1 **A dual nitrite isotopic investigation of chemodenitrification by**
2 **mineral-associated Fe(II) and its production of nitrous oxide**

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4 **Kalina C. Grabb^{1,2}, Carolyn Buchwald¹, Colleen Hansel¹, and Scott D. Wankel^{1,*}**

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6
7 ¹Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Woods
8 Hole, MA 02543

9
10 ²Earth and Planetary Sciences, Harvard University, Cambridge, MA 02138

11
12 [*sdwankel@whoi.edu](mailto:sdwankel@whoi.edu)
13
14
15
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ABSTRACT

Under anaerobic conditions, the environmental reduction of nitrate (NO_3^-) and nitrite (NO_2^-) to more reduced forms is widely regarded as being microbially catalyzed. However, the chemical reduction of oxidized nitrogen species by reduced iron (Fe(II)), whether mineral-bound or surface-associated, may also occur under environmentally relevant conditions. Here we examine the nitrogen (N) and oxygen (O) stable isotope dynamics of the chemical reduction of NO_2^- by mineral associated Fe(II) (chemodenitrification) and its production of the potent greenhouse gas nitrous oxide (N_2O). By shedding light on factors controlling kinetics of the reaction and its corresponding dual isotopic expression in the reactant NO_2^- and product N_2O , this work contributes to a growing body of work aiming to improve our ability to identify chemodenitrification in the environment.

Consistent with previous studies, we find that while homogenous reactions between aqueous NO_2^- and Fe(II) were kinetically slow, heterogeneous reactions involving Fe(II) -containing minerals often catalyzed considerable nitrite loss. In particular, rapid reduction of NO_2^- was catalyzed by the Fe-rich smectite clay mineral nontronite as well as the mixed Fe(II) - Fe(III) oxyhydroxide phase green rust. These minerals serve as both a source of reduced iron within the mineral structure as well as a surface for promoting the reactivity of Fe(II) . However, even in the presence of aqueous Fe(II) , experiments with low-Fe and non-Fe containing minerals showed little to no NO_2^- loss, perhaps suggesting a more dominant role for structural iron during chemodenitrification. When catalyzed by nontronite and green rust, N and O isotope effects for chemodenitrification ($^{15}\epsilon_{\text{cDNF}}$ and $^{18}\epsilon_{\text{cDNF}}$) ranged from 2 to 11‰ and 4 to 10‰, respectively, with lower values generally observed at higher reaction rates. Higher reaction rates were also linked to higher molar yields of N_2O (up to 31%), highlighting a strong potential for chemodenitrification to produce N_2O – especially relative to its production by microbial pathways, which typically exhibit yields <1%. The intramolecular ^{15}N site preference (SP) of the linear N_2O molecule (the difference in $\delta^{15}\text{N}$ between the central and outer atoms), reflective of different production mechanisms, was also measured for N_2O produced during green rust catalyzed chemodenitrification. Relative to values measured in other recent studies of chemodenitrification, SP values were consistently high ($+26.5\text{‰} \pm 0.8\text{‰}$), especially relative to N_2O produced via bacterial denitrification (SP $\sim 0\text{‰}$). Finally, the coupling of $^{18}\epsilon_{\text{cDNF}}$ and $^{15}\epsilon_{\text{cDNF}}$ at a ratio of ~ 1 during green rust catalyzed chemodenitrification contrasts distinctly with recently characterized bacterial nitrite reduction, potentially permitting disentangling of both processes under well-constrained conditions. This study contributes to the broader understanding of the potential relevance for mineral-derived Fe(II) to promote the reduction of nitrite and consequent production of N_2O , especially in iron-rich systems hosting dynamic redox oscillations, including hyporheic zones, estuarine sediments and groundwater aquifers.

1. INTRODUCTION

While reduction of nitrate and nitrite by denitrifying bacteria is generally regarded as the primary reactive nitrogen removal pathway of the global nitrogen (N) cycle (Gruber and Galloway, 2008), it has been suggested that denitrification may account for less than half of the total nitrogen reduction in natural ecosystems (Burgin and Hamilton, 2007). Indeed, a greater understanding of the complexity of the N cycle has led to further discoveries of biotic and abiotic processes leading to nitrogen reduction, with implications for the fate of nitrogen and the production of greenhouse gases including nitrous oxide (N₂O). Specifically, evidence has been mounting for both biotic and abiotic coupling of nitrate (NO₃⁻) and nitrite (NO₂⁻) reduction with iron (Fe) oxidation (Burgin and Hamilton, 2007; Picardal, 2012; Zhu-Barker et al., 2015) over a range of environmentally relevant conditions and reaction mechanisms (Buresh and Moraghan, 1976; Postma, 1993; Postma et al., 1991; Postma and Jakobsen, 1996; Straub et al., 1996). For example, a number of microbial cultures and/or enrichments have been identified that appear to couple the anaerobic oxidation of Fe(II) with the reduction of NO₃⁻ and/or NO₂⁻ (Carlson et al., 2012; Kappler and Straub, 2005; Straub et al., 1996; Weber et al., 2001). Nonetheless, it is also evident that a strong chemical potential exists between Fe(II) and oxidized forms of nitrogen, especially NO₂⁻, and that abiotic reduction - or 'chemodenitrification' - may occur at rates rivaling those of biologically catalyzed nitrogen transformations (Buchwald et al., 2016; Buresh and Moraghan, 1976; Hansen et al., 1996; Kampschreur et al., 2011; Kopf et al., 2013; Ottley et al., 1997; Sorenson et al., 1981). Indeed, it has been recently suggested that Fe(II) oxidation by some anaerobic Fe(II)-oxidizing bacteria may actually even stem from abiotic reaction with biologically produced NO₂⁻ (Klueglein and Kappler, 2012).

While numerous studies have shown that the homogeneous reduction of NO₂⁻ by aqueous Fe(II) can occur, Fe(II) in the environment generally exists in close association with mineral structures and surfaces, ligands and other complexes – associations which tend to increase its reactivity (Hansen et al., 1996; Kopf et al., 2013; Weber et al., 2001). Indeed, the reactivity of NO₂⁻ with Fe(II) in a variety of coordination environments is well documented including both mineral surface bound Fe(II) (Ernstsen, 1996) and ligand

bound Fe(II) (Kopf et al., 2013), as well as structural Fe(II) within primary silicates (Postma, 1990; Samarkin et al., 2010; Sorenson et al., 1981), phyllosilicates (Dong et al., 2009; Stucki et al., 2012), carbonates (Rakshit et al., 2008), sulfides (Summers, 2005) and oxides (Hansen et al., 1994; Weber et al., 2001). In particular, Fe(II) can be structurally incorporated into a variety of common clay minerals, such as smectites, illites, vermiculite, and kaolinite (Stucki et al., 2012) and, especially under fluctuating redox conditions, may occur in mixed-valence iron oxides such as magnetite and green rust (Hansel et al., 2003; Hansen et al., 1996). Occurrence of these Fe(II)-containing mineral forms is widespread in soil and sedimentary systems and represents a potentially important reductant for NO_2^- , particularly where active microbial communities rapidly are catalyzing reductive and oxidative transformations of nitrogen and iron.

