

ARTICLE

Nongrowing season N₂O and CO₂ emissions — Temporal dynamics and influence of soil texture and fall-applied manure¹

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Abstract: Nongrowing season (NGS) greenhouse gas (GHG) emissions may be significant in cold agricultural regions, but the influence of winter conditions, soil type, and fall manuring must be better documented. We monitored NGS N₂O and CO₂ fluxes and soil atmosphere composition from 2009 to 2013, on sandy loam and silty clay soils, with and without fall-applied pig slurry. Early-winter emission peaks indicated that soil respiration and biogenic N₂O production were stimulated during active soil freezing. Soil atmosphere GHG concentrations increased throughout winter, whereas O₂ concentration declined, especially when surface fluxes were low. Therefore, soil respiration and denitrification were not stopped by cold temperatures, and low surface fluxes were mainly due to restriction of gas diffusion. Nitrous oxide emissions varied from 0.4 to 8.5 kg N ha⁻¹ and were generally greater in the presence of pig slurry. Emissions were of similar magnitude between soil types, likely because O₂ restrictions on denitrification typically found in sandy soils during the growing season were eliminated by high soil moisture content in the NGS. This multiyear assessment highlights the need to include NGS GHG emissions to properly estimate yearly emissions and refine inventories in cold regions, particularly for sandy soils.

Key words: nongrowing season, N2O, CO2, soil type, manure.

Résumé : Les émissions de gaz à effet de serre (GES) hors saison de croissance peuvent être importantes dans les régions froides, mais l'influence des conditions hivernales, du type de sol et des apports de lisiers doit être mieux documentée. Nous avons suivi les émissions de N₂O et de CO₂ hors saison de croissance de 2009 à 2013 sur loams sableux et argiles limoneuses, avec et sans lisier de porc à l'automne. Des hausses d'émissions mesurées en début d'hiver ont indiqué que la respiration et la production biologique de N₂O étaient stimulées au cours du gel des sols. Les concentrations de GES dans l'air du sol ont augmenté au cours de l'hiver, alors que la concentration en O₂ diminuait, particulièrement en période de faibles émissions. Ceci indique que la respiration du sol et la dénitrification n'étaient pas inhibées par les basses températures et que de faibles flux de surface étaient attribuables à une restriction à la diffusion des gaz. Les émissions de N₂O ont varié de 0,4 to 8,5 kg N ha⁻¹ et étaient généralement plus élevées en présence de lisier. Ces émissions étaient de même magnitude pour les deux sols, vraisemblablement parce que les restrictions à la dénitrification par l'O₂, généralement observées en saison de croissance dans les sols sableux, étaient éliminées par une teneur en eau élevée en période hivernale. Cet essai pluriannuel met en lumière la nécessité d'inclure les émissions de GES hors saison de croissance afin d'estimer adéquatement les émissions annuelles et de préciser les inventaires en régions froides, en particulier pour les sols sableux.

Mots-clés: période hivernale, N2O, CO2, type de sol, lisier.

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Abbreviations: GHG, greenhouse gas; NGS, nongrowing season; SIEF, slurry-induced N2O emission factor.

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Introduction

Soil CO₂ and N₂O emissions have been observed in the nongrowing season (NGS) at subzero temperatures in natural (Sommerfeld et al. 1993; Brooks et al. 1997; Maljanen et al. 2010) and agricultural ecosystems (Wagner-Riddle and Thurtell 1998; van Bochove et al. 2001; Maljanen et al. 2009). While NGS CO₂ emissions generally account for less than 20% of yearly emissions in cold areas (Wei et al. 2010; Brooks et al. 2011; Miao et al. 2014), NGS N2O emissions may account for more than 50% (Wagner-Riddle and Thurtell 1998; Syväsalo et al. 2004; Virkajärvi et al. 2010; Yanai et al. 2011). Nongrowing season N₂O emissions may vary from near zero up to 5 kg N ha⁻¹ even within a given cropping system (e.g., Wagner-Riddle and Thurtell 1998; Syväsalo et al. 2004; Virkajärvi et al. 2010). Hence, gas fluxes must be measured over several years to adequately estimate the significance of NGS emissions in annual budget and to compare different soil types (Maljanen et al. 2009).

Nongrowing season soil CO₂ fluxes appear to be closely related to soil temperature, with a general decline as the temperature decreases in the winter, and a marked increase in spring when soil temperature rises again (Merbold et al. 2013; Miao et al. 2014). In contrast, soil N₂O fluxes follow more complex temporal patterns and may respond rapidly and markedly to freezing and thawing (Maljanen et al. 2009; Merbold et al. 2013; Pelster et al. 2013; Miao et al. 2014). Early-winter N2O fluxes have been reported in some studies (e.g., Maljanen et al. 2009; Virkajärvi et al. 2010) and could be due to constrained O₂ diffusion toward the unfrozen water films by the surrounding ice (Teepe et al. 2001), to the rise in substrate concentration in residual unfrozen water films as the soil solution freezes (Konrad and McCammon 1990), or to an increase in $N_2O/N_2O + N_2$ production ratio at lower temperatures (e.g., Holtan-Hartwig et al. 2002; Dörsch and Bakken 2004).

Teepe et al. (2001) demonstrated that N₂O production may occur during continuous soil freezing in the unfrozen water film present at the surface of soil particles, where the surrounding ice prevents N₂O release. In agreement, intense N₂O emissions during spring thaw have often been attributed to the physical release of accumulated greenhouse gases (GHGs) (e.g., Goodroad and Keeney 1984; Cates and Keeney 1987; Wagner-Riddle and Thurtell 1998), but others have evidenced that de novo production may also occur during thawing (Wagner-Riddle et al. 2008; Risk et al. 2014) in response to the release of carbon (C) and nitrogen (N) substrates from microbial cells and soil aggregates disruption during the freezing phase (Christensen and Christensen 1991; Mørkved et al. 2006). The apportionment of spring-thaw N2O fluxes into the two sources is still a matter of debate (Risk et al. 2014).

