Denitrification, anaerobic ammonium oxidation, and dissimilatory nitrate reduction to ammonium in an East African Great Lake (Lake Kivu)

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1. Abstract

We investigated anaerobic nitrogen (N) cycling in the water column of Lake Kivu, a deep meromictic tropical lake in East Africa. Data were collected at one station in the Northern Basin and one in the Southern Basin, during two sampling campaigns (June 2011—dry season, and February 2012—rainy season). Short-term incubations of sulfide-free water with ^{15}N-labeled substrates revealed high potential denitrification and dissimilatory nitrate reduction to ammonium (DNRA) rates (up to 350 and 36 nmol N produced L^{-1} h^{-1}, respectively), while anaerobic ammonium oxidation (anammox) was lower (up to 3.3 nmol N produced L^{-1} h^{-1}). However, anammox rates were 15 nmol N produced L^{-1} h^{-1} when $^{15}NH_4^+$ was added at depths where NH_4^+ concentrations were very low (<1 µmol L^{-1}). With the addition of 5 µmol L^{-1} of $^{15}NO_3^-$ and 10 µmol L^{-1} of H_2S, denitrification and anammox were stimulated in the Northern Basin, while the increase of DNRA rates was less notable. In the Southern Basin, the addition of H_2S decreased denitrification rates, probably because of competition with DNRA, which increased, while no effect was observed on anammox. This study puts into evidence the co-occurrence of denitrification, anammox and DNRA, for the first time in a great tropical lake, and underlines the spatial heterogeneity of these processes. Contrary to numerous reports in literature, we show that anammox can significantly occur in presence of H_2S, suggesting that the contribution of anammox in the N cycle may be underestimated.

2. Introduction

Nitrogen (N) cycle is disrupted by anthropogenic activities, and the detailed knowledge of the processes responsible for N-cycling is thus required.

Three anaerobic metabolisms are responsible for nitrate (NO_3^-) or nitrite (NO_2^-) reduction, with different impacts on N cycle[1]:

- Canonical **denitrification** (N loss, N_2O production), which can occur with different electron donor, such as organic matter or HS^-:

$$NO_3^- + OM \rightarrow N_2 + CO_2$$
$$NO_3^- + HS^- \rightarrow N_2 + SO_4^{2-}$$

- Anaerobic ammonium oxidation (**anammox**) (N loss):

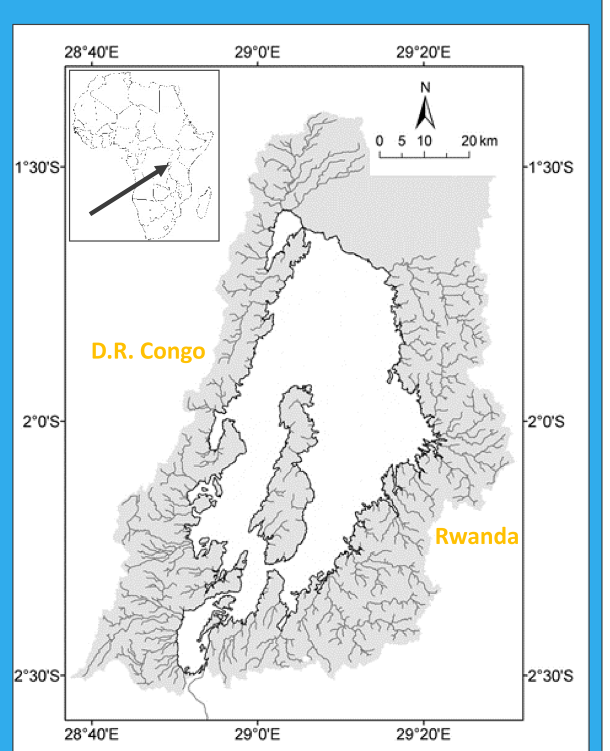
$$NH_4^+ + NO_2^- \rightarrow N_2$$

- Dissimilatory reduction of nitrate to ammonium (**DNRA**) (N retention):

$$NO_3^- + OM \rightarrow NH_4^+ + CO_2$$

Knowledge on the regulation of these competing processes is crucial for predicting, modeling, and managing the N-cycle, but they remained understudied in tropical freshwaters. We thus investigated one of the East-African Great Lakes, **Lake Kivu** (Rwanda-Democratic Republic of the Congo), a deep meromictic lake characterized by:

- Anoxic deep waters rich in NH_4^+, carbon dioxide, and methane [2],
- A nitrogenous zone at the base of the mixed-layer, forming in rainy season [3,4], where N_2O accumulates [5],
- A zone of sulfate (SO_4^{2-}) reduction below the oxic-anoxic interface, leading to HS^- accumulation to concentrations up to 200 µmol L^{-1} in the anoxic waters [4,6].



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3. Hypotheses and objectives

Three hypotheses:

1. Upward fluxes of HS^- from deep waters to nitrogenous zone could support denitrification coupled to HS^- oxidation,
2. Upward fluxes of NH_4^+ from deep waters to nitrogenous zone could support anammox
3. Competition could occur between the three processes

Three objectives:

1. To put in evidence the co-occurrence of heterotrophic denitrification, denitrification coupled to HS^- oxidation, anammox and DNRA
2. To test the effect of HS^- on anammox
3. To identify the competitive relationships

