Isotopic fractionation associated to nitrate attenuation by ferrous iron containing minerals

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Abstract. Biotic and abiotic laboratory experiments of nitrate and nitrite reduction by Fe-containing minerals were performed and the isotopic fractionation of the different reactions was calculated in order to determine whether it is possible to distinguish biotic and abiotic reactions involving N compounds. Results of biotic experiments showed nitrate reduction up to 96 % with transient NO₂⁻ accumulation and no significant N₂O production. No significant nitrate attenuation was observed in abiotic nitrate reduction experiments. Abiotic experiments of nitrite reduction showed a rapid decrease in nitrite concentrations in those experiments with added Fe²⁺ coupled with a significant N₂O production. Preliminary results of the N and O isotopic fractionation of the biotic experiments of nitrate reduction show differences in the ε¹⁵N(NO₃) and ε¹⁸O(NO₃) when different minerals were added. The abiotic experiments of nitrite reduction contrarily, showed similar ε¹⁵N(NO₂) in all the experiments.

1 Introduction

During the last years, numerous studies have pointed to the coexistence of biotic and abiotic reactions involving the N and Fe biogeochemical cycles in natural environments [1, 2]. Among the abiotic reactions, the NO₂⁻ reduction by Fe²⁺ oxidation has been well documented [3,4]. On the other hand, Fe²⁺ minerals have been suggested to enhance biotic denitrification through the NO₃⁻ dependent Fe²⁺ oxidation (NDFO) process, which is still not well constrained [5]. As the enzymatic Fe²⁺ oxidation by NDFO microorganisms has not yet been proven, it is unknown whether the Fe²⁺ oxidation is caused by an enzymatic activity, due to an abiotic reaction mediated by N intermediates from denitrification or a combination of both [1, 5, 6].

To trace N transformation processes, chemical and isotopic characterization have proved to be powerful tools. The enzymatic NO₃⁻ reduction provokes an enrichment in the heavy isotopes ¹⁵N and ¹⁸O of the unreacted substrate [7], unlike processes such as dilution that could lead to a concentration decrease without influencing the isotopic signature. The same pattern is expected throughout the reduction of all N intermediate products (e.g. NO₂⁻).

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or N₂O), which will be initially depleted in ¹⁵N and ¹⁸O with respect to the substrate. Information on the dual isotope systematics of NO₃⁻ and N₂O throughout the abiotic reduction by Fe²⁺ is still scarce [4, 8, 9]. Therefore, it is unknown in which extent the isotopic characterization of NO₃⁻, NO₂⁻ and N₂O might help in distinguishing biotic and abiotic reactions involving the N compounds.

The aim of this work was to determine at laboratory-scale the N and O isotopic fractionation (ε₁⁵N and ε₁⁸O, respectively) during biotic and abiotic NO₃⁻ and/or NO₂⁻ reduction by Fe²⁺ containing minerals. The selected Fe²⁺-containing minerals were magnetite (Mag), siderite (Sd) and olivine (Ol), and Mag was also tested nano-sized (Mag-NP) to quantify changes in reactivity. Special attention was directed on the generation of by-products (NO₂⁻ and N₂O) throughout the biotic process. Furthermore, the possible abiotic reactivity between the Fe²⁺-containing minerals and NO₃⁻ or NO₂⁻ and the possible N₂O generation through both the biotic and abiotic pathways were evaluated.

2 Methods

To accomplish the goal, five series of experiments were performed (Table 1). All series included replicates, a control without mineral and a blank (groundwater or synthetic water alone). The microcosms were set inside a glove box, using 20 mL serum bottles, crimp sealed with butyl rubber stoppers under an Ar headspace. Incubations were performed at 23 ºC and constant shaking in the darkness. The microcosms were sacrificed by turns at time intervals depending on denitrification dynamics. Control and blank microcosms were sacrificed at the end of the experiment.

