Influence of phytoplankton on fate and effects of modified zero-valent iron nanoparticles

Adeyemi S. Adeleye[*†,§,∥], *Louise M. Stevenson*[‡,§,∥], *Yiming Su*[†,ǂ,§,∥], *Roger M. Nisbet*[‡,§], *Yalei Zhang*[ǂ], *Arturo A. Keller*[**,†,§]

[†]Bren School of Environmental Science & Management, University of California, Santa Barbara, CA 93106-5131, USA

[‡]Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara, CA 93106-5131, USA

[ǂ]State Key Laboratory of Pollution Control and Resource Reuse, Tongji University, Shanghai 200092, China

[§]University of California Center for Environmental Implications of Nanotechnology, Santa Barbara, California, USA

Supporting Information

SUMMARY OF SUPPORTING INFORMATION (SI)

Methods

Tables

Figures

S1.0 Synthesis of FeSSi

Briefly, 250 ml mixture containing 7.6 g sodium borohydride (Oakwood Chemical), 1.5 g dithionite (Sigma-Aldrich), and 0.2 mL of colloidal silica (30 wt.% in water) was titrated into 250 ml solution containing 4.9 g $FeCl_3$ (Fisher Scientific). After reduction, nanoparticles were collected and triple-washed with nitrogen-purged deionized water (DI, 18.2 MΩ.cm, Barnstead Nanopure Diamond). A neodymium-iron-boron magnet was used to separate solids and liquids. Fresh FeSSi particles were stored in 30% ethanol at 4 °C until use.

S2.0 Culture Media and Characterization

To obtain media with algal natural organic matter (algal NOM), batches of 2-d and 11-d cultures were centrifuged (7500 *g*, 15 min, 4 °C, Sorvall RC-5B Plus) and then filtered (0.2 µm PES membrane, Thermo Scientific) in aseptic conditions to produce 2-d and 11-d media, respectively. Sterile COMBO media was used as 1-d media. The media were characterized by measuring dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) using a Shimadzu TOC-V with an attached TNM1 unit, pH (Oakton EW-35811-71, Cole Parmer), conductivity (HACH IntelliCAL CDC401), and dissolved oxygen (HACH LDO Model 2). Functional groups present in algal NOM were characterized by analyzing freeze-dried 11-d media (Labconco 7754042) via infrared spectroscopy using a Nicolet iS10 spectrometer with a diamond ATR crystal. Interferograms were obtained by taking 256 scans (resolution = 2 cm^{-1}).

Table S1. Composition of COMBO media (Adapted from Kilham *et al*.1998)

Compound	Concentration	
	(mg/L)	**(µmol/L)**
$CaCl_2.2H_2O$	36.76	250
$MgSO_4.7H_2O$	36.97	150
K_2HPO_4	8.71	50
$NaNO_3$	85.01	1000
$NaHCO_3$	12.60	150
$Na_2SiO_3.9H_2O$	24.42	100
H_3BO_3	24.00	388
KCl	7.45	100
$Na_2EDTA.2H_2O$	4.36	11.7
$FeCl_3.6H_2O$	1.00	3.7
$MnCl_2.4H_2O$	0.18	0.9
$CuSO_4.5H_2O$	0.001	0.004
$ZnSO_4.7H_2O$	0.022	0.08
$CoCl_2.6H_2O$	0.012	0.05
$Na_2MoO_4.2H_2O$	0.022	0.09
H_2SeO_3	0.0016	0.012
Na_3VO_4	0.0018	0.01
LiCl	0.31	7.3
RbCl	0.07	0.6
$SrCl_2.6H_2O$	0.15	0.57
NaBr	0.016	0.16
KI	0.0033	0.02
Vitamin B_{12}	0.00055	0.0004
Biotin	0.0005	0.002
Thiamin	0.1	0.3

S3.0 Aggregation kinetics of FeSSi

The initial aggregation rate constant (k) reflects doublet formation and is proportional to the initial rate of increase in the intensity-weighted hydrodynamic radius, $a_h(t)$, with time, t, and the inverse of initial number concentration of nanoparticles, N_0 (Eq. 1):

$$k \propto \frac{1}{N_0}\left(\frac{da_h(t)}{dt}\right)_{t\to 0} \quad (1)$$

k is obtained from the slope of the best fit line of $(da_h(t)/dt)_{t\to 0}$, which is determined via DLS using the Zetasizer. Attachment efficiencies (α) of FeSSi in 1-d, 2-d, and 11-d media were derived by normalizing the measured k by the diffusion-limited aggregation rate constant $(k)_{fav}$ (Eq. 2). $(k)_{fav}$ was determined in highly favorable aggregation conditions.

$$\alpha = \frac{\left(\frac{da_h(t)}{dt}\right)_{t\to 0}}{\left(\frac{da_h(t)}{dt}\right)_{t\to 0,\, fav}} \quad (2)$$

S4.0 FeSSi-Algal Model

The model state variables and equations are defined in Table 2. Parameters and their fitted values are in Table 3

Algal biomass

We used chlorophyll a concentration as a proxy for algal biomass. Algal growth is modeled using the logistic equation.

DOC concentration

DOC was used as a proxy for algal organic matter. As in Stevenson et al. (2013), we assumed that DOC production rate is the sum of rates proportional to algal growth rate and population size, since DOC excretion depends on photosynthesis, maintenance, and growth. We also included a loss term to account for DOC removal due to heteroaggregation of DOC with FeSSi. DOC could be lost from the measured DOC pool due to heteroaggregation if the DOC already bound to FeSSi may no longer be available to bind to other FeSSi particles, thus decreasing the concentration of available DOC; This latter hypothesis is supported by aggregation data showed that FeSSi can aggregate up to a micron in size within an hour (Figure S5).

Bioavailable and inactivated FeSSi

Bioavailable FeSSi is assumed to be inactivated when coated by DOC. In exponentially growing cultures that started at a very low cell concentration, all the added FeSSi particles were bioavailable when introduced into the cultures. However, FeSSi particles were a mix of bioavailable and inactivated particles when introduced to 11 day old cultures (see next section).

Parameter fitting and model development

We tested out a few other model formulations by fitting the models to our data and comparing the overall fits. The final two models considered were nested models – the reduced model (with fewer parameters) was identical to the full model (the final model described in this paper) except that it did not include β. We chose between these two models (with or without the parameter β) by calculating the negative log(likelihood) values of the final model functions and conducting a Likelihood Ratio Test. The test statistic, D, was 7.318 (two times the difference of the loglikelihood values) with 1 degree of freedom. We calculated a p-value assuming that the test statistic is approximately chi-squared distributed. This p-value was 0.0068 (less than 0.05), so we were able to reject the null hypothesis (reduced model) and continued our analysis using the full model defined in this paper.

All seven parameters in Table S3 were estimated simultaneously for all treatments by fitting the model to our chlorophyll a data (algal biomass) and DOC concentration measurements. We used the BYOM ("Bring Your Own Model") platform for parameter estimation, developed by Tjalling Jager for Matlab (http://debtox.info). BYOM finds parameters that minimize the value of the negative log(likelihood) function.

