the plant journal

SEB SOCIETY FOR EXPERIMENTAL BIOLOGY

The Plant Journal (2025) **122,** e70211 doi: 10.1111/tpj.70211

Shining light on Arabidopsis regulatory networks integrating nitrogen use and photosynthesis

Kithmee de Silva[1], Camila Coelho[2], Jenny Gao[2] and Matthew D. Brooks[3,*]

[1]*Department of Plant Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, USA,*
[2]*Department of Biology, New York University, New York, New York 10003, USA, and*
[3]*Global Change and Photosynthesis Research Unit, USDA ARS, Urbana, Illinois 61801, USA*

Received 18 October 2024; revised 6 March 2025; accepted 28 April 2025.
*For correspondence (e-mail matthew.d.brooks@usda.gov).

SUMMARY

Nitrogen and light availability are well-known to influence photosynthesis, having both individual and synergistic effects. However, the regulatory interactions between these signaling pathways, especially the transcription factors (TFs) that perceive and integrate these cues, remain to be elucidated. Arabidopsis grown in a matrix of nitrogen and light treatments exhibited distinct physiological and transcriptomic responses. Notably, the effect of nitrogen dose on biomass, nitrogen use efficiency, carbon-to-nitrogen ratio, and gene expression was highly dependent on light intensity. Genes differentially expressed across the treatments were enriched for photosynthetic processes, including the pentose-phosphate cycle, light-harvesting, and chlorophyll biosynthesis. TFs coordinating photosynthesis, carbon-to-nitrogen balance, and nitrogen uptake were identified based on motif enrichment, validated binding data, and gene regulatory network analysis. Dynamic light-by-nitrogen responses were found for TFs previously linked to either nitrogen or light signaling, which now emerge as regulatory hubs that integrate these signals. Among these TFs, we identified bZIP and MYB-related family transcription factors as pivotal players in harmonizing photosynthesis, nitrogen assimilation, and light responses. The transcription factors unveiled in this study have the potential to unlock new strategies for optimizing photosynthetic activity and nutrient-use efficiency in plants.

Keywords: photosynthesis, nitrogen, gene regulatory networks, Arabidopsis, light.

INTRODUCTION

Plant nitrogen and light responses have been extensively studied due to their significant impact on various aspects of metabolism, physiology, and developmental processes. Nitrogen is an essential macronutrient for plant growth and development and a key component of proteins and nucleic acids. It is particularly linked to photosynthesis as it is essential for synthesizing photosynthetic enzymes, chlorophyll, and other biomolecules involved (Evans & Clarke, 2019). Likewise, light serves as the energy source for photosynthesis, thus providing the energy necessary for physiological and biochemical processes in a plant (Whitmarsh & Govindjee, 1999), including nitrogen uptake and assimilation (Sakuraba & Yanagisawa, 2018; Yoneyama & Suzuki, 2020). Together, nitrogen and light availability influence photosynthetic capacity and efficiency through the accumulation and allocation of carbon and nitrogen (Grechi et al., 2007; Liang et al., 2022) and have a large effect on the plant carbon-to-nitrogen (C/N) ratio. This interaction is important for agricultural productivity, as shown in rice (Gu et al., 2017) and maize (Ren et al., 2023), where light and nitrogen relationships directly influence grain yield and content. Thus, the intricate relationship between nitrogen, light, and carbon ensures the optimal balance between nutrient uptake, photosynthesis, and energy allocation, ultimately shaping plant growth, development, and overall fitness.

To facilitate the perception and response to environmental signals, organisms evolve regulatory networks that are vital to their growth, development, and survival. Transcription factors (TFs) are pivotal in coordinating these networks and cellular responses, bridging changes in environmental signals and gene expression. Numerous TFs involved in nitrogen signaling and uptake have been identified. These include NLP6/7 (Castaings et al., 2009; Konishi & Yanagisawa, 2013), ANR1 (Zhang & Forde, 1998), LBD37/38/39 (Rubin et al., 2009), TGA1/4 (Alvarez et al., 2014), and CRF4 (Varala et al., 2018). Similarly, BBX22/24/25 (Gangappa & Botto, 2014) and PIF3/4/5 (Leivar & Monte, 2014) are instrumental in mediating responses to

light. Interestingly, a few TFs, namely bZIP1 (Obertello et al., 2010), HY5 (Jonassen et al., 2009), WRKY1 (Heerah et al., 2019), and TGA3 (Ruffel et al., 2021), have been identified as participants in both nitrogen and light responses.

Studies have investigated the reprogramming of transcriptional regulatory networks in response to fluctuations in light intensity coupled with shifts in nitrogen availability in rice and poplar (Pathak et al., 2020; Zhu et al., 2023). These findings highlight the interactions between nitrogen and light signaling pathways and imply potential crosstalk and shared downstream transcriptional regulation. Pathak et al. (2020) compared light-grown seedlings to etiolated rice seedlings under different nitrate treatments and observed distinct physiological and gene expression responses between the light conditions. While the relevance of nitrate responses in seedlings grown in complete darkness is debatable, they did observe that many of the nitrate-responsive genes and *cis*-binding motifs that were enriched were consistent with studies in Arabidopsis, demonstrating that core nitrogen signaling is likely conserved, as has been shown in maize–Arabidopsis studies (Cheng et al., 2021). Zhu et al. (2023) utilized a broader matrix of nitrogen and light treatments in poplar; however, their focus was on vascular multiomics to understand physiological characteristics of wood, and the regulation and signaling pathways in leaves were not addressed. Therefore, while the individual effects of nitrogen and light signals on gene regulation in plants have been characterized in some detail, there is still limited knowledge regarding how these signals are integrated and interact with each other.

This study aimed to uncover the central regulators involved in the genome-wide transcriptional control of photosynthesis and coordination with nitrogen assimilation in response to nitrogen and light signals. We grew plants in a matrix of nitrogen and light conditions, measuring physiological traits and identifying differentially expressed genes (DEGs) responding to nitrogen and light using a model reduction approach. From the gene expression data, we constructed a TF-directed gene regulatory network (GRN) and used it to predict regulatory connections within subnetworks consisting of nitrogen- and light-responsive TFs and the genes correlated to the traits. Network analysis enabled us to identify TFs that play a role in coordinating these photosynthesis and nitrogen assimilation pathways. Our results indicate that TFs belonging to bZIP and MYB-related families are particularly important to the transcriptional regulation of integrating nitrogen and light signals.

RESULTS

The interactions between nitrogen and light signals shape physiological traits

We investigated the combined effects of nitrogen and light on Arabidopsis (Col-0) using a full factorial design with four nitrate treatments (2, 5, 10, and 20 mM NO_3^-) and three light conditions (100, 250, and 500 $\mu mol\ m^{-2}\ sec^{-1}$, Figure 1A). Among the two major nitrogen sources, nitrate and ammonium, we selected nitrate as the sole nitrogen source as it is the most readily available form in aerobic soils (Prosser, 2005), and signaling responses to nitrate have been extensively studied (Vidal et al., 2020). Furthermore, ammonium has also been shown to negatively affect plant growth and photosynthesis, particularly under high light conditions (Guo et al., 2007), which could confound our experiment. The light conditions included low (100 $\mu mol\ m^{-2}\ sec^{-1}$) and mid (250 $\mu mol\ m^{-2}\ sec^{-1}$), which reflect recommended growing conditions for Arabidopsis (Rivero et al., 2014), as well as high light (500 $\mu mol\ m^{-2}\ sec^{-1}$), where plant growth was affected but without inducing stress responses.

To assess the effects of the combined nitrogen-by-light (N-by-L) treatments on plant physiology, we measured traits including biomass and photosynthetic parameters (chlorophyll fluorescence measurements of operating efficiency of PSII (Φ_{PSII}) and non-photochemical quenching [NPQ]), nitrogen uptake, and total plant C/N ratio (Table S1). The synergistic effect of nitrogen and light on plant growth and development was apparent in the physiological response (Figure 1A). We observed notable phenotypic effects of varying light levels on petiole elongation and leaf blade area, consistent with responses in shade avoidance (Hersch et al., 2014; Smith & Whitelam, 1997). The increase in biomass with the nitrogen dose at high light distinctly illustrates how nitrogen functions as a limiting factor for plant growth (Figure 1B). However, while there was a pronounced difference in biomass response to nitrogen level at high light, there was no significant effect of nitrogen dose at low light, highlighting the dependency of the nitrogen response on light level (Figure 1B).

The C/N ratio is altered in response to nitrogen and light availability

Photosynthetic carbon and nitrogen metabolism is tightly regulated in plants, suggesting the coupling of their sensing and signaling pathways (Champigny, 1995). To probe the coordination between carbon and nitrogen and the role played by nitrogen and light availability, we measured photosynthetic efficiency (Φ_{PSII}), non-photochemical quenching (NPQ), nitrogen uptake efficiency (NUpE), and C/N ratio. Measurements of NPQ and Φ_{PSII} were taken at six measuring light intensities ranging from 75 to 725 $\mu mol\ m^{-2}\ sec^{-1}$. As the measuring light intensity increases, the quantum yield of PSII decreases due to a greater proportion of PSII reaction centers closing under higher light intensities, thereby limiting the plant's ability to utilize the absorbed light for photochemistry (Maxwell & Johnson, 2000). Plants grown under low light conditions had a more rapid decline in Φ_{PSII} with increasing light

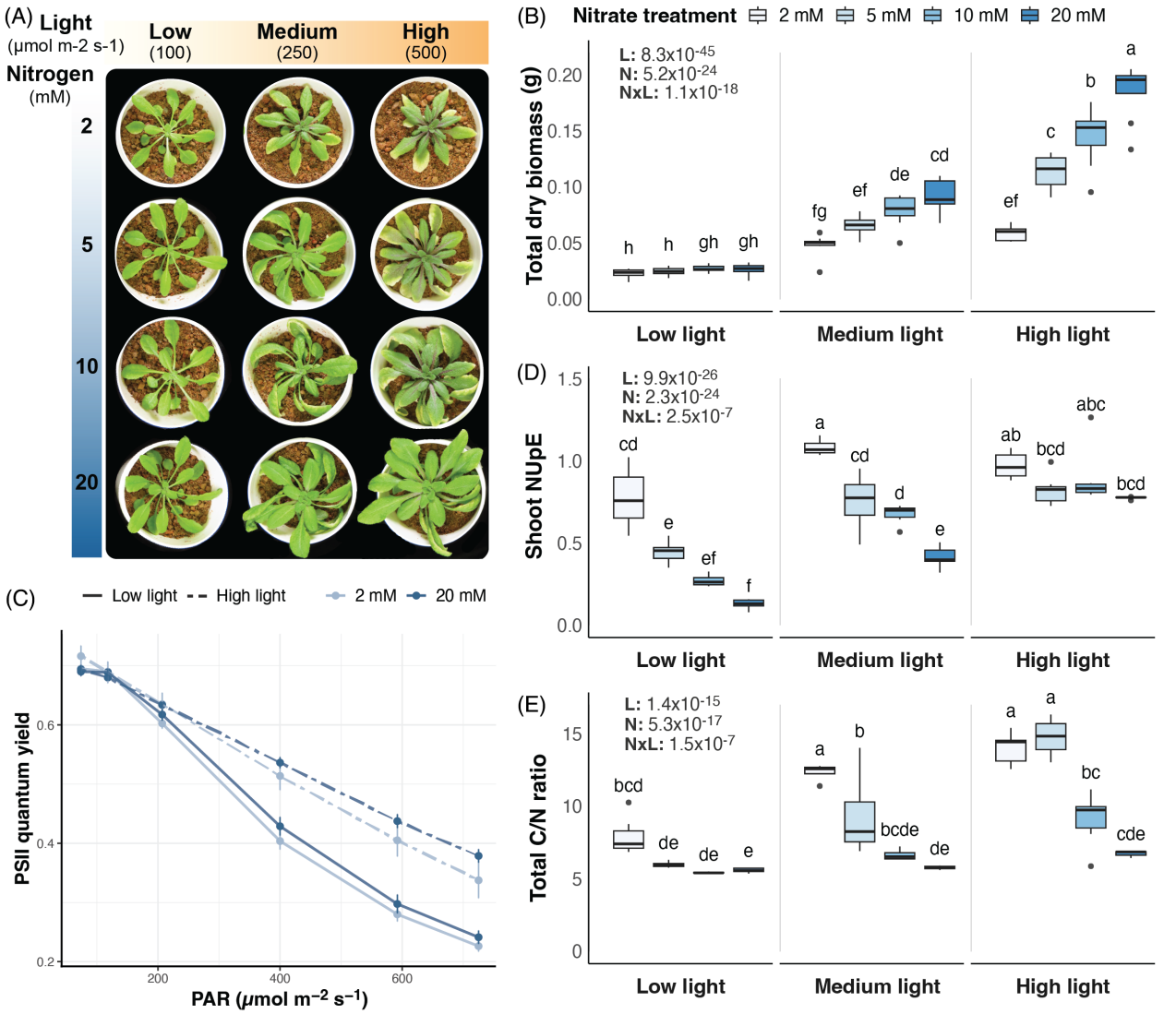

Figure 1. Interactive effects drive physiological responses to nitrogen and light treatments.
(A) Representative images of plants grown at each nitrogen and light condition.
(B) Total plant biomass.
(C) Photosystem II quantum yield (Φ_{PSII}) measured at different light intensities (photosynthetically active radiation [PAR]) of plants grown at the lowest and highest nitrogen doses under low and high light.
(D) Nitrogen uptake efficiency in the shoot [NUpE = shoot nitrogen (g)/nitrogen treatment (g)].
(E) C/N ratio. For (B, D, E), statistically significant differences indicated in lowercase letters were determined by two-way ANOVA followed by Tukey's test.

