Research Article

Methane Production and Consumption in Loess Soil at Different Slope Position

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Methane (CH$_4$) production and consumption and soil respiration in loess soils collected from summit (Top), back slope (Middle), and slope bottom (Bottom) positions were assessed in laboratory incubations. The CH$_4$ production potential was determined under conditions which can occur in the field (relatively short-term flooding periods with initially ambient O$_2$ concentrations), and the CH$_4$ oxidation potential was estimated in wet soils enriched with CH$_4$. None of the soils tested in this study emitted a significant amount of CH$_4$. In fact, the Middle and Bottom soils, especially at the depth of 20–40 cm, were a consistent sink of methane. Soils collected at different slope positions significantly differed in their methanogenic, methanotrophic, and respiration activities. In comparison with the Top position (as reference soil), methane production and both CO$_2$ production and O$_2$ consumption under flooding were significantly stimulated in the soil from the Middle slope position ($P<0.001$), while they were reduced in the Bottom soil (not significantly, by 6 to 57%). All upper soils (0–20 cm) completely oxidized the added methane (5 kPa) during 9–11 days of incubation. Soils collected from the 20–40 cm at the Middle and Bottom slope positions, however, consumed significantly more CH$_4$ than the Top soil ($P<0.001$).

1. Introduction

Methane (CH$_4$) is the most abundant hydrocarbon in the atmosphere, and it is an important greenhouse gas, which so far has contributed to an estimated 18–20\% [1, 2] of postindustrial global warming. Methane has environmental impacts beyond those of a direct greenhouse gas, through atmospheric chemistry that enhances the abundance of tropospheric ozone (O$_3$) and decreases that of hydroxyl radicals (OH) and hence the atmospheric lifetime of many other pollutants [3]. The atmospheric CH$_4$ concentration has risen from the background level from 700 to 1782 ppb in 2006, and the growth rate in CH$_4$ concentration was changing considerably; the very large and interannual variations in CH$_4$ concentration remain unexplained and present an important challenge to the research community [4, 5]. Estimated surface CH$_4$ emissions reach 643 Tg year$^{-1}$ [3]. Oxidation of atmospheric methane by well-drained soils accounts for about 10\% [6] or 6\% [4] of the global methane sink, that is about 30 Tg CH$_4$ per year. Other CH$_4$ sinks are the stratosphere (40 Tg year$^{-1}$) and tropospheric OH (445 Tg year$^{-1}$) [4].

Most methane on Earth is produced by Archaea through methanogenesis, the final step in fermentation of organic matter, which takes place in rice fields, the guts of animals, soils, wetlands, and landfills, as well as in freshwater and marine sediments. As a simple assumption, about 10–20\% of reactive organic material buried in soils and sediments is converted to methane [1]. The potential impact of methane on future global warming and an important role of soils in sorption of this gas have led to many terrestrial studies of methods and techniques to quantify CH$_4$ flux at the soil-atmosphere interface [7]. Numerous experimental data on emission of greenhouse gases are used in modelling of the local and global gas emissions, while some models were developed to determine abatement strategies to meet restrictions on emission and/or deposition levels at the least cost [8].

Soil saturation with water has dramatic consequences for gas diffusion processes in soil (as gases diffuse 10,000
faster in air than in water). Consequently, one of the main effects of flooding is a lower pool of available O₂ [9, 10] and a several-fold change in the activity of the oxidoreductases—intracellular enzymes involved in the oxidative metabolism of soil microorganisms [11]. Conventional knowledge states that water-saturated systems like wetlands (swamps, marshes) and paddy soils (rice fields) are net contributors of CH₄ to the atmosphere, whereas upland soils (with the exception of landfills) are generally sinks for CH₄ [12]. However, significant methane emission from field soils may also occur after normal precipitation if the soils remain saturated for a long enough period, since water occupation of soil voids may cause oxygen deficiency and development of reducing conditions. Even in unsaturated conditions, there may be anaerobic microsites capable of evolving methane. Little is known, however, about methane emission when usually well-drained soils become flooded for a short period [13]. In fact, soils can act as a source and a sink for CH₄, depending on their air-water conditions [7, 14].

