Effect of composts, nitrogen salts and NPK fertilizers on methane oxidation potential at different temperatures

Louis-B. Jugnia^{1†}, Yaseen Mottiar¹, Euphrasie Djuikom², Alexandre R. Cabral³ and Charles W. Greer¹

¹ National Research Council Canada, Biotechnology Research Institute, 6100
Royalmount Avenue, Montreal, Quebec, Canada, H4P 2R2

² University of Douala, Faculty of Sciences, P.O.Box: 24157 Douala, Cameroon

³ Department of Civil Engineering, Université de Sherbrooke, 2500 boulevard de
l'Université, Sherbrooke, Quebec, Canada, J1K 2R1

Jugnia, L-B. #, Mottiar, Y., Djuikom, E., Cabral, A.R., and Greer, C.W. (2012).
Effect of compost, nitrogen salts and NPK fertilizers on methane oxidation
potential at different temperatures. Applied Microbiology and biotechnology, 93:
2633-2643. DOI 10.1007/s00253-011-3560-4

Running title: Effects of compost, nitrogen salts and fertilizers on methanotrophic
activity

Keywords: Methanotrophs, potential activity, compost, nitrogen salts, NPK fertilizers,

[†] **Corresponding author**

Phone: + 1 (514) 496-0714; Fax: + 1 (514) 496-6265

Email: louis.jugnia@cnrc-nrc.gc.ca

Abstract

The effects of compost, nitrogen salts (N-salts) and nitrogen-phosphorous-potassium (NPK) fertilizers on the methane oxidation potential performance (MOP) of a landfill cover soil were assessed under various temperature regimes. For this, batch assays were conducted at 5, 15 and 25°C with microcosms containing soil slurries amended with different types and amounts of compost, N-salts and NPK fertilizers at different concentrations. Results indicated variable impacts dependent on the type of amendment and the incubation temperature. For a given incubation temperature, MOP varied from one compost to another and with the amount of compost added, except for the shrimp/peat compost. With this latter compost, independent of amount, MOP values remained similar and in general were significantly higher than those obtained with the other composts. N-salts addition indicated lack of inhibition or even stimulation of MOP. MOP with NPK fertilizer additions were amongst the highest of this study, the minimum value obtained with NPK (20-0-20) suggested, among others, the significance of P element for methanotrophs. MOP generally increased with temperature, with an indication that at high temperatures nutrients were no longer the major factor regulating methanotrophic activity. Overall, at each of the three temperatures tested, MOP with NPK fertilizer amendments provided the best results and were comparable to those observed with the addition of the shrimp/peat compost. Results of this study provide the first evidence on: 1) that compost addition to improve methanotrophic activity in a landfill cover soil requires considerations on the amounts and/or type of compost used, 2) the importance of using NPK fertilizers rather than N-salts, in enhancing this activity

53 principally at low temperatures with potentially beneficial impact for the vegetation

54 growth that is an advantage on this type of soil.

55

56

Introduction

Methane (CH_4) is a potent greenhouse gas that absorbs infrared radiation more effectively than CO_2 and has a global warming potential 25 times higher than that of CO_2 (IPCC 2007). Methane is the product of the anaerobic degradation of organic matter by methanogenic Archaea (Zinder 1993). Landfills represent a significant source of anthropogenic CH_4 emissions contributing as much as 17% (Wuebbles and Hayhoe 2002) of the 70% of CH_4 emissions attributed to human activities (IPCC 2007).

Landfills may play an important role in climate scenarios, as a source of CH_4 . When a landfill site is full and capped with soil, vertical migration brings CH_4 produced by methanogenic bacteria deep within the anaerobic region of the landfill, to the aerobic environment near the surface, where it may be subjected to microbiological oxidation by methane-oxidizing bacteria (methanotrophs) (Whalen et al. 1990; Jones and Nedwell 1993; Humer and Lechner 2001). Methane emissions from landfills can be mitigated by microbial methane oxidation in landfill cover soils (Boeckx et al. 1996; Hilger and Humer 2003; Kallistova et al. 2005) - known as biocovers. Several previous investigations have assessed the role of compost as biofilters in landfills cover soils (Humer and Lechner 1999; Straska et al. 1999; Wilshusen et al. 2004).

Composted organics are thought to promote good microbial growth by improving the porosity, water holding capacity, pH, and nutrient supply (Hilger and Humer 2003). Accordingly, there is an increasing use of compost in biocovers and biofilters to mitigate methane emissions from landfills and recent investigations in this direction have shown promising results (Humer and Lechner 2001; Barlaz et al. 2004; Maurice and Lagerkvist 2004; Wilshusen et al. 2004; Kettunen et al. 2006; Powelson et al. 2006; Jugnia et al.

2008; Aït-Benichou et al. 2009). Organic amendments to soil generally result in an increase in enzymatic activity (Bandick and Dick 1999; Garcia-Gil et al. 2000; Perucci et al. 2000). Also, Saison et al. (2006) reported that compost amendment affects the overall activity of the soil microbial community.

