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Methane oxidation in pig and cattle slurry storages, and effects of surface crust moisture and methane availability

Søren O. Petersen[1] and Per Ambus[2]
[1]*Department of Agroecology, Danish Institute of Agricultural Sciences, Tjele, Denmark;* [2]*Plant Research Department, Risø National Laboratory, Roskilde, Denmark; e-mail: soren.o.petersen@agrsci.dk*

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Abstract

Storages with liquid manure (slurry) may develop a surface crust of particulate organic matter, or an artificial crust can be established. Slurry storages are net sources of atmospheric methane (CH_4), but a potential for bacterial oxidation of CH_4 in surface crusts was recently suggested in a study of experimental storages. The present study was conducted to investigate methanotrophic activity under practical storage conditions. Surface crusts from slurry storages at two pig farms and four dairy farms were sampled in late autumn. Mixed samples (0–4 cm depth) were used to determine changes in CH_4, O_2 and CO_2 during incubation, while intact subsamples were used to characterize CH_4 oxidation as a function of CH_4 availability and moisture content. Methane oxidation was observed in all materials except for an expanded clay product (Leca) sampled from a pig slurry storage. Despite significant variation between replicate subsamples, there was a significant increase in methanotrophic activity when CH_4 concentrations increased from 500 to 50,000 ppmv. Maximum fluxes ranged from -1 to -4.5 g CH_4 m^{-2} d^{-1}. Surface crust samples were partly dried and then re-wetted in four steps to the original moisture content, each time followed by determination of CH_4 fluxes. Only one surface crust material showed a relationship between CH_4 fluxes and moisture content that would implicate gas diffusivity in the regulation of CH_4 oxidation. The occurrence of inducible CH_4 oxidation activity in slurry storage surface crusts indicates that there is a potential for stimulating the process by manipulation of gas phase composition above the stored slurry.

Introduction

Animal husbandry is a significant source of atmospheric CH_4 (>100 Tg yr^{-1}), a trace gas with a global warming potential that is 21-fold higher than that of CO_2 (Lelieveld et al. 1998). In 1999, agriculture accounted for 49% of anthropogenic CH_4 emissions in Western Europe, the main sources being enteric fermentation and manure management (Duchateau and Vidal 2003). Emissions from manure storages constituted around 10% of total agricultural emissions, and therefore this source is a relevant target for mitigation strategies.

Storages with liquid manure (slurry) may have a natural surface crust, depending on its dry matter properties, or an artificial crust can be established using, e.g., straw or Leca pebbles. A surface crust significantly reduces NH_3 volatilization (Sommer and Hutchings 1995), but this environment at the

interface between slurry and atmosphere also supports aerobic microbial transformations. It was recently demonstrated that these transformations include bacterial CH_4 oxidation, which was detected in selected surface crust materials taken from experimental storages with a natural surface crust and an artificial crust made of straw, respectively (Petersen et al. 2005). Although methanotrophic bacteria in slurry storage surface crusts have so far not been isolated and characterized, there is evidence to suggest that they are derived from the slurry and belong to the so-called Type II group of methanotrophs (Roslev and King 1994; Heyer et al. 2002). If the bulk slurry phase has the potential to colonize surface crusts with methanotrophs, then CH_4 oxidation in slurry storages could be widely occurring.

Ambient conditions with respect to temperature, O_2 gradients and CH_4 availability are likely to influence *in situ* methanotrophic activity in surface crusts. Seasonal patterns in precipitation influence the moisture content and thus redox conditions within the surface crust (Sommer et al. 2000). Petersen et al. (2005) hypothesized that covering slurry storages could stimulate CH_4 oxidation by stabilization of the surface crust moisture regime, but also by increasing the retention time of CH_4 in the air above the surface crust. The present study was conducted to examine the potential for CH_4 oxidation in practical slurry storages at pig and cattle farms, and to describe effects of wetness and CH_4 availability on CH_4 oxidation rates in intact surface crusts.

Materials and methods

Sampling and sub-sampling

Surface crust material was collected on 25 November 2003 from storages at two pig farms (PS1 and PS2) and four cattle farms (CS1–CS4) near Research Centre Foulum in Western Denmark (56° 29′ N, 9° 34′ E). Slurry storages were emptied during spring, hence the slurry had been collected, and the surface crust had developed, during a period of 7–8 months. The temperature in November 2003 had varied between 3 and 9° C, and the total precipitation up to the sampling day was 60 mm, including 13 mm of rain two days before sampling. Hence, the moisture content of the surface crusts was relatively high and ranged from 72 to 87% of the fresh weight except for an artificial crust of expanded clay (Leca) where it was 47%.

