Supplementary material

Exposure to TiO_2 nanoparticles induces shifts in the microbiota composition of *Mytilus galloprovincialis* hemolymph

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Supplementary tables

Table S1. Name and sequences of the primers used for the amplification of the V4 hypervariable region of the 16S rRNA gene.

Primer	Primer sequence
515F	GTGCCAGCMGCCGCGGTAA
802R	TACNVGGGTATCTAATCC
806R	GACTACHVGGGTWTCTATT

Table S2. Data summary and phylotype OTUs. Values under trimmed tags are the numbers of reads removed after chimeric and low-quality data trimming. The average length of the reads was calculated after data trimming. OTUs were calculated by comparing each tag to the SILVA database. CW = control seawater; TW= $nTiO_2$ treated seawater; C1-C4 = control mussels; T1-T4 = $nTiO_2$-exposed mussels).

Sample id	Total reads	Trimmed tags	Average length	OTUs
CW	60582	41977	263	18605
TW	95409	68539	272	26870
C1	53209	33246	279	19963
C2	136374	96830	278	39544
C3	109582	70489	277	39093
C4	96797	71875	278	24922
T1	116747	83120	276	33627
T2	88441	66448	278	21993
T3	20556	11551	281	9005
T4	93325	52011	282	41314

Table S3. Summary of the immunomodulatory effects of $nTiO_2$ exposure in *Mytilus galloprovincialis*. Data were obtained using the same type of $nTiO_2$ and experimental conditions of exposure of the present study.

$nTiO_2$ *In vivo* exposure 100 µg/L; 96h (from Barmo et al., 2013 (a); Balbi et al., 2014 (b))	Functional hemocyte and hemolymph parameters	Lysosomal membrane stability ↘ (a; b)
		Phagocytic activity ↘ (a; b)
		Extracellular ROS production ↗ (a)
		NO production ↗ (a; b)
		Apoptosis (a): - Mitochondrial membrane potential ↘ - Annexin V binding ↗
		Total hemocyte count ↘ (a)
		Lysozyme activity ↗ (b)
	Hemocyte Gene expression	↗ defensin, mytilin B (a) ↘ lysozyme, TLR-i (b)

References

Balbi, T., Smerilli, A., Fabbri, R., Ciacci, C., Montagna, M., Grasselli, E., Brunelli, A., Pojana, G., Marcomini, A., Gallo, G., Canesi, L., 2014. Co-exposure to n-TiO2 and Cd2+ results in interactive effects on biomarker responses but not in increased toxicity in the marine bivalve M. galloprovincialis. Sci. Total Environ. 493, 355–364. https://doi.org/10.1016/j.scitotenv.2014.05.146

Barmo, C., Ciacci, C., Canonico, B., Fabbri, R., Cortese, K., Balbi, T., Marcomini, A., Pojana, G., Gallo, G., Canesi, L., 2013. In vivo effects of n-TiO2 on digestive gland and immune function of the marine bivalve Mytilus galloprovincialis. Aquat. Toxicol. 132–133, 9–18. https://doi.org/10.1016/j.aquatox.2013.01.014

Supplementary figures

Figure S1. Relative abundance of the main bacterial genera in hemolymph samples of $nTiO_2$-exposed mussels with respect to samples of control mussels). Data are expressed in % variation. In green (left) and red (right) are represented the decrease and increase of different genera.

