Coupling Stable Isotope Analysis with Gas Push-Pull Tests to Derive In Situ Values for the Fractionation Factor $\alpha_{ox}$ Associated with the Microbial Oxidation of Methane in Soils

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DOI
10.2136/sssaj2016.11.0387

Publication date
2017

Document Version
Accepted author manuscript

Published in
Soil Science Society of America. Journal

Citation (APA)

Important note
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Coupling stable isotope analysis with gas push-pull tests to derive in-situ values for the fractionation factor $\alpha_{\text{ox}}$ associated with the microbial oxidation of methane in soils

KEYWORDS
Landfill cover soil, greenhouse gas emission, methane oxidation, isotopic fractionation

ABSTRACT
Prerequisite for the application of stable isotope fractionation for the quantification of the methane oxidation efficiency of landfill covers is that the fractionation factor $\alpha_{\text{ox}}$ is known or can be estimated with adequate accuracy. So far, $\alpha_{\text{ox}}$ has only been determined in laboratory experiments. For the first time, $\alpha_{\text{ox}}$ was determined under in situ conditions in the field by coupling two independent methods, gas push-pull tests and stable isotope analysis, to assess biological fractionation of methane isotopologues in landfill cover soils. On six landfills with nine points of investigation, 22 measurements were carried out, covering a wide range of environmental conditions such as soil temperature and moisture and observed oxidation rates. Values for $\alpha_{\text{ox}}$ varied between near 1, indicating only little fractionation, and 1.151. Correlation of $\alpha_{\text{ox}}$ with the methane oxidation rate found by gas push-pull tests revealed a clear asymptotic
relationship with low rates being associated with high values for $\alpha_{\text{ox}}$ and high rates resulting in only little fractionation. Values for $\alpha_{\text{ox}}$ varied between the different landfills, but also between the individual points of investigation on the same landfill. The latter is assumed to reflect the spatial variability of methanotrophic activity due to spatial variability in soil moisture and hence air-filled porosity, as well as the spatial variability of gas fluxes. Further, significant variation of $\alpha_{\text{ox}}$ was observed also for the same sampling point, presumably reflecting the temporal variability of factors influencing methanotrophic activity. These effects could include seasonally changing environmental conditions such as soil temperature and moisture, but also the temporal variability of gas fluxes through the landfill soil cover, changing exposure of methanotrophs to methane and oxygen and hence their activity. The quantification of the methane oxidation efficiency using fractionation of stable isotopes is very sensitive to $\alpha_{\text{ox}}$. Assuming a value constant in time and space and transferring this value from laboratory experiments to field settings entails significant uncertainty regarding the quantification of methane oxidation.
INTRODUCTION

Landfills are estimated to be the second to third largest source of anthropogenic methane emissions (EEA, 2009; USEPA, 2016). Sanitary landfilling including active extraction and subsequent treatment or energetic utilization of landfill gas is mandatory in many countries. The microbial oxidation of methane in optimized landfill covers, windows and filters is an option to complement technical measures and mitigate residual methane fluxes for which technical treatment is no longer feasible (Bogner et al., 2007; Huber-Humer et al., 2008; Scheutz et al., 2009). It is also an option for the many old landfills that are not equipped with a gas extraction system or for landfills where gas production is low from the start. The potential of biological treatment is widely recognized. Both operators and regulators, however, require accepted methods to prove and quantify the mitigation effect.

