



Figures and figure supplements

A guanosine tetraphosphate (ppGpp) mediated brake on photosynthesis is required for acclimation to nitrogen limitation in *Arabidopsis*

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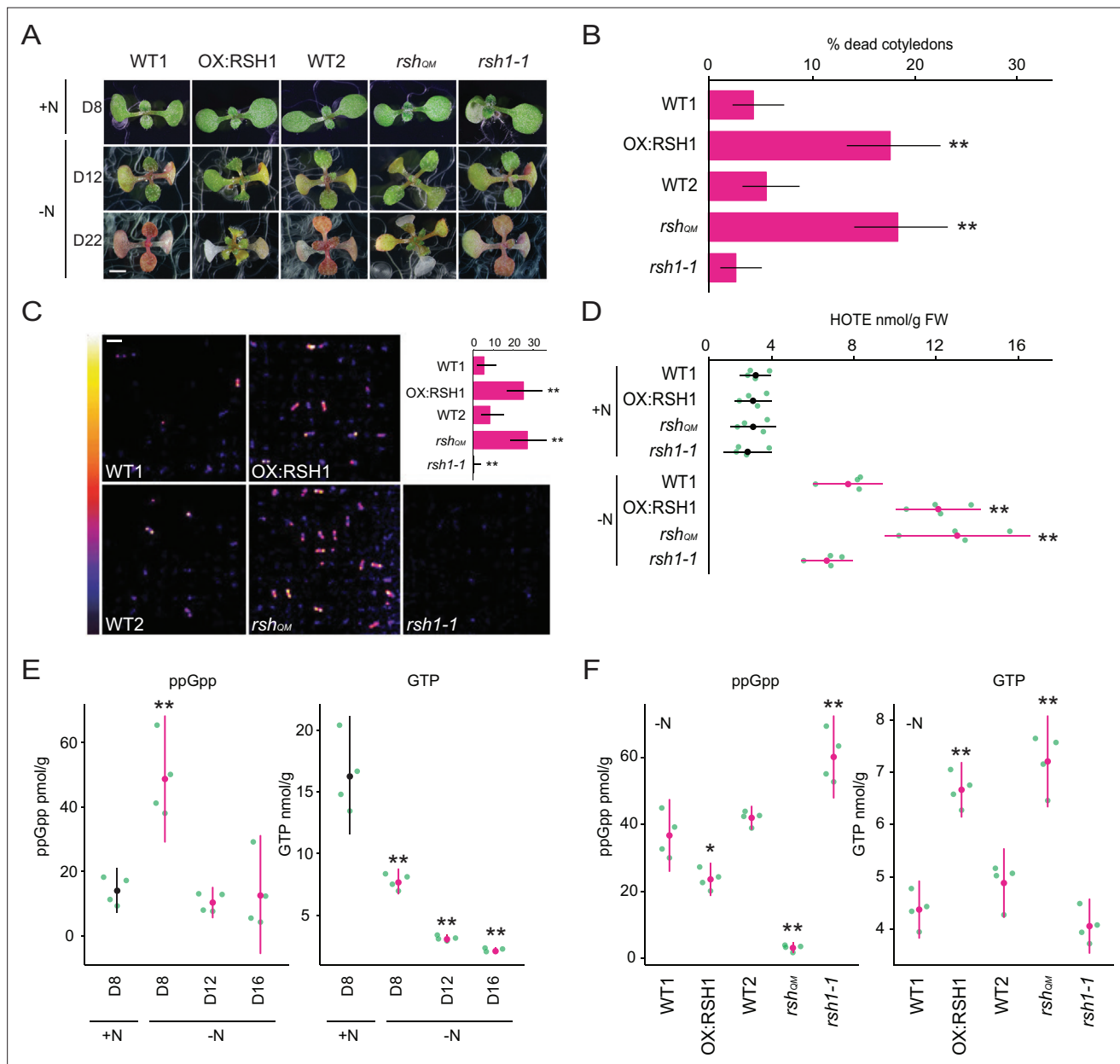


Figure 1. ppGpp is required for acclimation to nitrogen deprivation. **(A)** Images of seedlings grown on nitrogen replete (+N) or nitrogen limiting (-N) medium for 8 (D8), 12 (D12), and 22 (D22) days. Scale, 3 mm. WT1 (Col-0) and WT2 (*qrt1-2*) are the wild type for OX:RSH1 and *rsh_{OM}*/*rsh1-1*, respectively. See **Figure 1—figure supplement 1** for additional images. **(B)** Percentage of plants with dead cotyledons (completely white with collapsed tissue) for different genotypes grown on -N medium for 22 days. Three pooled experimental replicates, median \pm 95% confidence interval (CI), $n = 285$ – 298 seedlings per genotype. **(C)** Bioluminescence emission from lipid peroxides in seedlings grown on -N medium for 16 days. Inset graph shows median number of luminescent seedlings \pm 95% CI, $n = 100$ seedlings. Scale, 1.1 cm. **(D)** Quantification of hydroxy-octadecatrienoic acids (HOTEs) from seedlings grown in -N for 12 days or +N for 8 days where the developmental stage is similar. Mean \pm 95% CI, $n = 4$ experimental replicates. Concentrations of ppGpp and GTP in wild-type plants were determined **(E)** at the indicated time points during growth on +N and -N medium and **(F)** in different genotypes after 12 days of growth in -N medium (equivalent to 10 days in the experiment in panel E). The ppGpp/GTP ratio is shown in **Figure 1—figure supplement 2**. Mean \pm 95% CI, $n = 4$ experimental replicates. Statistical tests shown against respective wild-type controls, * $p < 0.05$, ** $p < 0.01$. Source data and statistical test reports are shown in **Figure 1—source data 1**.

