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METHANOGENIC POTENTIAL OF ARCHIVED SOILS

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Abstract: Methane (CH_4) is an important element of the biogeochemical carbon cycle. Methanogenic *Archaea* are strict anaerobes able to survive in dry and oxic soils, but not in liquid or agar slurry. Little is known about the mechanisms of their survival. The aim of this paper is to study the methanogenic potential of mineral soils stored as air-dry over 20 years. We tested the hypothesis that the recovery of CH_4 formation is strongly associated with soil textures. Samples of 16 mineral topsoils characterized by various C_{org}, pH and particle size distribution (PSD), and stored under air-dry conditions over 20 years were flooded with: i) water and incubated in N_2 atmosphere, or ii) with glucose solution without headspace gas exchange and incubated for 132 days. Gases were measured chromatographically, PSD by laser diffraction method. Microbial activity was restored in all tested soils, and CH_4 and CO_2 production started within a few days or weeks after flooding, depending on soil properties and incubation conditions. The glucose amendment resulted in a 2.8-fold increase in the total CH_4 and CO_2 release. However, in the presence of glucose, methanogens in three soils were outcompeted by other microorganisms, and required a long 132-d lag phase or did not start CH_4 production at all. The CH_4 positively correlated with the finer soil fractions, especially with fine silt and clay, while negatively with medium and coarse sand fractions. Consequently, silt loam soils showed approximately 5 and 2.5 times higher CH_4 production, than soils with coarser textures (sand and sandy loam soils, respectively). In contrast, CO_2 production was not influenced by soil texture. The C_{org} and moisture retention in dry soils showed even stronger correlations with CH_4 and CO_2, except for CH_4 released in the presence of glucose, where correlations with PSD were strongest. Most soil properties were associated with the first principal component (PC1), which explained 58.1% of the qualitative differences between the compared soils. The results stressed the significance of the inherent soil properties in determining the persistence of microorganisms responsible for CH_4 and CO_2 production over long storage in air-dry conditions. In fact, all analysed soil properties are related to each other and create specific habitats which allow microorganisms to persist in unfavourable conditions. Anaerobic incubations without C amendment resulted in CH_4 production in all tested soils, while in some glucose enriched sand or sandy loam soils methanogens were outcompeted by other microorganisms.

Keywords: soils, methanogens, persistence in dry soils, soil properties, particle size distribution

1. INTRODUCTION

Methane (CH_4) is the second most important greenhouse gas in the atmosphere after carbon dioxide (CO_2). Despite relatively low atmospheric concentration of CH_4 (1.782 ppm in 2006) and short atmospheric lifetime, it accounts for up to 20-30% of the global warming effect. Its global warming potential is up to 40 times higher than CO_2, mainly due to much higher efficiency in trapping radiation in the atmosphere (Shindell et al., 2009; Owens & Xu 2011), therefore it is crucial to understand the processes and factors affecting CH_4 fluxes. About 80% of the total CH_4 emitted to the atmosphere is microbially produced during methanogenesis, by a specific group of methanogens *Archaea*, in the absence of free oxygen though anaerobic decomposition of organic matter. CH_4 is formed in the last step of fermentation where methanogenic *Archaea* consume products of activity of the larger bacterial community including hydrolytic, fermenting, syntrophic and acetogenic bacteria (Le Mer & Roger 2001).

The ecology of methanogenic archaea is complex and not all aspects of soil methanogenic activity have been elucidated (Inubushi et al., 2003; Hatano & Lipiec 2004; Xu & Inubushi, 2009; Angel et al., 2011; Watanabe et al., 2011; Brzezińska et al., 2012). Methanogenesis is traditionally regarded to occur only in highly reduced, anoxic environments such as wetland, rice field soils, mud volcanoes, and landfills (Megonigal et al., 2004; Frunzeti et al., 2012; Watanabe et al., 2011). However, low quantities of CH_4 are also produced under unflooded conditions in various soils (Xu & Inubushi 2009; Angel et al., 2011; Watanabe et al., 2011). CH_4 consumption is conducted by methanotrophs, the aerobic *Proteobacteria.* Methanotrophy occurs under oxic conditions or at the oxic/anoxic boundaries of soils or sediments (Conrad 2002) where CH_4 arisen from methanogenesis or atmospheric CH_4 is consumed. Under experimental conditions the same soil may produce or consume CH_4, depending on the soil air-water and oxygen status present (Brzezińska et al., 2012).

CH_4 fluxes are known to respond quickly to seasonal soil moisture fluctuations and changes in the groundwater level when soil conditions change from oxic to anoxic (Hatano, 2011). Less is known about the CH_4 flux response to more extreme changes and longer term dry oxic conditions or flooding for typically aerated soils. According to current climatic predictions, prolonged dry spells and altered precipitation patterns causing flooding will become a very common feature of the temperate climate (Shindell et al., 2009; Owens & Xu 2011). The effect of drying and rewetting will become even more pronounced if soils become water-repellent and the water infiltration into the soil becomes restricted (Urbanek et al., 2007). The evermore extreme oscillations of soil moisture predicted are likely to alter the survival and activity of various microorganisms (De Nobili et al., 2006).

Drying and rewetting of the soil is known to change the soil status and influence the size and activity of soil microbial populations (Clark & Hirsch 2008; Chowdhury et al., 2011; Kim et al., 2012), including methanogenic archaea (Conrad 2002). In environments with regularly occurring prolonged dry conditions *e.g.* due to scarce precipitation, native soil microbial populations evolve to survive and reactivate in the rainy seasons (De Nobili et al., 2006). Strict anaerobic bacteria have the ability to survive under oxic conditions. Lin et al., (2004) observed that the anaerobic *Geobacter sulfurreducens* present in the anoxic interior zones of soil aggregates, in otherwise oxic soils, are periodically exposed to oxygen and are able to tolerate it but become the predominant microorganism once oxygen becomes limited. The methanogens also have the ability to endure desiccation, exposure to high levels of oxygen and can survive for long periods of time in largely dry and oxic soils (Liu et al., 2008) despite the fact that they do not form spores or other resting stages (Megonigal et al., 2004). Next generation molecular techniques used in the recent study of Angel et al., (2012) revealed that methanogenic microorganism are not only able to survive short-term oxic conditions, but are actually globally ubiquitous in aerated soils and become active once put under wet anoxic conditions. The discovery of Angel et al., (2012) about the abundance of methanogens in aerated soils raises the question whether soils with certain specific soil properties have a higher methanogenic potential than the others.