The fractionation of stable isotopes (SI), naturally occurring as a result of the oxidation process, offers one of the few approaches to assess the efficiency of microbial methane oxidation ($f_{ox}$) in situ (Chanton et al., 1999, 2011; Börjesson et al., 2001, 2007; Cabral et al., 2010; Abichou et al., 2011; Widory et al., 2012). The method is based on the fact that carbon naturally exists in the form of two stable isotopes ($^{12}$C and $^{13}$C) whose size, steric properties and diffusion coefficient differ, leading to unequal behavior at enzyme binding sites (Jahnke et al., 1999) and to different transport rates. Due to the preferential oxidation (Barker and Fritz, 1981) and faster diffusive transport (Tyler et al., 1994; Reeburgh et al., 1997; De Visscher et al., 2004) of the lighter isotope, the heavier isotope is enriched in the remaining gas phase. The factors by which oxidation and transport fractionate, $\alpha_{ox}$ and $\alpha_{trans}$, respectively, are used to calculate methane oxidation efficiency from the difference in isotopic signature between the landfill gas and the gas emitted from the soil surface or any other point of interest. In the past, values of $\alpha_{ox}$ have been
determined in laboratory under standardized conditions and have then been used to calculate methane oxidation efficiencies from field isotopic signature data. However, as $\alpha_{ox}$ is known to vary with oxidation rate (Templeton et al., 2006; Chanton et al., 2008), temperature (Chanton et al., 2008), and possibly the composition of the methanotrophic community (Jahnke et al., 1999; Templeton et al., 2006), the value was hypothesized to be subject to site-specific and seasonal variation.

In order to determine $\alpha_{ox}$ in situ, the oxidation efficiency must also be determined in situ. This is possible using the gas push-pull test (GPPT) methodology (Gómez et al., 2009; Urmann et al., 2009; Streese-Kleeberg et al., 2011). During a GPPT, a defined volume of the reactive gas of interest (in this case methane and oxygen) and a conservative tracer with similar transport properties (e.g. argon) are injected into the soil’s vadose zone. Following injection, the mixture of reactant, tracer and soil gas is extracted via the same tube. During extraction, the gas mixture is sampled periodically in order to obtain breakthrough curves of reactant and tracer. The reaction rate is calculated from the difference in the breakthrough curve of the reactive gas compared to the one of the tracer. The combination of GPPTs with SI analyses allows the in situ determination of $\alpha_{ox}$ by simultaneously collecting data on the change of the isotopic composition and on the oxidation efficiency. The aim of the study was to verify the range of fractionation factors previously published from laboratory experiments and to investigate the hypothesized in-situ variation of the fractionation factor resulting from to the variation in field oxidation rates.
MATHEMATICAL BACKGROUND OF THE STABLE ISOTOPE APPROACH

The shift in the isotopic ratio of $^{13}\text{C}$ to $^{12}\text{C}$ through microbial methane oxidation or through diffusive gas transport, signified in the delta notation as $\delta^{13}\text{C}$, is described by the following equation:

$$\delta^{13}\text{C} \text{ [%]} = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right) \times 1000$$  \hspace{1cm} \text{Eq. 1}$$

Where $R_{\text{sample}}$ = $^{13}\text{C}/^{12}\text{C}$ ratio in the sample

$R_{\text{standard}}$ = $^{13}\text{C}/^{12}\text{C}$ ratio of the carbon reference standard VPDB (Vienna Peedee Belemnite, $^{13}\text{C}/^{12}\text{C} = 0.0112372$).

The fractionation factors effective during the oxidation process and during gas transport are termed $\alpha_{\text{ox}}$ and $\alpha_{\text{trans}}$, respectively, and are defined as the oxidation or transport rate constant for $^{12}\text{CH}_4$ divided by the oxidation or transport rate of $^{13}\text{CH}_4$:

$$\alpha_{\text{ox, trans}} = \frac{k_{12}}{k_{13}}$$  \hspace{1cm} \text{Eq. 2}$$

No fractionation is assumed when gas transport is advective, then $\alpha_{\text{trans}}$ is equal to 1.

The exact factors for oxidative and transport fractionation need to be known to quantify methane oxidation efficiency from the measured shift in stable isotope ratios as follows (Blair et al., 1985):

$$f_{\text{ox}} = \frac{(\delta_s - \delta_{\text{anox}})}{1000 \times (\alpha_{\text{ox}} - \alpha_{\text{trans}})}$$  \hspace{1cm} \text{Eq. 3}$$
Where $f_{ox} = \text{fraction of methane load oxidized at the particular sampling location, also}$

\text{defined as methane oxidation efficiency}

$\delta_s = \delta^{13}C \text{ value in sample}$

$\delta_{anox} = \delta^{13}C \text{ value at the chosen point of reference, often the anaerobic zone of}$

methane production

$\alpha_{ox}, \alpha_{trans} = \text{factors for oxidative and diffusive fractionation.}$

As a fraction of 1 or a percentage (if multiplied by 100), $f_{ox}$ represents a relative value, not an

absolute oxidation rate.

