Interferences caused by the microbial methane cycle during the assessment of abandoned oil and gas wells

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Abstract.

In the global effort to reduce anthropogenic methane emissions, the millions of abandoned oil and gas wells are suspected to be prominent but so far often overlooked methane sources. Recent studies highlighted the hundreds of thousand undocumented abandoned wells in North America as sometimes strong methane emitters with up to several tons of methane per year. However, the majority of studies focused on abandoned wells with their surface installations still in place. Only a few studies examined cut and buried wells as their exact location are often unknown. In Germany, approximately 20,000 abandoned wells are described, which are well documented, and the data is publicly available. Here we present a methodological approach to assess methane emissions from such cut and buried abandoned wells. We sampled eight oil wells in a peat rich setting with four wells in a forest, three wells in an active peat extraction site, and one well on a meadow. All three areas have peat deposits underneath. At each site, we sampled a 30 x 30 m grid and a corresponding 20 x 20 m reference grid. Three of the eight well and reference sites showed net methane emissions. The highest emissions with up to ~110 nmol CH₄ m⁻² s⁻¹ were observed at one of the reference sites. All three methane-emitting sites were located within the active peat extraction area. Detailed soil gas characterization revealed no methane, ethane, and propane ratio typical for reservoir gas, but instead showed a typical biogenic composition and isotopic signature (mean δ¹³C-CH₄ = -63%). Accordingly, the escaping methane did not originate from the abandoned wells or the connected oil reservoir. In addition, isotopic signatures of methane and carbon dioxide suggest that the peat extraction site’s methane was produced by acetoclastic methanogens, whereas methane at the meadow site was from hydrogenotrophic methanogens. However, our genetic analysis showed that both types of methanogens were present at both sites and thus other factors were controlling the prevailing methane production mechanism. Subsequent molecular biological investigations highlighted that aerobic methanotrophs were also important and that they had the highest relative abundance at the peat extraction site. Furthermore, the composition of the methanotrophic community varied across sites and depth. The aerobic methane oxidation rates were highest at the peat extraction site potentially oxidizing a multiple of the emitted methane. Our findings underscore the necessity to combine methane emissions with the characterization of soil gases in comparison with a suitable reference site to survey cut and buried abandoned wells as a solely emission-based surveillance could misinterpret natural occurring emissions.
1 Introduction

The slow decay of global abandoned oil and gas infrastructure is a rising problem (Bowman et al. 2023, Williams et al. 2021, Riddick et al. 2020) which will intensify in the future during the transition to renewable energy sources. Depending on the country’s regulations, abandonment could mean to just close the well head but leave everything in place (Pekney et al. 2020, Williams et al. 2020), decommission the well and leave an open hole in the ground (Pekney et al. 2020, Lebel et al. 2020), or properly fill the well and cut and bury the remains (Schout et al. 2019, Davies et al. 2014). This resulted in varying situations around the world and a general call to action as anthropogenic methane emissions need to be cut (Saunois et al., 2020). Thus, authorities and scientists rush to identify super and mega emitters (Bowman et al 2023) as financial resources for proper well decommission are limited (Agerton et al. 2023). However, this straightforward methodology is only applicable for abandoned wells with visible remains at the surface. In countries with regulations to cut and bury wells (e.g., Germany, the Netherlands, and UK), single measurements at the wells location are insufficient (Schout et al. 2019). Even with correct coordinates, emissions can migrate away from the wells location (Dennis et al 2022, Forde et al. 2022), disperse through the soil and potentially be oxidized by methanotrophic microorganisms on its way to the atmosphere (Forde et al. 2022). In natural methane rich environments, however biogenic methane emissions could be mistaken for a leaking well. An example for such an environment are wetlands and peat rich areas that are associated with a large number of oil and gas wells in Germany.

Peat rich areas are former raised/ombrotrophic bogs, rich fens and other types of peat accumulating wetlands. In pristine ecosystems, the vegetation is taking up carbon dioxide from the atmosphere and producing biomass. Plant litter is only partially decomposed due to oxygen limitation in deeper layers. Once oxygen is depleted the microbial degradation of organic carbon is coupled to the reduction of a series of terminal electron acceptors depending on their half-cell potentials (Sikora et al., 2017). Organic carbon degradation is facilitated by a complex network of trophically linked microorganisms (i.e., intermediary ecosystem metabolism) ultimately resulting in methane production when alternative electron acceptors except for carbon dioxide are depleted (Whiticar 2020). Methanogenesis is mainly carried out by three types of anaerobic archaea in more than 30 genera: 1) acetoclastic methanogens converting acetate to methane and carbon dioxide, 2) hydrogenotrophic methanogens, reducing carbon dioxide to methane with hydrogen, and 3) methylotrophic methanogens disproportionating methyl groups to methane and carbon dioxide (Liu and Whitman, 2008). Although most methanogens are hydrogenotrophs, two-thirds of biologically produced methane is derived from acetate (Liu and Whitman, 2008). Is sufficient methane produced, it diffuses towards the surface and it is partially oxidized by anaerobic and aerobic methanotrophs to carbon dioxide along the way. Methanotrophs thereby act as an efficient methane filter and are associated with the regulation of methane fluxes from wetlands. However, these wetlands are still active net carbon sinks, since CO₂ fixation in plant biomass far exceeds biomass mineralization, and accumulate peat for millennials (Turetsky et al., 2014; Frolking et al., 2006). Thus, wetlands play a relevant role in the global C cycle as important terrestrial carbon pool (Belyea, 2013).
Most raised bogs in Central Europe were, however, drained in the past for agricultural use, forest cultivation, and peat extraction for fuel or horticultural purposes (Pfadenhauer and Klötzli, 1996; Laine et al., 2013). After drainage, most of these wetlands change from net carbon sinks to net carbon sources (Frolking et al., 2006). This is due to the remineralization of once stored organic matter to ultimately carbon dioxide (Abdalla et al., 2016). On the other hand, methane emission decreases drastically as the aerated soil enable aerobic methane oxidation to CO₂ and methanogenesis is restricted to deeper layers (Sundh et al., 1994; Abdalla et al., 2016). Nonetheless, the greenhouse gas balance changes with drainage and differs depending on land use (Abdalla et al., 2016). Methane emissions are thought to stop altogether in peatlands used for forestry or agriculture (Abdalla et al., 2016 and references therein). However, previous studies point towards substantial methane emissions from ditches, which are draining the peats and can even reach the magnitude of emissions from virgin peatlands (Sundh et al., 2000). The extraction of peat results in an accelerated carbon loss and increased greenhouse gas emissions as peat decomposition associated with end use comprises the majority of total emissions (e.g., combustion and use in horticulture), machinery for extraction and transportation add additional emissions (Cleary et al., 2005). In this complexity of methane und carbon dioxide related biogeochemical processes, one has to look closely to delicately allocate methane emission to natural or anthropogenic sources.

Worldwide only very few countries, i.e., the USA and Canada (Bowman et al., 2023) include emission from abandoned wells in their yearly greenhouse gas inventory. In a BGR project, we aim to fill this knowledge gap for Germany by studying a representative sub-set of wells over the course of five years. Here, we present a first detailed study of eight wells in a complex methane rich setting. In Germany ~ 2700 abandoned wells, which translates to roughly 15% of all abandoned wells (~20,000; NIBIS® Kartenserver 2014) are situated in an organic rich soil (mainly peats) setting (Wittnebel et al., 2023). Such soils are highly likely to produce and emit methane. We used this opportunity to test our methodological approach, a combination of geochemical and microbial techniques, to evaluate methane emissions from cut and buried abandoned wells. Our main focus was on the question of whether we could clearly identify the source of the methane. In addition, the microbiological methods enabled us to quantify the methane oxidation potential of the soil, i.e., the methanotrophic methane filter function, and identify key organisms feeding on the soil methane.