The animal houses all had fully or partly slatted floors. Some information concerning animal numbers and surface crust composition at the six farms are summarized in Table 1. Four sections of surface crust material (approx. $15\times15\times15$ cm^3) were excavated from different parts of each storage; a hydraulic lift was used to enable manual sampling. Undisturbed samples were obtained except from PS2 that was covered by a ca. 25 cm layer of Leca pebbles, from which only grab samples could be obtained. Each sample was stored in a container with a perforated lid at 2 °C until further processing.

From each of the four sections collected from each storage, both mixed and intact subsamples were obtained. Four intact sub-samples were isolated from each section and transferred to 36×36 mm^2 square frames of PVC with a height of 50 mm. The lower surface of the sub-samples that could be isolated was irregular, and the depth varied between 3.5 and 4.5 cm. However, care was taken that the upper surface remained undisturbed. The top and bottom of each frame was covered with Parafilm, and the bottom side was further covered with aluminum foil secured with tape to exclude oxygen entering from below. The mixed (non-structured) material was taken from the 0–4 cm depth interval around the intact samples and cut <10 mm.

The mixed material from each storage was analyzed for dry matter content and loss-on-ignition,

Table 1. Animal numbers and surface crust type at the six farms where surface crust materials were obtained.

Farm	Number			Surface crust
	Adults	Heifers	Slaughtering pigs	
PS1			500	Straw
PS2			1600	Leca pebbles
CS1	72	30		Natural[a]
CS2	85	35		Natural
CS3	106	104		Natural[b]
CS4	80	35		Natural

Slurry storages had been emptied during spring, hence the slurry had been collected over a period of 7–8 months.
PS – pig slurry storage; CS – cattle slurry storage.
[a] Natural crusts include some bedding material.
[b] Extra straw had been added to reinforce surface crust.

electrical conductivity (EC), pH, extractable mineral N and total N. Also, the first incubation experiment (see next section) was conducted with mixed material. The intact subsamples were used for incubation experiments with $^{13}CH_4$ (Ambus and Petersen 2005), and for the incubation experiments reported here.

Screening for methanotrophic activity (Expt. 1)

Ten-gram subsamples of mixed material ($n = 4$) were weighed into 320-ml glass bottles and pre-incubated in the dark at room temperature (22 ± 1 °C) for 72 h. Then the headspace of each bottle was flushed with moisturized air and closed with septum and screw cap, and CH_4 added to a final concentration of ca. 1.6% (vol vol^{-1}). Following analysis of headspace concentrations of CH_4, O_2 and CO_2, the samples were incubated at room temperature in the dark. Gas analyses were repeated after 1, 2, 3, 4 and 7 days.

Effect of headspace CH_4 concentration (Expt. 2)

A set of intact samples ($n = 4$) from three selected storages, PS1, CS2 and CS4, were pre-incubated at room temperature for 72 h. Then the upper Parafilm cover was removed and the samples transferred to 1-l Kilner glass jars equipped with a septum in the lid. A wet paper towel lined the jars to avoid water loss during incubation. The rubber seal was coated with vacuum grease and the septum filled with silicone to prevent gas leakages; an empty jar was included as a control of gas tightness.

The four replicates from each storage were then exposed to one of four CH_4 concentrations (calculated to be 500, 2490, 12350 and 47620 ppmv) during a sequence of 4-day trials. A large spatial heterogeneity was anticipated, and therefore each subsample was exposed to all four CH_4 concentrations; the order of exposure was randomized to avoid systematic errors associated with past exposures. Methane concentrations were determined after 30 min, and then daily until Day 4. Between the 4-day trials, all subsamples were removed from the jars, covered with Parafilm and left at room temperature for 72 h.

Effect of surface crust wetness (Expt. 3)

A new set of intact surface crust subsamples from slurry storages PS1, CS2 and CS4 was used for this experiment, in which CH_4 oxidation rates were determined after partial drying, and during a stepwise re-wetting to the original moisture content. This time the upper Parafilm was removed and the subsamples weighed, before they were left in a fume hood at low air-flow for 72 h to partly dry. After this pre-treatment, the samples were weighed again and then transferred to the 1-l Kilner jars and amended with 40 ppmv CH_4. This relatively low concentration was selected to obtain significant concentration changes during 24 h incubations.

