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Peat Particulate Organic Matter Accepts Electrons During In Situ Incubation in the Anoxic Subsurface of Ombrotrophic Bogs

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Key Points:

- Oxidized reference peat particulate organic matter (POM) was reduced in the anoxic subsurface of three ombrotrophic bogs over 1 year
- On average, 160 µmol electrons were transferred per gram reference POM across the three bogs
- The reduced POM was partially re-oxidized by dissolved oxygen, supporting that POM is a sustainable terminal electron acceptor

Supporting Information:

Supporting Information may be found in the online version of this article.

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Abstract Peat particulate organic matter (POM) in the anoxic subsurface of peatlands is increasingly recognized as an important terminal electron acceptor (TEA) in anaerobic respiration. While POM reduction has been demonstrated in laboratory peat-soil incubations and (electro-) chemical reduction assays, direct demonstration of POM reduction in peat soils under in situ, field conditions involving quantification of transferred electrons remain missing. Herein, we demonstrate that deployment of an oxidized reference POM in the anoxic, methanogenic subsurface of three ombrotrophic bogs, followed by one year incubation, resulted in the transfer of approximately 150–170 µmol of electrons per gram POM to the deployed reference POM. The capacity of this reduced POM to accept electrons was partially restored upon subsequent exposure to dissolved oxygen. These findings provide direct evidence for POM acting as regenerable and sustainable TEA for anaerobic respiration in temporarily anoxic parts of peat soils. Based on the number of electrons transferred to POM and thermodynamic considerations, we estimate that anaerobic respiration to POM may largely suppress methanogenesis in peat soils, particularly close to the oxic-anoxic interface across which POM is expected to undergo redox cycling.

Plain Language Summary In oxygen-depleted peat soil, microbes slowly break down peat material to CO_2 in a series of processes summarized under the term anaerobic respiration. These processes have in common that they require a chemical substance to which microbes can transfer electrons liberated in anaerobic respiration. Previous studies provided evidence that peat particulate organic matter (POM), the organic material primarily making up peat soils, can act as this electron acceptor. In this work, we buried a reference POM, placed in small mesh bags, in three Swedish peat soils. After 1 year, analysis of the recollected POM indeed showed that it had taken up electrons. Some of these electrons could be subsequently removed from the POM by bringing it in contact with dissolved oxygen. Taken together, our study supports that POM in peatlands accepts electrons, temporarily stores them, and then transfers them to oxygen when it becomes available. In this role of electron acceptor, POM may significantly decrease the formation of large amounts of methane, a gas that, when released from peatlands, largely contributes to warming of the atmosphere. Electron transfer to POM in peatland soils thus may play an important and desirable role in lowering natural methane release from peatland ecosystems.

1. Introduction

Peat particulate organic matter (POM) is increasingly recognized as an important terminal electron acceptor (TEA) for anaerobic microbial respiration in anoxic peat soils (Gao et al., 2019; Guth et al., 2022; Keller et al., 2009, 2023; Keller & Takagi, 2013; Rush et al., 2021). Accounting for POM as TEA is important given that the resulting respiration pathway may play a significant role in the carbon cycle of peatlands and affect the formation and emission of carbon dioxide (CO_2) and methane (CH_4) from peat soils. The use of POM as TEA allows for continuous substrate turnover to CO_2 by anaerobic respiration, even when canonical inorganic TEAs such as nitrate, sulfate, and ferric iron (dissolved, complexed, and/or mineral-associated) become depleted or are absent in peat soils. These inorganic TEAs are known to be present at only low concentrations in the pore water of ombrotrophic (rainwater-fed) bogs, which are hydrologically isolated from the surrounding groundwater

(Lindsay, 2016). In such systems, POM may be the predominant TEA for anaerobic respiration. Electron transfer to POM has also been implicated to lower pore-water concentrations of the potent greenhouse gas CH_4 via two pathways: competitive suppression of methanogenesis by thermodynamically more favorable anaerobic respiration to POM as a TEA, as well as anaerobic oxidation of methane (AOM), in which already formed CH_4 is oxidized to CO_2 with POM as TEA (Smemo & Yavitt, 2007, 2011). Both pathways have also been hypothesized to substantially lower CH_4 emissions from peatlands and thus are of direct relevance to global atmospheric CH_4 budgets, given that peatlands are a major natural source of CH_4 (Saunois et al., 2016).

Scientific evidence for anaerobic respiration to POM as TEA mainly stems from laboratory incubations of POM under anoxic conditions. In these incubations, methanogenesis of organic substrate molecules with a nominal oxidation state of carbon (NOSC) of zero (common for peat substrate organic matter (Boye et al., 2017; LaRowe & van Cappellen, 2011; Leifeld et al., 2020; Teickner et al., 2022; Yu et al., 2016)) is expected to result in the equimolar formation of CO_2 and CH_4 (Conrad, 1999). Yet, many of these studies reported CO_2:CH_4 molar formation ratios that exceeded unity by factors up to ten thousand (Corbett et al., 2013a, 2013b; Gabriel et al., 2017; Gao et al., 2019; Guth et al., 2022; Inglett et al., 2012; Keller et al., 2023; Keller & Takagi, 2013; Segers & Kengen, 1998; Yavitt et al., 1987). More importantly, the excess CO_2 formed could not be explained by anaerobic respiration to inorganic TEAs, which were present at too low concentrations, suggesting the presence of an unrecognized TEA. These findings led to the hypothesis that POM acted as the missing TEA.

The role of POM as TEA was strongly supported by studies demonstrating electron transfer to and from POM. For instance, oxidized POM was shown to accept electrons from chemical reductants (Gao et al., 2019; Guth et al., 2022; Joshi et al., 2021; Teickner et al., 2022). The reported number of electrons transferred from the reductants to a unit mass of POM (the so-called electron accepting capacities (EACs) of the POM) range from 90 to 600 μmol e^- (g dry POM)$^{-1}$. These EAC values are, however, likely overestimates of the number of electrons transferrable to POM by anaerobic respiration under in situ conditions in peat soils for two reasons. First, these EAC values were determined for POM specimen retrieved from anoxic peat soils, but subsequently transported and prepared under ambient (oxic) conditions (Gao et al., 2019; Guth et al., 2022; Teickner et al., 2022). This handling may have given rise to at least partial re-oxidation of the POM samples prior to EAC determination. Second, and more importantly, the chemical reduction of POM in these EAC assays is commonly carried out under highly reducing conditions and hence at chemical reduction potentials (E_H) much lower than the E_H values of organic electron-accepting moieties that are reducible in microbial anaerobic respiration (Aeschbacher et al., 2011; Klüpfel et al., 2014; Madigan et al., 2012; Straub et al., 2001). Consequently, the effective EAC of POM under in situ conditions is expected to be (substantially) smaller than EAC values determined on oxidized POM by (electro-)chemical reduction assays.