Along with parameter values, we also fitted the initial values of both stages of algal growth (within the range of observed starting populations within all cultures of the same age), the initial concentration of DOC in the Day 1 cultures (we know the initial concentration of DOC in the Day 11 cultures due to good overlap between DOC data from cultures that started on Day 1 and Day 11 of growth), and the starting concentration of bioavailable FeSSi particles in Day 11 culture exposures. We fit the initial value of bioavailable FeSSi in Day 11 cultures because the

stock suspension of FeSSi particles was added to media from 11-day-old algal cultures (with the algal cells removed) and mixed for 1 hour prior to exposure. This means that the algal-produced DOC had an hour to interact with the particles, during which time DOC may have begun inactivating FeSSi particles prior to exposure. Model parameterization leads us to believe this occurred – the model overestimates the initial toxic response of 180 mg-FeSSi/L to 11 day old cultures unless $C_{N11}(0)$ was allowed to be less than $6 * 10^{13}$ FeSSi particles/L (the model gives the best fit when 70% of the particles have been inactivated by DOC prior to dosing).

Parameters relating to algal growth and DOC production, r, K, h_{DN} and k_{DN} were initially fit to control data only. FeSSi-related parameters (kf, γ_{UN}, β and $C_{N11}(0)$) were fit to all treatments simultaneously with r, K, h_{DN} and k_{DN} fixed. Once we had good estimates for the parameters, we fit all parameters to all data sets simultaneously with different allowances – FeSSi-related parameters (kf, γ_{UN}, β and $C_{N11}(0)$) were given a wide range of potential values (large minimum and maximum parameter estimation limits) while parameters that were initially fit to control data only (r, K, h_{DN} and k_{DN}) were allowed to vary within a small range of the control fit values (minimum and maximum parameter values for these were +/- 5-15% of the control fit parameter estimate) for the final parameter set.

Potential model refinements

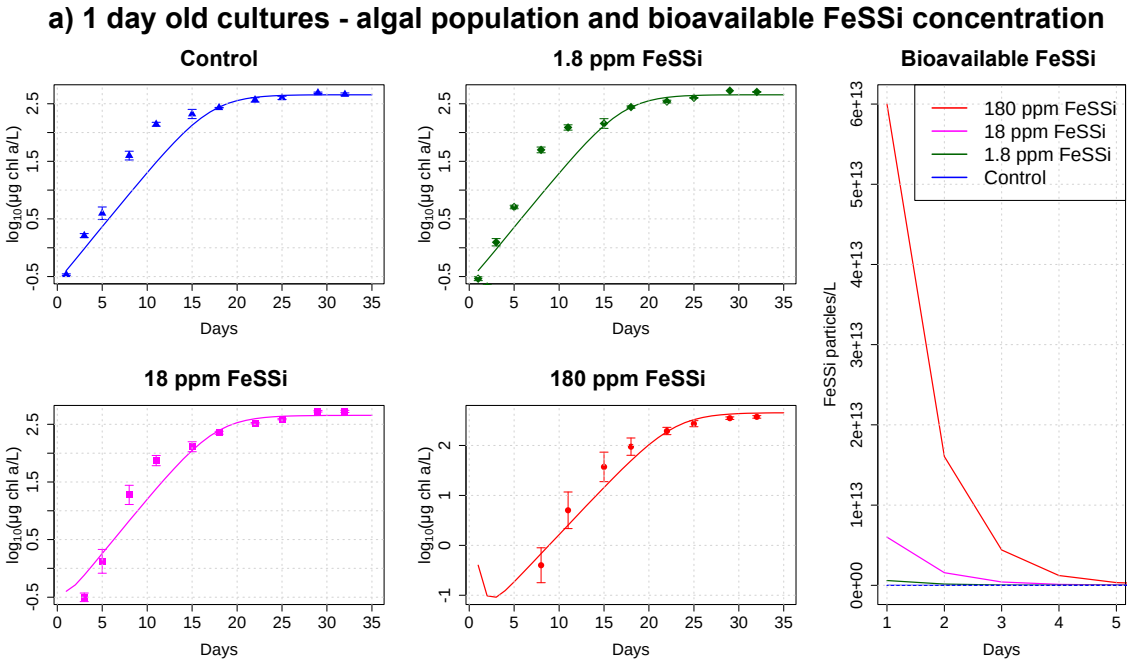
The model could be refined at the cost of adding more parameters, which did not seem prudent given the data we had available. Some potential model elaborations are:

1) *Mechanistic algal model* – we used the phenomenological logistic growth equation to model phytoplankton, which is an obvious oversimplification of algal growth. We could

use a mechanistic model of nutrient limited algal growth, but this would involve at least 3 additional parameters.

2) *DOC addition from algal mortality* – dead algal cells could provide a significant addition of DOC to algal cultures.

3) *Nanoparticle exposure altering DOC production* – nanomaterial exposure may alter algal DOC production, which could help explain the mismatch between our model's prediction and DOC concentrations of algal cultures in slowing growth phase exposed to the highest concentration of FeSSi.



