RESEARCH ARTICLE

Production pathways for CH₄ and CO₂ in sediments of two freshwater ecosystems in south-eastern Poland

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Abstract

This paper presents the results of research into pathways leading to the production of methane (CH₄) and carbon dioxide (CO₂) in sediments of two eutrophic reservoirs (Maziarnia and Nielisz), located in south-eastern Poland. In seeking to identify the pathways in question, use was made of analysis of stable carbon isotopes in CH₄ and CO₂ dissolved in pore water. This determined that CH₄ is mainly produced through acetate fermentation, though the hydrogenotrophic methanogenic process may also be of importance, especially in deeper layers of sediments. Both the presence of autochthonous organic matter and increased pH values are shown to favour acetate fermentation. In turn, methanogenesis in sediments is assessed as capable of accounting for the generation of a considerable amount of CO₂. Indeed, the role of methanogenesis in CO₂ production is increasingly important further down in the layers of sediment, where allochthonous organic matter is predominant.

Introduction

Carbon dioxide (CO₂) and methane (CH₄) are the two main greenhouse gases whose concentration in the atmosphere is growing steadily, causing an increase in average air temperature [1]. Among the sources of these gases in the atmosphere are reservoirs, in which decomposition proceeds in accumulated bottom sediments that are repositories of autochthonous and allochthonous organic matter. While CO₂ is among the end products where this process is ongoing under aerobic conditions, in anoxic conditions, the decomposition of organic matter by fermentation has both CO₂ and CH₄ as its gaseous end-products.

The two known mechanisms by which biogenic CH₄ is generated in aquatic environments are acetate fermentation [2] and CO₂ reduction [3]. It has been estimated that, in most freshwater ecosystems, acetate fermentation is 50–80% responsible for the production of CH₄ [4, 5]. Hydrogenotrophic methanogenesis becomes meaningful when other substrates for this process begin to run out and methanogens other than the obligatory methylotrophs begin to turn to the reduction of CO₂ [6].
CO₂ is produced in the aerobic water column during respiration, and in sediments via the processes of the mineralisation of organic matter, methanogenesis and the dissolution of carbonates. The gas is in turn consumed by methanogenesis (CO₂ reduction) and primary production (photosynthesis) [7].

To distinguish sources of CH₄ and CO₂, reference is made to carbon isotopic composition. During hydrogenotrophic methanogenesis, a preference is shown for the isotopically lighter carbon species with the result that the CH₄ produced via acetate fermentation has δ¹³C-CH₄ values in the range -65 to -50‰, whereas the δ¹³C of CH₄ produced by the reduction of CO₂ oscillates in the range -110 to -60‰ [8]. The δ¹³C values of the CH₄ and the CO₂ coexisting with it are also helpful in determining mechanisms by which CH₄ is produced. The distribution of the carbon isotopes between CO₂ and CH₄ can be presented as the fractionation factor α₃CH₄-CO₂. The values for α₃CH₄-CO₂ connected with methanogenesis in a marine environment, where the main pathway of CH₄ production is the reduction of CO₂, are in the range 1.05–1.1. In contrast, in the freshwater ecosystems where acetate fermentation predominates, the values for this factor range between 1.04 and 1.05 [6].

Reference to the isotopic composition of dissolved inorganic carbon (DIC) allows the main sources in water to be recognised, be these atmospheric CO₂, the mineralisation of organic matter, or the dissolution of carbonates. The latter processes in sediments result in the release to pore water of CO₂ isotopically similar to the sources, i.e., to the organic carbon in the sediments and to CaCO₃. In contrast, CO₂ released by methanogenesis is enriched in ¹³C as compared with the organic carbon in sediments [9].

The goal of the work described here was to examine pathways by which CH₄ and CO₂ are produced in the sediments of eutrophic reservoirs, and to identify the factors influencing them. This information enriches knowledge as regards the carbon cycle in aquatic ecosystems and the role in global warming of the reservoirs now present so commonly worldwide.

Materials and methods

Study area

Two eutrophic reservoirs [10] located in two provinces of south-eastern Poland were selected for the study (Maziarnia Reservoir– 50° 34’ N, 21° 93’ E, Nielisz Reservoir– 50° 80’ N, 23° 03’ E). The reservoirs selected differed in size and age as well as the influence of anthropogenic pollution. The characteristic parameters of the studied reservoirs are shown in Fig 1.

Put into operation in 1988 Maziarnia Reservoir was intended to provide water for the local water supply. Currently, the reservoir serves as retention, but due to the small capacity is unable to stop the flood peak.

Nielisz Reservoir was put into exploitation in 2008. The basic tasks of its include: protection against flooding, reduce fluctuations in water level during the period of breeding season of birds, the utilization of energy and the use for the purposes of recreation, leisure and amateur fishing.

Two stations of each reservoir as a whole were chosen for study. Station 1 was located near the dam, whereas station 2 was in the zone of the main tributary, immediately beyond the point of entry into the reservoir. The research station areas were lacking in vegetation. The locations of the sampling stations are as shown in Fig 1.

Sediment sampling and preparation

The studies were carried out during 2009, 2010, and 2011. Sediments were sampled 8 times for each reservoir, between May and October. Sediment cores were being taken from the littoral using a gravity sediment corer (KC Kajak of Denmark). The sediments cores with overlying
water were immediately transported to the laboratory of the Rzeszów University of Technology, the Department of Environmental and Chemistry Engineering, where were progressively pushed out from the bottom of Plexiglas tubes by a piston, and top (1 cm) layers of the sediment were placed in a modified pore water squeezer [11]. Three times for M2 station (in May, July, and September 2011), once for N1 station (July 2011) and twice for N2 station (July, and September 2011) pore water samples from deeper layers of sediment (1–3, 3–5, 5–10, and 10–15 cm) were also extruded. In the case of M1 and N1 stations, due to the sandy sediment structure and consequently low porosity, it has not always been possible to obtain pore water for study. The pore water was collected directly in gastight glass vials, in order for contact with the atmosphere to be avoided. Immediately after collection, the samples of water in the vials were acidified using 6 N HCl (final concentration ~ 50 mM) to quantitatively convert all carbonate anions into CO$_2$ [12]. Sediment samples were air dried and analyzed.

