Supporting Information

An Overlooked Effect Induced by Surface Modification: Different Molecular Response of *Chlorella pyrenoidosa* to Graphitized and Oxidized Nanodiamonds

Chaofan Zhang[a], Xiaochen Huang[b], Yuhao Chu[a], Nanqi Ren[a], Shih-Hsin Ho[a,*]

[a]*State Key Laboratory of Urban Water Resource and Environment, School of Environment, Harbin Institute of Technology, Harbin, 150090, P. R. China.*

[b]*School of Environment, Guangdong Key Laboratory of Environmental Pollution and Health, Jinan University, Guangzhou 510632, P. R. China*

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*** Corresponding author**

Shih-Hsin Ho, Ph.D., Professor

Tel.: +86-451-86418180

Email: stephen6949@hit.edu.cn; stephen6949@msn.com

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Figure S3. (a) The significantly enriched KEGG pathway (top 20) in response to ONDs exposure of *C. pyrenoidosa*. The vertical axis corresponds to the KEGG pathways, and the horizontal axis displays the enriched value expressed as the ratio of DEG and total gene number of the pathway. The size and color of dots indicates the DEG gene number and the FDR value, respectively. (b) Volcano plot for differentially expressed genes in response to ONDs exposure of *C. pyrenoidosa*. Plots in green and in red separately represented downregulated genes and upregulated genes. The data in Figure S2 were based on the microalgae free of nanoparticles as the control group.

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the horizontal axis displays the enriched value expressed as the ratio of DEG and total gene number of the pathway. The size and color of dots indicates the DEG gene number and the FDR value, respectively. (b) Volcano plot for differentially expressed genes in response to ONDs exposure of *C. pyrenoidosa*. Plots in green and in red separately represented downregulated genes and upregulated genes. The data in Figure S3 were based on the microalgae exposed to GNDs as the control group.

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■ Tables Content

Section S1. Preparation and Characterization of GNDs and ONDs:

GNDs were acquired by pyrolysis at 900 ^{o}C for 90 min under N_2 atmosphere with a heating rate of 15 ^{o}C/min. ONDs were prepared via mixture of H_2SO_4 and HNO_3 (3:1) at 80 ^{o}C with continuously stirring for 24 h. Then the acquired ONDs were washed repeatedly with 1 mM NaOH and 1 mM HCl until the suspension reached neutral pH. Finally, the ONDs were dried in the vacuum oven overnight. Fourier transform infrared spectroscopy (SPECTRUM one, PerkinElmer, USA) was used to identify the surface functional groups of GNDs/ONDs with a wavelength region of 500-4000 cm^{-1} at resolution of 1 cm^{-1}. Confocal raman spectroscopic system (Renishaw, InVia, UK) was applied to record the defects under the 532 nm laser with a wavelength region of 100-4000 cm^{-1}. X-ray powder diffraction (XRD, X'PERT Rro MPD, Panalytical, Holland) was used to examine the crystallinity of GNDs/ONDs over a 2θ collection range of 10-90°.

Section S2. Algae Growth Culture:

C. pyrenoidosa breeds were initially preserved on agar jelly. To activate the *C. pyrenoidosa*, the microalgae were rejuvenated in the classic BG11 under at 250 μmol/m^2·s illumination with continuous supply of 2% CO_2 until exponential growth. The BG11 medium was sterilized at 121 ^{o}C for 25 min and was composed as follow: 1.5 g/L $NaNO_3$, 0.04 g/L $K_2HPO_4{\cdot}3H_2O$, 0.075 g/L $MgSO_4{\cdot}7H_2O$, 0.036 g/L $CaCl_2{\cdot}2H_2O$, 0.02 g/L $NaCO_3$, 0.003 g/L $FeCl_3{\cdot}6H_2O$, 0.006 g/L citric acid and 1 mL of microelements composed of 2.86 mg/L H_3BO_3, 1.81 mg/L $MnCl_2{\cdot}4H_2O$, 0.22 mg/L

$ZnSO_4 \cdot 7H_2O$, 0.39 mg/L $Na_2MoO_4 \cdot 2H_2O$, 0.08 mg/L $CuSO_4 \cdot 5H_2O$ and 0.05 mg/L $Co(NO_3)_2 \cdot 6H_2O$.