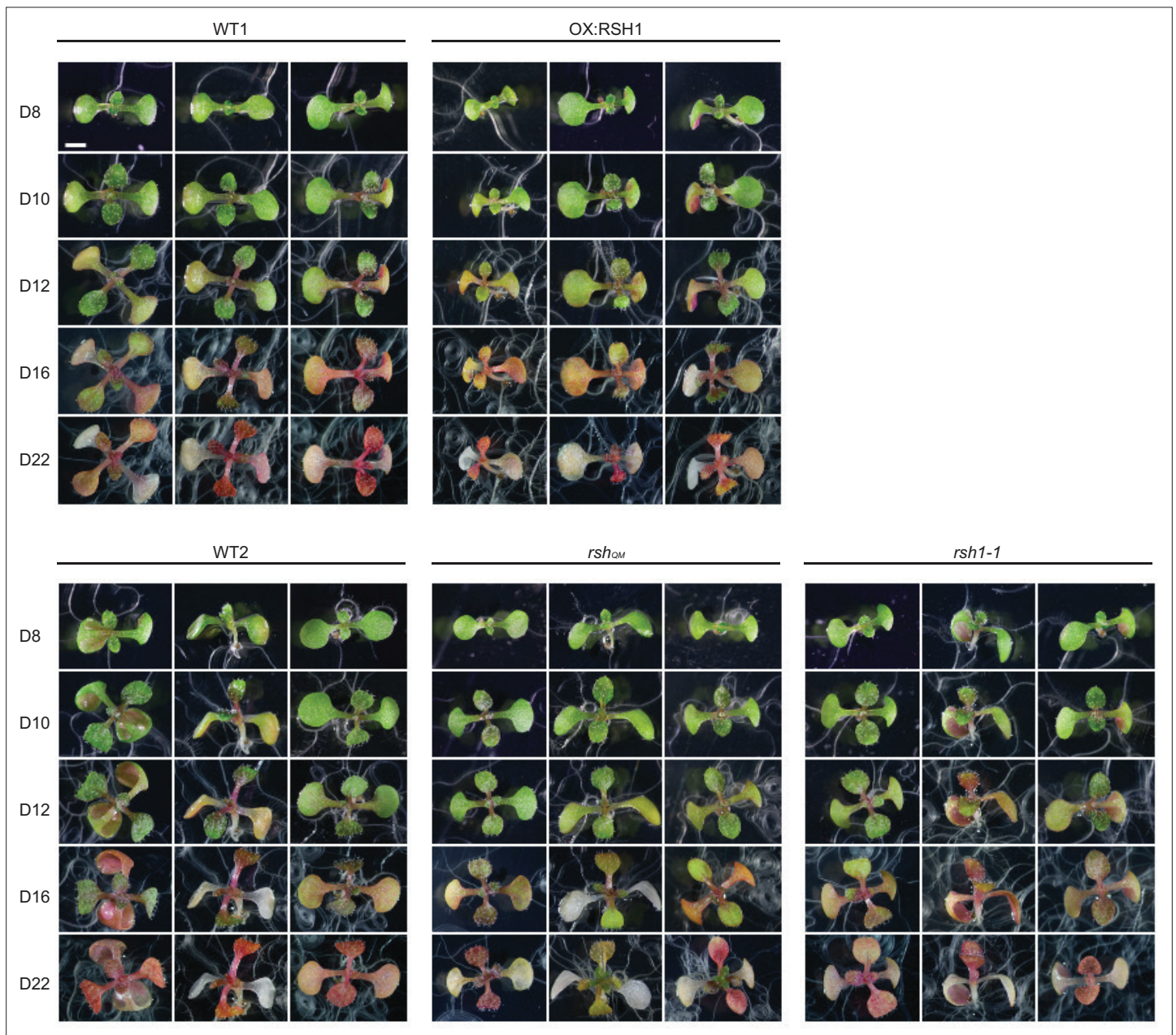


Figure 1—figure supplement 1. Extended timecourse of nitrogen deprivation. Images of seedlings grown on nitrogen limiting medium for the indicated number of days. Scale, 3 mm.

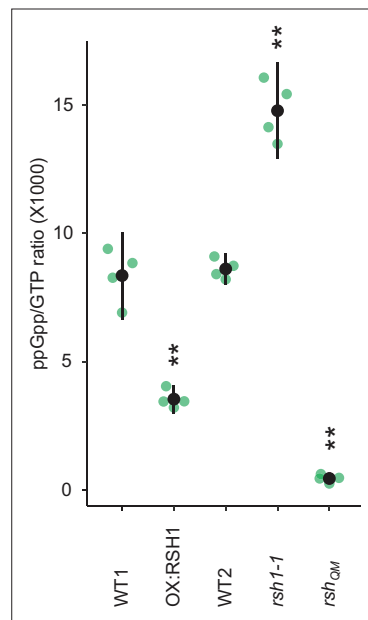


Figure 1—figure supplement 2. GTP/ppGpp ratios in RSH lines. The ratio of ppGpp to GTP concentration in seedlings grown for 10 days on nitrogen limiting media. Mean \pm 95% CI, $n = 4$ experimental replicates. Statistical tests shown against respective wild-type controls, ** $p < 0.01$.