Despite evidence suggesting a potentially important capacity for biogeochemical coupling of Fe and N cycling, the relevance of Fe in global biogeochemical cycling of nitrogen remains unclear. While it is understood that these reactions take place under a variety of environmental conditions, it has proven difficult to distinguish between abiotic and biotic processes under natural conditions (Picardal, 2012). To this end, stable isotopes offer a potentially unique perspective on these reactions and an improved understanding of the isotopic systematics of Fe(II) catalyzed NO_2^- reduction may help shed light on the extent of chemodenitrification in the environment. For instance, the leveraging of coupled isotope systems in nitrate (NO_3^-) has proven especially valuable for disentangling the biogeochemical complexity of nitrogen cycling in a range of aquatic environments including riverine, groundwater, estuarine and marine systems (Böhlke and Denver, 1995; Lehmann et al., 2003; Sebilo et al., 2006; Wankel et al., 2009). Comparatively, however, the isotope dynamics of nitrite are far less characterized, as its low accumulation in most environments has precluded its isotopic investigation. Nonetheless, recent investigations of nitrite isotopes in environments that permit some accumulation, including ocean oxygen minimum zones and brine lakes in Antarctica, have shed light on important biogeochemical dynamics (Buchwald et al., 2016; Casciotti and McIlvin, 2007; Peters et al., 2014; Samarkin et al., 2010) and the continued exploration of nitrite isotopes in other settings should also provide useful insight.

Here we examine the role of nitrite reduction coupled with anaerobic Fe(II) oxidation and its interactions with specific forms of mineral associated Fe(II) (including surface associated and mineral-bound) by measuring reaction rates as well as the stable N and O isotope systematics of the reactant NO_2^- . In a subset of experiments we also measured the isotopic composition of the product N_2O , which often harbors unique isotopic information that can be linked to specific transformation processes, including chemodenitrification (Buchwald et al., 2016; Heil et al., 2014; Jones et al., 2015). We expand on recent work exploring the nature of isotopic signatures of chemodenitrification, specifically those catalyzed by reactions involving common Fe-rich clay minerals and reactive mixed-valence iron oxides, such as green rust.

2. MATERIALS AND METHODS

2.1. Experimental setup

Before each experiment, all glassware, stoppers, and media solutions were autoclaved to eliminate biological contamination. Anoxic artificial seawater (AASW) was aliquotted into 160 ml serum bottles, followed by the addition of combinations of sodium nitrite (NaNO_2), mineral catalysts, and ferrous chloride (Table 1), with a resulting pH of ~ 6.5 for all reported experiments. Experimental conditions included 1) homogeneous reactions of NO_2^- with aqueous Fe(II), 2) heterogeneous reactions of NO_2^- with Fe(II) adsorbed onto either Fe(II)-bearing minerals or Fe(II)-free minerals, and 3) heterogeneous reactions of NO_2^- with structural Fe(II) contained in clay minerals (illite, Na-montmorillonite, and nontronite) or within the Fe(II)-Fe(III) oxyhydroxide mineral, green rust (chloride type).

All experimental preparation and sampling was performed in an anaerobic glove box (Coy, Inc.), filled with a 95/5% N_2/H_2 mix, with 100% N_2 used as the transfer chamber purge gas. Anaerobic conditions were maintained by use of two palladium catalyst scrubbers (Coy, Inc.). All plastic equipment remained within the glove box transfer chamber for at least one day prior to use to avoid oxygen contamination by O_2 adsorbed onto the surface of plastics. Anoxic solutions were created by boiling Milli-Q water ($\sim 1\text{L}$) for 10 minutes and continuously bubbling with O_2 -scrubbed nitrogen gas. In the glove box, aliquots of AASW were transferred into serum bottles and crimp-sealed,

leaving a headspace above the suspension which was bubbled/purged again with N₂ for 10 minutes. Anoxic stock solutions were stored crimped-sealed inside the glove box throughout the course of the experiments, while experimental bottles were incubated on a shaker outside the glove box. As a precaution, all experimental AASW solutions were filtered after addition of any FeCl₂, but before the addition of nitrite, to remove any iron minerals that may have precipitated due to minor levels of oxidation during transfer into the serum bottles. Corundum (Al₂O₃; Sigma-Aldrich, 11028) was used as an Fe-free mineral surface analog. Experiments were initiated by addition of anoxic NaNO₂ stock solution to final concentrations of ~200μM. At each time point, sample fluid was removed via syringe inside the glove box, filtered (0.22μm), and measured for concentration and isotopic composition.

2.2. Chemical Analyses

2.2.1. Iron concentrations

Dissolved iron concentrations were measured using the ferrozine method (Stookey, 1970), which quantifies aqueous Fe(II) or total dissolved Fe (after reduction of Fe(III) by hydroxylamine). 20μl samples were diluted in 80μl of 40mM anoxic sulfamic acid to prevent oxidation of iron upon removal from the glove box and also dissolve poorly crystalline solid phases (e.g., green rust) (Klueglein and Kappler, 2012). Fe content in silicates (e.g. clay minerals) cannot be measured by ferrozine, and instead we use the reported mineralogical composition of the clay mineral standards (see Section 2.4 below). Standards of Fe(II) ammonium sulfate were made and measured daily. Approximately 20 minutes after addition of 100μl of ferrozine reagent, Fe(II) was quantified in triplicate in 96 well plates by measuring absorption at 562nm. Total Fe was measured after mixing 80μl of boiling 1M hydroxylamine hydrochloride (HAHCl, to reduce Fe(III) to Fe(II)) with 20μl of standard or sample, followed by addition of 100μl ferrozine and measurement as described above. Reproducibility was typically within ±5%.

2.2.2. Nitrite concentration measurements

Nitrite concentrations were measured in triplicate using the Greiss-Islovay azo dye method (Pai et al., 1990). Nitrite samples were diluted 1:9 with artificial seawater (ASW) outside of the glovebox. 200µl of standard or ASW-diluted samples were pipetted into 96 well plates in triplicate. 20µl of sulfanilamide (SAN) was added and then allowed to react for five minutes, followed by addition of 20µl of naphthal-ethylenediamine (NED). After five minutes, the absorbance was measured at 543nm. Daily standard curves were prepared with bracketing standards of 0µM, 1µM, 5µM, 10µM, and 25µM of sodium nitrite (NaNO₂). Reproducibility was typically better than ± 2%.

2.2.3. Nitrous oxide (N₂O) concentration measurements

At the completion of a subset of experiments involving green rust, the accumulated nitrous oxide was measured by extracting 5ml of headspace gas and injecting into a crimp-sealed 20ml headspace vial pre-purged with N₂ gas (Table 2). During isotopic analysis of these samples (see below), the N₂O within each sample was flushed from the vial in a helium stream, purified and collected using a modified TraceGas purge and trap system (IsoPrime, Inc.) coupled to a Gilson headspace autosampler, before being quantified on an Isoprime 100 isotope ratio mass spectrometer (IRMS). The amount of N₂O in each 5ml headspace sample was then calculated from the linear relationship between IRMS peak area at m/z 44 and injections of known amounts of N₂O (derived from azide conversion of NO₂⁻ or injections of pure reference gas).

