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Individual internship report

4th year

Research assistant to the DrivNOS project: unravelling the Drivers of Nitrous Oxide emissions from Streams

2022-2023

Host Organisation supervisors:
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ACKNOWLEDGEMENTS

Thank you, Joachim, for providing me with this exceptional opportunity to delve into the world of research. You have taken complete responsibility for my integration while consistently monitoring the progress of our projects.

Mette, I am grateful for your continuous support throughout this internship. I feel privileged to have had clearly defined missions under your guidance, I learned a lot with you.

I am proud to have contributed to your project, and I hope it will lead to the adaptation of agricultural practices in the right direction by fostering a better understanding of natural processes.

Juan, Dennis, Chiara, Karina, Dorte, and all the researchers, technicians, and staff at the institute, I extend my gratitude to each of you. Your interactions with me have always been marked by humanity, kindness, and empathy. I have learned a great deal from all of you.

I would also like to express my appreciation to my family, who have consistently placed their trust in me and allowed me to dedicate significant time to my studies. Without them, I wouldn't be able to navigate through these experiences in the same manner.

Additionally, I would like to acknowledge the teacher-researchers and the administration at Polytech Tours. They make every effort to impart technical and theoretical knowledge in a coherent manner, enabling us to tackle the environmental challenges our society faces.

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ABBREVIATIONS

CO_2: Carbon dioxide

C:N: carbon:nitrogen

DrivNOS: Unravelling the Drivers of Nitrous Oxide emissions from Streams

DMC: Dry Matter Content

ECOS: The Department of Ecoscience

GC: Gas Chromatograph

GWP: Global Warming Potential

LOI: Loss On Ignition

N_2O: Nitrous Oxide

Nos : N_2O-reductase

TOC: Total Organic Carbon

TIC: Total Inorganic Carbon

INTRODUCTION

During my 4th year at Polytech Tours' Engineering School in the Urban and territorial planning and environment program, I had the opportunity to complete a 12-week placement as a research assistant at the Institute for Ecoscience, University of Aarhus in Denmark. Under the guidance of postdoctoral researcher Mette Vodder Carstensen and senior researcher Joachim Audet, I contributed to a research project focused on aquatic biogeochemistry. The project aimed to gain a better understanding of the factors influencing nitrous oxide (N_2O) emissions in small watercourses within natural and agricultural environments.

The research project, named DrivNOS (Unravelling the Drivers of Nitrous Oxide emissions from Streams), sought to develop new mitigation measures for reducing N_2O emissions and improving N_2O models and national inventories. The goal is to reduce the greenhouse gas footprint from agriculture and promote more sustainable land use practices.

This internship aligns perfectly with my objective of implementing practices that address concrete needs while respecting natural boundaries. Agricultural areas face a multitude of challenges, including the environmental impact of urbanization, food production practices, and the escalating energy demands. I firmly believe that a comprehensive understanding of the dynamic interactions between different environments is crucial for effectively addressing these pressures. This includes considering the biosphere, which encompasses the human sphere, hydrosphere, pedosphere, atmosphere, and lithosphere. In this context, biogeochemistry plays a pivotal role in this comprehension of interactions. As explained by Reddy & DeLaune (2008), biogeochemistry explores the exchange of materials between living and non-living components of the biosphere. It examines the intricate interplay of physical, chemical, and biological processes in ecosystems, ranging from small-scale particle exchanges to global-scale phenomena. Wetlands play a vital role in biogeochemical processes, involving microbial communities, periphyton, and vegetation. These processes quantify the movement of elements or compounds within wetlands, including their exchange with other systems such as the atmosphere.

My main responsibility in the research project was to assist in formulating an experimental protocol for testing the primary hypothesis of DrivNOS, which suggests that small rivers with an acidic water (pH < 7) would exhibit higher N_2O emissions compared to small rivers with sediments of neutral pH due to incomplete biotic denitrification or chemodenitrification in the acidic rivers. Furthermore, I made significant contributions by collecting and analysing sediment property data from various research sites. For a comprehensive list of completed assignments and the skills I acquired during this internship, please refer to the appendix.

1 HOSTING STRUCTURE: The Ecoscience Department of Aarhus University

1.1 A structure with diverse missions

The Department of Ecoscience (ECOS) is a world-renowned research institution that conducts both basic and applied research focused on nature and the environment. The Department of Ecoscience consists of approximately 275 employees with expertise covering marine, freshwater, and terrestrial ecosystems. Through cutting-edge research, the department generates valuable insights and knowledge that can help solve pressing societal challenges related to environmental sustainability.

In addition to conducting research, ECOS also provides consultancy services to government agencies and collaborates with businesses to translate research findings into actionable solutions. By sharing their expertise with key stakeholders, ECOS is helping to shape policy decisions and promoting environmentally responsible practices (Department of Ecoscience, 2021).

1.2 An internship in line with the structure's vision

The Department of Ecoscience strives to be the leading institution in Denmark for environmental research and consultancy related to ecology, dynamics, and the state of ecosystems. To achieve this goal, the department is committed to conducting excellent and solution-oriented research while also being the preferred consultancy and collaboration partner for both the public sector and businesses in Denmark and the Arctic region. Additionally, the department aims to play a central role in the global community by contributing to international efforts to protect and conserve the environment.

Over the course of the strategy period, the department will continue to develop and expand its course offerings at the Bachelor's, Master's, and Ph.D. levels, ensuring that students have access to cutting-edge research and training. The Department of Ecoscience is committed to providing a stimulating and supportive work environment for both Danish and international employees and students, attracting top talent from around the world.

Overall, the Department of Ecoscience is dedicated to making significant contributions to the field of environmental science and becoming a leader in research and consultancy related to the state of nature and the environment on land and in water. Through innovative research, partnerships, and education, the department is helping to address some of the most pressing environmental challenges of our time.

1.3 The Catchment Science and Environmental Management section's place in the Ecoscience department

At the heart of the organizational structure lie the sections, academic networks, and committees that form the backbone of the institution. These components play a vital role in shaping operations and fostering collaboration within the academic community. To facilitate effective research and knowledge sharing, the researchers at the Department of Ecoscience are organized into various sections (*Figure 1*). These sections serve as cohesive units, each with a distinct focus on a particular aspect of the biology field.

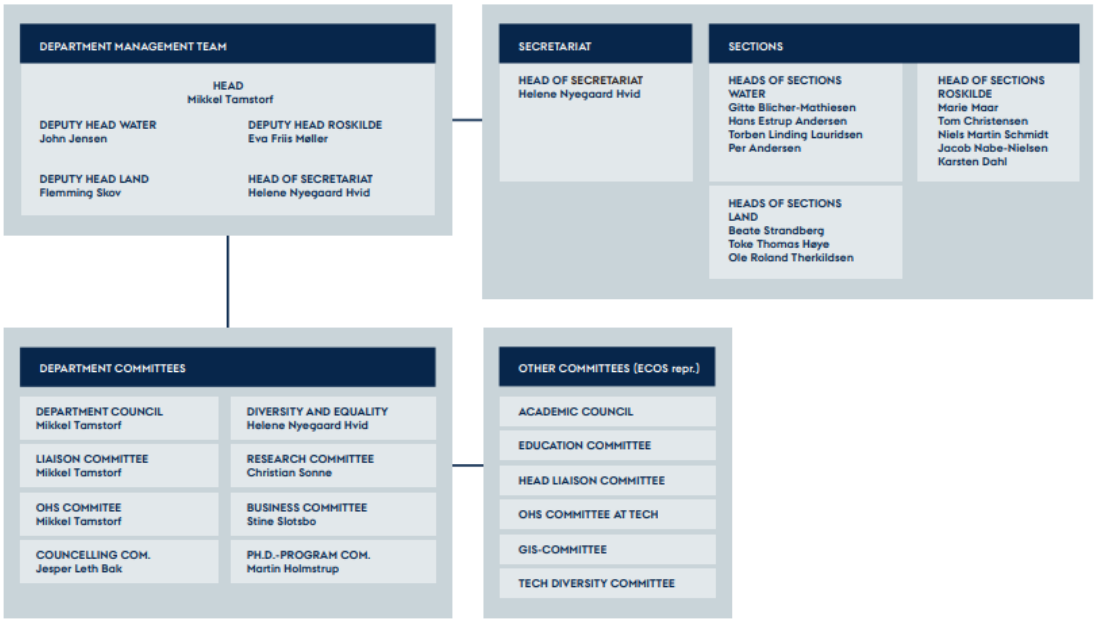

Figure 1: Aarhus University - Organisation chart for Ecoscience (Aarhus University, 2023)

The department embraces an ecosystem approach, evident in its division into four research fields that serve as both a structure for the sections and a reflection of its research focus. These research fields, namely Terrestrial ecosystems, Freshwater ecosystems, Marine ecosystems, and the Arctic, are not rigidly separated but rather exhibit significant overlap. This comprehensive framework allows the department to address a wide range of ecological aspects while maintaining a specialized focus on the unique challenges and opportunities presented by the Arctic region (Department of Ecoscience, 2023).

The Catchment Science and Environmental Management research area focuses on studying and addressing the ecological challenges faced by aquatic environments, such as lakes, streams, and fjords. These areas are constantly threatened by factors like increased nutrient input, hazardous substances from urban communities and agriculture, global climate change, and other human activities. Through their research, they aim to gain new knowledge and insights into effectively mitigating the negative consequences of these pressures. Research and consultancy activities revolve around four core areas. Firstly, they investigate nutrient dynamics and hydrology in aquatic ecosystems. Secondly, they develop tools and strategies to reduce nutrient loss to the aquatic environment. Thirdly, they study wetlands and their role in greenhouse gas emissions. Lastly, they delve into the field of stream ecology.

The wetland group is closely linked to the Catchment Science and Environmental Management research area. This team consists of interdisciplinary scientists dedicated to freshwater wetland research. Their expertise spans across multiple fields, including ecology, hydrology, biogeochemistry, and modelling. This group is actively engaged in various extensive projects at the national, Nordic, and European levels, with a primary focus on three key areas: riparian wetland restoration, restoration of low-lying organic soils, and the development of mitigation measures to address nutrient loads from agricultural catchments.

2 PRESENTATION OF MISSION - The DrivNOS project

2.1 The overall project

N_2O is a potent greenhouse gas with a global warming potential (GWP) approximately 300 times that of carbon dioxide (CO_2) over a 100-year period (Ciais et al., 2013). Moreover, N_2O is the primary ozone-depleting substance in the stratosphere, and its emissions can negatively affect the rate of ozone hole recovery (Ravishankara et al., 2009). Agriculture is the largest anthropogenic source of N_2O globally, accounting for approximately 60% of all anthropogenic N_2O emissions, or 4.1 Tg N/year (Ciais et al., 2014). Currently, there is an ongoing increase in atmospheric concentrations of N_2O, with levels reaching 332 ppb in 2019, showing an 8 ppb rise since 2011 (Masson-Delmotte et al., 2021). At a global level, the main human activity driving the heightened release of N_2O into the atmosphere is the intensive application of nitrogen (N) fertilizers in agricultural areas (Tian et al., 2020). N_2O emissions from drainage systems are considered hot spots for emissions (Reay et al., 2012) as well as the hyporheic zone from small streams which is the part beneath the stream bed where groundwater and stream water interact (*Figure 2)*, due to the large biochemically reactive surface area in this zone (Yao et al., 2020).