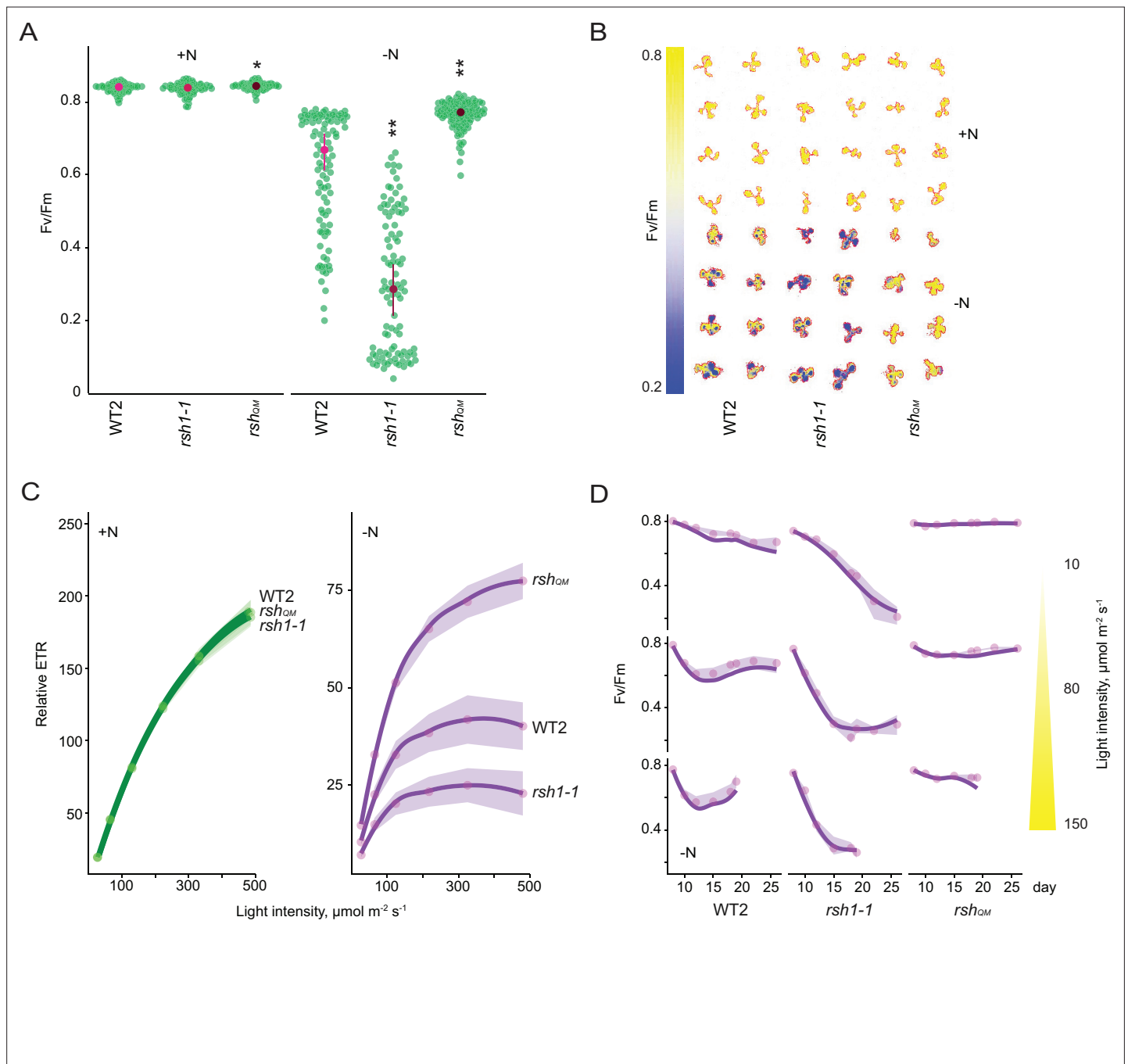


Figure 2. Nitrogen deprivation promotes a ppGpp-dependent drop in photosynthetic capacity. Seedlings were grown 8 days on nitrogen replete media (+N) or 12 days on nitrogen limiting (–N) media and **(A)** the maximal yield of PSII (Fv/Fm) measured by fluorescence imaging individual seedlings. Median \pm 95% confidence interval (CI), $n = 95$ –100 seedlings. **(B)** Fv/Fm images of whole seedlings grown on +N and –N media for 12 days. **(C)** Relative electron transport rate (ETR) measurements in different lines grown 8 days on +N media or 12 days on –N media. Median \pm 95% CI, $n = 95$ –100 seedlings. **(D)** Fv/Fm timecourses from seedlings grown on –N media and transferred to three different light intensities (photosynthetic photon flux density, 10, 80, and 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$) after 6 days. Median \pm 95% CI, $n = 95$ –100 seedlings. Tests shown against respective wild-type controls, * $p < 0.05$, ** $p < 0.01$. Additional supporting data are presented in **Figure 2—figure supplement 2**, **Figure 2—figure supplement 3** and **Figure 2—figure supplement 4**. Source data and statistical test reports are shown in **Figure 2—source data 1**.

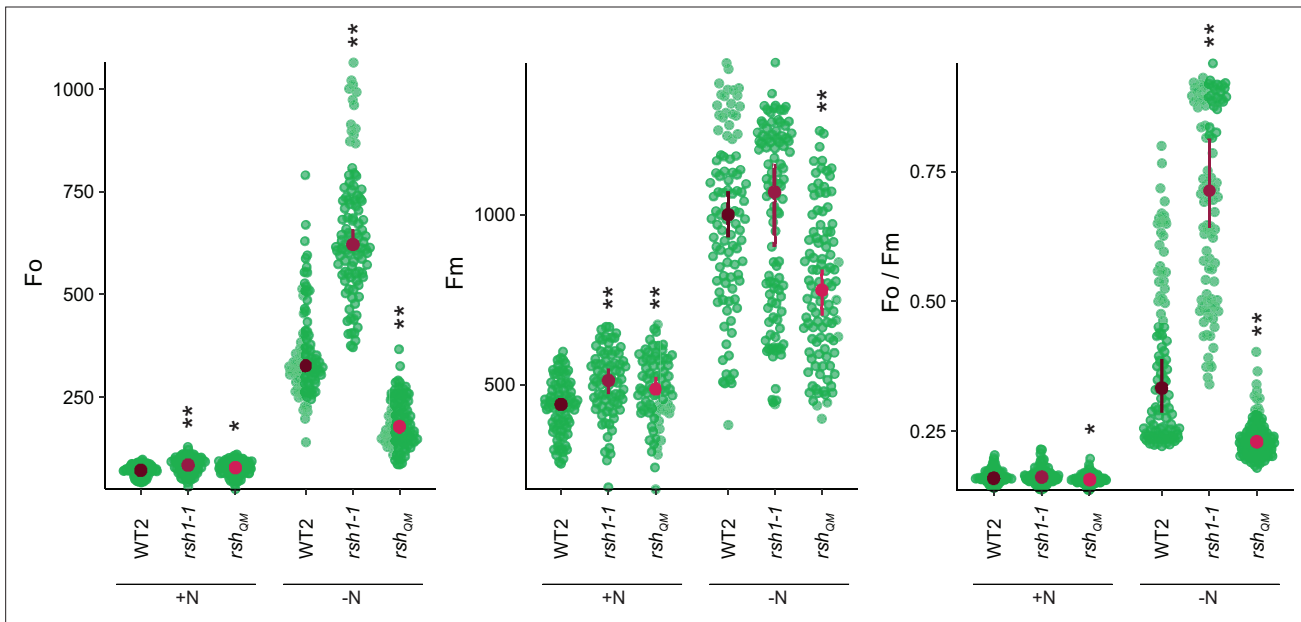


Figure 2—figure supplement 1. Changes in additional photosynthetic parameters (F_o , F_m , and F_o/F_m) for lines shown in panel 2A. Seedlings were grown 8 days on nitrogen replete media (+N) or 12 days on nitrogen limiting (-N) media. Median \pm 95% confidence interval (CI), $n = 95$ –100 seedlings. Statistical tests shown against respective wild-type controls, * $p < 0.05$, ** $p < 0.01$, Kruskal–Wallis test with post hoc Dunn tests.

