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journal homepage: www.elsevier.com/locate/soilbioLong-term effects of cropping system on N₂O emission potentialSøren O. Petersen^{a,*}, Per Ambus^b, Lars Elsgaard^a, Per Schjønning^a, Jørgen E. Olesen^a^a Department of Agroecology, Aarhus University, Tjele, Denmark^b Department of Chemical and Biochemical Engineering, Technical University of Denmark, Roskilde, Denmark

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ABSTRACT

The potential for N₂O emissions outside the main growing season may be influenced by long-term effects of cropping system. This was investigated by collecting intact soil cores (100 cm³, 0–4 cm depth) under winter wheat in three organic cropping systems and a conventional reference within a long-term crop rotation experiment. Average annual inputs of C in crop residues and manure ranged from 1.7 to 3.3 Mg ha⁻¹. A simulated freeze–thaw cycle resulted in a flush of CO₂ during the first 48 h, which could be mainly from microbial sources. Other samples were adjusted to approximately –10, –30 or –100 hPa and amended with excess ¹⁵NO₃⁻ prior to freezing and thawing. Denitrification was the main source of N₂O during a 72-h incubation at 22 °C, as judged from N₂O and total ¹⁵N evolution. Although the input of C in the conventionally managed cropping system was significantly less than in the organic cropping systems, it showed higher N₂O evolution at all three matric potentials. Estimates of relative gas diffusivity (*D_p/D₀*) in soil from the four cropping systems indicated that C input affected soil aeration. Soil from the two cropping systems with highest C input showed N₂O evolution at *D_p/D₀* in excess of 0.02, which is normally considered a threshold for development of anaerobic sites in the soil, presumably because the oxygen demand was also high. The study shows that cropping system affects both soil gas diffusivity and C availability, and that both characteristics significantly influence the N₂O emission potential.

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1. Introduction

Within arable agriculture, short-term N₂O emissions are stimulated by manure and fertilizer application (Röver et al., 1998; Rochette et al., 2008; Chantigny et al., 2010) and residue incorporation (Aulakh et al., 1991; Petersen et al., 2011). However, a significant part of annual N₂O emissions may not derive from recent amendments, but from soil organic matter (SOM) turnover, and occur partly outside the main growing season, for example after rainfall or, in mid-latitude regions, in connection with freezing and thawing cycles (Sexstone et al., 1985; Teepe et al., 2001; Matzner and Borken, 2008). SOM status will reflect cumulated effects of cropping system, as modified by soil type (Petersen et al., in press), and hence management could influence N₂O emissions caused by fluctuations in soil wetness and temperature.

Freeze–thaw events transiently stimulate soil respiration (Kim et al., 2012), and this has been explained by disruption of aggregates protecting SOM, or release of cell constituents from the soil

microbial biomass (Schimel and Clein, 1996; Christensen and Christensen, 1991; Deneff et al., 2001; Mørkved et al., 2006; Feng et al., 2007; Kim et al., 2012). The microbial biomass C of a cropping system is positively related to SOM (Anderson and Domsch, 1989), and these sources are therefore not easily distinguished.

Frequently, the flush in respiration is accompanied by biogenic N₂O emissions (Röver et al., 1998; van Bochove et al., 2000), possibly induced by the O₂ demand resulting from labile C turnover. Matzner and Borken (2008) discussed in some detail the various mechanisms potentially involved in promoting N₂O emissions after thawing; they concluded that the information available is inconclusive, and that different mechanisms may be involved depending on site conditions. It has been argued that freezing of soil water can impede gas exchange, resulting in accumulation of N₂O produced in unfrozen soil volumes which is then released in connection with thawing (Teepe et al., 2001; Elberling and Brandt, 2003). However, a field study with application of ¹⁵NO₃⁻ at two soil depths associated N₂O emissions with denitrification activity at 0–5 cm depth (Wagner-Riddle et al., 2008).

SOM is not only a driver of respiratory activity, but also interacts with minerals in the formation and maintenance of soil structure. Using X-ray computed tomography, Luo et al. (2010) found

* Corresponding author.

