Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supporting information

Different effect of micro-scale and nano-scale zero-valent iron particles on planktonic microorganisms from natural reservoir water

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CONTENTS

determine which pathways/enzymes are likely to be associated with a phylogenetically placed read. Paprica accepts reads pre-processed by Mothur software, allowing removal of low quality reads, short reads and contaminants (e.g. mitochondria, chloroplasts).

10. Figure S7. Microscopy images of planktonic microorganisms in reservoir water before the experiment started. (A) Green fluorescence of stained living bacteria, (B) phytoplankton under a bright field, (C, D) numerous free cells from broken cyanobacterial colonies (*Microcystis aeruginosa, Woronichinia naegeliana*) after 21 days (red arrows). The bacteria were stained using the Live/Dead BacLight kit (L7007, Life Technologies) in the dark for 15 min to distinguish viable and non-viable cells. Viable cells were then observed under a fluorescence microscope (AxioImager, Zeiss, Germany) with excitation at 470 nm and emission at 490–700 nm [2].

REFERENCES

1 J. S. Bowman and H. W. Ducklow, *PLoS One*, 2015, **10**, 1–18.

2 M. S. A. Darwish, N. H. A. Nguyen, A. Ševců, I. Stibor and Stoyan K. Smoukov, *Mater. Sci. Eng. C*, 2016, **63**, 88–95.

Table S1. Chemical composition of reservoir water (RW) obtained by iron chromatography (IC), inductively coupled plasma spectrometry (ICP-OES) and total organic carbon analysis (TOC). Samples obtained on 22.08.2016; pH = 8.3; oxidation-reduction potential (ORP) = 260 mV. Values measured in triplicate and standard deviation (mean) is provided in parentheses.

	mg L^{-1}	Detection limit (mg L^{-1})	Methods
Mg	3(0.1)	0.1	ICP-OES
Ca	15.7(0.2)	0.1	ICP-OES
Na	11.9(0.2)	1	ICP-OES
K	1.6(0.0)	0.1	ICP-OES
Fe	0.05(0.0)	0.02	ICP-OES
Mn	< 0.005	0.005	ICP-OES
Cr	< 0.005	0.005	ICP-OES
Al	0.05(0.0)	0.02	ICP-OES
Be	< 0.0002	0.0002	ICP-OES
Cu	< 0.01	0.01	ICP-OES
Ag	< 0.005	0.005	ICP-OES
P_{TOT}	0.02(0.0)	0.02	ICP-OES
Si	4.6(0.0)	0.1	ICP-OES
Cl	30.7(0.9)	2	IC
SO_4^{2-}	24.4(0.5)	10	IC
$N\text{-}NO_3^-$	1.2(0.0)	0.46	IC
$N\text{-}NO_2^-$	< 0.15	0.15	IC
$N\text{-}NH_4^-$	< 0.04	0.04	IC
$P\text{-}PO_4^-$	< 0.17	0.17	IC
TOC	6.6(2.4)	2	TOC analysis
Dissolved O_2	9.2		

Table S2. Number of (A) cultivable bacteria (CFU mL^{-1}), (B) cyanobacteria (cells mL^{-1}) and (C) algae (cells mL^{-1}). CFU: colony-forming unit, the values in the parentheses is standard deviation.

A	Bacterial number (CFU mL^{-1})					
Day	Control		+ nZVI		+ mZVI	
0	1157	(211)	1463	(1017)	1357	(476)
1	2023	(1451)	7920	(2813)	3993	(3180)
3	7167	(2967)	72900	(40084)	5187	(2901)
7	6533	(1258)	171333	(13317)	14000	(8000)
14	5100	(1945)	16667	(4000)	7257	(6738)
21	5183	(1919)	21700	(7708)	4910	(3708)

B	Cyanobacterial number (cells mL^{-1})					
Day	Control		+ nZVI		+ mZVI	
0	72850	(6718)	85600	(7920)	96100	(90368)
3	100850	(24819)	99450	(919)	111800	(94187)
7	111300	(37760)	108300	(26446)	198100	(179464)
14	179500	(20506)	176500	(48790)	355000	(264458)
21	139500	(41719)	82500	(22486)	219000	(142836)

C	Algal number (cells mL^{-1})					
Day	Control		+ nZVI		+ mZVI	
0	35450	(22132)	50650	(1626)	48850	(3041)
3	41450	(10394)	41950	(636)	44700	(15698)
7	26700	(19940)	12800	(2828)	28300	(3253)
14	24800	(15274)	24600	(5940)	22400	(566)
21	12900	(10041)	14900	(707)	10200	(849)

Table S3. Concentration of total Fe added in reservoir water (RW) and dissolved Fe at the beginning and end of the experiment. Analysed using inductively coupled plasma spectrometry (ICP-OES).