Besides demonstrating chemical reduction of POM in EAC assays, other work has shown that reduced POM transfers electrons to chemical oxidants. For instance, the capacity of POM to donate electrons to added Fe^{3+}-containing chemical oxidants was found to increase over the course of anoxic laboratory incubations, consistent with continuous POM reduction (Keller et al., 2023; Keller & Takagi, 2013; Roden et al., 2010; Rush et al., 2021). Similarly, anoxically sampled and stored POM reduced dissolved oxygen (DO) in laboratory experiments. Reduction of DO by reduced POM was recently also demonstrated directly in the anoxic subsurface of three ombrotrophic bogs in central Sweden (Obradović et al., 2023; Walpen, Lau, et al., 2018). The latter finding strongly suggests that re-oxidation of reduced POM by DO in situ regenerates oxidized POM and thus restores the capacity of POM to accept electrons under subsequent anoxic conditions. Such POM oxidation events may occur when DO enters the previously anoxic peatland subsurface, for example, through root and rhizome transport (Björn et al., 2022), recurring water-table fluctuations (Estop-Aragonés et al., 2012; Gabriel et al., 2017; Górecki et al., 2021), and strong oxygenated-water infiltration events (Rydin et al., 2013). Such redox cycling of POM implies that it can act as sustainable, long-term TEA in intermittently anoxic environments.

While there is compelling evidence for POM acting as a TEA, direct demonstrations of POM reduction in situ in the field, which provide a reliable estimate for the number of electrons transferrable to POM, are still missing. One possible experimental approach to obtain such data is to deploy air-oxidized reference POM with a known EAC in anoxic parts of peat soils across different peatlands, followed by in situ incubation for a prolonged time to allow for electron transfer to the deployed POM. Following incubation, the deployed POM can then be recollected anoxically and transferred back to the laboratory. Reduction of POM during in situ incubation would result in a lower EAC value of the retrieved, reduced reference POM as compared with the EAC of the deployed, air-

oxidized reference POM. Furthermore, exposure of the retrieved, reduced reference POM to DO under laboratory conditions would provide information on the reversibility of electron transfer to this reference POM. Deployment of the same reference POM in different peatland soils also allows to assess if the same POM is reduced to comparable extents across peatlands, and thus if these systems exhibit comparable reducing conditions.

The objective of this study was to demonstrate electron transfer to an oxidized reference POM under in situ anoxic conditions in peat soil, to quantify the number of electrons transferred to the POM during the incubation, and to assess the reversibility of this electron transfer by reacting the retrieved, reduced reference POM with DO. To reach this objective, we deployed an oxidized reference POM material contained in mesh bags in the anoxic subsurface of three ombrotrophic bogs in Central Sweden, Björsmossen (BM), Lungsmossen (LM) and Stohrultsmossen (SM), followed by 1 year incubation. We recollected the POM while maintaining anoxic conditions, determined its EAC, and compared it with that of the oxidized POM in the state in which it was originally deployed in the field. We further assessed reversibility of POM reduction by reacting the retrieved reduced POM with DO, followed by quantification of the resulting EAC increases. The use of mesh bags for our experiments is a well-established approach in other research fields, for instance to follow substrate transformation in natural systems (Barreto & Lindo, 2018; Belyea, 1996; Finér & Laine, 2000). We selected the above three bogs because we previously demonstrated anoxic conditions in their pore waters up to shallow depths close to the peat soil surfaces, that they contain only traces of inorganic TEAs, and that the natural POM in these bogs is present in reduced states (Joshi et al., 2021; Obradović et al., 2023; Walpen, Getzinger, et al., 2018; Walpen, Lau, et al., 2018). As part of this study, we further characterized these peat soils to establish that they are methanogenic at the depth at which the reference POM was deployed. These characterizations included investigating the presence of microbes that participate in methane cycling by determining methyl coenzyme M reductase gene (*mcrA*) abundance (Friedrich, 2005) (*mcrA* encodes the enzyme that catalyzes the final step of methanogenesis, but also the reversible first step in the anaerobic oxidation of methane (Scheller et al., 2010; Shima & Thauer, 2005; Thauer, 2019)), as well as quantification of peat pore-water CO_2 and CH_4 concentrations.

2. Materials and Methods

2.1. Chemicals

Analytical-grade potassium phosphate dibasic, potassium phosphate monobasic, and potassium chloride were obtained from Sigma Aldrich (Buchs, Switzerland). 4,4′-bipyridinium-1,1′-bis(2-ethylsulfonate) (zwitterionic viologen, ZiV) was synthesized according to Gorski et al. (2012). Distilled, deionized water (MQ, resistivity >18.1 MΩ·cm, Milli-Q IQ7000, Merck, Buchs, Switzerland) was used to prepare all solutions, which were sparged with N_2 (grade 5.0, 99.999%, Linde Gas, Dagmarsellen, Switzerland) for at least 2 hr prior to transferring them into an anoxic glovebox (pure N_2 atmosphere, <2.3 ppm O_2, MBraun, Garching, Germany). All experimental manipulations and measurements requiring anoxic conditions, except for anoxic experiments carried out directly in the field, were conducted in this glovebox.

2.2. Study Sites and Reference Peat Particulate Organic Matter (POM_{ref})

Experiments were conducted in three ombrotrophic peat bogs in Värmland county, Sweden: Björsmossen (BM, location 59.69272 N, 14.27713 E), Lungsmossen (LM, 59.54943 N, 14.23905 E), and Storhultsmossen (SM, 59.57176 N, 14.13208 E). The vegetation in these bogs was dominated by *Sphagnum* carpets and hummocks with few isolated trees, predominantly *Picea abies* and *Pinus sylvestris*, as described by Almquist-Jacobson and Foster (1995) for bogs in the larger area.

