PERGAMON

Soil Biology & Biochemistry 32 (2000) 1121–1130

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Variability of soil methane production on the micro-scale: spatial association with hot spots of organic material and Archaeal populations

Gisela Wachinger[a,*], Sabine Fiedler[a], Kornelia Zepp[b], Andreas Gattinger[c], Michael Sommer[a], Kurt Roth[d]

[a]*Institute of Soil Science 310, University of Hohenheim, Emil-Wolff-Str. 27, D-70599 Stuttgart, Germany*
[b]*EAWAG, Seestr. 79, CH-6047 Kastanienbaum, Switzerland*
[c]*GSF-National Research Center for Environment and Health, Institute of Soil Ecology, D-85764 Neuherberg, Germany*
[d]*Institute of Environmental Physics, Im Neuenheimer Feld 229, D-63125 Heidelberg, Germany*

Received 20 February 1999; received in revised form 2 December 1999; accepted 6 February 2000

Abstract

High temporal and spatial variability is a key problem when quantifying methane emissions from soils. Whereas the spatial variability on the landscape scale has been investigated in different studies, we investigated the spatial heterogeneity of CH_4 production on 1 cm scale, as well as the role of organic material as a relevant factor. Undisturbed soil cores (dia. 6 cm) of two mineral and one peaty wetland soils (Typic Humaquept, Aeric Endoaquept and Limnic Haplohemist) from the cool-humid region in southwest Germany were anaerobically incubated for 3 months. The time course of the CH_4 production rates was dependent on the water-table-level history of the incubated horizon and on the soil type. However, the absolute amounts of CH_4 production differed largely between parallel cores from each soil type, although they were obtained within 1 m^2. The native structures of the soil cores were determined by computed tomography. Fresh organic material was observed in all highly productive soil cores, whereas soil cores with low methanogenic activity included far less fresh organic material. The observed hot spots of fresh organic material were correlated to high amounts of Archaea, as analyzed by etherlipid analysis as well as by in situ hybridization using an Archaea-specific probe. The most dominant factor for the spatial variation in CH_4 production on the micro-scale is the distribution of fresh organic material, which activates and possibly attracts methanogenic Archaea (methanogens).

Keywords: Soil methane production; Spatial variability; Organic material; Computed tomography; In situ hybridization; Etherlipid analysis

1. Introduction

In recent years, great efforts have been undertaken to quantify methane emissions. However, a prospective calculation of CH_4 emission from different soil types has remained difficult because of the high temporal and spatial variability of CH_4 emissions (Granberg et al., 1997) at the landscape scale (Moore et al., 1990; Valentine et al., 1994) as well as at the m^2-scale (Adrian et al., 1994). In studies at three sites in the Allgäu-region, the high temporal variability of CH_4 emissions even exceeded the spatial variability on the landscape scale and there was no strong correlation to abiotic factors such as depth of water table, pH or soil temperature (Fiedler and Sommer, unpublished data).

Emission of CH_4 from soils is the net result of the anaerobic production and the aerobic consumption of CH_4. In soils with high amounts of gas-filled pores, where oxic processes take place, CH_4 oxidation will outweigh CH_4 production. The temporal variability of

* Corresponding author. Fax: +49-711-810-6405.
E-mail address: gisela@uni-hohenheim.de (G. Wachinger).

0038-0717/00/$ - see front matter
PII: S0038-0717(00)00024-9

CH_4 fluxes in soils with fluctuating water tables can be partly explained by the compensative effect of production and consumption (MacDonald et al., 1996). However even in strictly anaerobic soils CH_4 production potential is a function of depth (Hornibrook et al., 1997). Production as well as consumption rates vary by three orders of magnitude in different studies (Moore and Dalva, 1997; Segers, 1998; Wassman et al., 1998) and these were "weakly correlated with ecosystem type, incubation temperature, in situ aeration, latitude, depth and distance to oxic/anoxic interface" (Segers, 1998). In addition the variability of the collected data may even be highly dependent on the size of the soil samples (Brockman and Murray, 1997). On small spatial scales, denitrification rather than methanogenesis has been investigated. Parkin (1987) observed a high variability of denitrification on the "nugget-scale" (soil samples of about 5 g). High specific rates of denitrification were associated with particulate organic C material in the soil. Methane production also depends on substrate availability in the soil. The effects of the quantity as well as the quality of organic matter on CH_4 production have been shown by Joulian et al. (1996): Methanogenesis is influenced more by the availability and composition of the substrate than by the density of methanogens. Recently, Bergman et al. (1998) stressed that substrate availability — rather than abiotic factors like temperature and pH — is a predominant constraint for CH_4 productivity under field conditions.

The fact that variability on the landscape scale cannot be explained adequately from easily measurable factors makes it reasonable to ask whether it can be traced back to processes on smaller scales. Since biological processes may be non-linear it makes sense to look for variables on small scales (less than 1 m) that determine processes on larger scales. We present an approach to determine ruling factors of methane production at scales as small as 1 cm.

2. Material and methods

2.1. Site description

The sites investigated are situated in the Moraine Landscape "Allgäu" (area approx. 500 km^2) in southwest Germany, between 550 and 660 m a.s.l.. The mean annual precipitation is about 1300 mm, mean annual air temperature equals 6.5°C. For the measurement of methane emissions, we chose three representative land units: (i) an alluvial plain, (ii) a colluvial margin of a wet depression, and (iii) a peaty depression of the hummocky ground moraine. According to Soil Survey Staff (1998), the soils of the sites were classified as (i) Aeric Endoaquept (AE), (ii) Typic Humaquept (TH), and (iii) Limnic Haplohemist (LH). These soil types cover approx. 20% of the "Allgäu" area. The sites are farmed extensively (low input meadows).

2.2. Methane fluxes and environmental variables

Methane emissions were measured weekly from July 1996 to July 1998. The high temporal variability of CH_4 emissions exceeded even the spatial variability on the landscape-scale. A general trend of increasing water table, AE < TH < LH, can be observed from Table 1. Within the biologically most active zone at 0–20 cm depth a longer period of reducing conditions was registered in the TH compared to the LH (Fiedler and Sommer, unpublished data).