		Day 0	**Day 21**
Total Fe (mg L^{-1})	RW + nZVI	126.5(21.4)	121.8 (0.24)
	RW + mZVI	110.5(9.4)	157.7(0.01)
Dissolved Fe (mg L^{-1})	RW + nZVI	0.1(0.05)	< 0.01
	RW + mZVI	0.01(0.00)	< 0.01

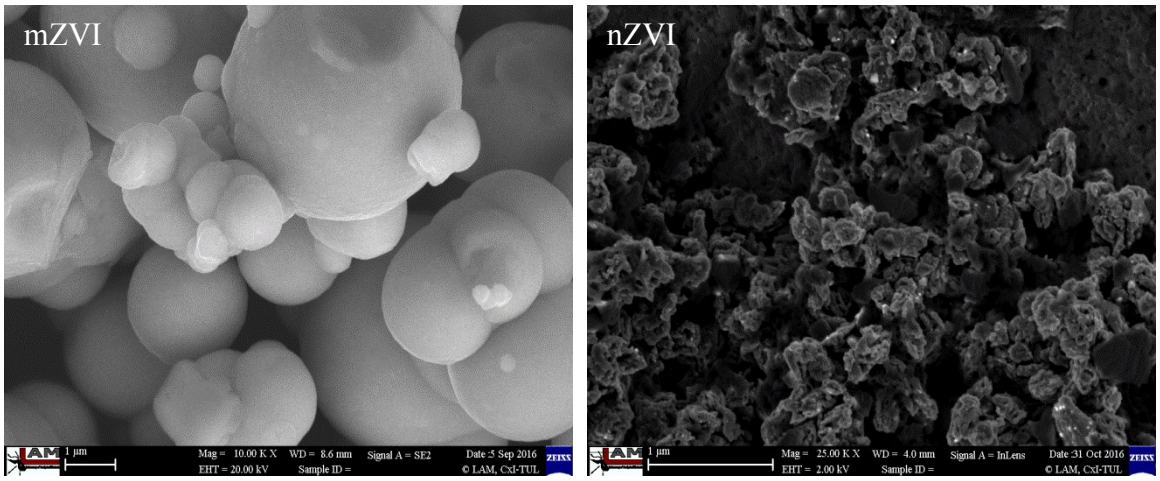


Figure S1. Scanning electronic microscopy (SEM) images of pristine mZVI and nZVI particles. Scale bar is 1 µm.