In the current study we hypothesise that the soil texture maybe strongly affecting the methanogenic potential of the soil. Specific particle size distribution (PSD) in soils creates unique physical conditions which affects soil structure, water relations, redox reactions etc., and therefore acts as soil microhabitats for diverse kinds of microbial biota. It is therefore very likely that the microbial community structure and consequently the methanogenic potential strongly depend on the PSD (Li et al., 2007; Zhang et al., 2007). In order to test the hypothesis, the methanogenic potential of soils with different origin and particle size distribution archived in dried conditions for over 20 years have been tested. The archived soils exhibit a very specific habitat for soil microorganisms due to the lack of external organic matter addition to the soil. Such isolation from water, external sources of microbial life and SOM should make the microbial activity in dry-stored soils more depended on the inherent properties of the soil rather than the environmental conditions.

2. MATERIALS AND METHODS

2.1. Soils Description

Sixteen mineral soils, which represent the main soil types in Poland, were selected for the study (Bieganowski et al., 2013). The samples were collected from the topsoil of various agricultural sites in Poland, air-dried without sieving, and stored for 23 years at room temperature (about 20^{o}C). Prior to further incubation trials, the air-dry soils were sieved through a 2mm mesh and basic soil properties were determined. The soils represent a wide range of textures, with the contents of sand, silt and clay within 11.3–90.8%, 8.67–76.0%, and 0.52–12.7%, respectively, the C_{org} from 0.49 to 2.64%, and pH (in KCl) from 4.14 to 6.87 (Tables 1-2)

Table 1. Basic characteristics of the tested soils

Soil No.	Soil type[a]	pH KCl	Moist.[b] (% w/w)	C_{org} (%)
1	Eutric Cambisol	4.44	0.65	0.67
2	Eutric Cambisol	6.19	0.58	0.49
3	Eutric Cambisol	4.14	1.06	0.97
4	Eutric Cambisol	4.45	0.83	0.60
5	Eutric Cambisol	4.85	1.08	0.94
6	Eutric Cambisol	5.09	1.13	1.43
7	Eutric Cambisol	4.64	1.30	1.41
8	Eutric Cambisol	4.45	1.18	1.16
9	Eutric Cambisol	6.17	1.28	0.77
10	Dystric Fluvisol	6.57	1.38	1.31
11	Dystric Fluvisol	5.34	1.76	1.05
12	Dystric Fluvisol	4.51	1.40	1.28
13	Haplic Phaeozem	6.87	1.48	1.06
14	Haplic Podzol	5.57	1.37	0.64
15	Haplic Podzol	6.54	1.07	0.86
16	Mollic Gleysol	5.34	3.07	2.64

[a] soil type according to FAO; [b] Moist. – moisture of air-dry soils before flooding

2.2. The Incubation Experiment

To measure the methanogenic potential of the soils, 10 g samples of soil material were prepared in 60cm^3 glass vials (three replicates per soil and per treatment), flooded with 10ml of deionized water or sterilized glucose solution, sealed with septa and metal caps, and exposed to two treatments:

1) The N_2-variant. Soil was flooded with water and the headspace was flushed with N_2 for three minutes to remove O_2. This treatment represents the anaerobic conditions without amendments of the carbon source, which in natural conditions occurs with a sudden flooding of the soil or extensive rainstorm with restricted gas exchange between soil and the atmosphere.

2) The Glucose-variant. Soil was flooded with glucose solution (5 mg glucose per gram of soil) and the atmospheric O_2 concentration was maintained in the headspace. The treatment represents soil areas with so called 'hot spots' which form around the easily available organic matter (*e.g.* decaying roots). The addition of easy available C source is expected to accelerate oxygen consumption, necessary for the activation of the methanogens.

The soils were incubated statically at 25°C in the dark for 132 days with frequent measurements (15 analytical days) of the concentration of the gases (CH_4, CO_2, O_2).

2.3. Methods for determination of gas concentration, PSD and other soil properties

The concentrations of CH_4, CO_2 and O_2 in the headspace were measured periodically with a Shimadzu GC-2014 (Japan) gas chromatograph equipped with a flame ionization detector (FID) for CH_4 measurements and a thermal conductivity detector (TCD) for CO_2 and O_2.

The detectors responses were calibrated using certified gas standards (Air Products) containing 20.9% O_2 in N_2, 10 ppm CH_4 and 1% CO_2, or 4% CH_4 and 10% CO_2 in He.

Table 2. Texture classes and particle size distribution of tested soils - percentage of main fractions and subfractions (in mm)

Soil No.	Soil texture class[a]	Sand	Sand subfractions					Silt	Silt subfractions		Clay
		2.0 – 0.05	2.0 – 1.0	1.0 – 0.5	0.50 – 0.25	0.25 –0.10	0.10 – 0.05	0.05 – 0.002	0.05 – 0.02	0.020 – 0.002	< 0.002
1	sand	87.7	15.3	33.8	26.8	8.4	3.4	11.3	5.1	6.2	1.0
2	sand	90.8	4.5	19.6	33.9	26.4	6.5	8.7	4.8	3.9	0.5
3	sand	87.0	1.1	18.9	39.2	22.6	5.2	11.9	6.0	5.9	1.0
4	sandy loam	50.8	3.9	14.1	16.1	9.3	7.4	44.7	16.8	27.9	4.5
5	silt loam	20.4	1.4	2.0	1.71	2.6	12.6	72.2	32.8	39.4	7.4
6	silt loam	14.1	0.0	0.0	0.0	0.9	13.2	75.6	36.7	38.9	10.3
7	silt loam	21.2	2.6	2.5	2.2	3.2	10.7	69.7	30.5	39.2	9.1
8	silt loam	11.3	0.0	0.0	0.0	1.6	9.6	76.0	26.9	49.1	12.7
9	silt loam	18.1	0.0	0.0	0.0	1.0	17.1	76.0	43.5	32.5	5.9
10	sandy loam	55.7	1.4	14.4	18.8	12.6	8.5	39.4	14.4	25.1	4.8
11	silt loam	17.5	0.7	1.1	2.2	4.3	9.2	74.8	31.2	43.7	7.7
12	silt loam	25.3	0.0	0.7	4.1	5.8	14.8	65.2	28.0	37.1	9.5
13	silt loam	19.5	0.0	0.0	0.0	0.6	18.9	73.2	43.5	29.7	7.2
14	sandy loam	69.6	3.7	11.9	20.3	23.6	10.0	26.7	9.2	17.6	3.7
15	silt loam	29.0	2.3	4.8	8.1	5.6	8.2	65.1	31.2	33.9	5.9
16	silt loam	34.0	0.6	3.0	7.6	12.7	10.1	58.6	18.2	40.5	7.3

