

1 **Final Technical Report for DE-SC0006681**  
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3 **Project Title:** Development of new and integrated isotope tools for characterizing nitrogen-  
4 uranium cycling in subsurface environments

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6

7 **Report Title:** Constraining the role of iron in environmental nitrogen transformations: Dual  
8 stable isotope systematics of abiotic  $\text{NO}_2^-$  reduction by Fe(II) and its production of  $\text{N}_2\text{O}$

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19 **ABSTRACT**

20 Despite mounting evidence for biogeochemical interactions between iron and nitrogen, our  
21 understanding of their environmental importance remains limited. Here we present an  
22 investigation of abiotic nitrite ( $\text{NO}_2^-$ ) reduction by Fe(II) or ‘chemodenitrification,’ and its  
23 relevance to the production of nitrous oxide ( $\text{N}_2\text{O}$ ), specifically focusing on dual (N and O)  
24 isotope systematics under a variety of environmentally relevant conditions. We observe a range  
25 of kinetic isotope effects that are regulated by reaction rates, with faster rates at higher pH (~8),  
26 higher concentrations of Fe(II) and in the presence of mineral surfaces. A clear non-linear  
27 relationship between rate constant and kinetic isotope effects of  $\text{NO}_2^-$  reduction was evident  
28 (with larger isotope effects at slower rates) and is interpreted as reflecting the dynamics of  
29 Fe(II)-N reaction intermediates. N and O isotopic composition of product  $\text{N}_2\text{O}$  also suggests a  
30 complex network of parallel and/or competing pathways. Our findings suggest that  $\text{NO}_2^-$   
31 reduction by Fe(II) may represent an important abiotic source of environmental  $\text{N}_2\text{O}$ , especially  
32 in iron-rich environments experiencing dynamic redox variations. This study provides a multi-  
33 compound, multi-isotope framework for evaluating the environmental occurrence of abiotic  $\text{NO}_2^-$   
34 reduction and  $\text{N}_2\text{O}$  formation, helping future studies constrain the relative roles of abiotic and  
35 biological  $\text{N}_2\text{O}$  production pathways.

## 36 INTRODUCTION

37 Evidence is mounting for the environmental importance of interactions between iron (Fe)  
38 and nitrogen (N) in biogeochemistry. For example, the reduction of Fe(III) coupled to the  
39 oxidation of ammonium ('feammox') has been recently demonstrated in soils <sup>1-3</sup>, while the  
40 reduction of nitrate or nitrite coupled to the oxidation of Fe(II) – or chemodenitrification – has  
41 been demonstrated across studies of both soils and bacterial cultures and/or enrichments <sup>4-9</sup>.  
42 Despite the potential importance of these processes in the fate of nitrogen, our understanding of  
43 their significance remains limited. In particular, a number of these reactions may be catalyzed  
44 both chemically and biologically and the resulting nitrogenous products vary widely, including  
45 ammonium (NH<sub>4</sub><sup>+</sup>), nitric oxide (NO), nitrous oxide (N<sub>2</sub>O) and dinitrogen (N<sub>2</sub>). The relative  
46 contribution of these reaction pathways, therefore, has wide implications for ecosystem function  
47 (e.g., N retention/loss) and production of potent greenhouse gases. Furthermore, distinguishing  
48 between biologically and chemically catalyzed pathways has important implications for  
49 geobiology, including an improved understanding of the evolution of iron and nitrogen based  
50 metabolic systems and a mechanistic understanding of biologically mediated transformations of  
51 nitrogen, iron and carbon across a host of modern and historical environments <sup>10</sup>.

52 Natural abundance isotopes of nitrogen and oxygen have proven useful as tools for  
53 disentangling complex networks of environmental nitrogen transformations <sup>11</sup>. In large part, these  
54 efforts rely on information gained from environmental samples and/or from experimental  
55 cultures grown under environmentally relevant conditions aimed at carefully constraining kinetic  
56 isotope effects (<sup>15</sup>ε and <sup>18</sup>ε, for N and O, respectively), as well as establishing the nature of  
57 coupling between isotope effects (<sup>18</sup>ε:<sup>15</sup>ε). Nevertheless, there remain important gaps in our  
58 understanding, in particular with respect to important isotope effects involving key nitrogen

59 intermediates including nitrite ( $\text{NO}_2^-$ ), nitric oxide (NO), nitrous oxide ( $\text{N}_2\text{O}$ ), and  
60 hydroxylamine ( $\text{NH}_2\text{OH}$ ). For example, although the dual isotope systematics for biologically  
61 mediated oxidative processes involving  $\text{NO}_2^-$  have been characterized (e.g.,  $\text{NO}_2^-$  formation by  
62  $\text{NH}_4^+$  oxidation<sup>12</sup>) and  $\text{NO}_2^-$  oxidation to  $\text{NO}_3^-$ <sup>13</sup>, information on the dual isotope systematics of  
63 reductive processes involving  $\text{NO}_2^-$ , whether biological or abiotic, is more limited<sup>14-17</sup>.

64 Specifically, there has been little investigation into the kinetic isotope effects of N and O  
65 catalyzed by abiotic chemical reduction of  $\text{NO}_2^-$  by Fe(II) – or ‘chemodenitrification’<sup>17, 18</sup>. These  
66 types of reactions may represent an important control on the dual  $\text{NO}_3^-$  and  $\text{NO}_2^-$  isotopic  
67 composition in reducing environments high in iron but low in organic carbon (e.g., aquifers) as  
68 well as in environments experiencing dynamic redox fluctuations (coastal sediments, estuaries,  
69 rivers). Moreover, although abiotic reactions are thought to contribute significantly to the  
70 production of the potent greenhouse gas  $\text{N}_2\text{O}$ , little is known about the controls on the N and O  
71 isotope composition of its production by these pathways, hindering their use in constraining  
72 global sources of  $\text{N}_2\text{O}$ . Thus, there is a need for a systematic investigation of N and O isotope  
73 effects catalyzed by chemical reactions with Fe before they can be used to constrain  
74 biological/abiotic interactions between N and Fe in the environment.

75 To this end, we investigated the stable N and O isotope dynamics of the abiotic reduction  
76 of  $\text{NO}_2^-$  by Fe(II) under a range of environmentally relevant conditions, including  
77 characterization of the yield and isotopic composition of the product  $\text{N}_2\text{O}$ . As more studies  
78 suggest a potential for anaerobic (a)biotic nitrogen transformations coupled with iron cycling, the  
79 N and O isotope effects determined here will aid in the application of dual isotopes of nitrite and  
80  $\text{N}_2\text{O}$  for deciphering the underlying biogeochemical mechanisms controlling the fate of N across

81 a host of environments including aquatic systems, aquifers, soils, sediments and wastewater  
82 treatment plants.

83

## 84 **MATERIALS AND METHODS**

85

### 86 **Nitrite reduction experiments**

87         Batch experiments were conducted anaerobically under reaction conditions outlined in  
88 Table S1 with 200  $\mu\text{M}$  nitrite and aqueous Fe(II) ranging from 0.5-8.9 mM in the presence and  
89 absence of goethite at room temperature. Experiments varied three primary parameters: Fe(II)  
90 concentration, solution pH, and the presence/absence of the iron-oxide goethite. All experiments  
91 were initiated in anoxic HEPES buffer (30mM) adjusted to the desired pH using NaOH. 140 mL  
92 of buffer was added to 160 mL serum bottles and purged with  $\text{N}_2$  gas for 30 minutes to remove  
93 any trace oxygen. Bottles were then transferred into an anaerobic glove box (5%  $\text{H}_2$ / 95%  $\text{N}_2$ ),  
94 where Fe(II) was added from a concentrated anoxic  $\text{FeCl}_2$  stock solution ( $\sim 1\text{M}$ ). Bottles were  
95 then pre-incubated by shaking for 3 days and any Fe precipitates were removed by filtration. In a  
96 subset of bottles, the Fe(III) oxyhydroxide goethite ( $\text{FeOOH}$ ) (as synthesized and fully  
97 characterized previously – see <sup>19</sup>) was added to a final concentration of 250  $\mu\text{M}$  Fe (in bottles  
98 with 1mM Fe(II) ) and 1mM Fe (in bottles with 5 or 9mM Fe(II)). Following nitrite addition,  
99 bottles were sampled  $\sim 4$  times within the first 6 hours and less frequently thereafter. Between  
100 sampling time points, the crimp-sealed bottles were incubated on an orbital shaker at 150 rpm.  
101 Samples were measured for Fe(II) and nitrite concentration using standard spectrophotometric  
102 methods (see Supplemental Materials). Subsamples were also measured for nitrite N and O  
103 isotopes as described below. Finally, 5ml samples of headspace gas composition were taken at

104 the end of the experiment and injected into pre-evacuated headspace vials (20 ml) for N<sub>2</sub>O  
105 concentration and isotopic composition analysis.

106 In addition to these batch incubations, a subset of these conditions was also run as a  
107 parallel 'N<sub>2</sub>O time-series' experiment (pH 8, no mineral addition, Fe(II) 1, 5 and 9mM), in which  
108 headspace N<sub>2</sub>O measurements were made over time using the autosampler of the IRMS system.  
109 For each condition, ten 20ml headspace vials were loaded with solutions as described above and  
110 placed immediately onto the headspace purge and trap system coupled to the IRMS. The amount  
111 and N and O isotopic composition of evolving N<sub>2</sub>O was monitored over time.

