Electronic Supplementary Information

Mechanistic insights into stress response and metabolic activity resilience of *Nitrosomonas europaea* cultures to CeO_2 nanoparticle long-term exposure

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Content

Supplementary Method

qRT-PCR

Three differentially expressed genes with the statistical difference (NE0925, *amoC3* and *ccmA*) were screened for the validation of microarray results on a ViiA 7 Real-time PCR System (Applied Biosystems, USA). The extracted RNA for microarray hybridization was also subject to qRT-PCR analysis. The specific primers were available in Table S3. The analysis procedures were explicated in previous study [1]. The amplification condition was: pre-denaturation at 95 ℃ for 10 min; denaturation at 95 ℃ for 10 s and annealing at 60 ℃ for 60 s (40 cycles). After application, the melting curve was obtained (60-99 ℃, ramp rate: 0.05 ℃/s) to confirm the non-specific amplicons. Finally, the target gene expressions were normalized to that of 16S rRNA genes.

Supplementary Result

Confirmation of microarray data

Three representative genes at different expression levels were subject to qRT-PCR for validation of microarray results. Gene NE0925 encoding cytochrome c (Complex IV) for electron transfer and proton-driven force generation was highly up-regulated with FC value (log2 transformed) of 2.82 and 4.16 after nano-CeO_2 exposure and 12-h recovery, respectively (Table S4). Gene *amoC3* encoding ammonia monooxygenase for ammonia oxidation was also highly regulated with FC value (log2 transformed) of 5.45 and 3.41 after NP exposure and 12-h recovery, respectively (Table S4). In addition, gene *ccmA* encoding for ABC transport was significantly differentially expressed (Table S4). Generally, their expressions obtained with qRT-PCR presented similar varying trend with microarray results although FC ratio was smaller than that obtained by microarray.

Supplementary Tables & Figures

Table S1 Variations of DLS size and ζ potential of 50 mg/L nano-CeO_2 in the medium during 6-h batch incubation.

DLS size (nm)			ζ potential (mV)		
0 h	**3 h**	**6 h**	**0 h**	**3 h**	**6 h**
281.5 ± 70.1	968.2 ± 161.7	1477 ± 354.6	-13 ± 6.2	-15.4 ± 5.3	-17.9 ± 6.2

Table S2 Concentrations of total and dissolved Ce (III) before and after NP removal treatment when the ARR declined by 10 %, 25 % and 40 % respectively.

Treatment	Total Ce (mg/L)			Dissolved Ce (III) (mg/L)		
	10 %	25 %	40 %	10 %	25 %	40 %
Before	42.94 ± 0.42	42.78 ± 0.44	43.02 ± 0.16	< 0.01	< 0.01	< 0.01
After	0.13 ± 0.01	0.11 ± 0.02	0.15 ± 0.02	< 0.01	< 0.01	< 0.01

Table S3 The oligonucleotide primers for selected genes used in qRT-PCR quantification.

Target Gene	Primer Sequence	References
16S rRNA	F:5’ CGTGTCGTGAGATGTTGGGT 3’ R:5’ CGTGCTTTCTGAGATTGGC 3’	[2]
NE0925	F:5' AGCGTCTTTATGTCCGTTCAGC 3’ R:5’ GGCATCAGCACCGATTTGTTT 3’	[2]
amoC3	F:5’ GGGGCTTCGTTATCCTGG 3’ R:5’ AGAATGGCTCTGTCCTGCTT 3’	this study
ccmA	F:5’ CCGTAAATCCCGGTGAGC 3’ R:5’ CAATCTGAGCAGGCTGGTTTT 3’	this study

Table S4 Microarray and qRT-PCR analysis of selected functional genes after 1.5-d exposure to 50 mg/L nano-CeO_2 and 12-h recovery incubation, respectively with the normal cells as the reference.

Locus tag	Gene Symbol	Product	Sample	Microarray fold-change (log_2 value)	*p*-value	qRT-PCR fold-change (log_2 value)
NE0925	-	Cytochrome c, class I	Exposure	2.82	5.68×10^{-3}	1.69 [a]
			Recovery	4.16	1.20×10^{-3}	3.21 [a]
NE1411	*amoC3*	ammonia monooxygenase subunit C	Exposure	5.45	1.10×10^{-8}	4.88 [a]
			Recovery	3.41	4.31×10^{-7}	3.01 [a]
NE0764	*ccmA*	ABC superfamily	Exposure	1.80	3.90×10^{-3}	1.46 [a]
			Recovery	0.89	6.32×10^{-3}	0.51

[a] Values were statistically significant according to the 95 % confidence.