2.3. Soil sampling

The three sites were sampled below the root mat (depth 5–15 cm) comprising the following horizons: Ah2 (AE), Ah2 (TH) and Oi (LH) according to Soil Survey Staff (1998). At the time of sampling, the sampled horizons of TH and LH were water saturated and anoxic, the Ah2 of the AE was oxic. Thirteen samples with intact soil structure were taken per horizon (each sample was 6 cm in diameter and 4 cm in height). The *Phragmites* stems in the cores from the TH were cut off above and below the core. All cores were kept at 4°C and transferred to the laboratory within 8 h, subsequently wetted (AE) and drained (all three soil types) to an air content of 9–15% (calculated from the water retention curve) in order to allow CH_4 to enter the gaseous phase by diffusion and convection. Three cores (controls) were sterilized by heat-denaturation or fumigation with chloroform for 48 h. Six additional undisturbed cores of each soil site were used for measurements of water content and water retention curve (Klute, 1986).

2.4. Incubation chambers for methane production

Chambers with minimal headspace (3 cm^3, volume of chamber 116 cm^3, volume of soil core 113 cm^3) for the anoxic incubation of soil cores with intact structures were constructed using polyoximethylene as a material tight for soil-gases and inert for CH_4. The drained soil cores were closed up within the chambers 48 h after sampling and incubated for 3 months at 15°C reflecting a mean temperature of the soil layer during summer. Methane concentration was measured individually in each chamber every 7 days by attaching a 20 ml vacutainer on the lower outlet, exhausting the gaseous phase in the chamber down to 40 kPa. Through the upper outlet the chambers were filled with N_2 after each measurement until normal atmos-

pheric pressure was re-established. As a control, chambers without soil, chambers with heat-denatured soil (of each soil type), and chambers with soil cores treated with chloroform in the gaseous phase for 48 h, were incubated in parallel. At the end of incubation, the cores were stored at −20°C. Of each soil type, six cores were pumped with 2 l ethanol prior to freezing for further use in computed tomography and in situ hybridization, two cores and the three sterilized control cores were frozen without ethanol treatment, and two were extracted for etherlipid analysis.

2.5. Gas analysis

Gas samples were analysed on a PE Autosystem XL (g.c. equipped with a f.i.d. operating at 400°C). Methane was separated on a Porapak Q column (80–120 mesh) with N_2 as carrier gas (45 ml min^{-1}) and with H_2 (45 ml min^{-1}) and synthetic air (400 ml min^{-1}) as auxiliary gases, under the following conditions: oven temperature 40°C, injector temperature 130°C.

2.6. Computed tomography

The structure of six cores of every soil type was analysed in a medical computer tomograph with a resolution of 1 mm in length as well as in depth (40 pictures for each soil core), 3D-visualization with volume rendering.

2.7. Quantification of Archaea by analysis of etherlipids

Each 113 cm^3 soil core was extracted with 70 ml phosphate buffer, 250 ml methanol, 250 ml chloroform and 125 ml distilled water for 2 h (Zelles and Bai, 1993). After phase separation the lower phase was recovered and concentrated in a rotary evaporator. The lipid material was fractionated into neutral lipids, glycolipids and phospho-(polar) lipids on a silica-bonded phase column (SPE-SI; Bond Elut, Analytical Chem. International, Calif. USA) by elution with chloroform, acetone and methanol, respectively. An aliquot of the phospholipid fraction was hydrolised with hydriodic acid (57%) for 18 h at 100°C for the cleavage of the ether bonds (Kates et al., 1965). The resulting alkyl iodides were reduced to the corresponding isoprenoid hydrocarbons following the procedure of Panganamala et al. (1971), with zinc powder in glacial acetic acid. Hydrocarbons were analyzed by gas chromatography mass spectrometry (Hewlett-Packard, 5971A MSD column), combined with a 5890 series II gas chromatography system. Nonadecylacidmethylester was used as an internal standard for the quantification of the hydrocarbons. The resulting phospholipid ether-linked isoprenoids concentrations were converted into number of Archaea cells per soil core by the following

Table 1
Site description, environmental variables and methane flux during the period from July 1996 to July 1998

Description	Alluvial plain	Colluvial margin of a wet depression	Peaty depression
Soil type	Aeric Endoaquept (AE)	Typic Humaquept (TH)	Limnic Halpohemist (LH)
Location (latitude and longitude)	Aichstetten (10°25′E 47°52.2′N)	Artisberg (9°51.5′E 47°43′N)	Wangen (9°50′E 47°40.5′N)
Dominating plant species	*Dactylis glomerata*, *Lolium perenne*, *Taraxacum officinale*, *Brachythecium rutabulum*	*Carex gracilis*, *Phragmites australis*, *Lysimachia thyrsiflora*, *Pleurozium schreberi*, *Climacium dendroides*	*Carex acutiformis*, *Potentilla erecta*, *Filipendula ulmaria*, *Pleurozium schreberi*, *Climacium dendroides*
Bulk density [g cm^{-3}]	0.81	0.23	0.1
pH ($CaCl_2$)	5.3	5.7	4.8
Soil organic carbon [% ± SD]	4.2 ± 0.1	11.7 ± 0.1	43.8 ± 2.3
C to N ratio in soil [% ± SD]	9.1 ± 0.02	12.2 ± 0.04	15.1 ± 0.28
C to N ratio in plants [% ± SD]	18.8 ± 2.0	26.9 ± 0.4	25.3 ± 0.8
Microbial C [μg C cm^{-3} ± SD]	1045 ± 92.6	286 ± 24.3	218 ± 21.3
Median groundwater table [m below surface ± SD][a,b]	1.00 ± 0.24/1.18 ± 0.23	0.1 ± 0.12/0.14 ± 0.16	0.08 ± 0.09/0.1 ± 0.17
CH_4-emission [g CH_4 m^{-2} y^{-1}][a,b]	0.1/0	92.5/37.5	15.3/10.6
Mean [g 10^{-3} CH_4 m^{-2} d^{-1}][a–d]	3/0.1/0.14/-0.13	244/96/17.7/51.8	42/31/9.7/8.6
Median [g 10^{-3} CH_4 m^{-2} d^{-1}][a,b]	0/-0.2	85/40	31/26

[a] weekly from 21 July 1996 to 21 July 1997.
[b] weekly from 21 July 1997 to 21 July 1998.
[c] 01 Dec 1997 (N = 3).
[d] 17 Mar 1998 (N = 3); SD = Standard deviation.

approximations: 1 mol etherlipid contains 2 mol phytane and biphytane, and methanogenic monocultures obtained from a variety of sources yield an average etherlipid concentration of 2.5 μmol g^{-1} dry weight of methanogenic cells (Nichols et al., 1987). One gram dry weight of methanogenic Archaea corresponds to 5.9×10^{12} cells (White et al., 1979).