112

### 113 **Isotopic Analyses**

114 Nitrogen and oxygen isotope ratios of nitrite (where  $\delta^{15}\text{N} = [({}^{15}\text{R}_{\text{sample}}/{}^{15}\text{R}_{\text{Air}})-1]*1000$  in  
115 units of ‰, and  ${}^{15}\text{R} = {}^{15}\text{N}/{}^{14}\text{N}$  and where  $\delta^{18}\text{O} = [({}^{18}\text{R}_{\text{sample}}/{}^{18}\text{R}_{\text{VSMOW}})-1]*1000$  in units of ‰,  
116 and  ${}^{18}\text{R} = {}^{18}\text{O}/{}^{16}\text{O}$ ) samples were measured by chemically converting 20 to 40 nmoles of NO<sub>2</sub><sup>-</sup> to  
117 N<sub>2</sub>O using the azide method in 20ml headspace vials<sup>20</sup>. The evolved N<sub>2</sub>O was purified and  
118 collected on a modified TraceGas (IsoPrime, Inc.) purge and trap coupled with a Gilson  
119 autosampler before isotopic analysis on an isotope ratio mass spectrometer (IsoPrime 100,  
120 Elementar Inc.). Internal nitrite isotope standards (WILIS 10, 11 and 20) were run in parallel at 3  
121 different sizes to correct for any variations in sample size and instrumental drift. Based on  
122 calibrations against isotope standards USGS 32, 34 and 35 for  $\delta^{15}\text{N}$ <sup>21</sup> and N23, N7373, and  
123 N10129 for  $\delta^{18}\text{O}$ <sup>22</sup>, the values of internal standards WILIS 10, 11, and 20 are -1.7, +57.1, and -  
124 7.8‰ for  $\delta^{15}\text{N}$  and +13.2, +8.6 and +47.6‰ for  $\delta^{18}\text{O}$ , respectively. All isotopic values are  
125 reported against the VSMOW (for  $\delta^{18}\text{O}$ ) or Air (for  $\delta^{15}\text{N}$ ) reference scales. Typical  
126 reproducibility for  $\delta^{15}\text{N}$  is  $\pm 0.2\text{‰}$  and for  $\delta^{18}\text{O}$  is  $\pm 0.2\text{‰}$ .

127           Headspace N<sub>2</sub>O isotopic composition was measured in duplicate by direct comparison  
128 against the N<sub>2</sub>O reference tank on the IRMS system. The composition of this tank ( $\delta^{15}\text{N}^{\text{bulk}} = -$   
129  $0.7\text{‰}$ ;  $\delta^{18}\text{O} = +39.1\text{‰}$ ; site preference (SP) =  $-5.3\text{‰}$ , where  $\text{SP} = \delta^{15}\text{N}(\alpha) - \delta^{15}\text{N}(\beta)$  and  $\alpha$  and  $\beta$   
130 refer to the central and outer N atoms in the linear N<sub>2</sub>O molecule, respectively) was calibrated  
131 directly against aliquots of two previously calibrated N<sub>2</sub>O tanks from the Ostrom Lab at  
132 Michigan State University. The molar amount of N<sub>2</sub>O in the headspace of each experimental  
133 bottle was calculated using the linear relationship between IRMS peak area at m/z 44 and  
134 injections of known amounts of N<sub>2</sub>O (derived from azide conversion of NO<sub>2</sub><sup>-</sup>). Reported values  
135 have been corrected for any size linearity of isotopic ratios (31/30, 45/44 and 46/44) by using a  
136 series of reference tank subsamples injected into 20ml headspace vials using a gastight syringe.  
137 Precision for replicate analyses of our reference gas analyzed as samples for  $\delta^{15}\text{N}$  is  $\pm 0.3\text{‰}$ , for  
138  $\delta^{18}\text{O}$  is  $\pm 0.4\text{‰}$  and for SP is  $\pm 0.8\text{‰}$ .

139

## 140 **Mineral Analysis**

141           The speciation of Fe was determined using synchrotron-based X-ray absorption  
142 spectroscopy (XAS)<sup>23</sup> (see Supporting Information).

143

## 144 **RESULTS AND DISCUSSION**

145

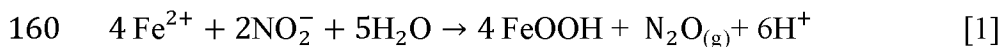
### 146 **Coupled Nitrite Reduction and Iron Oxidation**

147           Reaction between ferrous Fe and nitrite led to complete removal of 200  $\mu\text{M}$  nitrite under  
148 a range of geochemical conditions that varied in initial Fe(II) levels ( $\sim 0.5\text{-}9\text{ mM}$ ), pH (7, 8), and  
149 in the presence or absence of the mineral sorbent goethite (Figure 1). These findings are

150 consistent with previous studies demonstrating the ability of Fe(II) to chemically reduce nitrite  
151 over a large range of experimental conditions<sup>6, 24-26</sup>. Specifically, rapid reaction between high  
152 levels of nitrite (~2-43 mM) and ferrous Fe (~5-43 mM) has been documented over a wide pH  
153 range (4 to 8)<sup>17, 26-29</sup>.

154 Despite complete loss of nitrite, production of ammonium was never observed and thus  
155 nitrite was converted to gaseous products in all cases. Indeed, we observed production of N<sub>2</sub>O in  
156 all experiments (see below); yet, not enough to account for all of the reduced NO<sub>2</sub><sup>-</sup> likely  
157 pointing to N<sub>2</sub> as an additional product under our experimental pH range. As such, the primary  
158 net reactions operative in our experiments are represented by the following equations:

159



162

163 In comparison to changes in NO<sub>2</sub><sup>-</sup>, only a small percentage of dissolved Fe (II) was  
164 removed by Fe(II) oxidation in the majority of incubations (Figure S1). Assuming the  
165 stoichiometries of reactions R1 and R2, Fe(II) was always well in excess of nitrite in our  
166 experiments. An exception to this was the pH 8 experiment conducted at lower Fe(II) ≤1mM,  
167 where Fe(II) was completely oxidized over 100 hours, along with the full removal of nitrite.  
168 Consistent with the above reactions, the oxidation of Fe(II) led to rapid Fe(III) precipitation in all  
169 conditions except for the lowest initial Fe(II) level (0.5 mM) at pH 7. EXAFS spectral analyses  
170 (Figure S2) identified these minerals as goethite, ferrihydrite, and magnetite, which were present  
171 at varying proportions depending on conditions (Figure S3). At pH 7, dominant phases were  
goethite and magnetite with relative contribution of magnetite increasing at higher Fe(II) (Figure



172 S3a). This trend was inversed at higher pH (pH 8), with goethite and ferrihydrite increasing as  
173 Fe(II) increased. Interestingly, incubations conducted with exogenous goethite added initially led  
174 to the inhibition of magnetite formation under similar initial Fe(II) levels (Figure S3b).  
175 Ferrihydrite and goethite were also identified as secondary products in similar batch incubations  
176 but with higher nitrite and aqueous Fe(II) levels at pH 7<sup>17</sup>. In contrast to our findings, however,  
177 Jones and colleagues<sup>17</sup> did not observe formation of magnetite, and instead found precipitation  
178 of lepidocrocite particularly when nitrite was provided in excess of the initial aqueous Fe(II)  
179 concentration. This variability in precipitation patterns is most likely due to differences in the  
180 geochemical conditions of the incubations. In particular, when comparing pH 7 incubations the  
181 initial aqueous Fe(II) to nitrite ratio in our incubations ranged from 3 to 44 in contrast to a ratio  
182 of 0.13 to 4 in Jones et al (2015). These lower ratios are primarily due to the considerably lower  
183 nitrite concentrations used in our incubations (200  $\mu\text{M}$  versus 2.5-40 mM), leading to lower rates  
184 of reaction and likely allowing for more extensive Fe(II)-induced ripening and conversion of  
185 ferrihydrite to magnetite.

186         Indeed, the rate of aqueous Fe(II) loss and corresponding nitrite reduction varied as a  
187 function of pH, initial aqueous Fe(II) concentration, and exogenous goethite addition (Figure 2,  
188 Table S2). Initial reduction of nitrite by Fe(II) was linear with initial rates varying from 0.1 to 50  
189  $\mu\text{M h}^{-1}$  at pH 7 and 5.9 to 160  $\mu\text{M h}^{-1}$  at pH 8. Corresponding Fe(II) loss, a combination of both  
190 oxidation by nitrite and sorption/precipitation, exhibited initial rates ranging from 1 to 343  $\mu\text{M h}^{-1}$   
191 at pH 7 and 11 to 2300  $\mu\text{M h}^{-1}$  at pH 8 (see Figure S1). Our observed nitrite reduction and  
192 Fe(II) loss rates at pH 7 are generally comparable with previous studies using similar reaction  
193 conditions (e.g.,<sup>28, 30, 31</sup>), while lower than those rates documented at higher nitrite and Fe(II)  
194 levels (albeit at lower Fe(II):NO<sub>2</sub><sup>-</sup>;<sup>17, 26</sup>). For the 4 different scenarios employed here (pH 7 and

195 8, with/without goethite; Table S1), we observed a linear relationship between Fe(II)  
196 concentration and the first order rate constant ( $\text{hr}^{-1}$ ) (Figure 2; Table S2), highlighting the role of  
197 Fe(II) concentrations in regulating nitrite reduction rates.