Headspace CH_4 concentrations were determined five times during the 24 h trials. Then water corresponding to 25% of the water lost during drying was added dropwise to the surface of each replicate. The samples were then covered with Parafilm and left for 24 h to equilibrate, before CH_4 oxidation rates were determined again. This procedure was repeated three times, the final addition of water being based on actual weights rather than calculated values. The changes in water content during the experiment are shown in Figure 1. Finally, samples were incubated for an additional

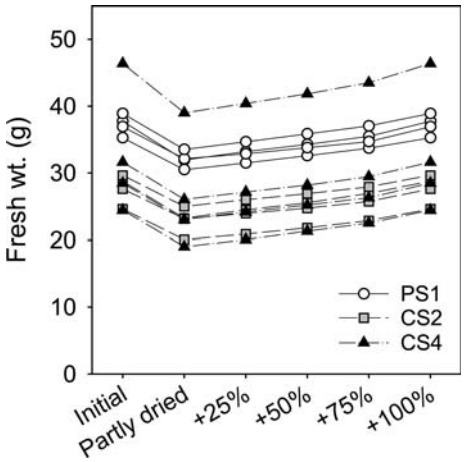

Figure 1. Fresh weights of each of the four individual subsamples from a pig slurry storage (PS1), and from two cattle slurry storages (CS2 and CS4). The plot shows fresh weights of these 12 samples ('Initial'), weights after partial drying ('Partly dried'), and weights after each re-wetting step, in which 25% of the water lost during the initial drying was returned ('25%', '50%', '75%' and '100%'). The variability among replicates was mainly due to an irregular lower boundary of the individual subsamples.

24-h period, but this time at atmospheric CH_4 concentration (1.7 ppmv) in the headspace.

Analytical techniques

Dry matter content was determined after drying at 105 °C for 24 h, and loss-on-ignition as weight loss after an additional 4 h at 450 °C. Electrical conductivity (EC) and pH was determined in slurries of field-moist surface crust material and deionized water (1:4, wt:vol). Extractable NH_4^+, NO_2^- and NO_3^- was determined in slurries of surface crust material and 1 M KCl (1:4, wt:vol); following 30 min extraction, the samples were filtered (0.75 μm) and frozen until analyzed colorimetrically (Keeney and Nelson 1982). Total N was determined by Kjeldahl digestion on a Kjeltec 1030 (Tecator, Höganäs, Sweden).

In Expt. 1, CH_4 and O_2 was analyzed on a Varian 3400 GC equipped with a column packed with molecular sieve 5A (60/80, 35 °C) and thermal conductivity detector (150 °C). The carrier gas was He at 45 ml min^{-1}. In Expts. 2 and 3, CH_4 was analyzed on a Shimadzu GC-14 equipped with a Porapak Q column (50 °C) and flame ionization detector (150 °C). The carrier was He at 60 ml min^{-1}. Carbon dioxide was analyzed on a GC from Mikrolab (Mikrolab Aarhus A/S, Højbjerg, Denmark) equipped with Porapak N (50 °C) and thermal conductivity detector (100 °C). The carrier was He at 35 ml min^{-1}.

Data analysis and statistics

Treatment effects on surface crust characteristics were evaluated by ANOVA followed by Tukey's HSD test. Correlations between individual properties were evaluated by a step-wise Bonferroni procedure to control the table-wise error rate (Rice 1989). In Expt. 1, respiratory quotients (RQ) were calculated as the molar ratio of [CO_2 production]/[O_2 consumption] for the 0–72 h period; the accumulation of dissolved carbonates was estimated using the pH values given in Table 2 (Lindsay 1979). In Expt. 2, CH_4 concentration changes during the 4-day trials generally followed first-order kinetics, and CH_4 oxidation rates were determined from linear regression of ln-transformed CH_4 concentrations. Concentration changes during the 24-h trials in Expt. 3 ranged from −65 to +40% and were approximately linear, and therefore these fluxes were estimated from regression of untransformed data. Methane fluxes in response to varying CH_4 concentration or moisture varied greatly among replicates and did not adhere to a normal distribution; Friedman's non-parametric test was employed for these results (Zar 1984). Methane fluxes are reported on an area basis.

Results and discussion

Surface crust characteristics

Table 2 summarizes information about the composition of the different surface crust materials. The Leca pebbles of PS2 had a higher dry matter (DM) content, and a lower proportion of organic material (OM) than the other surface crusts, as would be expected for this expanded clay product. The OM contents of the other surface crusts ranged from 56 to 78% of the dry weight. The OM content of slurry DM is typically around 80% (S.O. Petersen unpublished data), and that of

Table 2. Selected surface crust characteristics.

Farm	DM (% of fresh wt.)	OM (% of dry wt.)	pH	EC (dS m^{-1})	TAN (mg N kg^{-1} DM)	NO_2^- (mg N kg^{-1} DM)	NO_3^- (mg N kg^{-1} DM)
PS1	18.4bc	55.7b	8.29a	1.71a	3354a	1.9	887ab
PS2	53.4a	21.8c	8.89a	0.44d	423b	0.7	10.7b
CS1	21.3b	78.1a	8.86a	1.11b	3610a	0.2	6.0b
CS2	17.9bc	70.8a	8.06ab	0.78bc	112b	1.4	1281a
CS3	12.5d	76.8a	7.13b	0.55cd	251b	1.7	1198a
CS4	16.8c	71.7a	8.37a	0.96b	1055b	4.2	1227a

The data represent means of four independent subsamples; letters indicate significant differences ($P < 0.05$) within each column.
PS – pig slurry storage; CS – cattle slurry storage; DM – dry matter; OM – organic matter (loss-on-ignition); EC – electrical conductivity; TAN – total ammoniacal nitrogen.

straw is >95%, indicating different degrees of decomposition.