**Pore water analysis**

Gas concentrations and stable carbon isotopic compositions in the pore were analyzed using a headspace equilibration technique. Gases were extracted from the water into gastight glass vials, through the displacement of a known volume of water using helium. Water was equilibrated in the vials with added helium by means of 5 min of vigorous shaking. Then, gas samples were taken from headspace and analyzed for concentrations of CH$_4$ and CO$_2$ and $\delta^{13}$C-CH$_4$ and $\delta^{13}$C-CO$_2$. Concentrations of both CH$_4$ and CO$_2$ were measured using a Pye Unicam gas chromatograph with analytical error of ±5% (model PU-4410/19) equipped with a flame ionization detector (FID) and a stainless steel column packed with a Haye Sep Q, 80/100 Mesh, 6 ft. in length and of 2 mm ID. The GC was also equipped with a methanizer to detect
low levels of carbon dioxide. The carrier gas was helium at a flow rate of 30 cc/min. Gas concentrations were expressed in micromoles per decimeter of gas in the water.

The carbon isotopic compositions of CH₄ and CO₂ were determined using gas chromatograph combustion isotope mass spectrometry (GC-C-IRMS DELTAPLus Finnigan). The isotope ratios were expressed in δ notation (δ¹³C): δ¹³C = [(¹³C/¹²C (sample)/¹³C/¹²C(standard)−1] · 10³ [%], relative to the PeeDeeBelemnite (PBB) standard. The precision of measurement was about ±0.3‰ for δ¹³C-CO₂ and ±0.5‰ for δ¹³C-CH₄.

Sediment analysis
The pH of sediment in the suspension with 1 N KCl was determined potentiometrically with a MultiLine P5M (WTW, Germany). Before the analysis of total organic carbon (TOC) and δ¹³C-TOC, carbonates were removed from the samples by 72 h contact with the vapor of 30% HCl in desiccators [13]. The TOC concentrations were subsequently measured using an analyzer of carbon and nitrogen (CN Flash EA 1112, ThermoQuest) at 1,020˚C. Blank and standard samples with known elemental composition (sulfanilamide) were used for quality control. The precision of the method was about ±3%. Stable isotopic compositions of the organic carbon were determined using an IRMS DELTAPlus Finnigan on line with the analyzer of carbon and nitrogen (CN Flash EA 1112, ThermoQuest). The isotopic ratios were reported in δ¹³C [%], relative to the PDB standard. The method were calibrated using National Bureau of Standards 22 (NBS 22). The precision of measurements was ±0.1‰.

Calculations
Isotopic fractionation factor for conversion of CO₂ to CH₄ is defined as:

$$\alpha_{CH_4-CO_2} = \frac{\delta^{13}C-CO_2 + 1000}{\delta^{13}C-CH_4 + 1000}$$

where: δ¹³C-CO₂ and δ¹³C-CH₄ are the isotopic composition of CO₂ and CH₄, respectively [6].

Relative contribution of hydrogenotrophically derived CH₄ to total CH₄ was determined by mass balance equation [14]:

$$f_{CH_4,h} = \frac{(\delta^{13}C-CH_4 - \delta^{13}C-CH_{4,a})}{(\delta^{13}C-CH_{4,h} - \delta^{13}C-CH_{4,a})}$$

where: fCH₄,h is being the fraction of CH₄ formed by hydrogenotrophy, δ¹³C-CH₄ is the δ¹³C of total produced CH₄, and δ¹³C-CH₄,a and δ¹³C-CH₄,h are the δ¹³C of methane derived from acetoclastic and hydrogenotrophy methanogenesis, respectively. The δ¹³C-CH₄,a and δ¹³C-CH₄,h values were calculated using αCH₄-CO₂ obtained by Whiticar [6] and δ¹³C-CO₂. In this calculation, two different αCH₄-CO₂ values were used, with values of 1.04 and 1.07 for acetotrophy and hydrogenotrophy, respectively.

The calculations of sharing of CO₂ originating from methanogenesis were based on the isotopic mass balance. It was assumed that the process of fermentation of the organic matter deposited in bottom sediments would entail the generation of approximately similar amounts of CH₄ and CO₂: CH₃COOH → CO₂ + CH₄ [2], thus:

$$10^{13}C-TOC = 0.5 \delta^{13}C-CH_4 + 0.5 \delta^{13}C-CO_2_{(methanogenesis)}$$

where: δ¹³C-TOC is δ¹³C of the total organic carbon, δ¹³C-CH₄ is δ¹³C of the CH₄, and δ¹³C-CO₂_{(methanogenesis)} is δ¹³C of the CO₂ derived from methanogenesis.
By transforming the formula, it was possible to calculate the value of $\delta^{13}$C-CO$_2$ produced by methanogenesis. The fraction of CO$_2$ originating in the process of methanogenesis was determined using the mass balance equation [15]:

$$f = \frac{(\delta^{13}\text{C-CO}_2\text{(pore water)} - \delta^{13}\text{C-CO}_2\text{(OM decay)})}{(\delta^{13}\text{C-CO}_2\text{(methanogenesis)} - \delta^{13}\text{C-CO}_2\text{(OM decay)})}$$

(4)

where: $f$ is the participation of CO$_2$ derived from methanogenesis, $\delta^{13}$C-CO$_2$\text{(pore water)} is $\delta^{13}$C of CO$_2$ measured in pore water, and $\delta^{13}$C-CO$_2$\text{(OM decay)} is the value of $\delta^{13}$C for CO$_2$ originating through the mineralization of organic matter. In the calculations, it was assumed that $\delta^{13}$C-CO$_2$\text{(OM decay)} is equal to $\delta^{13}$C-TOC, because mineralization of organic carbon releases inorganic carbon into the pore water, this being isotopically similar to the source, i.e., to sedimentation organic carbon [9].

**Statistical analysis**

For the obtained results, basic descriptive statistics such as the minimum, maximum, mean, and standard deviation values were calculated using the MS Excel 2013 program. For linear relationships, coefficient of determination with the corresponding level of significance $p$ was calculated. It was performed using the Statistica 10 PL Statistical Package. Significances were defined as $p<0.05$.

