Supplementary information for

^{13}C-depletion and high concentration of carbon sources enhance carbon and nitrogen metabolism of *Nannochloropsis oceanica*

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Text S1. Atmospheric CO_2 carbon isotope composition analysis

Dalian Dayaowan Port located in the southeast of Jinzhou District, Dalian, China, and is the largest foreign trade container port in northeast China, with 450,000 tons of crude oil terminal, 400,000 tons of ore terminal and 200,000 tons of container terminal. The samples were collected at four sites, and the detailed information of sampling sites in Table S1. The $\delta^{13}C$ values of atmospheric CO_2 in Dayaowan Port was the average of the four sampling points.

Table. S1. Sampling points

Sampling sites	Longitude (°E)	Latitude (°N)
1	121.868	39.002
2	121.875	39.001
3	121.883	38.996
4	121.889	38.993

The sampling campaigns lasts for one week (March 30, 2023 - April 5, 2023), and typically near 7:00, 12:00, 16:00 and 21:00 of local time. All air samples were collected with a pump to fill a 1.5 L aluminum foil air bag (HongPu Experimental Technology Co., Ltd. Ningbo, China) for 10-15 min. Before sampling, each bag was evacuated to around 10^{-2} mbar in a pretreatment lab and purged with ambient air at the sampling site for 3 min in order to displace any remaining gas. The $\delta^{13}C$ values of atmospheric CO_2 were measured by GasBench isotope ratio mass spectrometer (GasBench-IRMS, GasBench II, 253 Plus, Thermo Fisher Scientific, Germany). The conventional standard is Vienna Pee Dee Belemnite (VPDB). The laboratory reference material (NBS-18, $\delta^{13}C$ = -5.014‰ ± 0.035 ‰) was used to calibrate $\delta^{13}C$. The analysis accuracy of $\delta^{13}C$ was ± 0.20‰. The carbon isotope composition was calculated by the Eq. (1):

$$\delta^{13}C\ (‰) = [(R_{sample}/R_{standard}) - 1)] \times 1000$$

where R_{sample} is the $^{13}C/^{12}C$ ratio of sample, $R_{standard}$ is the $^{13}C/^{12}C$ ratio of the VPDB.

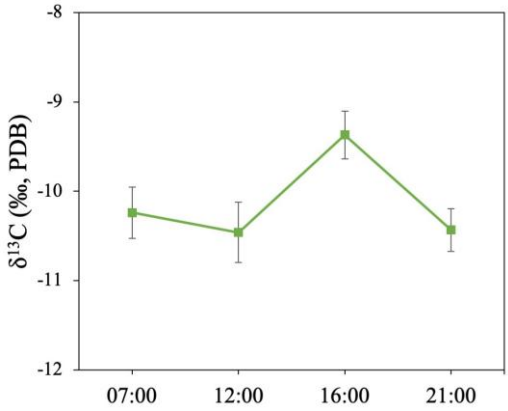

Fig. S1. $\delta^{13}C$ values of atmospheric CO_2 in Dalian Dayaowan Port

The $\delta^{13}C$ values of atmospheric CO_2 in Dalian Dayaowan Port ranged from -9.37‰ to -10.46‰.

Text S2. Dissolved inorganic carbon isotope composition analysis

The carbon isotope composition ($\delta^{13}C$) of dissolved inorganic carbon (DIC) were measured according to the method of (Remize et al., 2020). Briefly, 200 μL of phosphoric acid was added to the tube and flushed with ultra-pure helium gas for 5 min. Then 1 mL of water samples were injected to the reaction tube by a syringe and equilibrated for 24 h. The $\delta^{13}C$ values of DIC were measured by GasBench-IRMS. The conventional standard is VPDB. The laboratory reference material NBS-18 was used to calibrate the cylinder gas. Each sample was repeated three times, and one standard was inserted for every ten samples. The analysis accuracy of $\delta^{13}C$ was ± 0.20‰. The carbon isotope composition was calculated by the Eq. (1).

$$\delta^{13}C\ (‰) = [(R_{sample}/R_{standard}) - 1)] \times 1000 \quad (1)$$

where R_{sample} is the $^{13}C/^{12}C$ ratio of sample, $R_{standard}$ is the $^{13}C/^{12}C$ ratio of the VPDB.

