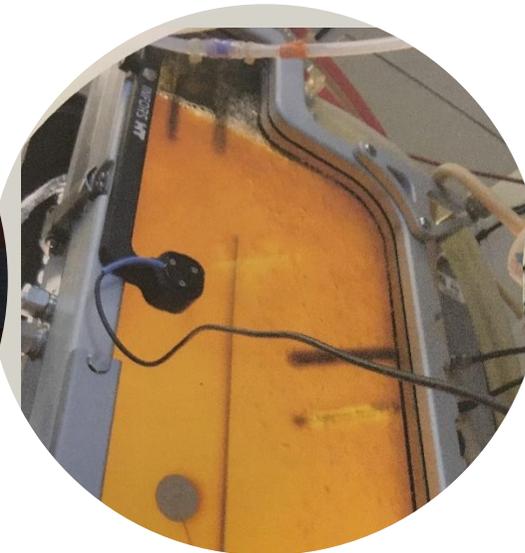

Highly controlled culturing parameters and “omics” analysis in the diatom *Phaeodactylum tricornutum* under N limitation

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Amsterdam, Dec 4-6, 2018



Presentation content



Background



Ilse Remmers, PhD

Remmers I., D'Adamo* S. et al. Nov 2018, 35, pp 33-49 Algal Research



DESIGN

Experimental set up



STORY

Major responses to N limitation

Physiological

Molecular



Conclusion and Perspectives



Phaeodactylum tricornutum a model diatom



- ✓ Enough genetic and physiological data available
- ✓ Emerging platform for genetic engineering
- ✓ TAG producer
- ✓ Natural source of PUFAs (such as EPA)
- ✓ Robust marine and industrially relevant strain
- ✓ Low silica requirements



Source:
<https://genome.jgi.doe.gov/Phatr2/Phatr2.home.html>



Understanding the nitrogen limitation effect under steady state growth and day night cycles



- State of art:

- ✓ -N promotes storage compound accumulation (lipids and sugars)
- ? Fragmented information about the molecular mechanisms

- Proposed solution:

- ✓ highly controlled culturing environment: PBR and steady state growth
- ✓ simultaneous and comprehensive analysis through “Omics” techniques



Nitrogen limitation- experimental set up



LED lamps warm spectrum,
light supply:
 $260 \mu\text{mol m}^2 \text{s}^{-1}$.

Light path: 0.02 m.

Air enriched with 1% CO_2

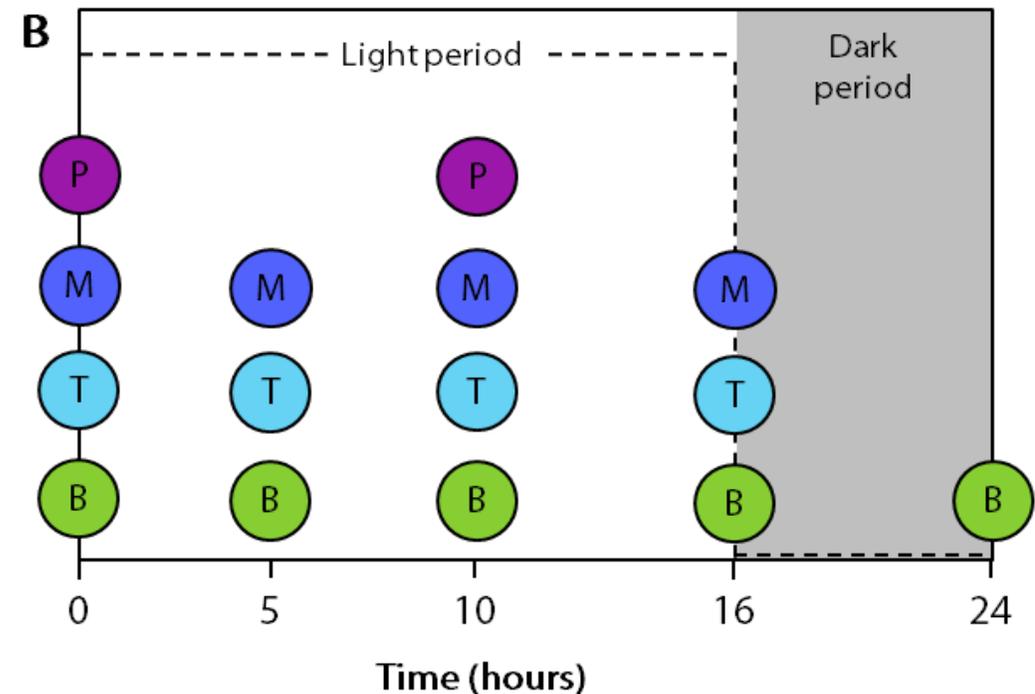
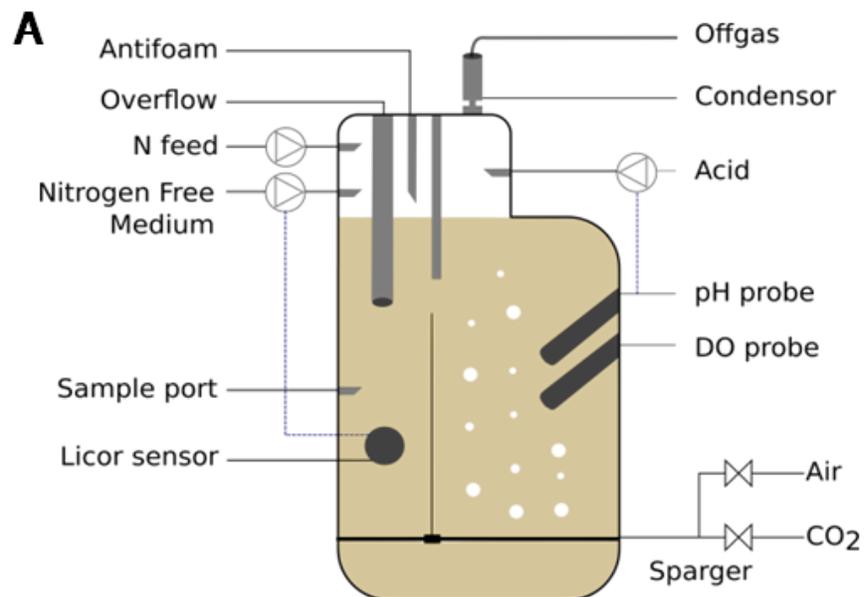
T 20C
pH 7.2