[a] soil texture class according to USDA classification

Particle size distribution (PSD) was determined using the Mastersizer 2000 (Malvern, UK) with a laser diffractometer within the size range of 0.02 μm to 2mm (Ryżak & Bieganowski 2010). The laser diffraction method involves measuring the intensity of laser light scattered on the analysed particles. Hydro G dispersion units were used with the pump speed set at 1750 rpm and the stirrer at 700 rpm. The soils were dispersed using ultrasound at 35W for 4 min. In case the obscuration exceeded 10–20%, it was lowered using the method described earlier. The intensity of laser light registered on the particular detectors of the measurement system was converted to particle size distribution according to the Mie theory, assuming the following values of the indices: refraction index 1.52 and absorption index 0.1 for the dispersed phase, and refraction index of 1.33 for water as the dispersing phase (Ryżak & Bieganowski 2010; Sochan et al., 2012). Along with the sand, silt and clay fractions (2–0.050, 0.050–0.002 and <0.002 mm, respectively), the percentage of subfractions were determined (Table 2). The textural triangle of the USDA classification scheme was used to determine the texture classes of tested soils.

The moisture retained in the air-dry soils was determined gravimetrically (24 hours at 105^{o}C). The C_{org} was determined by TOC-VCPH analyser (Shimadzu, Japan), and soil pH was measured in 1M KCl (1:2.5 w/w) after 24 h stabilization at room temperature. All measurements were conducted in triplicate and the results were expressed on an oven-dry weight basis (105°C, 24 h).

2.4. Data Processing and Statistical Analysis

The total cumulative methane released over 132 days was used as a measure of the soil methanogenic potential. Gases were expressed in mg CH_4-C kg^{-1} and mg CO_2-C kg^{-1} dry soil; the O_2 concentration in the headspace was presented as % (v/v). The concentrations of the gases were corrected for solubility in water by using published values of the Bunsen absorption coefficient (Gliński & Stępniewski, 1985). The reading for the α coefficient was made for the temperature of 25^{o}C, giving values of 0.029 and 0.829 for CH_4 and CO_2 respectively. The gas densities of 0.657 and 1.811mg cm^{-3} for CH_4 and CO_2 respectively, were used for calculation of the gas masses.

The statistical analysis was performed using Statgraphics Centurion XVI and STATISTICA. An analysis of variance (Fisher's LSD procedure) was used to indicate the effect of soil texture classes (sand, silt loam and sandy loam soils) and soil conditions established during the incubations (in the N_2-or Glucose-variants) on CH_4 and CO_2 production. This test also provided information on the significant factors affecting the methanogenic potential for examined soils. Simple regression procedures were performed to describe the correlations between the produced gases (CH_4 and CO_2) and the PSD, C_{org}, pH and moisture content retained in the soils. Due to the significant differences in gas concentrations for both variants, which could otherwise have been misleading for the interpretation of the results, only regression analyses conducted separately for each variant have been presented in the result.

The principal component analysis (PCA) was applied to determine the main trends in the data and the extent of differentiation of CH_4 and CO_2 production with regard to different soil properties. PCA allows the reduction of the dimensionality of a large number of potentially correlated variables with the least loss of information. The PCs were calculated based on the correlation coefficients matrix, with eigenvalues greater than 1 being extracted. In the PCA analysis, the following factors have been included: CH_4, CO_2, pH, C_{org}, moisture retained in archived soils, and six major PSD fractions instead of all subfractions. The selection was based on the results of the regression analyses (shown in Table 6) and only the fractions with the highest correlation coefficient were included.

3. RESULTS

3.1. CH_4 and CO_2 production during soil incubation

All soils incubated with N_2 in the headspace and without any C amendments (N_2-variant) express some level of methanogenic potential (Table 3). A lag period before the onset of CH_4 production ranged from 14 days in Eutric Cambisol soils No. 6 and 9, to 121 days in Eutric Cambisol No. 4. Most soils start to CH_4 release after 20-30 days of incubation. CO_2 evolution from all samples starts nearly immediately after incubation (Table 3).

The highest microbial activity can be observed in Mollic Gleysol No. 16 which, over the whole incubation period, evolved 594.0±18.5 mg CH_4-C kg^{-1} and 637.2±48.1 mg CO_2-C kg^{-1}. The lowest activity is shown by Eutric Cambisol No. 4 with 0.427±0.33mgCkg^{-1} and 87.6±5.86 mg C kg^{-1} of CH_4 and CO_2 produced respectively. Eutric Cambisol No. 2 showed an unexpectedly low production of CO_2 (69.6±1.66 mg C kg^{-1}), which is even lower than the amount of CH_4 (72.4±0.96 mg C kg^{-1}).

Table 3. Cumulative CH_4 and CO_2 over the incubation of 16 soils in N_2-variant. Seven analytical days were selected to present the recovery of the CH_4 production in rewetted soils after their long storage (means±SD, *n*=3)