Text S3. Determination of fatty acid contents

Fatty acid methyl esters (FAMEs) were prepared according to the method provided by Fan et al. (2024). After 5 days of cultivation, the collected algae were lyophilized for 48 h in a freeze-dryer (FD-1-50, Beijing Boyikang Experimental Instruments Co., Ltd., China). 10 mg of freeze-dried algae were weighed into a 10 mL colorimetric tube, followed by the addition of 2 mL of HCl: CH_3OH (14: 86, v/v). Then, 100 μL of methyl nonadecanoate (5 mg/mL in methanol) was added to the tube as an internal standard, and the mixtures were heated in a water bath at 70 °C for 1 h. After cooling to room temperature, 2 mL of n-hexane was added and vortexed for 1 min. The supernatant was collected in a brown injection bottle to measure fatty acid content.

The FAMEs were determined by gas chromatography-mass spectrometry (GC-MS, Trace GC Ultra, ISQ, Thermo Fisher Scientific, USA). The GC-MS parameters were as follows: a DB-5MS column (60 m × 0.25 mm × 0.25 μm, Agilent) was used with high-purity helium (purity > 99.99%) as the carrier gas at a flow rate of 1.2 mL/min. The injection volume was 1 μL with an injector temperature of 280 °C. The temperature program was set as follows: initial temperature of 70 °C held for 1 min, increased to 170 °C at a rate of 8 °C/min and held for 6 min, then raised to 280 °C at 4 °C/min and maintained for 10 min. The mass spectrometry parameters were configured as follows: transfer line temperature 250 °C, ion source temperature 230 °C, and electron impact (EI) energy of 70 eV.

Text S4. Determination of amino acid contents

The amino acids in *N. oceanica* were measured according to the method described by Zheng et al. (2024). After 5 days of cultivation, 20 mg of freeze-dried algae were added to colorimetric tubes containing 2 mL of 6 mol/L HCl, and reacted at 110 °C for 24 h. The cation exchange resin was closely placed in the chromatographic column. The supernatant collected by centrifugation (12000 rpm, 15 min) was poured into the chromatographic column and maintained for 10 min to allow the amino acid to fully bind to the cation exchange resin. Subsequently, the resin was slowly washed with 2 mL of methanol-water solution (8:1, v/v; repeated 3 times) to remove impurities such as pigments and lipids, and then eluted with 4 mL of 4 mol/L ammonia. The collected eluent was evaporated with N_2 in a water bath at 80 °C, and 1 mL of 0.1 mol/L HCl was added to redissolve the residue. Finally, the solutions were filtered by a 0.22 μm filter membrane. The quantitative analysis of amino acids was analyzed by ultraperformance liquid chromatography coupled with triple-quadrupole mass spectrometry (UPLC-MS, UltiMate 3000, TSQ Quantis, Thermo Fisher Scientific, USA).

A Hypersil GOLD C18 column (2.1 mm × 100 mm × 1.7 μm, Thermo Fisher Scientific) was used for separation of analytes, and the mobile phases were 0.1% formic acid aqueous solution (A) and acetonitrile (B). The mobile phase conditions were presented in Table. S2. The column temperature was 35 °C. The parameters of the H-ESI ion source are as follows: sheath gas flow rate 40 arb; aux gas flow rate 10 arb; sweep gas flow rate 0 arb; ion transfer tube temperature 300 °C; vaporizer temperature 350 °C.

Table. S2. Mobile phase conditions

Time (min)	Flow rate (μL/min)	A (%)	B (%)
0	300	98	2
3	300	98	2
6	300	40	60
8	300	40	60
9	300	98	2

Text S5. Transcriptomic analysis

After 3 days of cultivation, total RNA was extracted from *N. oceanica* cells using the cetyltrimethylammonium bromide (CTAB) reagent, following the manufacturer's instructions. RNA quality and quantity were analyzed by an Agilent 2100 Bioanalyzer (Agilent, CA, USA). Library preparation and RNA-Seq data processing were executed by BGI (high throughput laboratory in Wuhan, China). The raw data generated by sequencing were filtered using the SOAPnuke software (v1.5.6). Bowtie2 (v2.4.5) was applied to align the clean reads to the reference gene set. All genes were annotated by the BLAST program against six databases: Kyoto Encyclopedia of Genes and Genomes (KEGG) database, Gene Ontology (GO), NCBI non-redundant protein (NR), Swissprot database, Protein family (PFAM) and euKaryotic Ortholog Groups (KOG) database. Gene expression levels were calculated by RNA-Seq by Expectation Maximization (RSEM, v1.3.1). Differentially expressed genes (DEGs) were screened using DESeq2 (v1.34.0). Genes with a False Discovery Rate (FDR) < 0.001 and $|\log_2(\text{Fold Change})| > 1$ were identified as DEGs. KEGG enrichment analysis of DEGs was conducted by Phyper (Wang et al., 2023). The thumbnails of metabolic pathways were searched by KEGG database (https://www.kegg.jp/kegg/pathway.html).

