



Influence of atmospheric [CO₂] on growth, carbon allocation and cost of plant tissues on leaf nitrogen concentration maintenance in nodulated *Medicago sativa*

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Plant carbon (C) allocation and plant metabolic processes (i.e. photosynthesis and respiration) can be affected by changes in C availability, for example from changing atmospheric [CO₂]. In nodulated plants, C availability may also influence nitrogen (N) fixation by bacterioids. But C allocation and N fixation are often studied independently and hence do not allow elucidating interactive effects. We investigated how different atmospheric [CO₂] (Pleistocene: 170 ppm, ambient: 400 ppm and projected future: 700 ppm) influence plant growth, allocation to nodules, and the ratio of photosynthesis-to-respiration (R:A) as an indicator of C cost in *Medicago sativa* inoculated with *Ensifer meliloti*.

M. sativa grew c. 38% more nodules at 400 ppm and 700 ppm than at 170 ppm. However, ratios of above- and belowground plant biomass to nodule biomass were constant over time and independent of atmospheric [CO₂]. Total non-structural carbohydrate concentrations were not significantly different between plants grown at 400 and 700 ppm, but were four to five-fold higher than in 170 ppm plants. Leaf level N concentration was similar across treatments, but N-based photosynthetic rates were 82% and 93% higher in leaves of plants grown at 400 and 700 ppm, respectively, than plants grown at 170 ppm. In addition, leaf R:A was greater (48% or 55%) in plants grown at 170 ppm than plants grown at 400 and 700 ppm. Similarly, the greatest proportion of assimilated CO₂ released by root respiration occurred in rhizobial plants growing at 170 ppm. Our results suggest that C limitation in nodulated *Medicago sativa* plants did not influence C allocation to nodule biomass but caused a proportionally greater allocation of C to belowground respiration, most likely to bacterioids. This suggests that N tissue concentration was maintained at low [CO₂] by revving up bacterioid metabolism and at the expense of non-structural carbohydrate reserves.