A two-step process for improved biomass production and non-destructive astaxanthin and carotenoids accumulation in *Haematococcus pluvialis*

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SUPPLEMENTARY 1

Selection of the best cultivation conditions

Growth of *H. pluvialis* CCAP 34/1D was tested in 3N-BBM+V, BG11, MWC, JM, (www.CCAP.ac.uk) cultured at 20°C, 30 µmol photons m^{-2} s^{-1}, 12:12 hrs D/L photoperiod, 150 rpm over a 37 days period**.** The maximum growth rate was reached in the JM medium at 7.5 pH (Figure S1). The JM medium and 7.5 pH was therefore selected for *H. pluvialis* 34/1D cultivation in further experiments, 10 mM of HEPES buffer was supplied to control the pH variations.

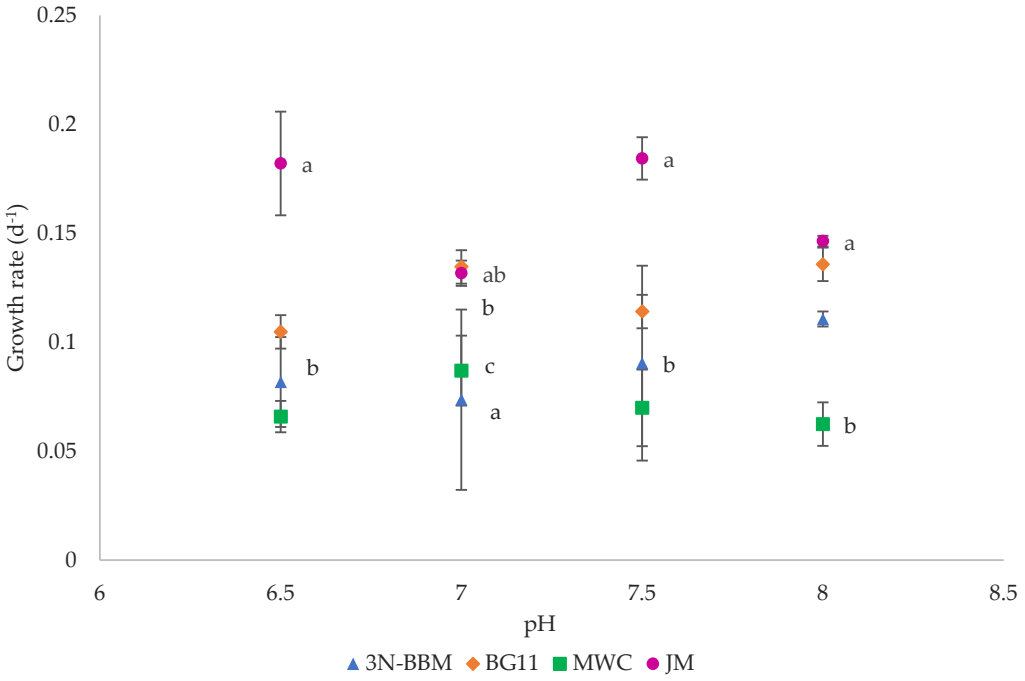


Figure S1_ Growth rate of cultures in each different medium 3N-BBM+V+V, BG11, MWC, JM at 6, 6.5, 7, 7.5, 8 pHs. Error bars are standard deviations of the means, *n*=3. The letters indicate significant differences between the treatments, at each pH value ($p < 0.05$).

The best N/P ratio based on growth rate and cell density (Figure S2) was the one originally present in the JM recipe, i.e., in the control.