During the growing season, sandy soils generally exhibit smaller N₂O fluxes than finer-textured soils

because of faster drainage of excess water and rapid return to an oxic status (e.g., Sexstone et al. 1985; Chantigny et al. 2007; Pelster et al. 2012). However, the few existing direct comparisons under freezing conditions showed little influence of soil texture (Syväsalo et al. 2004; Teepe et al. 2004), suggesting that the elevated and sustained soil moisture content during the NGS may alleviate constraints related to aeration status in sandy soils (Schürmann et al. 2002; Virkajärvi et al. 2010).

Fall application of animal slurries can increase NGS GHG emissions (Wagner-Riddle and Thurtell 1998; Chantigny et al. 2002; Virkajärvi et al. 2010; Kariyapperuma et al. 2012). Manure-induced emission factors (the proportion of N applied with manure that is released as N₂O) may be an order of magnitude greater in clayey than sandy soils during the growing season (e.g., Chantigny et al. 2007; Pelster et al. 2012). However, there is little information about emission factors in the NGS following fall applications, and the possible influence of soil texture is unknown. The objectives of this study were to determine (i) interannual variations in the temporal dynamics of N₂O and CO₂ productions in soil and surface emissions during the NGS and (ii) the influence of soil texture and fall manuring on the magnitude and variability of NGS GHG emissions.

Materials and Methods

Experimental site

The experimental site was located in the province of Quebec (46°73′N; 71°52′W) in a region with a mean annual temperature of 4.4 °C and mean annual precipitation of 1231 mm. In this region, soils are typically exposed to freeze-thaw conditions in November and April, and high soil moisture conditions with snow cover extending from December to March (Table 1). Two soil types were used: a well-drained sandy loam (Orthic Dystric Brunisol; 170 g clay kg⁻¹; 680 g sand kg⁻¹; 19 g C kg⁻¹; 1.5 g N kg⁻¹; pH 6.5) and an imperfectly drained silty clay (Gleyed Melanic Brunisol; 432 g clay kg⁻¹; 163 g sand kg⁻¹; 35 g C kg^{-1} ; 2.7 g N kg^{-1} ; pH 6.8). The two soil types are located about 500 m from each other, and the experiment was carried out in a 10 m \times 20 m zone (one for each soil type) that was cropped to spring barley (Hordeum vulgare L.) from 2009 to 2012. Each fall, the soils were harrowed twice (10 cm depth) in mid-October, crop residues were removed from the soil surface, and a new set of small replicate plots (30 cm \times 30 cm in size) was established. Each small plot either received pig slurry to provide 65 kg NH₄-N ha⁻¹ and to simulate local practices (Table 2), or was left unamended (control). The slurry was manually applied on the first week of November (third week of November in 2009) and immediately incorporated to the top 5 cm of soil with hand tools to minimize NH₄-N loss through ammonia volatilization. Experimental treatments were replicated five times, for a total of 10 small plots per soil type. The small plots were

Table 1. Climate normals (1981–2010) for the study area.^a

Parameter	November	December	January	February	March	April
Mean daily air temperature (°C)	-0.7	-8.6	-12.8	-10.6	-4.6	3.7
Mean daily max. temperature (°C)	2.9	-4.2	-7.9	-5.6	0.2	8.3
Mean daily min. temperature (°C)	-4.2	-12.8	-17.7	-15.9	-9.4	-1.0
Mean rainfall (mm)	69.1	31.7	22.7	15.2	30.2	67.5
Mean snowfall (cm)	32.7	72.4	71.9	63.6	46.4	13.2
Mean snow height (cm)	3	21	45	61	59	15

^aData obtained from the Jean-Lesage International Airport, 10 km northeast of the study site; detailed data available from Environment Canada at: http://climate.weather.gc.ca/climate_normals/results_1981_2010_e.html? searchType=stnName&txtStationName=qu%C3%A9bec&searchMethod=contains&txtCentralLatMin=0&txtCentralLatSec=0&txtCentralLongMin=0&txtCentralLongSec=0&stnID=5251&dispBack=1.

Table 2. Selected characteristics of the pig slurry applied in this experiment.

Characteristic	2009	2010	2011	2012
pН	7.9	8.0	7.2	7.4
Dry matter (g L ⁻¹)	52.0	51.6	120.4	125.2
Total N (g L ⁻¹)	4.1	4.3	8.3	8.3
NH ₄ -N (g L ⁻¹)	2.9	3.3	5.1	4.6

placed next to each other, and scaffolding was installed next to the plots so that flux measurements could be conducted without altering the snow cover. Scaffolding was installed downwind of the plots to minimize the possible influence on snow accumulation on the plots. Experimental plots were established only on the sandy loam in NGSs of 2009–2010 and 2010–2011 and on both soil types in the NGSs of 2011–2012 and 2012–2013.

Gas sampling

The N₂O and CO₂ fluxes were measured weekly from pig slurry application in early November to late March, with the exception of NGS 2009–2010, when measurements were carried out once every 2-3 wk. Gas fluxes were measured 1-2 times a week during spring thaw (late March to early May). Gas flux measurements were performed using static chambers. The base consisted of white polyvinyl chloride (PVC) pipe (16 cm i.d., 15 cm height), beveled at one end to facilitate insertion into the soil. One base was permanently inserted at 10 cm depth in the middle of each plot. Each base had one 6 mm hole pierced at 12.5 cm from the base top to receive probes for monitoring soil temperature and atmosphere composition within the chamber area, as described below; once the base was inserted in the soil, the hole was positioned at 7.5 cm depth.