**Results**

**Sediment characteristics**

The sediments analysed differed both between reservoirs and between sampling stations. Those collected from station M1 in Maziarnia Reservoir were sandy. The reaction of the top layer was slightly alkaline, ranging from pH 7.36 to 7.8 (Table 1).

Sediments from station M2 were characterised by a dark colour, and the top layer here was slightly acidic, with pH values in the 5.08–6.8 range. Further down into the sediment pH values were lower, declining to 4.34 (Table 1, Fig 2).

In Nielisz Reservoir, sediments at station N1 were sandy-clay. The reaction of the top layer was slightly alkaline, with pH values ranging from 7.4 to 7.99. In a sediment core collected during the summer, the reaction was slightly alkaline through the whole depth, but did not exceed pH 8 (Table 1, Fig 2). Sediments from station N2 were much darker than those at station N1. pH values noted there were indicative of sediments of a slightly alkaline character (Table 1, Fig 2).

The sediments investigated were characterised by a relatively low content of organic matter (OM), and consequently of total organic carbon (TOC). TOC accounted for approximately

|   | pH    | TOC [%]     | $\delta^{13}$C-TOC [%] |
|---|-------|-------------|-------------------------|
| M1 | 7.36  | 0.08        | -28.50                  |
| M2 | 7.08  | 0.98        | -29.76                  |
| N1 | 7.40  | 0.21        | -24.84                  |
| N2 | 7.20  | 1.03        | -26.53                  |
| Minimum | 7.36  | 0.08        | -28.50                  |
| Maximum | 7.80  | 0.98        | -24.84                  |
| Mean | 0.22  | 0.82        | -21.39                  |
| SD  | 0.27  | 0.80        | 3.03                    |
| n   | 8     | 8           | 8                       |

$n$—number of measurements, SD—standard deviation

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30% of OM. The TOC content in the top (1 cm) layer of bottom sediments in the studied reservoirs varied from 0.08 to 5.9% (Table 1). The lowest TOC value was noted at station M1 of the Maziarnia Reservoir and the highest at station M2. Low contents of TOC in sandy sediments were observed. Mean contents of TOC were in turn 0.22 and 0.82% at stations M1 and N1, respectively (Table 1). In deeper sediment layers at stations M2, N1, N2 it was usual to note a decrease of TOC content with depth. However, the decrease in TOC in the deeper layers of sediment cores from Nielisz Reservoir was much more limited than that characterising the cores from Maziarnia Reservoir (Fig 2).

The top layer of sediments from Maziarnia Reservoir was in turn characterised by values for δ¹³C-TOC between -29.76 and -26.99‰ (Table 1). In spring, in the analysed cores of the sediments (at station M2), an enrichment of organic carbon in ¹³C of approximately 2‰ was found between the top of the sediment and a depth of 10 cm. In summer, δ¹³C-TOC at full depth remained at a fairly constant level, with values in the range -28.60 to -28.08‰. In autumn, there was a downward trend for the δ¹³C-TOC value to a depth of 5 cm, beyond which values were higher again by about 1.5‰ (Fig 2). δ¹³C-TOC
values in the top sediment layer of Nielisz Reservoir varied over a wide range from -26.53 to -15.77‰ (Table 1). In summer 2011, the values for $\delta^{13}C$-TOC in the analysed 5 cm layers at station N1 revealed no unambiguously defined trends, with range being from -18.18 to -13.20‰. Sediment cores from station N2 were characterised by an almost-constant value of $\delta^{13}C$-TOC in the layers analysed (Fig 2).

**CH$_4$ and CO$_2$ concentrations and $\delta^{13}C$-CH$_4$ and $\delta^{13}C$-CO$_2$ values in pore water**

Characteristic values for CH$_4$ and CO$_2$ concentrations and for $\delta^{13}C$-CH$_4$ and $\delta^{13}C$-CO$_2$ in pore water from the layer 1 cm down into the analysed sediments are as presented in Tables 2 and 3, respectively. Fig 3 presents the relevant values in sediment cores.

CH$_4$ concentrations in pore water from the uppermost sediment varied across the range 0–360 μmol/dm$^3$. Stations located in upper parts of the reservoirs reported significantly higher values. Mean concentrations of CH$_4$ at stations M2 and N2 were high, at 140.58 and 213.33 μmol/dm$^3$ respectively (Table 2). CH$_4$ concentrations in the sediment cores of either reservoir (Fig 3).

CO$_2$ concentrations were much higher than those of CH$_4$, though also ranging widely, from 120 to 1906.66 μmol/dm$^3$. The lowest mean concentration in the pore water from the top layer of sediments, amounting to 433.33 μmol/dm$^3$, was noted at station M1. This contrasted with the highest value– 3089.17 μmol/dm$^3$ – recorded at station N1. In deeper sediment layers, CO$_2$ concentrations were shown to increase more or less steadily with depth (Fig 3).

Values for $\delta^{13}C$-CH$_4$ in the top layer of sediment ranged from -60.67 to -53.41‰. Mean values for $\delta^{13}C$-CH$_4$ were similar, and ranged from -57.63 to -56.27‰. Values of $\delta^{13}C$-CO$_2$ in turn ranged from -16.97 to -7.23‰. Mean values were in the range -11.65 to -10.16‰ (Table 3).

