**Nitrogen mineralization, not N2 fixation alleviates progressive nitrogen limitation – Comment on “Processes regulating progressive nitrogen limitation under elevated carbon dioxide: a meta-analysis” by Liang et al.**

Tobias Rütting1

1 Department of Earth Sciences, University of Gothenburg, Sweden, [tobias.rutting@gu.se](mailto:tobias.rutting@gu.se)

The responses of terrestrial ecosystems to increasing carbon dioxide (CO2) concentrations in the atmosphere are expected to be strongly affected by nitrogen (N) availability, due to its limiting effect on ecosystem productivity (Hungate et al., 2003; Wang and Houlton, 2009). A popular hypothesis is the progressive N limitation (PNL), which assumes that an enhanced net primary productivity (NPP) due to elevated CO2 leads to storage of not only carbon, but also N in long-lived ecosystems pools, such as woody biomass or soil organic matter (Luo et al., 2004). This storing of N is hypothesized to decrease the N availability for plant N uptake, posing a negative feedback to enhanced NPP. This negative feedback is expected to become progressively more pronounced through time. However, in the original formulation of the PNL hypothesis, soil processes are largely ignored, though they are mentioned as potential mechanisms that can delay or prevent the development of a PNL (Luo et al., 2004). Data on the development of NPP stimulation under long-term free air CO2 enrichment (FACE) experiments indicate that most ecosystems have not developed a PNL on decadal scale (Feng et al., 2015), although some experiments did show a clear decreased NPP stimulation due to PNL. Understanding why a PNL is often not (yet) present in most long-term FACE experiments requires a comprehensive assessment of processes that might alleviate, prevent or delay the development of a PNL.

In their recent meta-analysis, Liang et al. (2016) aimed at this and concluded that increased biological N2 fixation (BNF) together with decreased nitrate (NO3-) leaching “may potentially alleviate PNL under elevated CO2 conditions”. However, this conclusion is misleading due to at least two facts. First, there is a bias in the dataset towards experiments with dominance of symbiotic BNF, questioning the generality of the found increased in BNF in all terrestrial ecosystems. Second, one of the most crucial processes for N availability in terrestrial ecosystems has not been considered by Liang et al. (2016): the actual soil N mineralization measured as gross rates.

Biological N2 fixation can occur in most terrestrial ecosystems. The highest rates of BNF occur in ecosystems dominated by symbiotic N2 fixation, such as legumes (Evans and Barber, 1977; Cleveland et al., 1999). However, these ecosystems are limited in areal extend, for instance legumes cover only about 10% of the agricultural land globally (Smil, 1999). Moreover, in natural ecosystems symbiotic N2 fixation might be downregulated (Batterman et al., 2013). In their meta-analysis, Liang et al. (2016) found that BNF increased on average by 44 % under elevated CO2. However, this increase derived from a data set, having an overrepresentation of ecosystems dominated by plants with symbiotic N2 fixation relative to most ecosystems. Alternatively, when considering FACE and open top chamber (OTC) studies only, 15 of the 26 data points originated from experiments dominated by symbiotic N2 fixation. In those, the BNF was significantly increased under elevated CO2, by 56.3 % on average (95 % confidence interval: +25.1 % to +95.4 %; Fig. 1). In contrast, ecosystems not dominated by symbiotic N2 fixation showed no significant increase in the BNF (mean: +13.6 %; 95 % confidence interval: -8.6 % to +41.3 %). This contrast becomes even more clear if rice paddies were excluded from non-N2 fixing ecosystems (mean: +4.1 %; 95 % confidence interval: -20.2 % to +35.7 %), as these paddies have substantial biological crusts with N2 fixating cyanobacteria (Hoque et al., 2001).

None of the long-term FACE experiments for which biomass responses to elevated CO2 was investigated by Feng et al. (2015) or Liang et al. (2016) was dominated by plants with symbiotic N2 fixation. Therefore, enhanced BNF cannot explain why PNL has not developed after decade long exposure to elevated CO2 in the majority of these FACE experiments. The mechanism and conclusion presented by Liang et al. (2016; Fig. 5) represents a special case, limited to ecosystems dominated by plants with symbiotic N2 fixation.

What mechanism can then potentially prevent the development of or alleviate a PNL in the majority of terrestrial ecosystems? An increase in the N use efficiency (NUE) of plants could prevent the development of a PNL, which has indeed been observed in grassland species (Lee et al., 2011). However, among four forest FACE experiment, only in a poplar plantation NUE was increased under elevated CO2, while NUE was unchanged in the remaining three experiments (Finzi et al., 2007). Therefore, it is unclear if this mechanism is widespread in alleviating PNL. Liang et al. (2016) identified N mineralization, the transfer of organically bound N to mineral N, as a major source for plant available N. However, they failed to recognize that net N mineralization, which was the measure they looked at, does not represent the actual N mineralization dynamics in soils (Davidson et al., 1992) and, hence, not the supply capacity for plant available N. The actual soil N mineralization is measurable as the gross N mineralization rate (Davidson et al., 1992). We recently reviewed how gross N transformations are altered in ecosystems exposed to elevated CO2 (Rütting and Andresen, 2015). Although we found that overall, across all available data, the gross N mineralization was not affected by elevated CO2, rates were significantly increased in N limited ecosystems (Rütting and Andresen, 2015). The importance of that finding lies in the fact that only N limited ecosystems are expected to be at risk of developing a PNL (Hu et al., 2006). In fact, ecosystems dominated by plants with symbiotic N2 fixation are not N limited and not prone to develop a PNL (Hu et al., 2006). Therefore, in N limited ecosystems (prone to PNL), the observed increased gross N mineralization directly provides enhanced supply of plant available N, which can alleviate the PNL or prevent its development under elevated CO2.

