A FIELD STUDY COMPARING SOIL PROFILE N_2O CONCENTRATION WITH SURFACE FLUX UNDER DIFFERENT FARMING PRACTICES

By

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ABSTRACT

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Existing research has consistently demonstrated a significant increase in N_2O emissions during the spring thaw period. However, understanding the precise influence of farming practices such as cover crops and enhanced efficiency nitrogen fertilizers (EENFs) on N_2O dynamics—production, diffusion, consumption in the soil profile, and surface emissions—remains limited, particularly in clay soil in Manitoba. A two-year field study was conducted in 2022 and 2023 on clay soil at the TGAS-MAN research station in Manitoba to investigate the impact of different farming practices [cover crop and enhanced efficient nitrogen fertilizers (EENFs)] on soil profile N_2O concentrations at 5, 15, 30 and 60 cm of soil profile in relation to surface flux. The research site was divided into four 4-hectare fields, each measuring 200 meters by 200 meters. Six modified silicone diffusive equilibrium samplers were installed in each field to collect soil profile gas samples from different depths. The micrometeorological method was used to measure N_2O surface flux rates. The results indicated that growing winter rye as a cover crop significantly ($p<0.01$) decreased soil profile N_2O concentrations across all depths from September to December. Compared to non-cover crop, the cover crop treatment decreased the median N_2O concentrations at 5, 15, 30, and 60 cm. With the increasing temperature and soil moisture at spring–thaw, profile N_2O concentrations at all depths increased significantly. In contrast, no significant difference was observed between the non-cover and cover crop treatments during spring–thaw. While different fertilizer sources did not significantly affect N_2O concentrations, EENF demonstrated a capacity to reduce N_2O concentration in the 5 cm depth but did not have a discernible effect at deeper depths during the growing season 2023. The lowest N_2O concentrations were consistently observed at shallow depths (5 cm) throughout the study period, while peak N_2O concentrations were observed at deeper soil depths (30-60 cm). The peak N_2O concentration during spring–thaw 2022 and 2023 occurred at 30 cm of soil profile likely due to higher microbial activity, confirming that *de novo* production is the main mechanism driving N_2O peak emission during spring–thaw.

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FOREWORD

This thesis is prepared in manuscript format by the thesis guidelines of the Department of Soil Science, University of Manitoba. It is divided into three chapters. Chapter 1 provides a literature review of N_2O emissions from agricultural systems, with a focus on the mechanisms driving soil N_2O production during both the spring thaw and growing seasons. Chapter 2 assesses the effects of different farming practices, including enhanced efficiency nitrogen fertilizers (EENFs) and cover crops, on the temporal and spatial changes of soil profile N_2O concentration in relation to surface fluxes. Chapter 3 is a comprehensive synthesis that summarizes the key findings and recommendations from the entire study. Additionally, Chapter 2 is being prepared for submission to the Journal of Agriculture, Ecosystems & Environment.

AUTHOR'S CONTRIBUTIONS

Faezeh Parastesh performed the experiments, collected and analyzed the data, and wrote the thesis. Drs. Xiaopeng Gao and Mario Tenuta conceived the original idea, assisted in data interpretation, revised the thesis, and supervised the work. All authors discussed the results and contributed to the final version of the thesis.

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$\Delta[N2O]$	nitrous oxide vertical concentration gradient
°C	degrees Celsius
μL	microliter
AOA	ammonia-oxidizing archaea
AOB	ammonia-oxidizing bacteria
C	Carbon
cm	centimeter
CO_2	carbon dioxide
EEFs	Enhanced-efficiency fertilizers
EENFs	Enhanced-efficiency Nitrogen fertilizers
F_N	nitrous oxide flux
GHG	greenhouse gases
ha	hectare
k	turbulent transfer coefficient
K	potassium
KCl	potassium chloride
kg	kilogram
km	kilometer
L	Liter
m	meter
m^2	square meter
mg	milligram
ml	milliliter
mm	millimeter
N	nitrogen

N_2	nitrogen gas
N_2O	nitrous oxide
ng	nanogram
NH_4^+	ammonium
NIs	nitrification inhibitors
NO	nitric oxide
NO_2^-	nitrite
NO_2^-/NO_3^-	nitrate
NO_3^-	nitrate
NOB	nitrite-oxidizing bacteria
P	phosphate
PCFs	polymer-coated fertilizers
PE	polyethylene
ppb	parts per billion
ppm	parts per million
S	sulfur
TGAS-MAN	Trace Gas Manitoba
UIs	urease inhibitors
VMC	volumetric moisture content
z	vertical height difference

1. GENERAL INTRODUCTION

1.1 Background information

Agricultural lands play a crucial role in greenhouse gas emissions, with nitrous oxide (N_2O) being a significant contributor to climate change. Over the last decade, atmospheric N_2O concentrations have increased from 270 parts per billion (ppb) to 319 ppb (Haider et al., 2020; Thangarajan et al., 2013). Agricultural N_2O emissions account for approximately 72% of the annual N_2O emissions in Canada. Approximately 80% of N_2O emissions globally are attributed to agricultural activities, including the extensive use of chemical nitrogen (N) fertilizers (Shindell et al., 2013). This underscores the urgent need for sustainable agricultural practices to mitigate these emissions.

The relation between soil and N_2O is complex, soil can serve both as a source and a sink for this gas (Kool et al., 2009; Syakila et al., 2011). This dual role arises from the complex interactions of various physical, biological, and chemical processes within the soil. The balance between N_2O production, diffusion, and consumption in the soil is influenced by factors such as soil type, moisture levels, temperature, microbial activities, available N, and agricultural practices. A comprehensive understanding of these interactions is important for effectively reducing N_2O emissions and understanding their broader impact on global greenhouse gas levels and climate change. Therefore, this chapter explores N_2O production mechanisms in soil, shedding light on the factors that influence these processes.

1.2 Formation of N_2O in the soil

Approximately 70% of the world's N_2O emissions are linked to nitrification and denitrification processes (Tian et al., 2019). Both denitrification and nitrification are the fundamental microbial activities involved in the nitrogen cycle within the soil. Many

Microorganisms like anammox bacteria, certain fungi, and archaea participate in nitrification and denitrification processes (Zhang et al., 2011). Denitrification occurs under anaerobic conditions when soil microbes convert nitrate (NO_3^-) to nitrite (NO_2^-), then to nitric oxide (NO), and finally to N_2O and dinitrogen gas (N_2). It is important to note that describing these processes as sources of N_2O is an oversimplification, as microbial metabolic pathways encompass a range of processes that either generate or consume N_2O (Butterbach-Bahl et al., 2013).

Additionally, some abiotic processes, such as the chemical degradation of nitrite (chemodenitrification) and hydroxylamine, also contribute to N_2O production (Pandey et al., 2020). However, N_2O emissions produced from these non-biological pathways are usually minimal and insignificant (Grabb et al., 2017). Understanding the different N_2O production pathways is essential for developing effective strategies to minimize its emissions from soils.

1.2.1 Nitrification

Ammonia is converted to nitrite and subsequently to nitrate in two steps during nitrification. In the first step (1), ammonia is oxidized to nitrite by microorganisms known as ammonia-oxidizing archaea (AOA) and/or ammonia-oxidizing bacteria (AOB) (Kozlowski et al., 2016; Norton and Ouyang, 2019). In the second step (2), nitrite is further oxidized to nitrate by nitrite-oxidizing bacteria (NOB), during this process, N_2O can be produced as a byproduct. Both groups are collectively referred to as *Nitrobacteriaceae*. This partitioning of nitrification into two distinct functional groups has been recognized as a fundamental feature of the biogeochemical nitrogen cycle, as first described in 1890 (Stein and Klotz, 2016; Wrage-Mönnig et al., 2018):

$$(1)\ NH_4^+ + 1\frac{1}{2}O_2 \rightarrow NO_2^- + H_2O + 2H^+$$

$$(2)\ NO_2^- + \frac{1}{2}O_2 \rightarrow NO_3^-$$

In anaerobic conditions, the level of NO_2^-, a harmful compound, rises in the soil. This compound can potentially serve as an alternative electron acceptor for nitrifying microorganisms, leading to the production of N_2O and NO during the process of nitrification (1).

$$(1)\ NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$$

$$\downarrow$$

$$NO \rightarrow N_2O \rightarrow N_2$$

Van Kessel et al. (2015) found that it is possible for a single organism to fully oxidize ammonia into nitrate (known as complete ammonia oxidation or comammox) through a process that requires energy. This process likely occurs when conditions are optimal for microbial activity, allowing them to grow more efficiently but at a slower pace than ammonia-oxidizing microorganisms (1).

$$(1)\ NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O + 2H^+$$

1.2.2 Denitrification

In the facultative anaerobic process, nitrate (NO_3^-) is transformed into molecular nitrogen (N_2) as the end product and nitrous oxide (N_2O) as an intermediate gas. In this process, nitrate acts as an alternative electron acceptor (Yang et al., 2020). This process is comprised of four steps [nitrate (NO_3^-) to nitrite (NO_2^-) (1), NO_2^- to nitric oxide (NO) (2), NO to nitrous oxide (N_2O) (3), and N_2O to N_2 (4)] which is a stepwise reduction of NO_3^- to N_2. This metabolic pathway involves complex multisite metalloenzymes which catalyze each step along the reactions (Canfield et al., 2010). Each process stage is regulated by distinct reductases encoded by functional genes. These reductases include nitrate reductase such as *narG,* and *napA*, nitrite reductase such as *nirS,* and *nirK*, nitric oxide reductase such as *cnorB,* and *qnorB*, and nitrous oxide reductase like *nosZ.*

$$(1)\ NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O \text{ (Nitrate reductase)}$$

$$(2)\ NO_2^- + 2H^+ + e^- \rightarrow NO + H_2O \text{ (Nitrite reductase)}$$

$$(3)\ 2NO + 2H^+ + 2e^- \rightarrow N_2O + H_2O \text{ (Nitric-oxide reductase)}$$

$$(4)\ N_2O + 2H^+ + 2e^- \rightarrow N_2 + H_2O \text{ (Nitrous-oxide reductase)}$$

Denitrifying organisms usually prefer to respire oxygen instead of N-oxides or oxyanions because they rely on aerobic respiration to produce energy for cellular functions. Denitrification plays a role in the soil's nitrogen cycle, helping sustain a stable level of nitrogen availability within the ecosystem. Nitrate reductase, nitrite reductase, nitric oxide reductase, and nitrous oxide reductase are some of the enzymes that drive the denitrification process. In many mineral soils, the presence of nitrate is crucial for denitrification. When these soils turn anaerobic, the availability of organic material becomes the limiting factor for denitrifiers to use in reducing nitrate. Research on denitrification found that the accumulation of N_2O during the denitrification of nitrate in soils was promoted by high NO_3^- concentration and low pH when incubated in closed systems (Thomson et al., 2012). Some studies found that the soil's ability to convert N_2O to N_2 under anaerobic conditions depended largely on its NO_3^- availability and pH (Yoon et al., 2019).

When cells that are grown under anaerobic conditions for denitrification are exposed to oxygen, the proteins involved in denitrification lose their functionality. This can cause denitrification rates to decrease or completely inhibit the process. Denitrifying microorganisms that inhabit soils are typically aerobic bacteria that can reduce nitrogen oxides (NO_x) when exposed to anaerobic conditions, which results in the formation of N_2O and N_2.

1.2.3 Chemo-denitrification

Before 1970, research suggested that nitrite underwent chemical decomposition in neutral and acidic soils. This process resulted in the volatilization of nitrite-N and its fixation by soil organic matter (Wrage et al., 2001). These nitrite reactions in soils are similar to the biological denitrification process because they produce N_2O, N_2, and NO, commonly known as chemo-denitrification. Further studies investigating chemo-denitrification mechanisms and the factors that influence this process have revealed that soil pH and organic matter content were the primary factors controlling nitrite reduction in soils. Tropical peat soils provide optimal conditions for chemo-denitrification due to their low oxygen and pH, and high levels of Fe^{2+} and organic matter (Klueglein et al., 2014). The reduction process is typically associated with iron oxidation and can be represented by the equation (1) and (2) (Etique et al., 2014):

$$(1)\ NO_2^- + Fe_2^+ + 2H^+ \rightarrow NO + Fe_3^+ + H_2O$$

$$(2)\ 2NO + 2Fe_2^+ + 2H^+ \rightarrow N_2O + 2Fe_3^+ + H_2O$$

Small amounts of N_2O are produced by the chemical reaction of nitrite in soils compared to the amounts of NO and N_2 produced. There is no evidence indicating that substantial quantities of N2O are generated in soils through the chemical breakdown of nitrite created during the conversion of ammonium to nitrate by nitrifying bacteria or during the reduction of nitrate by denitrifying microorganisms (Van Cleemput and Samater, 1995).

1.3 Mechanisms Driving N_2O Emissions during Freeze-Thaw Cycles

Research has demonstrated that N_2O emissions throughout the spring–thaw period is an important contributor to the annual N_2O budget, accounting for more than half of the annual N_2O emissions for agricultural lands in temperate regions (Wagner-Riddle et al., 2017). Research by Dunmola et al. (2010) indicated that over 70% of the total annual N_2O emissions stem from the spring–thaw period. Using a meta-analysis on a global scale, Gao et al. (2017) reported that the freeze–thaw cycle can increase soil N_2O emissions by nearly 150%.

The first mechanism focuses on the physical aspects of soil spring–thaw dynamics. It involves the physical trapping and subsequent release of N_2O. During freezing conditions, N_2O produced in the soil can be trapped below ice or within snow cover, and upon thawing, the previously trapped N_2O is released (Van Bochove et al., 2001).

The second mechanism involved in this phenomenon is biological denitrification, driven by increased anaerobiosis and substrate availability during spring–thaw cycles. This process includes the release of substrates through the spring–thaw period on soil aggregates, microbial cell lysis, and residual NO_3^- and carbon (C) from crop residue. These factors collectively enhance the conditions for biological denitrification, leading to a significant rise in N_2O emissions during soil thawing (Wagner-Riddle et al., 2017).

Understanding the processes that evolve in this phenomenon is essential for accurately estimating the impact of soil freeze–thaw cycles on N_2O production and their contribution to global anthropogenic N_2O budgets. Mechanisms involving physical and biological processes, have been identified as main contributors to the N_2O bursts during spring–thaw and will be discussed in this section.

1.3.1 Release of Trapped N_2O through a Physical Barrier of Diffusion

Measurements of soil profile N_2O concentrations over time and at various depths can reflect the overall balance between the production, consumption, and transport of N_2O (Heincke and Kaupenjohann, 1999). Previous studies showed that in sub-zero air temperatures without substantial snow cover, ice formation can occur on the soil's surface and extend downwards depending on the severity of freezing (Risk et al., 2013).

Surface ice formation following brief thaw periods can melt snow and re-freeze. These ice layers act as diffusion barriers, restricting the permeability of gases and confining soil gases within the soil profile (Risk et al., 2013). Consequently, the formation of ice layers divides the soil profile into two sections: a frozen layer experiencing extreme temperature variations, and a region below where microbial processes can still produce N_2O. Within this frost-free area, extreme air temperature variations are mitigated, creating conditions conducive to the persistence and accumulation of N_2O production. When these "sealing caps" melt, any stored N_2O is subsequently released into the soil as it thaws (Libby et al., 2018).

When the temperature of the soil water is near zero, N_2O may accumulate at relatively high concentrations. If soil water becomes saturated with N_2O, the gas can be emitted from the soil when temperatures rise (Weeks and McMahon, 2007). Ice layers act as a barrier that inhibits air diffusion in soils. Once the ice begins to thaw, there appears to be a crucial moment during which N_2O rapidly diffuses upwards, leading to a significant drop in soil profile N_2O concentration and a concurrent flux burst. Risk et al. (2014) reported that "bursts" of N_2O can contribute to as much as 47% of thaw emissions in an Ontario cropland (Table 1.1).

Understanding the dynamics of N_2O emissions related to freeze-thaw cycles is a complex challenge. As mentioned by Wagner-Riddle et al. (2010), the question of whether N_2O is primarily generated in the frozen soil and released during winter, or if it is produced as the thawing process begins, has significant implications for models aiming to depict N_2O emissions during the thaw. A comprehensive understanding of the factors driving N_2O emissions during spring–thaw, coupled with the implementation of appropriate management strategies, is essential for mitigating greenhouse gas emissions from agricultural ecosystems and promoting sustainable farming practices. Moreover, there is a significant need for research to quantify the relative contributions of previously generated N_2O versus newly produced N_2O to emissions during the spring–thaw.

