



(a) Heatmap shows average protein expression of all genes contained within each proteome cluster (PC, n = 100), standardized first within treatment and then across all treatments. PC expression averages were then clustered hierarchically, shown by the represented dendogram. (b) The proportion of genes within each PC belonging to either of the two major RTs. Generally, PCs that were upregulated in N-replete conditions (i.e. PC57) were represented in RT102, while PCs that were upregulated in N-limited conditions (i.e. PC47) were more represented in RT184. Source data are provided as a Source Data file.



Supplementary Figure 2: Expression of transcription factors in the Nshort experiment.

Heatmap shows standardized transcript abundance of annotated transcription factors in *P. tricornutum* (Rayko et al. 2012). Dendogram shows hierarchical clustering (one-minus pearson, average linkage). Closed circles indicate nitrogen-replete time points, open circles indicate nitrogen-deplete time points. Black arrows indicate transcription factors implicated in the regulation of nitrogen metabolism (Matthijs et al. 2016) and the putative *NirA* homolog identified in *P. tricornutum*. Source data are provided as a Source Data file.



Supplementary Figure 3: Chloroplast localization of Fd-Nir and NAD(P)H-Nir in P. tricornutum.

Cells transformed with yellow fluorescent protein (YFP) tagged Fd-Nir and NAD(P)H-Nir were used to examine subcellular localization of both nitrite reductase isozymes in *P. tricornutum*. From left to right, micrographs show YFP fluorescence (yellow), location of chloroplast from detection of chlorophyll autofluorescence (red, Chl_{auto}), merged YFP-Chl_{auto}, and brightfield (BF).





YFP-Chl_{auto} merge



c Urease (mitochondria)



ΒF



YFP-Chl_{auto} Confocal



YFP-Chl_{auto} Confocal

Supplementary Figure 4: Subcellular localization of urea assimilation genes.

Cells transformed with yellow fluorescent protein (YFP) tagged (a) YFP-UT1, (b) YFP-UT3, and (c) YFP-urease were used to examine subcellular localization. (a,b) From left to right, micrographs show merged images of YFP fluorescence (yellow) with chlorophyll autofluorescence (red, Chl_{aub}), and brightfield (BF). (b,c) Composite 3D confocal image (far right in b), showing mitochondrial localization of UT3 (b) and urease (c).





(a) Cell concentration, C:N, and elemental composition of *P. tricornutum* grown in batch culture, TOC: Total organic carbon, TN: Total nitrogen (b) Metabolite composition of *P. tricornutum* from N_{short} experiment, max-normalized. Light grey values indicate metabolite was not detected. *Metabolites plotted in (c,d). (c) Isocaproic acid abundance from the N_{short} experiment. (d) Glutamine and α -ketoglutarate abundance from the N_{short} experiment. Source data are provided as a Source data file.



Supplementary Figure 6: Alanine transaminase phylogeny and subcellular localization predictions.

Expanded phylogeny of alanine transaminase (ALT) including diatom sequences from the MMETSP. There are two major diatom ALT clades, which are the result of a diatom-specific ALT duplication (indicated). Sequences from *P. tricornutum* are shown in red. Purple shows placement of an ALT domain trimmed from from an Aureococcus anophagefferens sequence, and is likely a contaminant. Pie charts to the right of each clade show the subcellular targeting predictions (TargetP) run on the untrimmed ORFs. Source data are provided as a Source Data file.

Supplementary Table 1: Molecular components of nitrogen response regulation from diverse eukaryotes and candidate homologs in *Phaeodactylum tricornutum*

Eukaryotic genes involved in the cellular response to nitrogen (also including *Synechococcus*) compiled from the literature. Accession sources are specified, including Uniprot, Joint Genome Institute (JGI, <u>https://genome.jgi.doe.gov/</u>), *Cyanidioschyzon merolae* Genome Project v3 (<u>http://czon.jp</u>), or the National Center for Biotechnology Information (NCBI, <u>https://www.ncbi.nlm.nih.gov</u>). Pfams associated with reference sequences were obtained from Uniprot, or by *de novo* search in the Pfam 32.0 database. IDs for candidate homologs in *P. tricornutum* (Phatr3 models) are listed when single-candidate or highly nitrate sensitive (HNS) or nitrate sensitive (NS) genes were identified (see methods). ND = not detected.