Mineral N concentrations in the surface crusts varied considerably between storages (Table 2). PS1 and CS1 had significantly higher concentrations of total ammoniacal N (TAN) than the other materials, whereas NO_3^- concentrations were more variable and NO_2^- concentrations were always low. Except for CS3, all surface crusts were characterized by a pH > 8, which means that significant pools of free NH_3 must have been present in the liquid phase of most surface crust materials. On average free NH_3, estimated by a modification of the Henderson-Hasselbalch equation, i.e., $[NH_3] = [TAN] \times 10^{pH-9.25} \times (1 + 10^{pH-9.25})^{-1}$ ranged from 41 to 283 mg N l^{-1} in PS1, PS2, CS1 and CS4. In contrast, the free NH_3 levels in CS2 and CS3 were 2 and 1 mg N l^{-1}, respectively. Concentrations of free NH_3 in excess of 50 mg N l^{-1} would certainly inhibit nitrification activity (Anthonisen et al. 1976), and the fact that PS1 and CS4 contained large amounts of NO_3^- therefore indicates that the mixed materials used for this incubation experiment represented a composite of spatially discrete zones within the undisturbed surface crusts.

Electrical conductivity ranged from 0.4 to 1.7 dS m^{-1} and was highest with PS1 and lowest with PS2 and CS3. Correlations between EC, TAN and NO_3^- were significant ($P < 0.05$), although not very strong with r^2 of 0.31–0.34 (data not shown). EC was recorded as an indicator for osmotic pressure which is a potential stress factor for methanotrophs (Schnell and King 1996). The EC values recorded in this study corresponded to osmotic potentials > – 0.1 MPa (Rawlins and Campbell 1986). This level of osmotic stress is not likely to have had a general negative impact on neither methanotrophs nor microbial activity in general (Polonenko et al. 1986), although zones within the intact surface crust with more extreme conditions may have existed.

Screening for CH_4 oxidation activity

Results from Expt. 1 where mixed surface crust materials were incubated, are shown in Figures 2 and 3. The time course of CH_4 oxidation (top panels) varied between the six surface crust materials. One material, the Leca pebbles (PS2), showed no activity within the 7-day period of this experiment. With CS2, CH_4 oxidation commenced immediately after incubation (Figure 3). In the remaining four surface crust materials, CH_4 oxidation was observed after a lag phase of 1–4 days.

The patterns of O_2 uptake and CO_2 evolution (bottom panels) were similar in the six treatments,