The chamber was made of a white PVC end cap (10 cm height) made to fit the PVC base. The end cap was equipped with two plastic tubes (Bev-A-Line, 3 mm i.d. \times 10 cm long: 0.7 mL dead volume) fitted on the top,

one of which was equipped with a septum male luer lock stopper (Vygon, Ecouen, France) for gas sampling, whereas the other one was kept as a vent to ensure constant pressure within the chamber during deployment. Once snow began to accumulate on the ground, collars were kept above the snow surface via 10 cm height PVC pipe sections, which were stacked on the base section and secured with wrapping plastic to form a tight seal around the joint. A new PVC section was installed the day before an expected snowfall (>10 cm). Section additions were managed to ensure that snow surface was as close as possible to the top of the base at time of flux measurements; snow height within the base was always measured just before chamber deployment to account for total above-snow headspace in calculations. The sections were gradually removed by cutting the wrapping plastic seal as snow melt progressed in the spring. This allowed for continued flux measurement with minimal disturbance of the snow cover.

At the time of chamber deployment, an acrylic ring slightly exceeding the base wall with closed-cell foam (6 mm thick; Lundell Manufacturing, Plymouth, MN, USA) glued on both sides was placed on the top of the base. The chamber was then placed on the ring with a 1 kg weight on the top to create a tight seal between the base and chamber. Gas samples were withdrawn from the headspace 0, 8, 16, and 24 min after chamber deployment with a 20 mL plastic syringe and immediately transferred into preevacuated glass vials (12 mL Exetainers, Labco, High Wycombe, UK) as described by Rochette and Bertrand (2008).

Ancillary measurements

Soil temperature was monitored in each PVC base using copper-constantan thermocouples inserted into the soil at 7.5 cm depth. Soil temperature was read every 5 min with a datalogger and averaged on an hourly basis. The soil atmosphere was sampled at 7.5 cm depth using plastic tubing (Bev-a-line, 3 mm i.d. \times 2.5 m long: 17.7 mL dead volume). The buried end of tubing was fitted to a mesh bag filled with glass beads, whereas the other end emerged from the soil and was fitted to a

septum for gas sampling as described for the chamber. The first 20 mL of withdrawn air was discarded to account for tubing dead volume; the second 20 mL sample was transferred to an evacuated vial. Soil atmosphere samples were collected at the same time as surface flux measurements. At times, some tubings became clogged with ice or by ice lenses forming in the soil, preventing sample collection. In particular, soil atmosphere composition could not be monitored in the sandy loam in NGS 2012–2013 and in the silty clay in NGS 2011–2012 because of extensive ice formation in early winter. As a result, statistical analyses were not carried out for data on soil atmosphere composition; nonetheless, these data are presented to support interpretation of surface fluxes over time.

Gas samples were analyzed within 48 h of sampling. The gas samples and quality control samples were analyzed for N₂O, CO₂, and O₂ (soil atmosphere only) concentrations with a gas chromatograph (model 450; Bruker Corporation Billerica, MA, USA) equipped with an electron capture detector (N2O) and a thermal conductivity detector (CO_2). The CO_2 concentration could be detected in samples at concentration up to 50% (v/v) with accuracy of ±0.001% without dilution, covering all cases in the present study. For N₂O, a calibration curve was made using eight gas standards ranging from 0.3 to 508 ppm, the upper limit of detection of the electron capture detector being 1000 ppm. Samples with N2O concentration up to 510 ppm were quantified as is, whereas samples with greater concentrations were diluted 1:12 by transferring gas subsample to a new vial containing helium. With this approach, all samples could be quantified using the aforementioned calibration curve, with accuracy of ±0.005 ppm at concentrations ≤10 ppm, and up to ±1 ppm at concentrations >100 ppm. Above-snow CO_2 (F_{CO_2} , mg m⁻² s⁻¹) and N_2O (F_{N_2O} , mg m⁻² h⁻¹) fluxes were calculated using equations proposed by Rochette and Bertrand (2008):

$$F_{CO_2}$$
 or $F_{N_2O} = dC/dt(V/A)Mm/Mv(1 - e_a/P)$

where dC/dt (mol mol⁻¹ h⁻¹) is the rate of change in CO_2 or N_2O concentration in chamber headspace in dry air at deployment time = 0, estimated using linear or nonlinear methods; V (m³) is the chamber headspace volume (above snow level), measured prior to each deployment; A (m²) is the surface area covered by the chamber; Mm (mg mol⁻¹) is the molecular weight of CO_2 or N_2O (44 000 for both gases); Mv (m³ mol⁻¹) is the molecular volume at the predeployment air temperature; e_a (kPa) is the predeployment partial pressure of water vapor; and P (kPa) is the barometric pressure.

We assumed that with the chambers used and considering the short deployment time, gas accumulation in the chamber headspace above the snow surface would not influence gas gradient in the snow profile. Therefore, gas fluxes were calculated using above-snow headspace in the chamber. Gas accumulation rate in

chambers was linear at all times when the slope was significantly greater than 0, supporting this assumption.

Calculations and statistical analyses

Cumulative CO₂-C and N₂O-N emissions were calculated by linear interpolation of fluxes between consecutive sampling dates and summing for the entire period of measurement (1 Nov. to 1 May). For the NGS 2009-2010, cumulative emissions extended from third week of November to 1 Apr. The cumulative NGS emissions were partitioned into "winter" (onset of measurements in November until onset of active snow melt) and "spring-thaw" (active snow melt until end of measurements) periods. The effect of fall application of pig slurry on the cumulative emissions in winter, spring-thaw, and whole NGS was tested for each soil type separately using mixed effects model (MIXED procedure, SAS, version 8), where manure addition was included as the fixed effect (control vs. manured), and replicates were considered a random effect. The effect of pig slurry addition was tested for individual years to assess interannual variations in the effect and in a second model with years as a random effect to assess the general effect.