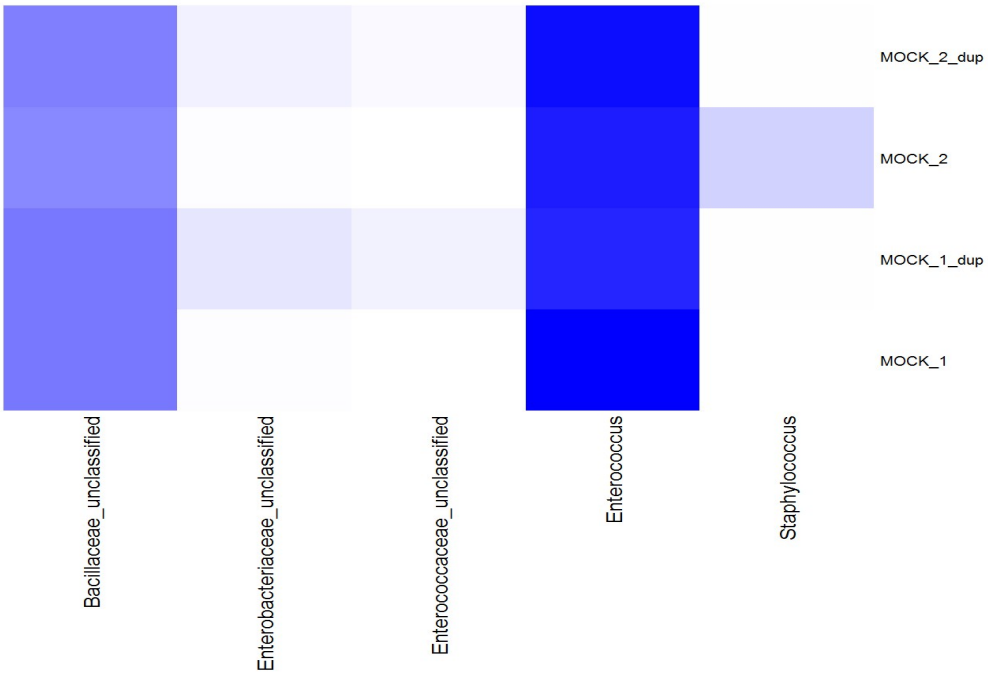


Figure S2. MOCK community samples. The quality of Mothur pipeline (see section 2.6) parameter settings was verified using in-house MOCK community samples (including *Enterococcus*, *Bacillus*, *Enterococcus*, *Escherichia-Shigella*, *Staphylococcus* and *Saccharomycotina*) added to each run. The results indicate that the taxonomic groups identified correspond with those expected. Note that *Saccharomycotina* are not included in the results as the primers were not designed to amplify Eukaryotes.

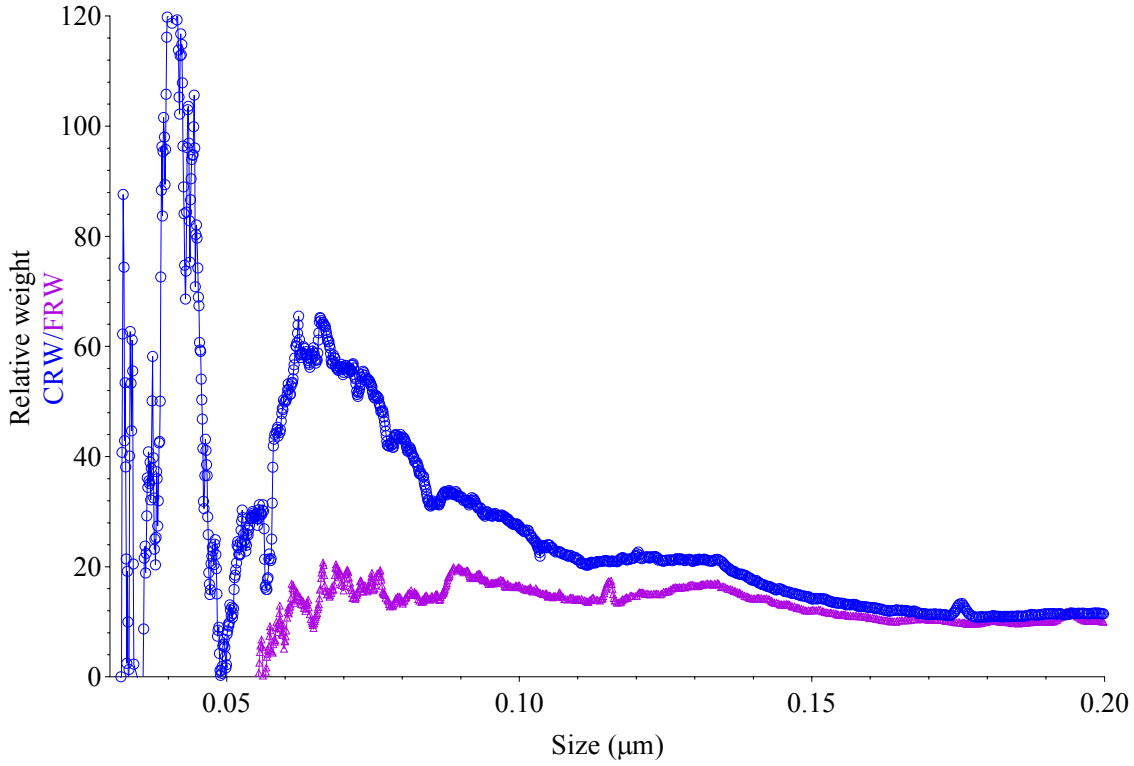


Figure S3. Reservoir water (filtered reservoir water [FRW] and centrifuged reservoir water [CRW]) colloidal particles of natural organic compounds obtained by centrifugal sedimentation (CPS) at 10000 rpm.