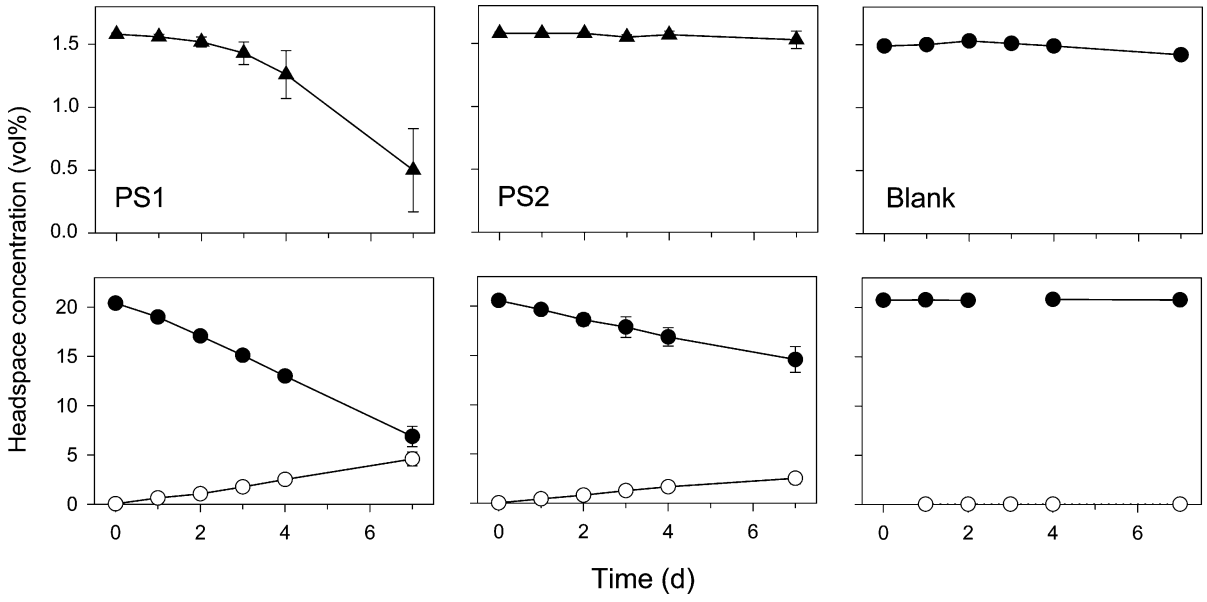

Figure 2. Concentrations of CH_4 (▲), O_2 (●) and CO_2 (○) during incubation of mixed surface crust material from two pig slurry storages (PS1 and PS2, see text) and a control without material The results represent mean ± standard error ($n = 4$).

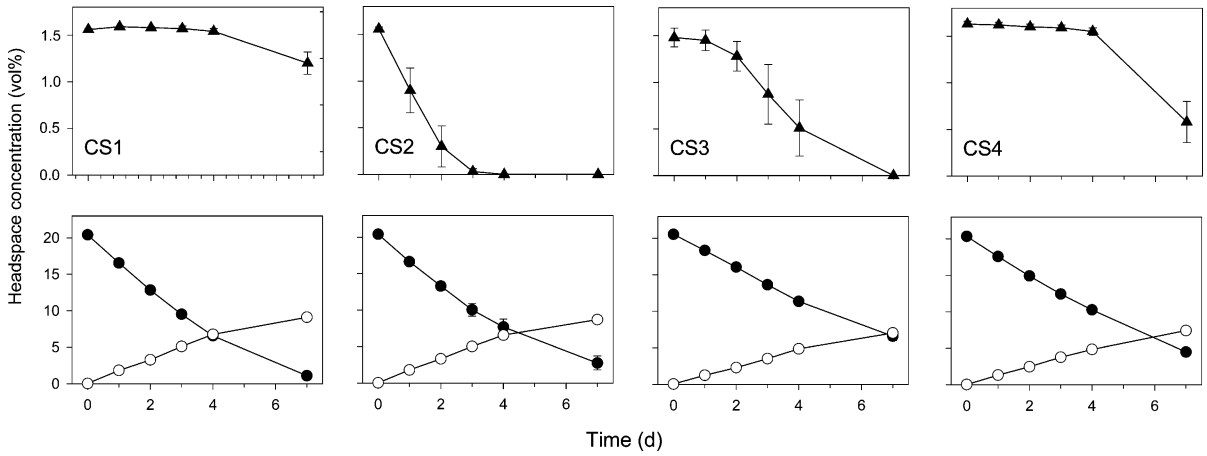

Figure 3. Concentrations of CH_4 (▲), O_2 (●) and CO_2 (○) during incubation of mixed surface crust material from four cattle slurry storages (CS1–CS4, see text). The results represent mean ± standard error (n = 4).

though quantitatively different. A decrease in respiration rates was indicated in all cattle slurry crusts towards the end of incubations when O_2 became scarce. The following estimates of respiratory quotients (RQ) were obtained for the 0–72 h period (mean ± standard error):

PS1: 1.0 ± 0.1
PS2: 2.5 ± 0.1
CS1: 3.8 ± 0.1
CS2: 1.0 ± 0.02
CS3: 0.6 ± 0.01
CS4: 1.7 ± 0.04

This range of RQ values correspond well with those observed for soils in response to changes in water-filled porosity (Rixon and Bridge 1968). The values at or below 1 observed for PS1, CS2 and CS3 would suggest that carbon metabolism was dominated by aerobic processes, whereas the high values recorded for PS2 and CS1 indicated a predominantly anaerobic environment. In accordance with this interpretation, PS2 and CS1 exhibited the least methanotrophic activity among the six surface crust materials (Figures 2 and 3) and contained little NO_3^- (cf. Table 2).

Effect of headspace CH_4 concentration

In Expt. 2, intact surface crust samples (n = 4) from PS1, CS2 and CS4 were exposed, in random order, to each of four CH_4 concentrations ranging from approximately 500–50,000 ppmv in batch incubations. Across all incubations, there were 33 cases of net CH_4 uptake, three cases of net CH_4 emission, while in 12 cases the CH_4 flux was not significantly different from 0. A dependency of CH_4 fluxes on past exposures could not be identified (data not shown).

Despite of a high level of variability, there was a statistically significant increase in net CH_4 oxidation rates with increasing CH_4 concentration with all three surface crust materials (Figure 4). Maximum fluxes in the three materials ranged from -1 to -4.5 g CH_4 m^{-2} d^{-1}.

