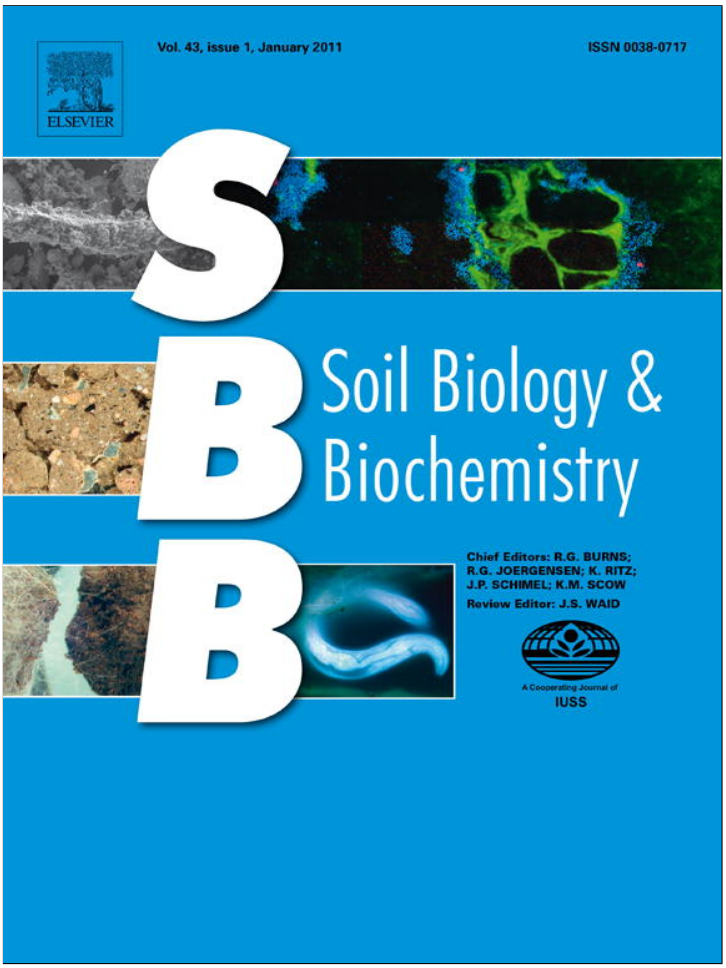

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Biogenic emissions of CO_2 and N_2O at multiple depths increase exponentially during a simulated soil thaw for a northern prairie Mollisol☆

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ABSTRACT

The fate of carbon (C) and nitrogen (N) belowground is important to current and future climate models as soils warm in northern latitudes. Currently, little is known about the sensitivity of microbial respiration to temperature changes at depths below 15 cm. We used whole-core (7.6 cm dia. × 90 cm) laboratory incubations to determine if temperature response quotients (Q_{10}) for CO_2 and N_2O varied with depth for undisturbed prairie while plants were senescent and clipped at the surface. We collected intact soil cores from an undisturbed prairie in central North Dakota and uniformly subjected them to freezing (5 to −15 °C) and thawing (−15 to 5 °C). We measured rates of CO_2 and N_2O emissions at 5 °C temperature increments at 0, 15, 30, 45, 60, and 75 cm depths. During freezing, active and sterilized core emissions occurred only between 0 and −10 °C. During thawing, a simple first-order exponential model, $E = \alpha e^{\beta T}$, fit observed CO_2 and N_2O emissions ($R^2 = 0.91$ and 0.99, respectively). Parameter estimates for β were not significantly different across depths for CO_2 and for N_2O ($Q_{10} = 4.8$ and 13.7, respectively). Parameter estimates for α (emissions when temperature is 0 °C) exponentially declined with depth for both gases for similar depth-response curves. Stepwise regressions of soil properties on α parameter estimates indicated emissions of CO_2 and N_2O at 0 °C during thawing were positively correlated ($R^2 > 0.6$) with soil porosity. Results indicate pedogenic properties associated with depth may not necessarily influence temperature response curves during thawing but will affect emissions at 0 °C for both CO_2 and N_2O.

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1. Introduction

Understanding controls on belowground trace gas emissions in regions where temperatures remain below 0 °C throughout much of the winter is needed to support multilayer models of CO_2 and N_2O emissions (Neff and Asner, 2001) and to assess potential feedbacks to climate change (Giardina and Ryan, 2000; Fang and Moncrieff, 2001; Davidson and Janssens, 2006; Öquist et al., 2009). At the surface of arctic tundra, winter respiration contributes a significant amount of CO_2 to the atmosphere, ranging from 69 to 189 g C m^{-2} yr^{-1}, with peak emissions during thaw (Oechel et al., 1997; Grogan and Chapin, 1999; Elberling and Brandt, 2003). Similarly, N_2O emissions at the surface of agricultural and prairie soils in winter can be greater than emissions during the growing season, particularly during thaw events (Goodroad and Keeney, 1984a; Cates and Keeney, 1987; Burton and Beauchamp, 1994; van Bochove et al., 2000a; Wagner-Riddle et al., 2007). Others have also observed comparable effects of soil thawing on CO_2 and N_2O emissions during laboratory incubations (Goodroad and Keeney, 1984b; van Bochove et al., 2000b; Priemé and Christensen, 2001; Elberling and Brandt, 2003), but reports of emissions during freezing are less common. While CO_2 and N_2O may be produced at sub-zero soil temperatures, transformation of liquid water to ice induces a moisture limitation that limits microbial respiration to a greater extent than temperature or substrate (Teepe et al., 2001; Grogan et al., 2004; Öquist et al., 2009; Harrysson Drotz et al., 2009). Explicit tests for the effect of temperature on biogenic emissions are problematic in the field because soils *in situ* are not isothermal. Temperature will vary with depth, and gases produced at warmer, deeper depth could diffuse to the surface (Burton and Beauchamp, 1994; van Bochove et al.,

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2000b; Yutse et al., 2007; Kellman and Kavanaugh, 2008). Laboratory incubations indicate how temperature affects emissions as soils thaw under controlled conditions (Fang and Moncrieff, 2001; Mikan et al., 2002; Elberling and Brandt, 2003), but soils incubated under laboratory condition often lack structure and pore connectivity found *in situ*, which influences gas diffusivity and microbial habitat (Schjønning et al., 1999; Fang et al., 2005; Risk et al., 2008). Experiments that control temperature while keeping the soil microenvironment intact (whole-core incubations) are needed to understand effects of freezing and thawing on CO_2 and N_2O emissions at multiple depths (Burton and Beauchamp, 1994; Schjønning et al., 1999; Risk et al., 2008).