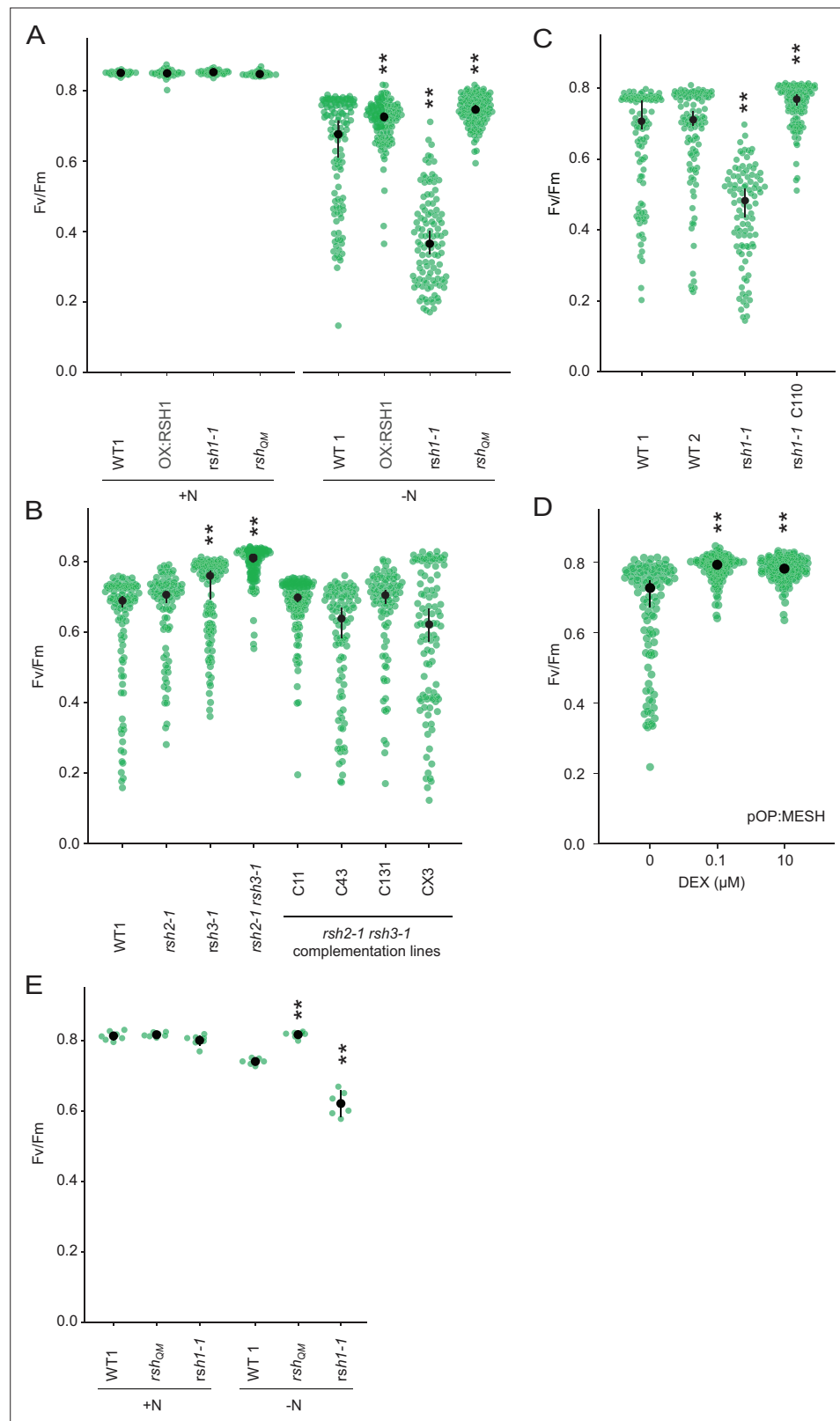


Figure 2—figure supplement 2. Role of ppGpp in the nitrogen deprivation induced decrease in PSII maximal yield. **(A)** Fv/Fm in seedlings of indicated lines grown 8 days on nitrogen replete media (+N) or 12 days on nitrogen limiting (–N) media. **(B–D)** Fv/Fm in seedlings of indicated lines grown 12 days on –N. *rsh1-1* C110 is complementation line complemented with the full-length *RSH1* (*pRSH1:RSH1*). pOP:MESH is a dexamethasone Figure 2—figure supplement 2 continued on next page

Figure 2—figure supplement 2 continued

(DEX) inducible line that expresses a chloroplast targeted ppGpp hydrolase MESH. **(E)** Fv/Fm in mature plants grown on quartz sand supplemented with nitrogen replete media (+N) or nitrogen-free (-N) media. Median \pm 95% confidence interval (CI), $n = 95\text{--}100$ seedlings **(A–D)**, 6 plants **(E)**. Statistical tests shown against respective wild-type controls, $**p < 0.01$.