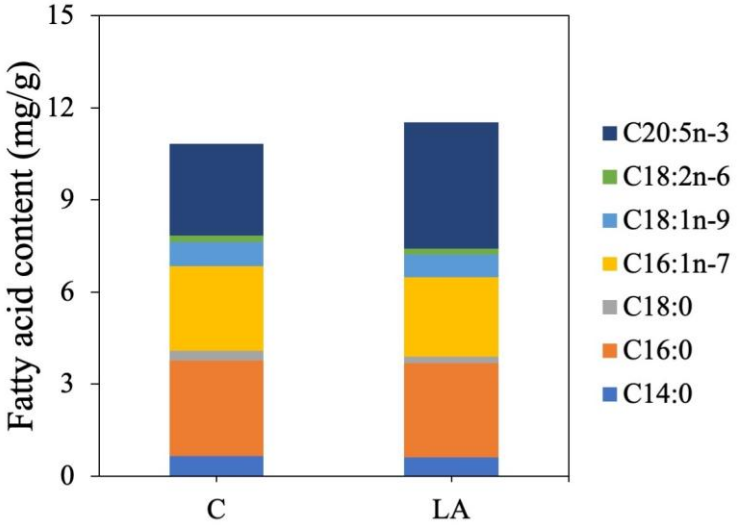

Fig. S2. The total fatty acids contents of *N. oceanica* under LA-HC conditions (C: control; LA: -18.42‰, 5 mmol/L $NaHCO_3$).

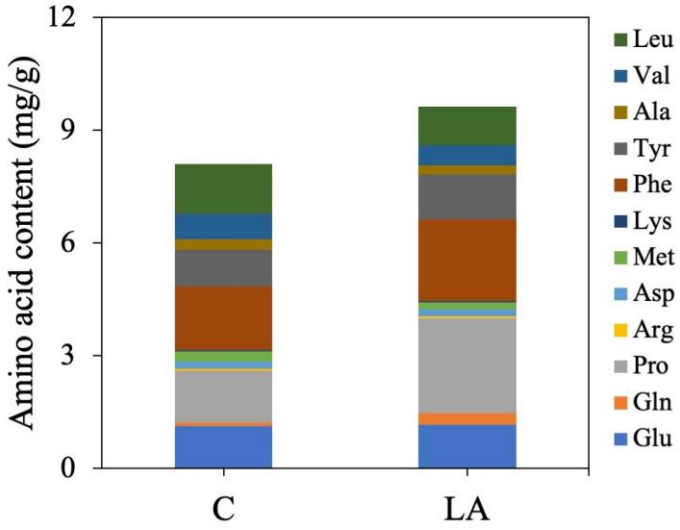

Fig. S3. The total amino acids contents of *N. oceanica* under LA-HC conditions (C: control; LA: -18.42‰, 5 mmol/L $NaHCO_3$).

Table S3. Gene abbreviations and full names of their encoded enzymes

Enzyme name	Abbreviation	Enzyme name	Abbreviation
glyceraldehyde 3-phosphate dehydrogenase	*GAPDH*	nitrate reductase	*NR*
phosphoglycerate kinase	*PGK*	nitrite reductase	*NirA*
2,3-bisphosphoglycerate-dependent phosphoglycerate mutase	*PGAM*	glutamine synthetase	*glnA*
pyruvate kinase	*PK*	glutamate synthase	*GLT1*
citrate synthase	*CS*	3-dehydroquinate synthase	*aroB*
aconitate hydratase	*ACO*	shikimate dehydrogenase	*aroDE*
isocitrate dehydrogenase	*IDH1*	shikimate kinase	*aroK*
2-oxoglutarate dehydrogenase E1 component	*sucA*	chorismate synthase	*aroC*
malate dehydrogenase	*MDH2*	arogenate dehydratase	*ADT*
fatty acid synthase	*FASN*	histidinol-phosphate aminotransferase	*hisC*
3-oxoacyl-(acyl-carrier-protein) synthase III	*fabH*	delta-1-pyrroline-5-carboxylate synthetase	*P5CS*
3-oxoacyl-(acyl-carrier protein) reductase	*fabG*		
long-chain acyl-CoA synthetase	*fadD*		

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