

# The influence of the aeration status (ODR, Eh) of peat soils on their ability to produce methane

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**Abstract** Methane is a greenhouse gas having well-known impacts on global radiative forcing. However, understanding the dynamics and thresholds of methane formation in peatlands, which undergo cyclic aeration and anoxia, is currently limited even though it is crucial for reliable estimation of methane emission to the atmosphere. The purpose of this study was to estimate the optimal range and thresholds of aeration conditions, which impact methane formation in upper layers of peat originating from a natural fen (East Poland). The experiment was conducted under laboratory conditions on two groups of differently treated soils which were incubated at field water capacity. One group was incubated in air and another in helium, both at temperatures of 5, 10 and 20 °C. Direct soil aeration estimation included measurements of redox potential and oxygen diffusion rate. The latter was used for the first time as an indicator of methane formation in soil. Both the aerated and anoxic groups showed that methane formation took place at ODR of below 20  $\mu\text{g O}_2 \text{ m}^{-2} \text{ s}^{-1}$  and Eh of below +240 mV which are the higher threshold levels for methanogenesis than previously reported. Maximum methanogenic activity amounted up to 71.86  $\text{mg CH}_4 \text{ kgDW}^{-1} \text{ d}^{-1}$ . Aeration was capable of reducing this

methanogenic activity by 28–100 %. Decreasing temperatures and aeration increased the time until the initiation of  $\text{CH}_4$  production ( $p < 0.05$ ,  $n = 72$ ).

**Keywords** Methanogenesis · Peat soil · Oxygen diffusion rate · Redox potential · Aeration thresholds

## Introduction

Methane ( $\text{CH}_4$ ) is one of the main greenhouse gases playing an important role in Earth's climate radiative forcing (Forster et al. 2007). Despite its lower concentration relative to  $\text{CO}_2$ , it is 25-times more efficient in infrared energy absorption over a 100-year time frame (Forster et al. 2007). Methanogens are microbes responsible for methane production and they belong to the Archaea domain. These microorganisms are regarded as strictly anaerobic and gain energy for growth by utilizing a limited number of chemical substrates such as hydrogen, carbon dioxide, formate, ethanol, methanol, methylamines and acetate in methane production (Demirel and Scherer 2008). Their metabolism involves many  $\text{O}_2$ -sensitive redox centres, such as Fe–S clusters present in almost every metalloenzyme involved in all of the three pathways of methanogenesis, i.e.  $\text{H}_2/\text{CO}_2$ , acetoclastic, and methylotrophic, used for electron transport and/or catalysis (Glass and Orphan 2012; Tholen et al. 2007). The presence of oxygen leads to formation of reactive

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oxygen species, which can damage cell membranes, DNA, and proteins within methanogens. Oxygen also causes an irreversible dissociation of the  $F_{420}$ -hydrogenase enzyme complex, a crucial electron transporter in methanogens (Angel et al. 2011). Various physico-chemical factors such as temperature, pH, substrate availability or aeration have been proposed for controlling the activity of methanogens in wetlands (Glass and Orphan 2012; Singh 2001).

When methanogenic activity is investigated in the context of sensitivity to oxygen, a good indicator of aeration is required. One of the parameters commonly used in investigations of methanogenic activity is redox potential (Eh), which expresses the tendency of environment to receive or supply electrons in the solution. Electron transfer between donors and acceptors is involved in various redox reactions. As long as the system is exposed to the ambient atmosphere,  $O_2$ , as an electron acceptor, is responsible for high Eh. In the absence of  $O_2$ , a decline in redox potential and reduction of oxidized forms ( $NO_3^-$ ,  $Mn^{4+}$ ,  $Fe^{3+}$ , and  $SO_4^{2-}$ ) takes place. Eh is widely regarded as a useful tool for environment quality research (Stępniewski et al. 2005; Yu et al. 2006). In peat and waterlogged soils, Eh below +350 mV is expected (Pezeshki 2001), while generally in well-aerated soils it can reach +700 mV (Bennicelli et al. 2006; Yu et al. 2006). In wetland soils, the range of Eh values commonly range from +700 to –300 mV. Soils with Eh above +300 mV are regarded as aerobic, while below +300 mV as anaerobic (Inglett et al. 2005). It is reported that reduced conditions are required for the activation of methanogens (Öquist and Sundh 1998; Singh 2001). Another way for assessing aeration state is measuring  $O_2$  concentration (Yu et al. 2006). Incubation experiments can use chromatographic measurements of oxygen concentration in the head-space over soil samples but this technique has challenges due to the different moisture of soils. These variations in soil moisture can have significant effects on soil aeration. Low moisture allows for easier access of the above-surface air to soil through pores, whereas at higher water content, diffusion of oxygen can become constrained due to the low solubility of oxygen in water (Kechavarzi et al. 2010). It is commonly known that diffusion of gases in water is approximately  $10^4$ -times slower than in air (Riutta 2008). At atmospheric pressure and temperature of 25 °C, air saturated water contains about 250  $\mu\text{mol } O_2$

per liter. Increase of temperature and salinity resulted in decrease of water saturation (Brune et al. 2000). In this context, oxygen diffusion rate (ODR) seems to be an accurate and useful tool for investigation of aeration in different soils, and can be used both in laboratory and in situ for  $O_2$  measurements (Minamikawa and Sakai 2005; Stępniewski et al. 2005; Bennicelli et al. 2006). Since methanogens are extremely sensitive to oxygen, ODR measurements can be useful for investigation of methanogenic activity in soils and particularly in organic soils such as peat. Typically an ODR value above 35  $\mu\text{g } O_2 \text{ m}^{-2}\cdot\text{s}^{-1}$  represents good soil aeration conditions, while below this value aeration it becomes limited (Stępniewski et al. 2000; Czyż 2004).