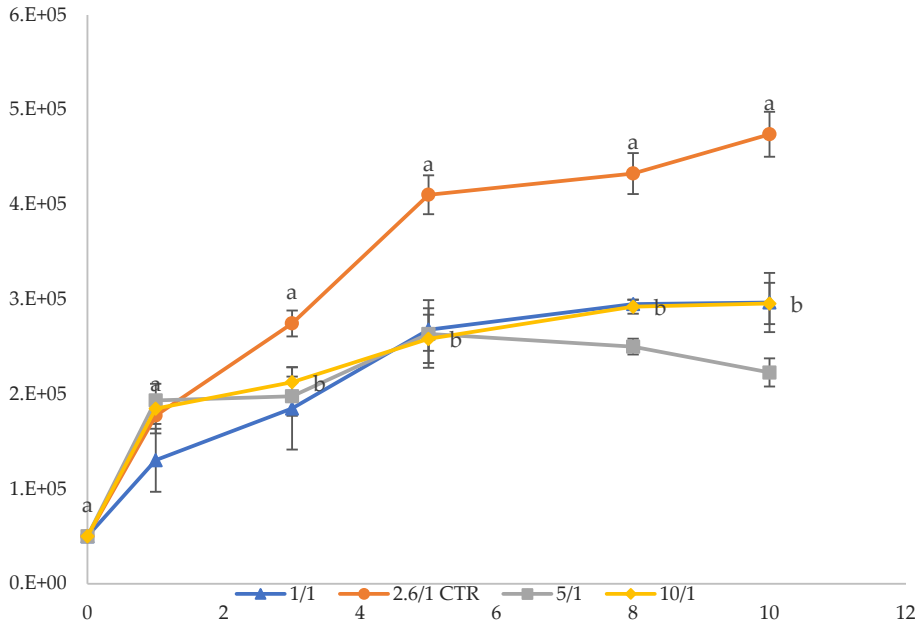


Figure S2_ *H. pluvialis* 34/1D grown in different N/P ratios: Control with $NaNO_3$ 2.6/1; 1/1; 5/1; 10/1. Error bars are standard deviations of the means, *n*=3. The letters indicate significant differences between the N/P ratios at each data point ($p < 0.05$)

SUPPLEMENTARY 2

Selection of the astaxanthin induction method

The experiment was performed in a 24 well plates with a starting inoculum of 10'000 cells/ml resuspended in a final volume of 2 ml of fresh JM at 7.5 pH. Seven different induction conditions were tested: salinity at different concentrations: 0%, 0.2%, 0.4%, 0.6%, 0.8%, 1%, 1.2% w/v; sodium acetate and sodium pyruvate at the following concentrations: 0%, 0.25%, 0.5%, 1%, 1.5%, 1.75%, 2%. Finally, $FeCl_3$ at 200 and 400 µM + 0,25% sodium acetate, and $FeCl_3$ at 200 and 400 µM + 0,25% pyruvate, were tested.

Furthermore, another variant was added: all stressors were applied under medium light (ML) (250 µmol photon $m^{-2}s^{-1}$) and high light (HL) (366 µmol photon $m^{-2}s^{-1}$).

Each treatment was tested in triplicate, controls were made with JM medium exposed to HL and ML.

Multi-well plates were maintained in a CT room and daily manual rotations were carried out to prevent clumping. The OD was measured at 480, 665, 649, 750 nm by the POLARstar Omega plate reader, to measure respectively carotenoids, chlorophyll *a* and *b* and cell growth, over a period of 11 days. The purpose of the experiment was to find the best combination of induction stressor to induce the astaxanthin accumulation and the biomass preservation.

Figure S3 shows the effect of sodium acetate on the carotenoids accumulation (OD_{480}) and cell growth (OD_{750}). Data refers to the 10th day of the experiment, when the maximum absorbance value at the two wavelengths was attained. Indeed, as discussed above, the addition of sodium acetate enhances not only the carotenogenesis but also the cell growth. Thus, a 0.25% w/v concentration was selected for the next experiments.

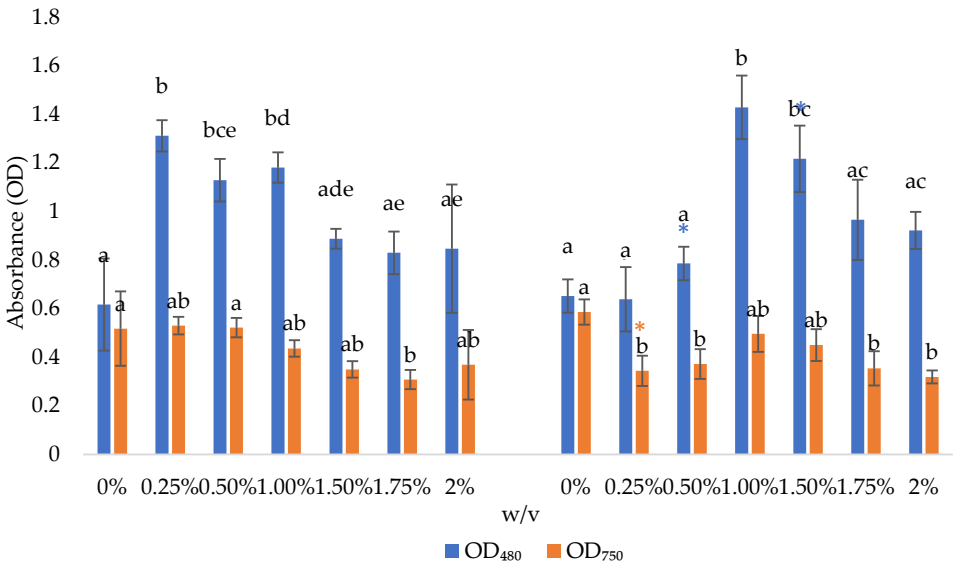


Figure S3_ Effect of sodium acetate 0%, 0.25%; 0.5%; 1%; 1.5%; 1.75%; 2% on carotenoids accumulation (OD_{480}) and cell density (OD_{750}) under ML and HL. Error bars are standard deviations of the means, *n*=3. The letters indicate significant differences between the treatments: OD_{480} ML, OD_{480} HL, OD_{750} ML, OD_{750} HL ($p < 0.05$). The asterisk indicates significant differences between the ML and HL treatments ($p < 0.05$)

The optical density at 750 nm, used to monitor algal growth, was compared with a *H. pluvialis* calibration curve to recover the number of cells/mL (Figure S4).