1.7L air lift flat panel PBR

Photoperiod: 16h Light : 8h
Dark

Steady state growth: the turbidostat-nutristat approach

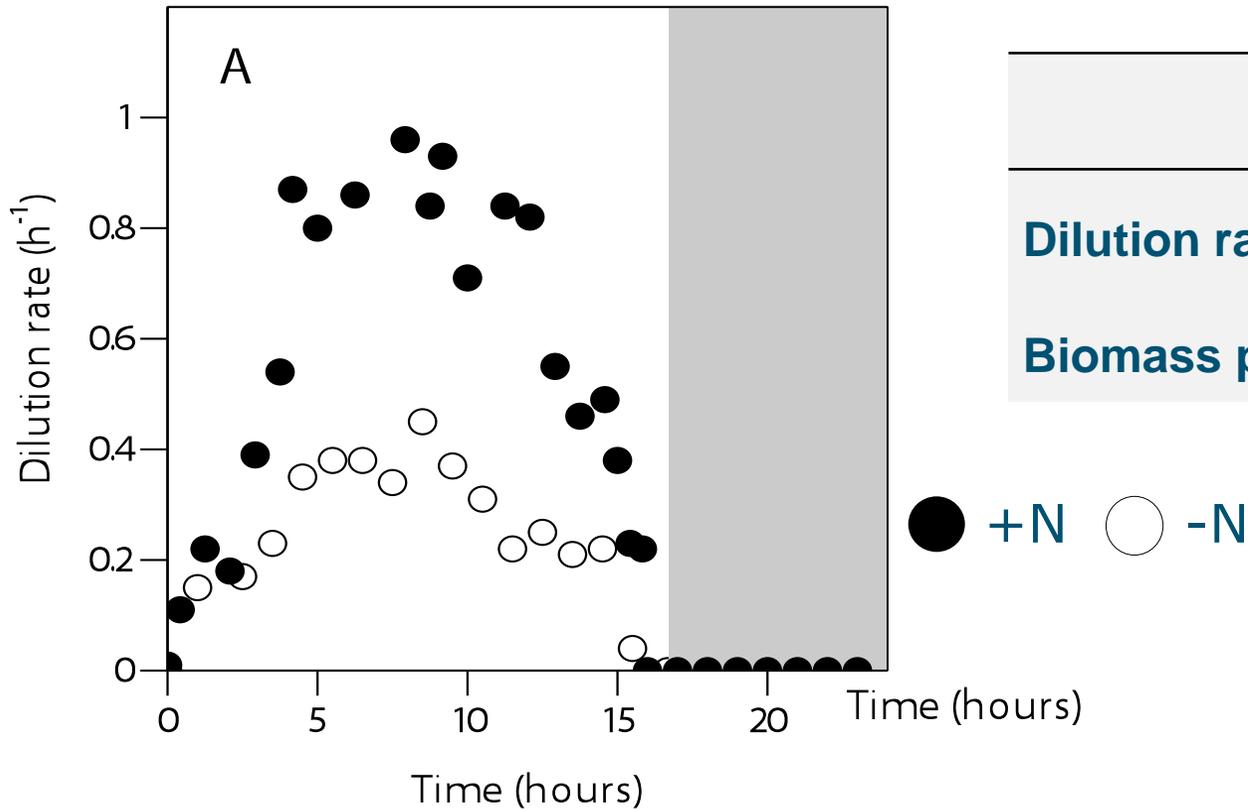
- Separate nitrogen (N) feeds: constant supply rate of 0.11 gN day^{-1} for N+ and 0.02 gN day^{-1} for N-
- Fresh medium valve (containing no nitrogen) opens when the outgoing light intensity dropped below $10 \mu\text{mol m}^2 \text{ s}^{-1}$.



Physiological response to nitrogen limitation



Reduced biomass productivity (↓ 3X)



Parameter	N +	N-
Dilution rate (day^{-1})	0.42 ± 0.02	0.11 ± 0.02
Biomass productivity ($g L^{-1} day^{-1}$)	0.68 ± 0.07	0.25 ± 0.04

