Ligand-enhanced abiotic iron oxidation and the effects of chemical vs. biological iron cycling in anoxic environments

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Derivation of Fe(II) and NO_2^- reaction equations:

$d[Fe(II)]/dt = -2\, k_{app}\, [Fe(II)][NO_2^-]$
$[Fe(II)] = [Fe(II)]_0 + \Delta[Fe(II)]$; $[NO_2^-] = [Fe(II)]_0 + ½\, \Delta[Fe(II)]$
➔ $d([Fe(II)]_0 + \Delta[Fe(II)]) / dt = -2\, k_{app}\, ([Fe(II)]_0 + \Delta[Fe(II)])\, ([Fe(II)]_0 + ½\, \Delta[Fe(II)])$
➔ $d\, \Delta[Fe(II)] / dt = -2\, k_{app}\, ([Fe(II)]_0 + \Delta[Fe(II)])\, ([Fe(II)]_0 + ½\, \Delta[Fe(II)])$ [eq. S1]

$d[NO_2^-]/dt = -k_{app}\, [Fe(II)][NO_2^-]$
$[NO_2^-] = [NO_2^-]_0 + 2\, \Delta[NO_2^-]$; $[NO_2^-] = [NO_2^-]_0 + \Delta[NO_2^-]$
➔ $d([NO_2^-]_0 + \Delta[NO_2^-]) / dt = -k_{app}\, ([NO_2^-]_0 + 2\, \Delta[NO_2^-])\, ([NO_2^-]_0 + \Delta[NO_2^-])$
➔ $d\, \Delta[NO_2^-] / dt = -k_{app}\, ([NO_2^-]_0 + 2\, \Delta[NO_2^-])\, ([NO_2^-]_0 + \Delta[NO_2^-])$ [eq. S2]

For 2:1 stoichiometry, $\Delta[Fe(II)] = -[Fe(II)]_{ox} = -([Fe(II)]_0 - [Fe(II)]_{obs})$ and $\Delta[NO_2^-] = -[NO_2^-]_{red} = -([NO_2^-]_0 - [NO_2^-]_{obs})$, and [S1] and [S2] integrate to yield:

$$Fe(II)_{obs}(t) = \frac{Fe(II)_0}{-1 + 2\, e^{Fe(II)_0\, k_{app}\, t}} \qquad NO_2^-(t) = \frac{NO_{2\,0}^-\, e^{NO_{2\,0}^-\, k_{app}\, t}}{-1 + 2\, e^{NO_{2\,0}^-\, k_{app}\, t}}$$

For 1:1 stoichiometry observed in the presence of NTA: The Fe-NTA-NO complex does not appear to be reactive towards NO_2^- such that [S1] describes Fe(II) oxidation even in the presence of NTA, with the caveat that measured concentrations of Fe(II) (which include the Fe(II)-NTA-NO^+ complex) require a correction for Fe-NTA-NO. Assuming all NO that is generated complexes with Fe(II)-NTA such that it no longer participates in a redox reaction with nitrite, but is still measured as Fe(II) by the ferrozine assay and assuming the reactions are coupled such that $[Fe(II)]_{ox} = [Fe(II)\text{-NTA-NO}]$, then $\Delta[Fe(II)] = -([Fe(II)]_{ox} + [Fe(II)\text{-NTA-NO}]) = -2\, [Fe(II)]_{ox} = -2\, ([Fe(II)]_0 - [Fe(II)]_{obs})$ and $\Delta[NO_2^-] = -[NO_2^-]_{red} = -([NO_2^-]_0 - [NO_2^-]_{obs})$. This leads [S1] to integrate to:

$$Fe(II)_{obs}(t) = \frac{Fe(II)_0\, e^{Fe(II)_0\, k_{app}\, t}}{-1 + 2\, e^{Fe(II)_0\, k_{app}\, t}}$$

SUPPORTING TABLES

Table S1

pH of reactant solutions at the beginning and end of kinetic Fe(II) oxidation experiments.

Condition	Start	End	Change
2mM Fe(II) + 2mM NO_2^-	7.03	6.88	-0.15
+ 2mM NTA	7.00	7.12	0.12
+ 300mg/L PPHA	6.99	7.03	0.04
+ 100µM Citrate	6.95	7.02	0.07
+ 500µM Citrate	6.97	7.07	0.10
+ 2mM Citrate	6.96	7.06	0.10
+ 2mM Citrate + 300mg/L PPHA	6.94	7.13	0.19

Table S2

Theoretical Fe(II) inorganic and organic speciation in bicarbonate-buffered freshwater medium at pH 7. Species with relative abundance < 0.01% for all experimental conditions are not shown. Species suggested to be relevant for Fe(II) oxidation by nitrite are highlighted in gray.