Peat soils are known as sources of atmospheric methane, and their oxygen status is an important factor influencing methane production rates. There are several publications reporting that methanogens can survive oxygen stress and remain active even in relatively oxygenated environments such as rice paddy soils, deserts, oxygenated water and peat soils (Freeman et al. 2002; Kim et al. 2008; Yuan et al. 2009; Angel et al. 2011; Grossart et al. 2011) and become active at the onset of anaerobic conditions. Temporal aeration of peatland influenced  $CH_4$  emission but also carbon release as a  $CO_2$ . Climate change results in drought in the summer or significant lowering of groundwater in many regions of the world. It is also the case in East and Central Europe, including Poland, and implies that there is more frequent decrease in water levels resulting in more frequent aeration of peats during the year. The drought could reduce the capacity of wetlands to serve as sinks of global carbon and turn them into sources of atmospheric carbon because of faster organic matter mineralisation, when oxygen plays a role of a major electron acceptor. Recent studies have shown that in some peats (UK) the  $CO_2$  emission during drought remains unchanged or increases even by 181 % and after re-submergence raise by 500 % in relation to the time before the drought (Fenner and Freeman 2011). Understanding these differential responses of carbon mineralization in peatlands is crucial for understanding their role in the global climate.

The four aims of this study were: (1) to investigate how methanogenic activity in a peatland is affected by the aeration state described by Eh, and ODR; (2) to find what are the threshold values of Eh and ODR for

methane formation initiation; (3) to determine how temperature impacts these threshold values; and (4) to reveal which levels of a peat profile are more sensitive to aeration.

## Materials and methods

### Study site description

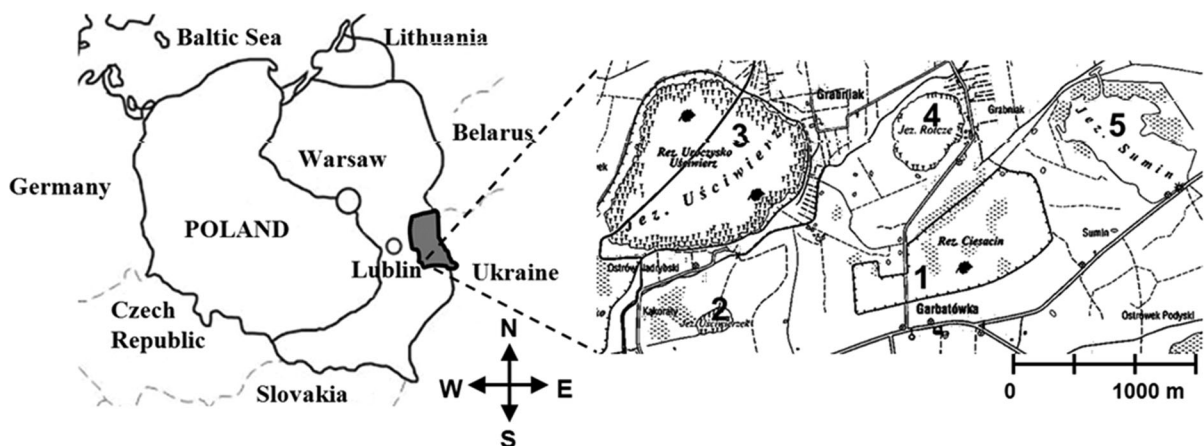
The experiment was carried out using peat material collected from a Garbatówka fen, a part of Ciesacin Reserve (Polesie Lubelskie, East Poland, 51°21'N, 23°6'E), characterized by a high groundwater table (+5 cm) resulting in high moisture and presence of numerous small water bodies. A 195 ha peatland area is situated in the catchment basins of eutrophic lakes: Uściwierzek, Uściwierz, Rotcze, and Sumin and borders agricultural soils in the south (Fig. 1). Vegetation is mainly composed of *Carex* spp., *Phragmites* spp., *Juncus* spp., *Schoenoplectus* spp., and some species of Bryophytes (Szafranek-Nakonieczna and Bennicelli 2010). Peat material was collected from three representative plots (according to prevailing vegetation, which was similar throughout the fen area), where four horizons of the peat profile layered at every 20 cm from the top to the depth of 80 cm (i.e. 0–20, 20–40, 40–60, 60–80 cm) were sampled. Samples were collected in September 2009, transported in tightly closed PVC containers, and stored in the laboratory at 4 °C for further analysis within

2–3 days. Further details on the soil treatment protocol are provided in Szafranek-Nakonieczna and Bennicelli (2010).