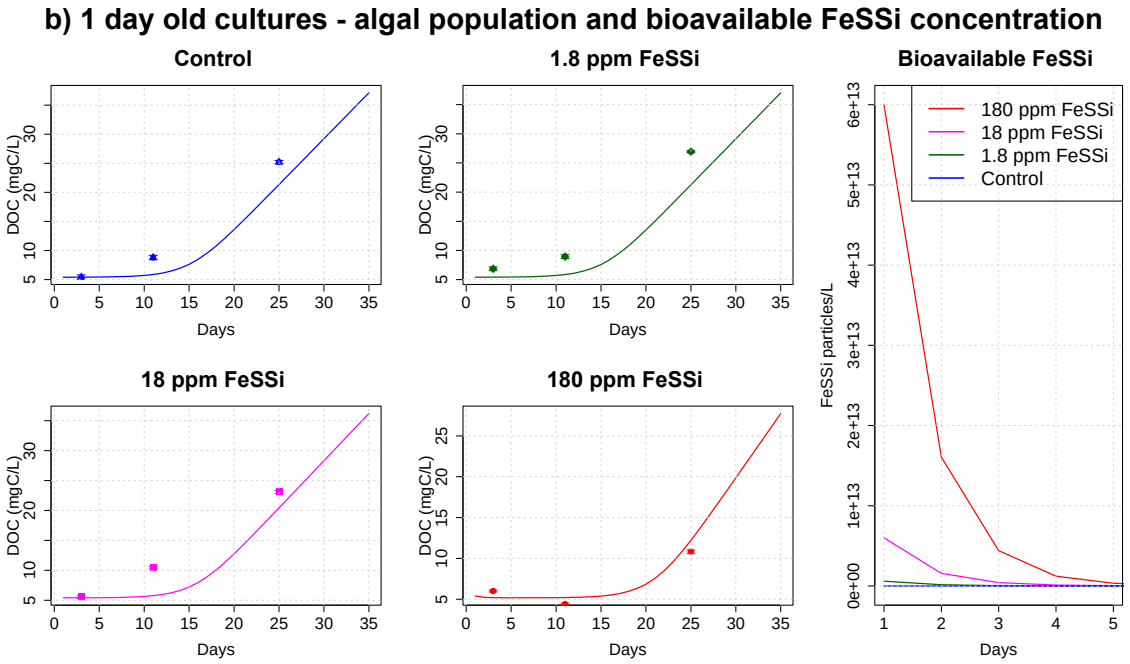
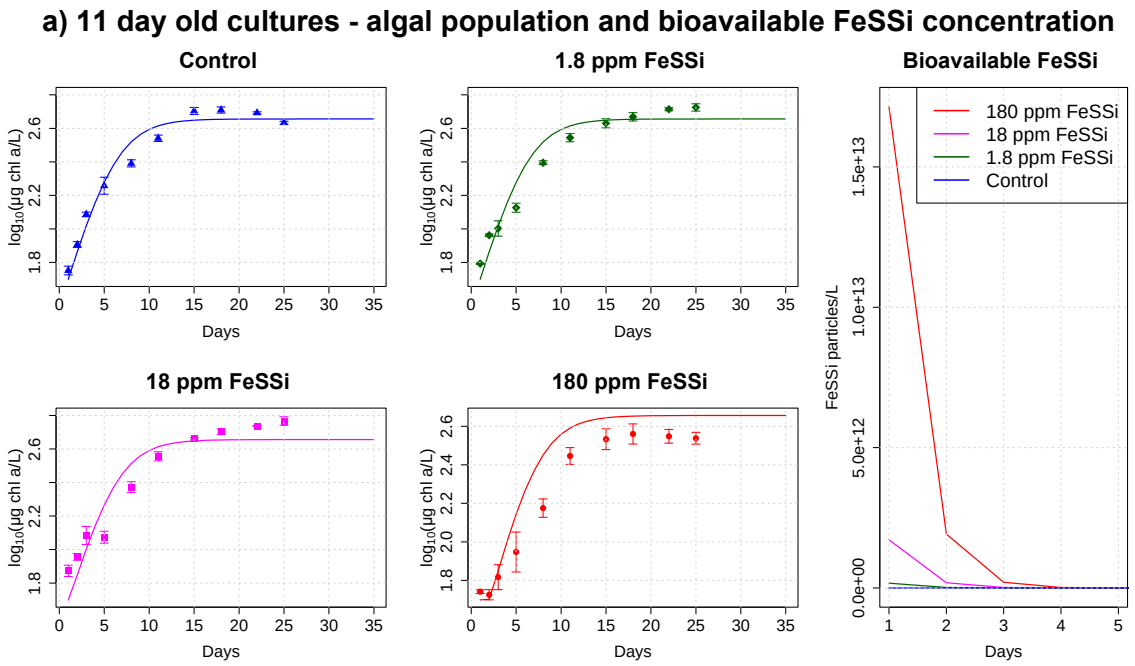


Figure S1. Fits of the FeSSi-Algal model to cultures in exponential growth phase (Day 1 cultures) to algal biomass (a) and DOC concentration (b) and the model predictions for the concentration of bioavailable FeSSi through time (up to day 5, at which time all of the FeSSi is inactivated)



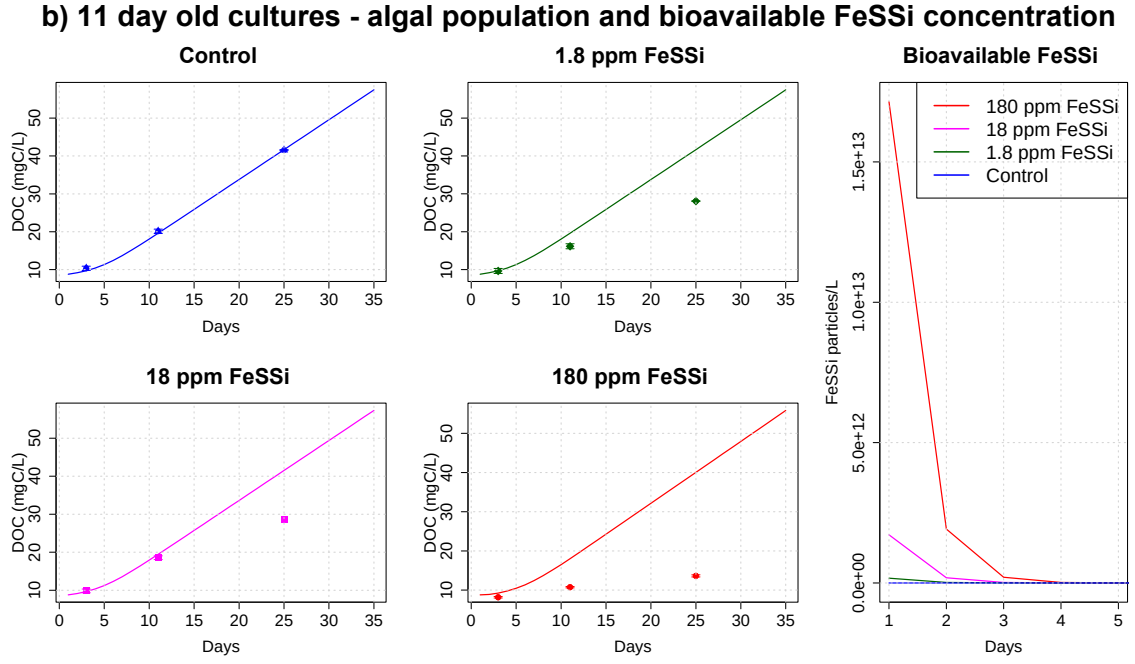


Figure S2. Fits of the FeSSi-Algal model to cultures in slowing growth phase (Day 1 cultures) to algal biomass (a) and DOC concentration (b) and the model predictions for the concentration of bioavailable FeSSi through time (up to day 5, at which time all of the FeSSi is inactivated)

Table S2. Dynamic FeSSi algal model

State Variables	
N	Algal biomass (μg chl a/L)
D	DOC concentration (mgC/L)
C_N	bioavailable FeSSi (FeSSi particles/L)
C_U	FeSSi inactivated by DOC (FeSSi particles/L)
Functions	
$J_{UN} = g_{UN} D C_N$	Inactivation of FeSSi by DOC
$P_D = k_{DN} N + h_{DN} \frac{dN}{dt}$	DOC production
$m = k_f C_N$	Mortality due to FeSSi exposure
Balance equations	
$\frac{dN}{dt} = rN\left(1 - \frac{N}{K}\right) - mN$	Algal biomass
$\frac{dD}{dt} = P_D - bJ_{UN}$	DOC
$\frac{dC_N}{dt} = - J_{UN}$	Bioavailable FeSSi
$\frac{dC_U}{dt} = J_{UN}$	Inactivated FeSSi

Table S3. Parameters for FeSSi-algal model

Parameters		
r	algal intrinsic growth rate	0.44 1/day
K	algal carrying capacity	453 μg chl a / L
k_f	FeSSi toxicity parameter	$5.576*10^{-6}$ (day * L) / FeSSi particles
k_{DN}	parameter in DOC production rate	0.003484 mgC / (ug chl a * day)
h_{DN}	parameter in DOC production rate	0.0072 mgC / ug chl a
γ_{UN}	FeSSi inactivation rate	0.2484 L / (mgC * day)
β	Loss of DOC due to heteroaggregation with FeSSi	$4.069*10^{-7}$ mgC / FeSSi particles
$C_{N11}(0)$	Initial concentration of bioavailable FeSSi exposed to 11 day old cultures	$1.75*10^{13}$ FeSSi particles / L

S5.0 Environmental Scanning Electron Microscopy (ESEM)

At the end of the experiments, 1 mL of 11-d *C. reinhardtii* cultures dosed with 180 mg/L of FeSSi was fixed with formalin (5%). The suspension was allowed to mix, deposited on a JEOL aluminum specimen mount, and then imaged using a Phillips FEI XL30 FEG ESEM equipped with a Bruker XFlash 6160 energy dispersive spectrometer (EDS). Imaging was done in wet mode at 3 Torr, 4 °C, and an accelerating voltage of 10 kV.