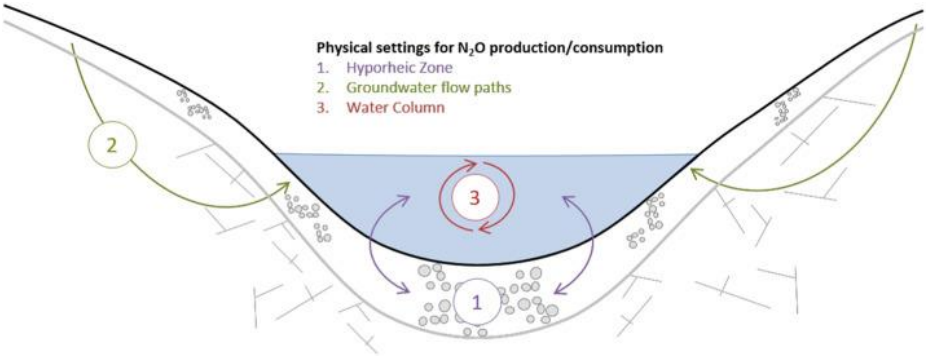

Figure 2: Cross section of a stream or river showing three possible physical settings for the generation of N2O (Quick et al., 2019)

The project ”Unravelling the Drivers of Nitrous Oxide emissions from Streams” also called DrivNOS aims to conduct a comparative analysis of the contribution of ”natural” (non-cultivated) and farmland areas to N_2O emissions in small streams (Strahler order: 1-2) (*Figure 3*). This is important because recent studies, such as that by Yao *et al.* (2020) have shown that small streams (below fourth-order) dominate global riverine N_2O emissions.

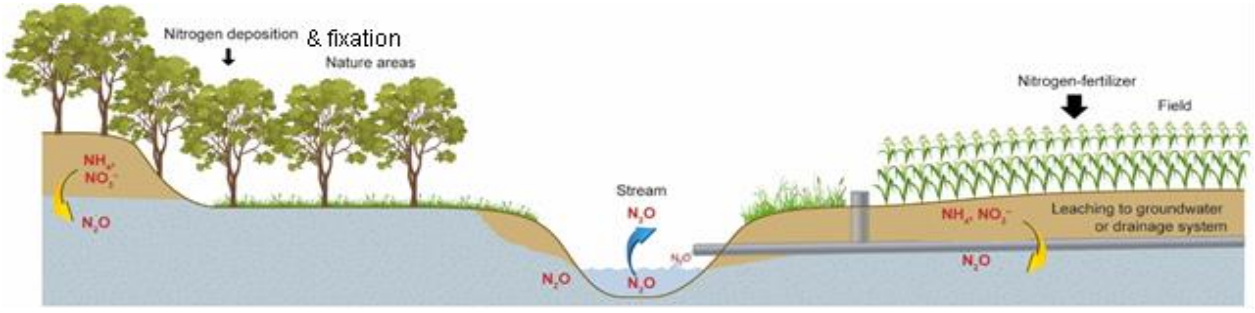

Figure 3: Conceptual figure of N2O production, transport and emission in streams. Left of the stream is the situation with a nature area; right of the stream is a drained agricultural area (©Vodder Carstensen, Audet, 2023)

In addition, the project aims to investigate the impact of nitrogen (N) pollution and soil properties, particularly iron content and pH, on N_2O emissions and the underlying processes driving them. For instance, low pH levels have been found to suppress N_2O-reductase (Nos) activity, partially inhibiting the reduction of N_2O to N_2, as highlighted by Bakken *et al.* (2012). Previous research by Audet *et al.* (2020) also demonstrated relatively high N_2O concentrations in forest streams in Sweden, despite lower nitrate (NO_3^-) concentrations than in agricultural areas. This could potentially be linked to the lower pH levels in forest streams, or to N_2O production through chemodenitrification (*Figure 4*), which involves the abiotic reduction of oxidized N species (i.e., NO_2^- and NO_3^-) by ferrous iron.

By identifying N_2O emission "hot spots" and understanding the processes driving N_2O emissions, the DrivNOS project aims to develop new mitigation measures to reduce N_2O emissions and improve N_2O models and national inventories. Ultimately, this will help to reduce the greenhouse gas footprint from agriculture and contribute to more sustainable land use practices.

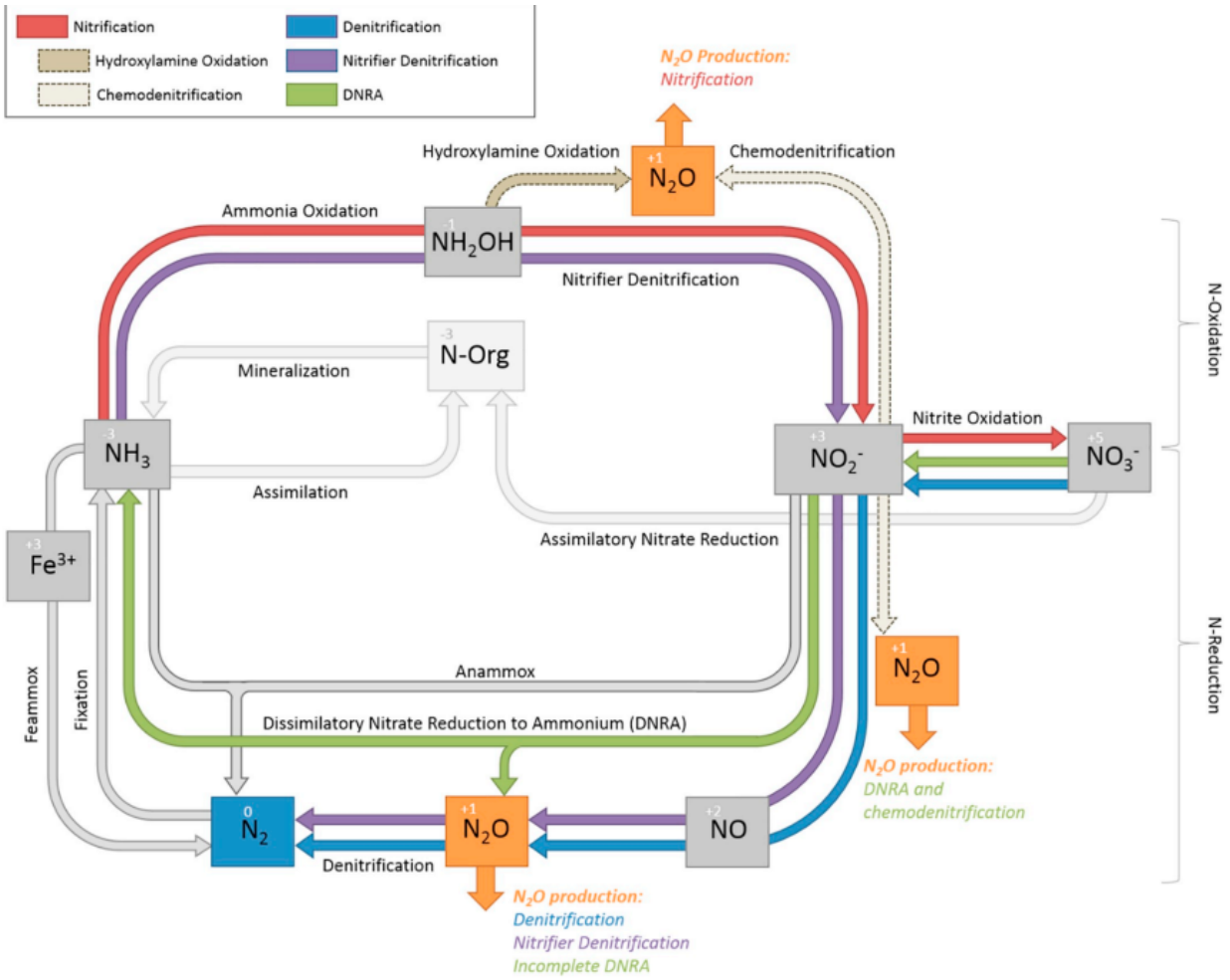

Figure 4: Nitrogen cycle processes, highlighting those that produce N_2O. Processes are detailed in Quick et al. (2019)

2.2 Contribution of the internship to the project

The internship aims to finish developing and finalizing the protocol for a large-scale study on N_2O emissions from sediments at various pH levels. This involves conducting pilot studies as preliminary small-scale experiments to test and refine procedures for the final experiment design. So, this internship offers the opportunity to contribute to the research process, from the finalization of the study design to data collection and even the start of analysis (*Figure 5*).

Figure 5: Diagram of the research process, the parts in which this internship takes part are circled in red (adapted from ©http://www.iedunote.com/)

First of all, a pilot study on pH adjustments aims to determine the optimal amount of base and acid required to adjust the pH of serum bottles for N_2O emissions measurements. pH is a crucial variable that can significantly impact the results of N_2O emissions measurements (Čuhel et al., 2010). Low pH can suppresses Nos activity (*Figure 6*) partially inhibiting reduction of N_2O to N_2 (Audet et al., 2020; Bakken et al., 2012). By adjusting the samples to a desired pH level, the pilot study will facilitate a comparison of N_2O emissions at neutral pH with those at natural pH. Neutral pH conditions are expected to be more conducive to certain biotic denitrification mechanisms (Rajta et al., 2020) .

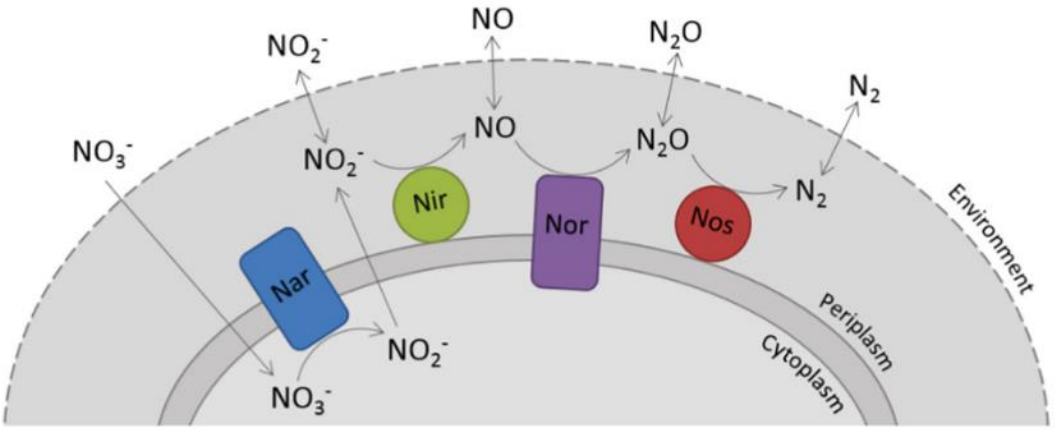

Figure 6: Microbial enzymes involved in denitrification. The enzymes, shown as circles and boxes, are nitrate reductase (Nar), nitrite reductase (Nir), nitric oxide reductase (Nor), and nitrous oxide reductase (Nos). These enzymes are encoded by the genes nar, nir, nor, and nos, respectively (Quick et al., 2019).

Then, the pilot study on sampling intervals aims to determine optimal sampling intervals for accurately measuring N_2O emissions from sediment samples using serum flask incubations. In order to achieve this, NO_3^- will be added to sediment samples collected from the field. By analyzing the quantitative evolution of N_2O emissions from the samples at different time increments using gas chromatography, the study aims to identify the most appropriate sampling times for measuring N_2O emissions from sediment samples.

