Effects of Preconditioning the Rhizosphere of Different Plant Species on Biotic Methane Oxidation Kinetics

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ABSTRACT

The rhizosphere is known as the most active biogeochemical layer of the soil. Therefore, it could be a beneficial environment for biotic methane oxidation. The aim of this study was to document - by means of batch incubation tests - the kinetics of methane oxidation in rhizospheric soils that were previously exposed to methane. Three CH_4 pre-exposure zones were sampled; the never-before pre-exposed, the moderately pre-exposed and the very pre-exposed. For each zone, 3 different plant species were considered. All of the samples were placed in Mason jars and submitted to the same initial CH_4 concentration. CH_4 consumption started in less than 3 days and ended in less than a week. The results showed that the fastest CH_4 consumption occurred for the rhizospheric soil that was moderately pre-exposed. However, no statistically significant differences were found in the CH_4 oxidation kinetic parameters (lag time and half-life) of all the rhizospheric soils, suggesting that methane oxidation did not depend on plant species or CH_4 pre-exposure levels, for the soils and plants tested herein. The oxidation rate values obtained were higher than those reported in the reviewed literature for unplanted landfill cover soils.

KEYWORDS: Rhizosphere, methane oxidation, kinetics, vegetation, preconditioning

26 **TOC/ABSTRACT ART.**

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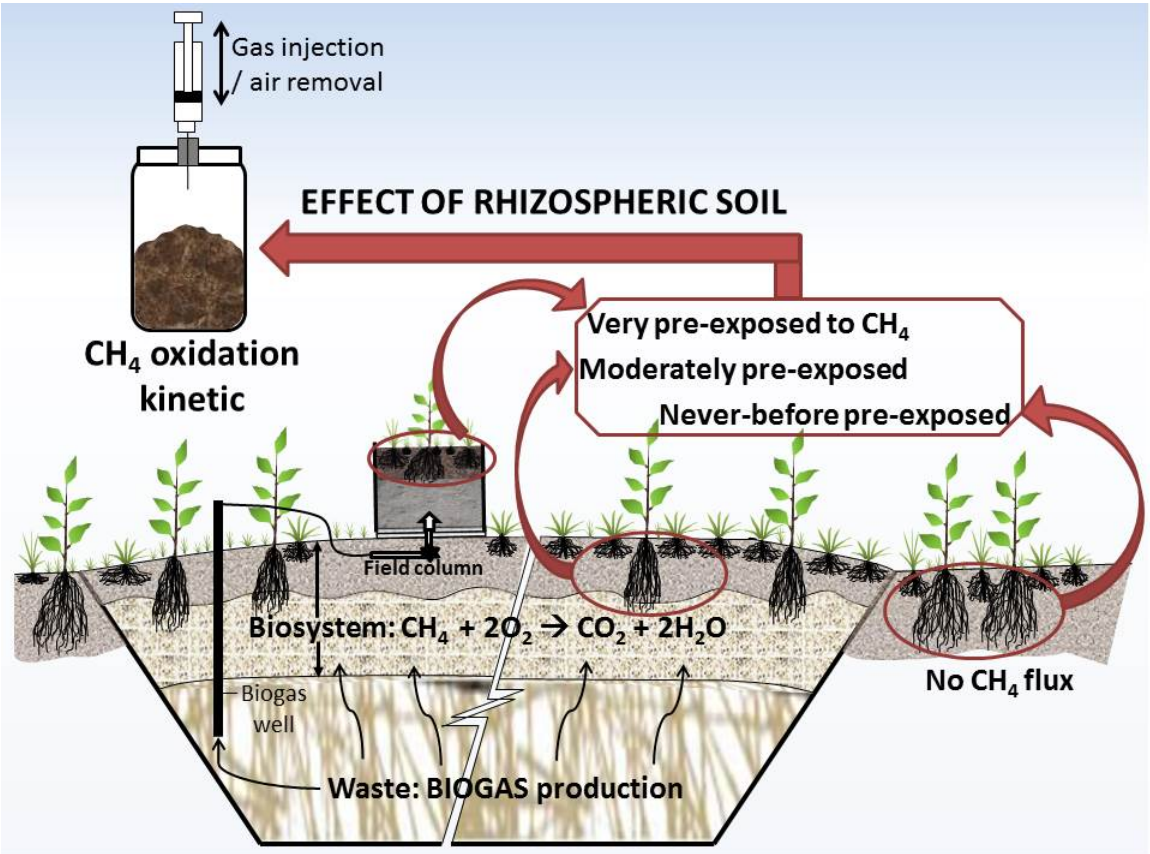

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INTRODUCTION

Biotic methane oxidation is a highly efficient biogeochemical process to reduce CH_4 emissions from landfills through the use of engineered biosystems. These biosystems are typically made up of a sequence of soil layers that promote the development of methane oxidizing bacteria, which use CH_4 as their energy source.[1]