The relationship between soil properties and aerobic biological activity, which is commonly measured as respiration of CO_2, is well-established with respect to soil depth (Paul and Clark, 1996; Fang and Moncrieff, 2001; Fierer et al., 2003a). Relatively abundant sources of high-quality organic substrates and roots near the soil surface are available to fuel greater rates of microbial activity, as compared to deeper soils (Fang et al., 2005). At deeper soil depths (subsurface), organic substrates are often lower in quality and quantity (Ajwa et al., 1999; Jobbágy and Jackson, 2000; Fierer et al., 2003a; Fang et al., 2005), leading to lower rates of microbial activity (Fierer et al., 2003b; Fang et al., 2005). While it is clear that temperature and substrate drive belowground biological activity (Yutse et al., 2007), it is not clear if temperature response curves for either CO_2 or N_2O vary between surface and subsurface soils during whole-core, thawing or freezing incubations.

Respiration is often represented in biogeochemical models with simple temperature dependence equations, such as Arrhenius or van't Hoff (van't Hoff, 1898). Application of these empirical models is often limited (Lloyd and Taylor, 1994; Kirschbaum, 1995) because other factors, such as substrate availability, are confounded with observed temperature variation (Davidson et al., 2006). While mechanistic models are needed to fully understand enzymatic controls to processes such as soil respiration, exponential temperature response data can help bridge the gap between trace gas emissions and physicochemical factors that vary with soil depth. A simple, first-order, exponential equation (van't Hoff, 1898) is commonly used to model emissions data collected at sub-zero soil temperatures. The relationship is expressed as $E = \alpha e^{\beta T}$; (linear transformation: $\ln E = \ln \alpha + \beta T$); where E is emission of CO_2 or N_2O (nmol CO_2 or pmol N_2O g^{-1} h^{-1}), T is measured soil temperature (°C), and α (emission of CO_2 or N_2O when $T = 0$) and β (units = 1/°C) are fitted parameters. The coefficient Q_{10} ($Q_{10} = e^{\beta \times 10}$) is a widely used index of temperature dependence which describes the proportional change in rate given a 10 °C change in temperature that is often referred to in the literature (Davidson et al., 2006). Model parameter α changes with physicochemical properties associated with soil quality (Mikan et al., 2002), which also change with soil depth (Ajwa et al., 1999; Fierer et al., 2003a). An exponential response in CO_2 or N_2O emissions to changes in soil temperature during freezing or thawing would support application of the van't Hoff model (Fang and Moncrieff, 2001; Mikan et al., 2002).

Intact core laboratory incubations are often used to evaluate microbial emissions of trace gases in soils while maintaining microsite integrity and pore connectivity (Parkin, 1987; Fang et al., 2005; Risk et al., 2008). Here, we describe a modified approach to include multiple depths within a 4100 cm^3 soil core and a sequence of temperature-step incubations. We aimed to determine temperature response curves for CO_2 and N_2O emissions for whole-cores evaluated at multiple (15-cm) depth increments during a short-term simulated freeze-up and thaw. Soil cores were not separated by depth but remained intact, so that root and aggregate structure, pore-space connectivity and diffusive transport were preserved. The objective of this study was to address two questions using soil cores collected at a northern prairie grassland site: 1) Does a simple, first-order exponential model fit the temperature responses observed for CO_2 and N_2O during a simulated soil thaw and soil freeze-up and 2) do model parameter estimates of α and β values vary with depth? Based on reports in the literature, we did not expect an exponential response to soil freezing. Instead, we hypothesized the exponential model would fit observed temperature responses during a simulated thaw for both CO_2 and N_2O.

2. Materials and methods

2.1. Site information and field sampling

A historically-native grassland enclosure (23 ha) with no history of tillage which was instrumented with eddy-covariance and belowground temperature-sensor equipment was selected at the USDA-ARS Northern Great Plains Research Laboratory (NGPRL) in south-central North Dakota (46°46′N, 100°55′W). Climate is semi-arid with an annual temperature of 5 °C and a mean annual precipitation of 41.2 cm. The site is managed for annual hay harvest and has not been grazed by cattle for 11 years. Soils are Temvik-Wilton silt loams (FAO: Calcic Siltic Chernozems; USDA: Fine-silty, mixed, superactive, frigid Typic and Pachic Haplustolls; Soil Survey Division Staff, 1993). The soil profile includes A, Bw, and Bt horizons, implying accumulated humified organic material, and weak illuviation of silicate clay. Soil structure within the profile transitions from subangular blocky to moderate prismatic structure with increasing depth (Schoeneberger et al., 1998). Soil C, N, and pH were 42 Mg ha^{-1}, 3.8 Mg ha^{-1} and 6.2, respectively at the 0–15 cm depth increment in spring 2009 (Phillips and Podrebarac, 2009).