Table 1. Experimental conditions of the batch experiments. R stands for the number or replicates.

| Experiment                      | Conditions                  | R | Code           |
|---------------------------------|-----------------------------|---|----------------|
| Biotic denitrif. (NO₃⁻) with groundwater | Groundwater (NO₃⁻) + sediment | 3 | BioSedGW-C     |
|                                 | Groundwater (NO₃⁻) + mineral + sediment | 10 (*) | BioSedGW-Min  |
| Biotic denitrif. (NO₃⁻) with synthetic water   | Synthetic water (NO₃⁻) + sediment | 3 | BioSedSW-C     |
|                                 | Synthetic water (NO₃⁻) + mineral + sediment | 3 (*) | BioSedSW-Min  |
| Abiotic denitrification (NO₃⁻) with Fe       | Synthetic water (NO₃⁻) FeCl₂   | 3 | AbFeNO₃-C      |
|                                 | Synthetic water (NO₃⁻) + mineral + FeCl₂ | 3 (*) | AbFeNO₃-Min  |
| Abiotic denitrification (NO₂⁻) with Fe       | Synthetic water (NO₂⁻) + FeCl₂   | 10 | AbFeNO₂-C      |
|                                 | Synthetic water (NO₂⁻) + mineral + FeCl₂ | 10 (*) | AbFeNO₂-Min  |
| Abiotic denitrification (NO₂⁻) | Synthetic water (NO₂⁻) + mineral | 3 (*) | AbNO₂-Min      |

(*) The number of replicates is for each mineral used (Mag, Ol, Sd, Mag-NP)

All samples were filtered through 0.2 μm Millipore® immediately when obtained and stored at 4 ºC until analysis. Chemical parameters were determined by standard analytical techniques. The δ¹⁵N-NO₃⁻, δ¹⁸O-NO₃⁻, δ¹⁵N-NO₂⁻ and δ¹⁸O-NO₂⁻ were determined following the cadmium and azide reduction method [10, 11]. N₂O was analyzed using a Pre-Con (Thermo Scientific) coupled to a Finnigan MAT 253 Isotope Ratio Mass Spectrometer (IRMS, Thermo Scientific). Notation is expressed in terms of δ‰ relative to the international standards AIR (Atmospheric N₂) for δ¹⁵N and V-SMOW (Vienna Standard Mean Oceanic Water) for δ¹⁸O.
3 Results and discussion

Biotic experiments (BioSedGw and BioSedSw) showed a decrease in NO$_3^-$ concentration during the first week of incubation (Figure 1), up to 42 % in the groundwater batch experiments and up to 64 %, in the synthetic water batch experiments. Attenuation was also observed in the control microcosm containing groundwater plus sediment with no mineral addition (BioSedGw-C up to 45 %). Transient NO$_2^-$ accumulation (not shown) was much higher in the microcosms containing synthetic water (up 50 % of the initial NO$_3^-$) compared to groundwater (up to 10 % of the initial NO$_3^-$). After the first week, no significant differences were observed in the microcosms except for the batch containing nano-sized Mag, where about 96 % of NO$_3^-$ reduction was achieved in 91 days (Figure 1). No significant NH$_4^+$ concentration was detected, discarding the occurrence of Dissimilatory Nitrate Reduction to Ammonium (DNRA). Headspace N$_2$O was below 1 % of the total reduced N, suggesting that the final gaseous product was N$_2$.