198 Addition of goethite consistently led to higher reaction rates between Fe(II) and nitrite at  
199 both pH values and regardless of initial Fe(II) concentration (Figure 2; Table S2). Multiple  
200 studies have shown that the presence Fe(III) oxyhydroxides and the sorption of Fe(II) to mineral  
201 surfaces or ligands increases rates of nitrite reduction by Fe(II), including reactions with 2-line  
202 ferrihydrite, goethite, biogenic magnetite, lepidocrocite, green rust (GR), siderite and wüstite<sup>5, 28,</sup>  
203<sup>30-34</sup>. These studies consistently indicate faster kinetics for heterogeneous than homogenous  
204 Fe(II)-nitrite reactions.

205 For the homogenous reaction conditions between aqueous Fe(II) and nitrite (absence of  
206 exogenous goethite), heterogeneous reactions likely also contributed to the observed reaction  
207 rates. The rapid formation of Fe(III) minerals upon Fe(II) and nitrite reaction provided a  
208 secondary and presumably faster nitrite reduction pathway. Thus, heterogeneous reactions would  
209 also be operative with ferrous Fe bound within magnetite (Figure S3) and/or Fe(II) adsorbed onto  
210 the secondary precipitates goethite and ferrihydrite.

211

### 212 *Nitrite Isotope Systematics*

213 The N and O stable isotope systematics of abiotic nitrite reduction can potentially be  
214 useful for distinguishing among nitrite reduction mechanisms in the environment. Here, the  $\delta^{15}\text{N}$   
215 and  $\delta^{18}\text{O}$  of the remaining nitrite increased during the reaction in all incubations, reflecting  
216 positive isotope effects for both N and O during nitrite reduction (Figure 3). Using a closed  
217 system Rayleigh model, the  $^{15}\text{N}$  isotope effect for nitrite reduction by Fe(II) (hereafter,  $^{15}\epsilon_{\text{NIR}}$

218 ranged from 6‰ to 45‰, while the  $^{18}\text{O}$  isotope effect (hereafter,  $^{18}\epsilon_{\text{NIR}}$ ) ranged between 6‰ and  
219 33‰ (Table 1). The ratio of  $^{15}\epsilon_{\text{NIR}}:^{18}\epsilon_{\text{NIR}}$ , was often lower than 1 (Table 1, Figure S4), reflecting  
220 a smaller isotope effect for oxygen compared to nitrogen. Oxygen isotopes of nitrite may also  
221 readily equilibrate with water as a function of pH and temperature, with faster equilibration  
222 occurring at lower pH and higher temperatures <sup>22</sup>. It is therefore possible that the observed  
223 oxygen isotope effect of  $\text{NO}_2^-$  reduction was influenced by oxygen isotope equilibration with  
224 water. No difference in  $^{15}\epsilon_{\text{NIR}}:^{18}\epsilon_{\text{NIR}}$  was observed between experiments at pH 7 and pH 8,  
225 suggesting perhaps this factor was not important in our experiments (Table 1). However, given  
226 the rapid consumption rates of our relatively small levels of  $\text{NO}_2^-$  it is also possible that our  
227 experiments were unable to catch the influence of this effect. Although, we would not predict  
228 high rates of oxygen exchange at pH 8, the very high  $\delta^{18}\text{O}$  values under high extents of  $\text{NO}_2^-$   
229 consumption would be particularly sensitive to even a small amount of isotopic exchange and  
230 may contribute to slightly lower observed  $^{18}\epsilon_{\text{NIR}}$  relative to that of  $^{15}\epsilon_{\text{NIR}}$ .

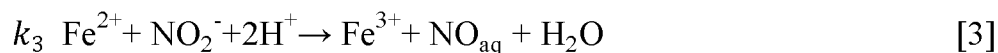
231 Although very little  $\text{NO}_2^-$  isotope data exist for chemical reduction, our data exhibit a  
232 wider range of  $^{15}\epsilon_{\text{NIR}}$  and  $^{18}\epsilon_{\text{NIR}}$  values than other recent studies (e.g., <sup>17, 35</sup>). While  $^{15}\epsilon_{\text{NIR}}$  and  
233  $^{18}\epsilon_{\text{NIR}}$  varied from 6 to 45‰ (Table 1) in our experiments, these values were consistent and  
234 reproducible under similar experimental conditions. Indeed, differences in the isotope effects  
235 correlate well with factors that directly influence reaction rate, including substrate concentration,  
236 pH and interactions with minerals (Figure 3, Table 1). In particular, variations in nitrite isotopic  
237 fractionation were inversely related to the reaction rate, with higher rates producing lower  $^{15}\epsilon$   
238 values (Figure 4).

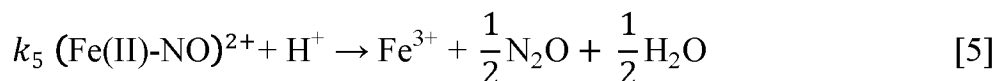
239 In our experiments differences in isotope effects between heterogeneous and homogenous  
240 reactions could not be explicitly addressed since even our ‘homogenous’ reactions exhibited

241 rapid mineral formation (Figures S2-S3), promoting heterogeneous surface reactions. Even at the  
242 lowest levels of mineral precipitation (pH 7) rates were also slower and it is therefore unclear  
243 whether the lower isotope effect stemmed from lower overall reaction rate or from reduced  
244 interaction with surface-bound Fe(II). If we assume that the rapid reactions were mostly  
245 catalyzed by interactions with surface associated Fe(II) and that the slower reactions were less  
246 influenced by surface-bound Fe(II), then it is possible that the heterogeneous reaction exhibits a  
247 smaller isotope effect for this process, leading to the lower observed net isotope effects at high  
248 reaction rates. Future studies should aim to tease apart the relative influence of rate versus  
249 mechanism in order to better understand the reaction mechanism.

250 The observed kinetic isotope effects in the reacting  $\text{NO}_2^-$  pool are governed by the  
251 combination of chemical reactions (e.g., bond forming/breaking) occurring during nitrite  
252 reduction. As such, changes in the relative proportions of different mechanisms/pathways  
253 (including back reactions) can change the observed isotope effects of the  $\text{NO}_2^-$  (and other N  
254 bearing intermediate) pools. While the net reaction results in the reduction of  $\text{NO}_2^-$  to  $\text{N}_2\text{O}$  (and  
255 and/or  $\text{N}_2$  (reactions [1] and [2] above)), the reaction proceeds through one or more nitrogenous  
256 and likely Fe-bound nitrogen species (e.g., nitrosyl ( $\text{Fe}(\text{NO})^+$  or dinitrosyl ( $\text{Fe}(\text{NO})_2^{2+}$ )  
257 intermediates, which may have limited stability under these reaction conditions<sup>5, 36-38</sup>.  
258 Specifically, we consider the involvement of an Fe(II) intermediate and isotope fractionation  
259 occurring at each of the reaction steps given below (reaction 3-5): the reduction of  $\text{NO}_2^-$  to NO  
260 [1], the complexation of NO with Fe(II) [4] and the reduction of the Fe-NO complex to  $\text{N}_2\text{O}$  (or  
261  $\text{N}_2$ ) [5].

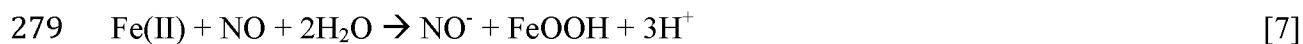
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263 While the forward reactions resulting in the formation of NO ( $k_3$  in R3) or the formation  
 264 of a nitrosyl complex ( $k_4$  in R4) may occur with a particular isotope effect, the backward  
 265 reactions ( $k_{-3}$  or  $k_{-4}$ ) may occur with distinctively different isotope effects. Indeed, recent  
 266 evidence indicates that the initial reduction of  $\text{NO}_2^-$  to NO (R3) may largely be controlled by an  
 267 equilibrium reaction <sup>26</sup>, in which case the forward/backward reactions give rise to an equilibrium  
 268 isotope effect. Additionally,  $\text{NO}_2^-$   $\delta^{18}\text{O}$  could be significantly affected by the incorporation of a  
 269 ‘new’ O atom (from  $\text{H}_2\text{O}$ ) during the reverse reaction [3] in the reverse direction. The influence  
 270 of this type of pathway reversibility on isotope dynamics has been well documented in the  
 271 sulfate reduction system <sup>39-43</sup>. Thus, we suggest that the relative ratio of forward to backward  
 272 reactions plays the key role in regulating our observed isotope effects in the reactant  $\text{NO}_2^-$  pool.  
 273 Also, to the degree that the net reaction is multi-step and/or proceeds through multiple (and  
 274 likely transient) intermediate species and/or through parallel pathways (e.g., reaction R8a vs.  
 275 R8b), changes in the relative rates of each step will contribute to changes in the net isotope effect  
 276 observed in the  $\text{NO}_2^-$  and product  $\text{N}_2\text{O}$  pools.

277



282

283 Likely, all of these factors contribute to our observed relationship between the kinetic  
284 isotope effect and reaction rate (Figure 4).