Soil samples were collected from an area dominated by big bluestem [*Schizachyrium scoparium* (Michx.) Nash] while plants were senescent in late Oct 2009. Soil cores were collected along a 30-m, north-south transect from six points spaced approximately 5 m apart. At each point, an intact core was collected using a hydraulic tractor press (Giddings Machine Co., Inc, Windsor, CO) with a plastic sleeve insert (7.6 cm dia. × 90 cm length). Plants were clipped at the surface and litter was removed prior to coring. The cores collected at each of the first five sites were used to measure gas emissions and soil physicochemical properties. Each was subjected to soil freezing, then a sequence of thawing temperature increments, and then a sequence of freezing temperature increments. We chose to follow the thawing response first because most the most significant biogenic emissions near 0 °C are reported during thawing rather than freezing (Wagner-Riddle et al., 2010). Following these incubations, cores were used for sampling bulk density, percent water-filled pore space (WFPS), porosity, carbon (C), nitrogen (N), nitrate–N (NO_3–N), ammonium–N (NH_4–N), phosphorous (P), and pH (see Section 2.4). Two cores were collected at the sixth site. One of these was reserved for measurement of non-biogenic emissions, so this was tightly wrapped and autoclaved for 1 h at 121 °C and allowed to cool for 2 days. The other was used for tracking soil temperature during the incubations. A soil temperature sensor (Omega Model HH21, Type *E*) was inserted 30 cm into the bottom of this core and attached to a datalogger (Campbell 21X, Logan, UT). Soil temperature measurements collected every 0.5 h at the site with type *E* thermocouple probes (model TCAV) at 6- and 8-cm depths (Campbell Sci., Logan, UT) indicated daytime soil temperature was 5 °C during core collection, and cores remained at 5 °C during transport and storage.

2.2. Intact core incubations using 5 °C temperature steps

Six incubation chambers were constructed using polyvinyl chloride (PVC) tubes (7.6 cm dia. × 95 cm length × 0.3 cm wall

thickness). At the top of each tube, a vented PVC cap was fitted with a septum for syringe sampling of the headspace for emission determination at the 0 cm depth (Fig. 1). The top of each core was placed 5 cm below the top of the tube. Threaded holes were tapped into each tube for sample ports at 15, 30, 45, 60, and 75 cm soil depths and the bottom capped. The five active cores and the one sterilized core were carefully transferred into the incubation chambers so the cores would remain intact. At each sample port, a small soil core was extracted (0.6 cm dia. × 5.5 cm length) to allow insertion of perforated Bev-Line® tubing (0.6 cm outside dia × 0.4 cm inside dia. × 5.5 cm length) where air samples would be collected. The tubing was attached to a threaded brass fitting that was screwed into each sample port and secured with silicone for an airtight connection. A septum for syringe sampling was inserted into the end of each fitting. Greater emission rates at the surface would be expected because porosity and area of soil sampled at the surface would facilitate greater diffusion to the headspace, as compared to samples collected belowground.

All cores were subjected to sequential, 5 °C temperature-step incubations for temperatures ranging from −15 to 5 °C during thawing and from 5 to −15 °C during freezing using a Queque Cryostar Model 7110 (Queue Systems, Parkersburg, WV) freezer for a total of one thawing and one freezing experiment. These temperatures are more common in northern prairie at surface than at subsurface layers; however, we needed to incubate all depths similarly to test for differences in Q_{10}. We started with the thawing experiment and allowed cores to equilibrate at −15 °C for 48 h prior to initiation of the first (−15 to −10 °C) incubation. Cores were vented by removing fittings at all ports and allowed to stabilize 12 h at −15 °C. Ports were then re-capped and gas samples drawn at each depth to determine the initial concentrations of CO_2 and N_2O. Gas samples (15 mL) were injected into evacuated, 12-mL exetainers (Labco Unlimited; Buckinhamshire, UK). As samples were pulled from the soil airspace, an equal volume of ultra-pure N_2 was backfilled into the port to maintain pressure equilibrium (Phillips, 2007; Phillips and Podrebarac, 2009). After sampling, freezer temperature was set to increase at 0.5 °C h^{-1} and allowed to stabilize at −10 °C for 2 h. Gas samples were subsequently drawn after 7 h to determine the concentrations of CO_2 and N_2O at the end of the incubation. Ports were then flushed with ultra-pure N_2 and vented (fittings removed from all ports) for 12 h at −10 °C.

The rates at which CO_2 and N_2O accumulated in the perforated tubing or chamber headspace (for the 0 cm depth) between the first and second time point were calculated (μmol h^{-1}) after correcting for dilution of the airspace with N_2 and the physical change in mols of gas resulting from a 5 °C temperature change (Phillips, 2007). This procedure was repeated for the remaining 5 °C temperature-step incubations (from −10 to −5 °C, from −5 to 0 °C, from 0 to 5 °C, from 5 to 0 °C, from 0 to −5 °C, from −5 to −10 °C, and from −10 to −15 °C). For the simulated thaw, the first data point represented net concentration change (emission) as soil temperature changed from −15 to −10 °C. For the simulated freeze-up, the first data point represented net emission as soil temperature changed from 5 to 0 °C. Incubations were completed within 28 d of core collection to minimize potential changes to microbial communities in the laboratory (Gödde and Conrad, 1999; Mikan et al., 2002). Our sample ports were not physically separated by depth, so the volume of soil contributing to emissions was estimated using bulk density measured (Section 2.4) for the 460 cm^3 of soil (46 cm^2 area × 10 cm depth) immediately surrounding each port (the nature of our whole-core incubation precluded quantification of the specific areas contributing to each port). We assumed the same volume for all depths, although CO_2 and N_2O may have traveled outside the defined volume to adjacent depths, (a problem with maintaining whole-core integrity). This was minimized with short-term incubations. We aimed to incubate the soils long enough to detect a measurable difference over time at these temperatures (Mikan et al., 2002) while minimizing time for diffusive transport outside of our defined soil volume. Effective diffusivity (D_s) area for both CO_2 and N_2O was estimated at <340 cm^2 (over the full 7-h incubation) using reference diffusivity (Pritchard and Currie, 1982) and tortuosity according to Tang et al. (2003).

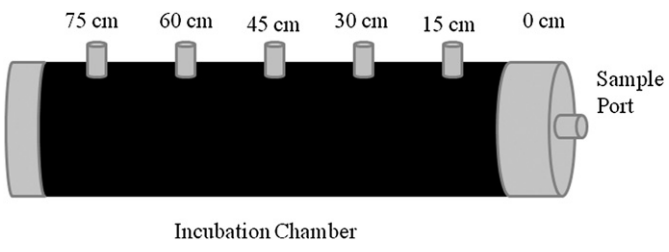

Fig. 1. Incubation chamber design for intact soil cores includes six sampling ports, as described in Methods.