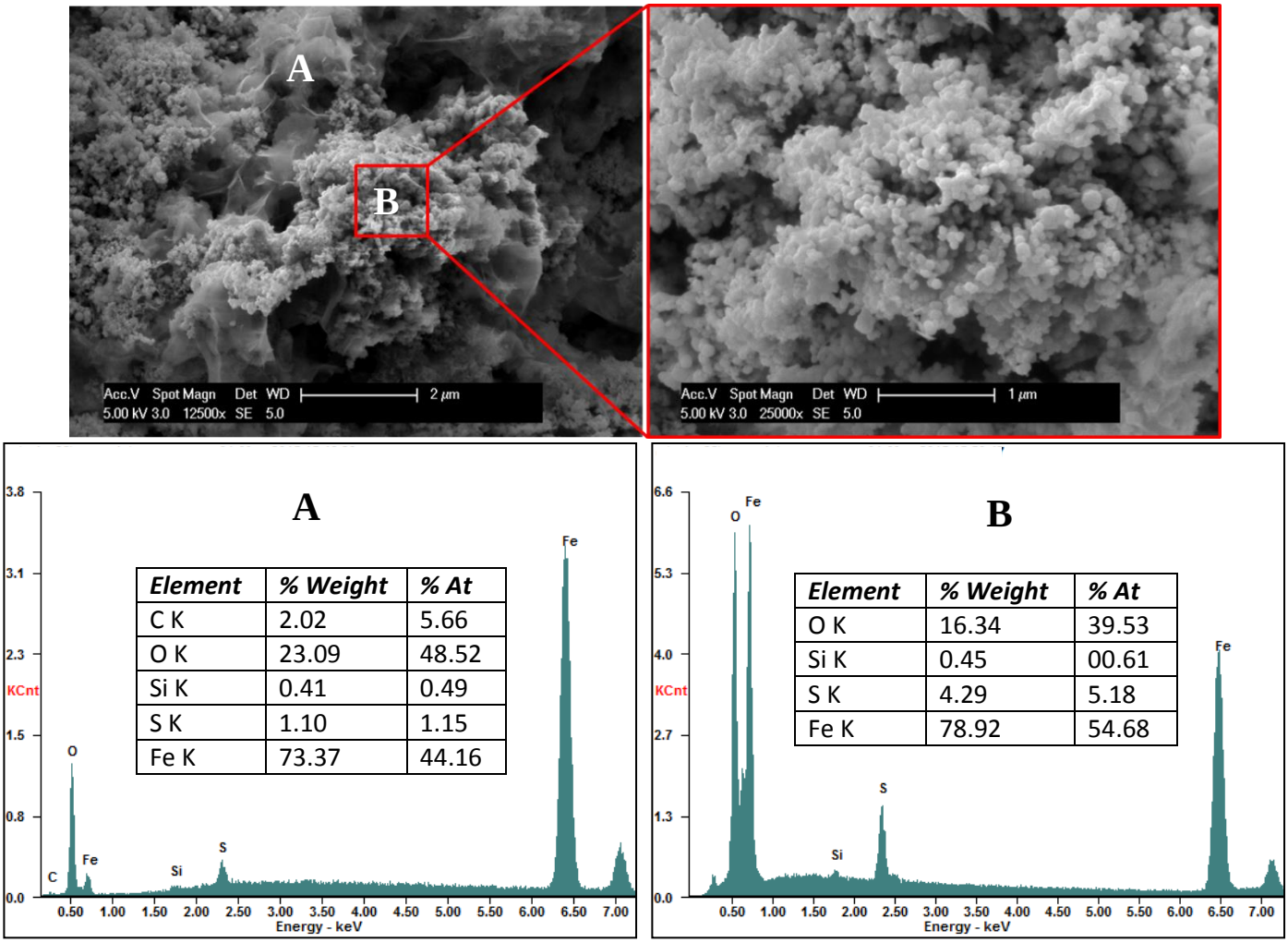


Figure S3. Scanning electron micrographs and EDS spectra of the surface of pristine FeSSi showing different states of iron, fully oxidized (A), and partially oxidized (B)

