

Interactive comment on “Impact of reactive surfaces on the abiotic reaction between nitrite and ferrous iron and associated nitrogen and oxygen isotope dynamics” by Anna-Neva Visser et al.

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First, we wish to thank the reviewer for his/her valuable inputs and comments on our manuscript.

L39-40: I'm surprised there are no older references to the role of iron.

Reply: We agree that indeed there are many more references regarding the role of iron in the environment. However, our choice can be considered as “best of” selection, covering a whole suite of different aspects: We choose (1) Expert et al., 2012 since

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they explicitly focus on the vital role of iron for all living organisms, its wide range of redox potentials and its catalytic role in various metabolic pathways; (2) Lovley et al., 1997, who reported on the importance of iron already in 1988, however, the publication chosen represents a nice “summary”, focusing also on various reactions and thus its “remediative” capabilities. Obviously, we wanted to limit the number of references, but if the reviewer has a specific publication in mind, we will be happy to include it. Again, in light of the many publications on the importance of iron available, and since our manuscript is already very long, we simply decided to pick two references that support the statement/sentence.

General experiment setup section: The conditions of the experiment are anoxia and the addition of iron and nitrogen in the form of nitrite. Under these conditions, in the environment, it is conceivable that dissimilative reduction of nitrite to ammonium may occur. Of course under perfect abiotic conditions DNRA should not occur. Did the authors measure ammonium concentrations throughout the experiment to ensure that no other processes than the one under study were taking place?

Reply: As the reviewer stated, DNRA should not occur under abiotic conditions. Considering that the abiotic experiments were all performed under laboratory conditions, using a medium that contains already high amounts of ammonium (5.61 mM NH₄Cl, see 2.1), ammonium concentrations were only checked sporadically for some setups. Since only (if at all) minor fluctuations were observed, no further efforts to determine ammonium concentrations were attempted.

L120-121: How long does it take from incubation to the measurement of concentrations and isotopes? Light is a factor that can generate abiotic reactions, which in turn can generate isotope fractionation. What about it?

Reply: Yes, light-induced reactions have to be considered. That was one reason why nitrite concentrations were measured via CFA immediately after the samples were taken (within one hour). After determining the nitrite concentrations, the azide method

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was applied (within max. 2-3 hrs). Samples were kept inside the glovebox in coloured (dark brown or blue) Eppendorf tubes, whereas the latter were chosen to inhibit potential photocatalytic reactions. The azide-treated headspace vials were stored in cardboard boxes at RT until measured. At this point, the sample is fixed (i.e., turned into N₂O). Therefore, we are rather confident that neither light nor (possibly) temperature could have influenced the values. However, one could argue that the blue coloured Eppendorf tubes might not suffice, since they are indeed partly translucent. Since during one of the experiments blue and brown vials were used, and still, the concentration values within the nine replicates were very similar (see Figure 1 A and C, note error bars), we are confident that the rapid processing and precautions taken to avoid light-induced reactions did indeed suffice.

L179-180: Two nitrite isotope standards have been used. What are the values of these standards? Do these values include those of the samples measured in this study? What is the analytical precision of the method (preparation + intrinsic analysis) for the determination of the isotopic composition of nitrite (¹⁵N and ¹⁸O)?

Reply: Standard N-7373 has a $\delta^{15}\text{N}$ value of -79.6‰ and a $\delta^{18}\text{O}$ value of +4.5‰. In contrast, standard N-10219 has a $\delta^{15}\text{N}$ value of +2.8‰ and a $\delta^{18}\text{O}$ value of +88.5‰. Using both standards allowed for a reliable correction using standard bracketing: The standard $\delta^{15}\text{N}$ range included the $\delta^{15}\text{N}$ values obtained for our samples perfectly. The $\delta^{18}\text{O}$ values measured fell only slightly below (-0.5 to 2.5‰ the range given by the standards, so that corrections are reliable. Based on replicate measurements of laboratory standards and samples, the analytical precision for NO₂- $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ analyses was $\pm 0.4\%$ and $\pm 0.6\%$ (1 SD), respectively.

L285-291: Rayleigh conditions allow the isotope fractionation factor to be easily determined by looking at the slope of the line on a representation $\ln C/C_0$ as a function of $d^{15}\text{N}$, but not C (with C the concentration at time t and C₀ the initial concentration). This paragraph is not clear to me. Moreover, doesn't the fact that there is first a decrease of ¹⁵N, i.e. an inverse isotopic fractionation, with a decrease of the amount

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of heavy isotope in the residual substrate, and then an enrichment, mean that several processes could take place and that process 1 takes place at the beginning of the experiment with a higher rate than the second process which either starts at the beginning of the experiment or when process 1 is completed? Very concretely, the trend line is calculated on the points starting from the lowest $d^{15}\text{N}$ values? I think it would be necessary to clarify this part.

