Increased N2O Production from Soil Organic Matter Following a Simulated Fall-Freeze-Thaw Cycle: Effects of Fall Urea Addition, Soil Moisture, and History of Manure Applications

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Increased N$_2$O production from soil organic matter following a simulated fall-freeze-thaw cycle: Effects of fall urea addition, soil moisture, and history of manure applications

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Abstract

Adding nitrogen substrates to soils can induce short-term changes in soil organic matter (SOM) transformations – a response termed the ‘priming effect’. However, it is unknown how priming effects on nitrous oxide (N$_2$O) emissions can be altered following a strong freeze-thaw cycle. A mesocosm experiment evaluated two soil managements: with and without history of manure applications. These soils were subjected to three moisture regimes: Low, Medium and High. Apart from the controls, which received no N, we banded $^{15}$N-labelled urea into these soils representing a typical fall fertilization, and subsequently simulated a wide fall-freeze-thaw cycle, with temperatures from +2, to -18, and finally +23°C, respectively. The overall highest N$_2$O production was observed 1 day after thawing. At that time, measurements of N$_2$O site preference indicated that denitrification produced 83% of the N$_2$O flux. Relative to the unamended controls (baseline), adding urea consistently triggered a 24% greater cumulative N$_2$O production specifically originated from SOM following thawing (245 vs. 305 μg N$_2$O-N kg$^{-1}$ soil, $P=0.022$). This substantiates a positive priming of SOM that manifested shortly after the rapid, wet thawing of the soils. Soils having a manure history or higher moisture also exhibited an augmented production of N$_2$O from SOM ($P<0.01$). Although the overall priming of SOM was positive, two weeks after thawing, negative priming of daily N$_2$O fluxes also occurred, but only in soils under High moisture. Besides urea additions, the propensity for primed N$_2$O emissions from SOM after thawing was influenced by increasing moisture and earlier manure applications.

Key words: priming effect, nitrous oxide, organic matter, denitrification, freeze, thaw.
1. Introduction

Nitrous oxide ($\text{N}_2\text{O}$) is a potent greenhouse gas – with even 300-fold higher global warming potential than carbon dioxide ($\text{CO}_2$) on mass basis (Parry et al. 2007, Intergovernmental Panel on Climate Change 2013). More than half of the anthropogenic sources of $\text{N}_2\text{O}$ are linked to agricultural landscapes (Parry et al. 2007, Intergovernmental Panel on Climate Change 2013, Chai et al. 2020), where manure and synthetic N fertilizers are recurrently applied (Lin et al. 2017, Grant et al. 2020, Thilakarathna et al. 2020). Such N additions not only provide substrates for $\text{N}_2\text{O}$ emissions directly, but they can also stimulate mineralization of pre-existing SOM, which would subsequently lead to additional $\text{N}_2\text{O}$ emissions indirectly – a response termed the ‘priming effect’ (Thilakarathna and Hernandez-Ramirez 2021). In other words, in the case of $\text{N}_2\text{O}$ emissions from soils, priming consists of the fertilizer-induced $\text{N}_2\text{O}$ emissions that originate from SOM mineralization. In further details, the magnitude and direction of $\text{N}_2\text{O}$ priming is a function of the short-term acceleration (positive priming effect) or retardation (negative priming effect) of SOM-derived $\text{N}_2\text{O}$ emissions from a soil receiving N compared to an unamended control as baseline. A comprehensive understanding of $\text{N}_2\text{O}$ priming effects can improve quantification, proactive mitigation and ability to predict $\text{N}_2\text{O}$ emissions from agricultural soils (Grant et al. 2020).

As climate change continues to take place, extreme fluctuations in the weather conditions can occur with increased frequency. Although soil freezing and thawing are already common phenomena in cold regions with relatively high latitude and altitude, the intensity and frequency of freeze-thaw cycles are gradually increasing as a feedback to escalating climate change.
(Goldberg et al. 2008, Easterling et al. 2017). This applies to large temperate regions located in North America and Eurasia where annual croplands typically dominate the landscape. Within this context, earlier laboratory and field studies have examined N₂O emissions taking place over freeze-thaw cycles (Wagner-Riddle et al. 2007, Goldberg et al. 2008, Wolf et al. 2010, Wu et al. 2010, Yanai et al. 2011, Abalos et al. 2016). These reports indicate that N₂O fluxes during spring thaw can typically account for 30-90% of the annual N₂O emissions. A recent field study in western Canada found that at least 67% of the annual N₂O emissions occurred during the spring thaw in soils that had received liquid manure in the previous fall (Lin et al. 2017, Grant et al. 2020). Soil thawing activates N₂O production not only because of fast increases in ambient temperature but also because of sudden increases in soil water content caused by snow and ice melting (Wolf et al. 2010, Thilakarathna et al. 2020). It has been documented that increased moisture influences N₂O production by displacing and reducing oxygen availability, which drives a shift towards microbial utilization of nitrate as terminal electron acceptor during denitrification (Davidson 1991, Ruser et al. 2006, Lin and Hernandez-Ramirez 2020).

Nevertheless, there is currently a paucity of knowledge on how freeze-thawing can influence the production of N₂O from SOM and the associated priming effects caused by labile N additions.

In addition to examining the priming of SOM, there is also a growing interest to assess the underlying processes of N₂O production. Both nitrification and denitrification contribute to N₂O fluxes from soils (Butterbach-Bahl et al. 2013). However, it is still unclear which of these processes is dominant during peak N₂O emissions in manured soils (Lin et al. 2017, Thilakarathna et al. 2020). As aforementioned an accelerated biological activity immediately
following thawing can critically deplete O$_2$ concentrations in the soil microsites, and hence it is hypoththesized that the contribution of bacterial denitrification to N$_2$O production would also increase (Yanai et al. 2011). The $^{15}$N isotope ratios at the central ($\alpha$; $^{14}$N-$^{15}$N-O) and terminal ($\beta$; $^{15}$N-$^{14}$N-O) positions within the N$_2$O molecule – known as site preference (SP) – can reveal the dominant process contributing to N$_2$O production (Toyoda and Yoshida 1999, Toyoda et al. 2011, Yamamoto et al. 2017). The difference between $\alpha$ and $\beta$ can be expressed as $\text{SP}=\delta^{15}\text{N}_\alpha - \delta^{15}\text{N}_\beta$ (Toyoda and Yoshida 1999, Yamamoto et al. 2017). When N$_2$O fluxes become sufficiently large, this SP analytical approach enables us to examine and apportion the major N$_2$O producing pathways (i.e., nitrification vs. bacterial denitrification) (Zimmerman et al. 2011, Daly and Hernandez-Ramirez 2020, Thilakarathna and Hernandez-Ramirez 2021).

