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## Nitrous oxide, methane and carbon dioxide patterns and dynamics from an experimental pig mass grave



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#### ABSTRACT

The objective of the three-year study was to examine spatial and temporal patterns of fluxes and soil pore air concentrations of methane (CH<sub>4</sub>), carbon dioxide (CO<sub>2</sub>) and nitrous oxide (N<sub>2</sub>O) from an experimental mass grave located in a temperate environment. The mass grave  $(5 \times 10 \text{ m})$  contained twenty pig carcasses at a depth of approximately 1 m was compared to a plot of the same dimensions containing only disturbed soil, as well as an undisturbed plot. Soil pore air CH<sub>4</sub> concentrations were sub-ambient (< 1.8 ppm) except at 75 and 100 cm depths at the mass grave in years 1 and 2 but decreased in year 3. The consumption of CH<sub>4</sub> within the aerobic soil resulted in small negative fluxes at the soil surface. Soil pore air CO<sub>2</sub> concentration showed an increase with depth in all three plots, with the largest increase (>100,000 ppm at 1 m) in the mass grave, though there was a marked decrease from years 1 to 3. Surface fluxes of CO<sub>2</sub> showed strong seasonal variations, peaking in summer. Soil pore air N<sub>2</sub>O concentration showed major increases in the mass grave, compared to the other two plots with the pattern maintained over the three years, resulting in larger surface fluxes of N<sub>2</sub>O. To establish the role of the carcasses in N<sub>2</sub>O dynamics, we incubated a soil sample containing carcass material which resulted in fast rates of N<sub>2</sub>O production and consumption. The maintenance of elevated pore air concentration and surface flux of N<sub>2</sub>O throughout the 3 years suggests that this is a long-term pattern and likely the best of the three gases to use to detect graves. Thus, we suggest that measurement of soil pore air concentrations, especially of N<sub>2</sub>O, could be a simple and effective approach to help determine the location of clandestine graves.

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#### 1. Introduction

Determining the location of clandestine burials is difficult and modern searches often rely on a variety of methods from cadaver dogs [1] to remote sensing [2]. Complementary analyses have also focused on characterizing the products of human cadaver and animal carcass decomposition [3–6]. Studies that describe the suite of volatile organic compounds (VOCs) released through the decomposition process of human remains also reference the production of gases such as methane (CH<sub>4</sub>), nitrous oxide (N<sub>2</sub>O) and carbon dioxide (CO<sub>2</sub>) [7,8].

Analysis of the utility of these inorganic gases for locating clandestine graves from large commingled burials and individual graves at different depths have shown these gases are reliable indicators of the presence of buried remains [9,10]. At a temperate climate study site with mass graves older than six years, soil CH<sub>4</sub> fluxes ranged from small rates of consumption in well-drained, non-grave sites (0 to  $-2 \text{ mg m}^{-2} \text{ d}^{-1}$ ) to large sources of emission  $(>1500 \text{ mg m}^{-2} \text{ d}^{-1})$  at open grave sites. Measurement of near surface air CH<sub>4</sub> concentration after stable meteorological conditions revealed spatial patterns that were related to the occurrence of graves [9]. These early results suggested that CH<sub>4</sub> biogeochemistry can be useful in detecting graves, though it may be more complex than VOCs because CH<sub>4</sub> production and consumption are heavily dependent on the mass, depth, age and nature of the burials as well as local environment factors, such as temperature and soil drainage [10]. More recent results examining both CO<sub>2</sub> and N<sub>2</sub>O produced by individual pig (Sus scrofa) burials at various depths, with and without plastic bags enveloping the body, indicate that in well-drained soils, CO2 and N2O are more useful indicators of buried remains because CH<sub>4</sub> was consumed in the soil profile [10].

Given the demonstrated ability of these gases for the detection of burials in well drained soils, we hypothesized that a mass grave

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in similar soil and climate conditions would show elevated soil pore air concentrations and fluxes of the three gases, compared to areas without buried remains.

#### 2. Site, materials and methods

#### 2.1. Site and materials

The study was established at the Flight Research Laboratory of the National Research Council of Canada near Ottawa, Ontario (45°19'N, 75°40'W). The climate is humid continental with a 30-year mean precipitation of 968 mm and mean monthly temperatures of -10.8 °C in January and 20.9 °C in July, recorded at the nearby Macdonald–Cartier International Airport.