Soil properties are a product of soil-forming factors including landscape variability, agroecosystem management, and climatic factors. Numerous studies were performed to measure the effect of landscape position and land management on physical, chemical and biological soil properties [15–21]. Soils developed from loess are fertile and show high erodibility [22]. Soil erosion results in heavy differentiation of a soil cover with natural pedons being reduced or overbuilt. Both eroded and colluvial soils differ from uneroded soils not only in morphological features but also in particle-size and pore distributions, organic matter content and plant nutrients, water retention, and bulk density [23]. Loess soils are among the most susceptible to the drop in redox potential under anaerobic conditions, which is followed by a rapid reduction of the oxidized inorganic soil components [24]. In consequence, periodical soil hypoxia changes soil respiration, which plays a fundamental role in the metabolism of the soil biota and promotes development of methanogenic microorganisms.

The objective of this study was to compare the CH₄ production and CH₄ consumption in slightly eroded loess soils taken at the summit, back slope, and bottom of a hill. The experiment was performed in laboratory under controlled temperature and air-water conditions. Initially, ambient O₂ concentrations were present in both flooded and wet soil incubations. Our intention was to determine the soil potential for methane production under conditions which occur in field (relatively short-term flooding periods for methanogenic activity) and for methane oxidation (soil enriched with CH₄).

2. Materials and Methods

2.1. Site and Soil Description. A loessial agricultural basin of the Ciemięga River (near Lublin, south-east part of Poland) is a region of the water erosion risk, including sediment transport and nutrient runoff, and is under intensive agricultural use [24, 25]. Soil samples were collected near Baszki village from two depths (0–20 cm and 20–40 cm) and three slope positions: at the summit (Top), back slope (Middle), and slope bottom (Bottom).

The slope is about 15 m high and 60 m long and is covered by natural grass vegetation; it is at the distance of about 150 m from the river. The annual precipitation in this region is 570 mm, and the average annual temperature is +7.5°C [25]. The basic characteristic of the tested brown loess soil (Eutric Cambisol) is shown in Table 1.

2.2. Incubation Experiment. For methanogenic activity measurements, 20 g portions of air-dry soils were placed into 60 cm³ glass vessels and flooded with 15 cm³ of distilled water. All the vessels were tightly closed with rubber stoppers and aluminium caps, and the flooded soils were incubated at 25°C for 28 days.

For methanotrophic activity measurements, 10 g portions of air-dried soils were placed into 60 cm³ glass vessels and 5 cm³ of distilled water was added. All the vessels were tightly closed with rubber stoppers and aluminium caps, and wet soils were enriched with 5% (v/v) CH₄ (5 kPa). The soil samples were incubated at 20°C for 21 days.

Initially, ambient O₂ concentrations were present in both incubations (20.5% v/v). Our intention was to determine the potential of soils for methane production under field conditions, with relatively short-term flooding periods, and for methane oxidation after soil enrichment with CH₄.

2.3. Methods. The concentrations of gases in the headspace were measured with gas chromatographs Shimadzu GC-14B and GC-14A (Japan) equipped with a flame ionization detector (FID) and a thermal conductivity detector (TCD), respectively. Methane was detected by the FID detector at 150°C. The gas components were separated on a column packed with a Porapak Q maintained at 80°C, and the temperature of the injector was 150°C. Carbon dioxide and O₂ were detected by TCD with the use of two 2 m columns.
The tested soils showed relatively large differences in their respiration under flooding. Both CO₂ evolution and O₂ consumption were more intensive in the upper rather than deeper soil layers (Figures 1(b) and 1(c)). The Middle soil produced 207.6 mg CO₂-C kg⁻¹ and consumed 19.14% (v/v), resp.), Final amounts of CH₄, CO₂, and O₂ were assessed by Student’s “t” test to determine the significance of the differences in gas production or consumption between soils. Correlations between total gases produced or consumed over time, and organic carbon in soils collected from different slope positions were tested with regression analysis.

### 3. Results

#### 3.1. Methanogenic Activity of Soils from Different Slope Positions

Position of soil in the slope strongly affected the capacity of CH₄ production. Methane was produced in flooded soils after a 17-day lag (Figure 1). The highest methanogenic activity was observed in the Middle soil. During 28-day incubation, the upper 0–20 cm soil evolved 3.17 mg CH₄-C kg⁻¹ at a rate of 0.304 mg CH₄-C kg⁻¹ d⁻¹ (Figure 1(a), Table 2). Soil sampled at the Top position produced only 0.359 mg CH₄-C kg⁻¹, while the Bottom soil evolved even less than 0.06 mg CH₄-C kg⁻¹.