Table 1. 1 Summary of studies showing N_2O bursts through soil physical barrier diffusion

Study Type	Methods	Location	Maximal N_2O concentration during spring–thaw	Key findings	References
Field	Closed chamber	Japan		N_2O built up during the winter and was smoothly released during the spring–thaw.	(Katayanagi and Hatano, 2012)
Field	Closed chamber	Quebec, Canada	35- 62 µL L^{-1}	The accumulation of N_2O was trapped beneath a solid, frozen soil layer.	(Van Bochove et al., 2001)
Field	Micrometeo rological	Ontario, Canada	25 µL L^{-1}	47% of N_2O emission at spring–thaw was attributed to physical release.	(Risk et al., 2014)
Forest	Closed chamber	Norway and Germany	26-50 µL L^{-1}	N_2O fluxes during spring–thaw were primarily attributed to the release of stored N_2O that originated in the subsurface soil.	(Goldberg et al., 2010)
Field	Closed soil cover box	Germany	-	The physical release of N_2O trapped below the soil surface and the microbiological production of N_2O throughout freeze–thaw cycles were responsible for N_2O spring–thaw emission.	(Kaiser et al., 1998)
Field	Chamber	New York, USA	-	The release of N_2O previously produced beneath ice layers was the reason for thaw emissions.	(Singurindy et al., 2009)
Lab	Soil Column	Germany	-	Microorganisms produced N_2O during soil freezing in unfrozen water on the soil matrix, where a layer of frozen water acts as a diffusion barrier, limiting oxygen supply and partially restraining N_2O release.	(Teepe et al., 2001)
Lab	Chamber	China	-	A key mechanism for N_2O emission was the physical isolation of previously created N_2O at the freezing stage.	(Peng et al., 2019)
Lab	Chamber	Quebec, Canada	-	Most of the released N_2O was likely a result of physical processes rather than biological reactions.	(Ejack and Whalen, 2021)

1.3.2 The release of newly produced (*de novo*) N_2O at the onset of the thaw

In contrast to the physical release mechanism, many studies suggest that N_2O production in response to increasing moisture and temperature is the dominant mechanism for the burst of N_2O at spring–thaw. During spring–thaw, there's an increase in soil nutrients, biological activity, lack of oxygen, and rapid temperature changes in the upper soil layer. These conditions are conducive to denitrification (Koponen and Martikainen, 2004). Previous studies have linked the release of N_2O during spring–thaw to the greater activity of microorganisms in the upper thawing layers of soil (Wagner-Riddle et al., 2008) (Table 1.2).

This newly generated N_2O through denitrification during soil thawing is primarily expected to result in the burst of N_2O at the soil surface known as newly produced N_2O or *de novo* (Nyborg et al., 2011). Although denitrification is still the primary predicted source of biologically started N_2O production in thawing soils, it's important to keep in mind that the possibility of ammonia oxidation and nitrifier denitrification in aerobic micro-environments cannot be completely ruled out (Kool et al., 2011; Li et al., 2016; Song et al., 2017). Furthermore, given the variations in environmental and management factors among research studies, it is still unclear under what conditions, and which proposed processes primarily contribute to the surge of spring-thaw N_2O emissions during freeze-thaw cycles (Wagner-Riddle et al., 2017; Chen et al., 2016). Consequently, additional research is required to explore these mechanisms across different climates and environments. There is a need for studies that assess the proportional impact of previously generated and newly generated N_2O on spring–thaw emissions.

Table 1. 2 Summary of studies showing newly produced N_2O during spring–thaw.

Study Type	Methods	Location	The highest N_2O flux rate during the spring–thaw	Key findings	References
Field	Micrometeorological Chamber	Ontario, Canada	202-83 ng N m^{-2} s^{-1}	N_2O release during the spring–thaw is largely attributed to N_2O newly generated within the surface layer.	(Wagner-Riddle et al., 2008)
Field	Static opaque chamber	Heilongjiang, China	199–257 μg m^{-2} h^{-1}	The rapid recovery of microbial biomass resulted in N_2O emission during the spring–thaw period.	(Zhe et al., 2018)
Field	Micrometeorological	Ontario, Canada	92-158 ng N m^{-2} s^{-1}	The variety of nitrifying and denitrifying bacteria significantly increased at spring–thaw indicating *de novo* was a primary mechanism contributing to N_2O emission.	(Smith et al., 2010)
Field	Chamber	Manitoba, Canada	-	Denitrification of near-surface soils is the source of thaw N_2O emissions.	(Tenuta et al., 2016)
Field	Chamber	Norway	20- 183 μg N_2O–N $m^{-1}h^{-1}$	*De novo* N_2O production was the main mechanism of spring–thaw emissions.	(Russenes et al., 2019)
Field	Chamber	Xinjiang, China	14.7 g N_2O-N $ha^{-1}d^{-1}$	The predominant source of N_2O emissions was the *de novo* production of gases, rather than a physical release.	(Yin et al., 2019)
Field	Chamber	Norway	107 μg m^{-2} h^{-1}	The heightened presence of denitrified post-thaw and the greater involvement of *nir* genes in N_2O emission provide evidence that *de novo* production can be the primary mechanism for N_2O generation.	(Kazmi et al., 2023)
Field	flux gradient method	Ontario, Canada	67 ng N m^{-2} s^{-1}	*De novo* denitrification played a role in the N_2O flux during the spring–thaw throughout the sampling duration.	(Németh et al., 2014)
Field	Sampler of Kammann et al. (2001)	Manitoba, Canada	3 μg m^{-2} s^{-1}	Denitrification contributed to the N_2O emission during the spring–thaw	(Rajendran, 2010)
Lab	Microcosm system with automatic sampling	Germany	20 mg N m^{-2} $period^{-1}$	Increased N_2O emissions during the freeze-thaw period were caused by high soil moisture content, available N, and high C content.	(Teepe et al., 2004)
Lab	-	United States	-	Both pre-synthesized and *de novo*-synthesized denitrification enzymes play roles in N_2O production, highlighting the significance of specific parameters in regulating N_2O dynamics.	(Zheng and Doskey, 2015)

1.4 Impact of Farming Practices on N_2O Production and Emissions

Understanding how different farming management practices influence soil N_2O emissions under specific climatic conditions is crucial for developing strategies to mitigate greenhouse gas emissions, and this importance is particularly pronounced for regions like Manitoba. The agricultural landscape of Manitoba, with its unique climatic and soil conditions, necessitates a focused study on N_2O emissions for several reasons. As Manitoba relies heavily on agriculture as a cornerstone of its economy, the environmental impact of agricultural practices, especially in terms of greenhouse gas emissions, has far-reaching consequences. The study of N_2O production and emissions under various practices is imperative for the region to design targeted strategies that align with both environmental sustainability and agricultural productivity goals.

Furthermore, strategies such as growing cover crops and utilizing enhanced efficient nitrogen fertilizer (EENF) have been widely recommended to address N_2O emissions during freeze-thaw cycles. In Manitoba's challenging climate, characterized by distinct freeze-thaw period in early spring, understanding the dynamics of N_2O emissions becomes particularly crucial. Adopting sustainable practices is essential not only for mitigating the environmental impact of agriculture but also for ensuring the resilience and long-term viability of the agricultural sector in Manitoba.

The optimization of nitrogen management while minimizing environmental impacts, as advocated by cover crops and EENF, directly aligns with Manitoba's agricultural goals. As Manitoba seeks sustainable farming practices that balance productivity with environmental stewardship, studying N_2O dynamics in soil profiles under different practices is instrumental in achieving this balance by providing insights into the efficacy of these strategies in a Manitoba-specific context.

1.5 Soil profile N_2O production

During the spring thaw in Ontario agricultural fields, Risk et al. (2014) identified the difference between new N_2O production from microbial denitrification and the physical release of N_2O that accumulated over the winter. This distinction seemed to depend on various soil management practices and conditions that affect N_2O production and consumption. In Ontario, soils experience multiple freeze-thaw cycles, creating a dynamic pattern of N_2O release influenced by various microclimatic factors.

While studies in Manitoba show a clear pattern in N_2O emissions during the spring thaw. The differences in weather between Ontario and Manitoba significantly impact N_2O emissions during the spring–thaw. N_2O emissions in Manitoba occur as a singular peak event within a relatively short timeframe, typically lasting less than a week (Tenuta et al., 2019). This coincides with a rapid temperature rise from above 0°C to around 5°C in early spring. These unique patterns highlight the need for region-specific approaches to address N_2O emissions. Understanding these variations helps develop targeted mitigation strategies and sustainable soil management practices tailored to the specific conditions of each region.

Wang et al. (2018) emphasized the role of soil N_2O consumption across various soil depths, highlighting the substantial N_2O production within the 0–5 cm and 5–15 cm soil layers, accounting for 80.4% and 6.6%, respectively, of the total surface N_2O emission. While these findings offer valuable insights into the spatial distribution of N_2O behavior within the soil profile, it's important to note that the contribution of N_2O consumption may vary based on location and soil management practices.

The accumulation of N_2O in the soil profile sometimes leads to surface emissions (Gao et al., 2014; Li et al., 2021). While surface emissions have received considerable attention, exploring the complex interplay of N_2O production, consumption, and diffusion within the soil profile unveils promising avenues for effectively managing and mitigating greenhouse gas emissions. This knowledge, particularly the understanding of regional differences, opens pathways for informed decision-making to minimize the environmental impact of N_2O emissions.

While numerous research studies have thoroughly investigated emissions at the soil surface, there is a notable gap in the scientific literature concerning the intricate relationship between these surface emissions and the production of greenhouse gases (GHGs) within the deeper layers of the soil profile. Our understanding of the interplay between surface emissions and GHG production in the deeper soil profile layers under different soil management is limited in cold regions like Manitoba, where specific climatic and soil conditions may influence these dynamics differently. Addressing this regional gap is crucial for developing comprehensive strategies encompassing diverse environmental contexts and management practices, thereby contributing to a more thorough understanding of greenhouse gas dynamics in soil ecosystems.

1.6 Trace Gas Manitoba Research Station

The Trace Gas Manitoba Research Station (TGAS-MAN) was established in 2005 to address environmental concerns in the Canadian Prairies. The research site adapts its focus to address emerging questions and technological advancements in farming methods. The primary goal is to provide clear empirical data guiding best management practices that effectively reduce greenhouse gas emissions without compromising profitability. The site utilizes the flux gradient micrometeorology method to measure each field's CO_2 and N_2O fluxes (Webb, 2023).

Studies have been conducted on this site to measure N_2O flux across diverse farming practices. It is worth mentioning that in this region, soil freezes every year to more than 10 cm depth. According to Tenuta et al. (2019), 15-20% of N_2O emissions happened during the spring-thaw period, while over 50% of emissions happened after planting and fertilizer application. Despite these valuable insights, there is a notable absence of information regarding the concentration of N_2O within the soil profile in this field. In Brandon, Manitoba, Rajendran (2010) reported that the highest amount of N_2O soli profile concentration was about 287.3 µg L^{-1}at a 15 cm soil depth, following the highest N_2O emission with about 0.1 µg N m^{-2} s^{-1}. Laboratory investigations revealed negligible N_2O emissions from deeper soils which were unfrozen. However, the highest N_2O emissions occurred during thaw events in the frozen soil surface between 0-5 cm of soil and at shallow depths between 10-15 and 30-35 cm of soil.

While N_2O flux measurements offer insights into immediate emissions, a comprehensive understanding of greenhouse gas dynamics requires knowledge of N_2O concentration throughout the soil profile. It is essential that this knowledge gap be filled since it may offer important insights into how N_2O is distributed across the soil profile, significantly enhancing our knowledge of the environmental effects of different farming methods in this region.

1.7 Objectives

Limited information exists regarding the relationship between surface emissions of greenhouse gases and their production within the soil profile, especially during the spring–thaw period under cold conditions in the clay soil of the Red River Valley, Manitoba, Canada. To address this knowledge gap, further research is essential to elucidate the influence of various agricultural practices, such as the utilization of cover crops and nitrogen fertilizers, on soil N_2O concentrations at different depths and their corresponding relationship with surface flux.

The primary aims of this study is to comprehensively examine the temporal and spatial changes in soil N_2O concentration throughout the spring–thaw period and crop growing season. These variations will be assessed in response to cover crop and fertilizer management practices. This research seeks to enhance our understanding of the intricate dynamics between soil N_2O production, depth-specific concentrations, and surface emissions, particularly in the context of various agricultural management strategies. By achieving these objectives, we aim to contribute valuable insights to agricultural sustainability and greenhouse gas mitigation.

1.8 Thesis Outline

The thesis is formatted in accordance with the standards set out by the University of Manitoba's Department of Soil Science. The thesis comprises three chapters: the first chapter provides an overall introduction to the research problem, objectives, and significance. The second chapter reports soil N_2O data from the Trace-Gas Manitoba (TGAS-MAN) research station in Manitoba over two years (October 2021 to September 2023), focusing on the impacts of cover crop and fertilizer N management on the temporal and spatial changes in soil profile atmosphere N_2O concentrations during spring–thaw and growing seasons. The third chapter serves as the overall synthesis, highlighting key research findings and suggestions for future works. Chapter 2 was prepared in manuscript format for submission to the Journal of Agriculture, Ecosystems & Environment.

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2. SOIL PROFILE N_2O CONCENTRATIONS IN RELATION TO SURFACE EMISSIONS: A TWO-YEAR ANALYSIS OF DIFFERENT AGRICULTURAL MANAGEMENT METHODS

2.1 Abstract

It remains unclear how different farming practices, such as cover crops and fertilizers, affect N_2O concentrations in the soil profile in cold regions like Manitoba. To address this knowledge gap, a two-year field study was conducted in 2022 and 2023 at the TGAS research station in southern Winnipeg, Manitoba. The objective was to investigate the impact of applying winter cover crops and employing enhanced efficient nitrogen fertilizers (EENF) on N_2O concentrations during the spring–thaw period within the soil profile. The study also aimed to explore the relationship between the soil profile N_2O concentrations and N_2O surface emissions. Soil profile gas samples at various depths (5, 15, 30, and 60 cm) were collected using modified silicone diffusive equilibrium samplers during the growing and non-growing seasons. The flux-gradient micrometeorological method was used to collect surface N_2O flux data. The lowest N_2O concentrations were consistently observed at shallow depths throughout the study period, while peak N_2O concentrations were at deeper depths of 30-60 cm. Nitrous oxide concentrations across soil profiles significantly increased during the spring–thaw period, coinciding with a concurrent surface flux event. Higher N_2O concentrations in deeper soil layers were attributed to increased microbial activity and nutrient availability during spring–thaw. These results suggest that the spring–thaw N_2O emissions were predominately induced through the *de novo* production, where N_2O is produced anew in the soil, potentially explaining the elevated N_2O levels in deeper soil layers during this critical period. Compared to the non-cover crop treatment, growing winter rye as a cover crop significantly decreased N_2O concentrations across soil profile N_2O concentrations

during the post-harvest period in 2021 but did not affect those during the spring–thaw period in 2022. Specifically, the cover crop treatment reduced median N_2O concentrations at 5, 15, 30, and 60 cm depths. This significant reduction in N_2O concentrations over the winter period highlights the effectiveness of winter rye cover crops in mitigating N_2O emissions from the soil. The effect of EENFs on N_2O concentrations in the soil profile during the spring–thaw period of 2023 was not significant in our study. Despite the lack of significant impact on N_2O concentrations, EENFs demonstrated the potential to reduce N_2O concentration at the 5 cm depth during the growing season, while no discernible effect was observed at deeper depths.

2.2 Introduction

About 84% of the anthropogenic N_2O emissions in the world are attributed to the agriculture sector (Smith et al., 2018; Tian et al., 2020; Wagner-Riddle et al., 2008). Understanding the production, consumption, and transformation mechanisms of N_2O is vital for predicting emissions and establishing effective methods to mitigate and adapt to climate change. In cold regions like Manitoba, N_2O emissions peak during two key agricultural periods: spring–thaw and fertilizer application (Tenuta et al., 2019).

Studies have shown that in the Canadian Prairies, these two events mainly influence N_2O emissions from fertilized croplands (Wood, 2024). The freeze-thaw cycles in cold climates have been shown to lead to substantial emissions of N_2O, contributing significantly to a soil's annual greenhouse gas emission budget (Risk et al., 2013). Research suggests that more than 50% of annual N_2O emissions stem from spring–thaw periods (Wagner-Riddle et al., 2017; Abalos et al., 2015). Neglecting the contribution of spring–thaw cycles to N_2O emissions could result in significant underestimations in global emissions (Wagner-Riddle et al., 2017).

Previous studies suggest that two main mechanisms drive N_2O production during spring–thaw. Firstly, N_2O is produced during the winter but remains trapped under a frozen soil layer. When the frozen layer thaws in early spring, a burst of gas is released (Van Bochove et al., 2001). Secondly, some studies propose that spring–thaw N_2O emissions are primarily attributed to '*de novo*' production, wherein newly produced N_2O during soil thawing contributes to the emission peak (Lemke et al., 1998). Understanding the main mechanism driving spring–thaw emissions is crucial for developing strategies to mitigate N_2O emissions from agricultural lands.

Several farming practices have been suggested to mitigate N_2O emissions. Implementing non-legume cover crops during non-cropping periods is one of the strategies. Cover crops could decrease soil available nitrate therefore reducing N_2O emissions (Reicks et al., 2021). Studies have shown that cover cropping with no-till practices is an effective conservation strategy to lower the environmental impact of agroecosystems, including reducing N_2O emissions (Webb, 2023). Recent research by Webb (2023) TGAS-MAN, where the current study was conducted, showed that cover cropping with fall rye (*Secale cereale* L.) reduced N_2O emissions during both the spring–thaw and post-fertilizer application periods compared to conventional methods. Fall rye can be a top choice for cover cropping in cold regions to mitigate N_2O emissions during spring–thaw. This recommendation is based on its rapid post-harvest establishment, resilience to extended periods of sub-zero temperatures in soil and air throughout winter, and capacity to persist and thrive into the subsequent spring (Larsen et al., 2018).