E-mail address: soren.o.petersen@agrsci.dk (S.O. Petersen).

a positive correlation between SOM and macroporosity across two soil types, two land uses and three soil depths. Pore size distribution affects soil water holding capacity (Mäder et al., 2002) and gas exchange (Schjøning et al., 2005) and, hence, SOM is a significant factor in defining both O_2 demand and O_2 supply of a given soil. This further implies that SOM will interact with soil moisture in determining when suboxic conditions develop to support denitrification (Smith and Tiedje, 1979; Matzner and Borken, 2008).

Denitrification and N_2O emissions associated with freeze–thaw cycles and rainfall have been related to individual management factors, such as tillage practice, crop species, and fertilizer type (Kim et al., 2012). However, long-term effects of management have until now not been evaluated at the level of cropping system, as represented by crop sequence, fertilizer strategy and residue management. In this study, we examined the potential for gaseous N losses from intact soil collected in three organically managed cropping systems and a conventionally managed reference within a long-term field experiment. Organic crop production relies exclusively on livestock manure and green manure crops for maintenance of soil fertility, and this generally results in higher levels of soil organic matter (Suddick et al., 2010; Gomiero et al., 2011). Soil cores were adjusted to different water potentials and exposed to a freeze–thaw cycle. Based on the considerations presented above we hypothesized that N_2O emission potentials outside the main growing season are influenced by long-term effects of cropping system. Secondly, since SOM promotes aggregation and macroporosity, different relationships between N_2O emission and soil moisture were expected for the four cropping systems investigated.

2. Materials and methods

2.1. Site description

Soil samples were collected within a long-term cropping system experiment at Flakkebjerg in Eastern Denmark (55°19'N, 11°23'E) that was initiated in 1997. The soil was a sandy loam (Typic Hapludult) with 780 g kg⁻¹ sand and 155 g kg⁻¹ clay, and with a pH (CaCl₂) of 7.4 (Chirinda et al., 2010). The experiment involved four (out of eight) cropping systems under organic (O) or conventional (C) management laid out in two randomized blocks, in which all four crops in the rotations were represented each year. The main crop sequences (Table 1) were identical except that O2 +CC had one

Table 1

Intact soil was sampled from the four cropping systems described below. The systems were under organic (O) or conventional (C) management, and with (+CC) or without (–CC) winter cover crops. The rotation O2 had grass-clover instead of faba bean in the 2nd year. For additional information about the experimental design, see Olesen et al. (2000) and Askegaard et al. (2011).

Cropping system	O2 +CC	O4 +CC	O4 –CC	C4 –CC
Crop 1	Spring barley:ley	Spring barley ^{CC}	Spring barley	Spring barley
Crop 2	Grass-clover	Faba bean ^{CC}	Faba bean	Faba bean
Crop 3	Potato	Potato	Potato	Potato
Crop 4	Winter wheat ^{CC}	Winter wheat ^{CC}	Winter wheat	Winter wheat
Soil organic C (g kg ⁻¹) ^b	9.9 b ^a	9.2 b	9.5 b	7.8 a
C input (Mg ha ⁻¹ yr ⁻¹) ^c	3.27 c	3.25 c	2.40 b	1.66 a

^a Values within a row followed by different letters are significantly different at the 95% confidence level.

^b Soil organic C at 0–25 cm depth was determined in 2008; data from Chirinda et al. (2010).

^c The annual C input in manure and above-ground residues were estimated as average for the period 1997–2006.

year of grass-clover for fertility building. The rotation C4 –CC was managed conventionally, i.e. with mineral fertilizers and pesticides. For details on field management, see Askegaard et al. (2011). Soil organic C, as well as average annual inputs of C during the period 1997–2007, are shown in Table 1.

2.2. Soil sampling

Soil sampling took place in winter wheat of each cropping system on 29 March 2007. The individual field plot was 13 m long and consisted of five 2.6-m strips, two of which were reserved for harvest, while the other three strips each contained seven pre-defined subplots. For the present experiment, three subplots per field plot were randomly selected in advance of soil sampling; only the two outer rows of subplots were considered in order to minimize disturbances during sampling. Fig. 1 summarizes the experimental design and sampling scheme.