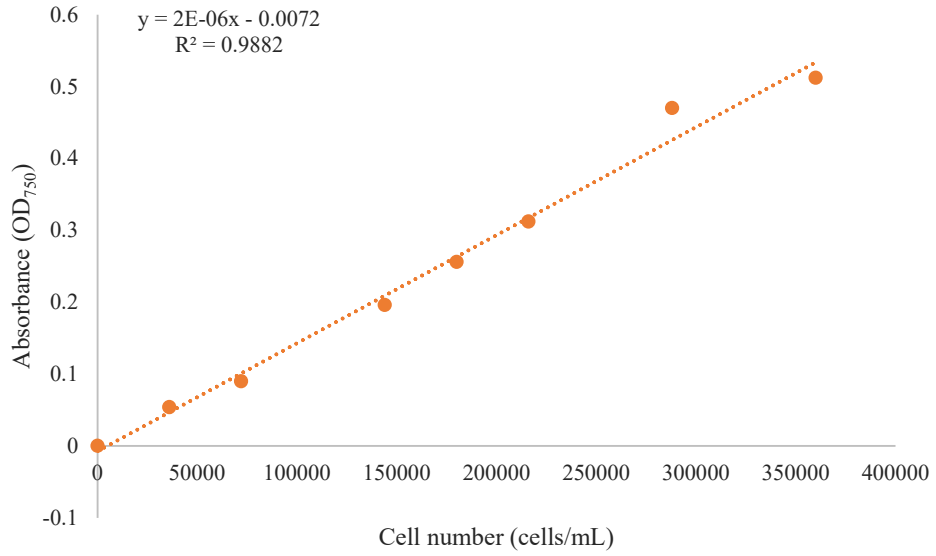


Figure S4_ *H. pluvialis* 34/1D calibration curve used for cell quantification (*n* = 3, error bars = 1 SD)

SUPPLEMENTARY 3

Biochemical analyses of the biomass

Biochemical analyses were performed on cultures during both their green and red stages of growth. Cultures were harvested on cultivation day 14 (prior to astaxanthin induction) and at day 21 (7 days following induction). In both instances, cells were centrifuged at 3000 g for 15 minutes; pellets of sub-samples were then flash frozen in liquid nitrogen, stored at -80°C, and then freeze-dried (ALPHA 1-2 LD plus freeze dryer, Christ).
All biochemical analyses were performed on lyophilized material. Proteins were extracted from 5 mg of dry weight (DW) biomass using a sequential acid and alkaline incubation and quantified using the Lowry assay, as described by Slocombe (2013). Protein concentration was estimated using a 0-5 mg L^{-1} BSA (Bovine serum albumin) calibration curve.

Total soluble carbohydrate fraction of the biomass was extracted and quantified using a modified phenol-sulphuric acid method according to Fournier (2001) derived from that by Dubois (1956).

Cell contents of proteins and carbohydrates were expressed as percentage of dry weight (% DW).

Table S1 shows the analysis carbohydrates and proteins content expressed in % DW of cultures grown under different nitrogen sources, during the green and the red phase. Carbohydrate concentration increased after the addition of 0.25% *w/v* sodium acetate in all the tested culturing regimes.

Table S1_Biochemical table reporting the mean value (% DW) ± S.D. within samples ($n = 3$). Values are reported as the mean value ($n = 3$) ± 1 S.D. Different letters indicate significant differences among the nitrogen sources within the green and the red phase, while the differences between the green and the red phase in each treatment are denoted by * ($p < 0.05$).