Enhanced-efficiency nitrogen fertilizers (EENFs) are another strategy for reducing N_2O emissions in agricultural systems. EENFs, including polymer-coated fertilizers (PCFs) and fertilizer products that are incorporated with nitrification inhibitors (NIs) and/or urease inhibitors (UIs), have been shown to manage nitrogen release rates, improving nitrogen availability for crops, enhancing nitrogen use efficiency, and reducing N_2O emissions in agricultural systems. In a soil column study conducted with a desert soil characterized by high pH and low organic carbon content, Kuang et al. (2019) found that N_2O accumulation in the soil profile was generally reduced by stabilized urea and polymer-coated urea compared to conventional urea, while N_2O surface emissions remained unchanged.

Soil is a dynamic system characterized by temporal and spatial variations, extending horizontally and vertically. Aerobic and anaerobic zones within the soil enable the production, consumption, and movement of N_2O in various directions, upward, downward, and horizontally (Congreves et al., 2018). Different patterns of N_2O concentrations across soil profiles result from these temporal and spatial changes (Gao et al., 2014). Previous research has explored the relationship between spring–thaw surface emissions and greenhouse gas production within the soil profile (Risk et al., 2014: Gao et al., 2014). The production of N_2O within the soil profile doesn't always lead to emissions at the surface.

In Ontario, Canada, where the soil undergoes multiple cycles of freezing and thawing, Risk et al. (2014) compared soil profile changes of N_2O concentrations with surface fluxes and suggested that up to 47% of spring–thaw N_2O fluxes resulted from the physical release of previously trapped N_2O. In contrast, N_2O emissions in Manitoba, Canada, occur as a peak event over a relatively short period. This coincides with a rapid temperature increase from above 0°C to around 5°C in early spring. These distinct emission patterns highlight the necessity for developing region-specific strategies to manage N_2O emissions effectively.

Therefore, this study aimed to understand the temporal and spatial variation of N_2O concentrations, especially during the spring–thaw and post-fertilizer periods, from agricultural lands under cold conditions in Manitoba. This current study addresses the following questions (1) Is there any correlation between N_2O flux and N_2O soil profile concentration? (2) Can EENF and cover crops reduce the subsoil N_2O concentrations during spring–thaw? Our findings will provide insights into soil N_2O profiles and increase understanding of cold region agroecosystem soils.

2.3 Materials and Methods

2.3.1 Site Description

A two-year field study was conducted at the TGAS-Man Research site, located at the University of Manitoba Glenlea research station (49.64° N, 97.16° W, 235 meters above sea level), about 16 km south of Winnipeg, MB, Canada (Figure 2.1).

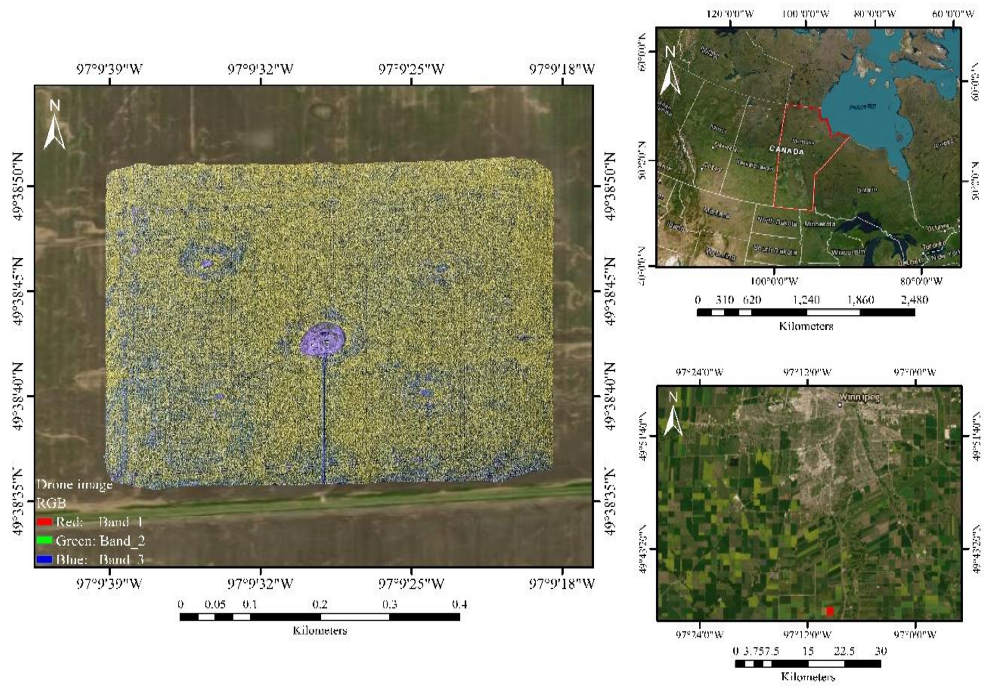

Figure 2.1 Location of the study site within Manitoba, Canada.

This site has a predominantly flat topography with a gentle incline ranging from 0 to 2 degrees. The soil, classified as imperfectly drained Gleyed Humic Vertisols (similar to a Dystric Vertisol), has a clay texture with 60% clay, 35% silt, and 5% sand. Surface soil (0-0.2 m) characteristics include a bulk density of 1.2 Mg m^{-3}, pH of 6.2 (measured using a 2:1 water-to-soil combination), and 32 g kg^{-1} organic carbon content, with no carbonate minerals present (Tenuta et al., 2019). In some areas of the fields (mostly between fields one and two) where the land is at a lower elevation, water may accumulate, leading to ponding. The TGAS-MAN research site, established in 2005, comprises four individual 4-hectare fields, each 200 m by 200 m, arranged in a 2×2 grid within a larger 30-hectare field (Tenuta et al., 2016) (Figure 2.2).

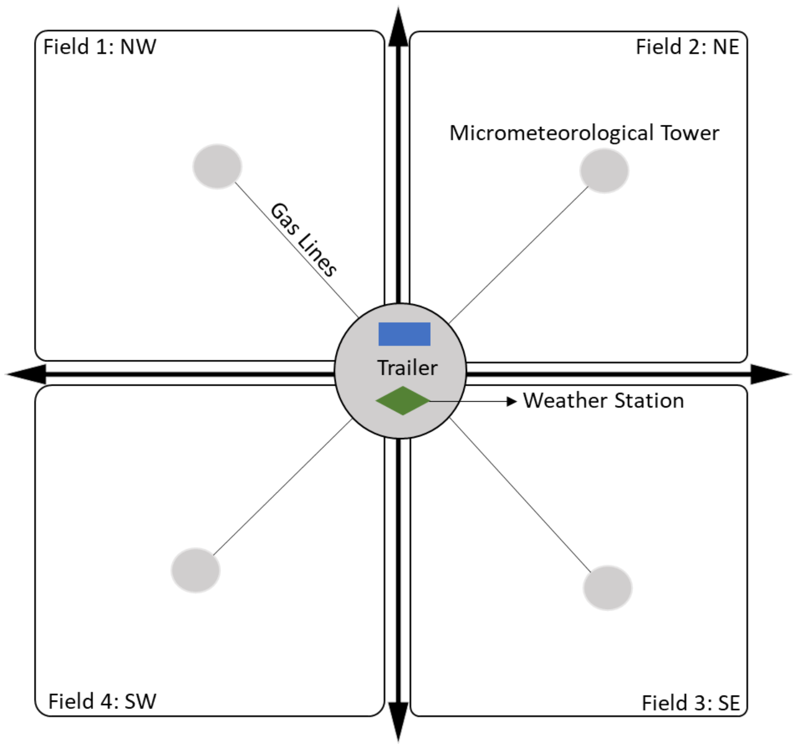

Figure 2.2 TGAS-MAN Experimental Site Layout Showing Four Divided Fields.

2.3.2. Agronomic History and Treatment Design

Fall Rye cover crops were planted in Fields 2 and 3, with a Case IH SDx30 seeder (rate of 63 kg ha^{-1}) on August 30, 2021, while the remaining Fields 1 and 4 were designated as control fields without cover crops. Glyphosate was applied at 0.67 L per acre to Fields 2 and 3 on June 10th, 2022, to remove the winter rye. Subsequently, on June 20th, 2022 late, all four fields were sown with spring wheat (*Triticum aestivum* L., cv 'AAC Viewfield'), banded with a starter fertilizer blend (N-P-S) of 22 kg ha^{-1} N, 22 kg ha^{-1} P, and 11 kg ha^{-1} S. Additionally, Fields 3 and 4, located on the south side, received deep banded 118 kg ha^{-1} of conventional urea (46-0-0), while fields 1 and 2, on the north side, were treated with 56 kg ha^{-1} deep banded of eNtrench-coated urea. The eNtrench is a commercial formulation developed by Corteva Agriscience. It contains a nitrification inhibitor called nitrapyrin. Nitrapyrin inhibits the activity of ammonia-oxidizing bacteria, thus reducing the oxidation of NH_4^+ to NO_3^-. This inhibition helps to retain nitrogen in the soil in the ammonium form for a longer period, consequently reducing nitrogen loss from leaching and denitrification (Wood et al., 2023).

Due to a technical problem with the fertilizer applicator, only half of the planned eNtrench was applied to the north fields, and the other half was planned to use Centuro. On June 22nd, Fields 1 and 2 received an additional 56 kg ha^{-1} of UAN (28-0-0) treated with Centuro (Koch Agronomic Services), dribble banded. As a nitrification inhibitor, Centuro prevents ammoniacal nitrogen from oxidizing to nitrate nitrogen, delaying the nitrification of urea and ammoniacal nitrogen fertilizers. It consists of 14% pronitridine and 86% other ingredients, with a recommended application rate of 6 L per metric tonne. All fields were sprayed with Velocity herbicide on July 13th. Spring wheat was harvested on October 5th, 2022.

No cover crop treatment was applied in the fall of 2022 due to the short growing season. On May 24, 2023, canola (*Brassica napus*, cv. 'L233P' (InVigor®), BASF) was planted on all fields. While Fields 1 and 2 in the north received the same quantity of conventional urea (46-0-0), Fields 3 and 4 in the south received 120 lbs N/ac of SuperU (46-0-0) on the same day. SuperU (46%N, Koch Agronomic Services) is a stabilized urea product that includes both a nitrification inhibitor (dicyandiamide) and a urease inhibitor (NBPT) (Wood et al., 2023).

Subsequently, the following herbicides were applied in all fields: glufosinate (1.5 L/ac) and clethodim (50 mL/ac) on June 9, 2023; glufosinate (1.35 L/ac) and quizalofop (200 mL/ac) on June 28; and Roundup Transorb (1 L/ac) on September 1. Canola was harvested on September

11th. Detailed agronomic management in each field over the two-year study period is provided in Table 2.1 and the treatment in each field is depicted in Figure 2.3.

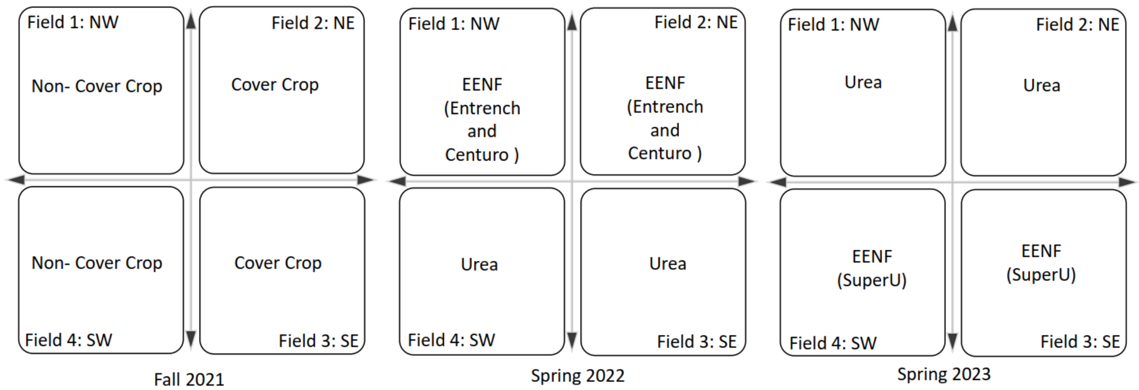

Figure 2.3 Treatment design in each of the four fields.

Table 2.1 Agronomic information during the study period

Date	Activity	Fields	Description
Aug 30th 2021	Cover crop seeded	Fields 2 and 3	Fall Rye cover crop seeded no-till
Sep 1st 2021	Cultivate	Fields 1and 4	West fields cultivated
June 10th 2022	Sprayed Glyphosate	Fields 2 and 3	0.67L/ac to kill fall rye
June 20th 2022	Wheat seeded	All Fields	20-20-0-10 starter fertilizer with seeding wheat (*Triticum aestivum* L., *cv 'AAC Viewfield'*),
June 20th 2022	Conventional fertilizer (urea) EENF (eNtrench + urea) *	Fields 3and 4 Fields 1and 2	118 kg ha^{-1} 56 kg ha^{-1}
June 22nd 2022	EENF (Centuro + UAN dribble banded)	Fields 1 and 2	56 kg ha^{-1}
July 13th 2022	Herbicide	All fields	Velocity herbicide (988 ml ha^{-1})
Oct 5th 2022	Crop harvest	All fields	
May 24th 2023	Seeding canola Starter fertilizer	All fields	Variety: *In vigour L233P*, Seeding rate: 4.4 lbs/ac Starter fertilizer 0-20-0-15 (20 lbs phos, 15 lbs sulfur)
May 24th 2023	Conventional fertilizer (urea) SuperU	Fields 3 and 4 Fields 1 and 2	120 lbs N/ac Urea (46-0-0) 120 lbs N/ac SuperU
June 9th 2023	Herbicide	All fields	Liberty/glufosinate at 1.5 L/ac (150 g/L) Centurion/Clethodim at 50 mL/ac (240 g/L)
June 28th 2023	Herbicide	All fields	Liberty/glufosinate at 1.35 L/ac (135 g/L) Assure II/Quizalofop at 200 mL/ac
Sept 1st 2023	Herbicide	All fields	Roundup Transorb at 1L/ac
Sept 11th2023	Crop harvest	All fields	

*There were issues with the fertilizer applicator on the north plots, the application rate might be different

2.3.3. Soil Gas Sampling

Modified silicone diffusive equilibrium samplers were used to collect soil gas samples. The samplers were made of polyethylene (PE) tubes with an outside diameter of 50.0 mm, an interior diameter of 40.8 mm, and a length of 65 cm. Four, 5cm silicone diffusive tubes, which allow gases through but not water, were included in each sampler to collect gas samples at 5 cm, 15 cm, 30 cm, and 60 cm below the soil's surface, respectively. One end of the silicone tube was sealed, and the other end was connected with a stainless-steel tube with an inner diameter of 0.6 mm, extended to the soil surface, and connected with a sample port equipped with a rubber septum (Kuang et al., 2019) (Figure 2.4 right). To prevent damage during installation and sampling, each silicone tube was covered by a 5 cm polyethylene (PE) pipe (40.8 mm i.d. and 50.0 mm o.d.). Six gas samplers were installed across the four fields to ensure comprehensive coverage (Figure 2.4 left).

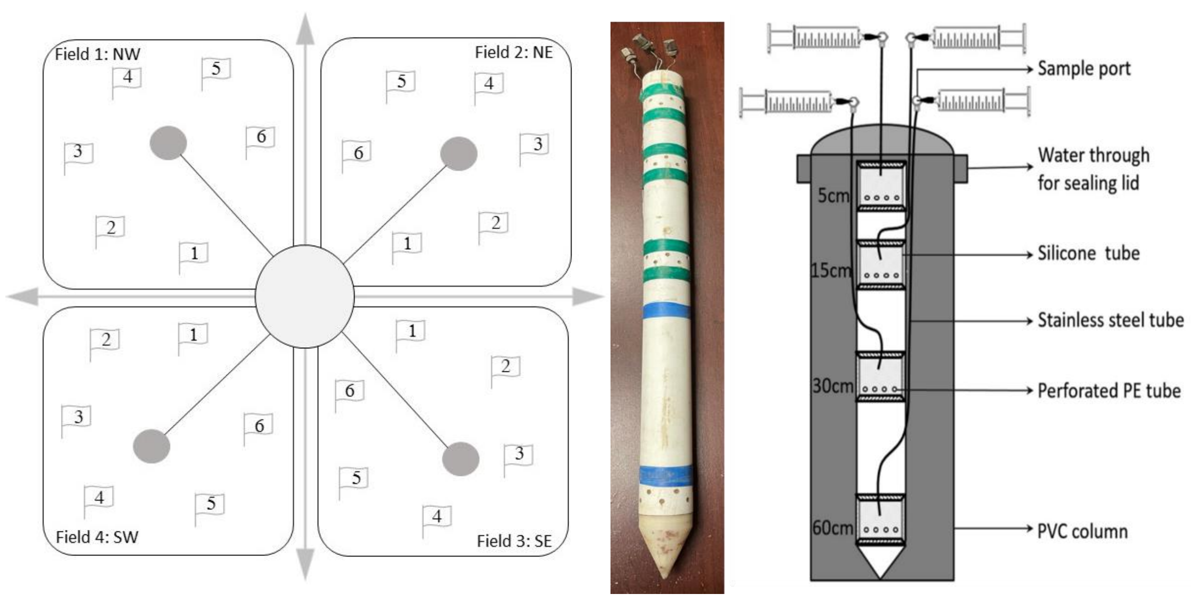

Figure 2. 4 Layout of gas sampling in the fields (left) and modified silicone diffusive equilibrium samplers (right) (modified from Kuang et al., 2019).