2.8. Quantification of Archaea and Eubacteria by in situ hybridization technique

Soil material was fixed in 99.8% ethanol immediately after sampling and stored at −20°C until used. Prior to hybridization soil samples were diluted in 0.1% sodiumpyrophosphate, spotted on gelatine coated slides (0.1% gelatine, 0.01% $KCr(SO_4)_2$) in 10 μl aliquots per well and dried at room temperature. After dehydration with 50, 80 and 99.8% ethanol for 3 min each, hybridization was carried out at 42°C for 2 h in 8 μl of hybridization buffer (0.9 M NaCl, 20 mM Tris–HCl, 10 mM EDTA, 0.01% SDS) and 1 μl of DAPI solution (200 ng μl^{-1}), in the presence of 20 or 30% formamide for hybridization with probes Arch915 or Eub338, respectively. Both probes were labeled with the fluorescent dye Cy3 (Amersham). After hybridization the slides were immersed in washing buffer for 15 min at 42°C (20 mM Tris–HCl, 10 mM EDTA, 0.01% SDS and 900 or 300 mM NaCl depending on the formamide concentration during hybridization, 20 or 30%, respectively), subsequently rinsed with destilled water and air-dried. Slides were mounted with Citifluor solution (Citifluor, Canterbury, UK) and examined with a Zeiss Axiolab microscope fitted for epifluorescence. Microbial cells were counted at 100× magnification. Twenty fields, randomly selected, covering an area of 0.01 mm^2 were examined from a sample distributed over two circular areas of 53 mm^2 each.

3. Results

3.1. Methane production

In all of the soil cores with intact structures CH_4 production was induced during anaerobic incubation at 15°C (Fig. 1a–c). In our experiments, in highly productive cores such as TH9, this rate reached up to 39 μ mol m^{-3} s^{-1} (equalling 53.9 g m^{-3} d^{-1}, see Table 2). In the cores taken from the soils with high annual mean groundwater levels in the field (TH and LH), CH_4 production started within 4–7 days after the beginning of anaerobic incubation (Fig. 1b and c). After 3 weeks of incubation, the exponential development of the weekly CH_4 production rates levelled off and a plateau phase was reached (Fig. 1b and c). The production rates remained high (up to 53.9 g m^{-3} d^{-1}) for about 2 months until the end of the experiment. In control soil cores CH_4 concentration stayed below 10 mg m^{-3} d^{-1}. A completely different pattern of CH_4 production rates was observed in the cores taken from the Ah2 horizon of the AE, which was oxic at the time of sampling. In these cores wetted prior to incubation, CH_4 production started slowly and production rates rose exponentially throughout the incubation (Fig. 1a).

The time course of CH_4 production represented the soil type and the conditions prior to sampling, whereas the absolute amount of CH_4 production varied largely between individual cores of one soil type, as is shown in the following section for all three soil types (Table 2). The high variability within the soil sites is

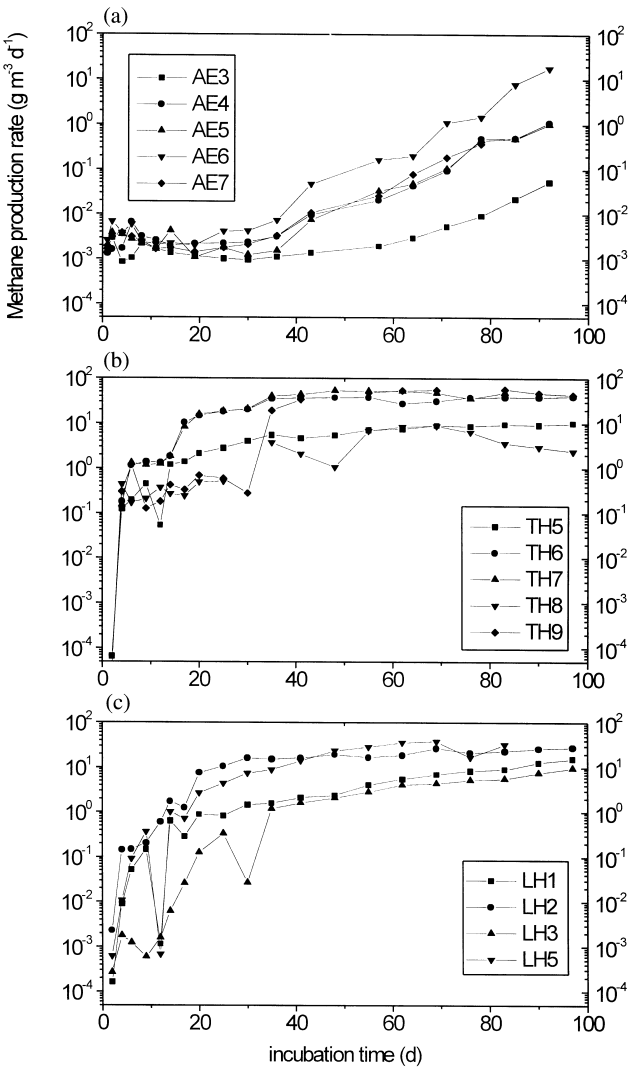

Fig. 1. Methane production rates in undisturbed incubated soil cores of the Aeric Endoaquept (a), the Typic Humaquept (b), and the Limnic Haplohemist (c). Note that methane production values are not cumulative and given on logarithmic scales.