		2mM Fe(II)							5mM Fe(II)
	Ligand	none	PPHA (300mg/L)	Citrate (0.1mM)	Citrate (0.5mM)	Citrate (2mM)	Citrate + PPHA (2mM+300mg/L)	NTA (2mM)	Citrate (10mM)
$[Fe(II)_{species}] / [Fe(II)_{total}]$	Fe^{2+}	26.66%	23.80%	25.75%	22.26%	11.49%	9.64%	1.89%	0.91%
	$Fe\text{-}OH^{+}$	0.06%	0.05%	0.06%	0.05%	0.03%	0.02%	< 0.01%	< 0.01%
	$Fe\text{-}HCO_3^{+}$	4.37%	3.91%	4.22%	3.64%	1.86%	1.57%	0.31%	0.13%
	$Fe\text{-}CO_{3\ (aq)}$	65.68%	58.82%	63.39%	54.60%	27.86%	23.44%	4.57%	1.93%
	$Fe\text{-}CO_3\text{-}OH^{-}$	0.15%	0.14%	0.15%	0.13%	0.07%	0.06%	0.01%	< 0.01%
	$Fe\text{-}(CO_3)_2^{2-}$	0.09%	0.08%	0.08%	0.07%	0.04%	0.03%	0.01%	< 0.01%
	$Fe\text{-}Cl^{+}$	0.09%	0.08%	0.08%	0.07%	0.04%	0.03%	0.01%	< 0.01%
	$Fe\text{-}NH_3^{2+}$	0.02%	0.02%	0.02%	0.01%	0.01%	0.01%	< 0.01%	< 0.01%
	$Fe\text{-}HPO_{4\ (aq)}$	0.32%	0.30%	0.32%	0.28%	0.16%	0.14%	0.03%	0.01%
	$Fe\text{-}H_2PO_4^{+}$	0.08%	0.07%	0.08%	0.07%	0.04%	0.03%	0.01%	< 0.01%
	$Fe\text{-}SO_{4\ (aq)}$	2.48%	2.24%	2.39%	2.06%	1.06%	0.90%	0.17%	0.07%
	$^{\#}Fe\text{-}L^{-}$			3.46%	16.73%	57.26%	55.67%	93.00%	96.79%
	$^{\#}Fe\text{-}HL$			0.01%	0.03%	0.09%	0.09%	< 0.01%	0.15%
	Fe-HA (complexed)		8.26%				7.04%		
	Fe::HA (weakly bound)		2.23%				1.33%		

#: $Fe\text{-}L^{-}$ = $Fe\text{-}NTA^{-}$ or $Fe\text{-}Citrate^{-}$, Fe-HL = Fe-HNTA or Fe-HCitrate

Table S3

Overview of rate constants reported for chemical oxidation of Fe(II) by NO_2^-.