Samplers remained in place throughout the year, continuously collecting soil gas samples to provide a comprehensive dataset. However, during critical field activities such as seeding, fertilizer application, and harvesting, the samplers were temporarily removed from the field to prevent any interference with the operations. This approach ensured that the farming machines were not interrupted by the samplers and also prevented the samplers from being damaged by the heavy farming machinery.

During the first study period, soil gas sampling began in October 2021 at 5, 15, 30, and 60 cm depths of the soil profile. Sampling occurred approximately three times a week until mid-December. The gas sampling was not feasible during winter and early spring due to the cold and harsh weather conditions. On April 10, 2022, gas sampling was initially scheduled to begin. However, frozen soil conditions from the winter and soil water saturation in early spring delayed the sampling process until May 16, 2022, which became the first sampling date after winter.

On June 9th, the samplers were removed from the soil due to the application of fertilizer and the seeding of spring wheat. They were reinstalled on July 25, although this process was delayed due to heavy rain and muddy soil conditions, which prevented earlier reinstallation. Subsequently, soil gas sampling was conducted until October 4th. The samplers were subsequently removed from the soil for harvesting, and they were reinstalled after harvest in preparation for the next study period and growing season.

The second-year soil gas sampling started on May 1st, 2023, and we conducted sampling approximately 1-2 days from spring–thaw until May 23rd. On this date, we removed the samplers from the soil in preparation for seeding and fertilizer application scheduled for May 24th. The samplers were then reinstalled in early June, and we endeavored to conduct sampling as frequently as possible until the harvest of canola in September 2023.

2.3.4. Soil Gas Analysis

At each sampling event, a 20 mL airtight syringe was used for collecting gas samples from each sampler into a pre-evacuated 12 mL vial. Before evacuation, each vial was purged twice with nitrogen until the final pressure reached less than 0.066 kPa. Gas analysis was performed using a gas chromatograph that was outfitted with a CombiPAL robotic sample introduction system and an electron capture detector (Varian CP-3800, Bruker Daltonics LTD) (Wood et al., 2023). Calibration gas was ordered from Innovare Industrial, pure N_2 as a zero reference, and a 'high standard' with known concentrations of N_2O was used. If the calibration error (coefficient of variance) exceeded 5%, the analytical runs were restarted, or the gas chromatograph columns were reconditioned by running new calibration samples.

2.3.5. N_2O Surface Flux Measurements

Surface N_2O flux was measured using the micrometeorological flux gradient method with methodology (Webb, 2023). Micrometeorological equipment, including a Tunable Diode Laser absorption spectrophotometer (Model TGA100A, Campbell Scientific Inc., Logan, UT, USA) trace gas analyzer (TGA), along with its associated hardware and electronics, was housed in a trailer situated in the middle of the fields. To calculate N_2O net exchange with the atmosphere, the flux-gradient micrometeorology method was used, involving a formula (1) with a turbulent transfer coefficient, vertical concentration gradient, and height difference (Figure 2.5) where *k* represents the turbulent transfer coefficient, Δ[N_2O] stands for the vertical concentration gradient, and Δz is the vertical height difference.

$$(1)\ F_N = -k\ \frac{\Delta[N_2O)]}{\Delta z}$$

2.3.6 Weather, environment, and soil sampling

Weather and soil conditions were obtained from a weather station in the center of the study site. Instruments were set up to measure different parameters, including the wind direction and speed (Wind Monitor Model 05103-10, R.M.); air temperature and humidity (Model HMP45C, Vaisala Inc., Woburn, MA, USA); soil temperature at different depths (5, 10, 20, and 50 cm) of soil (Thermistors Model 107 and 28 107B); and volumetric soil moisture contents at 10 and 30 cm of soil depths (Dielectric Aquameter Model EC-10 ECH$_2$O) (Webb 2023). The 30-year climate weather data for Glenlea, Manitoba, from 1981 to 2010, was collected from Environment Canada (Environment Canada, 2023; Webb.,2023)

Soil samples were collected, starting in the spring and continuing throughout each growing season. At each sampling event, six randomly chosen places within each field were used to gather these samples, which ranged in depth from 0 to 30 cm. Until they were ready for analysis, soil samples were kept at -20°C in a walk-in freezer. The samples were defrosted and well-mixed to ensure homogeneity before analysis. Sub-samples were oven-dried for 24 hours at 105°C to determine the gravimetric moisture content (GMC). Fresh soil samples were extracted using a 2 M KCl solution at a ratio of 1:5 soil to extractant, and the concentrations of ammonium (NH_4^+) and nitrite/nitrate (NO_2^-/NO_3^-) were measured using a Technicon Autoanalyzer II colorimetry (Pulse Instrumentation Ltd., Saskatoon, SK, Canada). Results were expressed on a dry weight base.

2.3.7 Statistical Analysis

The data were analyzed using the Prism software (version 10.2.2). Normality testing of both N_2O concentration data was conducted using the Shapiro-Wilk test, revealing non-normal distributions. The Wilcoxon signed-rank test, a nonparametric method suitable for non-normally distributed data, was employed to compare the median of the sample population (cover crop and

EENFs). Pearson correlation tests were performed to explore linear relationships between variables such as N_2O concentration at different depths, N_2O emission flux, and weather/soil variables. Statistical significance was determined based on calculated p-values, with significance set at $p < 0.05$.

2.4 Results

2.4.1 Environmental conditions

2.4.1.1. September 2021 to September 2022

From the start of cover crop seeding on August 30th until December 2021, the average air temperature was approximately 8.4°C. During this period, precipitation was approximately 89 mm, 58% lower than the 30-year average at the same period. This amount accounted for about 13% of the total annual precipitation from September 2021 to September 2022 (Table 2.2). The spring–thaw, defined as the period from the first date of daily average air temperature above 0°C to the first date of the soil temperature at a 5 cm depth above 5°C (Tenuta et al., 2019), began on March 20, 2022. Although the soil temperature was -1°C on this date, the first day of average soil temperature above 5°C was observed on May 4th. The average air and soil temperatures were approximately -0.7°C and 0.1°C during this transition period, respectively.

From June to September 2022, noticeable shifts in weather patterns were observed. The average air temperature significantly increased to approximately 19.1°C. These warmer conditions were accompanied by substantial precipitation, with approximately half of the annual precipitation occurring during these months. In June 2022 alone, 111.5 mm of precipitation were recorded. The total precipitation recorded from October 2021 to September 2022 amounted to 699 mm, 1.8 times greater than the 30-year normal (Figure 2.5).

2.4.1.2. October 2022 to September 2023

During this period, the average air temperature recorded between October and December 2022 was approximately -12.7°C. This temperature closely aligned with the 30-year average temperature for the same period and was notably colder than the previous year (Figure 2.6). The spring–thaw period in 2023 began on April 10^{th}, with soil temperatures rising above 0°C, and concluded on April 28^{th}, reaching 5°C at a soil depth of 5 cm. This period was notably shorter than the previous year, lasting approximately two weeks. Throughout the spring–thaw period, the average soil and air temperatures were recorded at 2.3°C and 0.9°C, respectively, similar to the previous year. However, precipitation during this period was lower than the 30-year average, totaling 18 mm.

The total precipitation recorded between October 2022 and September 2023 was 276 mm, approximately half the 30-year average precipitation. Comparing both study periods, the first year experienced about 698 mm of precipitation, while the second year had only 276 mm. Furthermore, compared to the 30-year average precipitation, the first study period was approximately 153% greater, whereas the second study period was 50% less than the 30-year average (Figure 2.6).

Table 2.2 Air, soil temperature at 5 cm, and precipitation from Sep 2021 until Sep 2022 and from Oct 2022 until Sep 2023.

	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sep	Ave/sum
							Average Air Temperature (°C)							
2021/2022	16	8.5	-2.8	-13.3	-19.6	-20.1	-8.3	-0.32	10.3	18.5	19.9	19	13.8	2.1
2022/2023	-	5.8	-4.55	-14.0	-14.2	-15.6	-11.2	-0.28	15.9	20.8	17.5	18	15.7	2.8
30 years Average	-		-5.3	-14.3	-17.2	-13.3	-6.0	4.4	12.2	17.0	19.4	18.8	12.5	2.8
							Total precipitation (mm)							
2021/2022	8	52	19.6	9.15	20.7	35.6	40.4	98.2	73.9	83	111.5	85.4	61.9	699
2022/2023	-	15	4.1	10.5	36.7	12.7	24	18.4	31.8	29.8	38.3	29.3	24.4	276
30 years Average	-	43	26.5	21.5	6.3	12.5	20.7	27.7	61.5	99.7	91.7	72.4	49.0	543
							Average Soil Temperature (°C)							
2021/2022	15.3	9.8	1.7	-3.2	-5.8	-6.3	-3	-0.002	8.9	15.5	19.8	19.1	14.3	5.9
2022/2023	-	7.3	1.6	-0.3	-0.6	-1.3	-1.7	-0.02	11.9	18.8	18.5	18.6	15.8	7.3
30 years Average	-	5.3	-0.2	-3.9	-5.9	-5.6	-3.2	1.5	8.6	14.9	17.8	17.0	11.7	4.8

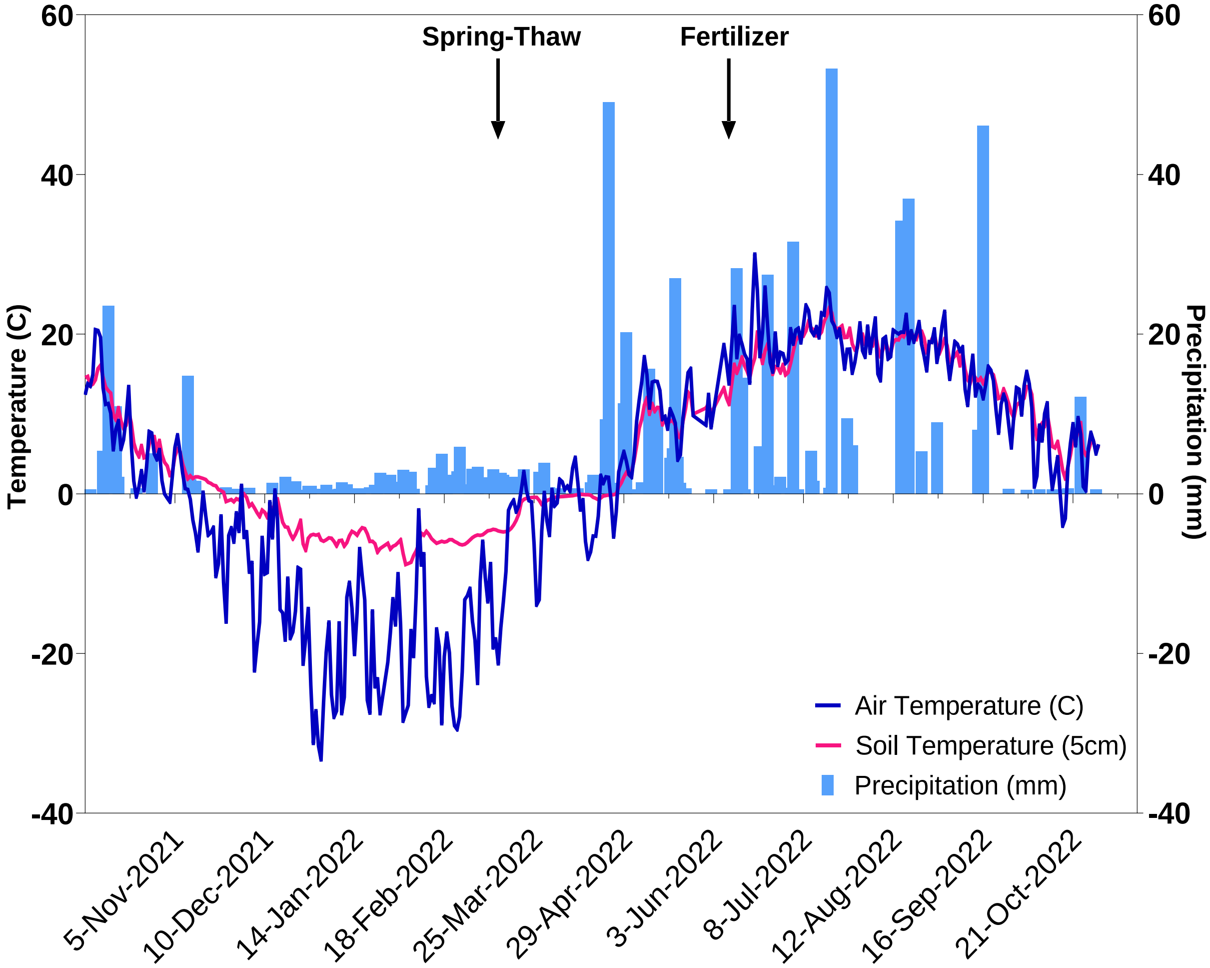

Figure 2. 5 Daily air and soil temperature (5 cm), and precipitation from October 2021 until October 2022. The vertical lines indicate the onset of spring–thaw and the date of fertilizer application, respectively.

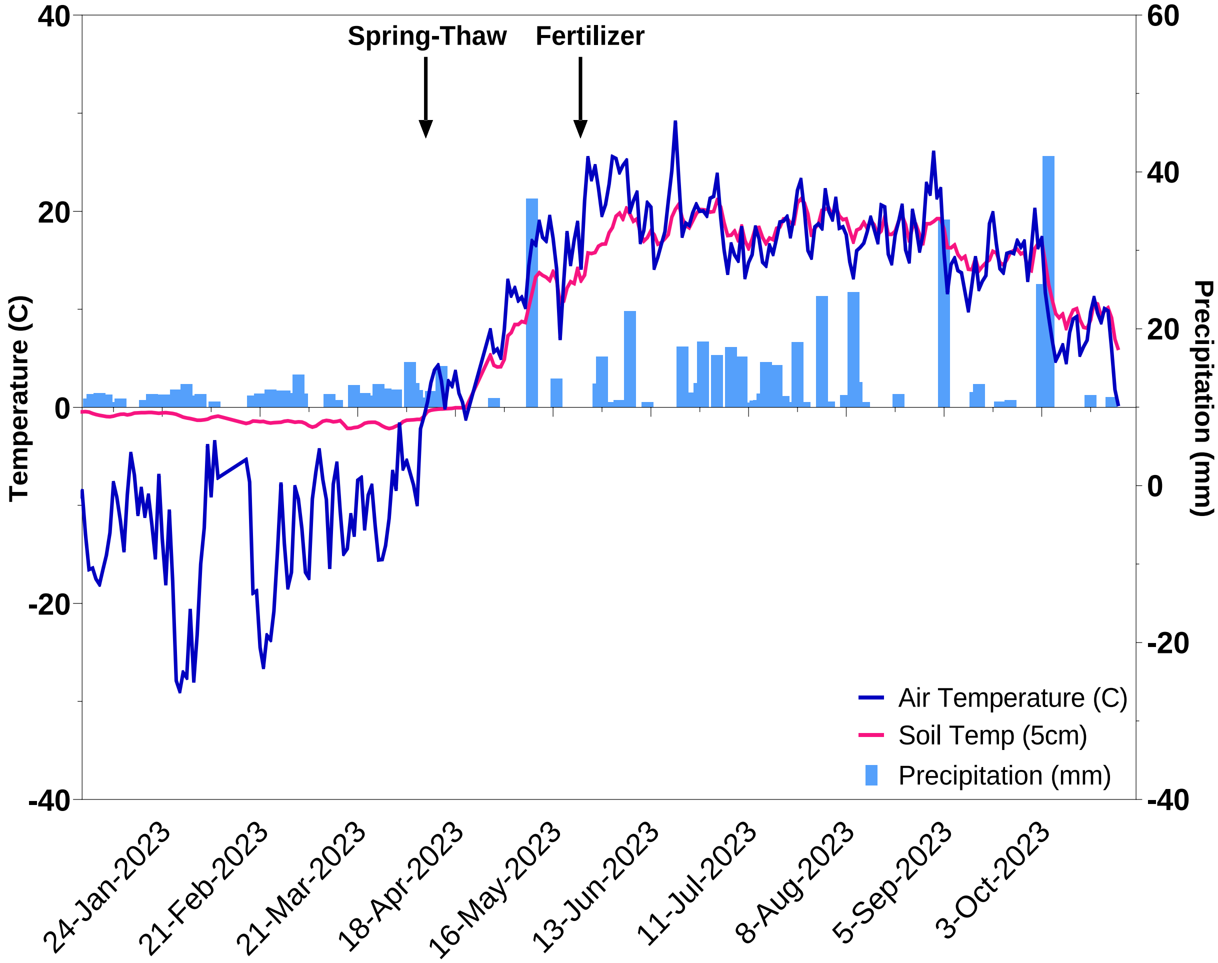

Figure 2. 6 Daily air and soil temperature (5 cm), and precipitation from January 2023 until October 2023. The vertical lines indicate the onset of spring–thaw and the date of fertilizer application, respectively.