Section S3. Crude Enzyme Extraction:

The microalgae cells were centrifuged at 4 °C and 5000 rpm for 10 min, and were washed by sterile phosphate buffer solution (PBS, pH = 7.4) for three times. Then the collected microalgae pellets were resuspended in PBS and ultrasonicated for 30 min (pulsed with 5 s interval) under ice-bath circumstances. Finally, the crude enzyme suspension was collected by centrifugation at 4 °C, 10000 rpm for 15 min.

Section S4. SOD, CAT and MDA Analysis:

The SOD activity was determined by the inhibition of nitroblue tetrazolium reduction at 560 nm. One unit of SOD activity was the amount of enzyme that decreased the rate of nitroblue tetrazolium reduction by 50%. The CAT activity was assayed by monitoring the degradation of H_2O_2 at 240 nm. One unit of CAT was defined as the amount of enzyme required to degrade 1 μmol of H_2O_2 per minute. The MDA content was measured using a thiobarbituric acid reactive substances (TBARS) assay at 535 nm.

Section S5. ROS Detection and Analysis:

Microalgal cells were centrifuged at 5000 rpm for 5 min and washed with sterile PBS for 3 times. Then, the microalgal cells were incubated with DCFH-DA (10 mM) under the dark condition at 25 °C for 30 min and then washed again with sterile PBS. The measurement and intensity of DCF fluorescence was analyzed using a fluorescence

microscope (Eclipse E200, Nikon, Japan) and fluorescence spectrophotometer (F-7000, Hitachi, Japan) with an excitation wavelength of 485 nm and an emission wavelength of 530 nm.

Section S6. Observation and Pretreatment of SEM Analysis:

After washed three times with sterile PBS, the microalgal cells were fixed in 2.5% glutaraldehyde (v/v) overnight. After fixation, all the samples were dehydrated with increasing concentrations of ethanol (30, 50, 70, 80, 90, 100%) and tert-butyl alcohol. Finally, these samples were gold-coated and observed using SEM (TM3030 plus, Hitachi, Japan) at a voltage of 15 kV.

Section S7. Observation and Pretreatment of TEM Analysis:

After washed three times with sterile PBS, the microalgal cells were fixed with 2.5% glutaraldehyde (v/v) for 4 d and then fixed with 1% osmium tetroxide (v/v) for 2 h. Subsequently, 50%, 75%, 90%, and 100% ethanol solutions were gradually added into samples for dehydration. A mixture of 100% ethanol and 100% acetone (v/v; 1:1) and 100% acetone were then successively added for 10 min at 4 °C and 25 °C, respectively. To permeate and embed samples, mixtures with different volume ratios of 100% acetone and epoxy resin (1:1, 1:2, and 1:3, respectively) were used. After staining ultra-thin samples (50-60 nm) with uranyl acetate and citrate, the observation from sample-loaded copper grids was performed using a TEM (H-7650, Hitachi, Japan) at a voltage of 100 kV.

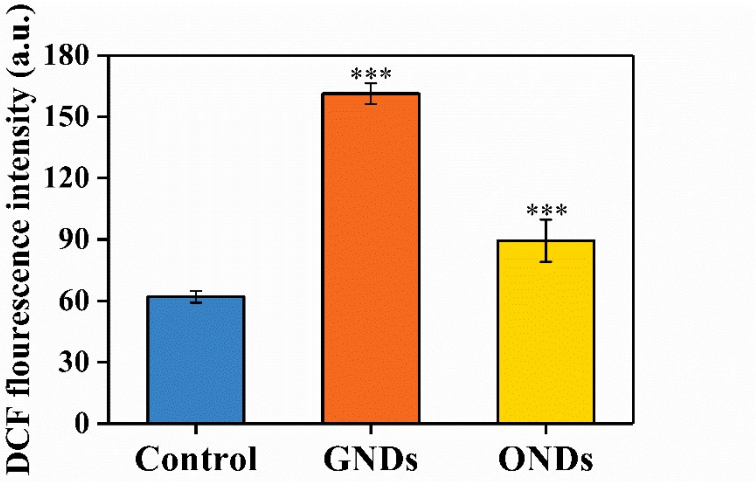
Section S8. EPSs Extraction and Analysis:

