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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_2 enrichment" *by* T. Watanabe et al.

Anonymous Referee #2

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General comments:

This work by Watanabe et al. reports on the effect of elevated CO2 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 and interpretation appear to be mostly sound. The data support the key messages that the reduced BNF under eCO2 was caused by the re-

duction in number of nifH genes, and that the relative input of N from fixation in the long term might be lower under future CO2 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 aCO2 and 0.16% P under eCO2 (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 eCO2 and 14.7 for aCO2) 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 aCO2 and 72.0% under eCO2, which was greater than 60% mentioned in the previous statement. So, seems that there was no P limitation in the present study?

- It is interesting that shoot biomass was significantly lower under eCO2 than aCO2 after 6 weeks, or shoot biomass remained unchanged between week 3 and week 6 under eCO2. However "there was no difference in N or P concentrations in shoot [between aCO2 and eCO2] (P. 9880, L17-18)". So, what potentially was limiting the shoot growth between weeks 3 and 6 under eCO2? Why couldn't the plant grown under eCO2 attain at least similar biomass as the plant grown under aCO2? How would this relate to the reduction in nifH genes under eCO2?

- The authors may consider discussing the potential causes of the decline in N fixation under elevated CO2 of the present study with respect to the potential causes observed

BGD
10, C4223–C4225, 2013

by others (e.g. Hungate et al. 2004; West et al. 2005), rather than only mentioning others also observed decline of N fixation under eCO2 several years later (P. 9882 and 9886).

Technical corrections:

P. 9873, L20: seems that total Kjeldahl N excludes both NO3– and NO2–

Title of Table 1: “(a)” missing

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)”?

Interactive comment on Biogeosciences Discuss., 10, 9867, 2013.