Soil-mediated CH_4 oxidation provides an important biological sink for both atmospheric and for *in situ* produced CH_4 (Amaral et al. 1998). Compost amendment to soil is a common practice generally used to improve soil fertility and physical structure. Numerous types of compost exist for this use and the amount applied varied from one site to site. However, the specific impact of compost amendments on methanotrophic capacity of soil is not well known and should be investigated insofar as, among other impacts, excessive use of organic amendment is likely to intensify non methanotrophic microbial activity, thus limiting the soil's CH_4 oxidation capacity.

The present study examined the combined effects of temperature and amendments of different types and amounts of compost on the CH_4 oxidizing potential of a soil, given that compost is increasingly used in landfill cover soil. For this, samples from a landfill cover soil that housed a well established community of methanotrophs (Aït-Benichou et al. 2009) was chosen. To our knowledge no previous work has compared the rates of CH_4 oxidation potential of a landfill cover soil amended with different types and amounts of compost under various temperature regimes. Similarly, we examined the effects of nitrogen salts and nitrogen-phosphorous-potassium fertilizers. Understanding changes in methanotrophic activity induced by these treatments is of economic and environmental importance to optimize the mitigation of the net flux of CH_4 from landfills.

Material and methods

Samples, soil slurries and CH₄ oxidation potential

Landfill cover soil samples were collected at the surface from an existing landfill located at Saint-Nicéphore, Quebec (Canada) and sieved to 2 mm. This soil had been exposed to methane and its granulometric composition was: 25.2% sand, 67.3% silt and 7.5% clay. Five different types of compost (green waste, biosolid waste, pulp and paper residue, manure and shrimp/peat) were obtained from a local producer (Les Composts du Québec) and sieved to 2 mm. Subsamples of soil and compost were sterilized by gamma irradiation at MDS Nordiom in Laval, Quebec (Canada). Sterilized and non-sterilized samples were stored in the dark at 4°C until use. Physico-chemical properties of this landfill cover soil together with those of the different types of composts that were used are presented in Table 1.

Microcosm experiments for the determination of CH₄ oxidation potential were performed with soils slurries in aerobic 120-ml glass serum bottles. Ten g of sample was used and three amounts (5, 10 and 25%) of compost amendment were tested. For this, 7.5 g of non-sterilized landfill cover soil was amended with either 0 g, 0.5 g, 1 g, or 2.5 g of sterile compost: sterilized landfill cover soil was used to bring the final weight to 10 g. Microcosm slurries were prepared by mixing 10 g of soil (control) or soil/compost mixture with 10 ml of deionized water. For experiments with nitrogen salts and nitrogen-phosphorous-potassium (NPK) fertilizers, stock solutions of NH₄Cl, (NH₄)₂SO₄, NH₄NO₃, NaNO₃, urea and NPK at rates of 20-20-20, 20-6-20 and 20-0-20 were prepared so that, the addition of 10 ml of either one of these solutions to 10 g of soil (7.5 g of non-sterilized landfill cover soil + 2.5 g of sterilized landfill cover soil), yielded 600 µg of

total nitrogen added in the microcosm. Ten ml of liquid was enough to form a slurry and thereby minimize variation in CH₄ oxidation potential caused by soil moisture.

The glass serum bottles were capped with Teflon-lined rubber stoppers and sealed with aluminum crimps. Pure CH₄ (0.5 ml) was added to each bottle (equivalent to approximately 5% V/V) after withdrawal of an equal volume of the gas phase. All treatments were prepared in triplicate for each incubation temperature (5°C, 10°C or 25°C). The CH₄ oxidation potential (MOP) was evaluated by measuring the headspace concentration of CH₄ over the course of the incubation period. Measurements were performed by gas chromatography on a SRI 8610 C gas chromatograph (SRI, Torrance, CA, USA) equipped as described in Roy and Greer (2000). For gas determinations, 0.2 ml of the headspace gas was injected into the GC system with simultaneous integration of peaks using the Peak Simple II software (SRI, Torrance, CA, USA).

Statistics

A well-adjusted linear regression equation describing the CH₄ oxidation in our microcosm was obtained independently with measurements taken using repeated sampling in the same triplicate microcosms through the analyzed period. Potential CH₄ oxidation rates were determined from the slope of the regression. For statistical analysis, general linear model with unbalanced repeated measures (type III) analysis was applied using the PASW statistics 18 package (formerly SPSS Statistics). The predicted means from the general linear model were compared by using the least significant difference method where a P value of < 0.05 was considered to be significant.

Results

Characteristics of the landfill cover soil and the different composts

Results of physico-chemical analysis of the landfill cover soil and the different composts are presented in Table 1. The pH of the landfill cover soil was alkaline and the different composts were neutral or slightly alkaline. The concentration of total nitrogen and phosphorus in the soil from the landfill cover differed considerably from those of the composts. This was also valid for bacterial assimilable forms of these chemicals (NH_4^+ , $\text{NO}_2\text{-NO}_3$ and PO_4^{3-}) that represented only a minor fraction of the total concentration available. Indeed, NH_4^+ concentrations were generally $< 5 \text{ mg of N kg}^{-1} \text{ DW}_{\text{soil}}$ (i.e. 0 to 0.2 % of total nitrogen) and lower than the $\text{NO}_2\text{-NO}_3$ concentration representing 0.2 to 1.86 % of total nitrogen, if one excludes the shrimp/peat compost with 4.5% of total nitrogen. With PO_4^{3-} , concentrations were $< 5 \text{ mg of P kg}^{-1} \text{ DW}_{\text{soil}}$ except for the manure compost that contained the highest concentration ($848 \text{ mg of P kg}^{-1} \text{ DW}_{\text{soil}}$, i.e. 8.23 % of total P).