	Experimental conditions			Kinetic parameters				Source
	pH	**Temp**	**buffer**	**Order**	**Rate constant (k)**		**d[Fe(II)]/dt =**	**Reference**
Oxidation by nitrite								
Fe(II) as siderite (10g/L ~ 80mM)	6	25C	MES/PIPES/HEPES	2nd	1.00E-04	$M^{-1} s^{-1}$	- 2 k $[FeCO_{3(s)}]$ $[NO_2^-]$	Rakshit et al. (2008)(*1*), Fig. 5
Fe(II) as siderite (10g/L ~ 80mM)	6.5	25C	MES/PIPES/HEPES	2nd	6.39E-05	$M^{-1} s^{-1}$	- 2 k $[FeCO_{3(s)}]$ $[NO_2^-]$	Rakshit et al. (2008)(*1*), Fig. 5
Fe(II) as siderite (10g/L ~ 80mM)	7.9	25C	MES/PIPES/HEPES	2nd	5.28E-05	$M^{-1} s^{-1}$	- 2 k $[FeCO_{3(s)}]$ $[NO_2^-]$	Rakshit et al. (2008)(*1*), Fig. 5
Fe(II) as goethite	6.8	30C	carbonate	1st	3.18E-06	s^{-1}	- k [Fe(II)]	Weber et al. (2001)(*2*), Table 3
Fe(II) as biogenic magnetite	6.8	30C	carbonate	1st	3.38E-05	s^{-1}	- k [Fe(II)]	Weber et al. (2001)(*2*), Table 3
Fe(II) as HFO	6.8	26-28	PIPES	3rd	3.83E+03	$M^{-2} s^{-1}$	- k $[Fe(II)_{diss}]$ $[Fe(II)_{bound}]$ $[NO_2^-]$	Tai et al. (2009)(*3*)
+2mM NTA	7	25C	carbonate	2nd	6.67E-03	$M^{-1} s^{-1}$	- 2 k [Fe(II)] $[NO_2^-]$	This study, Table 1
+2mM CIT	7	25C	carbonate	2nd	4.67E-03	$M^{-1} s^{-1}$	- 2 k [Fe(II)] $[NO_2^-]$	This study, Table 1
+10mM CIT, *P. denitrificans* spent medium	7	25C	carbonate	2nd	9.42E-03	$M^{-1} s^{-1}$	- 2 k [Fe(II)] $[NO_2^-]$	This study, Table 2
+10mM CIT, *P. denitrificans* culture	7	25C	carbonate	2nd	1.06E-02	$M^{-1} s^{-1}$	- 2 k [Fe(II)] $[NO_2^-]$	This study, Table 2
Oxidation by nitrate								
Fe(II) as green rust	8.25	25C	auto-titration	2nd	4.93E-05	$M^{-1} s^{-1}$	- 8 k $[Fe(II)_{GR}]$ $[NO_3^-]$	Hansen et al. (1996)(*4*), Table 1

SUPPORTING FIGURES

Figure S1

Anaerobic growth and concomitant Fe(II) oxidation of *Pseudogulbenkiania* sp. strain MAI-1 in freshwater medium amended with 10mM nitrate and different concentrations of Fe(II), NTA and acetate, and a headspace containing ~3% hydrogen. In the presence of NTA, up to 10mM Fe(II) is oxidized within 24hours (in yellow), however, in the absence of NTA, neither growth nor Fe(II) oxidation is observed (in green). Replicate culture (duplicates or triplicates) indicated with solid, dashed and dotted lines, respectively.