Transcriptional Regulators										
	Gene Name	Protein	Organism	Accession	PFam	Phatr3 Candidate	Reference(s)			
wHT H TF	ntcA	Global nitrogen regulator	SynechococcusA5GII0PF13545 HTHCRPsp.Uniprot(CL0123 Helix-turn-helix)		ND	Vega-Palas et al. 1992				
R2- R3 Myb	myb1	MYB-related protein	CyanidioschyzonCMJ282CPF00249 myb_DNA bindingmerolaeGenomePF13921 myb_DNA-bind_6(CL0123 Helix-turn-helix)		n = 21 n = 9	Imamura et al. 2009				
GATA-Type TF	Gln3	Nitrogen regulatory protein GLN3	Saccharomyces cerevisiae	P18494 Uniprot	PF00320 GATA (CL0167 Zinc beta ribbon)	ND	Ghaemmaghami et al. 2003			
	Gat1	Transcriptional regulatory protein GAT1	Saccharomyces cerevisiae	P43574 Uniprot	PF00320 GATA PF08550 DUF1752	ND ND	Ghaemmaghami et al. 2003			
	area	Nitrogen regulatory protein areA	Emericella nidulans	P17429 Uniprot	PF07573 AreA_N PF08550 DUF1752 PF00320 GATA	ND ND ND	Marzluf 1997 Todd 2016			
	nit-2	Nitrogen catabolic enzyme regulatory protein	Neurospora crassa	P19212 Uniprot	PF08550 DUF1752 PF00320 GATA	ND ND	Marzluf 1997 Todd 2016			
RWP- RK TF	Nit2	Nitrate assimilation regulatory protein	Chlamydomonas reinhardtii	Q2LGZ3 Uniprot	PF02042 RWP-RK (no CL)	ND	Sanz-Luque et al. 2015			
	Nlp7	Protein NLP7	Arabidopsis thaliana	Q84TH9 Uniprot	PF02042 RWP-RK	ND	Marchive et al. 2013			
Zn-clust TF	<i>nirA</i> Nitrogen assimilation transcription factor nirA		Emericella nidulans	P28348 Uniprot	PF04082 Fungal_trans PF00172 Zn_clust	ND n = 1 J50045	Marzluf 1997 Todd 2016			

Posttranscriptional/Posttranslational Regulators									
						Phatr3			
	Gene	Protein	Organism	Accession	PFam	Homolog	Reference(s)		
nmrA	nmrA	Nitrogen metabolite repression protein nmrA	Emericella nidulans	Q5AU62 Uniprot	PF05368 NmrA	- n = 5 J31822 - HNS	Marzluf 1997 Todd 2016		
	nmr	Nitrogen metabolite repression protein nmr	Neurospora crassa	P23762 Uniprot	PF05368 NmrA		Marzluf 1997 Todd 2016		
	PcNMRA4	Transcriptional regulator of biotrophy to necrotrophy transition	Phytophthora capsici	505845 Phyca11 JGI	PF05368 NmrA		Pham et al. 2018		
Gluta- redoxin	URE2	Transcriptional regulator URE2	Saccharomyces cerevisiae	P23202	PF00043 GST_CTER PF02798 GST_NTER	n = 7 n = 3	Coschigano & Magasanik 1991		
GC	CYG56	Soluble guanylate cyclase	Chlamydonomas reinhardtii	B6SE90 Uniprot	PF00211 Guanylate_cyc PF07700 HNOB PF07701 HNOBA	n = 29 ND ND	Sanz-Luque et al. 2014		
Tandem Zn- finger	CrNZF1	Nitrate Zinc Finger 1	Chlamydomonas reinhardtii	512330 Chlre4 JGI	PF00642 zf-CCCH	n = 8 J50624 - HNS	Higuera et al. 2014		
uncated noglobin	THB1	Putative truncated hemoglobin	ative truncated hemoglobin Chlamydomonas A8JAR4 reinhardtii Uniprot PF01152 Bac_globin		-n = 4	Sanz-Luque et al. 2015			
	HaNR2- 2/2HbN	Nitrate reductase, truncated hemoglobin fusion	Heterosigma akashiwo	JN831656 genbank	PF01152 Bac_globin	J37957 - HNS	Stewart and Coyne 2011		
Tr Hei	CsNR2- 2/2HbN	Nitrate reductase, truncated hemoglobin fusion	Chattonella subsalsa	JN831658 genbank	PF01152 Bac_globin				
Kinase	MPK6	Mitogen-activated protein kinase 6	Arabidopsis thaliana	Q39026 Uniprot	PF00069 Pkinase	n = 125 J21961 - HNS J15229 - HNS J5286 - NS J46233 - NS	Wang et al. 2011		
IId	GLB1	Nitrogen regulatory protein PII	Chlamydomonas reinhardtii	A8JI83 Uniprot	PF00543 P-II	ND	Uhrig et al. 2009		
ribosome binding GTPase	NOAI	NO-associated protein 1, chloroplastic/mitochondrial (note: role in NO production dubious)	Arabidopsis thaliana	Q66GP9 Uniprot	PF01926 MMR_HSR1	n = 28 J40200	Moreau et al. 2008, Vardi et al. 2008		