285

### 286 **Relationship between $\text{NO}_2^-$ reduction and $\text{N}_2\text{O}$ isotope dynamics**

287 The final amount and N and O isotopic composition of the  $\text{N}_2\text{O}$  accumulated in the batch  
288 experiment bottles were measured, including the intramolecular nitrogen isotopic composition,  
289 or site preference (SP). Complementary to these end product measurements,  $\text{N}_2\text{O}$  formation and  
290 isotopic composition was also monitored over time for a subset of these conditions (pH 8, no  
291 goethite, and starting Fe(II) concentrations of 0.9, 4.7 and 9.1mM). The endpoint composition of  
292 the  $\text{N}_2\text{O}$  in these ‘time series’ experiments (Figure S5) was consistent with the endpoint  
293 measurements of the batch experiment bottles (Figure S6). In general, lower amounts of  $\text{N}_2\text{O}$   
294 accumulated under conditions promoting slower rates of nitrite reduction (not shown). Molar  
295 yields of  $\text{N}_2\text{O}$  (e.g., the percentage of  $\text{NO}_2^-$  converted to  $\text{N}_2\text{O}$ ) ranged from ~11 to 52%,  
296 reflecting considerable variation in the relative magnitudes of reaction mechanisms involved in  
297 chemodenitrification. N and O isotopic composition of the final  $\text{N}_2\text{O}$  ranged from -19.8 to -3.0‰  
298 for  $\delta^{15}\text{N}_{\text{N}_2\text{Obulk}}$  and from +29.3 to +46.4‰ for  $\delta^{18}\text{O}_{\text{N}_2\text{O}}$  and were strongly correlated, with all but  
299 one outlier clustering between -7.4 to -3.0‰ and +38.4 to +46.4‰, respectively (Figure S6). The  
300 single outlier  $\text{N}_2\text{O}$  composition corresponds to the only case in which nitrite reduction was not  
301 complete, reflecting  $\text{N}_2\text{O}$  produced from only a partially reduced pool of  $\text{NO}_2^-$ .

302 Comparison of the starting composition of the reactant  $\text{NO}_2^-$  with the product  $\text{N}_2\text{O}$  offers  
303 important insights into reaction mechanisms. The elevated  $\delta^{18}\text{O}_{\text{N}_2\text{O}}$  values relative to  $\text{NO}_2^-$ ,  
304 relative to the starting  $\text{NO}_2^-$ , reflect the influence of branching effects by the preferential removal  
305 of  $^{16}\text{O}$  during reduction steps of both  $\text{NO}_2^-$  and  $\text{NO}^{16}$ . Together with the strong correlation

306 between the  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of product  $\text{N}_2\text{O}$  (Figure S6) this indicates a strong coupling of the  
307 kinetic isotope effect on N and the combined kinetic and branching isotope effects on oxygen  
308 during the formation of  $\text{N}_2\text{O}$ . The final  $\delta^{15}\text{N}$  of the accumulated  $\text{N}_2\text{O}$  was on average  $\sim 8\text{‰}$  lower  
309 than the  $\delta^{15}\text{N}$  of the starting  $\text{NO}_2^-$ , with higher  $\text{N}_2\text{O}$  yields exhibiting smaller differences  
310 (excluding the case in which  $\text{NO}_2^-$  did not fully react – Figure 1).

311 As all of the reacted  $\text{NO}_2^-$  was not accounted for in the product  $\text{N}_2\text{O}$ , another N-bearing  
312 pool must represent the mass balance complement to the  $\text{N}_2\text{O}$  pool, having a higher  $\delta^{15}\text{N}$  than the  
313 starting  $\text{NO}_2^-$ . Initial product  $\text{N}_2\text{O}$   $\delta^{15}\text{N}$  values during the time series experiments were lower  
314 than the N isotope effects calculated from the  $\text{NO}_2^-$  pool (Figure S5) reflecting production of a  
315 separate N bearing pool. At high  $\text{N}_2\text{O}$  yields ( $\sim 30\text{-}50\%$ ), end point  $\delta^{15}\text{N}$  values were on average  
316  $\sim 4$  to  $8\text{‰}$  lower than starting  $\text{NO}_2^-$   $\delta^{15}\text{N}$  values – and by mass balance imply production of an N-  
317 bearing pool at least several permil higher than the starting  $\text{NO}_2^-$   $\delta^{15}\text{N}$  values. Jones and others<sup>17</sup>  
318 observed similar results, suggesting that isotopically heavier N must have accumulated in the NO  
319 pool. Under similar reaction conditions (e.g. pH 7, very high  $\text{NO}_2^-$  and Fe(II)), Kampschreur and  
320 colleagues<sup>26</sup> observed complete recovery of  $\text{NO}_2^-$  as NO and  $\text{N}_2\text{O}$  – suggesting that the missing  
321 mass balance complement to the  $\text{N}_2\text{O}$  is likely to be found as NO. Under the higher pH  
322 conditions of our time series experiment (pH 8), however, NO was only observed at low levels  
323 (qualitatively observed as separate peaks while monitoring masses 30 and 31 on the IRMS  
324 during  $\text{N}_2\text{O}$  analyses) and only under low Fe(II) conditions (0.9mM) suggesting that, while  
325 possibly an important transient intermediate, gas phase NO did not appear to have been a  
326 significant end product ( $< \sim 5\%$ ). A lack of observed NO accumulation under our higher Fe(II)  
327 conditions also appears to highlight the role of Fe(II) in providing the forward kinetic drive  
328 (and/or complexation of NO) and likely promoting formation of Fe-bound nitrosyl species (as in

329 reaction R4 above). In comparing the likelihood of an NO vs N<sub>2</sub> pool as the missing mass  
330 balance complement of the low δ<sup>15</sup>N N<sub>2</sub>O, Jones and others (2015) suggested an NO product  
331 pool having a higher δ<sup>15</sup>N as a more parsimonious explanation since it is less reduced than N<sub>2</sub>O  
332 (making the assumption that an N<sub>2</sub> pool derived from an N<sub>2</sub>O precursor should be isotopically  
333 lower than the N<sub>2</sub>O). However, based on the apparently low observed accumulation of NO in our  
334 time series measurements at pH 8, together with the assumption that a high δ<sup>15</sup>N N<sub>2</sub> pool  
335 deriving from N<sub>2</sub>O reduction would be unlikely, we suggest instead that the production of N<sub>2</sub> and  
336 N<sub>2</sub>O may be occurring in parallel, competing reactions (as in R8a and R8b), under our  
337 experimental conditions (with the production of N<sub>2</sub> having a smaller isotope effect than the  
338 production of N<sub>2</sub>O). Alternatively, some proportion of NO may have remained bound in a  
339 nitrosyliron complex under the higher dissolved Fe(II) conditions. Indeed the difference in  
340 proportion of end products is related to differences in the formation kinetics and stability of  
341 (di)nitrosyl intermediates, which are sensitive to pH<sup>36, 38</sup>. Either way, whether the missing N  
342 pool is comprised of NO, as observed and inferred by others<sup>17, 26</sup>, or is comprised primarily of  
343 N<sub>2</sub> as appears to be the case in our experiments, the N isotopic composition of the N<sub>2</sub>O offers a  
344 useful perspective on the source of N and the isotope systematics of N<sub>2</sub>O release by  
345 chemodenitrification.

346         The intramolecular <sup>15</sup>N site preference of the product N<sub>2</sub>O also reflects differences in the  
347 governing reaction mechanisms. N<sub>2</sub>O SP values both from the batch experiments and the time  
348 series measurements ranged from -0.4 to +26.0‰ with endpoint values correlating with final  
349 concentration and yield of N<sub>2</sub>O (Figure 5). In our time series experiments, higher levels of Fe(II)  
350 lead to more consistently elevated SP values starting ~ +14.5‰ and increasing to ~ +26.0‰  
351 (Figure S5) – similar to previous observations of N<sub>2</sub>O SP by chemodenitrification falling



352 between +10 and +22‰<sup>17</sup>. In contrast, under lower Fe(II) conditions (~0.9mM) initial SP values  
353 were as low as 0‰, although eventually increased to 26.0‰ before reaching a final value of  
354 15.2‰ (Figure S5). Compared to  $\delta^{15}\text{N}_{\text{N}_2\text{O}_{\text{bulk}}}$  and  $\delta^{18}\text{O}_{\text{N}_2\text{O}}$ , which reflect the combined influence  
355 of both the composition of reactants (e.g.,  $\text{NO}_2^-$ ) and the kinetic isotope effects associated with  
356 the reaction pathways, SP is thought to be independent of the  $\delta^{15}\text{N}$  of the N source and instead  
357 reflect only formation pathway. Specifically, the combination of two NO precursor molecules to  
358 form  $\text{N}_2\text{O}$  in a singly catalyzed reaction should result in very little difference between the  $\delta^{15}\text{N}$  of  
359 the beta (outer) and alpha (inner) N atoms, such as is observed for nitric oxide reductases in  
360 denitrifying bacteria<sup>44, 45</sup>. In contrast, mechanisms whereby combination of two NO molecules  
361 proceeds via formation of an O-N=N-O intermediate favors breaking of  $^{14}\text{N}$ -O bond over a  $^{15}\text{N}$ -  
362 O bond – and promotes the  $^{15}\text{N}$  enrichment of the alpha position (e.g., elevated SP value), as is  
363 thought to occur during  $\text{NH}_2\text{OH}$  decomposition and  $\text{N}_2\text{O}$  production by ammonia oxidizing  
364 bacteria<sup>46, 47</sup> and denitrifying fungi<sup>48-50</sup>. Elevated SP values (+35‰) have in fact also been  
365 observed in chemical reactions involving  $\text{NH}_2\text{OH}$  and  $\text{NO}_2^-$  including in the presence of Fe  
366 catalysts – although the decomposition of  $\text{NH}_2\text{OH}$  may play the primary role rather than  $\text{NO}_2^-$ <sup>35</sup>.  
367 Results of chemical reduction of  $\text{NO}_2^-$  in experiments using other reductants (e.g.,  
368 trimethylamine-borane) have also yielded elevated SP values – suggesting an important role for  
369 an intermediate species<sup>44</sup>. Studies investigating reduction of  $\text{NO}_2^-$  by either aqueous Fe(II) or  
370 Fe(II)-containing primary minerals have also noted production of  $\text{N}_2\text{O}$  having positive SP values  
371 up to 22‰<sup>17, 51</sup>, although low SP values have also been reported<sup>18</sup>. We suggest that the range of  
372 SP values observed in field studies<sup>51</sup> and in lab studies such as ours and those of others<sup>17, 18</sup>,  
373 reflects shifts in the balance of at least two mechanisms of  $\text{N}_2\text{O}$  formation and specifically the  
374 relative involvement of intermediate nitrosyl and dinitrosyl species (and the factors regulating

375 their formation and stability; <sup>5, 36, 38</sup>). Indeed, in our experiments, higher N<sub>2</sub>O yields correspond  
376 with higher reduction rates and in turn higher concentrations of Fe(II). Thus, in summary, the  
377 high Fe(II) conditions of our experiments apparently favor the formation of nitrosyl-iron  
378 complexes as reaction intermediates and precursors for reactions yielding elevated SP values for  
379 product N<sub>2</sub>O. On the other hand, lower levels of Fe(II) and the correspondingly slower reduction  
380 of NO<sub>2</sub><sup>-</sup> apparently produce N<sub>2</sub>O having generally lower SP values (Figure 5; Figure S5).