Effect of moisture content

Studies of CH_4 oxidation in other environments have shown physical properties, such as diffusivity, air permeability, and texture, to be important for the extent and vertical distribution of methanotrophic activity (e.g., Whalen et al. 1990; Kruse et al. 1996; Ball et al. 1997a, b). Gas phase transport is orders of magnitude greater than diffusion in water, and Segers (1998) estimated that mass transfer limitations on CH_4 oxidation will be invoked when the diffusion path in water exceeds 1 mm. We therefore hypothesized that CH_4 oxidation in slurry storage surface crusts could be significantly influenced by the wetness of the material.

The variability of CH_4 fluxes was again considerable, though highly consistent between individual incubations of the same subsample, and

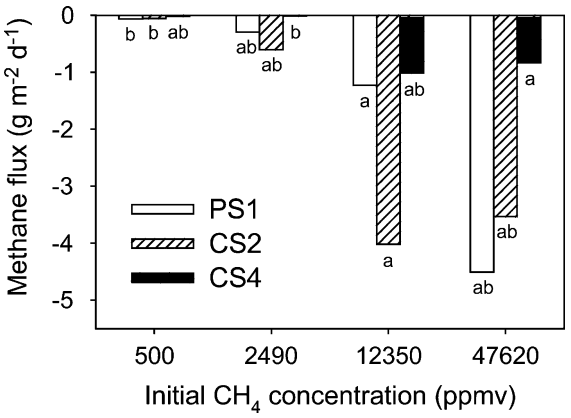

Figure 4. Fluxes of CH_4 during incubation of intact surface crust samples from a pig slurry storage (PS1) and two cattle slurry storage (CS2 and CS4) surface crusts at increasing initial headspace CH_4 concentrations. All of four replicates were incubated at each concentration in random order.

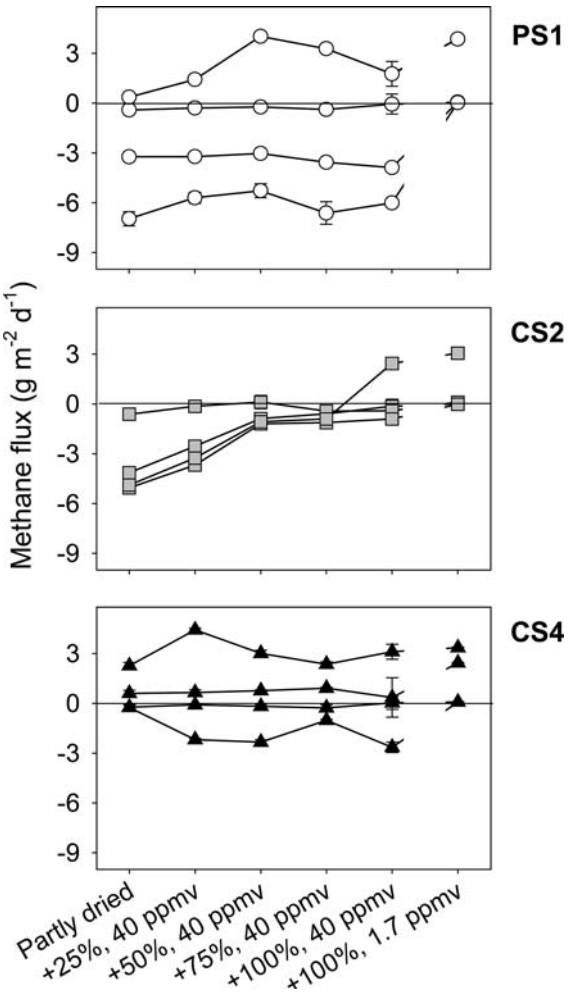

Figure 5. Methane fluxes from each of four intact surface crust samples from a pig slurry storage (PS1) and two cattle slurry storage surface crusts (CS2 and CS4) after drying and a stepwise addition of water to reestablish the water content at sampling. Finally, CH_4 fluxes were recorded at atmospheric CH_4.

the effect of moisture on fluxes differed both within and between surface crust materials (Figure 5). For PS1 there was no effect of moisture content within the range investigated here. In one subsample there was always a net emission of CH_4 (Figure 5, top); when incubated at 1.7 ppmv CH_4, net emission of CH_4 from this subsample increased, demonstrating that fluxes recorded at 40 ppmv CH_4 had included methanotrophic activity. In the other three subsamples of PS1 there was no CH_4 flux at atmospheric CH_4 in the headspace, implying that no CH_4 was produced in these samples. Two of these subsamples showed a considerable net uptake of CH_4 irrespective of moisture level provided CH_4 was available.