Table 2
Spatial variability of methane production rate after three different periods of incubation

Soil type		Aeric endoaquept (AE)			Typic humaquept (TH)			Limnic haplohemist (LH)		
Incubation time		30 day	64 day	92 day	30 day	62 day	90 day	30 day	62 day	90 day
	core				core			core		
Weekly methane production per soil core (g m^{-3} d^{-1})	AE1	0	0	0.02	TH1	0.22	7.40	19.9	LH1	1.45	5.49	12.7
	AE2	0	0	0.06	TH2	n.d.	16.3	33.5	LH2	16.4	18.8	26.1
	AE3	0	0	0.05	TH3	14.3	26.2	29.4	LH3	0.03	4.04	7.50
	AE4	0	0.04	1.09	TH4	n.d.	5.21	5.52	LH4	0.40	4.35	12.7
	AE5	0	0.05	1.01	TH5	3.96	7.51	9.31	LH5	7.33	35.4	n.d.
	AE6	0	0.20	18.0	TH6	20.25	27.4	38.7	LH6	0.27	3.96	18.7
	AE7	0	0.08	n.d.	TH7	20.73	52.2	n.d.				
	AE8	0	0	5.79	TH8	n.d.	8.43	2.92				
					TH9	0.28	53.9	47.2				
					TH10	n.d.	15.8	2.72				
Mean		0	0.05	3.25		10.0	22.0	21.0		4.3	12.0	15.5
Standard deviation		0	0.065	5.86		8.8	17.1	15.9		6.0	11.7	6.4
Coefficiant of variation (SD/mean, %)		n.d.	139.7	180.3		88.44	77.8	75.5		138.3	97.5	40.9

shown by the coefficient of variation (standard deviation in percent of mean), which often exceeds 100%.

The cores of the TH fall in two groups of productivity. Type 1 are the "highly productive" cores (TH3, TH6, TH7 and TH9) in which the weekly CH_4 production rates increased to a high value after a few days and remained constant until the end of the experiment. Type 2 are the "low productive" cores (TH1, TH2, TH4, TH5, TH8 and TH10) in which CH_4 production started within a few days, but it took about 90 days of incubation until some of the cores reached rates of CH_4 production which were comparable to those of the "highly productive" cores (Table 2).

The highly productive cores of the LH had a slightly lower rate of CH_4 productivity (around 30 g m^{-3} d^{-1}, see Table 2) than the highly productive cores of the TH (around 40–50 g m^{-3} d^{-1}). However the variability between different cores of LH was as high as in the

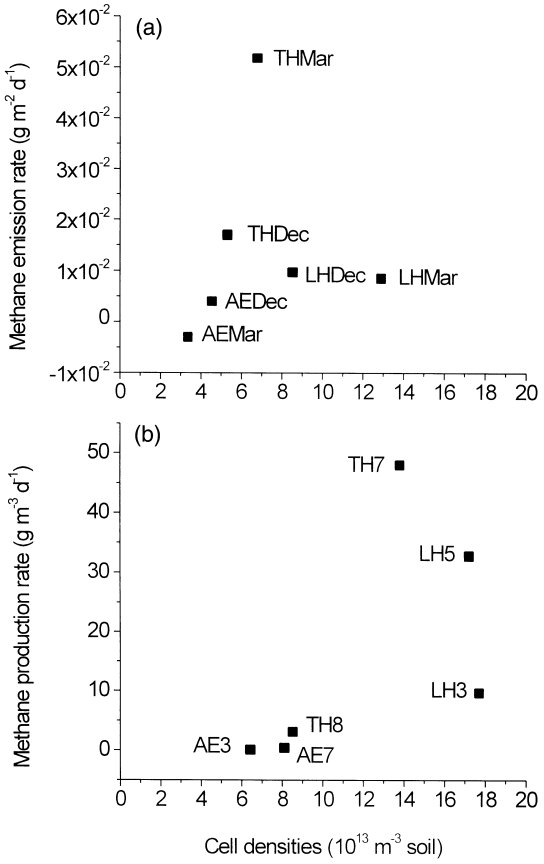

Fig. 2. (a) Methane emission rates of the field soil (g m^{-2} d^{-1}), plotted against Archaea densities (10^{13} m^{-3} soil) in samples of the same soils on the same days, calculated from the concentration of phytan and biphytan in the phospholipid fraction. (b) Methane production rates (g m^{-3} d^{-1}) of selected incubated soil cores (at the day of harvesting for etherlipid analysis) plotted against Archaea densities (10^{13} m^{-3} soil) in the soil cores, calculated from the concentration of phytan and biphytan in the phospholipid fraction of the incubated soil core.

TH. We found cores belonging to the "highly productive" type (LH2 and LH5) and others which were "low productive" (LH1, LH3, LH4, LH6).

Most of the cores of the AE showed much lower CH_4 production rates than the cores of TH and LH, but nevertheless all rates of the AE cores increased exponentially during incubation. The CH_4 production rate of one core of the AE was 18 g m^{-3} d^{-1} at the end of the incubation (AE6, Fig. 1a), almost as high as the maximal rates of the TH and the LH.

3.2. Quantification of Archaea in the soil cores based on etherlipid analysis

The CH_4 productivity of single cores was assumed to be represented by the numbers of methanogens in the cores. Methanogen population densities were calculated from the cell membrane etherlipid contents in soil extracts. In fact, in the highly productive soil cores (TH7, TH8, LH3 and LH5) cell densities were higher than those in the less productive AE cores (AE3 and AE7, Fig. 2b). The same trend could be shown for the population densities of the field samples of the wet soils (TH and LH) being higher than the densities of the AE-field samples (Fig. 2a). But a significant linear correlation was not found either between production rates and cell densities of the incubated cores or between emission rates and cell densities of the field samples. The incubated cores of all of the three soils show higher Archaea concentrations than the corresponding field samples, but during incubation the cell densities did not increase as prominently as CH_4 production (compare the scales in Fig. 2a and b).