3 MATERIALS AND METHODS

2.1 Site description

Investigations were carried out on five old non-sanitary municipal solid waste landfills (landfills A, D, L, H and K) in north western Germany, all filled in the 1960s to the 1980s. The landfills were part of a baseline study on methane oxidation in non-optimized cover soils, conducted within the framework of the MiMethox project (Rachor et al., 2009). The landfills were covered with whatever soil was available to the operator at landfill closure, mostly of sandy to loamy texture, cover thickness varied between 0.1 m and > 2 m. The vegetation that established since the placement of the soil cover consisted of grass, shrubs and small trees. None of the sites had a surface sealing so that landfill gas migrated freely from the waste body through the soil cover. Potential methane oxidation rates as determined in laboratory ranged between 25 and > 2000 g CH$_4$ m$^{-2}$ d$^{-1}$ (Gebert et al., 2016). For the purpose of the MiMethox project, all landfills were
equipped with gas probes, temperature and moisture sensors in 5, 15, 40, and 80 cm depth of the soil cover on three locations per landfill (details in Gebert et al., 2011a). These locations had been chosen based on a preliminary soil and soil gas survey. This study used the same locations. The composition of the methanotrophic community was dominated by *Methylocystis*, *Methylobacter* and *Methylococcus* species among type II, Ia and Ib methanotrophs, respectively, on all of the five investigated landfills (Gebert et al., 2009). Detailed information on the landfills, on the properties of the cover soils, and on the gas fluxes through and from the soil cover is given in Rachor (2012).

Further measurements were performed on two methane oxidation cover test cells (cells C and G) on a Dutch landfill (landfill W), constructed with a loamy sand (topsoil) above a loam (subsoil; classification according to FAO, 2006) and a forced gas load to the base of the methane oxidation layer of up to 41 g CH$_4$ m$^{-2}$ d$^{-1}$. The test cells had a size of 1.060 m$^2$ each. They served to investigate the combination methane oxidation and measures to limit drainage water infiltration. The latter was realized by a capillary barrier (cell C) and a drainage mat (cell G). Short grass vegetation was maintained on these test cells by regular mowing. More information on the test cell setup is given in (Röwer, 2016a; Geck et al., 2016).

### 2.2 Gas push-pull tests

The application of the gas push-pull test to quantify methane oxidation rates in landfill cover soils including a detailed description of the methodology and the nature and interpretation of results on 50 GPPTs is described in depth in Streeese-Kleeberg et al. (2011). This section summarizes the principal approach of the GPPT. A gas mixture of 30 l, containing methane, oxygen and argon (10% CH$_4$, 20% Ar in air), were injected at the designated soil depth (usually
between 40 and 60 cm below surface) at a flow rate of 2.5 l/min using a pump connected to a mass flow meter and a tube with a perforated tip. The depth was chosen so that the methane background concentration would not exceed 5% and the oxygen background concentration indicated oxidative conditions in the soil. Hereby, it was prevented that a high methane background confounded the GPPT results. Soil gas composition was verified beforehand by on-site analysis of the soil gas phase using a biogas analyser (BM2K2-E000, Geotechnical Instruments (UK) Ltd.) for measurement of CH₄ (infrared detector), CO₂ (infrared detector) and O₂ (electrochemical detector). The detection limit for all three gases was 0.1 vol.%, Instrument calibration was performed with certified calibration gases in the laboratory and validated with parallel gas-chromatographic analyses.