To address the abovementioned unknowns, we conducted a mesocosm experiment with the aim of investigating N$_2$O fluxes and sources (i.e., urea-N versus SOM-N) under increasing soil moisture regimes over a simulated fall season that included a urea addition followed by a strong freeze-thaw cycle in soils that had experienced contrasting histories of manure management. More specifically, this study focused on the dynamics of the N$_2$O priming effects caused by fall-added urea shortly after a sudden thawing. The following hypotheses were tested: i) compared with soils without urea addition (control baseline), adding urea would trigger a positive priming effect of N$_2$O emissions derived from SOM-N; ii) larger priming of N$_2$O production from SOM-N would occur in soils that had previously received recurrent manure applications; iii) increasing soil moisture would amplify SOM-derived N$_2$O production.
2. Materials and Methods

2.1 Soil collection

Soils (0-15 cm depth) were collected from experimental plots receiving spring manure (SW) and without a history of liquid manure injections (field control, CT). The soil was collected in October 2016 from a site located at the Edmonton Research Farm (53°29’30’’N, 113°31’53’’W), Alberta, Canada. The experimental site and field management have been described in Lin et al. (2017). The physical and chemical properties of the soils are shown in Table 1. Field moist soils were mixed and passed through an 8-mm sieve to homogenize and remove any large fragments and plant residue. After mixing, subsamples were oven-dried (105 °C) for 24 hours to measure the water content. Soils were stored at 2 °C until establishing the experiment.

2.2 Experimental setup

The experiment consisted of four sequential phases: i) an initial pre-conditioning phase, ii) a fall phase including urea addition, iii) a freezing phase, and iv) thawing phase. The experiment was established in 5.5-L plastic pots 21 cm in height and 19.8 cm in inner diameter at the top of the container. In each pot, 5.5 kg of soil (as oven-dry equivalent) were packed in increments up to 5.0 L (18.4 cm height) to a bulk density of 1.1 g cm$^{-3}$.

2.2.1 Pre-conditioning phase

To restore and resemble the soil condition as found in cropped fields, we conducted an initializing phase by growing wheat in all the pots in a greenhouse for 3 months. The air
temperature in the greenhouse averaged 23 °C (ranging from 12 to 36 °C). Twelve wheat seeds (AC Muchmore, Canadian Western Red Spring cultivar) (FP Genetics, Regina, SK, Canada) were planted to 4 cm soil depth in each pot in a circle about 1.5 cm from the pot edge. After germination, the number of wheat plants was reduced to eight per pot. Soil moisture was kept at 57% water-filled pore space (WFPS) by weighting the pots and adding water every two days.

Once wheat reached tillering stage, all pots began receiving a 0.5 g L\(^{-1}\) of dissolved fertilizer weekly (i.e., 20% N, 8% P\(_2\)O\(_5\), 20% K\(_2\)O, 0.5% Mg, 0.02% B, 0.05% Cu, 0.4% Fe, 0.05% Mn, 0.005% Mo, 0.05% Zn and 2.8% ethylene diamine tetra-acetate as chelating agent). Throughout the wheat growth period, each pot received an equivalent of 50.85 kg N ha\(^{-1}\) according to typical fertilizer recommendations (McKenzie et al. 2013). The aboveground plant biomass (> 5 cm height) was harvested and removed 3 months after seeding. To represent the crop residue, 5 g dry matter of straw biomass was added to the soil surface of each pot. Two pots from each soil were randomly selected for destructive soil sampling with the aim of measuring ammonium and nitrate concentrations as well as \(^{15}\)N isotopic composition in natural abundance. Six soil cores were taken from each of the two selected pots with an auger (3.5 cm diameter and 18.4 cm depth). The other pots were sealed with caps and stored at 2 °C until the beginning of the next experimental phase.

2.2.2 Treatment application and simulated fall phase

Prior to applying the moisture and urea treatments, all pots were removed from 2 °C to room temperature to facilitate air drying until reaching the target WFPS (e.g., 45%). For each of the
two soil managements (i.e., CT and SW), three moisture regimes and two N additions were applied as experimental treatments. The three soil moisture regimes were Low (i.e., WFPS of 45% over the fall, reaching 70% during freezing, and falling to 55% by the end of the thawing phase), Medium (i.e., 55-80-65% WFPS) and High (65-90-75% WFPS). The N addition treatments were urea (5 atom% $^{15}$N) (Sigma-Aldrich, St. Louis, MO, US) and control (without urea addition). The experimental design was a factorial with three replicates. In sum, the three experimental factors were: history of field manure injection (i.e., CT and SW), three moisture regimes (Low, Medium and High), and N addition (urea and unamended control). A total of 36 experimental pots were used for flux measurement during the experiment.

The moisture and N addition treatments were established on Day 0 of the fall phase which lasted for 27 days. The N addition consisted of 0.29 g of powder consistency 5 atom% $^{15}$N-urea per pot placed at 5 cm depth to represent fertilizer banding. This rate was equivalent to 85 kg N ha$^{-1}$, which simulates a common fall fertilization for a canola crop in the subsequent growing season. After applying the N treatment, room-temperature deionized (DI) water was added to achieve fall moisture levels of 45 (Low), 55 (Medium) and 65% (High) WFPS. As necessary, the WFPS was maintained by weighting the pots and adding DI water every day throughout the fall phase. All pots and glass flasks with DI water were kept at 2°C. Cardboard was placed 3-5 cm above the top of the pots to prevent rapid evaporation while still allowing air circulation.
2.2.3 Simulated freezing phase

On Day 28 after the beginning of the experiment, a freezing phase was started by moving all pots from a temperature of 2 to -18 °C. This freezing phase lasted for 27 days (i.e., Days 28 to 55 following the urea addition) which assured that the soil columns became completely frozen. Additionally, to simulate multiple water inputs that accumulate over a typical winter in Central Alberta (snow and ice precipitation), DI water (at 2 °C) was added during the freezing phase in three successive increments. These water additions were done incrementally in amounts equivalent to 8.3, 8.3 and 8.4% WFPS on Days 31, 37 and 45, respectively. Upon melting, this water input was calculated to increase the water content in the soil by a total of 25% WFPS, which is a typical increase from fall to early spring. For the Low moisture regime, this meant a change from 45 to 70% WFPS; for Medium moisture, from 55 to 80%; for High moisture, from 65 to 90%. In line with typical winter conditions, most of the water added over the frozen mesocosms solidified on the top of the soil surface and it remained frozen until the beginning of the succeeding thawing phase when it melted and infiltrated into the soil column.

2.2.4 Simulated thawing

On Day 56 after urea addition, all pots were moved from -18 °C to room temperature conditions to simulate a strong thawing. The room temperature averaged 23 °C, ranging from 20.4 to 25.9 °C as recorded with a HOBO UX100-001 data logger at 1 Hz (Onset® Computer Corporation, Bourne, MA, USA). After the rapid soil thawing on Day 56, soil moisture content was allowed to gradually decrease by 0.5% WFPS daily. The total moisture decrease was 15% WFPS evenly
distributed over 30 days (from Day 56 to 86 after the urea addition). Soil moisture content was monitored and adjusted daily by weighting the pots and adding room temperature DI water as necessary. On the last day of the experiment (Day 86), three soil cores were taken from each pot with an auger (3.5 cm diameter and 18.4 cm depth). These composite soil samples enabled us to determine ammonium and nitrate concentrations as well as $^{15}$N isotopic composition.

