

Point-by point response to reviewers:

Referee#1 (F. Dijkstra)

The exercise made on data from the Höglwald has been omitted from the paper. This does not alter the conclusion from the paper. Instead, a paragraph was added in which the amounts of additional N made available by different processes (N₂ fixation, N mineralization and N leaching) under elevated CO₂ are quantified, based on literature data. This demonstrates that stimulation of gross N mineralization provides a much larger amount of N than the other two processes.

The reduction in NO₃⁻ leaching under elevated CO₂ was calculated for N limited ecosystems alone. This reduction was with 21 % lower than the reduction of 42 % for the total data set. This information is added to the paper, when calculating the quantitative responses of the various processes (see above).

Referee#2 (D. Wårlind)

Increase in the N use efficiency (NUE) of plants is mentioned as an additional process that could prevent the development of a PNL. However, there is no general agreement if such an increase occurs, which is briefly discussed.

The suggested minor corrections by both referees were done. Also the reference list was updated.

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.

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The responses of terrestrial ecosystems to increasing 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. 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.

25 In their recent meta-analysis, Liang et al. (2016) aimed at this and concluded that increased biological N₂ fixation (BNF) together with decreased 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

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ecosystems has not been considered by Liang et al.: the actual soil N mineralization measured as gross rates.

Biological N₂ fixation can occur in most terrestrial ecosystems, highest rates often 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 extent, 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 fixing 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 ~~no~~-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) ~~rightly~~ 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 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, the rates were significantly increased in N limited ecosystems (Rütting and Andresen, 2015). The importance of that finding lies in the fact that only the 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 eCO₂.

~~Certainly, BNF also occurs in ecosystems not dominated by plants with symbiotic N₂ fixation. A question is, which of the different processes can provide a if this non-symbiotic BNF is 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 To provide some answer to this question, by different processes, N₂ fixation, N mineralization and N 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 (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.

5 I will discuss as a case, data from the spruce forest Höglwald, one of the most in detail studied ecosystems in terms of N cycling (Butterbach-Bahl and Gundersen, 2011), under current conditions together with likely responses to an elevated CO₂ level (Table 1), as already brought forward in my comment to the Liang et al. paper (Rütting, 2016). The current annual plant N uptake of the Höglwald forest is 100 kg N ha⁻¹ yr⁻¹, which is expected to
10 increase by 8 % or 8 kg N ha⁻¹ yr⁻¹ under eCO₂ (based on the average response in Feng et al., 2015). If we assume a reduction of N leaching by 42 % (as found by Liang et al., 2016) and an increase in gaseous N losses by 19 % (Van Groenigen et al., 2011), an additional amount of 1.5 kg N ha⁻¹ yr⁻¹ is needed to balance the enhanced plant N demand. If BNF should provide this, an increase by 75 % is needed, assuming the current day rate of 2 kg N ha⁻¹ yr⁻¹
15 (Table 1). This increase is well above the average increase in non-symbiotic plant communities (Fig. 1) and outside the 95 % confidence interval reported by Liang et al. (2016) (average = 14 %; range 9 % to 41 %). Therefore, it is unlikely that BNF can prevent the development of a PNL. On the other hand, a stimulation by as little as 0.3 % of gross N mineralization (currently 550 kg N ha⁻¹ yr⁻¹) can satisfy the enhanced plant N demand (Table
20 1) and prevent the development of a PNL. Such a small stimulation is much smaller than the average value we found for N limited ecosystem (+14 %) and the (non-significant) values for all ecosystems (+6 %) (Rütting and Andresen, 2015).

Unresolved is for how long an increase in gross N mineralization can satisfy the increased N demand under elevated CO₂. Even though that by this mechanism no N loss from the
25 ecosystem, 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
30 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 actually in agreement with the conceptual model by Rütting and Andresen (2015). However, in the majority of terrestrial ecosystems, with limited or no BNF, the enhanced carbon inputs to the soil stimulates 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.

