

## ***Interactive comment on “A reduced fraction of plant N derived from atmospheric N (%Ndfa) and reduced rhizobial nifH gene numbers indicate a lower capacity for nitrogen fixation in nodules of white clover exposed to long-term CO<sub>2</sub> enrichment” by T. Watanabe et al.***

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Anonymous Referee #2

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General comments: This work by Watanabe et al. reports on the effect of elevated CO<sub>2</sub> on biological N fixation in white clover and its association with rhizobia and nifH gene development. The work is generally well written and the experimental approach is solid. The methods are detailed and the analyses

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and interpretation appear to be mostly sound. The data support the key messages that the reduced BNF under eCO<sub>2</sub> was caused by the reduction in number of nifH genes, and that the relative input of N from fixation in the long term might be lower under future CO<sub>2</sub> atmospheres. This information provides major implications for future productivity of pasture systems, N management and ecosystem establishment in the region and other parts of the world. Therefore, the paper is timely and would be important for the scientific community and land managers. It would benefit the paper if the authors could expand a little more discussion on some findings of the study (see specific comments).

Specific comments:

Discussion section: - Authors mentioned “...our plant with 0.17% P under aCO<sub>2</sub> and 0.16% P under eCO<sub>2</sub> (Table 1a) were growing in a low P environment (P. 9882, L26-27)” and “we can conclude that the P availability was in the range where nutrient limitation might occur (P. 9883, L6-7)”, but “The N/P ratios we found (16.2 for eCO<sub>2</sub> and 14.7 for aCO<sub>2</sub>) were below the level of 20 suggested to indicate P limitation (Güsewell, 2004) and fell in the range that would suggest a contribution from BNF (%Ndfa) of about 60% (Almeida et al. 2000) (P. 9883, L18-21)”. . . A bit confusing as to whether there was P limitation in the present study? %Ndfa of the present study was 89.8% under aCO<sub>2</sub> and 72.0% under eCO<sub>2</sub>, which was greater than 60% mentioned in the previous statement. So, seems that there was no P limitation in the present study?

Author: The referee makes a good point about the %Ndfa in our study and that expected under P limitation. While this suggests there is no P limitation as does the fact there is no change in nodule number, we can see from our P and N/P values that we are at the lower end of P fertility for managed grassland although still well above those found in natural grasslands. Consequently, we cannot say definitively whether P limitation was present so the Discussion does not rule this out but considers other possible mechanisms.

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It is interesting that shoot biomass was significantly lower under eCO<sub>2</sub> than aCO<sub>2</sub> after 6 weeks, or shoot biomass remained unchanged between week 3 and week 6 under eCO<sub>2</sub>. However “there was no difference in N or P concentrations in shoot [between aCO<sub>2</sub> and eCO<sub>2</sub>] (P. 9880, L17-18)”. So, what potentially was limiting the shoot growth between weeks 3 and 6 under eCO<sub>2</sub>?

Why couldn't the plant grown under eCO<sub>2</sub> attain at least similar biomass as the plant grown under aCO<sub>2</sub>?

Author: Dry matter production at 3 weeks was insufficient to provide the sample size necessary for nutrient analysis so we cannot say whether the N/P was different prior to 6 weeks. If it was different this could explain the difference in biomass production. The Gentile reference we discuss shows a similar plant response in the same soil which the authors concluded was co-limitation by P and N.

How would this relate to the reduction in nifH genes under eCO<sub>2</sub>?

Author: If plant growth (or lack of it) is driving gene number it also needs to explain how nodule number remained unaffected. We don't feel able to separate cause and effect here.

The authors may consider discussing the potential causes of the decline in N fixation under elevated CO<sub>2</sub> of the present study with respect to the potential causes observed C4224 by others (e.g. Hungate et al. 2004; West et al. 2005), rather than only mentioning others also observed decline of N fixation under eCO<sub>2</sub> several years later (P. 9882 and 9886).

Author: The cause is clear in the Hungate reference but no cause is identified in the West reference. We will add this to the text.

Technical corrections: P. 9873, L20: seems that total Kjeldahl N excludes both NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup>

Author: Yes Kjeldhal N excludes both NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup>. Will change.

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Title of Table 1: “(a)” missing

Author: Will correct.

Fig. 2(b): upper end of confidence interval missing for week 0, or do you mean the upper end is 290 as indicated by “(290)”?

Author: Yes it is the upper limit – will add explanation to the Figure Legend.

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