Cumulative N₂O-N emissions were used to calculate slurry-induced N₂O emission factors (SIEF) as

$$SIEF = (CN_2O_{sl} - CN_2O_{ctl})/N_{sl} \times 100$$

where SIEF (%) is the proportion of applied N lost as N_2O , corrected for emission from the control treatment; CN_2O_{sl} and CN_2O_{ctl} are the cumulative N_2O -N emissions (kg ha⁻¹) from the slurry and control plot, respectively; and N_{sl} (kg ha⁻¹) is the amount of total N applied with the slurry.

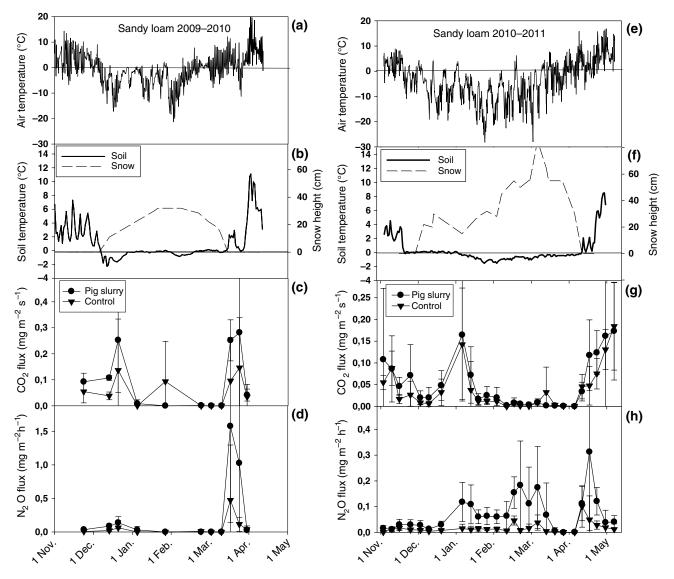
Results

NGS conditions

In general, daily mean air temperature was highly variable during the NGS, and was mostly below 0 °C from late November to early April (Figs. 1–3). Significant periods with temperature above 0 °C were recorded in December 2010 and January 2011 (Fig. 1e), December 2011 (Fig. 2a), and January 2013 (Fig. 3a). These warm periods were associated with rainfall, some causing a drop in snow height (Figs. 1f, 3b, 3f), and formation of ice lenses were observed at the soil surface as the rainy periods were followed by a rapid return to freezing temperatures.

Snow accumulation showed very different temporal patterns among years, which influenced the timing and intensity of soil frost during the winter months (Figs. 1b, 1f–3b, 3f). In general, snow cover appeared in early December, peaked at 30–50 cm height between late February and early March, and melted within 2–3 wk in late March to early April. The exceptions were in NGS 2010–2011, when snow accumulated rapidly in late November and peaked at 80 cm height in early March

Fig. 1. Nongrowing season (NGS) (2009–2010 and 2010–2011) air temperature (a, e), soil temperature and snow height (b, f), and snow surface $CO_2(c, g)$ and $N_2O(d, h)$ fluxes from a sandy loam with and without fall-applied pig slurry. Vertical bars in graphs c, d, g, and h indicate standard deviation of mean treatment values.

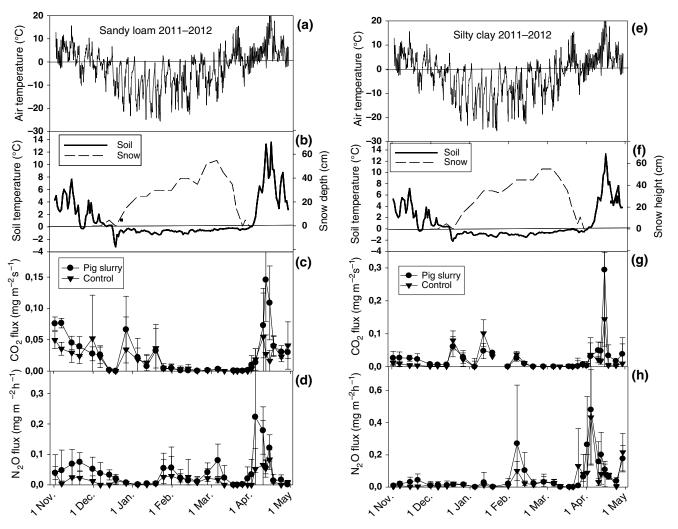


(Fig. 1f), and in NGS 2012–2013, when alternating midwinter snow and rain falls caused snow height to oscillate between 20 and 40 cm on the sandy loam (Fig. 3b) and between 10 and 30 cm on the silty clay (Fig. 3f). The difference in snow height between the soils in NGS 2012–2013 was attributed to windy conditions, with less snow accumulation on the more exposed silty clay site. Air temperature, snowfall, snow height, and the occurrence of rainfall events in the December–January period in the present study were found to be typical of the study area (Table 1), except in NGS 2010–2011 when snow height was higher than normal in February and March (Fig. 1f), and in NGS 2012–2013 when air temperature (Fig. 3a) and snow height (Figs. 3b, 3f) were lower than normal.

Soil temperature measured at 7.5 cm generally oscillated as a function of air temperature at the beginning

and end of the NGS but became decoupled from air temperature when snow height reached about 20 cm (Figs. 1b, 1f-1h, 2a-2h, 3b, 3f). During the first cold spells from mid-November to early December, when soils were not fully insulated by snow, soil temperature typically dropped below 0 °C and generally stayed between 0 and −2 °C after the snow cover developed. As an exception, snow accumulated before soils froze in winter 2010-2011, causing temperature in the sandy loam to remain around the freezing point for several weeks before falling gradually below 0 °C in early January (Fig. 1f). In contrast, the lowest soil temperatures were recorded in February 2013 (Fig. 3b: −4 °C in the sandy loam; Fig. 3f: −9 °C in the silty clay) and were attributable to a prolonged cold spell combined with shallow snow cover. Snow accumulation was similar for both soils in NGS 2011–2012 but was generally less on the silty clay in NGS

Fig. 2. Nongrowing season (NGS) (2011–2012) air temperature (a, e), soil temperature and snow height (b, f), and snow surface $CO_2(c, g)$ and $N_2O(d, h)$ fluxes from sandy loam (a-d) and silty clay (e-h) soils with and without fall-applied pig slurry. Vertical bars in graphs c, d, g, and h indicate standard deviation of mean treatment values.