Microalgal cells were harvested by centrifugation (8000 rpm for 5 min under 4 °C) and resuspended with sterile PBS to the original volume. Then, the microalgal pellets were heated at 60 °C under the water bath for 30 min. Finally, the EPSs extraction solution was obtained after filtering through a 0.22-μm membrane filter. The EEM spectra (F-7000, Hitachi, Japan) was subsequently scanned from 220 to 500 nm with an increment of 5 nm by varying the emission wavelength from 220 to 600 nm with an increment of 1 nm.

Section S9. Transcriptomic Analysis:

Transcriptomic analysis was performed at Novogene Bioinformatics Technology Co., Ltd., Beijing, China. Cells were centrifuged at 4 °C and 5000 rpm for 5 min and immediately frozen in liquid nitrogen. RNA degradation and contamination was monitored on 1% agarose gels. RNA purity was checked using the NanoPhotometer® spectrophotometer (IMPLEN, CA, USA). RNA integrity was assessed using the RNA Nano 6000 Assay Kit of the Agilent Bioanalyzer 2100 system (Agilent Technologies, CA, USA). A total amount of 1.5 μg RNA per sample was used as input material for the RNA sample preparations. Sequencing libraries were generated using NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA) following manufacturer's recommendations and index codes were added to attribute sequences to each sample. In order to select cDNA fragments of preferentially 250~300 bp in length, the library fragments were purified with AMPure XP system (Beckman Coulter, Beverly, USA). The clustering of the index-coded samples was performed on a cBot Cluster Generation

System using TruSeq PE Cluster Kit v3-cBot-HS (Illumia) according to the manufacturer's instructions. After cluster generation, the library preparations were sequenced on an Illumina Hiseq platform and paired-end reads were generated. Raw data (raw reads) of fastq format were firstly processed through in-house perl scripts. In this step, clean data(clean reads) were obtained by removing reads containing adapter, reads containing ploy-N and low quality reads from raw data. The no-reference-genome method of transcriptome data analysis was used. For quality control of the raw data and subsequent filtering, clean reads were assembled with the Trinity software program and redundant sequences were eliminated, producing non-redundant consensus reference sequences for subsequent pathway analysis.



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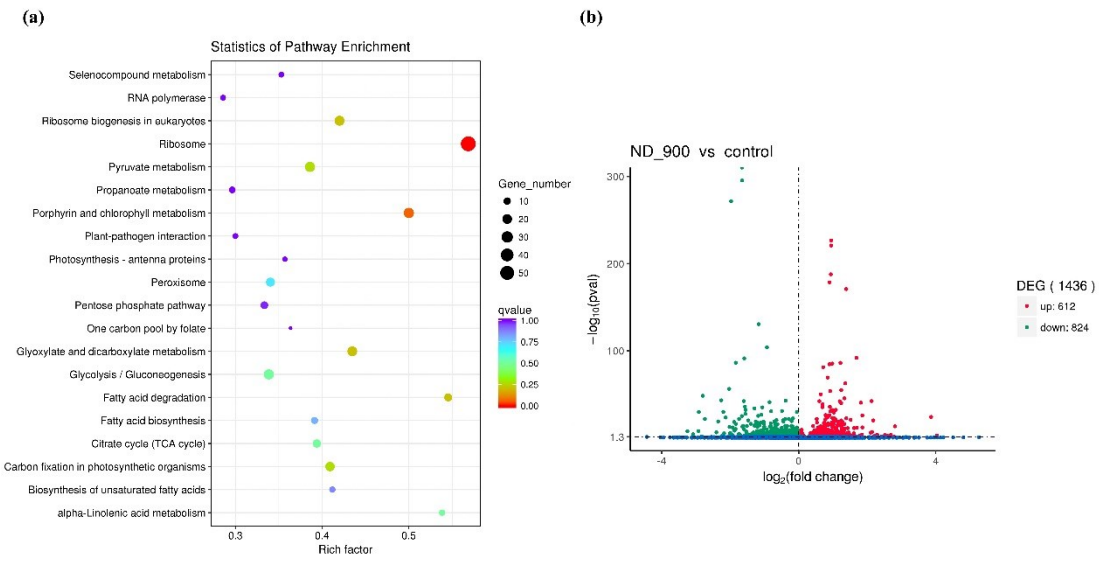