Soil No	CH_4 (mg C kg^{-1})						
	14 day	21 day	28 day	42 day	77 day	121 day	132 day
1	0.0	0.0	0.45±0.12	1.51±0.28	3.46±0.50	32.7±19.0	35.5±20.1
2	0.0	0.44±0.10	6.57±4.7	39.0±2.6	57.1±1.5	68.1±1.4	72.4±0.96
3	0.0	0.0	0.0	0.02±0.03	0.50±0.71	2.90±0.39	3.44±0.42
4	0.0	0.0	0.0	0.0	0.0	0.34±0.48	0.43±0.33
5	0.0	0.07±0.10	2.53±1.1	50.4±16.2	137.6±8.8	173.5±11.5	175.6±12.3
6	2.56±0.43	17.3±2.68	98.5±17.4	220.7±32.2	279.7±85.2	316.8±69.3	315.8±76.8
7	0.0	1.32±0.28	13.7±3.5	143.6±5.7	249.8±7.7	293.5±15.0	299.6±16.2
8	0.0	0.58±0.11	6.52±1.2	72.0±19.7	219.5±9.0	272.8±4.1	275.4±5.6
9	0.11±0.15	1.44±0.69	11.6±4.7	37.2±4.4	59.1±5.4	76.5±5.9	78.5±6.0
10	0.0	0.15±0.21	0.17±0.24	0.16±0.23	0.30±0.43	0.27±0.39	0.28±0.39
11	0.0	0.36±0.25	3.98±1.5	69.3±13.6	166.5±13.0	197.4±13.5	199.2±14.1
12	0.0	0.0	2.49±0.59	111.0±5.3	225.3±14.0	273.5±21.1	277.0±20.2
13	0.0	0.22±0.16	3.99±1.0	42.3±5.6	123.2±3.3	143.5±7.1	146.2±5.2
14	0.0	1.42±0.09	9.38±1.1	37.7±4.8	72.8±4.2	92.0±5.6	93.8±4.7
15	0.0	0.0	1.64±0.05	47.0±1.5	183.9±12.5	215.2±25.8	218.2±27.0
16	0.0	0.96±0.12	49.3±5.2	351.0±13.1	520.0±16.2	592.2±13.9	594.0±18.5
CO_2 (mg C kg^{-1})							
	14 day	21 day	28 day	42 day	77 day	121 day	132 day
1	42.7±1.6	49.9±2.9	56.8±3.4	68.9±4.8	82.1±7.2	107.4±26.2	111.9±29.9
2	27.9±0.94	30.8±0.93	34.3±0.99	43.5±2.1	57.6±1.3	67.1±1.8	69.6±1.7
3	62.2±2.7	72.3±4.0	82.3±5.2	98.7±5.5	111.6±3.2	131.9±5.4	135.2±7.0
4	40.7±1.0	50.5±1.4	54.1±2.7	64.2±3.9	77.4±0.81	86.6±5.9	87.6±5.9
5	142.7±5.3	156.6±6.3	161.9±7.4	192.3±7.1	260.3±23.5	293.2±24.3	298.4±23.0
6	201.7±23.0	239.2±32.5	269.7±27.0	335.3±42.6	402.9±47.5	429.5±53.1	432.0±50.7
7	192.1±1.6	210.4±1.4	218.3±1.5	279.6±8.8	353.1±5.9	386.5±13.9	395.0±10.0
8	177.9±1.3	205.6±2.4	217.9±2.9	265.4±4.2	351.3±4.9	389.9±5.0	395.1±3.1
9	73.8±2.1	83.8±1.7	88.1±3.2	103.1±2.2	129.0±3.1	143.9±2.6	146.3±2.5
10	117.3±16.5	129.4±18.9	134.5±20.2	147.0±26.6	169.7±28.3	206.6±6.6	211.0±6.8
11	160.8±5.9	184.4±8.2	199.4±11.3	238.0±16.3	309.1±8.0	345.1±15.4	351.4±18.8
12	183.9±17.1	209.5±19.1	215.6±23.3	273.6±28.3	356.9±47.1	394.6±54.4	399.5±54.9
13	98.8±1.2	105.1±1.5	108.0±2.7	128.1±1.5	160.6±1.3	172.3±6.8	174.8±6.7
14	91.6±1.0	103.2±0.78	109.3±0.77	124.9±2.4	160.2±7.5	177.4±7.7	180.4±8.7
15	63.5±4.2	69.2±5.1	68.9±5.3	95.0±9.6	137.5±12.3	155.5±15.4	150.8±11.3
16	307.4±13.7	340.3±14.8	359.0±12.5	472.8±14.1	572.0±42.3	628.3±43.9	637.2±48.1

In most soils incubated in Glucose-variant, CH_4 production starts after a 21 to 77–day lag period with the total CH_4 ranging from 400 to 863 mg CH_4-C kg^{-1} (Table 4). Despite the fact that some soils start CH_4 production more rapidly (e.g. Eutric Cambisol No. 6 and 9) the overall cumulative CH_4 production was not much different from other soils which show much longer lag period (i.e. >60 days in Haplic Podzol No.14 and Eutric Cambisol No. 2). However, only on the last incubation day can small amounts of CH_4 be observed in the Eutric Cambisol No. 4 (5.925 mg CH_4-C kg^{-1}), whereas no CH_4 is detected in two Eutric Cambisols enriched with glucose (No. 1 and No. 3) until the end of the incubation (Table 4). A rapid increase in CO_2 in the headspace is observed in all tested soils very soon after the start of the incubation (Table 4). The total released CO_2 lies in relatively narrow range for all soils (597 to 903 mg CO_2-C kg^{-1}). Oxygen concentration for most soils became low within 14–28 days of incubation (data not shown). In two soils, O_2 rapidly decreases below 1% (v/v) within 5–7 days (Mollic Gleysol No. 16 and Dystric Fluvisol No. 10), while in soils No. 3 and 4 it remains above 1% (v/v) for 77 days. All samples (except of soils No. 1 and 3) clearly show an increase in CH_4 production once the O_2 levels become very low (data not shown).

3.2. The effects of incubation conditions and soil texture on the methanogenic potential of archived soils

The methanogenic potential of archived soils presented as the mean CH_4 value from each incubation variant (N_2 -, Glucose-) and texture class (sand, sandy loam and silt loam) are presented in table 5. Both CH_4 and CO_2 concentrations are 2.8

times higher (statistically significant) in the Glucose-variant compared to N_2-variant.

As indicated by the F ratio, the effect of glucose addition is much lower for CH_4 than for CO_2 concentration (F = 52.4 vs. F = 332.4).

The silt loam soils show the highest methanogenic potential, which is approximately 5 and 2.5 times higher than in sand and sandy loam soils respectively (P<0.001). In turn, the average CO_2 production from silt loam soils is not significantly higher than from both sandy loam and sand soils.

3.3. The correlations between soil properties and CH_4 or CO_2 release

The correlations between the methanogenic potential and PSD, pH, C_{org} or moisture retention after storage are presented separately for two experimental variants in Table 6. For PSD, the direction of the relationships depends on the size of the particles in a given fraction, and is positive for particles smaller than 0.10 mm, while negative for particles larger than 0.10 mm.