2012–2013. As a result, soil temperature showed similar patterns between soil types in NGS 2011–2012 but tended to be lower in the silty clay in 2012–2013. Soil temperature typically started to rise in mid-February but stayed at or below 0 °C until snow melt was complete in April and then increased rapidly.

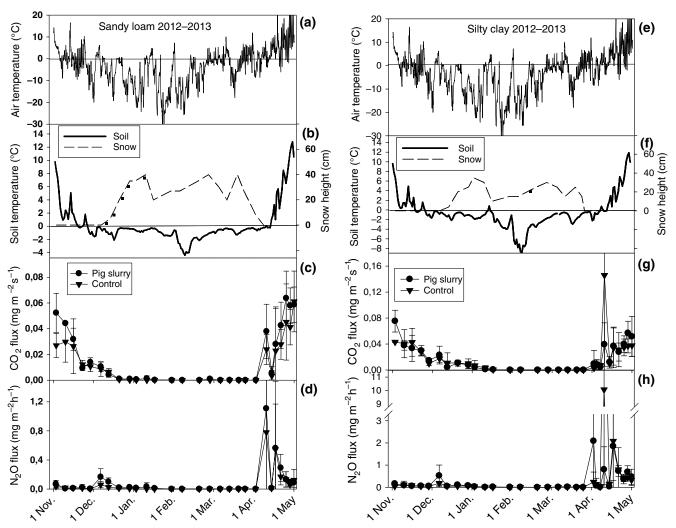
Winter surface gas fluxes and soil gas concentrations

Surface CO₂ and N₂O fluxes were generally moderate to low at the beginning of NGS in early November and typically declined to near zero in early December (Figs. 1–3). The gas flux rates remained generally low during winter (December to March), but periods of increased N₂O emissions were observed. In most years and in both soil types (Figs. 1d, 1h, 3d, 3h), N₂O fluxes peaked in mid-December or mid-January while soil temperatures dropped below 0 °C for the first or second time of the NGS. A similar peak was also observed in both soil types in 2011–2012 (Figs. 2d, 2h), but it occurred in late January or early February;

several weeks after soil temperatures had fallen below freezing. It is noteworthy that high N_2O fluxes were consistently measured for several weeks (January to March) in the sandy loam in NGS 2010–2011 (Fig. 1h). Winter time peaks were also observed on some years for CO_2 (Figs. 1c, 1g, 2c, 2g). The magnitude of winter CO_2 fluxes and N_2O fluxes was similar between soil types.

Fall application of pig slurry induced peak N₂O fluxes greater than the control during the winter period (Figs. 1–3), resulting in greater winter cumulative N₂O-N emission with the slurry on four out of six site years (Table 3), the other two site years showing similar trends. A positive response to slurry addition was also found for winter CO₂ fluxes (Figs. 1–3), resulting in a trend to greater CO₂-C emissions from the slurry plots (Table 3). Across years (general effect), pig slurry significantly increased winter emissions in both soil types for N₂O but only in the sandy loam for CO₂ even though a similar trend was found in the silty clay (Table 3).

Fig. 3. Nongrowing season (NGS) (2012–2013) air temperature (a, e), soil temperature and snow height (b, f), and snow surface $CO_2(c, g)$ and $N_2O(d, h)$ fluxes from sandy loam (a-d) and silty clay (e-h) soils with and without fall-applied pig slurry. Vertical bars in graphs c, d, g, and h indicate standard deviation of mean treatment values.



Soil CO₂ and N₂O concentrations started to increase in late January or early February and peaked in March (Figs. 4, 5), when snowmelt began and water logging restricted gas diffusion to the atmosphere. In general, CO₂ concentration peaked at values \leq 1.5%, and N₂O peaked at values \leq 100 μ L L⁻¹. These increases were mirrored by a decline in soil O₂, but O₂ concentrations generally remained above 14%. The largest changes in soil atmosphere composition were measured in the silty clay soil in NGS 2012–2013 (Fig. 5), when soil N₂O concentration rose above 1000 ppm from mid-January to snowmelt, and O₂ concentration fell below 5%. In general, the increase in CO₂ concentration was smaller than the corresponding decline in O₂ concentration (Figs. 4, 5).

Spring-thaw surface gas fluxes and soil gas concentrations

Surface gas fluxes typically dropped to zero during the active phase of snow melt in late March (Figs. 1–3).

During this period, soil CO₂ and N₂O concentrations peaked at their highest values, whereas O2 concentration was at its lowest (Figs. 4, 5). Snow melt occurred over 2-4 wk and was followed by a burst in CO₂ and N₂O fluxes which lasted for 2-6 wk, depending on the year (Figs. 1-3). The burst of N₂O was often characterized by the occurrence of two or more consecutive emission peaks (Figs. 2d, 2h, 3d, 3h). During the burst in GHG, soil atmosphere returned to background levels (Figs. 4, 5). Surface gas fluxes at spring thaw were generally much greater than during winter (Figs. 1-3). The largest spring-thaw N₂O fluxes were measured in NGS 2012-2013 in the two soils (Figs. 3d, 3h), after very cold soil temperatures had occurred in winter (Figs. 3b, 3h). This also corresponded to the NGS when late-winter soil N2O concentrations were the highest and O2 concentrations the lowest of the whole study (Fig. 5). As found in winter, spring-thaw CO₂ and N₂O fluxes were generally of the same magnitude between soil types.