2.3. Stable isotopic analyses

2.3.1. Nitrite

Nitrite isotopic composition was measured by chemically converting NO₂⁻ to N₂O using the sodium azide method (McIlvin and Altabet, 2005) described here in brief. All samples were prepared and measured in duplicate. Based on measured NO₂⁻ concentrations, 40 nmoles of each sample were pipetted into a 20ml headspace vial followed by addition of nitrite-free ASW to achieve a final volume of 2ml. Reagent variant controls (e.g., quality control standards) were prepared with 40 nmoles of an internal nitrite isotope standard (WILIS-10). The sodium azide reagent was prepared by combining equal volumes of 20% acetic acid and 2M sodium azide solutions, followed

by purging for 30 minutes with argon to remove any potential N₂O contamination generated by trace NO₂⁻ in the reagents. 100µl of Ar-purged sodium azide reagent was added per 2ml of sample, shaken, and allowed to react for 30 minutes. Afterwards, 100ul of 6M NaOH per 2ml of sample solution was added to neutralize the reaction. The evolved N₂O gas was purified and collected on a modified TraceGas (IsoPrime, Inc.) purge and trap coupled with a Gilson autosampler, before isotopic analysis on an isotope ratio mass spectrometer (IsoPrime 100, Elementar Inc.) using IonVantage software. Samples were analyzed with periodic sets of internal standards (WILIS-10, WILIS-11 and WILIS 20) to correct for any variations in size or instrument drift at the beginning and end of each run, as well as between every 10 samples.

2.3.2. Nitrous oxide

N₂O isotopic composition was measured on the same IRMS system as described above, taking advantage of a 5 collector array set up for quantification of mass/charge ratios of 46, 45 and 44 (for N₂O) and 31 and 30 (for the NO fragment of the N₂O molecule). Deconvolution of isotopologue abundance for calculation of elemental isotope ratios was carried out numerically using Matlab following an approach outlined previously for CO₂ and modified here for N₂O (Santrock et al., 1985). Sample N₂O was directly compared against the N₂O IRMS reference tank having the following values: $\delta^{15}\text{N}_{\text{bulk}} = -0.7\text{‰}$; $\delta^{18}\text{O} = +39.1\text{‰}$; site preference (SP) = -5.3‰, where $\text{SP} = \delta^{15}\text{N}(\alpha) - \delta^{15}\text{N}(\beta)$ and α and β refer to the central and outer N atoms in the linear N₂O molecule, respectively (Toyoda and Yoshida, 1999). This tank was calibrated directly against two previously calibrated N₂O tanks from the Ostrom Lab at Michigan State University. Reported values have been corrected for any size linearity of isotopic ratios (31/30, 45/44 and 46/44) by using a series of reference tank subsamples injected into 20ml headspace vials using a gastight syringe. Typical precision for replicate analyses of our reference gas analyzed as samples for $\delta^{15}\text{N}$ is $\pm 0.3\text{‰}$, for $\delta^{18}\text{O}$ is $\pm 0.4\text{‰}$ and for SP is $\pm 0.8\text{‰}$.

2.4. Reduction and treatment of clay minerals

Clay minerals were obtained from the Clay Mineral Society of America and have been thoroughly described elsewhere (Grathoff and Moore, 2002; Lear and Stucki, 1989;

Schulz and Grundl, 2004). Clays were chosen to represent a range of Fe content and phyllosilicate structure, including illite (1.59% total Fe) and two smectites, Na-montmorillonite (3.67% total Fe) and nontronite (37.42% total Fe). Expandable smectite clays, such as nontronite and Na-montmorillonite, are 2:1 phyllosilicates with one octahedral sheet sandwiched between two tetrahedral sheets. Fe in smectites is located both within sheets as well as at sheet edges allowing more exposure of the iron atoms and generally resulting in higher reactivity (Stucki and Kostka, 2006). In comparison, illites are non-expandable 2:1 phyllosilicates, with Fe atoms located only within sheets. Together with this intra-layer Fe location, the reduced expandability of illite layers generally minimizes Fe atom exposure and overall reactivity of illites.

The clays were chemically reduced following established protocols (Stucki et al., 1984). After homogenization by mortar and pestle, 200mg of clay mineral were reduced in 40ml of anoxic DI water combined with 10ml of anoxic citrate-bicarbonate buffer (8 parts 1M NaHCO₃, 1 part 0.3M sodium citrate (Na₃C₆H₅O₇-2H₂O)) and 1200mg of sodium dithionite. Suspensions were then crimp-sealed in serum vials and shaken at 30°C for two weeks to ensure complete reduction. Following the incubation, the clays were washed four times with an anoxic 0.005M NaCl solution. The solutions were centrifuged in serum bottles at 1500 RPM for 10 minutes and decanted through the septum by N₂-flushed syringe. The washed clays were then suspended as slurries in anoxic water and stored, crimp-sealed in the glovebox.

2.5. Synthesis of green rust

Green rust was synthesized by partial oxidation of a 1M FeCl₂ solution made up in 50ml of ASW. The solution was allowed to oxidize partially in air for 20 minutes, while being shaken, followed by titration to neutral with 6M NaOH causing precipitation. Immediately thereafter, the solution was placed into the transfer chamber, evacuated, flushed with 100% N₂ and allowed to react for an additional 3 hours. The serum bottle was then crimp-sealed inside the glove box to prevent further iron oxidation. This stock suspension was washed in AASW, centrifuged and decanted several times to remove free Fe(II) and to achieve desired concentrations of Fe(II) (Table 1), before being finally suspended as a slurry in AASW. Mineralogical composition of this stock suspension was confirmed by extended X-ray absorption fine structure (EXAFS) spectroscopy, which

indicated that the phase was purely a green rust chloride (GR1(Cl⁻); Figure S1). For use in experiments, aliquots of this stock were removed after re-suspending by gently shaking.

2.6. Solid-phase analysis

Samples for mineralogical analysis were dried in the anaerobic chamber, mounted onto Teflon holders with Kapton tape and stored anaerobically in an airtight container. Initial and reacted minerals were characterized by X-ray absorption spectroscopy (XAS), in particular X-ray absorption near edge spectroscopy (XANES) for average oxidation state and extended X-ray absorption fine-structure (EXAFS) spectroscopy for mineral identification. Iron K-edge XAS spectra were collected on beam line 11-2 at the Stanford Synchrotron Radiation Lightsource (SSRL) using a Si(220) monochromator ($\Phi=0^\circ$). To avoid oxidation during transport and analysis, samples were transferred from the glovebox to the beamline using portable anaerobic boxes and only exposed to air during the brief (~1 minute) transfer of the sample into a He-purged sample chamber under continuous He flow within the beamline hutch. Calibrations were made using a Fe(0) standard (7112 eV) run between the second and third in-line ionization chambers. Fluorescence data were collected with a 100-element solid-state Ge detector array with soller slits. Spectra were collected (2-3 scans per sample) at room temperature from -200 to approximately +1000 eV around the Fe K-edge. We confirmed that radiation damage was not occurring due to X-ray exposure by comparing successive scans during each sample analysis. XAS scans were averaged, background-subtracted, normalized, and deglitched, if necessary. The relative proportions of Fe(II) and Fe(III) were determined by fitting the edge position of the background subtracted, normalized XANES spectra. Deconvolution of the edge shape was carried out using Gaussian peaks at two fixed energies corresponding to primary Fe(II) (7122 eV) and Fe(III) (7126 eV) contributions over the energy range (~7120-7132 eV) (PeakFit software, SeaSolve Software Inc.). Confirmation of initial and identification of any secondary Fe phases was determined by fitting the k^3 -weighted Fe EXAFS of the unknown spectra with a linear combination of reference compounds as described in detail previously (Hansel et al., 2003). Linear combinations of the empirical model spectra were optimized where the only adjustable

parameters were the fractions of each model compound contributing to the fit. The goodness of fit was established by minimization of the *R*-factor (Newville, 2001). Data analysis of sample spectra was performed using the SIXPACK software program (Webb, 2005).