Finally, to accurately understand and quantify N_2O emissions, it is important to measure different variables that can influence them such as the carbon:nitrogen (C:N) ratio, moisture content or organic matter content. It is well known that other main environmental factors that can influence the $N_2O/(N_2O + N_2)$ ratio are organic carbon and NO_3^- availability, water content, and O_2 partial pressure (Čuhel et al., 2010). Carbon plays a significant role in natural systems' denitrification processes by creating anaerobic zones, particularly in hyporheic sediments (Quick et al., 2016). Denitrifiers are primarily heterotrophic bacteria, many of which are facultative anaerobes. They couple the oxidation of organic substrates to the reduction of NO_3^- to either N_2O or N_2 (Inglett et al., 2005). This reaction (1) takes place in moderately reduced environments where oxygen is absent and serves as a significant mechanism for nitrogen removal in aquatic systems (White & Reddy, 2009). This paths lead to formation of N_2 gas, but if the reaction is inhibited or incomplete, also nitrous oxide may be produced.

$$2.5\ \{CH_2O\} + 2\ NO_3^- + 2\ H^+ \rightleftarrows 2.5\ CO_2 + N_2 + 3.5\ H_2O \qquad (1)$$

3 PRESENTATION OF THE COURSE OF THE MISSION

3.1 Material and methods

3.1.1 Study sites and sampling

In this study, the researchers collected a total of 68 sediment samples for analysis (*Figure 7*). These samples were obtained from six main areas located in Jutland, Denmark. Jutland is a large peninsula that forms the mainland part of Denmark.

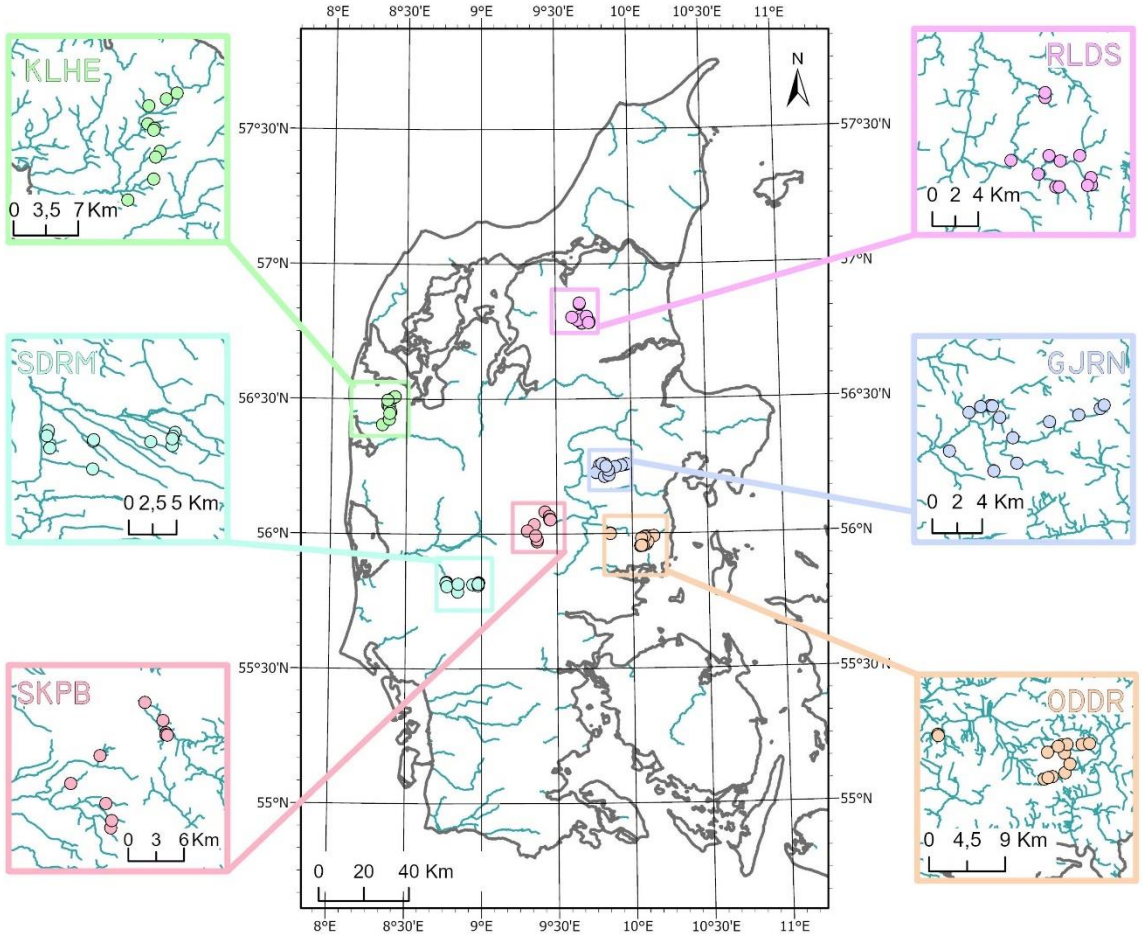

Figure 7: Map of a part of Denmark showing the locations of the different regions/catchments where N_2O samples were collected. The spatial distribution of sampling sites within each region or catchment is shown in the respective inserts. KLHE, Klosterheden; SDRM, SDR. Omme; SKPB, Skaerbaek; RLDS, Roldskov; GJRN, Gjern; ODDR, Odder

Each of these areas is further divided into multiple sample sites, depending on the specific streams of interest. Firstly, the sampling points needed to be situated on small, independent tributaries with a Strahler scale order of 1 or 2. These tributaries were chosen as they are part of monitored rivers, enabling the enrichment of data regarding NO_3^- concentrations within the entire river network. The range of streams selected covers a width of between 25 and 360 cm, have a NO_3^--N concentration of

between 0.01 and 12.50 mg N L^{-1}, a stream water temperature of between 0 and 19.5°C, and a water pH of between 5.0 and 8.3.

West and central Jutland has lower pH values in its topsoil and subsoil compared to the rest of Jutland and islands located east of the peninsula. According to Balstrøm *et al.* (2013), this aligns with the maximum extension of the Weichselian Young Baltic Ice Cap during the last glacial period (*Figure 8 & Figure 9*) (Houmark-Nielsen, 2011). Glacial processes and the deposition of acidic minerals could have had a significant influence on the low pH values observed in the soils of central and western Jutland, which were not covered by the ice cap.

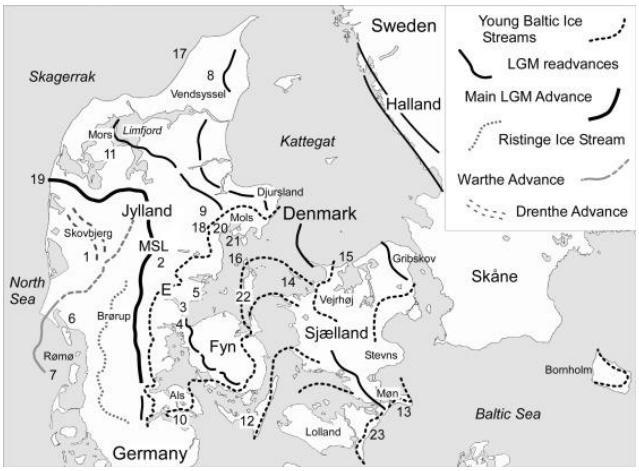

Figure 8: Key map of Denmark and surrounding countries and the distribution of end moraines of Saalian (Drenthe and Warthe), Middle Weichselian (Ristinge ice stream) and the Late Weichselian (Main advance and Young Baltic ice streams) ages (Houmark-Nielsen, 2011)

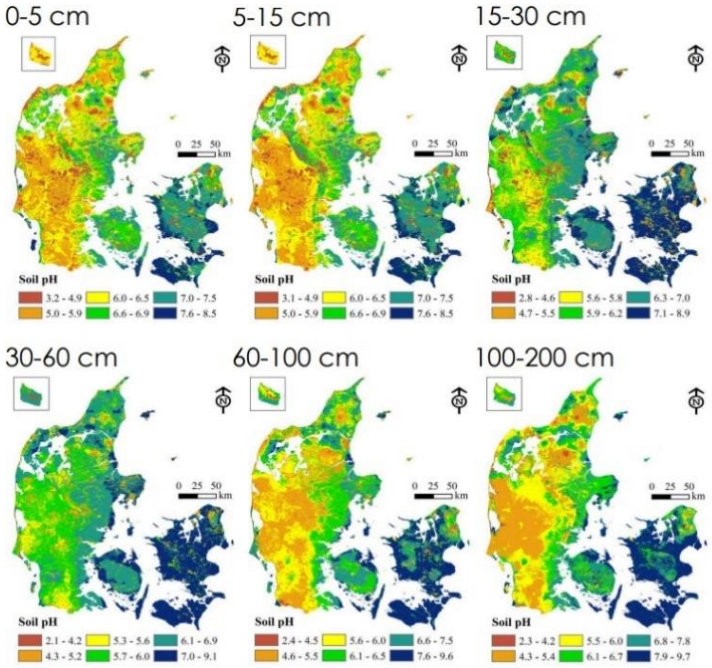

Figure 9: Map of soil pH at different soil depth for Denmark (Adhikari et al., 2014)

The pilot studies focused on two specific sites (*Figure 10*) of those 68 sites, chosen for their contrasting water pH levels but both located in agricultural area in the same monthly monitored area location called "Skaerbaek Plantage (SKBP)". The first site, identified as ID6, was selected due to its acidic water stream with a pH of 5.7. On the other hand, the second site, known as ID8, was chosen as it exhibited a nearly neutral pH of 7.

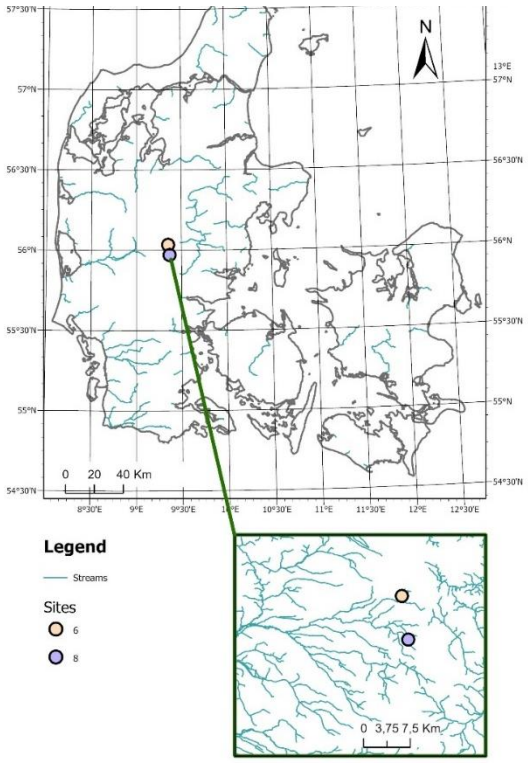

Figure 10: Map of Denmark showing the locations of the two catchments where N_2O samples were collected for pilot studies.

3.1.2 Investigate the effect of pH on N_2O production in stream sediment under anoxic conditions

3.1.2.1 Sediments samples and stream water properties collection

The top 20 centimetres of the river sediment were collected with a kajak core. Water samples were collected for nutrient analysis, filtered, and stored cold until analysis of NO_3^-, nitrit (NO_2), ammonia (NH_4^+), and dissolved organic carbon (DOC). Unfiltered samples were analysed for total nitrogen (TN) and total organic carbon (TOC). Separate water samples (50 mL) for N_2O were collected using a syringe. In the field 10 ml of air was added to the syringe and the syringe was shaken for 1 minute, hereafter the 10 mL headspace was added to a 5.9 mL vial, which were kept dark until analysis. Porewater samples from the stream sediment was extracted using a rhizonsystem (*Figure 11*) and analysed for NO_3^-, NH_4^+, and DOC. Temperature of water, dissolved oxygen, conductivity, and pH were measured using a portable water quality multiparameter instrument (YSI).

