

Interactive comment on “Processes regulating progressive nitrogen limitation under elevated carbon dioxide: a meta-analysis” by J. Liang et al.

Anonymous Referee #3

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The authors present an extensive meta-analysis on the effects of elevated atmospheric CO₂ levels on terrestrial N cycle processes. The aim is to investigate how the responses in various N cycle processes control the occurrence of progressive N limitation (PNL) under CO₂ perturbation. The major deductions from the analysis are that through increased biological N fixation (BNF) and decreased ecosystem N losses PNL may be alleviated, and that the soil NH₄⁺ to NO₃⁻ balance may shift.

I believe that a general assessment of terrestrial N process dynamics, in equilibrium and under perturbation, has the potential to be of tremendous value to biogeochemists, in particular to global modellers who perennially have to deal with lack of experimental evidence for their process hypotheses on large spatio-temporal scales. The effect of nutrient limitation on carbon sequestration responses to global change is (and has

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been) a very relevant issue in today's research, and I think the manuscript comes at a good time, and to a fitting journal. The suggested shift in soil NH₄/NO₃ seems like a novel hypothesis that could have some interesting follow-ups.

Unfortunately, I have several concerns with the presented manuscript detailed below. Based on these, I cannot recommend publication unless some of the fundamental issues are addressed.

Major concern:

The assertion that BNF significantly increased under elevated CO₂ is a very strong statement, and it has been my understanding thus far that this is not clear at all. I am not convinced that this hypothesis is supported by the presented meta-analysis for two reasons: (1) 15 of 29 studies included in the analysis of BNF responses have experiment durations of under 1 year. Or, by the authors' definition of <=3 years, all but 6 to 7 studies are short-term studies. Yet, the conclusions drawn from the analysis are used to speculate about the long-term (decade-scale!) controls on PNL. The Serraj & Sinclair (2003) experiment only lasted for a few days. The Hungate et al. (2004) paper shows why this is a problem, as their CO₂ effect on BNF diminished over the years. The effect of phenology and multi-year forest succession in natural ecosystems has long been part of the theory of controls on BNF (Vitousek & Howarth, 1991). I am assuming that Figure 2 is somewhat meant to address this issue, but I do not find it very helpful. (2) The mixing of agricultural experiments and experiments carried out in natural environments is problematic because of the very different nutrient regimes in these systems, which makes general conclusions about N cycle processes suspect. This is well illustrated by the Lam et al. 2012 BFS reference, where elevated CO₂ is reported to have increased BNF by 109 kg N ha⁻¹ over a span of 4 months. Such BNF rates are at least one order of magnitude above those in natural ecosystems. Even if only the response ratio was assimilated into the analysis, this does not make for a sound assessment of the BNF response in the terrestrial biosphere as a whole, which the manuscript is ultimately looking to provide.

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Since the BNF responses are a major part of the authors' arguments, I see this part as a strong weakness of the manuscript. I think that, if we want to do our understanding of BNF justice, an analysis should only include long-term experiments. It may well be that as of right now, we do not have enough experiments on BNF responses to eCO₂ to perform a meta-analysis. I would also like to encourage the authors to consider that vast parts of the terrestrial biosphere are not covered by N fixing vegetation, while obviously such species were always the subjects of the meta-analysed experiments.

Further concerns:

- Although the methodology is described in good detail in the MS, it is not clear how the individual studies contributed to the overall results. To this end, I agree with previous comments that Table S1 should show the individual effect sizes, not just markers. For example, I am unsure how the Hungate et al. (2004) response was treated. A mean+variation would certainly not be appropriate in this case.

- Many of the BNF experiments used the acetylene reduction method. This is very common to estimate BNF, however, as most studies point out, this method only determines Nitrogenase activity, which is not the same as BNF (Cleveland et al., 1999). This is a potential issue: Cabrerizo et al. (2001) report enhanced Nitrogenase activity, but no effect on BNF!

- I appreciate the differentiation between forests, grasslands, and croplands. However, since we are talking about the N cycle and nutrient limitation, there is strong reason to consider a zonal separation as well (boreal, temperate, tropical), see Vitousek & Howarth (1991). This may be offset by the fact that most experiments were carried out under controlled conditions (Greenhouses, growth chambers,...), but then again, how much can we expect to learn from these experiments about the overall terrestrial biosphere?

Minor concerns:

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- There are some inaccuracies in Table S1: I believe the Billings et al. (2003) experiment ran for 1 and 2 years, not 4. The Hofmockel & Schlesinger entry should read "2007", not "2002". The "Tobia" entry should read "Tobita".

- I strongly recommend an English language check for the entire MS for readability.

References not from the MS or supplement:

Vitousek, P. M., and R. W. Howarth (1991), Nitrogen limitation on land and in the sea: How can it occur?, *Biogeochemistry*, 13(2), 87-115.

Cleveland, C. C., et al. (1999), Global patterns of terrestrial biological nitrogen (N₂) fixation in natural ecosystems, *Global Biogeochemical Cycles*, 13(2), 623-645.

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