3. RESULTS

3.1 Nitrite reduction rates

Reduction of nitrite by Fe(II) varied widely as a function of experimental conditions (Table 1), especially in relation to the abundance of aqueous and/or mineral-bound Fe(II). All rate constants (*k*) were calculated as pseudo-first order with respect to NO₂⁻ concentration. Under homogenous reaction conditions, significant reduction of NO₂⁻ by 0.8mM aqueous Fe(II) was not observed; significant reduction was observed, however, with addition of 12mM Fe(II) (*k* = 0.0016 hr⁻¹) (Table 1, Figure 1a). By comparison, the presence of a non-Fe-bearing mineral surface (corundum) with 0.8mM aqueous Fe(II) was able to slowly catalyze nitrite reduction (*k* = 0.0002 hr⁻¹, Table 1, Figure 1b) and the presence of reduced Fe-bearing clays illite and Na-montmorillonite (in the absence of aqueous Fe(II)) also slowly catalyzed nitrite reduction (*k* = 0.0001 hr⁻¹, 0.0002 hr⁻¹, respectively, Table 1, Figure 1c,d). In contrast, reduction of nitrite by reduced nontronite proceeded much more rapidly (*k* = 0.003 to 0.005 hr⁻¹), with the addition of 0.8mM Fe(II) accelerating rates (*k* = 0.008 to 0.011 hr⁻¹) (Table 1, Figure 2). Finally, nitrite reduction by green rust yielded the fastest reduction rates, ranging from 0.76 to 6.4 hr⁻¹, with rates strongly correlated with total Fe(II) concentration (Table 1, Figure 3).

3.2 Nitrite isotopes

Nitrogen and oxygen stable isotopic compositions of the reactant nitrite pool ($\delta^{15}\text{N}$ and $\delta^{18}\text{O}$) were measured for those experimental conditions exhibiting extensive NO₂⁻ reduction (e.g., nontronite and green rust experiments) (Table 2). Increases in the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of the remaining NO₂⁻ pool were observed in all cases (starting $\delta^{15}\text{N}$ = -1.7‰, $\delta^{18}\text{O}$ = +13.2‰), reflecting normal kinetic isotope effects (¹⁵ ϵ and ¹⁸ ϵ) for nitrite reduction (e.g., ¹⁴N and ¹⁶O reacting slightly faster than ¹⁵N and ¹⁸O). Isotope

effects (ϵ) are equal to $(\alpha - 1) * 1000$, where α is equal to the ratio of light to heavy reaction rate constants (e.g., $^{14}k/^{15}k$). Values of $^{15}\epsilon$ and $^{18}\epsilon$ were calculated using a closed system Rayleigh model, in which ϵ is equal to the linear slope of change of the $\delta^{15}N$ value regressed against the natural logarithm of the fraction of NO_2^- remaining (Figure 4 and 5). In some cases the steepness of this slope decreased toward the end of reactions, in which case the isotope effect was calculated using only the initial linear portion of the data (Figure 5a, b). Kinetic isotope effects for nitrite reduction by nontronite (with and without amended Fe(II)) ranged from 2.3 to 11.1‰ and from 4.5 to 10.4‰ for $^{15}\epsilon$ and $^{18}\epsilon$, respectively (Table 2). Similarly, kinetic isotope effects for nitrite reduction by green rust ranged from 4.2 to 9.5‰ for $^{15}\epsilon$ and from 4.1 to 9.4‰ for $^{18}\epsilon$. Reported standard deviations in isotope effects include the combined error of parallel bottle incubations for each set of conditions shown in Tables 1 and 2. There was much tighter correspondence between $^{15}\epsilon$ and $^{18}\epsilon$ for the more rapid reduction of nitrite exhibited by green rust yielding $^{18}\epsilon: ^{15}\epsilon$ values ~ 1 (Figure 6). While $^{18}\epsilon: ^{15}\epsilon$ values for nitrite reduction by nontronite alone were also ~ 1 , values of $^{18}\epsilon: ^{15}\epsilon$ were ~ 2 for nitrite reduction with additional Fe(II) (Figure 6).

3.3 Nitrous oxide isotopic composition

The isotopic composition of product N_2O in the headspace of experimental vials was measured during seven experiments involving reaction of NO_2^- with green rust (Table 2). Composition of the product N_2O was very reproducible, having an average $\delta^{15}N_{bulk} = -3.5 \pm 0.8$, $\delta^{18}O = +36.5 \pm 0.8$ ‰ and SP value of $+26.7 \pm 0.7$ ‰. The yield of N_2O relative to the amount of NO_2^- reduced ranged from 7 to 31% and was positively correlated with total Fe(II) (Figure S2).

3.4 Solid-phase iron speciation

XANES spectra for green rust and nontronite reacted for <5 hours with 800 and for ~ 300 hours with 200 μM nitrite, respectively, are shifted to higher energies (between 0.5 to 1.5 eV) than the initial phases, indicating a higher overall oxidation state (Figure 7). Gaussian deconvolution of the absorption edge was successfully (standard error <

0.006 for all spectra) accomplished using two peaks at 7122 and 7126 eV corresponding to Fe(II) and Fe(III), respectively. Based on these peak fits, the initial chemically reduced nontronite had an Fe(II) to Fe(III) ratio of ~1:2 while the green rust Fe(II/III) ratio was 1:1. For duplicate nontronite samples reacted with nitrite the ratio of Fe(II) to Fe(III) increased to 1:3.1 and 1:3.5. For reacted green rust samples, the ratio similarly increased to 1:2.5 and 1:3. LCF of the K-edge Fe EXAFS did not indicate any iron secondary products (Figure S3). Peak fitting of the XANES absorption edge for the initial chemically reduced illite and montmorillonite revealed Fe(II) to Fe(III) ratios of ~1:15 and ~1:3, respectively.

4. DISCUSSION

Here we demonstrate the reduction of nitrite by Fe(II)-bearing minerals, which is broadly consistent with previous investigations highlighting the overall favorability of the reaction of Fe(II) and NO_2^- under circumneutral pH. Prior studies have demonstrated the propensity for Fe(II) to serve as an environmentally relevant reductant of NO_2^- , yet the role of this reaction as well as other ecosystem level nitrogen removal mechanisms under natural conditions remains unclear (Boyer et al., 2006; Burgin and Hamilton, 2007; Picardal, 2012; Seitzinger et al., 2006). For example, reports of nitrite reduction via homogenous reaction with Fe(II) span a wide range of experimental conditions, with studies variably concluding that both more alkaline (~ pH 8) and more acidic (< pH 6) conditions increase reaction rates (Moraghan and Buresh, 1977; van Cleemput and Baert, 1983; Wullstein and Gilmour, 1964). Reports of varying experimental conditions, including catalyst concentrations, buffers, pH, and differing aqueous and solid-phase Fe(II) abundances, make it difficult to directly compare results among experiments. In part, this complexity may stem from differences in secondary mineralization dynamics involving the production of a variety of Fe (hydr)oxide species (Buchwald et al., 2016; Hansen et al., 1994; Pantke et al., 2011; Weber et al., 2001).