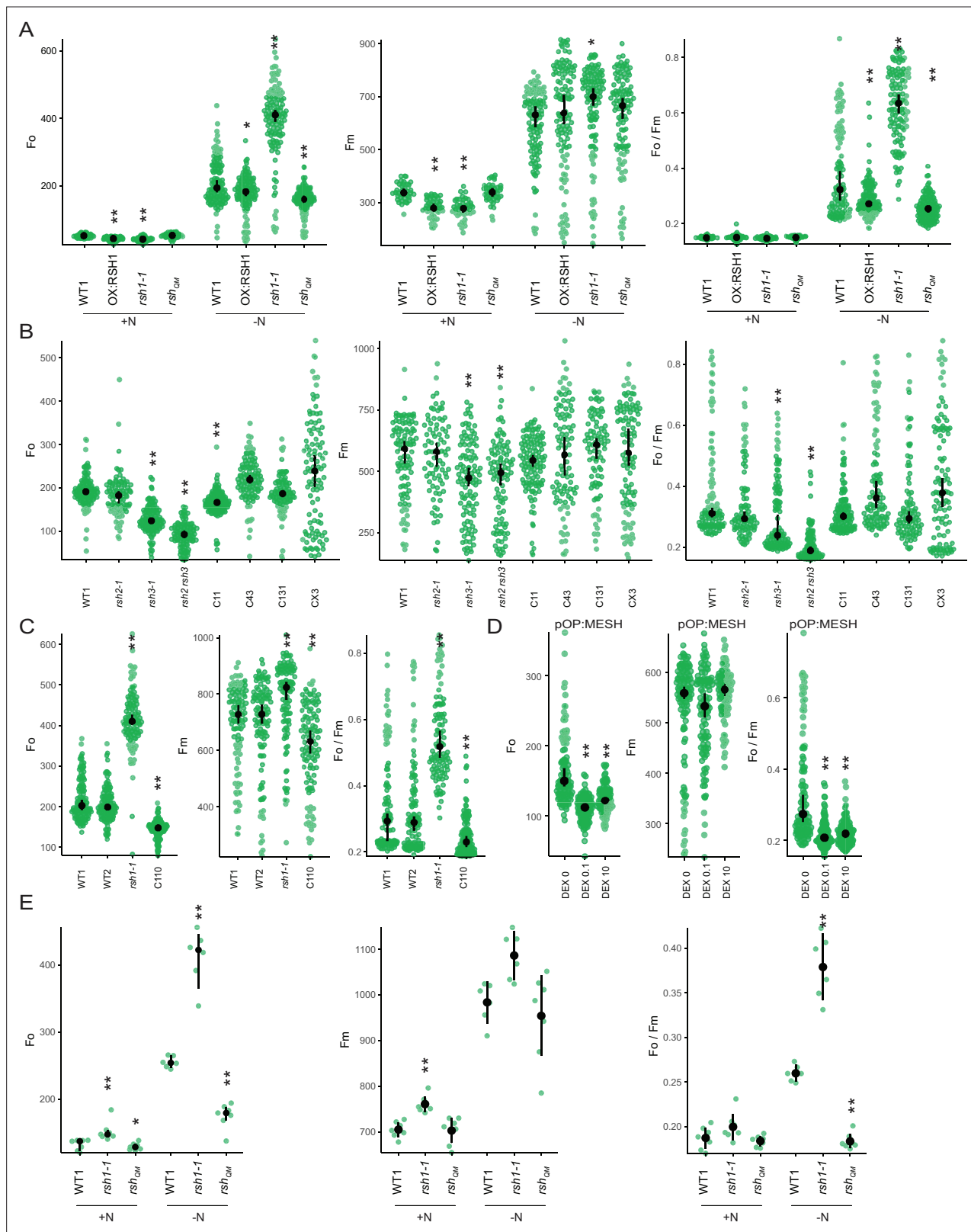


Figure 2—figure supplement 3. Changes in additional photosynthetic parameters (F_o , F_m , and F_o/F_m) for **Figure 2—figure supplement 2**. **(A)** In seedlings of indicated lines grown 8 days on nitrogen replete media (+N) or 12 days on nitrogen limiting (-N) media. **(B–D)** In seedlings of indicated lines grown 12 days on -N. C110 is an *rsh1-1* complementation line complemented with the full-length *RSH1* (*pRSH1:RSH1*). pOP:MESH is a dexamethasone (DEX) inducible line that expresses a chloroplast targeted ppGpp hydrolase MESH. **(E)** In mature plants grown on quartz sand **Figure 2—figure supplement 3 continued on next page**

Figure 2—figure supplement 3 continued

supplemented with nitrogen replete media (+N) or nitrogen-free (−N) media. Median \pm 95% confidence interval (CI), $n = 95$ – 100 seedlings (**A–D**), 6 plants (**E**). Statistical tests shown against respective wild-type controls, * $p < 0.05$, ** $p < 0.01$. Kruskal–Wallis test with post hoc Dunn tests (**A–D**), one-way analysis of variance (ANOVA) with post hoc Tukey test (**E**).