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Table 1: Exemplary data on the nitrogen fluxes ($\text{kg N ha}^{-1} \text{yr}^{-1}$) from the spruce forest Högwald (Butterbach-Bahl and Gundersen, 2011) with no symbiotic N_2 fixation under current conditions (aCO_2) and estimated fluxes under elevated CO_2 (eCO_2), based on expected changes:

-	aCO_2^{a}	eCO_2	Δ	Change
Plant N uptake	100	108	8	8% ^b
N leaching	20	11.6	-8.4	-42% ^c
N_{gas} emission	10	11.9	1.9	19% ^d
<i>Alternative pathways for balancing N budget</i>				
Biol. N_2 fixation	2	3.5		75%
Gross N mineralization	550	551.5	1.5	0.3%

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^a Butterbach-Bahl and Gundersen (2011)

^b Feng et al. (2015)

^c Liang et al. (2016)

^d van Groenigen et al. (2011), based on data of nitrous oxide emission

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.

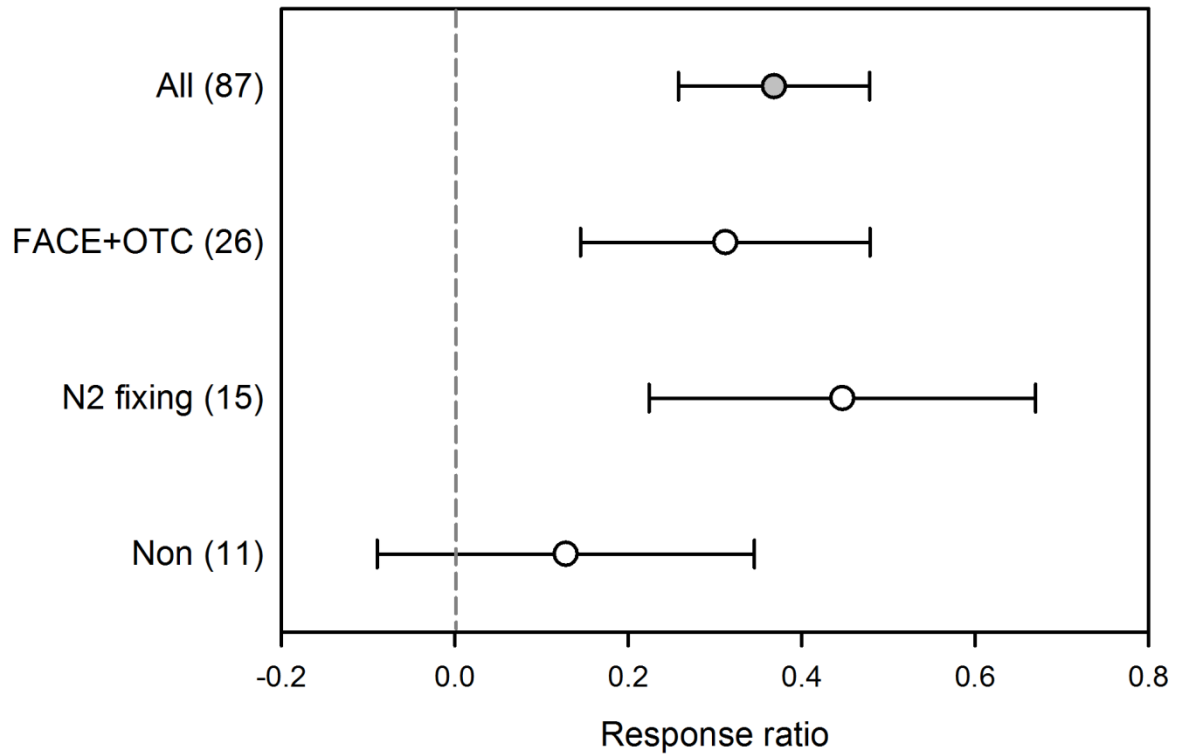


Figure 2: Conceptual model of the proposed mechanism preventing the development of or alleviating progressive nitrogen (N) limitation under elevated CO₂ (modified from Luo et al., 2004). Increased carbon inputs to soil stimulate in a priming like mechanism gross N mineralization (+), which keeps plant available N at high enough levels to sustain enhanced net primary productivity (NPP).