The relatively slow homogeneous reduction of nitrite by aqueous Fe(II) observed in our experiments is consistent with observations by others (Buchwald et al., 2016; Hansen et al., 1994; Jones et al., 2015; Weber et al., 2001) (Table 2, Figure 1). While elevated levels of Fe(II) increased reaction rate, the abiotic reduction of nitrite by Fe(II)

via this homogeneous reaction is likely kinetically inhibited under aqueous Fe(II) concentrations found in most environments and thus unlikely to be relevant in comparison with other microbial and surface catalyzed processes in most natural systems.

In contrast, reactivity of aqueous Fe(II) is generally enhanced in the presence of mineral surfaces (Picardal, 2012; Sorensen and Thorling, 1991; Tai and Dempsey, 2009; van Cleemput and Baert, 1983). In our experiments, however, little difference was observed between nitrite reduction rates via homogenous reaction with aqueous Fe(II) and those with Fe(II) in the presence of corundum (Al_2O_3). The slow rates of nitrite reduction suggest that adsorption of Fe(II) to mineral surfaces alone may not enhance rates under our experimental conditions (Table 2, Figure 1). Thus, although nitrite reduction may be coupled to oxidation of aqueous Fe(II) and/or mineral surface-associated Fe(II), here this process appears to be significantly faster in the presence of minerals specifically containing structural Fe(II) (Hansen et al., 1994; Picardal, 2012; Sorensen and Thorling, 1991).

Phyllosilicate clay minerals, such as smectites and illites, have been shown to play important roles in the efficient removal of contaminants, including nitrogen, from the natural environment, with differences in the Fe-content and mineral structure playing key roles in their reactivity (Ernstsen, 1996; Shelobolina et al., 2012; Stucki et al., 2012; Tor et al., 2000; Xu et al., 2001). While all three clays tested here contained structural iron (illite 1.6%, Na-montmorillonite 3.7% and nontronite 37.4%), XANES analyses revealed only partial reduction of mineral-bound iron by our reduction protocol, such that illite, Na-montmorillonite and nontronite contained 0.1%, 0.9% and 12.9% Fe(II), respectively. In the absence of aqueous Fe(II), the structural Fe(II) of both illite and Na-montmorillonite (at ~0.9 and 2.0mM, respectively) were unable to significantly reduce nitrite over the course of ~4.5 months (Table 2; Figure 1). This variability in nitrite reduction is a likely a consequence of differences in the location and/or concentration of iron within clays (Stucki et al., 2012). Further, while the addition of 0.8mM aqueous Fe(II) to these reduced clays did catalyze nitrite reduction, the slow rates would seem unlikely to outcompete biologically catalyzed nitrite reduction under most environmental conditions (Table 2). Significant enhancement of nitrite reduction beyond that observed for aqueous Fe(II) alone was only observed for nontronite – completely reducing nitrite

by 50 to 90% over several days – suggesting that the much higher structural Fe(II) content of nontronite was directly involved in nitrite reduction (Figure 2). In comparison to studies involving bacterial reduction of Fe within the clay structure (Dong et al., 2003; Ribeiro et al., 2009; Zhao et al., 2015), our chemical reduction procedure (using a citrate-dithionite buffer), may have partially collapsed the normally open smectite structure and reduced its reactivity (Ribeiro et al., 2009). Our results should therefore be considered conservative. In sum, the location of Fe(II) within the nontronite structure together with its higher mineral iron content relative to the two other clays, was sufficient to rapidly induce the chemical reduction of nitrite.

In comparison to the Fe(II)-bearing clay minerals, the rate of nitrite reduction by the mixed valence Fe oxide green rust proceeded rapidly, similar to previous observations (Hansen et al., 1994), with ~200µM nitrite reduced within <5 hours under all conditions (Table 1). In the presence of comparable amounts of structural Fe(II), reduction of nitrite was two to three orders of magnitude faster by green rust than by nontronite (Table 1), with elevated amounts of aqueous Fe(II) increasing rates (Figure 3). Mixed valence Fe oxides such as magnetite and green rust have previously been shown to be exceptionally reactive – including reactions with nitrite (Dhakal et al., 2013; Kappler and Straub, 2005; Klueglein et al., 2015; Kopf et al., 2013; Pantke et al., 2011; Rakshit et al., 2008). In the environment, these highly reactive phases often form as the result of rapid redox cycling of Fe, including the influence of both biotic and abiotic processes (Hansen et al., 1994; Pantke et al., 2011; Rakshit et al., 2008) and notably the oxidation of Fe(II) by reaction with nitrate or nitrite under various conditions has been shown to produce magnetite and/or green rust (Buchwald et al., 2016; Jones et al., 2015; Kappler and Straub, 2005; Miot et al., 2014; Picardal, 2012). The secondary precipitation of these mixed valence phases as well as (oxy)hydroxide phases including ferrihydrite, goethite and hematite clearly plays a strong role in increasing reactivity (Buchwald et al., 2016; Dhakal et al., 2013; Klueglein et al., 2015; Sorensen and Thorling, 1991). In fact, the oxidation of Fe(II) to Fe(III) by nitrite itself may result in auto-catalytic formation of green rust, promoting even greater reactivity, on time scales that could quite easily rival microbial nitrite reduction (Etique et al., 2014). Notably, green rust has even been directly observed

forming in cell cultures of anaerobic nitrate reducing, Fe-oxidizing bacteria (Etique et al., 2014; Pantke et al., 2011).

As expected, the mineral-mediated reduction of nitrite is accompanied by an increase in oxidation state within the Fe-bearing phases. We observed a substantial shift in the Fe absorption edge to higher energies (~1 eV) in the XANES spectra of the reacted nontronite and green rust, indicative of more oxidized iron with the structure (Figure 7). XANES spectral fitting indicated that the relative percentage of Fe(III) within reacted nontronite and green rust increased from 65% to 80% and from ~50% to 75%, respectively. LCF EXAFS did not indicate the presence of other secondary phases, such as ferrihydrite, indicating that secondary products were negligible (<3%) and instead the majority of the oxidized iron remained within the nontronite and green rust structure (Figure S3). Similarly, a recent study of nitrate reduction showed that the Fe(II) in phyllosilicate clays primarily remains within the clay mineral after oxidation to Fe(III), producing only a small amount of iron oxide in the form of ferrihydrite and magnetite (Zhao et al., 2013). In fact, this solid-state Fe redox cycling allows the Fe(III) to remain within the mineral acting as a source of iron that can be microbially re-reduced and recycled, continually reducing oxidized nitrogen species (Dong et al., 2009; Zhao et al., 2015).