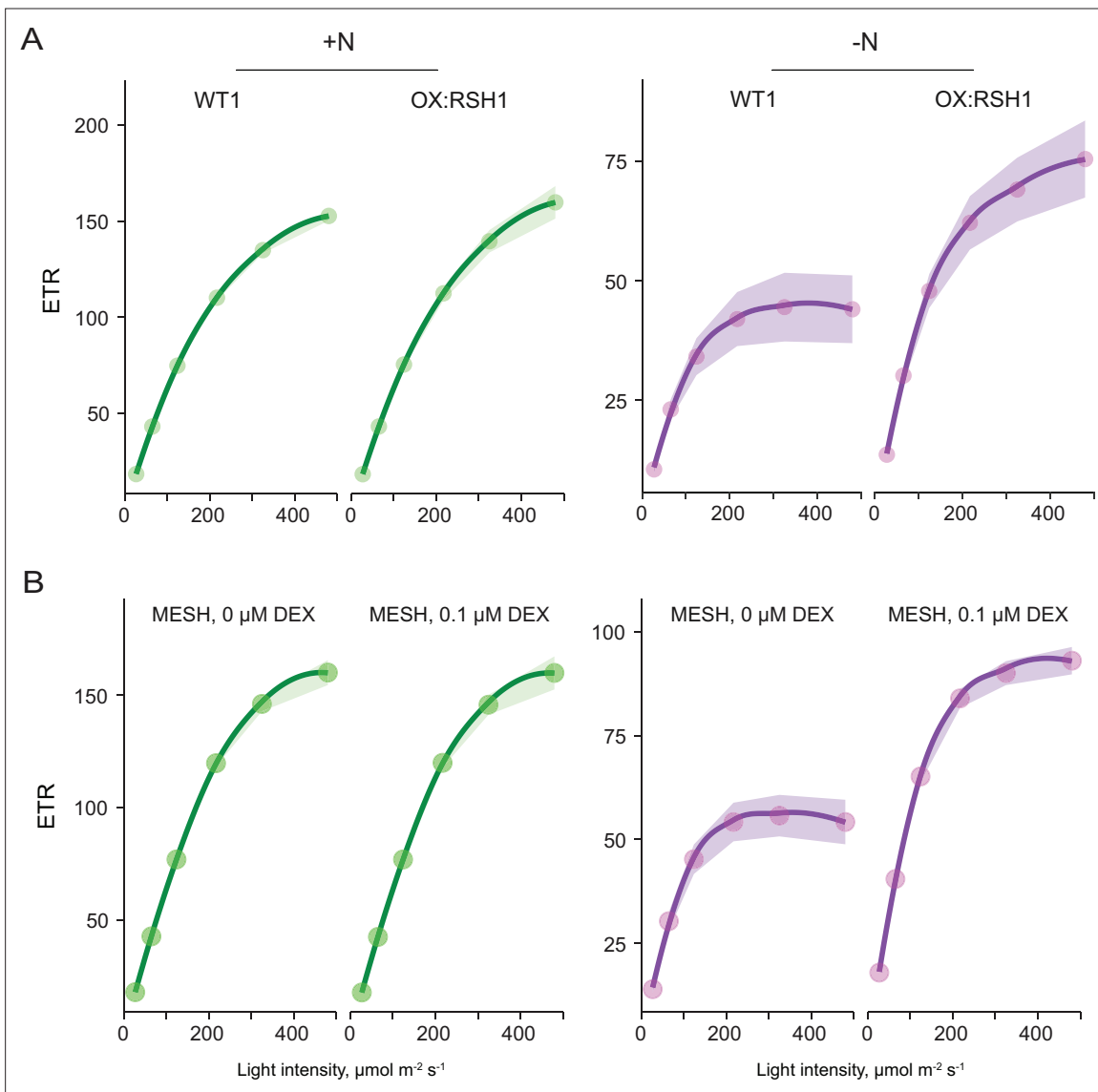


Figure 2—figure supplement 4. ppGpp is required for reducing electron transport rate (ETR) during nitrogen deprivation. Relative ETR measurements in different lines grown 8 days on +N media (left) or 12 days on -N media (right). **(A)** A comparison between OX:RSH1 and the wild-type control. **(B)** ETR in the dexamethasone (DEX) inducible pOP:MESH line grown on noninducing medium (0 μM DEX), or inducing medium (10 μM DEX). MESH is a chloroplast targeted ppGpp hydrolase. Median ± 95% confidence interval (CI), $n = 95-100$ seedlings.

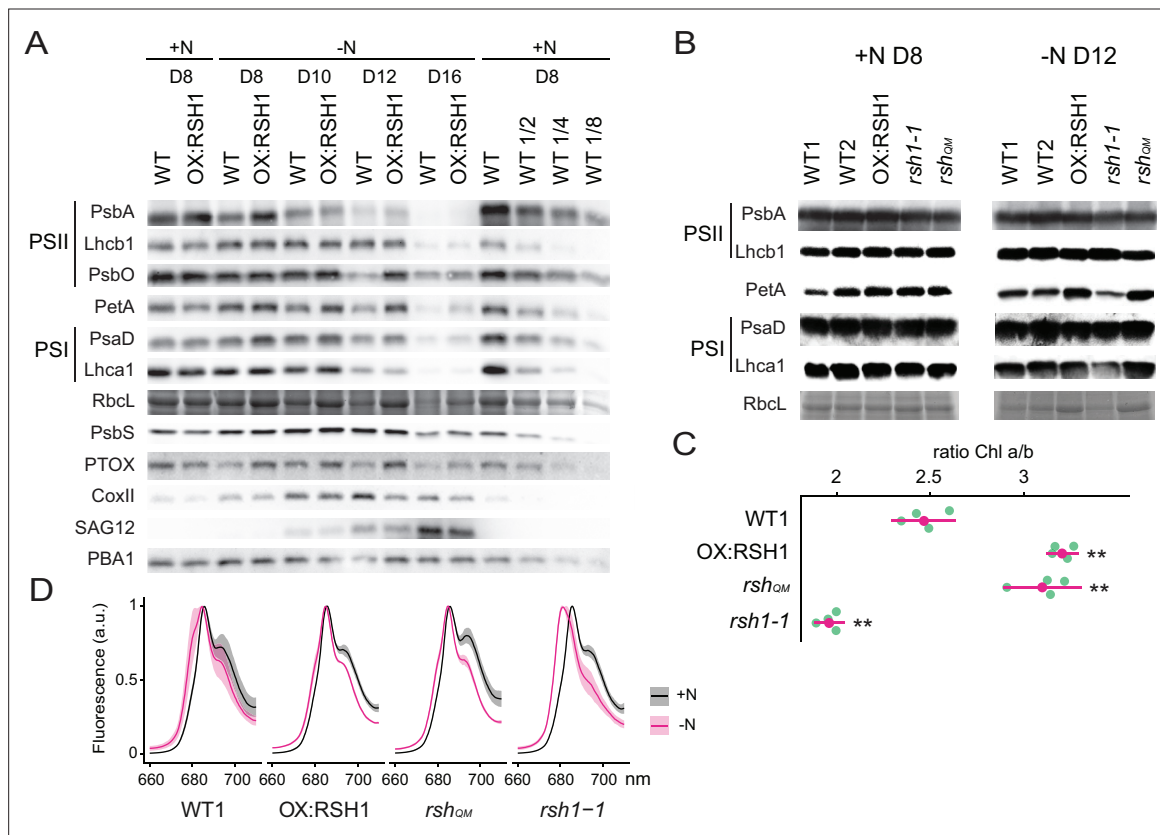


Figure 3. ppGpp-dependent alterations in the photosynthetic machinery during nitrogen deficiency. **(A)** Immunoblots showing evolution in abundance of the indicated proteins in seedlings grown in nitrogen replete (+N) or nitrogen limiting (–N) media for the indicated number of days. RbcL was revealed by Coomassie Brilliant Blue. Equal quantities of total proteins were loaded and PBA1, a subunit of the proteasome, was used as a protein normalization control **(B)**. Immunoblots showing the abundance of the indicated proteins in purified thylakoid membranes from seedlings grown in +N for 8 days or –N for 12 days. RbcL was revealed by Coomassie Brilliant Blue staining. Equal quantities of total chlorophyll were loaded. Immunoblots after ppGpp depletion by induction of chloroplastic MESH are shown in **Figure 3—figure supplement 1**. **(C)** Chlorophyll a/b ratios in extracts from seedlings subjected to –N for 12 days. Means \pm 95% confidence interval (CI), data from four experimental replicates. **(D)** Emission spectrum of chlorophyll fluorescence at 77 K between 660 and 720 nm, normalized to the PSII peak at 685 nm. The full spectra are shown in **Figure 2—figure supplement 2**. Measurements were made on seedlings grown in +N for 8 days or –N for 12 days. Means \pm 95% CI; data from four experimental replicates. Statistical tests, ** $p < 0.01$. Uncropped immunoblots are available in **Figure 3—source data 1** for 3A and **Figure 3—source data 2** for 3B. Numeric data and statistical test reports are shown in **Figure 3—source data 3**.