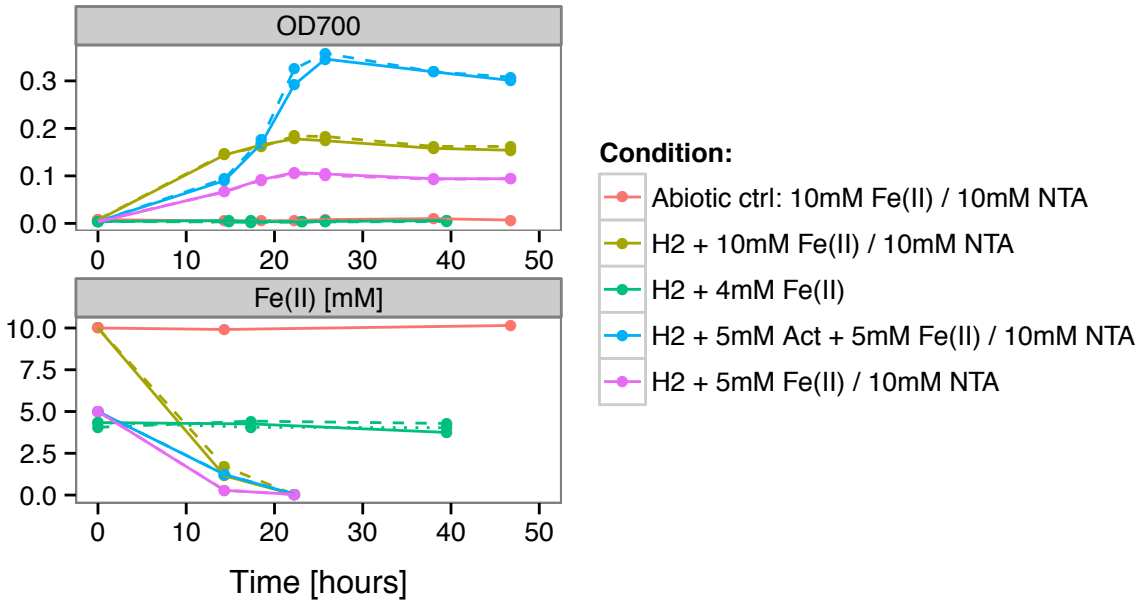


Figure S2

Growth of MAI-1 on various Fe(II) chelating ligands. The organism is grown aerobically in freshwater medium in a 96 well plate (OD_{600} is measured every 5 minutes) with different ligands as the sole carbon source. Citrate (Cit), humic acids (HA), acetate (Act) and diethylene triamine pentaacetic acid (DTPA) can all serve as growth substrates for MAI-1. The strain's ability to use siderophore desferioxamine (DFO) as a carbon source is ambiguous. No growth could be observed in the presence of nitrilotriacetate (NTA) as sole carbo source. This makes NTA a suitable choice for anaerobic growth experiments with MAI-1 as a chelator for Fe(II) that does not supply extra carbon. Replicate cultures indicated with dashed and solid lines, respectively.

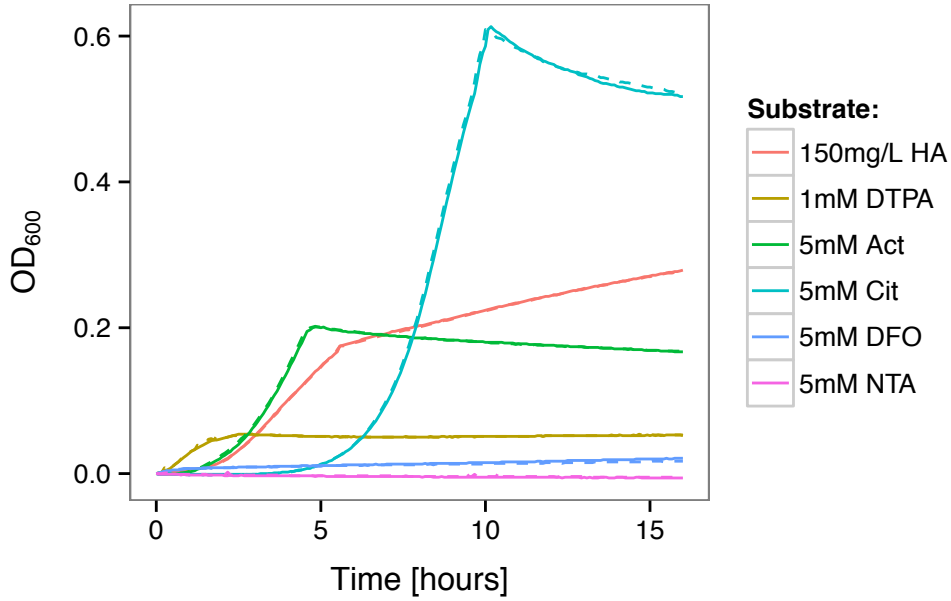


Figure S3

Oxidation of Fe(II)-NTA in spent MAI-1 growth medium. Triplicate cultures of *Pseudogulbenkiania* sp. strain MAI-1 (solid, dashed and dotted line) were grown in freshwater medium amended with 10mM nitrate and 1.25mM acetate, with ~3% H_2 present in the headspace. During growth of MAI-1 (upper left panel), significant amounts of nitrite accumulated in the medium (lower left panel). Accumulated nitrite was stable at the end of growth but upon addition of ~3mM Fe(II)-NTA to filer sterilized spent medium, Fe(II) oxidation and concomitant nitrite reduction could be observed (right panels).