intensity, indicating less efficient use of absorbed light than high-light grown plants, regardless of the nitrogen treatment (Figure 1C; Figure S1). Among the high-light grown plants, Φ_{PSII} decreased faster in plants treated with 2 mM nitrate compared to 20 mM nitrate, indicating that plant nitrogen supply affects the quantum yield of photosynthesis, as previously described (Lawlor et al., 1987). The decrease in Φ_{PSII} is accompanied by an increase in NPQ, as the absorbed light that cannot be utilized for photochemistry is dissipated to protect the photosynthetic apparatus (Maxwell & Johnson, 2000). This correlation is observed in the NPQ curves, mirroring the change across treatments observed in Φ_{PSII} (Figure S1). These results indicate that plants grown at high light and high nitrogen conditions utilize light most efficiently for photochemistry.

Typically, plants grown under low nitrogen conditions exhibit enhanced nitrogen uptake, transport, and assimilation efficiency, while those grown under high nitrogen conditions show reduced uptake efficiency (Glass, 2002; Wu, Luo, et al., 2019). Thus, nitrogen uptake efficiency (NUpE) is generally expected to decline with increasing nitrogen availability. While this trend is observed across low-light

and medium-light treatments, plants grown at high light did not show a decrease in efficiency as the nitrogen dose increased (Figure 1D). Similar trends are seen when looking at total NUpE, root NUpE, and nitrogen utilization efficiency (NUtE) or nitrogen use efficiency (NUE), which were each highly correlated between the shoot and root (Figure S2). The higher shoot NUpE at high nitrogen and high light is possibly due to an increase in the availability of reductant/energy from photosynthesis. The balance between nitrogen and carbon assimilation ultimately determines the C/N ratio in the plant (Lawlor, 2002). C/N ratios at lower nitrogen doses increased as the light level increased (Figure 1E), whereas across all light levels, the C/N ratio declined as the nitrogen dose increased.

Nitrogen and light have an interactive effect on the expression of a majority of genes

For each of the 12 N-by-L treatment conditions, we measured gene expression in leaf tissue using RNA-seq. To explore how gene expression can be influenced by nitrogen and light, we analyzed gene expression using a multivariate linear model ($\sim L + N + N \times L + N/L$) followed by model reduction as in Swift et al. (2019) and described in the "Methods" section. The four variables capture nitrogen-dependent (expression changes with nitrogen dose), light-dependent (expression changes with light intensity), N × L synergy (expression changes with the product of nitrogen and light), and nitrogen/light (N/L) ratio (expression changes relative to N/L ratio) (Figure S3). We identified 7762 differentially expressed genes (DEGs) (FDR adjusted $P < 1 \times 10^{-6}$), including 567 TFs (Table S2). Most DEGs belonged to composite models, i.e., different combinations of the four variables (Figure 2A), suggesting that the expression of genes is dynamic in response to N and L signals.

Gene ontology (GO) analysis of DEGs revealed the enrichment of multiple processes involved in photosynthesis, including reductive pentose-phosphate cycle, chloroplast organization, light-harvesting, chlorophyll biosynthesis, and protein import to the chloroplast stroma (Figure 2B; Table S3). Other significantly enriched GO terms were related to translation and transcription. This emphasizes the importance of nitrogen and light responses in coordinating photosynthesis and the machinery of cell growth and homeostasis. The absence of stress-related GO terms demonstrates that our experimental conditions effectively isolated nitrogen and light interactions, ensuring the observed gene expression changes were not confounded by unintended stress responses.

We compared these DEGs to a previous study that looked at nitrogen-by-carbon interactions on gene expression in hydroponically grown Arabidopsis using various sucrose and nitrate treatments (Gutiérrez et al., 2007). This comparison revealed that our DEGs captured 40% (2113/5322) of genes that responded to nitrogen and carbon treatments in that study (Figure 2C). Over 88% of these shared DEGs belong to a model with a significant N-by-L interaction and include 289 genes only responsive to nitrogen and 816 genes only responsive to carbon in the previous study. Focusing on genes within the photosynthetic and nitrogen assimilation pathways, we found that 58% (220/377) of genes involved in various photosynthesis processes and 52% (35/67) of genes in the nitrogen assimilation pathway were among the DEGs. A subset of these genes, visualized in Figure 3 (expression of all genes in Figures S4 and S5), illustrates their varied expression patterns in response to nitrogen and light availability. For the nitrogen assimilation pathway, genes involved in nitrate transport (e.g. NRT1.1) and reduction (e.g. NIR1) show a predominantly light-dependent expression pattern, where increased light availability induces their expression (Glass, 2002). The expression of genes involved in the latter steps of ammonium assimilation (GLN2, ASN2) and amino acid synthesis is dependent on both nitrogen and light availability, as observed by Zhu et al. (2023). Within the photosynthetic pathway, genes involved in light reactions showed dynamic regulation. Photosystem electron transport components (SPS2 and FD1) were upregulated in nitrogen-sufficient conditions with increasing light availability, potentially enhancing light harvesting and photoprotection. Carbon fixation genes (RBCS1A, SBPase, PGK1) expression patterns suggest coordination between nitrogen and carbon assimilation, with downregulation under low nitrogen and high light - likely due to nitrogen limitation constraining carbon assimilation at high light intensities. Chlorophyll biosynthesis genes (PORC, CH1, G4) showed differential expression of chlorophyll *a* and *b* synthesis similar to carbon fixation genes under varying nitrogen and light conditions, indicating the tight link between them.

DEGs correlated to photosynthetic traits suggest coordinated regulation of photosynthetic pathways, nitrogen uptake, and C/N balance

Pearson correlation was used to identify sets of genes, which we refer to as modules, that have expression patterns that track changes in the values of a trait across all the conditions (Table S4). For photosynthetic traits, only the measurements acquired at the lowest ($75\,\mu mol\,m^{-2}\,sec^{-1}$) and highest ($725\,\mu mol\,m^{-2}\,sec^{-1}$) measuring light intensities were used. This was done because the photosynthetic trait values measured at intermediate light intensities were highly correlated with each other (Figure S6) and therefore the modules for these traits had a large overlap. DEGs with a gene-trait coefficient of determination (r^2) > 0.36 (adjusted *P*-value less than 1×10^{-10}) were considered significantly associated with that trait (Figure 4A).

Each module of DEGs correlated to the different traits was enriched for genes with an N-by-L composite model

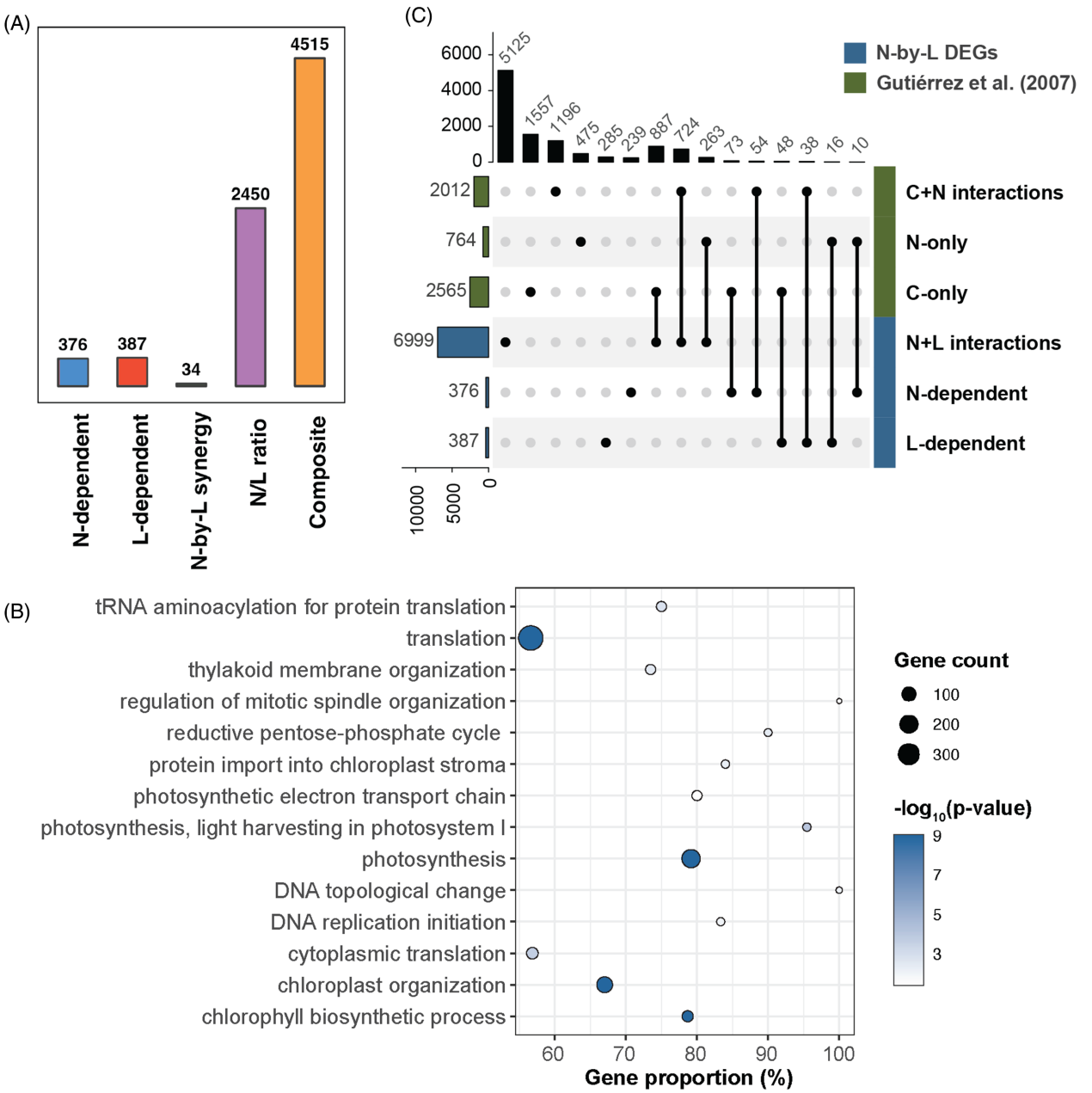

Figure 2. Differentially expressed genes (DEGs) are enriched for photosynthetic genes, and a majority of DEGs are influenced by the combined effect of nitrogen and light treatments.
(A) Distribution of DEGs across models of expression.
(B) Gene ontology enrichment of DEGs (adjusted $P < 0.05$).
(C) Overlap of DEGs with nitrogen and carbon-responsive genes identified by Gutiérrez et al. (2007).

of expression. Genes associated with NUpE, C/N ratio, NPQ, and Φ_{PSII} were enriched for the GO Biological processes response to light, photosynthetic light harvesting, and chlorophyll biosynthesis (Figure 4B; Table S3). This suggests that photosynthesis, nitrogen uptake, and C/N balance are coordinated, as previously observed (Brooks & Szeto, 2024; Halpern et al., 2022; Stitt & Krapp, 1999). NUpE-associated genes were uniquely enriched in response to nitrate and inorganic anion transmembrane transport.