Effect of compost types and amount on potential CH_4 oxidation performance at different temperatures

Of the different types of compost considered, CH_4 consumption was faster with samples amended with shrimp/peat compost, irrespective of the amount of this compost added to the soil sample, with the complete oxidation of CH_4 in microcosms lasting: 4 days at 25°C , between 7 and 12 days at 15°C , and < 30 days at 5°C (Fig. 1). This was not the case with the other composts for which, the complete oxidation of CH_4 required 6 to 8 days at 25°C , increasing to 21 to 30 days at 15°C or ≥ 30 days at 5°C (Fig. 1). Nevertheless, samples amended with biosolid waste and manure compost exhibited activities that were a bit faster and more considerable at 5 and 15°C with the increasing in the ratio of these composts in soil samples (Fig. 1).

Overall, the concentration versus time profiles of all the experiments in Fig. 1 indicated an impact of the amount of compost on CH₄ oxidation: at low temperatures, there was an increasing variability among the different composts dependent on the amount of compost added. Samples amended with shrimp/peat compost exhibited the highest MOP, with average values of 0.7, 2.0 and 5.7 $\mu\text{gCH}_4\cdot\text{g}^{-1}\text{DW}_{\text{soil}}\cdot\text{h}^{-1}$ at 5, 10 and 25°C, respectively. When excluding these values, the lowest MOP, achieved with 1.5% compost, was between 0.24 $\mu\text{gCH}_4\cdot\text{g}^{-1}\text{DW}_{\text{soil}}\cdot\text{h}^{-1}$ at 5°C and 2.45 $\mu\text{gCH}_4\cdot\text{g}^{-1}\text{DW}_{\text{soil}}\cdot\text{h}^{-1}$ at 25°C (Fig. 2). These values increased considerably with 25% compost and fluctuated between 0.32 $\mu\text{gCH}_4\cdot\text{g}^{-1}\text{DW}_{\text{soil}}\cdot\text{h}^{-1}$ at 5°C and 4.03 $\mu\text{gCH}_4\cdot\text{g}^{-1}\text{DW}_{\text{soil}}\cdot\text{h}^{-1}$ at 25°C (Fig. 2).

Effect of nitrogen salts and NPK fertilizers on potential CH₄ oxidation performance at different temperatures

CH₄ consumption was also improved when soils were amended with nitrogen salts or NPK fertilizers, although this was lower with urea and NPK. (20-0-20). With different nitrogen salts, the profiles of CH₄ consumption over time were close to each other on a time scale of < 5 days (at 25°C), to 63 to 80 days (at 15°C) or > 80 days (at 5°C) (Fig. 3). With fertilizers, NPK (20-20-20) and NPK (20-6-20) exhibited similar profiles of CH₄ consumption over time, with oxidation completed within 21 to 42 days at 15 and 5°C, while NPK (20-0-20) was > 79 days at 15 and 5°C (Fig. 3). For nitrogen salts and fertilizers, the time elapsed for complete CH₄ consumption increased with decreasing incubation temperature (Fig. 3), a trend that was observed with compost addition.

At a given temperature, changes in MOP were less significant between the different N-salts than the NPK fertilizers tested, but became more important with shifts in temperature, notably between 15 and 25°C (Fig. 4). Methane uptake rates ranged from

0.20 to 0.70 $\mu\text{gCH}_4\cdot\text{g}^{-1}\text{DW}_{\text{soil}}\cdot\text{h}^{-1}$ at 5°C, fluctuated between 0.31 and 0.97 $\mu\text{gCH}_4\cdot\text{g}^{-1}\text{DW}_{\text{soil}}\cdot\text{h}^{-1}$ at 15°C, and increased significantly to 2.35 to 7.18 $\mu\text{gCH}_4\cdot\text{g}^{-1}\text{DW}_{\text{soil}}\cdot\text{h}^{-1}$ at 25°C (Fig. 4). Except for urea, methane uptake rates at 25°C were similar for NPK fertilizers and the N-salts tested (Fig. 4). This was not the case at 5 and 15°C, since with NPK the MOP were greater than those obtained with N-salts: the maximum values (0.40 $\mu\text{gCH}_4\cdot\text{g}^{-1}\text{DW}_{\text{soil}}\cdot\text{h}^{-1}$) obtained with N-salts was similar to the minimum value (0.30 $\mu\text{gCH}_4\cdot\text{g}^{-1}\text{DW}_{\text{soil}}\cdot\text{h}^{-1}$) observed with NPK (20-0-20) (Fig. 4).

Under optimum conditions at 25°C, CH₄ oxidation with NPK fertilizers provided the best results, comparable to those observed with the shrimp/peat compost. They were followed in decreasing order by nitrogen salts and other types of compost. At 5 and 15°C, addition of NPK fertilizers provided the best results, and was followed by composts that were better than the nitrogen salts.