Figure 11: Photography of a rhizonsystem in A, a view of site 6 in B, and site 8 in C (©M. VODDER CARSTENSEN, 2023)

3.1.2.2 pH adjustments

Table 1: Comparison table of pilot test criteria for adjusting the pH of sediment slurries from 2 sites (ID6 & ID8)

ID Main pilot study on pH - Date	Criteria	ID6	ID8
1. 22/05/23	**Collection date of sediment slurries**	21-04-23	21-04-23
	pH of sediment slurry	6.55	6.82
	Treatment (base/acid)	**Na_2CO_3 (1M)**	**HCl (1M)**
	[NO_3^-] solution (mgN L^{-1})	**5**	
	pH measurement interval after the experiment (days)	+0, +6	
2. 01/06/23	**Collection date of sediment slurries**	21-04-23	21-04-23
	pH of sediment slurry	6.55	6.82
	Treatment (base/acid)	**Na_2CO_3 (1M)**	**MES**
	[NO_3^-] solution (mgN L^{-1})	**2**	
	pH measurement interval after the experiment (days)	+0, +1	

Experiments were performed in triplicate and controls. In the laboratory, 5 g of moist, homogenized soil was transferred to 20 mL vials. To each vial, 6 mL of NO_3^- (5 mgN L^{-1} or 2 mgN L^{-1}) was added.

In the first pilot pH adjustment experiment (*Table 1*), the pH of sediment from a stream (ID6) with acidic water (pH 5.7) was raised to near neutral (pH 7) by adding 20, 40, and 60 µl HCl (1 M) to each vial, and pH was measured after each application. The pH of the sediment from a stream (ID8) with near neutral water (pH 7) was lowered to about 5 by adding 20 µl Na_2CO_3 (1 M) to each vial, and pH was measured after each application. For control only NO_3^- solution was added.

The second pilot experiment aimed to observe in particular the evolution of the pH of the sediments of a stream (site 8) with near neutral water (pH 7), which was lowered to about 5 by adding 20, 40 and 60 µl of MES to each vial. MES (2-(N-morpholino)ethanesulfonic acid) is a widely used buffering agent in biology and biochemistry (Good et al., 1966). The acid was changed, because we suspected that HCl might affect denitrifiers, and that it is preferable to use a buffer that should keep the pH more stable over time.

After addition of a base or an acid, each vial was vacuumed and rinsed with N_2/He. After a defined number of days, the pH value was measured.

3.1.2.3 Calibration curve of nitrous oxide on gas chromatograph (SRI 310 C)

Gas chromatography is a technique used to separate volatile compounds, which are substances that readily vaporize at room temperature. This separation is achieved by exploiting the interactions between the molecules and two key components: the mobile phase and the stationary phase. Less volatile molecules have stronger interactions with the stationary phase in the column, causing them to move slowly, while more volatile molecules interact more with the mobile phase, enabling them to move faster (Littlewood, 2013). As the components exit the column, they are electronically detected and identified using an electron capture detector.

Figure 12: Photograph of the SRI 310C gas chromatograph

In the initial phase, a pilot study was conducted using the SRI 310C gas chromatograph (*Figure 12*) with manual injection. Calibration curves were generated using standards of known concentrations, enabling accurate quantification of N_2O concentrations in our samples during the experiment.

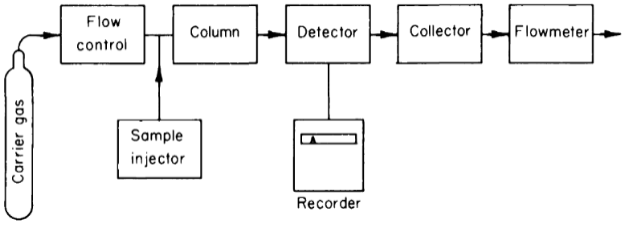

Figure 13: Block schematic diagram of apparatus for analytical gas chromatography (Littlewood, 1970)

It was observed that measurements of the same standard occasionally yielded different peak areas from one injection to another. This variation could be attributed to potential inaccuracies in the handling process. To mitigate any errors, a fresh calibration curve was constructed each time the chromatograph was restarted before any measurements were performed. An R^2 of at least 0.99 is expected in order to consider the calibration curve as good *(Figure 13*).

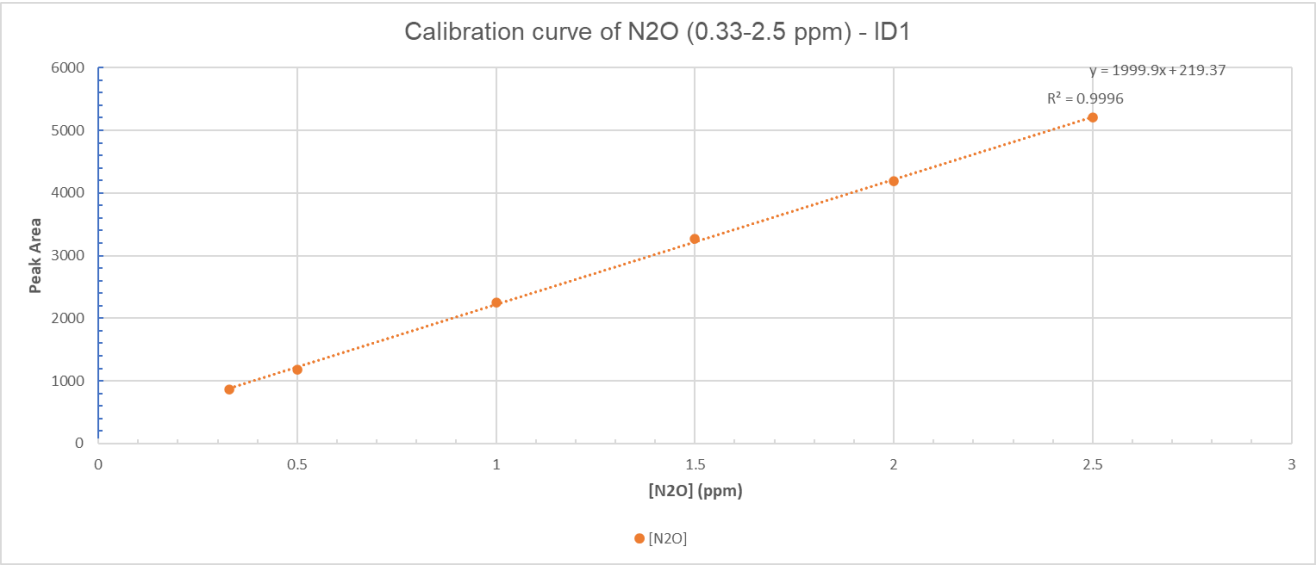

Figure 14: Calibration curve of N_2O on SRI 310C gas chromatograph

3.1.2.4 Sediment incubation experiments

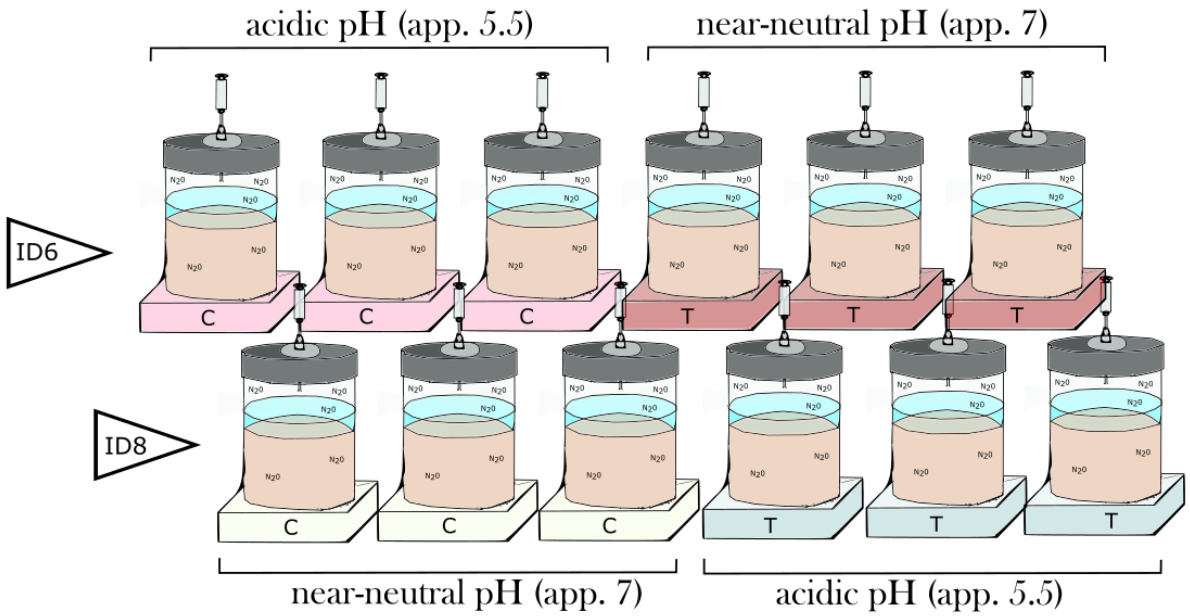

Figure 15: Simplified illustration of our measurement technique setup for N_2O: laboratory incubation of sediment and measurement of gaseous N_2O in headspace (C: Control, T: Treatment).

Table 2: Comparison table of pilot test criteria for sampling interval of air samples from pH adjusted incubations of sediments slurries from 2 sites (ID6 & ID8)

ID pilot study - Date	Criteria	ID6	ID8
1. 22/05/23	**Collection date of sediment slurries**	21-04-23	
	pH of sediment slurry	6.55	
	Treatment (base/acid)	**Na_2CO_3 (1M)**	
	[NO_3^-] solution (mgN L^{-1})	**5**	
	Air sample collection (hours after the start of the experiment)	**2, 4, 6, 8, 24, 30, and 126**	
	Air sample analysis - Gas chromatograph	**SRI 310 C – ECD detector (N_2O)**	
2. 14/06/23	**Collection date of sediment slurries**	13-06-23	13-06-23
	pH of sediment slurry	5.85	6.45
	Treatment (base/acid)	**Na_2CO_3 (1M)**	**MES**
	[NO_3^-] solution (mgN L^{-1})	**2**	

	Air sample collection (hours after the start of the experiment)	2, 4, 6, and 8	
	Air sample analysis - Gas chromatograph	**Agilent 7890A GC – ECD detector (N_2O), TCD (CO_2) and FID (CH_4)**	
3. 03/07/23	**Collection date of sediment slurries**	13-06-23	13-06-23
	pH of sediment slurry	5.81	5.58
	Treatment (base/acid)	**Na_2CO_3 (1M)**	**MES**
	[NO_3^-] solution (mgN L^{-1})	**2**	
	Air sample collection (hours after the start of the experiment)	0.5, 1, 2, 3, 4, 6, 8, 10, 24, 30	
	Air sample analysis - Gas chromatograph	**Agilent 8890 GC – ECD detector (N_2O), TCD (CO_2) and FID (CH_4)**	

On the first occasion (*Table 2*), incubation experiment was performed in triplicate and controls with sediments from ID6 collected in April 2023. To obtain sediment slurries, 5 g of fresh sediment were placed in a 20-ml glass vials in a glove bag (*Figure 16*) filled with N_2 to achieve anoxic conditions, and the headspace was purged with N_2 gas for at least 5 min. Experiments were started by adding 6 ml of a NO_3^- solution (5 mg N L^{-1}) and a base (Na_2CO_3, 1 M) to adjust the pH and then capped with a rubber stopper and sealed with an aluminum cap. To be sure of anoxic conditions, the headspace was vacuumed and then purged with N_2. Slurries were then placed on a shaking table (130 rpm) and incubated at 22 °C for 126 hours. Air samples were collected 2, 4, 6, 8, 24, 30, and 126 hours after the start of the experiment and injected directly into a SRI 310 C gas chromatograph (GC) to analyze N_2O concentration with an electron capture detector.