A question is which of the different processes can provide a large enough quantity of N to sustain the increased N demand under elevated CO2. Based on literature data, it is possible to quantify additional N supply by the different processes, N2 fixation, N mineralization and NO3- leaching, under elevated CO2. Annual N2 fixation rates in natural ecosystems, not dominated by symbiotic N2 fixing plants, are in the range 1.5 – 25 kg N ha-1 yr-1 (Cleveland et al., 1999). Assuming an increase by 14 % under elevated CO2 (as calculated for non-N2 fixing plant communities, Fig. 1), an additional amount of 0.2 – 3.5 kg N ha-1 yr-1 is provided. Annual NO3- leaching losses from temperate watershed are on average 0.3 kg N ha-1 yr-1 (range 0.02 – 2.3 kg N ha-1 yr-1; Brookshire et al., 2012). Liang et al. (2016) reported a decrease in NO3- leaching by 42 % under elevated CO2. However, when considering N limited ecosystem in their data-set (N=4), NO3- leaching decreased on average by 21 %. Therefore, reduced NO3- leaching under elevated CO2 is in the range of 0.004 to 0.48 kg N ha-1 yr-1 in N-limited temperate ecosystems. That means that the two processes identified by Liang et al. (2016) combined lead to an increase in N availability of at most 4 kg N ha-1 yr-1. Only few studies have reported annual gross N mineralization rates, which range between 120 and 450 kg N ha-1 yr-1 for temperate grasslands (Jamieson et al., 1999; Wang et al., 2016) and 800 – 1000 kg N ha-1 yr-1 in a Norway spruce forest (Rosenkranz et al., 2010). Assuming a 14 % increase in this rate under elevated CO2 (as found for N limited ecosystems by Rütting and Andresen, 2015), an additional amount of plant available N of 17 to 140 kg N ha-1 yr-1 will be produced annually. In other words, a small change in a large flux (i.e. gross N mineralization) might be more important than a large change in a small flux (i.e. N2 fixation and NO3- leaching) to alleviate a PNL.

Unresolved is for how long an increase in gross N mineralization can satisfy the increased N demand under elevated CO2. By this mechanism no N loss from the ecosystem occurs, but the redistribution of N from soil to vegetation could in the long-term diminish the pool of N in mineralizable organic matter, despite recycling via litter production. A recent multi-model study found that in several models the “mining” for N via enhanced mineralization was limited, due to changes in soil organic matter quality, and might only temporarily alleviate a PNL (Zaehle et al., 2014). On the other hand, a second study using the same set of models found an increased N mineralization rates due to larger soil organic matter N, which decreased N limitation (Walker et al., 2015). Experimental testing of the alternative model predictions is needed, which requires access to long term experiments with elevated CO2.

In conclusion, several processes can contribute to prevent the development of a PNL under elevated CO2. However, due to the limited spatial extend of plant communities dominated by symbiotic N2 fixation, the stimulation in BNF suggested by Liang et al. (2016) is a special case only, which is in agreement with the conceptual model by Rütting and Andresen (2015). In the majority of terrestrial ecosystems, with limited or no BNF, enhanced carbon inputs to the soil under elevated CO2 stimulate in a priming-like mechanism the gross N mineralization rate (Dijkstra et al., 2013; Rütting and Andresen, 2015), which can explain the absence of the development of a PNL in N limited ecosystems (Fig. 2). Further studies are needed to determine if this stimulation will be maintained in the long-term.

Acknowledgment:

This paper is a contribution to the strategic research area “Biodiversity and Ecosystem services in a Changing Climate – BECC” ([www.becc.lu.se/](http://www.becc.lu.se/)). The author declares no conflict of interests.

**References**

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

Brookshire, E. N. J., Gerber, S., Menge, D. N. L., and Hedin, L. O.: Large losses of inorganic nitrogen from tropical rainforests suggest a lack of nitrogen limitation, Ecology Letters, 15, 9-16, 2012.

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

Davidson, E. A., Hart, S. C., and Firestone, M. K.: Internal cycling of nitrate in soils of a mature coniferous forest, Ecology, 73, 1148-1156, 1992.

Dijkstra, F. A., Carrillo, Y., Pendall, E., and Morgan, J. A.: Rhizosphere priming: a nutrient perspective, Frontiers in Microbiology, 4, articel 216, 2013.

Evans, H. J., and Barber, L. E.: Biological nitrogen fixation for food and fiber production, Science, 197, 332-339, 1977.