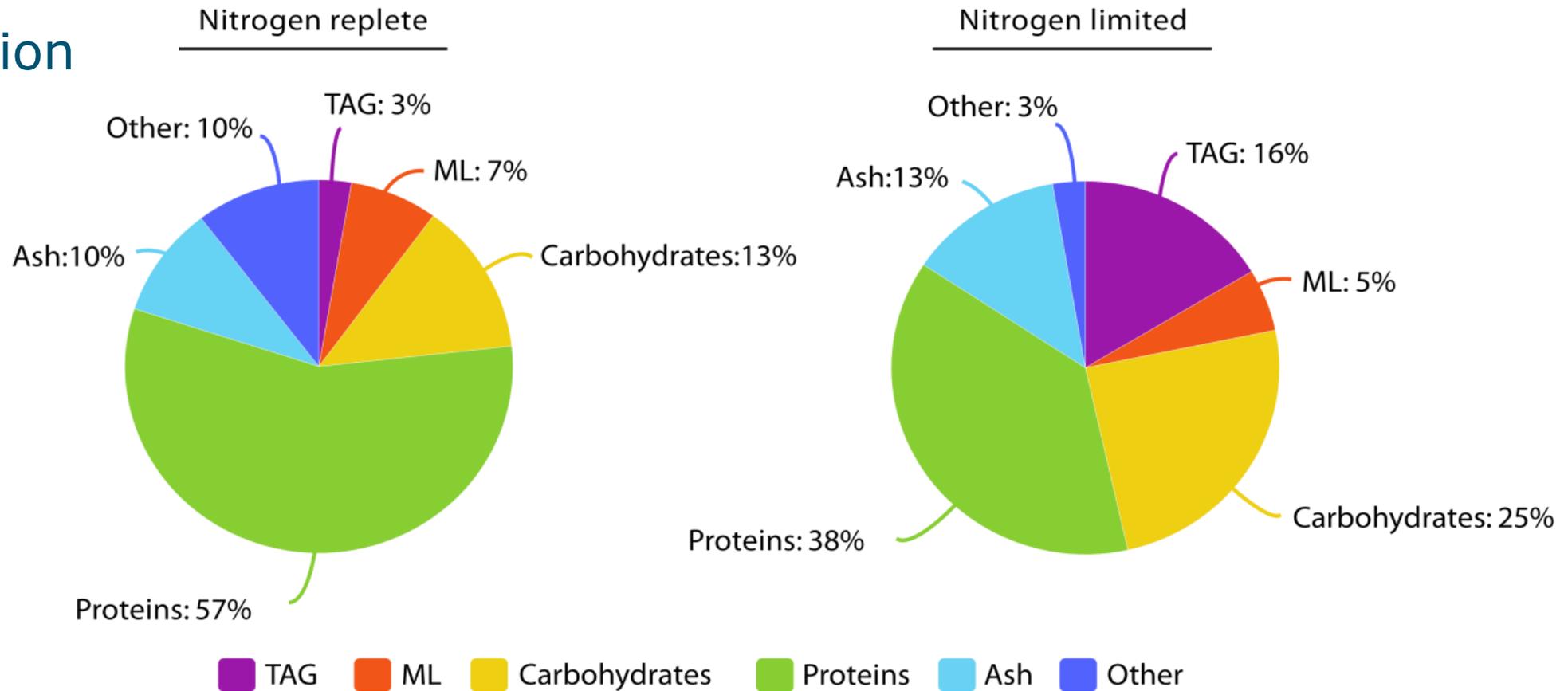
Oscillation pattern unaffected



Physiological response to nitrogen limitation



Biomass composition



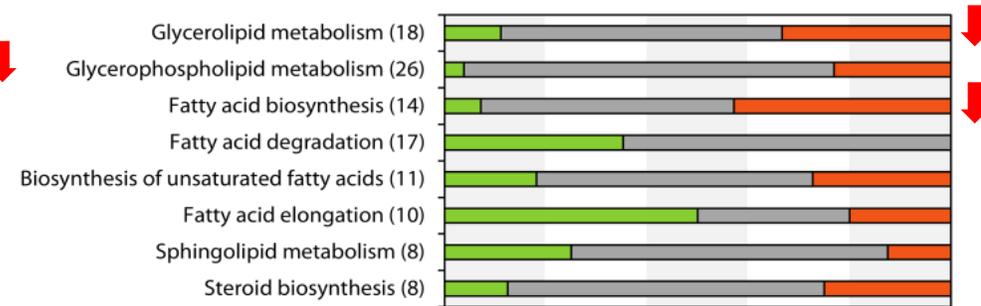
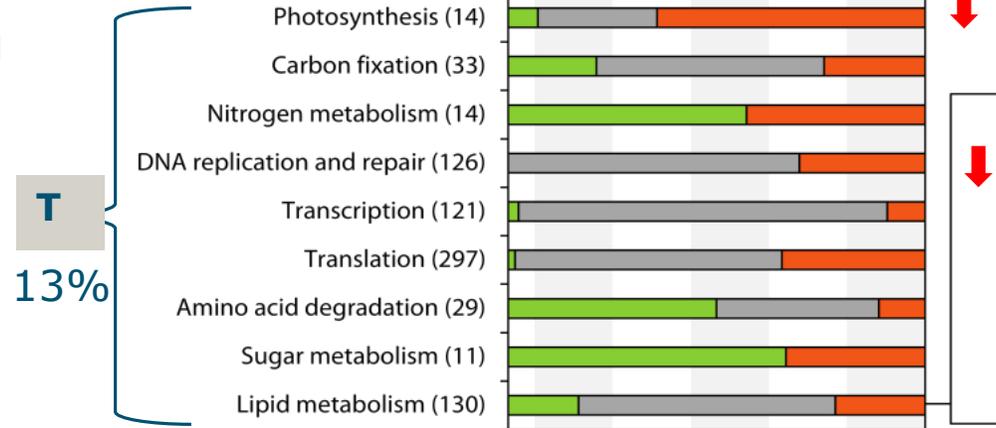
TAG and total carbohydrate content increased at the expense of protein and membrane lipids



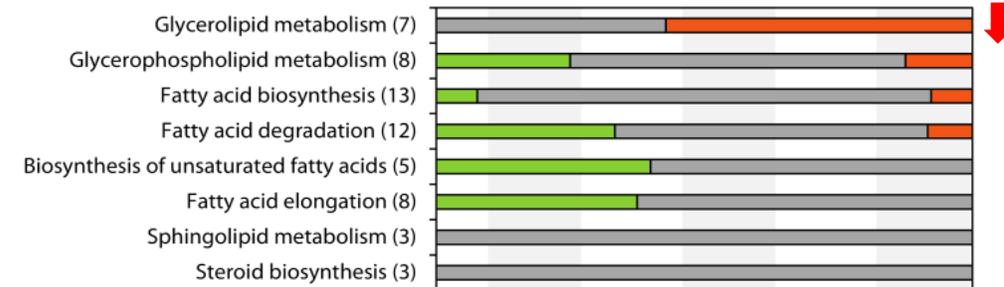
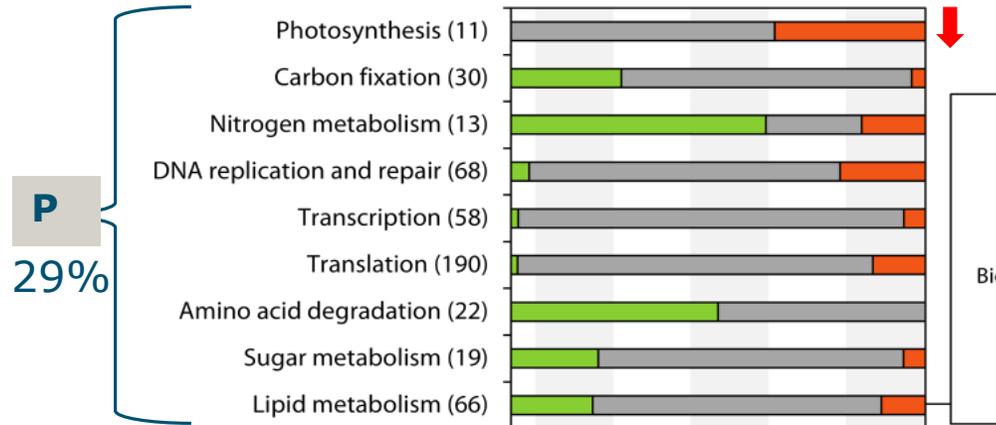
The effect of nitrogen limitation on the main cellular pathways (KEGG pathways)



- DNA replication photosynthesis downregulated



- Sugar and nitrogen metabolism upregulated



- Up regulated genes or proteins
- Not differential regulated genes or proteins
- Down regulated genes or proteins
- (#) Total number of annotated genes or proteins in KEGG pathway

