**2. “Predictive network modeling of the high-resolution dynamic plant transcriptome in response to nitrate**.” ***Relevant Pubs***: [[8](#_ENREF_8),[38](#_ENREF_38)] (*highly accessed*)**.** In previous work, we used time-series data to learn N-regulatory networks that can infer gene expression states at future time-points [[8](#_ENREF_8)], a central goal of Systems Biology [[38](#_ENREF_38)]. Because causality moves forward in time, fine-scale time-series experiments are a particularly valuable source of structure to derive predictive network models. To learn N-regulatory networks, we generated fine-scale time-series transcriptome data from nitrate-treated plants (0,3,6,9,12,15,20 min). This early time-series identified >200 new nitrate-regulated genes not identified in previously steady-state studies [[39-41](#_ENREF_39)]. Next, we used a machine-learning approach called “State-Space” analysis, a form of Dynamic Factor Graphs (DFG) [[42](#_ENREF_42)], with our adaptations [[8](#_ENREF_8)], to infer a dynamic regulatory network from this time-series transcriptome data of N-responses in roots. Briefly, State-Space models synthesize Bayesian and Markovian approaches (in which each gene’s expression value at time *t+1,* depends directly only on the state of potentially all the genes at time *t*). We added a noise-mitigation approach that uses hidden variables to represent an idealized, “true” sequence of gene expressions **z**(*t*) that would be measured if there were no noise in the data. The goal is to *learn* the function ***f*** that determines the change in expression of a target gene, as a combination of the expression of a relatively small number of TFs (typically under 10). ***To validate this approach***, we used the 0-15 min data (as a training set), and then used the “learned” network to predict the *direction* of gene expression change from 15🡪20 min (induced or repressed) and validated using the “left-out” 20 min experimental data. Our predictions of gene regulation at 20 min were correct for 74% of the genes in a sub-network of 76 genes (N-assimilation pathway genes and TFs) [[8](#_ENREF_8)]. This is significantly better (p-val<0.006) than the *"naive trend forecast" test*, correct for only 52% of the genes, just slightly better than random. Compared to other network inference approaches [[43-46](#_ENREF_43)], our adapted DFG method had a slight improvement in accuracy, and had a better signal-to-noise ratio using the same data.

 In the current grant, we will use metabolite quantity instead of expression values, but the computational method still applies.

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