Deeper soil layers (20–40 cm) showed significantly lower methanogenic activity (P < 0.001) with the highest production in the soil from the Middle position 1.35 mg CH₄-C kg⁻¹ and much lower in the other soils: less than 0.002 mg CH₄-C kg⁻¹ (Figure 1(d)).
(v/v). At the beginning of CH4 evolution in this soil after 17 days of incubation, there was only 3.21% (v/v) O2 left in the headspace. At the end of incubation, O2 was hardly depleted (1.36% v/v in the headspace). The Top and Bottom soils consumed 14.6 and 11.6% (v/v) O2, respectively, which yielded the final O2 concentration in the headspace of 5.88 and 8.85% (v/v), respectively.

The subsurface-flooded soils showed some lower respiration (Figures 1(e) and 1(f)). An exception was the Middle soil, which produced as much as 174.6 mg CO2-C kg$^{-1}$, while it consumed 18.5% (v/v) O2 (2% v/v O2 left in the headspace). The other soils followed the tendency observed in CH4 production; thus they evolved less CO2 and consumed communities and the factors which affect the accumulation and redistribution of water, nutrients, sediments, and organic matter. Soils on ridges and upper slopes will tend to loose soil and organic matter that will tend to accumulate on lower slopes and in depressions. Generally, soils in lower-slope positions will tend to have a wetter moisture regime for a longer period [33], while soil O2 concentrations may decrease significantly from ridges to valleys [34]. Methane emission from low-slope positions may be observed already one or three days after summer rainfall, depending on the intensity of precipitation [13]. It has been assumed that, in well-aerated soils, CH4 production in anaerobic microsites could be an important source of methane for methane oxidizing bacteria [35]. Little is known, however, about methane emission when usually well-drained soils become flooded for a short period [13]. The characteristics of CH4 oxidizing and producing communities and the factors which affect these characteristics as well as CH4 transport determine the magnitude of the surface CH4 flux to the atmosphere [36]. Our studies with loess soil collected from different slope positions and incubated under laboratory conditions showed that the slope position significantly affected the soil

### Table 2: CH4 production, CO2 evolution and O2 uptake of soils collected from three slope positions, and incubated for 28 days under flooding (average values ± standard error, $n = 3$).

| Slope position | Soil depth (cm) | CH4 production | O2 uptake % (v/v) |
|---------------|----------------|----------------|------------------|
|               |                | Total (mg C kg$^{-1}$) | Rate (mg C kg$^{-1}$ d$^{-1}$) | CO2 evolution (mg C kg$^{-1}$) |               |
| Top           | 0–20           | 0.3595 ± 0.118 | 0.0316           | 138.1 ± 2.56 | 14.61 ± 0.85 |
|               | 20–40          | 0.0017 ± 0.001 | 0.0001           | 110.2 ± 2.19 | 8.73 ± 0.04 |
| Middle        | 0–20           | 3.1679** ± 0.140 | 0.3042           | 207.6** ± 2.34 | 19.14** ± 0.10 |
|               | 20–40          | 1.3538** ± 0.129 | 0.1162           | 174.6** ± 0.48 | 18.49** ± 0.29 |
| Bottom        | 0–20           | 0.0584 ns ± 0.011 | 0.0065           | 106.7** ± 0.13 | 11.65** ± 0.12 |
|               | 20–40          | 0.0018 ns ± 0.001 | 0.0003           | 47.8** ± 0.59  | 7.71 ns ± 0.13 |

*, **, ***, different from the Top position (reference soil) at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively, according to Student’s t-test; ns—not significant difference.

3.3. Relations between Measured Soil Properties. The amount of methane produced in flooded soils showed a close relationship with the amount of organic C modified by the soil position in the slope (Figure 3(a)). Similar significant relations were observed for CO2 produced and O2 consumed during incubation of flooded and wet soils (i.e., in the course of methane production and oxidation, resp.) versus Corg (Figures 3(a) and 3(b)). Such correlations for methane oxidation were not shown.

