Doctoral thesis defense

University of Liège (Belgium) **24.11.2020**

Isotopic and microbiologic evidence of greenhouse gases transformation mechanisms in groundwater

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Inspiration

Background of the study

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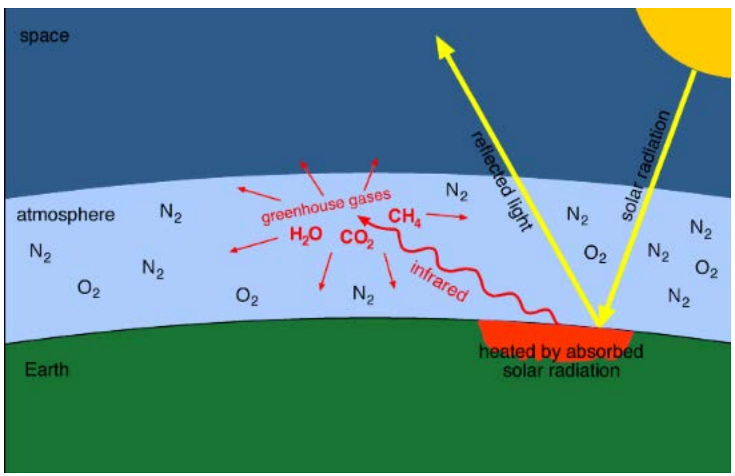
- H_2O
- CO_2
- CH_4
- O_3
- N_2O

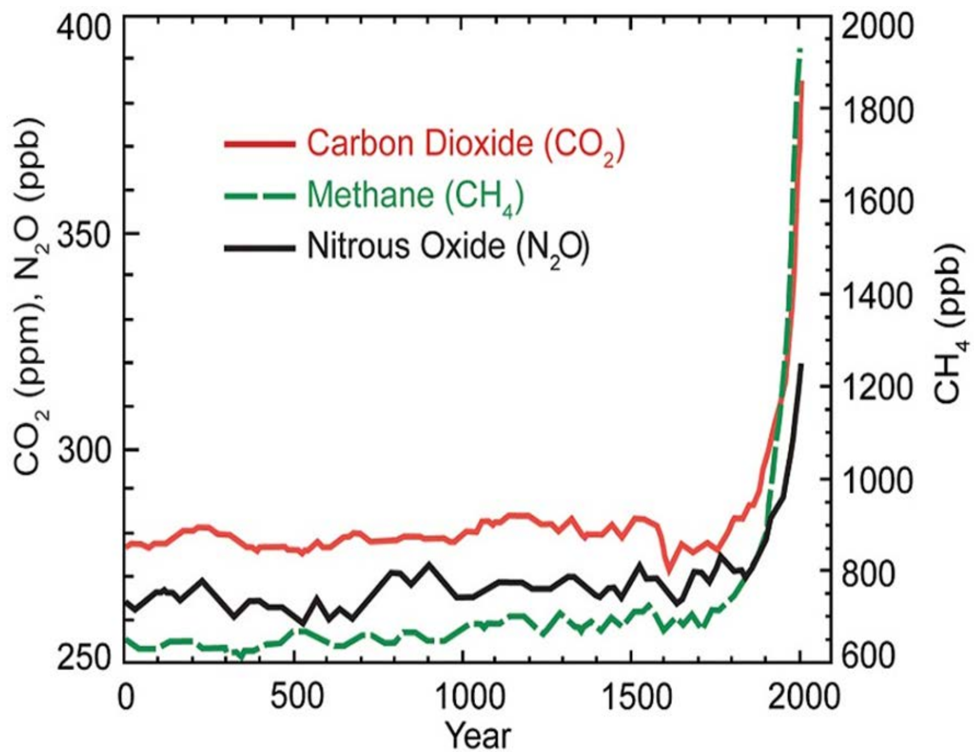
Most significant greenhouse gases (**GHGs**)

Strong ozone depleting substance



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

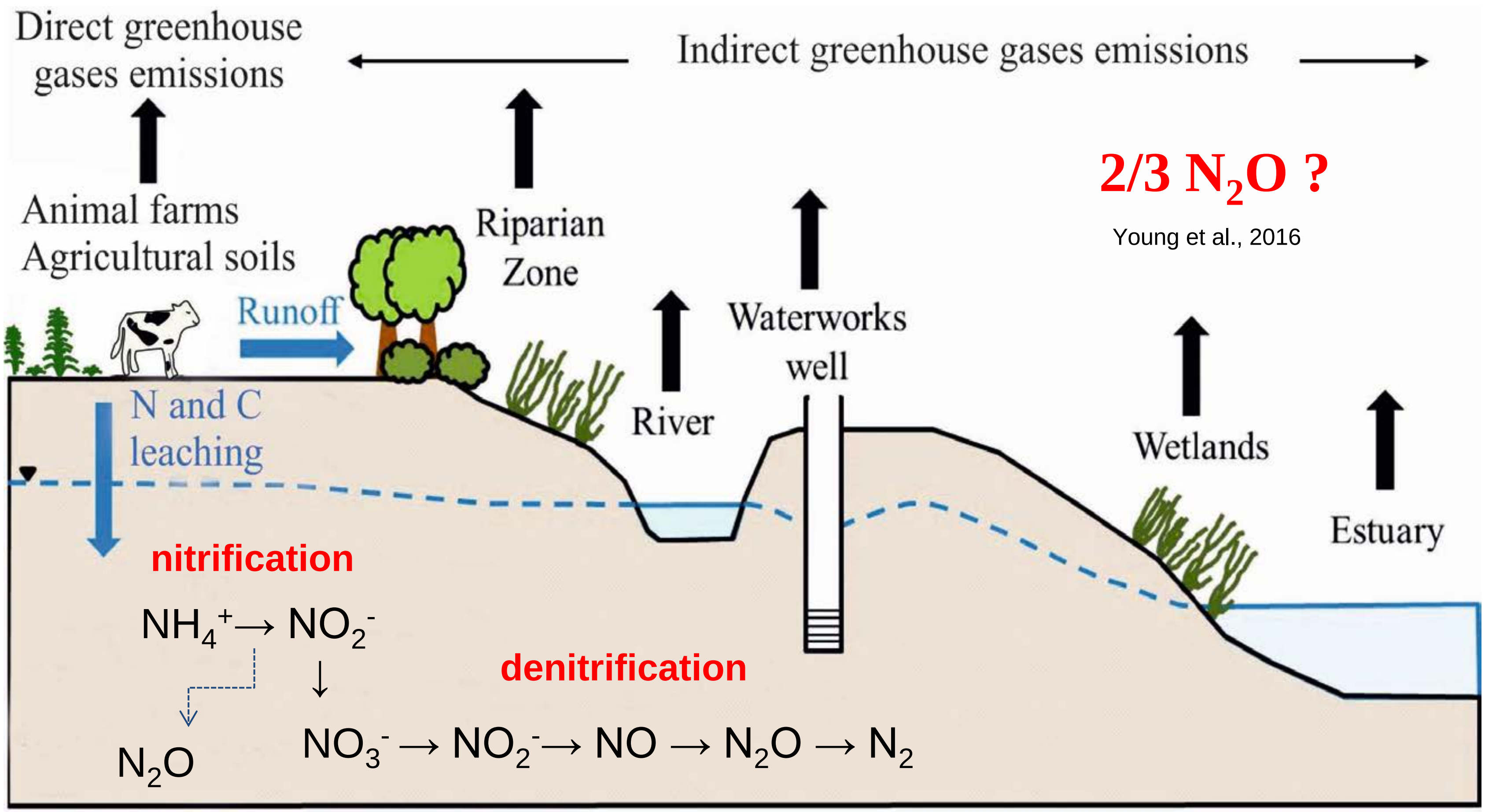
Fig. 1. The greenhouse effect of solar radiation on the Earth's surface