Following injection, the mixture of reactant, tracer and soil gas were extracted from the same tube. The extraction flow rate was 0.3 to 0.5 l/min. During the extraction phase the gas was streamed through 40 ml glass vials with septum caps which were exchanged every 1 to 4 minutes. The time interval depended on the rate of decline of the methane concentration. This was continuously measured in the extracted gas stream with an infrared cell. These samples were analysed for methane and argon concentrations. The vials contained a barrier solution (200 g NaCl l⁻¹ and 5 g citric acid l⁻¹ in water) to prevent gaseous diffusion through the vial septa and were stored in an inverted position until analysis. In addition, gas samples intended for carbon stable isotope analyses were collected from the gas stream using a syringe and injected into vials containing a supersaturated salt solution. Supersaturation served to prevent dissolution of gas into the liquid phase. Vials were also stored in an inverted position until stable isotope analyses took place. Three to eleven stable isotope samples were retrieved during each GPPT. The average δ¹³C signature of the injected gas was -41.56 ± 0.89‰ (standard deviation).
Data evaluation of the GPPT was performed according to Yang et al. (2007). First, reactant and tracer were corrected for background concentrations for each sampling point. The corrected concentrations were then normalized to the concentration of the injected reactant and tracer. Thereafter, the normalized concentrations were plotted over time elapsed since the commencement of the extraction phase. From the normalized tracer concentrations, the mixing factor was obtained for each sampling point as follows:

\[ f = \frac{(C_{tr} - C_{tr}^b)}{(C_{tr}^0 - C_{tr}^b)} \]  

Eq. 4

Where \( f \) = mixing factor

\( C_{tr} \) = current tracer concentration

\( C_{tr}^b \) = background tracer concentration

\( C_{tr}^0 \) = injected tracer concentration.

All concentrations are to be expressed in the same unit.

The normalized concentrations decrease at a higher rate than those of the tracer and so-called net mass transfer takes place if the reactant is consumed, i.e. in this case is microbially degraded. Net mass transfer thus represents the change in reactant (methane) concentration that cannot be attributed to dilution and therefore represents methane consumption. The decrease in concentration is due to dispersion in the soil only if the slopes of reactant and tracer are similar.

The net mass transfer is calculated as follows:

\[ NMT = C_r - [f \times C_r^0 + (1 - f) \times C_r^b] \]  

Eq. 5
Where \( NMT \) = net mass transfer

\[ C_r = \text{current reactive gas concentration} \]

\[ f = \text{mixing factor} \]

\[ C_r^0 = \text{injected reactive gas concentration} \]

\[ C_r^b = \text{background reactive gas concentration}. \]

All concentrations are to be expressed in the same unit, the net mass transfer then assumes this unit.

Prerequisite for the validity of the approach is that reactant and tracer gases exhibit similar transport characteristics in the soil gas phase. In the case of methane, argon meets this condition (Gómez et al., 2009). The methane oxidation rate is derived as a zero order kinetic constant from the slope of the net mass transfer over time:

\[ k_0 = \frac{NMT}{t} \]

Where \( k_0 \) = zero order rate constant and \( t \) = time.

The zero order rate constant yields the methane oxidation rate in mass unit CH\(_4\) per volume unit soil air per unit time. It has to be considered that this rate is to be interpreted as a potential rate, as both oxygen and methane are supplied at optimum concentration levels during the test.

### 2.3 Analysis of stable isotopes

The \( \delta^{13}C \) ratio was analysed in triplicate using a GC-IRMS (gas chromatography isotope ratio mass spectrometry, Delta Plus, ThermoScientific, Dreieich, Germany) equipped with a 25 m
capillary column (Poraplot, 0.32 mm). The reference standard NGS3 8561 ($\delta^{13}C_{VPDB}$-NGS3 8561 = -73.27‰; NIST, Gaithersburg, MD, USA) was used to express the methane-derived $\delta^{13}C$ ratio relative to the VPDB (Vienna Pee Dee Belemnite) standard. Analytical replicate precision was < 0.2‰.