In addition to the perspectives provided by the kinetic and mineralogical analyses, an improved understanding of the N and O stable isotope systematics of abiotic nitrite reduction can potentially aid in determining the relative importance of Fe-induced nitrogen transformations in complex natural environments (Buchwald et al., 2016; Jones et al., 2015; Peters et al., 2014; Samarkin et al., 2010). In our experiments, kinetic isotope effects of NO₂⁻ reduction (Table 2), as calculated from the evolving reactant pool, ranged from 2.3 to 16.5‰ and from 4.1 to 10.4‰ for N and O, respectively, (including ¹⁵ε values for montmorillonite and illite) falling toward the lower end of the range of values previously reported for NO₂⁻ reduction by Fe(II) (Buchwald et al., 2016; Jones et al., 2015). Notably, however, previous studies focused primarily on homogenous reactions between NO₂⁻ and Fe(II) and secondary precipitation of iron oxide minerals (Buchwald et al., 2016; Jones et al., 2015), and to our knowledge these are the first reported isotope data for reduction of NO₂⁻ by mineral bound Fe(II) in clays and green rust.

Observed isotope effects (or ‘net isotope effects’) can be affected by a variety of factors relating to reaction conditions including pH, speciation of precipitated minerals, and/or the relative concentrations of reactants. For nontronite and green rust we observed a strong relationship between reaction rate and kinetic isotope effects, with faster reaction rates leading to lower values of $^{15}\epsilon$ and $^{18}\epsilon$ (Figure 5c, d). Thus, higher Fe(II) concentrations, which lead to faster nitrite reduction, also corresponded to lower $^{18}\epsilon$ and $^{15}\epsilon$ values, consistent with other recent observations (Buchwald et al., 2016). Changes in the expression of isotope effects in the reactant NO_2^- pool suggest that multiple processes may be contributing to isotope fractionation – and that the factors regulating reaction rates are also contributing to the relative expression of the resultant net isotope effects.

Thus, factors governing the accessibility of Fe(II) to reaction with nitrite may be a key factor. For example, to the degree that reactions occur with inner layer Fe(II), changes in apparent isotope effects could reflect the relative proportion of reactions with inner layer Fe(II) versus surface associated Fe(II). However, higher rates (and lower ϵ values) corresponded with higher aqueous Fe(II) (and a lower presumed proportion of inner layer Fe(II) involved in NO_2^- reduction). Therefore control of the observed relationship between rate and $^{15}\epsilon$ is not easily explained by limitation of nitrite diffusion to reactive inner layer sites, which would be expected to lead to a reduced expression of an intrinsic isotope effect associated with the redox reaction (Brandes and Devol, 1997; Farquhar et al., 1982; Lehmann et al., 2004). Alternatively, reaction of NO_2^- with surface associated or aqueous Fe(II) likely proceeds via the formation of nitrosyl-iron complexes (Bonner and Pearsall, 1982; Kopf et al., 2013; Pearsall and Bonner, 1982), which would not be as likely to form with Fe(II) bound within a mineral lattice. If indeed the NO_2^- reaction mechanism is different for structural and surface-associated or aqueous Fe(II), then the relationship between reaction rate and observed isotope effect may be controlled by the relative reactivity of these two Fe(II) pools. This would suggest that the isotope effect for reaction with aqueous and/or surface-bound Fe(II) must be lower than the isotope effect for reduction by structural Fe(II). Indeed similar stable isotope dynamics were observed in a recent study with the homogenous reaction of NO_2^- and aqueous Fe(II) under varying Fe(II) concentrations and pH (Buchwald et al., 2016), and it was speculated that formation/decomposition of a nitrosyl-iron species may have played an

important role. Monitoring of stoichiometric changes during reactions can help to inform reaction pathways and kinetic relationships (Tai and Dempsey, 2009); yet because our experiments were generally conducted in a large relative excess of Fe, the small changes resulting from reactions with nitrite were insufficient for stoichiometric calculations. Future work focused on teasing apart the reaction mechanisms and their associated isotope effects might benefit from more direct consideration of reaction stoichiometry.

During the reduction of oxyanions, such as nitrate, sulfate, sulfite, and perchlorate, diagnostic relationships typically exist between the isotope effects of O and the other elements involved (Brunner et al., 2005; Granger et al., 2008; Hatzinger et al., 2009; Lehmann et al., 2003). Indeed, the strength of a dual isotope approach often leverages deviations from these relationships as evidence for occurrence of other simultaneous processes or back reactions (Casciotti and McIlvin, 2007; Sigman et al., 2005; Wankel et al., 2007). In our experiments, the relationship between nitrite N and O isotope effects was generally tightly coupled for all reactions with green rust ($^{18}\epsilon:^{15}\epsilon = 0.98 \pm 0.1$; Figure 6b). For the experiments with nontronite $^{18}\epsilon:^{15}\epsilon$ values were also near 1 when structural Fe(II) within the nontronite was the only Fe source (0.94), but much higher (~2) when aqueous Fe(II) was added. The reason for this higher $^{18}\epsilon:^{15}\epsilon$ relationship remains unclear. Interestingly, the coupled $^{18}\epsilon:^{15}\epsilon$ for microbial (enzymatic) nitrite reduction, however, was recently shown to diverge strongly from 1:1, whether by the Fe-bearing nitrite reductase *nirS* ($^{18}\epsilon:^{15}\epsilon \sim 0.75$) or by the Cu-containing *nirK* ($^{18}\epsilon:^{15}\epsilon \sim 0.1$) (Martin and Casciotti, 2016). Thus, based on our results here and those of other recent studies (Buchwald et al., 2016), an intriguing distinction emerges: chemodenitrification appears to impart a stronger nitrite oxygen isotope effect relative to its concurrent nitrogen isotope effect than does its enzymatic counterpart (e.g., $^{18}\epsilon:^{15}\epsilon$ slopes are higher for chemical reduction than for enzymatic reduction). Thus, given adequate constraints, it would be theoretically possible to estimate a minimum amount of nitrite consumption by chemodenitrification using dual nitrite isotope dynamics. As nitrite is a reactive intermediate of multiple microbial N cycling processes (e.g., nitrate reduction, ammonium oxidation), however, this will undoubtedly prove challenging under most natural settings. In circumstances where microbial activity can be ruled out, chemodenitrification has already been shown to be responsible for abiotic N₂O

production (e.g., (Ostrom et al., 2016; Peters et al., 2014; Samarkin et al., 2010)). Under most conditions the detection of chemodenitrification will also require careful attention to other N pools (especially NO_3^- , NH_4^+ and N_2O) to provide more quantitative constraint. Indeed, N cycling studies are increasingly addressing multi-process complexity by turning to multi-compound, multi-isotope approaches for disentangling the relative influence of various (and cryptic) processes (Buchwald et al., 2015; Casciotti et al., 2013; Casciotti and McIlvin, 2007; Granger and Wankel, 2016). With the information presented here, future studies will be better informed to more quantitatively address the likelihood of chemodenitrification. Of course, the isotopic signature of chemodenitrification (both in NO_2^- as well as N_2O) is based on only a handful of studies – and future work may reveal further complexity. In particular, it is clear that $\delta^{18}\text{O}$ values in some reactions (Figure 6a) were influenced by equilibration of nitrite oxygen isotopes with water (Casciotti et al., 2007), with reaction conditions promoting partial equilibration during the longer reaction times of the nontronite experiments. It is also possible that $^{18}\epsilon$ dynamics were influenced by back-reactions, in which a nitrosyl-iron gains an O atom (from water) – also promoting the ingrowth of a water O isotope signal into the NO_2^- pool and decreasing observed $^{18}\epsilon$ values. Both of these would lead to a suppression of the apparent $^{18}\epsilon$ values by incorporation of water having a lower relative $\delta^{18}\text{O}$. Notably, however, the distinction between chemical and enzymatic reduction ($^{18}\epsilon$: $^{15}\epsilon$) remains robust. Regardless, interpretation of nitrite $\delta^{18}\text{O}$ must carefully consider oxygen isotope equilibration with water and future experiments might explicitly target oxygen as an indicator of reaction mechanisms (for example, using water with different $\delta^{18}\text{O}$ values).