2.3 Measurements of N$_2$O fluxes and isotopic composition

The mixing ratios of $^{14}$N-$^{14}$N-$^{16}$O, $^{14}$N-$^{15}$N-$^{16}$O ($\alpha$) and $^{15}$N-$^{14}$N-$^{16}$O ($\beta$) (Ostrom et al. 2021) were quantified in a continuous mode via direct absorption spectroscopy at wavenumber of 2188 cm$^{-1}$. Briefly, the analyzer was an Aerodyne (Aerodyne Research, Inc., Billerica, MA, USA) with a thermoelectrically-cooled, mid-infrared quantum cascade laser, equipped with 200-m path length analytical cell (2 L volume at 30 Torr vacuum), and Nafion tubing (Perma Pure, Lakewood, New Jersey, USA). Temperature (20 °C) and sample flow rate (1.5 standard L min$^{-1}$) were kept constant in the instrumentation. TDLWintel software provided system control as well as data acquisition and recording at 1 Hz resolution (Daly and Hernandez-Ramirez 2020).

Aerodyne analyzer was coupled with a flow-through, recirculation, non-steady-state chamber. A custom-made cylindrical polyvinyl chloride chamber system consisted of a chamber base and a chamber top. The cross-sectional area of the chamber was 184 cm$^2$ (15.3 cm diameter). The chamber base was installed 3 cm inside the soil, leaving 7 cm above the soil surface. These chamber bases were installed at the center of each pot at the beginning of the fall phase. The chamber top (5 cm in height) was equipped with two tubing connection ports for gas...
recirculation (one for inlet and another one for outlet), a stainless capillary tubing (3/16” in inner
diameter, 10 cm in length) on the wall for the purpose of pressure equilibration, and rubber seals
fitted to the chamber top to ensure headspace closure. The total chamber headspace was 2.2 L.
The chamber enclosure and sample recirculation with the Aerodyne lapsed for 3 minutes.

During every flux measurement, air temperature and pressure were recorded by a HOBO
UX100-001 data logger and a Testo 511 barometer (Testo Inc., Lenzkirch, Germany),
respectively.

2.4 Measurements of CO₂ fluxes

During the fall and freezing phases, CO₂ fluxes from the same soil pots were determined by a
simple system, which included a Picarro G2508 cavity ring-down spectroscope (CRDS) with a
105 mL analytical cell at a constant 140 Torr pressure and at a temperature of 45 °C (Picarro,
Santa Clara, CA, USA), a low-leak diaphragm A0702 pump (Picarro, Santa Clara, CA, USA)
and the custom-made chamber described above. Similar to the N₂O measurements with the
Aerodyne, a vacuum pump enabled the re-circulation of gas sample flow through the chamber
headspace at a rate of 240 standard mL min⁻¹ during an enclosure time of 3 min.

After soil thawing (following Day 56 after the urea addition), CO₂ fluxes were measured
with an automated chamber system, which included the CRDS described above, and an eosMX
multiplexer connected to 12 eosAC automated chambers (Eosense Inc., Dartmouth, NS, Canada)
(Roman-Perez and Hernandez-Ramirez 2021). The total headspace of the automated chamber
system was 2.8 L. Each flux measurement lapsed 10 min.
2.5 Flux calculation

The daily fluxes of \( \text{N}_2\text{O} \) and \( \text{CO}_2 \) were calculated as follows:

\[
F = \left( \frac{dC}{dt} \right) \times \left( \frac{V}{S} \right) \times \left( \frac{P}{R \times T} \right) \times M \times k
\]  

[1]

where \( F \) is the gaseous flux (\( \mu \text{g kg}^{-1} \text{d}^{-1} \)), \( \frac{dC}{dt} \) is the slope of a simple linear regression or as the first derivative of a quadratic regression at \( t_0 \) (\( \mu \text{L L}^{-1} \text{s}^{-1} \)), \( V \) is the headspace volume of the gas chamber (L); \( S \) is the dry soil weight (kg), \( P \) is the pressure in the chamber headspace during measurement (atm), \( R \) is the gas constant (atm \( \mu \text{L K}^{-1} \mu \text{mol}^{-1} \)), \( T \) is the temperature at chamber headspace during measurement (K), \( M \) is the molar mass of N within \( \text{N}_2\text{O} \) (28 g mol\(^{-1}\)), or C within \( \text{CO}_2 \) (12 g mol\(^{-1}\)), and \( k \) is a conversion factor for the flux unit (from \( \mu \text{g kg}^{-1} \text{s}^{-1} \) to \( \mu \text{g kg}^{-1} \text{d}^{-1} \)).

2.6 Calculations of \( \text{N}_2\text{O} \) derived nitrification and denitrification

With the aim of examining the contributions of nitrification and bacterial denitrification processes to the total \( \text{N}_2\text{O} \) production, the \( \text{N}_2\text{O} \) measurements conducted 1 day after thawing were used to estimate the site preference (SP) under natural abundance. This is because the large \( \text{N}_2\text{O} \) production on this day improved the accuracy of isotopic ratio measurements (Waechter et al. 2008).

Calculations for \( ^{15}\alpha \text{R}, ^{15}\beta \text{R}, \delta^{15}\alpha \text{N}_2\text{O}, \delta^{15}\beta \text{N}_2\text{O}, \) and \( \delta^{15}\text{bulk} \text{N}_2\text{O} \) were as follows:
\[ \frac{^{15}iR}{^{14}N} (i = \alpha \text{ or } \beta) \]  

\[ \delta^{15i}N_2O = \left( \frac{^{15i}R_{sample}}{^{15i}R_{std}} - 1 \right) \times 1000 \ (i = \alpha \text{ or } \beta) \]

\[ \delta^{15\text{bulk}}N_2O = \frac{\delta^{15\alpha}N + \delta^{15\beta}N}{2} \]

where \( ^{15}\alpha N, \ ^{15}\beta N \) and \( ^{14}N \) are the mixing ratios of \( ^{15}\alpha N-N_2O, \ ^{15}\beta N-N_2O \) and \( ^{14}N-N_2O \) in the sample as measured with Aerodyne, respectively; \( ^{15}\alpha R \) is the isotopic ratio of \( ^{15}\alpha N \) to \( ^{14}N \); \( ^{15}\beta R \) is the ratio of \( ^{15}\beta N \) to \( ^{14}N \); \( ^{15}R_{std} \) is the isotopic ratio in the atmospheric dinitrogen (\( ^{15}N_2 \)) (\( ^{15}R_{std} = 0.003676 \)).

The \( \delta^{15\alpha}N_2O \) and \( \delta^{15\beta}N_2O \) emitted from each experimental pot during chamber enclosure was obtained from the intercept of Keeling plots (i.e., from a linear regression of \( \delta^{15\alpha}N_2O, \delta^{15\beta}N_2O, \) or atom\% \( ^{15}N_2O \) as y-axis vs. 1/total \( N_2O \) as x-axis including 180 data points for each chamber measurement of each replicated soil pot separately) (Harris et al. 2017, Thilakarathna and Hernandez-Ramirez 2021).