2 Methods

2.1 Study site

The sampling and field measurements were conducted in March and April 2022 near Steimbke (Lower Saxony, Northern Germany), an area with ongoing and historical industrial peat production. Additional samples were taken in April 2023 from the peat extraction site and November 2023 from reference sites. Three oil fields were located around Steimbke. From these three, we focused on field Steimbke-Nord. Data including location, depth, date of drilling, etc. of wells related to this oil field as well as the other ~20,000 wells in Germany (abandoned, producing, and exploration) and data on the oil and gas fields are publicly available via NIBIS map server (NIBIS® Kartenserver 2014). We used this database to locate about 200 wells in the...
vicinity of Steimbke-Nord including 159 abandoned production wells. The oil-bearing horizons were located in the Malm and Dogger (both Jurassic) in 500 and 700 m depth covering an area of about 1.5 km². The wells were drilled between 1942 and 1950 and are typically 570 to 695 m deep. In total $3 \times 10^8$ t oil and $2.9 \times 10^9$ m³ oil associated gas were produced until 1964 (https://nibis.lbeg.de/cardomap3/?permalink=WeOGYg3, accessed 03.05.2024). We studied and sampled eight abandoned wells, each with respective reference measurements (Figure 1). These well sites can be grouped into three area types. Three out of eight wells (R-WA 254, R-WA 264, R-WA-272) lie in an active peat extraction site (from here on “Peat” sites), four (R-WA 209, R-WA 211, R-WA 273, R-WA 274) in a woodland area (from here on “Forest”), and one (R-WA 275) on a meadow (from here on “Meadow” site). In case of the Forest and Meadow sites, the top soil above the peat layer was sampled, whereas at the Peat sites the peat was sampled directly. Regarding pH of the Peat site, Welpelo et al. (2024) published an pH of approximately 3.5 for a nearby rewetted part of the peat extraction area, about 2.5 km away as well as additional physicochemical parameters. Residues from the drilling and/or production were only visible in the forest area. Here, cement residues likely from the rig cellar or associated infrastructure, sand from the backfill procedure, and small depressions were signs of former activity. No residues of the former well itself, like wellheads, old horsehead pumps, or any kind of piping were visible. All sample sites are situated in a peat rich area and the majority of sites include about 1.5 m of peat or more raised-bog peat either below the topsoil (Forest, Meadow) or as bare peat at the Peat site (https://nibis.lbeg.de/cardomap3/?permalink=1baQ8yzX, accessed 03.05.2024). Peat depth in this area was taken from a geological exploration in 1983. An exemplary soil profile is shown in Fig. 2d, this profile was drilled near our peat reference site (~50 m west). These profiles show a peat thickness of ~1.9 m up to 2.6 m for the peat sites with about 1 m and more already extracted since ~2019. For sites R-WA 273, R-WA 274, R-WA 275 the state agency (https://nibis.lbeg.de/cardomap3/?permalink=1uIMU2yt, accessed 03.05.2024) estimated a peat thickness of more than 2 m, however, for sites R-WA 211 and R-WA 209 peat was confirmed with more than 30 cm depth but its entire thickness is unknown.

Table 1: Overview of surveyed well locations and selected meta data. All wells were used for oil production in the past. *peat is present in all areas (Peat = active peat extraction site)

<table>
<thead>
<tr>
<th>name</th>
<th>short name</th>
<th>north</th>
<th>east</th>
<th>drilling completed</th>
<th>depth (m)</th>
<th>area*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rodewald-WA-211</td>
<td>R-WA 211</td>
<td>5836503</td>
<td>32525924</td>
<td>26.10.1942</td>
<td>635.5</td>
<td>Forest</td>
</tr>
<tr>
<td>Rodewald-WA-209</td>
<td>R-WA 209</td>
<td>5836399</td>
<td>32526148</td>
<td>27.08.1942</td>
<td>570.5</td>
<td>Forest</td>
</tr>
<tr>
<td>Rodewald-WA-273</td>
<td>R-WA 273</td>
<td>5836338</td>
<td>32525761</td>
<td>03.08.1950</td>
<td>682.7</td>
<td>Forest</td>
</tr>
<tr>
<td>Rodewald-WA-274</td>
<td>R-WA 274</td>
<td>5836299</td>
<td>32525835</td>
<td>04.07.1950</td>
<td>680</td>
<td>Forest</td>
</tr>
<tr>
<td>Rodewald-WA-275</td>
<td>R-WA 275</td>
<td>5836302</td>
<td>32525931</td>
<td>21.07.1950</td>
<td>670</td>
<td>Meadow</td>
</tr>
<tr>
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<td>R-WA 272</td>
<td>5836374</td>
<td>32525686</td>
<td>15.06.1950</td>
<td>700</td>
<td>Peat</td>
</tr>
<tr>
<td>Rodewald-WA-254</td>
<td>R-WA 254</td>
<td>5836366</td>
<td>32525498</td>
<td>15.12.1948</td>
<td>695</td>
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</tr>
<tr>
<td>Rodewald-WA-264</td>
<td>R-WA 264</td>
<td>5836323</td>
<td>32525566</td>
<td>03.06.1950</td>
<td>660</td>
<td>Peat</td>
</tr>
</tbody>
</table>
Figure 1: Overview of the study site in Steimbke with the well sites and reference site measuring grids each with 17 and 9 measuring points, respectively. Abandoned wells are depicted in white dots. The rough dimensions of the oilfield Steimbke-Nord are outlined by the yellow dotted line. Coordinates are stated in UTM-32U (WGS84) with easting and northing planar coordinates in meter. Green indicates negative methane emission (methane sink) whereas red indicates positive methane emission (methane source) for each flux measuring point. The multiple measurements of reference sites are shown in a white box. The map was created using QGIS (v.3.22.3) and © Google Earth satellite images from 2015 as background.

2.2 Sampling method and grid

We studied well and reference sites for methane emission (positive and negative), soil gas composition and microbial community analysis (Figure 2c). The reference sites were placed in a distance of 15–100 m to any well on the same terrain. The position of the wells was extracted from the NIBIS® MAPSERVER (NIBIS® Kartenserver 2014), and a handheld GPS device (Garmin, etrex Vista Hcx) was used to navigate in the field. Due to the burial of abandoned wells in the working area, our study relied on the coordinates of the wells. Discussion with the LBEG, the local public as well as indications (e.g., color changes, remnants of roads/pathways) from recent and historical Google Maps images supported the correctness of the well positions.

The central measuring point was placed directly above the well. We positioned the other 16 measuring points around the well pointing north with the help of two measuring tapes and a compass. The distance between these 16 points was 10 m from point...
to point aiming at a broad coverage of potential methane emission areas above the buried wells. In total, the well site grid covered an area of 30 x 30 m and 17 measuring points (Figure 2a). Soil gas samples were taken in the central five positions of the well as indicated in Fig. 2a. Soil samples for microbial analyses were usually taken at three positions starting at the center towards one of the corners. In case of high methane emissions, additional soil gas and microbial samples were taken at the respective spots.

The reference grids consisted of nine measuring points covering an area of 20 x 20 m (Figure 2b). Measuring reference grids is necessary to determine and account for potential natural background variations for each abandoned well. Reference grids were typically located in a distance of 50–150 m from the well site and on similar soil conditions and vegetation, and were investigated immediately after the measurement of the well grid. Three soil gas samples were usually taken in a diagonal pattern and the soil sample for microbial analysis in the center of the grid (Figure 2b). To estimate the emission’s spatial variability, we sampled a transect through a point with high emission at the Peat reference site. The measuring points along the 12 m transect were 1 m apart.

**Figure 2:** Sampling scheme for emission measurements for well (a) and close-by reference sites (b), both with likely similar biogeochemistry and vegetation, as well as a schematic display of a buried abandoned well (c). Additional soil gas samples (stars) and soil samples for microbial analysis and methane oxidation rates determination (square) were taken at the marked positions. The shift of the symbols towards the upper right was made for graphical reasons. Samples were directly taken on the numbered positions. The well position is marked in red.

A simplified profile of a pedological well (# 54315, source LBEG) drilled in 1983 before peat extraction initiated. Coordinates RW: 32525578, HW: 5836405 (EPSG 4647), close (50 m west) to the reference grid in the peat extraction site. SMS: medium sandy fine sand, Hsw: diffuse or in nests enriched with unconsolidated sesquioxides. Link to map: https://nibis.lbeg.de/cardomap3/?permalink=2RfGItuF.
2.3 Methane and CO₂ emission

Methane emissions from the soil surface into the atmosphere were measured with an optical feedback – cavity enhanced absorption spectroscopy trace gas analyzer (LI-COR 7810) coupled to a portable hydraulic chamber (LI-COR smart chamber) following the closed chamber principal. The measurement was conducted as instructed by the manufacturer, which is described in the following: First, defined plastic collars with a diameter of 20.3 cm and height of 12.4 cm (outside diameter 8.4”, height 4.5”) were positioned at each of the measuring points and were pushed few centimeters into the soil to guarantee a complete closure of the smart chamber with the underlying soil profile. Since the exact penetration depths of the collars were needed for the calculation of fluxes (dead volume of the ring) each insertion depth were measured individually.