### Table 2. Concentrations of CH$_4$ and CO$_2$ in pore water (1 cm into the sediment layer) of the reservoirs.

| Station | CH$_4$ [μmol/dm$^3$] | CO$_2$ [μmol/dm$^3$] |
|---------|----------------------|----------------------|
|         | M1       | M2       | N1       | N2       | M1       | M2       | N1       | N2       |
| Minimum | 0.00     | 37.33    | 0.00     | 30.66    | 120.00   | 493.33   | 1233.33  | 2133.33  |
| Maximum | 205.33   | 320.00   | 360.00   | 346.66   | 960.00   | 1906.66  | 11480.00 | 3453.34  |
| Mean    | 26.42    | 140.58   | 87.92    | 213.33   | 433.30   | 1072.07  | 3089.17  | 2827.79  |
| SD      | 72.31    | 104.58   | 159.90   | 267.33   | 515.09   | 3437.96  | 479.61   |
| n       | 8        | 8        | 8        | 8        | 8        | 8        | 8        |

**Table 2**—standard deviation; n—number of measurements

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### Table 3. The values of $\delta^{13}C$-CH$_4$ and $\delta^{13}C$-CO$_2$ in pore water (1 cm into the sediment layer) of the reservoirs.

| Station | $\delta^{13}C$-CH$_4$ [%o] | $\delta^{13}C$-CO$_2$ [%o] |
|---------|-----------------------------|-----------------------------|
|         | M1       | M2       | N1       | N2       | M1       | M2       | N1       | N2       |
| Minimum | -        | -60.67   | -57.35   | -58.05   | -15.19   | -15.30   | -16.97   | -15.89   |
| Maximum | -        | -54.36   | -56.22   | -53.41   | -8.66    | -7.23    | -9.18    | -9.90    |
| Mean    | -        | -57.63   | -56.73   | -56.27   | -11.42   | -10.16   | -11.65   | -12.51   |
| SD      | -        | 2.40     | 0.57     | 1.60     | 2.42     | 2.89     | 2.79     | 2.20     |
| n       | 0        | 6        | 3        | 8        | 7        | 7        | 8        |

**Table 3**—standard deviation; n—number of measurements

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Analysis of the changes in δ^{13}C-CH\textsubscript{4} values in deeper sediment make it clear that, with a few exceptions, increasing depth is associated with a depletion of the carbon isotope. At station M2 in Maziarnia Reservoir, a greater degree of δ^{13}C carbon depletion was observed in the spring, the difference between the top layer and that 10 cm down then being of little more than 2.5‰. In summer, the δ^{13}C-CH\textsubscript{4} value at a depth of 15 cm was lower by approx. 1.5‰ than that characterising the top layer. In July 2001, at Nielisz Reservoir (at station N2), the decline in δ^{13}C-CH\textsubscript{4} amounted to approx. 2‰. In autumn depletion of CH\textsubscript{4} in δ^{13}C was observed to a depth of 5 cm, while at 10 cm δ^{13}C-CH\textsubscript{4} was back up values similar to those in the uppermost layer of sediment (Fig 3).

δ^{13}C-CO\textsubscript{2} was usually found to present a trend opposite to that characterising δ^{13}C-CH\textsubscript{4}, with increasing depth enrichment of CO\textsubscript{2} in δ^{13}C. At station M2 on Maziarnia Reservoir the differences in the δ^{13}C-CO\textsubscript{2} values from the top to a depth of 10 cm were of approx. 6 and 7‰ in spring and summer respectively. At Nielisz Reservoir (at station N2), in summer, a descent to a depth of 15 cm was associated with changes in the value of δ^{13}C-CO\textsubscript{2} in pore water in the range -16.42 to -12.60‰. The enrichment of CO\textsubscript{2} in δ^{13}C at a depth of 15 cm in relation to the top layer was negligible, at approx. 0.2‰. In autumn, depletion of CO\textsubscript{2} in δ^{13}C was of approx. 2.5‰ between the top and a depth of 15 cm (Fig 3).

Production pathways of CH\textsubscript{4} and CO\textsubscript{2}

Table 4 shows calculated values for (α\textsubscript{CH\textsubscript{4}-CO\textsubscript{2}}) fractionation coefficients in pore water, as well as a calculation for the contribution of CO\textsubscript{2} reduction to the production of CH\textsubscript{4} based on the isotopic mass balance equation.
Values of $\alpha_{\text{CH}_4-\text{CO}_2}$ were in the range 1.04 to 1.07. They were lower in Nielisz Reservoir than at Maziarnia. In the latter reservoir, the values for the fractionation coefficients $\alpha_{\text{CH}_4-\text{CO}_2}$ twice (in autumn 2010 and spring 2011) already amounted to 1.06 in the top sediment layer. In other cases, they were of 1.05. An increase in $\alpha_{\text{CH}_4-\text{CO}_2}$ values from 1.06 to even 1.07 was observed with increasing depth. In Nielisz Reservoir, the values for the $\alpha_{\text{CH}_4-\text{CO}_2}$ fractionation coefficients amounted to 1.04 or 1.05 in the analysed period. There was no increase in their value with increasing depth.

In Maziarnia Reservoir, from 43 to 82% of CH$_4$ was produced via acetate fermentation. The largest contributions made by hydrogenotrophic methanogenesis were to be observed in September 2010 and May 2011. During other periods the acetate fermentation pathway predominated.

In the top sediment layer of Nielisz Reservoir estimated contributions of hydrogenotrophic methanogenesis were of between 0 and slightly over 30%. There was no clear relationship between season of the year and production pathways involving CH$_4$.

The deeper sediment layer in Maziarnia Reservoir showed greater importance of CO$_2$ reduction at greater depth, in both spring and summer (Table 4). An increase in the role of acetate fermentation in summer as compared with spring was also observed. In deeper sediment layers, the contribution made to total methanogenesis by hydrogenotrophic methanogenesis was a large one, in some cases even approaching 90%.

In Nielisz Reservoir, hydrogenotrophic methanogenesis only assumed lesser importance where the production of CH$_4$ was concerned. The highest most major contribution of the hydrogenotrophic methanogenesis made by this process was the 18% of the total noted in July 2011 for the layer of sediment 1–3 cm down. In September, it was only once possible to note (in the 3–5 sediments layer) a small contribution of this pathway to the production of CH$_4$. In other layers, CH$_4$ was formed by acetate fermentation only (Table 4).