Figure 16: Experimental manipulation in a glove bag under anoxic conditions (©M. VODDER CARSTENSEN, 2023)

In a second experiment (*Table 2*), a different GC instrument (Agilent technologies 7890A) solution was used to focus on the first hours of incubation, with the aim of reaching the nitrogen limits faster by reducing the concentration in the solution. The sediments for this study were collected in the day before the experiment (in June), and in this experiment vials with sediment from ID8 was also included. The setup was the same as stated above, despite that 6 mL of a NO_3^- solution of 2 mg N L^{-1} was used. For ID 8, 60 µL MES was used to lower the pH. After sealing, the headspace was vacuumed and then purged with helium gas (He). Air samples were collected 2, 4, 6, and 8 hours after the start of the experiment and injected directly into a GC (Agilent 7890A GC), which uses an electron capture detector for N_2O concentration analysis, flame ionization detector for methane detection and a thermal conductivity detector for CO_2 analysis.

In a third experiment (*Table 2*), a different GC instrument (Agilent technologies 8890A) was used. The aim was to have more air sample collection during first hours of incubation but also after 8 hours, always with the aim of reaching the nitrogen limits faster by keeping a low concentration of NO_3^-. The sediments for this study were collected in June (13/06/2023). The setup changed for 1L bottles filled with 150g of sediments and 110 mL of a NO_3^- solution of 2 mg N L^{-1}. For ID 8, 250 µL MES was used to lower the pH but also 300 µL Na_2CO_3 in "controls" bottles to increase pH to near-neutral because sediment pH was lower than expected. For ID 6, 350 µL Na_2CO_3 was used to increase pH. After sealing, the headspace was vacuumed and then purged with helium gas (He) for 10 minutes. Air samples were collected 0.5, 1, 2, 3, 4, 6, 8, 10, 24 and 30 hours after the start of the experiment and injected directly into a GC (Agilent 8890A GC).

At the end of incubations, pH and NO_3^- were measured. NO_3^- concentration was analyzed using ion chromatography.

3.1.2.5 Statistics

To assess the significance of the concentration difference between the control and treatment samples, we employ the null hypothesis (H0): the data distributions for both cases (control and treatment) are similar. In other words, altering the sediment pH does not result in a significant variance in N_2O emissions under anoxic conditions.

On the other hand, the alternative one-sided hypothesis (Hα) suggests that the data distributions of the control and treatment cases are dissimilar. This implies that adjusting the sediment pH leads to a notable difference in N_2O emissions under anoxic conditions.

The objective of our study is to compare two related quantitative variables with a sample size (N) of less than 30, which is the case for our study with a N of 4 for experiment n°2 (*Table 2*). Each site yields a pair of samples (control and treatment) obtained from the same sediment location, establishing a matched comparison. To analyze this data, we employ the Wilcoxon paired test, a non-parametric statistical test for comparing two samples. The chosen significance level (α) was 0.05, indicating the threshold for accepting or rejecting the null hypothesis.

3.1.3 Dry matter content and loss on ignition determination

The 68 sediment samples collected in April were stored in a cold and dark room in a zip lock bag until analysis. For each sample, approximately 5 g of wet, homogenized soil was oven dried at a temperature of 105 °C for 48 hours, then cooled in a desiccator (*Figure 17*) and weighed for dry matter content (DMC) calculated as follows:

DMC (%) = [soil weight after oven dried / initial soil weight] × 100

Moisture content (%) = 100 - DMC

The loss on ignition (LOI) method is commonly used to determine the organic content of soil or sediment samples. To calculate LOI, the dried samples were then burned in a muffle furnace (*Figure 17*) at 550 ◦C for a period of 2 hours. After combustion, the samples were again cooled in a desiccator and weighed.

The following equation (Schulte & Hopkins, 1996) was used to calculate the percentage of soil organic matter determined by the loss-on-ignition method (SOM_{LOI}):

SOM_{LOI} = [(soil weight after combustion - oven-dry soil weight) / oven-dry soil weight] × 100

Figure 17: Photography of a desiccator in A, sediments samples in the oven for dry mass in B and the muffle furnace in C

3.1.4 Carbon-to-nitrogen (C:N) ratio determination

For each site, 20 g of sediments were oven-dried for 2 days at 105°C and milled using the Retsch ® oscillating mill MM400 for 2 min at 30 Hz within seconds. For each site, triplicates of 10 mg sub-samples were weighed in tin capsules and then analyzed for total N (TN) and total C (TC) using a Thermo Scientific FlashSmart elemental analyzer. The total organic carbon (TOC) determined by oxidation with HCl.

4 RESULTS AND DISCUSSION

4.1 Influence of pH on N_2O production in stream sediment under anoxic conditions

4.1.1 Experiment number 1

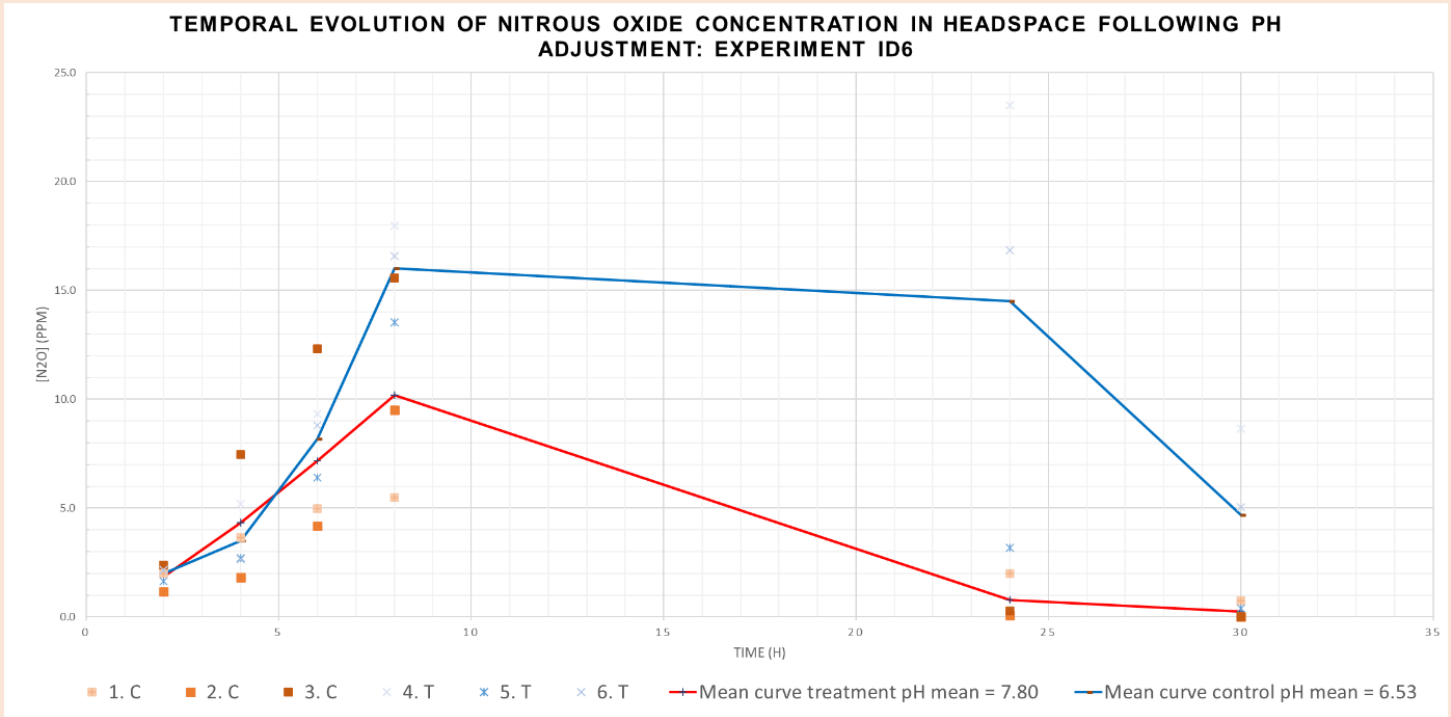

Figure 18: Temporal evolution of nitrous oxide concentration means following pH adjustment (T) and no pH adjustment (C) of sediments from site ID6

We observed a faster increase in N_2O concentration at a more acidic pH (control samples) between 2 and 8 hours, with a decrease in concentration in both cases between 8 and 24 hours (*Figure 18*). This decrease was lower at acidic pH (controls) compared to basic pH (treatments). We noted some heterogeneity among the triplicates in terms of concentration.

It could be assumed that NO_3^- became limiting between 8 and 24 hours in both cases. The kinetics of NO_3^- transformation into N_2O did not seem to present the same trend at these two different pH levels. Quantitatively, at a lower pH, the N_2O concentration appeared to have significantly increased in the first 8 hours, which could have meant that N_2O accumulated in the interstice but was not rapidly transformed into N_2, unlike at a higher pH. Therefore, it would be interesting to decrease the concentration of the NO_3^- solution so that this reagent would become limiting more quickly, and then precisely observe the dynamics of N_2O conversion to N_2 at different pH levels.

4.1.2 Experiment number 2

The results obtained from both sites indicate that N_2O concentrations were consistently higher at more acidic pH levels (*Figure 19*). Notably, low pH samples from site 6 (controls) exhibited lower emissions compared to those from site 8 (treatments) (*Figure 19*). What is also evident for both sites were the substantial variation in emissions observed among the triplicate samples (*Figure 20 & Figure 21*).

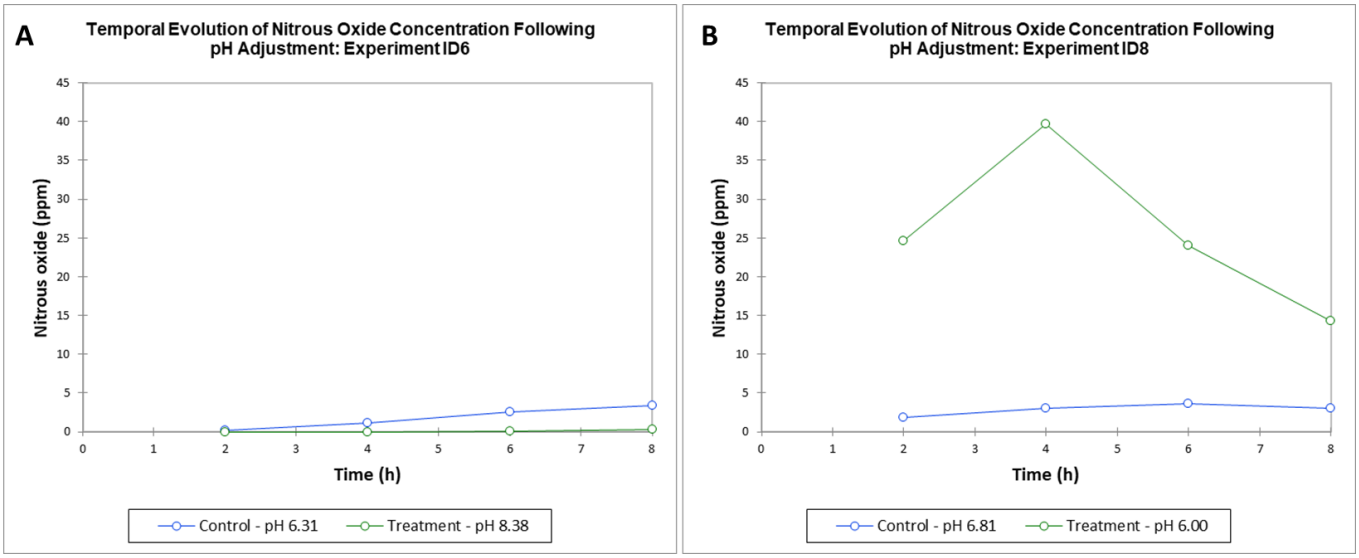

Figure 19: Comparison between ID6 (A) and ID8 (B) of temporal evolution of N_2O concentration means following pH adjustment (treatment) in experiment no. 2.