Although methane oxidation (CH_4-ox) has been extensively documented, there are a limited number of studies on the effects of vegetation on methanotrophic activity and CH_4 oxidation efficiency in landfills. Among these studies, Bohn et al.[2] and Reichenauer et al.[3] assessed the impact of several types of plant covers on CH_4 oxidation. Their studies concluded that CH_4 oxidation efficiencies of the tested biosystems differed, and that vegetation enhanced biotic CH_4 oxidation. Wang et al.[4] evaluated how *Chenopodium album* L. affected methanotrophic activity. They observed a significant increase in the total number of soil culturable bacteria in soils seeded with *C. album* L. and exposed to landfill gas. According to Wang et al.[4], the total number of methanotrophic bacteria in the seeded soils exposed to landfill gas was significantly higher than in soils either not exposed to landfill gas or seeded. In a recent study, Ndanga et al.[5] reported the impact of *Trifolium repens* L., *Phleum pratense* L., and a mixture of both on aerobic CH_4 oxidation. Ndanga et al.[5] performed column studies in the laboratory and in the field and concluded that the influence of vegetation on methane oxidation was not noticeable for loadings up to approximately 100 g m^{-2} d^{-1}. In fact, these authors observed that the bare soil performed as well as the 3 vegetated covers they analyzed. In the above mentioned

51 studies, it was concluded that a positive impact of vegetation on biotic CH_4 oxidation
52 could be attributed to enhanced nutrient supply through the root system and gas diffusion.

53 The effect of vegetation on CH_4 oxidation in landfills may also be assessed through the
54 efficiency of the rhizospheric soil in oxidizing CH_4. This is another subject that remains
55 poorly documented in the technical literature relating to biosystems incorporated in
56 landfill final covers. The rhizosphere is the zone of contact between root and soil that
57 supports high levels of bacterial activity. Through root exudations (rhizodeposition),
58 plants release organic compounds such as amino acids and sugars, which serve as energy
59 substrates for soil heterotrophic microorganisms. This, in turn, may stimulate soil nutrient
60 turnover with positive effects on methanotrophic populations. Accordingly, the
61 abundance of micro-organisms in the rhizosphere is generally 5 to 20 times greater than
62 that found in non-vegetated soil.[6, 7] Therefore a well-developed rhizospheric soil would
63 accelerate CH_4 oxidation. The variability in microbial composition and activity in the
64 rhizosphere generally depends on the quality and quantity of root exudations, which vary
65 according to plant species, age and vigor, as well as site specific factors such as soil
66 chemical properties and climatic conditions.[8]

67 The aim of the present study was to assess the importance of the rhizosphere in methane
68 oxidation kinetics for a limited number of plant species commonly found at the Saint-
69 Nicephore landfill in Quebec, Canada. We hypothesized that preconditioning the
70 rhizospheric soils (therefore the microflora) through exposure to CH_4 (in fact landfill gas)
71 might lead to faster development of CH_4 oxidizing bacteria and, as a consequence, to
72 faster and earlier CH_4 oxidation, in comparison with unconditioned rhizospheric soils.

73 This experimental plan was designed to document CH_4 oxidation within the rhizosphere
74 layer of the biosystems tested. The rhizospheric soils associated with 3 levels of root
75 development, and for different plant species, were exposed to 3 levels of CH_4
76 preconditioning. As schematized in Figure 1, the lowest, medium and highest levels of
77 root development were found in the very pre-exposed, moderately pre-exposed and
78 never-before pre-exposed soils respectively. The kinetic approach, which has been
79 commonly employed to assess the capacity of soil materials to oxidize CH_4, was adopted
80 herein. It involves batch incubation for determining the kinetic parameters, such as the
81 potential rate of CH_4 oxidation and its associated half-life ($t_{1/2}$). To determine the CH_4-ox
82 kinetic parameters, rhizospheric soil samples were collected from 2 selected locations
83 within a covered landfill and 1 from its surroundings, each associated with 1 of the 3
84 levels of preconditioning.

85 One practical outcome of this investigation was the possibility of assessing whether or
86 not some level of exposure of the rhizospheric soil to methane, prior to construction of
87 the biosystem, might result in a faster - and more substantial - reduction in fugitive
88 methane fluxes from landfills.