Source: IPCC AR5 WGIII, 2014

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

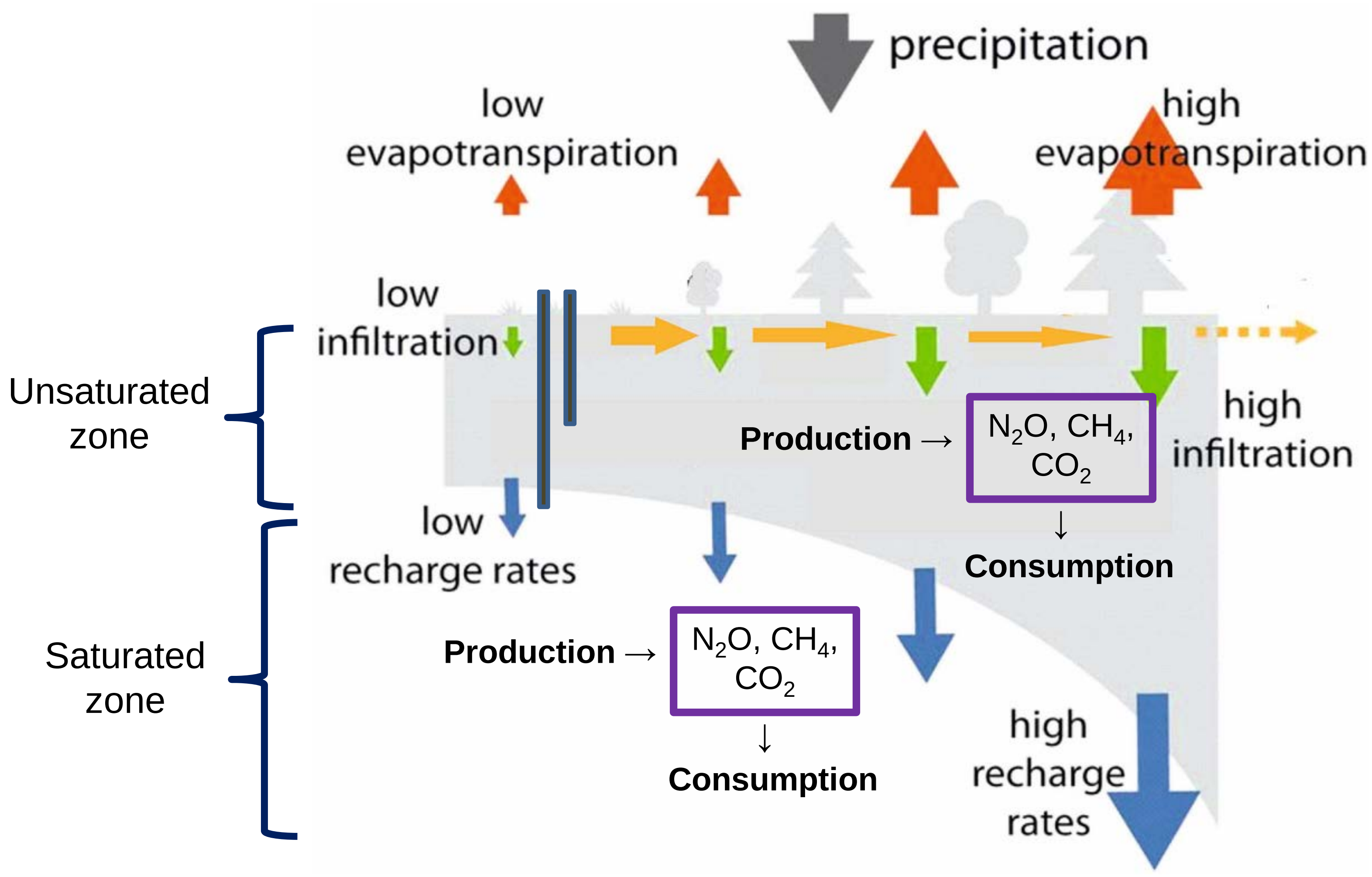
Background of the study



Source: Jurado et al., 2017

Fig. 3. Types of GHGs emissions from agricultural areas

Main challenges



Source: Hartmann et al., 2017

Fig. 4. Heterogeneous subsurface

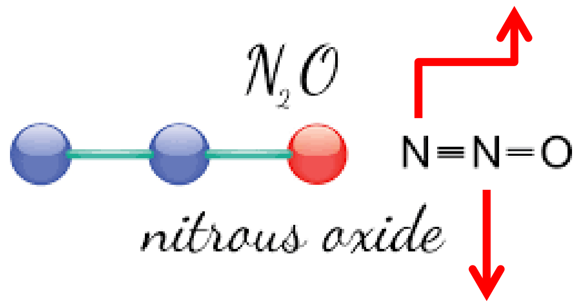
Approaches applied

- Stable isotope and isotopomer analysis.

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

production ← decrease — Site preference = $\delta^{15}N^{\alpha} - \delta^{15}N^{\beta}$ — increase → consumption

peripheral (β) position ($^{15}N^{14}NO$)

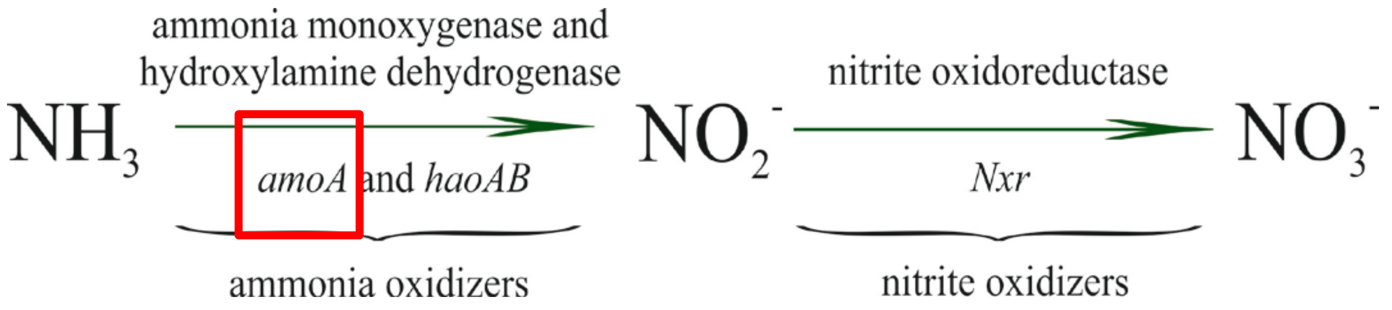


central (α) position ($^{14}N^{15}NO$)

Fig. 5. N_2O isotopomer representation

Microbiological studies

- **Nitrification**



- **Denitrification**

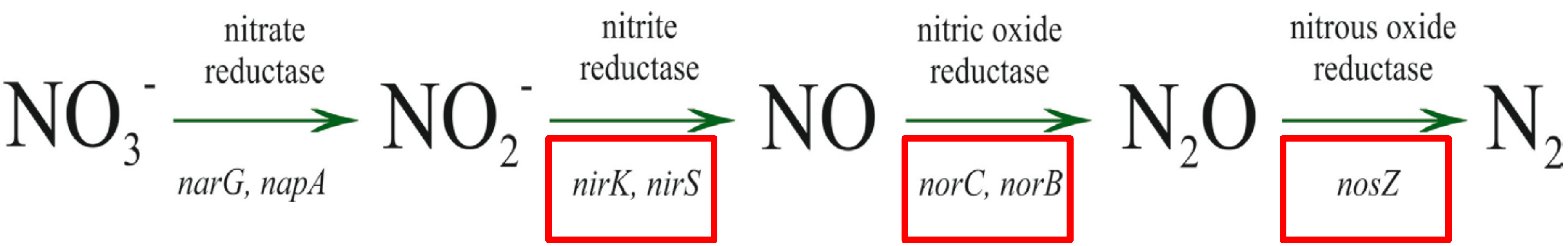
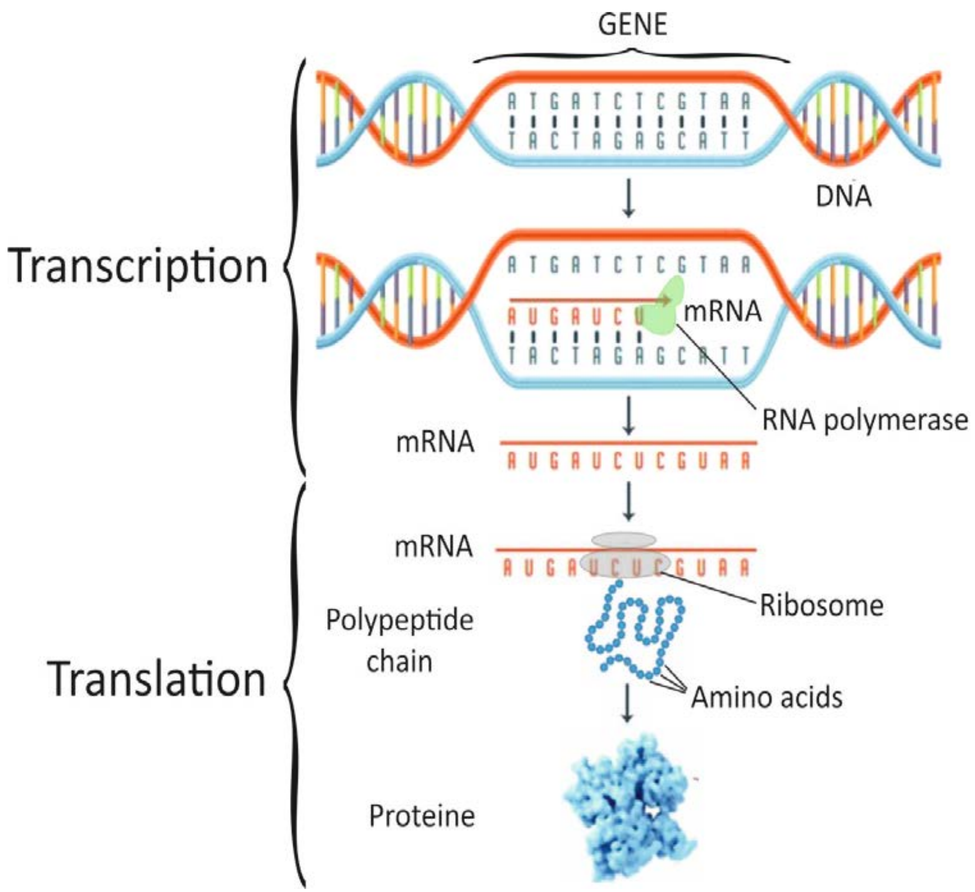


Fig. 6. **Biotic nitrification and denitrification pathways**

Approaches applied



Source: Campbell et al., 2019

Fig. 7. Schematic representation of a protein formation

Objectives of the study

Theoretical part:

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

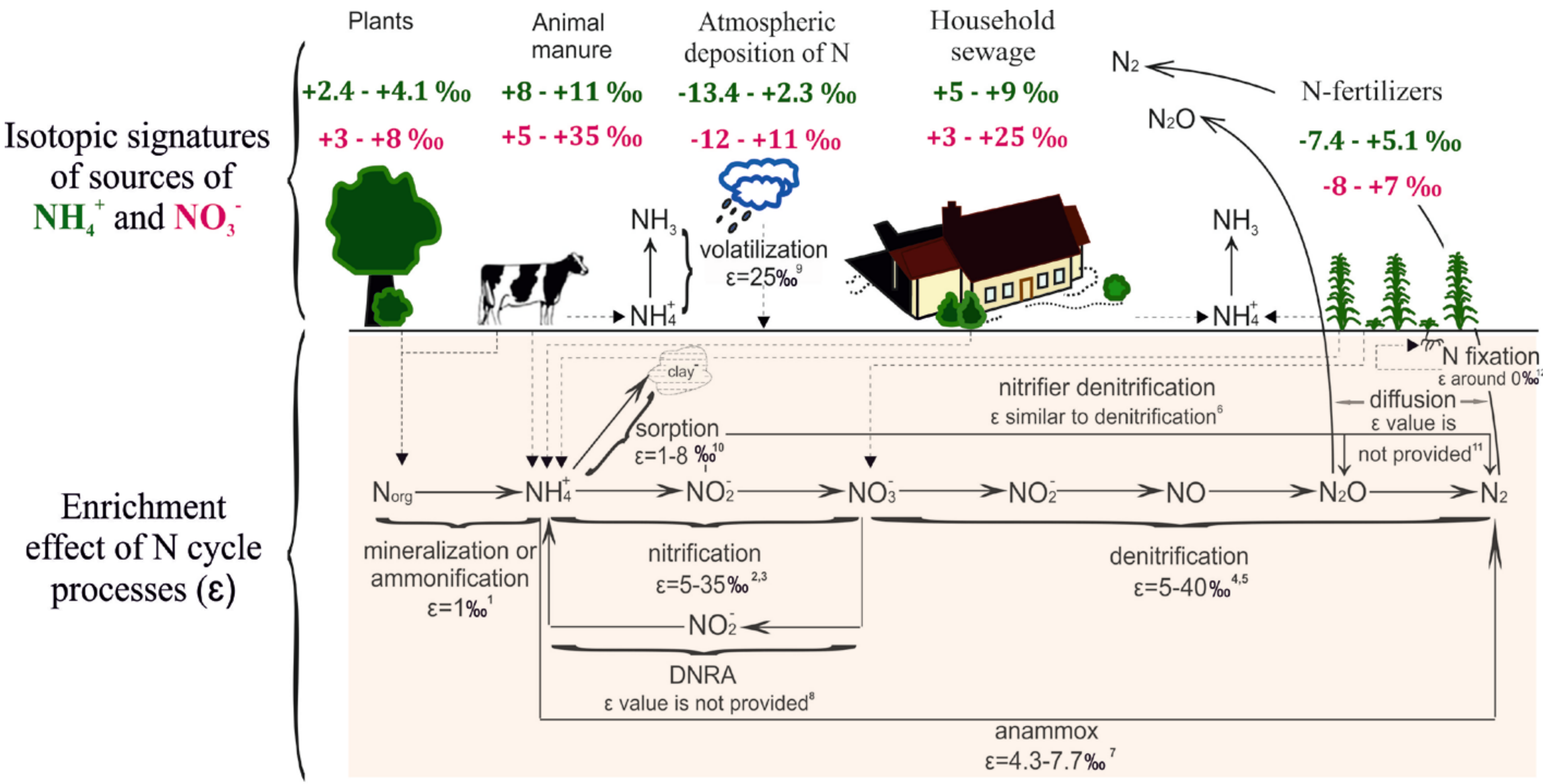
Practical part:

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

2. to **identify the N_2O** production and consumption **processes** and reveal **conditions** that govern **N_2O accumulation** in groundwater;

3. to **collect in situ evidence** about the **SP ranges of N_2O** and **activity of bacteria involved into N_2O production and consumption** processes.

N stable isotopes



Source: Nikolenko et al., 2018

Fig. 8. **N sources and transformation processes that affect N species in the subsurface**

N stable isotopes

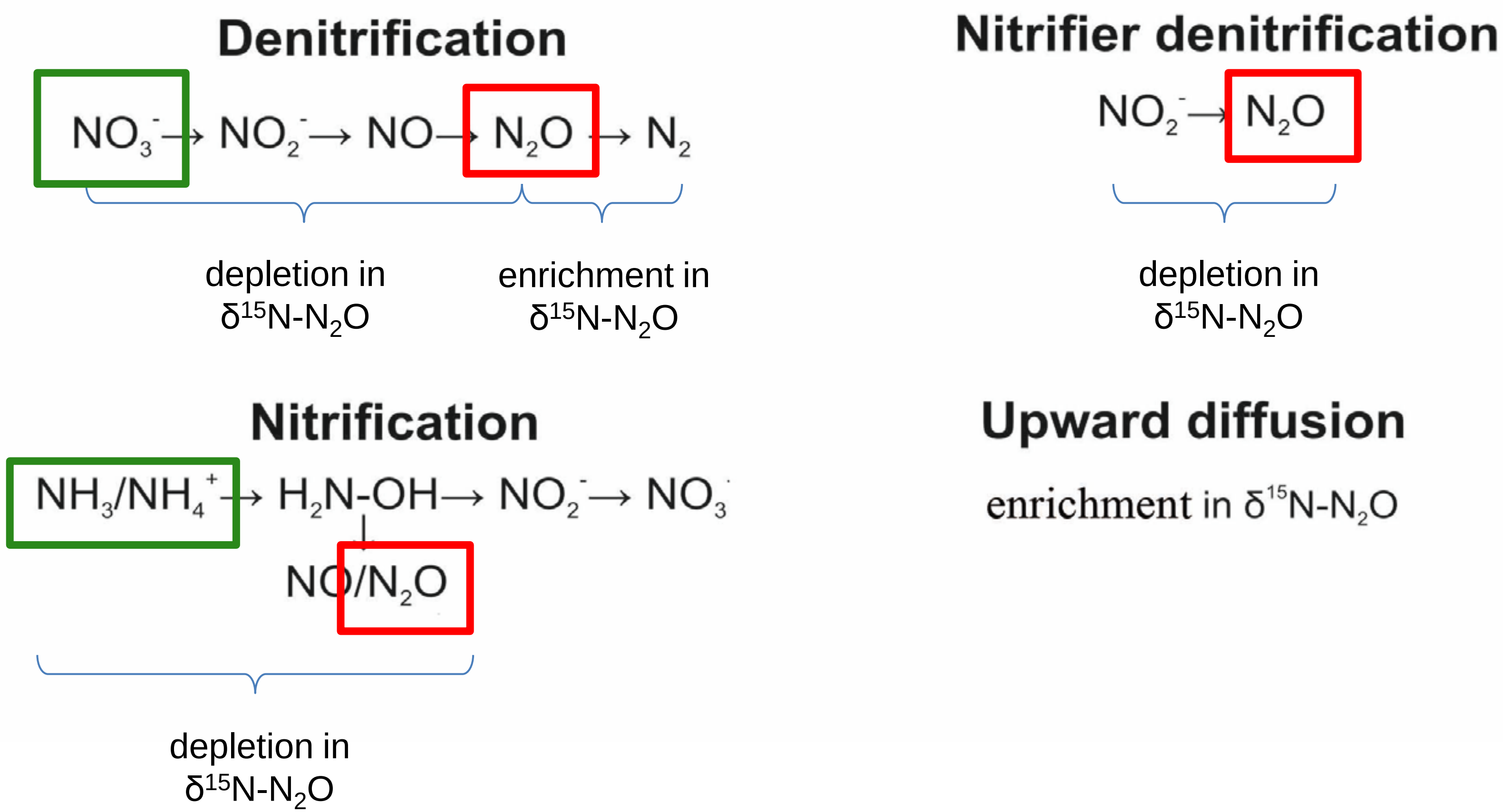


Fig. 9. **N Sources, processes and factors that influence $\delta^{15}N\text{-}N_2O$ values**

Complementary isotope studies: O, B, S, C

$\delta^{18}O-NO_3^-$

Nitrification:

$\delta^{18}O_{nitrate} = 1/3 * \delta^{18}O_{O2} + 2/3 * \delta^{18}O_{H2O}$

Denitrification:

$\delta^{18}O-NO_3^-$ ↑ and **$\delta^{15}N-NO_3^-$** ↑

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

$\delta^{11}B$

δ11B of sewage: -7.7‰ to +12.9‰

δ11B of manure: +14.5‰ 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 N_2O production and consumption processes within the studied aquifer using in situ and laboratory designed hydrogeological, isotope and microbiological experiments.