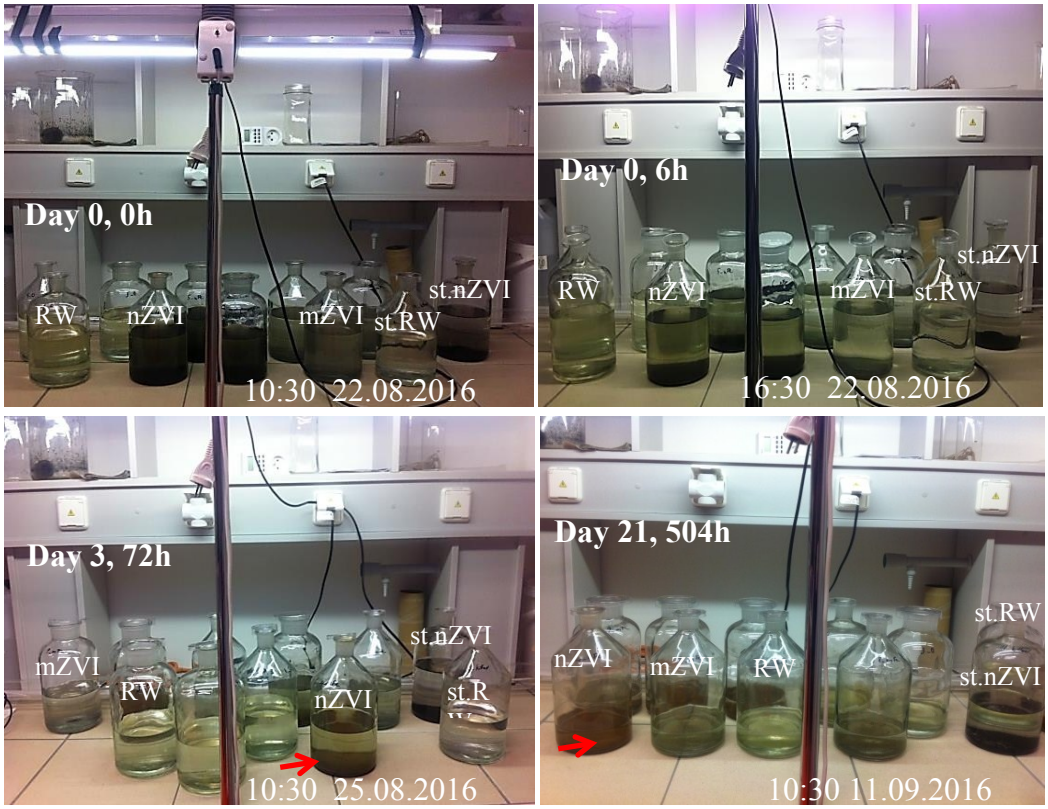


Figure S4. Experimental set-up. The reddish brown oxidised colour in bottles containing nZVI (indicated by a red arrow) first observed after day 3. The position of the bottles was changed over the experiment. Abbreviations: reservoir water (RW), reservoir water with nZVI (nZVI), reservoir water with mZVI (mZVI), sterilised reservoir water (st.RW) and sterilised reservoir water and nZVI (st.nZVI).