After both devices, analyzer and chamber, reached operation modus, a startup measurement was conducted to ensure stable condition of the instrument. Each grid position was sampled in triplicates at least 1 h after placing the respective collar. Therefore, the chamber stayed closed for 120 s to record continuously (1 Hz) the change in methane and carbon dioxide concentrations in the loop headspace, which was open to the soil surface. Between measurements, the chamber stayed open for 60 s to enable equilibration with atmospheric CH₄ and CO₂ concentrations. Gas fluxes were computed after a dead band time of 40 s after chamber closing applying a linear regression of the concentration data for each singular measurement, subsequently averaging the triplicate measurements. The standard deviation of the triplicate measurements is tabulated in supplementary data (Table S2).

Additional measurements at each site included soil moisture (SWC) and bulk conductivity measurements (EC) using a Stevens HydraProbe sensor with 6 cm long measuring rods. The sensor was not particularly calibrated for the organic (peat) rich soils at the study site and used the default “sand” settings for data evaluation. Thus, reported SWC and EC data in Table S2 are only indicative data. Since the short rods measured the temperature effectively directly below the soil surface with all potential bias to solar radiation, we applied an additional 25 cm long temperature probe (Omega, Type E) to better constrain soil temperatures.

2.4 Soil gas sampling and compositional analysis

Gas samples were acquired using soil gas probes. The probes are made of stainless steel with an outer diameter of 6 mm and inner diameter of 3 mm with a total length of 1.5 m. To prevent the probes from becoming blocked while pushing into the ground, a pin has been attached to the front of the probe. This pin remains in the ground after the desired depth is reached and the probe was lifted by a few cm. The lances are usually driven into ground with a moveable anvil. However, at the study site with soft unconsolidated sediments they could easily be pushed into the maximum depth of 1 m. Locations sampled are indicated in Fig. 2 and wherever methane emission was detected. Due to the shallow ground water table, the probes often had to be lifted close to the surface to be able to sample the gas phase of the vadose zone, thus giving a true indication of the actual
water level (sampling depths are listed in Table S1). A septum port is attached to the end of the probe, which allows sampling with a syringe. Before sampling, the dead volume of the soil gas probe was flushed twice with soil gas immediately after placement with a 20 mL syringe and then rested at least for 1 h to equilibrate. Afterwards 20 mL soil gas was extracted and stored in crimped vials pre-filled with saturated NaCl as sealing solution. Vials were stored upside down for further gas analysis in the laboratory.

Stored gas samples were analyzed in the lab using a Trace 1310 GC (Thermo Fischer Scientific, USA) equipped with a heated valve system and column switching. One milliliter of sample was then injected into the sample loops. The individual components were quantified in parallel on three channels.

On channel 1, pre-separation of hydrocarbons (C₁ through C₆) from a 500 µL sample was performed on a non-polar polysiloxane polymer column (Restek MX-1, 15 m, 0.28 mm ID, film thickness 3 µm). Molecular weight components >C₇ were back-flushed. Full separation was performed on the main 50 m Al₂O₃ capillary column (0.32 mm ID, film thickness 5 µm). Both columns were operated non-isothermally starting at 30 °C and ending at 180 °C. All components were detected on a Flame Ionization Detector (FID) with helium (He) as carrier gas.

On channel 2, the sample was injected via a 500 µL sample loop. CO₂ was separated from other components by a pre-column (30 m Hayesep Q, 0.53 mm ID, film thickness 20 µm) and directly detected after bypassing the Molsieve column on the thermal conductivity detector (TCD). All other components (Ne, H₂, Ar, O₂, N₂, CH₄, and CO) were chromatographically separated on the main analytical Molsieve column (80 m 5 Å, 0.53 mm ID, film thickness 50 µm). Carrier gas on this channel was He.

For better sensitivity for helium and hydrogen, these compounds were analyzed on a channel 3 with argon as carrier gas. The sample loop used had a volume of 125 µL. CO₂ and higher molecular weight carbon-components were retained and back-flushed on a packed pre-column (2 m Hayesep Q, mesh 100/120, 1 mm ID). Separation of He, Ne, H₂, O₂, and N₂ components was performed on a 5 Å packed molecular sieve column (3 m, mesh 80/100, 1 mm ID) and subsequently detected on a TCD.

2.5 Isotopic analysis of methane and carbon dioxide

For samples with concentrations (>200 ppm) carbon isotope signatures of CH₄ (δ¹³C-CH₄) and CO₂ (δ¹³C-CO₂) were determined after injecting into a continuous flow GC-IRMS system (Agilent GC coupled to a Thermo Fisher Scientific MAT 253 via a GC-Combustion interface II/III). The different compounds were separated on a 25 m Porapak column and methane was combusted to CO₂ at a temperature of 960 °C. Low concentration samples (2 – 200 ppm CH₄) were measured applying a cryo-focusing with liquid nitrogen of methane on a 1 m 1/16 packed column installed in an Agilent 6890 GC likewise coupled to a Thermo Fisher Scientific MAT 253 via a GC-Combustion interface II/III. Deuterium isotope signatures of methane (δ²H–CH₄) were determined by a similar GC-IRMS system (Trace GC and Isolink/ConFlow IV coupled to a MAT 253) if methane concentrations were above 2000 ppm. Methane was reduced to molecular H₂ at a temperature of 1420 °C. The reproducibility
for δ^{13}C is ± 0.3‰ and for δ^{2}H–CH₄ ± 3‰. δ^{13}C/δ^{12}C and δ^{2}H/δ^{1}H ratios are presented in the standard δ-notation versus the reference standards Pee Dee Belemnite (VPDB) and Standard Mean Ocean Water (VSMOW), respectively (Coplen, 2011).

2.6 Methane oxidation rates

In the field, shallow soil samples (down to 20 cm) were obtained using a stainless steel push core with an inner Plexiglas liner. Exact coordinates and sampling depth are listed in the supplementary data (Table S4). Deeper samples (40–100 cm) were retrieved with the help of an Edelman auger as a 20 cm composite sample. Samples were kept, transported, and stored at 4–7 °C until further processing. As next step, samples were homogenized and 5 g subsamples were collected and stored at −20 °C for DNA extraction. For determination of potential aerobic methane oxidation rates (MOx) each sampled was divided into seven aerobic incubations (100 mL vials), with ~10 g homogenized soil sample in each. Three parallels were incubated with 1% methane in the headspace, four without methane with one being autoclaved prior to incubation. Headspace methane and carbon dioxide concentration were determined regularly with a 610C gas chromatograph (SRI Instruments Europe GmbH, Bad Honnef, Germany) equipped with a flame ionization detector (FID) and a copper methanizer to convert CO₂ to CH₄. At the end of the incubations, active bottles were subsampled for DNA extraction again (s. section DNA extraction) and then the remaining sample dried at 80 °C to calculate soil water content.

2.7 DNA extraction

DNA was extracted from soil samples (~0.5 g) using the FastDNA SPIN kit for soil (MP Biomedicals, Illkirch, France). The extraction followed manufacturer’s instructions with modifications as previously described Webster et al. (2003): (1) the addition of 200 μg of poly(adenylic acid) (Roche Diagnostics International Ltd., Rotkreuz, Switzerland) prior to bead beating; (2) two bead beating steps of 45 s at 6.5 m s⁻¹ were performed on a FastPrep-24 system (MP Biomedicals); and (3) DNA was eluted in TE-buffer and quantified with the Quantifluor dsDNA chemistry using a Quantus fluorometer (Promega GmbH, Walldorf, Germany).

2.8 Sequencing bacterial and archaeal community via 16S rRNA genes

Following DNA extraction, samples were sequenced by Microsynth AG (Balgach, Switzerland) using MiSeq Illumina technology for microbial community analysis. Both Bacteria and Archaea were sequenced from the same DNA extractions and analyzed separately by targeting the 16S rRNA gene. For bacteria primer pair 515F / 806R (GTG CCA GCM GCC GCG GTAA; GG ACT ACH VGG GTW TCT AAT; Caporaso et al. 2011) and for archaea 340F / ARCH806R (CCC TAY GGG GYG CAS CAG; GGA CTA CVS GGG TAT CTA AT; Takai and Horikoshi 2000; Gantner et al. 2011) were used. Sequences will be deposited in the European Nucleotide Archive (ENA) and the accession number will be published in the final version of the manuscript. Sequences were processed following a bioinformatics pipeline (USEARCH, Edgar 2010; Cutadapt, Martin et al. 2017).
2011; MOTHUR, Schloss et al. 2009) previously described by Dohrmann and Krüger (2023) to generate zero-radius OTUs (ZOTUs, Edgar 2016). Potentially methanotrophic ZOTUs were identified according to the pmoA database taxonomy (Yang et al., 2016) and known methanotrophic genera (Knief, 2015, 2019 and references therein). Relative abundances of a methanotrophic genus or family were calculated as the share of all methanotrophic genera or families in the respective sample pool.