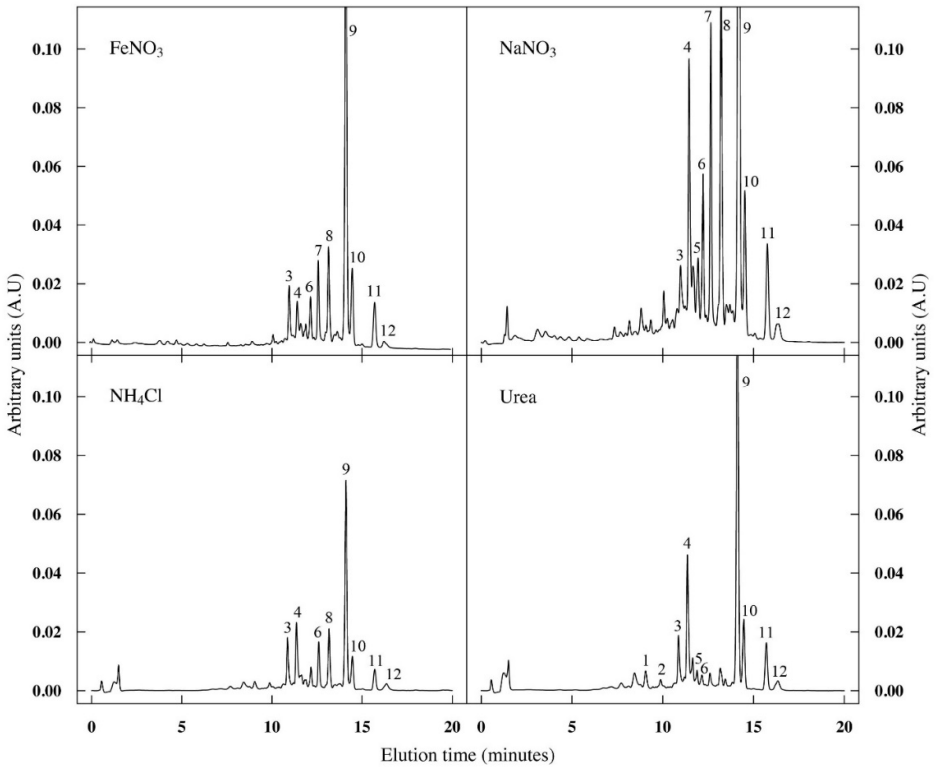
.	GREEN		RED	
%DW	***Carbs***	*Proteins*	*Carbs*	*Proteins*
$NaNO_3$	10.72±0.34 ^{a}	12.66±0.29 ^{a}	*23.74±0.7 ^{a}	12.60±0.26 ^{a}
$FeNO_3$	41.98±1.07^{c}	12.47±0.46 ^{a}	*51.80±0.61^{c}	12.59±1.15 ^{a}
Urea	38.61±0.1^{d}	19.24±0.56^{b}	*43.22±0.69^{d}	*14.00±0.9 ^{a}
NH_4Cl	45.25±1.39^{b}	10.95±0.14 ^{a}	*61.52±1.15^{b}	9.91±0.94^{b}

Cultures grown in $FeNO_3$ and NH_4Cl as N-source, started to encyst before the induction process, where the iron and the ammonium probably acted as stressors as it is suggested by the higher amount of carbohydrates accumulated in the green phase, in comparison with the cultures grown in urea and in the $NaNO_3$ control (Table 3).

Under these induction conditions, the macromolecular profile of the cells, in particular the carbohydrate pool, changed according to the nitrogen source and the growth phase.

The protein amount remained nearly constant, thanks to the presence of nitrogen in the medium, except for the cells grown in urea, while the ratio carotenoids/chlorophylls increased in all the treatments.

HPLC Chromatograms


FeNO3
NaNO3
NH4Cl
Urea
Arbitrary units (A.U)
Elution time (minutes)

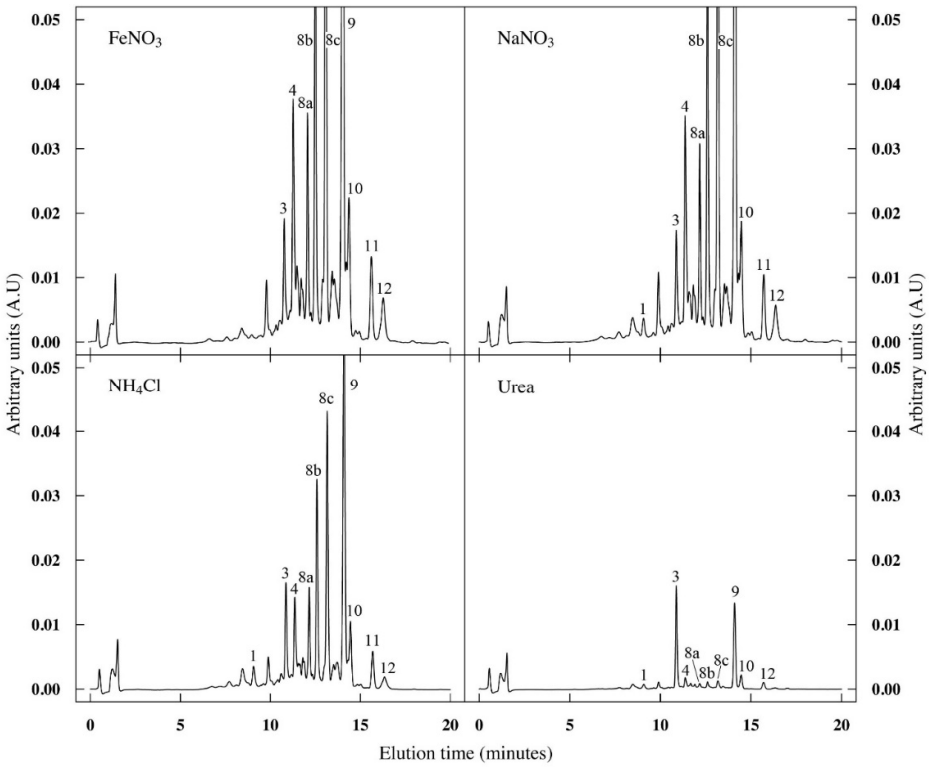

FeNO3
NaNO3
NH4Cl
Urea
Arbitrary units (A.U)
Elution time (minutes)