Table 3. Cumulative N_2O -N and CO_2 -C emissions for winter, spring-thaw, and whole nongrowing season (NGS), and N_2O emission factors, as a function of soil type and year.

		N_2O				CO_2		
	Treatment	Winter (mg N m ⁻²)	ST (mg N m ⁻²)	NGS (mg N m ⁻²)	SIEF (%)	Winter (g C m ⁻²)	ST (g C m ⁻²)	NGS (g C m ⁻²)
Yearly effect	ı							
Sandy loam								
2009-2010	Pig slurry	54a	272a	326a	2.22	125a	88a	213a
	Control	26a	96b	122b	_	115a	41a	156b
2010-2011	Pig slurry	148a	57a	205a	1.86	138a	76a	214a
	Control	28b	19b	47b	_	100a	62a	162a
2011–2012	Pig slurry	70a	39a	109a	0.65	67a	35a	102a
	Control	26b	14b	40b	_	52a	20a	72b
2012–2013	Pig slurry	47a	120a	167a	0.47	21a	21a	42a
	Control	17b	95a	112b	_	15b	16b	31b
Silty clay								
2011–2012	Pig slurry	73a	81a	154a	0.46	53a	40a	93a
	Control	35a	65a	100b	_	29b	34a	63b
2012–2013	Pig slurry	291a	276b	567a	NA	32a	19a	51a
	Control	99b	755a	854a	_	31a	26a	57a
General effec	ct ^b							
Sandy loam	Pig slurry	80a	122a	202a	_	88a	55a	143a
	Control	24b	56b	80b	_	70b	35b	105b
Silty clay	Pig slurry	182a	178b	360a	_	42a	30a	72a
- •	Control	67b	410a	477a	_	30a	30a	60a

Note: ST, spring thaw; NGS, whole nongrowing season; SIEF, slurry-induced emission factor; NA, not applicable, as value of the control was greater than value of the manured soil. Different lowercased letters for cumulative emission values within a given soil type and year indicate a significant effect of pig slurry addition (P < 0.05).

Fall application of pig slurry increased spring-thaw CO₂ and N₂O fluxes (Figs. 1–3), except on one sampling date in April 2013 when fluxes in the control plots were four times greater than in pig slurry plots for CO₂ (Fig. 3g) and an order of magnitude greater than pig slurry plots for N₂O (Fig. 3h). As a result, cumulative CO₂-C and N₂O-N emissions at spring thaw were generally greater where pig slurry had been applied (Table 3), with the exception of the silty clay soil in NGS 2012–2013 when cumulative N₂O-N emission was greater in the control treatment. Across years (general effect), pig slurry increased spring-thaw N₂O and CO₂ emissions in the sandy loam but had no influence on CO₂ emissions and decreased N₂O emissions in the silty clay.

Cumulative GHG emissions during the NGS

The cumulative GHG emissions varied greatly among NGSs (Table 3). Cumulative N₂O-N and CO₂-C losses over the whole NGS were of similar magnitude between soil types and generally greater for soils receiving fall-applied pig slurry. Across all study years, pig slurry significantly increased NGS GHG emissions in the sandy loam soil (Table 3, general effect). Due to contradictory results between the spring-thaw periods of NGS

2011–2012 and NGS 2012–2013, a similar conclusion could not be reached for the silty clay soil. Cumulative emissions during the winter period accounted for 12%–72% of whole NGS $\rm N_2O$ emissions and 46%–74% of NGS $\rm CO_2$ emissions, depending on year.

Discussion

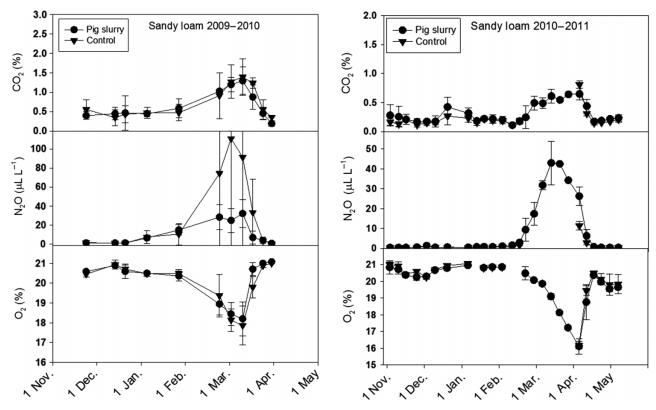
Temporal dynamics of surface fluxes and soil gas accumulation

The peaks of N_2O fluxes that were observed during the winter period of all NGSs in both soil types indicate that N_2O production was stimulated by freezing, in agreement with findings in other cold regions (van Bochove et al. 2001; Maljanen et al. 2009). Our results also support a study by Tatti et al. (2014), at the same experimental site as in the present study, showing that soil denitrifier communities are present, active, and highly dynamic throughout the winter period in the 0–7.5 cm top soil layer. The occurrence of CO_2 flux peaks and decline in soil O_2 concentration in winter in the present study also point to a biogenic origin of N_2O , most likely through denitrification (Müller et al. 2002; Öquist et al. 2004; Mørkved et al. 2006; Wagner-Riddle et al. 2008).

^aThe slurry effect was tested separately for each year to assess interannual variations in the effect.

^bYears were included as a random effect in the mixed statistical model.

Fig. 4. Soil CO_2 , N_2O , and O_2 concentrations (7.5 cm depth) in a sandy loam with and without fall-applied pig slurry. Measurements were made in the 2009–2010 (left graphs) and the 2010–2011 (right graphs) nongrowing seasons (NGSs). Vertical bars in graphs indicate standard deviation of mean treatment values.