Gas samples stored in the exetainers were analyzed for CO_2 and N_2O within 24 h of collection using a Varian Model 3800 Gas Chromatograph with Combi-Pal autosampler. In this system, the sample is auto-injected into a 1 mL sample loop, then loaded into columns and routed through two detectors: a ^{63}Ni electroncapture detector (ultra-pure 95% Argon/5% CH_4 carrier gas) and a thermal conductivity detector (ultra-pure He carrier gas). The gas chromatograph was calibrated with commercial blends of CO_2 and N_2O balanced in N_2 (Scott Specialty Gases) following verification of stated concentrations with standards from the National Institute of Standards and Technology. The precision of analysis, expressed as a coefficient of variation for 10 replicate injections of low and high concentration standards was consistently <2% for all gases. The minimum detectable concentration change was 10 μL L^{-1} for CO_2 and 7 nL L^{-1} for N_2O (Phillips et al., 2009).

2.3. Soil property analyses

Following the thawing and freezing incubations, each core was analyzed for physiochemical properties by cutting each core into 10-cm sections. Field soil water content was determined for each sample using a 12–15 g subsample by measuring the difference in mass before and after drying at 105 °C for 24 h (Gardner, 1986). Bulk density was calculated for each core section using inputs for sample mass at field soil water content, measured gravimetric water content, and known volume of each core section (Blake and Hartge, 1986). Expansion was restricted by the incubation chamber, so volume was considered constant for both frozen and thawed soil. Values for WFPS and porosity were calculated using bulk density and a particle density of 2.65 g cm^{-3}. Soils were then dried at 35 °C for 4 d and passed through a 2.0-mm sieve by hand with care to remove identifiable plant material (>2.0-mm diameter, >10-mm length). Soil NO_3–N and NH_4–N were measured using a 1:10 soil/2 mol L^{-1} KCl extracts using Cd reduction followed by a modified Griess-Ilosvav method and idophenol blue reaction (Mulvaney, 1996). Available P was estimated by bicarbonate extraction (Olsen et al., 1954). Soil pH was estimated from a 1:1 soil/water mixture (Watson and Brown, 1998; Whitney, 1998). Soil texture was determined using the hydrometer method with 40 g air-dried soil (Gee and Bauder, 1986). Soils were powder-ground to <53 μm with a roller mill for C and N analysis via dry combustion (Carlo Erba, Thermo Scientific, Waltham, MA, USA). As the pH was <7.2 for all depths sampled, total C was considered equivalent to organic C

(Nelson and Sommers, 1996). All data were expressed on an oven-dry basis prior to statistical analysis.

2.4. Statistical analyses

Rates of CO_2 and N_2O emissions [mol h^{-1} and mol h^{-1} g dry weight (g^{-1})] during the simulated soil thaw and freeze-up were analyzed to determine if the temperature responses for both CO_2 and N_2O were exponential at all depths using SAS/STAT software Version 9.2 (SAS System for Windows, © 2002–2008, SAS Institute Inc., Cary, NC, USA). The first-order exponential equation ($E = \alpha e^{\beta T}$) was fit to rate of CO_2 emission and then to rate of N_2O emission using Proc Nlin to estimate parameters α and β for each core-depth. Modeled CO_2 and N_2O emission estimates were compared to observed CO_2 and N_2O emissions and R^2 values reported. A stepwise linear regression was used on soil property data (porosity, NO_3–N, NH_4–N, pH, total C, total N, C:N ratio, D_s, and WFPS) to identify the property that showed the greatest potential relationship (highest R^2) with CO_2 and N_2O α parameter estimates. Further, we fitted a mixed, random coefficients regression model to the soil property identified by the stepwise regression. For soil property data, differences with depth were determined using one-way analysis of variance followed by student's *t*-tests (Jmp version 4.0.4, © 2001, Division of SAS, SAS Institute Inc., Cary, NC, USA). Level of significance was set at $P < 0.05$.

3. Results

3.1. Temperature response curves for CO_2 and N_2O during freeze and thaw

During the simulated thaw, active core average emissions declined with depth and were greater than the sterile core for both CO_2 and N_2O (Fig. 2). During freezing, active core emissions did not decline with depth and were not greater than the sterile core for both CO_2 and N_2O (Fig. 2). Emissions calculated per hour and on a mass basis followed similar trends (Fig. 2), so rates per g of soil are reported. During the simulated thaw, average CO_2 emissions ranged from 6.0 nmol CO_2 g^{-1} h^{-1} at the surface to 1.7 nmol CO_2 g^{-1} h^{-1} at 75 cm, while average N_2O emissions ranged from 0.30 pmol N_2O g^{-1} hr^{-1} at the surface to 0.06 pmol N_2O g^{-1} h^{-1} at 75 cm. Sterile core average emissions during thawing were <0.01 nmol