Abiotic experiments of NO$_3^-$ reduction (AbFeNO3) showed no significant decrease in NO$_3^-$ concentration after 220 days (results not shown). With regards to abiotic experiments of NO$_2^-$ reduction also no significant reduction was observed in microcosms containing synthetic groundwater and micro-sized minerals (AbNO2). However, a rapid NO$_2^-$ reduction was observed in the experiments in which microcosms contained synthetic groundwater with NO$_2^-$ and micro-sized minerals plus dissolved Fe$^{2+}$ (AbFeNO2, Figure 2). The beginning of the reaction seemed to be immediate, NO$_2^-$ complete removal was achieved in microcosms containing mineral plus dissolved Fe$^{2+}$ or dissolved Fe$^{2+}$ alone. An increased reduction rate (~ 50 hours) was observed in the microcosms containing micro-Sd compared to the others (~ 175 hours). A possible explanation could be an increased dissolution rate of Sd that increased dissolved Fe$^{2+}$ availability. N$_2$O accumulated at the headspace of the AbFeNO2 microcosms, mass balance results suggested that N$_2$O was the end product, other authors [8] previously observed a large N$_2$O accumulation during NO$_2^-$ abiotic reduction by Fe$^{2+}$ oxidation.

Fig. 1. Percentage of nitrate remaining in the biotic experiments. Experiments with sediment + groundwater + minerals (A) and experiments with sediment + synthetic water + minerals (B).
Preliminary results of the isotopic characterization of some of the batch experiments were obtained. Biotic experiments (BioSedGw) showed different isotopic fractionation depending on the added minerals. The higher $\varepsilon^{15}$N$_{NO_3}$ ($-28.5\%$) was obtained in the control experiments with no added minerals, decreasing $\varepsilon^{15}$N$_{NO_3}$ was observed for Mag-NP, Mag, Sd, and OI experiments ($-25.4\%$, $-15.4\%$, $-11.2\%$ and $-8.7\%$ respectively) (Figure 3). Similarly, the $\varepsilon^{18}$O$_{NO_3}$ obtained for the control experiment was $-20.7\%$, and also decreasing values were observed for Mag-NP, Sd, Mag, and OI experiments ($-17.9\%$, $-10.3\%$, $-8.2\%$ and $-2.6\%$ respectively, results not shown). The $\varepsilon^{18}$O$_{NO_3}/\varepsilon^{15}$N$_{NO_3}$ ratio calculated is $0.3$ (O1), $0.5$ (Mag), $0.7$ (Mag-NP), $0.7$ (Control) and $0.9$ (Sd). This ratio is similar (except for Sd) to what is observed in most groundwater studies ($0.5$) but differs from the ratio observed in laboratory experiments with cultures of denitrifying bacteria (close to 1).

Abiotic experiments of nitrite reduction (AbFeNO2) showed a similar isotopic fractionation in all the batch experiments, with an $\varepsilon^{15}$N$_{NO_2}$ between $-14.1\%$ and $-17.8\%$, and no oxygen fractionation (data not shown).
4 Conclusions

Results of the biotic experiments showed similar percentages of nitrate reduction regardless the addition of different Fe\textsuperscript{2+} containing minerals. The highest reactivity was observed in the experiments using magnetite nanoparticles since those experiments reached almost complete NO\textsubscript{3} \textsuperscript{-} reduction. Transient NO\textsubscript{2} \textsuperscript{-} accumulation and no significant N\textsubscript{2}O production were observed in all the biotic experiments. No significant nitrate attenuation was observed in abiotic nitrate reduction experiments after 220 days. Abiotic experiments of nitrite reduction showed a rapid decrease in nitrite concentrations in those experiments with added Fe\textsuperscript{2+}, and the final product of NO\textsubscript{2} \textsuperscript{-} reduction was gaseous N\textsubscript{2}O. Preliminary results of the N and O isotopic fractionation of the biotic experiments of nitrate reduction show differences in the $\epsilon^{15}$N\textsubscript{NO\textsubscript{3}} and $\epsilon^{18}$O\textsubscript{NO\textsubscript{3}} when different minerals were added. The abiotic experiments of nitrite reduction contrarily, showed similar $\epsilon^{15}$N\textsubscript{NO\textsubscript{2}} in all the experiments. The $\epsilon^{18}$O\textsubscript{NO\textsubscript{3}}/$\epsilon^{15}$N\textsubscript{NO\textsubscript{3}} ratio in the biotic experiments showed values around 0.7 similar to values commonly found in groundwater.

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