### Physicochemical analysis of soil material

Dry weight of the peat material and its water content (three replicates for each layer) were determined by weighing peat before and after drying at 105 °C for 48 h. The decomposition degree of peat was classified as from H1 (peat being pristine, undecomposed, and fibrous) to H10 (fully decomposed, humified peat) on the basis of the von Post index (Glatzel et al. 2004). Total organic carbon (TOC) was determined in dry peat samples by combustion and the analysis of evolved CO<sub>2</sub> by means of TOC-V<sub>CSH</sub> with SSM-5000A module (Shimadzu, Japan). Bioavailable forms of N and P were measured in extracts: peat-water for nitrite and nitrate (35 g soil in 100 ml of water), peat-NaCl (35 g soil in 100 ml of 0.2 M NaCl) for ammonium, and peat- NaHCO<sub>3</sub> (5 g soil in 100 ml of 0.5 NaHCO<sub>3</sub>) for phosphorus using an AA3 autoanalyzer, (Braun + Luebbe, Germany), after filtering through filter paper (Munktell, grade 390, Germany) according to the method described by Banach et al. (2009).

Redox potential (Eh) and pH were measured directly after opening the incubation bottles by means of a multifunctional potential meter pIONeer 65 equipped with a combined platinum and Ag/AgCl (reference) electrode or with a glass electrode



**Fig. 1** Location of the tested fen—Garbatówka (1), and surrounding water bodies: Uściwierzek Lake (2), Uściwierz Lake (3), Rotcze Lake (4), Sumin Lake (5)

(E31M004 and Cartrode pH E16M340, Radiometer Analytical S.A., France). Final Eh was corrected for pH values according to a pattern reported by Bennicelli et al. (2006).

ODR was determined by the amperometric method elaborated by Lemon and Erickson (1952). The method consists of measurement of the amount of oxygen diffusing to the surface of a platinum wire electrode, where it is reduced electrochemically. The platinum electrode is therefore a model of center absorbing oxygen, and the intensity of oxygen flux to the electrode indicates the maximum amount of oxygen that would be available for soil microorganisms. The value of the oxygen reduction current on the platinum electrode rate is expressed as the oxygen diffusion rate (ODR):

$$\text{ODR } [\mu\text{g O}_2 \text{ m}^{-2} \text{ s}^{-1}] = 8.29 \times 10^{-5} \frac{i \text{ } [\mu\text{A}]}{A \text{ } [\text{mm}^2]} \quad (1)$$

where:  $i$  current in  $\mu\text{A}$ ,  $A$ —area of the electrode in  $\text{mm}^2$  (Stepniewski et al. 2005).

#### Incubation experiment

Twenty-cm layers from 3 cores of peat were mixed together. Peat material was placed in dark sterile bottles (60 mL), tightly closed (with a septum and aluminium cap) and incubated in dark conditions, under two treatments: aerated (AT) with a natural water content combined with ambient air and anoxic (AN) without oxygen treatment, where soil was incubated in helium (AN). An anoxic environment was created by replacement of air with helium (purity 99.999 %, Praxair, Poland). The peat samples from each layer were incubated under AT and AN conditions at three temperatures: 5, 10, and 20 °C for up to 300 days. The incubation temperatures were selected on the basis of mean seasonal temperatures (spring 12 °C, autumn 5 °C and summer 19 °C) in the investigated region (Szafranek-Nakoneczna and Bennicelli 2010). Sixty replicates for each treatment were prepared in order to allow all necessary measurements in three replicates to minimize the random error.

The concentration of methane in the headspace was determined by the gas chromatography technique (Varian GC 3800, USA) with a flame ionization detector—FID (Szafranek-Nakoneczna and Bennicelli

2010). Methane production was recorded in mg of methane per gram of dry peat material ( $\text{mg CH}_4 \text{ gDW}^{-1}$ ). During incubation of the peat samples, the methane concentration in the headspace as well as aeration parameters (ODR, Eh) were measured up to 300 days (at the beginning, on the 3rd, 7th day, and next every 14–21 days, a total of 20-times, always in three replicates. After determination of the Eh and ODR values, the samples were no longer incubated and were eliminated from the investigations.

#### Statistical analysis

Collected data were statistically evaluated using a Statistica 9.1 (Systat Soft Inc., USA) package. Prior to the analysis all data were tested for the normality (Shapiro–Wilk test) and homogeneity of variances (Levene's test). If necessary a  $\ln(x + 1)$  transformation was applied. In order to explore variation of peat characteristics by depth a one-way ANOVA test with Tukey procedure as post hoc was used.

The dynamics of methane concentration, Eh, and ODR were visualised using the Sigma Plot 10.0 software (Systat Soft Inc., USA) where regression models were applied to data and resulted in regression equations and the goodness of fit statistics ( $R^2$ ) with their significance. The methane concentration observed during the experiment (as a cumulative concentration) was expressed in mg methane per g of dry peat material. The relationships between concentration of formed methane and aeration indicators were assessed using a correlative approach: first, a correlation matrix was generated and significant correlations were further studied by means of correlation coefficient (Pearson's or Spearman's rho depending on data normality).

The final analysis revealed which of experimental variables (depth in profile, temperature and aeration conditions) play a significant role in methane generation (in terms of activation time and methanogenic activity). For this study a three-way ANOVA with Tukey within-group test for each factor was used. For better clarity of understanding separate effects of studied factors were omitted for any possible interactions. The significance level of all tests was accepted at  $p < 0.05$ . The results were presented as means of non-transformed data.

The methanogenic activity (MGA) rate was calculated by a linear regression of the increase of methane concentration in time and expressed as  $\mu\text{g}$  of produced

methane per mass of dry peat material and per day ( $\mu\text{g CH}_4 \text{ kgDW}^{-1}\cdot\text{d}^{-1}$ ) (Yu et al. 2007; Freitag and Prosser 2009).