2.4.2 Temporal Changes in Soil Profile N_2O Concentrations

The result of N_2O concentration was divided into five sub-periods based on different factors, including weather, field management practices, and treatments. Below, soil profile N_2O concentrations during each category sub-period, representing a specific timeframe within the research study, are explained in detail.

2.4.2.1. Post-harvest (Oct 2021-Dec 2021)

This phase starts from the first sampling date from October 2021 until mid-December, including 13 sampling times. During this period, soil N_2O concentrations generally remained low but varied across all fields at different depths. N_2O concentrations were typically lower in shallow depths compared to deeper ones. Field 2 recorded the highest N_2O concentration at a depth of 5 cm, approximately 1.6 µL N_2O L^{-1}, while the lowest concentrations, around 0.39 µL N_2O L^{-1}, were found in Field 3. Field 1, experienced the highest N_2O concentration at a depth of 15 cm, recorded at about 7.1 µL N_2O L^{-1}. In contrast, the lowest concentration was observed in Fields 2 and 3 with approximately 0.46 µL N_2O L^{-1} for both fields (Figure 2.8-A and 2.9-A).

The peak N_2O concentration among all fields and depths was observed on October 16 at a depth of 30 cm in Field 1, being 9.6 µL N_2O L^{-1}. Additionally, this field exhibited the highest N_2O concentration at a depth of 60 cm, with approximately 6.7 µL N_2O L^{-1} on October 19^{th}. In total, the highest N_2O concentration was observed in field one with 0.6, 2.8, 3.9, and 2.9 µL N_2O L^{-1} for 5, 15, 30, and 60 cm of soil depth respectively.

2.4.2.2. Spring–thaw to pre-planting (April 2022- June 2022)

Only four gas samplings were done during spring–thaw to pre-planting (May 2022 - June 2022). However, more sampling wasn't possible during the spring–thaw period due to excessive water and clay soil. This period started on March 20, which marked the beginning of spring–thaw, defined as the first day with air temperatures rising above 0°C. As temperatures and moisture increased during this period, soil profile N_2O concentrations at all depths rapidly increased. At a depth of 5 cm, N_2O concentrations increased across all fields compared to the previous period (Figures 2.8-A, 2.9-A, 2.10-A, and 2.11-A). Field 4 exhibited the highest concentration, peaking at approximately 6.3 µL N_2O L^{-1} on May 26, while Field 1 had the lowest concentration of 2.7 µL

N_2O L^{-1} on May 24. On May 16, Field 4 recorded the highest N_2O concentration at a depth of 15 cm in the soil profile, reaching approximately 16.6 µL N_2O L^{-1}, followed by Field 3 and Field 2, showing the lowest concentration.

Similarly, on May 16, Field 4 displayed the highest N_2O concentrations at 30 cm and 60 cm depths, measuring approximately 30 µL N_2O L^{-1} and 33 µL N_2O L^{-1}, respectively. In contrast, Field 1 exhibited the lowest concentrations at these depths. Across the 4 fields, average N_2O concentrations over the spring–thaw to pre-planting period were 1.2 µL N_2O L^{-1}, 3.4 µL N_2O L^{-1}, 5.2 µL N_2O L^{-1}, and 6.5 µL N_2O L^{-1} for depths of 5 cm, 15 cm, 30 cm, and 60 cm, respectively. Additionally, the average N_2O concentrations in shallow depths were higher in May compared to June. In total, the highest N2O concentrations in shallow depths were in field two with 3.6 and 5.5 for 5 and 15 cm µL N_2O L^{-1} respectively while the highest N_2O concentrations in deeper depth (30 and 60) were in field four with 8.1 and 10.9 for 30 and 60 cm µL N_2O L^{-1} respectively.

2.4.2.3. Growing Season (June 2022- Oct 2022)

This sub-period began after fertilizer application and crop seeding on June 22 and continued until crop harvesting with five samples. The N_2O concentration peaked on August 4, 2022. On this date, the highest N_2O concentrations were observed at depths of 15 cm and 30 cm, being approximately 10.3 µL N_2O L^{-1} and 8.1 µL N_2O L^{-1}, respectively. During this sub-period, the highest concentration of 22 µL N_2O L^{-1} was recorded at a depth of 60 cm in Field 1. Meanwhile, the peak N_2O concentration at a depth of 5 cm occurred on August 26 in Field 4, reaching 4.4 µL N_2O L^{-1}. Additionally, the highest average N_2O concentration was observed at a depth of 15 cm in Fields 2, with 3.6 µL N_2O L^{-1}. Fields 1 and 4 displayed their highest concentrations at a depth of 60 cm in the soil profile, being 7.2 µL N_2O L^{-1} and 5.3 µL N_2O L^{-1}, respectively. The total N_2O concentration decreased compared to spring thaw 2022 in this period.

2.4.2.4. Spring–thaw (March 2023-May 2023)

The spring thaw for this study year began on April 10. Sampling, however, commenced on May 1, with a total of 10 sampling dates. Sampling dates were recorded throughout May, indicating a notable increase in N_2O concentration across all depths compared to previous periods. At a depth of 5 cm, on May 1, the highest N_2O concentration was observed in Fields 1 and 2, measuring 5.8 µL N_2O L^{-1} and 4.0 µL N_2O L^{-1}, respectively (Figures 2.7-A, and 2.8-A).

Meanwhile, on May 3, Field 3 and 4 recorded their highest N_2O concentrations, reaching approximately 6.1 µL N_2O L^{-1} and 25.1 µL N_2O L^{-1}, respectively. Moving to 15 cm depth in the soil profile, Field 1 exhibited an N_2O concentration peak of approximately 17.9 µL N_2O L^{-1} on May 1, while Field 2 recorded a concentration peak of approximately 63.1 µL N_2O L^{-1} on May 4. Similarly, Field 3 and 4 had their highest concentrations at 15 cm on May 3, being 17 µL N_2O L^{-1} and 47 µL N_2O L^{-1}, respectively (Figures 2.9-A and, 2.10-A).

The highest N_2O concentration observed at a depth of 30 cm in the soil profile occurred on May 3 in Field 4, measuring 71 µL N_2O L^{-1}. This concentration stands out as the peak recorded throughout the two-year research period, underscoring the significance of this observation. Subsequently, on May 4, Field 2 recorded a concentration of 62.4 µL N_2O L^{-1}, reinforcing the notable levels of N_2O production during this period. Furthermore, on May 3, Field 1 and Field 3 exhibited their highest N_2O concentrations at a depth of 30 cm, measuring 33.0 µL N_2O L^{-1} and 17.8 µL N_2O L^{-1}, respectively, indicating consistent trends across multiple fields.

Moving deeper into the soil profile, on May 3, Field 1 displayed the highest N_2O concentration at a depth of 60 cm, measuring 14.3 µL N_2O L^{-1}. In contrast, Fields 2 and 3 showed their highest concentrations at the same depth on May 9, recording 29 µL N_2O L^{-1} and 13 µL N_2O L^{-1}, respectively. Field 4 recorded its highest concentration on May 1 and May 4, being 30 µL N_2O

L^{-1} at a depth of 60 cm in the soil profile. At 60 cm depth, Fields 2 and 4 consistently had the highest concentrations. Overall, N_2O concentrations were higher across all fields, with the peak concentration consistently observed at a depth of 30 cm compared to the secondary peak at 15 cm in the soil profile. The highest N_2O concentrations in Fields 1, 2, 3, and 4 were 19.4, 37.3, 11.05, and 41.99 µL N_2O L^{-1}, respectively, observed at 30 cm of soil profile.

2.4.2.5. Growing Season (May 2023 - September 2023)

During this period, N_2O concentrations were notably higher following seeding and fertilizer application in June 2023 compared to July and August but remained lower compared to the spring–thaw period. 18 samples were collected in this period. On June 8, the first sampling date in this period, the highest N_2O concentration at a depth of 5 cm in the soil profile was recorded in Fields 1 and 4, being approximately 6.4 µL N_2O L^{-1} for both respectively. Conversely, Fields 2 and 3 exhibited lower concentrations of about 2 µL N_2O L^{-1} at this depth on June 8 and 14, respectively. At a depth of 15 cm, Field 4 displayed the highest N_2O concentration, with around 28 µL N_2O L^{-1} on June 8. Subsequently, on the same date, Fields 1 and 2 recorded concentrations of 9.1 µL N_2O L^{-1} and 8.9 µL N_2O L^{-1}, respectively, while field three measured around 9.1 µL N_2O L^{-1} on June 14.

All fields exhibited the highest N_2O concentration at 30 cm in the soil profile on June 8, with about 19.7 µL N_2O L^{-1} for Field 1, 27.7 µL N_2O L^{-1} for Field 2, 10.9 µL N_2O L^{-1} for Field 3, and 47.4 µL N_2O L^{-1} for Field 4. However, N_2O concentration at a depth of 60 cm was higher on June 13 in Field 1, 2, and 4, observing about 20 µL N_2O L^{-1}, 28 µL N_2O L^{-1}, and 43 µL N_2O L^{-1}, respectively, while Field 3 recorded its highest concentration on June 8 with about 31.8 µL N_2O L^{-1}.

Overall, the average N_2O concentration during this period increased with depth. The highest N_2O concentrations in Fields 1, 2, 3, and 4 were 8.4, 7.9, 7.2, and 10.9 µL N_2O L^{-1}, respectively, recorded at a depth of 60 cm in the soil profile. These concentrations decreased by 56%, 78%, 34%, and 74%, respectively, compared to the average peak N_2O concentration during the spring–thaw period, which was observed at a depth of 30 cm in the soil profile.

2.4.3 Temporal Changes in N_2O Flux

Throughout the study period, temporal changes in N_2O surface flux rates were observed across different stages and seasons.

2.4.3.1. Post-harvest (Oct 2021 - Dec 2021)

N_2O flux during this period for Fields 3 and 4 was not recorded due to technical issues. The average N_2O flux rate from October to December was approximately 0.003 kg N/ha/day for Field 1 and 0.001 kg N/ha/day for Field 2 (Figures 2.7-B, 2.8-B, 2.9-B, and 2.10-B). The highest N_2O flux emissions occurred on November 10 for Field 1, reaching 0.016 kg N/ha/day, and on November 30 for Field 2, which recorded 0.013 kg N/ha/day.

2.4.3.2. Spring–thaw to pre-planting (April 2022 - June 2022)

During the spring–thaw to the pre-planting period from March 2022 to June 2022, N_2O flux emissions were monitored across four distinct fields, each displaying varying average rates and peak emissions. Field 1 exhibited an average N_2O flux emission rate of 0.016 kg N/ha/day, with a peak emission of 0.091 kg N/ha/day recorded on May 9th. Field 2 displayed a slightly higher average flux rate of 0.018 kg N/ha/day, with a peak emission of 0.066 kg N/ha/day, also occurring on May 9th. Field 3 showed the highest average emission rate at 0.025 kg N/ha/day, with a peak emission of 0.100 kg N/ha/day recorded on May 3rd. Meanwhile, Field 4 surpassed the others with an average emission rate of 0.033 kg N/ha/day and a peak emission event of 0.220 kg N/ha/day on May 6th.

2.4.3.3. Growing Season (June 2022 - Sep 2022)

The growing season generally exhibited higher N_2O flux emissions across all fields compared to the spring–thaw to pre-planting period. In Field 1, the average N_2O flux rate was 0.114 kg N/ha/day, with the highest emission recorded at 1.039 kg N/ha/day on June 25th after fertilizer application. Field 2 exhibited a slightly higher average flux rate of 0.141 kg N/ha/day, with a peak emission of 3.156 kg N/ha/day on June 25th following fertilizer application. Field 3 had an average flux rate of 0.121 kg N/ha/day, with the highest recorded at 1.703 kg N/ha/day on July 6th after heavy rain. Similarly, Field 4 had an average flux rate of 0.143 kg N/ha/day, with the highest emission recorded at 2.43 kg N/ha/day on July 6th following a heavy rain event. Field 2 consistently exhibited higher average flux rates than the other fields, with the average and highest peak emission events recorded during the growing season. Similarly, Field 4 consistently displayed the highest peak emission events in both periods.

2.4.3.4. Spring–thaw (March 2023 - May 2023)

Field 3 exhibited the lowest average N_2O flux emission rate at 0.01 kg N/ha/day, with the highest emission recorded at 0.1 kg N/ha/day on May 2nd. Field 1 displayed a slightly higher average emission rate of 0.011 kg N/ha/day, with the highest emission recorded at 0.11 kg N/ha/day recorded on May 23rd. Field 4 had a similar average flux emission rate of 0.013 kg/ha/day, with the highest emission recorded at 0.09 kg N/ha/day on April 18. Meanwhile, Field 2 had the highest average emission rate of 0.017 kg N/ha/day, with the highest emission recorded at 0.16 kg N/ha/day on May 23. Comparing these findings with previous periods, it's evident that the spring–thaw period generally exhibits lower average N_2O flux emissions than the spring–thaw period of 2022. Also, peak emission events during the spring–thaw period seem to occur earlier in the season than in other periods, with the highest emissions typically recorded in April or early May.

2.4.3.5. Growing Season (May 2023 - September 2023)

Field 1 had an average N_2O flux emission rate of 0.07 kg N/ha/day, with the highest emission reaching 0.9 kg N/ha/day on July 28. Field 2 displayed a slightly lower average emission rate of 0.06 kg/ha/day, with a peak emission of 0.43 kg N/ha/day recorded on June 12. Field 3 showed an even lower average emission rate of 0.016 kg N/ha/day, with the highest emission recorded at 0.17 kg N/ha/day on June 9. Meanwhile, Field 4 exhibited the lowest average emission rate of 0.011 kg N/ha/day, with the highest emission recorded at 0.14 kg N/ha/day on June 13.

Comparing these findings with the spring–thaw period and previous growing seasons reveals notable trends. During the growing season, average N_2O flux emissions remain relatively high across all fields, except for Field 1, which averaged 0.11 kg N/ha/day. This is compared to 0.07 kg N/ha/day, 0.06 kg N/ha/day, 0.016 kg N/ha/day, and 0.11 kg N/ha/day for Fields one, two, three, and four, respectively, during the spring–thaw period. Additionally, peak emission events during the growing season exhibit variability, with no consistent pattern in terms of timing or magnitude compared to other periods.

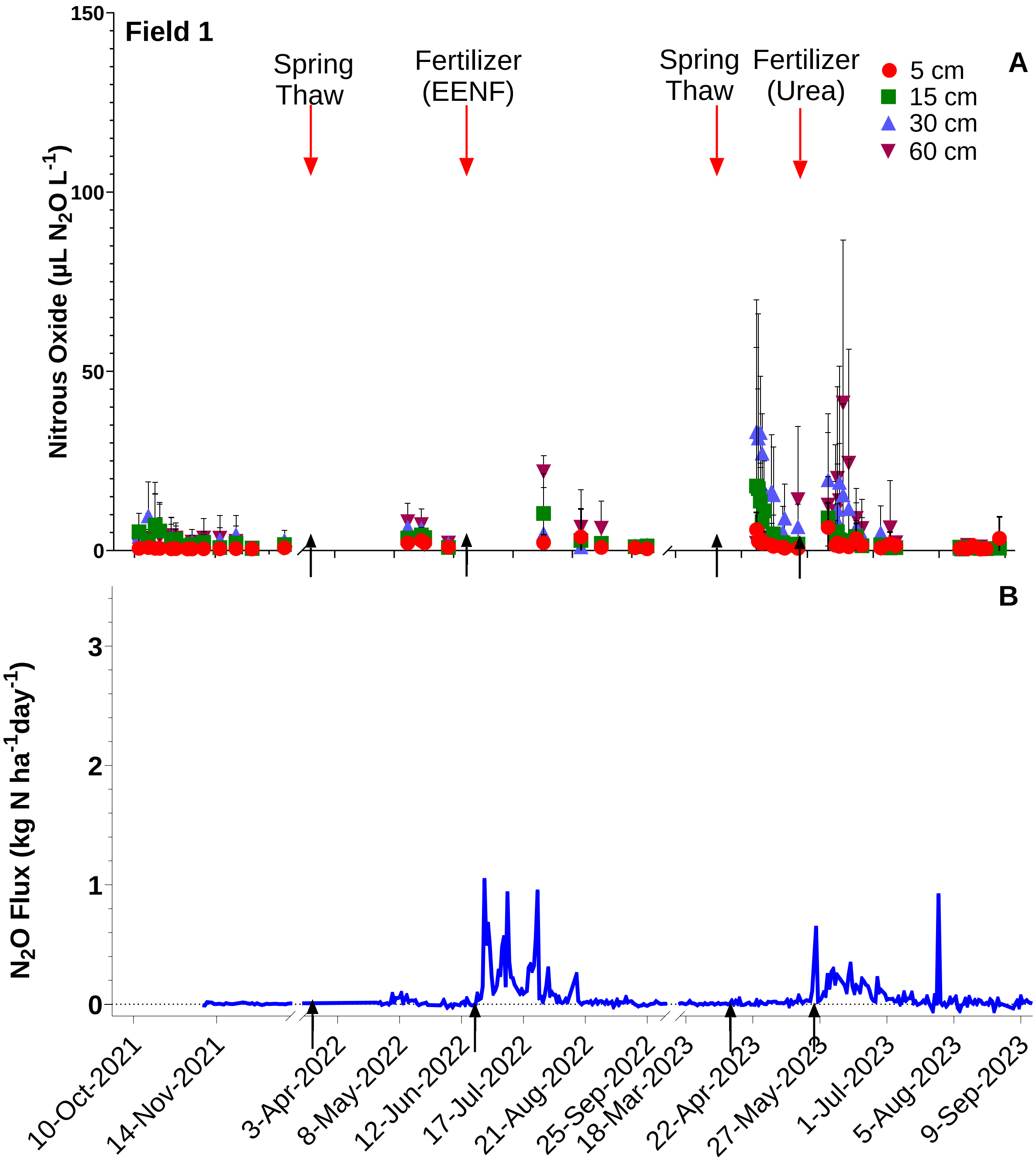

Figure 2. 7 N_2O concentrations in soil profiles (A) and surface flux rates (B) from 2021 October to 2023 September on Field 1.