2.9 Quantification of methane oxidizing bacteria by pmoA-gene targeted quantitative PCR

Using a quantitative PCR (qPCR) assays to targeting both, general bacterial 16S rRNA gene and the pmoA gene encoding for the β subunit of the particulate methane monooxygenase expressed by methane oxidizing bacteria (MOB), we were able to determine the methanotrophic abundances.

The qPCR targeting the 16S rRNA gene (primer pair 341F/ 805R; forward: 5′-GTGCCAGCMGCGCCGGTTAA-3′, reverse: 5′-GACTACHVGGGTWTCTAAT-3′) was performed as described previously (Hedrich et al., 2016). The pmoA gene targeting qPCR (primer pair 189F/ mb661r; forward: 5′-GGNGACCGGGATTTCTGG-3′, reverse: 5′-CAGGMGCAACGTCTTACC-3′; Costello and Lidstrom 1999) was performed in a CFX Connect real-time PCR system (Bio-Rad, Hercules, CA) in a final volume of 10 µl, consisting of 5 µl 2x Luna Universal qPCR Master Mix (New England BioLabs GmbH, Frankfurt am Main, Germany), 0.7 µl forward and reverse primers each (10 µM), 0.5 µl bovine serum albumin (1 %), 1.1 µl nuclease-free water and 2 µl template DNA. The thermal profile consisted of an initial denaturation step at 95 °C for 5 min, 40 cycles of denaturation at 95 °C for 30 s, annealing at 62 °C for 30 s, elongation at 72 °C for 45 s, and an additional data acquisition step at 79 °C for 8 s, followed by final elongation at 72 °C for 5 min. The template DNA was used in five times or ten times dilution and spiked with the standard to a concentration of 10^5 copies per µl to correct for inhibition.

Standards consisted of a dilution series (10ⁱ – 10⁶ pmoA gene copies) of a PCR product flanking the pmoA gene of Methylomonas rhizoryzae GJ1 (Japan Collection of Microorganisms, JCM 33990) amplified with a designed primer pair (forward: 5′-GTACGCATACGCATGAACGC-3′, reverse: 5′-GTTTCCCGTGCGTTTGACTG-3′). The amplicon specificity was confirmed using a melt curve and agarose gel electrophoresis. Samples that did not show this specificity, i.e., Forest samples, were not considered to calculate pmoA abundances.
3 Results

3.1 Methane emission

To investigate methane emissions related to abandoned onshore wells eight cut and buried wells in the south-eastern part of the oil field Steimbke-Nord covering an area of ~ 0.2 km² (Figure 1) were targeted. Three wells (R-WA 272, R-WA 254, R-WA 264) are located in the western part of the area where active peat mining is ongoing. One well (R-WA 275), ~ 350 m to the east, is located on a meadow which is temporarily grazed and possibly fertilized with liquid manure. Before the peat extraction in the active area began, the Peat site was also an agricultural meadow that was probably regularly fertilized with manure. Two of the four wells from the Forest area (dominated by birch trees and pines) are located between the active Peat site and the Meadow (R-WA 273, R-WA 274), the remaining two in a larger forested area ~ 225 m to the north and northeast, respectively.

For these eight wells, we established four different reference sites R1 to R4 (Figure 1). The reference site R4 for the abandoned well on the Meadow was measured once and the two reference sites for the wells in the Forest area (R1 and R2) were each measured twice on consecutive days (Table 2). The single reference site for the three wells in the Peat area (R3) was thus surveyed three times within one week.

In total 64 out of 206 single measurement points showed methane emissions to the atmosphere, however, only the flux of 32 were higher than 1 nmol CH₄ m⁻² s⁻¹ and 31 of these were on the Peat site. The absolutely highest flux was 540 nmol CH₄ m⁻² s⁻¹ on the Peat site (position 16, site R-WA 264) and the lowest ~4.4 nmol CH₄ m⁻² s⁻¹ at the Forest site R-WA 273 position 14 (Table S2).

Thirteen out of 17 measuring points of the grid above well R-WA 275 on the Meadow and all corresponding reference measuring points were a methane sink (up to ~1.2 nmol CH₄ m⁻² s⁻¹). Only the four southernmost grid points at the well depict small methane emissions between 0.08 and 0.3 nmol CH₄ m⁻² s⁻¹.

All grid points of the wells R-WA 273 and R-WA 274 in the Forest between the Peat site and the Meadow were a methane sink ranging from ~4.4 to ~0.03 nmol CH₄ m⁻² s⁻¹. Only the reference site (R2.1 and R2.2) showed methane emissions on three grid points at the southwestern edge consecutively on both measuring day (~ 0.2 nmol CH₄ m⁻² s⁻¹, Table S2, Figure 1). Well sites and the reference site in the northern Forest do not reveal a pattern of methane emissions rates. A few grid points of the well areas (5 out of 34) revealed methane emissions with a singular value exceeding 1 nmol CH₄ m⁻² s⁻¹.

Results from the well sites and single reference site on the Peat area are more variable. At well R-WA 264 high methane emission rates have been determined at the southwestern corner including the highest flux rate of 540 nmol CH₄ m⁻² s⁻¹. The two nearest grid points to the west still revealed flux rate of ~ 30 nmol CH₄ m⁻² s⁻¹, the highest flux was 540 nmol CH₄ m⁻² s⁻¹, whereas all other points showed slightly positive to negative values. Four well grid points of R-WA 272 revealed high emission rates ranging from 40 to 160 nmol CH₄ m⁻² s⁻¹, the highest flux was 540 nmol CH₄ m⁻² s⁻¹, these are located at the northwestern part of the grid close to the reference grid ~ 50 m to the northwest. Similar to R-WA 264 all other points showed slightly positive to negative values. Two grid points of well R-WA 254 showed slightly elevated fluxes of ~7 nmol CH₄ m⁻²
s⁻¹, the remaining positive emissions being below 0.25 nmol CH₄ m⁻² s⁻¹ and all other measurements proving a strong methane sink, up to −2 nmol CH₄ m⁻² s⁻¹.

The reference grid on the Peat site showed always (on three different measuring campaigns) substantial positive methane emissions ranging from 15 to 380 nmol CH₄ m⁻² s⁻¹ but only at the northern and middle transect lines. The southern three point always represented a sink or the emissions were lower than 0.2 nmol CH₄ m⁻² s⁻¹.

As a simple first approximation, we averaged all measuring points of the individual well and reference grids (mean and median, Table 2). However, this should not be directly compared to more sophisticated emission techniques, e.g. long term eddy covariance studies, but rather as a snapshot of our study site for internal comparison of wells/references and different grounds (Forest, Meadow, Peat).

Mean and median values that are close to each other are typical for symmetrical distributions with minimal outliers. This holds for the data from the Forest and Meadow for both well and reference site (Table 2, Figure 3a, d). The data from the Peat site show means that are much higher than medians indicating positively skewed data i.e. outliers on the high end (compare histogram Figure 3g). However, as such outlier can control emissions of an area the mean is more suitable. On the other hand, the difference between these two indicate the huge variation in emissions at the Peat site. This is particularly evident at R-WA 264 with one grid point showing 560 nmol CH₄ m⁻² s⁻¹ and only two additional points with 30 nmol CH₄ m⁻² s⁻¹. All other 14 values are negligible small positive or representing a sink, thus the median of this grid is negative whereas the mean is positive (38 nmol CH₄ m⁻² s⁻¹).

Summarizing, all three well sampling grids, for which we observed overall methane emissions based on the mean values of 17 grid points covering an area of 900 m² around the well, were located in the Peat area. At wells R-WA 254 and R-WA 264 highly localized methane emissions with high flux rates occur. These singular grid points with high emissions are not spatially correlated with the well location. Moreover, averaged methane emissions (both mean and median) were even consistently higher at the Peat reference site than well sites in the Peat area (Table 1). All four Forest wells were a stronger sink than the corresponding reference sites at the day of measurement. The Forest’s acted as a higher methane sink than the Meadow site.
Table 2: Summary of the sampled oil well and reference sites.