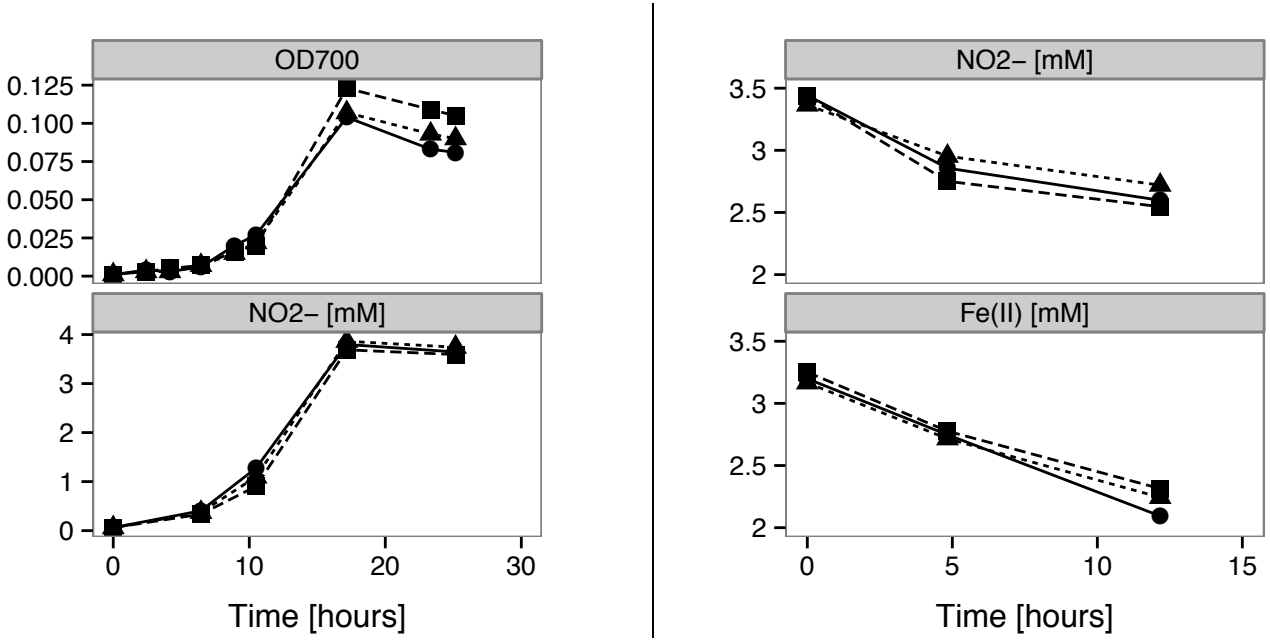


Figure S4

Oxidation test of Fe(II) in the presence of nitrite during sample dilution for the ferrozine(*5*) assay. The ferrozine assay often includes an acid dilution step prior to spectrophotometric determination of Fe(II) with the ferrozine reagent. Acidification aids in the desorption of strongly coordinated Fe(II) from mineral surfaces and other strong sorption sites and is an important preparative step for environmental samples. However, at acidic pH, nitrite is protonated (pKa=3.4) to nitrous acid, which can self- decompose to form reactive N-oxides(*6*) as well as oxidize Fe(II) directly(*7*, *8*). To assess the effect of acidification in the presence of nitrite for our experimental setup, an anoxic freshwater solution containing ~650µM Fe(II) and ~1mM NO_2^- was diluted 1:10 with 1M HCl, and Fe(II) concentrations were measured after varying time intervals using the ferrozine assay (depicted in grey). Within 10 seconds of acidification, >20% of Fe(II) was oxidized and could no longer be detected by the ferrozine assay. After 1 minute, >60% of Fe(II) was lost. Without the acidification step (e.g. by direct dilution of the sample with the ferrozine reagent), Fe(II) concentrations did not significantly decrease within several minutes (black line). Since our experimental conditions included relatively high concentrations of nitrite, but little to no risk of sorptive loss of Fe(II), all ferrozine measurements were conducted without acidification.

