

# Nitrate and Nitrite Attenuation by Fe(II) Minerals: Biotic and Abiotic Reactions

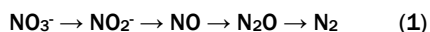
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## INTRODUCTION

Nitrate (NO<sub>3</sub><sup>-</sup>) pollution of groundwater has become a relevant issue and an environmental priority as it is related to ecological and human health problems (Rivett et al. 2008) and its concentration is still above the threshold limit of 50 mg/L in many areas (Nitrate Directive, 91/676/EEC). Contamination sources of NO<sub>3</sub><sup>-</sup> are linked to extensive use of fertilizers, inappropriate placement of animal waste and spills from septic system effluents.

Among the techniques proposed for NO<sub>3</sub><sup>-</sup> polluted water remediation, induced denitrification is one of the most promising treatments. Denitrification (Eq.1) is an effective process driven by autotrophic or heterotrophic bacteria by which NO<sub>3</sub><sup>-</sup> (electron acceptor) is reduced to N<sub>2</sub> under anaerobic conditions while an electron donor is oxidized (e.g. organic C or inorganic compounds such as Fe(II) or reduced S).



Laboratory and field studies have already demonstrated that adding organic carbon as an external electron donor source is adequate to induce NO<sub>3</sub><sup>-</sup> groundwater denitrification (Vidal-Gavilan et al. 2013; Carrey et al. 2014; Critchley et al. 2014). However, these treatments can generate some drawbacks as NO<sub>2</sub><sup>-</sup> accumulation, which is in fact, more toxic than NO<sub>3</sub><sup>-</sup>.

In the search for alternative economical and environmentally safe electron donors, Fe(II) minerals are suggested to be potentially valuable in promoting autotrophic denitrification. In this context, pyrite has successfully proven to enhance NO<sub>3</sub><sup>-</sup> attenuation (Torrentó et al. 2010). Therefore, the use of other Fe(II) bearing minerals can be a promising approach to remove NO<sub>3</sub><sup>-</sup>

from contaminated water. Besides, the relevance of the interactions between microbial and chemical reactions involved in NO<sub>3</sub><sup>-</sup> dependent Fe(II) oxidation (NDFO) has been recently emphasized. Nitrite (NO<sub>2</sub><sup>-</sup>) has been found to be a chemical oxidant for Fe(II) (Buchwald et al. 2016; Dhakal et al. 2013), especially when Fe(II) associates to mineral surfaces. Therefore, it is hypothesized that NDFO is the sum of biotic and abiotic processes whereby Fe(II) is chemically oxidized by NO<sub>2</sub><sup>-</sup>, which is generated throughout NO<sub>3</sub><sup>-</sup> biological reduction (Melton et al. 2014). However, the relevance of this process remains unclear.

Isotopic studies are a useful tool to trace N compounds sources and transformation processes. Throughout denitrification, unreacted residual NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> become enriched in the heavy isotopes <sup>15</sup>N and <sup>18</sup>O and therefore, biological attenuation can be distinguished from other processes such as dilution. However, isotopic fractionation has scarcely been reported in studies assessing NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> chemical reduction. Characterizing the stable isotope evolution of N compounds involved in NDFO might help in distinguishing biotic and abiotic reactions.

## OBJECTIVES

In this context, this work aims to evaluate the potential of three different Fe(II) minerals to promote NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> biological and chemical reduction. The second goal is to compare the mineral reactivity in the presence or absence of dissolved Fe(II). The last purpose is to characterize the δ<sup>15</sup>N evolution of the residual dissolved NO<sub>2</sub><sup>-</sup> and also of the produced N<sub>2</sub>O, during induced NO<sub>2</sub><sup>-</sup> reduction by Fe(II) oxidation. Selected minerals for research were: magnetite (Mag), olivine (Ol) and siderite (Sd).

## METHODS

The study was executed at laboratory scale by means of three series of batch experiments, all of them conducted in 20 mL glass bottles, sealed with butyl rubber stoppers under an Ar headspace. Each flask contained 10mL of synthetic water (1.1 mM N-NO<sub>2</sub> or N-NO<sub>3</sub>) and the pertinent Fe(II) supply given the following different possibilities: mineral alone (5g/L Mag, Ol or Sd), dissolved Fe(II) alone (5mM FeCl<sub>2</sub>·4H<sub>2</sub>O) or a combination of both sources. Incubation was performed at 20°C in darkness and constant vibratory shaking. Original Mag, Ol and Sd minerals were previously milled using a Tungsten carbide grinding set in a vibratory disc mill and sieved to obtain particles below 30 µm diameter.

Approximate NO<sub>2</sub><sup>-</sup> or NO<sub>3</sub><sup>-</sup> concentration in the flasks was periodically checked using semi-quantitative test strips. When significant differences were observed, a subset of flasks was sacrificed by turns, until a complete NO<sub>2</sub><sup>-</sup> removal was achieved, to characterize chemical and isotopic evolution during NO<sub>2</sub><sup>-</sup> reduction. Samples from sacrificed flasks were analyzed for NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup> and cations concentration and δ<sup>15</sup>N-NO<sub>2</sub><sup>-</sup> and δ<sup>15</sup>N-N<sub>2</sub>O determination.