For almost all the samples, the oxidation rate increased with increasing temperature, especially between 15 and 25°C (Figs. 3 and 4). Also for all the samples, the optimum temperature was 25°C, the highest temperature tested during our investigations.

Discussion

The results indicated that CH₄ oxidation performance in microcosms was generally improved by amendment with compost, nitrogen salts and NPK fertilizers. These findings are similar to those reported by others for landfill covers soils (Hilger et al. 2000; Humer and Lechner 2001; De Visscher and Van Cleemput 2003; Barlaz et al. 2004; Maurice and Lagerkvist 2004; Aït-Benichou et al. 2009; Albanna and Fernandes 2009; Lee et al. 2009).

The CH₄ oxidation capacity of landfill cover soils is quite variable. Albanna and Fernandes (2009) reported values of CH₄ oxidation capacities of landfill cover soils incubated at 3 different temperatures (5, 22 and 35°C), ranging from 3.3 to 12.3 µg CH₄.g⁻¹_{DWsoil} h⁻¹ with a clayey silt soil, and from 3.5 to 10.5 µg CH₄ g⁻¹_{DWsoil} h⁻¹ with a clay soil. Using engineered soil with a mixture of sewage sludge compost and de-inking waste amended with sand (SDS soil) or bark chips (SDB) incubated at 4-6°C, Kettunen et al (2006) reported CH₄ oxidation rates of 3.75 and 2.45 µg CH₄ g⁻¹_{DWsoil} h⁻¹, respectively. Einola et al. (2007) found CH₄ consumption rates ranging between 0.48 and 3.68 µg CH₄ g⁻¹_{DWsoil} h⁻¹ for landfill cover soils incubated between 1 and 6°C. Compared to others, our results fall within the lower range of those previously published for landfill cover soil, probably because we added water in our microcosms to form a slurry and therefore minimize variation caused by soil moisture. Whalen et al. (1990) and Czepiel et al. (1996) confirmed that gas diffusion at soil saturation is limited by the diffusion coefficient of CH₄ in water, which is 4 orders of magnitude lower than in the air.

Effect of compost type and percentage on potential CH₄ oxidation performance

The MOP values from our experimental microcosms amended with compost were generally higher than those observed in control microcosms that contained only the landfill cover soil. This is an indication that compost addition to this landfill cover soil provided some required missing factors that fueled the activity of indigenous methanotrophs. Among these factors are organic matter and nutrients. Previous studies established a clear relationship between methanotrophic activity and/or diversity and organic matter concentration (Christophersen et al. 2000; Bin et al. 2009). Our results agree with both field observations at landfills and results from laboratory studies that

have demonstrated that the CH₄ oxidation potential in mineral soils can be enhanced by adding organic material, e.g. sewage sludge (Kightley et al. 1995) and compost (Humer and Lechner 2001; Barlaz et al. 2004; Maurice and Lagerkvist 2004; Aït-Benichou et al. 2009). Also, Kettunen et al (2006) and Wilshusen (2004) reported that compost should be capable of enhancing the oxidation of CH₄ at rates two to three times higher than that of mineral soils.

MOP observed within each group of samples treated separately with the same amounts of the different composts under study at a given incubation temperature, varied from one type of compost to another (Fig. 2). This suggests differences between the type of compost that was evaluated, with indices regarding the quantity and quality of the elements in the compost that powered methanotrophic activity. The observed increase in MOP with increasing amounts of compost, except with shrimp/peat compost, is evidence of this quantitative effect. Moona et al. (2010) reached the same conclusion using increasing ratios of earthworm cast to improve methanotrophic activity in a filter bed material consisting of a mixture of earthworm cast and rice paddy soil in a biocover. In contrast, results with shrimp/peat compost tend to highlight the difference among composts in terms of quality. With the shrimp/peat compost and at each of the three temperatures examined, MOP values remained similar despite differences in the amount of this compost added.

MOP values obtained with shrimp/peat compost were generally higher than those observed with the other composts. Small differences, principally at 5 and 15°C, were detected between composts with, in most cases, the highest MOP values attributed to manure and/or biosolid composts. As such, shrimp/peat compost followed by manure and

or biosolid composts could be considered the most effective additives. However, since excessive use of organic amendments is likely to intensify non-methanotrophic microbial activity, thus limiting the soils CH_4 oxidation capacity, shrimp/peat could be considered the most effective compost. From this study, the highlighted differences between composts in terms of quality and quantity, in relation to their ability of enhance methanotrophic activity, are pretty new information that had never been reported with landfill cover soil, although previous studies focused on compost types.

At all of the three temperatures considered in this study, MOP values with the lowest amount of shrimp/peat compost provided similar results to the highest amounts of other composts. The main physico-chemical property of shrimp/peat compost that differed from the other composts was the relatively high concentration of bioavailable N, in the form of $\text{NO}_2\text{-NO}_3$. This prompted us to examine how N elements may impacted MOP in the landfill cover soil under study.