Figure S5. Representative HPLC chromatograms of *H. pluvialis* green (A) and red (B) phases of growth, when cultivated under $FeNO_3$, $NaNO_3$, NH_4Cl, or Urea as the principal nitrogen source in Jaworski's Media. **Identified peaks: 1. Antheraxanthin; 2. Echinenone; 3. Internal Standard; 4. Lutein; 5. Neoxanthin; 6. Carotenoid-like; 7. Canthaxanthin; 8a, 8b, 8c. Astaxanthin isomers; 9. Chlorophyll *a*; 10. Chlorophyll *a* epimer; 11. Pheophytin *a*; 12. β-carotene.**

Statistics

Table S2. Ordinary one-way ANOVA and Tukey's multiple comparison test, with a single pooled variance were performed for *H. pluvialis*. *H. pluvialis* CCAP 34/1D maximum growth rate and cell density in different NS. ANOVA test was performed for growth rate (a) and cell density (b).

a

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	0.02340	3	0.007800	F (3, 8) = 29.59	P=0.0001
Residual (within columns)	0.002109	8	0.0002636		
Total	0.02551	11			

b

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	153913807379	3	51304602460	F (3, 8) = 9.854	P=0.0046
Residual (within columns)	41651433356	8	5206429169		
Total	195565240735	11			

Table S3. The relative change of *H. pluvialis* CCAP 34/1D cell number based on the number of cells/ml at the beginning and end of the 7 day induction process. Thereby representative of cell number change during the red phase alone. Ordinary one-way ANOVA and Tukey's multiple comparisons test, with a single pooled variance were performed.

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	2528	3	842.6	F (3, 8) = 13.47	P=0.0017
Residual (within columns)	500.6	8	62.58		
Total	3028	11			

Table S4. Quantitative pigment composition (in mg/g DW), obtained by HPLC analysis, of *H. pluvialis* cells at the end of the green phase, in cultures previously supplied with equimolar concentrations of nitrogen from different sources. Ordinary one-way ANOVA and Tukey's multiple comparisons test, with a single pooled variance were performed.

HPLC green

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	q	DF
LUTEIN								
$NaNO_3$ vs. $FeNO_3$	0.07843	0.06883	0.009595	0.3072	1	1	0.04417	54.00
$NaNO_3$ vs. UREA	0.07843	0.2778	-0.1993	0.3072	1	1	0.9177	54.00
$NaNO_3$ vs. NH_4^+	0.07843	0.07202	0.006408	0.3072	1	1	0.02950	54.00
$FeNO_3$ vs. UREA	0.06883	0.2778	-0.2089	0.3072	1	1	0.9619	54.00
$FeNO_3$ vs. NH_4^+	0.06883	0.07202	-0.003187	0.3072	1	1	0.01467	54.00
UREA vs. NH_4^+	0.2778	0.07202	0.2057	0.3072	1	1	0.9472	54.00
ASTAXANTHIN								