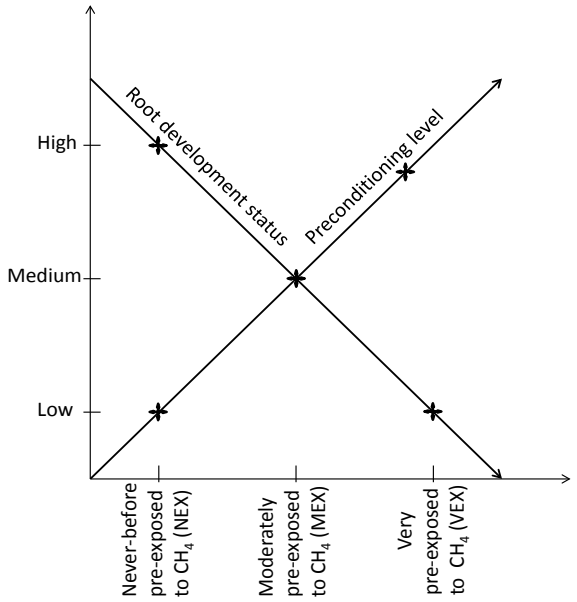


Figure 1. Schematic framework of the study

MATERIALS AND METHODS

Study site

Samples were obtained at a landfill site located in the municipality of Drummondville (Saint-Nicephore), Quebec, Canada. Sampling regions were chosen based on plant cover and CH_4 pre-exposure level. 3 CH_4 pre-exposure zones were selected: 1) never-before pre-exposed zone (NEX), located < 30 m away from the landfill site, so as to obtain the same plant species found on the final cover; 2) moderately pre-exposed zone (MEX), located in an area where the final cover was placed approximately 8 years ago, and for which CH_4 emissions oscillate between 0-200 ppm; 3) very pre-exposed zone (VEX). The latter was not actually a zone, but our own field column experiments, described in Ndanga et al.[5]

For each CH_4 pre-exposure zone, 3 plant species were considered for the study: clover (*Trifolium spp.* L.), timothy grass (*Phleum pratense* L.) and perennial ryegrass *(Lolium perenne* L.). These are commonly found species in the area within and around the landfill. However, for the VEX-soil, instead of ryegrass, a mixture of clover and timothy grass was used. As shown in Figure 2, all 9 treatments (3 CH_4 pre-exposure levels x 3 plant species) were carried out in 5 replicates, or blocks, for a total of 45 samples.

For all the CH_4 pre-exposure levels, sampling points were defined based on the premise that, in a delimited area of 1.5 x 1.5 m, a sampling point was retained if the plant species of interest for the study covered at least 75% of total plant cover in the area. The sampling points chosen were geo-referenced using a GPS.

Soil sampling

Rhizospheric soil is defined as the soil that adheres to the roots after gently shaking them. Rhizospheric soil samples were collected in the upper 15-cm layer in July 2013, placed in sterilized plastic bags and kept at approximately 4°C until further processing. The soils were classified as fine sand and their natural physical characteristics (water content and organic matter) were measured. CH_4 incubation tests were performed without any adjustment of these initial values (Table 1).

121 **Table 1.** Physical characteristics of rhizospheric soil

Soil type	Plant species	Water content (w/dw %)[1]	Organic matter (%)[1]
NEX	Clover	11.3 ± 3.2	3.9 ± 0.7
	Timothy grass	13.9 ± 1.9	4.4 ± 0.7
	Ryegrass	10.0 ± 3.4	3.2 ± 1.5
MEX	Clover	20.9 ± 2.5	4.9 ± 1.4
	Timothy grass	21.2 ± 5.3	4.6 ± 1.4
	Ryegrass	26.2 ± 8.6	5.6 ± 0.9
VEX	Clover	21.3 ± 13.4	10.6 ± 3.0
	Timothy grass	27.8 ± 7.8	10.3 ± 1.7
	Mixture	22.7 ± 9.2	8.9 ± 1.8

122 [1] Data are the mean ± standard deviation of the 5 replicates of the rhizospheric soil.

123 **CH_4-ox kinetic measurements**

124 After rhizospheric soil samples were removed from the refrigerator, they were left
125 overnight at ambient temperature before incubation. Approximately 100 g of fresh soil
126 samples were incubated in 500-ml Mason jars tightly closed. The jars were previously
127 tested to confirm their air-tightness (no loss of gas). A rubber septum was fitted to the cap
128 of the Mason jars to allow gas injection and sampling using a syringe.

129 Methane was injected into the jars so as to reach an initial CH_4 concentration of ~ 45%.
130 Before CH_4 injection, an equal amount of air was removed from the jar to equilibrate the
131 pressure. The first gas measurement was taken within the first 2 minutes after gas
132 injection. CH_4, CO_2 and O_2 concentrations in the headspace were monitored using a
133 Micro GC Agilent Technologies 3000A gas chromatograph. Incubation in each jar
134 continued until the CH_4 concentration decreased below 1%.

Analyses were performed one block (Figure 2) at a time and 3 parameters were defined in order to describe the CH_4-ox kinetics: the lag time, which is the time required for the rapid acceleration in CH_4 consumption to occur; the oxidation rate, k, and the CH_4 oxidation half-life (related to the oxidation rate). The latter two are respectively the average slope of CH_4 consumption versus time plot, and the time required for the CH_4 concentration to decrease by one-half in the headspace.

The oxidation rate was obtained by fitting the measured CH_4 concentrations to the typical zero-order reaction equation, i.e. $-k = dC/dt$, where C is the concentration. k was normalized to the soil dry weight and its unit was μmol CH_4 /g d.w. /h.