2.4 Calculation of fractionation factor $a_{ox}$

Values for $a_{ox}$ were obtained using GPPT and methane-derived $\delta^{13}C$-data based on the approach derived by Coleman et al. (1981) from Rayleigh (1896):

$$\delta^{13}C_t \cong 1000 \times \left( \frac{1}{a_{ox}} - 1 \right) \times \ln \left( \frac{M}{M_0} \right) + \delta^{13}C_{t=0}$$

Eq. 7

Where

$$\delta^{13}C_t = \delta^{13}C \text{ value of CH}_4 \text{ remaining at time } t \text{ at the particular soil depth during the GPPT}$$

$$M/M_0 = \text{fraction of CH}_4 \text{ (corrected for dilution) remaining at time } t \text{ at the particular soil depth related to the initial methane concentration } M_0 \text{ (also corrected for dilution)}$$

$$\delta^{13}C_{t=0} = \delta^{13}C \text{ value of CH}_4 \text{ at the initial time, i.e. of the mixture injected into the soil.}$$

The slope of the line $\delta^{13}C_t - \delta^{13}C_{t=0}$ versus $\ln(M/M_0)$ (see also Figure 2) is equal to $1000 \times (1/a_{ox} - a_{trans})$, so that $a_{ox}$ was calculated as follows:

$$\frac{1}{a_{ox}} = 1 + \frac{(\delta^{13}C_t - \delta^{13}C_{t=0})}{\ln(M/M_0) \times 1000}$$

Eq. 8

Pushing and pulling the gas into and out of the soil results in advective gas transport. Methane concentrations can be assumed to be spatially uniform within the injected and extracted volume a
given point in time. Hence, gas transport driven by a concentration gradient was assumed to be insignificant within the time frame of the test. Therefore, fractionation due to diffusive gas transport (Barker and Fritz, 1981) was neglected and $\alpha_{\text{trans}}$ was assumed to be equal to 1. Values for $\alpha_{\text{ox}}$ were only accepted when the correlation coefficient of the slope was significant on a 99% confidence level.

4 RESULTS AND DISCUSSION

Table 1 summarizes the methane oxidation rates derived from the GPPTs, the details of the respective environmental conditions, the soil properties as well as the calculated values $\alpha_{\text{ox}}$. Some of the information can also be found in Streese-Kleeberg et al. (2011) who introduce the GPPT method adapted for landfill cover soils and present data on more than 50 GPPTs. The following sections discuss the data on environmental conditions, soil properties, and the variability of $\alpha_{\text{ox}}$ in relation to the oxidation rate.

<table>
<thead>
<tr>
<th>Site ID notation</th>
<th>Temp.</th>
<th>moist.</th>
<th>Soil properties, environmental conditions, fractionation factors and oxidation rates for each site. n.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>n.d. = not determined. Temp. = temperature, moist. = moisture. Site ID notation: H, K, D, L, A = different MSW landfills in north western Germany; WC, WG = test cell C and test cell G on landfill W in The Netherlands. P1-5 = individual sampling points.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.1 Environmental conditions, soil properties and methane oxidation potential

Both the environmental conditions and soil properties such as porosity varied between the individual points of investigation but also for the same point over time. Over all landfill sites and points of investigation, soil temperature spanned between 1.0 and 20 °C, and soil moisture ranged between 3.5 and 26.1 vol%. Air-filled porosity, predominantly determining the soil’s gas transport properties, varied between 10.2 and 32.9 vol%.
The seasonal variation of soil temperature and moisture influences the microbial process of methane oxidation (e.g. Gebert et al., 2003; Spokas and Bogner, 2011) and hence also emission (e.g. Tecle et al., 2009; Rachor et al., 2013; Geck et al., 2016). The variability of texture, compaction and moisture strongly impacts soil gas diffusivity and gas conductivity (Mølndrup et al., 2000; Gebhardt et al., 2009; Gebert et al., 2011b; Röwer et al., 2016b) and thereby modulates the methane oxidation rate in landfill soil covers by influencing the ingress of atmospheric air.

Further variability of methane oxidation rates is introduced by the varying level of exposure of the methanotrophic community to methane (Röwer et al., 2011; Schroth et al., 2012), leading to methane oxidation rates spanning several orders of magnitude on one or between different landfill sites (Spokas and Bogner, 2011; Gebert et al., 2016).