### Table 4. Calculated $\alpha_{\text{CH}_4-\text{CO}_2}$ factors and the contribution of hydrogenotrophic methanogenesis to total methanogenesis [%] in the sediments of Maziarnia (M2) and Nielisz (N1, N2) Reservoirs.

| Date/Depth | 0–1 cm | 1–3 cm | 3–5 cm | 5–10 cm | 10–15 cm | 0–1 cm | 1–3 cm | 3–5 cm | 5–10 cm | 10–15 cm |
|------------|--------|--------|--------|---------|---------|--------|--------|--------|---------|---------|
| M2 IX 2009 | 1.05   | -      | -      | -       | -       | 18     | -      | -      | -       | -       |
| IX 2010    | 1.06   | -      | -      | -       | -       | 55     | -      | -      | -       | -       |
| V 2011     | 1.06   | 1.06   | 1.07   | 1.07    | -       | 57     | 60     | 84     | 88      | -       |
| VI 2011    | 1.05   | -      | -      | -       | -       | 38     | -      | -      | -       | -       |
| VII 2011   | 1.05   | 1.05   | 1.06   | 1.06    | 1.06    | 36     | 47     | 51     | 59      | 56      |
| VIII 2011  | 1.05   | -      | -      | -       | -       | 27     | -      | -      | -       | -       |
| N1 VI 2010 | 1.05   | -      | -      | -       | -       | 32     | -      | -      | -       | -       |
| VII 2010   | 1.05   | -      | -      | -       | -       | 34     | -      | -      | -       | -       |
| IX 2010    | 1.05   | -      | -      | -       | -       | 33     | -      | -      | -       | -       |
| N2 VI 2010 | 1.05   | -      | -      | -       | -       | 29     | -      | -      | -       | -       |
| VII 2010   | 1.05   | -      | -      | -       | -       | 30     | -      | -      | -       | -       |
| IX 2010    | 1.05   | -      | -      | -       | -       | 34     | -      | -      | -       | -       |
| V 2011     | 1.04   | -      | -      | -       | -       | 15     | -      | -      | -       | -       |
| VI 2011    | 1.05   | -      | -      | -       | -       | 33     | -      | -      | -       | -       |
| VII 2011   | 1.04   | 1.05   | 1.04   | 1.04    | 1.05    | 9      | 18     | 16     | 13      | 17      |
| VIII 2011  | 1.05   | -      | -      | -       | -       | 24     | -      | -      | -       | -       |
| IX 2011    | 1.04   | 1.05   | 1.04   | 1.04    | -       | 0      | 0      | 0      | -       | 0       |

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On the basis of data obtained for the uppermost sediments layer a statistically significant correlation was obtained, showing that the type of CH$_4$ production pathway depends on TOC content (Fig 4).

Additionally, it was possible to note a statistically significant negative correlation between the contribution of hydrogenotrophic methanogenesis and the pH of sediments (Fig 5).
The isotope mass balance (Eq 4), was used to estimate the participation of methanogenesis-derived CO\(_2\) in sediments. Table 4 results for the calculations. At Maziarnia Reservoir’s station M2, between 59 and 75% of CO\(_2\) in the top sediment layer was produced via methanogenesis, with the mean value being 68%. In the deeper sediment layers the contribution of methanogenesis to CO\(_2\) production was even of 88%, though there was no clear relationship between depth and amounts of CO\(_2\) produced by methanogenesis (Table 5).

In the top sediment layers of Nielisz Reservoir, the contributions methanogenesis made to the production of CO\(_2\) ranged from 23 to 53%. Mean values at stations N1 and N2 were 30 and 40%, respectively. In deeper sediment layers at station N2, more CO\(_2\) was produced by methanogenesis during autumn than summer. At greater depths proportionately more CO\(_2\) came from processes other than methanogenesis. This was particularly evident in September.

On the basis of data from the top and deeper sediment layers it is shown that, the lower the value for \(\delta^{13}\text{C}-\text{TOC}\) in sediments, the more CO\(_2\) is derived from methanogenesis (Fig 6).

### Discussion

Analysis of the carbon isotopic composition in CH\(_4\) and CO\(_2\) dissolved in pore water helped define mechanisms by which these gases are produced in reservoirs.

The isotopic composition of CH\(_4\) is affected by: carbon source, availability of substrates and production pathway [16]. The values for \(\delta^{13}\text{C}-\text{CH}_4\) measured in the pore water of the top sediment layer in Maziarnia and Nielisz Reservoirs (Table 3) are within the range characteristic for fresh waters that are poor in sulphate [6, 17–21]. As noted above, CH\(_4\) is formed mainly by the twin processes of acetate fermentation and CO\(_2\) reduction. While \(\delta^{13}\text{C}-\text{CH}_4\) resulting from acetate fermentation reaches values in the -65 to -50‰ range, that arising from hydrogenotrophic methanogenesis has \(\delta^{13}\text{C}\) values of -110 to -60‰ [6, 22–23]. The obtained values of \(\delta^{13}\text{C}-\text{CH}_4\) in the range -60.67 to -53.41‰ are thus indicative of acetate fermentation being the main pathway of CH\(_4\) formation, though CO\(_2\) reduction might also have a role to play (in Maziarnia Reservoir in particular).

### Table 5. Amount of CO\(_2\) from methanogenesis [%] in the sediment of Maziarnia Reservoir (M2) and Nielisz Reservoir (N1 and N2).

| Date/Depth | 0–1 cm | 1–3 cm | 3–5 cm | 5–10 cm | 10–15 cm |
|------------|--------|--------|--------|---------|---------|
| **M2**     |        |        |        |         |         |
| X 2009     | 65     | -      | -      | -       | -       |
| IX 2010    | 73     | -      | -      | -       | -       |
| V 2011     | 73     | 65     | 75     | 74      | -       |
| VI 2011    | 75     | -      | -      | -       | -       |
| VII 2011   | 64     | 76     | 75     | 88      | 74      |
| VIII 2011  | 59     | -      | -      | -       | -       |
| **N1**     |        |        |        |         |         |
| VI 2010    | 23     | -      | -      | -       | -       |
| VII 2010   | 39     | -      | -      | -       | -       |
| IX 2010    | 29     | -      | -      | -       | -       |
| **N2**     |        |        |        |         |         |
| VI 2010    | 45     | -      | -      | -       | -       |
| VII 2010   | 48     | -      | -      | -       | -       |
| IX 2010    | 53     | -      | -      | -       | -       |
| V 2011     | 36     | -      | -      | -       | -       |
| VI 2011    | 41     | -      | -      | -       | -       |
| VII 2011   | 23     | 30     | 21     | 17      | 20      |
| VIII 2011  | 37     | -      | -      | -       | -       |
| IX 2011    | 34     | 32     | 29     | 23      | -       |