Statistical analyses

The results were analysed in two different ways. The first was an analysis of the effect of the 9 different treatments (each representing a combination of CH_4 pre-exposure level and plant species; Figure 2) on lag time and CH_4 oxidation half-life responses. It was impossible to dissociate the effect of all the plant species studied herein from the effect of CH_4 pre-exposure, due to the fact that the plant species were not always the same for each level of CH_4 pre-exposure. A one-way ANOVA test was performed for each response variable of the 9 treatments. The second analysis (two-way ANOVA) tested the effect of CH_4 pre-exposure level and plant species on CH_4 oxidation half-life. Only timothy grass and clover, the 2 plant species found in all 3 zones of CH_4 pre-exposure, were considered in the second analysis. Post hoc Scheffé tests were performed after each ANOVA to identify differences between the treatments. For each statistical analysis, the statistical significance threshold was 95%.

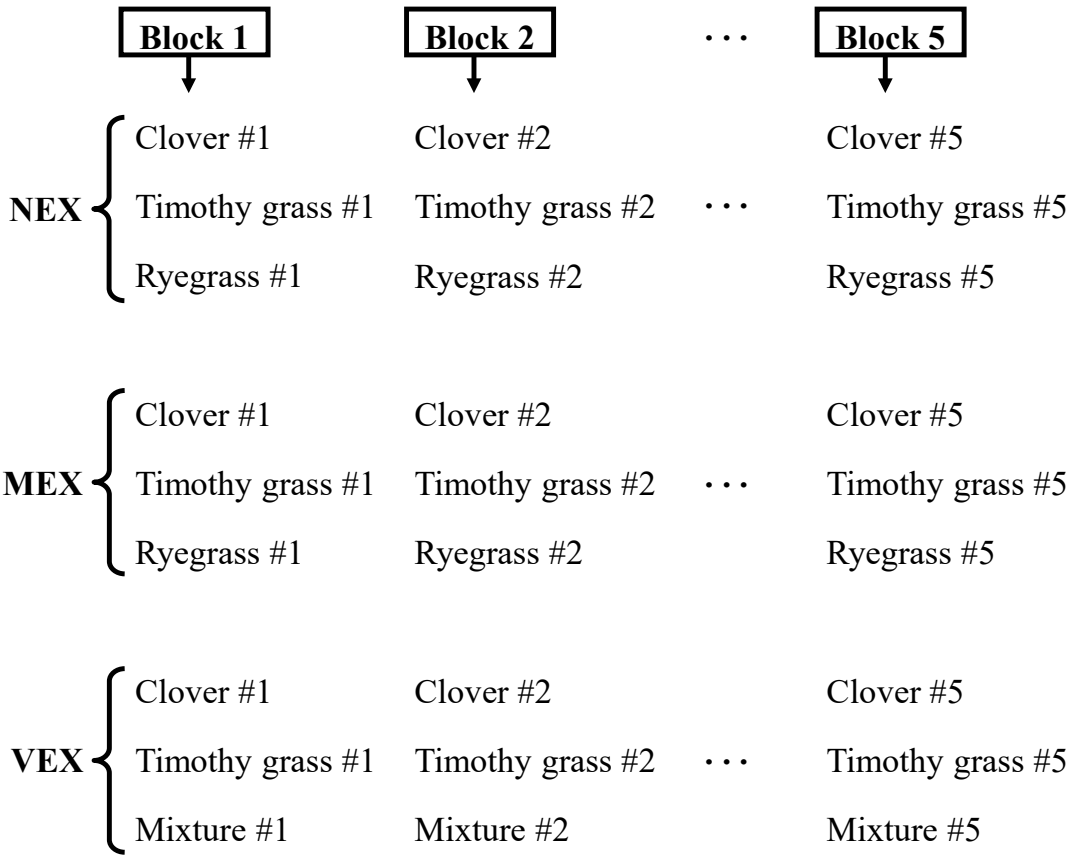
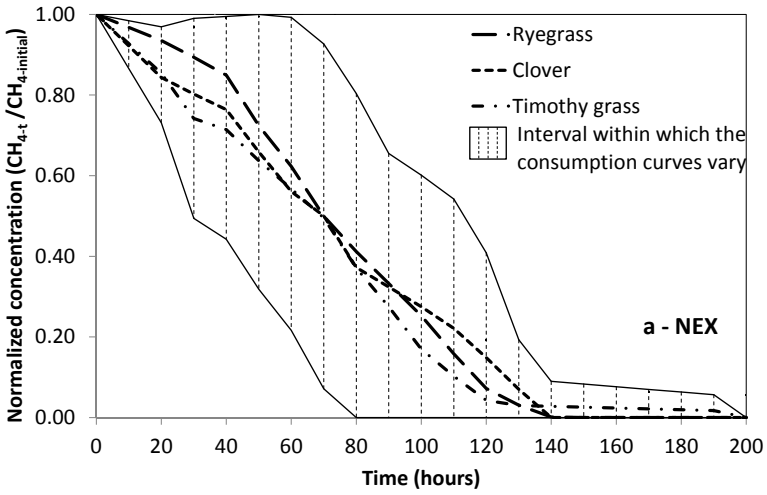