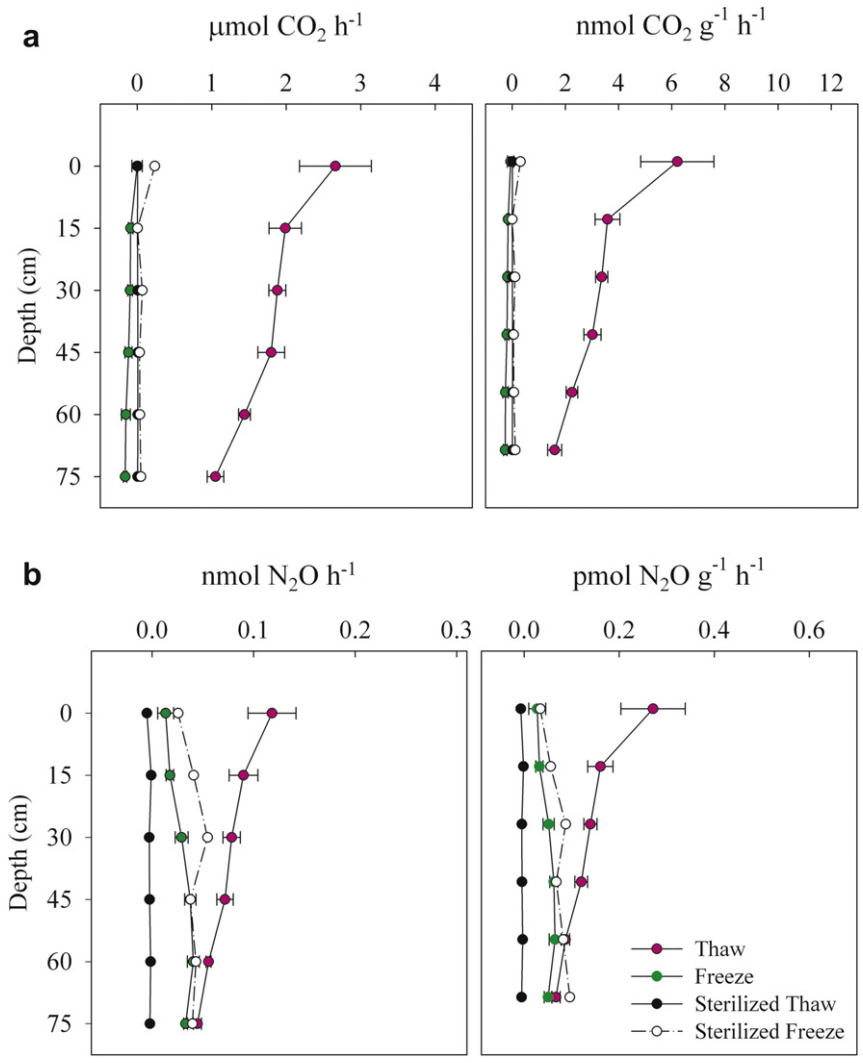

Fig. 2. a) Average (±standard error) observed rates of CO_2 emitted at 15-cm depth increments in μmol h^{-1} and in nmols g^{-1} h^{-1} for five active cores during simulated freezing (from 5 to −15 °C) and thawing (from −15 to 5 °C), and b) average observed rates of N_2O emitted across all temperature steps at 15-cm depth increments in nmols h^{-1} and in pmols g^{-1} h^{-1}. Also shown are observed rates determined for a sterilized core subjected to the same conditions.

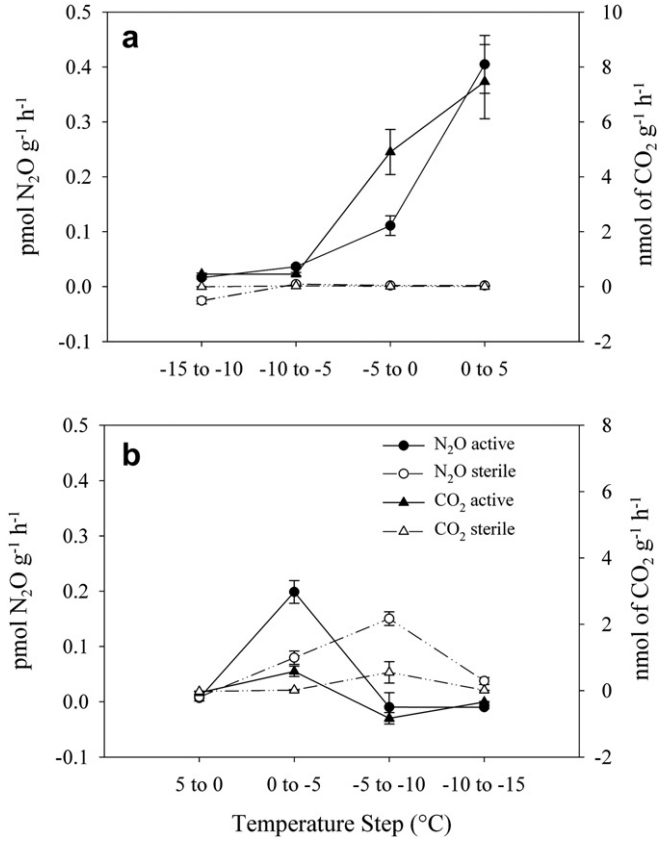

Fig. 3. a) Average (±standard error) observed rates of CO_2 and N_2O emitted across all depth increments at each temperature step (nmols CO_2 g^{-1} h^{-1} and in pmols N_2O g^{-1} h^{-1}) for active and sterile cores during thawing and b) during freezing.

CO_2 g^{-1} h^{-1} and <0.01 pmol N_2O g^{-1} h^{-1}. During the simulated freeze-up, both active and sterile cores emitted single pulses of CO_2 (0.1 nmol CO_2 g^{-1} h^{-1}) and N_2O (0.2 pmol N_2O g^{-1} h^{-1}) at temperatures below 0 °C (Fig. 3), with no differences among depths (Fig. 2). Sterilized core emissions of both CO_2 and N_2O during freezing occurred only at one temperature step (between −5 and −10 °C) and not during thawing (Fig. 3). Active core emissions during freezing occurred only at the 0 to −5 °C temperature step (Fig. 3). Analyses of CO_2 and N_2O data during the simulated freeze-up indicated emissions did not vary exponentially and were not analyzed further.

Analyses of data collected during the simulated thaw indicated observed emissions of CO_2 and N_2O responded exponentially to soil temperature as it increased from −15 to 5 °C. Modeled emissions (Fig. 4) fit observations reasonably well, with R^2 values of 0.91 for CO_2 and 0.99 for N_2O. For CO_2, parameter estimates for β were not significantly different among soil depths and ranged from 0.1 to 0.2. The average Q_{10} (±std. error) for CO_2 during the simulated soil thaw was 4.8 (±1.4). For N_2O, parameter estimates for β were not significantly different among soil depths and ranged from 0.2 to 0.3. Average Q_{10} for N_2O was 13.7 (±2.0). While β values were comparable across depths for both CO_2 and N_2O, parameter estimates for α varied with depth for both gases (Fig. 5). Alpha declined exponentially with depth for CO_2 (R^2 = 0.96) from an average of 6.9 (±1.3) at the surface to 1.5 (±0.2) at 75 cm. Alpha also declined exponentially with depth for N_2O (R^2 = 0.93), from an average of 0.3 (±0.05) at the surface to 0.05 (±0.01) at 75 cm. Similar to CO_2, the decrease in α for N_2O was also most evident between the surface and 15 cm depth (Fig. 5).