## Results and discussion

### Physicochemical properties of peat soils

Soil physicochemical characteristics are summarized in Table 1. All depths in the investigated profile differed significantly with respect to moisture and total organic carbon (TOC) content. The highest moisture was observed in the deepest layer while the highest TOC at depth 40–60 cm. Decomposition degree was somewhat low in levels 0–20 and 20–40 cm, described as H2 by the von Post index (below 40 cm as H3). In case of biogenic N,  $\text{NO}_3^-$  forms, the concentration in depths 40–80 cm was, by a factor of two, greater than in the 0–40 cm levels. Distribution of other forms of N and P was similar in all profiles as was pH which was almost perfectly neutral in a narrow range of 6.77–6.93.

### Changes in aeration conditions (Eh and ODR results)

Changes of Eh and ODR during experiment are described by exponential regressions (Fig. 2, equations in Table 2). During incubation, decreasing Eh values were noted in each of the treatments. A more sudden decrease (maximally by 200 mV) under the AN was found, while under the AT treatment, the change in Eh was 10-times smaller (Fig. 2). This was the effect of lack of molecular oxygen under the AN conditions and its rapid utilization by aerobic microorganisms in peat soil, while  $\text{O}_2$  diffusion in the AT treatment was very slow but it did occur. Therefore, at the end of the experiment a higher drop of redox

potential (to  $-100$  mV) was found in peat material incubated without oxygen than in that incubated in air, where Eh decreased to 0 mV (Fig. 2). The low Eh values have a positive effect on the activity of methanogens (reflected as an increase of methane concentration in the headspace) in all the methanogenically active samples (exponential model,  $y = a \exp(-b x)$ ,  $p < 0.05$ , Table 3, Fig. 2) This was earlier also reported by other researchers in rice paddies fields and peats (e.g. Minamikawa and Sakai 2005; Bennicelli et al. 2006, Yu et al. 2006).

Generally in wetland soils critical values of Eh limiting the activity of methanogens seems to be well investigated. But in the case of peat soil there is little available data. Paddy soils appear to be the most recognized. The researchers provide a diverse range of values for the Eh thresholds which is as wide as  $+400$  to  $-300$  mV (see Table 4 for the comparative summary). The differences in Eh thresholds are observed often within the same kind of soils such as paddy soils, but also in natural wetlands as well as in medium cultures. However, it is still commonly used as an indicator of soil methanogenesis (Yao et al. 1999; Lyimo et al. 2002; Yu et al. 2006).

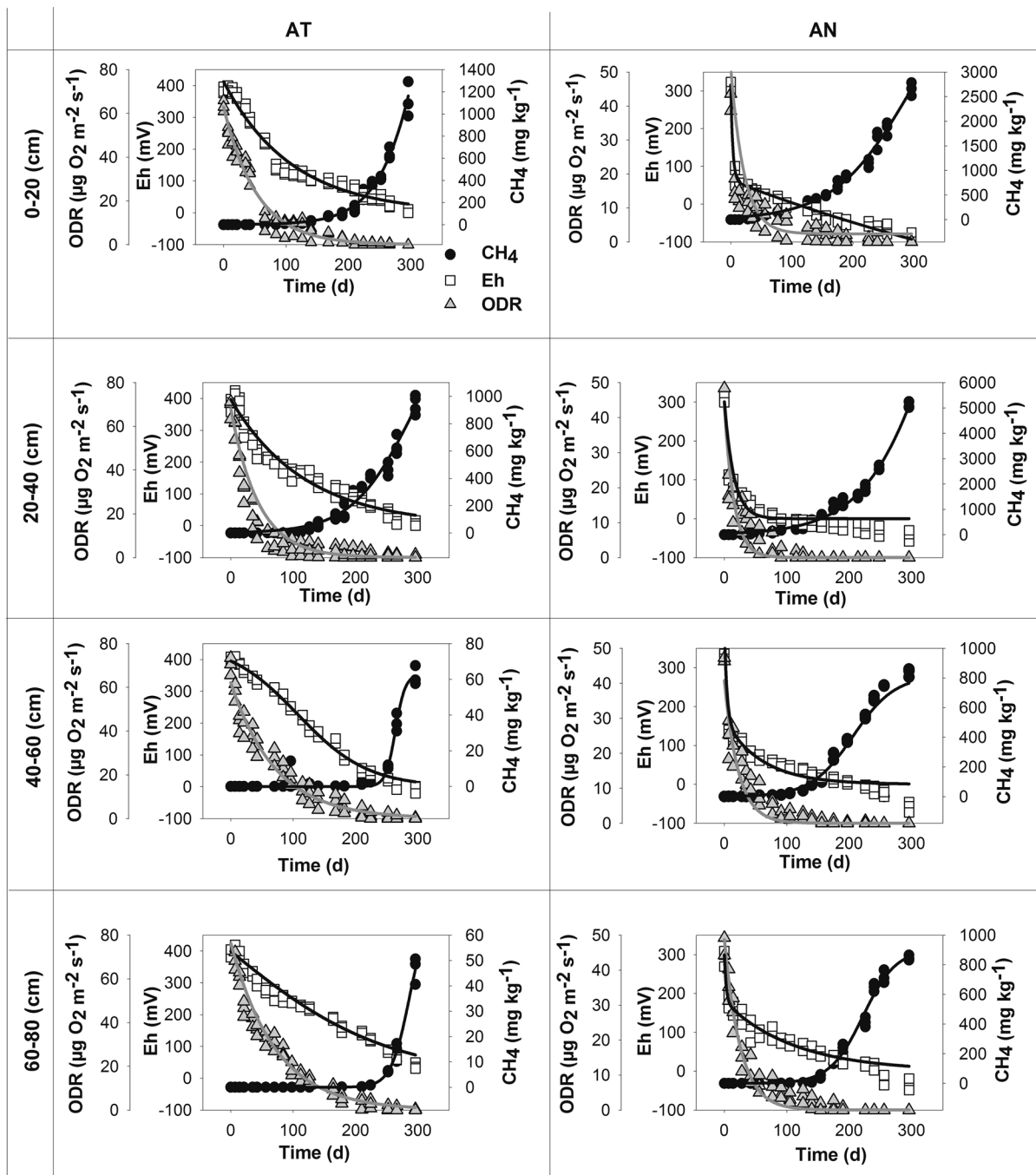
In investigated peat soils, methane (at the concentration of 2 ppm) in the headspace was detected when Eh dropped to  $+240$  mV under AT and  $+112$  mV under AN treatments (Table 3; Fig. 3), and the values were generally higher than those found in natural wetlands (Table 4). The differences in Eh conditions suitable for activation of methanogens in both the AT and AN treatments (Fig. 2) suggest that methanogens can adapt to aerated conditions (i.e. presence of molecular oxygen). This seems possible particularly when the time of methanogenesis activation is compared in the investigated profiles, where under AT treatment the lag time was 8- to 10-times longer than in AN (Fig. 4). The structure of peat provides protective