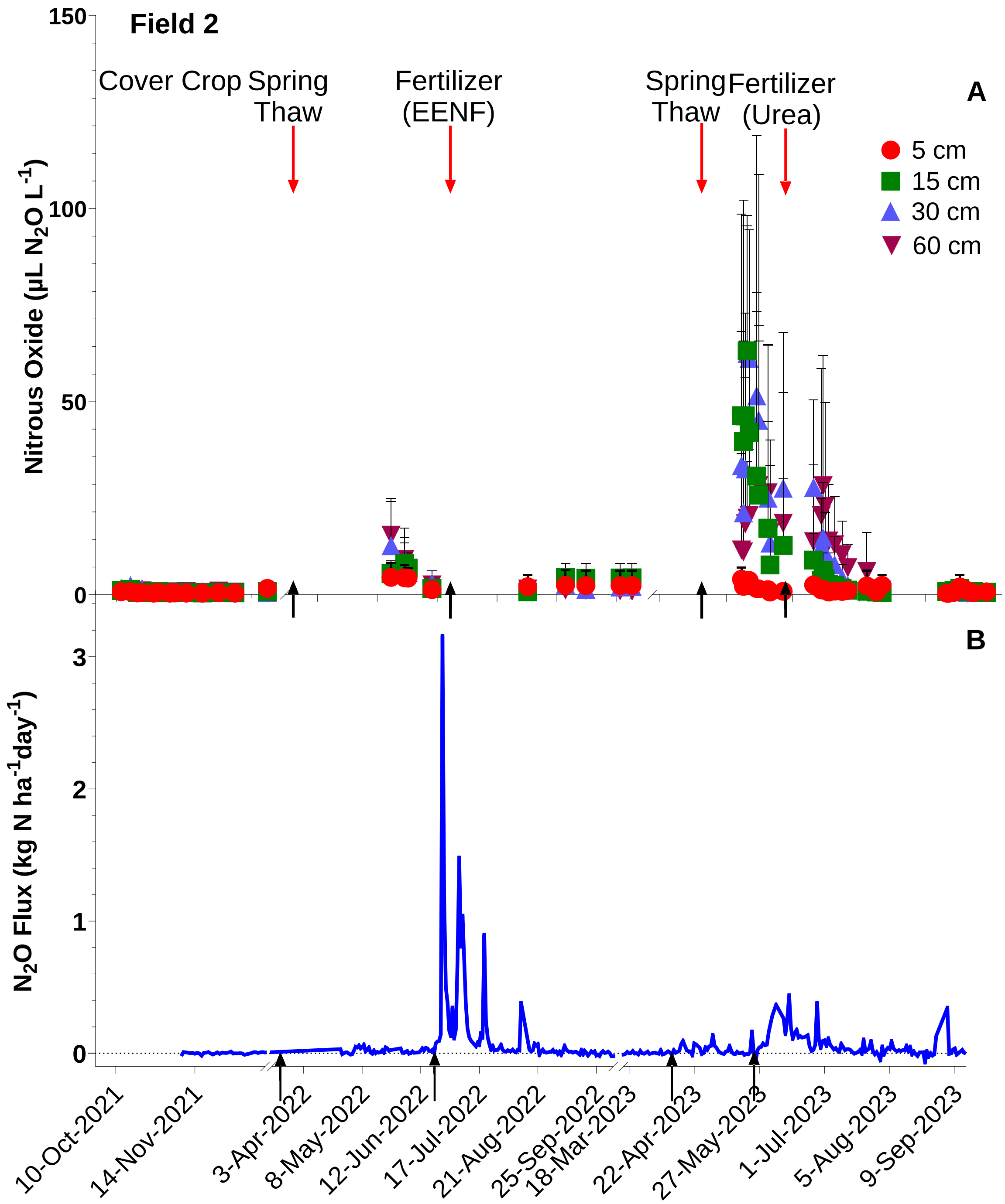

Figure 2. 8 N_2O concentrations in soil profiles (A) and surface flux rates (B) from 2021 October to 2023 September on Field 2.

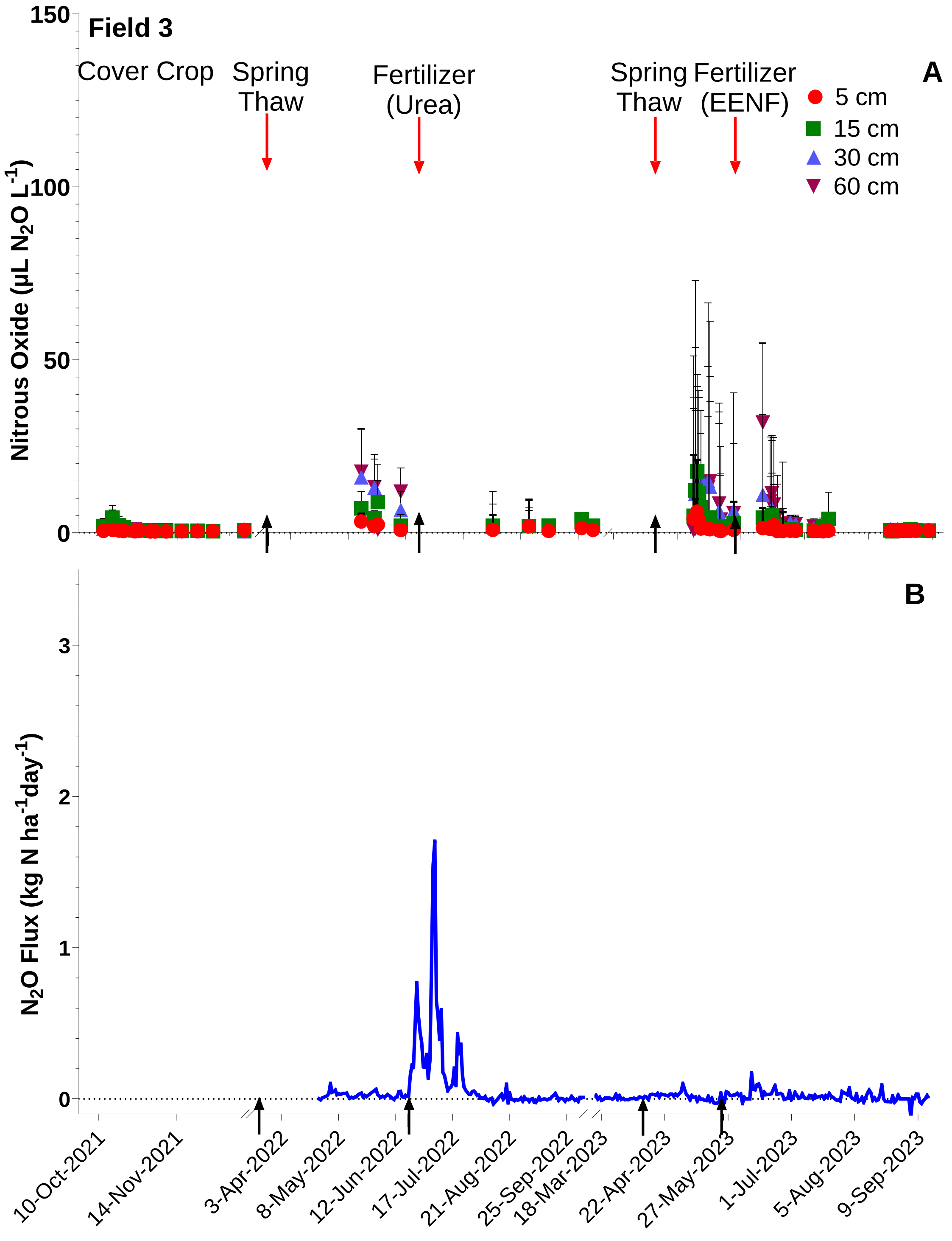

Figure 2. 9 N_2O concentrations in soil profiles (A) and surface flux rates (B) from 2021 October to 2023 September on Field 3.

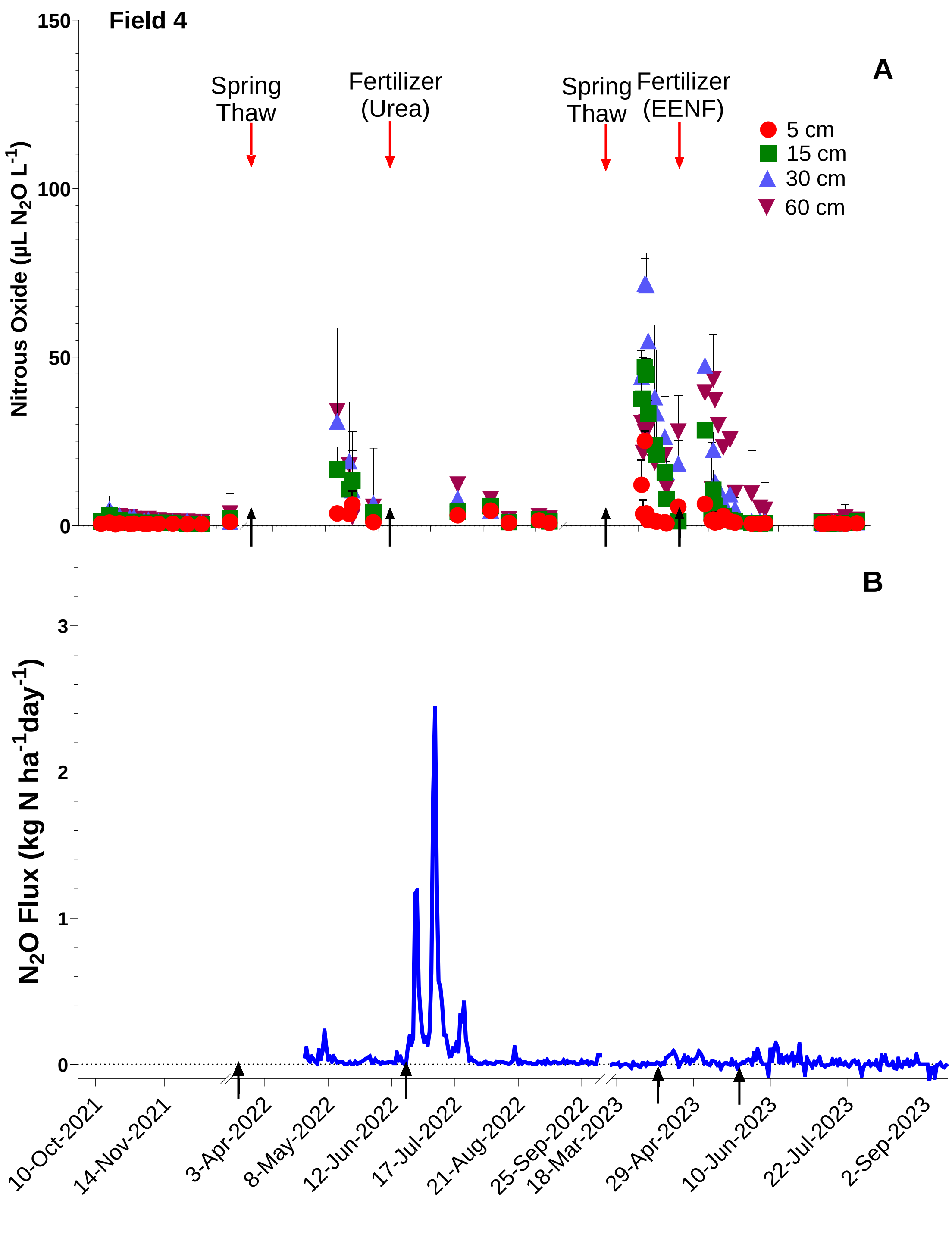

Figure 2. 10 N_2O concentrations in soil profiles (A) and surface flux rates (B) from 2021 October to 2023 September on Field 4.

2.4.4 The Effect of Management Practices on Soil Profile N_2O Concentrations at Different Depths

2.4.4.1 Cover Crop (post-harvest and spring–thaw)

We used the Wilcoxon signed rank test to analyze the impact of cover crop treatments on N_2O concentration at different depths during the post-harvest and the spring–thaw period. Results indicated significant effects of cover crop treatment on N_2O concentration across all depths (5, 15, 30, and 60 cm) during post-harvest (Table 2.3). Despite generally low concentrations during the post-harvest period, cover crop treatments significantly ($P<0.001$) reduced N_2O concentrations at all depths, with median N_2O concentrations of 0.43, 0.57, 0.63, and 0.65 μL N_2O L^{-1} at 5, 15, 30, and 60 cm depths, respectively, compared to 0.48, 0.96, 1.37, and 1.21 μL N_2O L^{-1} for non-cover crop controls.

From October to December 2021 (post-harvest period), N_2O concentrations were higher at all depths in non-cover crop treatments than in cover crop treatments. Average N_2O concentrations during this period in cover crop treatments were approximately 0.57, 0.92, 1.04, and 0.86 μL N_2O L^{-1} at depths of 5, 15, 30, and 60 cm, respectively. Conversely, N_2O concentrations in fields without cover crops exceeded those with cover crops, with average concentrations of approximately 0.59, 2.02, 2.9, and 2.4 μL N_2O L^{-1} at depths of 5, 15, 30, and 60 cm, respectively, in the soil profile.

The highest N_2O concentration at a depth of 5 cm was observed on December 14, 2021, being 1.25 μL N_2O L^{-1} for cover crop treatments and 0.96 μL N_2O L^{-1} for non-cover crop treatments (Figure 2.11). At a depth of 15 cm, the highest N_2O concentration for cover crop treatments occurred on October 16, with an approximate value of 2.8 μL N_2O L^{-1}, and for non-cover crop treatments on October 19, with about 3.9 μL N_2O L^{-1} (Figure 2.12). Peak N_2O concentration in the 30 cm soil profile was observed during the post-harvest period, occurring on October 16 for both cover crop and non-cover crop treatments, with concentrations of approximately 2.9 μL N_2O L^{-1} and 7.2 μL N_2O L^{-1}, respectively (Figure 2.13). At 60 cm, cover crop treatments exhibited the highest N_2O concentration of 1.54 μL N_2O L^{-1} on October 16, while non-cover crop treatments reached the peak concentration of 4.2 μL N_2O L^{-1} on October 19 (Figure 2.14).

Following the spring-thaw period in 2022, N_2O concentration sampling was conducted on May 16, 24, and 26. Despite generally high concentrations in May, cover crops did not result in significantly different N_2O concentrations compared to non-cover crop treatments at any depth (Table 2.3). The median N_2O concentrations were 2.3, 5.4, 7.9, and 7.1 μL N_2O L^{-1} at depths of 5, 15, 30, and 60 cm for the cover crop treatment, respectively, compared to 2.8, 5.9, 7.3, and 5.9 μL N_2O L^{-1} for the non-cover crop control. N_2O concentration increased with depth in this period until 30 cm and then decreased at the 60 cm depth of the soil profile.

During this period, N_2O concentration levels were numerically higher in the non-cover crop treatments than in the cover crop treatments. At a depth of 5 cm in the soil profile, both treatments recorded approximately 3.4 μL N_2O L^{-1}, while at 15 cm depth, concentrations were 6.7 μL N_2O L^{-1} for cover crop treatments and 8.7 μL N_2O L^{-1} for non-cover crop treatments. Subsequently, concentrations increased to approximately 11.2 μL N_2O L^{-1} and 12.7 μL N_2O L^{-1} for cover and non-cover crop treatments, respectively. Finally, concentrations at the deepest sampling depth decreased to approximately 10.1 μL N_2O L^{-1} and 11.8 μL N_2O L^{-1} for cover crop and non-cover crop treatments, respectively.