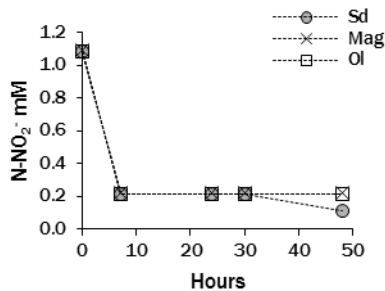
## RESULTS AND DISCUSSION

First series of assays aimed to determine NO<sub>2</sub><sup>-</sup> attenuation potential by Fe(II). Results showed that a rapid NO<sub>2</sub><sup>-</sup> reduction occurred in flasks containing mineral plus dissolved Fe(II), whereas no attenuation was detected in the experiments without dissolved Fe(II) (flasks were incubated for two months), probably due to an extremely low Fe(II) release from the studied minerals. In flasks containing only dissolved Fe(II), in the absence of mineral, a significant NO<sub>2</sub><sup>-</sup> reduction was also observed but at a lower rate, confirming previous

**palabras clave:** Desnitrificación, Oxidación de Fe(II), Minerales, Isótopos estables.

**key words:** Denitrification, Fe(II) Oxidation, Minerals, Stable Isotopes.

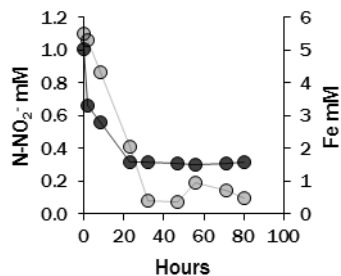
reported results, stating that Fe(II) reactivity increases when it is associated to mineral surfaces. The  $\text{NO}_2^-$  reduction observed using the three different minerals was attributed to an abiotic reaction and therefore, autotrophic denitrification occurrence was discarded.



**fig 1.** Qualitative evolution of  $\text{N-NO}_2^-$  concentration in batch assays using different Fe(II) minerals plus dissolved Fe(II).

Qualitative  $\text{NO}_2^-$  concentration decrease in the batch experiments testing different minerals plus dissolved Fe(II) showed no relevant differences when comparing Mag, Ol and Sd (Fig.1). Sd was chosen as the first of the three minerals to perform a more detailed study of the attenuation process.

Chemical and isotopic characterization of  $\text{NO}_2^-$  reduction by Fe(II) oxidation in the presence of Sd was provided by the second series of experiments.  $\text{NO}_2^-$  chemical reduction by Fe(II) oxidation was almost complete in 30 hours (Fig. 2) and no significant  $\text{NH}_4^+$  production was detected, suggesting that  $\text{N}_2\text{O}$  and  $\text{N}_2$  might be the end products of the transformation process.



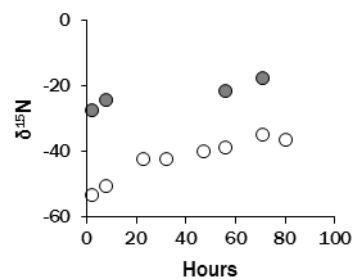
**fig 2.** Evolution of  $\text{N-NO}_2^-$  (light circles) and dissolved Fe (dark circles) concentration over time in  $\text{NO}_2^-$  attenuation experiments using Sd and dissolved Fe(II).

As expected, total dissolved Fe, determined by ICP-OES analysis, showed a decrease coupled to  $\text{NO}_2^-$  attenuation

(Fig. 2). As Fe(II) is oxidized, Fe(III) precipitates and it becomes unavailable for detection given that samples are filtered before being analysed.

In addition, no trace metals released from original Sd were detected in the ICP-OES results.

Isotopic analysis results showed a  $\delta^{15}\text{N}$  enrichment of residual  $\text{NO}_2^-$  and of newly produced  $\text{N}_2\text{O}$  throughout the chemical reaction (Fig. 3).



**fig 3.**  $\delta^{15}\text{N}$  Evolution of remaining  $\text{NO}_2^-$  (dark circles) and produced  $\text{N}_2\text{O}$  (empty circles) over time in  $\text{NO}_2^-$  attenuation experiments using Sd and dissolved Fe(II).

Last series of batch trials, using synthetic water containing  $\text{NO}_3^-$  instead of  $\text{NO}_2^-$  permitted to discard a rapid  $\text{NO}_3^-$  chemical reaction by Fe(II). No  $\text{NO}_3^-$  decrease was observed after 24h incubations in any of the following conditions: Sd alone, dissolved Fe(II) alone or Sd plus dissolved Fe(II). Moreover,  $\text{NO}_3^-$  autotrophic denitrification was discarded after two-month incubation, suggesting that indigenous denitrifiers were not present in the minerals or in the synthetic water.

## CONCLUSIONS

Overall results reinforce the relevance of Fe(II) mediated  $\text{NO}_2^-$  chemical reduction. The presence of Fe(II) minerals such as Mag, Ol or Sd increase dissolved Fe(II) reactivity probably due to adsorption on its surface. Minerals themselves do not promote a fast  $\text{NO}_2^-$  chemical reduction although delayed activity can not be discarded.  $\delta^{15}\text{N}$  evolution of residual  $\text{NO}_2^-$  and newly produced  $\text{N}_2\text{O}$  point to the occurrence of isotopic fractionation events during the  $\text{NO}_2^-$  abiotic reduction. In the case of  $\text{NO}_3^-$ , no reactivity has been detected to occur associated to Fe(II) minerals in two months. Lacking biological reactions might be due to the necessity to provide a source of

autotrophic bacteria to promote NDFO.

Further studies are needed to accomplish all scheduled objectives and thus to go into detail on N and Fe biogeochemical cycles interactions. Pending batch experiments will assess chemical and isotopic evolution throughout  $\text{NO}_2^-$  chemical reduction by Mag and Ol in the presence of dissolved Fe(II).  $\text{NO}_3^-$  chemical reactivity with these minerals will be also checked. Furthermore, batch experiments using groundwater and sediments from  $\text{NO}_3^-$  contaminated areas containing indigenous bacteria will assess Mag, Ol and Sd potential to promote autotrophic denitrification.

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