Reply: We agree, the title of the x-axis of Figure 5 might be misleading. Of course, the values of the x-axis represent the \ln of the substrate fraction remaining (as mentioned in the caption below the figure). Hence, it is the $\ln(f)$ whereas f is C/C_0 . We will change the title of the x-axis to avoid future confusions. With regards to the second comment, i.e., that the data presented might simply reflect that two different processes are at work, we also agree. However, since it is hard to explain which processes might be at work and if this is indeed a clear inverse effect, we decided to calculate the isotope effect using the lowest $\delta^{15}\text{N}$ values observed (i.e. for the experimental period where we show a clear decline in nitrite concentration with a net increase in $\delta^{15}\text{N}$). We will clarify that there is putative evidence for multiple processes occurring in the incubations, and that this has implications for the Rayleigh approach.

L296-302: Is it not possible to envisage that the variations in ¹⁸O are due solely to an exchange between the oxygen of the nitrite and the oxygen of the water? By the way, what is the isotopic composition of water? Is it constant during the experiment?

Reply: Unfortunately, the isotopic composition of the water was not measured, and we can only assume its $\delta^{18}\text{O}$ (the water used in Tübingen has a $d^{18}\text{O}$ of roughly 11‰). It is possible that the variations in $\delta^{18}\text{O}$ are partially attributable to oxygen atom exchange dynamics with the matrix (see e.g. L504-516). However, considering that the observed drop in $\delta^{18}\text{O}$ values in both experiments occurs more or less simultaneously with the drop in $\delta^{15}\text{N}$ might be indicative of other dynamics (e.g. sorption, complexation?).

L309-313: The authors have done a significant analytical work. Why not show the

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variations in N₂O concentration as a function of nitrite concentrations? Before any interpretation with isotopes or isotopologists, it seems to me useful and necessary to work on the concentrations and in particular to make mass balances.

Reply: The proposed graph could be added to the supplementary material. However, particularly for the mineral only setups, this way of visualizing the data does not help much (see Figure 1 ¹⁵N₂O vs NO₂⁻ concentrations in (A) mineral plus dead biomass and (B) mineral only experiments). Also, for the main manuscript we had severe concerns with regards to its length. Therefore, we chose to present only graphs that really help to understand the main messages of this project. With regards to the mass balance: The initial objectives of this project included mass balance considerations since it was supposed to lay the ground for a following study on nitrate-dependent Fe(II) oxidation in selected microbial strains. Unfortunately, we did not have the capacities to also analyse the N₂ samples, so a proper mass balance is unfortunately not possible.

L314-315: The authors do not discuss the very negative SP value, which is very distinct from the other points. Is this an analytical problem?

Reply: We assume that the reviewer is referring to the observed drops in SP values (-120 to -80‰, occurring at t1 for samples taken from the mineral + dead biomass setup at pH 6.2 and mineral only at pH 5.8. After another thorough check of the raw data, we have to admit that for those particular samples the peak areas of the data obtained via CF-IRMS were much higher (compared to standards), possibly causing an extreme linearity or contamination effect that is affecting the data. We re-checked the entire data set again and removed these outliers (see revised Figure 2 "Site Preference (SP; A, C) and $\delta^{15}\text{N}_{\text{bulk}}$ (B, D) values of N₂O produced in experiments amended with mineral + dead biomass (red) and mineral-only (grey)"). The bulk of the data is not compromised, as we have good agreement between the standard and the sample peak areas.

L326: There is no figure S6. But mentioned in S5 section figure 3.

Reply: We thank the reviewer for pointing this out and apologize for the mistake. Figure

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S5 mentioned in L322 actually corresponds to Figure S4 in the supplements, while S6 in L 326 refers to S5! We will change this in the re-submitted version of the MS.

L484-486: Large variations of $\delta^{15}\text{N}$ are not associated with variations of $\delta^{18}\text{O}$. While these are measurements made on the residual substrate. The drop in $\delta^{18}\text{O}$ at the beginning of the experiment is more likely due to an isotopic exchange with the oxygen in the water than evidence of a process. Reply: Whether the drop is solely caused by the O isotopic exchange or, maybe partially, by interactions with the mineral surface, is not really clear. The drop observed in $\delta^{18}\text{O}$ occurs almost simultaneously with the e.g. the decrease in $\delta^{15}\text{N}$ for the mineral + dead biomass experiment. This might be indicative of other processes playing indeed a certain role. However, as we tried to explain in L496ff in the original MS, we assume that the main effect is the oxygen exchange with the water of the medium, which simply takes time and thus results in "fluctuations" (especially for the mineral only experiments) until the entire system is equilibrated.