Figure 2. Flowchart of the CH_4-ox kinetic analyses

RESULTS AND DISCUSSION

CH_4 consumption with time

The comparison of normalized CH_4 concentration depletion in the rhizosphere of different plant species and for different levels of CH_4 pre-exposure is presented in Figure 3. The normalized CH_4 concentration is the ratio of CH_4 concentration at time t (monitored frequently) and the initial CH_4 concentration in the headspace (45%). The curves represent the average of the 5 replicates of the rhizospheric soils and the hatched zone defines the area within which the CH_4 consumption curves of the 5 replicates vary.



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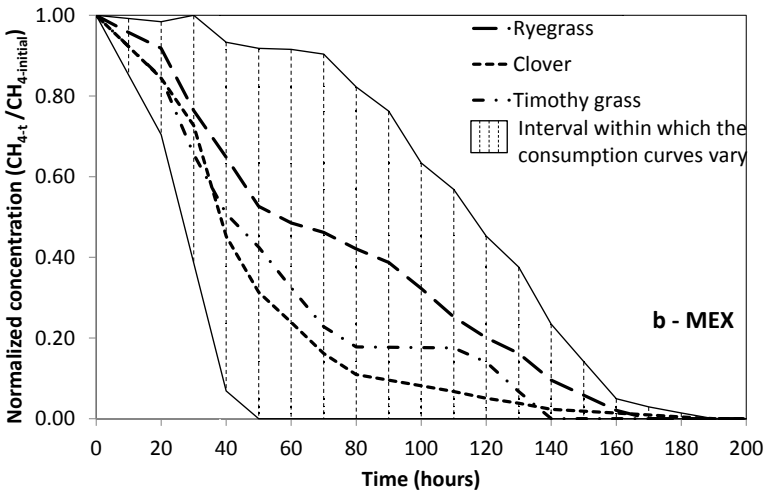

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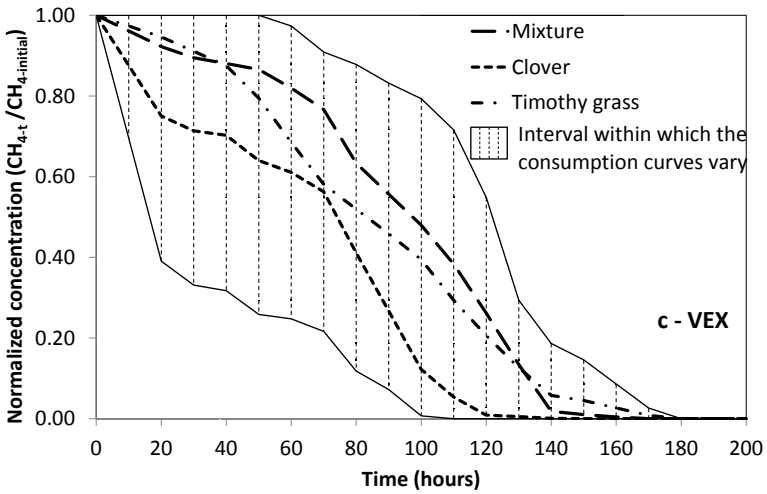

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171 **Figure 3.** Variation of average normalized CH_4 concentrations with time within the

172 rhizospheres of (a) never-before pre-exposed; (b) moderately pre-exposed; and (c) very

173 pre-exposed soils.

For all the plant species studied herein, it took 50 to 200 hours for the rhizospheric soil to oxidize all the CH_4. It is noteworthy that the average normalized methane concentration curves started to level off approximately at the same time in all cases. The average time for total CH_4 consumption was 140 hours. Regardless of plant species, the average curves for the NEX soils nearly superimposed, suggesting that the plant species studied herein did not affect the CH_4 response of NEX soils, as far as CH_4 oxidation is concerned. However, for the MEX and VEX soils, the curves obtained for the clover rhizosphere soil were below the others, suggesting that the CH_4 consumption in this rhizosphere was faster compared to timothy grass, ryegrass or mixture rhizospheres.