We observed that up to 31% of reactant NO_2^- was converted to N_2O during reactions with green rust (Figure S2), consistent with yields observed in other studies (Buchwald et al., 2016; Jones et al., 2015; Peters et al., 2014; Samarkin et al., 2010). Further, the N_2O yield was positively correlated with reaction rate and thus Fe(II) level ($r^2 = 0.78$; $p < 0.00001$). Formation of N_2O during chemodenitrification reactions has been well documented (Buchwald et al., 2016; Buresh and Moraghan, 1976; Heil et al., 2014; Jones et al., 2015; Kampschreur et al., 2011). Foremost, these results underscore the strong relative potential of chemodenitrification as mechanism of N_2O production in environments hosting active redox cycling of iron and nitrogen. In our experiments, the

end product N_2O (after all NO_2^- was reacted) had a lower $\delta^{15}\text{N}$ ($\sim -3.5\text{‰}$) than the reactant NO_2^- pool (-1.7‰), indicating production of an additional N pool having a higher $\delta^{15}\text{N}$ as the mass balance complement. Although we did not measure concentrations or isotopic composition of other N bearing pools, based on results of previous studies the most likely other products are dinitrogen and/or nitric oxide (NO) (Buchwald et al., 2016; Kampschreur et al., 2011). Indeed future studies specifically targeting N isotopic mass balance could shed even more light on underlying reaction mechanisms. The elevated $\delta^{18}\text{O}_{\text{N}_2\text{O}}$ values relative to NO_2^- , reflect the influence of branching effects by the preferential abstraction of ^{16}O during reduction steps of both NO_2^- and NO (Casciotti et al., 2002). The site preference of the product N_2O (ranging between $+25$ to 30‰) is at the higher end of the range observed in similar experiments (Buchwald et al., 2016; Jones et al., 2015; Peters et al., 2014; Samarkin et al., 2010). Jones and colleagues (Jones et al., 2015), reported chemodenitrification based N_2O yielding SP values between 10 - 22‰ , while a more recent study examining a somewhat broader range of reaction conditions reported a wider span of SP values – from ~ 0 to 26‰ (Buchwald et al., 2016). Although not well understood – it has been speculated that the variability in chemodenitrification based N_2O SP values may stem from variations in the formation mechanisms associated with involvement of Fe(II) -complexes with NO . The consistently high SP values we observe at rapid reaction rates with green rust (Table 2) may therefore suggest the dominance of a single formation mechanism. This pathway may proceed with the formation of a dinitrosyl intermediate ($\text{Fe}-(\text{O}-\text{N}=\text{N}-\text{O})$), followed by preferential breaking of the $^{14}\text{N}-\text{O}$ bond and a resulting ^{15}N enrichment of the central alpha atom in the product N_2O (e.g., high SP).

Clearly nitrite reduction by Fe(II) can be catalyzed under environmentally relevant conditions, given sufficient levels and redox cycling of nitrogen and mineral-associated Fe. In heterogeneous environments hosting dynamic redox oscillations, a number of interrelated physical, chemical, and biological processes lead to a complex network of feedbacks that directly or indirectly influence reaction favorability. Since green rust often forms under fluctuating redox conditions (and the co-occurrence of Fe(II) and Fe(III)) such as those often observed in soils and estuarine, wetland and even hydrothermal vent sediments (Bourrie et al., 1999; Trolard et al., 1997; Wankel et al.,

2012), its formation is also likely to coincide with environments favoring active biological redox cycling of N species – including the reductive and oxidative formation of NO_2^- . Indeed the formation and flux of N_2O from chemodenitrification in such environments has become increasingly considered in terrestrial ecosystems (Heil et al., 2015; Zhu-Barker et al., 2015). Similarly, Fe-rich smectite clays such as nontronite are common in marine environments as the weathering product of seafloor basalt and the precipitation product of Fe-rich hydrothermal fluids in the deep sea (Alt, 1988; De Carlo et al., 1983; Toner et al., 2012) and could easily serve as a driver for chemodenitrification. Chemodenitrification could also be an important process at redox boundaries in Fe-rich marine/estuarine sediments, and along with biological transformations could play a major role in promoting the release of gaseous forms of nitrogen to the atmosphere, including trace gases such as NO and N_2O (Heil et al., 2014; Kampschreur et al., 2011; Zhu-Barker et al., 2015). Consideration of chemodenitrification in the production and flux of N_2O could also help to explain the highly episodic and heterogeneous nature typifying N_2O releases across many ecosystems. Thus, while chemodenitrification may help to mitigate impacts of reactive nitrogen on aquatic ecosystems, it may also promote the release of climatically active greenhouse gases – and its role in N_2O fluxes should be better understood. Finally, nontronite is also one of the major phyllosilicate minerals found on the surface of Mars (Mustard et al., 2008; Poulet et al., 2005). In the search for evidence of past or extant life on Mars, it has been suggested that we “follow the nitrogen” (Capone et al., 2006). To this end, the stable isotopic composition of nitrogen-bearing pools, including NO_3^- , NO_2^- and N_2O , clearly represents a sensitive tool for investigating the history of N cycling on Mars. It is also evident, however, that abiotic reactions between Fe-rich minerals and oxidized N species cannot be ignored – and may complicate such interpretation. By expanding our understanding of the controls on chemodenitrification as well as its stable isotope dynamics, this study contributes to the body of work aiming to improve our ability to track these reactions across a wide range of environments harboring active redox cycling of reactive iron and nitrogen species.

FIGURE CAPTIONS

Figure 1. Reaction progress of NO_2^- a) in homogeneous reaction with aqueous Fe(II), b) with Fe(II) sorbed onto corundum and c) with reduced illite and d) with reduced Na-montmorillonite. In the absence of aqueous Fe(II) no significant reduction occurred, while the presence of 0.8mM Fe(II) together with a mineral surface catalyzed measurable reduction of nitrite.

Figure 2. Reaction progress of NO_2^- with the Fe-rich clay nontronite, illustrating faster reduction in the presence of aqueous Fe(II). Circles and squares denote repeated experiments.

Figure 3. Pseudo-first order reaction rate constants (with respect to NO_2^-) as related to total Fe(II) concentrations in experiments involving green rust.

