Isotopic and microbiologic evidence of greenhouse gases transformation mechanisms in groundwater

by Olha Nikolenko

Supervisor: Serge Brouyère
Co-supervisor: Alberto V. Borges
Background of the study

Fig. 1. The greenhouse effect of solar radiation on the Earth's surface

Source: http://www.ehso.com/climatechange/climatechangecauses-greenhouseeffect.php

Fig. 2. The historical change in the atmospheric concentrations of GHGs

Source: IPCC AR5 WGIII, 2014

Most significant greenhouse gases (GHGs)

H₂O  CO₂  CH₄  O₃  N₂O

Strong ozone depleting substance

Source: http://www.ehso.com/climatechange/climatechangecauses-greenhouseeffect.php
Background of the study

Fig. 3. Types of GHGs emissions from agricultural areas

Source: Jurado et al., 2017
Main challenges

Fig. 4. Heterogeneous subsurface

Source: Hartmann et al., 2017
Approaches applied

- Stable isotope and isotopomer analysis.

Isotopomers are molecules having the same number of each isotopic atom but differing in their positions.

![Diagram of N₂O isotopomer representation](image)

Site preference = \( \delta^{15}N^\alpha - \delta^{15}N^\beta \)

Fig. 5. \( \text{N}_2\text{O} \) isotopomer representation
Microbiological studies

- **Nitrification**
  - ammonia monooxygenase and hydroxylamine dehydrogenase
  - $NH_3$ to $NO_2^-$ (amoA and haoAB)
  - $NO_2^-$ to $NO_3^-$ (Nitrite oxidoreductase, Nxr)
  - Ammonia oxidizers

- **Denitrification**
  - $NO_3^-$ to $NO_2^-$ (nitrate reductase, narG, napA)
  - $NO_2^-$ to NO (nitrite reductase, nirK, nirS)
  - NO to $N_2O$ (nitric oxide reductase, norC, norB)
  - $N_2O$ to $N_2$ (Nitrous oxide reductase, nosZ)

**Fig. 6.** Biotic nitrification and denitrification pathways
Fig. 7. Schematic representation of a protein formation

Source: Campbell et al., 2019
Objectives of the study

Theoretical part:
1. review available information about the variability of $^{15}$N isotopes in groundwater under agricultural areas.

Practical part:
1. to estimate the variability of GHGs concentrations in groundwater under different hydrogeological, hydrochemical and land management conditions;

2. to identify the $\text{N}_2\text{O}$ production and consumption processes and reveal conditions that govern $\text{N}_2\text{O}$ accumulation in groundwater;

3. to collect in situ evidence about the SP ranges of $\text{N}_2\text{O}$ and activity of bacteria involved into $\text{N}_2\text{O}$ production and consumption processes.
Fig. 8. N sources and transformation processes that affect N species in the subsurface

Source: Nikolenko et al., 2018
Fig. 9. N Sources, processes and factors that influence $\delta^{15}$N-$N_2O$ values
**Complementary isotope studies: O, B, S, C**

\[ \delta^{18}O - NO_3^- \]

**Nitrification:**

\[ \delta^{18}O_{\text{nitrate}} = \frac{1}{3} \delta^{18}O_{O_2} + \frac{2}{3} \delta^{18}O_{H_2O} \]

**Denitrification:**

\[ \delta^{18}O - NO_3^- \uparrow \quad \text{and} \quad \delta^{15}N - NO_3^- \uparrow \]

1:2 (Kendall & Aravena, 2000), 1:1 (Koba et al., 2009) etc.

\[ \delta^{11}B \]

\[ \delta^{11}B \text{ of sewage: } -7.7\% \text{ to } +12.9\% \]

\[ \delta^{11}B \text{ of manure: } +14.5\% \text{ to } +42.5\% \]

\[ \delta^{13}C - DIC \]

- heterotrophic denitrification

\[ \delta^{34}S - SO_4^{2-} \]

- autotrophic denitrification
Practical part

> regional scale investigations:

The aim:

1) examination of the distribution and accumulation of GHGs in different parts of the studied aquifer across its lateral and vertical dimensions;
2) collecting information about hydrochemical conditions of the subsurface.