The winter of 2006–07 was unusually warm, and there was no snow on the ground at time of sampling. Due to the warm winter, plants had developed about five tillers at the time of sampling. In C4 –CC, a topdressing of pelletized NS mineral fertilizer with 12 kg ha⁻¹ NH₄⁺-N and 12 kg ha⁻¹ NO₃⁻-N had been applied two days prior to soil sampling, but was still visible as pellets at the soil surface, probably because there had been no rainfall during the 48-h period between the time of application and sampling (based on hourly registrations from a nearby climate station). No manure had been applied recently prior to sampling. Four intact soil cores (100 cm³) were sampled from 0 to 4 cm depth between crop rows in each of the three pre-selected subplots within each field plot ($n = 24$ per system). Six additional samples were collected 1–2 m from the boundary of one of the C4 –CC field plots for a pre-trial to evaluate CO₂ evolution as an index of C turnover following freezing and thawing of this soil. Soil samples were transported to the laboratory in a cooler and stored at 2 °C.

2.3. Laboratory incubations

The six soil cores collected for the pre-trial were subjected to freezing at –10 °C for 16 h and then, while still frozen, transferred to 1-L glass containers equipped with a septum for gas sampling and placed either in an incubator at 10 °C ($n = 3$) or at room temperature at approx. 22 °C ($n = 3$). Each container was connected to a gas chromatograph via a six-port multi-position valve (Cheminert Model C25Z; VICI Valco Instr., Schenkon, Switzerland). Headspace CO₂ concentration was analysed every hour in one of the six glass containers, i.e., each sample was analysed every six hours; monitoring was continued until CO₂ evolution rates were constant, after approximately 100 h.

One intact soil sample from each subplot was used for determination of soil NO₃⁻. The other three samples were randomly assigned to batches that were adjusted to one of three matric potentials (ψ_m) as previously described (Petersen et al., 2008); these potentials were selected using a water retention curve previously determined for the same field site, albeit for soil at 6–10 cm depth (Schjøning et al., 2007), so that subsequently 2 mL K¹⁵NO₃ (50 atom% excess, final concentration 10 mg NO₃-N kg⁻¹ soil) could be added drop-wise to the soil surface, bringing final ψ_m to approx. –10, –30 or –100 hPa. With excess NO₃⁻, any treatment effects on N_2O and N_2 evolution were assumed to reflect C availability and O_2 supply, as modified by cropping system and soil water content.

Nitrate-amended samples were frozen overnight and then transferred to 1-L glass containers as described above. The headspace atmosphere was replaced by a He:O₂ mixture to increase sensitivity of ¹⁵N gas analyses, but adding 5 mL L⁻¹ N₂ to ensure