Given the above, significant spatial and temporal variability of the methane oxidation rate in landfill cover soils can be expected \textit{a priori} and was also observed during the gas push-pull tests conducted in this study (Table 1). For the six investigated landfill cover soils the range of oxidation rates found with the GPPTs ranged between 2.3 and 144 g m$^{3}_{\text{soil air}}$ h$^{-1}$, corresponding to 0.32 and 18 g m$^{3}_{\text{soil}}$ h$^{-1}$ when values for total porosity and soil moisture at the time of measurement were considered. These oxidation rates relate to efficiencies of 3\% and 100\% at the end of the GPPT. An extraordinarily high rate of 1110 g m$^{3}_{\text{soil air}}$ h$^{-1}$ or 354 g m$^{3}_{\text{soil}}$ h$^{-1}$ was found for location WC1. Sites WC1 and WC4 are located within 10 m of each other on a test cell investigating the suitability of biocovers for the mitigation of landfill methane. As indicated by gas profile measurements at both sites (data not shown), WC1 receives high concentrations of methane whereas due to spatial heterogeneity of gas fluxes the exposure to landfill gas at WC4 is low. As a result, WC1 developed a very high potential activity of 1110 g CH$_{4}$ m$^{3}_{\text{soil air}}$ h$^{-1}$, compared to only 50 g CH$_{4}$ m$^{3}_{\text{soil air}}$ h$^{-1}$ at WC4. Evolution of methanotrophic potential strongly
depends on the supply of the substrate methane (Röwer et al., 2011; Spokas and Bogner, 2011; Schroth et al., 2012) and can hence span orders of magnitude in relation to the spatial heterogeneity of fluxes. In line with this, GPPT-based oxidation potentials for the cover soil on a Swiss municipal solid waste landfill were found to be as high as 210 g m$^{-3}$ soil h$^{-1}$ for sites where high methane concentrations were measured in the soil gas phase. The oxidation potential was below the GPPT’s detection limit where soil methane concentrations were very low (Schroth et al., 2012).

4.2 Shift of the methane $\delta^{13}$C signature with oxidation efficiency

During the time course of each GPPT, the $\delta^{13}$C signature in the sampled soil gas phase shifted towards less negative values, i.e. the heavier isotopologue $^{13}$CH$_4$ became enriched as the lighter isotopologue ($^{12}$CH$_4$) was preferentially consumed by the methanotrophic bacteria. In Figure 1, the change in isotopic signature is plotted versus the oxidation efficiency ($f_{ox}$) determined for each individual sampling point during each of the 22 GPPTs. For the entire data set, the efficiency ranged between near 0% and near 100%. The maximum observed enrichment in $\delta^{13}$C was 62‰ (from -41 to +21‰ for GPPT no. 26 at site HP5, compare also Table 1), with large shifts suggesting vigorous methane oxidation activity. As expected, the degree of enrichment generally increased with increasing oxidation efficiency. However, some data points showed little enrichment in relation to the oxidation efficiency while for others, the extent of enrichment was substantially higher than would be expected from the relationship between $f_{ox}$ and $\delta^{13}$C for the bulk data, indicating different extents of isotopic fractionation during the oxidation process.

Figure 1: Relationship between methane oxidation efficiency $f_{ox}$ and respective shift in carbon isotopic signature of methane relative to the injected gas. Data from 22 individual GPPTs with three to six individual points per GPPT (compare also Table 1).
Figure 2 shows how the change in isotopic signature over time ($\delta^{13}C_t - \delta^{13}C_{t=0}$) relates to the natural logarithm of the remaining methane ($\ln(M/M_0)$), using an example of a GPPT yielding a low oxidation rate (#51, site DP1) and one yielding a high oxidation rate (#59, also site DP1). According to Eq. 8, values for $\alpha_{ox}$ were calculated from the slope of this line, if significant on a 99% level.

Figure 2: Relationship between the fraction of methane remaining during the course of a GPPT, expressed as $\ln(M/M_0)$, and the change in isotopic signature, expressed as $\delta^{13}C_t - \delta^{13}C_{t=0}$, for GPPTs 51 (left panel, point DP1) and 59 (right panel, also point DP1). Line = linear fit.

