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## **Increasing atmospheric CO<sub>2</sub> levels may reduce extraction of nutrients from soil microbes in plant roots**

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### **Background/Question/Methods**

The rhizophagy cycle is a nutritional process where nutrients are extracted from microbes oxidatively in roots. In the rhizophagy cycle, microbes alternate between a free-living phase in soil and a plant-dependent protoplast phase inside plant root cells. Microbes acquire nutrients (protein and minerals) in the soil phase, and nutrients are extracted from microbes in plant root cells through exposure to reactive oxygen (superoxide) produced on the root cell plasma membrane. In the endophytic protoplast phase microbes are also increased in numbers prior to expulsion into the soil from elongating root hairs. Carbon dioxide has been shown to be a universal inhibitor of reactive oxygen. We hypothesize that increasing levels of CO<sub>2</sub> in the atmosphere will reduce the efficiency of nutrient extraction from microbes by inhibiting the production of reactive oxygen in roots. To test this hypothesis, seedlings of wheat (*Triticum aestivum*) and tomato (*Lycopersicon esculentum*) were germinated on agarose then placed in gas chambers that contained air with approximately 0.04% CO<sub>2</sub> (current level in atmosphere) or air with significantly elevated levels of CO<sub>2</sub> (0.06%). After several days, Petri dishes containing seedlings were flooded with stains nitro blue tetrazolium (to detect superoxide) or 3, 3-diaminobenzidine (to detect hydrogen peroxide) and then incubated for 15 hours at lab ambient temperature. Seedling roots were then examined microscopically to detect microbes in root cells and presence of reactive oxygen staining around microbes.

### **Results/Conclusions**

Seedlings of both species grown in chambers containing air with 0.06% CO<sub>2</sub> showed reduced numbers of microbe protoplasts in roots and reduced staining for superoxide and hydrogen peroxide around microbes compared to seedlings grown in chambers containing air with 0.04% CO<sub>2</sub>. These results suggest that increasing CO<sub>2</sub> levels in the air may reduce efficiency of nutrient acquisition in the rhizophagy cycle by suppressing reactive oxygen secretion in plant roots around microbes, resulting in failure to strip cell walls from microbes and replicate them prior to expulsion from root hairs. Recently, experiments on several food crops have shown that enriching the air with CO<sub>2</sub> results in increased photosynthesis, but levels of proteins and minerals (iron and zinc) are reduced. Our experimental results point to CO<sub>2</sub> inhibition of the rhizophagy cycle as a possible explanation of reduced nutrient absorption in crop plants subjected to increased levels of CO<sub>2</sub>.