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

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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 ($^{15}\varepsilon_{cDNF}$ and $^{18}\varepsilon_{cDNF}$) 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 $\delta^{15}N$ 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‰ ± 0.8‰), especially relative to N_2O produced via bacterial denitrification (SP ∼ 0‰). Finally, the coupling of $^{18}\varepsilon_{cDNF}$ and $^{15}\varepsilon_{cDNF}$ 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.

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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 (N_2O). Specifically, evidence has been mounting for both biotic and abiotic coupling of nitrate (NO_3^-) and nitrite (NO_2^-) 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 et al., 1991; Postma, 1993; 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 NO_3^- and/or NO_2^- (Straub et al., 1996; Weber et al., 2001; Kappler and Straub, 2005; Carlson et al., 2012). Nonetheless, it is also evident that a strong chemical potential exists between Fe(II) and oxidized forms of nitrogen, especially NO_2^-, and that abiotic reduction – or 'chemodenitrification' – may occur at rates rivaling those of biologically catalyzed nitrogen transformations (Buresh and Moraghan, 1976; Sorenson et al., 1981; Hansen et al., 1996; Ottley et al., 1997; Kampschreur et al., 2011; Kopf et al., 2013; Buchwald et al., 2016). 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 NO_2^- (Klueglein and Kappler, 2012).

While numerous studies have shown that the homogeneous reduction of NO_2^- 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; Weber et al., 2001; Kopf et al., 2013). Indeed, the reactivity of NO_2^- 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 (Sorenson et al., 1981; Postma, 1990; Samarkin et al., 2010), 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 (Hansen et al., 1996; Hansel et al., 2003). 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 (Casciotti and McIlvin, 2007; Samarkin et al., 2010; Peters et al., 2014; Buchwald et al., 2016) 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 (Heil et al., 2014; Jones et al., 2015; Buchwald et al., 2016). 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