Effect of nitrogen salts and NPK fertilizers on potential CH_4 oxidation performance

As the methanotrophic community developed, the mineral N content of the soil decreased (De Visscher et al. 1999), and results of other studies reported an increase of methane oxidation rates possibly due to a relief of nitrogen limitation (Hilger et al. 2000; De Visscher and Van Cleemput 2003; Lee et al. 2009). We hypothesized that the impact of N elements may change with the type of nitrogen salt used, and therefore a range of nitrogen salts were tested together with NPK fertilizers.

Our results, in general, indicated that there was a lack of inhibition and even CH_4 oxidation stimulation, following addition of the different nitrogen salts. This is consistent with previous studies (Bodelier et al. 2000; Hilger et al. 2000; Sitaula et al. 2000; De

287 Visscher et al. 2001; De Visscher and Van Cleemput 2003), but on the other hand
288 somewhat contrasts with results by Kightley et al. (1995) reporting that NO_3^- had an
289 inhibitory effect on CH_4 oxidation in landfill cover soils. De Visscher (2001) observed
290 that amending the landfill cover soil with nitrifying sludge or compost initially inhibited
291 methane oxidation, followed by a stimulation after a few days. In general, the reported
292 information about methane oxidation in the presence of nitrogen salts is often
293 contradictory (Hütsch et al. 1994; Dunfield et al. 1995; Willison et al. 1995; Delgado and
294 Mosier 1996; Bodelier and Laanbroek 2004). In the present study, exactly which species
295 of the N salts was the most important in the stimulation of soil mediated CH_4 oxidation
296 remained unclear principally at 5 and 10°C, since at 25°C and with the exception of urea,
297 all the N-salts tested similarly increased activity. Urea is a NH_4^+ -producing compound
298 and its inhibitory effect on methanotrophs has been demonstrated (Dubey and Singh
299 2000; Zheng et al. 2008). However, the mechanism responsible for this effect on CH_4
300 uptake is complex because it involves both methanotrophs and nitrifiers. Others (Bédard
301 and Knowles 1989) hypothesize that NH_4^+ is a competitive inhibitor of CH_4
302 monooxygenase.

303 At 5 and 15°C, the highest methane uptake rates using N-salts matched the lowest
304 rates observed with NPK fertilizers, suggesting that NPK fertilizers were more efficient
305 than N-salts in improving methanotrophic activity. Although the increase in methane
306 oxidation rates can be due to relief of nitrogen limitation, Zheng et al. (2008) reported
307 that potassium (K) fertilizer may play an important role in maintaining methanotrophic
308 metabolic processes, and a field experiment by Babu et al. (2006) suggested amendment
309 with K to stimulate the activity of methanotrophic bacterial populations. The fact that the

lowest CH₄ uptake rates at 5 and 15°C were obtained with NPK (20-0-20) amendment suggests the importance of P element in the metabolism of the indigenous methanotrophic community. Of interest, methanotrophs rely on intracellular polyphosphate (Trotsenko and Shishkina 1990), which in our case might have been accumulated during growth of methanotrophs of our samples in the landfill cover soil. However, the importance of P appears to be less relevant at 25°C, given that all forms of the NPK fertilizer addition stimulated CH₄ uptake to similar extents. At high temperatures, rather than nutrients limiting methanotrophic activity, other complex processes that occur in the environment at these temperatures (organic matter degradation) contribute to releasing, amongst other components, appropriate nutrients that can sustain methanotrophic activity. This points to the role of temperature in enhancing methanotrophic activity, as substantiated by the fact that differences observed at 5 and 15°C following additions of NPK fertilizer and/or N-salts, were not noticeable at 25°C except for urea.

Effect of temperature on methane oxidation

Temperature has a direct impact on biological reactions. The response to temperature of the CH₄ consumption rate was similar to that generally observed: CH₄ consumption increases with increasing temperature (Czepiel et al. 1996; Christophersen et al. 2000; Gebert et al. 2003; Börjesson et al. 2004; Park et al. 2005; Jugnia et al. 2006; Kettunen et al. 2006; Jugnia et al. 2008). This generally occurs when the CH₄ concentration, among other factors, is not limiting (Pawlowska and Stepniewski 2006; Einola et al. 2007). The rate of CH₄ consumption by methanotrophs in soil is affected by the rate of substrate (CH₄ and O₂) supply as well as the amount and activity of CH₄ monooxygenase enzymes that catalyze the process. Accordingly, Boeckx et al. (1996) observed a low response of

CH₄ consumption to temperature (average Q₁₀ 1.9) in landfill cover soil incubate at 10 µl l⁻¹ CH₄ in contrast to the higher Q₁₀ values (1.9-7.26) in previous studies at >1% CH₄ concentrations (Czepiel et al. 1996; Christophersen et al. 2000; De Visscher et al. 2001; Börjesson et al. 2004), as well as in the present study (Q₁₀ 2.54-3.43). Our observation that at high temperatures nutrients were no longer the major factor regulating methanotrophic activity, is consistent with a recent report by Albanna (2009) that the temperature effect on CH₄ oxidation process was so significant that it masked the effect of moisture content and nutrients.