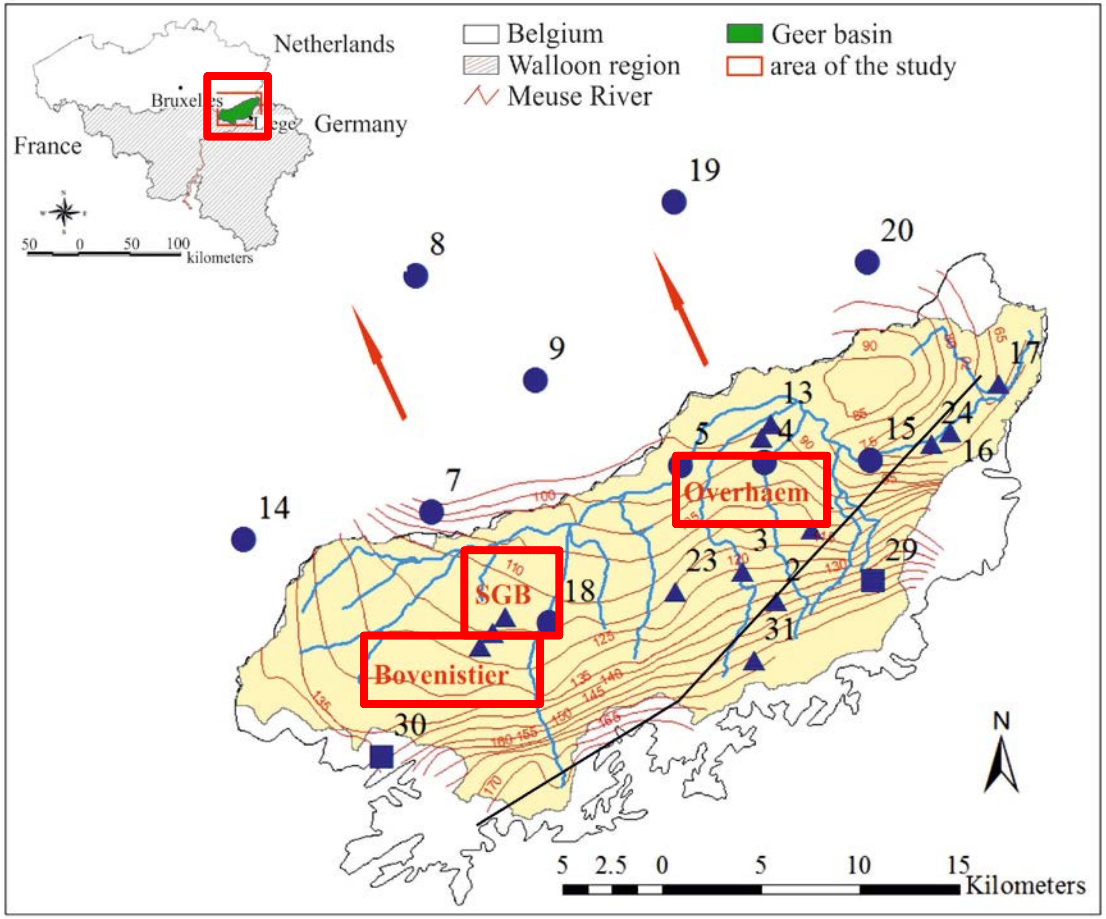
Regional studies: objectives

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



Source: Nikolenko et al., 2019

Fig. 10. Map of the studied area in the Geer basin

Peculiarities of the studied area:

- area: 480 km^2;
- 65% of agricultural activities;
- high fracturing of chalk aquifer;
- unconfined – the South;

  semi-confined – near the Geer river;

  confined – the North-West.

Regional studies: analyzed parameters

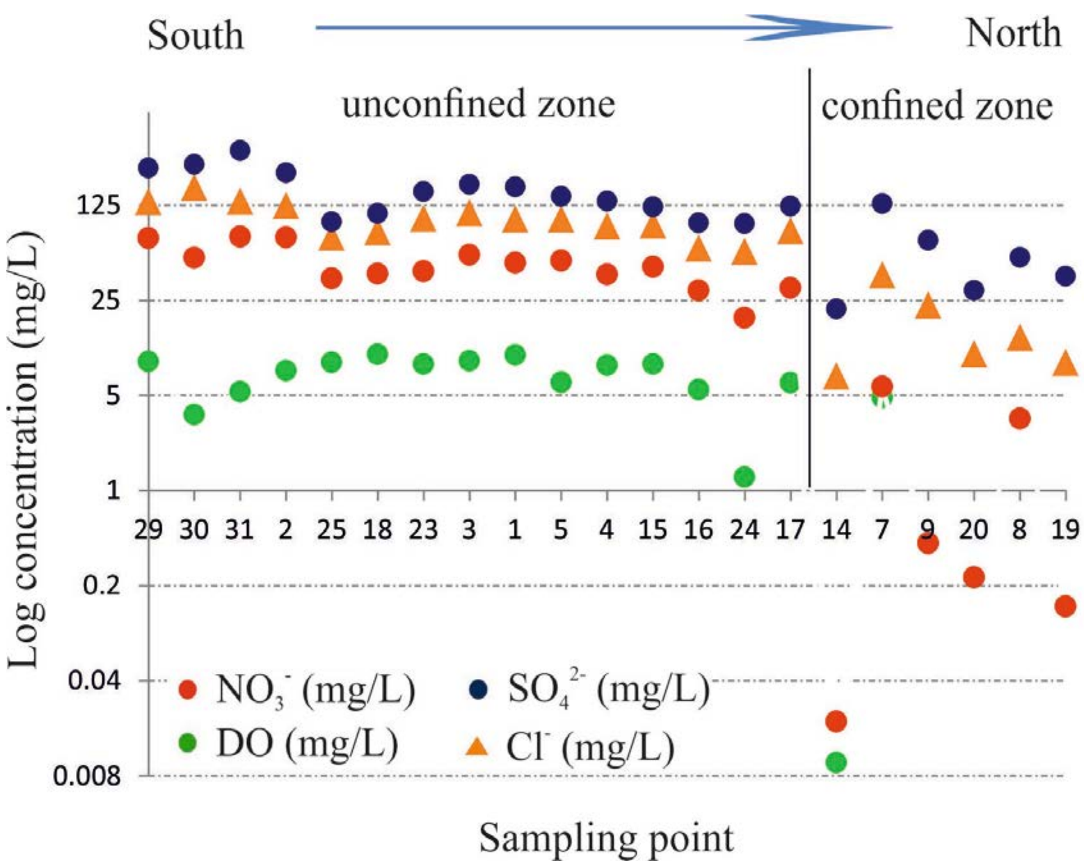


Fig. 11. Distribution of NO_3^-, DO, Cl^-, SO_4^{2-} along groundwater flow

- hydrogeochemical controls (DO, DOC, SO_4^{2-}, HCO_3^-, pH etc.);
- concentrations of N-species (NO_3^-, NH_4^+, NO_2^- and N_2O);
- isotope signatures of N_2O, NO_3^-, SO_4^{2-}, ^{11}B, ^{3}H.