Supplementary Table 2: MEME Output

MEME version 5.0.4 ALPHABET= ACGT strands: + -Background letter frequencies (from unknown source): A 0.270 C 0.230 G 0.230 T 0.270 MOTIF 1 log-odds matrix: alength= 4 w= 13 E= 9.1e-018 31 34 62 -369 143 -247 -114 -111 155 -346 -188 -111 -88 112 -11 -111 139 -1223 -88 -47 -1223 -1223 123 76 -1223 203 -247 -369 -89 186 -1223 -369 -369 -247 203 -1223 -1223 -1223 212 -1223 -47 -1223 -1223 163 189 -1223 -1223 -1223 -52 -188 166 -369 letter-probability matrix: alength= 4 w= 13 nsites= 48 E= 9.1e-018 0.333333 0.291667 0.354167 0.020833 0.729167 0.041667 0.104167 0.125000 0.791667 0.020833 0.062500 0.125000 0.250000 0.125000 0.500000 0.125000 0.000000 0.125000 0.166667 0.708333 0.000000 0.000000 0.541667 0.458333

0.000000 0.000000 0.541667 0.458333 0.000000 0.937500 0.041667 0.020833 0.145833 0.833333 0.000000 0.020833 0.020833 0.041667 0.937500 0.000000 0.000000 0.000000 1.000000 0.000000 0.833333 0.166667 0.000000 0.000000 1.000000 0.000000 0.000000 0.187500 0.062500 0.729167 0.020833 MEME version 5.0.4

ALPHABET= ACGT

strands: + -

Background letter frequencies (from unknown source): A 0.270 C 0.230 G 0.230 T 0.270

MOTIF 2

log-odds	matrix	: alen	gth= 4	w =	13	E =	2.5e-013
46	106	-264	-128				
99	-164	17	-106				
-1240	117	-18	4				
-1240	-205	-205	172				
89	-1240	106	-386				
189	-1240	-1240	-1240				
-1240	198	-1240	-154				
-6	-264	-264	131				
-286	-1240	206	-1240				
-286	-363	-1240	181				
104	-1240	95	-1240				
189	-1240	-1240	-1240				
60	-131	69	-106				

letter-probability matrix: alength= 4 w= 13 nsites= 54 E= 2.5e-013 0.370370 0.481481 0.037037 0.111111 0.537037 0.074074 0.259259 0.129630 0.000000 0.518519 0.203704 0.277778 0.000000 0.055556 0.055556 0.888889 0.500000 0.000000 0.481481 0.018519 1.000000 0.000000 0.000000 0.000000 0.000000 0.907407 0.000000 0.092593 0.259259 0.037037 0.037037 0.666667 0.037037 0.000000 0.962963 0.000000 0.037037 0.018519 0.000000 0.944444 0.555556 0.000000 0.444444 0.000000 1.000000 0.000000 0.000000 0.000000 0.407407 0.092593 0.370370 0.129630

Supplementary Table 3: Sample details, library names, and corresponding accessions for transcriptomes from the N_{short} experiment.