L531-538: It might be interesting to look at $\delta^{18}\text{O}$ variations of N₂O during the experiment. And see if it correlates with that of nitrite. This would also be an opportunity to confirm or deny whether there is an isotope exchange between the oxygen in the nitrite and the oxygen in the water. Reply: Indeed, using the $\delta^{18}\text{O}$ variations of N₂O might help to better understand the isotope exchange processes within the system. However, since N₂O is definitely not the only product and possibly further reduced (resulting in a branching effect caused by the removed O atoms, which is further affecting the O dynamics within the system), this approach would be biased.

L551-552: if N₂O is considered to accumulate, it can be considered to be the accumulated product in the case of a Rayleigh distillation. In this case, and taking into account the isotope fractionation associated with nitrite reduction, it is easy to calculate what the expected $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of the N₂O produced. It would then be interesting to compare the measured values with the expected values.

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Reply: We agree that it is indeed possible to estimate the predicted value of $\delta^{15}\text{N}$ by using the accumulated product equation. An epsilon value calculated from the $\delta^{15}\text{N}$ - NO_2^- data could be used to estimate the predicted $\delta^{15}\text{N}$ - N_2O values, which would be different since N_2O is clearly not the single product. However, for $\delta^{18}\text{O}$ this approach would not work due to the branching effect occurring during reduction. Hereby, the O atoms get plucked off and lost along the reaction, which is also affecting the dynamics. At the editor's discretion, and if the manuscript is not already considered too long, we would be happy to add the "predicted" $\delta^{15}\text{N}$ - N_2O values with a short explanation.

Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2020-73>, 2020.

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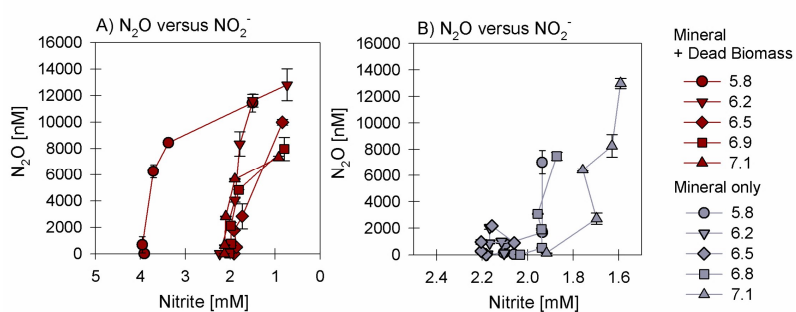


Fig. 1. N_2O vs NO_2^- concentrations in (A) mineral plus dead biomass and (B) mineral only experiment

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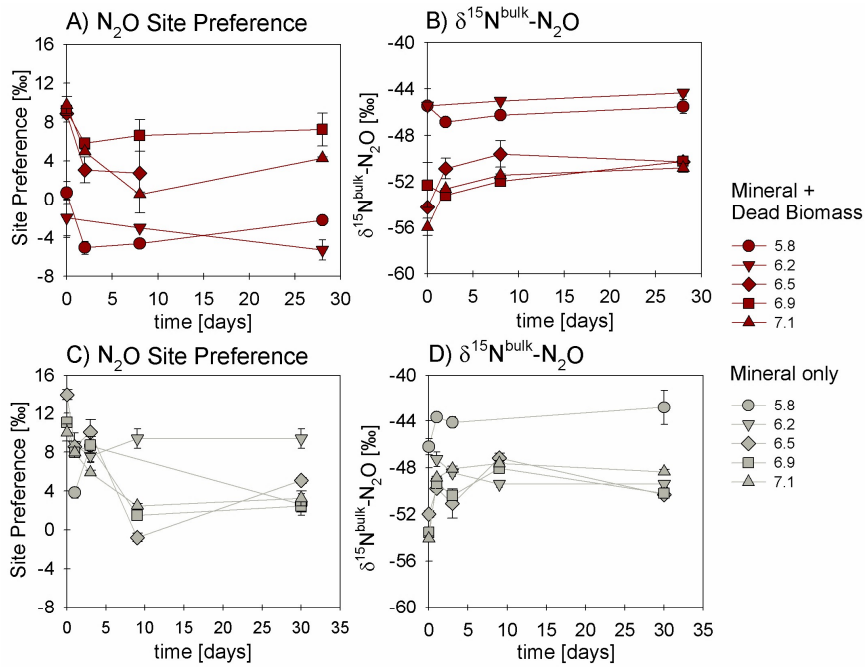


Fig. 2. Site Preference (SP; A, C) and δ¹⁵N^{bulk} (B, D) values of N₂O produced in experiments amended with mineral + dead biomass (red) and mineral-only (grey)