Regional studies: distribution of GHGs

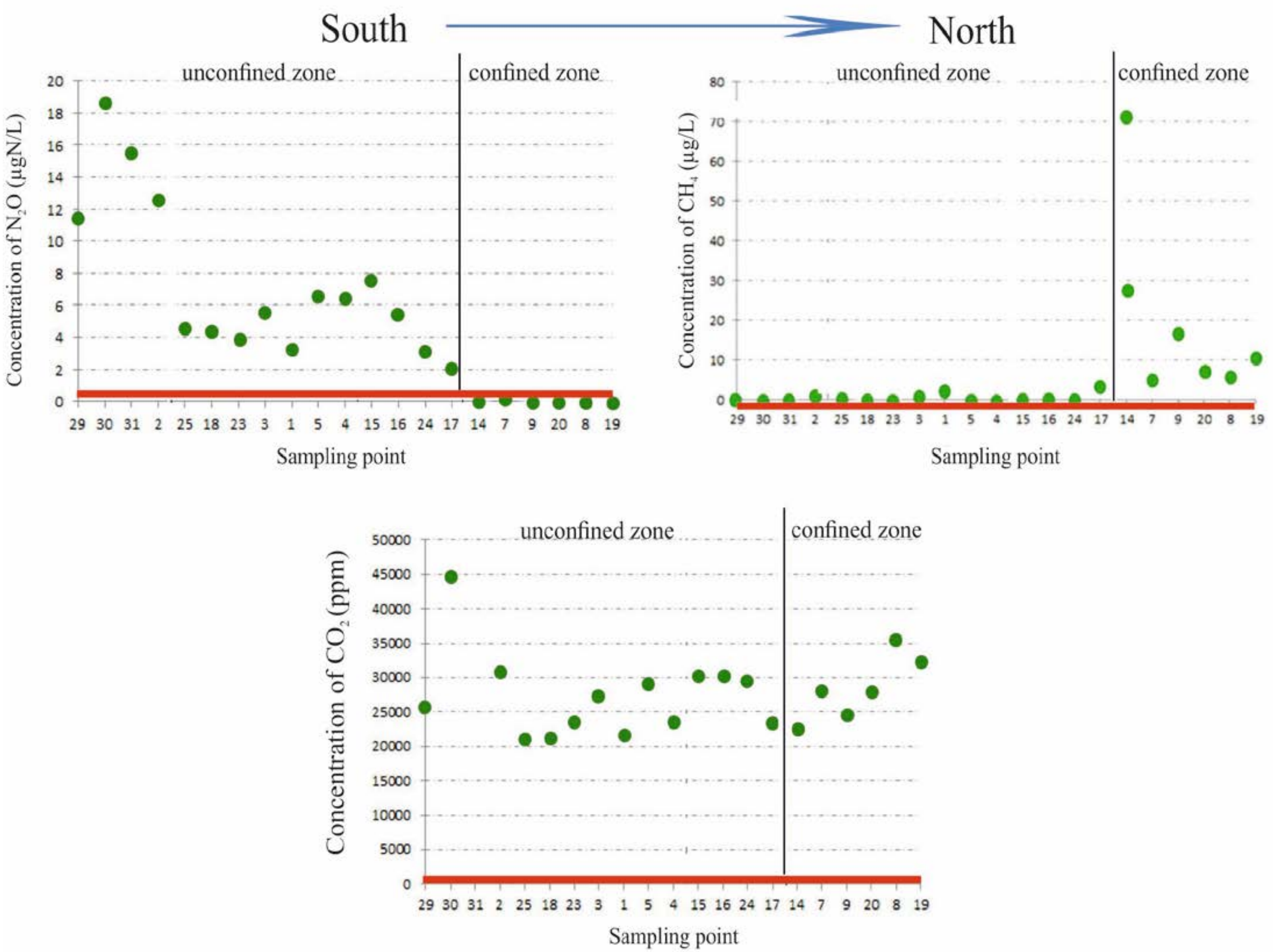
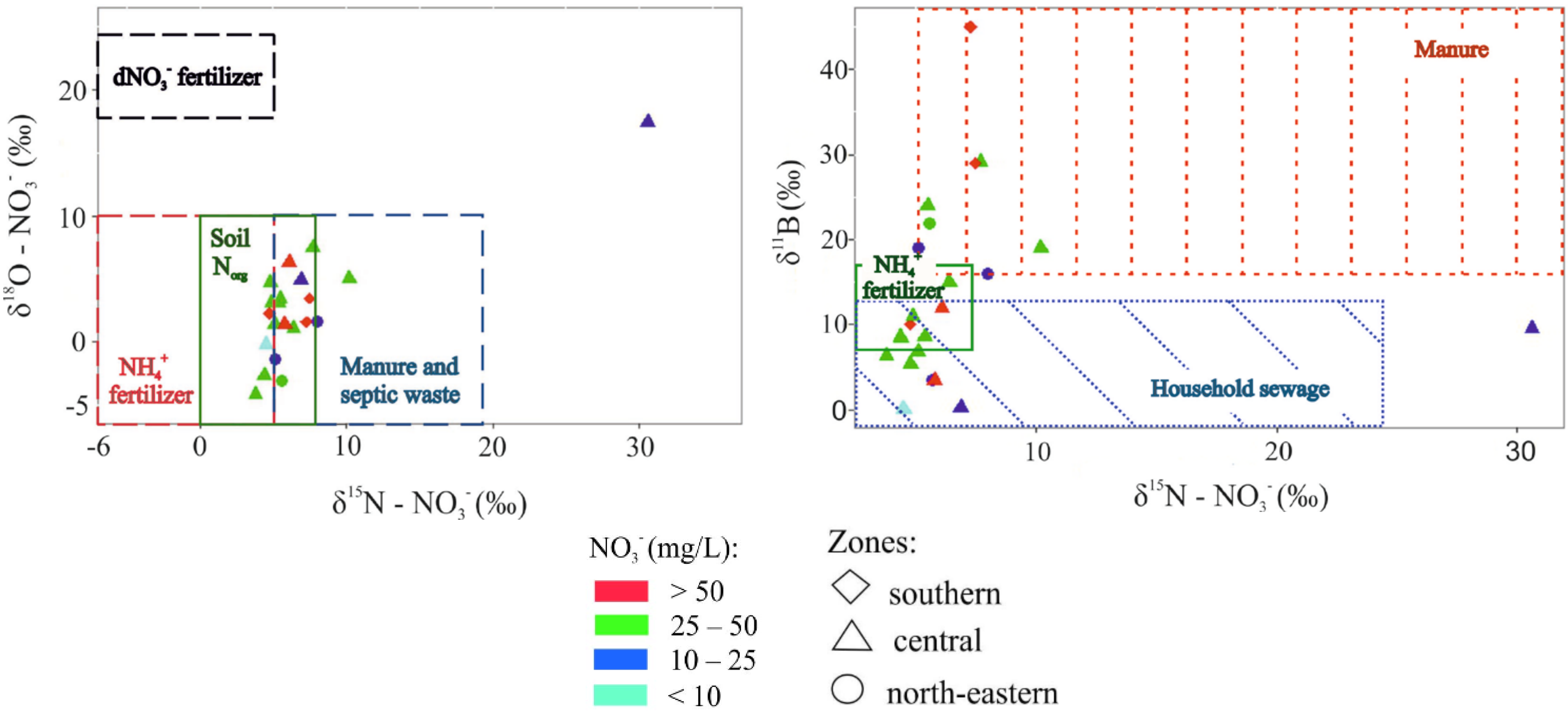


Fig. 12. **Distribution of N_2O, CH_4 and CO_2 along groundwater flow**

Regional studies: N sources



Source: Nikolenko et al., 2019

Fig. 13. Sources of N loading across the aquifer

Regional studies: N sources

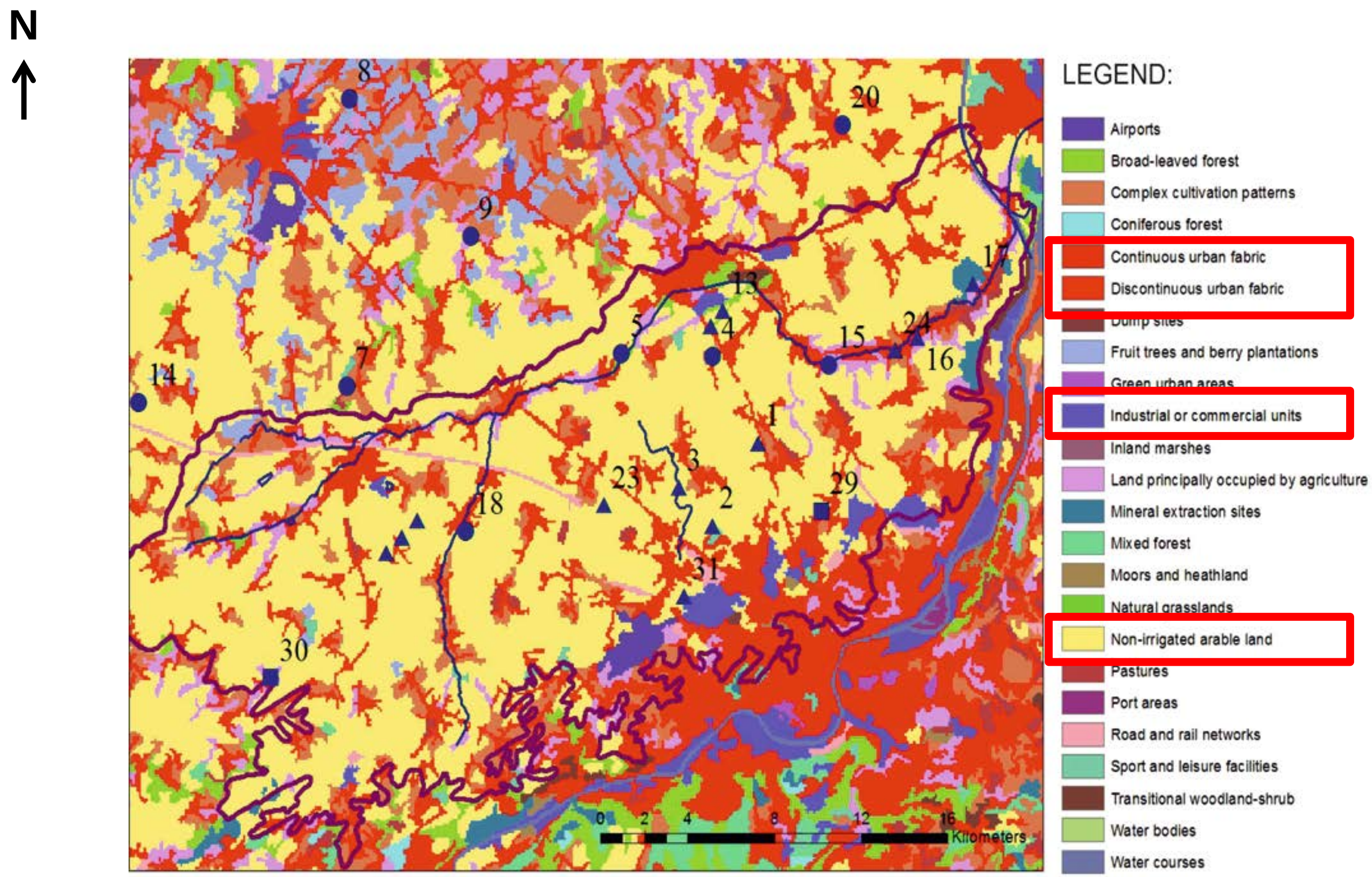


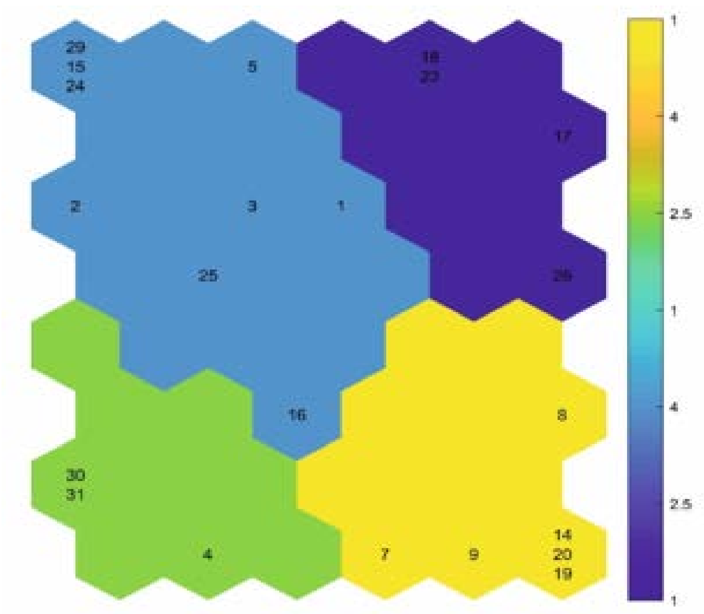
Fig. 14. **Land use map of the studied area**