As reference POM we obtained commercially available, air-dried, and sieved (<2 mm) POM (hereafter referred to as POM_{ref}) from Stockås Torvströ (Torv and Maskinentreorad AB, Mullhyttan, Sweden; Joshi et al., 2021). The POM_{ref} is a mixture of dried and sieved peat soil collected from three *Sphagnum* spp.-dominated ombrotrophic bogs in Värmland and Örebro counties, Sweden. We mixed a large amount (10 kg) of POM_{ref} to minimize any physical or chemical heterogeneity, and then divided this stock into smaller batches used for subsequent experiments. For the burial experiments described herein, we used POM_{ref} material from a single batch. As POM_{ref} was extensively exposed to air and thus oxygen during processing and storage by the manufacturer and by us, we considered that redox-active moieties in POM_{ref} were present in an extensively oxidized state.

2.3. Characterization of Study Sites

2.3.1. Peat Soil and Pore Water Collection

In each of the three bogs, we collected peat soil in ten 500 mL jars at ∼50 cm depth below the water table, which coincided with the bog surface at the time of sampling. First, we removed the top layer of *Sphagnum* mosses (up to approximately 10 cm depth) and then dug a hole by hand vertically into the peat soil. The hole was large enough to place a single 500 mL jar below the water table and let it fill with pore water, thereby replacing the air within. We then pushed the jar to a depth of ∼50 cm and hand-packed peat soil into the jar. The jar was brought back to the peat surface and immediately sealed air-tight with a screw-on lid, which was fixed in place with tape. We subsequently stored the jars at 4°C until further use.

We also collected pore-water samples at three locations in the direct vicinity of each of the peat-soil sampling locations. At each location, we inserted three custom-built drive-point samplers (Walpen, Getzinger, et al., 2018; Walpen, Lau, et al., 2018) into the peat soil to depths of 30, 40 and 50 cm below the surface. Each sampler consisted of a hollow stainless-steel tube (50 cm length, 3 mm inner diameter) fitted with a perforated head with stainless steel screen (90 µm pore size) to facilitate manual pore-water extraction using an air-tight plastic syringe attached to the sampler top. For each of the three locations, we transferred 8 mL of the pore-water samples collected at a given depth into separate N_2-sparged gas chromatography (GC) vials (20 mL) each of which contained 3 g NaCl. The resulting saturated salt solution resulted in complete partitioning of CO_2 and CH_4 from the solution into the headspace of the vials. Upon return to the laboratory, we quantified CO_2 and CH_4 concentrations in the pore water as described below.

2.3.2. DNA Extraction From Peat Soil and Quantitative Polymerase Chain Reaction

Samples for DNA extraction were taken from two different jars per peat bog, with four individual subsamples per jar. Total DNA was extracted using the DNeasy® PowerSoil® Pro Kit (Qiagen N.V., Venlo, Netherlands) according to the manufacturer's protocol (i.e., 250 mg wet soil per extraction). DNA concentrations were quantified using a Qubit™ 4 Fluorometer and a NanoDrop 8000-GL Spectrophotometer (both from Thermo Fisher Scientific AG, Reinach, Switzerland). All preparation steps were performed in an Ultraviolet light-sterilized PCR workstation (Peqlab Biotechnologie GmbH, Erlangen, Germany), which was cleaned with RNase AWAY™ Surface Decontaminant (Thermo Fisher Scientific AG) and exposed to UV light for 30 min before use. We amplified the bacterial and archeal 16S rRNA genes and the *mcrA* gene by quantitative polymerase chain reaction (qPCR) with the extracted DNA as template. Copy numbers of the gene were normalized per gram of dry peat soil, which was determined after drying peat-soil samples at 50°C for 6 days. More information on qPCR and the primers that were used are provided in Text S1 in Supporting Information S1.

2.3.3. Quantification of Pore-Water CH_4 and CO_2 Concentrations

Headspace CH_4 and CO_2 concentrations in the GC vials were quantified using a GC-Methanizer system (SRI 8610C GC, SRI Instruments Europe, Bad Honnef, Germany). The GC oven was operated at 40°C and was equipped with a Porapak Q and a HayeSep D column connected in series. We used N_2 (grade 5.0) as carrier gas and H_2 (5.0, all gases from Linde Gas) as fuel gas for the methanizer detector (a modified flame-ionization detector). In this detector, CH_4 is quantified directly, but CO_2–to allow its detection and quantification–is first reduced to CH_4 by H_2 on a Ni catalyst operated at 380°C. We then converted the obtained CH_4 and CO_2 concentrations (in µL L^{-1}) from GC vials to molar concentrations (µmol L^{-1}) using the ideal gas law. Taking the headspace and pore-water sample volumes into account, we report total CO_2 and total CH_4 concentrations present in the peat pore-water samples. More information on CO_2 and CH_4 quantification is provided in Text S2 in Supporting Information S1.

2.4. Field Burial Experiment to Assess In Situ Electron Transfer to POM_{ref}

2.4.1. Mesh-Bag Preparation and Field Deployment

In the laboratory we transferred 1.5 g of POM_{ref} into each of the self-made rectangular PET mesh bags (8 × 6 cm from pieces cut out of 100 µm mesh size, Sefar AG, Heiden, Switzerland; heat sealed on three sides) and closed the bags by heat sealing of the fourth side using a heat sealer (Wu-Hsing Electronics Co., Ltd., Taiwan). This ensured that we deployed reproducible POM_{ref} amounts, ruled out mixing of the deployed reference POM with