The intramolecular \( ^{15}N-N_2O \) SP was calculated as follows:

\[ SP = \delta^{15\alpha}N_2O - \delta^{15\beta}N_2O \]

The isotopic fractionation effect of the transformation from \( N_2O \) to \( N_2 \) was accounted for based on relationships between \( \delta^{15}N_2O \) and SP (Yamamoto et al. 2017, Congreves et al. 2019). The
resultant changes in SP magnitude were minor (Daly and Hernandez-Ramirez 2020), extending from negligible to -2.5‰ in only 6% of the individual measurements.

The contributions of nitrification and bacterial denitrification to N₂O production were calculated as follows:

\[ F_{ni}(\%) = \frac{SP}{33} \times 100 \]  \[ F_{deni}(\%) = \frac{33 - SP}{33} \times 100 \]

where \( F_{ni} \) and \( F_{deni} \) are the proportional contributions of nitrification and denitrification, respectively. This assumes that the SPs of the nitrification and denitrification sources are 0 and 33 ‰, respectively (Sutka et al. 2006).

### 2.7 Calculation of the N₂O derived from SOM-N and the priming effects

As our study used urea labelled with \(^{15}\)N, a mass balance based on isotopic composition of the emitted N₂O (atom%) was conducted to separate the contributions of two N pools (i.e., added urea-N vs. existing SOM-N sources) to the overall N₂O flux using the entire dataset over the thawing phase. \( \text{Atom}^{15} \text{N}_{2}O \) is the isotopic percentage of \(^{15}\)N in N₂O as follows:

\[ \text{Atom}^{15} \text{N}_{2}O = \frac{\frac{^{15}\alpha N + ^{15}\beta N}{2}}{\frac{^{15}\alpha N + ^{15}\beta N + ^{14}N}{2}} \times 100 \]
Similar as for the SP derivation described above, the atom%\textsuperscript{15}N\textsubscript{2}O emitted from each experimental soil pot during each chamber enclosure was obtained from the Keeling plot intercepts.

The fractions of N\textsubscript{2}O production derived from added \textsuperscript{15}N-urea and from SOM-N were calculated as follows:

\[ FN_{2O_{\text{15N-urea}}} (\%) = \frac{\text{Atom%}^{15}N_{2O_{\text{15N-urea}}} - \text{Atom%}^{15}N_{2O_{\text{control}}}}{5\% - \text{Atom%}^{15}N_{2O_{\text{control}}}} \] \hspace{1cm} [9]

\[ FN_{2O_{\text{SOM}}} (\%) = \frac{5\% - \text{Atom%}^{15}N_{2O_{\text{15N-urea}}}}{5\% - \text{Atom%}^{15}N_{2O_{\text{control}}}} \] \hspace{1cm} [10]

\[ N_{2O_{\text{SOM}}} = FN_{2O_{\text{SOM}}} \times N_{2O \text{ flux from urea amended soil}} \] \hspace{1cm} [11]

where \( FN_{2O_{\text{15N-urea}}} \) and \( FN_{2O_{\text{SOM}}} \) are the fractions of N\textsubscript{2}O production derived from added \textsuperscript{15}N-urea and from existing SOM-N, respectively; \( \text{Atom%}^{15}N_{2O_{\text{15N-urea}}} \) and \( \text{Atom%}^{15}N_{2O_{\text{control}}} \) are the isotopic percentages of \textsuperscript{15}N in N\textsubscript{2}O emitted from the experimental pots with and without added urea, respectively; \( N_{2O_{\text{control}}} \) is the N\textsubscript{2}O flux from the control soils (without urea).

The priming effect of daily N\textsubscript{2}O fluxes was calculated as follows:

\[ N_{2O \text{ priming effect}} = N_{2O_{\text{SOM}}} - N_{2O_{\text{control}}} \] \hspace{1cm} [12]

In the Eq. [12], \( N_{2O \text{ priming effect}} > 0 \) corresponds to a positive priming effect caused by added urea, whereas \( < 0 \) indicates a negative priming effect. More specifically, daily negative
priming was identified when the mean daily SOM-derived N₂O flux from a urea-amended soil was one standard error below the zero baseline (which was defined as the corresponding control without urea addition). Results of N₂O priming were expressed as magnitude and also in relative basis as a percentage of the total flux for each soil pot receiving urea.

Cumulative fluxes of total N₂O and CO₂ as well as urea-derived N₂O, SOM-derived N₂O and primed N₂O after thawing were calculated by linear interpolations of the consecutive daily flux measurements.

2.8 Measurements of soil properties

Soils were air dried and passed through a 2 mm mesh prior to analyses. Soil extractable NH₄⁺ and NO₃⁻ in the filtrate (2M KCl) were determined by using a SmartChem 200 Discrete Wet Chemistry Analyzer (Westco Scientific Instruments, Inc., Brookfield, CT, US) (McKeague 1978, Carter and Gregorich 2007). The soil organic C and total N were determined by a dry combustion method in a Costech Model EA 4010 Elemental analyzer (Costech International Strumatzone, Florence, Italy). The clay, silt and sand percentages were determined by the rate of settling in a solution with a hydrometer (McKeague 1978, Carter and Gregorich 2007). Soil pH was determined in a mixture with a soil-to-water ratio of 1:2 (McKeague 1978, Carter and Gregorich 2007).

With the aim of measuring ¹⁵N isotopic composition in the soil at natural abundance (without addition of labelled urea), soil samples were oven-dried at 60 °C and ball-ground to a fine consistency to ensure homogeneity for isotope analysis. The soil δ¹⁵N was determined by using a
Flash 2000 Elemental Analyzer (Thermo Fisher Scientific, Delft, Netherlands) to dry combust
the soil sample converting all N to N$_2$. This analyzer was interfaced online to a Finnigan Delta V
Plus isotopic ratio mass spectrometer (Thermo Electron, Bremen, Germany) to detect the $^{15}$N
isotope composition.

Based on the $^{15}$N isotopic compositions of soil N and N$_2$O emitted from control soils, isotope
discrimination ($\varepsilon$) was calculated as follows:

$$\varepsilon \text{ (‰)} = \left( \frac{15R_{N2O}}{15R_{soilN}} - 1 \right) \times 1000$$ [13]

where $^{15}R_{N2O}$ is the isotopic ratio of N$_2$O emitted on Day 57 (1 day after thawing) and $^{15}R_{soilN}$ is
the isotopic ratio of soil N. A positive $\varepsilon$ implies the enrichment of $^{15}$N during the processes of
transforming soil N to N$_2$O emissions; a negative $\varepsilon$ implies a depletion of $^{15}$N during this
conversion from soil N to emitted N$_2$O. This $\varepsilon$ estimation was based on the premise that the
transformation from the SOM-N pools into the emitted N$_2$O pool was unidirectional.