3.2. Effect of soil depth on CO_2 and N_2O emissions at 0 °C

Most soil properties varied significantly with depth (Table 1), particularly near the interface between the mollic epipedon (0–40 cm) and subsoil (40–80 cm). The mollic epipedon was underlain by combined B horizons with angular blocky structure

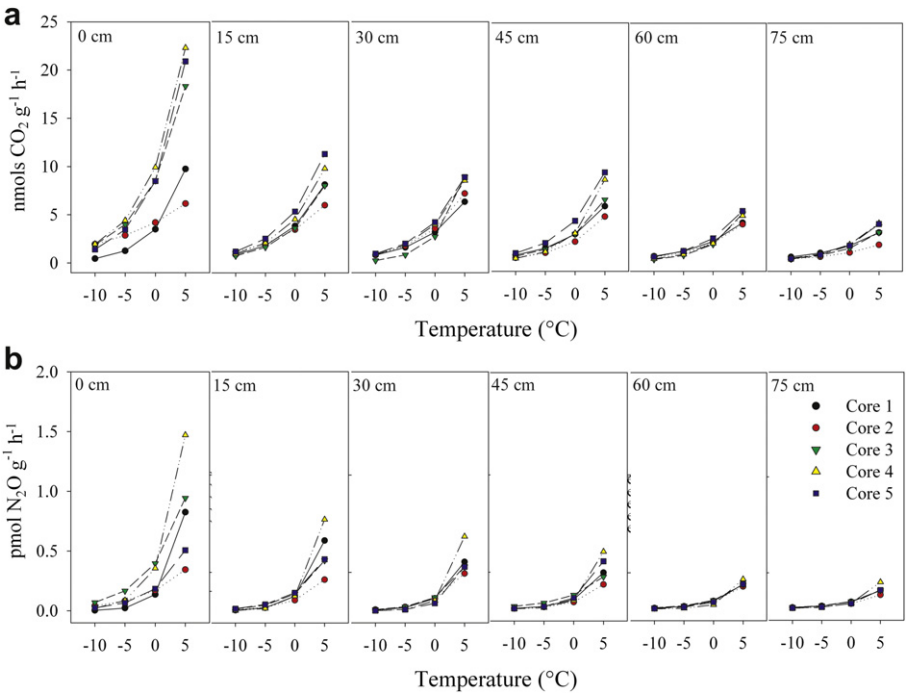

Fig. 4. Fitted temperature response curves for emissions of a) CO_2 and b) N_2O during a simulated thaw 15-cm depth increments for each experimental soil core using a simple first-order exponential model.

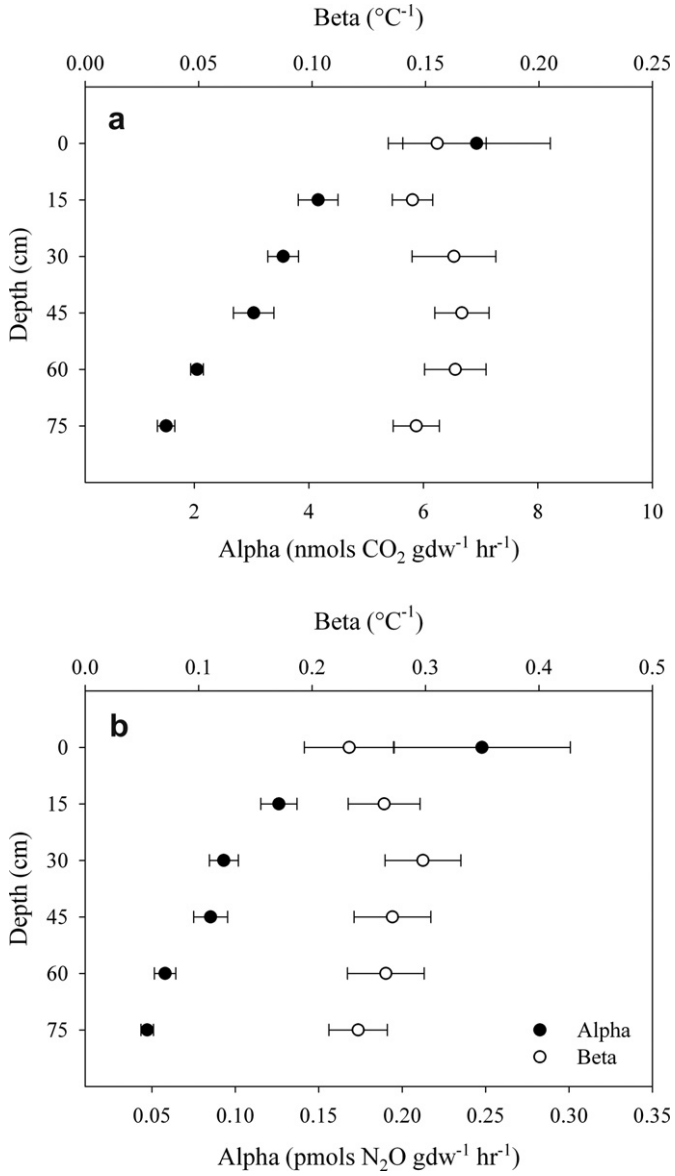

Fig. 5. Average (±standard error) model parameter estimates for α and β for a) CO_2 and b) N_2O during the simulated thaw.

through the remainder of the core. Macropores were observed throughout the epipedon, while very fine tubular pores were observed throughout the B horizon (Soil Survey Division Staff, 1993). Sand and silt content were similar across depths, while soils within the epipedon contained less clay than subsoil material (Table 1). Porosity decreased significantly with depth (ranging from 37 to 66%), but not WPFS, which averaged 55.6 (±4.0) across all depths (Table 1). Soil pH increased significantly with depth throughout the epipedon and remained similar throughout subsoil depths. Bulk density increased with soil depth (0.91–1.65 g cm^{-3}; Table 1). Both NO_3–N and available P decreased with depth, while NH_4–N increased with depth (Table 1). Soil C and N pools (indicating substrate quantity) decreased gradually with depth within the epipedon and then remained similar throughout the subsoil horizon (Table 1). Soil C:N ratios (indicating the level of substrate decomposition) declined steadily with each 10 cm depth from (12.1 to 10.3) until 40 cm; below the mollic epipedon, C:N remained steady at 10.0.