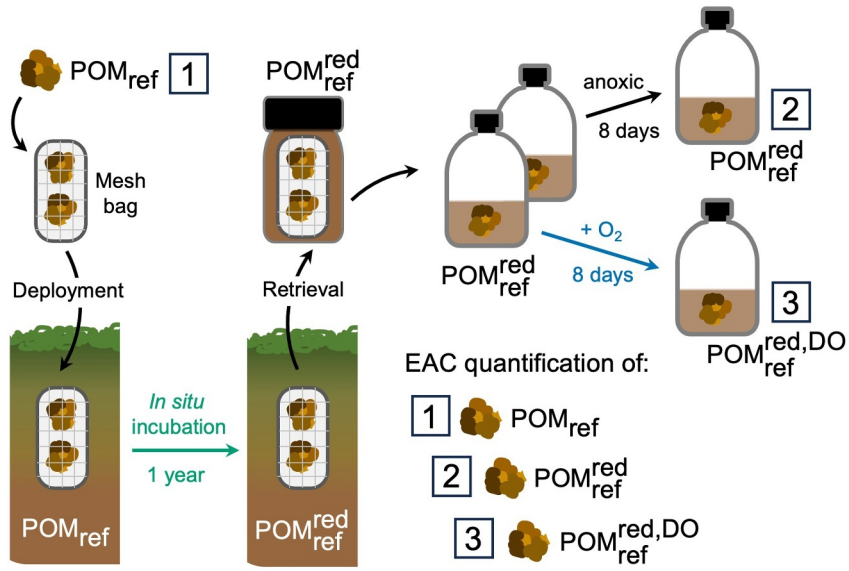

Figure 1. Schematic of the reference peat particulate organic matter (POM_{ref}) [1] being deployed in the peat soils, incubated under in situ anoxic conditions in the field for 1 year, and retrieved as reduced POM_{ref} (POM_{ref}^{red}). Following transfer to the laboratory, we quantified the electron accepting capacity (EAC) of POM_{ref}^{red} without any exposure to dissolved oxygen (DO) [2], as well as after exposure to DO for 8 days (i.e., EAC of $POM_{ref}^{red,DO}$) [3]. These EAC values were compared to that of the POM_{ref} [1].

the reduced native POM from the bogs in situ, and ensured the retrieval of the deployed POM_{ref} after incubation and the relocation of POM_{ref} in its entirety. In July 2019, we buried five mesh bags in each bog (i.e., BM (59.692683 N, 14.277200 E), LM (59.549367 N, 14.238533 E), and SM (59.571783 N, 14.131900 E)) at ~40 cm depth below the bog surface (equivalent to 40 cm below the water table at the time of deployment, Figure 1). In addition, we buried five mesh bags at a depth of ~10 cm in BM (a depth that was assumed to be temporarily oxic, as compared to permanently anoxic 40 cm depth). In July 2020, following one year of in situ incubation, we retrieved the mesh bags anoxically (i.e., without exposure to air). To this end, we lowered 150 mL glass jars (Bormoli Rocco, Fidenza, Italy) filled with anoxic pore water at a shallow soil depth to the depth at which the mesh bag was deployed. We then transferred the bags by hand into the jar together with the surrounding peat soil and pore water. Following retrieval of the jars to the peat surface, we immediately sealed them air-tight with a screw-on lid, which we fixed in place with tape (Figure 1). We subsequently stored the jars containing the mesh bags at 4°C until we transferred them into the anoxic glovebox in the laboratory for sample preparation and analysis.

2.4.2. Laboratory Manipulations of Mesh-Bag POM Samples

Inside the anoxic glovebox, we opened the jars (20 in total), retrieved the mesh bags, and cut them open. We suspended the content (i.e., the POM_{ref}^{red}) of each bag in 100 mL MQ water to obtain a POM suspension concentration of ~15 g L^{-1} (hereafter referred to as stock suspension). In total, we had 20 POM_{ref}^{red} stock suspensions, five from each of the three peatlands where mesh bags were buried at 40 cm depth and an additional five suspensions from the samples deployed in BM also at 10 cm depth. In addition, we also prepared four 10 g POM_{ref} L^{-1} stock suspensions inside the glovebox from the same oxidized POM_{ref} batch from which we filled the mesh bags.

For the 20 POM_{ref}^{red} and four POM_{ref} stock suspensions we prepared diluted suspensions at the native pore water pH (average pH 4.54, Text S3 in Supporting Information S1). For each stock suspension, we transferred 10 mL aliquots into a pair of 35-mL serum bottles, stoppered and crimped them with aluminum caps, and transferred them out of the glovebox. Of each pair of serum bottles, one suspension remained anoxic as a negative control, whereas the second suspension was exposed to dissolved oxygen (DO) to assess the reversibility of electron transfer to POM (Figure 1). Exposure to DO was accomplished by aerating the respective serum bottle daily by

removing the bottle stopper for 5 min. All suspensions were stirred at 350 rpm at 15°C on a multipoint magnetic stir plate (Variomag, Daytona Beach, USA) for 8 days. After 8 days period, we sparged the POM suspensions in all bottles with N_2 gas for 2 hr, followed by transferring the bottles back into the glovebox for EAC determination (Figure 1).

We also conducted independent experiments to determine the extent of POM_{ref}^{red} re-oxidation by DO at pH 7 instead of at native pH. These experiments were conducted only on POM_{ref}^{red} stock suspensions from BM (mesh bags buried at 40 cm depth) and followed the same setup as outlined above, except that 9 mL aliquots of the POM_{ref}^{red} stock suspensions were combined with 1 mL pH-buffered solution (pH 7, 1 M potassium phosphate) in each pair of 35-mL serum bottles. The serum bottles containing pH-adjusted, diluted POM_{ref}^{red} suspensions were then stoppered and crimped before taking them out of the glovebox, and treated in similar fashion as described for re-oxidation at native pH (see above; i.e., of each pair of serum bottles, one was opened for DO-exposure while the other remained closed and thus anoxic).

We quantified the POM concentrations in each suspension by taking five 0.5 mL sub-aliquots in the glovebox, transferring them out of the glovebox, followed by drying them for 24 hr at 50°C, and weighing the dry POM mass on an analytical balance (Mettler Toledo, Greifensee, Switzerland) outside of the glovebox.

2.4.3. Quantification of EAC of Suspended POM

We quantified EACs of suspended POM according to Joshi et al. (2021). Briefly, from each serum bottle, we took triplicate aliquots of 0.5 mL. To each aliquot, we subsequently added 7.5 mL of MQ water, 1 mL of pH-buffered solution (1 mL; pH 7, final concentrations of 0.1 M potassium phosphate and 0.1 M KCl), and 1 mL of a solution that contained the chemical reductant zwitterionic viologen (i.e., one-electron reduced $ZiV^{\bullet-}$ radical; final concentration 280 μM). The chemical reductant was obtained by bulk electrochemical reduction of oxidized ZiV^0. The EAC measurements were performed at pH 7 because of optimal stability of $ZiV^{\bullet-}$ at this pH and expected complete reduction of electron-accepting moieties in POM (Joshi et al., 2021). After 24 hr of reaction of the POM with $ZiV^{\bullet-}$, we filtered suspension aliquots (1.5 mL) of each triplicate reactor using 0.22 μm cellulose acetate filters (BGB) and quantified the residual $ZiV^{\bullet-}$ concentration spectrophotometrically at 603 nm (UV5 Bio, Mettler-Toledo, Switzerland). Two additional sets of triplicate controls were run in parallel to POM/$ZiV^{\bullet-}$ triplicates. The first set contained only $ZiV^{\bullet-}$ and buffer and was used to determine the extent of slow $ZiV^{\bullet-}$ oxidation over 24 hr in the absence of POM. The second set contained only POM and buffer, without $ZiV^{\bullet-}$. These control vials served to account for solution absorbance at 603 nm that originated from dissolved organic matter (DOM) released from POM. The EAC values were then calculated using Equation 1 (Joshi et al., 2021):