Fig. S1 Long-term effects of 1 and 50 mg/L nano-CeO_2 on ARR in the chemostat

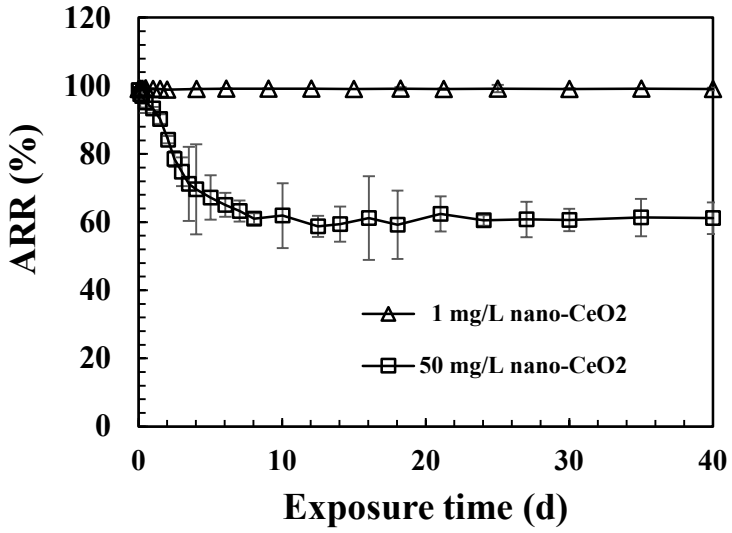


Fig. S2 Long-term effects of 1 and 50 mg/L nano-CeO_2 on DLS size (A) and ζ potential (B) of cell-NP complex cultures