Several of the measured soil properties varied with depth, particularly between epipedon and subsoil, including pH, C:N ratio, NO_3–N, NH_4–N, P, clay content, and porosity. However, stepwise regressions selected porosity as the property with the greatest potential relationship with α parameter estimates during the simulated soil thaw (Fig. 5). Alpha parameter estimates were highly correlated with porosity for both CO_2 ($R^2 = 0.62$) and N_2O ($R^2 = 0.61$). The mixed, random intercept model also indicated α parameter estimates were significantly affected by porosity for CO_2 ($F_{1,26.2} = 79.56$; $P < 0.0001$) and for N_2O ($F_{1,27.8} = 52.83$; $P < 0.0001$).

4. Discussion

4.1. Temperature response curves for CO_2 and N_2O during freeze and thaw

Figs. 2 and 4 indicate how the simple first-order exponential model fit our observed temperature responses for whole-core CO_2 and N_2O emissions during the simulated soil thaw, as observed by others for CO_2 (Kirschbaum, 1995; Mikan et al., 2002). During the simulated freeze-up, however, emissions of CO_2 and N_2O were low and did not follow an exponential trend (Figs. 2 and 3). Similar freezing results were reported by Teepe et al. (2001), where emissions were attributed to CO_2 being forced out of the growing ice structure. Like Teepe et al. (2001), emissions at specific temperature steps below 0 °C for both sterilized and active cores suggest these may have been due to the physical disruption caused by the formation of ice in soil pores (Edwards and Cresser, 1992) as opposed to biogenic production (Figs. 2 and 3). The freezing point temperature for the sterilized core may have dropped even further than the active cores because high temperature and pressure during autoclaving would increase solute concentration and effectively lower the initial osmotic potential of soil solution (Harrysson Drotz et al., 2009).

The exponential model fit during thawing (Fig. 4) is supported by other CO_2 studies for soils near 0 °C (Kirschbaum, 1995; Fang and

Table 1
Soil properties with depth (0–80 cm) for an undisturbed Mollisol in Mandan, ND. Nitrate–N (NO_3^-), ammonium–N (NH_4^+), available phosphorous (P), whole soil carbon (SOC), whole soil nitrogen (N), whole soil C:N ratios, sand, silt clay, bulk density, water-filled pore space (WFPS), and porosity. Significant differences ($p < 0.05$) across depth for each parameter are indicated with different letters.

Soil depth (cm)	pH (−log[H^+])	NO_3^- (mg kg^{-1})	NH_4^+ (mg kg^{-1})	P (mg kg^{-1})	Whole soil C (Mg ha^{-1})	Whole soil N (Mg ha^{-1})	Whole soil C:N (ratio)	Sand (g kg^{-1})	Silt (g kg^{-1})	Clay (g kg^{-1})	BD (Mg m^{-3})	WFPS (%)	Porosity (%)
0–10	6.2 bc	6.1 a	5.1 bc	5.4 a	45.6 a	3.78 a	12.1 a	348	570	82 c	0.91 f	47	66 a
10–20	6.2 c	7.1 a	4.9 c	3.4 b	38.4 b	3.24 b	11.9 ab	328	570	102 c	1.14 ef	58	57 b
20–30	6.3 bc	5.4 ab	4.9 c	2.6 bc	32.0 c	2.77 c	11.5 b	322	576	102 c	1.19 e	59	55 bc
30–40	6.4 ab	4.3 b	4.7 c	2.2 bc	27.4 c	2.42 c	11.3 b	320	542	138 bc	1.27 d	59	52 c
40–50	6.6 a	2.5 bc	5.2 bc	2.2 bc	18.7 d	1.81 d	10.3 c	296	534	170 ab	1.37 c	53	48 d
50–60	6.6 a	1.1 c	5.1 bc	1.6 c	17.3 d	1.72 d	10.0 c	334	472	194 a	1.56 b	53	41 e
60–70	6.6 a	0.9 c	6.2 ab	2.0 c	18.0 d	1.78 d	10.1 c	298	486	216 a	1.67 a	57	37 ef
70–80	6.6 a	0.9 c	6.9 a	2.0 c	17.1 d	1.70 d	10.0 c	342	462	196 a	1.65 ab	57	38 f

Moncrieff, 2001; Mikan et al., 2002). Q_{10} values reported in the literature are highly variable (Davidson and Janssens, 2006), but average Q_{10} for CO_2 during our simulated thaw was similar to reports for thawing tundra soil (Mikan et al., 2002) and boreal forest soil (Öquist et al., 2009), but slightly higher than forests soil incubated at above freezing temperatures (Maag and Vinther, 1996; Boone et al., 1998). Waldrop et al. (2010) studied the CO_2 temperature response between −5 and 5 °C and reported a Q_{10} of 7.5 for the active soil layer above permafrost. Like Waldrop et al. (2010), we crossed over the freezing point of water, where both microbial activity and substrate availability likely changed during thaw. Unlike Waldrop et al. (2010) but similar to Teepe et al. (2001), we evaluated emissions for both freezing and thawing and found temperature direction clearly affected temperature response. In addition, we found the depth-response curves during thawing for α were nearly identical for both gases (Fig. 5). These data suggest pedogenic properties associated with depth may control emissions at 0 °C during thaw.