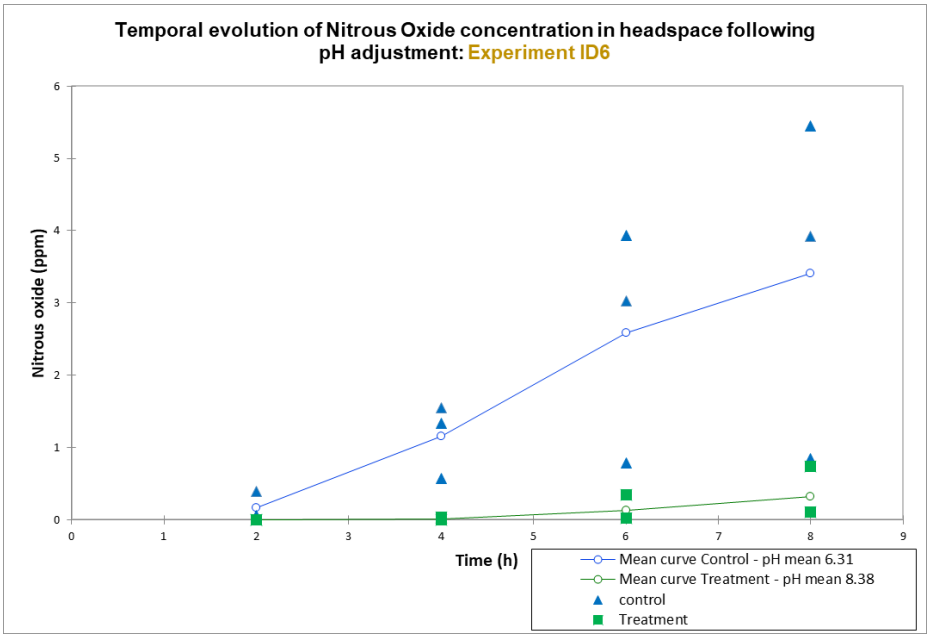

Figure 20: Temporal evolution of N_2O concentration means following pH adjustment (treatment) and no pH adjustment (control) of sediments from site ID6

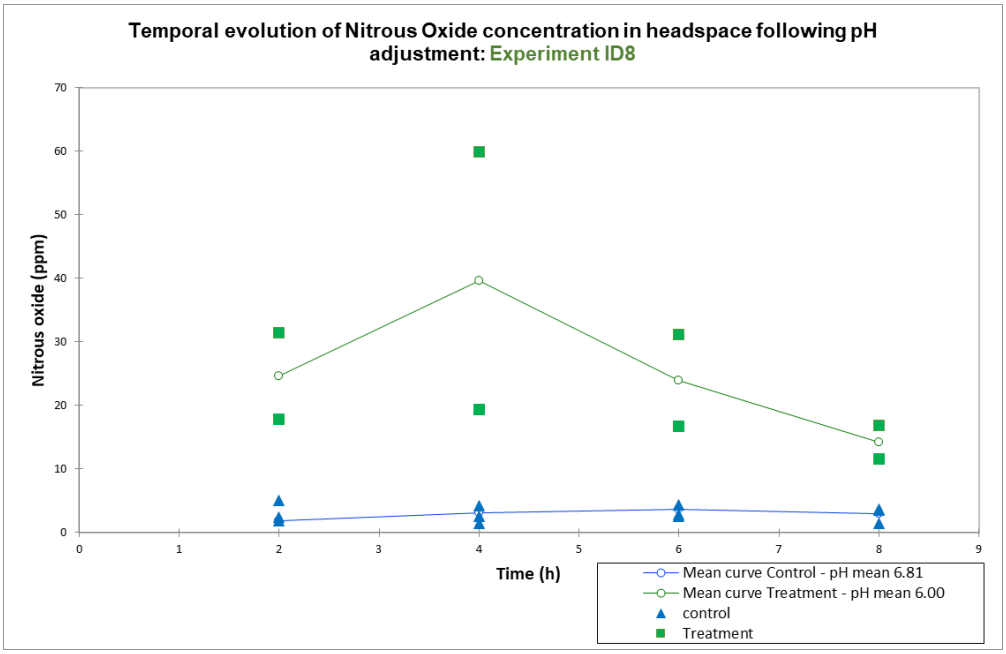

Figure 21: Temporal evolution of N_2O concentration means following pH adjustment (treatment) and no pH adjustment (control) of sediments from site ID8

Furthermore, after an 8-hour incubation period, N_2O concentrations continued to rise for sediments in site 6 (*Figure 20*). For this site, we can also observe negligeable N_2O emissions during the initial 6-hour period at basic pH (approximately 8.38 as a mean). Specifically, at site 8, the N_2O concentration exhibited an increasing trend until approximately 4 hours, whereas for the control sample at a more neutral pH, a slight increase was observed until around 6 hours (*Figure 21*). However, after these respective time points, a subsequent decrease in N_2O concentrations was observed.

Table 3: Wilcoxon signed-rank test performed with XLSTAT

Sediment ID	Variable\Subsamples	Sign test	Wilcoxon signed-rank test
ID6	control - Treatment	0.125	0.125
ID8	control - Treatment	0.063	0.063

Given that p(obs) > α (*Table 3*), we accept the null hypothesis (H_0), indicating that the adjustment of sediment pH does not lead to a significant difference in N_2O emissions under anoxic conditions. However, it is worth considering that the lack of significance may be attributed to the limited number of samples (n=4), raising questions about the statistical robustness. To enhance future data acquisition, it is recommended to improve the protocol by measuring the concentration before the 2-hour mark to observe the kinetics at the start of incubation. Additionally, extending the measurement duration beyond 8 hours is advisable, as the N_2O formation kinetics might not be complete for a NO_3^- concentration of 2 mg N L^{-1}. We can suggest that the NO_3^- is still being consumed, as indicated by the continuously increasing concentration of N_2O after 8 hours.

Despite the lack of statistical significance, it is noteworthy that higher N_2O emissions were observed for samples with low pH in both cases, suggesting an incomplete denitrification process. These results indicate that soil pH may influence the $N_2O/(N_2+N_2O)$ ratio by impeding or delaying the expression of

N_2O reductase of denitrifiers, partially inhibiting reduction of N_2O to N_2 (Audet et al., 2020; Bakken et al., 2012). The N_2O-reductase enzyme is typically located in the periplasm of the cell, which is considered a weakly buffered zone. As a result, its functionality and synthesis are hindered or reduced at low acidic pH levels (Qu et al., 2014).

Table 4: Average change in pH of samples from incubation experiment (no. 2)

Soil ID	pH start (after adding base or acid and nitrate)	pH end (2 days after the end of the experiment)
6 - Control	6.31	6.35
6 - Treatment	8.38	7.41
8 - Control	6.81	6.68
8 - Treatment	6.00	6.14

As explained by Rajta *et al.* (2020), neutral to alkaline pH (7–8) is considered most optimal for denitrification, which could explain low N_2O emissions in treatment samples from site 6 in comparison with site 8 because pH started and ended basic (*Table 4*). Variation in pH from this range (7–8) decreases the denitrification efficiency of bacteria.

We observed elevated N_2O concentration in the acidic treatment samples from sediment 8 compared to the relatively acidic pH control samples from sediment 6 (*Figure 19*). It is not surprising considering that N_2O is generated through biological processes, that emission rates can be influenced by different limiting factors in different environments (*Table 5*).

Table 5: Streams properties of sampling points ID 6 and ID 8

	ID6 – 13/06/23	ID8– 13/06/23
Type of area	Agricultural Area	Agricultural Area
Strahler order	2	1
[NO_3^- -N] (mg N L^{-1})	1.3272	0.2233
pH Water	5.32	6.45
DMC (%)	75.98	55.57
LOI (%)	1.02	3.33

In fact, measurements conducted on sediment samples in June revealed different NO_3^- concentration and organic matter content in stream 6 and 8 (*Table 5*). According to Wrage *et al.* (2001) the N_2O/N_2 ratio increases when NO_3^- is abundant, as NO_3^- serves as a preferential electron acceptor compared to N_2O. In the treatment sample 8, it can be assumed that a significant portion of the NO_3^- has been consumed after approximately 4 hours, and the N_2O in the interstitial space, under the

experimental conditions, may begin to be utilized and reduced to N_2, fulfilling its role as an intermediate. This raises questions about the inhibition of Nos reductase. As suggested by Jones *et al.* (2014), pH may indirectly influence the emission ratio by regulating the composition of soil denitrifying communities.

The observed variability in emissions among triplicate samples can be attributed to heterogeneities in sediment properties within the same site. These variations in sediment characteristics likely contribute to the differences in N_2O emissions observed, highlighting the importance of considering the spatial but also temporal heterogeneity of sediments properties when analyzing and interpreting emission data.

4.1.3 Experiment number 3

This new experiment confirmed the trends observed in experiment number 2. Higher concentrations of N_2O were observed at more acidic pH values for both sites (blue curves) (*Figure 22 & Figure 23*). However, the average concentration curves at different pH values showed similar trends of evolution. For example, a sharp decrease in N_2O concentration was observed between 6 and 8 hours for site 6 (*Figure 22*) and between 2 and 3 hours for site 8 (*Figure 23*). After 8 and 6 hours, respectively, the average N_2O concentrations at different pH values were relatively similar. It appeared that the kinetics of N_2O decrease was faster at acidic pH for both sites (*Figure 22 & Figure 23*).

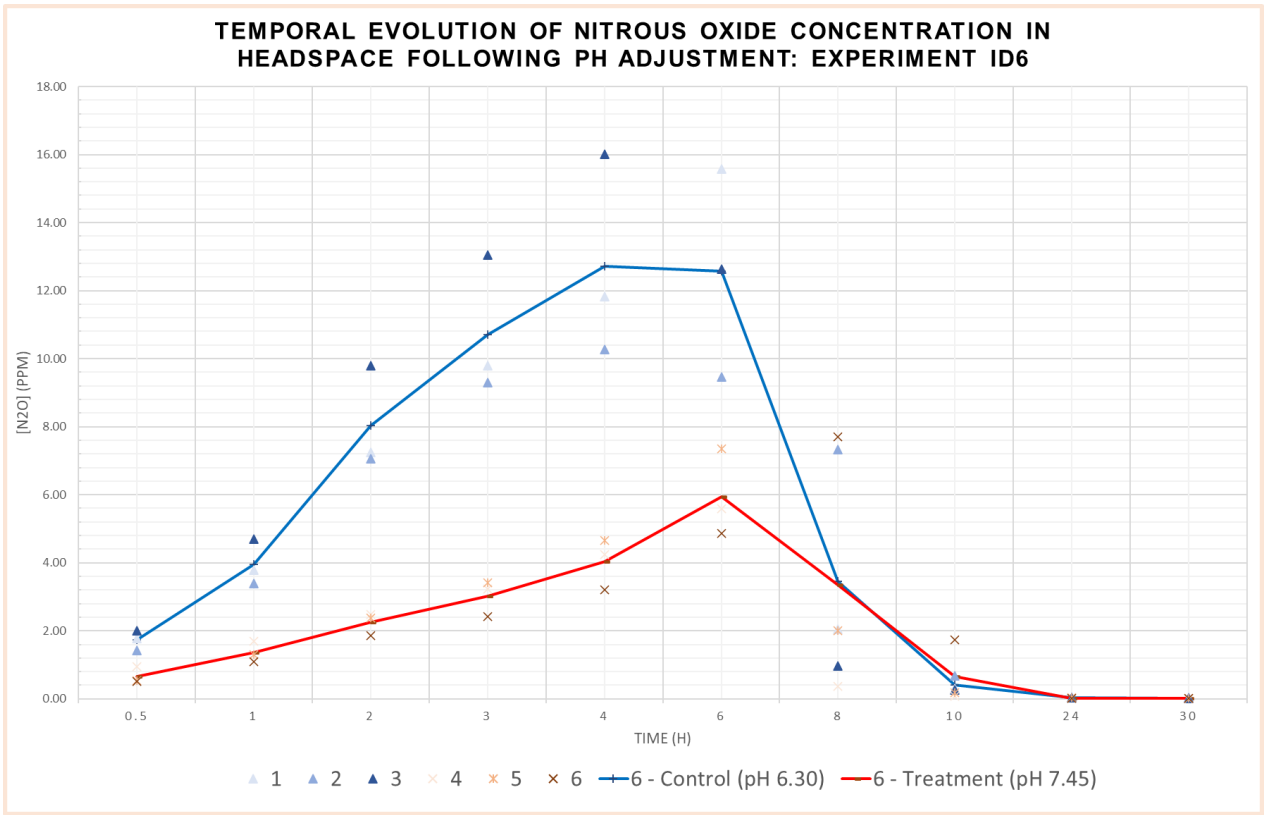

Figure 22: Temporal evolution of N_2O concentration following pH adjustment (treatment) and no pH adjustment (control) of sediments from ID6, curves represent means of triplicates measurements.