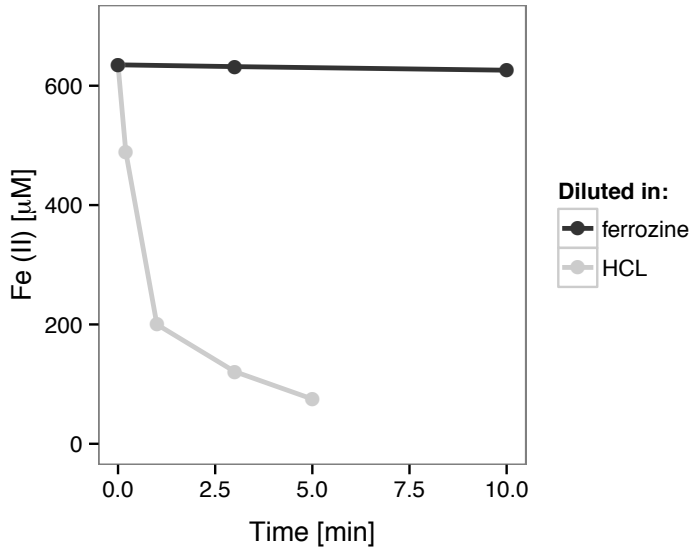


Figure S5

Reduction test of nitrite in the presence of Fe(II) during incubation with sulfanilamide in phosphoric acid for the nitrite assay used in this study. To assess the effect of free and chelated Fe(II) on the assay, an anoxic freshwater solution containing ~1.7mM nitrite was amended with 2mM Fe(II) and no ligand / 2mM citrate / 2mM EDTA / 2mM NTA / 300mg/L PPHA, and immediately diluted 1:10 with 1% sulfanilamide in 5% phosphoric acid for diazodization. Nitrite concentrations were determined colorimetrically after varying time intervals by addition of 0.1% N-1-napthylethylenediamine. The true concentration of nitrite measured in the absence of Fe(II) is indicated as a grey band with 95% confidence intervals. As previously observed(*9*), the presence of Fe(II)-EDTA leads to rapid disappearance of nitrite and significant underestimation of nitrite concentrations by this assay. The addition of Fe(II) without a ligand, as well as with the ligands used in this study did not significantly affect the determination of nitrite by this assay (all measurements were conducted within 3 minutes of sulfanilamide addition to prevent nitrite loss).

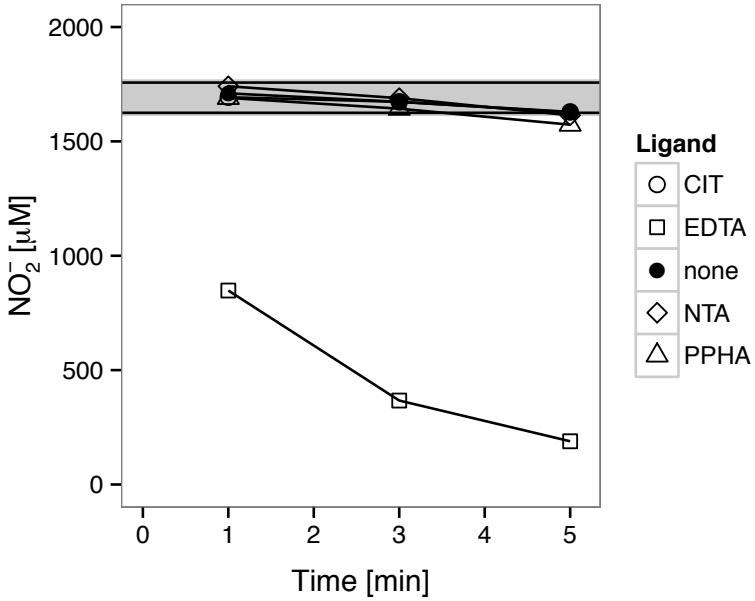


Figure S6

NO_2^- production by *P. denitrificans* (B) during anaerobic growth on succinate (A). Samples for Fe(II) oxidation assays (Figure 3) were taken after accumulation of ~5mM NO_2^- for each biological replicate, respectively (grey shaded area indicated by arrow in panel B). Experiment conducted in biological triplicates. All data are shown.

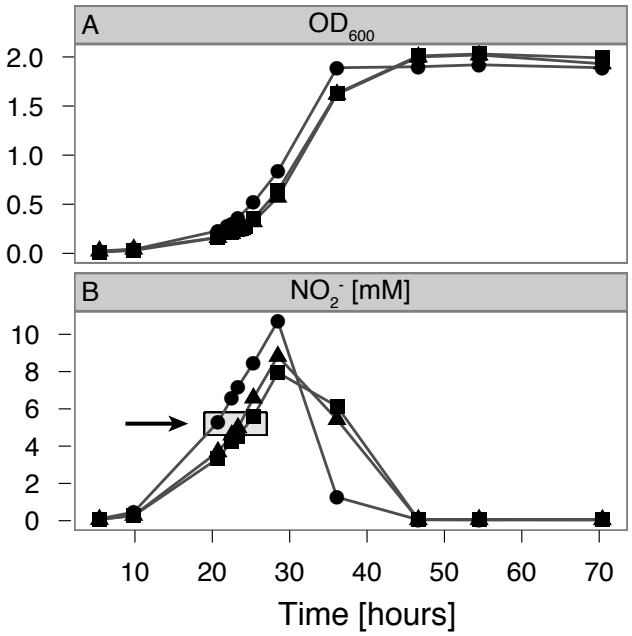


Figure S7

Model fits for abiotic Fe(II) oxidation by nitrite. Low citrate, no ligand, PPHA are best described by a zero-order (i.e. linear) reaction model (linear least squares fit illustrated for these conditions instead of 2nd order decay).