Feng, Z., Rütting, T., Pleijel, H., Wallin, G., Reich, P. B., Kammann, C. I., Newton, P. C. D., Kobayashi, K., Luo, Y., and Uddling, J.: Constraints to nitrogen acquisition of terrestrial plants under elevated CO2, Global Change Biology, 21, 3152-3168, 2015.

Finzi, A. C., Norby, R. J., Calfapietra, C., Gallet-Budynek, A., Gielen, B., Holmes, W. E., Hoosbeek, M. R., Iversen, C. M., Jackson, R. B., Kubiske, M. E., Ledford, J., Liberloo, M., Oren, R., Polle, A., Pritchard, S., Zak, D. R., Schlesinger, W. H., and Ceulemans, R.: Increases in nitrogen uptake rather than nitrogen-use efficiency support higher rates of temperate forest productivity under elevated CO2, PNAS, 104, 14014-14019, 2007.

Hoque, M. M., Inubushi, K., Miura, S., Kobayashi, K., Kim, H. Y., Okada, M., and Yabashi, S.: Biological dinitrogen fixation and soil microbial biomass carbon as influenced by free-air carbon dioxide enrichment (FACE) at three levels of nitrogen fertilization in a paddy field, Biology and Fertilty of Soils, 34, 453-459, 2001.

Hu, S. J., Tu, C., Chen, X., and Gruver, J. B.: Progressive N limitation of plant response to elevated CO2: a microbiological perspective, Plant and Soil, 289, 47-58, 2006.

Hungate, B. A., Dukes, J. S., Shaw, M. R., Luo, Y., and Field, C. B.: Nitrogen and climate change, Science, 302, 1512-1513, 2003.

Jamieson, N., Monaghan, R., and Barraclough, D.: Seasonal trends of gross N mineralization in natural calcareous grassland, Global Change Biology, 5, 423-431, 1999.

Lee, T. D., Barrott, S. H., and Reich, P. B.: Photosynthetic responses of 13 grassland species across 11 years of free-air CO2 enrichment is modest, consistent and independent of N supply, Global Change Biology, 17, 2893-2904, 2011.

Liang, J., Qi, X., Souza, L., and Luo, Y.: Processes regulating progressive nitrogen limitation under elevated carbon dioxide: a meta-analysis. Biogeosciences, 13, 2689-2699, 2016.

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

Rosenkranz, P., Dannenmann, M., Brüggemann, N., Papen, H., Berger, U., Zumbusch, E., and Butterbach-Bahl, K.: Gross rates of ammonification and nitrification at a nitrogen-saturated spruce (*Picea abies* (L.) Karst.) stand in southern Germany, European Journal of Soil Science, 61, 745-758, 2010.

Rütting T., and Andresen L. C.: Nitrogen cycle responses to elevated CO2 depend on ecosystem nutrient status. Nutrient Cycling in Agroecosystems, 101, 285-294, 2015.

Smil, V.: Nitrogen in crop production: an account of global flows, Global Biogeochemical Cycles, 13, 647-662, 1999.

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

Wang, C., Chen, Z., Unteregelsbacher, S., Lu, H., Gschwendtner, S., Gasche, R., Kolar, A., Schloter, M., Kiese, R., Butterbach-Bahl, K., and Dannenmann, M.: Climate change amplifies gross nitrogen turnover in montane grasslands of Central Europe in both summer and winter seasons, Global Change Biology, 22, 2963-2978, 2016.

Wang, Y.-P., and Houlton, B. Z.: Nitrogen constraints on terrestrial carbon uptake: Implications for the global carbon-climate feedback, Geophysical Research Letters 36, L24403, 2009.

Zaehle, S., Medlyn, B. E., De Kauwe, M. G., Walker, A. P., Dietze, M. C., Hickler, T., Luo, Y., Wang, Y.-P., El-Masri, B., Thornton, P., Jain, A., Wang, S., Warlind, D., Weng, E., Parton, W., Iversen, C. M., Gallet-Budynek, A., McCarthy, H., Finzi, A., Hanson, P. J., Prentice, I. C., Oren, R., and Norby, R. J.: Evaluation of 11 terrestrial carbon-nitrogen cycle models against observations from two temperate Free-Air CO2 Enrichment studies, New Phytologist, 202, 803-822, 2014.

Figure 1: Response ratio of biological N2 fixation to elevated CO2 (mean ± 95 % confidence interval). Data from Liang et al. (2016), separated into different groups: FACE = Free Air CO2 Enrichment, OTC = Open Top Chambers; N2 fixing = plant communities dominated by plants with symbiotic N2 fixation; Non = plant communities not dominated by symbiotic N2 fixation. Number in parenthesis is the number of data points.



Figure 2: Conceptual model of the proposed mechanism for preventing a progressive nitrogen (N) limitation under elevated CO2 (modified from Luo et al., 2004; used with permission). Increased carbon (C) inputs to soil stimulate in a priming like mechanism (red dashed line) gross N mineralization (blue arrows), which keeps plant available N (labile soil N) sufficient to satisfy the enhanced plant N demand (uptake), thereby preventing the negative feedback on net primary productivity (NPP).