**Table 1** Characteristics of the peat materials

Depth (cm)	pH ( $\text{H}_2\text{O}$ )	Moisture ( $\text{g g}^{-1}$ )	TOC (%)	Decomposition degree (von Post index)	(mg $\text{kgDW}^{-1}$ )			
					$\text{NO}_2^-$	$\text{NO}_3^-$	$\text{NH}_4^+$	$\text{PO}_4^{3-}$
0–20	6.93a	5.93c	42.7c	H2	0.67a	4.34a	5.62a	14.47a
20–40	6.80a	5.30a	39.2a	H2	0.46b	4.79a	5.84a	11.95a
40–60	6.83a	5.82b	44.9d	H3	0.59ab	8.36b	5.63a	11.14a
60–80	6.77a	6.67d	41.8b	H3	0.51ab	9.90b	5.18a	10.13a

Values followed by the same letter are not significantly different (ANOVA,  $p < 0.05$ ),  $n = 3$





**Fig. 2** Dynamics of methane formation, variation of ODR and Eh at 20 °C during aerated (AT) and anoxic (AN) treatment at particular depths ( $n = 60$ )

conditions for methanogens, or methanogens possess their own protective mechanisms; e.g. antioxidant enzymes to protect them against oxygen (Erkel et al. 2006; Yu et al. 2007; Morozova and Wagner 2007).

The high values of ODR and Eh recorded at the beginning of the experiment (in the AN treatment), were related to the short time sufficient for oxygenation of peat samples during preparation of incubation

**Table 2** Equations of regression describing the change of methane concentration, redox potential (Eh) and oxygen diffusion rates (ODR) in time at aerated (AT) and anaerobic (AN) treatments

Depth	Parameter	AT		AN	
		Equation	R <sup>2</sup>	Equation	R <sup>2</sup>
0–20	CH <sub>4</sub>	$y = 355.66/(1 + \exp(-(x-358.25)/41.59))$	0.98	$y = 5155.86/(1 + \exp(-(x-292.3)/61.67))$	0.98
	Eh	$y = 419.593 \exp(-0.0097x)$	0.97	$y = 281.313 \exp(-0.0489x)$	0.50
	ODR	$y = 62.9197 \exp(-0.0187x)$	0.97	$y = 35.6012 \exp(-0.0460x)$	0.77
20–40	CH <sub>4</sub>	$y = 730.1/(1 + \exp(-(x-290.36)/48.26))$	0.97	$y = 19418.21/(1 + \exp(-(x-358.21)/60.1))$	0.99
	Eh	$y = 396.574 \exp(-0.0084x)$	0.96	$y = 300.549 \exp(-0.066x)$	0.95
	ODR	$y = 68.0830 \exp(-0.0226x)$	0.97	$y = 43.3931 \exp(-0.0647x)$	0.89
40–60	CH <sub>4</sub>	$y = 2.7/(1 + \exp(-(x-63.9)/7.86))$	0.97	$y = 816.96/(1 + \exp(-(x-206.37)/33.48))$	0.88
	Eh	$y = 439.9872 \exp(-0.0076x)$	0.94	$y = 246.0573 \exp(-0.0278x)$	0.81
	ODR	$y = 62.1742 \exp(-0.0140x)$	0.97	$y = 40.7713 \exp(-0.0448x)$	0.92
60–80	CH <sub>4</sub>	$y = 69.46/(1 + \exp(-(x-285.69)/14.53))$	0.99	$y = 906.64/(1 + \exp(-(x-222.1)/26.53))$	0.99
	Eh	$y = 406.5303 \exp(-0.0057x)$	0.97	$y = 227.2747 \exp(-0.0128x)$	0.87
	ODR	$y = 76.3911 \exp(-0.0140x)$	0.96	$y = 48.6916 \exp(-0.039x)$	0.98

