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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.

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With predictions that gaseous CO2 levels will continue to rise, the effect on BNF is ofgreat importance. Little is understood about the magnitude of BNF in pastures andrangelands that cover a large proportion of managed landscapes, however, productivityof these systems rely almost entirely on inputs from BNF. Although previous

studieshave indicated an increase in BNF with increased CO2, these have been short-termstudies and the authors have provided evidence that activity decreases over the longerterm. The authors have provided sound evidence that the decrease in BNF is notrelated to strain variation but rather to a decrease in nifH gene copy and nifH genetranscription over six weeks of growth of white clover at elevated CO2. The authorsconclude that cycling of amino acids which drive N2 fixation in the symbiosis may beaffected by elevated CO2. The outcomes have been discussed carefully and with consideration to a number of potential factors. I just have a few points relating to the discussion for the authors' consideration when reviewing the manuscript.

1. When comparing nutrient limitation effects on plant tissue concentrations, nodulation and nitrogen fixation it would be helpful to know how others have done these analyses in particular measurement of nitrogen fixation and stage of plant growth. For example at what stage of plant growth were measurements taken in the studies by Høgh-Jensenet al. (2002) and Edwards et al. (2006) and how was BNF measured? Author: We will add these details to the revised manuscript on p 9882 l 21 onwards.

2. You conclude that P was low when tissue levels are considered on their own but not limiting when considered relative to N (ie. N/P ratio). Are you suggesting that the N/P ratio is the more critical factor? Author: We are not in a position to say which is the most critical factor P or N/P. We include both in our discussion because we have literature that relates BNF to both P and N/P in white clover. If this is the case then could the observed changes in nifH gene copy and expression be related to differences in N/P ratio between eCO2 and aCO2 treatments? As cited, Edwards et al. (2006) found a substantial increase (31%) in BNF when the N/P ratio was decreased from 21.4 to 11.8. Are your ratios of 16.2and 14.7 different enough to be the cause of the observed change in BNF? How does the percentage decrease in eCO2 in your study compare with the change reported in Edwards et al. (2006)? Author: We can't say whether an N/P of 16.2 is sufficiently different from 14.7 to be the cause of the 17% observed change in BNF. However, the referee makes an important point about N/P and we will

include some text in the Discussion to cover potential effects of P limitation on N making use of a recent paper on this topic p 9885 l 15. Sulieman S, Ha CV, Schulze J, Tran L-SP (2013) Growth and nodulation of symbiotic Medicago truncatula at different levels of phosphorus availability. Journal of Experimental Botany 64:2701-2712.

3. You have reported an increase in tissue Cu concentration and soil B. Is there likely to be any toxic effect of elevated Cu or B? Why do you think these elements as well as sulfur are higher in the eCO2 treatment? Author: The plant concentrations of B and Cu are in the 'adequate' range according to NZ pasture guidelines. We cannot say why Cu, B and S were higher under elevated CO2 but increased Cu uptake has been observed in a recent paper and we will reference this in the changed ms. Tian S, Jia Y, Ding Y, Wang R, Feng R, Song Z, Guo J, Zhou L (2013) Elevated Atmospheric CO2 Enhances Copper Uptake in Crops and Pasture Species Grown in Copper-Contaminated Soils in a Micro-Plot Study. CLEAN – Soil, Air, Water:n/a-n/a. Soil B level in both soils were low therefore B toxicity can be ruled out.

4. Can you suggest how mechanisms could be investigated in the future? What improvements could be made to experimental design to determine contributions from nutrient effects and amino acid cycling? Author: Control of the plant N/P ratio and better monitoring of amino acid fluxes would be valuable.

Minor corrections to text 1. P9877, line 8 – change 'hypochloride'to 'hypochlorite' 2. Author: will change. Table 1 (plant analysis) – change 'By' to 'B' in column heading 3. Author: will change. Fig 2 (b) – what does '(290)' represent on the figure? Upper limit of 95% CI Author: Yes it is the upper limit – will add explanation to the Figure Legend.

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