$$EAC = \frac{A_{\text{only ZiV}^{\bullet-}} - (A_{\text{POM \& ZiV}^{\bullet-}} - A_{\text{only POM}})}{\varepsilon \times m_{\text{POM}}} \times V_{\text{reaction}} \quad (1)$$

where $A_{\text{only ZiV}^{\bullet-}}$ (AU) is the average absorbance of $ZiV^{\bullet-}$-containing solutions from the triplicate POM-free controls, $A_{\text{POM \& ZiV}^{\bullet-}}$ (AU) is the average absorbance of $ZiV^{\bullet-}$containing solution from the triplicate POM-containing vials, $A_{\text{only POM}}$ (AU) is the average solution absorbance determined in the POM-containing but $ZiV^{\bullet-}$-free triplicate controls, ε (14,740 ± 52 AU L mol^{-1}) is the molar absorption coefficient of $ZiV^{\bullet-}$, m_{POM} (g) is the average mass of POM pipetted into the triplicate suspensions, and V_{reaction} (L) is the reaction volume of the triplicates (10 mL each).

Finally, we calculated the differences in EAC (i.e., ΔEAC) for each POM_{ref} and POM_{ref}^{red} sample that resulted from re-oxidation of one of the sample duplicates by subtracting the EAC of the sample without exposure to DO from the EAC of the sample exposed to DO.

3. Results and Discussion

3.1. Characterization of Peat Soils for In Situ POM_{ref} Incubations

We characterized the peat soils at the sites that we selected for deployment and incubation of the POM_{ref}. The characterization included determining the genetic potential of the microbial community for methanogenesis, as well as the quantification of pore-water CO_2 and CH_4 concentrations, both as indicators for in situ

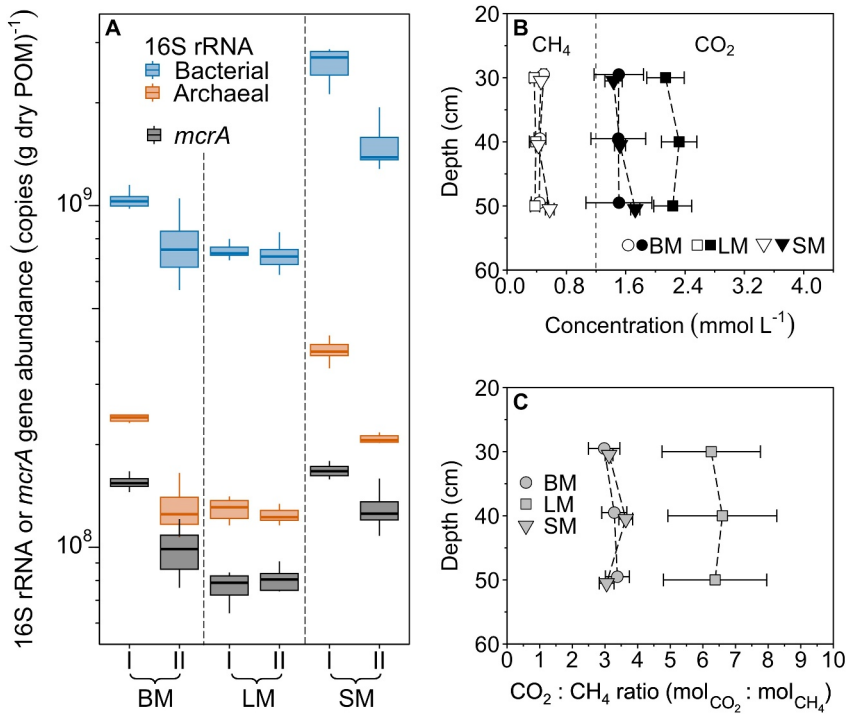

Figure 2. Characterization of peat soils in the three selected ombrotrophic bogs Björsmossen (BM), Lungsmossen (LM), and Storhultsmossen (SM) for in situ incubations of reference peat particulate organic matter (POM_{ref}). (a) Gene copy numbers of the archeal and bacterial 16S rRNA genes, as well as *mcrA* gene, per gram dry POM sampled at a depth of 50 cm, with I and II indicating duplicate jars filled with peat soil from each of the three sampling locations. (b) Depth profiles of pore-water concentrations of dissolved carbon dioxide (CO_2) and dissolved methane (CH_4), and (c) the resulting molar CO_2:CH_4 concentration ratios. Data points correspond to means of triplicate samples ($n = 3$) and the error bars represent standard deviations. The slight vertical offset between the data points at the same depth was created to avoid overlapping data points.

methanogenesis. While selecting incubation sites with anoxic conditions would suffice to assess microbial respiration to POM_{ref}, we aimed to deploy samples in methanogenic systems given that anaerobic respiration to POM has been implicated in the decrease of pore-water CH_4 concentrations.

Each of the *mcrA* and bacterial and archeal 16S rRNA genes, amplified from samples collected at ~50 cm depth below the peat surface and reported as copy numbers per gram of dry POM soil, showed comparable abundance across the three bogs (Figure 2a). The number of bacterial 16S rRNA gene copies (between approximately 0.7 and 2.6×10^9 copies (g dry POM)$^{-1}$) outnumbered the archeal 16S rRNA gene copies 6-fold on average. The ratio of *mcrA* gene copies to archeal 16S rRNA gene copies was 0.62 ± 0.1. This finding indicates that a large fraction of the archeal community has the genetic capability to produce or anaerobically consume methane. The *mcrA* gene copy numbers for the three bogs are comparable to the highest *mcrA* gene copy numbers (6×10^8 *mcrA* copies per gram dry soil) detected in three different *Sphagnum*-dominated ombrotrophic peats in Sweden and Denmark, using the same *mcrA* primers and a similar qPCR protocol (Martí et al., 2015).