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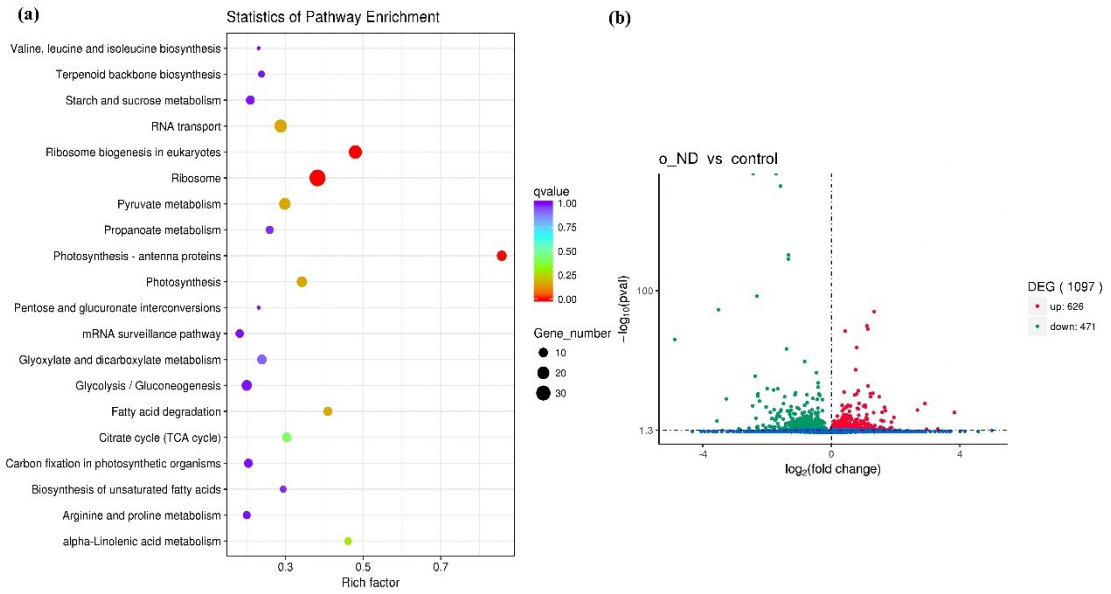