Motif enrichment and binding data implicate bZIP11 and MYB-related TFs as regulators of C/N ratio and photosynthesis

We looked at motif enrichment in the 1 kb promoter regions of genes within each module. We observed 20

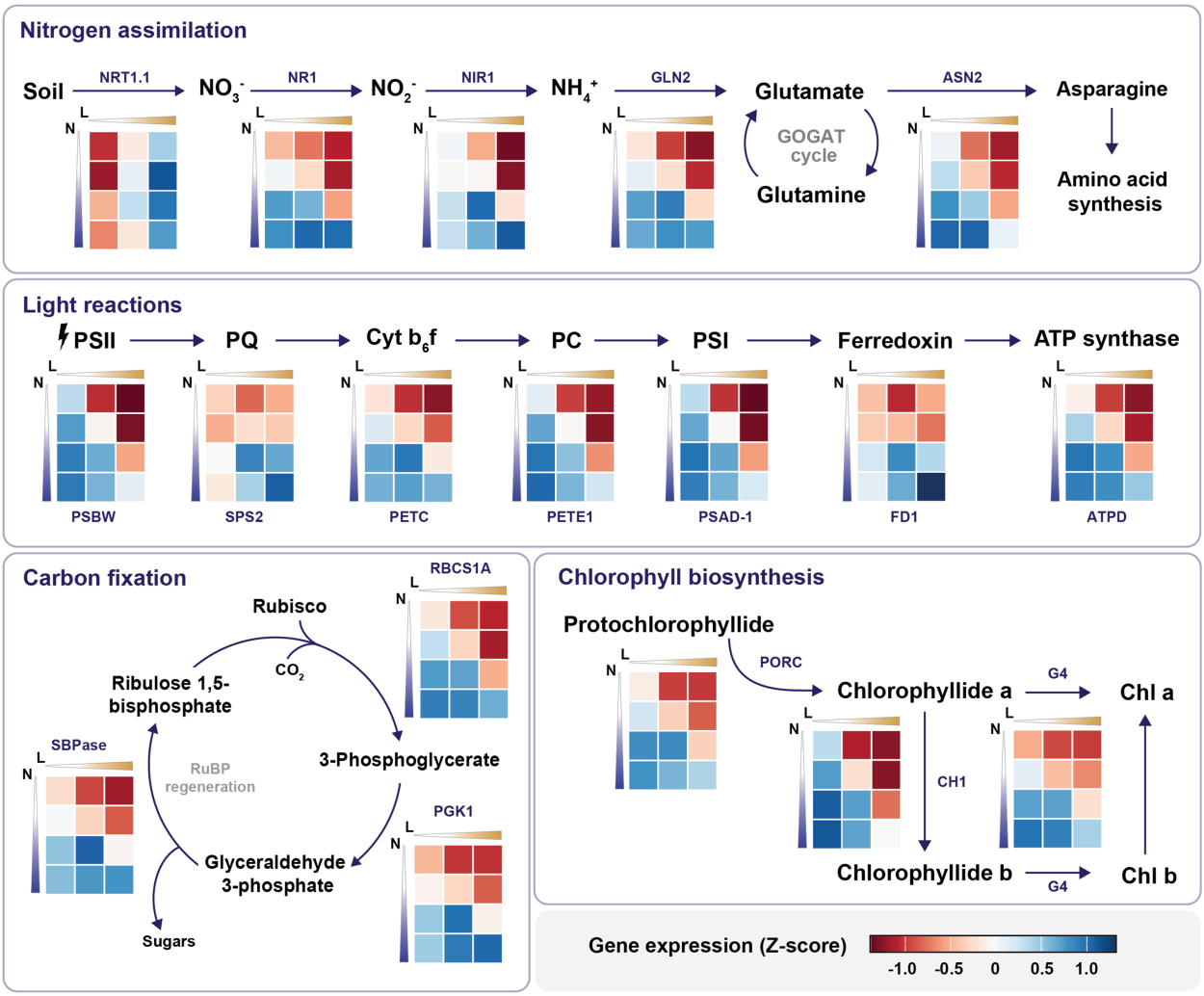

Figure 3. The expression of genes in the nitrogen assimilation and photosynthesis pathways highlights the synergistic effect of nitrogen and light interactions. Gene expression was *Z*-score normalized across all samples, as indicated by colors ranging from red (low expression) to blue (high expression). Gene names are as follows. Nitrogen assimilation: NRT1.1, nitrate transporter 1; NR1, nitrate reductase; NIR1, nitrite reductase 1; GLN2, glutamine synthetase 2; ASN2, asparagine synthetase 2. Light reactions: PSBW, photosystem II reaction center W; SPS2, solanesyl diphosphate synthase 2; PETC, photosynthetic electron transfer C; PETE1, plastocyanin 1; PSAD-1, photosystem 1 subunit D-1; FD1, ferredoxin 1; ATPD, ATP synthase δ-subunit. Carbon fixation: RBCS1A, ribulose bisphosphate carboxylase small chain 1A; PGK1, phosphoglycerate kinase 1; SBPase, sedoheptulose-bisphosphatase. Chlorophyll biosynthesis: PORC, protochlorophyllide oxidoreductase C, CH1, chlorophyll A oxygenase, G4, chlorophyll synthase.

different significantly enriched TF *cis*-binding motifs for genes associated with traits C/N ratio, NPQ, Φ_{PSII}, and NUpE (Table S5). Among these enriched motifs, 8 were from bZIP family TFs, 10 were MYB-related family TFs, and 2 were from the GeBP family. Of these TFs with enriched *cis*-binding motifs, four MYB-related TFs (MYBS1, MYBH, AT5G56840, and AT1G19000) and three bZIP TFs (HY5, GBF3, bZIP68, bZIP44, and bZIP11) were DEGs (Figure 5A). Another MYB-related TF, AT1G74840, has a significantly enriched motif and is a paralog of AT1G19000, sharing 60% protein sequence identity (Emms & Kelly, 2022). Additionally, bZIP11 binding motifs were found in the promoter regions of over 30% of genes enriched for photosynthesis and response to light stimulus in the C/N and $\Phi PSII_{75}$ subnetworks (Figure 5B).

In vitro TF-binding data from DNA affinity purification sequencing (DAP-seq) (O'Malley et al., 2016) were also used to identify potential regulators of genes within trait modules. To do this, we tested the overlap between module genes and TF targets based on DAP-seq binding data. Out of the 555 TFs present within our N-by-L DEGs, binding data were available for 109 TFs. Among these, 51 TFs were significantly enriched in one or more modules ($Q < 0.05$) (Figure 5C). TFs from the bZIP and MYB/MYB-related families were overrepresented, with many consistently associated with multiple traits.

Gene network analysis identifies bZIP family TFs as master regulators of trait subnetworks

Gene regulatory networks (GRNs) were built from the expression profiles of the DEGs to further identify potential regulators, including those that lack a known motif or DAP-seq binding data. We used four inference approaches: ARACNE (Margolin et al., 2006), CLR (Faith et al., 2007), GENIE3 (Huynh-Thu et al., 2010), and OutPredict (Cirrone et al., 2020) to infer networks (Figure S7). A consensus network was constructed by retaining edges present in at least three of these networks. This network was pruned for high-confidence TF-target gene interactions based on precision-recall analysis using ConnecTF (Brooks et al., 2021). The validated data used for pruning was cell-based TF regulation data for 26 TFs (Alvarez et al., 2020; Brooks et al., 2019, 2021; Varala et al., 2018). The resulting N-by-L GRN comprised 20987 TF–target gene interactions between 6018 genes (5493 target genes and 525 TFs) (Figure S7; Table S6). We used the gene-trait correlation modules (Table S4) to create trait-specific subnetworks, where each subnetwork included genes associated with a given trait and their predicted TF regulators (Figure 6A; Table S6). The C/N, $\Phi PSII_{75}$, and NPQ_{75} subnetworks were the largest with 5554, 4582, and 4089 TF-target gene interactions, respectively.

For each subnetwork, the most influential TF regulators (Figure 6B) were identified based on (i) the connectedness (outdegree) of the TF, (ii) the correlation of the TF-target genes to a particular trait, and (iii) the enrichment of the TF-target genes within the subnetwork relative to the full network (Table S7). Among the 268 TFs present within the trait subnetworks, 40 TFs were recognized as the most influential (Figure 6B; Table S8), including seven TFs with validated targets that were enriched for the trait modules based on DAP-seq binding data (Figure 5C). TFs previously found to be involved in signaling pathways related to carbon/energy [bZIP11 (Ma et al., 2011), nitrogen (LBD38/39; Rubin et al., 2009) and NLP8 (Yan et al., 2016)], carbon-light signaling in hypocotyl growth [BEE2 (Singh et al., 2017)], carbon-nitrogen partitioning [NF-YC4 (Li et al., 2015)], and root nitrogen-carbon/energy interactions [TGA3 (Ruffel et al., 2021)] were consistently among the top regulators in each subnetwork. Almost all of the TFs (39 out of 40) found to be most influential for the trait modules exhibit a composite N-by-L model of expression. Notably, two of the TFs with enriched *cis*-binding motifs, bZIP11 and the MYB-related TF AT1G19000, emerged as important regulators of C/N, $\Phi PSII_{75}$ and NPQ_{75} subnetworks, and NPQ_{75} subnetwork, respectively. bZIP family TFs were also recognized as regulators of photosynthesis-related genes within the subnetworks (Table S8).

The predicted TF-target gene interactions were compared to *in vitro* TF-binding data (O'Malley et al., 2016). DAP-seq data were available for 51/268 of all TFs associated with trait subnetworks and 7/40 TFs identified as key regulators among them (Table S7). The experimental data significantly supported our predicted TF-target interactions for the key regulators AT1G19000 and bZIP11 (Fisher's exact test, $P < 0.05$).

To understand how these TFs regulate the expression of their target genes, we examined the expression patterns of TFs and their targets in trait subnetworks. The expression profiles of AT1G19000 and its targets in the NPQ_{75} module were largely similar, with gene expression of both TF and target genes being lower at higher light levels and generally increasing with nitrogen dose (Figure 6C). This suggests that AT1G19000 may function as an inducer of its target genes. In contrast, for bZIP11, the expression profiles of its targets in the $\Phi PSII_{75}$ module exhibited two distinct patterns when clustered based on changes in expression across treatments, with cluster I showing a negative correlation to bZIP11 expression and cluster II showing a positive correlation (Figure 6D). These results suggest that bZIP11 may act as both a transcriptional activator and repressor. For example, bZIP11 appears to repress genes involved in photosynthetic light harvesting while inducing genes related to polysaccharide catabolism and starvation response (i.e., at high light, the decreased expression of bZIP11 corresponds with an increased expression of photosynthetic genes). A similar potential dual regulatory role was observed for NLP8 and TGA3 (Figure S8). Notably, the expression of these TFs is influenced not only by light levels but also by nitrogen availability and their synergistic interaction (Table S2).