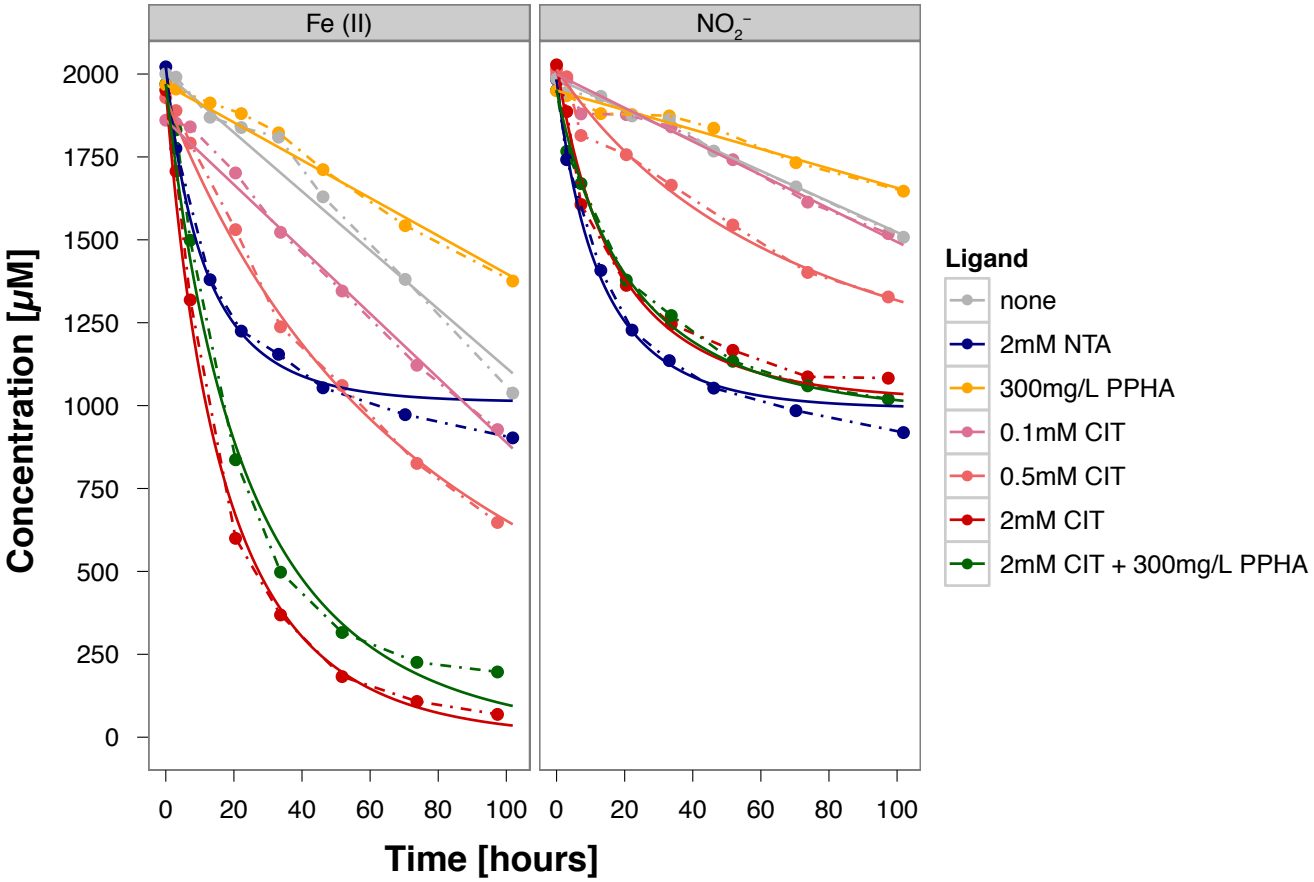


Figure S8

Evolution of N_2O in the headspace of sealed septum bottles during the reaction of 5mM nitrite with ~3mM Fe(II) complexed by citrate vs. NTA (peaks normalized to Ar). Retention times of the gases in the headspace were 2.2min (Ar), 3.0min (N2), 10.8min (N_2O) and 12-13min (CO_2, poorly resolved). The accumulation of N_2O (gray band) as a reaction product could only be observed in the presence of citrate, but not in the presence of NTA. Varying trace amounts of N_2 were present in the Ar/CO_2 headspace of the reaction vessels at the start of the experiment but did not change significantly with reaction progress.