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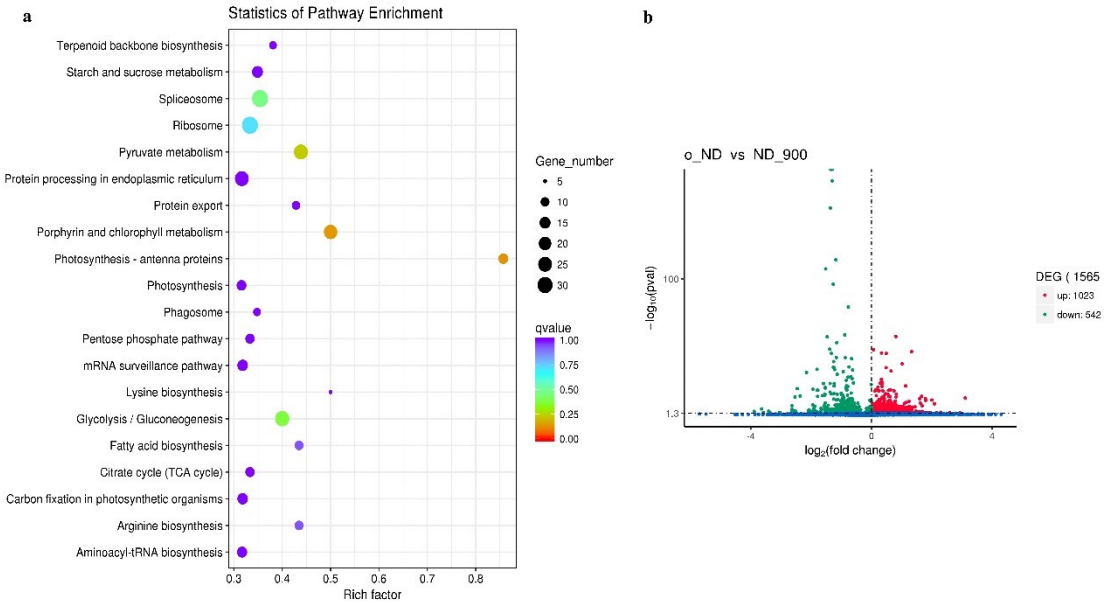

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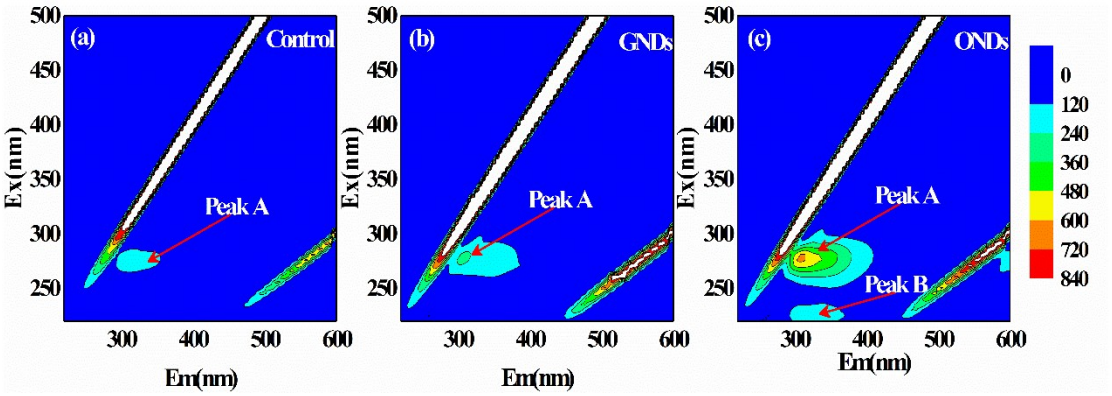

Figure S5. EEM distribution of EPS extraction of (a) control, (b) GNDs and (c) ONDs treatments.

Table S1. Gene name and gene function of the transcript, encoding enzymes for global changes of cellular metabolic pathways in response to GNDs exposure of *C. pyrenoidosa* based on the microalgae free of nanoparticles as the control group.