For CS2 there was a consistent effect of moisture level (Figure 5, center), even though only the difference between 'Partly dried' (cf. Figure 1) and '100%' was statistically significant. With one exception (at 75% of original moisture content), the net uptake of CH_4 decreased with increasing moisture content. One of the four subsamples had a lower net CH_4 uptake than the other three subsamples, but the net emission observed at 1.7 ppmv CH_4 suggested that methanotrophic activity in the four subsamples was in fact of the same magnitude, and that the low net uptake of the first subsample at 40 ppmv CH_4 represented the sum of CH_4 uptake and CH_4 production in different parts of the subsample. In an isotope tracer study, Ambus and Petersen (2005) also found evidence for concomitant CH_4 production and oxidation in this crust material.

In CS4 there was little effect of moisture content (Figure 5, bottom). One subsample showed net emission of CH_4 across all moisture contents, and the activity at 100% moisture and 40 ppmv CH_4 was similar to that at 100% moisture and 1.7 ppmv CH_4, which demonstrated that there was no methanotrophic activity in this subsample. Two other subsamples showed little CH_4 flux at all moisture levels and 40 ppmv CH_4, but differed in the response to incubation at 1.7 ppmv CH_4. One

of the two subsamples still revealed no flux activity at atmospheric CH_4, whereas the other showed a net emission of CH_4. Hence, one was characterized by a combination of CH_4 production and consumption, whereas the other showed neither production nor consumption.

In summary, only CS2 exhibited a reduction of CH_4 uptake with increasing water-filled porosity that would implicate gas diffusivity as an important limitation on CH_4 oxidation. The lack of moisture effects with PS1 and CS4 could mean that methanotrophic activity in these materials was restricted to external surfaces and/or regulated by factors other than gas diffusion. The materials PS1 and CS4 were characterized by higher NO_2^- and especially TAN concentrations than CS2 (Table 2), and so an interaction between N transformations and CH_4 oxidation could have occurred (King and Schnell 1994; Whalen 2000).

General discussion

The six surface crust materials collected from slurry storages at local farms differed considerably with respect to physical and chemical properties, probably as a result of site-specific conditions with respect to animal composition, use of bedding material etc. Four of the six materials, PS1, CS2, CS3 and CS4, showed a significant accumulation of NO_3^- (Table 2), but not NO_2^-, indicating that these surface crust environments sustained both NH_4^+ oxidation and NO_2^- oxidation. The RQ values observed with PS1, CS2 and CS3 corresponded to well-aerated conditions in the mixed material from 0 to 4 cm depth. In view of the known sensitivity of NO_2^- oxidizers towards environmental stresses such as free NH_3 (Anthonisen et al. 1976; Smith et al. 1997), the absence of NO_2^- was an indication that aerobic parts of these surface crust materials were favorable for microbial activity.

The screening for methanotrophic activity showed that, except for the Leca material of PS2, there was a potential for this process in all storages (Figures 2 and 3). The high RQ value observed with PS2 indicated that conditions in this material were largely anaerobic and, furthermore, free NH_3 concentrations in the mixed material may have been inhibitory to methanotrophs (Megraw and Knowles 1987). The capillary rise of water in Leca is < 20 mm (www.leca.com), and CH_4 oxidation activity in the upper parts of the intact Leca layer at the time of sampling can therefore not be excluded.

A lag phase of CH_4 oxidation was generally observed in the mixed surface crust materials. Methanotrophs form exospores or cysts (King 1992), and a lag phase could thus be due to methanotrophic bacteria in these storages being inactive at the time of sampling. Alternatively, the mixing of surface crust material prior to Expt. 1 could have caused a temporary inhibition of methanotrophs, for example by increasing the level of free NH_3, or by changing substrate availabilities (Amaral et al. 1995; van Bodegom et al. 2001). The interactions between CH_4 oxidation and mineral N are extremely complex. Both NH_4^+ and NO_3^- have been shown to inhibit CH_4 oxidation (King 1992) but, according to Bodelier and Laanbroek (2004), adverse effects may be due to osmotic stress rather than a direct effect of the N species, since addition of mineral N at moderate concentrations often stimulates CH_4 oxidation. Nitrite has a strong inhibitory effect on CH_4 oxidation, and NO_2^- production by methanotrophs has been implicated in the effect of NH_4^+ (King and Schnell 1994).