Looking at the most differentially regulated genes and proteins



Feature ID	T 0	T 10	P 0	P 10h	Annotation/ Predicted domain
Phatr3_J48383	9.24	5.52		0.49	Predicted protein, diatom specific
Phatr3_EG01955	8.13	6.40			CC1/ammonium transporter
Phatr3_J50149	6.82	5.28			sodium/dicarboxylate transporter (aminoacid transporter)
Phatr3_J55010	6.79	5.82	10.53	12.28	coccolith scale associated protein
Phatr3_J50390	6.49	5.26			cell wall regeneration glycoprotein
Phatr3_J38074	6.13	5.91			predicted protein
Phatr3_EG01401	6.13	4.94			
Phatr3_J51797	6.07	5.80	4.61	4.19	FRU3/frustulin
Phatr3_EG00333	5.99	6.92	10.24	8.83	FAD/NAD(P)-binding domain superfamily: Flavin containing amine oxidoreductase
Phatr3_EG01690	5.93	5.23	4.70		integral component of membrane/ TRAM/LAG1/CLN8 homology domain
Phatr3_EG02032	5.72	4.70			AdoMEt domain protein
Phatr3_J32906	5.66	4.18			predicted protein
Phatr3_J47571	5.58	5.48			*probably misassembled, signal peptide
Phatr3_J47573	5.48	5.25			*probably misassembled, signal peptide
Phatr3_J47572	5.37	5.80			*probably misassembled, signal peptide
Phatr3_J47569	5.28	4.96			*probably misassembled, truncated signal peptide
Phatr3_J50084	5.24	4.84	8.52	7.15	peroxiredoxin/thioredoxin
Phatr3_J49734	5.23	5.02			ado-Met domain containing
Phatr3_J49200	5.15	5.39			predicted protein, no homologies, signal peptide
Phatr3_J44641	5.15	3.92	4.99	4.49	bHlCmyc containing n ter domain
Phatr3_J54686	5.09	0.81	1.20	1.62	1,3-beta-D-glucan phosphorylase
Phatr3_J10852	5.05	4.91			Fatty acid hydroxylase domain/sterol metabolism
Phatr3_EG01364	5.02	5.56			Galactose-3-O-sulfotransferase
Phatr3_J44347	5.00	6.23			predicted protein, conserved
Phatr3_J49567	4.37	5.49			HSF-type DNA-binding;
Phatr3_EG01952		5.46			CLC voltage-gated chloride channel.
Phatr3_J40228	4.02	5.15			possible porine
Phatr3_J48357	3.56	5.08			nodZ of ucosylation
Phatr3_J44100		4.94			predicted protein
Phatr3_J50401	4.74	2.77	7.30	7.15	amidase domain containing
Phatr3_J13951		3.10	5.31	5.61	Glutamate dehydrogenase
Phatr3_J37719		3.91	2.45	4.75	5.36 Acetamidase/formamidase
Phatr3_J1379		3.18	1.68	3.86	4.49 leucine-rich repeat receptor-like protein kinase
Phatr3_EG01099		2.31	2.23	5.60	4.49 a/b hydrolase
Phatr3_EG02356			5.33	5.36	

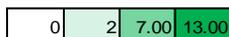
Cell wall/membrane components

Glycosilation

N metabolism

Feature ID	T 0	T 10	P 0	P 10h	Annotation/ Predicted domain
Phatr3_EG01529	-6.25	-5.82			Predicted protein
Phatr3_J37038	-5.98	-3.04			redox protein
Phatr3_J12902	-5.30		-0.46		ferredoxin nitrite reductase
Phatr3_J49286	-5.13	-4.90	-4.05	-3.92	Predicted protein
Phatr3_J50738	-4.54			-0.77	Triosephosphate isomerase
Phatr3_J47674	-4.47	-6.30	-5.78	-5.97	ISIP3/iron-starvation-induced proteins
Phatr3_J32984	-4.12	-3.86			Predicted protein
Phatr3_J54465	-4.11	-4.78	-4.66	-4.87	ISIP2A/iron-starvation-induced proteins
Phatr3_J54987	-4.09	-9.46			ISIP2B/iron-starvation-induced proteins
Phatr3_EG00885	-4.04	-5.34			
Phatr3_J28056	-3.95	-3.91			Cobalamin-independent methionine synthase (MetE)
Phatr3_J55031		-7.66	-1.93	-5.31	ISIP1/ iron-starvation-induced proteins
Phatr3_J23658	-1.10	-6.54			flavodoxin
Phatr3_J51183		-6.41	-1.16	-2.11	FMN binding domain
Phatr3_J54986	-2.15	-6.10	-1.34	-4.07	cell surface protein/ similar to metallo beta lactamase
Phatr3_J52498	-1.72	-4.43			cell surface protein/similar to GPI-anchored Fasciclin-like arabinogalactan protein
Phatr3_J46929		-4.39	-2.11	-4.34	abc transporter
Phatr3_J48527	-2.92	-4.32			
Phatr3_J15815	-2.97	-4.09			H ⁺ -Ppases (pyrophosphate proton pump)
Phatr3_J9040			-4.02		ZDS/Zeta-carotene desaturase
Phatr3_J5928	-1.74		-3.72	-1.63	ZEP2/zeaxanthin epoxidase
Phatr3_J45845				-1.91	ZEP1/zeaxanthin epoxidase
Phatr3_J36048			-2.24		VDL1/violaxanthin deepoxidase-like protein
Phatr3_J41423		-2.67		-3.97	FABC5/aldolase
Phatr3_EG00319				-3.77	Predicted protein
Phatr3_J40199	-1.93			-3.53	immunophilin? DUF 1995 containing protein
Phatr3_J43701			-1.94	-3.24	APC7/PEP-CTERM system TPR-repeat lipoprotein
Phatr3_J48921				-5.48	Apolipoprotein