4.3 Calculated values for $\alpha_{ox}$ and their relationship to the methane oxidation rate

The literature reports values for $\alpha_{ox}$ between 1.018 and 1.049 (Chanton et al., 1999; Chanton and Liptay, 2000; Börjesson et al., 2001, 2007; De Visscher et al., 2004; Capanema and Cabral, 2012), a range also represented by 64% of the measurements in this study (Table 1, Figure 3). Two GPPTs showed lower and six of the 22 GPPTs higher isotopic fractionation. The relationship between the in-situ methane oxidation rate derived from the GPPTs and the values for $\alpha_{ox}$ is shown in Figure 3. Values for $\alpha_{ox}$ were high at low oxidation rates and decreased nonlinearly with increasing methane oxidation rate, approximating the value of $\alpha_{ox} = 1$ (i.e. no fractionation) at very high rates (sampling point WC1). Hardly any discrimination of the heavier isotopologue was observed under these conditions of high methanotrophic activity (GPPTs #53 at site KP2, #103 at site WC3). Vice versa, a low activity allows for a high extent of discrimination (e.g. GPPTs #41 at site DP1, #44 at site LP1). The observed fractionation factor not only varied between the different sites but also for different GPPTs conducted at the same landfill and the same sampling point, presumably reflecting the temporal variability of process controls (e.g. temperature, moisture and hence oxygen supply) over the seasons. The maximum
span of values for $\alpha_{ox}$ observed for one individual point of investigation was 1.035 to 1.100 (point DP1, Table 1, Figure 3).

Figure 3: Relationship between methane oxidation rate and fractionation factor $\alpha_{ox}$, n = 22. For site IDs see heading of Table 1.

The general relationship between environmental conditions, the methane oxidation rate and the fractionation factor $\alpha_{ox}$ can be deduced from the largest single data set available from site DP1 (n = 7), shown in Figure 4. It is seen that cooler conditions in the winter coincide with increased soil moisture, which is typical for the temperate European climate with the lower methane oxidation potentials and with higher values for $\alpha_{ox}$. Vice versa, the highest oxidation potentials and the lowest extent of fractionation are found when the soil is warmer and dryer. The same seasonal pattern is apparent for sites HP5 and KP2. All in all, the individual data sets are too small for a multivariate statistical analysis of the factors of influence, which, however, have been previously described (e.g. Scheutz et al., 2009).

Figure 4: Relationship between environmental parameters, methane oxidation rate and fractionation factor $\alpha_{ox}$ as found at site DP1 (data from Table 1).

The data from sampling point DP1 were also used to assess the effect of the variability of $\alpha_{ox}$ on calculated values for the oxidation efficiency $f_{ox}$, by example of GPPT #28. To this end, $f_{ox}$ was calculated for the change in isotopic signature observed for this particular test (-40.36‰ to -16.54‰) employing the seven individual values for $\alpha_{ox}$ determined during the seven GPPTs conducted at site DP1 (1.036 to 1.100, see Table 1). The resulting oxidation efficiencies varied between -2.5% and 66%, indicating that it is not possible to assume $\alpha_{ox}$ to be constant even in the very same sampling location (in this case, DP1).

In another sensitivity analysis, Capanema and Cabral (2012) varied the value of $\alpha_{ox}$ by ±0.5% of the value determined in the laboratory on their own samples. The resulting methane oxidation
efficiencies ($f_{ox}$) varied between 53 and 88% for the same data set. Increasing $\alpha_{ox}$ by 0.5% led to implausible oxidation efficiencies greater than 100%. All examples show that the sensitivity of the method to the correct value of $\alpha_{ox}$ is hence very high and that hence the exact value of $\alpha_{ox}$ needs to be determined for each individual combination of conditions under which the methane oxidation efficiency of a landfill cover is assessed.