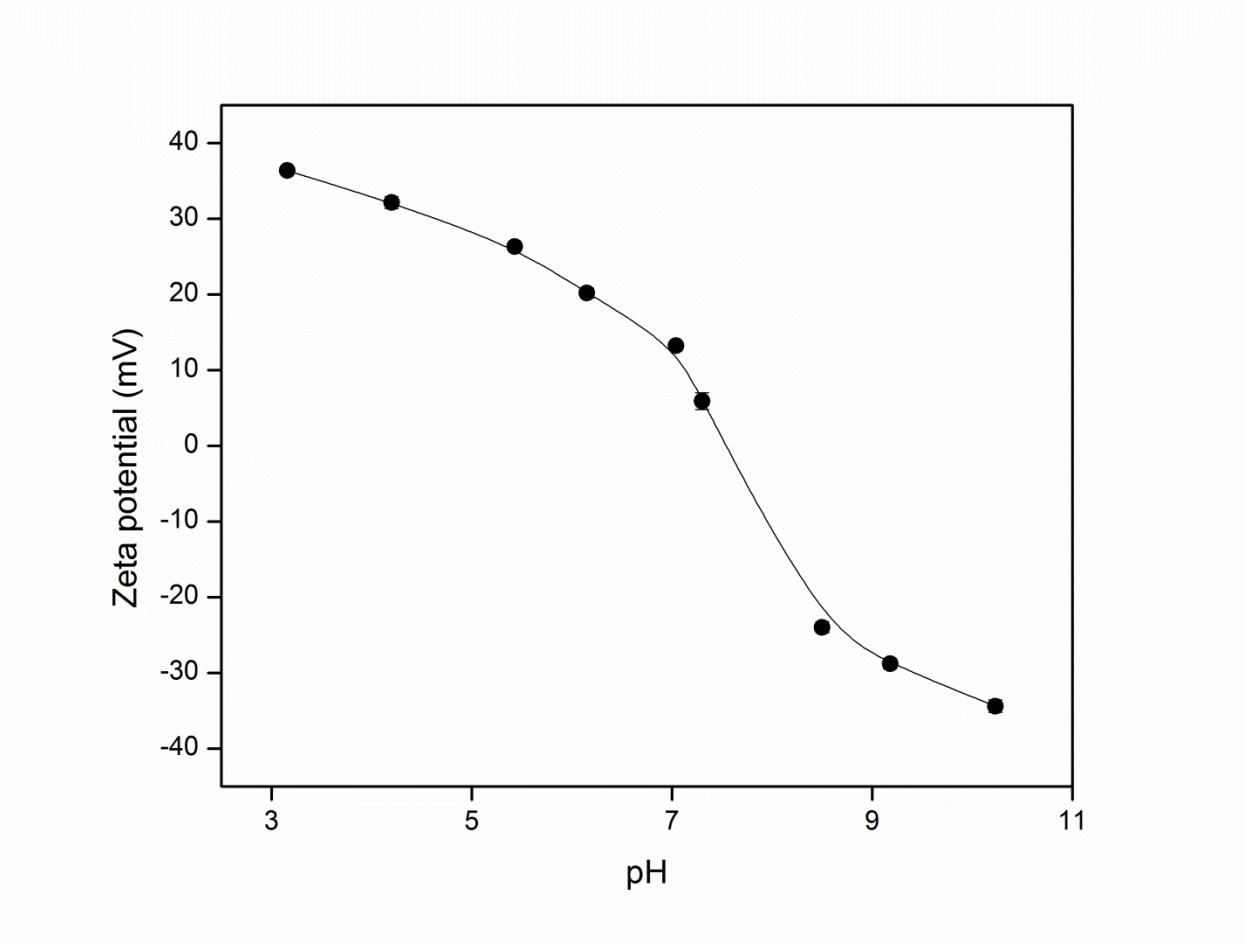


Figure S4. Effect of pH on zeta potential of FeSSi. Isoelectric point (IEP) ≈ pH 7.4

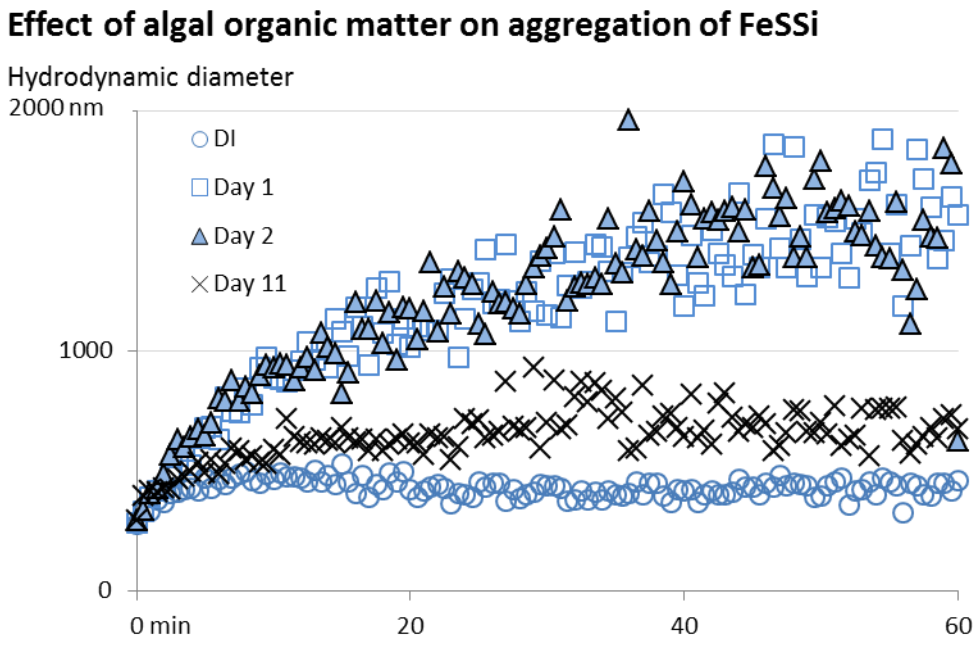


Figure S5. Aggregation kinetics of FeSSi in DI, Day 1 (1-d), Day 2 (2-d), and Day 11 (11-d) media

Table S4. Properties of media with and without *C. reinhardtii* cells

Property	**Day 1 media**	**Day 2 media**		**Day 11 media**	
		With cells	Without[a] cells	With cells	Without[a] cells
Dissolved organic carbon (mg/L)	1.83	Nd	2.19	nd	5.58
Total dissolved nitrogen (mg/L)	18.8	Nd	16.3	nd	6.30
Dissolved oxygen (mg/L)	8.43	8.98	8.47	9.42	8.92
Phosphorus (mg/L)	4.50	Nd	4.10	nd	0.07
pH	7.6	7.8	7.7	10.5	10.4
Conductivity (µS/cm)	272	284	283	284	298

[a] Cells were removed by centrifugation followed by 0.2 µm filtration under sterile conditions

nd = Not determined

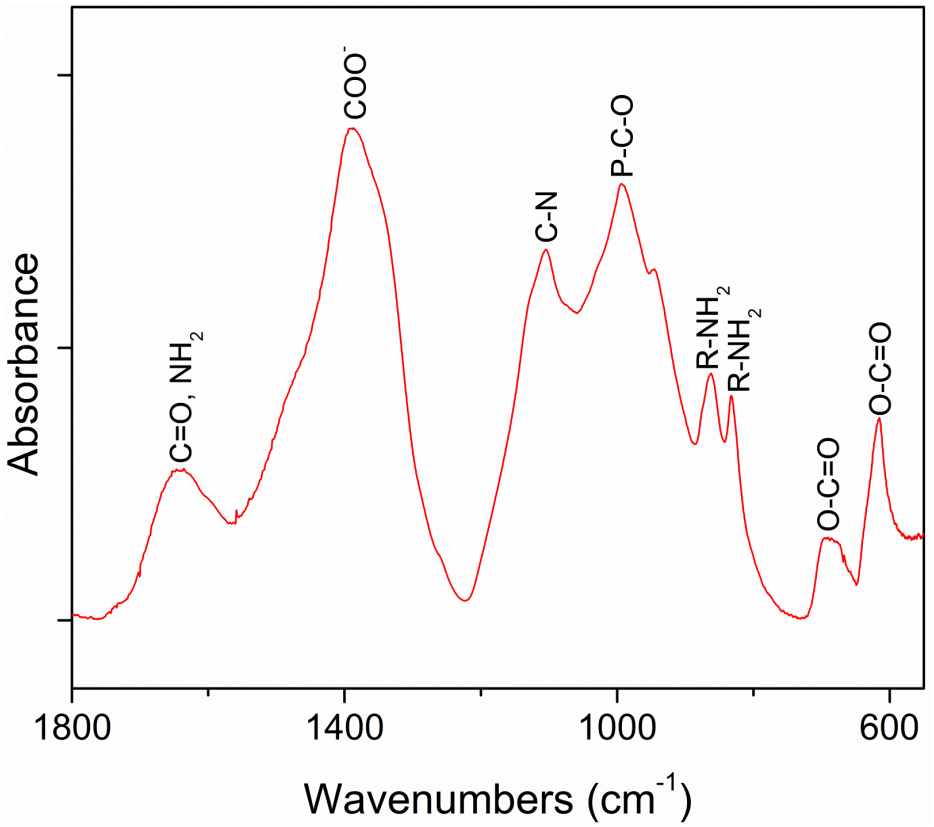


Figure S6. FTIR spectrum of organic matter produced by *Chlamydomonas reinhardtii*. Detailed band assignments are provided in SI Table S2