$NaNO_3$ vs. $FeNO_3$	0.2270	2.714	-2.487	0.3072	1	1	11.45	54.00
$NaNO_3$ vs. UREA	0.2270	-6.661e-016	0.2270	0.3072	1	1	1.045	54.00
$NaNO_3$ vs. NH_4^+	0.2270	0.2993	-0.07229	0.3072	1	1	0.3328	54.00
$FeNO_3$ vs. UREA	2.714	-6.661e-016	2.714	0.3072	1	1	12.50	54.00
$FeNO_3$ vs. NH_4^+	2.714	0.2993	2.415	0.3072	1	1	11.12	54.00
UREA vs. NH_4^+	-6.661e-016	0.2993	-0.2993	0.3072	1	1	1.378	54.00
CHL A								
$NaNO_3$ vs. $FeNO_3$	2.682	2.722	-0.04013	0.3072	1	1	0.1848	54.00
$NaNO_3$ vs. UREA	2.682	2.212	0.4703	0.3072	1	1	2.165	54.00
$NaNO_3$ vs. NH_4^+	2.682	1.132	1.550	0.3072	1	1	7.135	54.00
$FeNO_3$ vs. UREA	2.722	2.212	0.5104	0.3072	1	1	2.350	54.00
$FeNO_3$ vs. NH_4^+	2.722	1.132	1.590	0.3072	1	1	7.320	54.00
UREA vs. NH_4^+	2.212	1.132	1.080	0.3072	1	1	4.970	54.00
B CAROTENE								
$NaNO_3$ vs. $FeNO_3$	0.01667	0.03083	-0.01416	0.3072	1	1	0.06518	54.00
$NaNO_3$ vs. UREA	0.01667	0.08992	-0.07325	0.3072	1	1	0.3372	54.00
$NaNO_3$ vs. NH_4^+	0.01667	0.01321	0.003461	0.3072	1	1	0.01593	54.00
$FeNO_3$ vs. UREA	0.03083	0.08992	-0.05909	0.3072	1	1	0.2720	54.00
$FeNO_3$ vs. NH_4^+	0.03083	0.01321	0.01762	0.3072	1	1	0.08112	54.00
UREA vs. NH_4^+	0.08992	0.01321	0.07671	0.3072	1	1	0.3531	54.00
PHEO A								
$NaNO_3$ vs. $FeNO_3$	0.3760	1.083	-0.7072	0.3072	1	1	3.256	54.00
$NaNO_3$ vs. UREA	0.3760	0.3246	0.05145	0.3072	1	1	0.2369	54.00
$NaNO_3$ vs. NH_4^+	0.3760	0.1655	0.2106	0.3072	1	1	0.9695	54.00
$FeNO_3$ vs. UREA	1.083	0.3246	0.7587	0.3072	1	1	3.493	54.00
$FeNO_3$ vs. NH_4^+	1.083	0.1655	0.9178	0.3072	1	1	4.225	54.00
UREA vs. NH_4^+	0.3246	0.1655	0.1591	0.3072	1	1	0.7326	54.00
ANTERA								
$NaNO_3$ vs. $FeNO_3$	-3.331e-016	-4.441e-016	1.110e-016	0.3072	1	1	5.111e-016	54.00
$NaNO_3$ vs. UREA	-3.331e-016	0.2939	-0.2939	0.3072	1	1	1.353	54.00
$NaNO_3$ vs. NH_4^+	-3.331e-016	6.661e-016	-9.992e-016	0.3072	1	1	4.600e-015	54.00
$FeNO_3$ vs. UREA	-4.441e-016	0.2939	-0.2939	0.3072	1	1	1.353	54.00
$FeNO_3$ vs. NH_4^+	-4.441e-016	6.661e-016	-1.110e-015	0.3072	1	1	5.111e-015	54.00
UREA vs. NH_4^+	0.2939	6.661e-016	0.2939	0.3072	1	1	1.353	54.00
CAROTENOID-LIKE								
$NaNO_3$ vs. $FeNO_3$	1.690	2.260	-0.5700	0.3072	1	1	2.624	54.00
$NaNO_3$ vs. UREA	1.690	3.090	-1.400	0.3072	1	1	6.445	54.00
$NaNO_3$ vs. NH_4^+	1.690	0.3900	1.300	0.4344	1	1	4.232	54.00
$FeNO_3$ vs. UREA	2.260	3.090	-0.8300	0.3072	1	1	3.821	54.00
$FeNO_3$ vs. NH_4^+	2.260	0.3900	1.870	0.4344	1	1	6.088	54.00
UREA vs. NH_4^+	3.090	0.3900	2.700	0.4344	1	1	8.790	54.00

Table S5. For canthaxanthin, a t-test was used to determine if there is a significant difference between the means of two groups, $NaNO_3$ and $FeNO_3$.

Table Analyzed	**Canthaxanthin**
Column B	$NaNO_3$
vs.	vs.
Column A	$FeNO_3$
Unpaired t test	
P value	0.4961
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed

t, df	t=0.7479, df=4

Table S6. Quantitative pigment composition (in mg/g DW), obtained by HPLC analysis, of *H. pluvialis* cells at the end of the red phases stationary phase, in cultures previously supplied with equimolar concentrations of nitrogen from different sources. Ordinary one-way ANOVA and Tukey's multiple comparisons test, with a single pooled variance were performed.