DISCUSSION

The close relationship between nitrogen assimilation, light availability, and photosynthetic activity has been recognized and studied at the physiological level for more than 50 years (Evans, 1989; Fahl et al., 1994; Fu et al., 2017; Makino et al., 1997; Terashima & Evans, 1988). As in other species, we found that nitrogen and light treatments had a strong combined effect on the physiological traits in Arabidopsis, including biomass, nitrogen uptake, and C/N ratio (Figure 1). Despite this known synergy, nitrogen and light are still primarily studied independently at a molecular level, particularly with regard to the signaling networks that control gene expression. This study addressed how photosynthesis and nitrogen assimilation are regulated in response to nitrogen and light availability. Notably, there was a significant interaction between nitrogen and light on the expression of 90% of DEGs (Figure 2A), which mirrored the interactive effect of nitrogen and light on physiological traits (Figure 1). Among the DEGs, there was an enrichment of genes associated with the GO biological processes "Photosynthesis" and "Translation," as seen previously (Palenchar et al., 2004; Scheible et al., 2004), emphasizing

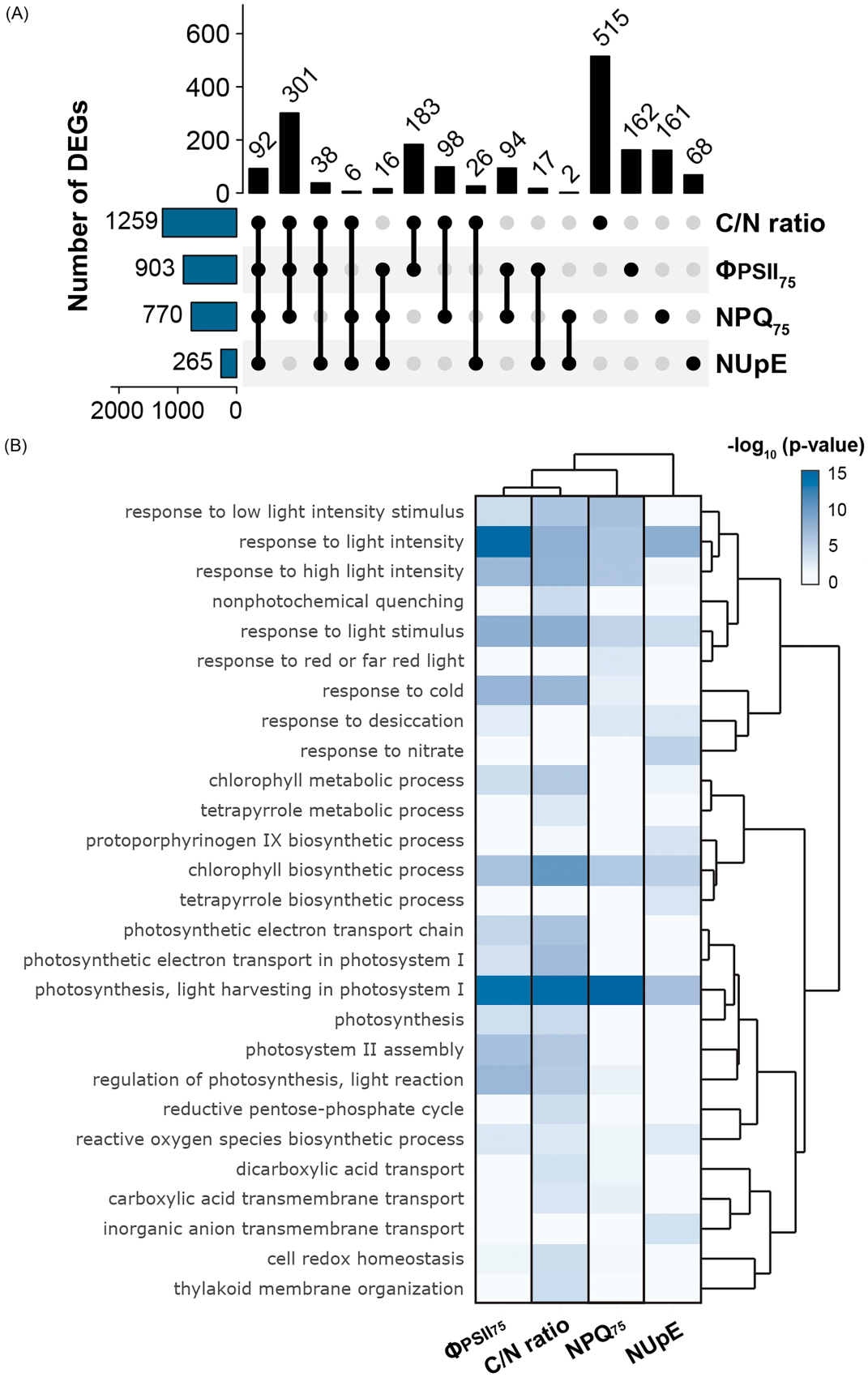

Figure 4. Gene–trait correlation reveals that the genes in the trait modules are enriched for photosynthesis and related processes.
(A) UpSet plot showing the overlap of genes significantly correlated with traits.
(B) Gene ontology enrichment for genes correlated to traits Φ_{PSII}, NPQ, NUpE, and C/N ratio ($P < 1 \times 10^{-3}$).

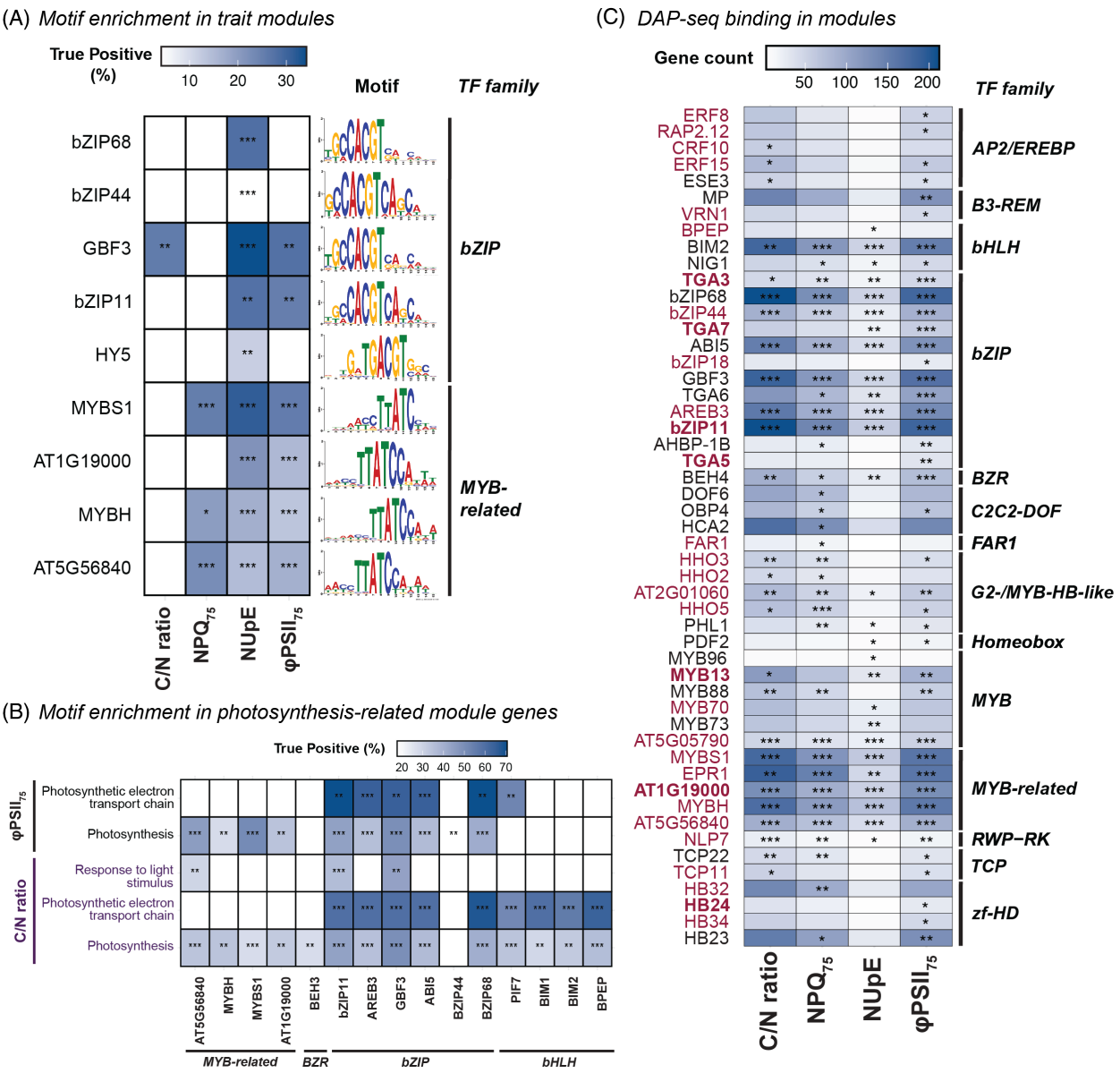

Figure 5. Motif enrichment and *in vitro* binding data implicate bZIP and MYB TFs as regulators of trait modules.
(A) Motif enrichment for the set of genes within each trait module.
(B) Motif enrichment of the genes associated with specific GO terms within the modules.
(C) Potential regulators of trait modules based on DAP-seq binding data. TFs in red were also identified within trait subnetworks through GRN analysis. TFs in bold represent key regulators of traits. (*Q*-values: *<0.05, **<0.01, ***<0.001).

the importance these signals have on plant energetics and growth. The combined effects of nitrogen and light on gene expression are exemplified in the control of genes in the nitrogen assimilation and photosynthetic pathways (Figure 3; Figures S3 and S4), where the light intensity can have a large impact on nitrogen response, and likewise, nitrogen can dramatically impact light responses. Specifically, our results indicate that bZIPs, in particular bZIP11, and TFs from the MYB-related family are important regulators of these interactions.

(A)

Network	Interactions	TFs	Genes
N-by-L GRN	20,987	525	6,018
$\Phi PSII_{75}$	4,582	260	789
NPQ_{75}	4,089	268	667
C/N ratio	5,554	292	1,115
NUpE	1,464	149	245

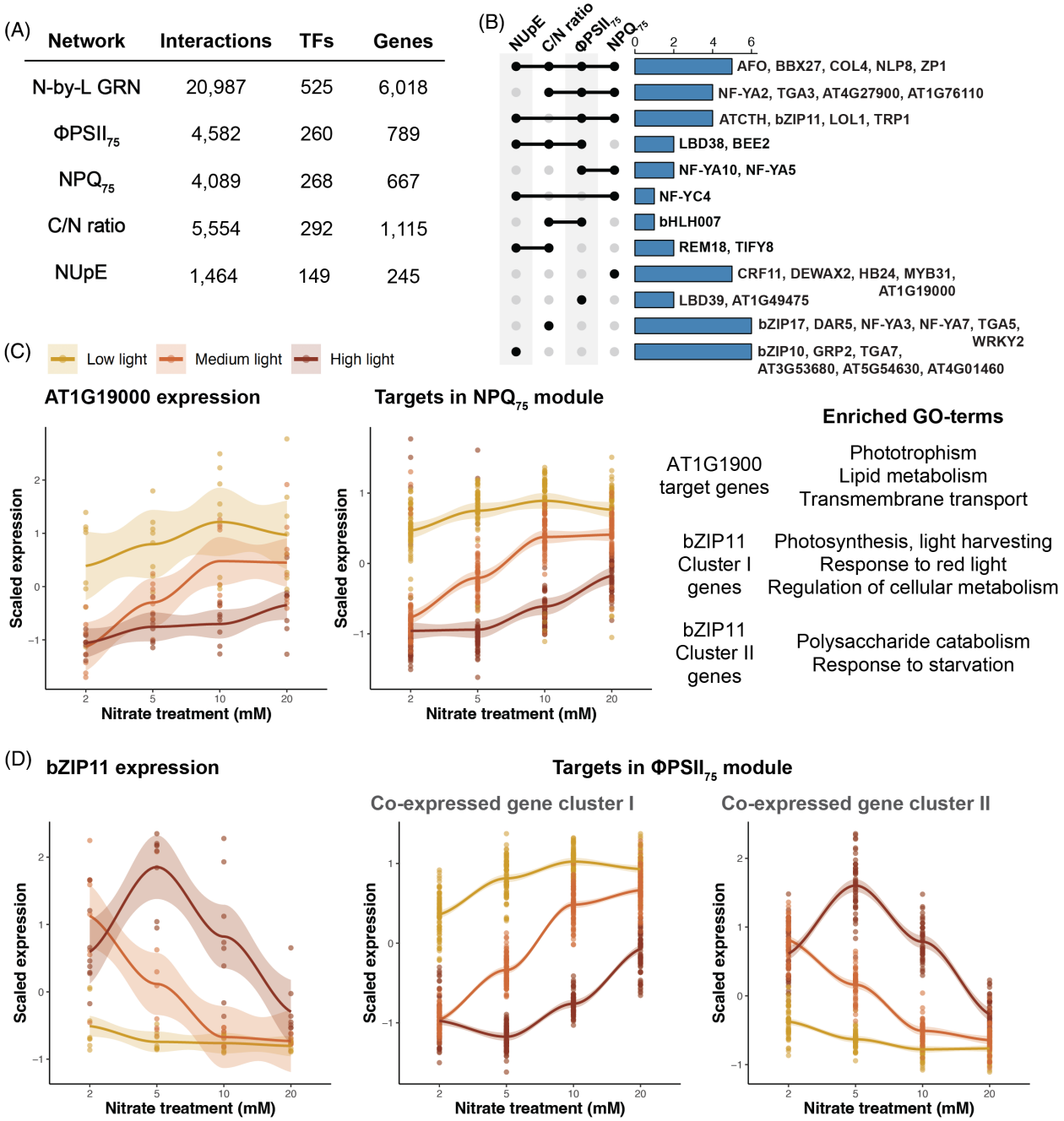

Figure 6. Inferred nitrogen-by-light gene regulatory network reinforces the role of bZIP and MYB TFs and identifies other candidate regulators lacking a *cis*-binding motif or validated data.
(A) Overview of the nitrogen-by-light GRN and the trait subnetworks.
(B) TFs identified as key regulators of trait subnetworks based on outdegree, correlation of target genes to the trait, and enrichment of target genes for that trait (Table S8).
(C) Expression levels of AT1G19000 and its predicted targets in the NPQ_{75} module.
(D) Expression levels of bZIP11 and its predicted targets in the $\Phi PSII_{75}$ module. For bZIP11 targets, hierarchical clustering of expression profiles identified two distinct co-expressed gene clusters. Potential functions of targets were inferred through GO biological processes analysis.