Another important finding of this study is our observation that amendment with NPK fertilizers (containing [P] >0) improved CH₄ oxidation better than N-salts, notably at 5 and 15°C. At the best of our knowledge, the outcome of such a comparison is something that has not been reported so far for landfill cover soil, and this information would be of great interest for an application under cold climates by landfill owners. On the other hand, this has implications for landfill cover soil where it would be possible to provide nutrients to promote growth and maintain the vegetation system. A good vegetation system minimizes erosion by stabilizing the surface of the cover and it contributes to the aeration and moisture control of the soil.

Acknowledgements:

We gratefully acknowledged financial support of this work by the National Research Council Canada (NRC) through Biotechnology Research Institute (BRI). Y.M. was a summer student from University of Ottawa granted by NRC-BRI. E.D. was supported by

355 the fellowship program “Professeur Invité du sud” from AUF (Agence Universitaire de la
356 francophonie).

357

358 **References**

- 359 Aït-Benichou, S., Jugnia, L.-B., Greer, C.W. and Cabral, A. (2009) Methanotrophs and
 360 methanotrophic activity in engineered landfill biocovers. *Waste Manage* **29**, 2509-
 361 2517.
- 362 Albanna, M. and Fernandes, L. (2009) Effects of temperature, moisture content, and
 363 fertilizer addition on biological methane oxidation in landfill cover soils. *Pract*
 364 *Periodical of Haz, Toxic, and Radioactive Waste Mgmt* **13** 187-195
- 365 Amaral, J., Ren, T. and Knowles, R. (1998) Atmospheric methane consumption by forest
 366 soils and extracted bacteria at different pH values. *Applied and Environmental*
 367 *Microbiology* **64**, 2397-2402.
- 368 Babu, Y.J., Nayak, D.R. and Adhya, T.K. (2006) Potassium application reduces methane
 369 emission from a flooded field planted to rice. *Biol Fertil Soils* **42**, 532-541.
- 370 Bandick, A.K. and Dick, R.P. (1999) Field management effects on soil enzyme activities.
 371 *Soil Biol Biochem* **31**, 1471-1479.
- 372 Barlaz, M.A., Green, R.B., Chanton, J.P., Goldsmith, C.D. and Hater, G.R. (2004)
 373 Evaluation of a biologically active cover for mitigation of landfill gas emissions.
 374 *Environ Sci Technol* **38**, 4891-4899.
- 375 Bédard, C. and Knowles, R. (1989) Physiology, biochemistry, and specific inhibitors of
 376 CH₄, NH₄⁺, and CO oxidation by methanotrophs and nitrifiers. *Microbiol Rev* **53**, 68-
 377 84.
- 378 Bin, L., Monreal, C.M., Tambong, J.T., Miguez, C.B. and Carrasco-Medina, L. (2009)
 379 Phylogenetic analysis of methanotrophic communities in cover soils of a landfill in
 380 Ontario. *Can J Microbiol* **55**, 1103-1112.

- 381 Bodelier, P.L.E. and Laanbroek, H.J. (2004) Nitrogen as a regulatory factor of methane
382 oxidation in soils and sediments. *FEMS Microbiol Ecol* **47**, 265–277.
- 383 Bodelier, P.L.E., Roslev, P., Hencke, T. and Frenzel, P. (2000) Stimulation by ammonium-
384 based fertilizers of methane oxidation in soil around rice roots. *Nature* **403**, 421–424.
- 385 Boeckx, P. and Van Cleemput, O. (1996) Methane oxidation in a neutral landfill cover soil:
386 influence of moisture content, temperature, and nitrogen-turnover. *J Environ Qual* **25**,
387 178–183.
- 388 Boeckx, P., Van Cleemput, O. and Villaralvo, I. (1996) Methane emission from landfill and
389 the methane oxidizing capacity of its covering soil. *Soil Biol Biochem* **28**, 1397–1405.
- 390 Börjesson, G., Sundh, I. and Svensson, B.H. (2004) Microbial oxidation of CH₄ at different
391 temperatures in landfill cover soils. *FEMS Microbiol Ecol* **48**, 305–312.
- 392 Christophersen, M., Linderod, L., Jensen, P.E. and Kjeldsen, P. (2000) Methane oxidation
393 at low temperatures in soil exposed to landfill gas. *J Environ Qual* **29**, 1989–1997.
- 394 Czepiel, P.M., Mosher, B., Crill, P.M. and Harriss, R.C. (1996) Quantifying the effects of
395 oxidation on landfill methane emissions. *Journal of Geophysical Research* **101**,
396 16721–16729.
- 397 De Visscher, A., Schippers, M. and Van Cleemput, O. (2001) Short-term kinetic response
398 of enhanced methane oxidation in landfill cover soils to environmental factors. *Biol*
399 *Fertil Soils* **33**, 231–237.
- 400 De Visscher, A. and Van Cleemput, O. (2003) Induction of enhanced CH₄ oxidation in
401 soils: NH₄⁺ inhibition patterns. *Soil Biol Biochem* **35**, 907–913.
- 402 De Visscher, D., Boeckx, T.P. and Van Cleemput, O. (1999) Methane oxidation in
403 simulated landfill cover soil environments. *Environ Sci Technol* **33**, 1854–1859.