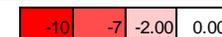
Redox process



Log 2 fold

No significantly differentially expressed

Not present



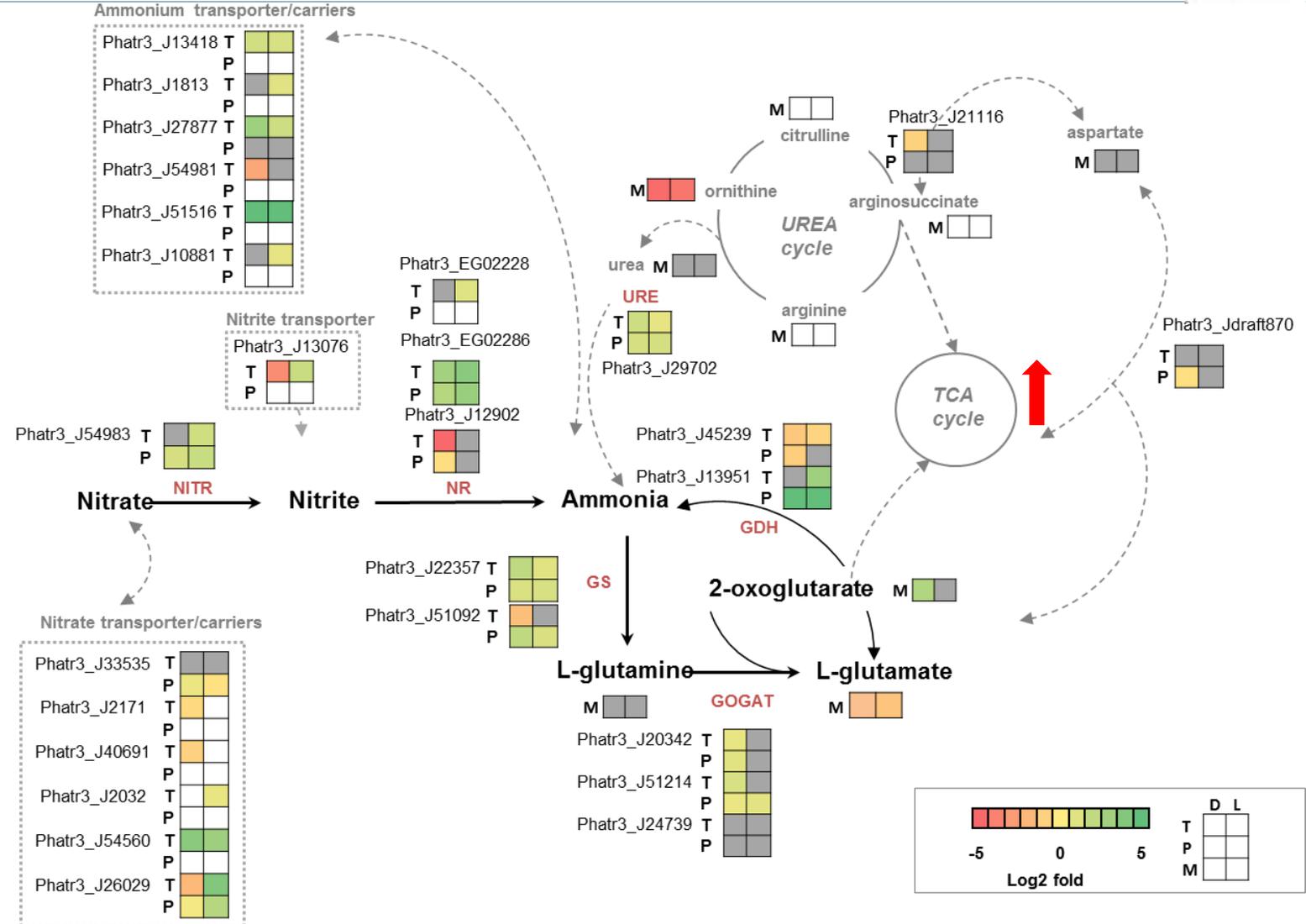
Log 2 fold

T=transcript, P=protein, 0h (Dark), 10h (Light)



Cells in need of nitrogen: scavenger mechanisms

- Nitrogen metabolism-related transcripts and proteins upregulated
- Coupling between nitrogen assimilation, OUC and carbon metabolism via TCA cycle



No significantly differentially expressed
 Not present

T=transcript, P=protein, 0h (Dark), 10h (Light)

Carbon central metabolism and mitochondrial engagement



- Intricate carbon metabolism and regulation
- Mitochondrion possibly engaged in the carbon allocation during nitrogen limitation

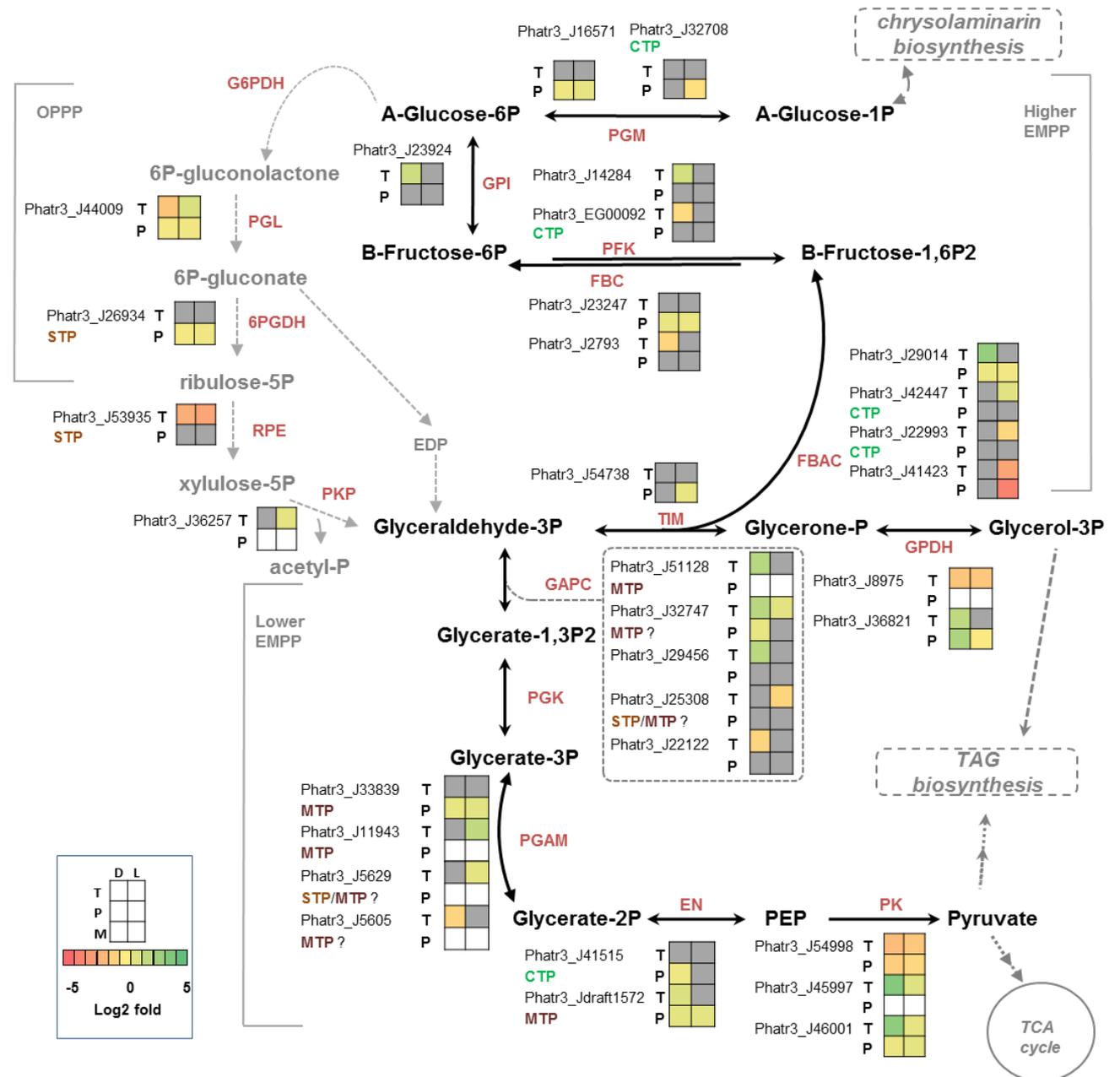
No significantly differentially expressed