HPLC red

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	q	DF
LUTEIN								
$FeNO_3$ vs. NH_4^+	0.07600	0.04300	0.03300	0.2118	1	1	0.2203	48.00
$FeNO_3$ vs. UREA	0.07600	0.003000	0.07300	0.2118	1	1	0.4874	48.00
$FeNO_3$ vs. $NaNO_3$	0.07600	0.04600	0.03000	0.2118	1	1	0.2003	48.00
NH_4^+ vs. UREA	0.04300	0.003000	0.04000	0.2118	1	1	0.2671	48.00
NH_4^+ vs. $NaNO_3$	0.04300	0.04600	-0.003000	0.2118	1	1	0.02003	48.00
UREA vs. $NaNO_3$	0.003000	0.04600	-0.04300	0.2118	1	1	0.2871	48.00
ASTAXANTHIN								
$FeNO_3$ vs. NH_4^+	1.353	0.4860	0.8670	0.2118	1	1	5.788	48.00
$FeNO_3$ vs. UREA	1.353	0.3130	1.040	0.2118	1	1	6.943	48.00
$FeNO_3$ vs. $NaNO_3$	1.353	0.7110	0.6420	0.2118	1	1	4.286	48.00
NH_4^+ vs. UREA	0.4860	0.3130	0.1730	0.2118	1	1	1.155	48.00
NH_4^+ vs. $NaNO_3$	0.4860	0.7110	-0.2250	0.2118	1	1	1.502	48.00
UREA vs. $NaNO_3$	0.3130	0.7110	-0.3980	0.2118	1	1	2.657	48.00
CHL A								
$FeNO_3$ vs. NH_4^+	1.664	1.396	0.2680	0.2118	1	1	1.789	48.00
$FeNO_3$ vs. UREA	1.664	0.1810	1.483	0.2118	1	1	9.901	48.00
$FeNO_3$ vs. $NaNO_3$	1.664	0.8120	0.8520	0.2118	1	1	5.688	48.00
NH_4^+ vs. UREA	1.396	0.1810	1.215	0.2118	1	1	8.112	48.00
NH_4^+ vs. $NaNO_3$	1.396	0.8120	0.5840	0.2118	1	1	3.899	48.00
UREA vs. $NaNO_3$	0.1810	0.8120	-0.6310	0.2118	1	1	4.213	48.00
B CAROTENE								
$FeNO_3$ vs. NH_4^+	0.02900	0.02300	0.006000	0.2118	1	1	0.04006	48.00
$FeNO_3$ vs. UREA	0.02900	0.003000	0.02600	0.2118	1	1	0.1736	48.00
$FeNO_3$ vs. $NaNO_3$	0.02900	0.01700	0.01200	0.2118	1	1	0.08012	48.00
NH_4^+ vs. UREA	0.02300	0.003000	0.02000	0.2118	1	1	0.1335	48.00
NH_4^+ vs. $NaNO_3$	0.02300	0.01700	0.006000	0.2118	1	1	0.04006	48.00
UREA vs. $NaNO_3$	0.003000	0.01700	-0.01400	0.2118	1	1	0.09347	48.00
PHEO A								
$FeNO_3$ vs. NH_4^+	0.2320	0.5600	-0.3280	0.2118	1	1	2.190	48.00
$FeNO_3$ vs. UREA	0.2320	0.000	0.2320	0.2118	1	1	1.549	48.00
$FeNO_3$ vs. $NaNO_3$	0.2320	0.1070	0.1250	0.2118	1	1	0.8345	48.00
NH_4^+ vs. UREA	0.5600	0.000	0.5600	0.2118	1	1	3.739	48.00
NH_4^+ vs. $NaNO_3$	0.5600	0.1070	0.4530	0.2118	1	1	3.024	48.00
UREA vs. $NaNO_3$	0.000	0.1070	-0.1070	0.2118	1	1	0.7144	48.00
ANTERA								
$FeNO_3$ vs. NH_4^+	0.000	0.05200	-0.05200	0.2118	1	1	0.3472	48.00
$FeNO_3$ vs. UREA	0.000	0.01500	-0.01500	0.2118	1	1	0.1001	48.00
$FeNO_3$ vs. $NaNO_3$	0.000	0.01000	-0.01000	0.2118	1	1	0.06676	48.00
NH_4^+ vs. UREA	0.05200	0.01500	0.03700	0.2118	1	1	0.2470	48.00
NH_4^+ vs. $NaNO_3$	0.05200	0.01000	0.04200	0.2118	1	1	0.2804	48.00
UREA vs. $NaNO_3$	0.01500	0.01000	0.005000	0.2118	1	1	0.03338	48.00