### 2.9 Statistical analyses

Statistical analyses were performed in R 3.1.3 (R Core Team 2014) at alpha critical value of
0.05. The data were transformed to meet the assumptions of normality and homoscedasticity as
necessary. The effects of manure history (CT vs. SW soils), N (urea vs. control) and soil water
content (Low, Medium vs. High) treatments on soil NH$_4^+$, NO$_3^-$, cumulative N$_2$O, SOM-derived
N$_2$O and cumulative CO$_2$ were examined by three-way analysis of variance (ANOVA) for a
fixed-effect model with interaction analysis. We run two-way analysis ANOVA for a fixed-
effect model to determine the effects of manure history and water content on primed N$_2$O and
urea-derived N$_2$O as well as the differences in the contributions of nitrification and
denitrification to the N$_2$O emitted 1 day after thawing. Tukey’s Honest Significant Difference
(HSD) test was used to compare the difference further in cases where the treatment effects
described above were significant.

3. Results

3.1 Daily and cumulative fluxes of total N$_2$O production

Throughout the fall and freezing phases, N$_2$O production was relatively low (Fig. 1). Shortly
after the urea and water treatments were established at the beginning of the fall phase, the
average daily N$_2$O flux rose up to 0.75 ± 0.20 µg N$_2$O-N kg$^{-1}$ d$^{-1}$ on Day 3, then dropped to 0.17
± 0.01 µg N$_2$O-N kg$^{-1}$ d$^{-1}$ by Day 9, followed by a gradual increase up to 1.23 ± 0.40 µg N$_2$O-N
kg$^{-1}$ d$^{-1}$ on the last day of the fall phase (Fig. 1). During the freezing phase, the average daily
N$_2$O fluxes were consistently low at 0.22 ± 0.02 µg N$_2$O-N kg$^{-1}$ soil d$^{-1}$ (Fig. 1).

Robust N$_2$O fluxes occurred after soil thawing (Fig. 1). Overall, daily N$_2$O fluxes reached a peak
of 71.44 ± 7.08 µg N$_2$O-N kg$^{-1}$ d$^{-1}$ one day after thawing (on Day 57 of the experiment). More
specifically, soils under the Low and Medium moisture regimes peaked on Day 57, whereas soils
under High moisture showed an even larger peak on Day 58. After that, fluxes quickly declined
to 17.66 ± 3.90 µg N$_2$O-N kg$^{-1}$ d$^{-1}$ 5 days after thawing (Day 61 of the experiment) (Fig. 1).
Subsequently, daily N\(_2\)O fluxes continued to decrease gradually. The fluxes on the last day of the experiment (Day 86) averaged 0.76 ± 0.18 µg N\(_2\)O-N kg\(^{-1}\) d\(^{-1}\) (Fig. 1).

Following thawing, cumulative N\(_2\)O emissions were significantly impacted by the history of manure applications (SW > CT), soil water regime (High > Medium > Low) and urea addition (urea-N > control) (Table 2, Fig. 2a).

### 3.2 N\(_2\)O production derived specifically from SOM-N and priming effects

In parallel with the results of total N\(_2\)O emissions, the main effects of urea addition, moisture content, and history of manure applications showed separate, significant impact on the cumulative N\(_2\)O production derived from SOM during the period after thawing (Table 2, Fig. 2a). It is noted that the interactions amongst these three experimental factors were not significant. Irrespective of soil moisture and manure history effects, soils subjected to fall-banded urea were consistently higher in cumulative SOM-derived N\(_2\)O emissions after thawing than the control soils by a difference of 24% (305 vs. 245 µg N\(_2\)O-N kg\(^{-1}\) soil, \(P = 0.022\); Fig. 2a); this substantiates a positive priming effect of SOM that took place following the rapid, wet thawing of the soils. Furthermore, increasing moisture also significantly increased SOM-derived N\(_2\)O emissions (Low: 197 µg N\(_2\)O-N kg\(^{-1}\) soil, Medium: 292, vs. High: 473, \(P < 0.001\)). Likewise, having a history of manure application (SW soil) showed to raise the SOM-derived N\(_2\)O emissions by 39% above those of the CT soil (374 vs. 268 µg N\(_2\)O-N kg\(^{-1}\) soil, \(P = 0.002\)). Specifically, there was a tendency for the SW soil to have a numerically higher positive priming
effect compared with the CT soil in both magnitude (Fig. 2b) and relative (+17 vs. +6% Fig. 2c) basis.

Most of the daily \( \text{N}_2\text{O} \) fluxes following soil thawing showed positive priming (Fig. 3). Soils under Low moisture regime showed a peak of primed daily \( \text{N}_2\text{O} \) fluxes 1 day after thawing (Fig. 3a). In the case of soils under Medium and High moisture regimes, the peak of positive priming in daily \( \text{N}_2\text{O} \) fluxes occurred 1 day later (i.e., 2 days after thawing) (Fig. 3b and Fig. 3c). Across the three moistures in the SW soil, the peak of daily positive priming was greater at the two higher moisture contents (29.44 µg \( \text{N}_2\text{O-N kg}^{-1} \text{d}^{-1} \) at Low vs. 62.95 at Medium and 52.82 at High moisture content). Overall, peak primings were greater for SW than for CT soil under both Medium and High soil moisture regimes (Fig. 3). In general, following these early peaks, primed \( \text{N}_2\text{O} \) fluxes gradually dropped back to approximately the zero baseline.

Interestingly, about 2 weeks after soil thawing, negative priming of daily \( \text{N}_2\text{O} \) fluxes was clearly observed (i.e., SOM-derived \( \text{N}_2\text{O} \ll \text{control N}_2\text{O} \)). These episodes of evident negative primings occurred only under High soil moisture for both SW and CT soils (Fig. 3c). The negative priming effect began slightly earlier in the SW soil (Day 70 of the experiment) than in the CT soil (Day 71) (Fig. 3c). The last day that registered negative priming effect in SW soil was Day 86 of the experiment; in CT soil, it was Day 85. Towards the end of the experiment, the magnitude of the priming effects returned to zero or became minor. Collectively, the results indicate that higher moisture generated more dynamic priming activity.
Contrary to the wide responses of cumulative and daily SOM-derived N$_2$O fluxes to urea addition, history of manure applications and soil moisture as aforementioned, the direct contribution of the urea-N source to cumulative N$_2$O fluxes (urea-derived N$_2$O) was consistent across all assessed experimental factors and treatment combinations, with no significant effects caused by manure history or soil moisture (Table 2, Fig. 2a).