<table>
<thead>
<tr>
<th>short name</th>
<th>date</th>
<th>area</th>
<th>CH4 flux [nmol m^{-2} s^{-1}]</th>
<th>mean soil CH4 [ppm]</th>
<th>mean δ^{13}C-CH4 [%]</th>
<th>mean δ^{2}H-CH4 [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-WA 211</td>
<td>09.03.2022</td>
<td>Forest</td>
<td>-0.47</td>
<td>1.4</td>
<td>-51</td>
<td>-49.6</td>
</tr>
<tr>
<td>R-WA 209</td>
<td>10.03.2022</td>
<td>Forest</td>
<td>-0.35</td>
<td>1.6</td>
<td>-56.3</td>
<td>-49.6</td>
</tr>
<tr>
<td>R-WA 273</td>
<td>30.03.2022</td>
<td>Forest</td>
<td>-1.31</td>
<td>1.4</td>
<td>-48.3</td>
<td>-56.1</td>
</tr>
<tr>
<td>R-WA 274</td>
<td>31.03.2022</td>
<td>Forest</td>
<td>-1.41</td>
<td>20.3</td>
<td>-61</td>
<td>-61</td>
</tr>
<tr>
<td>R-WA 275</td>
<td>21.04.2022</td>
<td>Meadow</td>
<td>-0.2</td>
<td>3,695</td>
<td>-85.4</td>
<td>-222.8</td>
</tr>
<tr>
<td>R-WA 272</td>
<td>20.04.2022</td>
<td>Peat</td>
<td>25.38</td>
<td>376,918</td>
<td>-58.4</td>
<td>-338</td>
</tr>
<tr>
<td>R-WA 254</td>
<td>27.04.2022</td>
<td>Peat</td>
<td>0.25</td>
<td>286,312</td>
<td>-66.1</td>
<td>-332.1</td>
</tr>
<tr>
<td>R-WA 264</td>
<td>28.04.2022</td>
<td>Peat</td>
<td>37.56</td>
<td>537,317</td>
<td>-64</td>
<td>-314.1</td>
</tr>
</tbody>
</table>

R1.1 09.03.2022 Forest -0.12 -0.09 2.1 -49.6
R1.2 10.03.2022 Forest -0.08 -0.05 2.1 -49.6
R2.1 30.03.2022 Forest -0.76 -0.87 5.2 -56.1
R2.2 31.03.2022 Forest -0.51 -0.43 6.7 -58
R4 21.04.2022 Meadow -0.1 -0.1 4,467 -99.1 -181.8
R3.1 20.04.2022 Peat 50.07 15.42 181,802 -64.9 -306.9
R3.2 27.04.2022 Peat 0.25 -0.08 286,312 -66.1 -332.1
R3.3 28.04.2022 Peat 109.03 55.79 369,909 -63.1 -316.3
R3.1 20.04.2022 Peat 37.56 -0.05 537,317 -64 -314.1
R3.2 27.04.2022 Peat 50.5 20.91 290,555 -65.9 -304.1
Figure 3: Methane flux (a, d, g), soil gas methane concentration (b, e, h), and potential methane oxidation rates (MOx; c, f, i) depicted as histograms for well (blue) and reference sites (yellow) at the three areas forest (a, b, c), meadow (d, e, f), and peat extraction site (g, h, i). The red line in a, d, g indicates zero flux, sites left of the line acted as net methane sinks and at the right as net methane sources.
Figure 4: Depth profiles of $O_2$ (a), $CH_4$ (b), $CO_2$ (c), and $N_2$ (d) soil gas concentrations, as well as $\delta^{13}C$-$CH_4$ (e) and $\delta^{13}C$-$CO_2$ (f) values for Forest (brown diamonds), Meadow (light green squares) and Peat (dark grey circles) sites. Note the logarithmic scales in a to f. Isotopic composition of methane (d) and carbon dioxide (e) is depicted as difference to the Vienna Pee Dee Belemnite (VPDB) standard.
3.2 Soil gas geochemistry

Soil gas samples were taken from up to 95 cm depth and analyzed in the laboratory for gas compositions including gaseous hydrocarbons (C₁–C₆) as well as carbon and hydrogen isotopic composition if possible. Depths of the soil gas sampling differed and were determined by the groundwater table. Generally, the sampling depth was closely above the groundwater and is, thus, an indirect measure of the lowest interval of the vadose zone at the time of sampling. The soil methane concentrations between the sampled areas were clearly distinct, with Forest soils showing the lowest methane concentrations compared to Meadow and Peat (extraction site) soil gases (Figure 3b, e, h and 4b). The majority of methane concentrations at the Forest sites were around or below atmospheric concentrations (Table S1), however, two samples had with ~93 ppm and ~64 ppm elevated methane concentrations. These Forest sites did not emit substantial amounts of methane (Table 1). The overall mean for samples from Forest soil was ~7.5 ppm methane (Table S1), the median however was ~2.1 ppm, which corresponds to atmospheric concentrations. Soil methane concentrations in samples from the nearby Meadow site started at ~1.8 ppm and reached up to 9,200 ppm. The respective mean methane concentration was ~1,960 ppm and the median ~710 ppm. Soil gas samples from the Peat (extraction site) showed both, the highest overall concentration with nearly 65% methane (~645,000 ppm) and with ~315,000 ppm (mean) and 282,000 ppm (median) the highest mean and median concentration, respectively. The general differences in the soil gas composition between the three sampling areas becomes also clear from the plot of all data on O₂, CH₄, CO₂, and N₂ concentrations with depth (Figure 4a–d).

We also analyzed the δ¹³C-CO₂, δ¹³C-CH₄, and δ²H-CH₄ for most samples (Table S1). Methane concentrations in the Forest soil were, however, too low to determine δ²H-CH₄. As for δ¹³C-CO₂, isotopic compositions of Forest and Meadow soil gases were similar, ranging both between ~21.7‰ and ~24.9‰, with means of ~23.3‰ and ~23.5‰, respectively. Soil gases from the Peat site, on the contrary, were much more ¹³C-enriched with δ¹³C values up to ~1.8‰ and a mean of ~11.6‰. Thus, while for the Forest and Meadow area δ¹³C-CO₂ in the soil gas was relatively uniform and typical for common soil gas, variations at the Peat extraction sites were high, indicative for different controls on soil CO₂ in this area (Figure 4f). The δ¹³C-CH₄ signatures differed between all three areas with the methane in the Meadow soil being most ¹³C-depleted with a mean δ¹³C value of ~86.6‰ (~104.8‰ to ~49.5‰), in the Forest soil of ~57.4‰ (~84.1‰ to 46.4‰), and in Peat soil of ~63.8‰ (~79‰ to ~52.2‰; see also Figure 4e). The mean hydrogen isotopic composition of methane differed strongly between the Meadow and Peat soil gases with δ²H-CH₄ of ~270‰ and ~320‰, respectively (Table S1). All isotope data from the reference and well sites did not show any relevant differences.

3.3 Methane oxidation rates

Methane oxidation rates were determined to investigate the soils’ potential to mitigate methane emissions. In total 27 positions were sampled in up to two depths, resulting in 46 methane oxidation rates. Following the incubations, the potential oxidation rates were determined per gram dry soil. In addition, we calculated methane oxidation rates for a square meter of wet soil with
20 cm height, which was the maximum aggregated depth for a homogenized soil sample. This way, we got interpolated methane oxidation rates for the sampled soil column that are considered better comparable with measured and published methane fluxes (e.g. calculated per well).

Mean methane oxidation rates per g dry soil (Table 3) were lowest for Forest soils (~ 0.04 nmol g\(^{-1}\) s\(^{-1}\)) and highest for soils from the Peat sites (~18.3 nmol g\(^{-1}\) s\(^{-1}\)) with intermediate values for Meadow soils (1.4 nmol g\(^{-1}\) s\(^{-1}\)). Potential methane oxidation rates per g wet soil sample (single measurements: Table S4) ranged between 2 nmol 0.2 m\(^3\) s\(^{-1}\) and ~266 nmol 0.2 m\(^3\) s\(^{-1}\) in Forest soils, ~11 nmol 0.2 m\(^3\) s\(^{-1}\) and ~8383 nmol 0.2 m\(^3\) s\(^{-1}\) at the Meadow, and ~81 nmol 0.2 m\(^3\) s\(^{-1}\) and ~150,000 nmol 0.2 m\(^3\) s\(^{-1}\) at the industrial Peat site. The respective mean oxidation rates per g wet soil sample (Table 3) for the three studied areas increased from ~47 nmol 0.2 m\(^3\) s\(^{-1}\) (Forest) over ~3100 nmol 0.2 m\(^3\) s\(^{-1}\) (Meadow) to ~14,100 nmol 0.2 m\(^3\) s\(^{-1}\) (Peat extraction site). Mean dry MOx are listed in Table 3.