381

## 382 **Environmental Implications**

383 Here we have shown that abiotic reduction of nitrite by Fe(II) is rapid at environmentally  
384 relevant pH and Fe(II) concentrations. Indeed, we demonstrate that factors regulating the rates of  
385 this chemical process in the environment include reactant concentrations, surface interactions  
386 and pH. Further, these factors appear to control the relative proportions of reaction pathways,  
387 with strong implications for the isotopic evolution of reactant NO<sub>2</sub><sup>-</sup> as well as the isotopic  
388 composition and yield of product N<sub>2</sub>O. Specifically, even though elevated levels of Fe(II)  
389 increase reaction rate, the homogeneous reaction of NO<sub>2</sub><sup>-</sup> with aqueous Fe(II) is kinetically slow  
390 under our experimental conditions compared to biological reduction (e.g., <sup>52</sup>). Nevertheless, in  
391 most natural environments at circumneutral pH, aqueous Fe(II) is found adsorbed onto mineral  
392 surfaces and/or bound as ligands. Thus, while the homogenous reaction of aqueous Fe(II) with  
393 NO<sub>2</sub><sup>-</sup> may be kinetically inhibited, the heterogeneous reaction will most likely drive  
394 environmental chemodenitrification. In fact, our data also demonstrate dramatically increased  
395 reactivity of NO<sub>2</sub><sup>-</sup> with surface associated Fe(II). In all cases exhibiting Fe-oxide formation, rates  
396 of nitrite reduction were dramatically higher (Figure 2) as well as corresponding yields of N<sub>2</sub>O.  
397 Thus, under redox conditions promoting production of Fe(II) and conditions enabling sorption of

398 Fe onto mineral surfaces (e.g., soils, porewaters, permeable sediments, riparian zones), the  
399 kinetic drive for abiotic  $\text{NO}_2^-$  reduction by Fe(II) is substantial – as is the potential for its  
400 significance as an abiotic source of  $\text{N}_2\text{O}$  to the atmosphere. Notably, recent work with a culture  
401 of nitrate-reducing iron oxidizing bacteria also suggests that anaerobic Fe oxidation actually  
402 occurs as a chemical side reaction upon the intracellular production of  $\text{NO}_2^-$  and Fe oxides,  
403 raising the possibility that anaerobic Fe oxidation by other nitrate reducing microbes may also  
404 stem primarily from chemical interactions rather than direct enzymatic catalysis<sup>5, 53</sup>.

405         The sensitivity of the nitrite isotope effects and the product  $\text{N}_2\text{O}$  to reaction conditions  
406 will complicate interpretation of natural abundance isotope values for detecting reactions with  
407 Fe(II). Accordingly, environmental studies will need to fully account for factors including pH  
408 and Fe(II) concentrations/fluxes, abundance and form of Fe-bearing minerals, and  
409 concentrations/fluxes of nitrite. Constraining pH will be especially crucial to account for  
410 potential oxygen isotope exchange with water, which is rapid at pH values  $< 7$ <sup>22</sup>. Many of the  
411 ecosystems in which nitrite accumulation may be important such as groundwater, estuaries and  
412 coastal sediments may also exhibit dynamic changes in pH (during tidal flushing of an estuary,  
413 or a storm runoff event, for example). Currently, rates of nitrite-water oxygen isotope  
414 equilibration have only been quantified in seawater<sup>54</sup>. Thus, in order to fully exploit  $\text{NO}_2^-$   
415 oxygen isotopes in other ecosystems, future work on exchange rates across a range of salinity  
416 will be necessary.

417         With this improved understanding of controls on  $\text{N}_2\text{O}$  production by abiotic nitrite  
418 reduction, future studies should focus on establishing the importance of chemodenitrification in  
419 the environment especially under environmental conditions in which it may outcompete  
420 biological nitrite reduction. For example, in environments exhibiting rapidly fluctuating redox

421 conditions, such as estuarine sediments and permeable coastal and shelf sediments, high fluxes  
422 of Fe(II) released by iron-reducing bacteria and/or by abiotic reduction by sulfur intermediates  
423 are often closely juxtaposed with elevated nitrogen concentrations in overlying water. Such  
424 conditions could represent prime hotspots for abiotic reactions between  $\text{NO}_2^-$  and Fe(II) and the  
425 abiotic formation of  $\text{N}_2\text{O}$ .

426 **Table 1.** Observed N and O isotope effects for abiotic reduction of nitrite by Fe(II). In some  
 427 cases reactions were too fast for reliable measurement of NO<sub>2</sub><sup>-</sup> isotopes. ND = not enough nitrite  
 428 detected.

429

<b>pH</b>	<b>Starting [Fe(II)] (mM)</b>	<b>Goethite</b>	<b><sup>15</sup>ε (‰)</b>	<b><sup>18</sup>ε (‰)</b>	<b><sup>18</sup>ε: <sup>15</sup>ε</b>
7	0.5	No	33.9±24.8	24.8±15.9	0.7
7	4.7	No	25.1±2.5	18.1±2.1	0.7
7	8.4	No	6.1±1.0	7.8±0.2	1.3
8	0.6	No	22.6±1.0	14.4±1.6	0.6
8	4.2	No	6.6±1.1	5.7±1.3	0.9
8	6.3	No	N.D.	N.D.	N.D.
7	0.8	Yes	44.8±9.7	33.0±8.3	0.7
7	4.8	Yes	11.8±0.6	11.2±0.3	0.9
7	7.9	Yes	5.9	5.2	0.9
8	1.0	Yes	15.1±0.5	11.2±0.6	0.7
8	4.5	Yes	N.D.	N.D.	N.D.
8	8.9	Yes	N.D.	N.D.	N.D.

430

431

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443 **CONFLICT OF INTEREST**

444 The authors declare no competing conflict of interest.

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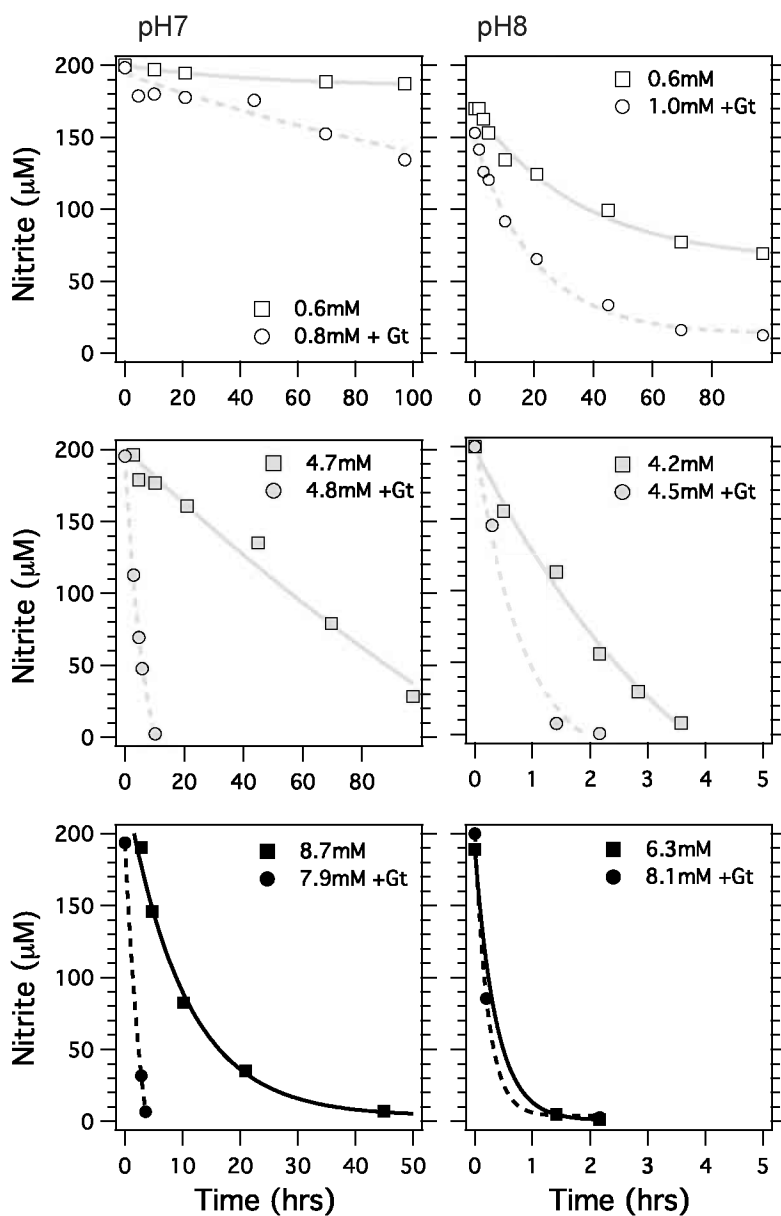