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Analysis of the $\delta^{13}$C-CH$_4$ in sediment cores (Fig 3) found values in the range -63.32 to -53.11‰, while the CH$_4$ produced in deeper sediment layers was usually depleted of $^{13}$C. Higher values for $\delta^{13}$C-CH$_4$ in the top layer of sediments may reflect the oxidation of CH$_4$. Admittedly, bottom sediments below 2–5 mm have anaerobic conditions [20, 24], but a large amount of CH$_4$ can be oxidised to CO$_2$ by sulphate-reducing bacteria before diffusing into the interface between the sediment and the overlying water. This process called “anaerobic oxidation” takes place in line with the equation CH$_4$ + SO$_4^{2-}$ $\rightarrow$ HCO$_3^-$ + H$_2$O + HS [25]. In marine environments, even more than 76% of the methane diffusing into the above interface can be oxidised anaerobically in sediments [26]. In line with the Rayleigh equation, $^{12}$CH$_4$ is oxidized faster than $^{13}$CH$_4$ [23], with the result that CH$_4$ is enriched in the heavier isotope of carbon. At the same time a declining $\delta^{13}$C-CO$_2$ value and an increased CO$_2$ value are to be observed [27].

As the sediments of freshwater ecosystems are usually characterised by a low content of sulphate, an impact of oxidation on the $\delta^{13}$C-CH$_4$ value seemed rather unlikely. The dynamics to the isotopic composition of C-CH$_4$ can be explained rather by a change in the production pathways for CH$_4$ in deeper layers of sediment [19, 28–30]. Similar results for the $\delta^{13}$C-CH$_4$ distribution in sediments were noted by Nüsslein and others [20]. Hornibrook and others [16] reports that such a distribution is characteristic where CO$_2$ reduction increases in importance at the expense of the acetate fermentation pathway. This can reflect limited availability of labile organic matter (OM). In marine sediments, it is usual to observe a different distribution of $\delta^{13}$C values, with the depth of both CH$_4$ and CO$_2$ being dominated by the heavier isotope of carbon, thanks to the limited availability of substrates for methanogenesis [16].

In the defining of the CH$_4$ production pathway, the $\delta^{13}$C values of co-existing CO$_2$ prove helpful. The partitioning of carbon isotopes between CO$_2$ and CH$_4$ can be expressed as an isotopic fractionation factor $\alpha_{\text{CH}_4-\text{CO}_2}$, which, in marine environments (where the main pathway leading to methane production is entails the reduction of CO$_2$) [31], varies in the range 1.05 to 1.1 [6]. In turn, in freshwater ecosystems, where acetate fermentation is mainly responsible [32], values for $\alpha_{\text{CH}_4-\text{CO}_2}$ are in the 1.04–1.05 range [6]. According to Conrad [33], calculation
of $\alpha$CH$_4$-CO$_2$ fast if imprecise way of determining the pathway leading to the production of CH$_4$. Calculated on the basis of both $\delta^{13}$C-CH$_4$ and $\delta^{13}$C-CO$_2$, the fractionation factor for the reservoirs studied was in the range 1.04–1.07 (Table 4), with characteristic values for both pathways of methanogenesis therefore being reached [32]. Noted $\alpha$CH$_4$-CO$_2$ values show that hydrogenotrophic methanogenesis was not observed in Nielisz Reservoir as a whole, the, while in Maziarnia Reservoir there is increased $\alpha$CH$_4$-CO$_2$ with sediment depth, suggesting different pathways of CH$_4$ production. In the top sediment layer of Maziarnia Reservoir, values characteristic for the pathway involving the reduction of CO$_2$ were recorded. Conclusions drawn from analysis of the calculated $\alpha$CH$_4$-CO$_2$ were values were thus consistent with those reached through $\delta^{13}$C-CH$_4$ analysis.

An increase in the role of the CO$_2$ reduction mechanism in the overall process by which methane is generated in the deeper parts of bottom sediments has also been observed by other researchers [9, 15, 20, 32, 34–36].

Confirmation of the above was provided by reference to of the isotope mass balance (Eq 2) contribution of hydrogenotrophically-derived CH$_4$ to total CH$_4$ within the different sediment layers (Table 4). In the top layer of sediment in Maziarnia Reservoir, it was acetate fermentation that was found to be the main. Though not only, pathway leading to CH$_4$ production. The maximum observed contribution of hydrogenotrophic methanogenesis was of 57%, the minimum of 18%. In turn, in the top layer of sediment in Nielisz Reservoir, the typical contribution made by acetate fermentation was close to 70%, though values of almost 100% were also recorded. In both analysed reservoirs, the relative importance of hydrogenotrophic methanogenesis were observed to be greater deeper down into the sediment, with the peak contribution at around 90%.

The majority of publications show that acetate fermentation predominates over hydrogenotrophic methanogenesis by a ratio of 2:1 [14, 37]. However, there are works indicating acetate fermentation vs. CO$_2$ reduction at proportions much higher than the theoretical ones, e.g. with a difference that is threefold [38] or even up to eightfold [37]. This shows that the top layer of sediment in Maziarnia Reservoir has an acetate fermentation contribution close to the theoretical value. However, this pathway is found to be of lesser importance deeper down into the sediment.

Analysis of data for the top (1 cm) layers of sediment did not reveal statistically significant correlations between temperature and pathways leading to the production of CH$_4$. Nevertheless, data for the sediment cores did indicate seasonal change as regards the various pathways of CH$_4$ production. In Maziarnia Reservoir acetate fermentation was more important in summer than in spring, for example, while in Nielisz Reservoir the contribution made by CO$_2$ reduction was greater in summer than in autumn (Table 4). The effect of temperature on pathways leading to the production of CH$_4$ is ultimately therefore unambiguous.