Pathway	EC number	Transcripts	Gene Name	Gene Function
Pentose Phosphate Pathway	EC:1.1.1.49	Cluster-3132.5109	*G6PD*	glucose-6-phosphate 1-dehydrogenase
	EC:1.1.1.44	Cluster-3132.2868	*PGD*	6-phospho-gluconate dehydrogenase
Glycolysis	EC:5.3.1.9	Cluster-3132.4748	*pgi*	glucose-6-phosphate isomerase
	EC:2.7.1.11	Cluster-3132.5113	*pfk*	6-phospho-fructokinase 1
	EC:4.1.2.13	Cluster-3132.6079 Cluster-3132.2453	*ALDO*	fructose-bisphosphate aldolase
	EC:1.2.1.9	Cluster-3132.6635	*gapN*	glyceraldehyde-3-phosphate dehydrogenase
	EC:2.7.2.3	Cluster-3132.6066	*pgk*	phosphoglycerate kinase
	EC:5.4.2.12	Cluster-3132.3559	*gpmI*	phosphoglycerate mutase
	EC:4.2.1.11	Cluster-3132.4040	*eno*	enolase
	EC:2.7.1.40	Cluster-3132.5274	*pyk*	pyruvate kinase
Tricarboxylic Acid Cycle	EC:2.3.3.1	Cluster-3132.4753	*CS*	citrate synthase
	EC:4.2.1.3	Cluster-3132.4636	*ACO*	aconitate hydratase
	EC:1.1.1.42	Cluster-3132.356	*icd*	isocitrate dehydrogenase
	EC:1.2.4.2	Cluster-3132.3410	*OGDH*	2-oxoglutarate dehydrogenase
	EC:1.3.5.1	Cluster-	*SDHA*	succinate

		3132.7608		dehydrogenase
	EC:1.2.4.1	Cluster-3132.6272 Cluster-3132.5842	*aceE*	pyruvate dehydrogenase
	EC:1.8.1.4	Cluster-3132.5344	*DLD*	dihydrolipoamide dehydrogenase
Oxidative Phosphorylation	EC:7.1.2.1	Cluster-3132.5238	*PMA*	H+-transporting ATPase
	EC:3.6.1.1	Cluster-3132.6284	*ppa*	inorganic pyrophosphatase
Oxidative Stress	PEX5	Cluster-3132.3608	*PEX5*	peroxin-5
	PMP70	Cluster-3132.2175	*PMP70*	ATP-binding cassette
	EC:1.11.1.6	Cluster-3132.6109	*CAT*	catalase
	EC:1.15.1.1	Cluster-3132.6101	*SOD1*	superoxide dismutase
	EC:1.11.1.9	Cluster-3132.3807	*pgx*	glutathione peroxidase

Table S2. Gene name and gene function of the transcript, encoding enzymes for global changes of cellular metabolic pathways in response to ONDs exposure of *C. pyrenoidosa* based on the microalgae free of nanoparticles as the control group.

Pathway	EC number	Transcripts	Gene Name	Gene Function
Pentose Phosphate Pathway	EC:1.1.1.49	Cluster-3132.5109	*G6PD*	glucose-6-phosphate 1-dehydrogenase
	EC:1.1.1.44	Cluster-3132.2868	*PGD*	6-phospho-gluconate dehydrogenase
Glycolysis	EC:2.7.2.3	Cluster-3132.6066	*pgk*	phosphoglycerate kinase
	EC:2.7.1.40	Cluster-3132.5274	*pyk*	pyruvate kinase
Tricarboxylic Acid Cycle	EC:2.3.3.1	Cluster-3132.4753	*CS*	citrate synthase
	EC:4.2.1.3	Cluster-3132.4636	*ACO*	aconitate hydratase
	EC:1.1.1.42	Cluster-3132.356	*icd*	isocitrate dehydrogenase
	EC:1.1.1.37	Cluster-3132.1836	*MDH1*	malate dehydrogenase
	EC:2.3.1.12	Cluster-3132.1498	*DLAT*	pyruvate dehydrogenase
	EC:1.2.4.2	Cluster-3132.3410	*OGDH*	2-oxoglutarate dehydrogenase
Oxidative Phosphorylation	EC:7.1.1.2	Cluster-3132.2649	*nuoA*	NADH-quinone oxidoreductase
	EC:7.1.1.8	Cluster-3132.3545 Cluster-3132.5014	*petA*	ubiquinol-cytochrome c reductase
	EC:3.6.3.14	Cluster-3132.5838 Cluster-3132.4716 Cluster-3132.2544	*atpB*	F-type H+-transporting ATPase