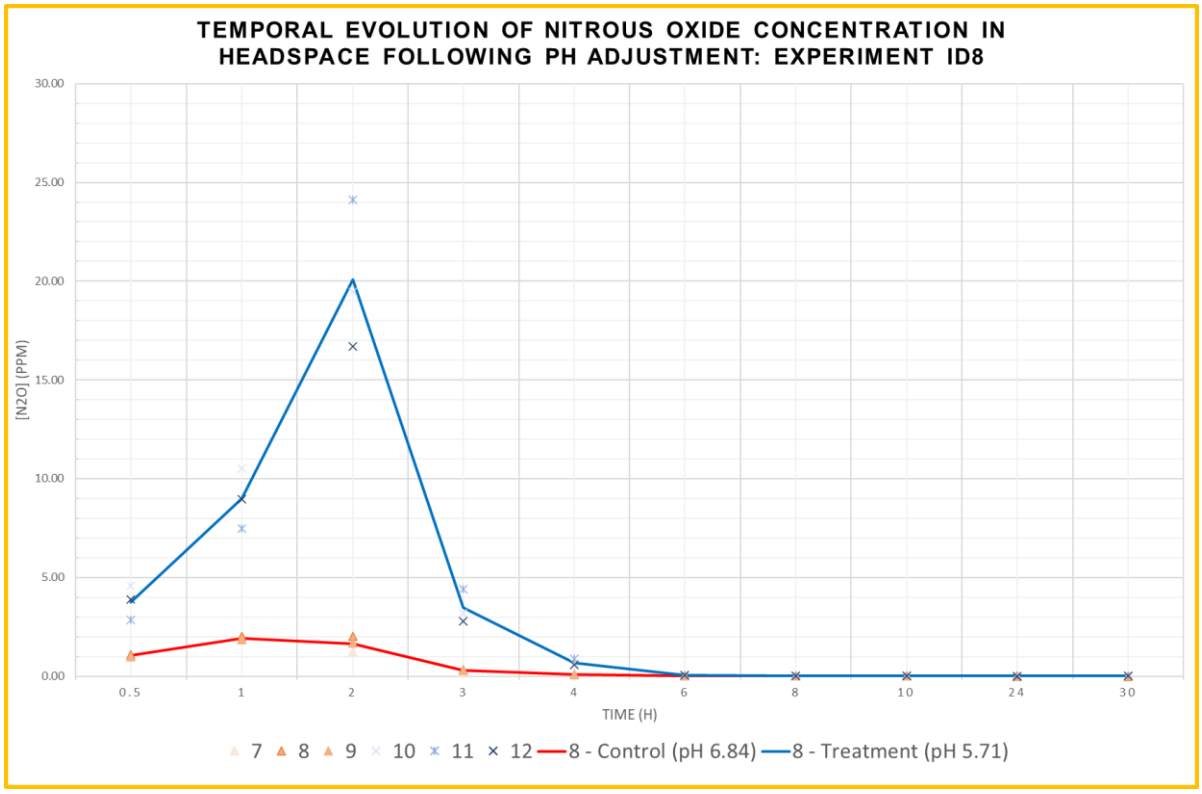

Figure 23: Temporal evolution of N_2O concentration following pH adjustment (treatment) and no pH adjustment (control) of sediments from ID8, curves represent means of triplicates measurements.

Regarding the pH variation of the samples between the beginning and the end of the experiment, most of them became more acidic, except for the acidic samples from sites 10-12, which became more alkaline (*Table 6*).

Table 6: pH variation of the different samples at the start and end of the experiment (1-3: site 6 controls; 4-6: site 6 treatments, 7-9: site 8 controls; 9-12: site 8 treatments)

Sample ID	pH start (after adding base or acid and nitrate)	pH end (mean)	pH variation
1	6.36	6.01	-0.35
2	6.31	5.95	-0.36
3	6.24	5.98	-0.26
4	7.53	7.11	-0.42
5	7.32	6.97	-0.35
6	7.49	7.01	-0.48
7	7	6.60	-0.40
8	6.72	6.62	-0.10
9	6.81	6.61	-0.20
10	5.77	5.99	0.22
11	5.65	6.04	0.39
12	5.7	6.06	0.36

Respiratory activity (measured by CO_2 accumulation) was lower at acidic pH values (blue curves) compared to more alkaline pH values (red curves) (*Figure 25 & Figure 24*).

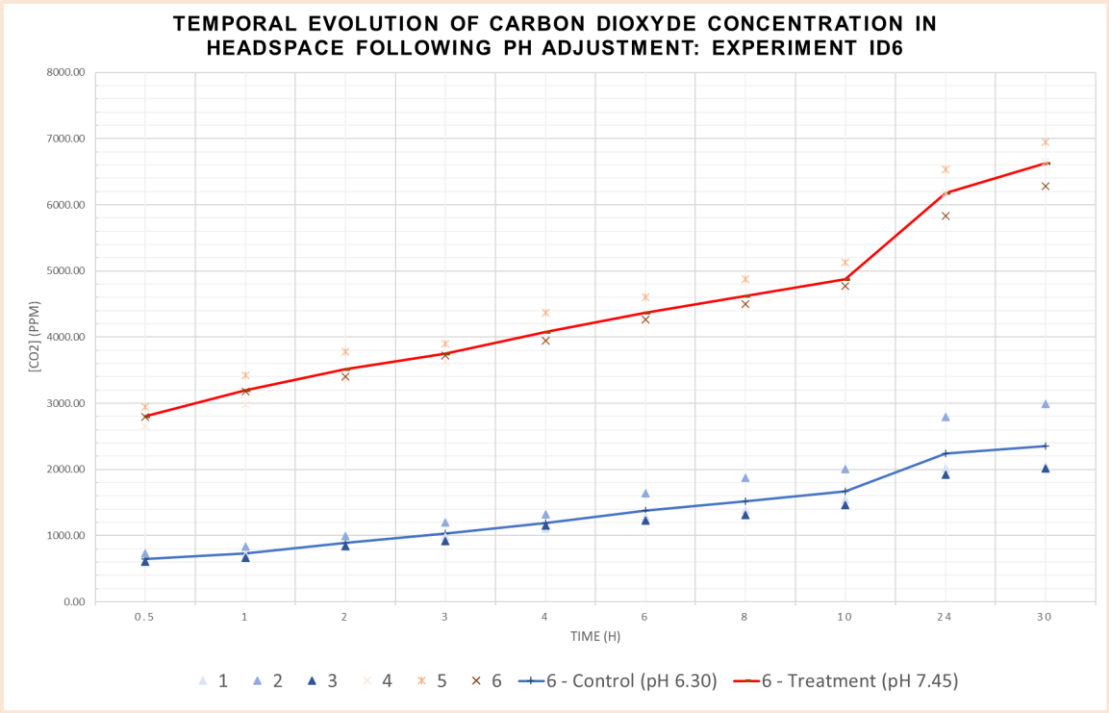

Figure 25: Temporal evolution of CO_2 concentration following pH adjustment (treatment) and no pH adjustment (control) of sediments from ID6, curves represent means of triplicates measurements.

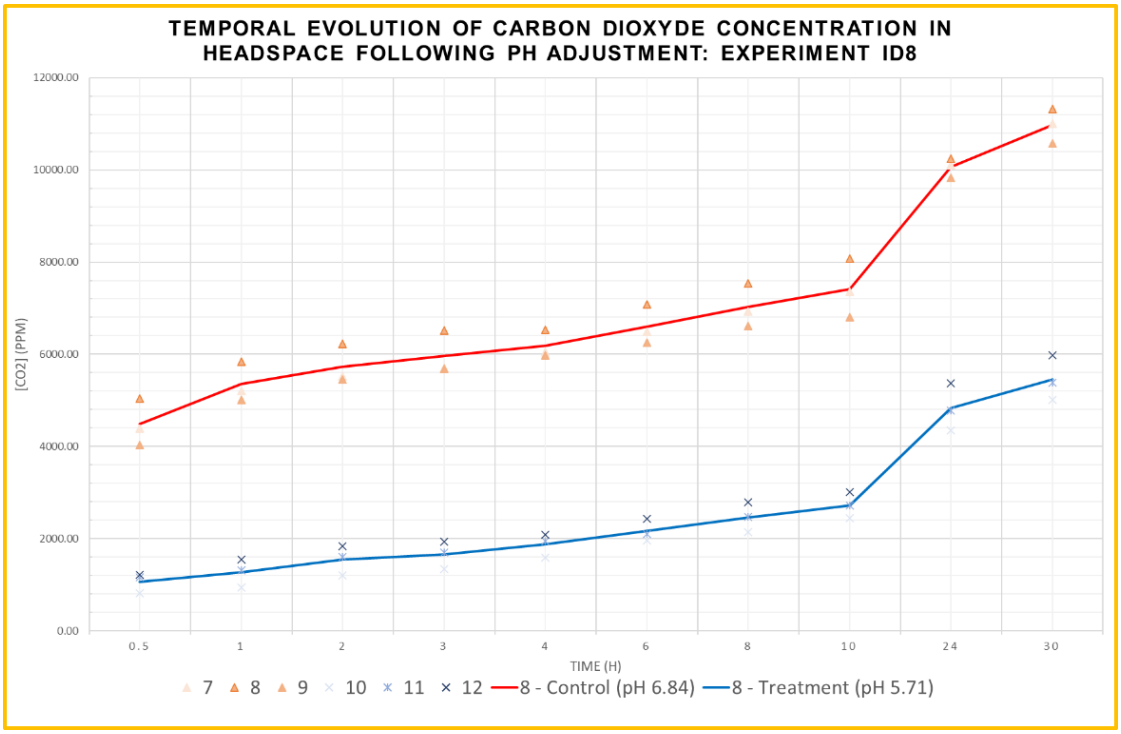

Figure 24: Temporal evolution of CO_2 concentration following pH adjustment (treatment) and no pH adjustment (control) of sediments from ID8, curves represent means of triplicates measurements.

Samples with lower pH values showed a lower NO_3^- concentration at the end of the experiment compared to more neutral/alkaline samples. This trend was more pronounced for site 8.