- 404 Delgado, J.A. and Mosier, A.R. (1996) Mitigation alternatives to decrease nitrous oxide
405 emissions and urea-nitrogen loss and their effect on methane flux. *J Envir Quality* **25**,
406 1105-1111.
- 407 Dubey, S.K. and Singh, J.S. (2000) Spatio-temporal variation and effect of urea
408 fertilization on methanotrophs in a tropical dryland rice field. *Soil Biol Biochem* **32**,
409 521-526.
- 410 Dunfield, P.F., Topp, E., Archambault, C. and Knowles, R. (1995) Effect of nitrogen
411 fertilizers and moisture content on CH₄ fluxes in a humisol: Measurements in the field
412 and intact soil cores. *Biogeochemistry* **29**, 199-222.
- 413 Einola, J.-K.M., Kettunen, R.H. and Rintala, J.A. (2007) Responses of methane oxidation
414 to temperature and water content in cover soil of a boreal landfill. *Soil Biology &*
415 *Biochemistry* **39**, 1156-1164.
- 416 Garcia-Gil, J.C., Plaza, C., Soler-Rovira, P. and Polo, A. (2000) Long-term effects of
417 municipal solid waste compost application on soil enzyme activities and microbial
418 biomass. *Soil Biol Biochem* **32**, 1907-1913.
- 419 Gebert, J., Grönröft, A. and Miehlich, G. (2003) Kinetics of microbial landfill methane
420 oxidation in biofilters. *Waste Manage* **23**, 609–619.
- 421 Hilger, H. and Humer, M. (2003) Biotic landfill cover treatments for mitigating methane
422 emissions. *Environ Monit Assess* **84**, 71-84.
- 423 Hilger, H.A., Barlaz, M.A. and Wollum, A.G. (2000) Landfill methane oxidation response
424 to vegetation, fertilization, and liming. *J Envir Quality* **29**, 324–334.

- 425 Humer, M. and Lechner, P. (1999) Methane oxidation in compost cover layers of landfills.
426 In *Proceeding of the Sardinia 99, 7th International Waste Management and Landfill*
427 *Symposium*. Cagliari, Italy.
- 428 Humer, M. and Lechner, P. (2001) Microbial methane oxidation for the reduction of
429 landfill gas emissions. *Journal of Solid Waste Technology and Management* **27**, 146–
430 151.
- 431 Hütsch, B.W., Webster, C.P. and Powlson, D.S. (1994) Methane oxidation in soil as
432 affected by land-use, soil-pH and N-fertilization. *Soil Biology & Biochemistry* **26**,
433 1613–1622.
- 434 IPCC. (2007) *Climate Change 2007: The Physical Science Basis*. Cambridge, United
435 Kingdom: Cambridge University Press.
- 436 Jones, H.A. and Nedwell, D.B. (1993) Methane emission and methane oxidation in landfill
437 cover soil. *FEMS Microbiol Ecol* **102**, 185-195.
- 438 Jugnia, L.-B., Cabral, A.R. and Greer, C.W. (2008) Biotic methane oxidation within an
439 instrumented experimental landfill cover. *Ecol Eng* **33**, 102-109.
- 440 Jugnia, L.B., Roy, R., Pacheco-Oliver, M., Planas, D., Miguez, C.B. and Greer, C.W.
441 (2006) Potential activity and diversity of methanotrophs in forest soil, peat, and
442 sediments from hydro-electric reservoir (Robert Bourassa) and lakes in the Canadian
443 taiga. *Soil Science* **171**, 127-137.
- 444 Kallistova, A.Y., Kevbrina, M.V., Nekrasova, V.K., Glagolev, M.V., Serebryanaya, M.I.
445 and Nozhevnikova, A.N. (2005) Methane oxidation in landfill cover soil.
446 *Microbiology* **74**, 608-614.

- 447 Kettunen, R.H., Einola, J.-K.M. and Rintala, J.A. (2006) Landfill methane oxidation in
448 engineered soil columns at low temperature. *Water, Air & Soil Pollution* **177**, 313-
449 334.
- 450 Kightley, D., Nedwell, D.B. and Cooper, M. (1995) Capacity for methane oxidation in
451 landfill cover soils measured in laboratory-scale soil microcosms. *Appl Environ*
452 *Microbiol* **61**, 592–601.
- 453 Lee, S.-W., Im, J., Dispirito, A.A., Bodrossy, L., Barcelona, M.J. and Semrau, J.D. (2009)
454 Effect of nutrient and selective inhibitor amendments on methane oxidation, nitrous
455 oxide production, and key gene presence and expression in landfill cover soils:
456 Characterization of the role of methanotrophs, nitrifiers, and denitrifiers *Appl*
457 *Microbiol Biotechnol* **85**, 389-403
- 458 Maurice, C. and Lagerkvist, A. (2004) Assessment of methane oxidation capacity of soil.
459 *Waste Manage Res* **22**, 42-48.
- 460 Moona, K.-E., Lee, S.-Y., Lee, S.H., Ryu, H.W. and Cho, K.-S. (2010) Earthworm cast as a
461 promising filter bed material and its methanotrophic contribution to methane removal
462 *J Hazard Mater* **176**, 131-138
- 463 Park, J.R., Moon, S., Ahn, Y.M., Kim, J.Y. and Nam, K. (2005) Determination of
464 environmental factors influencing methane oxidation in a sandy landfill cover soil.
465 *Environmental Technology* **26**, 93–102.
- 466 Pawlowska, M. and Stepniewski, W. (2006) An influence of methane concentration on the
467 methanotrophic activity of a model landfill cover. *Ecol Eng* **26**, 392-395.