> local scale explorations:

The aim:

1) identification and quantification of the rates of $\text{N}_2\text{O}$ production and consumption processes within the studied aquifer using in situ and laboratory designed hydrogeological, isotope and microbiological experiments.
Regional studies: objectives

1) explore the variability of GHGs concentration along groundwater flow;

2) reveal the sources of N and C loads across the aquifer;

3) identify the processes that govern biogeochemistry of GHGs under different environmental settings.
Regional studies: description of the area

Peculiarities of the studied area:
- area: 480 km²;
- 65% of agricultural activities;
- high fracturing of chalk aquifer;
- unconfined – the South;
  - semi-confined – near the Geer river;
- confined – the North-West.

Source: Nikolenko et al., 2019

Fig. 10. Map of the studied area in the Geer basin
Regional studies: analyzed parameters

- hydrogeochemical controls (DO, DOC, SO$_4^{2-}$, HCO$_3^-$, pH etc.);
- concentrations of N-species (NO$_3^-$, NH$_4^+$, NO$_2^-$ and N$_2$O);
- isotope signatures of N$_2$O, NO$_3^-$, SO$_4^{2-}$, $^{11}$B, $^3$H.

Fig. 11. Distribution of NO$_3^-$, DO, Cl$^-$, SO$_4^{2-}$ along groundwater flow
Regional studies: distribution of GHGs

Fig. 12. Distribution of \( \text{N}_2\text{O}, \text{CH}_4 \) and \( \text{CO}_2 \) along groundwater flow
Regional studies: N sources

Fig. 13. Sources of N loading across the aquifer

Source: Nikolenko et al., 2019
Regional studies: N sources

Fig. 14. Land use map of the studied area
Regional studies: CO$_2$ and CH$_4$ biochemistry

- tendency towards accumulation of CO$_2$
  - the subsurface dynamics of CO$_2$ is governed by two processes:
    1) dissolution of carbonate minerals;
    2) degradation of DOC derived from the mineralization processes in the soil.

- CH$_4$ accumulation
  - northern zone is characterized with the higher tendency towards CH$_4$ accumulation;
  - presence under aerobic conditions in southern, central and north-eastern zones suggests its thermogenic origin.
Regional studies: \( \text{N}_2\text{O} \) biochemistry

<table>
<thead>
<tr>
<th>Group</th>
<th>( \text{N}_2\text{O} (\mu g \text{ N/L}) )</th>
<th>( SP (%) )</th>
<th>( DO (\text{mg/L}) )</th>
<th>( \text{NO}_3^- (\text{mg/L}) )</th>
<th>Processes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>3.4 ± 1.2</td>
<td>11.2 ± 1.6</td>
<td>8.2 ± 1.9</td>
<td>28.7 ± 3.8</td>
<td>nitrification and incomplete denitrification</td>
</tr>
<tr>
<td>Group 2</td>
<td>13.6 ± 6.3</td>
<td>26.1 ± 3.4</td>
<td>5.7 ± 2.4</td>
<td>48.7 ± 18.7</td>
<td>nitrification and complete denitrification</td>
</tr>
<tr>
<td>Group 3</td>
<td>6.7 ± 3.4</td>
<td>19.1 ± 6.7</td>
<td>7.2 ± 2.6</td>
<td>39.6 ± 16.2</td>
<td>nitrification and incomplete denitrification</td>
</tr>
<tr>
<td>Group 4</td>
<td>0.1 ± 0.1</td>
<td>not available</td>
<td>1.5 ± 2.1</td>
<td>0.2 ± 0.4</td>
<td>complete denitrification</td>
</tr>
</tbody>
</table>