Files are available at the NCBI Sequence Read Archive (SRA) under the identifier PRJNA311568. Replicate experiments are indicated (A or B). Sample ID corresponds to label in Supplementary Dataset 1.

Rep.	Time point	N Source	Sample ID	Library	Accession
А	Pretreatment 1 (exp)	NH4+	Pre 1 A	<u>NH4 (NH4A-PE-IL1-1)</u>	SRX1922057
А	Pretreatment 2 (2 hr)	N-	Pre ² A	N-free (NFREEA-PE-IL2-1)	SRX1922055
Α	Pretreatment 3 (90 min)	NO3-	Pre_3_A	NO3 spike (NO3SPIKEA-PE-IL3-1)	SRX1922059
Α	15 min	NO3-	NO34_A	T15 NO3 (T15NO3A-PE-IL5-1)	SRX1922074
А	15 min	NO2-	NO2- 4 A	T15 NO2 (T15NO2A-PE-IL6-1)	SRX1922065
Α	15 min	NH4+	NH4+4A	T15 NH4 (T15NH4A-PE-IL7-1)	SRX1922063
Α	15 min	N-	N4_A	T15 N-free (T15NFREEA-PE-IL4-1)	SRX1922061
Α	45 min	NO3-	NO35_A	T45 NO3 (T45NO3A-PE-IL9-1)	SRX1922093
Α	45 min	NO2-	NO25_A	T45 NO2 (T45NO2A-PE-IL10-1)	SRX1922091
Α	45 min	NH4+	NH4+_5_A	<u>T45 NH4 (T45NH4A-PE-IL11-1)</u>	SRX1922089
Α	45 min	N-	N5_A	T45 N-free (T45NFREEA-PE-IL8-1)	SRX1922076
Α	18 hr	NO3-	NO36_A	T-end NO3 (TENDNO3A-PE-IL26-1)	SRX1922107
Α	18 hr	NO2-	NO26_A	T-end NO2 (TENDNO2A-PE-IL27-1)	SRX1922105
Α	18 hr	NH4+	NH4+_6_A	T-end NH4 (TENDNH4A-PE-IL28-1)	SRX1922097
Α	18 hr	N-	N6_A	T-end N-free (TENDNFREEA-PE-IL12-1)	SRX1922095
В	Pretreatment 1 (exp)	NH4+	Pre_1_B	<u>NH4 (NH4B-PE-IL1-1)</u>	SRX1922058
В	Pretreatment 2 (2 hr)	N-	Pre_2_B	N-free (NFREEB-PE-IL2-1)	SRX1922056
В	Pretreatment 3 (90)	NO3-	Pre_3_B	NO3 spike (NO3SPIKEB-PE-IL3-1)	SRX1922060
В	15 min	NO3-	NO34_B	T15 NO3 (T15NO3B-PE-IL5-1)	SRX1922075
В	15 min	NO2-	NO24_B	T15 NO2 (T15NO2B-PE-IL6-1)	SRX1922073
В	15 min	NH4+	$NH4+_4B$	T15 NH4 (T15NH4B-PE-IL7-1)	SRX1922064
В	15 min	N-	N4_B	T15 N-free (T15NFREEB-PE-IL4-1)	SRX1922062
В	45 min	NO3-	NO35_B	T45 NO3 (T45NO3B-PE-IL9-1)	SRX1922094
В	45 min	NO2-	NO25_B	T45 NO2 (T45NO2B-PE-IL10-1)	SRX1922092
В	45 min	NH4+	$NH4+_5_B$	<u>T45 NH4 (T45NH4B-PE-IL11-1)</u>	SRX1922090
В	45 min	N-	N5_B	T45 N-free (T45NFREEB-PE-IL8-1)	SRX1922088
В	18 hr	NO3-	NO36_B	T-end NO3 (TENDNO3B-PE-IL26-1)	SRX1922108
В	18 hr	NO2-	NO26_B	T-end NO2 (TENDNO2B-PE-IL27-1)	SRX1922106
В	18 hr	NH4+	$NH4+_6_B$	T-end NH4 (TENDNH4B-PE-IL28-1)	SRX1922098
В	18 hr	N-	N6_B	T-end N-free (TENDNFREEB-PE-IL12-1)	SRX1922096

Supplementary Table 4: Sample details and corresponding accessions for proteome from the N_{short} experiment.