All regressions significant at  $p < 0.05$ ,  $n = 60$

(in atmospheric air), but an immediate decrease was observed after flushing with helium. At the beginning of the experiment, the ODR values were between 55 and 70  $\mu\text{g O}_2 \text{ m}^{-2} \cdot \text{s}^{-1}$  irrespective of the peat sampling depth and achieved final levels of 0  $\mu\text{g O}_2 \text{ m}^{-2} \text{ s}^{-1}$  under both AT and AN conditions (Fig. 2). The values of ODR below 26  $\mu\text{g O}_2 \text{ m}^{-2} \text{ s}^{-1}$  at which methanogenesis processes started (Table 3) were lower than 35  $\mu\text{g O}_2 \text{ m}^{-2} \text{ s}^{-1}$  reported in the literature as the limiting threshold of good aeration conditions (Stępniewski et al. 2000; Czyż 2004). However, the intensification of methane formation took place when ODR was around 0  $\mu\text{g O}_2 \text{ m}^{-2} \cdot \text{s}^{-1}$ , indicating a complete lack of oxygen in soil pores. Methane production potential was negatively correlated with the ODR values (Table 3), which was expected. We have confirmed that methanogens in the tested fen soils can be active irrespective of the strictly anoxic conditions reflected by ODR values e.g. higher than 0  $\mu\text{g of O}_2 \text{ per m}^2 \text{ s}^{-1}$  (Table 3). The average values of ODR for methane formation (to the headspace concentration of 2 ppm) at 20 °C under the AT treatment were ca. 20  $\mu\text{g O}_2 \text{ m}^{-2} \cdot \text{s}^{-1}$ , while under the AN treatment, they were slightly lower (<17  $\mu\text{g O}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) (Fig. 3).

There are currently no data about the relation of ODR to methanogenic activity. To study the effect of peat aeration, drainage or rewetting on methanogenesis most researchers used oxygen concentration measurements either in the headspace of investigated samples or in situ chambers as an indicator or by manipulation of water level (Freeman et al. 2002; Yu et al. 2006; Dowrick et al. 2006). Taking into account soil properties, small size of measuring electrodes and possibility of direct measurement, ODR is a convenient alternative for the existing methods of Eh. We found significant negative relationships ( $p < 0.001$ , Table 3) between ODR and cumulative methane in the headspace of the investigated peat soils in the tested treatments (except at 5 °C under the AT conditions, where no methane was detected). Positive and statistically significant relationships ( $p < 0.001$ ) between ODR and Eh were also found (Table 3) suggesting that these two parameters can be considered for approximating methane emission potentials.

#### Methane formation and methanogenic activity

Directly after flushing the bottles, the composition of gases was assessed and no methane in the headspace of

**Table 3** The methane detection time (days, MD) and associated values of aeration parameters (ODR, Eh)

	T (°C)	Depth (cm)	MD (day)	ODR ( $\mu\text{g O}_2 \text{ m}^{-2} \text{ s}^{-1}$ )	Eh (mV)	M-Eh	M-ODR	Eh-ODR	MGA ( $\mu\text{g CH}_4 \text{ kgDW}^{-1} \cdot \text{d}^{-1}$ )
AT	5	0–20	–	42a	389b	–	–	0.30**	0.0
		20–40	–	49b	384a	–	–	0.85***	0.0
		40–60	–	40a	383a	–	–	0.83***	0.0
		60–80	–	42a	382a	–	–	0.72***	0.0
	10	0–20	217a	14a	194c	0.83***	0.71***	0.74***	5.6b
		20–40	231b	15a	169a	0.88***	0.58***	0.48***	6.6c
		40–60	238b	16a	184b	–	–	0.48***	1.8a
		60–80	259c	17a	190c	0.74***	0.79***	0.83***	7.8d
	20	0–20	65b	12a	225a	0.87***	0.93***	0.96***	15610.0d
		20–40	35a	26c	240c	0.86***	0.91***	0.95***	7875.0c
		40–60	70b	24c	240c	0.91***	0.88***	0.97***	1755.0b
		60–80	84c	19b	231b	0.62***	0.66***	0.85***	2720.0a
AN	5	0–20	65b	6a	64a	0.83***	0.87***	0.88***	20.3a
		20–40	70c	5a	82b	0.92***	0.90***	0.92***	244.8b
		40–60	64b	6a	94b	0.69***	0.79***	0.92***	385.6c
		60–80	49a	6a	107c	0.62***	0.98***	0.96***	512.0d
	10	0–20	28a	17b	70a	0.90***	0.87***	0.71***	209.9c
		20–40	28a	13a	90b	0.69***	0.90***	0.96***	1179.4d
		40–60	35b	14a	100c	0.88***	0.85***	0.85***	678.4a
		60–80	49c	13a	112c	0.77***	0.85***	0.94***	443.8b
	20	0–20	3a	15a	90a	0.20*	0.92***	0.67***	21560.0b
		20–40	3a	18b	104b	–	0.96***	0.45***	71860.0c
		40–60	7b	16a	111c	0.36***	0.88***	0.76***	20920.0b
		60–80	14c	18b	104b	0.81***	0.85***	0.81***	20250.0a

Correlation coefficients ( $R^2$ ) between the concentration of formed methane (M,  $\text{mg CH}_4 \text{ g DW}^{-1}$ ) and the oxygen diffusion rate (ODR) as well as redox potential (Eh) ( $n = 60$ ) and methanogenic activity (MGA) at aerated (AT) and anaerobic (AN) treatments. Values followed by the same letter in particular treatments and temperatures across depth are not significantly different (ANOVA,  $p > 0.05$ ).