Not present

T=transcript, P=protein, 0h (Dark), 10h (Light)

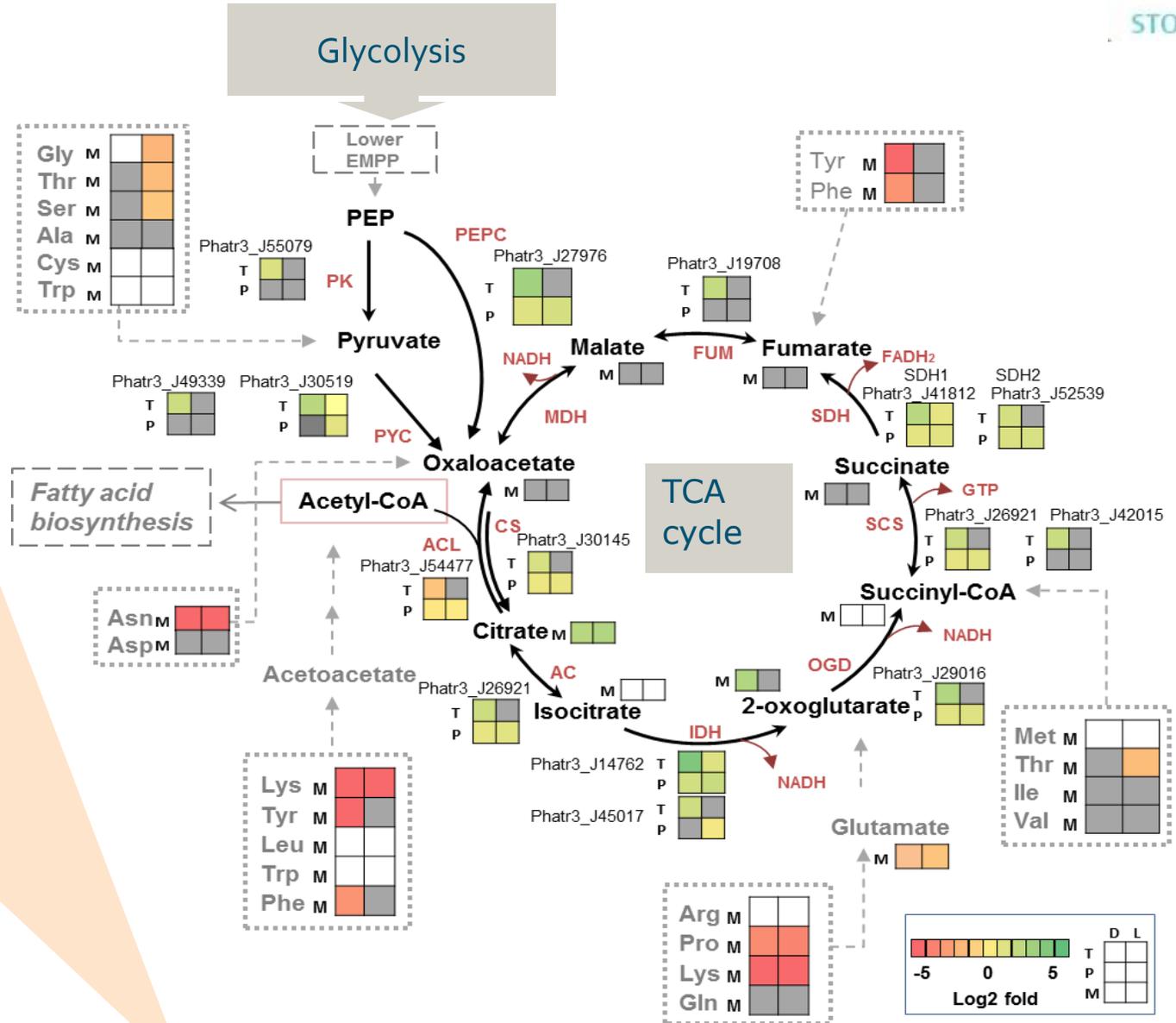
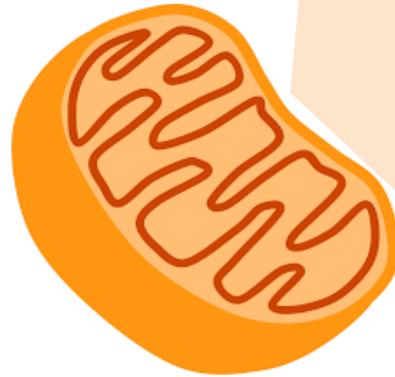