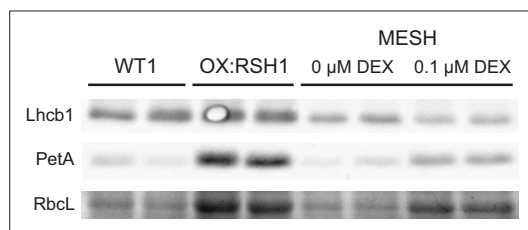


Figure 3—figure supplement 1. ppGpp depletion by MESH also affects abundance of chloroplast proteins. Immunoblots showing the abundance of the indicated proteins in extracts from 100 bulked seedlings grown in $-N$ for 12 days from two experimental replicates. MESH seedlings were induced by inclusion of dexamethasone (DEX) or not in the growth medium. RbcL was revealed by Coomassie Brilliant Blue. Equal quantities of total protein were loaded. Uncropped immunoblots are shown in **Figure 3—source data 3**.

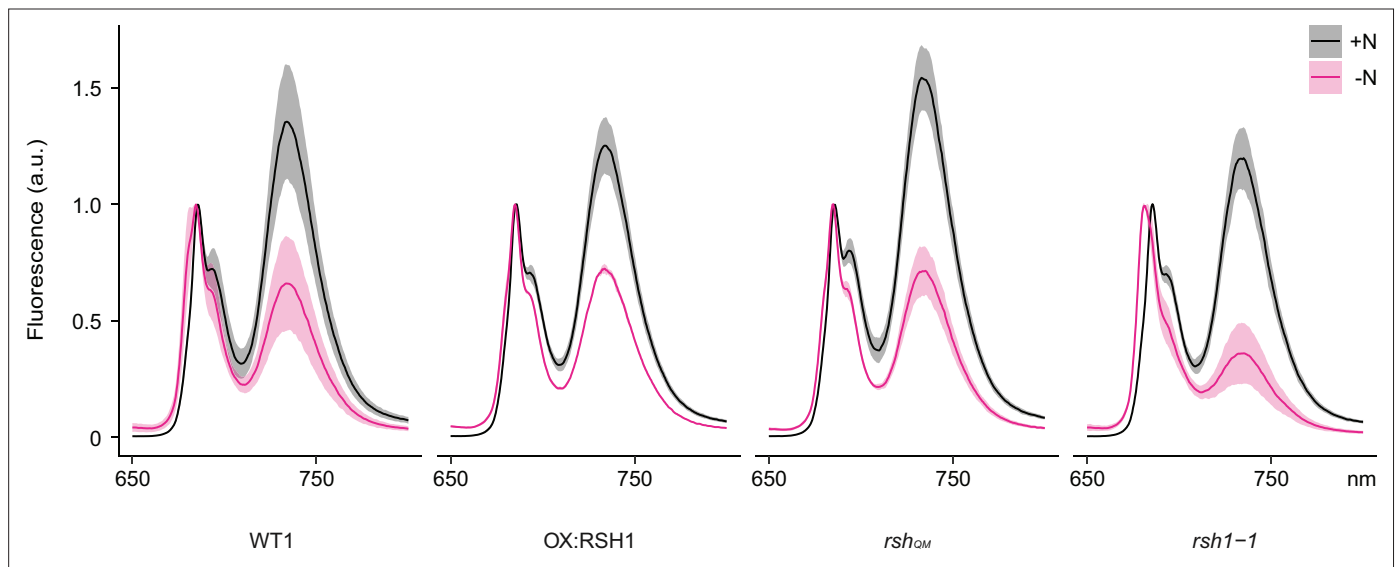


Figure 3—figure supplement 2. Full 77°K chlorophyll fluorescence spectra under nitrogen deprivation. Emission spectrum of chlorophyll fluorescence at 77°K normalized to the PSII peak at 685 nm. Measurements made on 8- or 12-day-old seedlings grown under +N or -N. Means \pm 95% confidence interval (CI); data from four experimental replicates.