Wu and others [39] indicated a key role for temperature where anaerobic fermentation is concerned, given the effect on the supply of substrates for methanogenesis, and hence indirect control over the CH$_4$ production pathway. At low temperature, the acetate fermentation prevails, while at an average temperature CH$_4$ formation by both pathways proceeds equally. In turn, at high or very high temperatures it is hydrogenotrophic methanogenesis that prevails. In eutrophic Lake Dagow, CO$_2$ reduction was found to be the more important source of CH$_4$ at high temperature, but it was less significant at 4°C than 30°C [40]. However, such findings are not consistent with the results of research carried out in oligotrophic, ice-covered Lake Untersee (Eastern Antarctica) [41], where the calculated contribution of CH$_4$ from the reduction of CO$_2$ ranged from 93.1 to 99.2%. This suggests that the mechanism in question may be dominant in freshwater ecosystems, even at very low temperatures. In turn, in Lake Bled, hydrogenotrophic methanogenesis was the dominant pathway for the production of CH$_4$ at low...
temperatures (6°C) [35]. However, even at high temperatures (of approx. 30°C), Amazonian lakes are characterised by a situation in which the reduction of CO$_2$ outweighs acetate fermentation, being 53–63% responsible for the formation of CH$_4$ [42].

In a study of the isotopic composition of CH$_4$ bubbles produced in the sediments of Lake Moszne (Poland), Jedrzejek [43] found a weak correlation between values for $\delta^{13}$C-CH$_4$ and the temperature of deposits. The higher the latter, the more CH$_4$ was seen to be enriched in the $^{13}$C isotope, indicating production by acetate fermentation. However, in tropical climates, where the vertical and annual temperature variation in sediments is negligible, observed values of $\delta^{13}$C-CH$_4$ are low, and significantly lower at greater depths [28], suggesting that temperature is not the factor directly responsible for the isotopic composition of CH$_4$.

The same author presents the results of research on the circadian variation to CH$_4$ isotopic composition in three Polish lakes [18,19], which shows that night and early-morning methane is characterised by lower values of $\delta^{13}$C-CH$_4$ than that produced in the afternoon. These results indicate that CH$_4$ is rather formed by acetate fermentation at higher temperatures. In the case of Lake Bled (Slovenia), it was calculated that the dominant mechanism generating methane in the spring is acetate fermentation, which accounts for about 65% of the total. 95% of autumn CH$_4$ was generated through the reduction of CO$_2$, while in summer the two mechanisms were equally responsible for the production of this gas [30]. The authors suggest that the seasonal variations to the origin of CH$_4$ were rather the result of qualitative differentiation characterising OM. Nüsslein and others [20] also found that temperature is not a factor underpinning the type of mechanism involved in CH$_4$ production.

In light of the above, it can be concluded that temperature is not a factor affecting the source of CH$_4$ in freshwater environments directly, with an important role in this probably being played by the type of OM deposited in sediments.

There is no doubt that the origin of OM will affect rates of CH$_4$ production, as is confirmed by the results of published studies showing how freshwater ecosystems with high primary production are characterised by conditions more favourable to the process of methanogenesis [44]. Algae decompose to CH$_4$ and CO$_2$ ten times faster than lignocellulose [45], suggesting that autochthonous organic material is a better substrate for methanogenesis [46].

As was mentioned above, the type of degradable OM can also have an impact on the mechanism of CH$_4$ production. In deeper layers poorer in decomposable OM, the CH$_4$ production pathway moves over to the reduction of CO$_2$ [16, 34, 36]. The presented results of calculations show that $\alpha_{\text{CH}_4-\text{CO}_2}$ coefficient values for the deeper layers of sediment reach values characteristic for hydrogenotrophic methanogenesis. Moreover calculated contribution to overall CH$_4$ production also increased with depth.

Research conducted by Murase and Sugimoto [47] likewise confirms the importance of the type of OM impact on the CH$_4$ production pathway. The results obtained by these authors for both $\delta^{13}$C-CH$_4$ and $\alpha_{\text{CH}_4-\text{CO}_2}$ in the lake sediments studied were more typical for marine than freshwater environments, and indicated that CH$_4$ was produced as a result of CO$_2$ reduction. It should be emphasised that the lake investigated was oligo/mesotrophic and only therefore alimented by autochthonous OM to a limited extent. Study of methanogenesis in oligotrophic and mesotrophic peatlands has shown that, under oligotrophy, hydrogenotrophic methanogenesis accounts for more than 75% of production, while in the circumstances of mesotrophy acetate fermentation dominated, accounting for 54–59% of production [48]. In Lake Bled, during the spring when the acetate fermentation was dominant, sediments were rich in easily-degradable planktonic OM [30]. Mandić-Mulec and others [35] and Gruca-Rokosz and Tomaszek [36] also confirmed that an increase in hydrogenotrophic methanogenesis in the deeper layers of sediment was associated with a lack of labile OM.
To confirm the above hypothesis concerning the impact of type of OM on the mechanism of \(\text{CH}_4\) formation, the relationship between the calculated contribution made to overall methanogenesis by the \(\text{CO}_2\) reduction pathway and indicators used to identify the OM source was researched. The statistically significant correlation shown in Fig 4 was identified. As the value of \(\delta^{13}\text{C-TOC}\) increased, the importance of the hydrogenotrophic methanogenesis pathway decreased. This correlation confirmed a previous view that OM of autochthonous origin is conducive to acetate fermentation, with \(\text{CO}_2\) reduction becoming important when the prevailing, decomposition-resistant OM is of land origin.

Another factor limiting the pathway via which \(\text{CH}_4\) is produced may be the pH of deposits. When this is low, acetate fermentation seems to be the dominant mechanism, while high pH values favour hydrogenotrophic methanogenesis. This may reflect the adaptability of bacterial consortia in different pH ranges [49]. However, the analysis of the results obtained provides different information. The contribution \(\text{CO}_2\) reduction made to \(\text{CH}_4\) formation reached its highest values in Maziarnia Reservoir, for which the profiles of sediment are characterised by pH values lower than those noted in Nielisz Reservoir (Table 1, Fig 2). A statistically significant correlation was then obtained (Fig 5), with the importance of \(\text{CO}_2\) reduction found to decrease significantly where pH values are higher.