	EC: 7.1.2.1	Cluster-3132.5238	*PMA*	H+-transporting ATPase
Oxidative Stress	PEX5	Cluster-3132.3608	*PEX5*	peroxin-5
	EC:1.15.1.1	Cluster-3132.6101	*SOD1*	superoxide dismutase
	EC:1.11.1.9	Cluster-3132.5343	*gpx*	glutathione peroxidase

Table S3. Gene name and gene function of the transcript, encoding proteins for photosynthetic mechanism in response to ONDs exposure of *C. pyrenoidosa* based on the microalgae exposed to GNDs as the control group.

Pathway	Transcripts	log2	Gene Name	Gene Function
Light Harvesting Complex	Cluster-3132.4709	0.26747	*Lhca1*	light-harvesting complex I
	Cluster-3132.5562	0.56418	*Lhca3*	
	Cluster-3132.5691	0.32026	*Lhca4*	
	Cluster-3132.5813	0.20443		
	Cluster-3132.5820	0.57116	*Lhca5*	
	Cluster-3132.7868	0.31416	*Lhcb1*	
	Cluster-3132.6202	0.32916	*Lhcb2*	light-harvesting complex II
	Cluster-3132.6603	0.62195		
	Cluster-3132.6617	0.61617		
	Cluster-3132.6290	0.48496		
	Cluster-3132.6200	0.48496	*Lhcb4*	
	Cluster-3132.5980	0.54156	*Lhcb5*	
Photosystem II	Cluster-3132.5617	0.16056	*PsbO*	photosystem II oxygen-evolving enhancer protein
	Cluster-3132.5747	0.25216	*PsbP*	
	Cluster-3132.5099	0.49741	*PsbR*	photosystem II 10kDa protein
	Cluster-3132.4729	0.23359	*Psb27*	photosystem II Psb27 protein
Photosystem I	Cluster-3132.5502	0.27935	*PsbK*	photosystem I subunit X
	Cluster-3132.6089	0.19708	*PsaD*	photosystem I subunit II
	Cluster-3132.6067	0.31936	*PsaF*	photosystem I subunit III
	Cluster-3132.4540	0.20365	*PsaG*	photosystem I subunit V
	Cluster-3132.2387	0.40317	*PsaL*	photosystem I subunit XI
Photosysthetic Electron Transport	Cluster-3132.4729	0.23359	*PetH*	ferredoxin-NADP+ reductase
	Cluster-3132.5502	0.31859	*PetF*	ferredoxin

Table S4. Gene name and gene function of the transcript, encoding proteins for protein synthesis mechanism in response to ONDs exposure of *C. pyrenoidosa* based on the microalgae exposed to GNDs as the control group.

Pathway	EC number	Transcripts	Gene Name	Gene Function
Glycolysis	EC:5.3.1.9	Cluster-3132.4748	*pgi*	glucose-6-phosphate isomerase
	EC:2.7.1.11	Cluster-3132.4546	*pfk*	6-phospho-fructokinase
	EC:2.7.2.3	Cluster-3132.6066	*pgk*	phosphor-glycerate kinase
	EC:1.2.1.12	Cluster-3132.8027	*gapA*	glyceraldehyde 3-phosphate dehydrogenase
	EC:5.4.2.12	Cluster-3132.3559	*gpmI*	2,3-bisphosphoglycerate-independent phosphoglycerate mutase
	EC:4.2.1.11	Cluster-3132.4040	*eno*	enolase
	EC:2.7.1.40	Cluster-3132.3153	*pyk*	pyruvate kinase
	EC:1.2.4.1	Cluster-3132.4765	*aceE*	pyruvate dehydrogenase
	EC:2.3.1.12	Cluster-3132.1498	*aceF*	pyruvate dehydrogenase
	EC:2.3.3.8	Cluster-3132.6398	*ACLY*	ATP citrate (pro-S)-lyase
Nitrogen Metabolism	Nrt	Cluster-3132.6233	*NRT*	nitrate/nitrite transporter
	NR	Cluster-3132.5007	*NR*	nitrate reductase
	EC:6.3.1.2	Cluster-3132.2891	*glnA*	glutamine synthetase
Amino Acid Synthesis	EC:2.6.1.45	Cluster-3132.7036 Cluster-3132.6481	*AGXT*	serine-glyoxylate transaminase
	EC:4.2.1.20	Cluster-3132.4183	*trpA*	tryptophan synthase
	EC:4.1.3.27	Cluster-3132.5219	*TRP3*	anthranilate synthase