Several studies have investigated GRNs controlling plant responses to nitrogen (reviewed in Shanks et al., 2024; Vidal et al., 2020). A majority of these studies have focused on juvenile plants and rapid responses within a few hours of nitrogen treatment (Brooks et al., 2019; Gutiérrez et al., 2008; Krouk et al., 2010; Pathak

et al., 2020; Varala et al., 2018). In contrast, we compared plants exposed to different nitrogen and light treatments over a much longer period (40 days). Despite the difference in experimental design, several regulators previously identified as influential in nitrogen signaling also respond to our treatments and are hubs in our GRN. This includes LBD38 (Rubin et al., 2009), NLP8 (Yan et al., 2016), TGA3 (Ruffel et al., 2021), BT2 (Araus et al., 2016; Sato et al., 2017), and AT1G74840 (Gutiérrez et al., 2008). Another group of TFs are hubs in our subnetworks and have been previously shown to respond to nitrogen treatment but lack a defined role in nitrogen response, such as NF-YA7 (Bi et al., 2007) and bZIP11 (Canales et al., 2014). Finally, we also found a set of hub TFs that, to our knowledge, have not previously been implicated in nitrogen signaling and may be specifically important for long-term acclimation to nitrogen dose rather than rapid nitrogen signaling.

It is notable that previous studies on nitrogen signaling networks in Arabidopsis have been performed at light levels of 100–250 $\mu mol\, m^{-2}\, sec^{-1}$, the range typically recommended for growing Arabidopsis (Rivero et al., 2014). In this study, 4468/7375 (57%) of DEGs that respond to nitrogen would have been missed if the high light treatment had been excluded, e.g., there was no significant nitrogen response between low and medium light (Table S2). This set of genes includes genes involved in translation, plastid organization, and organic nitrogen compound metabolic processes, as well as 103/383 photosynthesis-related genes. Within the recommended range of growth light intensities, between low ($100\, \mu mol\, m^{-2}\, sec^{-1}$) and medium light ($250\, \mu mol\, m^{-2}\, sec^{-1}$), differences are also seen in nitrogen responses (Figure 1B). These results emphasize the importance of carbon and energy status on nitrogen responses and nitrogen signaling. How would our current understanding of nitrogen signaling networks differ had prior experiments been done at multiple light levels or if there was a better representation of higher light treatments? Nitrogen signaling networks are also known to be influenced by other nutrients (Krouk, 2017; Krouk & Kiba, 2020; Tsay et al., 2011), abiotic/biotic stresses (Fagard et al., 2014; Swift et al., 2019), and hormones (Vega et al., 2019). While more affordable DNA sequencing now makes it possible to explore more combinations of treatments, exploring all possible permutations is impossible, and it will be important to determine if there are general principles that can be learned and applied to build GRNs that transcend specific conditions tested.

Two recent studies have built GRNs related to photosynthesis and chloroplast development by leveraging published datasets (Frangedakis et al., 2023; Halpape et al., 2023). Several known chloroplast developmental TFs emerged as hubs in these networks, including GLK1/2, CGA1, and GNC. Both studies also identified MYBS1 as an important regulator of photosynthetic gene expression. Each of these TFs, except GLK1 and GNC, is a DEG in our N-by-L response network (Table S2), but they are not hubs in our photosynthesis-related or other trait subnetworks (Table S7). This potentially highlights the difference between TFs involved in chloroplast biogenesis and photomorphogenesis versus those TFs involved in the integration of light and nitrogen signals in fully developed leaves.

Only a few studies have looked specifically at nitrogen and light or nitrogen and carbon interaction regulatory networks in model or crop plants (Gutiérrez et al., 2008; Pathak et al., 2020; Ruffel et al., 2021). Typically, in these studies, the carbon and light treatments were extreme and not conditions that plants are likely to experience in nature. This includes growing plants in high concentrations of sugars or exposing them to continuous light/dark. For example, Pathak et al. (2020) explored how light modulates the nitrogen response by transcriptome profiling of nitrogen-treated light-grown and etiolated rice seedlings. The etiolated samples exhibited an enrichment of stress-related genes, suggesting that excluding light from the experimental design likely introduced confounding factors (Yoneyama & Suzuki, 2020). Our approach in this study enabled us to identify integrators of nitrogen and light signaling under conditions a plant is more likely to experience in nature and thus identify TFs specifically related to integrating nitrogen and light signals, rather than stress responses.

Another recent study investigated TFs regulating root nitrate transporters in response to nitrogen and light/photosynthesis (Ruffel et al., 2021). In that work, microarrays were used to look at gene expression in root samples from nitrogen treatments performed in combination with different light or CO_2 treatments. The goal was to delineate the effect of light and photosynthesis in the shoot on nitrogen uptake in the roots. Three TFs were found to be important in this signaling pathway in the root, with TGA3 and MYC1 responding to nitrogen deprivation in a light-dependent manner and bHLH093 responding to light independent of the nitrogen treatment. In our study, TGA3 and bHLH093 were both nitrogen- and light-responsive in shoots, while MYC1 was not a DEG. Furthermore, TGA3 was identified as a TF that was among the top hubs in our subnetworks, underscoring its role in integrating nitrogen and light signals in both the root and shoot of Arabidopsis. Among the other top TFs was bZIP11, a TF known to respond to sugars (Ma et al., 2011) and implicated in nitrogen responses via control of amino acid metabolism (Hanson et al., 2008). Another top hub was LBD38, one of the most consistently nitrogen-responsive genes (Canales et al., 2014).

Studies have demonstrated that coordinated control of nitrogen and photosynthesis is a promising approach for optimizing crop yield (Wei et al., 2022; Wu et al., 2019). However, a major question with any Arabidopsis study is

how the results might translate to crop improvement. Cheng et al. (2021) showed that genes that are evolutionarily conserved for nitrogen response between Arabidopsis and maize are more predictive of trait outcomes in the field. It is therefore notable that Pathak et al. (2020) also specifically identified bZIPs and MYBs, including AT5G56840 (Figure 5), as being important for nitrogen by light interactions in rice, despite significant differences in experimental design. Additionally, 16 of the top TF regulators we identified, including LBD37/38 and NLP5/8, have been shown to be conserved in Arabidopsis and maize nitrogen response (Cheng et al., 2021). Other TFs we identified have been implicated in more focused studies, such as NF-YC4, a conserved transcriptional regulator that influences carbon and nitrogen partitioning, leaf starch, and seed protein composition (Li et al., 2015). The conservation of NF-YC4 across eukaryotes has enabled promoter optimization to enhance seed protein content across species, including in Arabidopsis, rice, and soybean (Li et al., 2015; Wang et al., 2025). Thus, our approach provides a framework for identifying regulators of light/carbon/energy and nitrogen signaling, which has the potential to identify targets for crop improvement.

METHODS

Plant materials, growth conditions, and phenotypic measurements

Surface-sterilized *Arabidopsis thaliana* (Col-0) seeds were vernalized for 72 h at 4°C and plated on 0.5 × Murashige and Skoog (MS) media (Research Products International, Mt. Prospect, IL, USA) with 1% sucrose. The seedlings were transferred to 3′ pots containing a 1:1 mixture of coarse calcined clay (Turface MVP, PROFILE, Buffalo Grove, IL, USA) and fine clay (Greens Grade, PROFILE, Buffalo Grove, IL, USA), 10 days after germination. After transplanting, the plants were treated with 30 ml of 0.5 × modified nitrogen-free Hoagland solution with nitrate concentrations of 2, 5, 10, and 20 mM (using KNO_3). The full-strength Hoagland solution composition was (mg L^{-1}): $CaCl_2$ (554.9), KCl (372.7), $MgSO_4$ (240.325), KH_2PO_4 (136.025), Fe-EDTA (33) with 1 ml L^{-1} of micronutrients (H_3BO_3 2.86], $CuCl_2$ [0.045], $MnCl_2$ [1.81], $Na_2MoO_4{\cdot}2H_2O$ [0.025], and $ZnCl_2$ [0.11]). The plants were grown under three light levels with photon flux densities of 100, 250, and 500 μmol m^{-2} sec^{-1} under an 8/16 h day/night cycle and a 23/21°C day/night temperature regime. The experiment consisted of 12 treatments with eight replicates for each treatment. To maintain uniform soil moisture content throughout the experiment, the plants were watered every 2 days until each pot reached its initial weight. Two leaf punches were taken from each plant and immediately frozen in liquid nitrogen 30 days after transplanting for RNA extraction. Chlorophyll fluorescence was measured using a pulse-amplitude modulated (PAM) fluorometry imaging system (FluorCam, Photon Systems Instruments, Drasov, Czech Republic) the day before harvesting. Chlorophyll fluorescence was determined by exposing plants that were dark-adapted for 30 min to actinic light pulses of increasing intensities (75, 120, 200, 400, 600, and 725 μmol m^{-2} sec^{-1}), a light response curve as described previously (Brooks & Niyogi, 2011).

Biomass and elemental analysis

Plant shoot and root were harvested separately and dried at 65°C for 48 h for biomass quantification. The dried tissue was ground to a fine powder, and 0.9–2 mg of each sample was analyzed for carbon and nitrogen content using an elemental analyzer (Costech 4010 CHNSO Analyzer; Costech Analytical Technologies, Inc., Valencia, CA, USA). The following equations were used to calculate nitrogen use based on Menz et al. (2018);

$$\mathrm{NUE} = (\text{Dry shoot biomass (g)})/(\text{Nitrogen treatment (g)})$$

$$\mathrm{NUtE} = (\text{Dry shoot biomass (g)})/(\text{Shoot nitrogen (g)})$$

$$\mathrm{NUpE} = (\text{Shoot nitrogen (g)})/(\text{Nitrogen treatment(g)})$$

$$\mathrm{C/N\ ratio} = (\text{Shoot carbon (g)} + \text{Root carbon (g)})/(\text{Shoot nitrogen (g)} + \text{Root nitrogen (g)})$$

RNA extraction, library preparation, and sequencing

Frozen leaf samples were powdered in 2 ml tubes with 8–12 beads of Matrix D (MP Biomedicals, Irvine, CA, USA) with the FastPrep-24 (MP Biomedicals, Irvine, CA, USA) cryo rotor using the following program (2 × 5.5 m sec^{-2} × 60 sec). RNA extraction, construction of the RNAseq libraries, and sequencing were performed at the Roy J. Carver Biotechnology Center at the University of Illinois at Urbana-Champaign. RNA from ground plant tissues was extracted with the MagMAX Plant RNA Isolation kit (ThermoFisher, Hanover Park, IL, USA) following the manufacturer protocol, which includes treatment with DNAse. Individual libraries were generated using the Kapa HyperPrep mRNA kit (Roche, Basel, Switzerland) and were barcoded with Unique Dual Indexes (UDI's). The adaptor-ligated double-stranded cDNAs were amplified with the Kapa HiFi polymerase (Roche, Basel, Switzerland). The barcoded RNAseq libraries were sequenced on one S4 lane on NovaSeq 6000 for single-end 100 bp reads. The fast files were generated and demultiplexed with the bcl2fastq v2.20 conversion software (Illumina, San Diego, CA, USA).