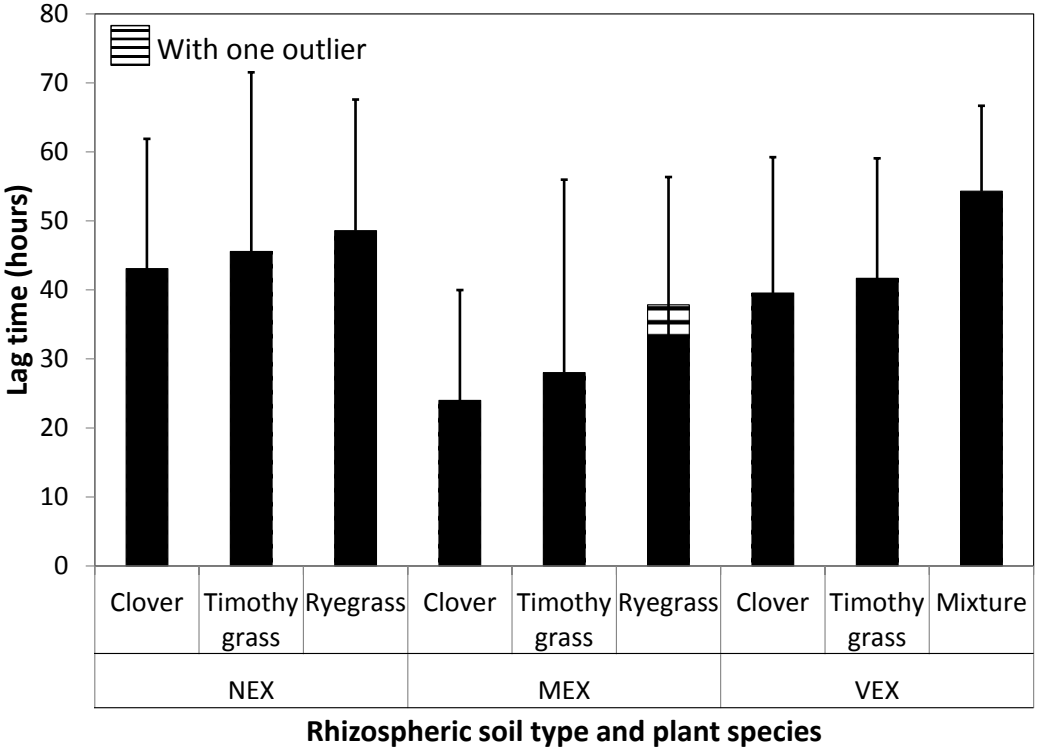
As expected, the normalized CH_4 concentration curves varied widely between replicates (hatched zone). This variation was attributed to the fact that the samples were not homogeneous. Variations could therefore be a consequence of the specific characteristics of the samples, such as water content and organic matter content. For timothy grass, for example, it took 50 hours for one replicate to consume all the CH_4, whereas for another replicate, it took 190 hours.

Figure 3 reveals 3 phases of the consumption curves: lag time, high CH_4 consumption and residual consumption. The lag time, whose values are presented in Figure 4, is the time required for the soil to accelerate CH_4 consumption. The high CH_4 consumption phase corresponds to a period of accelerated CH_4 consumption, i.e. where the oxidation rate (k) is the highest. The residual consumption phase is associated with the slowdown in the CH_4 consumption curve. The lag time for MEX soils (29.9±22.2 h) was shorter than those for the NEX and VEX soils (45.7±21.3 h and 45.2±16.5 h, respectively). Moreover, Figure 3 shows that the CH_4 consumption was faster for MEX than for the two

other soils. Indeed, after 60 hours, 50 to 75% of CH_4 was consumed in MEX soils, while only 20 to 40% was consumed in NEX and VEX soils. NEX and MEX samples were taken from places where the vegetation had been established for several years. Although no microbial analyses were performed for this project, one would expect to find well-established root zones and microbial biomass in these soil samples. The fact that MEX soils had been pre-exposed to CH_4 for several years led to shorter lag times (Figure 4), while lag times for the NEX samples were longer since the samples had never been pre-exposed to CH_4. In the case of VEX soils, the rhizosphere was sampled from field tests 4 months after the bare soil had been seeded. At the time of sampling, the vegetation was well grown and the rhizosphere had been exposed for more than one week to a CH_4 loading of approximately 200 g CH_4 $m^{-2}d^{-1}$; a very high loading that could characterize a hotspot. Since vegetation was only 4 months old for VEX samples, one cannot expect the root zone to be as mature as in the NEX and MEX samples. Consequently, the VEX microbial biomass was not as developed, despite the fact that the methanotrophic community was well developed.

An ANOVA performed on lag time data revealed that there was not a significant difference in lag time between the 9 treatments ($p>0.05$). However, a significant difference was found between the clover-MEX and the mixture-VEX soils. Indeed, the shortest lag time was obtained for the clover-MEX soil (23.98±16.02 h) while the longest was observed for the mixture-VEX soil (54.31±12.37 h). This result suggests that the rhizosphere of the clover and timothy grass mixture studied herein requires more time to develop a methanotrophic community and to accelerate CH_4 oxidation than all the other treatments. In a study evaluating the CH_4 oxidation efficiencies (MOE) of biosystems

220 covered with different types of plants, Ndanga et al.[5] found that the lowest MOE was
221 obtained for biosystems covered with the mixture of plants used in the present study,
222 rather than more uniform vegetation (such as timothy grass).