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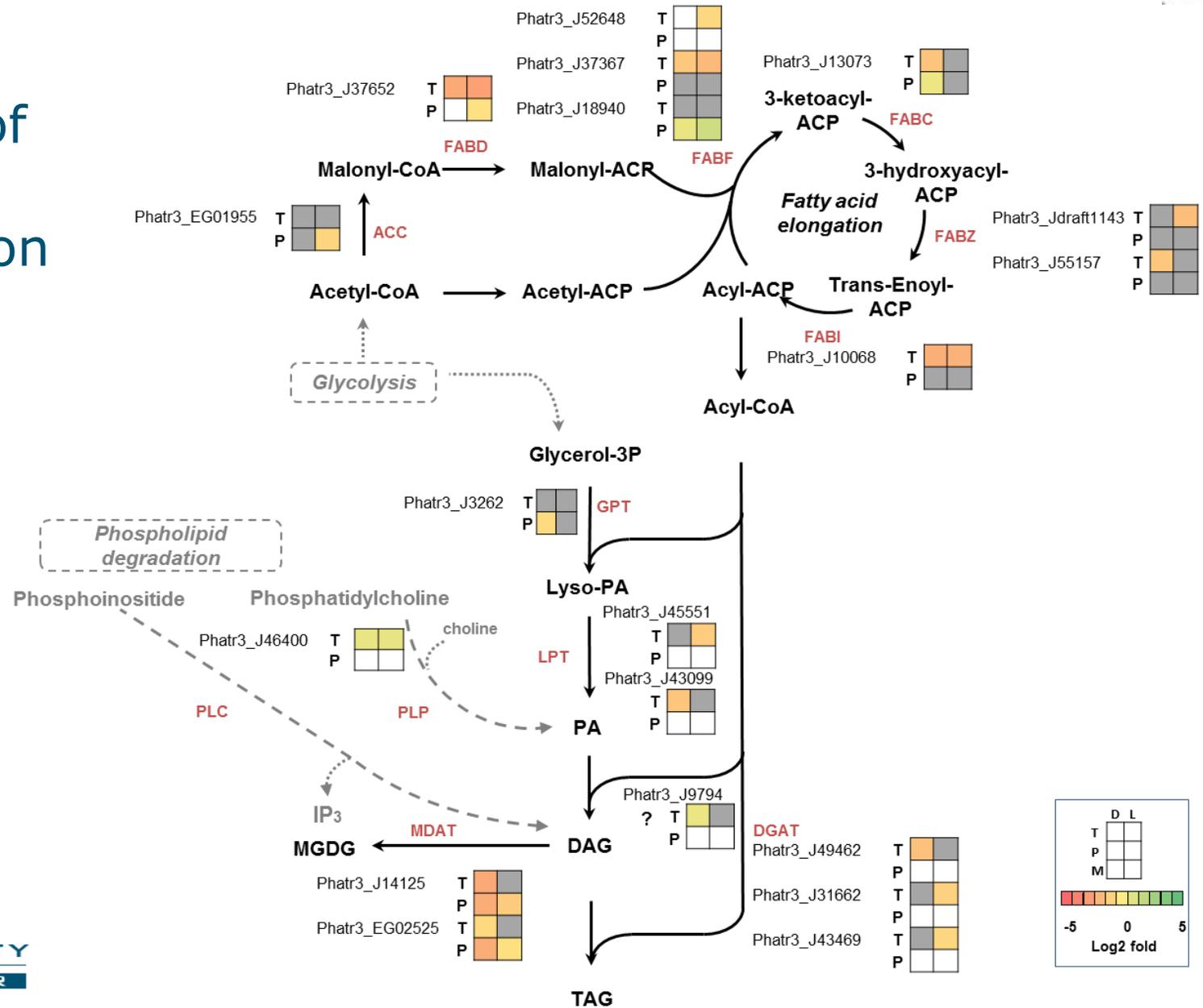
Carbon metabolism and mitochondrial engagement

- Cell metabolism is directed to recycle components and, to use the energy and reducing power through TCA cycle



Lipid metabolism and lipid rearrangements

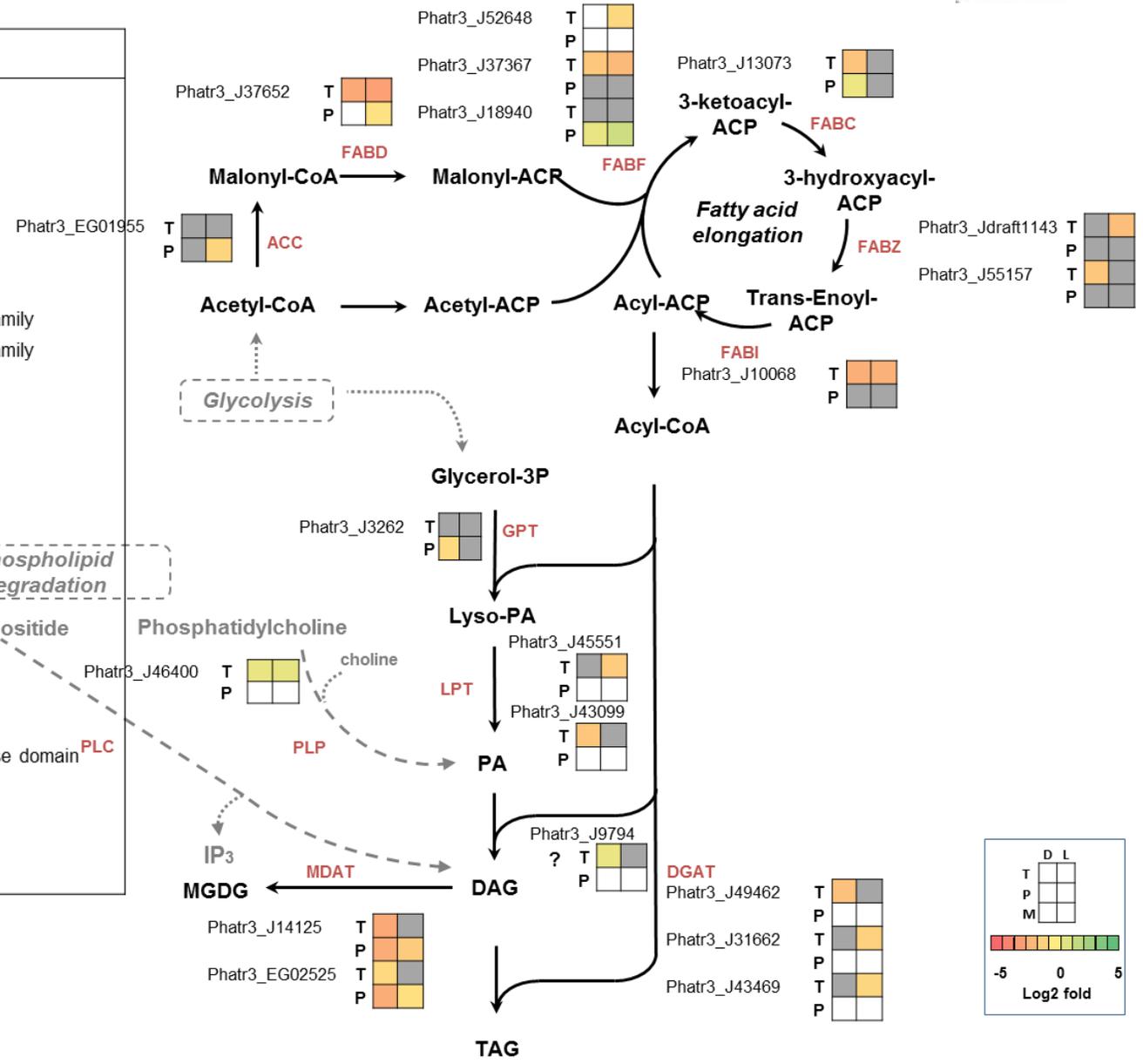
- No clear upregulation of genes and proteins related to the conversion of acyl-CoA into TAGs