3.3. Structure of the soil cores

Dense soil particles, pores, fissures, clefts and roots seemed to be distributed equally in high and low productive cores. No difference in the micro-structures of the soil matrices was detected between highly and low productive cores of one soil type, whereas differences were observed with respect to larger structural elements (Figs. 3 and 4): In TH3, TH6, TH7 and TH9 (highly productive, see Table 2), the structure of a *Phragmites* stem was detected (shown for TH9 [3D] in Fig. 3a and for TH6 [cross-section] in Fig. 4b). In the

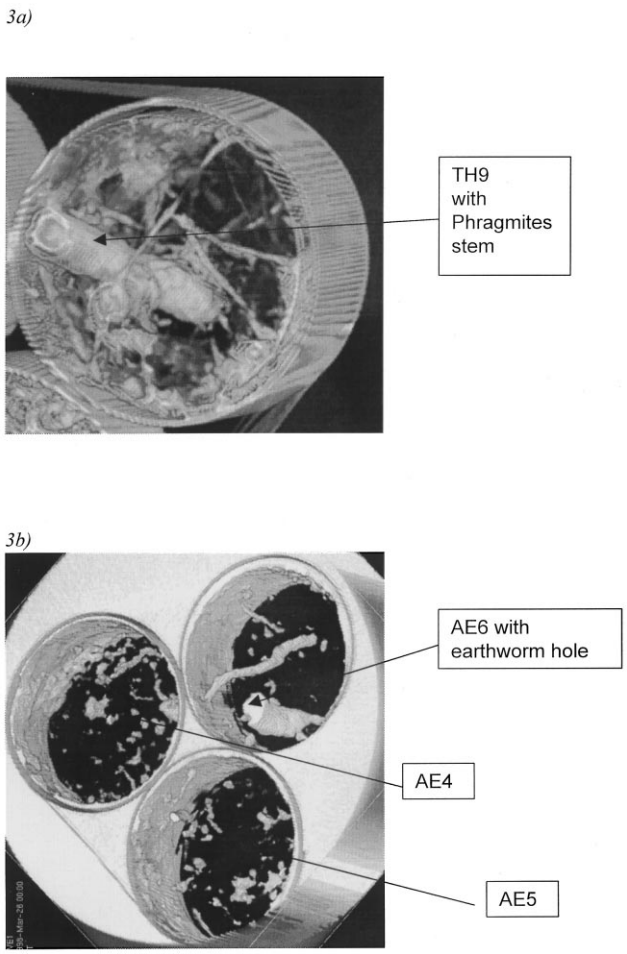

Fig. 3. 3D-visualization of two highly productive cores (with included organic material) and two low productive cores. Pictures generated by computed tomography, diameter of cores: 6 cm. (a) Structure of a *Phragmites* stem within the core TH9 of the Typic Humaquept. (b) Structure of a hole that was left after degradation of a dead earthworm in core AE6 of the Aeric Endoaquept (upper right) and two cores without such structures (AE4 and AE5).

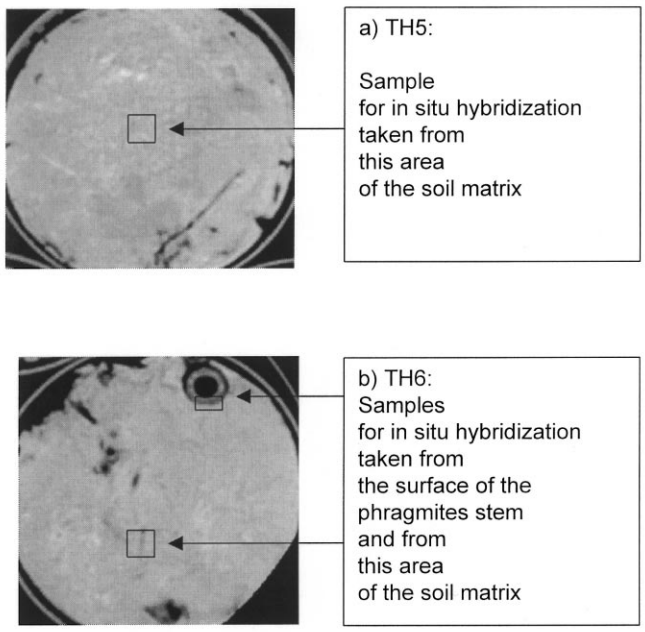

Fig. 4. Localization of the sampling for in situ hybridization (Table 3) within two incubated cores taken from the Typic Humaquept. (a) TH5 (low productive core without fresh organic material) (b) TH6 (highly productive core with *Phragmites* stem included, visible on the upper right side). Horizontal core cross-sections were generated by computed tomography, diameter of core: 6 cm.

highly productive AE6 a hole was observed that corresponded to the earthworm which had been included during sampling and died in the core during the incubation experiment (Fig. 3b 3D). Such structures were not found in the low productive cores (shown for AE4 and AE5 [3D] in Fig. 3b, and for TH5 [cross-section] in Fig. 4a).

3.4. Quantification of Archaea within two soil cores of the TH based on in situ hybridization

To confirm whether high CH_4 productivity within single soil cores was in fact due to methanogenic activity that is associated with the included organic material, two of the TH cores (TH6 with high productivity and TH5 with low productivity) were tested for the amount of Archaea cells in specific regions of the core. Samples were taken from the cover layer on the epidermis of the *Phragmites* stem (TH6), as well as from the soil matrix (TH5 and TH6, Fig. 4 and Table 3). Whereas the numbers of Eubacteria (in % of total cell count after DAPI-staining) did not vary much between the *Phragmites* epidermis and the soil matrix of TH6, the number of cells hybridized with the Archaea-probe was five times higher on the *Phragmites* epidermis compared to soil matrix samples which were about 4 cm apart (Fig. 4b and Table 3) and 80 times higher than in the matrix of the low productive TH5. In TH5 the number of Archaea in the soil matrix was below 1% of all cells as quantified by DAPI-staining (Fig. 4a and Table 3).