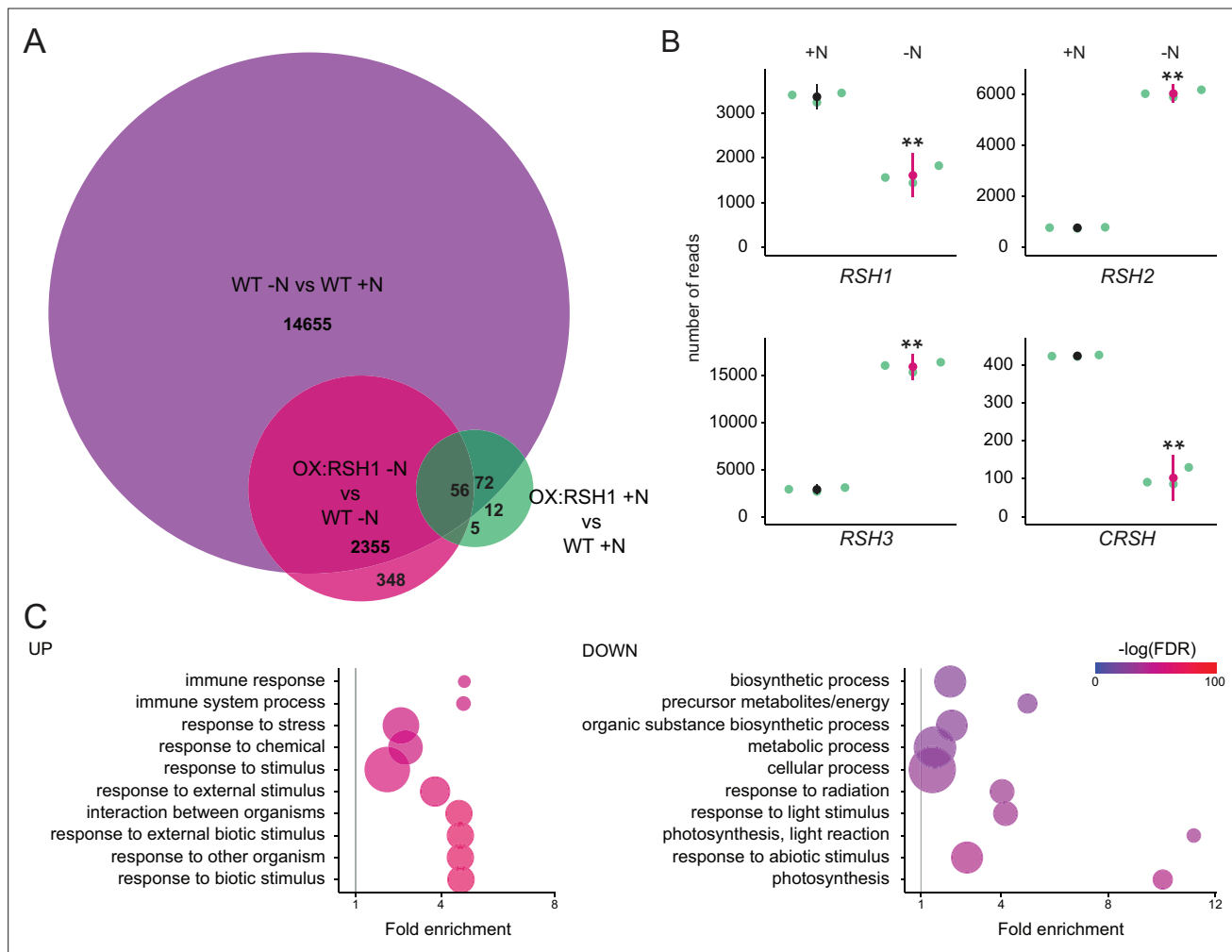


Figure 4. ppGpp plays a major role during acclimation to nitrogen deprivation. RNA-seq experiments were performed on WT and OX:RSH1 seedlings grown 8 days on nitrogen replete media (+N) or 12 days on nitrogen limiting (–N) media, $n = 3$ experimental replicates. **(A)** Venn diagram for transcripts showing differential accumulation for each of three comparisons. All differentially accumulating transcripts are listed in **Figure 4—source data 1**. **(B)** RNA-seq transcript levels for the four *RSH* genes in the WT, +N vs –N. Mean \pm 95% confidence interval (CI), $**p < 0.01$. **(C)** Enriched gene ontology terms among significantly up- and downregulated transcripts in OX:RSH1 vs WT under –N. The 10 most significant terms are shown, point size is proportional to gene number. FDR, false discovery rate. The full GO analysis is presented in **Figure 4—source data 2**. Source data and statistical test reports are shown in **Figure 4—source data 3**.

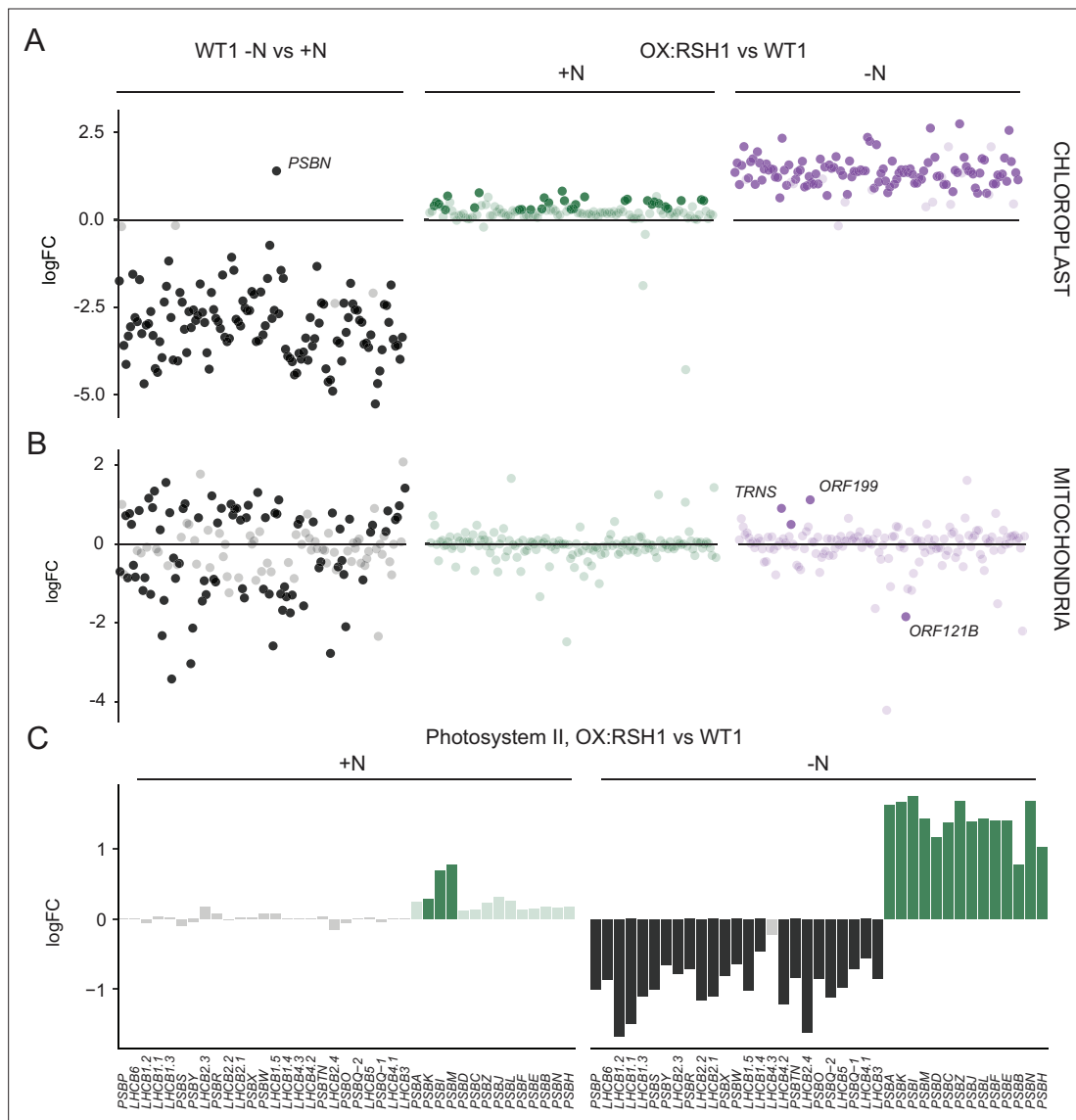


Figure 5. ppGpp is required for the downregulation of chloroplast gene expression during nitrogen deficiency. **(A)** The differential expression of chloroplast transcripts ordered along the chloroplast genome (**Figure 5—source data 1**). **(B)** The differential expression of mitochondrial transcripts ordered along the mitochondrial genome (**Figure 5—source data 2**). **(C)** Relative transcript levels in OX:RSH1 vs the wild-type control for nuclear (black) and chloroplast (green) genes encoding subunits of the photosystem II complex. Solid colors indicate significantly different changes in expression ($p < 0.05$), transparent colors indicate nonsignificant changes. Source data for 5C are shown in **Figure 5—source data 3**.