### 3.3 Contributions of denitrification to the peak of N$_2$O fluxes

The very large N$_2$O fluxes that occurred 1 day after soil thawing provided the opportunity to measure and allocate the N$_2$O produced from nitrification and denitrification sources in all unamended control soils (i.e., under natural $^{15}$N abundance conditions) and across the three moisture contents (Fig. 4). The results for $^{15}$N-N$_2$O SP ranged from 1.0 ‰ in the CT soil under High moisture to 5.7 ‰ in the SW soil under Medium moisture ($P > 0.05$) (Fig. 4c). This suggests that denitrification dominated the vigorous N$_2$O production, with 83% contribution in the case of the SW soil under Medium moisture and up to nearly all the N$_2$O produced in the case of the CT soil under High moisture (97%) (Fig. 4b). When averaging across the three moistures, the relative contributions of denitrification to N$_2$O production in the CT soil were marginally significantly larger than those in the SW soil ($P= 0.06$; Fig. 4b).

### 3.4 Isotopic depletion of $^{15}$N-N$_2$O relative to soil N

There was a consistently negative depletion of $^{15}$N ($\varepsilon$) during the transformation from the soil N pool to the emitted N$_2$O pool across all soil management histories and water regimes (Table 3).
3.5 Inorganic soil N concentrations

There were no significant effects of experimental factors on the NH$_4^+$ concentrations (Table 2). There was a significant interaction of manure history and water content on soil NO$_3^-$ concentrations (Table 2, Fig. 5). Specifically, irrespective of urea addition, soil NO$_3^-$ concentration was significantly lower in the treatment combination of CT soil at Low moisture than most of other treatments, with the only exception of the treatment combination of CT soil at Medium moisture (data not shown). The NO$_3^-$ concentration apparently increased with increasing soil moisture content in CT soils, but this pattern was not found in SW soils (Fig. 5b). The NO$_3^-$ concentration was in general greater in the SW soil than the CT soil (Fig. 5b). As expected, soils receiving added urea had greater increments in the NO$_3^-$ concentration than the soil without urea (i.e., CT + urea > CT control; SW + urea > SW control, Fig. 5b). These increased nitrate concentrations indicate the occurrence of nitrification in these soils. Furthermore, both NH$_4^+$ and NO$_3^-$ concentrations increased over time from the beginning to the end of the experiment, including in the control soils; therefore, this indicates that active mineralization and ammonification from SOM-N also took place over the experimental period.

3.6 Soil CO$_2$ fluxes

Within most of the fall and freezing phases, CO$_2$ fluxes were generally low and relatively stable across all treatment combinations. Over the fall phase, CO$_2$ fluxes averaged 1.29 ± 0.13 µg CO$_2$-C kg$^{-1}$ d$^{-1}$ (Fig. 1c). Afterwards, CO$_2$ fluxes steadily decreased to 0.57 ± 0.14 µg CO$_2$-C kg$^{-1}$ d$^{-1}$ on Day 6 of the freezing phase, and then became negligible (Fig. 1c).
Similar to N\textsubscript{2}O fluxes, most of the dynamics of CO\textsubscript{2} fluxes took place shortly after soil thawing (Fig. 1). Three days after thawing, the average CO\textsubscript{2} flux across all treatments sharply peaked at $8.65 \pm 0.29 \mu g \text{CO}_2\text{-C kg}^{-1} \text{d}^{-1}$. Thereafter, CO\textsubscript{2} fluxes slowly decreased over time, reaching $1.94 \pm 0.16 \mu g \text{CO}_2\text{-C kg}^{-1} \text{d}^{-1}$ on the last day of the experiment (Fig. 1c). It is noted that there was a strong correlation between daily CO\textsubscript{2} and N\textsubscript{2}O fluxes following thawing ($r=0.968$, $P<0.001$; Supplementary Fig. 1 and Fig. 1), with the exception of the first 3 days after thawing when the N\textsubscript{2}O fluxes were decoupled and disproportionally larger than the measured CO\textsubscript{2} fluxes.

Over the entire experiment and specifically in the period after thawing, the cumulative CO\textsubscript{2} emissions significantly increasing with higher soil moisture in the SW soil that had not received fall-urea (data not shown; Table 2).

4. Discussion

4.1 Added urea triggered primed N\textsubscript{2}O emissions derived from SOM-N

Results suggest that fall-applied N fertilizer induces a net positive priming effect from SOM-N at the onset of the subsequent spring thaw. This is consistent with earlier studies showing that fall N applications lead to large thaw-associated N\textsubscript{2}O emissions (Burton et al. 2008, Lin et al. 2017). This is the first time in the literature that the direction and magnitude of potential priming effects on augmented N\textsubscript{2}O emissions shortly after thawing has been quantified (Fig. 2, Fig. 3). These results suggest that mineralization of SOM increases over a strong freeze-thawing cycle because of the indirect influence of an earlier fall-banded urea, leading to large gaseous N losses at the
onset of thawing. Shortly after thawing, the input of extra N substrates from added urea in conjunction with heat and moisture activated microbial activity, collectively accelerating SOM-N availability (Curtin et al. 2012, Curtin et al. 2014). Such additional mineralized SOM-N in the soils amended with urea would become available for nitrifiers and denitrifiers, consequently producing extra SOM-derived N$_2$O fluxes consistently above the unamended baseline soils.

Recent studies have postulated a stoichiometry-based hypothesis with the aim to explain how an addition of labile N (e.g., urea) could prime SOM mineralization (Chen et al. 2014, Roman-Perez and Hernandez-Ramirez 2021). This hypothesis is centered on stoichiometric pre-requirements for SOM decomposition where adding labile N to soils rich in SOM satisfies microbial requisites for undertaking faster decomposition and mineralization of the existing SOM.

**4.2 Influence of manure history on N$_2$O emissions derived from SOM-N**

In addition to the priming of SOM-N caused by labile N additions, soils can be predisposed to exhibiting inherent priming because of the legacy effects from earlier management choices (Ginting et al. 2003, Blagodatskaya et al. 2007, Thilakarathna and Hernandez-Ramirez 2021). It is plausible that the manured soils (SW) in our study showed a more intense response of primed N$_2$O dynamics to the fall-applied urea because the previous field manure injections in this soil had increased the easily decomposable SOM. It is noted that the SW soil showed a tendency for higher organic C concentrations than CT (Table 1). Conversely, it is acknowledged that increasing SOM concentrations have shown to lead to microbial immobilization of the available N in certain studies (Hou et al. 2000, Zimmerman et al. 2011). Moreover, availability of labile organic C could also reduce the N$_2$O priming in urea-amended soils. This is explained by the
increased conversion of N₂O into N₂ as driven by heterotrophic utilization of organic C that
enhances the last step of bacterial denitrification (Daly and Hernandez-Ramirez 2020). Future
research focusing on these drivers of N₂O priming would help to deepen our understanding of C
and N turnover in soils, particularly in agricultural systems that experience high, frequent
nutrient outputs and inputs such as croplands that receive heavy manure additions. We
hypothesize that in environments that are N-rich and even N-saturated, coupling availabilities of
C and N could reduce and even cancel the potential priming effects on N₂O emissions derived
from SOM-N.