Source: Nikolenko et al., 2019

Fig. 15. Clustering of the groundwater samples using SOM algorithm
Local studies: description of the area

Fig. 16. Piezometers and sampling depths at the Bovenistier and SGB sites
Local studies: vertical profile SGB

Fig. 17. Vertical distribution of N compounds, their isotopes and DO
Fig. 18. Vertical distribution of N compounds, their isotopes and DO
Local studies: isotope labeled experiment

Fig. 19. Scheme of laboratory $^{15}$N stable isotope labeled experiment
Local studies: summary

- $\text{N}_2\text{O}$ produced by nitrification and denitrification processes occurring within the other parts of the aquifer

OR

- discrepancy between real aquifer conditions and laboratory experiments.
Microbiological studies

Biomass from groundwater samples

RNA
- amoA
- nirK, nirS
- norC, norB
- nosZ

DNA
- bacterial population
Fig. 20. Results of PCR analysis aimed to reveal nirS gene expression in groundwater samples.
## Microbiological studies

<table>
<thead>
<tr>
<th>Location</th>
<th>amoA</th>
<th>nirK_3</th>
<th>nirK_5</th>
<th>nirS_3</th>
<th>nirS_5</th>
<th>norB_4</th>
<th>norC_2</th>
<th>norC_3</th>
<th>nosZ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PCR</td>
<td>SSA</td>
<td>PCR</td>
<td>SSA</td>
<td>PCR</td>
<td>SSA</td>
<td>PCR</td>
<td>SSA</td>
<td>PCR</td>
</tr>
<tr>
<td>1 (Pz13)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 (Pz12 bot)</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (Pz12 top)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 (PzCs bot)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 (PzCs top)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 (SGB1 bot)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 (SGB1 top)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 (SGB3 bot)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 (SGB3 top)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Legends:**

- **nitrifiers genes**
- **denitrifiers genes**
- **N₂O production**
- **N₂O consumption**
Conclusions

1. The concentration of $\text{N}_2\text{O}$ is the most variable which is attributed to the fact that its production/consumption pathways within the studied aquifer are controlled to a large extent by microbiological metabolism:
   - consequently, the total flux of $\text{N}_2\text{O}$ originating from the given aquifer is associated with high level of uncertainty, particularly in comparison to the other GHGs.

2. The concentration of $\text{CO}_2$ does not change significantly in groundwater which might be explained by:
   - equal distribution of organic matter across the studied area;
   - aquifer geology controls the amount of $\text{CO}_2$ dissolved in groundwater.

3. $\text{CH}_4$ is accumulated despite oxic subsurface conditions which might be related to the presence of natural sources of this gas:
   - coal formations below the aquifer.
4. within the framework of this study it was not possible to obtain the complete understanding about dynamics of $\text{N}_2\text{O}$ within the aquifer:

- nevertheless, there is evidence that show that isotopic signatures of $\text{N}_2\text{O}$ in the aquifer are affected by ongoing denitrification.

5. application of isotope/isotopomer mapping approach together with hydrochemical evidence can give the general idea about the occurrence of $\text{N}_2\text{O}$ production and consumption mechanisms but it cannot differentiate which exactly microbiological processes occur in the aquifer:

- the observed $\text{N}_2\text{O}$ isotopic signatures are affected by mixing between different subsurface compartments.

6. in order to identify the processes occurring in situ it necessary to complement subsurface findings with the study of enzyme activities.
Further steps in studying $\text{N}_2\text{O}$ dynamics:

1. comparison of GHGs transformation processes in the “soil – unsaturated zone – aquifer” system;

2. studying GHGs production and consumption processes within the riparian zones and river sediments;

3. comparison of $\text{N}_2\text{O}$ fluxes in the areas of similar hydrogeological conditions but different sources of N loads;

4. comparison of GHGs fluxes occurring in contrasting hydrological/meteorological conditions and under different agricultural management practices.
THANK YOU!

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 675120.