Table S5. Band assignments for FTIR spectrum of organic matter produced by *Chlamydomonas reinhardtii*

Wavenumber (cm^{-1})	**Functional group/assignment**
530	NO_2 deformation
615	C=O out-of-plane bend in amides
690	O-C=O bending in carboxylic acids
833	NH_2 wag primary amines
862	NH_2 wag primary amines
945	P-O-C antisym stretch in organophosphorus compounds
993	P-O-C antisym stretch in organophosphorus compounds
1104	C-O, C-O-C stretching of polysaccharides
1387	COO- symmetric stretch
1636	C=O stretch and NH_2 deformation in primary amides

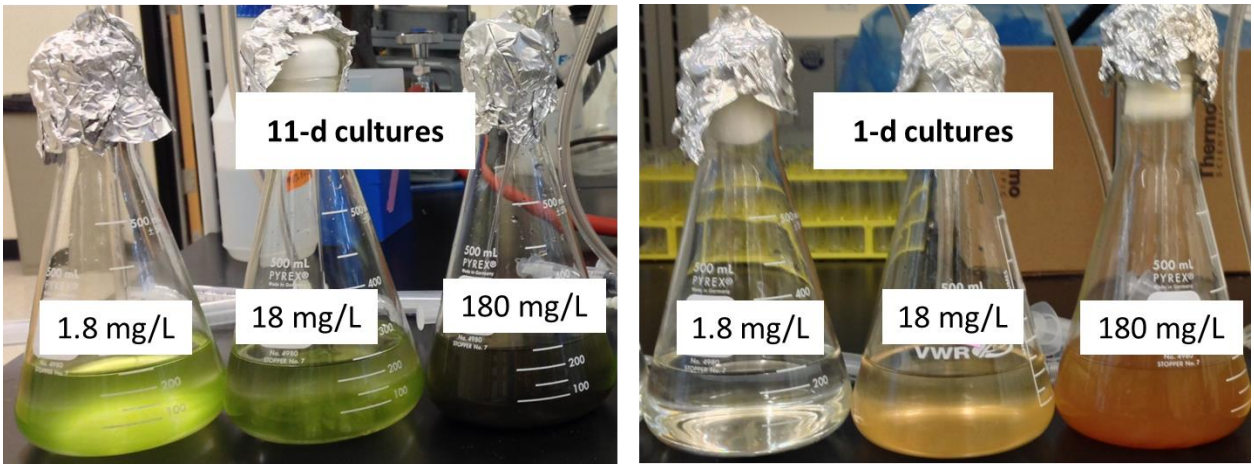


Figure S7. FeSSi in Day 1 and Day 11 *C. reinhardtii* media right after dosing with nanoparticles. FeSSi stock was stirred in either Day 1 or Day 11 media before dosing

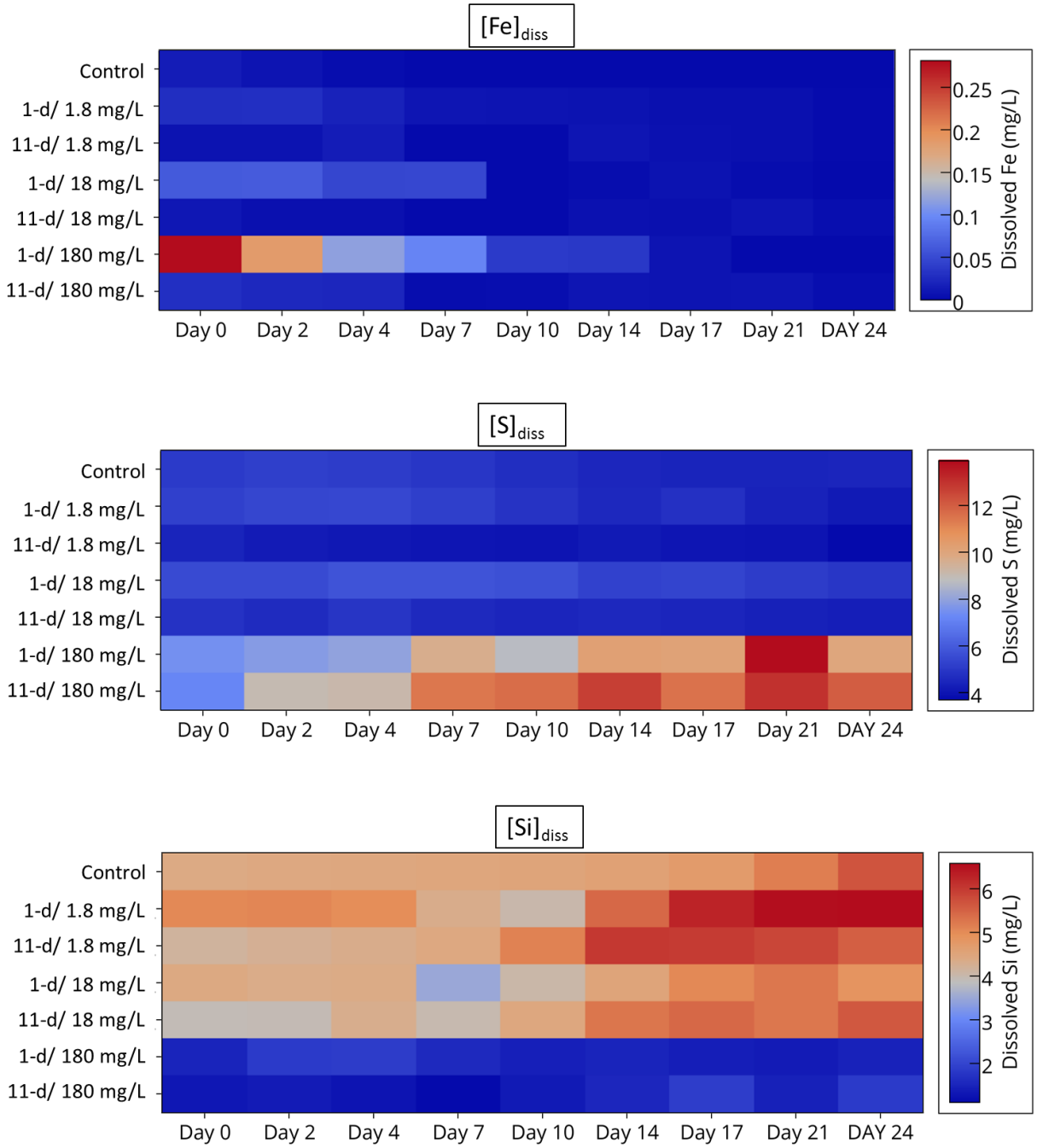


Figure S8. Concentrations of dissolved Fe, S, and Si detected in 1-d and 11-d cultures of *C. reinhardtii* in the presence of 1.8, 18, and 180 mg/L of FeSSi particles