4.3 Moisture regime altered the N₂O produced from SOM-N

In addition to the effects of contrasting manure history on N₂O priming following thawing, soil
moisture clearly affected the dynamics of primed N₂O fluxes as well. Although the overall
priming was positive across all experimental combinations, only soils under High moisture
experienced negative N₂O priming of daily fluxes and also longer-lasting priming effects as
noted above (Fig. 3). The temporal shift of daily priming effects from positive to negative and
eventually back to zero priming at High moisture could be explained by the mechanism of
preferential substrate utilization. The hypothesis of preferential substrate utilization states that
when given a variety of nutrient supplies, microorganisms prefer easily available and highly
accessible substrates over recalcitrant substrates (Cheng 1999, Cheng and Kuzyakov 2005,
Blagodatskaya and Kuzyakov 2008). Within the context of our study, it could be postulated that
at the onset of thawing, soil microbes initially utilized the easily available substrates, and then
switched to consuming more complex substrates (e.g., wheat straw residues and roots) in
conjunction with any available inorganic N, and eventually started utilizing the recalcitrant
SOM. When the soil microbes switch to decomposing plant residues, they would need to uptake
inorganic N available from the soil solution because of the high C:N ratios of wheat straw and
roots (Gan et al. 2011); this would induce a temporary net N immobilization, which may have
generated the temporal negative priming of N₂O production observed in the urea-amended soils
under High moisture. It is noted that although all assessed soils could possibly experience this
phenomenon, this is particularly crucial in the soils that had received urea because there was
more inorganic N available to conduct this temporal immobilization. Additionally, this short-
term immobilization could even entail a pool substitution of urea-N in lieu of native inorganic N.
These episodes of negative priming could have also become more evident under higher water
content because increasing moisture has been found to favor greater SOM-N mineralization and
nitrification (Stanford and Epstein 1974, Paul et al. 2003, Curtin et al. 2012, Curtin et al. 2014).
The higher N availability with increasing water content is partly shown by the tendency of
increased NH₄⁺ and NO₃⁻ concentrations with higher moisture in the CT soils at the end of the
experiment (Fig. 5). In other words, with the decrease in the availability of more easily-
decomposable substrates, soil microorganisms can progressively utilize recalcitrant SOM to
sustain their ongoing metabolism and growth, which subsequently causes additional N
mineralization coupled with a gradually-diminishing negative priming of N₂O production, and
this can finally shift the soil system to steadily approach neutral priming.

Provided that climate change predictions include higher precipitation over the fall and winter
seasons in the North American Plains (Easterling et al. 2017), our study demonstrates the
potential for exacerbated N₂O emissions in the early spring soon after a wet thawing, which was primarily driven by increased N₂O production from SOM in fertilized, near water-saturated soils (Fig. 2a). This interpretation is consistent with previous studies that evaluated the driving effects of increasing moisture on N₂O peak emissions (Hou, A. et al. 2000, Lin and Hernandez-Ramirez 2020, Roman-Perez and Hernandez-Ramirez 2021). Increasing moisture and microbial activity immediately after soil thawing can have led to the depletion of O₂ concentrations in the soil microsites, and hence mediating an increased N₂O production from denitrification (Yanai et al. 2011).

4.4 Main processes producing N₂O shortly after soil thawing

Irrespective of different soil manure history and moisture levels, the consistent negative isotope discrimination (ε) indicated ¹⁵N depletion during the transformation of the SOM-N to the major N₂O fluxes just emitted 1 day after thawing (Table 3). This further suggests that the SOM-N pool was the dominant source for substantial N₂O production in our study because several SOM-N transformations in soils such as mineralization, nitrification and denitrification are known to fractionate against the heavier isotope (i.e., ¹⁵N), resulting in ¹⁵N depletion in the N₂O product compared to the remaining SOM-N substrate (Högberg 1997). However, it is acknowledged that this specific data were available only one time over the experiment while the key processes responsible for N₂O production (nitrification vs. bacterial denitrification) can fluctuate within a few hours or days. If we consider denitrification to be the main source of significant peak N₂O production following the simulation of a strong soil thawing (Fig. 3), abundant soil NO₃⁻ (and including the intermediate nitrite) pool inexorably played a role as the primary N substrate.
contributing to the large N$_2$O emissions instead of NH$_4^+$ (and the intermediate hydroxylamine substrate). On the other hand, nitrification could become a key source for N$_2$O emissions in soils under lower water contents or in years when spring thawing is mild (Davidson 1991, Ruser et al. 2006).

5. Conclusions

Adding urea asymmetrically increase the primed N$_2$O emissions specifically derived from SOM-N. Results indicated that annual croplands receiving fall-banded urea followed by a strong freeze-thaw cycle can manifest accelerated SOM transformations that intensify N$_2$O emissions in the early spring. In addition to these priming effects triggered by the added urea, N$_2$O production from SOM was further amplified in soils that have had a recent history of manure applications or experienced increasing moisture during spring thawing.
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8. Conflict of interest

The authors declare that they have no conflict of interest.
9. References

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Table 1. Soil physical and chemical properties at the 0-15 cm depth increment of the soils with (SW) and without (CT) history of liquid manure injections. Note that only organic carbon showed a magnitude difference between these two soil managements, with SW slightly higher than CT (P > 0.05).

| Soil properties                  | Black Chernozem | SW          | CT          |
|----------------------------------|-----------------|-------------|-------------|
| Classification†                 |                 |             |             |
| Bulk density (g cm\(^{-3}\))    | 1.11 ± 0.06     |             |             |
| pH                              | 6.1 ± 0.2       |             |             |
| Texture                         | Clay            |             |             |
| Clay(%)                         | 47.0 ± 1.2      |             |             |
| Silt(%)                         | 36.0 ± 0.3      |             |             |
| Sand(%)                         | 17.0 ± 1.0      |             |             |
| Organic C (g C kg\(^{-1}\))    | 63.7 ± 5.0‡     | 61.6 ± 4.3  |             |
| Total N (g N kg\(^{-1}\))      | 5.9 ± 0.3       | 5.9 ± 0.4   |             |

†Based on the Canadian System of Soil Classification (Soil Classification Working Group 1998).
‡Standard error of the mean.
Table 2. P-values of ANOVA models for soil NH$_4^+$ and NO$_3^-$ concentrations as well as cumulative N$_2$O and CO$_2$ emissions after thawing (Days 56 – 86).

| Experimental factor                      | NH$_4^+$ | NO$_3^-$ | Cumulative N$_2$O | SOM-derived N$_2$O | Urea-derived N$_2$O | Primed N$_2$O | Cumulative CO$_2$ |
|------------------------------------------|----------|-----------|-------------------|--------------------|---------------------|---------------|------------------|
| History of manure application (Soil)†   | n.s.     | 0.002     | 0.006             | 0.002              | n.s.                | n.s.          | n.s.             |
| Water content (Water)‡                  | n.s.     | 0.035     | <0.001            | <0.001             | n.s.                | n.s.          | <0.001           |
| Nitrogen addition (Nitrogen)§           | n.s.     | <0.001    | <0.001            | 0.022              | N/A                 | N/A           | n.s.             |
| Soil × Water                            | n.s.     | 0.042     | n.s.              | n.s.               | n.s.                | n.s.          | n.s.             |
| Soil × Nitrogen                         | n.s.     | n.s.      | n.s.              | n.s.               | N/A                 | N/A           | n.s.             |
| Water × Nitrogen                        | n.s.     | n.s.      | n.s.              | n.s.               | N/A                 | N/A           | n.s.             |
| Soil × Water × Nitrogen                 | n.s.     | n.s.      | n.s.              | n.s.               | N/A                 | N/A           | n.s.             |