- 468 Perucci, P., Dumontet, S., Bufo, S.A., Mazzatura, A. and Casuci, C. (2000) Effects of
469 organic amendment and herbicide treatment on soil microbial biomass. *Biol Fertil*
470 *Soil* **32**, 17-23.
- 471 Powelson, D.K., Chanton, J., Abichou, T. and Morales, J. (2006) Methane oxidation in
472 water-spreading and compost biofilters. *Waste Manage Res* **24**, 528-536.
- 473 Roy, R. and Greer, C.W. (2000) Hexadecane mineralization and denitrification in two
474 diesel fuel-contaminated soils. *FEMS Microbiol Ecol* **32**, 17-23.
- 475 Saison, C., Degrange, V., Oliver, R., Millard, P., Commeaux, C., Montange, D. and Le
476 Roux, X. (2006) Alteration and resilience of the soil microbial community following
477 compost amendment: Effects of compost level and compost-borne microbial
478 community. *Env Microbiol* **8**, 247-257.
- 479 Sitaula, B.K., Hansen, S., Sitaula, J.I.B. and Bakken, L.R. (2000) Methane oxidation
480 potentials and fluxes in agricultural soil: effects of fertilisation and soil compaction.
481 *Biogeochemistry* **48**, 323–339.
- 482 Straska, F., Crha, J., Musilova, M. and Kuncarova, M. (1999) LFG-biofilters on old
483 landfills. In *Proceeding of the Sardinia 99, 7th International Waste Management and*
484 *Landfill Symposium*. Cagliari, Italy.
- 485 Trotsenko, Y.A. and Shishkina, V.N. (1990) Studies on phosphate metabolism in obligate
486 methanotrophs. *FEMS Microbiol Rev* **87**, 267-272.
- 487 Whalen, S.C., Reeburgh, W.S. and Sandbeck, K.A. (1990) Rapid methane oxidation in a
488 landfill cover soil. *Appl Environ Microbiol* **56**, 3405-3411.

- 489 Willison, T.W., Webster, C.P., Goulding, K.W.T. and Powlson, D.S. (1995) Methane
490 oxidation in temperate soils-effects of land-use and the chemical form of nitrogen-
491 fertilizer. *Chemosphere* **30**, 539–546.
- 492 Wilshusen, J.P., Hettiaratchi, J.P.A. and Stein, V.B. (2004) Long-term behavior of
493 passively aerated compost methanotrophic biofilter columns. *Waste Manage* **24**, 643-
494 653.
- 495 Wuebbles, D.J. and Hayhoe, K. (2002) Atmospheric methane and global change. *Earth-*
496 *Science Reviews* **57**, 177–210.\
- 497 Zheng, Y., Zhang, L.M., Zheng, Y.M., Di, H. and He, J.Z. (2008) Abundance and
498 community composition of methanotrophs in a Chinese paddy soil under long-term
499 fertilization practices. *J Soils Sediments* **8**, 406-414.
- 500 Zinder, S.H. (1993) Physiology ecology of methanogens. In *Methanogenesis* ed. Ferry, J.G.
501 pp.35-80. London, UK: Chapman & Hall.
- 502
- 503

504 Table 1: Results of some physico-chemical analysis of samples from the the landfill cover soil and the different composts
 505 understudied

	landfill	Composts					
Parameters	Cover soil	Green waste	Biosolid	Pulp paper	Manure	Shrimp/peat	
pH	8.6	7.7	7.6	7.6	7.8	7.0	
Organic matter (%)	1.14	22.68	20.01	21.87	25.27	20.26	
*NH ₄ ⁺	< 5	< 5	< 6	< 5	< 5	< 5	
*NO ₂ -NO ₃	1.1	< 0.8	24.1	184	< 0.8	752	
*PO ₄ ³⁻	< 0.5	< 4.3	< 5.0	< 2.5	848	< 3.2	
*Total N	241	11400	10700	9890	11800	15700	
*Total P	595	1780	6100	5080	10300	9810	
*	Units	are	in	mg.Kg ⁻¹	of	dry	weight

Figure legends

Fig. 1

Fig. 1: Profiles of CH_4 consumption over the time from our microcosms amended with variables levels of different composts and incubated under different temperatures. Data are averages of triplicates with error bars equivalent to 1 SD

Fig. 2

Fig. 2: Potential activity of methane oxidation rates ($\pm$ standard error) from our microcosms. At a given temperature, means with the same letter are not significantly different.

Fig. 3

Fig. 3: Profiles of CH₄ consumption over the time from our microcosms amended with different N-salts and NPK fertilizers and incubated under different temperatures. Data are averages of triplicates with error bars equivalent to 1 SD

Fig. 4

Fig. 4: Potential activity of methane oxidation rates ($\pm$ standard error) from our microcosms. At a given temperature, means with the same letter are not significantly different.