4. Discussion

Our experiments were focused on microbial processes in soil cores with undisturbed structure. A simple and effective method is provided which allows anoxic incubation of soil cores with native structural properties. When supplying N_2 to the gaseous phase of the incubated chambers, anoxic conditions can be established without destroying the soil structure. The weekly rates of CH_4 production (Table 2) are within the range of the potential CH_4 production rates reported by different authors, which are typically between 10^{-2} and 10^{1} μ mol m^{-3} s^{-1} (see Segers, 1998, for review). The ranking of the mean CH_4 production rates was TH > LH ≫ AE at every sampling time during laboratory incubation. The same ranking was shown for the mean CH_4 emission rates in the field (Table 1). Although the laboratory conditions of the incubation experiment (constantly anaerobic, constant temperature and constant water saturation) are quite different from the situations in the field, the CH_4 productivity of the soil cores reflected the CH_4 emissions in the field.

The time course of the CH_4 production rates reflected the histories of the horizons prior to sampling (Fig. 1): As in the hypothetical case of a pre-incubation, the almost continuous anaerobic conditions in the field on the sites TH and LH shortened the lag phase of CH_4 production in the incubated samples to a period of about 1 week. We found different classes of production patterns as did Wassman et al. (1998): TH and LH are related to the ''instantaneous'' type, which reached high productivity rates within the first 2 weeks of incubation; AE belongs to the ''delayed'' type of Wassman et al. (1998) with a steady increase of CH_4 production over several weeks. On the day of sampling, the surface of AE was not flooded. In the incubated AE samples, CH_4 production developed very slowly and the exponential development of the CH_4 production rates continued throughout the incubation time (3 months). This pattern is similar to the classical growth curves of microbial cultures. However without additional information on the cell number of the methanogens it is not possible to conclude whether the exponential development of CH_4 production rates is due to an increase in methanogenic population, or to an activation process of methanogenic pathways, or both. Although methanogenesis is an anaerobic process and pure cultures of methanogens do not tolerate O_2 (Kiener and Leisinger, 1983), it is possible to recover methanogens from naturally oxic habitats and to induce methanogenesis by incubation of even desert

Table 3
Numbers of Archaea and Eubacteria in subcompartments of single incubated cores taken from the Typic Humaquept. For localization of the samples see cross-sections in Fig. 4. Cell numbers were calculated after hybridization to the specific gene probe, as percentages of DAPI-stained cells. Total cell counts are presented as means, standard deviations are given in brackets

Core	Organisms	Matrix without *Phragmites*	Biofilm on *Phragmites epidermis*
TH5 (low productive)	DAPI-stained organisms (cell number g^{-1} fresh weight)	2×10^9 (0.3) (=100%)	n.d.
	Percentage active Archaea	< 1	n.d.
	Percentage active Eubacteria	101	n.d.
TH6 (highly productive)	DAPI-stained organisms (cell number g^{-1} fresh weight)	5×10^9 (2.1) (=100%)	11×10^9 (4.4) (=100%)
	Percentage active Archaea	7	35
	Percentage active Eubacteria	56	66

soils and other oxic soil samples (Peters and Conrad, 1995; Wagner and Pfeiffer, 1997).

In our study the number of Archaea (detected by etherlipid analysis) seems to reflect the increase in CH_4 productivity during incubation of all of the three soil types, but not in a proportional fashion. Previous most probable number (MPN) studies reported much fewer methanogens (10^3 g^{-1} soil dry weight) in arable soils than in paddy soils (3×10^5 g^{-1} soil dry weight), and showed an increase in cell densities by three–four orders of magnitude when forest or arable soil was submerged (Mayer and Conrad, 1990) or during anaerobic incubation of desert, savanna or cultivated soils (Peters and Conrad, 1996). In our study, the exponential development of CH_4 production rates in the chambers of the AE was not associated with a corresponding growth of methanogens. Like Asakawa et al. (1998), we used the etherlipid assay for calculating archaeal population densities. We analyzed field samples as well as incubated soil cores. The representative CH_4 emission/production data were plotted against these population density values. In both cases there was no significant linear relationship between emission/production rates and cell densities (Fig. 2a and b). The overall impression given by the presented data however suggests that higher productivity coincides with higher population density. We conclude that activation/inhibition processes may contribute to the non-linear relationships between population densities and emission/production rates. Absolute cell numbers alone do most likely not allow a good prediction of CH_4 productivity for two reasons: First, the variability between different soil cores is too high to extrapolate a development of cell densities for each soil from two individual cores per soil, and second, a physiological shift could overlie the growth function of the micro-organisms and therefore activation processes should be taken into account.

The large differences in CH_4 production between single soil cores, even if taken from the same soil horizon within 1 m^2, reflected the landscape scale variability mentioned in Section 1. Standard deviation often exceeds 100% of the mean of all cores of one soil site (Table 2). Moore and Knowles (1990) observed a very high spatial variability of CH_4 production rates in fen, bog and swamp peatlands. The precision of the mean was very low in general, even with 40 chambers selected per site (Moore et al., 1994). CH_4 production potentials of peat soils spanned up to four orders of magnitude (Moore and Dalva, 1997). The authors suggested that "CH_4 exchange is probably one of the most variable microbially-mediated processes in soil". On the other hand our experiments show that the temporal development of the CH_4 production rates within single cores is rather stable (Table 2 and Fig. 1). This suggests that the spatial variability of CH_4 production within each of the three soil sites is not an experimental artefact, but rather represents the natural situation. The phenomenon of small scale variability has been found in each of the three soil types. Similarly, Wagner and Pfeiffer (1997) found high variances of CH_4 production rates in incubated undisturbed soil cores, whereas homogenization of the soil samples resulted in a smaller variance of CH_4 measurements. Correspondingly, high as well as low productive cores are found in each of the three soil sites we investigated, although the histories of the three horizons and the development of the rates were completely different between the "wet" soil horizons of TH and LH and the "dry" horizon of AE.