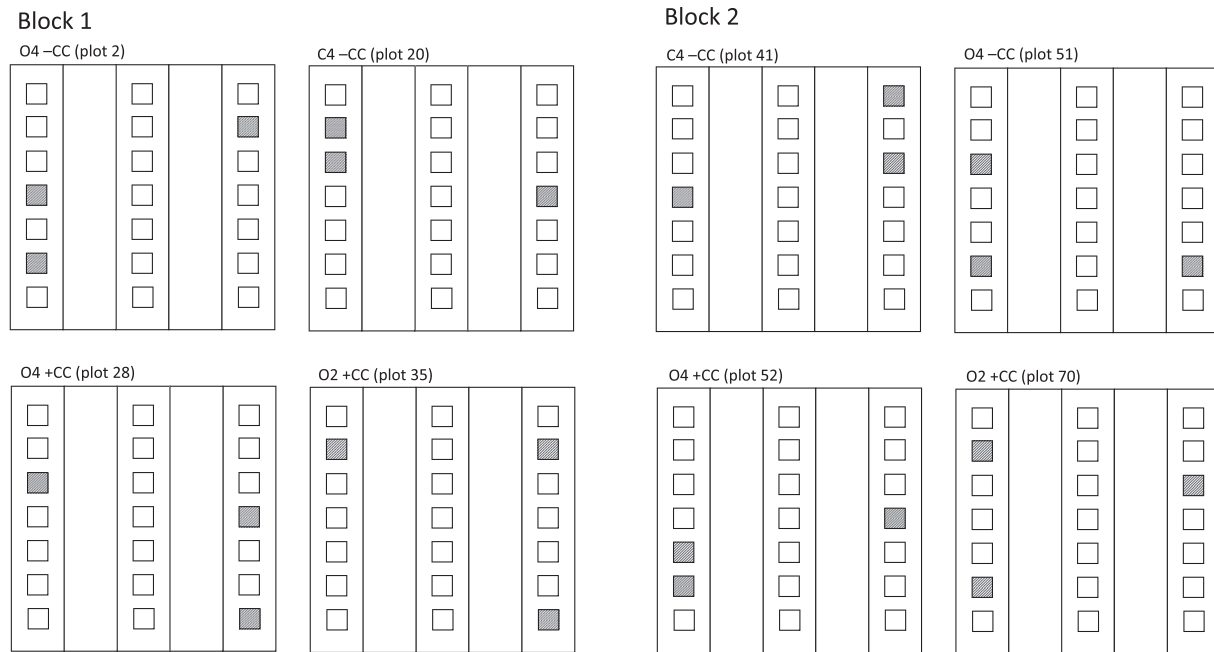


Fig. 1. A schematic overview of the winter wheat plots where intact soil cores were sampled for the laboratory experiment. Sampling took place in three randomly selected miniplots (hatched areas). The four cropping systems, i.e., O2 +CC, O4 +CC, O4 –CC and C4 –CC, are described in Table 1.

a pool of nitrogen for mass spectrometric analysis. Initial concentrations of N_2 and O_2 were 4.7 ± 0.9 and $17.0 \pm 0.7\%$ (mean \pm SD, $n = 38$), respectively. The soil cores were incubated at room temperature (22°C) for 72 h. By the end of incubation, gas samples were taken for analysis of N_2O and ^{15}N gas accumulated during incubation.

2.4. Analytical methods

Soil mineral N was extracted in 1 N KCl and filtered extracts analysed colorimetrically (Keeney and Nelson, 1982). Headspace concentrations of N_2 , CO_2 and O_2 were determined with a dual-channel Agilent 3000 micro GC configured as described by Petersen et al. (2009). Nitrous oxide was analysed on a Chrompack 9001 GC (Chrompack; Middelburg, Netherlands) with settings as described by Petersen et al. (2008). Gas samples were analysed for ^{15}N abundance as previously described (Carter and Ambus, 2006) using an elemental analyser (EA 1110, Carlo Erba, Milano, Italy) coupled in continuous flow mode to an isotope-ratio mass spectrometer (IRMS; Finnigan MAT Delta, Bremen, Germany). Total $N_2O + N_2$ derived from $K^{15}NO_3$ was calculated from m/z 28, 29 and 30 assuming 50% ^{15}N enrichment of the substrate pool.

2.5. Data analysis

The effect of temperature on CO_2 evolution rates was calculated from the Arrhenius relationship:

$$\ln(k_2/k_1) = E_A/R(1/T_1 - 1/T_2), \quad (1)$$

where k_1 and k_2 are CO_2 evolution rates ($\text{mg C m}^{-2} \text{d}^{-1}$) at the lower (T_1 , K) and higher temperature (T_2 , K), respectively, E_A is the apparent activation energy (J mol^{-1}), and R is the universal gas constant ($8.314 \text{ J mol}^{-1} \text{K}^{-1}$). Rates of CO_2 evolution were derived from cumulated CO_2 . For each time interval, E_A was calculated from CO_2 evolution rates at 10 and 22°C using Equation (1), and then Q_{10} ($=k_2/k_1$) was then calculated for the 10– 20°C temperature range.

Soil gas diffusivity (D_p) relative to air (D_0), i.e., D_p/D_0 , was calculated for individual samples with the empirical model of Moldrup et al. (2005), using soil porosity and air-filled pore space at the respective water contents and at -100 hPa matric potential (ψ_m). Effects of cropping system, ψ_m , and cropping system $\times \psi_m$ on N gas evolution and D_p/D_0 were determined with a mixed model using SAS 9.2 (SAS Institute Inc., Cary, NC, USA). The relationship between D_p/D_0 and N_2O evolution across all treatments was further described by an exponential relationship, i.e., $N_2O = a(D_p/D_0)^b$, where a and b are empirical fitting parameters.