Comparing the correlation coefficients (r) within particular incubation variants, the highest positive coefficients are observed for clay and fine silt fractions. The coefficient values range from r = 0.416 to r = 0.720 and have higher values for fine silt in the case of CH_4 and for clay in the case of CO_2. In turn, the strongest negative correlations (except for CO_2 in Glucose-variant) are found for CH_4 and CO_2 in the coarse sand subfraction (in the range from r=–0.586 to r = –0.795). In the N_2-variant, the correlation coefficients with the PSD are slightly higher for CO_2 than for CH_4. In the Glucose-variant, positive correlation between CO_2 production and clay or fine silt are found, but in case of other particles size fractions no significant correlation can be detected.

Table 4. Cumulative CH_4 and CO_2 over the incubation of 16 soils in Glucose-variant. Seven analytical days were selected to present the recovery of the CH_4 production in rewetted soils after their long storage (means ± SD, n = 3)

Soil No	CH_4 (mg C kg^{-1})						
	7 day	21 day	28 day	35 day	77 day	98 day	132 day
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	3.2±0.5	14.5±15.8	446.6±93.7
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.0	0.0	0.0	0.0	0.0	0.0	5.92±4.8
5	0.0	0.0	0.0	0.9±1.1	274.1±30.1	420.4±59.8	587.6±30.6
6	0.0	4.38±0.92	23.8±6.9	138.0±61.6	570.2±15.4	634.0±8.5	712.1±3.6
7	0.0	0.0	0.32±0.23	4.66±0.40	455.1±10.3	552.0±11.6	648.7±4.0
8	0.0	0.0	0.0	0.4±0.6	371.7±20.0	503.4±40.8	630.6±5.8
9	0.0	0.57±0.18	13.4±2.7	157.2±32.2	490.0±10.0	501.7±8.2	540.9±1.5
10	0.0	0.50±0.19	4.70±2.0	28.9±12.9	297.0±30.0	349.6±30.1	400.3±25.9
11	0.0	0.43±0.08	4.48±0.85	28.3±4.2	413.7±10.1	532.8±27.7	578.6±18.5
12	0.0	0.0	0.09±0.13	3.31±2.2	404.1±11.0	546.9±8.2	640.9±17.9
13	0.0	0.0	1.7±0.4	20.7±4.0	477.3±10.1	529.4±4.3	579.1±5.0
14	0.0	0.0	0.0	0.0	43.0±5.0	124.5±65.4	573.9±7.2
15	0.0	0.0	0.46±0.40	5.06±3.8	483.3±15.0	570.6±13.9	630.3±5.7
16	0.0	0.0	0.0	3.6±1.0	488.3±20.3	725.0±45.0	863.4±54.3
	CO_2 (mg C kg^{-1})						
	7 day	21 day	28 day	35 day	77 day	98 day	132 day
1	538.8±9.7	615.6±44.2	646.0±45.7	698.2±12.7	739.0±12.7	756.4±13.4	765.4±18.7
2	492.9±3.9	586.5±21.1	623.4±5.2	619.3±14.1	620.0±5.5	616.2±5.7	596.9±3.8
3	466.2±31.1	546.8±69.7	602.7±122.7	622.9±114.3	716.0±50.0	745.4±51.9	758.2±41.9
4	478.6±20.3	586.1±29.6	632.9±29.8	672.2±25.9	695.3±20.0	698.2±22.9	695.5±22.1
5	514.7±18.9	712.3±0.13	739.3±3.2	752.9±13.4	758.0±7.0	732.2±19.2	717.6±18.8
6	581.0±8.11	668.6±9.1	722.3±6.03	726.9±11.1	788.0±5.0	803.0±22.5	810.2±35.8
7	555.7±3.8	680.3±7.9	724.4±3.3	726.9±1.3	776.0±3.0	778.8±6.3	790.5±10.1
8	520.9±7.3	717.8±4.0	751.1±6.0	790.5±15.8	805.3±5.0	795.0±8.3	782.8±7.5
9	461.2±1.1	539.2±10.3	597.7±3.8	604.1±11.0	656.7±4.8	649.8±12.9	648.9±12.1
10	655.1±6.9	668.2±10.9	670.2±8.2	669.5±17.4	695.3±15.0	688.3±18.8	691.0±18.0
11	514.5±4.2	689.6±7.5	723.6±5.4	757.3±3.4	775.7±7.1	786.0±16.5	786.9±14.1
12	577.1±1.9	713.6±13.3	748.0±10.8	760.0±11.0	787.3±6.0	786.0±4.6	801.3±6.7
13	483.1±3.3	589.0±7.4	647.2±4.8	658.7±6.6	664.3±9.0	662.3±9.9	669.5±14.4
14	500.4±3.4	625.8±3.4	686.5±13.8	700.9±9.1	688.3±6.0	681.2±6.7	675.8±9.1
15	437.3±4.7	529.5±12.5	571.5±18.8	574.5±22.9	600.4±15.1	605.0±5.8	605.0±5.8
16	732.5±2.2	819.1±7.4	859.2±5.2	881.9±19.0	907.0±8.0	912.1±13.6	903.2±11.8

Table 5. Mean CH_4 and CO_2 values (±standard error) accumulated during 132-d incubations of soils flooded after long storage. Two factor ANOVA (Fisher's LSD procedure) with *F*-ratios and *P*-values for both factors: experimental variant determining incubation conditions and soil texture class (total n=96). Values within a column followed by the same letter (for a given factor) do not differ significantly at $P < 0.05$.

Factor	CH_4 (mg C kg^{-1})	CO_2 (mg C kg^{-1})
Experimental variant	F=52.4; $P < 0.001$	F=332.4; $P < 0.001$
N_2 -variant	174.2 ± 22.5 [b]	260.9 ± 22.8 [b]
Glucose -variant	489.9 ± 37.3 [a]	731.2 ± 12.0 [a]
Soil texture	F=24.1; $P < 0.001$	n.s.
Sand	93.0 ± 40.2 [b]	406.2 ± 74.5 [a]
Sandy loam	179.1 ± 54.8 [b]	423.5 ± 64.7 [a]
Silt loam	449.7 ± 29.1 [a]	544.8 ± 31.2 [a]

Table 6. Comparison of the correlation coefficients (*r*) obtained for CH_4 or CO_2 released vs. C_{org}, moisture and PSD subsfractions (the ranges of subfractions given in mm) in soils incubated for 132 days in two experimental variants (n=48 for each variant). The data in bold represent the highest positive and the highest negative correlation coefficients within a given column.