<table>
<thead>
<tr>
<th>MOX dry [nmol CH(_4) g(^{-1}) s(^{-1})]</th>
<th>MOX dry [nmol CH(_4) 0.2 m(^3) s(^{-1})]</th>
<th>MOX wet [nmol CH(_4) 0.2 m(^3) s(^{-1})]</th>
<th>16S rRNA gene [10(^9) g(^{-1}) dry wt.]</th>
<th>pmoA [10(^9) g(^{-1}) dry wt.]</th>
<th>pmoA abundance [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forest</td>
<td>0.04</td>
<td>85</td>
<td>47</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Meadow</td>
<td>1.4</td>
<td>2475</td>
<td>3106</td>
<td>16</td>
<td>30</td>
</tr>
<tr>
<td>Peat</td>
<td>18.3</td>
<td>18199</td>
<td>14114</td>
<td>4.6</td>
<td>14</td>
</tr>
</tbody>
</table>

For a selected experiment on the methane turnover in the Peat area the carbon isotopic fractionation of methane during aerobic methane oxidation was determined in the laboratory (see supplement S2). Using a calculation from (Feisthauer et al., 2011) this resulted in an epsilon (ε) of ~31.3‰ (Table S2).

3.4 MOB abundance and identification

We determined MOB abundances by targeting both, the general 16S rRNA gene and \(pmoA\) gene using qPCR (Table 3). The Peat sites had with ~4.6 x 10\(^9\) copies g\(^{-1}\) dry weight about three times lower 16S RNA gene copies than the other two sites with 1.3 x 10\(^10\) (Forest) and 1.6 x 10\(^10\) copies g\(^{-1}\) dry weight. The \(pmoA\) abundances were similar at Meadow and Peat site, with 3.0 x 10\(^7\) and 1.4 x 10\(^7\) copies g\(^{-1}\) dry weight, respectively. The relative abundance of the \(pmoA\) was highest in the Peat (~0.30%) reaching up to 0.89%, followed by the Meadow (0.19%). However, there were huge differences between the samples in each area (Table S5).

We used DNA-based microbial analyses to identify changes in bacterial community over depth and identify potential methanotrophic key player. Bacterial 16S rRNA gene sequencing revealed between ~1.5 x 10\(^4\) and ~1.35 x 10\(^5\) sequences per sample with a median of ~8.5 x 10\(^4\) sequences and a mean library coverage C of >98.5% (data not shown). In total ~22 x 10\(^4\)
ZOTUs were determined. A comparison on genus level with published taxa known to contain the pmo operon sequences resulted in up to 151 potential methanotrophic ZOTUs, grouping into 15 methanotrophic genera and 5 families (Table S6). The most abundant putative methanotrophic family in amplicon libraries was *Methylacidiphilaceae*, with 71 uncultured ZOTUs followed by *Beijerinckiacaeae*. The most abundant genera were *Methylocystis* and the uncultured cluster SH765B-TzT-35 from the *Methylomirabilaceae* family (hereafter referred to as SH765B-TzT-35). In the following, we grouped the ZOTUs belonging to the same genera together in order to simplify the dataset and make changes between the areas better visible.

Most reads affiliating with known methanotrophic taxa reads were found at the Peat site, whereas, Forest and Meadow had about half as much reads. In Forest samples, most of such reads were found in the top layer. On the contrary, they increased with depth for the Meadow site until a depth of 8–13 cm and 15–20 cm at the Peat site and decreased afterwards in both cases slightly (Table S6). The top layer at Forest and Meadow sites was with regard to methanotrophic taxa dominated by an uncultured *Methylacidiphilaceae* genus, which relative share in reads decreased with depth but was still the most abundant genus (Figure 5). A member of the genus *Methylocystis*, however, dominated the peat site, its relative abundance first increased to a depth of 20 cm and the abruptly declined at a depth of more than 40 cm. In samples of 40 cm and below SH765B-TzT-35 dominated the methanotrophic community (Figure 5).

In addition to bacterial 16S RNA gene sequencing, we used archaeal primer to identify methanogenic key player. Sequencing resulted into ~9.3 x 10^3 and ~1.2 x 10^5 reads per sample with a coverage of >99.9% (data not shown). Overall, 798 ZOTU were identified and a comparison with known methanogenic genera revealed 132 potential methanogenic ZOTU (Table S7). These could be grouped into 11 genera and 9 families (Figure 5). The most abundant genera were *Methanosarcina*, followed by *Methanoregula*, which was almost exclusive present in Peat samples, and third *Methanosaeta*. Together with *Methanobacterium* they account for 96% of methanogenic reads over all samples.
Figure 5: a) Relative abundance of potential methanotrophic genera estimated at three depth intervals detected in the Forest, Meadow, and Peat areas. b) Potential methanogenic genera detected at the three areas Forest, Meadow, and Peat depicted as relative abundance. Reads are displayed as relation to the sum of all reads associated with methanotrophic taxa in the respective sample pool.
4 Discussion

4.1 Evaluation of the methodological approach

Abandoned wells in Germany are generally decommissioned and buried (Landesamt für Bergbau, Energie und Geologie (LBEG), 1998). This includes plugging and backfilling of the well, cutting, and removing of the shallow casings, and reconditioning of the area (e.g. for agricultural use). Hence, it is not possible to use the same methods to detect methane from such wells as for wells with visible surface installations, like partly in the US and Canada (Williams et al. 2021, Lebel et al. 2020). As Schout et al. (2019) pointed out, gas leakage of buried wells maybe easily missed by surface measurements alone. In a study with a strategy comparable to ours, Schout et al. (2019) studied potentially leaking (buried) wells in the Netherlands. While different in some aspects, we, however, also used a tandem approach to detect both methane emissions and methane concentrations in the soil gas closer to the buried wells. The combination of both methods is necessary, as high soil gas concentrations did not necessarily correspond to high methane emissions at the same spot (Table S1, S2). Probably due to the high methane oxidation potential of soils in the presence of methanotrophs, as shown previously (Kolb and Horn, 2012; Ho et al., 2019; Guerrero-Cruz et al., 2021). In our case, even measuring points with soil methane concentrations of ~45% of biogenic methane at 20 cm depth, e.g., site WA-264, position 2, were a methane sink at the surface (Table S1, S2). A similar situation was also observed by Schout et al. (2019), who were unable to detect any methane emissions into the atmosphere above a leaking borehole that was detectable at a depth of 2 meters below the soil surface.

Differences between the areas in our study were more pronounced in soil methane concentrations than in methane emissions. These emissions on the other hand, tended to change from source to sink between two measuring points and, thus, on short distances and eventually over time. We therefore conducted a second sampling campaign at the Peat extraction reference site with flux measurements only one meter or less apart to better understand variations on a smaller scale than that usually chosen in our study (10 x 10 m). The transect was chosen to pass through a point with high emissions (Figure 6). The resulting methane fluxes varied more than two orders of magnitude over the distance of less than one meter, whereas CO2 emissions showed fewer changes and varied in total only by a factor of ~2. This displays the high spatial heterogeneity of the methane emissions and is in agreement with other soil studies (Davidson et al., 2002; Savage et al., 2014; Ambus and Christensen, 1995; Le Mer and Roger, 2001). To address temporal variation, we revisited reference sites in the Forest and Peat up to three times (Figure 1). The overall flux-pattern at the Peat site changed from one week to another (Figure S4b, d, f) and fluxes at the same spot differed in part greatly (Table 4), whereas fluxes of two consecutive days differed less. However, soil methane concentration did not vary as much (Table S1). Compared to methane, CO2 fluxes at the same spots were much more stable and did not show a time dependent variation (Table 4). This temporal data and other data above underline the importance of the use of individual reference measurements. In addition, we propose with regard to our results that a single measurement is not sufficient to evaluate background emissions properly. Both, spatial and temporal variability could be explained by changes in soil compaction (Flechard et al., 2007), differences in moisture content (Basiliko et al., 2007), fluctuating macropores (Schwen et
al., 2015), differing floras (Jentzsch et al., 2024) and microforms (Welpelo et al., 2024) and fauna (Lubbers et al., 2013). Furthermore, occurrence of precipitation and air pressure variations between two consecutive measurements could result in different emission pattern and rates as well (Blagodatsky and Smith, 2012).

Despite the high spatial variation of methane fluxes, we are confident to detect relevant leakage from a well with our strategy due to (1) the combination of flux and soil gas measurements as well as (2) relying on a 17-point grid instead of single measurements. For the grid, we used a distance of 10 m from point to point and ~7 m to the position above the wells at the center of the grid (Figure 2a). These distances are in between the ones used by Sechman (2022) and Schout et al. (2019) who used similar methodical approaches to evaluate the well integrity of buried petroleum and gas wells, respectively.

Considering the main conclusions from our methodological approach, particularly at Peat sites, high methane concentration in addition to methane flux at a well or close-by site does not automatically imply a leaking well, as the methane can also origin from shallow methanogenesis. Thus, we determined the methane’s isotopic composition δ^{13}C-CH_{4} and δ^{2}H-CH_{4} to distinguish between thermogenic (in our case oil-associated) and biogenic methane emissions (Whiticar, 1999; Milkov and Etiope, 2018).