3. Results

Following overnight freezing at -10°C and subsequent incubation at 10 or 22°C , CO_2 evolution was monitored to describe the turnover of labile C from undisturbed 100-cm^3 soil samples (Fig. 2). CO_2 evolution and Q_{10} values were calculated for each 24-h period (Table 2). At both temperatures the last two 24-h periods had very similar CO_2 evolution rates, indicating that the flush of CO_2 had largely ceased within 48 h. Cumulated CO_2 evolution during 96 h was significantly ($P < 0.001$) higher at 22°C . Apparent activation

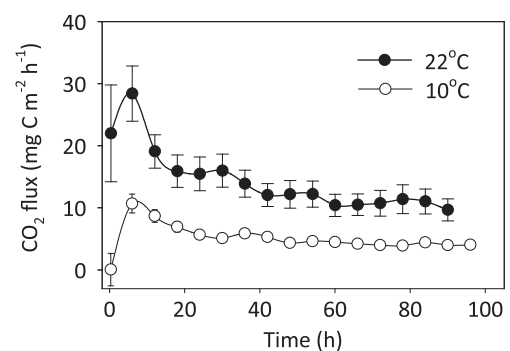


Fig. 2. Carbon dioxide evolution from intact, field moist soil cores (100 cm^3), collected at 0–4 cm depth in a winter wheat field in early spring, after freezing at -10°C overnight, followed by incubation at 10°C or room temperature (approximately 22°C).

Table 2

CO₂ evolution from undisturbed soil (0–4 cm depth) following overnight freezing and incubation at two temperatures. An Arrhenius relationship was used to derive apparently activation energy (E_A) and Q_{10} for each 24-h period and the full period. Data shown are mean \pm SE ($n = 3$).

Period	CO ₂ (mg C m ⁻² d ⁻¹)		E_A (kJ mol ⁻¹)	Q_{10}
	10 °C	22 °C		
0–24 h	192 (23)	473 (75)	51.9 (3.1)	2.13 (0.10)
24–48 h	132 (15)	344 (56)	55.1 (4.2)	2.24 (0.14)
48–72 h	105 (12)	271 (47)	54.5 (1.8)	2.21 (0.06)
72–96 h	98 (11)	257 (49)	55.5 (2.4)	2.24 (0.08)
0–96 h	528 (59)	1344 (215)	54.3 (1.4)	2.20 (0.05)

energies were nearly identical in all 24-h period, averaging 54.3 kJ mol⁻¹. Similarly, Q_{10} values were within a narrow range of 2.13–2.24 during and after the post-freezing flush of CO₂.

In the three organic cropping systems, *in situ* concentrations of NO₃⁻ under winter wheat were low (<1 mg N kg⁻¹), whereas in samples from C4 –CC there was between 14 and 193 mg NO₃⁻ N kg⁻¹ (theoretical value: 30 mg NO₃⁻ N kg⁻¹) as a result of the dissolution of fertilizer pellets during extraction. Fertilizer-derived NO₃⁻ was probably lost via leaching or denitrification during adjustment of matric potentials, but any fertilizer-derived NO₃⁻ remaining would have diluted the ¹⁵NO₃⁻ introduced.

The accumulation of N₂O (Fig. 3A) ranged from <0.5 to 5 mg N₂O–N kg⁻¹ and increased with ψ_m ($P < 0.001$). Trends for higher emissions