4. Material and methods

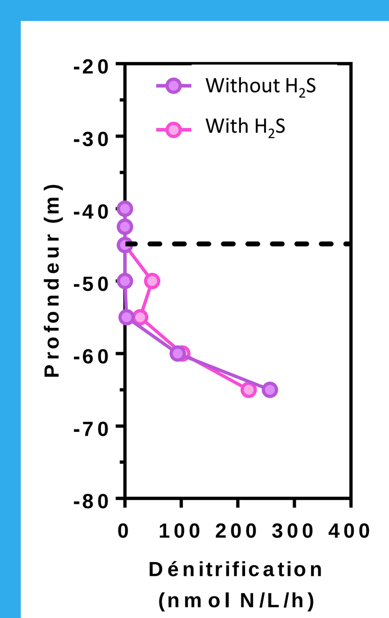
Two different **N stable labeling experiments** were performed:

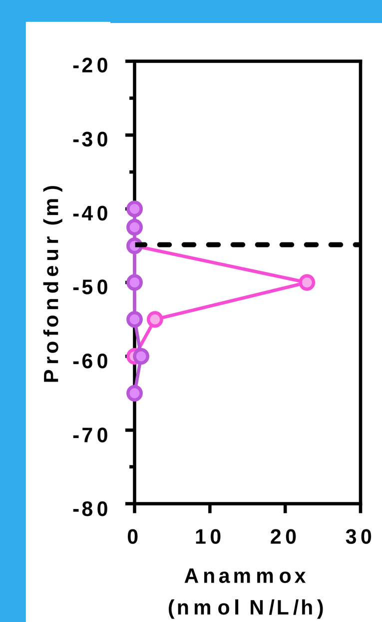
1) Determination of denitrification, anammox and DNRA by amending water with $^{15}NO_3^-$,
2) Determination of denitrification, anammox and DNRA by amending water with $^{15}NO_3^- + H_2S$.

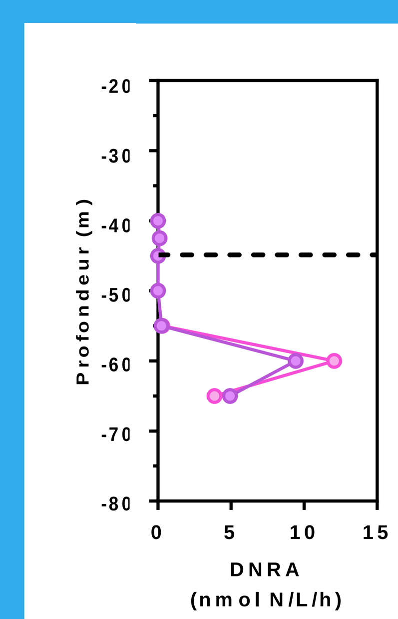
For both experiments, a time course was established by arresting samples at 6h, 12h, 18h, 24h and 48h.

5. Results and conclusions

5.1 Co-occurrence of denitrification, anammox and DNRA, and stimulating effect of HS^-

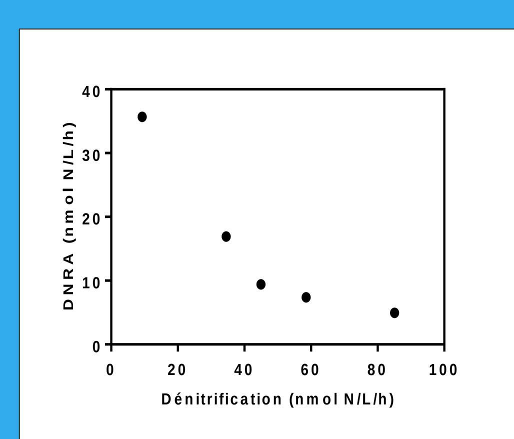




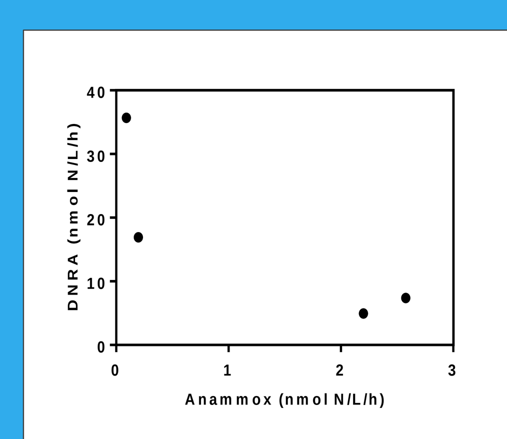


Dashed line = oxic-anoxic interface

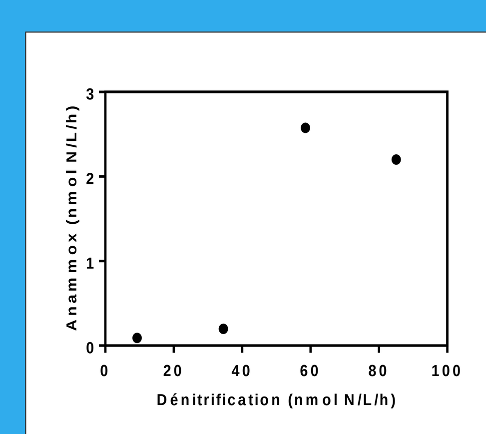
5.2 Competitive relationships



Competition for:
Organic matter
NO_3^- or NO_2^-



Competition for:
NO_3^- or NO_2^-



« Symbiosis » for:
NO_2^-
Detoxification

5.3 Conclusions

Our results show:

1. The **co-occurrence of denitrification, anammox and DNRA** in the water column of a tropical lake;
2. The **link between denitrification and HS^- oxidation**, probably when organic matter is limiting;
3. The **occurrence of anammox with HS^-**, and even a stimulation of anammox by the addition of HS^-;
4. That **anammox is not stimulated in the presence of DNRA** in the water column of Lake Kivu, probably because NH_4^+ is not limiting;
5. That **anammox is favored in the presence of denitrification**, probably because denitrification create a detoxified zone for anammox microorganisms.

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