Figure 4. Closed system Rayleigh isotope plots for a) N and b) O isotopes in NO_2^- for chemodenitrification catalyzed by reduced nontronite. The addition of aqueous Fe(II) increased reaction rates and decreased the observed isotope effects for both N and O. Note that later time points exhibited strong influence of O isotope exchange and were omitted from the calculation of isotope effects (symbols with dotted outlines).

Figure 5. Nitrogen and oxygen isotope dynamics for nitrite reduction by green rust. Closed system Rayleigh isotope plots for (a) $\delta^{15}\text{N}_{\text{NO}_2}$ and (b) $\delta^{18}\text{O}_{\text{NO}_2}$ showing the dependence of isotope effect of total Fe(II) (color scale). Where slopes of the evolving reactant pool decrease over time, isotope effects are calculated using the initial slope. Calculated isotope effects for nitrogen (c) and oxygen (d) as a function of reaction rate and total Fe(II) (color scale).

Figure 6. Dual N and O isotope plot of the reactant nitrite pool during chemodenitrification catalyzed by reduced nontronite (a) and green rust (b). Note that partial equilibration was observed between nitrite oxygen isotopes and ambient water during slower/late portions of reactions involving nontronite (symbols with dotted outlines) and were not included in calculation of $^{18}\epsilon$.

Figure 7. a) Overlain Fe K-edge XANES spectra of initial (black) and reacted (gray) nontronite and green rust to illustrate the edge shift to higher energies upon reaction with 0.8mM nitrite (for green rust) and with 0.2mM nitrite (for nontronite). Gray lines reflect duplicate samples for each experimental treatment. b) XANES spectra of the initial and reacted iron phases (black line) along with model Gaussian peak fits (gray circles) to the absorption edge (~7120-7132 eV).

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Table 1. Experimental conditions evaluated for reactions noting the concentrations of reactants involved. Rate constants only reported where statistically significant reaction progress was observed (p <0.05).

Experiment	Initial [NO₂⁻] (μM)	Initial [Fe(II)](aq) (mM)	Initial [Fe(II)](s) (mM)[†]	Rate Constant k1 (h⁻¹), (95% C.I.)
Control + Fe	213	0.8	0.0	*NS
Control + high Fe	183	12.0	0.0	0.0016, (0.0072, -0.0049)
Corundum	197	-	0.0	*NS
Corundum + Fe	205	0.8	0.0	0.0002 (0.0002, 0.0001)
Illite	172	-	0.9	*NS
Illite + Fe	176	0.7	0.9	0.0001 (0.0001, 0.0001)
Montmorillonite	121	-	2.0	*NS
Montmorillonite + Fe	118	0.8	2.0	0.0002 (0.0002, 0.0001)
Nontronite	130	-	32.9	0.003 (0.004, 0.002)
Nontronite + Fe	117	0.8	32.9	0.008 (0.011, 0.005)
Green Rust #1	168	0.9	2.9	1.03 (1.24, 0.82)
Green Rust #2	187	1.1	3.0	1.27 (1.45, 1.09)
Green Rust #3	191	0.4	3.3	0.76 (0.85, 0.67)
Green Rust #4	213	0.9	3.6	1.28 (1.41, 1.16)
Green Rust #5	197	0.3	3.6	1.07 (1.40, 0.75)
Green Rust #6	179	1.1	4.0	1.15 (1.59, 0.71)
Green Rust #7	206	0.4	6.9	1.57 (1.66, 1.48)
Green Rust #8	185	1.1	8.9	3.06 (3.43, 2.69)
Green Rust #9	101	0.7	10.6	3.08 (3.38, 2.67)
Green Rust #10	100	1.3	12.0	4.13 (5.25, 2.66)
Green Rust #11	964	2.3	12.4	6.28 (6.68, 5.88)
Green Rust #12	934	1.1	12.6	6.30 (7.24, 5.36)
Green Rust #13	180	1.5	13.5	6.30 (7.24, 5.36)
Green Rust #14	219	1.0	14.1	6.43 (7.18, 5.67)

*NS = no significant reaction observed

† Solid phase Fe(II) content for clay minerals was quantified using XANES together with reported total Fe content

† Solid phase Fe(II) content for green rust was measured by ferrozine.

Table 2. Isotope effects for nitrite reduction by Fe(II) and the isotopic composition of the endproduct N₂O

Experiment	¹⁵ e _{cDNF(NO₂)}			¹⁸ e _{cDNF(NO₂)}			¹⁸ e: ¹⁵ e	d ¹⁵ N _{N₂O} (‰) [†]			d ¹⁸ O _{N₂O} (‰) [†]			SP _{N₂O} (‰)			N ₂ O yield (%)
Control + Fe	^			^				^			^			^			^
Control + high Fe	^			^				^			^			^			^
Corundum	^			^				^			^			^			^
Corundum + Fe	11.7	±	1.0	^				^			^			^			^
Illite	^			^				^			^			^			^
Illite + Fe	9.1	±	0.0	^				^			^			^			^
Montmorillonite	^			^				^			^			^			^
Montmorillonite + Fe	16.5	±	0.7	^				^			^			^			^
Nontronite	11.1	±	0.8	10.4	±	1.6	0.94	^			^			^			^
Nontronite + Fe	2.3	±	0.1	4.5	±	0.2	1.98	^			^			^			^
Green Rust #1	9.4	±	0.7	9.4	±	0.4	1.00	^			^			^			^
Green Rust #2	9.0	±	0.1	9.0	±	0.1	1.01	^			^			^			^
Green Rust #3	9.5	±	0.2	8.6	±	0.4	0.91	-3.0	±	0.9	37.4	±	1.8	27.5	±	0.4	7.2
Green Rust #4	8.4	±	0.3	8.4	±	0.8	0.99	-3.4	±	1.0	37.0	±	1.6	27.0	±	0.2	8.7
Green Rust #5	9.1	±	0.6	9.1	±	0.6	1.01	-3.5	±	1.1	36.8	±	1.7	26.9	±	0.3	9.5
Green Rust #6	8.1	±	1.0	8.4	±	0.3	1.04	^			^			^			^
Green Rust #7	7.2	±	0.3	8.4	±	0.4	1.17	-8.9	±	0.1	28.3	±	0.0	30.2	±	0.1	9.9
Green Rust #8	7.0	±	0.1	8.0	±	0.2	1.14	-3.9	±	0.4	35.0	±	0.7	26.6	±	0.6	16.5
Green Rust #9	5.4	±	0.6	5.7	±	0.7	1.06	-3.6	±	0.3	36.0	±	0.3	25.3	±	0.0	28.5
Green Rust #10	5.9	±	0.9	5.8	±	0.9	0.98	-4.1	±	0.4	35.3	±	1.0	26.0	±	0.2	31.3
Green Rust #11	5.4	±	0.3	5.9	±	0.3	1.09	^			^			^			^
Green Rust #12	4.9	±	0.8	5.3	±	1.0	1.09	^			^			^			^
Green Rust #13	4.2	±	0.1	5.3	±	0.2	1.27	^			^			^			^
Green Rust #14	4.3	±	0.2	4.1	±	0.0	0.96	^			^			^			^

^not measured

† = starting NO₂⁻ composition was d¹⁵N = -1.7‰; d¹⁸O = +13.2‰

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7