Average Q_{10} for N_2O emission during our simulated thaw was greater than Q_{10} values reported at temperatures above 0 °C (Parkin and Kaspar, 2006). Few reports for N_2O temperature sensitivities are available for frozen soil, but Wagner-Riddle et al. (2010) found an exponential temperature response for N_2O fluxes measured at the surface from −10 to 5 °C that was not evident at temperatures above 5 °C. This was attributed to a measured increase in apparent heat capacity due to changes in water content resulting from phase change. While Q_{10} was not reported by Wagner-Riddle et al. (2010), the exponential N_2O temperature response curve near 0 °C at the surface paralleled our N_2O data collected at the 0 cm depth (Fig. 4). Similar to CO_2, the exponential temperature response for N_2O (Fig. 4) point to the potential for a common driving variable (such as unfrozen water content) that also increases exponentially during soil thawing but not during soil freezing (Koopmans and Miller, 1966). The lack of emissions during desiccation induced by freezing would differ from desiccation relieved during thawing. Unfrozen water content was not measured here, but previous work indicates unfrozen water content does not depend on initial moisture conditions prior to freezing (Watanabe and Wake, 2009). The exponential emissions response may have been driven in part by the exponential increase in liquid water that occurs as temperatures approach 0 °C (Koopmans and Miller, 1966). If unfrozen water content is a primary controller for CO_2 emissions below 0 °C, as demonstrated by Öquist et al. (2009), then unfrozen water content might also be a primary controller for N_2O emissions below 0 °C.

4.2. Effect of soil depth on CO_2 and N_2O emissions at 0 °C

We found clear difference between epipedon (0–40 cm) and subsoil (40–80 cm) properties, with properties following trends commonly described with depth in prairie Mollisols (Stevenson and Cole, 1999; Fierer et al., 2003a,b). Water-filled pore space did not vary with depth but was within the range of ideal conditions for gas emissions at low temperatures (Del Prado et al., 2006). Organic substrate quantity (as expressed by C and N) followed the commonly observed classic decline with depth (Paul and Clark, 1996), where 67% of total C observed was contained in the mollic epipedon (0–40 cm). A review of grassland organic matter worldwide indicated the upper 20 cm contain 42% of total C in the soil profile (Jobbágy and Jackson, 2000), and we report 39% in the upper 20 cm. An estimated 55–80% of ecosystem respiration occurs in the upper 10–20 cm of soil (Jobbágy and Jackson, 2000; Neff and Asner, 2001), and most data supporting this estimate were collected at temperatures above 0 °C. Emissions of CO_2 at depths below 20 cm are estimated to contribute 20–45% of the observed surface fluxes worldwide. Few data are available to support multilayer models for respiration (Neff and Asner, 2001). In our case, depth increments only represent a sample of the pore space and not cumulative flux. Considering the seven depth samples per core, two of these sample depths (surface and 15 cm) contributed most (52%) of the total CO_2 and most (54%) of the total N_2O measured during thawing.

Differences in α parameter estimates with depth for both CO_2 and N_2O (Fig. 5) suggest soil properties affect emissions at 0 °C (Mikan et al., 2002; Fierer et al., 2003a), but this has only been postulated for CO_2 and not N_2O. We measured a number of soil properties and found α was most strongly related to porosity for both gases. Greater porosity, great root density, and soil organic matter near the surface of grasslands has been found to enhance hydraulic conductivity (Tokumoto et al., 2010; Stadler et al., 1997), retention of unfrozen water (Öquist et al., 2009), gas diffusion (Tang et al., 2003), and delivery of water and solutes required for respiration and nitrification (Öquist et al., 2009; Tilston et al., 2010). As soils thawed, hydraulic transport of substrate dissolved in unfrozen water coupled with greater gas diffusion through more porous media in the epipedon likely enhanced production of CO_2 and N_2O at 0 °C, as compared to subsoil (Tilston et al., 2010). However, porosity could simply be a proxy for multiple biotic and abiotic properties that also occur with increased pore space. Attributes associated with porosity that also change with depth, such as aggregate, root and microbial community distribution, may be more predictive of biogenic gas emissions near 0 °C (Fierer et al., 2003a; Sey et al., 2008; Watanabe and Ito, 2008; Waldrop et al., 2010) but would require further investigation.

While a frozen core manipulation experiment is convenient for studying temperature responses with depth, isothermal conditions rarely occur in the field (Wagner-Riddle et al., 2010). Instead, northern prairie subsurface soil tends to thaw before the epipedon, where temperatures may remain just below 0 °C for several days (Phillips, unpublished data). Under isothermal conditions, emissions were evident for epipedon and subsoil, although most biogenic activity occurred in the epipedon. Some authors have concluded that N_2O emitted from thawing soil may actually represent release of gases previously trapped by the freezing process (Teepe et al., 2001; Koponen et al., 2004). Our results corroborate previous studies that indicate biogenic N_2O and CO_2 are produced below 0 °C (Panikov et al., 2006; Öquist et al., 2009; Wagner-Riddle et al., 2010). We suggest that emission peaks observed by others at the surface of soil *in situ* following spring thaw represent cumulative production by organisms belowground as well as de novo synthesis near the surface.

5. Conclusions

Intact cores incubated under isothermal conditions indicate epipedon and subsoil responded exponentially to temperature increases during thawing for both epipedon and subsoil, but not during freezing. The exponential increase in CO_2 and N_2O during thawing and hysteresis during freezing mirror reported effects of thawing and freezing on unfrozen soil water content. Observed Q_{10} values during thawing were likely influenced more by temperature-induced changes in unfrozen water content than by pedogenic properties. Both CO_2 and N_2O emissions at 0 °C declined exponentially with depth and were most strongly correlated with porosity, pointing to the importance of maintaining soil structure during laboratory investigations of trace gas exchange. Pore size, root and microbial community distribution affect substrate availability and microbial activity during thawing, and these need to be explored further while maintaining soil structure to more fully understand the full suite of factors potentially contributing to differences in CO_2 and N_2O emissions with depth.

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