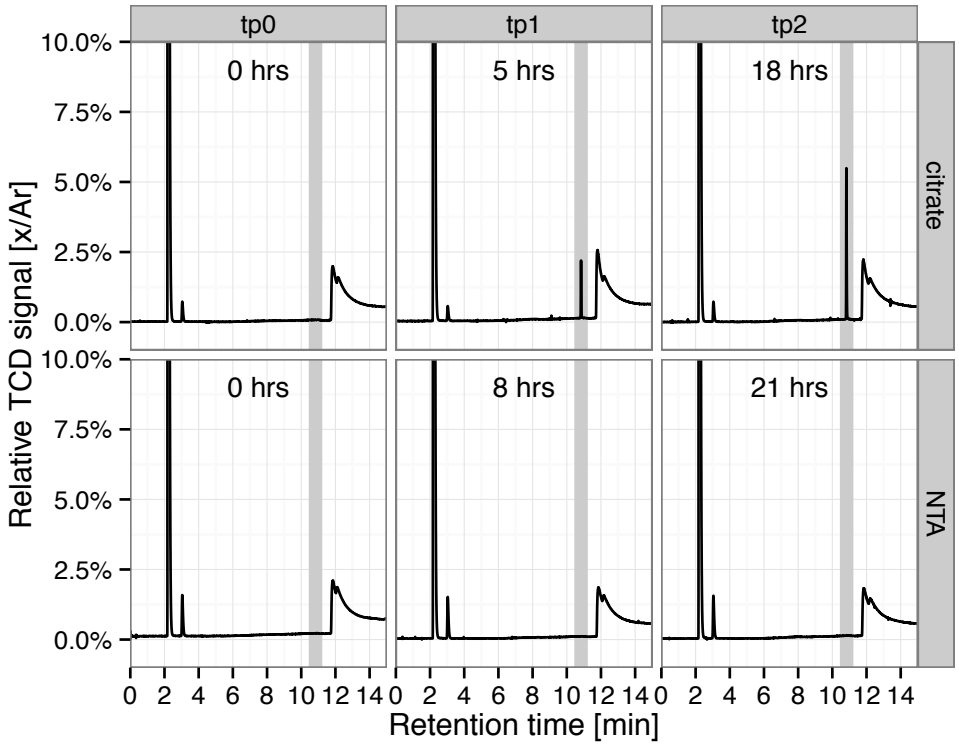
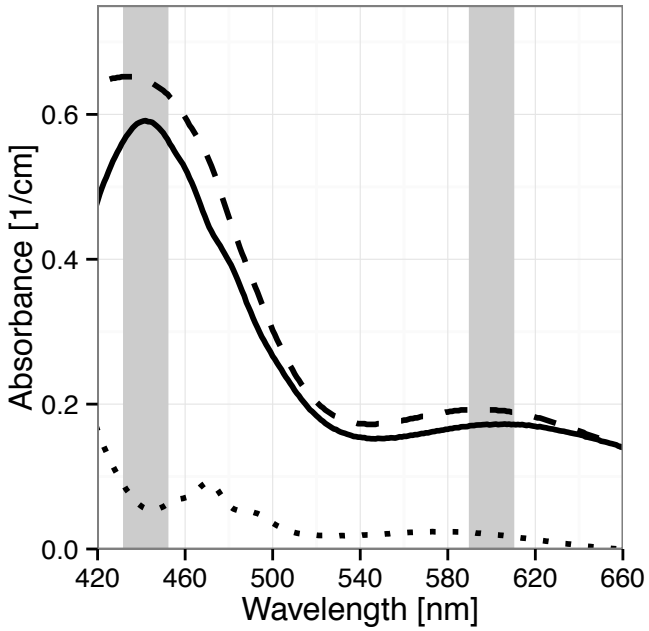


Figure S9

Absorption spectrum of a ~3mM Fe(II)-NTA solution (dashed line) after 950µM NO_2^- was lost by abiotic oxidation of 1086µM Fe(II) (21hrs data point in S3). Fe(II)-NTA by itself does not absorb in this wavelength range. The oxidized Fe forms a complex with NTA that absorbs light weakly with a characteristic peak at 470nm(dotted line). Residual light absorption (solid line) after accounting for the effect of Fe(III)-NTA in solution is indicative of Fe(II)-NTA-NO^- complex formation. Characteristic absorption peaks of the Fe(II)-NTA-NO^- complex (440nm and 600nm)(*10*) are indicated in gray.



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