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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 CO₂ enrichment” by T. Watanabe et al.

Anonymous Referee #1

Received and published: 20 August 2013

With predictions that gaseous CO₂ levels will continue to rise, the effect on BNF is of great importance. Little is understood about the magnitude of BNF in pastures and rangelands that cover a large proportion of managed landscapes, however, productivity of these systems rely almost entirely on inputs from BNF. Although previous studies have indicated an increase in BNF with increased CO₂, these have been short-term studies and the authors have provided evidence that activity decreases over the longer term. The authors have provided sound evidence that the decrease in BNF is not

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related to strain variation but rather to a decrease in nifH gene copy and nifH gene transcription over six weeks of growth of white clover at elevated CO₂. The authors conclude that cycling of amino acids which drive N₂ fixation in the symbiosis may be affected 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-Jensen et al. (2002) and Edwards et al. (2006) and how was BNF measured?
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? 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 16.2 and 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)?
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?
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?

Minor corrections to text

1. P9877, line 8 – change 'hypochloride' to 'hypochlorite'
2. Table 1 (plant analysis) – change 'By' to 'B' in column heading
3. Fig 2 (b) – what does '(290)' represent on the figure?

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