We complemented the quantification of gene abundances with quantifications of the concentrations of dissolved CO_2 and CH_4 in the pore water of BM, LM and SM for depths of 30, 40 and 50 cm (Figure 2b). Neither dissolved CO_2 nor CH_4 concentrations showed an apparent depth dependence. The concentrations were 1.4–2.3 mM CO_2 and 0.36–0.58 mM CH_4, which translated to a narrow range of CO_2:CH_4 molar ratios of approximately 3–6.5 mol CO_2: mol CH_4 (Figure 2c). The presence of pore-water dissolved CH_4 is indicative of methanogenesis at these depths across all three peat soils, suggesting that there likely were insufficient TEAs, including oxidized POM, to sustain anaerobic respiration at a level that would have completely suppressed methanogenesis. The pore-water concentrations of CO_2 and CH_4 were 19–31 and 3–4 times lower than the water solubilities of CO_2 (44.73 mM) and CH_4 (1.62 mM) at the given temperature of 15°C, respectively (Speight & Lange, 2017).

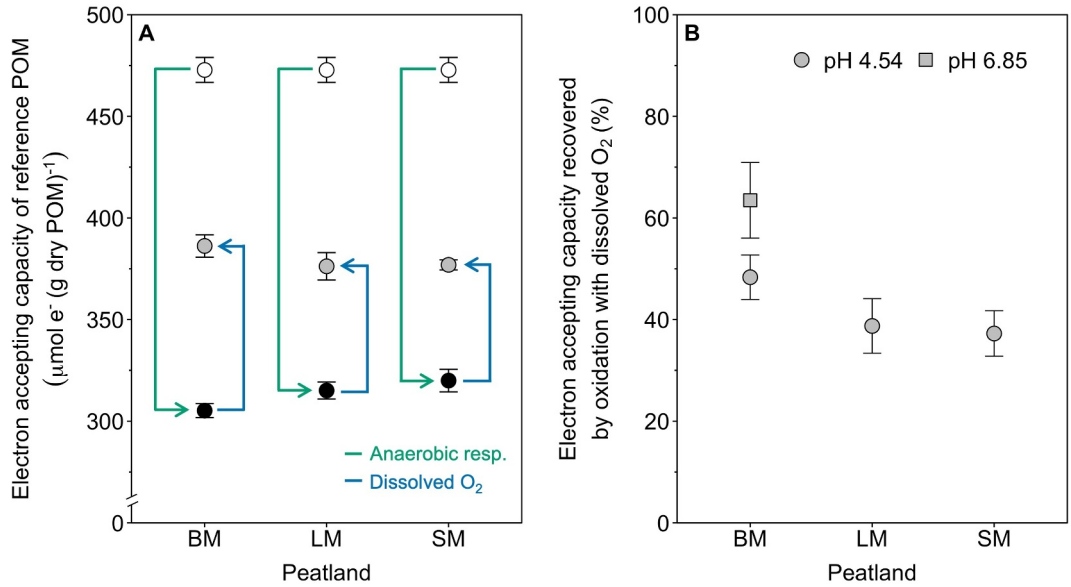

Figure 3. Changes in the electron accepting capacities (EACs) of reference peat particulate organic matter (POM) samples resulting from 1 year in situ incubations in the peat soils of Björsmossen (BM), Lungsmossen (LM), and Storhultsmossen (SM) and from subsequent laboratory exposure to dissolved oxygen (DO). (a) EAC values of oxidized, non-buried POM_{ref} (white circles) and partially reduced POM_{ref} (POM_{ref}^{red}) (black circles) retrieved from peat soils after the 1-year in situ incubation. Exposure of the POM_{ref}^{red} to DO for 8 days at the native pH of the peat soils (Text S3 in Supporting Information S1) resulted in an increase in EAC values and thus a partially re-oxidized $POM_{ref}^{red,DO}$ (gray circles). (b) The EAC restored by DO re-oxidation of POM_{ref}^{red}, expressed in percent of the respective decreases in EAC from POM_{ref} to POM_{ref}^{red} during incubation in BM, LM and SM. The re-oxidation by DO was additionally performed at neutral (buffered) pH for POM samples incubated in BM. Data is shown with one standard error margin ($n = 5$, except for POM_{ref} with $n = 4$; Text S3 in Supporting Information S1).

Taken together, our site characterization in the anoxic subsurface of the three ombrotrophic bogs indicates that methanogenesis (and/or AOM) is not only an important metabolic trait within the native microbial communities, but also has taken place, resulting in significant pore-water CH_4 concentrations. As such, the peat soils fulfilled the criterion of being methanogenic that we set for the incubation of POM_{ref} and the assessment to what extent it was reduced as TEA in anaerobic microbial respiration.

3.2. Electron Transfer to POM_{ref} During Incubation in the Anoxic Peat Subsurface

We assessed the use of POM as TEA in anaerobic microbial respiration by deploying oxidized POM_{ref} in the anoxic peat soils of BM, LM and SM, followed by in situ incubation for 1 year. The redox state of POM_{ref} upon deployment was indeed oxidized as evident from a high electron accepting capacity of 473 ± 6 µmol e^- (g dry POM)$^{-1}$ ($n = 4$) (Figure 3a). This EAC value was higher than the reported EAC for a similar reference POM used in our previous work (Joshi et al., 2021), likely reflecting differences in the composition of the used POM_{ref} batches. We did not further explore the inter-batch EAC variability in the reference POM. However, for the work presented herein, we used a single batch of POM_{ref} to ensure that its chemical composition and thus EAC values were reproducible. We also added a relatively large amount of 1.5 g of POM_{ref} to each bag to minimize possible sample-to-sample variations resulting from heterogeneity in the POM material. More importantly, the EAC of POM_{ref} fell in the range of EAC values of 90–600 µmol e^- (g dry POM)$^{-1}$ determined for artificially oxidized POM from various peatlands globally (Teickner et al., 2022; Guth et al., 2022; note that we assumed a carbon content of the POM analyzed in these studies of 50 weight % to convert reported values per g carbon to g POM).

