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**Program Area:**  
1. Responding to Climate and Energy Needs; Sub-goal: Responding to Climate Variability

**Priority Area:**  
2. Global Climate Effects on Agriculture  
Program Code Name: Climate Change Mitigation and Adaptation in Agriculture  
Program Code – A3141

**Title:** Enhancing Nitrogen Use Efficiency (NUE) of Crops under Rising CO2

**Rationale:** Changes in nitrogen acquisition determine crop responses to rising atmospheric CO2 concentrations. Because CO2 enrichment inhibits NO3– assimilation into protein in the shoots of C3 plants, it will be necessary to breed crops that have enhanced NUE under increasing CO2. In this proposal, we will identify mechanisms that optimize NUE under high CO2 using a combination of physiological, genomic, and systems biology approaches. Thus, the proposed project will address the Priority Issue: “*Understand the physiological basis of adaptation to abiotic and biotic stresses likely resulting from climate change, especially the effects of stressors created by extremes in…carbon dioxide.*”

**Overall hypothesis or goal:** The proposed research will exploit cross-species “network knowledge” to sustain or enhance NUE in C3 crops under rising CO2. The research will examine both NO3–or NH4+ , because NH4+ enjoys several advantages over NO3– as a N source: its conversion into protein *is not* inhibited by CO2 enrichment and requires less energy, it does not leach rapidly from soils and contaminate groundwater, and its efficient use minimizes agricultural N2O emissions.

**Specific objectives:** 1) Identify genes that determine efficient use of NO3–or NH4+ under elevated CO2. 2) Characterize NO3– vs. NH4+ responsive gene networks using transcriptomics in NUE variants of Arabidopsis and wheat. 3) Identify conserved network modules to enable molecular breeding of NUE in C3 crops.

**Approach:** **Aim 1. Identification of genes associated with NUE under elevated CO2.** Arabidopsis (96 different ecotypes) will receive NO3–or NH4+ as sole N sources in controlled environmental chambers at ambient or elevated CO2 concentrations. We will *a*) assess NUE from biomass, 15N-absorption and assimilation, and protein accumulation; *b*) identify candidate genes that influence NUE using SNP-based GWAS (Genome-Wide Association Studies); and *c*) evaluate these candidate genes in a similar chamber experiment on 10 wheat genotypes that differ in NUE.

**Aim 2. Systems integration of physiological and genomic responses to elevated CO2**. We will grow Arabidopsis and wheat NUE genotypes in soils of different fertilities in CO2-controlled greenhouses at ambient or elevated CO2. We will evaluate *a*) plant NO3– vs. NH4+ usage via the ratio of shoot CO2 to O2 fluxes; *b*) shoot vs. root NO3– assimilation via xylem sap analyses; *c*) NUE via uptake, partitioning, biomass, seed production, and seed quality; [Dennis thinks one can stop here:] *d*) N-responsive gene networks using transcriptomics (RNA-seq); and *e*) multivariate networks that link genes to NUE traits.

**Aim 3. Cross-species Network inspired Biomarkers for NUE breeding.** We will perform cross-species network analysis to identify core network modules associated with NUE under elevated CO2.

**Potential impact and expected outcomes:** Wheat provides about 20% of the protein in the human diet, but its protein yield declines under CO2 enrichment as a result of inhibited NO3– assimilation. *This research and its network-inspired biomarker approach will identify the genetic basis of improved NO3– and NH4+ utilization, an understanding that will guide the breeding of crops better suited to the conditions anticipated in the face of elevated CO2.*