Regional studies: CO_2 and CH_4 biochemistry

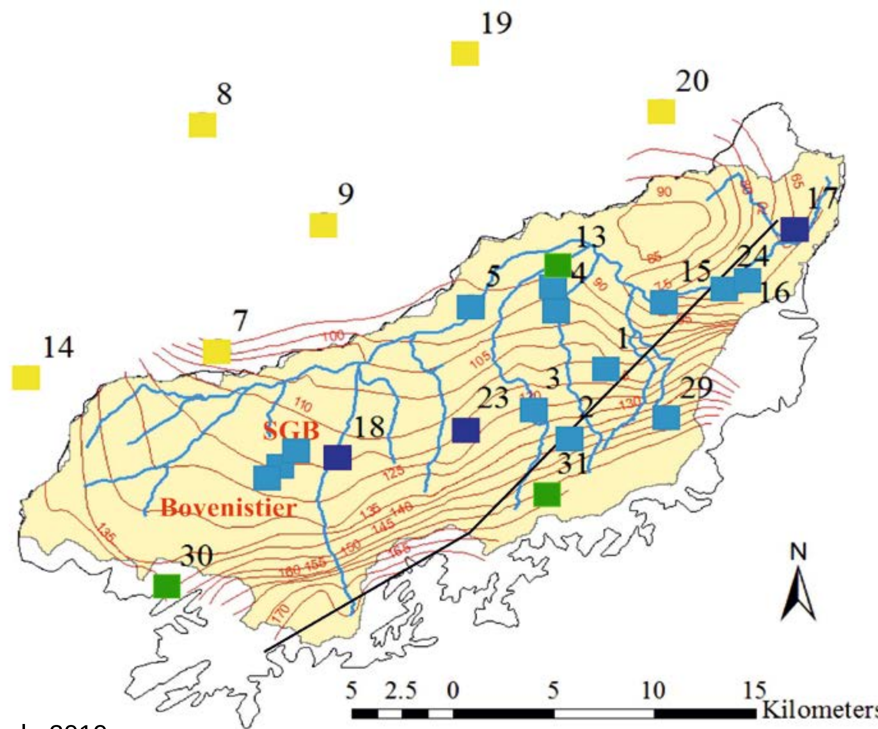
- tendency towards accumulation of **CO_2**
- ✓ the subsurface dynamics of CO_2 is governed by two processes:
1) dissolution of carbonate minerals;
2) degradation of DOC derived from the mineralization processes in the soil.

- **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: N_2O biochemistry



Group 1 – dark blue, group 2 – green, group 3 – blue and group 4 – yellow.



Source: Nikolenko et al., 2019

Fig. 15. **Clustering of the groundwater samples using SOM algorithm**

Group	*N_2O (µg N/L)*	*SP (‰)*	*DO (mg/L)*	*NO_3^- (mg/L)*	*Processes*
Group 1	3.4 ± 1.2	11.2 ± 1.6	8.2 ± 1.9	28.7 ± 3.8	nitrification and incomplete denitrification
Group 2	13.6 ± 6.3	26.1 ± 3.4	5.7 ± 2.4	48.7 ± 18.7	nitrification and complete denitrification
Group 3	6.7 ± 3.4	19.1 ± 6.7	7.2 ± 2.6	39.6 ± 16.2	nitrification and incomplete denitrification
Group 4	0.1 ± 0.1	not available	1.5 ± 2.1	0.2 ± 0.4	complete denitrification