Soil characteristics			N_2-variant		Glucose-variant	
			CH_4	CO_2	CH_4	CO_2
C_{org}			**0.802*****	**0.839*****	0.531***	**0.712*****
pH (KCl)			n.s.	n.s.	0.341*	**-0.595*****
Moisture retention			0.729***	0.744***	0.589***	0.582***
PSD subfractions:		mm				
Sand	very coarse	2.0-1.0	-0.364*	-0.424**	-0.585***	n.s.
	coarse	1.0-0.5	**-0.589*****	**-0.586*****	**-0.795*****	n.s.
	medium	0.50-0.25	-0.556***	-0.561***	-0.704***	n.s.
	fine	0.25-0.10	-0.319*	-0.347*	-0.349*	n.s.
	very fine	0.10-0.05	0.285*	n.s.	0.590***	n.s.
Silt	coarse	0.05-0.02	0.341*	0.304*	0.571***	n.s.
	fine	0.02-0.002	0.654***	0.710***	**0.694*****	0.416**
Clay		< 0.002	0.644***	0.720***	0.692***	0.457**

*, **, *** – significant at $P < 0.05$, 0.01, and 0.001, respectively; n.s. – not significant;

Only the very fine sand subfraction (0.10–0.05 mm) is positively correlated with CH_4 production, while all larger sand subfractions (>0.10 mm) are negatively correlated (negative *r* values). This very fine sand subfraction is, however, included in some texture classifications as a silt fraction. A strong positive correlation (P<0.001) is detected between both CH_4 and CO_2 production and C_{org} content, as well as the gravimetric water content in air-dry soil samples (Table 6). The *r* values are even slightly higher for samples incubated in the N_2-variant, than in the Glucose-variant. Correlation with soil pH exists only for the CO_2 production in the Glucose-variant.

3.4. Results of PCA analysis

The PCA generated three principal components (PCs) that account for 88.3% of the underlying variability. The first principal component (PC1) with an eigenvalue of 7.56 explains 58.1% of the variance and has a high positive loading for fine silt, clay, C_{org}, moisture content, CO_2 produced in the N_2-variant and CH_4 produced in both variants (Fig. 1a; Table 7). High negative loadings are obtained for coarse and medium sand subfractions. The second principal component, PC2 (eigenvalue of 2.56) explains 19.7% of the variance and has a negative loading for CO_2 produced in the Glucose-variant. The principal subsequent component PC3 (eigenvalue of 1.36) explains much less of total variability (10.5%), and shows a positive correlation with pH (Table 7).

Figure 1b illustrates scores for all tested soils. Positive PC1 scores are dominated by silt loam soils (e.g. Mollic Gleysol No. 16, Eutric Cambisols No. 6 and 8), whereas sandy soils (e.g. Cambisols No. 1 and 3) have high negative PC1 scores. The Mollic Gleysol No. 16 is also characterized by high negative PC2 scores.

4. DISCUSSIONS

Since soil microbial populations have been recently highlighted as a major players in the regulating of major greenhouse gas emissions (e.g. CO_2 and CH_4) (Owens & Xu 2011), it is important to know how they will react to environmental changes forecast by climatic predictions like intensive drying or flooding.

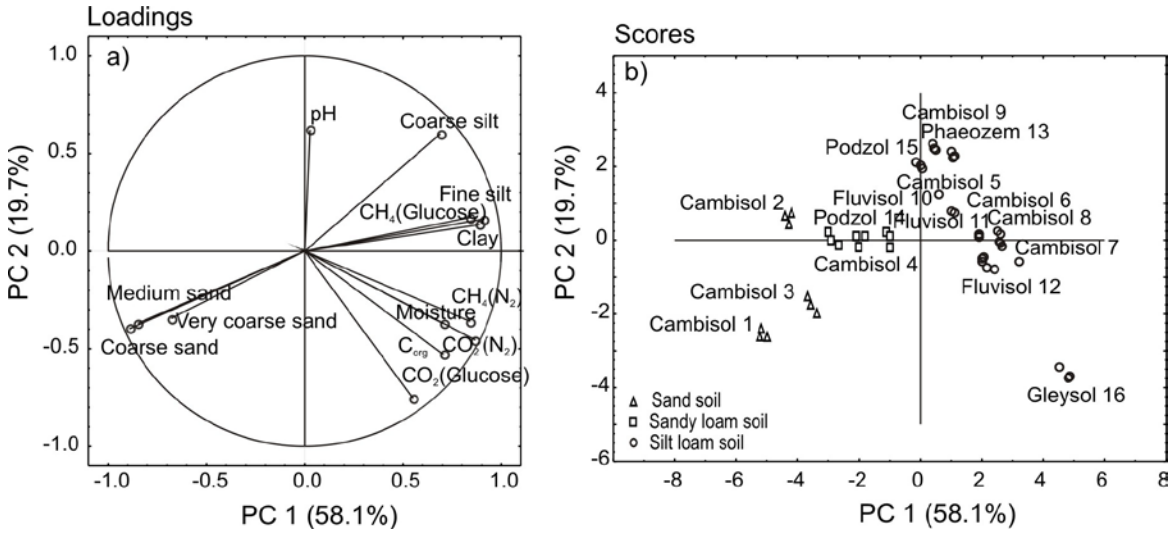

Figure 1. The results of principal component analysis: PCA loadings (a) and scores (b) of all tested soils.

Table 7. Loadings for each variable along PC1, PC2 and PC3 resulting from principal components analysis.