	EC:4.1.1.20	Cluster-3132.4869	*lysA*	diaminopimelate decarboxylase
	EC:2.7.2.4	Cluster-3132.5010	*lysC*	aspartate kinase
	EC:2.6.1.2	Cluster-3132.6088	*ALT*	alanine transaminase
	EC:4.3.2.1	Cluster-3132.1832	*ASL*	arginine-succinate lyase
	EC:2.1.3.3	Cluster-3132.3623	*OTC*	ornithine carbamoyl-transferase
Aminoacyl-tRNA Biosynthesis	EC:6.1.1.17	Cluster-3132.5606	*gltX*	glutamyl-tRNA synthetase
	EC:6.1.1.18	Cluster-3132.2905	*glnS*	glutaminyl-tRNA synthetase
	EC:6.1.1.7	Cluster-3132.3757	*alaS*	alanyl-tRNA synthetas
	EC:6.1.1.14	Cluster-3132.2406 Cluster-3132.3221	*glyQ*	glycyl-tRNA synthetase
	EC:6.1.1.11	Cluster-3132.6426	*serS*	seryl-tRNA synthetase
	EC:6.1.1.9	Cluster-3132.4574	*valS*	valyl-tRNA synthetase
	EC:6.1.1.4	Cluster-3132.4687	*leuS*	leucyl-tRNA synthetase
	EC:6.1.1.6	Cluster-3132.2999	*lysK*	lysyl-tRNA synthetase
	EC:6.1.1.19	Cluster-3132.4691	*argS*	arginyl-tRNA synthetase
	EC:6.1.1.20	Cluster-3132.3522	*pheS*	phenylalanyl-tRNA synthetase
Ribosome Biogenesis	-	Cluster-3132.2689	*UTP13*	U3 small nucleolar RNA-associated protein
	-	Cluster-3132.3353	*UTP21*	
	-	Cluster-3132.4746	*Real*	midasin
	-	Cluster-3132.5207	*CRM1*	exportin-1
	EC:3.6.1.-	Cluster-	*LSG1*	large subunit GTPase

		3132.6367		
Translocation	-	Cluster-3132.4985	*SEC61α*	protein transport protein
	-	Cluster-3132.327	*SEC63*	translocation protein
	-	Cluster-3132.1652	*SRP72*	signal recognition
	-	Cluster-3132.1598	*SRP68*	
	-	Cluster-3132.6475	*SRP54*	
	-	Cluster-3132.4703	*TatA*	sec-independent protein translocase protein
Protein Processing	EC:3.2.1.207	Cluster-3132.961	GlcII	mannosyl-oligosaccharide glucosidase
	-	Cluster-3132.1413	CNX	calnexin
	-	Cluster-3132.7363		
	-	Cluster-3132.2943	OSTs	Oligosaccharyl-transferase complex
	-	Cluster-3132.4000		
	-	Cluster-3132.1627	NEF	hypoxia
	-	Cluster-3132.7142	GRP94	heat shock protein
	-	Cluster-3132.6683	Sec13/31	protein transport protein
	-	Cluster-3132.6517		
	-	Cluster-3132.6387	Sec22/24	
	-	Cluster-3132.5744		
	EC:3.4.21.112	Cluster-3132.4180	S1P	membrane-bound transcription factor protease