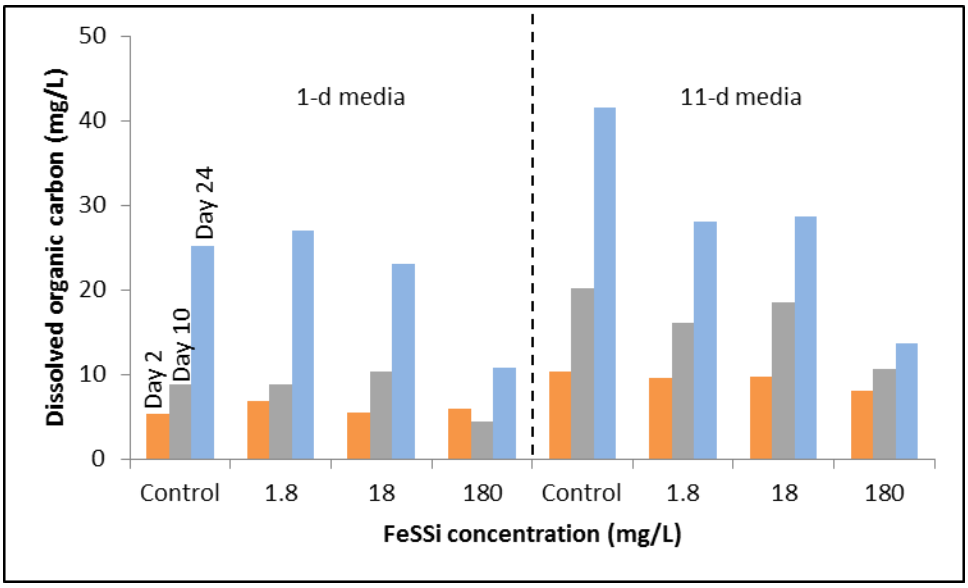


Figure S9. Dissolved organic carbon (DOC), a proxy for algal organic matter, in aqueous phase of *C. reinhardtii* cultures

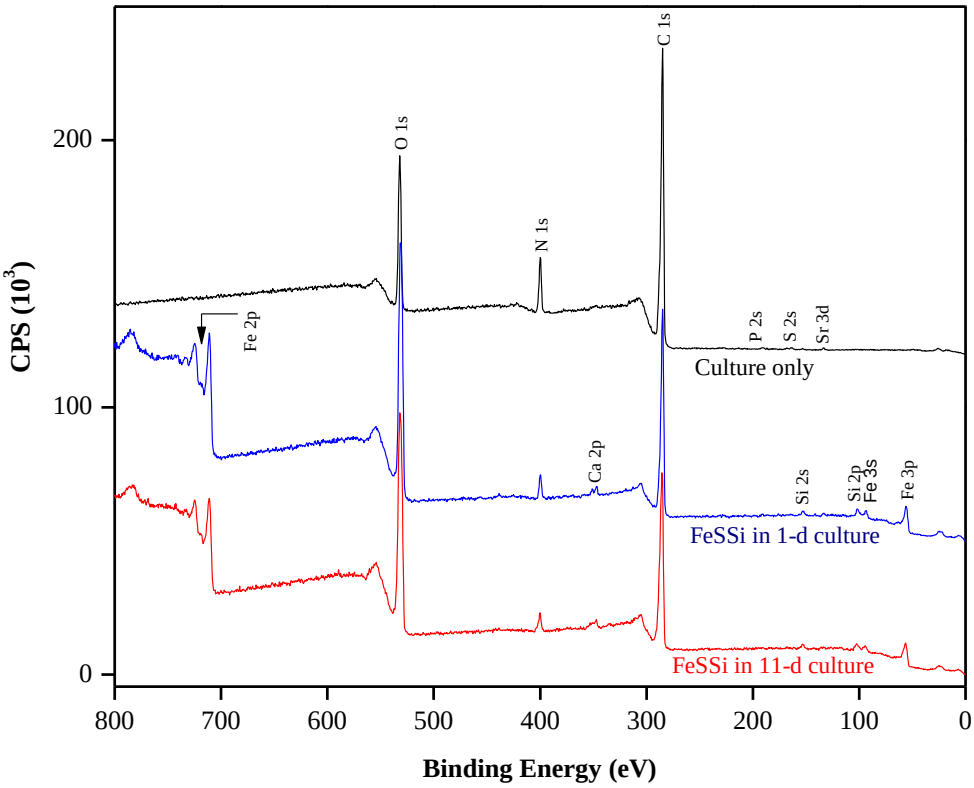


Figure S10. XPS spectra of *C. reinhardtii* culture cells in the presence and absence of FeSSi

Table S6. Elemental composition of solid phase via XPS analyses

% Mass concentration

Name	Culture	FeSSi + 11-d cultures			FeSSi + 1-d cultures		
	Day 10	Day 2	Day 10	Day 30	Day 2	Day 10	Day 30
Fe 2p	0	38.9	11.88	10.51	21.23	22.56	11.67
O 1s	20.84	39.73	44.32	37.68	53.78	54.62	38.82
Si 2s	0	2.79	1.93	1.5	3.5	3.25	2.21
C 1s	71.68	15.85	36.62	45.87	17.16	16.57	41.99
Ca 2p	0	0.94	1.65	1.26	0.84	0.82	1.21
P 2s	0.47	0.44	0.49	0.56	1.92	0.63	0.54
N 1s	6.53	0.72	2.62	2.11	0.28	0.21	2.78
Sr 3d	0.24	0.45	0.29	0.38	1.11	1.34	0.78
S 2p	0.23	0.18	0.2	0.14	0.19	0	0