On May 16, the highest N_2O concentration was observed at all depths for the cover and non-cover crop treatments. Significant increases were observed at a depth of 15 cm in the soil profile for both cover crop and non-cover crop treatments, with concentrations reaching approximately 6.2 μL N_2O L^{-1} and 10.1 μL N_2O L^{-1}, respectively. The concentration then remained steady at 30 cm on May 16 before increasing at a depth of 60 cm in the soil profile for both cover crop and non-cover crop treatments, reaching approximately 16.5 µL N_2O L^{-1} and 21.0 µL N_2O L^{-1}, respectively.

Table 2.3 Wilcoxon signed rank test comparing soil profile N_2O concentrations at different depths during the post-harvest and spring–thaw periods between the cover and non-cover crop treatments.

Depth	Period	Cover Crop			Non-Cover Crop				
5 cm		Mean	Median	SE	Mean	Median	SE	n	P
	Post-harvest (Oct 2021-Dec 2021)	0.57	0.43	0.040	0.59	0.48	0.02	156	0.0039**
	Spring–thaw (Apr 2022-May 2022)	3.4	2.35	0.43	3.4	2.85	0.4	36	0.59
15 cm	Post-harvest (Oct 2021-Dec 2021)	0.95	0.57	0.100	2.03	0.96	0.28	156	<0.0001***
	Spring–thaw (Apr 2022-May 2022)	6.7	5.4	0.74	8.7	5.9	1.3	36	0.65
30 cm	Post-harvest (Oct 2021-Dec 2021)	1.04	0.63	0.09	2.94	1.37	0.31	156	<0.0001***
	Spring–thaw (Apr 2022-May 2022)	11.2	7.9	1.5	12.7	7.3	2.2	36	0.79
60 cm	Post-harvest (Oct 2021-Dec 2021)	0.86	0.65	0.06	2.43	1.21	0.29	156	<0.0001***
	Spring–thaw (Apr 2022-May 2022)	10.1	7.1	1.6	11.8	5.6	2.7	36	0.87

*, **, and *** indicate significance at P = 0.05, 0.01, and 0.001, respectively.

SE represents standard error, n represents sample size, and P represents the p-value.

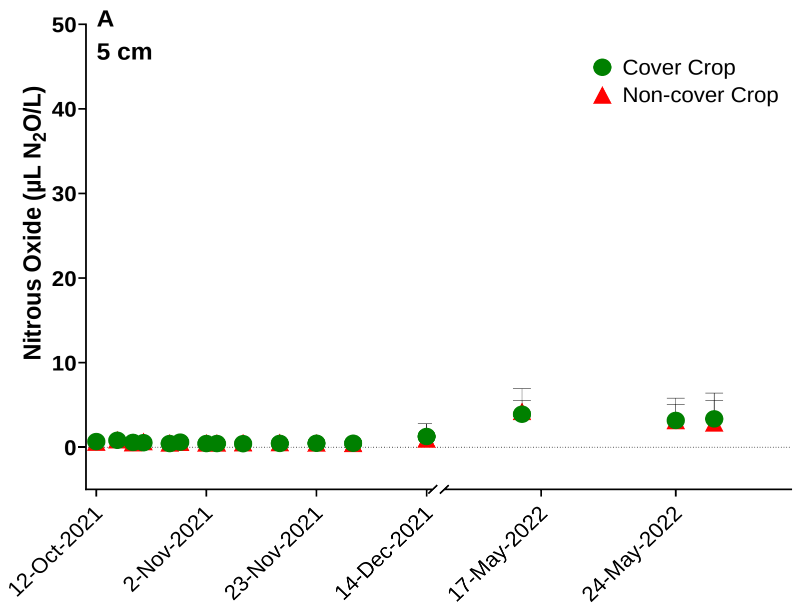

Figure 2.11 N_2O soil concentration at a soil depth of 5 cm for the cover and non-cover crop treatments during the post-harvest and spring–thaw periods. Bars are +1 standard error of the means.

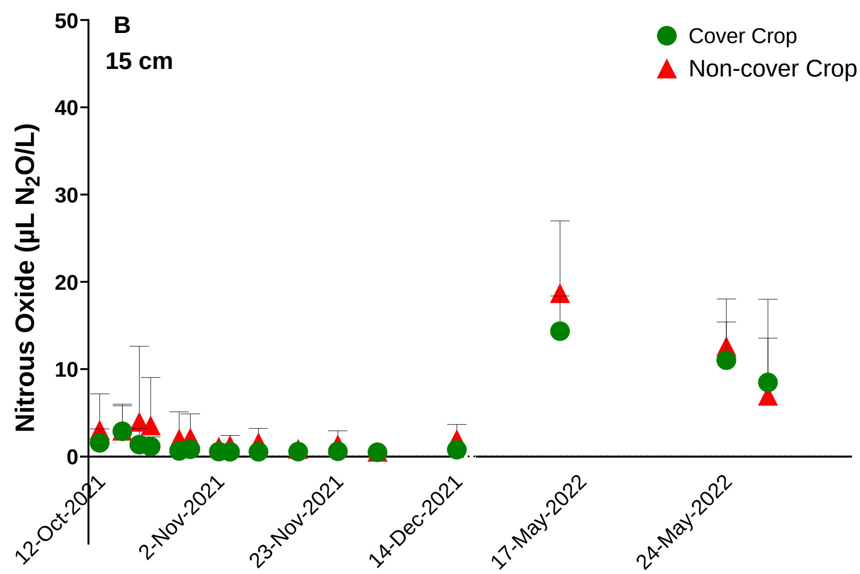

Figure 2.12 N_2O soil concentration at a soil depth of 15 cm (B) for the cover and non-cover crop treatments during the post-harvest and spring–thaw periods. Bars are +1 standard error of the means.

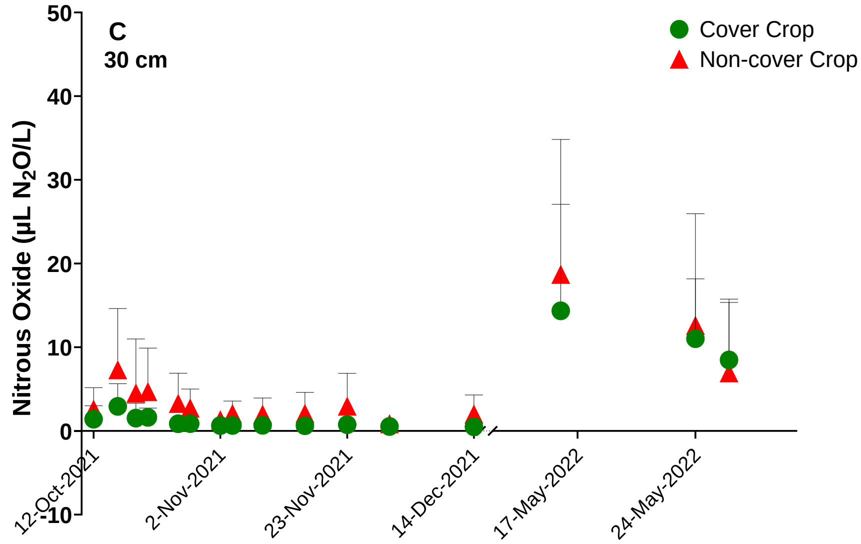

Figure 2.13 N_2O soil concentration at a soil depth of 30 cm (C) for the cover and non-cover crop treatments during the post-harvest and spring–thaw periods. Bars are +1 standard error of the means.

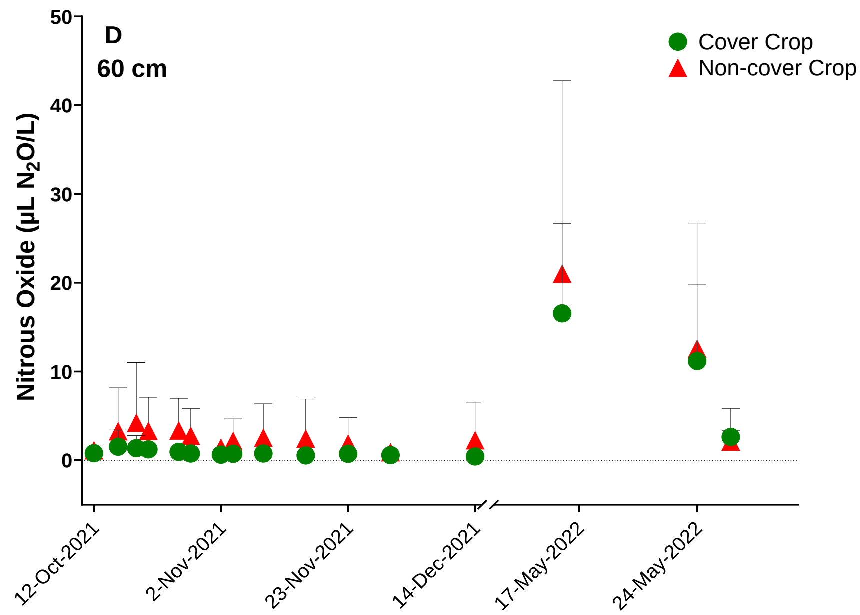

Figure 2.14 N_2O soil concentration at a soil depth of 60 cm (D) for the cover and non-cover crop treatments during the post-harvest and spring–thaw periods. Bars are +1 standard error of the means.

2.4.4.2 Enhanced Efficiency Nitrogen Fertilizer

The Wilcoxon signed-rank test was used to compare the treatment medians and examine the effect of different fertilizer treatments on N_2O concentration at various depths during the spring–thaw period (May 1 until May 10, 2023) with a 7-date sampling period and growing season 2023. The results revealed that the overall effect of different fertilizer treatments on N_2O concentration at all depths (5, 15, 30, and 60 cm) was not significant during spring–thaw (Table 2.4). During the spring–thaw period, the average N_2O concentration during this period at 5 cm of the soil profile was about twice as high in the conventional fertilizer treatment compared to EENF. The highest N_2O concentration on EENF treatments at 5 cm depth was observed on May 1 with about 4.9 μL N_2O L^{-1} while the highest N_2O concentration for EENF treatments was recorded on May 3 with about 15.6 μL N_2O L^{-1}.

The N_2O concentration at 15 cm of soil profile was higher in EENF treatments rather than conventional. The average N_2O concentration was about 26.3 μL N_2O L^{-1} and 21.8 μL N_2O L^{-1} for EENF and conventional treatments, respectively. On May 4, the highest N_2O concentration was observed, with about 35.5 for EENF treatment. The N_2O concentration at 30 cm showed a similar trend as those at 15 cm, being generally higher with EENF (34.3 μL N_2O L^{-1}) compared to conventional fertilizers (32.3 μL N_2O L^{-1}). The highest N_2O concentration for EENF was observed on May 4, with about 44 μL N_2O L^{-1}. The average N_2O concentration at 60 cm was higher in conventional treatments compared to EENF, being 15.3 and 12.2 μL N_2O L^{-1} for conventional treatments and EENF treatments, respectively. The highest N_2O concentration was about 16 μL N_2O L^{-1} for both treatments.

However, at a depth of 5 cm, the effect of fertilizers on N_2O concentration was significant, with EENF showing lower N_2O concentrations compared to conventional fertilizers. This significant difference was not observed at other depths (15, 30, and 60 cm) in the soil profile during the growing season of 2023 (Table 2.4). In the growing season of 2023, the median N_2O concentration for EENF was approximately 0.66, whereas for the conventional (urea) treatment, it was approximately 0.87. N_2O concentrations increased from 5 cm to 60 cm depth. The highest concentrations during this period were observed at a depth of 60 cm in the soil profile for both EENF and urea. At a depth of 30 cm in the soil profile, the N_2O concentration did not show a significant difference between treatments, although the median concentration with urea was higher than with EENF. A similar pattern was observed at a depth of 15 cm, where there was also no significant difference in N_2O concentration between treatments, with urea showing a higher median concentration compared to EENF.

Table 2.4 Wilcoxon signed rank test comparing soil profile N_2O concentrations at different depths during the spring–thaw periods and growing season 2023 between EENF and Conventional (Urea) treatments.

Depth	Period	EENF			Conventional (Urea)				
		Mean	Median	SE	Mean	Median	SE	n	P
5 cm	Spring–thaw (2023)	2.14	1.20	0.21	4.0	1.1	0.97	120	0.75
	Growing Season (2023)	1.2	0.66	0.12	1.56	0.87	0.14	276	0.0003***
15 cm	Spring–thaw (2023)	20.63	2.64	3.62	16.85	2.77	3.17	120	0.51
	Growing Season (2023)	3.64	0.95	0.53	2.87	1.3	0.26	276	0.08
30 cm	Spring–thaw (2023)	1.975	9.36	0.45	1.64	9.86	0.38	120	0.26
	Growing Season (2023)	7.09	2.13	0.87	8	2.2	0.82	276	0.99
60 cm	Spring–thaw (2023)	12.50	2.27	2.29	14.62	1.51	2.34	120	0.82
	Growing Season (2023)	12.48	2.1	1.1	11.51	1.8	1.2	276	0.69

*, **, and *** indicate significance at P = 0.05, 0.01, and 0.001, respectively.

SE represents standard error, n represents sample size, and P represents the p-value.

2.4.5 Relationship of Soil N_2O Concentrations with Surface Flux and Environmental Factors

Pearson correlation analysis was conducted to examine the relationship of N_2O concentration at different soil depths with surface flux rate, NO_3^-, NH_4^+, air temperature, soil temperature (5,10,20 and 50 cm), and VMC (volumetric moisture content at 10 and 30 cm) (Table 2.5). At shallow depths of 5 cm and 15 cm, N_2O concentration was positively correlated with volumetric moisture content (VMC), while showing no significant correlation with other variables (Table 2.5). In contrast, at a depth of 30 cm, N_2O concentration displayed a negative correlation solely with soil temperature, suggesting a potential influence of soil temperature on N_2O dynamics at this depth. Furthermore, at a depth of 60 cm, N_2O concentration exhibited a negative correlation with VMC, indicating a potential role of soil moisture in regulating N_2O concentrations even at greater depths within the soil profile. These findings highlight the complex interplay between N_2O concentration and environmental variables across different soil depths, underscoring the importance of considering multiple factors in understanding N_2O dynamics in soil ecosystems.

Table 2.5 Pearson Correlation coefficient (r) of N_2O concentration at different depths with soil NO_3^-, NH_4^+, air temperature, soil temperature, volumetric water content (VWC), and surface flux rate.

Depths	NO_3^- – N (mg/Kg)	NH_4^+- N (mg/Kg)	Air Temperature (C)	Soil Temp (5cm)	Soil Temp (10 cm)	Soil Temp (20 cm)	Soil Temp (50 cm)	VMC 10	VMC 30	Flux
$[N_2O]$-5 cm	-0.019	0.001	0.10	0.03	-	-	-	0.17*	-	0.15
$[N_2O]$-15 cm	-0.06	-0.04	0.03	-	-0.11	-	-	0.18**	-	-0.05
$[N_2O]$-30 cm	-0.02	-0.02	0.01	-	-	-0.15*	-	-	-0.07	-0.05
$[N_2O]$-60 cm	0.04	0.06	-0.04	-	-	-	-0.12	-	-0.28***	0.18

* Indicates significance at P = 0.05. ** Indicates significance at P = 0.01. *** Indicates significance at P = 0.001.

2.5 Discussion

2.5.1 Temporal and Spatial Variation of Soil Profile N_2O Concentrations

2.5.1.1 post-harvest

During the post-harvest period, from October to December, lower concentrations of N_2O were observed compared to the whole study period. The highest N_2O concentration during the post-harvest period was in Field 1, which had a lower latitude than other fields and was aligned with the findings of Rajendran (2010). It might be due to water accumulation causing an anaerobic environment, thereby increasing the rate of denitrification. A slight increase in N_2O concentrations at all depths occurred across all fields in October which might be due to a 45 mm precipitation from October 8 to 14 that can lead to higher levels of moisture in the aerated pores between soil aggregates, inhibiting O_2 exchange and making anoxic situation which leads to denitrification process (Chapuis-Lardy et al., 2007; Qin et al., 2020).

Our findings showed higher N_2O concentrations than previous research by Rajendran (2010), who analyzed N_2O concentrations at different depths under different topography in clay loam soils at the Manitoba Zero-Tillage Research Association's farm in Brandon, Manitoba. They reported the lowest N_2O concentrations were observed in the fall with about 1.32 $\mu L\ L^{-1}$ at 35 cm of soil profile. They found that during the post-harvest period, N_2O concentrations were lower in the Upper and Middle landscape sections compared to Riparian areas, likely due to increased soil oxygen and decreased moisture. In another study conducted in Ontario, Canada, Risk et al. (2014) reported that soil N_2O concentrations remained low during the post-harvest and winter periods, with values below 1.97 $\mu L\ N_2O\ L^{-1}$ at all depths (10 cm, 15 cm, 30 cm, and 60 cm), which was closely aligned with our findings.