Transcriptome analysis and identification of DEGs

Reads were filtered and trimmed using BBDuk (BBtools; Joint Genome Institute), and reads were aligned using the STAR aligner (v2.5.3a) (Dobin et al., 2013) to the Arabidopsis TAIR10/Araport11 (Cheng et al., 2017) genome. Gene-level read counts were quantified using featureCounts (Liao et al., 2014). Chloroplast and mitochondrial reads were removed. DESeq2 (v1.40.1) (Love et al., 2014) was used to identify DEGs (FDR-adjusted P-value < 0.05) with the independent variables as nitrogen-dependent (N), light-dependent (L), N × L synergy, and N/L ratio. A model reduction approach was used to fit the expression profiles of genes to the simplest multivariate linear model (Swift et al., 2019). The full model included all four variables (N + L + N × L + N/L). For each gene, the likelihood ratio test (LRT) was used to compare the fit of the full model to all of the possible three-factor models. If the model fit was significantly different (P-adjusted < 1×10^{-6}) in all cases, this gene was deemed fit by the full model and removed from remaining steps. For the remaining genes, the fit for each of the possible three-factor models was compared to the corresponding two-factor models in which a single variable was removed. The gene was deemed fit by a three-factor model if the adjusted p-value was less than the cutoff in all three comparisons. The process was repeated to determine genes that were fit by two-factor- and one-factor models. Genes with a best-fit model that included more than one variable were categorized as "composite" models.

Gene–trait correlation

Modules of genes important to the measured traits were identified using Pearson correlation. The significant gene–trait connections filtered at $r^2 \geq 0.36$ and adjusted P-value $< 1 \times 10^{-10}$ for correlation were used for analysis.

GRN construction and analysis

Four GRN inference approaches ARACNE (Margolin et al., 2006), CLR (Faith et al., 2007), GENIE3 (Huynh-Thu et al., 2010), and OutPredict (Cirrone et al., 2020) were used to determine the TF-target interactions among the DEGs using the normalized expression values. The resulting GRNs were merged to build a consensus network, retaining only the edges present in at least three of the four individual networks. Precision-recall analysis (Brooks et al., 2019, 2021) was done on the ConnecTF platform (Brooks et al., 2021) to generate a high-confidence consensus GRN. The validated data for pruning was from cell-based TF regulation data for 26 TFs (Alvarez et al., 2020; Brooks et al., 2019, 2021; Varala et al., 2018), and a precision cut-off of 0.14 was used. Subnetworks were created for each trait by extracting the trait-associated DEGs (based on gene-trait correlation) and their regulators from the pruned network. The importance of TFs within each subnetwork was assessed by using three parameters: (i) degree of connectivity (outdegree), (ii) statistical significance of the overlap between GRN-predicted targets and trait-associated genes using a hypergeometric probability function followed by a false discovery rate (FDR) correction, and (iii) averaged gene-trait correlation coefficient for each TF's targets using a modified Bayesian average. TFs were identified as important regulators if they ranked within the top 20 for any of these three metrics.

$$\text{Outdegree} = \text{No. of edges directed from the TF}$$

$$P(x) = (m_x)\,(n_{k-x})/(m + n_k)$$

$$\text{Bayesian average} = \frac{(\text{mean}(r) \times X) + (r_{\min} \times w)}{X + w}$$

where r: gene–trait correlation coefficients for the TF's target genes for a given trait, w: weighting factor ($w = 10$ in our analysis), $r_{\min}$: prior minimum correlation (across all genes), m: total number of genes in the background set (i.e., DEGs), k: number of trait-associated genes, X: number of overlapping genes between the TF's predicted targets and the trait-associated genes and n: total number of predicted targets of the TF.

Functional enrichment of genes

GO enrichment analysis was conducted using topGO (Alexa & Rahnenführer, 2009) (v.2.52.0), employing Fisher's exact test. Redundant GO terms were identified by assessing semantic similarity among GO terms using Wang's method (Wang et al., 2007). The enriched terms were visualized using ViSEAGO (v1.14) (Brionne et al., 2019).

Motif analysis of gene promoters and statistical analysis of binding data

Analysis of Motif Enrichment (AME) (v5.5.5) (Bailey et al., 2009) was used to look for over-representation of Arabidopsis TF binding motifs (O'Malley et al., 2016) within 1 kb promoter regions upstream of the coding sequence of trait subnetwork genes. Regulators of modules based on *in vitro* binding data (O'Malley et al., 2016) enrichment were assessed by testing the significance of the overlap between validated TF target genes and the genes within each trait module relative to the background of all DEGs. This was accomplished using Fisher's exact test implemented in the R package GeneOverlap (v1.3.6.0) (Li Shen, 2017).

AUTHOR CONTRIBUTIONS

MDB conceptualized the project. MDB, CC, and JG developed the experimental approach. KdS performed the experiments and analyzed the data. KdS and MDB wrote the manuscript.

ACKNOWLEDGMENTS

We thank Gloria Coruzzi for her extensive support and advice throughout the life of this project. We also acknowledge and appreciate Michael Masters for performing the elemental analysis and the group at the Roy J. Carver Biotechnology Center DNA services core facility for processing and sequencing samples. Any opinions, findings, and conclusions or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the views of the US Department of Agriculture (USDA). Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA. USDA is an equal opportunity provider and employer. This work was supported by the USDA Agricultural Research Service and an NIH NIGMS Fellowship F32GM116347 to M.D.B.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Sequencing data from this project have been deposited in the National Center for Biotechnology Information Gene Expression Omnibus (GEO) database, with accession number GSE276461. The data supporting the findings of this study are available in the supplementary material of this article. The code used for analyzing transcriptomic data, trait correlation, and network inference is available on GitHub at https://github.com/desilvakithmee/A.thaliana_GRNanalysis.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Figure S1. Photosynthetic activity of Arabidopsis under varying nitrogen doses is strongly influenced by light intensity. Shown are the effects on PSII efficiency (left) and NPQ (right).

Figure S2. Total, shoot, and root nitrogen use efficiency (NUE), nitrogen utilization efficiency (NUtE), and NUpE traits show similar trends, with strong correlations observed among total, shoot, and root values.

Figure S3. Hypothesized models of gene expression in response to N-by-L treatment.

Figure S4. The expression patterns of genes associated with photosynthesis show a degree of consistency across functional categories. Each column represents the average expression for one N-by-L treatment condition (across eight replicates), with annotations at the top of each column. Each row corresponds to a gene, with shaded colors indicating the normalized expression levels –

red for low expression and blue for high expression. The genes are grouped by the processes with their respective functions detailed in the legend.

Figure S5. Nitrogen assimilation genes exhibit a stronger nitrogen dependency under higher light conditions. Each column represents the average expression for one N-by-L treatment condition (across eight replicates), the treatment indicated by annotations at the top of each column. Each row corresponds to a gene, with shaded colors indicating the normalized expression levels – red for low expression and blue for high expression. The genes are grouped by their functional category with their respective functions detailed in the legend.

Figure S6. Variations in physiological traits with the N-by-L treatment share similarities. (a) Scaled values of physiological traits under the experimental conditions. (b) Graphical summary of two-way ANOVA results for traits, with nitrogen and light as independent variables (Model: N + L + N/L). Significance levels are denoted by asterisks. (c) Correlation heatmap of physiological traits. The color and sizes of the circles represent the Pearson correlation coefficient.

Figure S7. Constructing a high-confidence TF-directed gene regulatory network with N-by-L DEGs by integrating networks inferred from different approaches. Network performance was assessed using only the top 100000 edges of each network using ConnecTF (Brooks et al., 2021).

Figure S8. Expression profiles of two candidate key regulators (TGA3 and NLP8) and their targets within a trait subnetwork/module. Co-expressed gene clusters were identified through hierarchical clustering of expression profiles. Potential functions of targets were inferred through GO biological processes analysis.

Table S1. Physiological traits of plants under N-by-L treatment.

Table S2. N-by-L differentially expressed genes using the model reduction approach.

Table S3. Significant GO enrichment of network genes (P-adj < 0.05).

Table S4. Gene-trait association based on Pearson correlation.

Table S5. Over-represented TF binding motifs present within the 1 kb upstream promoter regions of module genes.

Table S6. N-by-L pruned GRN and subnetwork composition.

Table S7. Transcription factor connectivity in the full GRN and subnetworks.

Table S8. Top regulators of pathways of interest within the subnetworks.

REFERENCES

Alexa, A. & **Rahnenführer, J.** (2009) Gene set enrichment analysis with topGO. *Bioconductor Improvement*, **27**, 1–26.

Alvarez, J.M., **Riveras, E.**, **Vidal, E.A.**, **Gras, D.E.**, **Contreras-López, O.**, **Tamayo, K.P.** ***et al.*** (2014) Systems approach identifies TGA1 and TGA4 transcription factors as important regulatory components of the nitrate response of *Arabidopsis thaliana* roots. *Plant Journal*, **80**, 1–13. Available from: https://doi.org/10.1111/tpj.12618

Alvarez, J.M., **Schinke, A.-L.**, **Brooks, M.D.**, **Pasquino, A.**, **Leonelli, L.**, **Varala, K.** ***et al.*** (2020) Transient genome-wide interactions of the master transcription factor NLP7 initiate a rapid nitrogen-response cascade. *Nature Communications*, **11**, 1157. Available from: https://doi.org/10.1038/s41467-020-14979-6

Araus, V., **Vidal, E.A.**, **Puelma, T.**, **Alamos, S.**, **Mieulet, D.**, **Guiderdoni, E.** ***et al.*** (2016) Members of BTB gene family regulate negatively nitrate uptake and nitrogen use efficiency in *Arabidopsis thaliana* and *Oryza sativa*. *Plant Physiology*, **171**, 1731. Available from: https://doi.org/10.1104/pp.15.01731

Bailey, T.L., **Boden, M.**, **Buske, F.A.**, **Frith, M.**, **Grant, C.E.**, **Clementi, L.** ***et al.*** (2009) MEME SUITE: tools for motif discovery and searching. *Nucleic Acids Research*, **37**, W202–W208. Available from: https://doi.org/10.1093/nar/gkp335

Bi, Y.-M., **Wang, R.-L.**, **Zhu, T.** & **Rothstein, S.J.** (2007) Global transcription profiling reveals differential responses to chronic nitrogen stress and putative nitrogen regulatory components in Arabidopsis. *BMC Genomics*, **8**, 281. Available from: https://doi.org/10.1186/1471-2164-8-281

Brionne, A., **Juanchich, A.** & **Hennequet-Antier, C.** (2019) ViSEAGO: a bioconductor package for clustering biological functions using gene ontology and semantic similarity. *Biodata Mining*, **12**, 16. Available from: https://doi.org/10.1186/s13040-019-0204-1

Brooks, M.D., **Cirrone, J.**, **Pasquino, A.V.**, **Alvarez, J.M.**, **Swift, J.**, **Mittal, S.** ***et al.*** (2019) Network walking charts transcriptional dynamics of nitrogen signaling by integrating validated and predicted genome-wide interactions. *Nature Communications*, **10**, 1569. Available from: https://doi.org/10.1038/s41467-019-09522-1

Brooks, M.D., **Juang, C.-L.**, **Katari, M.S.**, **Alvarez, J.M.**, **Pasquino, A.**, **Shih, H.-J.** ***et al.*** (2021) ConnecTF: a platform to integrate transcription factor–gene interactions and validate regulatory networks. *Plant Physiology*, **185**, 49–66. Available from: https://doi.org/10.1093/plphys/kiaa012

Brooks, M.D. & **Niyogi, K.K.** (2011) Use of a pulse-amplitude modulated chlorophyll fluorometer to study the efficiency of photosynthesis in Arabidopsis plants. In: Jarvis, R.P. (Ed.) *Chloroplast research in Arabidopsis, methods in molecular biology*. Totowa, NJ: Humana Press, pp. 299–310. Available from: https://doi.org/10.1007/978-1-61779-237-3_16

Brooks, M.D. & **Szeto, R.C.** (2024) Biological nitrogen fixation maintains carbon/nitrogen balance and photosynthesis at elevated CO_2. *Plant, Cell & Environment*, **47**, 2178–2191. Available from: https://doi.org/10.1111/pce.14873

Canales, J., **Moyano, T.C.**, **Villarroel, E.** & **Gutiérrez, R.A.** (2014) Systems analysis of transcriptome data provides new hypotheses about Arabidopsis root response to nitrate treatments. *Frontiers in Plant Science*, **5**, 22. Available from: https://doi.org/10.3389/fpls.2014.00022

Castaings, L., **Camargo, A.**, **Pocholle, D.**, **Gaudon, V.**, **Texier, Y.**, **Boutet-Mercey, S.** ***et al.*** (2009) The nodule inception-like protein 7 modulates nitrate sensing and metabolism in Arabidopsis. *Plant Journal*, **57**, 426–435. Available from: https://doi.org/10.1111/j.1365-313X.2008.03695.x