4. Discussion

Position in the landscape affects the accumulation and redistribution of water, nutrients, sediments, and organic matter. Soils on ridges and upper slopes will tend to loose soil and organic material that will tend to accumulate on lower slopes and in depressions. Generally, soils in lower-slope positions will tend to have a wetter moisture regime for a longer period [33], while soil O2 concentrations may decrease significantly from ridges to valleys [34]. Methane emission from low-slope positions may be observed already one or three days after summer rainfall, depending on the intensity of precipitation [13]. It has been assumed that, in well-aerated soils, CH4 production in anaerobic microsites could be an important source of methane for methane oxidizing bacteria [35]. Little is known, however, about methane emission when usually well-drained soils become flooded for a short period [13]. The characteristics of CH4 oxidizing and producing communities and the factors which affect these characteristics as well as CH4 transport determine the magnitude of the surface CH4 flux to the atmosphere [36].
Table 3: CH₄ consumption, CO₂ evolution, and O₂ uptake in soils collected from three slope positions and incubated with 5 kPa methane for 21 days (average values ± standard error, n = 3).

| Slope position | Soil depth (cm) | CH₄ consumption | CO₂ evolution | O₂ uptake |
|---------------|----------------|----------------|--------------|-----------|
|               | Total (mg C kg⁻¹) | % of initial CH₄ | Rate (mg C kg⁻¹ d⁻¹) | % (v/v) Total (mg C kg⁻¹) | % of initial CH₄ |
| Top          | 0–20 | 130.84 ± 2.31 | 100 | −16.08 | 186.7 ± 11.4 | 11.14 ± 0.53 |
|              | 20–40 | 17.80 ± 2.62 | 14 | −0.799 | 83.9 ± 2.86 | 2.55 ± 0.11 |
| Middle       | 0–20 | 121.36* ± 0.01 | 100 | −20.66 | 198.9 ns ± 3.57 | 11.77 ns ± 0.34 |
|              | 20–40 | 131.71* ± 0.80 | 100 | −12.26 | 224.1*** ± 5.26 | 13.63*** ± 0.32 |
| Bottom       | 0–20 | 130.96 ns ± 43.7 | 100 | −17.58 | 151.9 ns ± 5.34 | 9.44 ns ± 0.38 |
|              | 20–40 | 116.49*** ± 12.2 | 92 | −7.954 | 104.1** ± 3.14 | 6.99*** ± 0.50 |

*, **, *** different from the Top (reference) soil at P < 0.05, P < 0.01, and P < 0.001, respectively, according to Student’s t-test; ns—not significant difference.

Figure 2: Changes of CH₄, CO₂, and O₂ over time in loess soils collected from three slope positions and incubated with added methane, 5 kPa (methanotrophic potential). Top: summit, Middle: back slope, Bottom: bottom of the slope. Upper graphs (a–c) upper soil depth of 0–20 cm; lower graphs (d–f) lower soil depth of 20–40 cm. (a) and (d) cumulative CH₄ production; (b) and (e) cumulative CO₂ production; (c) and (f)—changes in O₂ in the headspace. Points represent triplicate-means with standard error.

In the course of methanogenesis, soil respiration underwent modification similar to that observed for the methanogenic potential. In the Middle slope position, CO₂ production and O₂ uptake were significantly stimulated as compared with the Top soil (by 50% and 30%, resp., P < 0.001 and P < 0.01). Soil collected at the Bottom position showed lower respiration than the Top soil (both CO₂ and O₂ less by about 20%, P < 0.01). In the deeper soil layer, the changes were generally more pronounced.

Changes in soil respiration in the course of methanotrophy were apparently dependent on soil depth. In comparison with the Top site, the upper Middle and Bottom soils were not changed, as all soils consumed comparable amounts of methane. However, soils sampled from a depth of 20–40 cm...
respired at a significantly higher rate than the Top soil (up to 5 times) \( (P < 0.001) \).

In the experiments of \cite{21}, water-stable aggregates were significantly different among landscape positions and decreased from lower > middle > summit landscape position. However, in some soils, the landscape effect was insignificant, for example, for enzyme activities or emission of CH4 and CO2 \cite{19, 21}. Fang et al. \cite{17} observed that neither potential net N mineralization nor nitrification was differentiated by the slope position, nor was accumulative emissions of N2O or CO2 from incubated soils in laboratory; in contrast, the ability to oxide CH4 appeared to decrease from the bottom to the top.