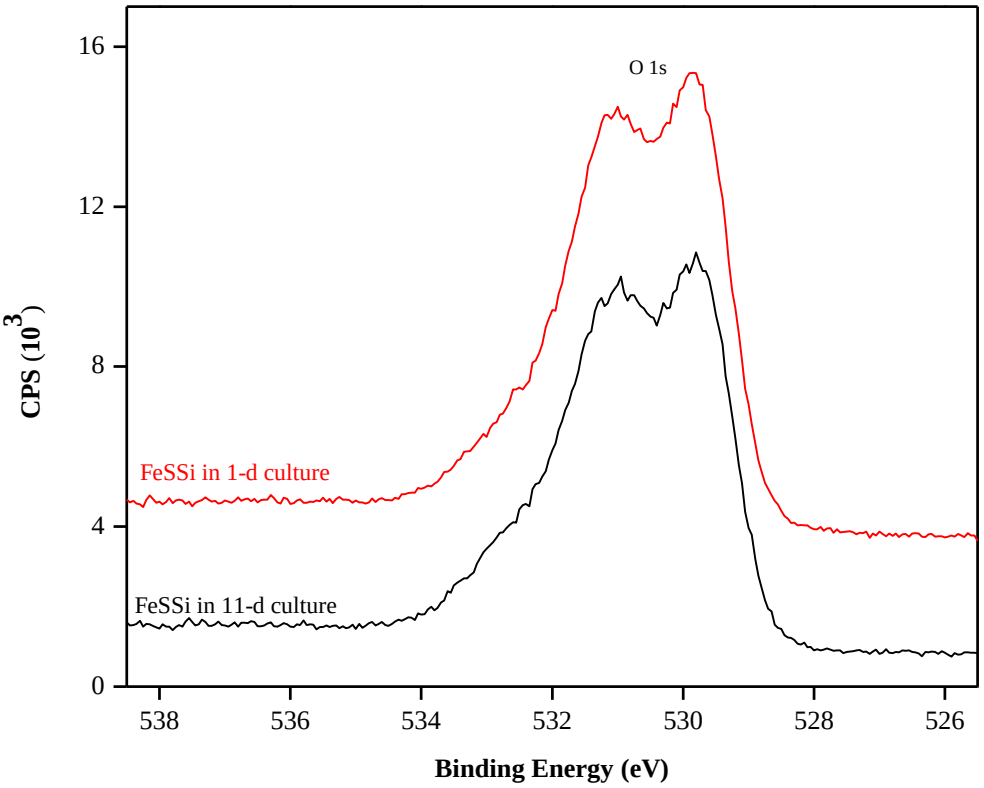


Figure S11. High resolution scans of O 1s in 1-d and 11-d cultures

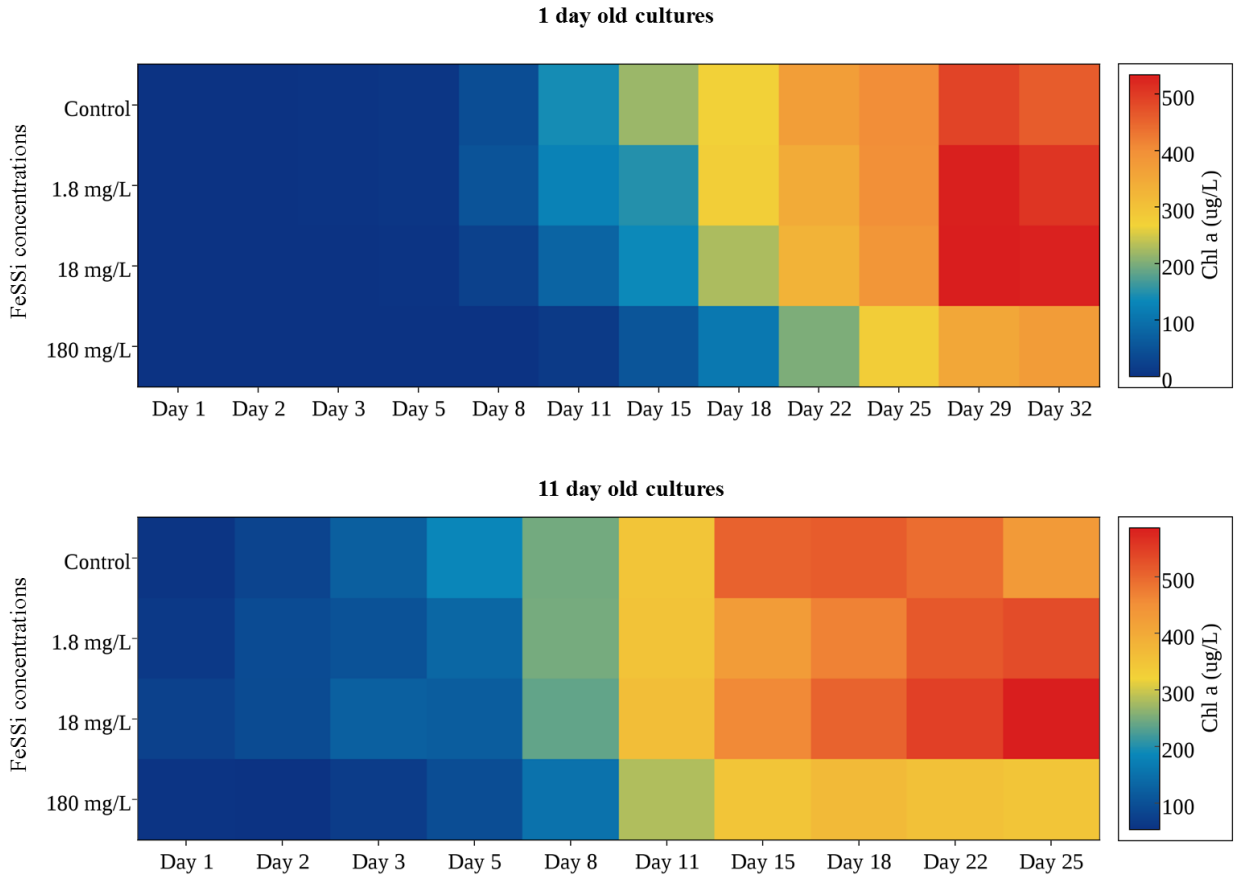


Figure S12. Nominal concentrations of chlorophyll a (Chl a) detected after exposure of FeSSi to *C. reinhardtii* at exponential (1-d) and slowing growth (11-d) phases

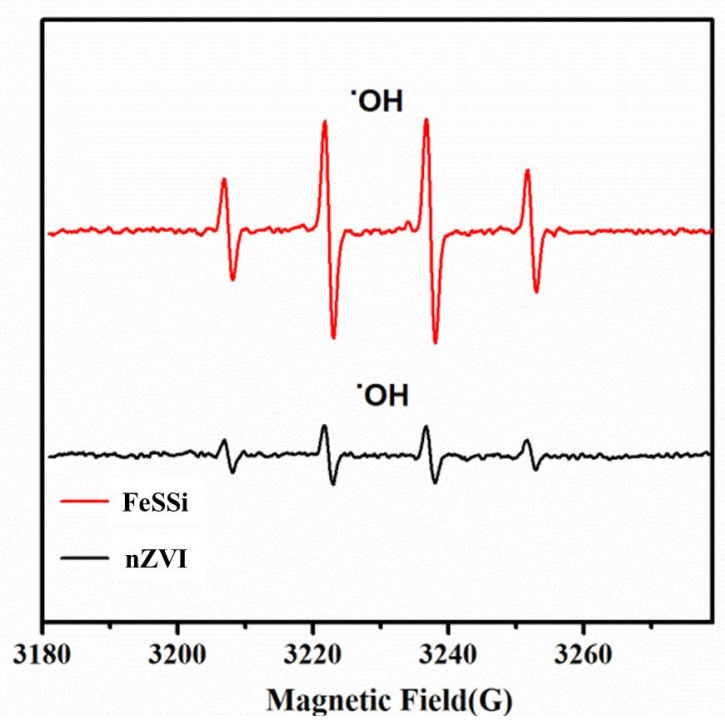


Figure S13. Electron spin resonance (ESR) spectra of FeSSi and nZVI showing the production of reactive oxygen species (ROS).