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224 **Figure 4.** CH_4 oxidation lag times in rhizosphere of NEX, MEX and VEX soils, for
225 different plant species. *Error bars* are standard deviations from experiments on 5
226 replicate soil samples

227 **Effect of plant species and CH_4 pre-exposure on CH_4-ox kinetics**

228 Table 2 shows the average of the oxidation rates (k) of the 5 replicates, and the standard
229 deviation. The constant k represents the rate of CH_4 consumption with time. It is the
230 average slope along the CH_4 oxidation experiment (until $[CH_4] \sim 0\%$). Therefore, the
231 greater the value of k, the faster the reaction. In order to determine the reaction order,

232 linear regression analyses were performed using the results of consumption tests
233 performed with the 5 replicates of each of the 9 treatments. First and zero order kinetic
234 models were tested. The limiting condition was the initial CH_4 concentration, which was
235 set at 45%. Most of the treatments were fitted with the zero order kinetic model (R^2 was
236 statistically significant when considering $p<0.05$). Accordingly, oxidation rates were
237 calculated using the zero-order reaction model. This corroborates what is usually
238 obtained in cases of high initial CH_4 concentration.[9-13]

239 **Table 2.** Average methane oxidation rates in never-before pre-exposed, moderately pre-
240 exposed and very pre-exposed rhizospheric soils.

Soil type	Plant species	Oxidation rate (k) (µmol CH_4 /g d.w./h)[1]
NEX	Clover	0.75 ± 0.29
	Timothy grass	0.97 ± 0.35
	Ryegrass	0.80 ± 0.30
MEX	Clover	1.21 ± 0.60
	Timothy grass	1.05 ± 0.48
	Ryegrass	0.79 ± 0.54
VEX	Clover	0.94 ± 0.65
	Timothy grass	0.63 ± 0.21
	Mixture	0.53 ± 0.12

241 [1] Data are the mean ± standard deviation (*n = 5*).

242 The oxidation rate values obtained in this study are very close to those reported in the
243 literature for landfill cover soils. In a review paper, Scheutz et al.[14] reported oxidation
244 rates for landfill soils in the same range as those presented above, i.e. 0.5 to 1.2 µmol
245 CH_4/g.d.w./h (8 to 19 µg CH_4/g.d.w./h).

246 It can be observed in the present study that all the k values fall within the same order of
247 magnitude (Table 2). This suggests that for the soil materials studied herein, CH_4
248 oxidation was not affected by CH_4 pre-exposure level and plant species. In order to
249 confirm this observation, a one way ANOVA was performed using the methane oxidation
250 half-life, which represents the time required for the concentration of a chemical to
251 decrease by one-half. The half-life is a direct function of the oxidation rate and is
252 calculated according to the following equation:

$$t_{1/2} = \frac{0.5 \times [CH_4]_{initial}}{k} \qquad [1]$$

253 Figure 5 presents the average $t_{1/2}$. The result of the ANOVA is presented in Table 3.
254 Similar to lag time, the MEX-soil had the lowest half-life values, suggesting that CH_4
255 consumption was the fastest in this rhizospheric soil. Indeed, the highest oxidation rates
256 were obtained in the MEX-soil (Table 2); specifically the clover-MEX which had the
257 shortest lag time and half-life and the highest oxidation rate. However, the statistical
258 analysis performed in all samples, including the outlier half-life value obtained for the
259 ryegrass-MEX soil, revealed that the differences between half-lives of the 9 treatments
260 were not significant ($p>0.05$), i.e. any difference in oxidation rate cannot be attributed to
261 plant species combined to CH_4 pre-exposure, whether or not the outlier is considered. In
262 the present case differences in half-life would be attributed to factors such as initial water
263 content of the samples, organic matter content, microbial biomass and stage of root
264 development.

265 In order to distinguish the possible effect of plant species from the effect of the CH_4 pre-
266 exposure on the value of $t_{1/2}$, a two-way ANOVA was performed with clover and timothy

grass for the NEX, MEX and VEX soils. This analysis shows that the two factors (plant species and CH_4 pre-exposure) do not have a significant effect on $t_{1/2}$ ($p>0.05$). An interaction test between these two factors on $t_{1/2}$ also showed that the plant species did not interact ($p>0.05$) with CH_4 pre-exposure, as far as CH_4 oxidation half-life was concerned.