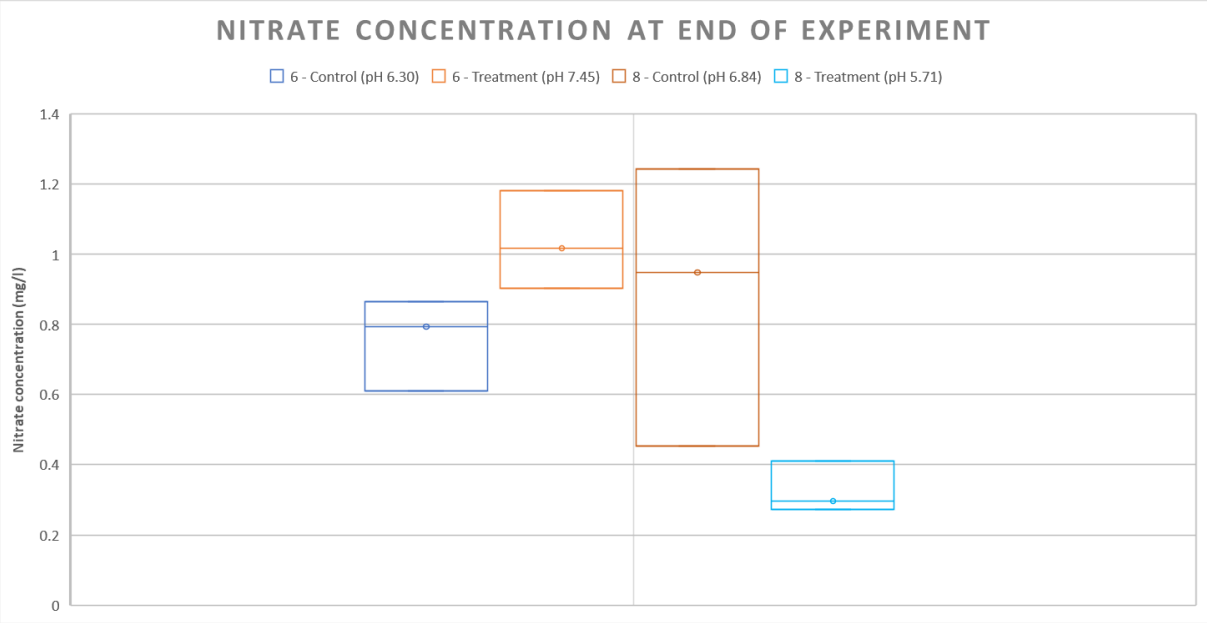

Figure 26: Box plots showing a comparative analysis of final NO_3^- concentrations in experiment samples

This study indicates that pH seems to directly control the $N_2O/(N_2O + N_2)$ ratio of denitrification and, consequently, N_2O emission rates from sediments. A higher accumulation of intermediates at low pH is suggested, which is consistent with previous studies that found clear effects of pH on the accumulation of intermediates in denitrification (Bergaust et al., 2010; Liu et al., 2010, 2014).

Overall, it appears that denitrifying activity in the sediments maintains a significant level of functionality over a wide pH range, suggesting a high functional versatility of the underlying microbial community. To verify this, it would be relevant to measure the changes in population size and diversity of microbial communities in the sediments during the experiment. Reduction of N_2O into N_2 seems to be operative for the treatment samples from site 8 at acidic pH, suggesting the presence of bacterial communities capable of carrying out the reduction. Thus, the occurrence of N_2O reduction in acidic samples can be explained by denitrification activity in neutral microsites as proposed by Liu *et al.* (2014) or by the presence of acid-tolerant denitrifiers in neutral soils. This agrees well with the functional redundancy hypothesis that distinct species perform similar roles in communities and ecosystems under different environmental conditions, and may therefore be substitutable with little impact on ecosystem processes (Rosenfeld, 2002).

The temporal difference in N_2O increase and decrease between sites can also be explained by the organic carbon content. When organic carbon is insufficient as an electron donor, NO_3^-, which is more oxidizing than N_2O, becomes an electron acceptor, preventing N_2O from being reduced to N_2 (Wrage et al., 2001).

The temporal dimension can also play an important role in the variability of responses between sites. The nosZ gene encoding N_2O reductase plays an important role in controlling the reduction of N_2O to N_2 (Shan et al., 2021). Chen *et al.* (2012) found that long-term application of high NO_3^- fertilizer increased the abundance of nosZ genes. Therefore, the temporal study over years and seasons of NO_3^- exposure in the hyporheic zone is a potential key factor that can significantly influence denitrifying microbial communities' compositions and their capacity to reduce N_2O. It can be hypothesized that

the hyporheic zone of site 8 is more exposed to high and regular NO_3^- levels, resulting in a genetically more adapted bacterial community, a more diverse population of microbes that are capable of sustaining a process (e.g. N_2O reduction) over a range of conditions (Samad et al., 2016).

4.2 Prospects for applying the research

The calculation of N_2O emissions from nitrogen leaching and runoff in Denmark is currently based on the IPCC model and a national model. Nitrogen, which is transported through the soil, can be transformed into N_2O. The IPCC recommends using an N_2O emission factor of 0.0075 kg N_2O-N/kg N, with 0.0025 for leaching to groundwater, 0.0025 for transport to watercourses (referred to as rivers in the IPCC definition), and 0.0025 for transport to the sea (IPCC, 2006). The N_2O emission from nitrogen leaching is the sum of emissions for the three parts, calculated as follows (Nielsen et al., 2022):

N_2Oleaching = (Nleachground * EFleach + Nleachrivers * EFrivers + Nleachestuaries * EFestuaries) * 44/28

These studies, along with further research, will eventually allow for the refinement of these three different N_2O emission factors by considering the water pH. Ultimately, this can contribute to a more accurate inventory of N_2O emissions in Denmark. This may also lead to localized regulations regarding the application of NO_3^- in areas where streams have lower pH levels. This would particularly apply to the central and western regions of Jutland, for example. Therefore, there is a prospect of improving agricultural practices.

5 REFLECTIVE FEEDBACK ON THE EXPERIENCE

5.1 Approach, methods used, results produced

The implementation of pilot studies proved to be interesting due to the multitude of parameters influencing denitrification, making it challenging to anticipate certain kinetics. This justifies the progressive adjustments made to determine the ideal time intervals for gas sample collection and optimal NO_3^- concentration to observe the process variations at different pH levels most effectively. This allowed to test and compare different experimental approaches.

Regarding the experiment itself, it is highly likely that most of the changes induced by pH adjustments occur at the physiological, compositional, or size levels of the microbial communities in the samples. Therefore, it would be interesting to study the ecology of the species performing these transformations at the core of denitrification. For instance, observing the adaptability of sediment microbial populations over a longer period (weeks, months, years) to NO_3^- input and pH changes would help determine whether the new ecological niche created by acidification or alkalization promotes the emergence of new populations or adaptations that fulfill similar functions as the original populations. The results suggest that denitrification functionality is not completely inhibited by pH modification but rather slowed down. However, it remains to be seen if this slowdown is likely to result in massive gas emissions into the atmosphere which depends on gas diffusion in the hyporheic zone in real conditions, necessitating further information on the parameters influencing N_2O diffusion in these compartments of streams. According to Chapuis-Lardy *et al.* (2007), N_2O consumption tends to increase under conditions that hinder N_2O diffusion in soils.

Another potential bias in our experiment arises from the fact that sediment samples were collected from a range of depths and then mixed. This mixing may have brought together denitrifying microbial communities that exploit different niches and subjected them to conditions that are not their natural habitats. For examples, it has been showed that strict aerobes possessing functional nosZ from Clade II, may only start reducing N_2O after transitioning from oxic to anoxic conditions (Park et al., 2017). This N_2O reduction would serve as an electron sink that allows aerobic organisms to survive transient anoxic periods when oxygen is unavailable (Park et al., 2017). Changes in conditions such as temperature and oxygen can, therefore, lead to different results compared to those observed in real conditions. This bias is mainly due to the practical impossibility of conducting this experiment in-situ. Thus, this experiment may not only study the influence of pH on denitrification but also the adaptation of communities to new conditions. However, it can be reassuring to consider that this disturbance is similar for all samples. Nevertheless, it is challenging to assert that what we observe in our new conditions will also occur in real conditions.

5.2 Projection in a profession

I have a strong interest in this dimension of investigation that encompasses the spirit of research and understanding of the initial environment to apply appropriate solutions. I have also observed that teamwork is the key to well-being, if everyone is committed, and communication is good.

What struck me particularly in research field is the complexity and the need for time and resources to understand such mechanisms. This reality evokes a deep sense of injustice when I see how quickly certain chemicals or practices are allowed, making understanding even more complex and demonstrating the harmfulness of certain practices even more challenging.

In my opinion, the reasoned use of technology is crucial to preserve the purpose of research. I have noticed that many studies now rely on data provided by new satellites, enabling a better understanding of biological processes. However, I also perceive a danger in this trend, which legitimizes a dangerous and destructive space market, resulting in significant environmental costs associated with satellite deployment. This internship has reinforced the reflection I nurture for several years about the place of technology. For instance, during GC work, I faced two technological choices. The first chromatograph was an easily repairable model, relatively easy to handle, which could be easily mastered but allowed for more human error. On the other hand, there were fully automated models where humans only need to program but cannot be repaired in case of issues, requiring expensive intervention from the manufacturer's technician. That's why the functional economy model can be interesting in this case. The company would provide chromatographs, and the laboratory would pay for each use of the service. Thus, the company would be responsible for maintenance, proper functioning, and adequate user training. They would have every interest in minimizing breakdown frequency and striving for machine durability.

As I shape my professional trajectory, I am now eager to engage in projects within a company or organization involved in the development of soil restoration initiatives in both urban and rural settings, encompassing project management and project owner assistance. The values I want prioritize revolve around the utilization of appropriate technologies that foster nature-based solutions and promote solidarity. Soils, as a vital ecosystem, offer compelling professional opportunities given the myriad challenges they pose in realms such as agriculture, housing, transport, energy, and beyond.

CONCLUSION - ASSESSMENT OF THE INTERNSHIP

This internship has confirmed my ability to apply the technical and theoretical skills I acquired during my academic studies in a professional context. I am proud and grateful to have had the opportunity to contribute to the DrivNOS project. A better understanding of the pH's influence on nitrous oxide emissions from hyporheic zones in small streams will enable the adaptation of agricultural practices and refine national greenhouse gas inventories. It makes sense to me in a context where humans need to reduce their impact on the environment, and that's what motivated me to actively contribute to this project.

In practical terms, I utilized my skills in geographic information systems (GIS) tools, field and laboratory measurement instruments, and laboratory analysis to acquire and visualize data. I also began analyzing this data and comparing it with existing scientific literature to derive meaningful information.

From a human perspective, this international experience allowed me to work on cultivating open-mindedness, perseverance, initiative, and risk-taking while enhancing my language skills. It is now evident to me that curiosity, coupled with a solid theoretical foundation and awareness of possibilities, is the key to integrating into any project.

I am certain that this experience in biogeochemistry will enhance my holistic approach by providing a better understanding of certain cycles. I believe this perception is crucial for driving the evolution of practices while avoiding the transfer of impacts. Through the implementation of an experimental method during this internship, I have laid the foundation for conducting future projects with rigor and thoroughness, aiming to promote ecosystem resilience.

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ABSTRACT

During my 4th year internship at Polytech Tours' Engineering School, I worked as a research assistant at the Institute for Ecoscience, University of Aarhus in Denmark. Under the guidance of researchers Mette Vodder Carstensen and Joachim Audet, I contributed to a project studying N_2O emissions in small streams. The project DrivNOS aims to understand the factors affecting emissions and develop mitigation measures. My role involved helping to formulate and process an experimental protocol to verify a hypothesis and collecting sediment property data from research sites. This experience aligns with my goal of implementing sustainable practices in agriculture and understanding biogeochemical processes in ecosystems.

KEYWORDS:

Biogeochemistry / Nitrous oxide / Streams / Land use / Sediments properties / Wetlands

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