Furthermore, we included measurements of all parameters at reference sites to determine the natural biogeochemical background. This approach (see below) helps to get information on whether migration of shallow biogenic methane along the well takes place (e.g., Vielstädte et al., 2015; 2017) or whether natural biogenic methane sources and processes are responsible for methane fluxes.

**Table 4:** Flux measurements at the Peat reference site (ref.) at the exact same coordinates at different time points.

<table>
<thead>
<tr>
<th>grid-position</th>
<th>CH_{4} [nmol m^{-2} s^{-1}]</th>
<th>CO_{2} [µmol m^{-2} s^{-1}]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ref. 3.1 20.04.2022</td>
<td>ref. 3.2 27.04.2022</td>
</tr>
<tr>
<td>1</td>
<td>273.80</td>
<td>190.43</td>
</tr>
<tr>
<td>2</td>
<td>15.42</td>
<td>381.75</td>
</tr>
<tr>
<td>3</td>
<td>0.21</td>
<td>–0.37</td>
</tr>
<tr>
<td>4</td>
<td>–0.19</td>
<td>–0.28</td>
</tr>
<tr>
<td>5</td>
<td>0.02</td>
<td>0.13</td>
</tr>
<tr>
<td>6</td>
<td>31.19</td>
<td>201.39</td>
</tr>
<tr>
<td>7</td>
<td>1.16</td>
<td>55.79</td>
</tr>
<tr>
<td>8</td>
<td>96.14</td>
<td>41.06</td>
</tr>
<tr>
<td>9</td>
<td>32.89</td>
<td>111.38</td>
</tr>
</tbody>
</table>
Figure 6: Methane (blue) and CO\textsubscript{2} (red) fluxes on a meter scale over a 12 m transect at the peat reference site. The fluxes were measured over the course of 3 h. Data is listed in Table S3.

### 4.2 Assessing contribution of abandoned wells to the methane emissions in the studied areas

Using our gas geochemical approach, we could identify three well sites and their respective reference measurements with net methane emissions (Table 1), all of which were located at the Peat site. The first indication that the methane emissions were not well-related was that, the reference site emitted more methane than the corresponding well sites (Table 1). All soil gases contained >5% methane with a median of ~35% with no recognizable trend between sites (Table S1). The use of the combination of isotope data on carbon and hydrogen in methane is an established method to identify the methane’s source (Whiticar, 1999; Schoell, 1980). Thermogenic gases, which are produced during the maturation of organic material and which occur in natural gases and oil-associated, are characterized by relatively high $\delta^{13}$C values ($\sim -50\%$). In combination with $\delta^2$H values of the methane, thermogenic origins (natural gas or oil-associated) can be well recognized in $\delta^{13}$C/$\delta^2$H diagrams (Figure 7a). None of the isotopically analyzed methane samples from Steimbke showed a signature for thermogenic methane. This excludes the ebullition of relevant amounts of natural gases from the oil reservoir to the atmosphere or upper soils. Further and supporting this conclusion, oil-associated gases and natural gas contain ethane and other higher hydrocarbons, which were also not found in the analyzed gases (Table S1).
Methane concentrations were not sufficient for δ²H analyses in all gas samples, so that the following conclusion does not necessarily hold for low concentrated samples. Our data show that other, distinct biogenic, sources for the methane are likely: methanogenesis using acetate (methyl fermentation; acetoclastic) or CO₂-reduction (Figure 7b). While our approach cannot exclude well integrity problems in general, our data argue against methane leakage into the upper soil and/or atmosphere from the reservoir for the studied eight wells in the Steimbke-Nord oil field. Furthermore, the high methane emissions at both, well and reference sites, argues against the migration of shallow biogenic methane along the wells (methane concentrations were not higher in the well grid than in the reference grid samples).

Another previous proposed test for well leakage from underground CO₂-storage sites (Romanak et al., 2017; Romanak et al., 2014) focusses on soil gas composition. The authors argue that oxygen and carbon dioxide concentrations in soil gases, driven by normal microbial respiration, should sum-up to around 21% (Romanak et al., 2012). We observed this in the Forest soil gases (Figure 7c). Single Forest measurements and the majority of the Meadow followed, however, a conversion of 2:1 oxygen to CO₂, which corresponds to the stoichiometry of aerobic methane oxidation (Romanak et al. 2012; and references therein). The Peat soil gas compositions spread between both processes and conversions. About half of the samples, however, were enriched in CO₂ (up to 33%). According to Romanak et al. (2012), this indicates an addition of CO₂ or oxidation of exogenous CH₄. The relation of CO₂ to N₂/O₂ is another indication of methane oxidation at the meadow site, however Peat samples indicated excessive CO₂, too (Figure 7e). Following this narrative, only soil gases at the Peat sites were depleted in N₂ (Figure 7f), which indicates leakage or addition of another deeper gas source displacing the atmospheric nitrogen (Romanak et al., 2012). These findings would point to leakage from the abandoned wells at Steimbke, however as we demonstrated above, isotopic compositions as well as the lack of ethane and propane excludes a thermogenic gas source questioning the possibility to use the model for the interpretation of our study site with a highly active methane cycle. In our view, the drastically increased CO₂ levels in soil gases could be best explained by an extensive microbial degradation of peat via acetate by methanogenesis, which releases methane and CO₂. This is supported by the Peat’s high methane and CO₂ concentrations (Figure 7d). This methane is than oxidized by MOB to CO₂, which further complicates the soil gas interpretation. In addition, if methane oxidation would be the sole source for the excessive CO₂, this should be visible in the isotopic signature of methane. However, the in the laboratory determined fractionation factor would resulted in the more enriched δ¹³C CH₄ and lighter δ¹³C CO₂. δ¹³C CO₂ was in fact, however, even heavier at the Peat site, which contradicts the interpretation following Romanak et al 2012. While the soil gas approach by Romanak et al. (2014, 2017) would suggest CO₂ or CH₄ leakage, we were able to disprove this hypothesis in our case. Furthermore, we used this data to look into the apparent differences in methane cycling between the three sites, which will be discussed in the following.
4.3 Natural methane-cycling at the study sites

In natural environments, methane emissions are the result of the interplay between production and consumption, and the biotic regulation of emissions can occur at the methanogenic and methanotrophic side. Regarding methane production, previous studies discussed these possible factors to control methanogenesis (1) availability of acetate due to acetate-oxidizing bacteria outcompeting CO₂-reducing methanogens (Kotsyurbenko, 2005), (2) phenolic compound concentrations which might limit peat degradation in the Forest and Meadow sites (Freeman et al., 2001), (3) and temperature (Brauer et al., 2006). We consider one or more of these differences also likely to hold for our studied Peat and Meadow areas with the likely different predominating methanogenic pathways. Our genetic analysis of the methanogenic community suggests a higher methanogenic potential at the Peat site (Table S7). Methanosarcina and Methanosaeta are known acetoclastic methanogens whereas Methanoregula and Methanobacterium are hydrogenotrophic methanogens (Conrad, 2020). At all sites both acetoclastic and hydrogenotrophic methanogens were present (Figure 5). Soil temperatures were similar at the point of sampling with ~10 °C.
which supported the growth of both acetoclastic and hydrogenotrophic methanogens. The isotopic data shown in Fig. 7a, b underline differences between the sites and suggest that methane was produced via two methanogenic pathways. Namely, the methane at the Peat site is mostly derived from acetate whereas CO₂ reduction is the main methanogenesis pathway at the Meadow site. This is underlined by the higher mean δ¹³C-CO₂ in Peat soil gases (−12‰) compared to soil gases from Meadow and Forest sites (−23.5‰). One explanation for the site dependent dominant methanogenic pathway could be the differences in peat degradation progression due to the removal of vegetation for peat extraction. The drainage of peatlands is known to lead to decomposition of peat and results in substantial losses of carbon (Couwenberg, 2011). This may in part explain the higher methane emissions from the active peat extraction site as the drainage of the whole investigate area started already decades ago. However, only recently (starting 2017/18) the peat extraction started at this site. Thus, we expect that the decomposition of deeper peat layers and the remaining peat intensified after the start of the extraction. Furthermore, about 1 m of peat was extracted from the whole area used for extraction. In addition, this led as we observed to a higher water table, which is one of the main factor for higher methane emission (Abdalla et al., 2016) as it limits oxygen penetration into deeper layers (Basiliko et al., 2007). For our gas geochemical study and related sampling strategy, however, a respective in-depth understanding of the drivers for the individual methane-formation pathways was beyond the scope of our study and thus we will focus on the microbial methane filter towards the atmosphere in the following.