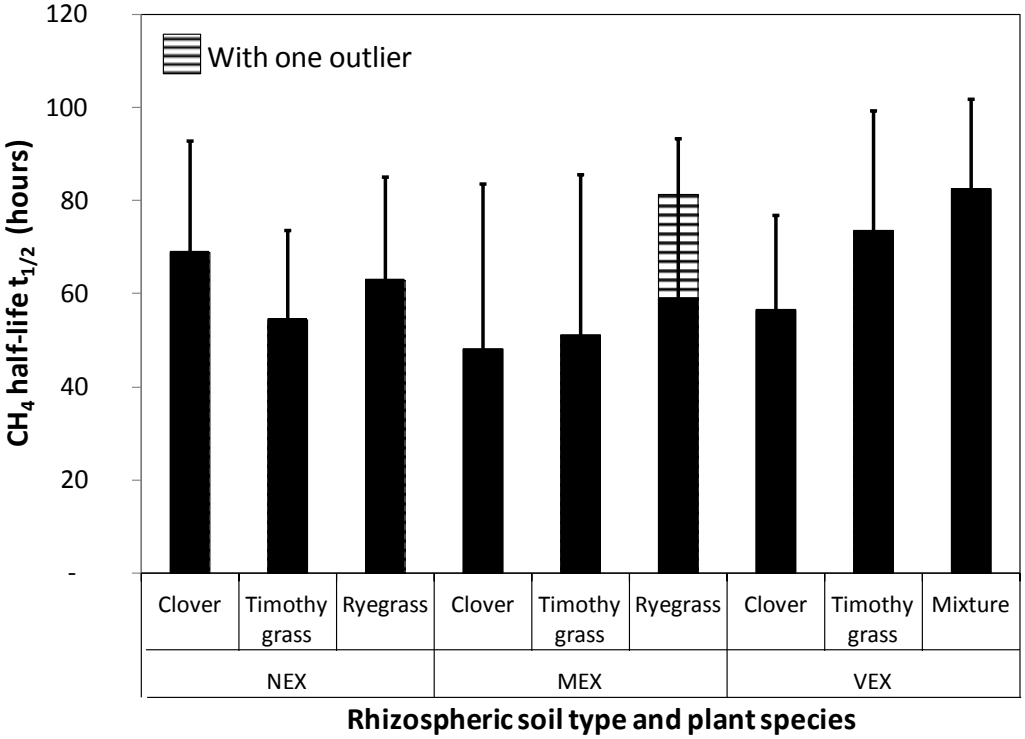

Figure 5. CH_4 oxidation half-lives in the rhizosphere of NEX, MEX and VEX soils for different plant species. *Error bars* are standard deviations from test results from 5 replicates.

Table 3. Results of the ANOVA evaluating the effects of plant species and level of CH_4 pre-exposure on half-life.

Source	*df*	Sum of squares	Mean Square	F statistic	p value
Block	4	6563.05	1640.76		
Model	8	6640.88	830.11	0.9426	0.496
Treatment	8	6640.88	830.11	0.9426	0.496
Residual error	32	28180.60	880.64		
Corrected total	44	41384.53			

Further discussions and practical aspects

The results presented above show that there is no clear relationship between the following parameters: 1) type of plant species considered herein (and their associated rhizospheric soil); 2) potential CH_4 oxidation in landfill covers; and 3) level of preconditioning the soil microbial communities with CH_4. In addition, Ndanga et al.[5] concluded that root depth did not influence the rate of methane oxidation in biosystems, for the same plant species studied herein. These results do not corroborate those obtained by Epp and Chanton[15], Watson et al.[16] and Nouchi et al.[17] among others, who concluded that there is a positive correlation between methane oxidation, root depth and plant species in wetlands, peat and other anoxic media.

In a study comparing the CH_4-ox kinetics of a rhizospheric clayey soil (planted) and a non-rhizospheric clayey soil (unplanted), both collected from a landfill cover, Wang et al.[4] found no significant difference in oxidation rates, except for one sampling day. They concluded that "plant growth plays an integrated role in enhancing the number and activity of soil methanotrophic bacteria". Furthermore, several authors have reported maximum k values for unplanted sandy loam and forest soils lower than those obtained in

this study during the high CH_4 consumption phase (obtained from the maximum slopes of the curves presented in Figure 3).[18-24] This suggests that rhizospheric soils may have promoted CH_4 oxidation by providing an environment conducive for microbial growth and activity. However, for mature compost Mor et al.[25] and Scheutz et al.[11] reported oxidation rate values two to five times greater. These findings are not surprising given that mature well-structured compost was identified and recognized as the best soil for CH_4 uptake.[14]

It is noteworthy that for the high initial CH_4 concentrations applied (45%), the lag time for all soils was less than 3 days. The lowest average lag times were obtained for moderately pre-exposed rhizospheric soil to CH_4. The latter may have created a favourable environment for methanotrophic development. There would be interest in further documenting the role of pre-exposure, because it could lead to novel strategies (or complementary measures) to mitigate hotspots in final covers, such as using previously exposed soils around hotspots.

The main limitations of this study were the number of plant species tested. It would be relevant to further study the interactions between vegetation and methane oxidation including complex phenomena such as changes in gas diffusion patterns, changes in the physical and chemical characteristics of soils due to plant growth, etc.

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Note: The authors declare no competing financial interest.

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