In order to scale down, single soil cores with different productivity rates were analyzed with respect to their inner structure, and visualized by computed tomography. No difference of the soil matrices was found between the highly and the low productive cores (Fig. 3). Nevertheless there was a structural phenomenon occurring exclusively in the highly productive cores: In all of the highly productive cores which were visualized by computed tomography, large inclusions of particulate organic material were detected: A *Phragmites* stem was found in the highly productive cores of the TH, and in the very highly productive core of the AE the hole of an earthworm was detected that had decomposed during incubation. In both cases the material was present as living organism while sampling and became accessible to microbial degradation during anaerobic incubation.

The high productivity in cores of the TH coincided with an inclusion of a *Phragmites* stem (Fig. 3a and Table 2). In core TH6 the epidermis of this *Phragmites* carries a biofilm with an Archaea-content higher than that found in the soil matrix of these highly productive cylinders (Fig. 4b and Table 3). The coincidence of high productivity, inclusion of fresh organic material and high density of Archaea suggests a causal relationship. Substrate accessibility seems to be a predominant factor for spatial distribution of micro-organisms, as shown for example for the growth of bacteria in a biofilm (Moller et al., 1996; Beveridge et al., 1997; Amann et al., 1992) or in close vicinity to plant cell walls (Ladd et al., 1993). Methanotrophic bacteria even colonize the interior of rice plants, especially roots and culms, where CH_4 and O_2 concentrations are optimal due to aerenchymatic transport processes (Bosse and Frenzel, 1997). *Phragmites* stems play an important role in gas exchange between the soil and the atmosphere (Armstrong et al., 1996; Brix et al., 1996). Comparison of the above mentioned results with these observations from the literature strongly suggests that methanogens grow at the border of plant surfaces and soil, or in decaying organisms. The commonly assumed

pattern of CH_4 flux in soils with heterogeneous water content (CH_4 production in deep anoxic layers, transport to the surface directly or mediated by plants, CH_4 oxidation at the border to oxic zones) will therefore have to be modified by introducing more structural details at smaller scales: Obviously there is a ruling factor for CH_4 production on the sub-horizontal scale, which is also suggested by the high spatial variability of the CH_4 production. This ruling factor was found on the structural level: *Phragmites* stems are the sites of the high productivity as was shown for the stem-carrying cores in the Typic Humaquept. Their function as a substrate for methanogens could be verified by high cell numbers of Archaea within a biofilm on the *Phragmites* epidermis.

We assume that the distribution of methanogenic organisms is ruled by fresh organic material, and that this is the case not only on the scale of soil cores as shown in this paper, but also on the microscopic scale. With respect to many processes, soil may be considered as a hierarchical heterogeneous system with structures on many different scales. This is in particular the case for the production of CH_4. The actual microbiological processes operate at scales of a few micrometers. At scales of a few millimetres, there exist very strong gradients, e.g., for the O_2 partial pressure, because of the soil's porous structure and root O_2 release. At still larger scales, CH_4 production in soils is determined by the hydrological regime and by the pattern of soil variables, which themselves are reflected in soil types. Obviously, all process-oriented measurements have to refer to the scale in which a factor-process-junction can be observed which is, in the case of microbial processes, a very small scale. From the perspective of environmental quality, however, the phenomenology at large scales is of primary interest. Therefore a path for translating small-scale understanding into large-scale phenomenology is required. In our view, such a path needs to include information about the hierarchical structure of soil. We suggest to identify, at each scale of interest, homogeneous subregions whose effective properties are either measured directly or are recursively determined by considering the next smaller scale. The effective phenomenology at the scale of interest is then calculated from the measured geometry of the homogeneous subregions and from their effective properties. Evidently, this procedure can be repeated at the next larger scale and thus will provide a path that leads to larger scales.

In the work presented here, we provide initial steps on the path outlined above. In particular, we demonstrate that CH_4 production is strongly correlated to the presence of fresh organic carbon. At the scale relevant for the microbiological processes, organic carbon exhibits a very high spatial variability, but, as we demonstrate, a rather high temporal persistence. Since the spatial structure of organic carbon can be measured directly, it is suggested to be taken as a proxy for identifying uniform subregions at a scale of a few centimeters (or may be even smaller) which allows the first step to upscaling.

Acknowledgements

We thank Angelika Gassama for expert assistance in constructing the incubation chambers and measuring gas production. Hans-Jörg Vogel (University of Heidelberg) is a pioneer in using computed tomography technique in soil science and is therefore gratefully acknowledged, as well as Claus D. Claussen, Michael Schaich and Herbert Schwarz (University Hospital of Tbingen) for their generous support in performing computed tomography of soil cores. We would like to thank the anonymous peer reviewers of this paper for critically reading the manuscript and substantially contributing to the presentation stile. This project was funded by Deutsche Forschungsgemeinschaft (Graduiertenkolleg).

References

Adrian, N.R., Robinson, J.A., Suflita, J.M., 1994. Spatial variability in biodegradation rates as evidenced by methane production from an aquifer. Applied and Environmental Microbiology 60, 3632–3639.

Amann, R., Stromley, J., Devereux, R., Key, R., Stahl, D.A., 1992. Molecular and microscopic identification of sulfate-reducing bacteria in multispecies biofilms. Applied and Environmental Microbiology 58, 614–623.

Armstrong, J., Armstrong, W., Beckett, P.M., Halder, J.E., Lythe, S., Holt, R., Sinclair, A., 1996. Pathways of aeration and mechanisms and beneficial effects of humidity- and venturi-induced convections in *Phragmites australis* (CAV. Trin. ex. Steud.). Aquatic Botany 54, 177–197.

Asakawa, S., Akagawa-Matsushita, M., Koga, Y., Hayano, K., 1998. Communities of methanogenic bacteria in paddy field soils with long-term application of organic matter. Soil Biology and Biochemistry 30, 299–303.

Bergman, I., Svensson, B.H., Nilsson, M., 1998. Regulation of methane production in a Swedish acid mire by pH, temperature and substrate. Soil Biology and Biochemistry 30, 729–741.

Beveridge, T.J., Makin, S.A., Kadurugamuwa, J.L., Li, Z., 1997. Interactions between biofilms and the environment. FEMS Microbiology Reviews 20, 291–303.