The CH_4 oxidation rates of up to 4.5 g m^{-2} d^{-1} observed here in slurry storage surface crusts (Figure 4) are in the same order of magnitude as the rates summarized by Le Mer and Roger (2001) for wetlands (median: 0.017 g m^{-2} d^{-1}, range: 0–70 g m^{-2} d^{-1}) and landfill cover soil (median: 45 g m^{-2} d^{-1}, range: 7–170 g m^{-2} d^{-1}). Net fluxes of CH_4 from open and semi-covered experimental storages with cattle slurry or digested cattle slurry were 3–10 g CH_4 m^{-2} d^{-1} during summer storage and 0.2–0.5 g CH_4 m^{-2} d^{-1} during winter storage (Clemens et al. 2005). Another experiment with open storage of cattle slurry showed maximum emissions of 18 g CH_4 m^{-2} d^{-1} (Sommer et al. 2000). Recently, a long-term study of CH_4 emissions from practical, un-covered storages with cattle slurry from organic or conventional farms showed average annual emissions of 31 and 28 g CH_4 m^{-2} d^{-1} (F. Beline pers. comm. 2004). These observations indicate that, while CH_4 oxidation in surface crusts may mitigate net emissions from open storages, there is a large scope for improving CH_4 retention via optimized storage conditions, if technically feasible.

The significance of CH_4 oxidation under practical storage conditions is difficult to assess, as the actual substrate availabilities *in situ* are not known. At near-ambient concentrations of CH_4, methanotrophic activity is probably insignificant, as indicated by the absence of negative fluxes at 1.7 ppmv CH_4 in Figure 5. Typical apparent half-saturation constants (K_{app}) reported for CH_4 oxidation in peat soil and sediments are in the range 2–10 μM (King 1992; Hanson and Hanson 1996). These concentrations of dissolved CH_4 correspond to gas phase concentrations of 1500–7000 ppmv (Wiesenburg and Guinasso 1979). The response to CH_4 depicted in Figure 4 is consistent with K_{app} for surface crust materials in this range and suggests that methanotrophic activity could be stimulated by increasing storage headspace concentrations of CH_4, for example by reducing the air exchange rate. In a previous study, a simple wooden lid on experimental storages with untreated or anaerobically digested cattle slurry resulted in 15–30% reductions in CH_4 emissions during both winter and summer storage (Clemens et al. 2005). Also, Williams and Nigro (1997) varied the air flow above stored slurry to simulate increasing cover tightness; they found that CH_4 emissions during a one-month laboratory storage experiment were reduced by up to 90% by lowering the air exchange rate.

The large variability observed among intact subsamples from the same storage may reflect an underlying spatial heterogeneity in the distribution of methanotrophs or methanotrophic activity. One potential source of heterogeneity in the distribution of organisms would be macropores or crevices with reduced resistance against diffusion or convectional gas flow. Methane release *via* ebullition is quantitatively significant in many environments (Chanton and Whiting 1995) and is also known to occur in slurry storages (Husted 1994). Preferential transport of CH_4 through crevices would probably lead to an enrichment of methanotrophs near the surface of such channels provided that they are temporally stable and O_2 is available. The heterogenous distribution of activity could also have been caused by a variable level of inhibition by mineral N among the surface crust subsamples. Nitrogen was not analyzed following flux measurements, but coefficients of variation for NH_4^+ and $NO_2^- + NO_3^-$ in bulk samples were 5–114% and 16–122%, respectively. Although methanotrophic activity will depend on conditions in micro-sites that do not necessarily reflect average conditions, the observed variability of NH_4^+ and $NO_2^- + NO_3^-$ concentrations does suggest that N inhibition could have influenced the distribution of CH_4 oxidation.

In terms of greenhouse gas mitigation, establishment of a surface crust with or without an additional cover appears to be cost-effective (Petersen et al. 2005), but high removal efficiences are likely to require additional measures. Melse and van der Werf (2005) studied the properties of a biofilter for removal of CH_4; with the assumption made, 40 m^3 of filter volume would be required to achieve a 75% CH_4 removal at 22 g m^{-3} (33 000 ppmv) CH_4 in the air leaving the storage. Provided the surface crust should act as such a biofilter, the corresponding height of crust material with methanotrophic activity would be 10–15 cm for a 20-m diam. storage with 1000 m^3 slurry which produces 40 g CH_4 m^{-3} d^{-1} (cf. Melse and van der Werf, 2005). Future research should explore the properties of natural and artificial surface crusts, and the potential for manipulating these in order to achieve the methanotrophic activity required, the attraction being that investment costs compared to a separate biofilter unit could be lower.

Conclusions

This study demonstrated a potential for CH_4 oxidation in naturally developed surface crusts from practical slurry storages, although the specific activity varied considerably both within and between storages. There was a positive response to increasing CH_4 concentrations indicating that control of the air exchange rate, and thus steady-state CH_4 concentrations above the stored slurry, will stimulate methanotrophic activity and thereby mitigate atmospheric emissions. High removal efficiencies may require manipulation of surface crust properties. The effect of moisture was complex and suggested that in some surface crusts gas diffusibility was not the limiting factor.

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