The goal of the NutriNet project is to identify network-connected biomodules used as markers to predict and/or generate high nutrient use efficiency (NUE) crops. Such network modules will be derived by integrating genomic and phenotypic NUE data from genetically diverse lines with protein:protein network-knowledge from Arabidopsis, in order to derive network modules associated with high NUE in a model (Arabidopsis) and crop (Maize) species. This network-oriented approach has several advantages over current gene-centric approaches in molecular breeding: i) it exploits the large amount of network knowledge on data on gene and protein interactions in Arabidopsis to inform analysis of data-poor and poorly annotated crop genomes, ii) it provides a focus for experimental studies in model species (Arabidopsis) that have direct relevance to crops enhancing translational research, and iii) by combining genes that are functionally linked, network modules should be more robust diagnostic tools both across crop species and varieties. Importantly, this approach and its associated tools can be broadly applied in molecular breeding programs to enhance selection and model-to-crop translation for any trait or crop of interest.

We apply this NutriNet approach to improving NUE, because in current agricultural practice, there been the strong selection of crop varieties for yield with excess levels of nutrients supplied by fertilizer, rather than efficient production under nutrient levels that promote climate, resource, and energy sustainability. We propose to uncover mechanisms underlying NUE and develop network-module biomarkers by combining the following aims

**Aim 1. Exploiting Genetic Diversity in NUE (Coruzzi/Moose)**. We will exploit phenotypic variation for NUE in Arabidopsis (Coruzzi) and Maize (Moose), by collecting genomic data for nitrogen-regulated genes across an initial test set of 5-10 genetic variants. This data will be used to define gene-to-phenotype associations, that will fuel the generation of network modules in Aim 3. (Our European collaborators provide breadth in Brassica and wheat (Hawkesford) as well as metabolite data).

**Aim 2. Exploring root-shoot N-signaling (Coruzzi)**. A key determinant of NUE we will use to drive network module construction is the influence of plant demand (e.g. in shoots) on feedback mechanisms controlling nutrient uptake and assimilation (e.g. in roots). Using a split-root experimental design, we will quantify root nutrient foraging and 15N-assimilation across 20 lines of Arabidopsis and Maize. This data will be used to select the most efficient NUE lines for transcriptomics analysis of local and systemic signalling, based on the biomass/root foraging “cost”. Gloria: I have to say it’s not clear that split root is the only or best way to answer this question.

**Aim 3. Network Module Construction: Tool development and application (Shasha/Coruzzi).** This aim combines gene-to-phenotype, gene-to-gene, protein-to-protein, and gene-to-metabolite interactions into a pipeline analysis that will result in the construction of Network modules from Arabidopsis and Maize. Network modules generated using data from 10 strains, will be tested for their ability to predict NUE based on gene expression data using data from 10 additional strains not used to construct the modules. Further, the model will predict which genes and gene network modules most influence network use efficiency.

**Aim 4. Nutri-Net Validation: GWAS studies and Field testing**. (Coruzzi/Moose) The network modules identified in Aim 3 will be further validated and tested in field trials in Aim 4. This will include using the network modules as a focus for GWAS analysis in 96 Arabidopsis ecotypes for which NUE phenotypes are available and also in field trials in Maize. (Steve – do you want to add something about ozone here????)