It is well known that methane fluxes are strongly regulated by the presence or absence of methanotrophs (CH4 oxidizers), which are generally found in the upper (0–20 cm) soil \cite{37}. On the other hand, methanogens (CH4 producers) use labile carbon compounds that were produced in the root zone and are less abundant with increasing distance from the soil surface, and rates of potential CH4 production decline with depth below the aerobic zone \cite{38}. However, deeper soil also contributes to CH4 emission. In our experiment, both upper (0–20 cm) and lower (20–40 cm) soil layers of the Middle position evolved CH4. The process started relatively fast, after 17 days of flooded incubation at 25°C, and CH4 reached 3.16 mg CH4-C kg\(^{-1}\) (0.35% v/v of CH4 in the headspace) over 28-day incubation. Similarly, Mayer and Conrad \cite{39} observed a rapid increase in CH4 production within 25 days of flooding an upland agricultural soil and a forest soil. It is possible that, in our experiment, CH4 emissions did not occur in the other soils since O2, which is the most thermodynamically favourable electron acceptor \cite{29, 37}, was still in the headspace. Methanogenesis is evidently inhibited by O2 this is apparent from field studies that show no overlap in the depth distributions of O2 penetration in soils or sediments and net CH4 production \cite{38}. However, the lack of CH4 production in the presence of O2 \textit{in situ} may be due to a combination of factors, of which O2 toxicity is just one. For example, methanogens are more sensitive to desiccation than O2 exposure in a paddy

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**Figure 3:** Relationships between total gases produced or consumed over time and organic carbon (Corg) in soils collected from different slope positions. (a) CH4 produced in flooded soils versus Corg; (b) CO2 produced and O2 consumed in flooded soils versus Corg \( (y = 7.60 \cdot \text{Ln}(x) + 14.1, R^2 = 0.92^{***}) \) and \( (y = 88.4 \cdot \text{Ln}(x) + 138.7, R^2 = 0.93^{***}) \); respectively, (c) CO2 produced and O2 consumed in wet soils enriched with CH4 soils versus Corg \( (y = 78.1 \cdot \text{Ln}(x) + 165.2, R^2 = 0.74^{***}) \) and \( (y = 4.38 \cdot \text{Ln}(x) + 4.60, R^2 = 0.92^{*}) \), respectively. Points present mean values. ** and *, \( P < 0.001 \) and \( P < 0.05 \), respectively.
with the top soil (Table 1), stimulated methane production
of organic soils (field and
in agriculture, “Global methane emissions
of land use on soil aggregate stability, ” International Agrophysics
activity and accompanying processes in organic soils (field and
systems,” Journal of Environmental Quality
on soil properties in a loessial landscape. They observed that slight, moderate, and severe erosion has an
in soil containing more sand, because \( \text{O}_2 \) is necessary for monooxynogen enzyme which catalyzes methane oxidation [42]. Coarse-textured soils have been documented as supporting \( \text{CH}_4 \) oxidation by enhancing gas diffusion (\( \text{CH}_4 \) and \( \text{O}_2 \)) into the soil [43].

Paluszek and Žembrowski [44] present their findings from a long-term study designed to explore effect of accelerated erosion on soil properties in a loessial landscape. They observed that slight, moderate, and severe erosion has an adverse effect on soil physical properties. The clay content and bulk density in Ap horizons of eroded soils are on the increase whereas the content of organic matter, content of water-stable aggregates, field water capacity, and retention of water useful for plants decrease. In the consequence, soil porosity, air capacity, and air permeability deteriorate. By contrast, in very severely eroded soils whose Ap horizons developed from carbonate loess, pore-size distribution, field water capacity and retention of water useful for plants are favourable and comparable to those in noneroded soils [44].

High organic C content in the Middle soil, as compared with the Top soil (Table 1), stimulated methane production under soil hypoxia. Probably, small differences in \( C_{\text{org}} \) between Top and Bottom soils may explain insignificant differences in methanogenic activity. Nevertheless, high correlation coefficients obtained for relationships between \( C_{\text{org}} \) and produced \( \text{CH}_4 \), evolved \( \text{CO}_2 \), and consumed \( \text{O}_2 \) confirm their universal character (Figure 3). However, better explanation of the changes observed in our experiment needs more information on the properties of tested soils at different slope positions.

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