The site included a mass grave (MG),  $5 \times 10 \text{ m}$  with a depth of  $\sim 1 \text{ m}$ , in which the soil was dug up and replaced by a backhoe, and an adjacent plot (RD) of the same dimensions where the soil was also dug up and replaced, but no carcasses were buried (Figs. 1A, B and 2). A plot with no disturbance was also established (U). On June 26th, 2013, 20 food-grade pig carcasses dressed in cotton clothing (to simulate human burial) with a total mass of 1977 kg (mean 99 kg) were buried in the MG. The carcasses were placed in the northern and southern ends of the grave with a 3.7 m gap (G) separating the two groups of carcasses (Figs. 1 C and 2). The gap was created to allow for high carcass density at either end of the grave and to allow for examination of lateral movement of gases within the grave.

The soils are well-drained, sandy loam Brunisols (Inceptisols) of the Leitrim series (Fig. 1B) with a parent material containing fragments of granite, gneiss, limestone and dolomite [11]. The texture within the soil profile is variable, with gravel, sand and clay layers [12]. The soil pH ranges from 5.5 to 8.1 with a bulk density of 0.7–1.0 g cm<sup>-3</sup>. The soils are well-drained Brunisols (Inceptisols) of the Leitrim series (Fig. 1B) with a parent material containing fragments of granite, gneiss, limestone and dolomite [11]. The soil profiles at the MG and RD plots are similar, with variable textures, ranging from gravel and sand to clay and variable thicknesses of the layers [12]. The dark brown topmost layer of soil is  $\sim 20 \,\mathrm{cm}$  thick consisting of organic-rich materials with clasts ranging in diameter from 0.05 to 3 cm. This is underlain by a brown, sandy layer, varying in thickness from 20 to 40 cm with clasts 1-5 cm in diameter. The third layer in the profile is 20-30 cm thick and comprises poorly sorted, white sand with clasts up to 10 cm in diameter. The basal layer consists of grey clay, varying in thickness from 5 to 20 cm, at a depth of  $\sim$ 75 cm and forms a layer impeding drainage below both MG and RD. The layers became mixed when the soil was replaced in the MG and RD plots by the backhoe, following the digging.

#### 2.2. Gas flux, soil pore air concentration and ancillary measurements

Gas fluxes were measured at two collars located at each end of the MG (C1, 2, 4 and 5) and one in the central G section (C3) from 2013 to 2015 (Fig. 2). A collar was placed in RD (C6) in 2015. Gas fluxes were measured by the static chamber technique using grooved water sealed collars (25 cm diameter) placed permanently into the soil to a depth of 5–10 cm. Chambers (9L) covered with aluminum foil to prevent overheating were placed on the collars and 20 mL air samples collected every 15 min for a period of 1 h by mixing the air within the chamber with a 60 mL syringe.

Soil pore air samples were taken from 6 mm diameter stainless steel tubes installed permanently at depths of 10, 30, 50, 75 and 100 cm. There were 8 sampling locations in MG, 6 overlying carcasses at the north and south ends (P1–P3 and P6–P8) and 2 in G (P4–P5; Fig. 2). Samples from the same depths were collected from the RD (P9) and U plots (P10). The tubes had 3–4 mm slits along the bottom 4–5 cm covered with Nytex mesh to prevent clogging



**Fig. 1.** The site and the mass grave (MG) after establishment (A), soil profile at MG (B) and placement of clothed pig carcasses with a 3.7 m gap between carcasses at either end of MG (C).

during sampling and at the top were fitted with Tygon tubing and 2-way stopcocks to facilitate sample withdrawal.

Gas flux and soil pore air measurements were taken weekly in 2013 and at two to three week intervals in 2014 and 2015. Winter samples of soil pore air were taken once per month in January, February and March of 2014 and 2015 from 100 cm depth at the north and south sections of MG.

To determine the spatial variability of pore air  $CH_4$ ,  $CO_2$  and  $N_2O$  concentrations in MG/G, 9 transects with 4 sampling points each for a total of 36 locations were established in June 2015. Pore air tubes were installed at 1 m ( $\pm$ 10–20 cm) intervals at depths of 50 and 75 cm and sampled bi-weekly from June to November 2015.

Gas samples were analyzed within 24–96 h of collection, using a Shimadzu GC-2014 Greenhouse Gas Analyzer fitted with a flame ionization detector (FID) for CH<sub>4</sub>, a methanizer with a 0.7 m column of Shimalite (Ni reduced 100/180 mesh) catalyst for CO<sub>2</sub>

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