Local studies: description of the area

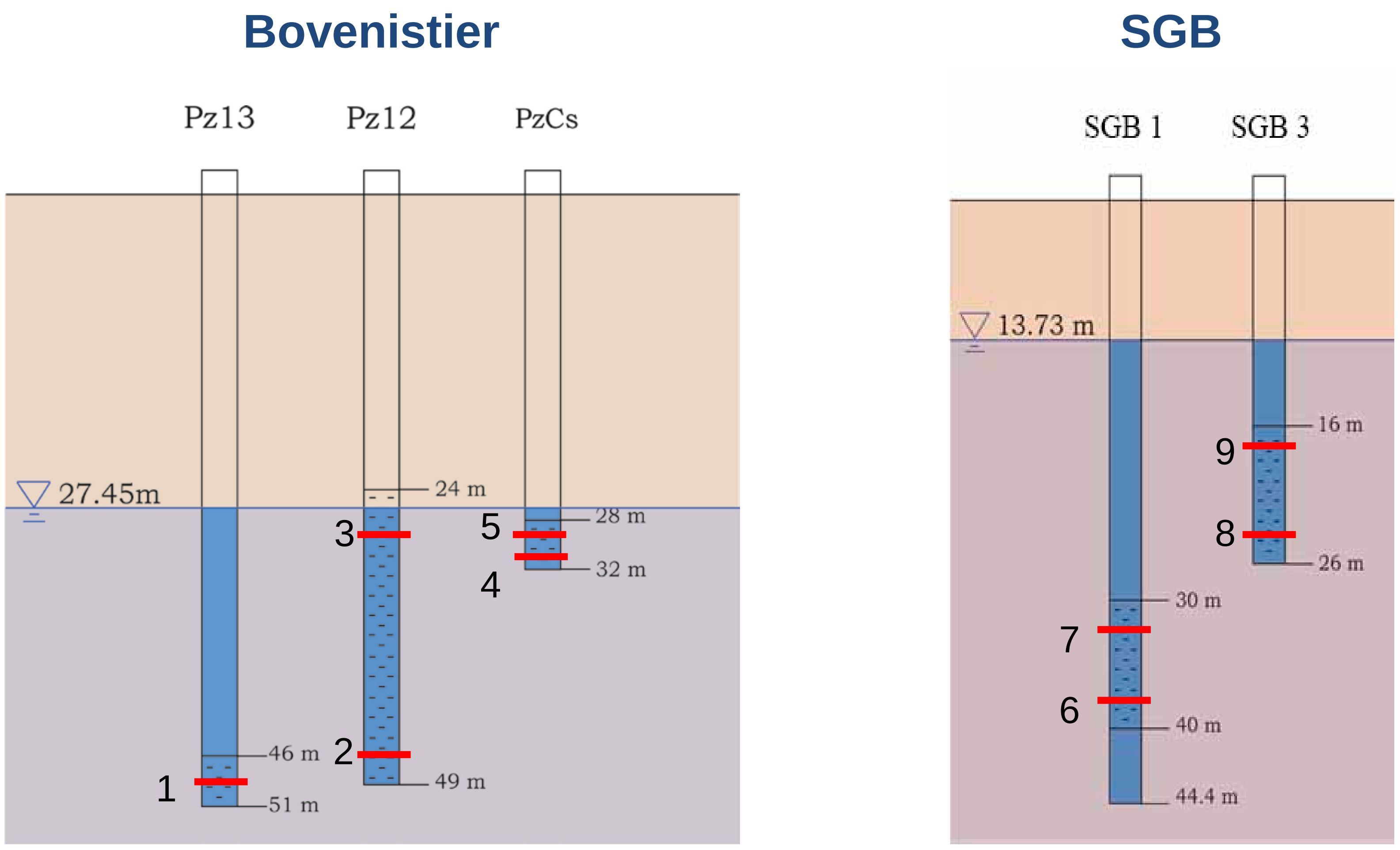


Fig. 16. **Piezometers and sampling depths at the Bovenistier and SGB sites**

Local studies: vertical profile SGB

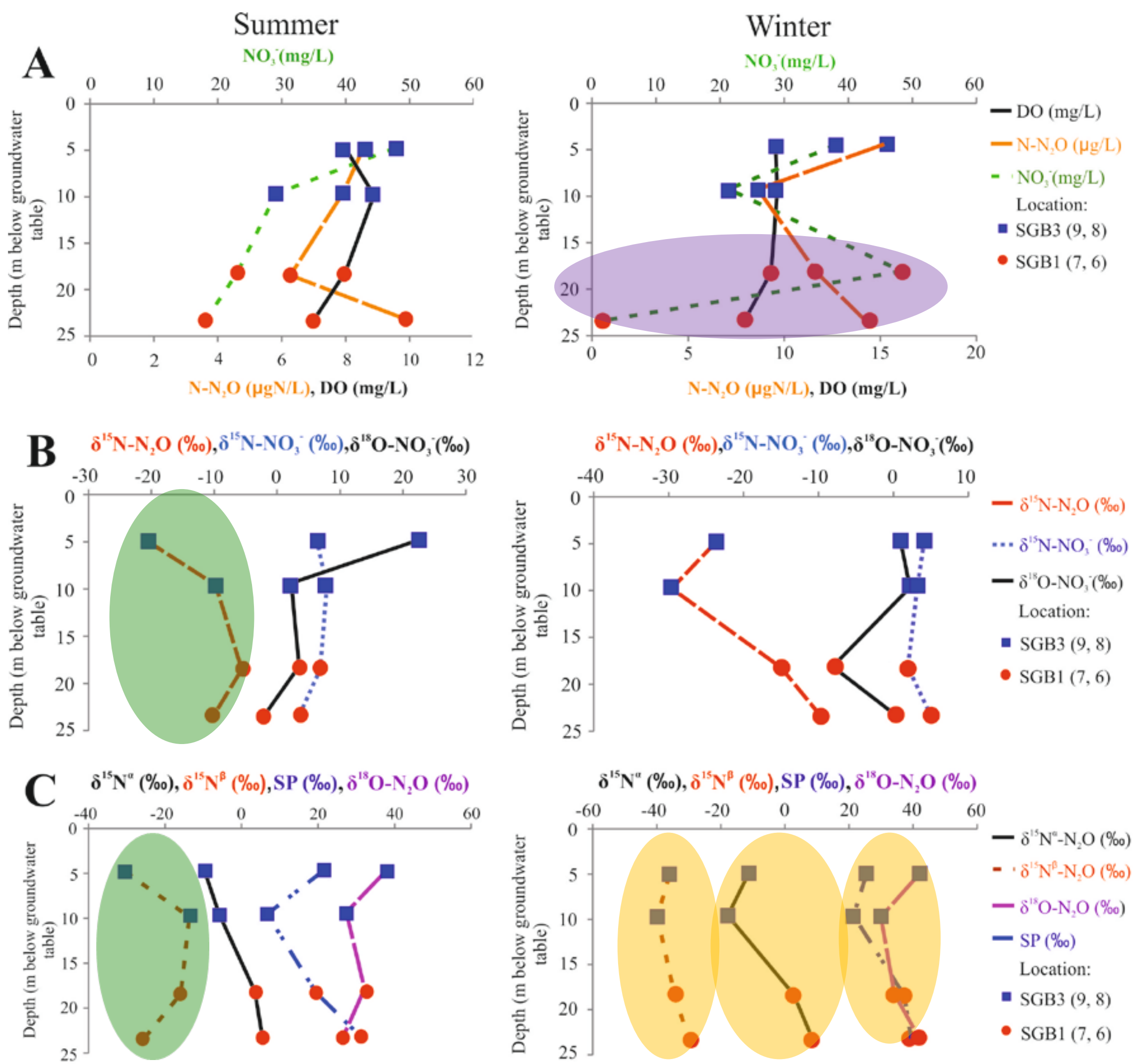


Fig. 17. **Vertical distribution of N compounds, their isotopes and DO**

Local studies: vertical profile Bovenistier

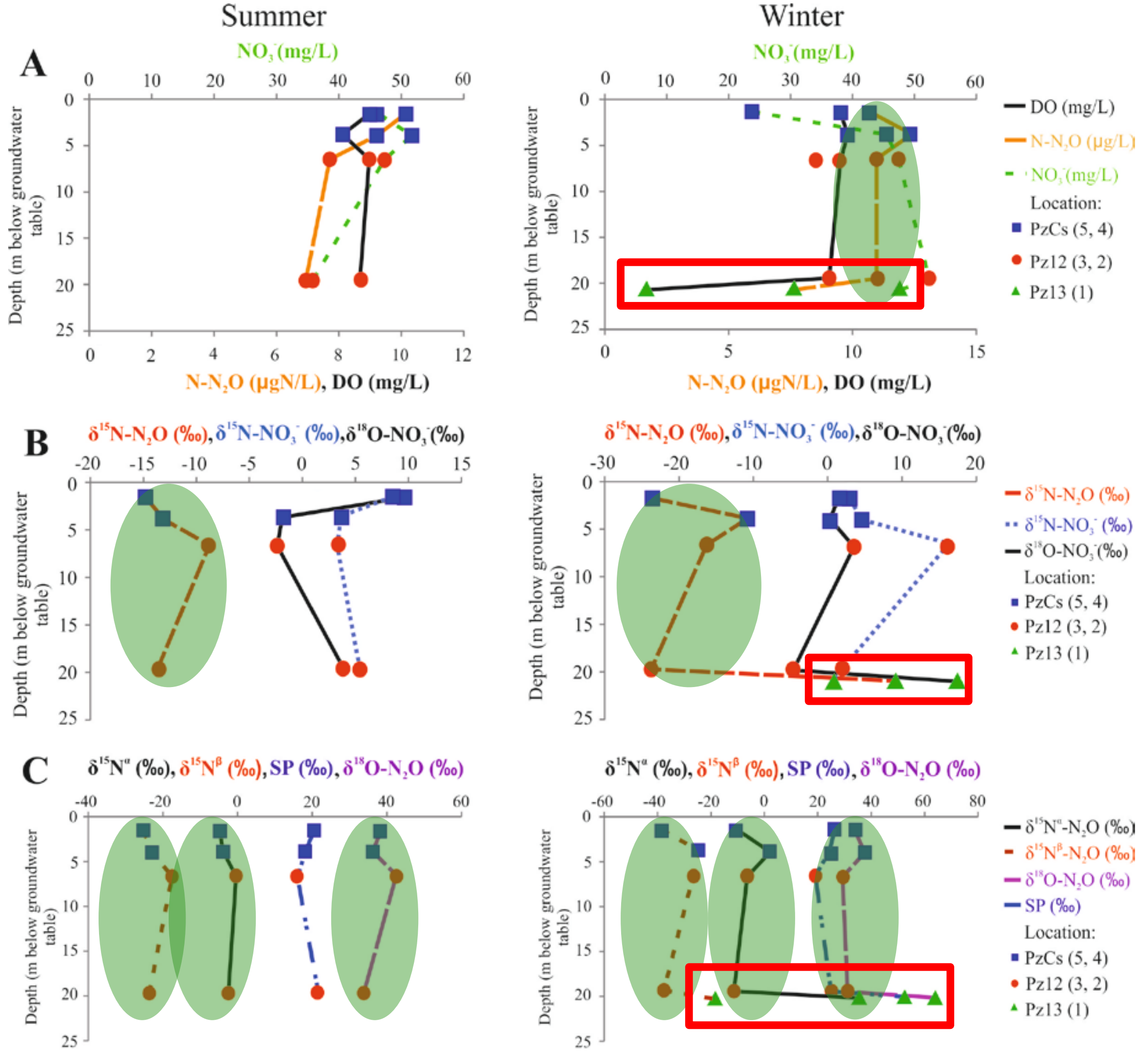


Fig. 18. **Vertical distribution of N compounds, their isotopes and DO**

Local studies: isotope labeled experiment

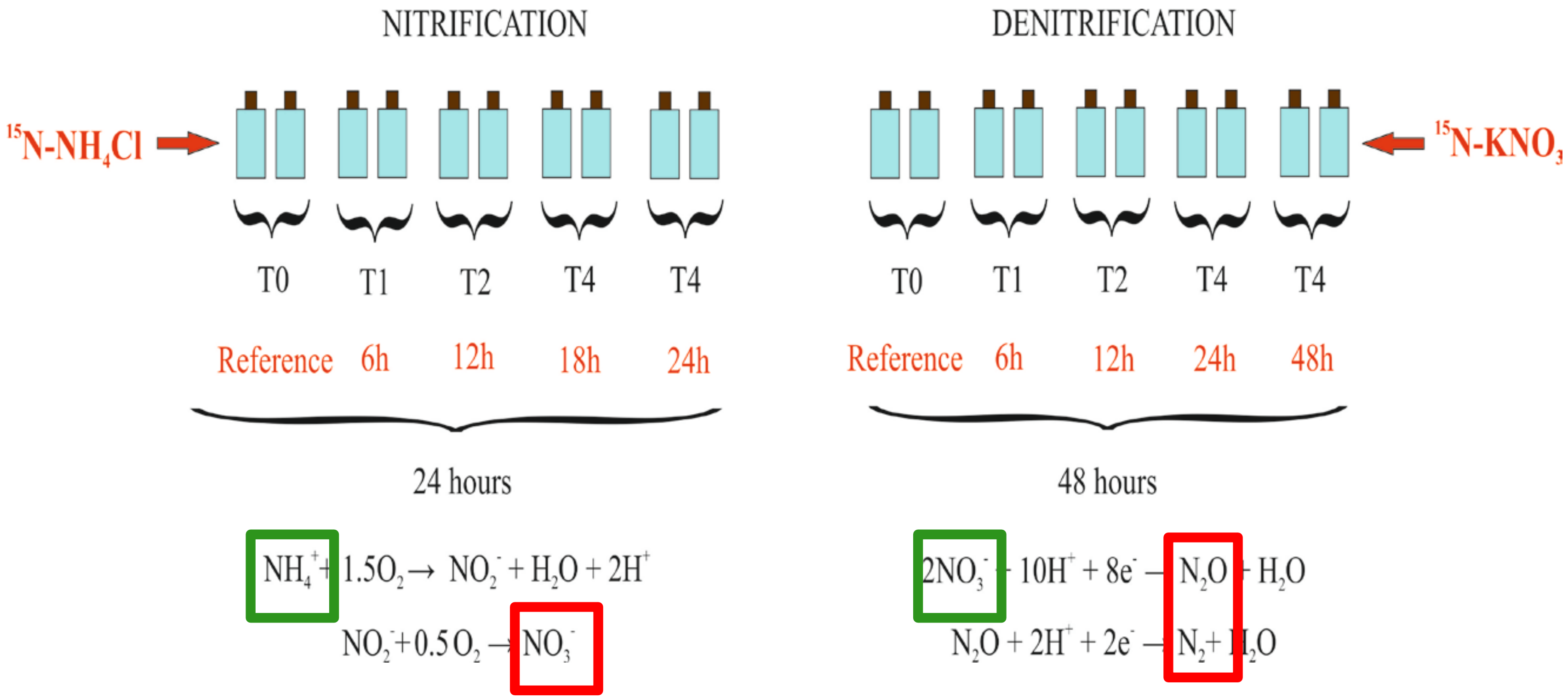


Fig. 19. **Scheme of laboratory ^{15}N stable isotope labeled experiment**

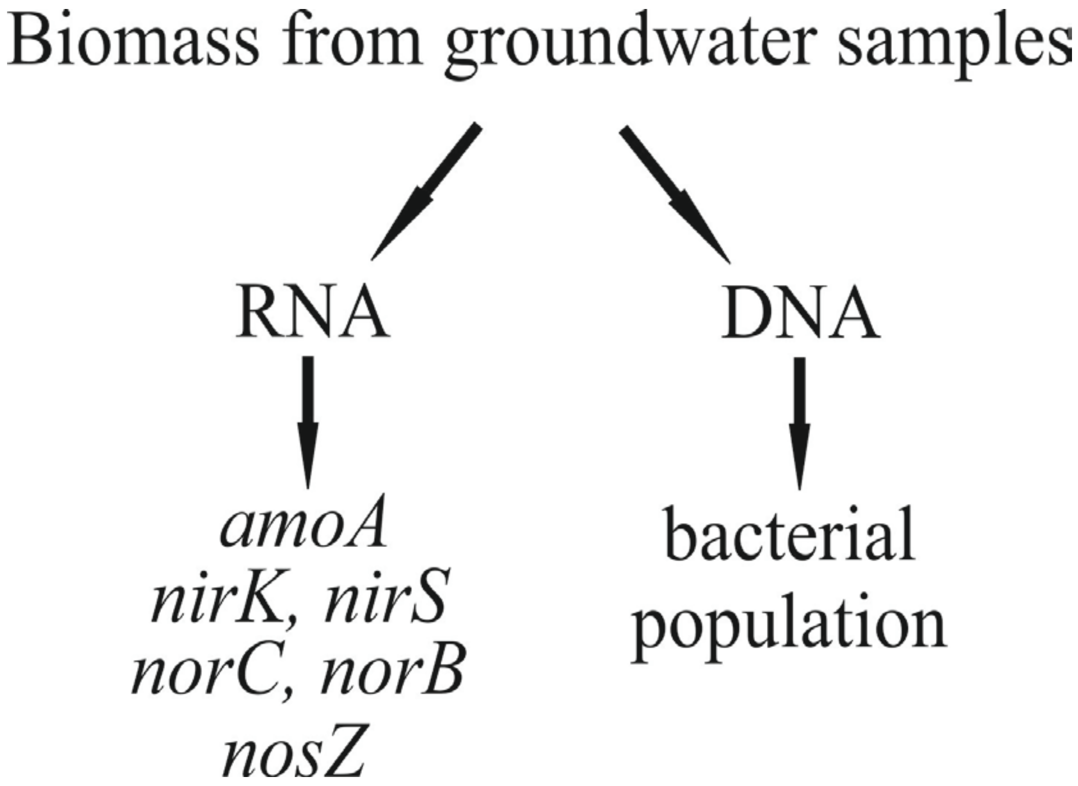
Local studies: summary

- N_2O produced by nitrification and denitrification processes occurring within the other parts of the aquifer

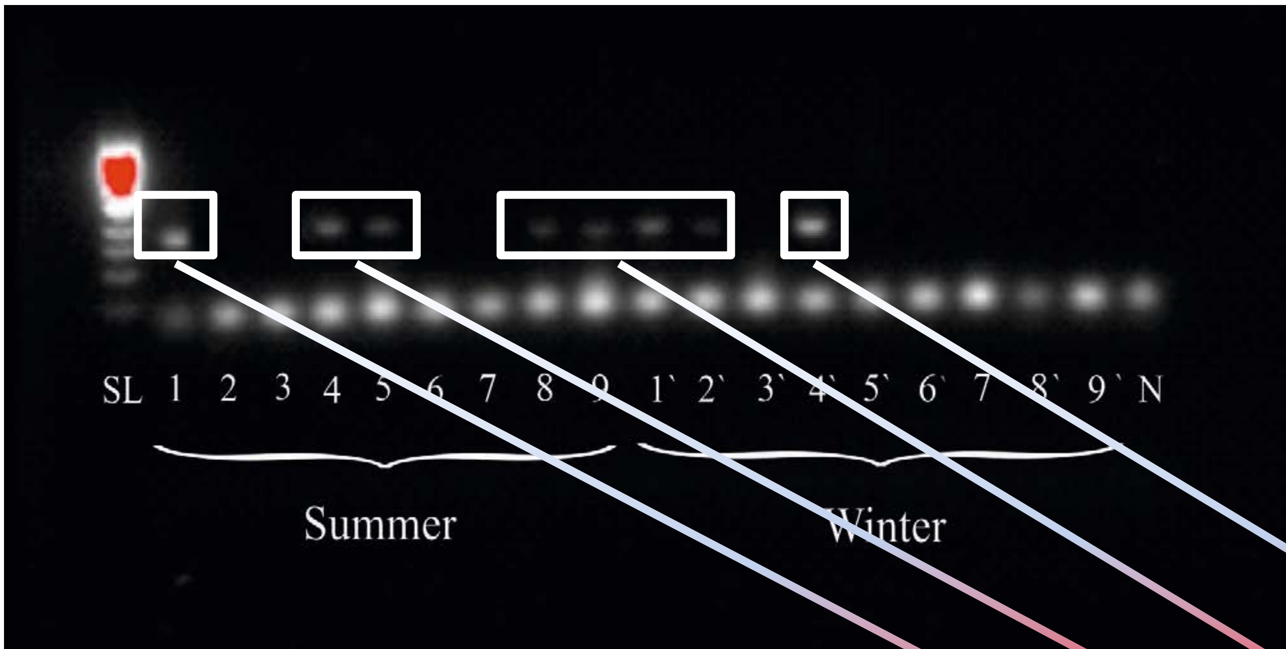
OR

- discrepancy between real aquifer conditions and laboratory experiments.

Microbiological studies



Microbiological studies



RNA

↓

cDNA synthesis

Fig. 20. **Results of PCR analysis aimed to reveal nirS gene expression in groundwater samples**

Sanger sequence analyses of PCR products

Microbiological studies

Location (S/W)	amoA		nirK_3		nirK_5		nirS_3		nirS_5		norB_4		norC_2		norC_3		nosZ	
	PCR	SSA	PCR	SSA	PCR	SSA	PCR	SSA	PCR	SSA	PCR	SSA	PCR	SSA	PCR	SSA	PCR	SSA
1 (Pz13)					+	+			+	+	+	+	+				+	+
2 (Pz12 bot)	+																	
3 (Pz12 top)					+	+					+	+						
4 (PzCs bot)					+	+			+	+	+	+	+	+				
5 (PzCs top)									+									
6 (SGB1 bot)					+	+												
7 (SGB1 top)			+	+	+	+					+	+	+	+				