The previous studies generally attributed the stimulation of denitrification and high N₂O production following freeze-thaw cycles to the disruption of soil aggregates and microbial cells during freezing, followed by the mobilization of substrates and development of anoxic conditions during the thawing phase (e.g., Christensen and Christensen 1991; Mørkved et al. 2006; de Bruijn et al. 2009; Pelster et al. 2013; Risk et al. 2014). However, this freeze-thaw effect cannot explain the early-winter N2O peaks observed during soil freezing in the present study (Figs. 1-3), because there were no obvious thawing events in early winter in our study, and the ice formed during early freezing likely restricted solute diffusion throughout the soil (Tilston et al. 2010; Tucker 2014). An increase in concentration of available C and N in the unfrozen water films of frozen or freezing soils (Konrad and McCammon 1990; Teepe et al. 2001) would more likely explain early-winter N₂O and CO₂ flux peaks due to stimulation of soil heterotrophs.

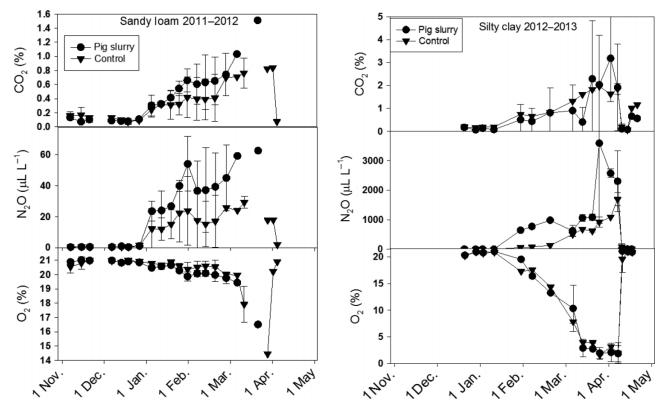
Alternatively, early-winter N_2O fluxes could be due to a shift in the N_2O -to- N_2 production ratio rather than an increase in total denitrification. The selective inhibition of the N_2O reductase by cold temperatures was proposed to explain increased N_2O fluxes in winter (Holtan-Hartwig et al. 2002; Dörsch and Bakken 2004). It is also hypothesized that soil denitrifiers that have the capacity to synthetize N_2O reductase are selected against by cold

conditions, as was demonstrated to occur under decreasing soil pH conditions (Philippot et al. 2006).

The high N₂O fluxes measured for several consecutive weeks in the sandy loam in NGS 2010–2011 (Fig. 1h) are generally not observed during the growing season, when N₂O emissions typically decline a few hours to a few days after rainfall as water drains and soil aeration status rapidly returns to an oxic regime (e.g., Sexstone et al. 1985; Chantigny et al. 2007; Pelster et al. 2012). In addition to the generally high soil moisture content in the NGS, the early onset of a deep snow cover (>30 cm) in NGS 2010–2011 maintained soil temperature close to a relatively warm 0 °C (Fig. 1f), which likely created sustained favourable conditions for soil microorganisms (Brooks et al. 2011), including denitrifier communities.

The accumulation of N₂O and CO₂ and the disappearance of O₂ in the soil atmosphere occurred during the coldest part of winter, indicating that denitrification and heterotrophic respiration were not stopped by even the coldest temperatures (e.g., Figs. 3b, 3f vs. Fig. 5). Such accumulation during winter was previously reported for N₂O and CO₂ (Cates and Keeney 1987; Burton and Beauchamp 1994; van Bochove et al. 2001; Maljanen et al. 2009) and was mainly attributed to the entrapment of gases under ice lenses in and on the soil surface. In the present study, the late fall to early winter period (November–December) was characterized by a

Fig. 5. Soil CO₂, N₂O, and O₂ concentrations (7.5 cm depth) in sandy loam (left graphs) and silty clay (right graphs) soils with and without fall-applied pig slurry. Measurements were made in the 2011-2012 nongrowing seasons (NGSs) for the sandy loam and in the 2012-2013 NGS for the silty clay soil. Vertical bars in graphs indicate standard deviation of mean treatment values.



gradual decline in surface gas fluxes and low soil CO₂ and N2O concentrations and was followed by a gradual rise in soil CO₂ and N₂O concentrations from January to early March. This temporal dynamic suggests that the decline in temperature and early freeze-thaw cycles in November-December could have negatively affected soil microbes, which had to adapt to the changing conditions. This may be possible through physiological adaptation at the cell level (e.g., production of antifreeze molecules) and through the development of psychrophilic microbial communities (Angilletta 2009; Hall et al. 2010; Crowther and Bradford 2013; Tatti et al. 2014, 2015). Alternatively, denitrification and respiration rates at subzero temperatures may be mainly limited by extracellular barriers (ice) to substrate diffusion (Mikan et al. 2002), as controlled by unfrozen water content (Tilston et al. 2010; Tucker 2014). It is also possible that gases were produced at depth, where the soil was not as cold and gradually migrated and accumulated in the frozen surface horizon (e.g., Burton and Beauchamp 1994; Wagner-Riddle et al. 2008).

Soil O₂ concentration has rarely been measured in studies on overwinter GHG emissions. In the present study, the substantial reduction in O2 concentration during winter, especially in the silty clay soil in NGS 2012-2013 (Fig. 5), indicated that restriction to O_2 diffusion was developed in the top soil and was maintained for a long period of time, further supporting the assumption that denitrification was the main source of N₂O in the soils (Yanai et al. 2011).

The increase in CO₂ concentration in soil atmosphere was not equivalent to the decline in O_2 . This is in agreement with Rochette et al. (2006) who found that the increase in CO₂ in frozen soil atmosphere explained only 50%-70% of the decrease in O₂. They suggested that in the absence of living roots, O2 consumption was attributable to soil heterotrophic respiration. They also suggested that the discrepancy was caused by the greater solubility of CO₂ than O₂ in water (CO₂ is 80 times more soluble), and the portion of soil CO₂ present in the water phase was therefore much greater than for O_2 . Accordingly, Chantigny et al. (2002) estimated that at 0 °C, the ratio of dissolved to gaseous O₂ in soil was 0.047, compared with 1.64 for CO₂. Thus, the discrepancy between O2 and CO2 accumulation most likely reflected differences in gas solubility, rather than an additional O₂ sink beyond heterotrophic activity.