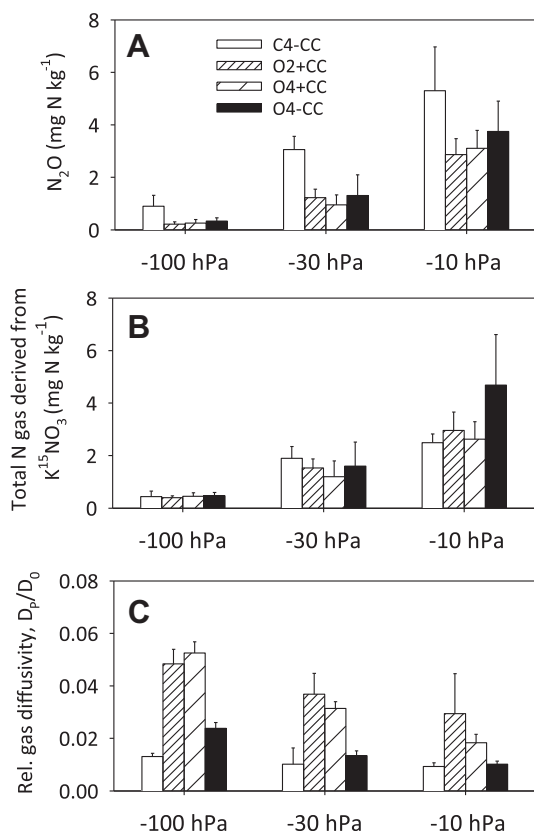


Fig. 3. The figure shows (A) N₂O emissions, (B) total gaseous N loss (N₂ + N₂O) derived from K¹⁵NO₃, and (C) relative gas diffusivity (D_p/D_0) of intact soil cores from four cropping systems (cf. Table 1). The soil cores were collected at 0–4 cm depth under winter wheat, adjusted to one of three matric potentials, amended with K¹⁵NO₃, and then exposed to overnight freezing at –10 °C, followed by incubation at room temperature for 72 h. Relative gas diffusivity was calculated from specific bulk density and soil water content of each sample using an empirical model (see text). The results represent mean \pm SE ($n = 3$).

from C4 –CC compared to O4 +CC and O2 +CC were not significant ($0.05 < P < 0.1$). The evolution of total N gases (N₂ + N₂O) was calculated assuming that K¹⁵NO₃ was the only source. Total gaseous N, like N₂O, showed a significant effect of ψ_m ($P < 0.001$), but no effects of cropping system (Fig. 3B). Relative gas diffusivities are shown in Fig. 3C. Effects of cropping system and ψ_m , but not their interaction, on D_p/D_0 were significant ($P < 0.01$). The –10 and –30 hPa matric potentials both differed significantly ($P < 0.05$) from –100 hPa with respect to D_p/D_0 . In a pair-wise comparison of cropping systems, O2 +CC and O4 +CC were similar, and differed significantly from C4 –CC and O4 –CC ($P \leq 0.01$).

The relationship between D_p/D_0 and N₂O evolution was described by an exponential model, $N_2O = a(D_p/D_0)^b$; the results are shown as double-logarithmic plots in Fig. 4, where b then corresponds to the slope for each system. The stimulation of N₂O emissions with declining D_p/D_0 for O4 –CC and O4 +CC appears to be less than for O2 +CC and C4 –CC, but slopes were not significantly different. In contrast, the intercepts of the regression lines with the x -axis, corresponding to $N_2O = 1$ mg N kg⁻¹ in the log–log plot, differed significantly ($P < 0.01$) between cropping systems with and without winter cover crops in the rotation, i.e., C4 –CC = O4 –CC < O4 +CC = O2 +CC. Hence, the two systems with cover crops had, for a given D_p/D_0 , significantly higher rates of N₂O emission than those without cover crops.

4. Discussion

Organic farming systems are highly diverse, and there can be large differences in the amounts and quality of organic matter inputs which may, in turn, impact soil N transformations via effects on, e.g., water holding capacity and soil microbial biomass and activity (Mäder et al., 2002; Gomiero et al., 2011; Petersen et al., *in press*). Here, we used a long-term crop rotation experiment with three organic cropping systems and a reference under conventional management to evaluate the potential for N₂O emissions outside the growing season. Intact soil cores were exposed to a freeze–thaw cycle, a disturbance that will generally stimulate soil respiration and denitrification activity in arable soil (Matzner and Borken, 2008). In comparison with natural ecosystems, such as forests, arable soils tend to show greater N₂O emissions after freeze–thaw cycles, most likely due to a lower C-to-N ratio of fertilized soil (Matzner and Borken, 2008).