In the results we point out, that the Forest sites were characterized as a methane sink with soil methane concentrations at atmospheric levels with one sample at ~100 ppm. In addition, Forest soils showed only minor laboratory methane oxidation rates, one explanation for this could be that the present MOB are specialized for low methane concentrations (Bengtson et al., 2009; Kolb, 2009). The Peat sites on the other hand, showed locally prominent methane fluxes to the atmosphere and methane soil gas concentrations between 1% and 65%. Methane oxidation rates were moderate except for the highest measured rate of 150 μmol s⁻¹ g⁻¹ (wet soil). The Meadow sites acted as net methane sink, soil methane concentrations were mostly above the atmospheric concentration but below 1%, and oxidation rates were moderate. Methane oxidation rates at both Peat and Meadow sites were, thus, higher than at the Forest site, which coincides with MOB abundance (Table 3). In addition, the higher methane concentrations at the Peat site could enable the growth of low affinity methanotrophs (Christiansen et al., 2014). These methanotrophs require higher methane concentrations (>100 ppm) but are characterized by higher Michaelis–Menten kinetics (Whiticar 2020, and references therein).

The community analysis revealed a shift from uncultured Methylacidiphilaceae at the Forest to members of the Methylocystis genus in Peat samples with an additional increase in abundance of Sh765B-TzT-35 (Figure 5). The genus Sh765B-TzT-35 thereby increased with depth and was most abundant in the likely anaerobic layers in the peat at a depth of 40 cm or more.

Other members of the Methylomirabilaceae family are known to oxidize methane under anaerobic conditions by internal oxygen production from nitrite reduction to dinitrogen (Ettwig et al., 2010; Versantvoort et al., 2018). Although this was so far not shown for members of Sh765B-TzT-35, a previous study showed their ability to anaerobically oxidize methane (Nakamura, 2019). Our phylogenic data suggests that members of the Methylacidiphilaceae family promote atmospheric methane oxidation whereas Methylocystis, Methylobacter, and Sh765B-TzT-35 oxidize ascending methane. Kaupper et al.
(2021) who compared pristine and restored peatlands previously observed a similar shift from *Methylocystis*. Simplified, one can content that the microbial community at our studied Forest site was similar to that of a pristine peatland. The active peat extraction site, on the other hand, showed similarity to the restored site in Kaupper et al. (2021). This indicates that starting with peat drainage the methanotrophic community shifts but remains active throughout the extraction process. However, it takes the microbial community decades to restore pristine-like diversity and complexity (Kaupper et al., 2021). Geochemical and molecular microbial work also underline the differences between all sites. Our phylogeny analysis were supported by phospholipid fatty acid (PLFA) analyses of selected samples from the Peat site, which indicate that *Methylocystis heyeri*, a Type II (α-Proteobacteria), was likely involved in methane oxidation at these sites (Figure S1). We also found these PLFA in incubation samples and observed a significant increase after methane addition. Methane oxidation rates were highest in samples with elevated soil methane concentrations (>4000 ppm), which is in concordance with previous studies (Basiliko et al., 2007; Moore and Dalva, 1997). It was previously shown, that in addition to substrate availability (here methane concentration), the methanotrophic community can be influenced by physico-chemical parameters and land use (Kaupper et al., 2022) references therein. Kaupper et al. (2022) showed that the environmental parameters, total C and N content, and electrical conductivity, indicative of salinity, affected the active bacterial community. This suggests that the methanotrophic communities can adapt to different methane regimes and, as speculation, could mitigate a potential leakage over time.

Converting our values for mean methane emissions to enable the comparison with literature data, we observed an emission rate of ~23 g m⁻² yr⁻¹ for the Peat sites. These numbers are in our case without emissions from ditches, which Sundh et al. (2000) showed can be substantial. An in-depth study on the influence of vegetation on methane emission conducted by Welpelo et al. (2024) at a rewetted peat site about 3 km north-west from our study area, estimated yearly emission between 7.1 and 36.1 g m⁻² year⁻¹. As our field campaign was conducted in April 2022, and we observed comparable methane emissions to their combination of measurement and modeling for the same season, our yearly estimation seems plausible, although, the Peat’s water table was comparably lower. The emissions at the Peat site’s (our study) were about twice (Strack et al., 2016) to more than hundredfold (Wilson et al., 2016) higher than from pristine peat sites and about tenfold from a restored peatland (Strack et al., 2014). Since carbon dioxide emissions were not elevated, one can assume that the Peat site acted emission wise more like a wetland without vegetation than a drained peatland. In addition, it is possible that the progressed peat extraction provided a different type and quality of organic precursor substrates than the Forest and Meadow sites as suggested from and observed in other peat sites (Alstad and Whiticar, 2011). Our data suggest that active peat extraction sites do not necessarily emit less methane than rewetted ones as stated in literature (Welpelo et al., 2024; Bieniada and Strack, 2021; Rankin et al., 2018; Abdalla et al., 2016) and that these areas can be significant methane sources.

Overall, we conclude that there is no connection between the methane emissions detected and the abandoned wells investigated. Furthermore, the factors discussed above suggest that the level of disturbance can be considered as the major driving force for the here shown methane emissions. Thus, the anthropogenic influences play a key-role for methane formation and emission in such altered ecosystems.
5 Conclusion

In the worldwide efforts to mitigate anthropogenic methane emissions, which are a key factor for climate change, a comprehensive approach is needed. One of these sectors is the oil & gas industry and although research targeting abandoned wells developed momentum, financial resources to backfill additional wells are limited (Raimi et al., 2021) and progression advances slowly. Especially orphaned and abandoned wells in the USA and Canada are an active area of research as they are mostly not properly decommissioned and thus prone to leakage. In several studies, high methane emissions (several tons per year) from such wells were observed (i.e., Boutot et al., 2022; Bowman et al., 2023; Hachem & Kang, 2023). Plugged and buried abandoned wells on the other hand were so far poorly studied, and data on location and history of these wells is often limited. In this study, we demonstrate a methodological approach to survey such cut and buried abandoned wells to unravel the methane emissions origin in a complex peat rich setting with a distinctive methane cycle containing several abandoned wells. The approach combined methane flux measurements spanning an area of 30 x 30 m around the well location and a 20 x 20 reference area with the characterization of soil gas samples, and determination of the methane’s isotopic composition, respectively. In total, we sampled eight well site, out of which three showed net methane emissions to the atmosphere, all located in an active peat extraction site. However, similar methane abundances at related reference site as well as soil gas and isotopic composition revealed a biogenic origin, thus confirming that the surveilled abandoned wells did not emit methane to the upper soil and atmosphere originating. The methane emission patterns exhibited a substantial spatial variability. Methane concentrations in the soil gas on the other hand were much more homogenous. Subsequent microbial analyses showed substantial methane oxidation capacities at the Peat site. In combination with phylogenetic data, we suggest that established methanotrophic communities act as an efficient aerobic methane filter and may pose as a potential barrier for small leakages. However, further research is necessary to determine their mitigation potential and respective work is ongoing.

It remains unclear to what extent natural microbial oxidation capacities for methane could degrade methane in the ground from theoretical leaks in the event of a broken well. However, such processes could be highly relevant for Germany, as 15 % of abandoned wells in Germany are located in areas with highly organic-rich soils such as peat (mostly in Northern Germany). Furthermore, our data showed mean methanotrophy capacities of (wet) peat samples in our lab up to ~14,000 nmol CH₄ 0.2 m⁻³ s⁻¹ (Table 3; = 0.8 g 0.2 m⁻³ h⁻¹). To put this in perspective, methane leakage rates from plugged wells in two regions in Canada ranged between 0.04 to 1 g CH₄ well⁻¹ h⁻¹ (Bowman et al., 2023).

Exclusively emission-based approaches, such as the use of emission chambers, survey cars, specialized cameras etc., are not suited for buried wells as they would be susceptible to the misinterpretation of natural methane emissions. For a conclusive surveillance of cut and buried abandoned wells, the here presented multilayered strategy determining methane emissions, soil gas composition and isotopic signatures, ideally together with microbiological techniques in comparison with carefully selected reference sites is necessary.
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7 Data availability

Measured and derived data supporting the findings of this study are available in the supplementary data sheet.

8 Author contributions

M.B., S.S., S.F.A.J., and M.K. conceived and designed the experiments. S.F.A.J., S.S., M.B., M.K. conducted the fieldwork and performed the experiments. S.F.A.J. and T.H. performed qPCR and processed the data. Main data interpretation was performed by S.F.A.J. in cooperation with the co-authors. S.F.A.J. wrote the main manuscript text with input from M.B., S.S., M.K., T.H., M.A.H. All authors read and approved the final version of the manuscript.

9 Competing interests

The authors declare no competing interests.
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