Champigny, M.L. (1995) Integration of photosynthetic carbon and nitrogen metabolism in higher plants. *Photosynthesis Research*, **46**, 117–127. Available from: https://doi.org/10.1007/BF00020422

Cheng, C., **Krishnakumar, V.**, **Chan, A.P.**, **Thibaud-Nissen, F.**, **Schobel, S.** & **Town, C.D.** (2017) Araport11: a complete reannotation of the Arabidopsis thaliana reference genome. *Plant Journal*, **89**, 789–804. Available from: https://doi.org/10.1111/tpj.13415

Cheng, C.-Y., **Li, Y.**, **Varala, K.**, **Bubert, J.**, **Huang, J.**, **Kim, G.J.** ***et al.*** (2021) Evolutionarily informed machine learning enhances the power of predictive gene-to-phenotype relationships. *Nature Communications*, **12**, 5627. Available from: https://doi.org/10.1038/s41467-021-25893-w

Cirrone, J., **Brooks, M.D.**, **Bonneau, R.**, **Coruzzi, G.M.** & **Shasha, D.E.** (2020) OutPredict: multiple datasets can improve prediction of expression and inference of causality. *Scientific Reports*, **10**, 6804. Available from: https://doi.org/10.1038/s41598-020-63347-3

Dobin, A., **Davis, C.A.**, **Schlesinger, F.**, **Drenkow, J.**, **Zaleski, C.**, **Jha, S.** ***et al.*** (2013) STAR: ultrafast universal RNA-seq aligner. *Bioinformatics*, **29**, 15–21. Available from: https://doi.org/10.1093/bioinformatics/bts635

Emms, D.M. & **Kelly, S.** (2022) SHOOT: phylogenetic gene search and ortholog inference. *Genome Biology*, **23**, 85. Available from: https://doi.org/10.1186/s13059-022-02652-8

Evans, J.R. (1989) Photosynthesis and nitrogen relationships in leaves of C3 plants. *Oecologia*, **78**, 9–19. Available from: https://doi.org/10.1007/BF00377192

Evans, J.R. & **Clarke, V.C.** (2019) The nitrogen cost of photosynthesis. *Journal of Experimental Botany*, **70**, 7–15. Available from: https://doi.org/10.1093/jxb/ery366

Fagard, M., **Launay, A.**, **Clement, G.**, **Courtial, J.**, **Dellagi, A.**, **Farjad, M.** ***et al.*** (2014) Nitrogen metabolism meets phytopathology. *Journal of Experimental Botany*, **65**, 5643–5656. Available from: https://doi.org/10.1093/jxb/eru323

Fahl, J.I., **Carelli, M.L.C.**, **Vega, J.** & **Magalhães, A.C.** (1994) Nitrogen and irradiance levels affecting net photosynthesis and growth of young coffee plants (*Coffea arabica* L.). *Journal of Horticultural Science*, **69**, 161–169. Available from: https://doi.org/10.1080/14620316.1994.11515262

Faith, J.J., **Hayete, B.**, **Thaden, J.T.**, **Mogno, I.**, **Wierzbowski, J.**, **Cottarel, G.** ***et al.*** (2007) Large-scale mapping and validation of *Escherichia coli* transcriptional regulation from a compendium of expression profiles. *PLoS Biology*, **5**, e8. Available from: https://doi.org/10.1371/journal.pbio.0050008

Frangedakis, E., **Yelina, N.E.**, **Billakurthi, K.**, **Schreier, T.**, **Dickinson, P.J.**, **Tomaselli, M.** ***et al.*** (2023) Synergistic control of chloroplast biogenesis by MYB-related and Golden2-like transcription factors. *bioRxiv*. Available from: https://doi.org/10.1101/2023.08.11.552970

Fu, Y., **Li, H.**, **Yu, J.**, **Liu, H.**, **Cao, Z.**, **Manukovsky, N.S.** ***et al.*** (2017) Interaction effects of light intensity and nitrogen concentration on growth, photosynthetic characteristics and quality of lettuce (*Lactuca sativa* L. var. *youmaicai*). *Scientia Horticulturae*, **214**, 51–57. Available from: https://doi.org/10.1016/j.scienta.2016.11.020

Gangappa, S.N. & **Botto, J.F.** (2014) The BBX family of plant transcription factors. *Trends in Plant Science*, **19**, 460–470. Available from: https://doi.org/10.1016/j.tplants.2014.01.010

Glass, A.D.M. (2002) The regulation of nitrate and ammonium transport systems in plants. *Journal of Experimental Botany*, **53**, 855–864. Available from: https://doi.org/10.1093/jexbot/53.370.855

Grechi, I., **Vivin, P.**, **Hilbert, G.**, **Milin, S.**, **Robert, T.** & **Gaudillère, J.-P.** (2007) Effect of light and nitrogen supply on internal C:N balance and control of root-to-shoot biomass allocation in grapevine. *Environmental and Experimental Botany*, **59**, 139–149. Available from: https://doi.org/10.1016/j.envexpbot.2005.11.002

Gu, J., **Chen, Y.**, **Zhang, H.**, **Li, Z.**, **Zhou, Q.**, **Yu, C.** ***et al.*** (2017) Canopy light and nitrogen distributions are related to grain yield and nitrogen use efficiency in rice. *Field Crops Research*, **206**, 74–85. Available from: https://doi.org/10.1016/j.fcr.2017.02.021

Guo, S.-W., **Zhou, Y.**, **Gao, Y.-X.**, **Li, Y.** & **Shen, Q.-R.** (2007) New insights into the nitrogen form effect on photosynthesis and photorespiration. *Pedosphere*, **17**, 601–610. Available from: https://doi.org/10.1016/S1002-0160(07)60071-X

Gutiérrez, R.A., **Lejay, L.V.**, **Dean, A.**, **Chiaromonte, F.**, **Shasha, D.E.** & **Coruzzi, G.M.** (2007) Qualitative network models and genome-wide expression data define carbon/nitrogen-responsive molecular machines in Arabidopsis. *Genome Biology*, **8**, R7. Available from: https://doi.org/10.1186/gb-2007-8-1-r7

Gutiérrez, R.A., **Stokes, T.L.**, **Thum, K.**, **Xu, X.**, **Obertello, M.**, **Katari, M.S.** ***et al.*** (2008) Systems approach identifies an organic nitrogen-responsive gene network that is regulated by the master clock control gene CCA1. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 4939–4944. Available from: https://doi.org/10.1073/pnas.0800211105

Halpape, W., **Wulf, D.**, **Verwaaijen, B.**, **Stasche, A.S.**, **Zenker, S.**, **Sielemann, J.** ***et al.*** (2023) Transcription factors mediating regulation of photosynthesis. *bioRxiv*. Available from: https://doi.org/10.1101/2023.01.06.522973

Halpern, M., **Yermiyahu, U.** & **Bar-Tal, A.** (2022) Photosynthetic acclimation and elevated [CO_2] induced nitrogen deficiency: two related phenomena that limit positive plant responses to elevated [CO_2]. *Advances in Agronomy*, **176**, 1–34. Available from: https://doi.org/10.1016/bs.agron.2022.07.001

Hanson, J., **Hanssen, M.**, **Wiese, A.**, **Hendriks, M.M.W.B.** & **Smeekens, S.** (2008) The sucrose regulated transcription factor bZIP11 affects amino acid metabolism by regulating the expression of ASPARAGINE SYNTHETASE1 and PROLINE DEHYDROGENASE2. *The Plant Journal*, **53**, 935–949. Available from: https://doi.org/10.1111/j.1365-313X.2007.03385.x

Heerah, S., **Katari, M.**, **Penjor, R.**, **Coruzzi, G.** & **Marshall-Colon, A.** (2019) WRKY1 mediates transcriptional regulation of light and nitrogen signaling pathways. *Plant Physiology*, **181**, 1371–1388. Available from: https://doi.org/10.1104/pp.19.00685

Hersch, M., **Lorrain, S.**, **De Wit, M.**, **Trevisan, M.**, **Ljung, K.**, **Bergmann, S.** ***et al.*** (2014) Light intensity modulates the regulatory network of the shade avoidance response in Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America*, **111**, 6515–6520. Available from: https://doi.org/10.1073/pnas.1320355111

Huynh-Thu, V.A., **Irrthum, A.**, **Wehenkel, L.** & **Geurts, P.** (2010) Inferring regulatory networks from expression data using tree-based methods. *PLoS One*, **5**, e12776. Available from: https://doi.org/10.1371/journal.pone.0012776

Jonassen, E.M., **Sévin, D.C.** & **Lillo, C.** (2009) The bZIP transcription factors HY5 and HYH are positive regulators of the main nitrate reductase gene in Arabidopsis leaves, NIA2, but negative regulators of the nitrate uptake gene NRT1.1. *Journal of Plant Physiology*, **166**, 2071–2076. Available from: https://doi.org/10.1016/j.jplph.2009.05.010

Konishi, M. & **Yanagisawa, S.** (2013) Arabidopsis NIN-like transcription factors have a central role in nitrate signalling. *Nature Communications*, **4**, 1617. Available from: https://doi.org/10.1038/ncomms2621

Krouk, G. (2017) Nitrate signalling: calcium bridges the nitrate gap. *Nature Plants*, **3**, 17095. Available from: https://doi.org/10.1038/nplants.2017.95

Krouk, G. & **Kiba, T.** (2020) Nitrogen and phosphorus interactions in plants: from agronomic to physiological and molecular insights. *Current Opinion in Plant Biology*, **57**, 104–109. Available from: https://doi.org/10.1016/j.pbi.2020.07.002

Krouk, G., **Mirowski, P.**, **LeCun, Y.**, **Shasha, D.E.** & **Coruzzi, G.M.** (2010) Predictive network modeling of the high-resolution dynamic plant transcriptome in response to nitrate. *Genome Biology*, **11**, R123. Available from: https://doi.org/10.1186/gb-2010-11-12-r123

Lawlor, D.W. (2002) Carbon and nitrogen assimilation in relation to yield: mechanisms are the key to understanding production systems. *Journal of Experimental Botany*, **53**, 773–787. Available from: https://doi.org/10.1093/jexbot/53.370.773

Lawlor, D.W., **Boyle, F.A.**, **Young, A.T.**, **Keys, A.J.** & **Kendall, A.C.** (1987) Nitrate nutrition and temperature effects on wheat: photosynthesis and photorespiration of leaves. *Journal of Experimental Botany*, **38**, 393–408. Available from: https://doi.org/10.1093/jxb/38.3.393

Leivar, P. & **Monte, E.** (2014) PIFs: systems integrators in plant development. *Plant Cell*, **26**, 56–78. Available from: https://doi.org/10.1105/tpc.113.120857

Li, L., **Zheng, W.**, **Zhu, Y.**, **Ye, H.**, **Tang, B.**, **Arendsee, Z.W.** ***et al.*** (2015) QQS orphan gene regulates carbon and nitrogen partitioning across species via NF-YC interactions. *Proceedings of the National Academy of Sciences of the United States of America*, **112**, 14734–14739. Available from: https://doi.org/10.1073/pnas.1514670112

Li Shen, M.S. (2017) GeneOverlap. https://doi.org/10.18129/B9.BIOC.GENEOVERLAP

Liang, Y., **Cossani, C.M.**, **Sadras, V.O.**, **Yang, Q.** & **Wang, Z.** (2022) The interaction between nitrogen supply and light quality modulates plant growth and resource allocation. *Frontiers in Plant Science*, **13**, 864090. Available from: https://doi.org/10.3389/fpls.2022.864090

Liao, Y., **Smyth, G.K.** & **Shi, W.** (2014) featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics*, **30**, 923–930. Available from: https://doi.org/10.1093/bioinformatics/btt656

Love, M.I., **Huber, W.** & **Anders, S.** (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, **15**, 550. Available from: https://doi.org/10.1186/s13059-014-0550-8

Ma, J., **Hanssen, M.**, **Lundgren, K.**, **Hernández, L.**, **Delatte, T.**, **Ehlert, A.** ***et al.*** (2011) The sucrose-regulated Arabidopsis transcription factor bZIP11 reprograms metabolism and regulates trehalose metabolism. *The New Phytologist*, **191**, 733–745. Available from: https://doi.org/10.1111/j.1469-8137.2011.03735.x