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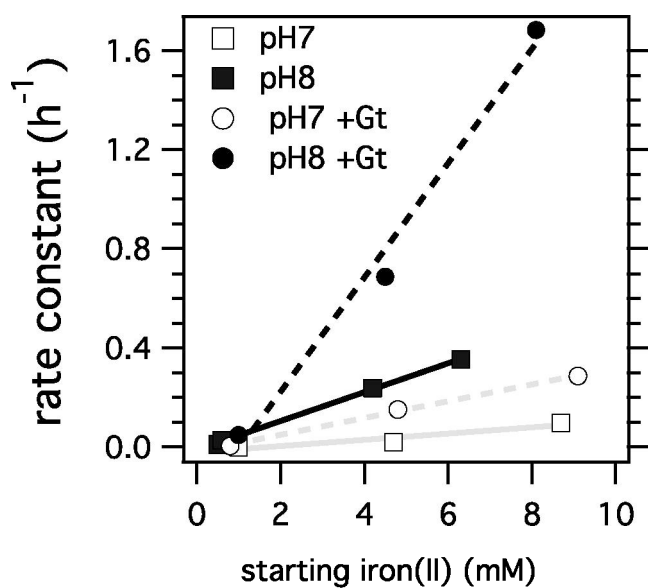
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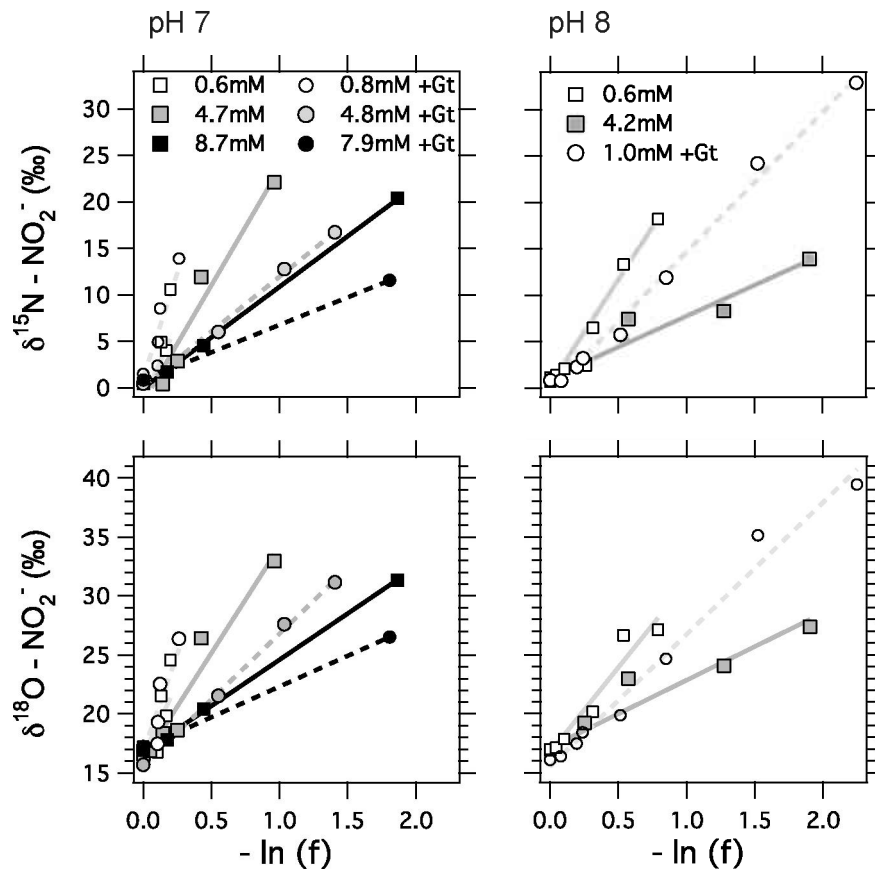




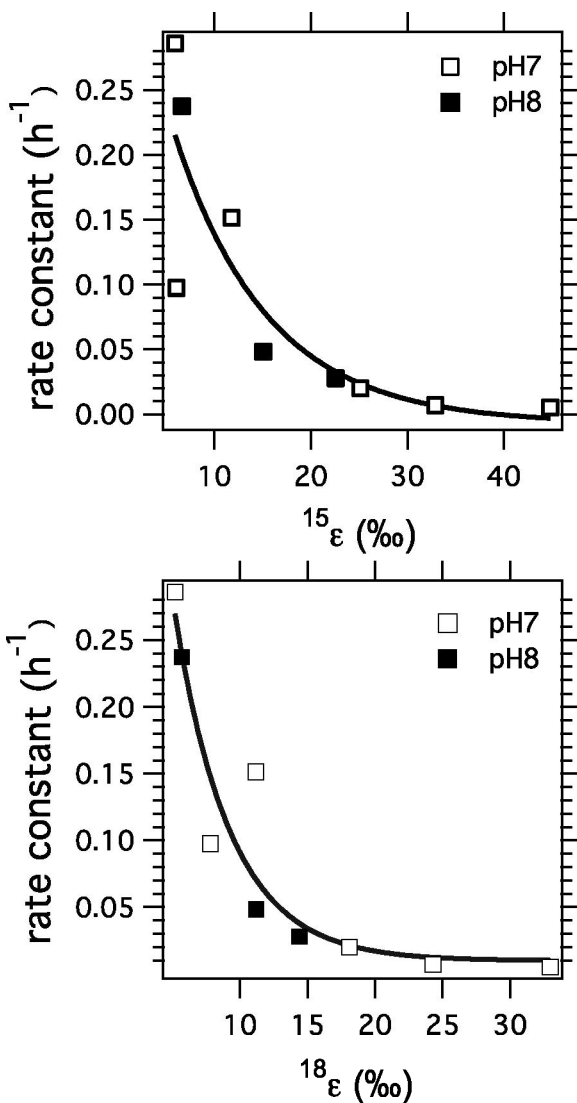
**Figure 1. Reaction of nitrite with aqueous Fe(II) as a function of time.** Rates of nitrite reduction were faster at higher Fe(II) concentrations, higher pH and in the presence of exogenous goethite. Note the time scale change at higher iron and high pH.



**Figure 2.** Pseudo-first order rate constants (with respect to  $\text{NO}_2^-$ ) varied linearly with starting Fe(II) concentration, with faster rates at higher pH and in the presence of an FeOOH (goethite) mineral surface. Rate constants were calculated assuming a pseudo-first order rate with Fe(II) in excess of nitrite, and exponential fitting of nitrite concentration over time.

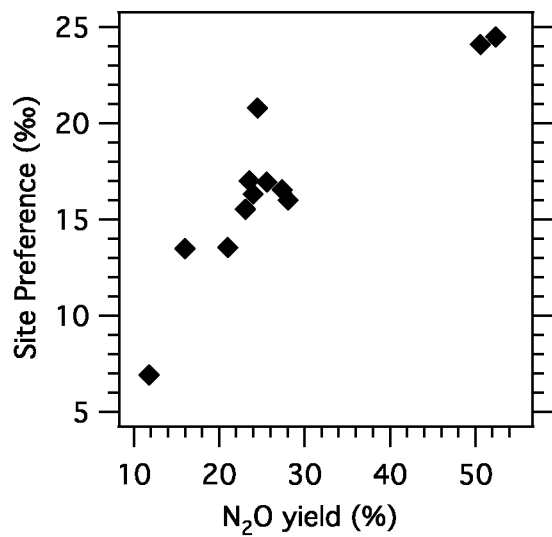


**Figure 3.** Closed system Rayleigh plots illustrating differences in the observed isotope effects of nitrite reduction by Fe(II) for  $\delta^{15}\text{N}-\text{NO}_2^-$  (top) and  $\delta^{18}\text{O}-\text{NO}_2^-$  (bottom) at pH 7 (right) and pH 8 (left). Dotted lines indicate experiments containing amended goethite.



**Figure 4.** The relationship between kinetic isotope effect (<sup>15</sup>ε<sub>NIR</sub> (top) and <sup>18</sup>ε<sub>NIR</sub> (bottom)) and pseudo-first order rate constant. The empirical relationship with the best fit is an exponential fit with the following equations:  $k = 0.0007 + 0.222 \cdot e^{-(^{15}\epsilon - 5.93)/9.68}$  and  $k = 0.0009 + 0.260 \cdot e^{-(^{18}\epsilon - 5.20)/4.13}$ . Changes in observed kinetic isotope effects as a function of reaction rate likely reflect complex shifts in reaction mechanisms, pathways and intermediates.





**Figure 5.** Site preference as related to the N<sub>2</sub>O yield from reduction of NO<sub>2</sub><sup>-</sup> by Fe(II). Changes in SP reflect differences in N<sub>2</sub>O production mechanisms and likely reflect the formation and reactivity of nitrosyl-iron intermediates.