The observed variation of isotopic fractionation with oxidation rate corroborates findings from earlier studies. Templeton et al. (2006) showed that most important factor determining fractionation of methane isotopologues was the fraction of total methane oxidized per time unit, with values for $\alpha_{ox}$ decreasing with increasing turnover rates and increasing cell densities. Chanton et al. (2008) found $\alpha_{ox}$ to decline nonlinearly with increasing maximum methane oxidation rates ($v_{max}$), indicating less discrimination at higher turnover rates when the temperature is constant.

In summary, it was shown that values for $\alpha_{ox}$ varied at the same sampling point, between different sampling points on the same landfill, and between different landfills. Further, the oxidation rate strongly affected the extent of discrimination of the heavy isotopologue and hence the magnitude of $\alpha_{ox}$. Low rates caused a high extent of fractionation whereas at very high rates $\alpha_{ox}$ approximated the value of one, i.e. no preferential oxidation of the lighter isotopologue was observed. In conclusion, the exact value for $\alpha_{ox}$ and its variability in time and space should be known to reduce uncertainty in the determination of the methane oxidation efficiency. Since soil temperature and moisture as well as the spatial and temporal heterogeneity of gas fluxes through landfill covers result in continuously changing environmental conditions, the extent of both transport and biological fractionation will vary correspondingly. Adopting $\alpha_{ox}$ values from the literature or assigning the same $\alpha_{ox}$ value to the same measurement point over time or assigning...
the same value to different points on one or several landfills can lead to misjudgment of methane oxidation efficiencies. In order to avoid this, $\alpha_{ox}$ would have to be determined for each individual campaign using undisturbed soils samples at the prevailing moisture content. Depending on the desired level of accuracy, these limitations affect the applicability of the stable isotope approach as a robust, practical and economic method for the exact quantification of methane oxidation efficiencies.

FUNDING SOURCES

The research was funded by the German Federal Ministry for Education and Research (BMBF) within the framework of the project MiMethox (Microbial Methane Oxidation in Landfill Covers).

ACKNOWLEDGMENT

The authors would like to thank Dr. Ingke Rachor, Dr. Kim-Karen Kleeberg, Volker Kleinschmidt, Cindy Streblow, Simon Klauke and Dr. Christian Knoblauch for their assistance in field and laboratory measurements.
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Table 1: Soil properties, environmental conditions, fractionation factors and oxidation rates for each site. n.d. = not determined. Temp. = temperature, moist. = moisture. Site ID notation: H, K, D, L, A = different MSW landfills in north western Germany; WC, WG = test cell C and test cell G on landfill W in The Netherlands. P1-5 = individual sampling points.
Figure 1: Relationship between methane oxidation efficiency $f_{ox}$ and respective shift in carbon isotopic signature of methane relative to the injected gas. Data from 22 individual GPPTs with three to six individual points per GPPT (compare also Table 1).
Figure 2: Relationship between the fraction of methane remaining during the course of a GPPT, expressed as $\ln(M/M_0)$, and the change in isotopic signature, expressed as $\delta^{13}C_t - \delta^{13}C_{t=0}$, for GPPTs 51 (left panel, point DP1) and 59 (right panel, also point DP1). Line = linear fit.

Oxidation rate
3.6 g m$^{-3}$ soil air h$^{-1}$

Oxidation rate
46.2 g m$^{-3}$ soil air h$^{-1}$

GPPT 51
slope = -46.70
$r = -0.995$

GPPT 59
slope = -56.87
$r = -0.997$
Figure 3: Relationship between methane oxidation rate and fractionation factor $\alpha_{\text{ox}}$. $n = 22$. For site IDs see heading of Table 1.
Figure 4: Relationship between environmental parameters, methane oxidation rate and fractionation factor $\alpha_{ox}$ as found at site DP1 (data from Table 1).
Table 1: Soil properties, environmental conditions, fractionation factors and oxidation rates (potentials) for each site. n.d. = not determined. Temp. = temperature, moist. = moisture. Site ID notation: H, K, D, L, A = different MSW landfills in north western Germany; WC, WG = test cell C and test cell G on landfill W in The Netherlands. P1-5 = individual sampling points.

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<th>Background CH₄ (vol.%</th>
<th>Background O₂ (vol.%</th>
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