Makino, A., **Sato, T.**, **Nakano, H.** & **Mae, T.** (1997) Leaf photosynthesis, plant growth and nitrogen allocation in rice under different irradiances. *Planta*, **203**, 390–398. Available from: https://doi.org/10.1007/s004250050205

Margolin, A.A., **Nemenman, I.**, **Basso, K.**, **Wiggins, C.**, **Stolovitzky, G.**, **Favera, R.D.** ***et al.*** (2006) ARACNE: an algorithm for the reconstruction of gene regulatory networks in a mammalian cellular context. *BMC Bioinformatics*, **7**, S7. Available from: https://doi.org/10.1186/1471-2105-7-S1-S7

Maxwell, K. & **Johnson, G.N.** (2000) Chlorophyll fluorescence—a practical guide. *Journal of Experimental Botany*, **51**, 659–668. Available from: https://doi.org/10.1093/jexbot/51.345.659

Menz, J., **Range, T.**, **Trini, J.**, **Ludewig, U.** & **Neuhäuser, B.** (2018) Molecular basis of differential nitrogen use efficiencies and nitrogen source preferences in contrasting Arabidopsis accessions. *Scientific Reports*, **8**, 3373. Available from: https://doi.org/10.1038/s41598-018-21684-4

Obertello, M., **Krouk, G.**, **Katari, M.S.**, **Runko, S.J.** & **Coruzzi, G.M.** (2010) Modeling the global effect of the basic-leucine zipper transcription factor 1 (bZIP1) on nitrogen and light regulation in Arabidopsis. *BMC Systems Biology*, **4**, 111. Available from: https://doi.org/10.1186/1752-0509-4-111

O'Malley, R.C., **Huang, S.C.**, **Song, L.**, **Lewsey, M.G.**, **Bartlett, A.**, **Nery, J.R.** ***et al.*** (2016) Cistrome and epicistrome features shape the regulatory DNA landscape. *Cell*, **165**, 1280–1292. Available from: https://doi.org/10.1016/j.cell.2016.04.038

Palenchar, P.M., **Kouranov, A.**, **Lejay, L.V.** & **Coruzzi, G.M.** (2004) Genome-wide patterns of carbon and nitrogen regulation of gene expression validate the combined carbon and nitrogen (CN)-signaling hypothesis in plants. *Genome Biology*, **5**, R91. Available from: https://doi.org/10.1186/gb-2004-5-11-r91

Pathak, R.R., **Jangam, A.P.**, **Malik, A.**, **Sharma, N.**, **Jaiswal, D.K.** & **Raghuram, N.** (2020) Transcriptomic and network analyses reveal distinct nitrate responses in light and dark in rice leaves (*Oryza sativa* indica var. panvel1). *Scientific Reports*, **10**, 12228. Available from: https://doi.org/10.1038/s41598-020-68917-z

Prosser, J.I. (2005) Nitrogen in soils: nitrification. In: Hillel, D. (Ed.) *Encyclopedia of soils in the environment*. Oxford: Elsevier, pp. 31–39. Available from: https://doi.org/10.1016/B0-12-348530-4/00512-9

Ren, H., **Zhou, P.**, **Zhou, B.**, **Li, X.**, **Wang, X.**, **Ge, J.** ***et al.*** (2023) Understanding the physiological mechanisms of canopy light interception and nitrogen distribution characteristics of different maize varieties at varying nitrogen application levels. *Agronomy*, **13**, 1146. Available from: https://doi.org/10.3390/agronomy13041146

Rivero, L., **Scholl, R.**, **Holomuzki, N.**, **Crist, D.**, **Grotewold, E.** & **Brkljacic, J.** (2014) Handling Arabidopsis plants: growth, preservation of seeds, transformation, and genetic crosses. In: Sanchez-Serrano, J.J. & Salinas, J. (Eds.) *Arabidopsis protocols, methods in molecular biology*. Totowa, NJ: Humana Press, pp. 3–25. Available from: https://doi.org/10.1007/978-1-62703-580-4_1

Rubin, G., **Tohge, T.**, **Matsuda, F.**, **Saito, K.** & **Scheible, W.-R.** (2009) Members of the LBD family of transcription factors repress anthocyanin synthesis and affect additional nitrogen responses in Arabidopsis. *Plant Cell*, **21**, 3567–3584. Available from: https://doi.org/10.1105/tpc.109.067041

Ruffel, S., **Chaput, V.**, **Przybyla-Toscano, J.**, **Fayos, I.**, **Ibarra, C.**, **Moyano, T.** ***et al.*** (2021) Genome-wide analysis in response to nitrogen and carbon identifies regulators for root AtNRT2 transporters. *Plant Physiology*, **186**, 696–714. Available from: https://doi.org/10.1093/plphys/kiab047

Sakuraba, Y. & **Yanagisawa, S.** (2018) Light signalling-induced regulation of nutrient acquisition and utilisation in plants. *Seminars in Cell & Developmental Biology*, **83**, 123–132. Available from: https://doi.org/10.1016/j.semcdb.2017.12.014

Sato, T., **Maekawa, S.**, **Konishi, M.**, **Yoshioka, N.**, **Sasaki, Y.**, **Maeda, H.** ***et al.*** (2017) Direct transcriptional activation of BT genes by NLP transcription factors is a key component of the nitrate response in Arabidopsis. *Biochemical and Biophysical Research Communications*, **483**, 380–386. Available from: https://doi.org/10.1016/j.bbrc.2016.12.135

Scheible, W.-R., **Morcuende, R.**, **Czechowski, T.**, **Fritz, C.**, **Osuna, D.**, **Palacios-Rojas, N.** ***et al.*** (2004) Genome-wide reprogramming of primary and secondary metabolism, protein synthesis, cellular growth processes, and the regulatory infrastructure of Arabidopsis in response to nitrogen. *Plant Physiology*, **136**, 2483–2499. Available from: https://doi.org/10.1104/pp.104.047019

Shanks, C.M., **Rothkegel, K.**, **Brooks, M.D.**, **Cheng, C.-Y.**, **Alvarez, J.M.**, **Ruffel, S.** ***et al.*** (2024) Nitrogen sensing and regulatory networks: it's about time and space. *Plant Cell*, **36**, 1482–1503. Available from: https://doi.org/10.1093/plcell/koae038

Singh, M., **Gupta, A.**, **Singh, D.**, **Khurana, J.P.** & **Laxmi, A.** (2017) Arabidopsis RSS1 mediates cross-talk between glucose and light signaling during hypocotyl elongation growth. *Scientific Reports*, **7**, 16101. Available from: https://doi.org/10.1038/s41598-017-16239-y

Smith, H. & **Whitelam, G.C.** (1997) The shade avoidance syndrome: multiple responses mediated by multiple phytochromes. *Plant, Cell & Environment*, **20**, 840–844. Available from: https://doi.org/10.1046/j.1365-3040.1997.d01-104.x

Stitt, M. & **Krapp, A.** (1999) The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. *Plant, Cell & Environment*, **22**, 583–621. Available from: https://doi.org/10.1046/j.1365-3040.1999.00386.x

Swift, J., **Adame, M.**, **Tranchina, D.**, **Henry, A.** & **Coruzzi, G.M.** (2019) Water impacts nutrient dose responses genome-wide to affect crop production. *Nature Communications*, **10**, 1374. Available from: https://doi.org/10.1038/s41467-019-09287-7

Terashima, I. & **Evans, J.R.** (1988) Effects of light and nitrogen nutrition on the organization of the photosynthetic apparatus in spinach. *Plant & Cell Physiology*, **29**, 143–155. Available from: https://doi.org/10.1093/oxfordjournals.pcp.a077461

Tsay, Y.-F., **Ho, C.-H.**, **Chen, H.-Y.** & **Lin, S.-H.** (2011) Integration of nitrogen and potassium signaling. *Annual Review of Plant Biology*, **62**, 207–226. Available from: https://doi.org/10.1146/annurev-arplant-042110-103837

Varala, K., **Marshall-Colón, A.**, **Cirrone, J.**, **Brooks, M.D.**, **Pasquino, A.V.**, **Léran, S.** ***et al.*** (2018) Temporal transcriptional logic of dynamic regulatory networks underlying nitrogen signaling and use in plants. *Proceedings of the National Academy of Sciences*, **115**, 6494–6499. Available from: https://doi.org/10.1073/pnas.1721487115

Vega, A., **O'Brien, J.A.** & **Gutiérrez, R.A.** (2019) Nitrate and hormonal signaling crosstalk for plant growth and development. *Current Opinion in Plant Biology*, **52**, 155–163. Available from: https://doi.org/10.1016/j.pbi.2019.10.001

Vidal, E.A., **Alvarez, J.M.**, **Araus, V.**, **Riveras, E.**, **Brooks, M.D.**, **Krouk, G.** ***et al.*** (2020) Nitrate in 2020: thirty years from transport to signaling networks. *Plant Cell*, **32**, 2094–2119. Available from: https://doi.org/10.1105/tpc.19.00748

Wang, J.Z., **Du, Z.**, **Payattakool, R.**, **Yu, P.S.** & **Chen, C.-F.** (2007) A new method to measure the semantic similarity of GO terms. *Bioinformatics*, **23**, 1274–1281. Available from: https://doi.org/10.1093/bioinformatics/btm087

Wang, L., **O'Conner, S.**, **Tanvir, R.**, **Zheng, W.**, **Cothron, S.**, **Towery, K.** ***et al.*** (2025) CRISPR /Cas9-based editing of NF-YC4 promoters yields high-protein rice and soybean. *The New Phytologist*, **245**, 2103–2116. Available from: https://doi.org/10.1111/nph.20141

Wei, S., **Li, X.**, **Lu, Z.**, **Zhang, H.**, **Ye, X.**, **Zhou, Y.** ***et al.*** (2022) A transcriptional regulator that boosts grain yields and shortens the growth duration of rice. *Science*, **377**, eabi8455. Available from: https://doi.org/10.1126/science.abi8455

Whitmarsh, J. & **Govindjee**. (1999) The photosynthetic process. In: Singhal, G.S., Renger, G., Sopory, S.K., Irrgang, K.-D. & **Govindjee** (Eds.) *Concepts in photobiology*. Dordrecht: Springer Netherlands, pp. 11–51. Available from: https://doi.org/10.1007/978-94-011-4832-0_2

Wu, J., **Lawit, S.J.**, **Weers, B.**, **Sun, J.**, **Mongar, N.**, **Van Hemert, J.** ***et al.*** (2019) Overexpression of zmm28 increases maize grain yield in the field. *Proceedings of the National Academy of Sciences of the United States of America*, **116**, 23850–23858. Available from: https://doi.org/10.1073/pnas.1902593116

Wu, Z., **Luo, J.**, **Han, Y.**, **Hua, Y.**, **Guan, C.** & **Zhang, Z.** (2019) Low nitrogen enhances nitrogen use efficiency by triggering NO_3^- uptake and its long-distance translocation. *Journal of Agricultural and Food Chemistry*, **67**, 6736–6747. Available from: https://doi.org/10.1021/acs.jafc.9b02491

Yan, D., **Easwaran, V.**, **Chau, V.**, **Okamoto, M.**, **Ierullo, M.**, **Kimura, M.** ***et al.*** (2016) NIN-like protein 8 is a master regulator of nitrate-promoted seed germination in Arabidopsis. *Nature Communications*, **7**, 13179. Available from: https://doi.org/10.1038/ncomms13179

Yoneyama, T. & **Suzuki, A.** (2020) Light-independent nitrogen assimilation in plant leaves: nitrate incorporation into glutamine, glutamate, aspartate, and asparagine traced by ^{15}N. *Plants*, **9**, 1303. Available from: https://doi.org/10.3390/plants9101303

Zhang, H. & **Forde, B.G.** (1998) An Arabidopsis MADS box gene that controls nutrient-induced changes in root architecture. *Science*, **279**, 407–409. Available from: https://doi.org/10.1126/science.279.5349.407

Zhu, D., **Li, Z.**, **Deng, S.**, **Liu, Q.**, **Wu, J.**, **Chen, X.** ***et al.*** (2023) Transcriptomic dissection underlying physiological and anatomical characteristics of poplar wood in response to changes in light intensity and nitrogen availability. *Environmental and Experimental Botany*, **206**, 105186. Available from: https://doi.org/10.1016/j.envexpbot.2022.105186