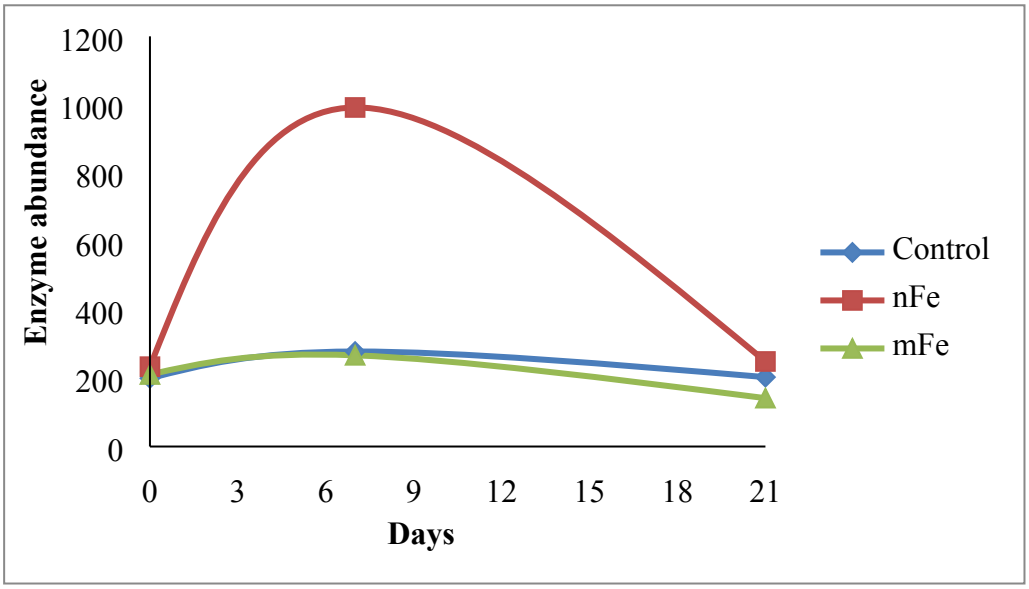


Figure S5. Changes in ferroxidase enzyme (EC1.16.3.1) abundance over the 21-day experiment.