**Supporting Information for:**

**Final Technical Report for DE-SC0006681**

**Project Title:** Development of new and integrated isotope tools for characterizing nitrogen-uranium cycling in subsurface environments

**PI: David Johnston**

**Report Title:** Constraining the role of iron in environmental nitrogen transformations: Dual stable isotope systematics of abiotic  $\text{NO}_2^-$  reduction by Fe(II) and its production of  $\text{N}_2\text{O}$

**Report Authors:**

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**METHODS**

**Chemical Analyses**

***Nitrite***

Nitrite concentration was measured using the Greiss-Islovay spectrophotometric method <sup>1</sup>. Reactions were prepared in 1cm cuvettes in the glove box and diluted as needed with anoxic HEPES buffer.  $\text{NaNO}_2$  standards of 0, 25, 50, 250  $\mu\text{M}$  were run in parallel. All samples and standards received 100  $\mu\text{L}$  sulfanilamide (SAN) and 100  $\mu\text{L}$  naphthyl ethylene diamine (NED). Absorbance was recorded at a wavelength of 543 nm.

***Fe(II)***

Fe(II) concentration measurements were made using ferrozine <sup>2</sup>. All reactions were conducted within the glove box followed by immediate measurement on the spectrophotometer at 562 nm. Reactions were done in 1cm cuvettes with 2.7 mL of ferrozine, and 0.3 mL of sample. At higher Fe concentrations samples were diluted as necessary with anoxic HEPES buffer.

## Mineral Analysis

The speciation of Fe was determined using synchrotron-based X-ray absorption spectroscopy (XAS)<sup>3</sup> (see Supporting Information). Samples were anaerobically mounted on a Teflon plate and sealed with Kapton polyimide film to prevent moisture loss and oxidation while minimizing X-ray absorption. XAS was performed at the Stanford Synchrotron Radiation Lightsource (SSRL) on beamline 11-2 using a He-purged sample chamber. Spectra were acquired from -200 to approximately 1000 eV around the K-edge of Fe (7111 eV). The mineralogical composition of the sediments was obtained using the extended region of the XAS spectra (EXAFS region). Percentages of various Fe phases were determined by linear combination fitting (LCF) of  $k^3$ -weighted EXAFS (LC-EXAFS) spectra with a set of reference standards as described in detail previously<sup>3</sup> using the fitting program SIXPack<sup>4</sup>.

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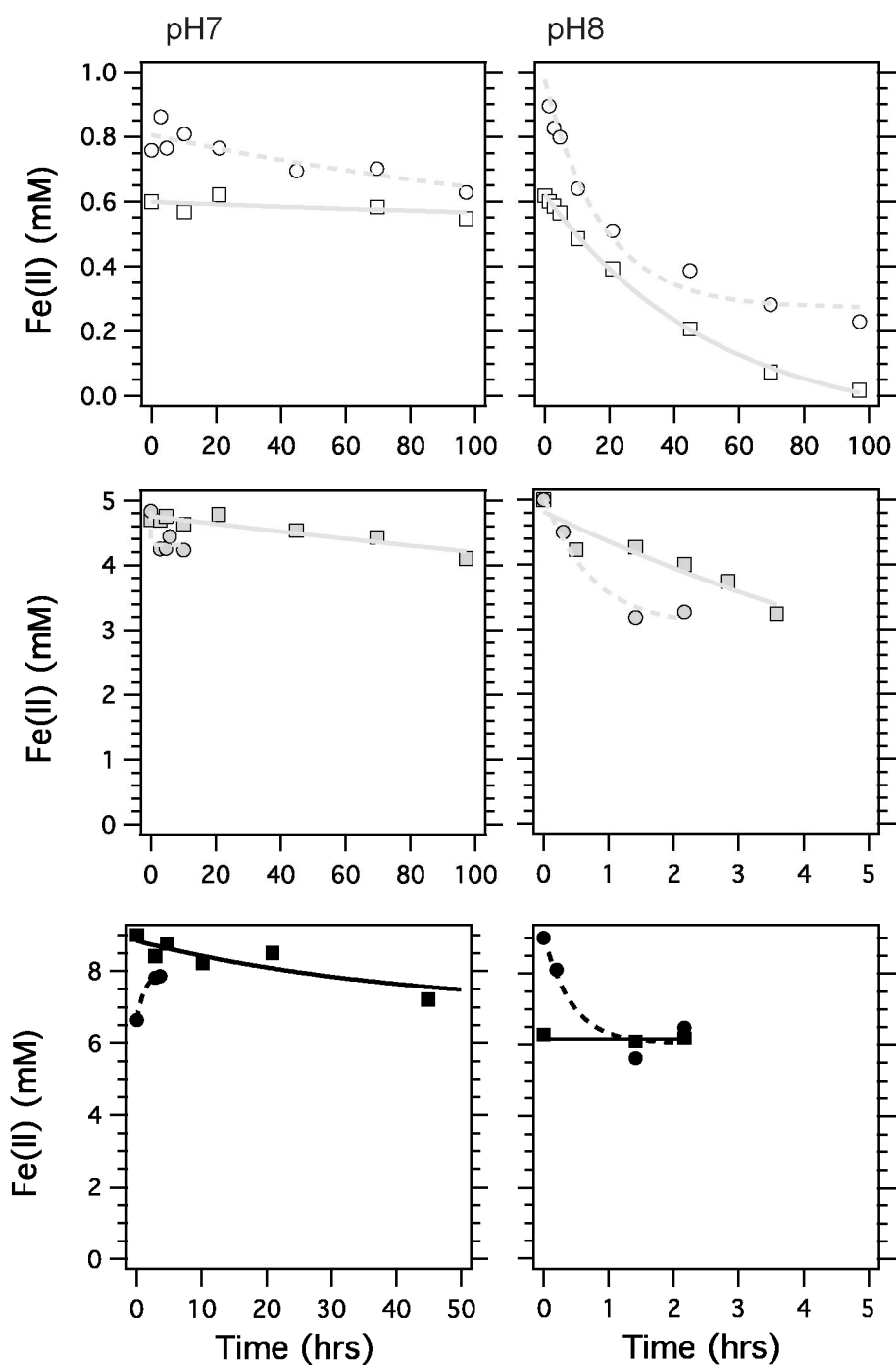
## Supplementary Figures and Tables

**Table S1.** Experimental conditions for each incubation.

<b>pH</b>	<b>Starting [NO<sub>2</sub>] (<math>\mu</math>M)</b>	<b>Starting [Fe(II)] (mM)</b>	<b>Goethite added</b>
7	200	0.5	No
7	200	4.7	No
7	200	8.4	No
8	200	0.6	No
8	200	4.2	No
8	200	6.3	No
7	200	0.8	Yes
7	200	4.8	Yes
7	200	7.9	Yes
8	200	1.0	Yes
8	200	4.5	Yes
8	200	8.9	Yes

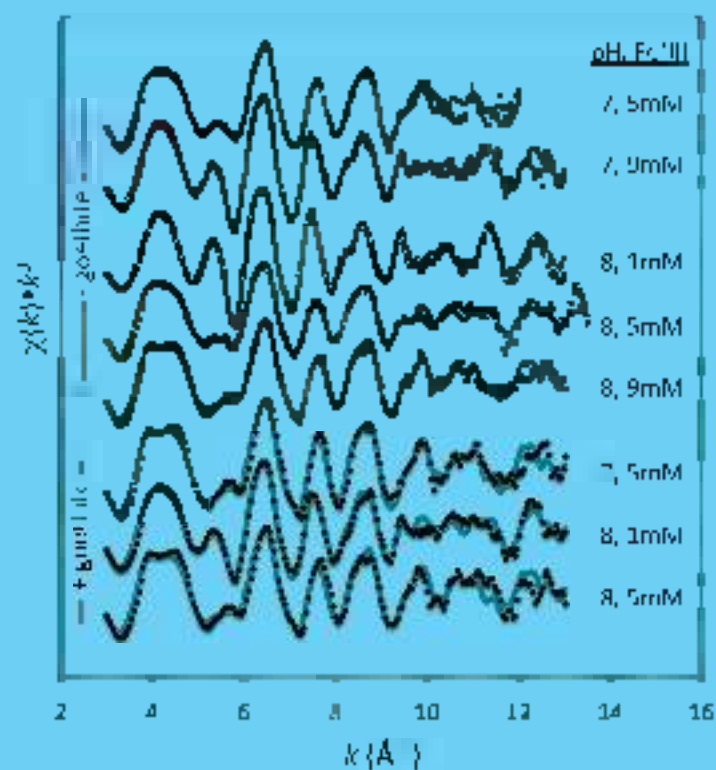
**Table S2.** First-order rate constants for nitrite reduction at pH 7 and pH 8

pH	Starting [Fe(II)] (mM)	Goethite added	$k_1$ ( $\text{h}^{-1}$ )	Initial Rate ( $\mu\text{M h}^{-1}$ )
7	0.5	No	<b>0.0007</b>	<b>0.1</b>
7	4.7	No	<b>0.02</b>	<b>4.0</b>
7	8.4	No	<b>0.10</b>	<b>19</b>
8	0.6	No	<b>0.03</b>	<b>5.9</b>
8	4.2	No	<b>0.23</b>	<b>41</b>
8	6.3	No	<b>0.35</b>	<b>60</b>
7	0.8	Yes	<b>0.005</b>	<b>1.0</b>
7	4.8	Yes	<b>0.15</b>	<b>28</b>
7	7.9	Yes	<b>0.29</b>	<b>50</b>
8	1.0	Yes	<b>0.05</b>	<b>9.8</b>
8	4.5	Yes	<b>0.69</b>	<b>99</b>
8	8.9	Yes	<b>1.68</b>	<b>160</b>