\* Significant at  $p < 0.05$ , \*\* Significant at  $p < 0.01$ , \*\*\* Significant at  $p < 0.001$

any investigated peat samples was found. In AT treatment methane at 20 and 10 °C was detected between the 35th and 259th days. At 5 °C, despite the 300 days of experiment, no methane was found (Table 3). In AN samples the overall lag time for initiating methane production was significantly shorter (8–10-times) in comparison to AT (Fig. 4). Once methane production was detected, a further increase in its concentration in the headspace was observed (see Table 2 and Fig. 2). Furthermore, the incubations at 20, 10, and 5 °C indicated that for the lower temperatures, methanogens required less oxygen (lower values of ODR and Eh) to initiate methane production ( $p < 0.001$ , Table 3; Fig. 3). Decreasing temperatures

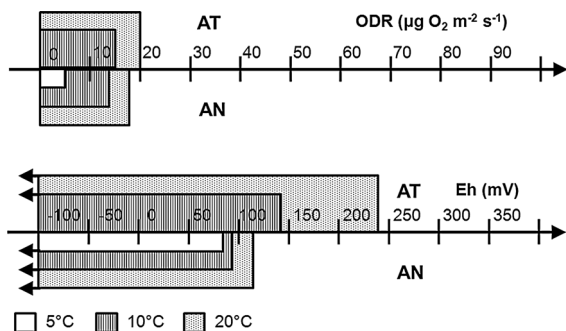
and aeration increased the time until the initiation of  $\text{CH}_4$  production ( $p < 0.05$ ,  $n = 72$ , Fig. 4). Lowering of temperature inhibits enzymatic activity and may also cause microbes to undergo physiological changes that result in reduced carbon and oxygen use efficiency (Frey et al. 2013). It is also possible that some groups of microorganisms cope better with temperature stress, deplete oxygen and create conditions (Eh and ODR values defined as critical) for methane production even at low temperatures.

Methanogenic activity (MGA) of investigated peats under AN treatment ranged from 20.3 (5 °C) to 71.860 (20 °C)  $\text{mg CH}_4 \text{ kgDW}^{-1} \cdot \text{d}^{-1}$  and was influenced by temperature, treatment and depth (three-way ANOVA

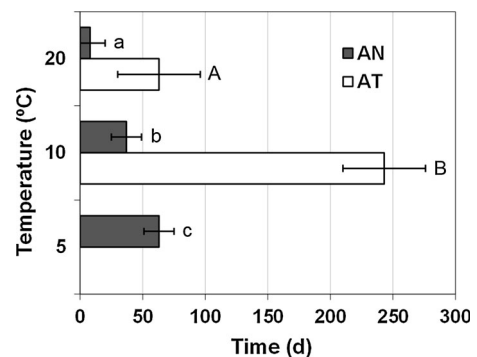


**Table 4** Threshold Eh values for starting methane formation and methanogenic activity in different samples

Origin of the samples	Threshold Eh values for CH <sub>4</sub> production	Methanogenic activity		Authors
		Flux from surface (mg CH <sub>4</sub> m <sup>-2</sup> d <sup>-1</sup> ) <sup>a</sup> or concentration in gas phase (μg l <sup>-1</sup> ) <sup>b</sup>	By weight of the sample (μg kg <sup>-1</sup> d <sup>-1</sup> ) <sup>c</sup>	
Wetland soils (Alluga region, Germany)	-75	15–244 <sup>a</sup>	–	Fiedler and Sommer (2000)
Organic sediments (Denmark)	-90	–	23,040	Grünfeld and Brix (1999)
Mangrove Sediment (Mzinga creek, Tanzania)	-150 to -300	200 <sup>a</sup>	–	Lyimo et al. (2002)
Forest soil (Louisiana, USA)	+300	1.6–177.0 <sup>b</sup>	–	Yu et al. 2006
Water bodies (Lucknow, India)	-150	91.2–1241.6 <sup>a</sup>	–	Singh (2001)
Rice paddy soils (Louisiana, USA)	+300	–	24–12,082	Yu and Patrick (2003)
Paddy soils (Japan)	-150	–	495.36	Minamikawa and Sakai (2005)
Rice soils: Arkansas, California, Louisiana, Texas (USA); Hangzhou (China), Java (Indonesia)	+400	–	To 240	Yu et al. (2007)
<i>Methanothermobacter thermautotrophicus</i> in medium culture	-200	–	–	Hirano et al. (2013)
<i>Methanosarcina barkeri</i> in medium culture	+50 to +420	–	–	Fetzer and Conrad (1993)
Garbatówka fen (east Poland)	+240 to +112	–	1.8–71,860	This study

<sup>a</sup> Flux from the surface<sup>b</sup> Concentration in gas phase<sup>c</sup> Data recalculated to common unit**Fig. 3** The favourable ranges of ODR and Eh for methane production at 5, 10 and 20 °C under aerated (AT) and anoxic (AN) treatments

test,  $p < 0.001$ ). It was higher than in an organic sediment or rice paddy soils (Table 4) as well as a natural bog from Québec, Canada (130 μg CH<sub>4</sub> kgDW<sup>-1</sup>·d<sup>-1</sup>; Glatzel et al. 2004), acid bog from Germany (6,400–27,200 μg CH<sub>4</sub> kgDW<sup>-1</sup>·d<sup>-1</sup>; Horn et al. 2003), freshwater marshes from Sanjiang Mire Wetland in China (2–200 μg CH<sub>4</sub> kgDW<sup>-1</sup>·d<sup>-1</sup>; Liu et al. 2011) but was 3-times lower than in