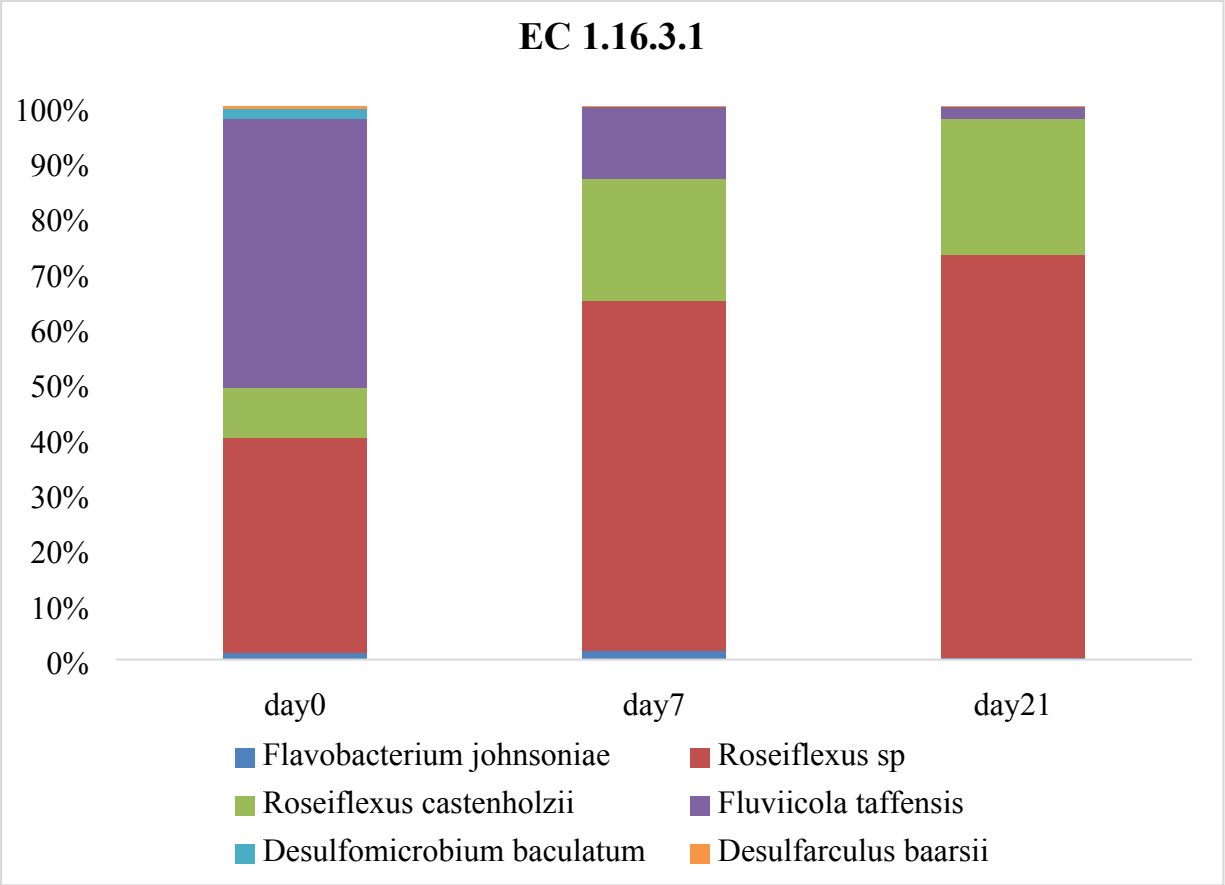
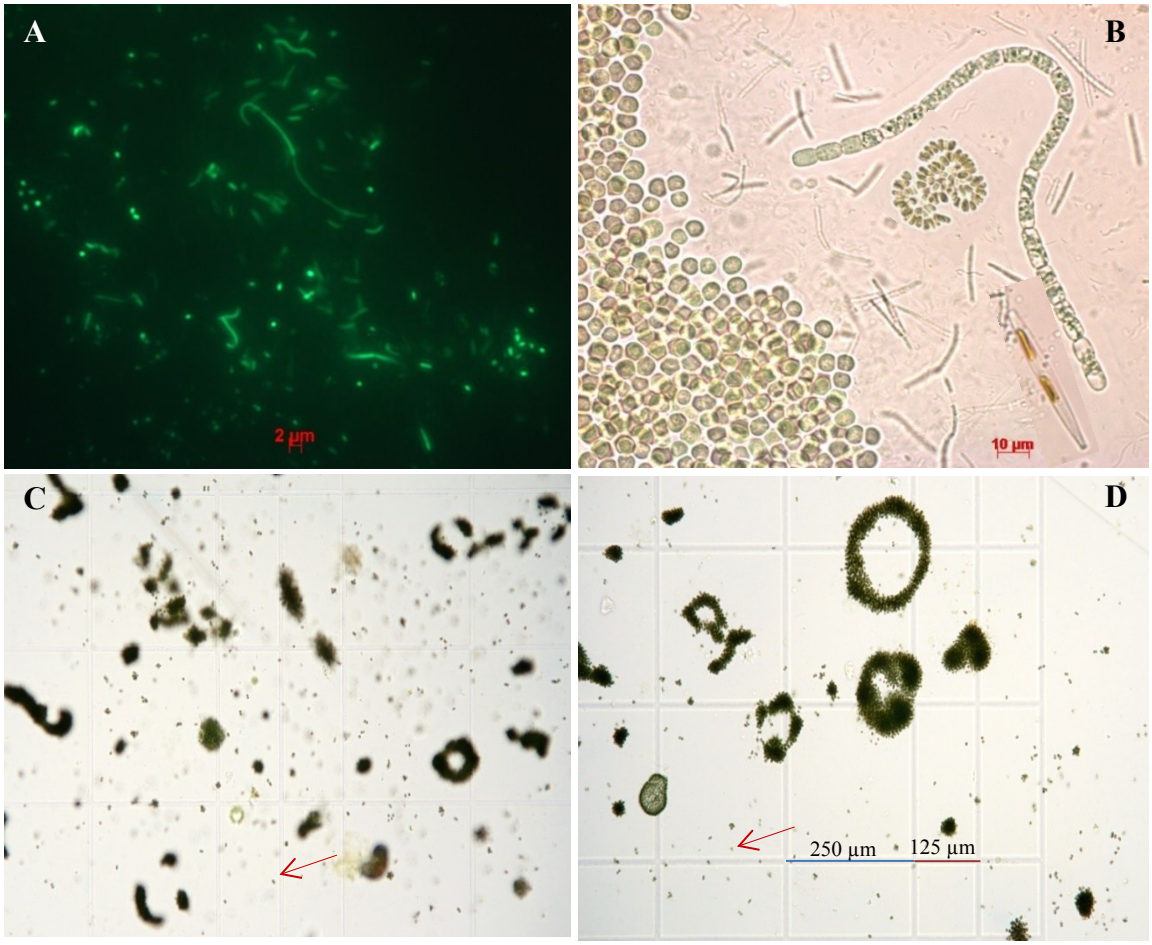


Figure S6. Abundance of planktonic bacteria associated with ferroxidase enzyme (EC1.16.3.1) when exposed to nZVI. The data was obtained by metabolic inference on (preferably, but not exclusively, by next-generation sequencing [NGS]) 16S rRNA gene libraries using phylogenetic placement in Paprica software [1]. Paprica uses pathways/enzymes shared between the members of all clades on a reference tree to determine which pathways/enzymes are likely to be associated with a phylogenetically placed read. Paprica accepts reads pre-processed by Mothur software, allowing removal of low quality reads, short reads and contaminants (e.g. mitochondria, chloroplasts).



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