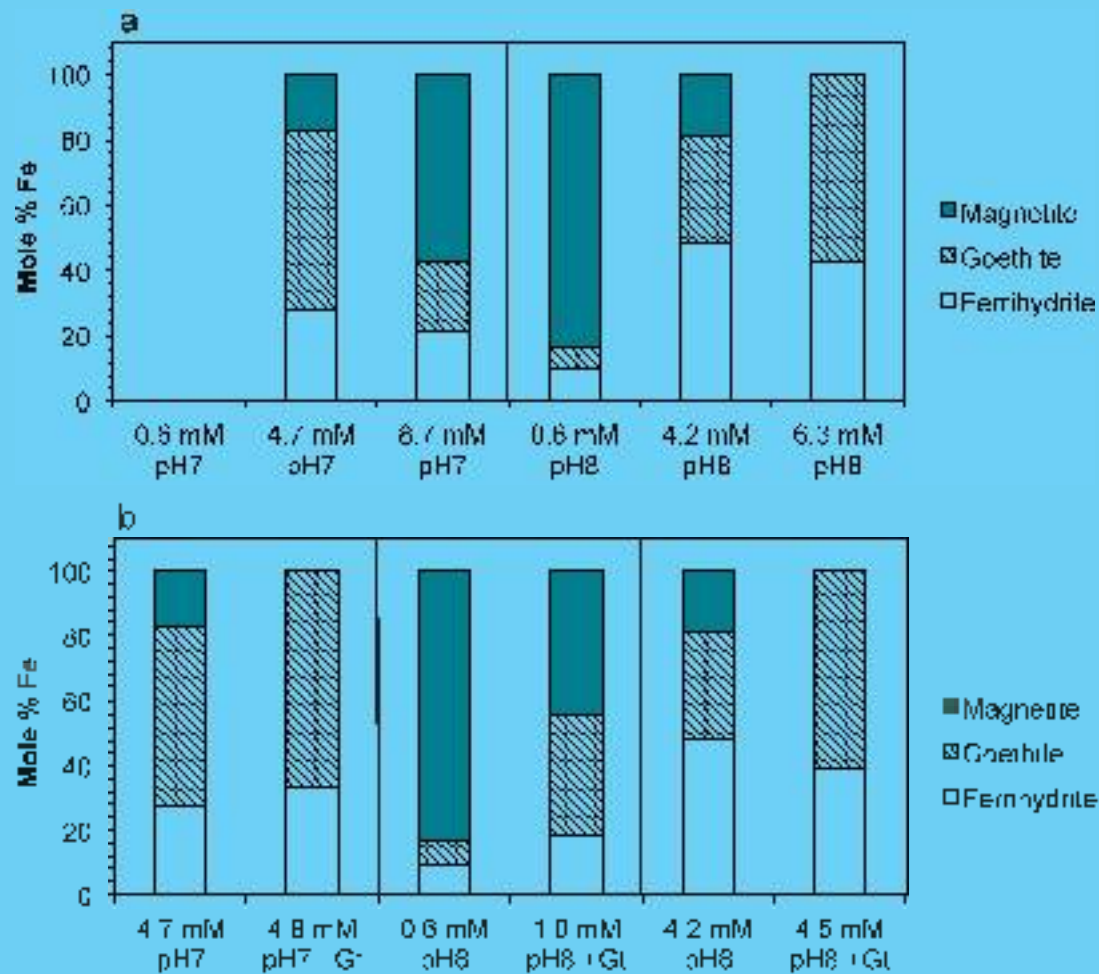


**Figure S1.** Dissolved Fe(II) concentration as a function of time, shown in panels by pH and Fe(II) concentration (note the time scale change at higher iron and high pH). Circles refer to experiments in which incubations were amended with goethite. A change in the aqueous Fe(II)

levels in the goethite incubation at the highest Fe(II) concentration at pH 8 was not observed, likely due to the rapid and extensive sequestration of Fe(II) within the solid phase

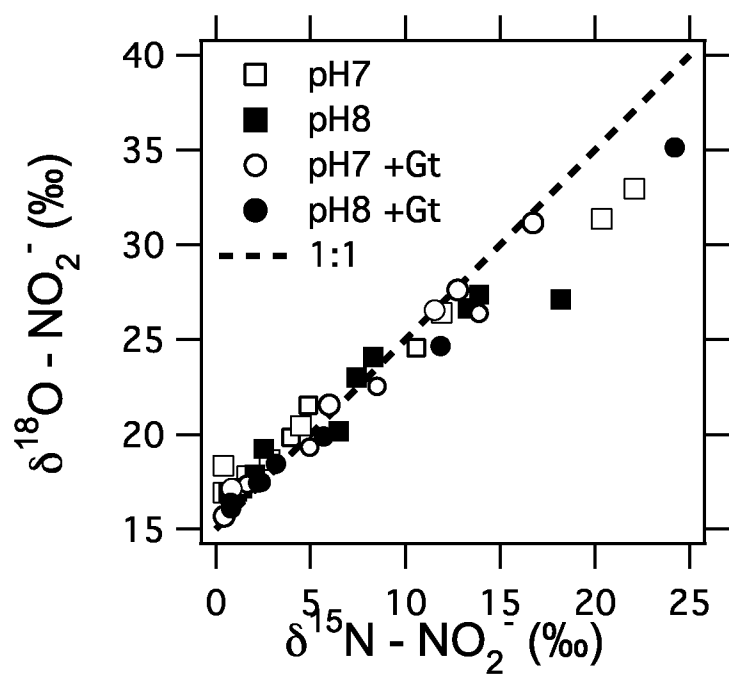


**Figure S2**  $k^3$ -weighted EXAFS spectra (dotted black line) and linear combination fits (solid gray line) for the mineral percentage shown in Figure S2 obtained for incubations containing 200  $\mu\text{M}$  nitrite, ferrous Fe(II) (1, 5, or 9 mM) at pH 7 or 8 and in the presence (+) or absence (-) of goethite

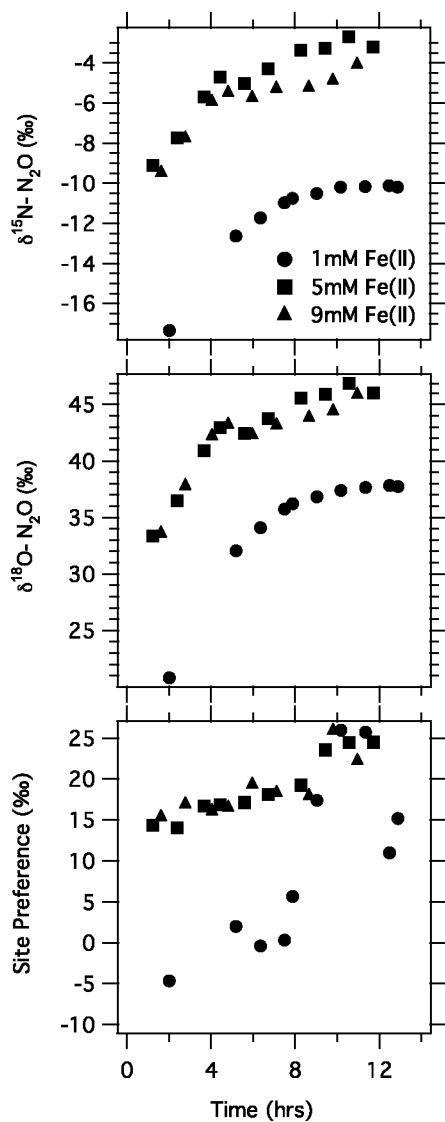


**Figure S3.** Summary of the final secondary minerals found at the end of the Fe(II)-nitrate incubations. Mineral proportions were obtained via linear combination EXAFS shown in Figure S2.

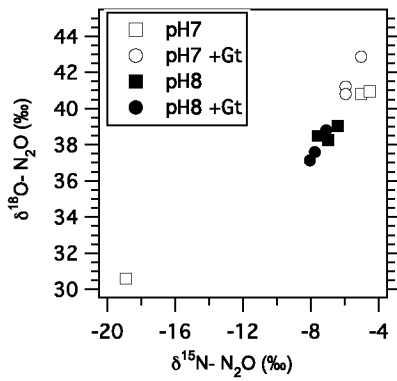




**Figure S4.**  $\delta^{18}\text{O} - \text{NO}_2^-$  and  $\delta^{15}\text{N} - \text{NO}_2^-$  in all incubations. The dotted line indicates a 1:1 relationship.



**Figure S5.** Time series  $\text{N}_2\text{O}$  experiments illustrating the evolution of  $\text{N}_2\text{O}$  in the sample vials over the course of the time course of the reaction. Higher  $\text{Fe(II)}$  concentrations resulted in faster reactions, higher  $\text{N}_2\text{O}$  yields (not shown) and higher  $\delta^{15}\text{N}$  (top),  $\delta^{18}\text{O}$  (middle) and site preference values (bottom) in comparison to reactions at  $\sim 1\text{mM Fe(II)}$ .



**Figure S6.** The dual N and O isotopic composition of  $\text{N}_2\text{O}$  produced during nitrite reduction by Fe(II). The single outlier having the lowest  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  represents  $\text{N}_2\text{O}$  produced from a partially consumed  $\text{NO}_2^-$  pool, while all others reflect  $\text{N}_2\text{O}$  produced after complete  $\text{NO}_2^-$  reduction.