Sampling took place in winter wheat, which was represented in all four cropping systems, at a time where the soil had not been disturbed for several months. The experimental treatments were realistic in that soil was sampled from shallow depth (0–4 cm) where diurnal temperature fluctuations are greatest, and at

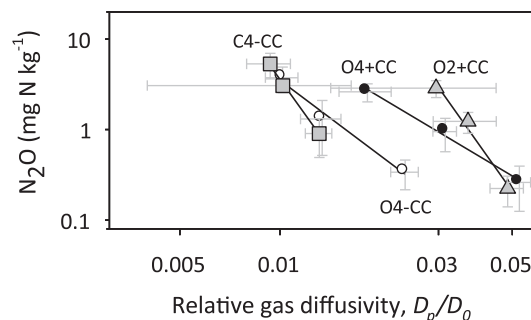


Fig. 4. The relationships between relative gas diffusivity, D_p/D_0 , and N₂O evolution rates are presented in a double-logarithmic plot. The slopes of the four cropping systems were not different, whereas the x -axis intercepts of systems with cover crops were significantly higher than those of systems without cover crops. The results represent mean \pm SE ($n = 3$).

a time of year where temperature fluctuations may be high (Henry, 2007). However, the temperature shift, from $-10\text{ }^{\circ}\text{C}$ to $22\text{ }^{\circ}\text{C}$, was deliberately large to induce measurable effects. For reference, temperature changes from $-5\text{ }^{\circ}\text{C}$ or below to $5\text{ }^{\circ}\text{C}$ or above within 24 h occurred just four times between 1988 and 2011, and shifts from -4 to $4\text{ }^{\circ}\text{C}$ around 20 times (based on data from a climate station at the experimental site).

4.1. Flush of CO_2 after freeze–thaw cycle

In the preliminary freeze–thaw test to evaluate the time course of labile C (and N) turnover, the flush of CO_2 occurred within the first 48 h (Table 2). However, CO_2 evolution remained higher at $22\text{ }^{\circ}\text{C}$ compared to $10\text{ }^{\circ}\text{C}$, suggesting that this represented basal respiration. The post-freezing flush has been explained by i) break-up of soil aggregates exposing previously protected SOM (van Bochove et al., 2000); ii) microbial decay (Herrmann and Witter, 2002); or iii) a release of substrates from the microbial biomass such as osmolytes from organisms living in unfrozen water films (Panikov et al., 2006). Average E_A ($54.3 \pm 1.4\text{ kJ mol}^{-1}$) and Q_{10} (2.20 ± 0.05) were consistent with previous studies on the effect of temperature on soil respiration (Kätterer et al., 1998), and remarkably constant in all four 24-h periods, suggesting a common origin of CO_2 . Herrmann and Witter (2002) linked 65% of the flush in CO_2 after a freeze–thaw cycle to microbial sources. Schimel and Clein (1996) exposed boreal soils to repeated freezing and thawing and concluded that C and N released after the first cycle, but not after subsequent cycles, was mainly of microbial origin. In the present experiment, some microbial decay may have occurred due to freezing, but a microbial source of CO_2 could also be adaptation to the post-freezing incubation temperature. This has been shown to include an increase in metabolic quotient and alterations in cell membrane composition (Petersen and Klug, 1994; Feng and Simpson, 2009). As temperature fluctuations are dampened with soil depth, it implies that O_2 consumption due to microbial adaptation is largest near the soil surface. This would be in accordance with the observations of Wagner-Riddle et al. (2008) that denitrification at 0–5 cm depth was the main source of N_2O during spring thaw.

4.2. N_2O and ^{15}N evolution

The time course of CO_2 evolution was taken to indicate the phase where also soil N transformations, including N_2O emission, were stimulated. The time frame of 48–72 h agrees with the results of Tenuta and Sparling (2011). A recent literature review calculated, for six laboratory studies with simulated freezing and thawing, a wider range of 2–11 d during which N_2O emissions were stimulated (Kim et al., 2012), but also pointing to availability of labile C as the main driver. Availability of electron acceptor was non-limiting in this incubation study due to addition of excess NO_3^- , but in general denitrification activity in arable soil is controlled by the maintenance of anaerobic microsites via decomposer activity, rather than by NO_3^- availability (Myrold and Tiedje, 1985).

For the three organically managed rotations, the total gaseous ^{15}N losses were comparable to the amounts of N_2O evolved, suggesting that denitrification based on K^{15}NO_3 was the main source of N_2O , and that N_2O was the main product of denitrification. Previous reports have also concluded that denitrification is the main source of N_2O during thawing (Müller et al., 2002; Phillips, 2008; Wagner-Riddle et al., 2008). The predominance of N_2O in the present study may have been biased by the addition of excess NO_3^- , since a high availability of NO_3^- relative to metabolizable C will shift the $\text{N}_2\text{O}:\text{N}_2$ ratio towards N_2O (Tiedje, 1988). Tenuta and Sparling (2011) found

that $\text{N}_2\text{O}:\text{N}_2$ ratios of gas emitted after a freeze–thaw cycle changed dynamically and never exceeded 2.45, corresponding to 70% N_2O .