One year of in situ incubation in BM, LM, and SM peat soils resulted in a significant decrease in the EACs of the POM_{ref} from 473 ± 6 µmol e^- (g dry POM)$^{-1}$ to values of 305 ± 3, 315 ± 4 and 320 ± 6 µmol e^- (g dry POM)$^{-1}$, respectively (Figure 3a; *t*-test, degrees of freedom (df) = 7, $p << 0.05$, significance level (α) = 0.05). This EAC decrease indicates that approximately 150–170 µmol e^- (g dry POM)$^{-1}$ were transferred to POM_{ref} during the incubation, resulting in a partially reduced reference POM, hereafter referred to as POM_{ref}^{red}. Thus, approximately one third of the EAC of POM_{ref} was utilized as TEA over the 1 year of in situ incubation. The utilization of POM

as TEA in anaerobic microbial respiration is supported by previous laboratory studies demonstrating microbial POM and also DOM reduction (Gao et al., 2019; Guth et al., 2022; Keller & Takagi, 2013; Klüpfel et al., 2014; Lovley et al., 1996, 1998).

The finding that POM_{ref}^{red} exhibited non-zero EAC values implies that it still contained redox-active moieties that are chemically reducible in the EAC assay and, therefore, that in situ incubation resulted in only partial reduction of POM_{ref}. We cannot completely rule out that incomplete reduction of POM_{ref} resulted from partial re-oxidation in the peat soils during the incubation due to an oxidation event. Yet, we consider this possibility highly unlikely given the burial depth of 40 cm below the peat-soil surface, and thus incubation of the samples substantially below the oxic-anoxic interface in the peat soil at about 5–10 cm depth. Instead, partial reduction of POM_{ref} likely reflected thermodynamic constraints on microbial respiration to POM, as previously demonstrated for anaerobic respiration to DOM. For instance, the facultative anaerobe *Shewanella oneidensis* was shown to utilize electron-accepting moieties in respiration only down to reduction potentials of approximately $E_H = -250$ mV at pH 7 (Klüpfel et al., 2014). By comparison, the chemical reductant $ZiV^{\bullet-}$ used in the EAC assay had a substantially lower initial E_H of −460 mV (and only slightly increased during the reaction), suggesting that $ZiV^{\bullet-}$ transferred electrons also to reducible moieties in the POM with E_H values much lower than the E_H corresponding to the thermodynamic limit to which electrons can be transferred by anaerobic microbial respiration (Figure 4). We cannot rule out that slow electron transfer kinetics to POM_{ref} also contributed to only partial reduction, possibly due to low abundances and/or activities of anaerobically respiring microorganisms in these permanently anoxic peat soils, or due to constraints in the transfer of electrons from microbial cells to the POM. The finding that only a fraction (approximately a third) of the EAC of POM_{ref} determined in assays decreased during extensive in situ incubation implies that (electro-)chemically determined EAC values of POM may substantially overestimate microbially accessible EAC of POM in situ (Figure 3).

The extents of POM_{ref} reduction and thus the attained redox states of POM_{ref}^{red} were comparable across the three tested bogs (Figure 3a). This finding strongly suggests that there were comparable thermodynamic constraints on microbial respiration in the peat pore waters across the three bogs and, therefore, likely comparable reducing conditions (i.e., pH and reduction potentials E_H), resulting in a comparable subset of the total reducible moieties in the POM that were available for microbial reduction. We did not attempt to determine E_H values in situ in the three peatlands given the well-documented redox-nonequilibria in potentiometric E_H measurements using redox electrodes (Lindberg & Runnells, 1984; Morris & Stumm, 1967; Sander et al., 2015; Stumm, 1984). Future studies may use added chemical mediators for mediated E_H measurements to confirm comparable reductive conditions. Such measurements would, however, require sampling of POM and transfer to the laboratory as the addition of chemical mediators to the peat subsurface would result in chemical contamination of the peat soils and hence is not viable in the field. Combining pore-water E_H measurements with chemical analyses of the naturally present POM across peatlands are expected to be needed to explain possible differences in the microbially available EAC of POM in different peatlands.

3.3. Electron Transfer From POM_{ref}^{red} to Dissolved Oxygen in Laboratory Incubations

Under anoxic conditions in peatland soils, POM is expected to undergo continuous reduction until its capacity to accept electrons from anaerobic microbial respiration is depleted. For POM to act as a sustainable and long-term TEA, two conditions therefore need to be fulfilled: (a) electron transfer to the POM needs to be (at least partially) reversible and thus occur to redox-active moieties in the POM with reversible electron transfer chemistry, and (b) the reduced POM needs to undergo periodic re-oxidation in the presence of an oxidant. Arguably the most relevant oxidant in peat soils is DO, which is present at the oxic-anoxic interface. We have previously demonstrated oxidation of native, reduced POM by DO both in situ and in the laboratory (Joshi et al., 2021; Obradović et al., 2023; Walpen, Lau, et al., 2018). We herein assessed the reversibility of electron transfer to POM_{ref} during its in situ incubation by exposing part of the collected POM_{ref}^{red} to DO in the laboratory and quantifying the resulting changes in its EAC.

Exposure of POM_{ref}^{red} retrieved from BM, LM, and SM to DO (hereafter referred to as $POM_{ref}^{red,DO}$) for 8 days resulted in increased EAC values of 386 ± 6, 376 ± 7 and 377 ± 3 μmol e^- (g dry POM)$^{-1}$, respectively (Figure 3a). We chose this long reaction time of 8 days to account for slow oxidation kinetics of reduced POM with DO (Obradović et al., 2023; Walpen, Lau, et al., 2018). By comparison, exposure of non-incubated POM_{ref} to DO over the same period had no significant effect on its EAC (*t*-test, $df = 6$, $p = 0.92$, $\alpha = 0.05$, Text S3 in

