Isotopic fractionation associated to nitrate attenuation by ferrous iron containing minerals

Rosanna Margalef-Martí¹, Raul Carrey¹, Albert Soler¹, and Neus Otero^{1,2,*}

¹Grup MAIMA, SGR Mineralogia Aplicada, Geoquímica i Geomicrobiologia, Departament de Mineralogia, Petrologia i Geologia Aplicada, Facultat de Ciències de la Terra, Universitat de Barcelona (UB), Martí i Franquès s/n, 08020 - Barcelona (Spain).

²Serra Hünter Fellowship, Generalitat de Catalunya, Spain.

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 NO_2^- accumulation and no significant N_2O 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 Fe^{2+} coupled with a significant N_2O production. Preliminary results of the N and O isotopic fractionation of the biotic experiments of nitrate reduction show differences in the $\epsilon^{15}\text{N}_{\text{NO}_3}$ and $\epsilon^{18}\text{O}_{\text{NO}_3}$ when different minerals were added. The abiotic experiments of nitrite reduction contrarily, showed similar $\epsilon^{15}\text{N}_{\text{NO}_2}$ 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 NO_2^- reduction by Fe^{2+} oxidation has been well documented [3,4]. On the other hand, Fe^{2+} minerals have been suggested to enhance biotic denitrification through the NO_3^- dependent Fe^{2+} oxidation (NDFO) process, which is still not well constrained [5]. As the enzymatic Fe^{2+} oxidation by NDFO microorganisms has not yet been proven, it is unknown whether the Fe^{2+} 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 NO_3^- reduction provokes an enrichment in the heavy isotopes ^{15}N and ^{18}O 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. NO_2^-

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* Corresponding author: notero@ub.edu

or N₂O), which will be initially depleted in ¹⁵N and ¹⁸O with respect to the substrate. Information on the dual isotope systematics of NO₂⁻ 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 °C 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. (NO ₃ ⁻) with groundwater	Groundwater (NO ₃ ⁻) + sediment	3	BioSedGW-C
	Groundwater (NO ₃ ⁻) + mineral + sediment	10 (*)	BioSedGW-Min
Biotic denitrif. (NO ₃ ⁻) with synthetic water	Synthetic water (NO ₃ ⁻) + sediment	3	BioSedSW-C
	Synthetic water (NO ₃ ⁻) + mineral + sediment	3 (*)	BioSedSW-Min
Abiotic denitrification (NO ₃ ⁻) with Fe	Synthetic water (NO ₃ ⁻) FeCl ₂	3	AbFeNO ₃ -C
	Synthetic water (NO ₃ ⁻) + mineral + FeCl ₂	3 (*)	AbFeNO ₃ -Min
Abiotic denitrification (NO ₂ ⁻) with Fe	Synthetic water (NO ₂ ⁻) + FeCl ₂	10	AbFeNO ₂ -C
	Synthetic water (NO ₂ ⁻) + mineral + FeCl ₂	10 (*)	AbFeNO ₂ -Min
Abiotic denitrification (NO ₂ ⁻)	Synthetic water (NO ₂ ⁻) + mineral	3 (*)	AbNO ₂ -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 °C 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_2O was below 1 % of the total reduced N, suggesting that the final gaseous product was N_2 .

Fig. 1. Percentage of nitrate remaining in the biotic experiments. Experiments with sediment + groundwater + minerals (A) and experiments with sediment + synthetic water + minerals (B).

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_2O accumulated at the headspace of the AbFeNO2 microcosms, mass balance results suggested that N_2O was the end product, other authors [8] previously observed a large N_2O accumulation during NO_2^- abiotic reduction by Fe^{2+} oxidation.

Fig. 2. Percentage of nitrite remaining in the abiotic experiments. Experiments with synthetic water + minerals (A) and experiments with synthetic water + minerals + Fe²⁺ (B), note the different timescale in the x-axis.

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 $\epsilon^{15}\text{N}_{\text{NO}_3}$ (-28.5 ‰) was obtained in the control experiments with no added minerals, decreasing $\epsilon^{15}\text{N}_{\text{NO}_3}$ was observed for Mag-NP, Mag, Sd and Ol experiments (-25.4 ‰, -15.4 ‰, -11.2 ‰ and -8.7 ‰ respectively) (Figure 3). Similarly, the $\epsilon^{18}\text{O}_{\text{NO}_3}$ obtained for the control experiment was -20.7 ‰, and also decreasing values were observed for Mag-NP, Sd, Mag, and Ol experiments (-17.9 ‰, -10.3 ‰, -8.2 ‰ and -2.6 ‰ respectively, results not shown). The $\epsilon^{18}\text{O}_{\text{NO}_3}/\epsilon^{15}\text{N}_{\text{NO}_3}$ ratio calculated is 0.3 (OI), 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 $\epsilon^{15}\text{N}_{\text{NO}_2}$ between -14.1 ‰ and -17.8 ‰, and no oxygen fractionation (data not shown).

Fig. 3. Isotopic fractionation $\epsilon^{15}\text{N}_{\text{NO}_3}$ in the biotic experiments of nitrate reduction with groundwater + sediment + minerals (A). Isotopic fractionation $\epsilon^{15}\text{N}_{\text{NO}_2}$ in the abiotic experiments on nitrite reduction with synthetic water + minerals + Fe²⁺ (B).

4 Conclusions

Results of the biotic experiments showed similar percentages of nitrate reduction regardless the addition of different Fe^{2+} containing minerals. The highest reactivity was observed in the experiments using magnetite nanoparticles since those experiments reached almost complete NO_3^- reduction. Transient NO_2^- accumulation and no significant N_2O 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^{2+} , and the final product of NO_2^- reduction was gaseous N_2O . Preliminary results of the N and O isotopic fractionation of the biotic experiments of nitrate reduction show differences in the $\varepsilon^{15}\text{N}_{\text{NO}_3}$ and $\varepsilon^{18}\text{O}_{\text{NO}_3}$ when different minerals were added. The abiotic experiments of nitrite reduction contrarily, showed similar $\varepsilon^{15}\text{N}_{\text{NO}_2}$ in all the experiments. The $\varepsilon^{18}\text{O}_{\text{NO}_3}/\varepsilon^{15}\text{N}_{\text{NO}_3}$ ratio in the biotic experiments showed values around 0.7 similar to values commonly found in groundwater.

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