8 (SGB3 bot)			+	+	+	+	+		+	+	+	+	+	+	+			
9 (SGB3 top)					+	+			+	+								
1`(Pz13)	+								+	+	+	+					+	+
2`(Pz12 bot)					+				+		+	+	+	+				
3`(Pz12 top)			+	+							+	+						
4`(PzCs bot)	+				+		+		+	+	+	+			+			
5`(PzCs top)					+	+					+	+	+	+	+			
6`(SGB1 bot)					+													
7`(SGB1 top)	+				+		+				+	+			+			
8`(SGB3 bot)					+		+											
9`(SGB3 top)					+										+			

nitrifiers genes

denitrifiers genes

N_2O production

N_2O consumption

Conclusions

1. The concentration of **N_2O** 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 **N_2O** originating from the given aquifer is associated with **high level of uncertainty**, particularly in comparison to the other GHGs.

2. The concentration of **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 **CO_2** dissolved in groundwater.

3. **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.

Conclusions

4. within the framework of this study **it was not possible** to obtain the **complete** understanding about dynamics of **N_2O** within the aquifer:

➢ nevertheless, there is evidence that show that isotopic signatures of **N_2O** 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 N_2O production and consumption** mechanisms **but it cannot differentiate** which exactly **microbiological processes** occur in the aquifer:

➢ the observed **N_2O** 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.

Perspectives

Further steps in studying N_2O 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 N_2O 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!

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