The values of \(\delta^{13}\text{C-CO}_2\) obtained for top layers of sediment are in the -16.97 to -7.23‰ range (Table 3). In deeper layers, \(\delta^{13}\text{C-CO}_2\) ranged from -18.32 to -1.32‰, while enrichment of \(\text{CO}_2\) with the heavier isotope at greater depth was also observed (Fig 3).

\(\text{CO}_2\) in sediments can come from OM mineralisation, methanogenesis or the dissolution of carbonates. Obtained values for \(\delta^{13}\text{C-CO}_2\) thus reflect mixing of \(\text{CO}_2\) originating from these sources. It can be assumed that, in the case of \(\text{CO}_2\) from OM mineralisation, the isotopic composition of carbon values will be close to that in TOC deposited in the sediments, because the process entails the release of dissolved inorganic carbon into pore water—which is similar isotopically to the source [9]. In the case of the studied reservoirs, the \(\delta^{13}\text{C-TOC}\) values ranged from about -29 to about -13‰, and were in most cases significantly lower than recorded values for \(\delta^{13}\text{C-CO}_2\), suggesting that the mineralisation of OM was not the dominant process behind \(\text{CO}_2\) formation. Higher values of \(\delta^{13}\text{C-CO}_2\) may indicate that sediment \(\text{CO}_2\) came from the carbonate dissolution process or from methanogenesis. \(\text{CO}_2\) released by methanogenesis is enriched in the isotope \(^{13}\text{C}\)—with respect to the organic carbon in sediment [9]; and the carbonates which may be a source of \(\text{CO}_2\) [30] are also characterised by high values of \(\delta^{13}\text{C}\) [9, 20, 32]. Had the source of \(\text{CO}_2\) been the dissolution of carbonates, a negative correlation between \(\delta^{13}\text{C-CO}_2\) and pH [32] ought to have been observed, but was not. It is therefore hypothesised that methanogenesis played a major role in the production of \(\text{CO}_2\) in sediments, especially in the deeper layers.

To confirm this hypothesis, the contribution of methanogenesis to the production of \(\text{CO}_2\) in the deeper layers of sediment was determined (Table 5). In the top layer, 23% to even 75% of \(\text{CO}_2\) originated from methanogenesis. In sediment cores taken from Maziarnia Reservoir neither a significant increase in \(\text{CO}_2\) from methanogenesis with depth, nor a significant seasonal difference was to be noted. In turn, in Nielisz Reservoir, methanogenesis was less important in generating \(\text{CO}_2\) in the deeper layers of sediment than at the surface and less important in summer than autumn.

A depth-related increased contribution of methanogenesis to the production of \(\text{CO}_2\) has also been observed by other researchers. Kelly and others [51] found that \(\text{CO}_2\) produced during methanogenesis accounted for as much as 70–80% of the total. Lojen and others [30] reported 43%, and Ogrinc and others [9] figures in the range 38–78% and the process clearly predominated in deeper sediment layers and anaerobic areas of the lake. Corbet and others [15] concluded that the proportion of \(\text{CO}_2\) from methanogenesis increased further down
in sediments, accounting for 36% at a depth of 10 cm and 61% at a depth of 50 cm. In the top sediment layer the aforementioned Ogrinc and others [9] only observed a preponderance of CO$_2$ from the process of methanogenesis in summer, when the temperature of the sediment was higher and there was more labile OM derived from microalgae and phyto-plankton. In deeper, anaerobic sediments the impact of the season was not shown to be of consequence.

Analysis of the influence type of OM has on the contribution methanogenesis to the generation of CO$_2$, revealed a statistically significant correlation between the $\delta^{13}$C-TOC and that contribution (Fig 6), thereby showing that CO$_2$ production via methanogenesis is more pronounced with OM deposited in sediments more depleted of the heavier C isotope and of allochtonous origin. These results differ from those obtained by Ogrinc and others [9].

Such a relationship may reflect incomplete decomposition of OM originating on land. For complete methanogenic degradation of OM an equimolar production of CH$_4$ and CO$_2$ is expected, but these proportions are often distorted and higher production of CO$_2$ than CH$_4$ is observed. As Fig 4 shows, the contribution due to hydrogenotrophic methanogenesis was greater where $\delta^{13}$C-TOC was lower, denoting that, where degraded OM was allochthonous in origin, this was especially a property of deeper layers of sediment. As was discussed earlier, a growing importance of CO$_2$ reduction at greater depths has also been identified by other researchers, suggesting that OM does not undergo complete degradation deeper down in sediment [32]. According to Galand and others [48], in the case of incomplete degradation of OM the higher rate of production of CO$_2$ may be due to the mutual oxidation of certain organic substances. Certain humic substances are of high redox potential [48]. It should be noted that the sediments from station M2 in Maziarnia Reservoir (associated with methanogenesis making the greatest contribution to in the production of CO$_2$), stood out in a near black colour indicative of a high content of humic substances.

**Conclusion**

Despite the common opinion that acetate fermentation is the dominant mechanism of methane production in freshwater ecosystems, our work has shown that CO$_2$ reduction may constitute an equally important mechanism of particular significance in the deeper layers of bottom sediment. Temperature is not found to be a factor directly affecting the mechanism of CH$_4$ production in freshwater environments, and any seasonal influence is rather a reflection of the qualitative diversity characterising organic matter. Autochthonous organic matter produced during warm and sunny days via the process of photosynthesis creates favourable conditions for acetate fermentation, and hydrogenotrophic methanogenesis plays a greater role in the case of the less-readily-decomposable matter originating on land. Sediment reaction is another significant factor affecting the mechanism of methane production, as an increase in pH is favourable to acetate fermentation. CO$_2$ in sediment derives, not only from the mineralisation of organic matter and carbonate dissolution, but also (in considerable quantities) from methanogenesis. In deeper layers of sediment the importance of methanogenesis to the production of carbon dioxide is even greater.

**Supporting information**

S1 Dataset. Results of conducted studies.
(XLSX)
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