In samples from the conventional rotation (C4 –CC), N_2O evolution exceeded total ^{15}N gas loss, indicating that K^{15}NO_3 was not the only source of gaseous N (Fig. 3). N_2O could have been produced via nitrification of fertilizer-derived, unlabelled NH_4^+ (Wrage et al., 2004), but significant nitrifier activity during early spring is not likely (Smith et al., 2010). Alternatively, fertilizer-derived NO_3^- could have diluted the $^{15}\text{NO}_3^-$ pool, violating the assumption that K^{15}NO_3 was the only significant source of ^{15}N gases. The magnitude of this error is difficult to assess, because the equilibration between fertilizer-derived NO_3^- and $^{15}\text{NO}_3^-$ could have been incomplete due to diffusion limitations (Laegdsmand et al., 2012), but most likely the amounts of N_2O observed with soil from all four cropping systems represented total denitrification activity under the experimental conditions used.

4.3. Relative gas diffusivity

The exponential relationships between D_p/D_0 and N_2O emissions confirmed the importance of gas diffusivity as a driver for denitrification. However, within each matric potential N_2O emissions from the four cropping systems were comparable despite very different gas diffusivities, which highlights the involvement of labile C. N_2O emissions from soil cores of O2 +CC and O4 +CC occurred mainly at D_p/D_0 values above 0.02, although this is normally considered to be a threshold for development of anaerobiosis (Stepniewski, 1981). Average annual inputs of crop residues in these two cropping systems were significantly higher than in the two cropping systems without cover crops (Table 1), and release of labile C during and after the freeze–thaw cycle could thus have induced an O_2 demand that lead to suboxic conditions and denitrification activity, even at relatively high air-filled porosity and hence gas diffusivity.

In this study, D_p/D_0 was used as an index of soil aeration. Water-filled pore space (WFPS) is another widely used proxy for soil aeration (Linn and Doran, 1984; Smith et al., 2003). There was a quadratic relationship ($r^2 = 0.963$) between D_p/D_0 and WFPS, and both indices of soil aeration would probably lead to the same conclusions regarding the regulation of N_2O emissions with this data set. However, gas and solute diffusivity have been found to be better descriptors of, respectively, CO_2 evolution and net nitrification activity compared to soil water- and air-filled porosity across soil types (Schjøning et al., 2003). Further, a laboratory study with intact soil cores (Petersen et al., 2008) found a better explanation of N_2O emissions across seven matric potentials and two depths when using D_p/D_0 rather than WFPS to explain the effect of soil moisture.

As mentioned above, a water retention curve for soil collected at 6–10 cm depth was used as reference for adjustment of matric potentials, which introduces a potential error with respect to the true ψ_m levels used in this experiment. For a similar soil type under conventional tillage that was also sampled in winter wheat during early spring, air-filled porosities and D_p/D_0 at 0–4 and 14–18 cm depth were nearly identical in the range of matric potentials investigated here (Schjøning et al., 2011), which indicates that under these soil conditions there will be little difference in soil properties within the plough layer. Despite this uncertainty, the results clearly indicate the importance of soil water regime for soil aeration.

4.4. Conclusion

In conclusion, a freeze–thaw event, representing off-season fluctuations in climatic conditions, influenced potential N_2O emissions in a complex way. Denitrification was probably the main

source of N₂O. There were indeed consistent long-term effects of cropping system on “background” N₂O emissions, as hypothesized, but higher organic inputs via crop residues and manure in cropping systems O2 +CC and O4 +CC with cover crops did not result in higher N₂O emission potentials; highest rates tended to be in C4 –CC having the least SOM concentration and average annual input of C. Presumably the stimulation of N₂O production by C availability (O₂ demand) in the systems with cover crops was counter-balanced by improved soil aeration (O₂ supply), as evidenced by N₂O emissions occurring at comparatively high relative gas diffusivities.

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