Files are available at the Proteomics IDEntifications (PRIDE) database under the identifier PXD015061. Samples are from N_{short} biological replicate experiment B. Pre-treatment samples: N-Free +2h B and NO3 SPIKE B. Nitrogen treatments correspond to N-free B, NO2 B, NO3 B, and NH4 B for nitrogen free, nitrite, nitrate, and ammonium respectively. T-15, T-45, and T-end correspond to 15 min, 45. Min, and 18 hr sampling times respectively.

Dataset	Accession	113 ion	114 ion	115 ion	116 ion	117 ion	118 ion	119 ion	121 ion
Andy0016_1_11Jul13_Pippin_12-12-39	112779	16, N-Free +2h B	18, NO3 SPIKE B	20, T-15 N-free B	22, T-15 NO3 B	28, T-45 N-Free B	30, T-45 NO3 B	36, T-end N-free B	38, T-end NO3 B
Andy0016_2_11Jul13_Pippin_12-12-39	112778	16, N-Free +2h B	18, NO3 SPIKE B	20, T-15 N-free B	22, T-15 NO3 B	28, T-45 N-Free B	30, T-45 NO3 B	36, T-end N-free B	38, T-end NO3 B
Andy0016_3_11Jul13_Pippin_12-12-39	112777	16, N-Free +2h B	18, NO3 SPIKE B	20, T-15 N-free B	22, T-15 NO3 B	28, T-45 N-Free B	30, T-45 NO3 B	36, T-end N-free B	38, T-end NO3 B
Andy0016_4_11Jul13_Pippin_12-12-39	112788	16, N-Free +2h B	18, NO3 SPIKE B	20, T-15 N-free B	22, T-15 NO3 B	28, T-45 N-Free B	30, T-45 NO3 B	36, T-end N-free B	38, T-end NO3 B
Andy0016_5_11Jul13_Pippin_12-12-39	112776	16, N-Free +2h B	18, NO3 SPIKE B	20, T-15 N-free B	22, T-15 NO3 B	28, T-45 N-Free B	30, T-45 NO3 B	36, T-end N-free B	38, T-end NO3 B
Andy0016_6_11Jul13_Pippin_12-12-39	112787	16, N-Free +2h B	18, NO3 SPIKE B	20, T-15 N-free B	22, T-15 NO3 B	28, T-45 N-Free B	30, T-45 NO3 B	36, T-end N-free B	38, T-end NO3 B
Andy0016_7_11Jul13_Pippin_12-12-39	112775	16, N-Free +2h B	18, NO3 SPIKE B	20, T-15 N-free B	22, T-15 NO3 B	28, T-45 N-Free B	30, T-45 NO3 B	36, T-end N-free B	38, T-end NO3 B
Andy0016_8_11Jul13_Pippin_12-12-39	112786	16, N-Free +2h B	18, NO3 SPIKE B	20, T-15 N-free B	22, T-15 NO3 B	28, T-45 N-Free B	30, T-45 NO3 B	36, T-end N-free B	38, T-end NO3 B
Andy0016_9_11Jul13_Pippin_12-12-39	112774	16, N-Free +2h B	18, NO3 SPIKE B	20, T-15 N-free B	22, T-15 NO3 B	28, T-45 N-Free B	30, T-45 NO3 B	36, T-end N-free B	38, T-end NO3 B
Andy0016_10_11Jul13_Pippin_12-12-39	112785	16, N-Free +2h B	18, NO3 SPIKE B	20, T-15 N-free B	22, T-15 NO3 B	28, T-45 N-Free B	30, T-45 NO3 B	36, T-end N-free B	38, T-end NO3 B