Lipid metabolism and lipid rearrangements



Lipid process	T T P P				annotation/domain
	0	10	0	10	
Phatr3_J28797					D9, Delta 9 desaturase
Phatr3_J29488					D6, Delta 6 fatty acid desaturase
Phatr3_J22274					ELO6B_1, Elongation of fatty acids protein
Phatr3_J34485					ELO5B, Elongation of fatty acids protein
Phatr3_J9255					Elongation of fatty acids protein
Phatr3_J20143					ACS1, Long chain acyl-coa synthetase
Phatr3_J46275					Fatty acid desaturase domain
Phatr3_Jdraft1611					PLC-like phosphodiesterase, TIM beta/alpha-barrel domain superfamily
Phatr3_J46908					PLC-like phosphodiesterase, TIM beta/alpha-barrel domain superfamily
Phatr3_J45895					Alpha/Beta hydrolase fold
Phatr3_J50397					Alpha/Beta hydrolase fold
Phatr3_EG01987					Ribonuclease H/alpha-beta hydrolase
Phatr3_EG01635					Alpha/beta hydrolase
Phatr3_EG02610					Alpha/beta hydrolase
Phatr3_J31492					Alpha/beta hydrolase
Phatr3_J41624					Alpha/beta hydrolase
Phatr3_J44028					Alpha/beta hydrolase
Phatr3_J44622					Fatty acid desaturase
Phatr3_J45660					Acyl transferase/acyl hydrolase/lysophospholipase
Phatr3_EG00720					Acyl transferase/lysolipase
Phatr3_J46193					Acyl transferase/acyl hydrolase/lysophospholipase
Phatr3_EG02619					Cytochrome 5like-heme steroid binding domain/fatty acid desaturase domain
Phatr3_J8860					Lecithin:cholesterol/phospholipid:diacylglycerol acyltransferase
Phatr3_J28009					3-oxo-5-alpha-steroid 4-dehydrogenase, c-terminal
Phatr3_J54971					Lipoprotein, type 6
Phatr3_J3262					Glycerol-3-phosphate o-acyltransferase, chloroplast



Desaturases and lipases upregulated



Conclusions and perspectives



- Nitrogen limitation in *P. tricornutum* suppresses photosynthesis, ribosomal and protein synthesis.

- Increased mitochondrial activity and TCA cycle.

What is the mitochondrial role?

- Evident membrane remodelling

- Improvements in genome/pathway annotation could help in identifying novel candidates for genetic engineering.

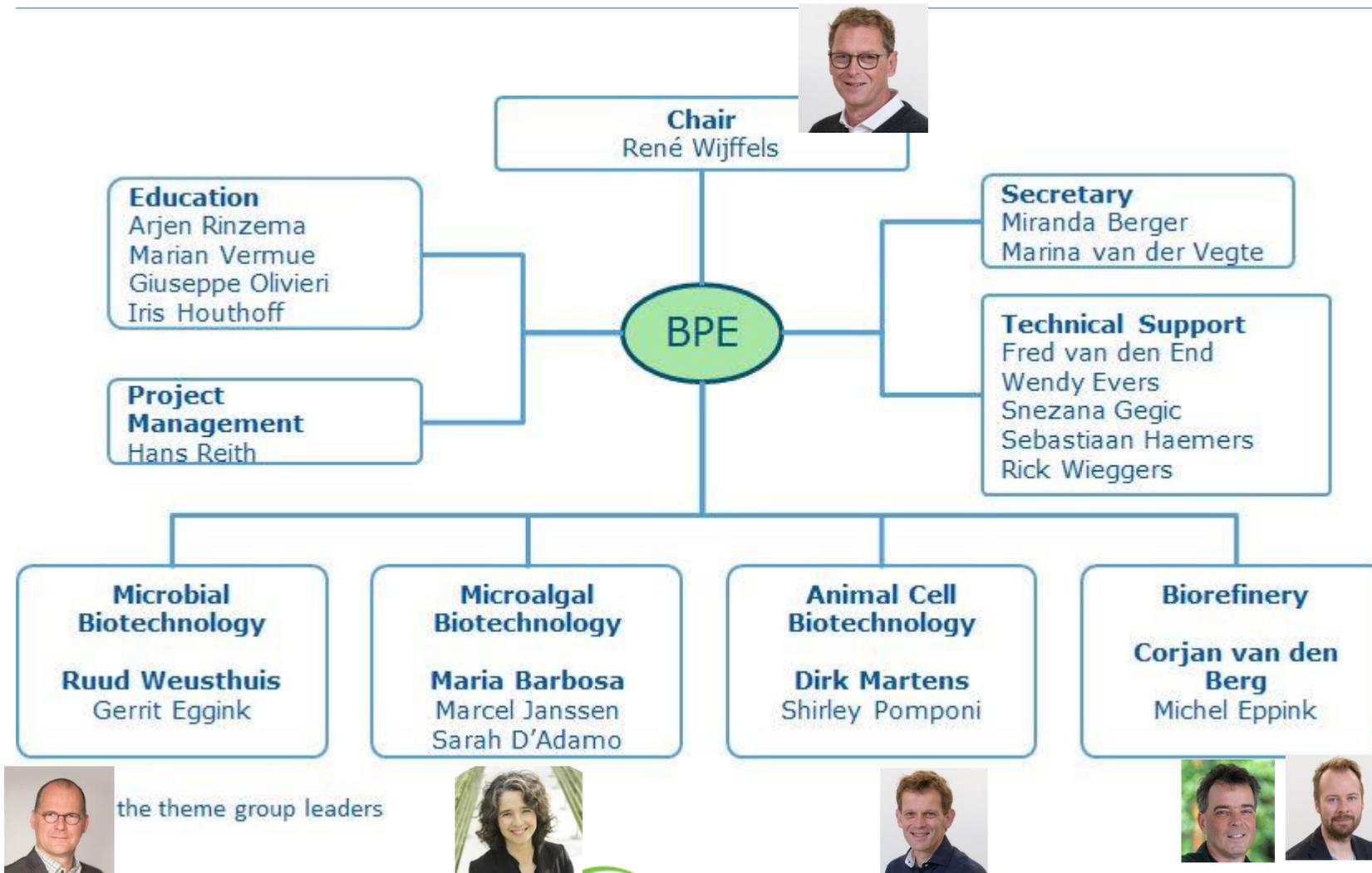


Acknowledgement

- Dr. Ilse Remmers
 - Prof. Dirk Martens
 - Dr. Ric de Vos
 - Dr. Packo Lamers
 - Prof. Rene' Wijffels
- Bioprocess Engineering
- Dr. Roland Mumm,
 - Dr. Antoine America,
 - Dr. Jan Corderwener,
 - Dr. Linda Bakker,
 - Dr. Sander Peters
- BU Bioscience



Acknowledgement –BPE GROUP



Thank you!