Bosse, U., Frenzel, P., 1997. Activity and distribution of methane-oxidizing bacteria in flooded rice soil microcosms and in rice plants (*Oryza sativa*). Applied and Environmental Microbiology 63, 1199–1207.

Brix, H., Sorrell, B.K., Schierup, H., 1996. Gas fluxes achieved by in situ convective flow in *Phragmites australis*. Aquatic Botany 54, 151–163.

Brockman, F.J., Murray, C.J., 1997. Subsurface microbiological heterogeneity, current knowledge, descriptive approaches and applications. FEMS Microbiology Reviews 20, 231–247.

Granberg, G., Mikkelä, C., Sundh, I., Svensson, B.H., Nilsson, M.,

1997. Sources of spatial variation in methane emission from mire in northern Sweden: a mechanistic approach in statistical modeling. Global Biogeochemical Cycles 11, 135–150.

Hornibrook, E.R.C., Longstaffe, F.J., Fyfe, W.S., 1997. Spatial distribution of microbial methane production pathways in temperate zone wetland soils: stable carbon and hydrogen isotope evidence. Geochimica et Cosmochimica Acta 61, 745–753.

Joulian, C., Ollivier, B., Neue, H.-U., Roger, P.A., 1996. Microbiological aspects of methane emission by a ricefield soil from the Camargue (France). Part 1: Methanogenesis and related microflora. European Journal of Soil Biology 32, 61–70.

Kates, M., Yengoyan, L.S., Sastry, P.S., 1965. A diether analog of phosphatidyl glycerophosphate in *Halobacterium cutirubrum*. Biochimia Biophysica Acta 98, 252–268.

Kiener, A., Leisinger, T., 1983. Oxygen sensitivity of methanogenic bacteria. Systematic and Applied Microbiology 4, 305–312.

Klute, A., 1986. Water retention: laboratory methods. In: Klute, A. (Ed.), Methods of Soil Analysis, Part 1: Physical and Mineralogical Methods. Soil Science Society of America, Madison, pp. 635–662.

Ladd, J.N., Foster, R.C., Skjemstad, J.O., 1993. Soil structure: carbon and nitrogen metabolism. Geoderma 56, 401–434.

MacDonald, J.A., Skiba, U., Sheppard, L.J., Hargreaves, K.J., Smith, K.A., Fowler, D., 1996. Soil environmental variables affecting the flux of methane from a range of forest, moorland and agricultural soils. Biogeochemistry 34, 113–132.

Mayer, H., Conrad, R., 1990. Factors influencing the population of methanogenic bacteria and the initation of methane production upon flooding of paddy soil. FEMS Microbiology Ecology 73, 103–112.

Møller, S., Pedersen, A.R., Poulsen, L.K., Arvin, E., Molin, S., 1996. Activity and three-dimensional distribution of toluene-degrading *Pseudomonas putida* in a multispecies biofilm assessed by quantitative in situ hybridization and scanning confocal laser microscopy. Applied and Environmental Microbiology 62, 4632–4640.

Moore, T.R., Dalva, M., 1997. Methane and carbon dioxide exchange potentials of peat soils in aerobic and anaerobic laboratory incubations. Soil Biology and Biochemistry 29, 1157–1164.

Moore, T.R., Knowles, R., 1990. Methane emissions from fen, bog and swamp peatlands in Quebec. Biogeochemistry 11, 45–61.

Moore, T., Roulet, N., Knowles, R., 1990. Spatial and temporal variations of methane flux from subarctic/northern boreal fens. Global Biogeochemical Cycles 4, 29–46.

Moore, T.R., Heyes, A., Roulet, N.T., 1994. Methane emissions from wetlands, southern Hudson Bay lowland. Journal of Geophysical Research 99, 1455–1467.

Nichols, P.D., Mancuso, C.A., White, D.C., 1987. Measurement of methanotroph and methanogen signature phospholipids for use in assessment of biomass and community structure in model systems. Organic Geochemistry 11, 451–461.

Panganamala, R.V., Sievert, C.F., Cornwell, D.G., 1971. Quantitative estimation and identification of o-alkyl glycerols as alkyl iodides and their hydrocarbon derivatives. Chem. Phys. Lipids 7, 336–344.

Parkin, T.B., 1987. Soil microsites as a source of denitrification variability. Soil Science Society of America Journal 51, 1194–1199.

Peters, V., Conrad, R., 1995. Methanogenic and other strictly anaerobic bacteria in desert soil and other oxic soils. Applied and Environmental Microbiology 61, 1673–1676.

Peters, V., Conrad, R., 1996. Sequential reduction processes and initiation of CH_4 production upon flooding of oxic upland soils. Soil Biology and Biochemistry 28, 371–382.

Segers, R., 1998. Methane production and methane consumption: a review of underlying wetland methane fluxes. Biogeochemistry 41, 23–51.

Soil Survey Staff, 1998. Keys to Soil Taxonomy. Washington DC. 8th ed. www.statlab.iastate.edu/soils/ssl/natch_data.html.

Valentine, D., Holland, E.A., Schimel, D., 1994. Ecosystem and physiological controls over methane production in northern wetlands. Journal of Geophysical Research 99, 1563–1571.

Wagner, D., Pfeiffer, E., 1997. Two temperature optima of methane productions in a typical soil of the Elbe marshland. FEMS Microbiology Ecology 22, 145–153.

Wassman, R., Neue, H.U., Bueno, C., Lantin, R.S., Alberto, M.C.R., Buendia, L.V., Bronson, K., Papen, H., Rennenberg, H., 1998. Methane production capacities of different rice soils derived from inherent and exogenous substrates. Plant and Soil 203, 227–237.

White, D.C., Davis, W.M., Nickels, J.S., King, J.D., Bobbie, R.J., 1979. Determination of the sedimentary microbial biomass by extractable lipid phosphate. Oecologia 40, 51–62.

Zelles, L., Bai, Q.Y., 1993. Fractionation of fatty acids derived from soil lipids by soil phase extraction and their quantitative analysis by GC–MS. Soil Biology and Biochemistry 25, 130–134.