† History of manure application factor included two levels: soils with spring manure (SW) and without a history of liquid manure injections (field control, CT).
‡ Water content factor included three levels: Low, Medium, and High water contents.
§ Nitrogen factor included two levels: with and without urea additions. Soils without urea are also referred as controls.
Table 3. The $^{15}$N isotope discrimination [$\varepsilon$, mean $\pm$ SE (%), n=3] of soil N$_2$O emitted 1 day after thawing (the time of peak emissions in the study) relative to the soil N pool in both field control (CT) and manure-treated (SW) soils at various water contents. Calculation based on Eq. [13].

| Soil | Water content | Soil × Water Content | Soil |
|------|---------------|----------------------|------|
|      | Low           | -10.19 $\pm$ 11.21   |      |
| CT   | Medium        | -7.68 $\pm$ 2.76     | -11.73 $\pm$ 3.86 |
|      | High          | -17.34 $\pm$ 4.46    |      |
| SW   | Low           | -18.78 $\pm$ 3.60    |      |
|      | Medium        | -15.45 $\pm$ 4.94    | -15.26 $\pm$ 2.30 |
|      | High          | -11.56 $\pm$ 3.65    |      |
Fig. 1. Daily nitrous oxide (N\(_2\)O) and carbon dioxide (CO\(_2\)) fluxes from soils over the entire experiment. In the case of N\(_2\)O, fluxes are shown in two separate panels as subsets (a) without and (b) with added urea. Fluxes of CO\(_2\) are averaged across all treatment combinations. SW and CT stand for soils with and without a history of manure additions,
respectively. Low, Med, and High correspond to moisture regimes where Med stands for Medium. Error bars correspond to one standard error of the mean.
Fig. 2. (a) Cumulative N₂O emissions allocated to urea and soil organic matter (SOM) sources, (b) magnitude priming and (c) relative priming caused by urea addition following soil thawing. SW and CT stand for soils with and without a history of manure additions,
respectively. Low, Med, and High correspond to moisture regimes where Med stands for Medium. In Panel a, N and C acronyms correspond to the urea-N addition treatment and the zero-N addition (control) treatment, respectively. In Panel a, different letters indicate significant difference in total cumulative N\textsubscript{2}O (uppercase), SOM-derived N\textsubscript{2}O (lowercase) and urea-derived (italic) N\textsubscript{2}O emissions after thawing ($P < 0.05$). In Panels b and c, N\textsubscript{2}O primings were respectively shown as magnitudes and also in relative basis as percentages of the total fluxes (shown in Panel a) of soil pots receiving urea. Error bars correspond to one standard error.
Fig. 3. Primed daily N$_2$O fluxes following soil thawing. SW and CT stand for soils with and without a history of manure additions, respectively. Low, Med, and High correspond to moisture regimes where Med stands for Medium. Positive and negative primed daily N$_2$O fluxes represent positive and negative priming effects, respectively. Error bars correspond to one standard error of the mean.
Fig. 4. (a) Magnitude and (b) relative contributions of nitrification and denitrification, as well as (c) site preference for the N₂O fluxes emitted 1 day after thawing (Day 57 of the
experiment). SW and CT stand for soils with and without a history of manure additions, respectively. Low, Med, and High correspond to moisture regimes where Med stands for Medium. In Panels a and b, numbers in the columns are respectively the flux magnitude and percentage of N₂O emissions produced via denitrification or nitrification. Error bars correspond to standard error of the mean.
Fig. 5. Soil (a) ammonium and (b) nitrate concentrations at the end of the experiment for the soils with (SW) and without (CT) history of manure additions at Low, Medium (Med) and High moisture regimes. Horizontal lines (with one standard error) across moisture
contents are the concentrations of (a) ammonium and (b) nitrate of the two soils at the beginning of the experiment (prior to urea addition and establishment of the three moisture regimes). Different letters indicate significant differences among treatment combinations ($P < 0.05$). Error bars correspond to one standard error of the mean. n.s. = not significant.
Daily nitrous oxide (N2O) and carbon dioxide (CO2) fluxes from soils over the entire experiment. In the case of N2O, fluxes are shown in two separate panels as subsets (a) without and (b) with added urea. Fluxes of CO2 are averaged across all treatment combinations. SW and CT stand for soils with and
without a history of manure additions, respectively. Low, Med, and High correspond to moisture regimes where Med stands for Medium. Error bars correspond to one standard error of the mean.

Figure 2

(a) Cumulative N2O emissions allocated to urea and soil organic matter (SOM) sources, (b) magnitude priming and (c) relative priming caused by urea addition following soil thawing. SW and CT stand for soils with and without a history of manure additions, respectively. Low, Med, and High correspond to
moisture regimes where Med stands for Medium. In Panel a, N and C acronyms correspond to the urea-N addition treatment and the zero-N addition (control) treatment, respectively. In Panel a, different letters indicate significant difference in total cumulative N2O (uppercase), SOM-derived N2O (lowercase) and urea-derived (italic) N2O emissions after thawing (P < 0.05). In Panels b and c, N2O primings were respectively shown as magnitudes and also in relative basis as percentages of the total fluxes (shown in Panel a) of soil pots receiving urea. Error bars correspond to one standard error.
Primed daily $N_2O$ fluxes following soil thawing. SW and CT stand for soils with and without a history of manure additions, respectively. Low, Med, and High correspond to moisture regimes where Med stands for Medium. Positive and negative primed daily $N_2O$ fluxes represent positive and negative priming effects, respectively. Error bars correspond to one standard error of the mean.

Figure 4
(a) Magnitude and (b) relative contributions of nitrification and denitrification, as well as (c) site preference for the N2O fluxes emitted 1 day after thawing (Day 57 of the experiment). SW and CT stand for soils with and without a history of manure additions, respectively. Low, Med, and High correspond to moisture regimes where Med stands for Medium. In Panels a and b, numbers in the columns are respectively the flux magnitude and percentage of N2O emissions produced via denitrification or nitrification. Error bars correspond to standard error of the mean.

Figure 5
Soil (a) ammonium and (b) nitrate concentrations at the end of the experiment for the soils with (SW) and without (CT) history of manure additions at Low, Medium (Med) and High moisture regimes. Horizontal lines (with one standard error) across moisture contents are the concentrations of (a) ammonium and (b) nitrate of the two soils at the beginning of the experiment (prior to urea addition and establishment of the three moisture regimes). Different letters indicate significant differences among treatment combinations ($P < 0.05$). Error bars correspond to one standard error of the mean. n.s. = not significant.

### Supplementary Files

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