NH_4^+/NO_3^-
Short-term
Long-term
Without N
With N
Forest
Grassland
Cropland
-80 -40 0 40 80 120 160
-80 -40 0 40 80 120
-80 -40 0 40 80 120
Response to CO_2 enrichment (%)
Response to CO_2 enrichment (%)
Response to CO_2 enrichment (%)

Figure 4.

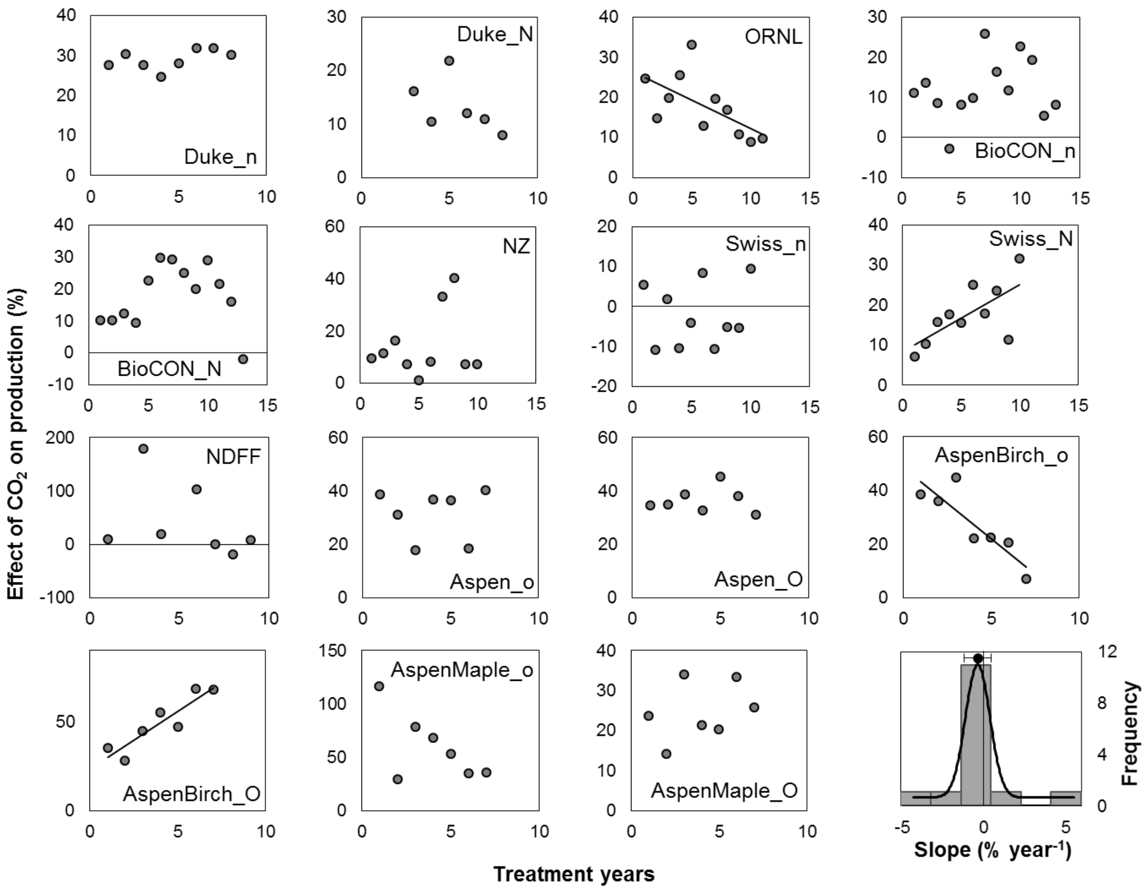
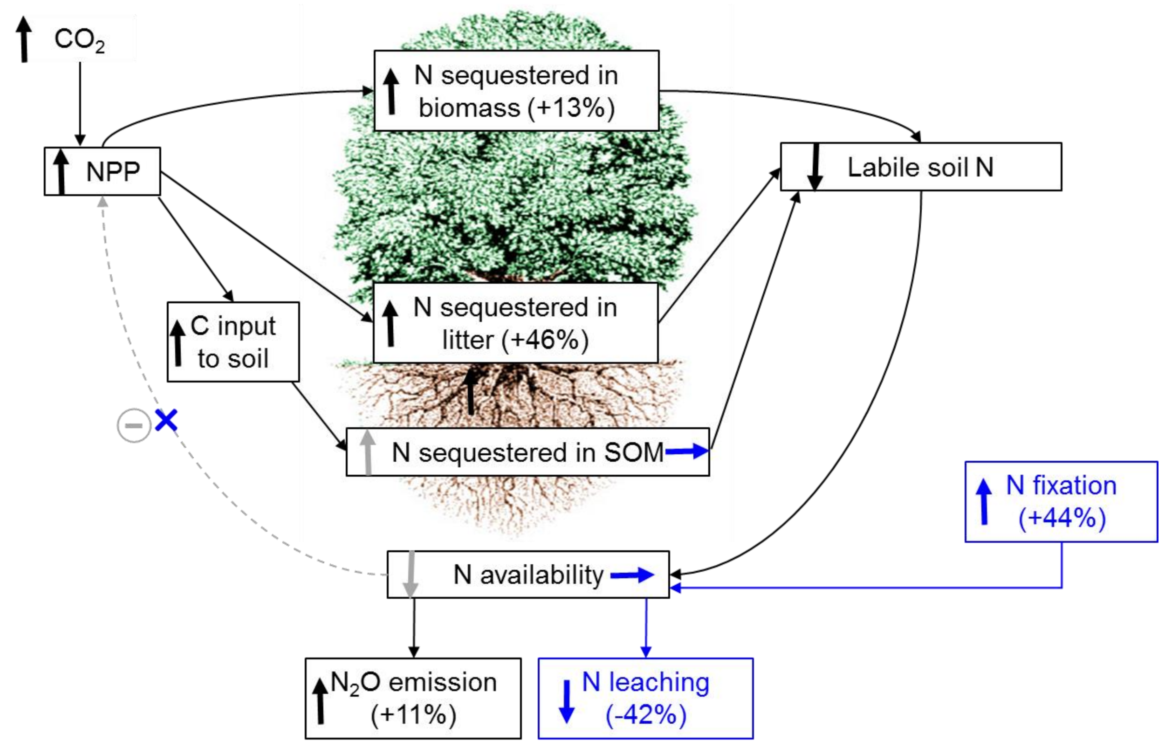

Duke_n
Duke_N
ORNL
BioCON_n
BioCON_N
NZ
Swiss_n
Swiss_N
NDFF
Aspen_o
Aspen_O
AspenBirch_o
AspenBirch_O
AspenMaple_o
AspenMaple_O
Effect of CO2 on production (%)
Treatment years
Slope (% year-1)
Frequency

Figure 5.


CO_2
NPP
N sequestered in biomass (+13%)
Labile soil N
C input to soil
N sequestered in litter (+46%)
N sequestered in SOM
N fixation (+44%)
N availability
N_2O emission (+11%)
N leaching (-42%)