Smith et al. (2017) found that N_2O production was notably reduced during the post-harvest period due to decreased microbial activity and limited availability of organic matter. Additionally, Köbke et al. (2018) reported that N_2O emissions from agricultural fields were minimal during the post-harvest period, attributing this phenomenon to reduced nitrogen inputs and lower soil temperatures. Their study showed that lower soil temperatures correlated with decreased N_2O emissions, aligning with our observations.

2.5.1.2 Spring–thaw

During the spring–thaw period, N_2O concentrations increased across all depths. This rise in N_2O concentrations in response to higher temperatures and increased moisture has been well-documented (Gao et al., 2014; Risk et al., 2014). The highest concentrations were observed at depths of 15 and 30 cm, predominantly at 30 cm, indicating that the majority of N_2O was produced from this layer of soil and subsequently diffused into the surface soil. In contrast, Wang et al. (2013) highlighted that N_2O production induced by fertilizer primarily occurs in the topsoil layer (0-30 cm) due to heightened microbial activity. Previous research studies conducted in Manitoba, Canada, also found significant N_2O production specifically in the surface soil (0-5 cm) following the application of urea ammonium nitrate solution (Gao et al., 2014). The difference in N_2O concentration distribution between our research and others could be due to variations in different farming practices, soil types, and weather conditions.

The relatively higher N_2O concentration in the soil profile occurred mostly between the depth of 15-30 cm during both spring–thaw periods similar to previous results in western Manitoba (Rajendran, 2010). In contrast, lower values of N_2O concentration were mostly found in the surface layer (5 cm). Our findings in this study indicate that newly produced N_2O through denitrification is the primary mechanism of N_2O production during this period. We observed the highest N_2O

concentrations at a depth of 30 cm in the soil profile, coinciding with peak microbial activity. Interestingly, N_2O production remained low during fall and winter, challenging the hypothesis that N2O produced during these seasons is trapped beneath the ice surface layer and released during spring–thaw periods.

Moreover, saturated soil conditions during spring–thaw can limit gas diffusion, potentially leading to the accumulation of N_2O within the subsurface region, as suggested by previous research (Risk et al., 2014). Overall, the observed increase in N_2O production during the spring–thaw 2023 underscores the complex interplay of environmental factors influencing soil microbial processes and greenhouse gas emissions.

The dominance of microbial activity in N_2O production underscores the dynamic nature of soil processes during freeze-thaw cycles, where microbial communities respond to changing environmental conditions by initiating denitrification processes and releasing N_2O. This emphasizes the importance of understanding and investigating the intricate interplay in soil microbial dynamics, environmental factors, and greenhouse gas emissions to accurately assess the impact of freeze-thaw cycles on soil N_2O dynamics. Further research is needed to delve into the mechanisms through which microbial communities drive N_2O production during spring–thaw events, shedding light on the complexities of soil biogeochemical processes in response to changing climatic conditions.

In 2022, despite an increase in N_2O concentration across all depths, the actual N_2O emission was not significant during spring thaw. This could be because the soil was overly wet and saturated during that period. Saturated soil conditions limit the movement of N_2O, within the soil. Saturated soil conditions restrict the diffusion of N_2O, which in turn limits their upward movement to the shallow soil surface (Clunes et al., 2020; Kuang et al., 2019). This phenomenon aligns with findings from studies such as Smith et al. (2010), which emphasize the influence of soil moisture on N_2O emissions. Additionally, under anaerobic conditions, microbial processes such as dissimilatory nitrate reduction to ammonium (DNRA) or reduction of N_2O to N_2 may become more prevalent. These processes can mitigate the net release of N_2O to the atmosphere (Wrage et al., 2001).

During the spring–thaw of 2023, the N_2O concentration in the soil profile was notably higher compared to the previous year. This increase in N_2O concentration can be attributed to several factors, including moisture levels, increased organic matter and microbial biomass carbon content, and increased activity of denitrifying enzymes (Tenuta and Sparling, 2011). Another reason would be because the precipitation and moisture level during spring–thaw 2022 was much higher than spring–thaw 2022 and it was reported that when soil moisture levels are extremely high, the production of N_2O decreases. This phenomenon happens because microbial activity may decline when the soil becomes waterlogged, obstructing airflow in the soil pores and reducing oxygen availability. Initially, increased soil moisture boosts microbial activity, but excessive moisture suppresses it (Signor and Cerri, 2013; Schlüter et al., 2024). According to Liu et al. (2011), N_2O emissions showed an initial increase followed by a decrease as soil moisture levels were increased.

2.5.1.3 Growing Season

After fertilizer application in 2022, due to heavy rain, we couldn't install sampling probes immediately following fertilizer application. However, after installing samplers, we noticed a significant increase in N_2O concentration. This surge in soil N_2O concentrations after fertilizer application was likely due to the abundance of nitrogen available for microorganisms. Moreover, in 2023, we managed to install probes and observed a considerable peak in N_2O concentration.

This suggests that more nitrogen available after fertilizing likely enhanced microbial activity, causing the peak in N_2O concentration. As a result, we noticed higher surface N_2O emissions after applying fertilizer in 2023.

During the 2022 growing season, elevated N_2O concentrations were detected at a depth of 60 cm in soil profiles in Fields 1 and 4. This could be explained by the diffusion of N_2O to deeper layers of the soil. Factors like increased precipitation may have facilitated the downward movement of N_2O within the soil profile. In the 2023 growing season, higher N_2O concentrations were found at a depth of 60 cm in all fields, indicating that N_2O was leached to deeper soil profiles. This highlights the need to understand how N_2O moves through the clay soil and its possible effects on nitrogen loss.

2.5.2 Relation between Soil Profile N_2O Concentration and Surface N_2O Flux

The lack of a significant relationship between soil profile N_2O concentration and surface flux rate suggest a complex interplay of factors influencing greenhouse gas dynamics in soil systems. Studies have shown that N_2O emissions are influenced by various processes beyond simple concentration levels, including gas diffusion across soil profile, and soil physical properties such as texture and moisture. The decoupling of N_2O concentration and surface flux highlights the need to consider multiple interacting variables when assessing greenhouse gas dynamics in soil environments. Moreover, studies have demonstrated that N_2O production and consumption can happen simultaneously in soils, complicating how we interpret N_2O concentration and emission rates. Understanding the mechanisms driving N_2O emissions requires a comprehensive analysis of the underlying biogeochemical processes and environmental conditions that drive gas fluxes in soil systems.

2.5.3 Agricultural Management

2.5.3.1 Cover Crop

Studies showed that cover crop application as a farming practice can decrease N_2O emissions from croplands due to mineral N availability uptake by plants for N_2O-producing processes (Eagle and Olander, 2012; McSwiney et al., 2010). Few studies focus on cover crop farming practices on N_2O concentration at different depths across soil profiles. A recent study was conducted at the TGAS to measure the effect of cover crops on N_2O flux emission over five years and reported that growing winter rye reduced N_2O emissions (Webb, 2023). In the current study, we found that the N_2O concentrations at different depths were significantly reduced by the cover crop during post-harvest, which was aligned with previous findings (Webb, 2023) and supported the idea that cover crops play a significant role in mitigating N_2O emissions.

This can be attributed to several factors. First, cover crops can improve soil structure by promoting root growth (Haruna et al., 2020). Better soil structure enhances water infiltration and aeration, reducing the likelihood of anaerobic conditions favouring N_2O production during denitrification. Second, cover crops can take up excess soil nitrate, a precursor for N_2O production. By reducing nitrate availability in the soil, cover crops can limit the substrate available for denitrification, thus reducing N_2O emissions (Adetunji et al., 2020; Webb, 2023).

Additionally, Basche et al. (2014) highlighted the potential of cover crops to decrease soil water through transpiration, influencing N_2O-producing microbial processes. Also, Kaye et al. (2019) emphasized the role of cover crops as agricultural nitrogen regulators, reducing leaching and delivering N to subsequent cash crops, potentially impacting N_2O emissions. These studies collectively suggest that cover crops have the potential to influence N_2O concentrations through their impact on soil properties, N cycling, and microbial processes. Cover crops may delay soil freezing in the fall, leading to improved soil insulation, and reduced freezing intensity, which could contribute to reductions in spring–thaw N2O concentration (Webb, 2023).

While Webb (2023) reported a significant decrease in N_2O emissions from the soil surface due to cover crops during the spring–thaw of 2022, the current study did not find a concurrent decrease of N_2O concentrations throughout the soil profile at all depths during spring–thaw. Although the effect of cover crop and non-cover crop treatments on N_2O soil profile concentration during the spring–thaw was not statistically significant, on average, N_2O concentration was numerically higher for non-cover crop treatments compared to cover crop treatments during spring–thaw. Petersen et al. (2011) investigated the emissions of N_2O and concentration in different tillage treatments with a winter cover crop and reported that cover crop residues decreased N_2O emissions.

2.5.3.2 Different N Fertilizer Sources on Soil N_2O Concentration During Spring–Thaw and Growing Season

Using EENF reduced the average N_2O concentration at the 5 cm depth during the spring–thaw compared to conventional urea, but it was not statistically significant. This reduction was attributed to EENF's ability to delay the conversion of NH_4^+ to NO_3^-, resulting in an extended residence time of applied urea N in the form of NH_4^+. Our observation aligns with the results of Chen et al. (2021), who reported a reduction in N_2O emissions with EENFs compared to conventional fertilizers during the spring–thaw. Wood et al. (2024) conducted a study across six site-years in Manitoba using different fertilizer sources and found that eNtrench was effective in mitigating cumulative N_2O emissions by 47%–64%.

The spring–thaw N_2O concentration increased at the 15 and 30 cm soil profile depths in our study when using EENF. This finding challenges the conventional understanding of EENF benefits in reducing N_2O emissions. It is worth considering the difficulties and challenges of using EENF during the 2022 growing season, which may have influenced this finding. Therefore, further research and sampling are needed to understand the effect of eNtrench and Centrue on clay soil at different depths during spring–thaw.

The effect of superU during the growing season of 2023 in N_2O concentration was significant at the 5 cm soil depth, indicating that fertilizer application had the highest impact on N_2O concentration in the surface soil profile. SuperU significantly decreased N_2O concentration at the 5 cm soil depth but this effect was not significant at the 15 cm, 30 cm, and 60 cm soil depths. However, when comparing the median N_2O concentrations of superU and urea, superU showed lower N_2O concentrations than urea at the 15 cm and 30 cm soil depths.

During a three-year field study conducted by Wood et al. (2024) in Manitoba, it was observed that SuperU decreased N_2O emissions by 37%–57% in three out of six instances across different fields each year. In a plot-based cotton field study conducted in northwestern China by Li et al. (2021), the effects of applying polymer-coated urea (ESN), conventional urea (Urea), and SuperU (with a rate of 120 and 240 kg N ha^{-1}) on N_2O concentrations at soil depths of 5 cm, 15 cm, 30 cm, and 60 cm were investigated. They found that the average N_2O concentrations throughout the growing season were not significantly influenced by nitrogen fertilizer type. However, further studies and research are needed to examine the effects of different sources of nitrogen fertilizers on N_2O transport, consumption, and production in the soil profile at different depths.

2.6 Conclusion

Our study showed an average fluctuation in N_2O concentrations across various depths, ranging from 0.5 to 42 µL N_2O L^{-1}. Throughout the study period, the lowest N_2O concentrations were consistently found at shallow depths, whereas peak concentrations were observed at deeper soil depths. The mechanism influencing N_2O concentrations during spring–thaw may involve *de novo* production, potentially explaining higher N_2O levels in deeper soil layers due to increased microbial activity. Notably, there was no significant correlation observed between N_2O concentrations in the soil profile and N_2O flux emissions.

The presence of cover crop had a significant impact on N_2O concentrations during the post-harvest period. Additionally, although not statistically, cover cropping numerically reduced N_2O concentrations in the soil profile during the spring–thaw period, coinciding with a decrease in surface N_2O flux. These findings suggest that cover crops could potentially serve as an effective strategy to mitigate N_2O production within the soil profile.

While different fertilizer sources did not significantly impact N_2O concentrations, EENF showed the potential to decrease N_2O levels specifically in the 5 cm depth, with no noticeable effect observed at deeper depths. This underscores the importance of examining depth-specific responses to fertilizer applications when managing N_2O emissions. Continued monitoring is recommended to further investigate the relationships between N_2O concentrations in the soil profile, providing valuable insights for sustainable agricultural practices.

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3. OVERALL SYNTHESIS

3.1 Study Findings and Implications

Research on the effects of different farming practices on N_2O concentrations in soil profiles is limited in the Canadian Prairies. Understanding the balance between N_2O production and consumption processes within the soil profile is crucial for identifying effective strategies to mitigate N_2O emissions. While most of the research has predominantly focused on surface emissions, there was a significant gap in understanding the correlation between surface emissions and greenhouse gas production in deeper soil layers, especially under varied management practices during the spring–thaw period. This understanding is essential for developing region-specific mitigation strategies.

This study indicates significant variability in N_2O concentrations at different soil depths, ranging from 0.5 to 42 μL N_2O L^{-1}. Lower N_2O concentrations were observed at shallow depths throughout the study period, while peak concentrations were predominantly found in deeper soil layers. This pattern suggests a possible connection between depth and N_2O concentrations, potentially influenced by increased microbial activity in 15-30 cm of soil profile, during spring–thaw periods which could trigger *de novo* production mechanisms as a main mechanism driving N_2O production during the spring–thaw period. Also, no correlation between N_2O concentrations in the soil profile and N_2O flux emissions was observed. This suggests that factors other than just N_2O concentration and production levels might affect N_2O emissions.

The impact of cover crops, especially winter rye (*Secale cereale* L.), on soil N_2O concentrations during the post-harvest period was significant, suggesting their effectiveness in mitigating N_2O production within the soil profile. However, their influence on spring–thaw N_2O concentration was not significant, highlighting the necessity for additional research to explore underlying mechanisms that could be contributing. While various fertilizer sources did not significantly affect N_2O concentrations during the spring thaw, Enhanced Efficiency Nitrogen Fertilizer (EENF) demonstrated a significant difference in reducing N_2O concentration at a depth of 5 cm of soil profile during the growing season. However, this effect was not observed at deeper depths, indicating the importance of considering depth-specific responses to fertilizer applications in managing N_2O emissions effectively. Based on these findings, it is recommended to continue monitoring the relationships between N_2O concentrations in soil profiles and N_2O emission. These insights will be crucial for implementing sustainable agricultural practices effectively.

3.2 Study Recommendations

This study represents a pioneering investigation into the impact of agricultural practices on N_2O concentrations at various depths in the Canadian Prairies. The results underscore the diverse behavior of N_2O within the soil profile under the influence of different farming practices, with a specific focus on cover crops and EENF. To enhance our understanding of greenhouse gas emissions in agricultural systems, future studies should explore the influence of soil physical properties on N_2O dynamics. Properties such as soil texture, structure, and moisture content play significant roles in N_2O emissions (Buchkina et al., 2013). During the spring–thaw of 2022, the statistical significance of cover crops on N_2O concentration was not demonstrated due to challenging field access and limited sampling occasions. Using silicone diffusive equilibrium samplers with extended wires could improve sampling reliability under such conditions.

Installing silicone diffusive equilibrium samplers may affect microbial activity and soil physical properties. These samplers might alter nutrient availability, moisture levels, and oxygen diffusion, impacting soil properties like porosity, compaction, microbial activity, and water retention capacity. These changes potentially influence soil processes, including nutrient cycling, organic matter decomposition, and N_2O concentration behavior. Therefore, understanding the implications of samplers on microbial activity and soil physics is crucial. In order to fully evaluate the environmental impact of agricultural practices, our study highlights the necessity of taking indirect emissions into account in addition to direct emissions. In this study, we observed N_2O leaching into deeper soil profiles, suggesting an indirect production pathway. This highlights the need for further investigation to develop more research in indirect N_2O concentration in the soil profile.

Another suggestion is using ^{15}N-labeled to specifically trace nitrogen transformations (e.g., nitrification, denitrification) in the system. The most accurate and precise method for tracking N_2O contributions from various sources and calculating the N process turnover is the ^{15}N-labeling technique (Li et al., 2024). This method can be used to calculate the percentage of N_2O emissions that originate from each source. To fully evaluate the environmental impact of agricultural practices, our study suggests the necessity of taking indirect emissions into account in addition to direct emissions by applying this method.

Our study demonstrated a reduction in post-harvest N_2O production within the soil profile through the cover crop implementation. This aligns with findings by Webb (2023) highlighting the significant role of cover crops in mitigating N_2O emissions. Conversely, the impact of EENF on N_2O concentrations at various depths did not show significant effects in our study. However, challenges in fertilizer application in the growing season of 2022 may have limited the accurate assessment of EENF's influence on N_2O concentration. Further investigations are recommended to explore the specific effects of EENF on N_2O concentration across different soil depths. This additional research will provide valuable insights into optimizing fertilizer practices to effectively mitigate N_2O emissions and advance our understanding of greenhouse gas dynamics in agricultural systems.

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