Variable	PC1	PC2	PC3
CH_4 (N_2-variant)	**0.843**	-0.360	0.145
CO_2 (N_2-variant)	**0.868**	-0.455	0.001
CH_4 (Glucose-variant)	**0.844**	0.173	0.295
CO_2 (Glucose-variant)	0.551	**-0.754**	-0.231
C_{org}	**0.707**	-0.531	0.342
pH (KCl)	0.027	0.622	**0.734**
Moisture retained in dry soils	**0.705**	-0.372	0.482
Very coarse sand (2.0-1.0 mm)	**-0.680**	-0.344	-0.354
Coarse sand (1.0-0.5 mm)	**-0.892**	-0.398	-0.262
Medium sand (0.50-0.25 mm)	**-0.850**	-0.370	-0.188
Coarse silt (0.05-0.02 mm)	**0.690**	0.601	0.243
Fine silt (0.02-0.002 mm)	**0.911**	0.165	0.056
Clay (< 0.002 mm)	**0.890**	0.136	-0.139
PC1, PC2 and PC3 – first, second and third principal component, respectively; The highest contribution of each variable is highlighted in bold characters			

Soil air-water conditions strongly influence the physicochemical status of soil and regulate the size and activity of soil microbial populations (Stępniewski & Stępniewska, 2009; Brzezińska et al., 2011a; Brzezińska et al., 2011b; Stępniewski, 2011; Włodarczyk et al., 2011). The rewetting of dry soils represents an abrupt step change in soil biophysical conditions, with critical implications for biogeochemical cycling; it increases the availability of soil water, rehydrates microbial cells, increases microbial metabolism, and mobilizes nutrients (Kim et al., 2012). De Nobili et al., (2006) reported on the reactivation of aerobic microorganism's respiration in soils stored in air-dry conditions for up to 103 years. Moreover, Clark & Hirsch (2008) demonstrated that air-dried soils can protect microbial DNA (Deoxyribonucleic acid) for more than 150 years. The processes of air-drying greatly reduced bacterial viability, whilst DNA yields declined less and may be preserved by desiccation. The higher clay content can probably offer more protection to the released DNA, while the higher organic matter content may harbour or protect bacterial cells (Clark & Hirsch, 2008). Our study has shown that methanogenic microorganisms are able to become active in soils after 20 years of dry storage. The methanogenic activity can be restored within a few days or weeks following flooding.

As can be expected, methanogenesis did not occur immediately after exposure to anoxic conditions, but a certain amount of time was necessary for the microorganisms to respond. In most soils, CO_2 was present in the headspace nearly immediately following incubation, while a lag phase ranging from 14 to 132 days was observed for CH_4 production. It should be noted that in other experiments reported in the literature, such a lag period (14 days) has also been observed for soils tested directly after sampling, i.e. incubated without long storage (Angel et al., 2011; Brzezińska et al., 2012).

The immediate presence of CO_2 in the sample headspace can be assigned to bacterial respiration. Additionally, the CO_2 presence at the beginning of the

incubation can be partly assigned to the removal of gases accumulated in soil pores during the dry storage time (Chowdhury et al., 2011). The lag phase in the CH_4 detection can be due to time it takes for oxygen and potentially other alternative electron acceptors to be depleted, as well as the recovery and growth of the methanogenic population (Angel et al., 2011). It is also important to remember that the activity of methanogenic microorganisms is strongly affected by other microbial populations such as fermenting bacteria, iron reducers, syntrophic bacteria etc. that influence the availability of methanogenic substrates (Conrad 2002). The activity of other microorganisms may be especially important for the recovery of methanogenic microorganisms in soils exposed to dry conditions for longer periods of time.

4.1. Influence of different incubation variant on methanogenic potential

The response in CO_2 and CH_4 production in our study was largely dependent on the characteristic of the individual soil, but also the type of incubation variant (N_2- or Glucose-variant) which simulated different environmental conditions. The quickest and the highest response in CH_4 and CO_2 production was observed in soils incubated with glucose, which conforms to expectations as organic amendments are well known for the stimulation of soil redox transformations (Gliński et al., 1996) and methanogenesis (Wang et al., 2013). Glucose addition to the soil provides a highly labile substrate for microbial respiration and glucose rather than the native (more recalcitrant) soil organic matter present in soil was preferentially used as a carbon source. Stimulation of both CH_4 and CO_2 production was about 2.8-fold greater as compared with the N_2-variant. The significance of this effect was much stronger for CO_2, because all tested soils followed similar trend of the changes, while the CH_4 response to glucose addition was more diverse (Tables 3-5). However, as regression analysis revealed, native C_{org} strongly determined CH_4 and CO_2 release, especially in N_2-variant, but also in the Glucose-variant (Table 6). It is often considered that native soil organic matter dominated by recalcitrant forms of carbon is less accessible for the microorganisms (Hooker & Stark 2012). However, in the study of the microbial activity of soils stored for up to 103 years, De Nobili et al. (2006) observed that the prolonged soil storage doubled the concentration of water soluble organic carbon (as compared to air-dried fresh soil), and this large increase in C content might actually account for the observed larger CO_2-C evolution, indicating that this material was bioavailable. The incubation of soils with N_2 in the headspace created anaerobic conditions at the start of incubation, but soil organic matter present in the soil (native) acted as the only carbon source available for the microorganisms. It is clear that the lack of an easily available carbon source was one of the main limitations in the growth of all microorganisms, when compared with the glucose variant. Though, an important constraint was also the deficiency of electron acceptors in the soil as well as the effectiveness of the anaerobic bacteria having survived the dry period to reproduce and reactivate.

In general, CH_4 production in the incubation with glucose started later than in the N_2-variant, likely caused by the presence of oxygen at the beginning of incubation. In most soils, CH_4 release began once the O_2 concentration in the sample headspace became very low (0.5–0.87% v/v). Methane evolution in flooded soil has been reported to start when O_2 concentration dropped below 2.5% v/v (Megonigal et al., 2004). In some soils incubated with glucose however, CH_4 production started just before the end of the incubation or did not even start, regardless of the low O_2 concentration. This can indicate that more time was needed to enable methanogenic populations to overcome the competition with other anaerobes.

4.2. Effect of soil properties on methanogenic potential

There has been a clear variability in the methanogenic potential of individual soils incubated after long storage, which cannot solely be assigned to the incubation variant. A clear significant correlation has been found between CH_4 and CO_2 production and the PSD. In both experimental conditions, the methanogenic potential was much higher in silt loam soils than sandy loam and sand soils (Table 5). Mean values of total CH_4 production in silt loam soil was approximately 5 and 2.5 times higher than in sand and sandy loam soils respectively. Meanwhile the mean total CO_2 production in silt loam was only 1.3 times higher than in coarse textured soils. Despite the fact that the laser diffraction method used for PSD determination may underestimate the content of the clay fraction (Dobrowolski et al., 2012), a very strong positive correlation has been detected between both CH_4 and CO_2 production and the content of the finest particle fractions like clay and fine silt (Table 6). Negative correlations were observed between CH_4 and CO_2 production and the sand subfractions. It is therefore evident that the methanogenic potential increases concurrently with fine soil particle content and that they play an important role in protection of methanogens over long, dry and oxic soil conditions.