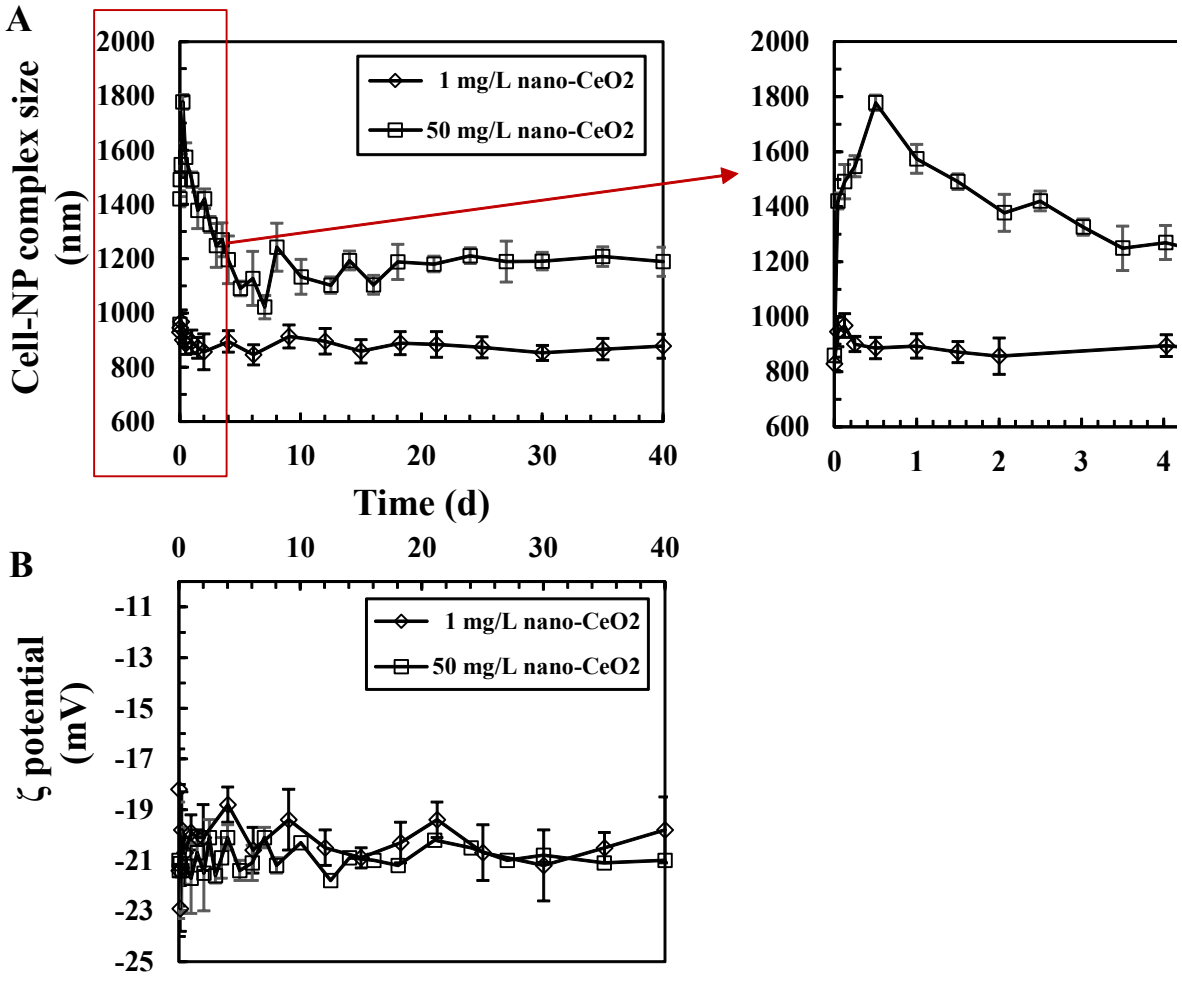
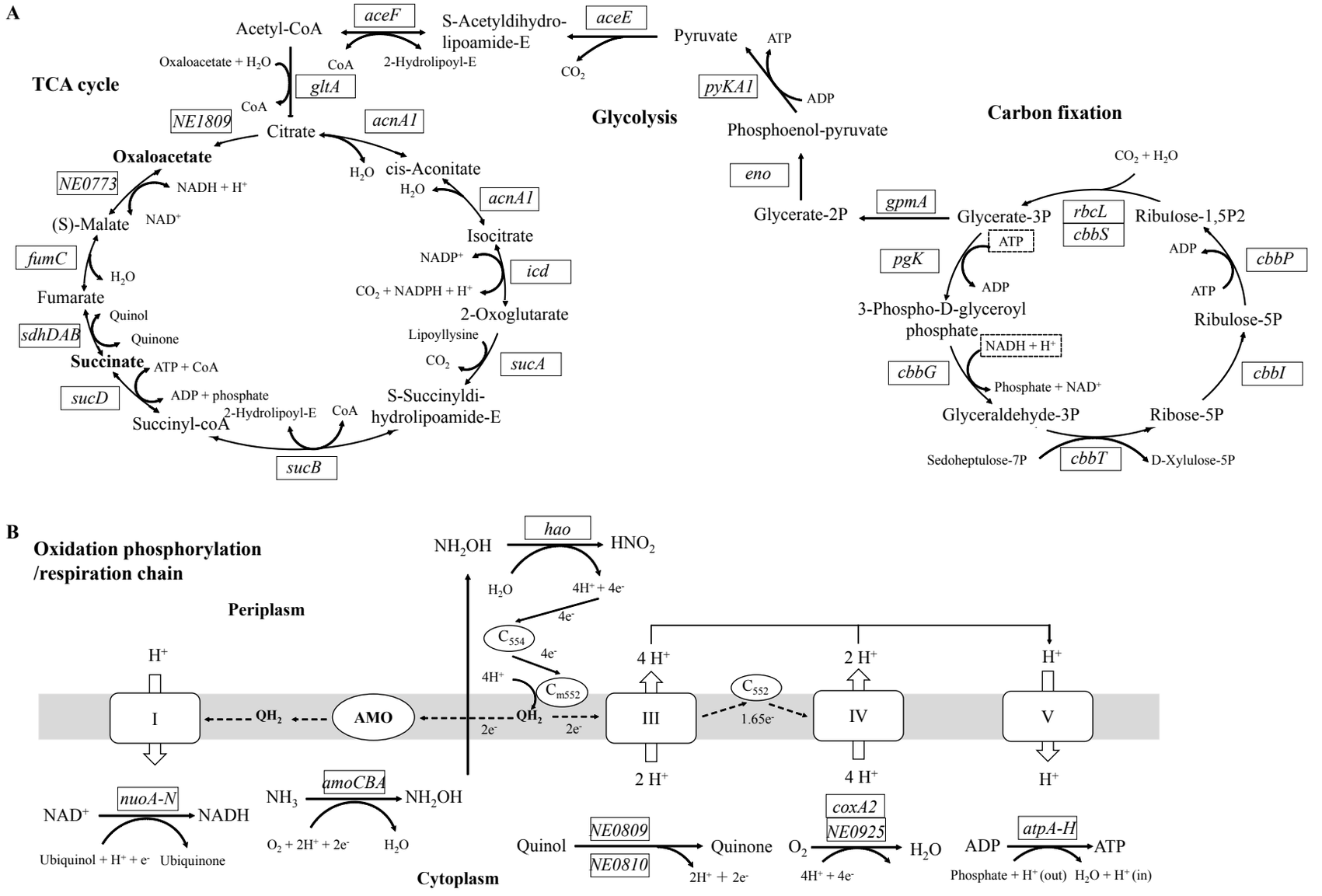


Fig. S3 KEGG pathways of carbon assimilation (A) and oxidation phosphorylation (B); Complex I, NADH dehydrogenase; Complex III, cytochrome bc1 oxidase; Complex IV, cytochrome c oxidase; Complex V, ATP synthase.



Supplementary references:

[1] R. Yu, J. Wu, M. Liu, G. Zhu, L. Chen, Y. Chang, H. Lu, Toxicity of binary mixtures of metal oxide nanoparticles to *Nitrosomonas europaea*, Chemosphere 153 (2016) 187-197.
[2] J. Wu, H. Lu, G. Zhu, L. Chen, Y. Chang, R. Yu, Regulation of membrane fixation and energy production/conversion for adaptation and recovery of ZnO nanoparticle impacted Nitrosomonas europaea, Appl. Microbiol. Biotechnol. 101 (2017) 2953-2965.