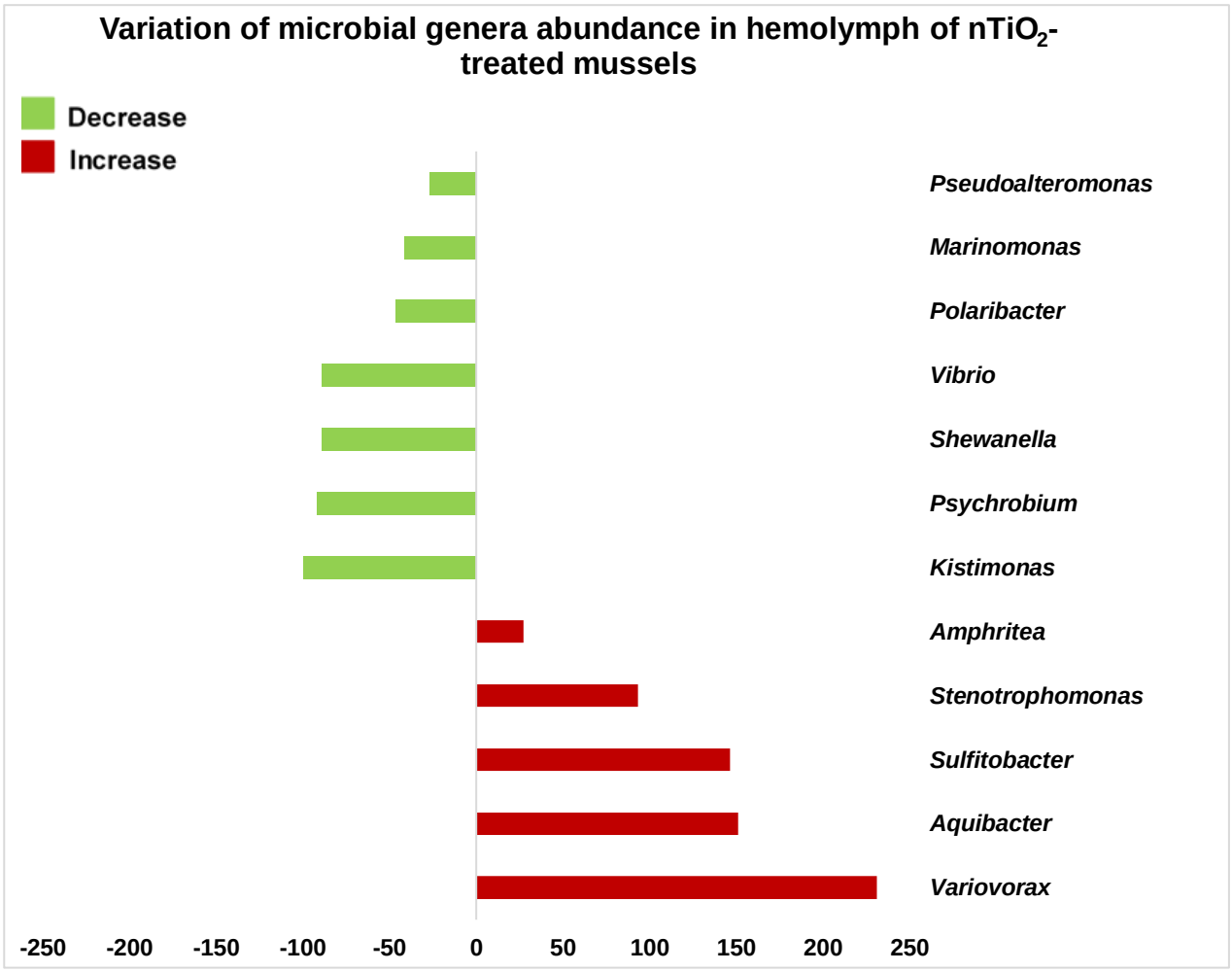
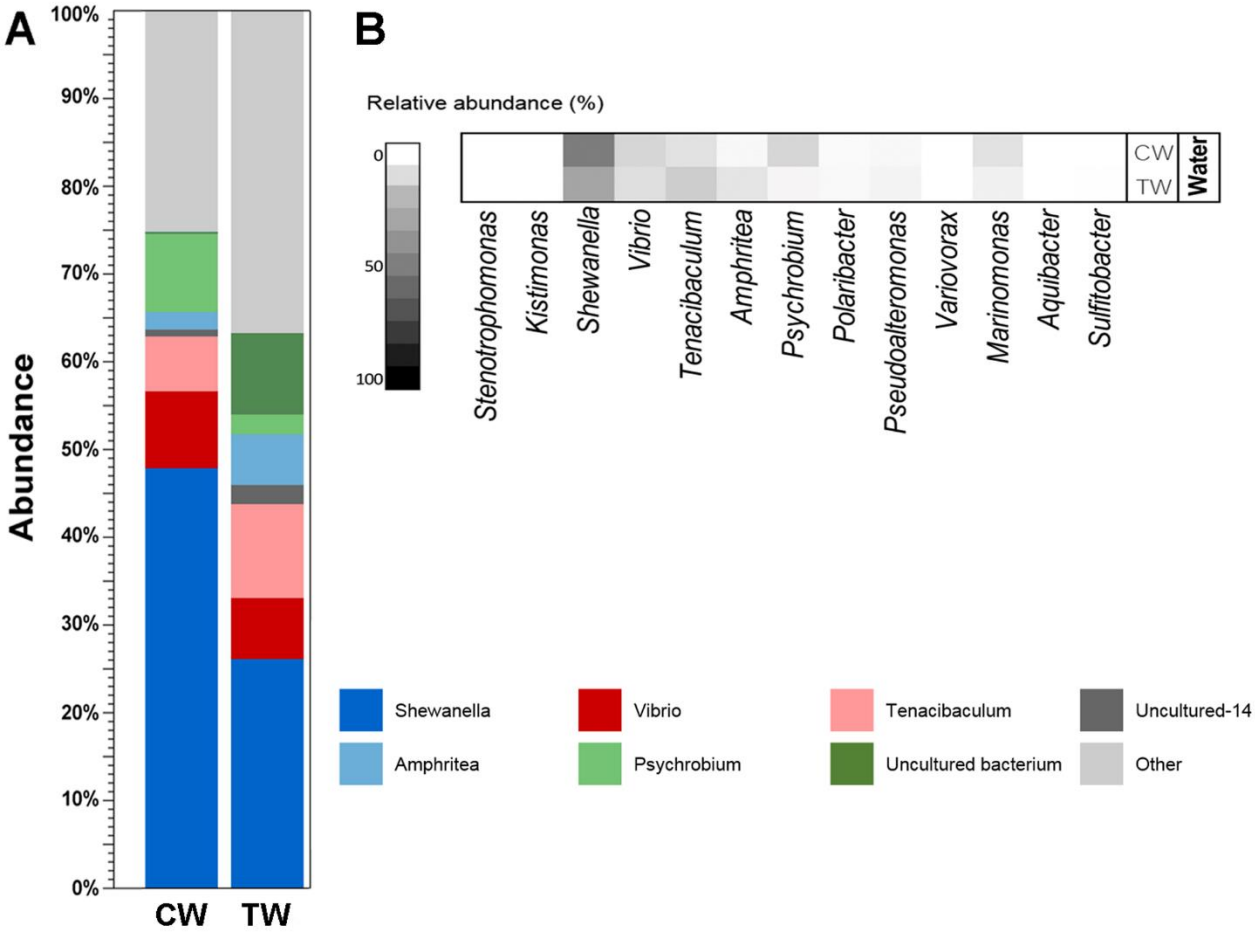
Figure S2. Microbiome analysis in seawater medium sampled at the end of exposure (96 h) from tanks of control (CW) and $nTiO_2$-exposed mussels (TW). A) Relative abundance of bacterial genera. Data represent cumulative abundance expressed in %. B) Same data represented by Heatmap analysis Data represent only taxa with abundance greater than 1% of total.