**Fig. 4** Time of methanogenic activation at 5, 10, and 20 °C ( $n = 72$ ). Different letters above the bars refer to significant differences in the lag time among methanogenically active samples separately of AT and AN treatments ( $p < 0.05$ )

ombrotrophic upland blanket peat at Lake Vyrnwy in the United Kingdom (228,480 μg CH<sub>4</sub> kgDW<sup>-1</sup>·d<sup>-1</sup>; Freitag and Prosser 2009).

Soil aeration lowered the methanogenic activity of investigated peats from 28 to 100 % ( $p < 0.001$ , Table 3). The stronger reduction at 5 and 10 °C (by

98–100 %) while weaker (by 28–92 %) at 20 °C was noted. Similarly in mid-Wales wetland (UK) methane production after drought was reduced by 89 % (Freeman et al. 2002) and after 4 week the lowering of the surface emission was from 1.21 to 0.28 mg m<sup>-2</sup> h<sup>-1</sup> (Dowrick et al. 2006). A short period of aeration (1 week) can either stimulate (by stimulation organic matter decomposition in presence of oxygen and increase of availability simple substrate for methanogenesis) or does not affect the methane production as was found in Dutch and Germany peats (Fenner and Freeman 2011; Brouns et al. 2014). But the longer the drought the higher reduction in methane emission was observed. The fact that methanogens can survive the periods of drought (e.g. 4 weeks) in some bogs, fens and riparian wetlands has been confirmed by molecular analysis. There were no differences in the diversity and composition of the microbial communities between control and drought (Kim et al. 2008). However, in rice paddy soils the growth of methanogenic populations, as revealed by *mcrA* gene abundance, was suppressed during the second dry/wet cycle (Ma et al. 2012).

Our results also revealed that in optimal temperature (20 °C), surface (0–20 cm, AT), and subsurface (20–40 cm, AN) depths were associated with the highest methanogenic potential of 15,610 µg CH<sub>4</sub> kgDW<sup>-1</sup>·d<sup>-1</sup> and 71,860 µg CH<sub>4</sub> kgDW<sup>-1</sup>·d<sup>-1</sup>, respectively ( $p < 0.001$ , Table 3). In 10 and 20 °C peat from level 0–20 cm was more resistant to oxygenation and the reduction of methanogenic activity was 87 and 28 % (Table 2). The surface level of peatlands is often exposed to air due to groundwater table fluctuations, therefore the present methanogenic microorganisms could be adapted to the presence of oxygen, which could be transported also by plant root system e.g. *Carex* spp. and *Phragmites* spp. overgrowing investigated fen. Plants can also enhance methane production in surface layers by supplying fresh litter and exudates as a substrates for methanogenic microorganisms (Roura-Carol and Freeman 1999). Deeper layers of peat are more stable, permanently anoxic and probably inhabited mostly by microorganisms highly sensitive to oxygen (Glass and Orphan 2012; Knoblauch et al. 2008; Tholen et al. 2007).

## Conclusions

We conducted research on peat soils under aerated and anoxic conditions and found that aeration parameters

such as Eh or ODR are tightly coupled to methane production by methanogenic microbial communities. Methane concentration was negatively correlated with both Eh and ODR ( $p < 0.05$ ). Our data suggest that methanogens inhabiting natural peats can survive the exposure to oxygen stress (ODR > 60 µg O<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>, Eh > + 400 mV). We determined the Eh upper threshold of +240 mV, for methane production in this peat. The corresponding threshold for ODR was 20 µg O<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>. These threshold values are higher than reported previously. We also showed that the temperature has significant influence on the aeration parameters. When the incubation temperature decreased (from 20 to 10 or 5 °C), the aeration thresholds required for methane synthesis also decreased. The time needed for creation conditions suitable for methane production varied between 3 and 259 days, depending on the temperature and treatment. These results suggest that methanogens survived stress caused by oxygen and temperature with exception of the 5 °C AT treatment when no methane was found during the 300 days of the experiment. Stress caused by aerated conditions resulted in a decrease in the methanogenic activity from 28 to 100 % relative to the anoxic treatment. Finally, we found that methanogenic activity was dependent on the depth in the peat soil profile. Approximately four times higher activity was observed in the surface (0–40 cm), compared with the deeper (40–80 cm) layers.

To our knowledge, this is the first study which directly links ODR to methane production potential. ODR was found to be a useful technique for estimating the methanogenic potential of organic soils, and showed statistically significant negative relationships between ODR and methane production as well as between ODR and Eh ( $p < 0.001$ ). The measurement of O<sub>2</sub> availability in soils by ODR can provide further insight into the dynamics of methane cycling in the field. The future studies should be conducted in order to confirm the usefulness of ODR technique in other types of wetlands. The relationships reported here between ODR/Eh and methanogenic activity can be potentially incorporated into predictive models for methane emission from peat soils and be useful for testing future methane emission scenarios based on Eh and ODR predictions in the changing climate.

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