Table S7. Pigments variation between the green and the red phase

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	SE of diff.	N1	N2	q	DF
Lutein										
$NaNO_3$ vs. $NaNO_{3\ red}$	0.03243	-0.6597 to 0.7246	No	ns	>0.9999	0.2234	1	1	0.2053	96
$FeNO_3$ vs. $FeNO_{3\ red}$	-0.00717	-0.6993 to 0.6850	No	ns	>0.9999	0.2234	1	1	0.04538	96
UREA vs. Urea red	0.2748	-0.4174 to 0.9669	No	ns	0.9209	0.2234	1	1	1.74	96
NH_4^+ vs. $NH_4^{+\ red}$	0.02902	-0.6631 to 0.7212	No	ns	>0.9999	0.2234	1	1	0.1837	96
Astaxanthin										
$NaNO_3$ vs. $NaNO_{3\ red}$	-0.484	-1.176 to 0.2081	No	ns	0.3809	0.2234	1	1	3.065	96
$FeNO_3$ vs. $FeNO_{3\ red}$	1.361	0.6693 to 2.054	Yes	****	<0.0001	0.2234	1	1	8.62	96
UREA vs. Urea red	-0.313	-1.005 to 0.3791	No	ns	0.8545	0.2234	1	1	1.982	96
NH_4^+ vs. $NH_4^{+\ red}$	-0.1867	-0.8789 to 0.5054	No	ns	0.9906	0.2234	1	1	1.182	96
Chla										96
$NaNO_3$ vs. $NaNO_{3\ red}$	1.87	1.178 to 2.562	Yes	****	<0.0001	0.2234	1	1	11.84	96
$FeNO_3$ vs. $FeNO_{3\ red}$	1.058	0.3663 to 1.751	Yes	***	0.0002	0.2234	1	1	6.702	96
UREA vs. Urea red	2.031	1.339 to 2.723	Yes	****	<0.0001	0.2234	1	1	12.86	96
NH_4^+ vs. $NH_4^{+\ red}$	-0.2635	-0.9557 to 0.4286	No	ns	0.9358	0.2234	1	1	1.669	96
B-carotene										
$NaNO_3$ vs. $NaNO_{3\ red}$	-0.00033	-0.6925 to 0.6918	No	ns	>0.9999	0.2234	1	1	0.002058	96
$FeNO_3$ vs. $FeNO_{3\ red}$	0.001833	-0.6903 to 0.6940	No	ns	>0.9999	0.2234	1	1	0.01161	96
UREA vs. Urea red	0.08692	-0.6052 to 0.7791	No	ns	>0.9999	0.2234	1	1	0.5504	96
NH_4^+ vs. $NH_4^{+\ red}$	-0.00979	-0.7019 to 0.6824	No	ns	>0.9999	0.2234	1	1	0.06196	96
Pheophytin a										
$NaNO_3$ vs. $NaNO_{3\ red}$	0.269	-0.4231 to 0.9612	No	ns	0.9287	0.2234	1	1	1.703	96
$FeNO_3$ vs. $FeNO_{3\ red}$	0.8512	0.1591 to 1.543	Yes	**	0.0058	0.2234	1	1	5.39	96
UREA vs. Urea red	0.3246	-0.3676 to 1.017	No	ns	0.8297	0.2234	1	1	2.055	96
NH_4^+ vs. $NH_4^{+\ red}$	-0.3945	-1.087 to 0.2976	No	ns	0.6439	0.2234	1	1	2.498	96
Antheraxanthin										
$NaNO_3$ vs. $NaNO_{3\ red}$	-0.01	-0.7021 to 0.6821	No	ns	>0.9999	0.2234	1	1	0.06332	96
$FeNO_3$ vs. $FeNO_{3\ red}$	0	-0.6921 to 0.6921	No	ns	>0.9999	0.2234	1	1	0	96
UREA vs. Urea red	0.2789	-0.4132 to 0.9710	No	ns	0.9149	0.2234	1	1	1.766	96

Sidak's multiple comparisons test		SE of diff.	N1	N2	t			DF	
car/chl green - car/chl red									
$NaNO_3$		0.1373	1	1	6.159			16.00	
$FeNO_3$		0.1373	1	1	1.145			16.00	
UREA		0.1373	1	1	11.26			16.00	
NH_4^+		0.1373	1	1	0.6738			16.00	
NH_4^+ vs. $NH_4^{+\ red}$	-0.052	-0.7441 to 0.6401	No	ns	>0.9999	0.2234	1	1	0.3292	96

Table S8. Ordinary two-way ANOVA and Sidak's multiple comparison test, with individual variances computed for each comparison, were performed.

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
car/chl green								
$NaNO_3$ vs. $FeNO_3$	0.12	1.033	-0.9134	0.1373	1	1	6.654	16
$NaNO_3$ vs. UREA	0.12	0.2993	-0.1793	0.1373	1	1	1.306	16
$NaNO_3$ vs. NH_4^+	0.12	0.3401	-0.2201	0.1373	1	1	1.603	16
$FeNO_3$ vs. UREA	1.033	0.2993	0.7342	0.1373	1	1	5.348	16
$FeNO_3$ vs. NH_4^+	1.033	0.3401	0.6933	0.1373	1	1	5.05	16
UREA vs. NH_4^+	0.2993	0.3401	-0.04082	0.1373	1	1	0.2974	16
car/chl red								
$NaNO_3$ vs. $FeNO_3$	0.9655	0.8762	0.08931	0.1373	1	1	0.6506	16
$NaNO_3$ vs. UREA	0.9655	1.845	-0.8795	0.1373	1	1	6.407	16
$NaNO_3$ vs. NH_4^+	0.9655	0.4326	0.5329	0.1373	1	1	3.882	16
$FeNO_3$ vs. UREA	0.8762	1.845	-0.9688	0.1373	1	1	7.057	16
$FeNO_3$ vs. NH_4^+	0.8762	0.4326	0.4436	0.1373	1	1	3.231	16
UREA vs. NH_4^+	1.845	0.4326	1.412	0.1373	1	1	10.29	16

Table S9. Carotenoids/Chlorophylls ratio analysis between the green and the red phase

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	Mean 1	Mean 2	Mean Diff.
car/chl green - car/chl red								
$NaNO_3$	-0.8455	-1.230 to -0.4606	Yes	****	<0.0001	0.1200	0.9655	-0.8455
$FeNO_3$	0.1572	-0.2277 to 0.5421	No	ns	0.7143	1.033	0.8762	0.1572
UREA	-1.546	-1.931 to -1.161	Yes	****	<0.0001	0.2993	1.845	-1.546
NH_4^+	-0.09250	-0.4774 to 0.2924	No	ns	0.9424	0.3401	0.4326	-0.09250