Several mechanisms can account for such

behaviour. Under field conditions, fine textured soils have poor drainage and therefore are in general more prone to anaerobiosis and are expected to favour methanogenesis (Le Mer & Roger 2001). Sessitsch et al., (2001) found aerobic and strictly anaerobic bacteria species to be associated with the clay-sized fraction and only aerobic species to sand-sized particles. Small-sized fractions contain the most microbial biomass in different soils as clay-sized particles have a higher surface area than coarser particles, thus facilitating bacterial growth as well as attachment and protection of microorganisms and extracellular enzymes (Kögel-Knabner et al., 2008). Fine textured soils also tend to form aggregates which create very distinct conditions for water and organic matter storage (Urbanek et al., 2011) and therefore allow survival of microorganisms during prolonged storage. Soil texture is also important for soil organic matter transformations. The association of SOM with fine particle fractions, silt and clay, is considered to play an important role in its protection (Nicolás et al., 2012). Soil microorganisms are more abundant in smaller fractions because of the protection offered by microaggregates (Lagomarsino et al., 2009). Methanogens may be able to survive in small anaerobic microsites imbedded in dry soils, or they may be protected from O_2 by reactive soil minerals (Megonigal et al., 2004). Additionally, fine textured soils have the ability to retain more water at higher suctions, than the coarse textured soils, which can significantly benefit the survival of microorganisms (Bieganowski & Ryżak, 2011). On the other hand, the clay fraction is an important source of trace elements, including heavy metals (Sîrbu-Rădăşanu et al., 2013). Nevertheless, Liu et al., (2008) reported that a liquid culture of *Methanobacterium formicicum* could remain viable when mixed well with fresh or sterile soil, but not when cultured without soil, or with agar slurry. This suggests that indigenous methanogens localize within soil compartments to protect themselves from the damage caused by gradual drying under an oxic atmosphere.

In contrast to our results, Zhang et al., (2007) observed, for fertilized paddy soils (but not stored as air-dry), that CH_4 was predominantly produced in the coarser fractions (which may be contributing to the storage of labile organic carbon in these fractions), while more species and a higher diversity of bacteria survived in the clay sized fraction due to the vicinity between microbes, access to carbon resource outside the microaggregates and smaller pore size as protective agent suitable habitats for microbes rather than high C_{org}. Fertilizer application caused more change to the bacterial community in the clay fraction and greatly increased bacterium and methanogen activity in coarser fractions, but only a slight effect on the methanogenic archaeal community in the particle size fractions was observed (Zhang et al., 2007). Apparently, long term storage of air-dry soils resulted in a shift in the distribution of microorganisms among soil particles and changed the response of methanogens to soil rewetting.

Next to the textural properties, the C_{org} of archived soils correlated closely with the total CO_2 and CH_4 production (Table 6). Considerably better correlations were observed between C_{org} and CO_2 production, rather than with CH_4, suggesting that the availability of organic matter stored in archived soils is more essential for the activity of other microorganisms than methanogens. The results directly correspond with other studies which also report that the intensity of reductive processes in flooded soils depends on the content and nature of organic matter, the ability of the microflora to decompose SOM as well as availability and nature of electron acceptors (Le Mer & Roger 2001; Watanabe et al., 2011). Nevertheless with the exception of CH_4 released in the Glucose-variant, where fine silt and clay contents were more important, among soil characteristics, C_{org} showed the best correlations with CH_4 and CO_2 released.

A positive correlation was also found between the residual water content retained in soils (before incubations) and the total CO_2 and CH_4 production (Table 6). Such a relation can be indirectly linked to the specific textures of each soil, given that coarse (sand and sandy loam) soils with larger pores have lower residual water and C_{org} contents than finer soils (silt loam), which are able to store higher amounts of water, even at high soil suction. The residual water associated with fine soil particles and organic matter can therefore create zones for the survival of microorganisms. The PCA analysis well illustrate the specific properties of two extremes: less active sand soils (Cambisols No. 1, No. 3 characterized by low C_{org}, residual moisture, clay and silt contents) with their low methanogenic potential were located at negative PC1 score, while the most active silt loam soil (Mollic Gleysol No. 16, characterized by high residual moisture, C_{org}, and relatively high clay and silt contents) – at high positive PC1 score, at a distinct distance from other silt loam soils (Fig. 1b).

5. CONCLUSIONS

Based on the current study, which determined the methanogenic potential of 16 mineral soils stored in air-dry conditions over 20 years, it can be concluded that even after prolonged dry oxic conditions, soils are able to quickly restore microbial activity and start methane production within few days

or weeks after flooding. Although we can not present direct evidence that tested soils retained active populations of methanogenic *Archaea*, it was documented that archived soils do possess potential for CH_4 production without any amendment of the carbon source. Methanogenic potential shows a strong positive correlation with finer particle size fractions like fine silt (0.02-0.002 mm) and clay (<0.002 mm). The coarse soil fractions like medium and coarse sand (0.50-0.25 and 1.0-0.5 mm, respectively), on the other hand, show strong negative correlations with the methanogenic potential. Silt loam soils show significantly, and approximately 5 and 2.5 times, higher CH_4 production than soils with coarser textures (sand and sandy loam soils, respectively).Therefore finer textured soils are more likely to become a high source of methane upon flooding compared to course textured soils.

The production of CO_2 on the other hand, is not affected by the soil texture if labile carbon source like glucose has been made available in the soil. Only in the case when the microorganisms relied on the native carbon source in the soil (N_2-variant) was both CH_4 and CO_2 production correlated with particular PSD fractions. However, in glucose enriched soils, a significant correlation for CO_2 production was obtained only with the finest but not with coarser fractions, whereas for CH_4 formation all PSD fractions were important. Nevertheless, the C_{org} and retained moisture showed even stronger correlations with methanogenic potential, except for CH_4 released in the presence of glucose (where the correlation coefficients for C_{org} and moisture were lower than for PSD fractions). In fact, all analysed soil properties are related to each other and all create specific habitats which allow microorganisms to persist in long unfavourable conditions. By applying PCA we can clearly discriminate between sand soils showing low methanogenic potential, and silt loam soils expressing high methanogenic potential. Most soil properties were associated with the first principal component (PC1), which explained 58.1% of the qualitative differences between compared soils.

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