Andy0016_11_11Jul13_Pippin_12-12-39	112773	16, N-Free +2h B	18, NO3 SPIKE B	20, T-15 N-free B	22, T-15 NO3 B	28, T-45 N-Free B	30, T-45 NO3 B	36, T-end N-free B	38, T-end NO3 B
Andy0016_12_11Jul13_Pippin_12-12-39	112784	16, N-Free +2h B	18, NO3 SPIKE B	20, T-15 N-free B	22, T-15 NO3 B	28, T-45 N-Free B	30, T-45 NO3 B	36, T-end N-free B	38, T-end NO3 B
Andy0017_1_8Jul13_Pippin_12-12-39	112772	16, N-Free +2h B	18, NO3 SPIKE B	24, T-15 NO2 B	26, T-15 NH4 B	32, T-45 NO2 B	34, T-45 NH4 B	40, T-end NO2 B	42, T-end NH4 B
Andy0017_2_8Jul13_Pippin_12-12-39	112783	16, N-Free +2h B	18, NO3 SPIKE B	24, T-15 NO2 B	26, T-15 NH4 B	32, T-45 NO2 B	34, T-45 NH4 B	40, T-end NO2 B	42, T-end NH4 B
Andy0017_3_8Jul13_Pippin_12-12-39	112789	16, N-Free +2h B	18, NO3 SPIKE B	24, T-15 NO2 B	26, T-15 NH4 B	32, T-45 NO2 B	34, T-45 NH4 B	40, T-end NO2 B	42, T-end NH4 B
Andy0017_4_Rerun_11Jul13_Pippin_12-12-39	112794	16, N-Free +2h B	18, NO3 SPIKE B	24, T-15 NO2 B	26, T-15 NH4 B	32, T-45 NO2 B	34, T-45 NH4 B	40, T-end NO2 B	42, T-end NH4 B
Andy0017_5_8Jul13_Pippin_12-12-39	112771	16, N-Free +2h B	18, NO3 SPIKE B	24, T-15 NO2 B	26, T-15 NH4 B	32, T-45 NO2 B	34, T-45 NH4 B	40, T-end NO2 B	42, T-end NH4 B
Andy0017_6_8Jul13_Pippin_12-12-39	112782	16, N-Free +2h B	18, NO3 SPIKE B	24, T-15 NO2 B	26, T-15 NH4 B	32, T-45 NO2 B	34, T-45 NH4 B	40, T-end NO2 B	42, T-end NH4 B
Andy0017_7_8Jul13_Pippin_12-12-39	112793	16, N-Free +2h B	18, NO3 SPIKE B	24, T-15 NO2 B	26, T-15 NH4 B	32, T-45 NO2 B	34, T-45 NH4 B	40, T-end NO2 B	42, T-end NH4 B
Andy0017_8_8Jul13_Pippin_12-12-39	112781	16, N-Free +2h B	18, NO3 SPIKE B	24, T-15 NO2 B	26, T-15 NH4 B	32, T-45 NO2 B	34, T-45 NH4 B	40, T-end NO2 B	42, T-end NH4 B
Andy0017_9_8Jul13_Pippin_12-12-39	112792	16, N-Free +2h B	18, NO3 SPIKE B	24, T-15 NO2 B	26, T-15 NH4 B	32, T-45 NO2 B	34, T-45 NH4 B	40, T-end NO2 B	42, T-end NH4 B
Andy0017_10_8Jul13_Pippin_12-12-39	112780	16, N-Free +2h B	18, NO3 SPIKE B	24, T-15 NO2 B	26, T-15 NH4 B	32, T-45 NO2 B	34, T-45 NH4 B	40, T-end NO2 B	42, T-end NH4 B
Andy0017_11_8Jul13_Pippin_12-12-39	112991	16, N-Free +2h B	18, NO3 SPIKE B	24, T-15 NO2 B	26, T-15 NH4 B	32, T-45 NO2 B	34, T-45 NH4 B	40, T-end NO2 B	42, T-end NH4 B
Andy0017_12_8Jul13_Pippin_12-12-39	112790	16, N-Free +2h B	18, NO3 SPIKE B	24, T-15 NO2 B	26, T-15 NH4 B	32, T-45 NO2 B	34, T-45 NH4 B	40, T-end NO2 B	42, T-end NH4 B