The rapid increase in N₂O and decline in O₂ in the soil atmosphere during active snow melt (in March) and the absence of surface gas fluxes during the same period (just before the spring-thaw burst) indicated that soil respiration and denitrification continued, but that gases were trapped in the soil by the surface water front. As soon as the soil had thawed and drained, accumulated

Table 4. Comparison of range of cumulative N₂O-N emissions during the growing season^a and the nongrowing season (NGS) for silty clay and sandy loam soils with and without pig slurry.

	Growing seas	on	Nongrowing season		
	Without PS (kg N ha ⁻¹)	With PS (kg N ha ⁻¹)	Without PS (kg N ha ⁻¹)	With PS (kg N ha ⁻¹)	
Sandy loam Silty clay	0.3–0.4 2.2–3.3	0.5–1.3 4.4–7.6	0.4–1.1 1.0–8.5	1.1–3.3 1.5–5.7	

^aMeasurements for the growing season were carried out from May to October 2009 and 2010, and data were published in Pelster et al. (2012).

gases were released, as evidenced by the fast return of soil atmosphere CO_2 , N_2O , and O_2 to background levels. The concurrent burst in surface N2O and CO2 fluxes indicated that passive degassing of accumulated gases made a significant contribution to the spring-thaw burst, in agreement with earlier studies (Goodroad and Keeney 1984; Burton and Beauchamp 1994; Teepe et al. 2001). However, other authors demonstrated that N₂O is also produced during the thawing period (Wagner-Riddle et al. 2008; Pelster et al. 2013; Risk et al. 2014). Two or more consecutive peaks in N₂O were often observed during the spring-thaw N2O burst in the present study. Such a temporal dynamic was also reported by Wagner-Riddle et al. (2008), Maljanen et al. (2009), and Risk et al. (2014). They argued that the first peak was mainly attributable to the degassing of gases accumulated in soil, whereas the second peak was caused by de novo N₂O production.

Influence of soil type and fall-applied manure

Surface N₂O fluxes and cumulative N₂O-N emissions were of the same magnitude between soil types. This contrasts with losses reported from the same soils during the growing season, which were up to an order of magnitude greater for the silty clay than the sandy loam (Pelster et al. 2012), in agreement with other studies in the same region (Chantigny et al. 2007, 2010). This differential response between seasons could be explained by different moisture regimes. Soil moisture is generally lower in coarse- than in fine-textured soils during the growing season, resulting in greater O2 availability and limited denitrification and N2O production in coarse-textured soils (Sexstone et al. 1985). In contrast, the generally high soil moisture content in the NGS, due to high water table and the absence of evapotranspiration, likely alleviates the texture-related limitation of denitrification (Schürmann et al. 2002; Virkajärvi et al. 2010). This influenced the cumulative N₂O-N emission in the NGS as a function of soil type, which was of the same range in the growing season and in the NGS for the silty clay soil, but greater in the NGS than in the growing season for the sandy loam (Table 4). Moreover, the SIEF calculated for the silty clay soil in NGS 2011– 2012 (Table 3) was smaller than values reported by Pelster et al. (2012) for the same soil during the growing

season with spring-applied pig slurry, whereas SIEFs calculated for the sandy soil were an order of magnitude greater in the NGS than values reported in the same soil during the growing season. These findings indicate that in the silty clay soil, the risk of N₂O-N loss in the NGS following fall-applied pig slurry was lower than in the growing season following spring-applied slurry, whereas the risk was greater with fall-applied than spring-applied slurry in the sandy soil.

The generally greater cumulative N_2O-N and CO_2-C emissions found with pig slurry than in control soils likely reflected the addition of fresh organic C and mineral N with the slurry (Chantigny et al. 2002; Kariyapperuma et al. 2012). Moreover, the greater GHG fluxes measured throughout the NGS indicate that C and N substrates applied with the slurry in late fall could be readily used by soil heterotrophs, including denitrifier communities, under frozen conditions. However, large fluxes were measured also in the control silty clay during spring thaw in April 2013, indicating that high N₂O emission during thawing could occur without amendment in this soil, corroborating results of Pelster et al. (2013) under laboratory conditions with the same soil. These authors argued that freezing mobilized a large amount of substrates already present in this organic matter-rich soil. In the present study, the very cold conditions encountered in the silty clay soil in NGS 2012-2013 (Fig. 3f) may have induced the release of a large amount of C and N substrates from the soil reserve at spring thaw. There was no simple biological explanation for the smaller emission with pig slurry than the control, and it is possible that high fluxes from soils with pig slurry occurred between sampling dates.

When comparing NGS emissions with those of the growing season (Table 4), we estimated that NGS N₂O emissions accounted for 16%–79% of yearly N₂O-N losses for the silty clay and 46%–87% for the sandy loam soil. These values are in the range reported by previous studies (Wagner-Riddle and Thurtell 1998; Maljanen et al. 2009; Virkajärvi et al. 2010; Yanai et al. 2011) and further emphasize the need to account for NGS emissions in yearly GHG budgets. It was not possible to evaluate the

significance of NGS CO₂-C emissions on a yearly basis as emissions for the growing seasons were not measured.

This multiyear field assessment indicated that significant GHG emissions may occur at any time during the NGS. The significance of cumulative N₂O-N losses during the winter period (up to 74% of whole NGS emissions) emphasizes the need to better document GHG emissions throughout the NGS (also during winter), to get realistic estimates of yearly GHG emissions in cold regions and to decrease uncertainty in national GHG inventories, particularly for sandy soils.

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