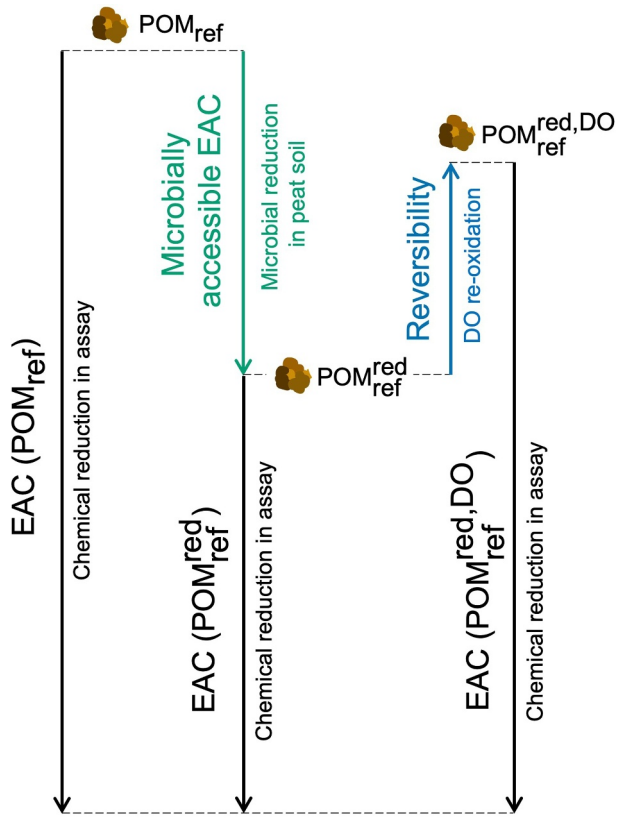

Figure 4. Schematic representation of different redox states of reference peat particulate organic matter (POM_{ref}), with their respective electron accepting capacities (EACs) quantified by the chemical reduction assay in the laboratory (black arrows). The different redox states of POM_{ref} were the oxidized starting material which also was deployed in the field, EAC (POM_{ref}), the reduced reference POM_{ref} (i.e., POM^{red}_{ref}) retrieved from field soils after 1 year of incubation, and the partially re-oxidized POM_{ref} after exposure to dissolved oxygen (DO) for 8 days, $POM^{red,DO}_{ref}$. The difference between EAC(POM_{ref}) and EAC (POM^{red}_{ref}) corresponds to the number of electrons transferred to the reference POM in the peat soil and thus corresponds to an EAC that is microbially accessible (green arrow). The difference between EAC ($POM^{red,DO}_{ref}$) and EAC (POM^{red}_{ref}) represents the electrons that were reversibly transferred to DO (blue arrow).

Supporting Information S1), consistent with POM_{ref} being extensively oxidized as purchased. Reaction of POM^{red}_{ref} with DO therefore removed between 57 and 81 μmol e^- (g dry POM)$^{-1}$ from the POM, which corresponds to about 37%–48% of the EAC decreases during incubation in BM, LM, and SM (Figure 3b). Electron transfer to POM_{ref} during the incubation was therefore at least partially reversible when exposed to DO. Such intermittent oxygenation events are expected to occur frequently across the oxic-anoxic interface in peat-soil profiles. The finding of partial electron transfer reversibility to the reference POM material used herein is consistent with our previous work demonstrating that exposure of native reduced POM collected from anoxic parts of BM and LM soils to DO in the laboratory for 8 days resulted in similar increases in EACs of 90 ± 40 μmol e^- (g dry POM)$^{-1}$ (Joshi et al., 2021). However, we previously found no increase in the EAC upon DO exposure of native POM collected from the anoxic subsurface of SM, suggesting that this POM was not present in a reduced state (Joshi et al., 2021). Given that POM^{red}_{ref} retrieved from SM showed increased EAC when reacted with DO in the present study implies that the conditions in the SM peat soil in principle allowed for POM reduction. There are two plausible explanations for this seemingly contrasting finding. First, POM_{ref} used herein may have had very different redox characteristics (i.e., a larger number of redox-active moieties with reversible electron transfer properties) than the native POM that we previously collected and analyzed from SM. This explanation is supported by the low degree of decomposition of the native POM collected from SM (Joshi et al., 2021). Second, the conditions at the sites where mesh bags with POM_{ref} were buried could have been more reducing than at the locations from which we previously retrieved native POM from SM (Joshi et al., 2021). Systematic studies assessing these factors across a given peatland and between different peatlands are warranted.

The increased EAC of $POM^{red,DO}_{ref}$ as compared to POM^{red}_{ref} implies at least partial reversibility of electron transfer to POM_{ref} and, therefore, the presence of redox-active moieties in POM_{ref} that reversibly accept and donate electrons over recurring reduction and re-oxidation cycles. Yet, the regeneration of the reducible moieties in the POM remained incomplete given that the EAC of $POM^{red,DO}_{ref}$ was smaller than of POM_{ref}. This finding can have two explanations. First, some of the reducible moieties in POM_{ref} possibly were reduced irreversibly during the in situ incubation and thus could not undergo re-oxidation by DO. While the chemical nature of moieties undergoing irreversible reduction remains elusive, it was previously proposed that double bonds in peat DOM may accept electrons in biohydrogenation reactions under anoxic conditions (McGivern et al., 2024; Wilson et al., 2017). While these reactions indeed would be irreversible, our analytical EAC method is insensitive to such groups (i.e., the one-electron reduced $ZiV^{\bullet-}$ radical does not hydrogenate double bonds). Thus, biohydrogenation reactions, if occurring, cannot explain the decrease in EAC of POM during the in situ incubation–and therefore cannot explain the incomplete regeneration of EAC when exposing the reduced POM to DO. The second, much more likely explanation for incomplete re-oxidation of POM^{red}_{ref} by DO is slow oxidation kinetics of some of the reduced moieties with DO. Slow oxidation kinetics with DO are well established for reduced reference DOM samples and for model hydroquinones (Aeschbacher et al., 2011, 2012), and have been ascribed to the thermodynamically unfavorable (i.e., endergonic) transfer of the first electron from reduced hydroquinones (and hydroquinone moieties in DOM) to O_2 to form a semiquinone radical and superoxide (Eyer, 1991; Roginsky & Barsukova, 2000). In fact, this first electron transfer is even more endergonic at acidic pH (the pH of the tested POM suspensions) than at circumneutral pH for which it was previously described (Aeppli et al., 2022; Aeschbacher et al., 2011, 2012). Slow oxidation kinetics of POM^{red}_{ref} by DO in our experiments at pH 4.54 are supported by previously reported very slow in situ re-oxidation of reduced native POM by DO in the subsurface of LM over a period of 12 days (Obradović et al., 2023). Similarly, column breakthrough

experiments in the laboratory showed that native reduced POM from BM, LM, and SM packed into the columns continued to react with DO beyond 24 hr (Obradović et al., 2023).

We experimentally tested for slow POM oxidation kinetics under native, acidic pH conditions by additionally reacting the POM_{ref}^{red} retrieved from BM with DO for 8 days at a solution pH of 6.85 (Figures 3a and 3b). We chose to test only POM_{ref}^{red} from BM given that incubations in all three bogs resulted in comparable POM reduction extents. Consistent with the above kinetic explanation, reaction of POM_{ref}^{red} with DO at the higher pH resulted in a significantly larger EAC increase of the POM of 106 ± 12 μmol e^- (g dry POM)$^{-1}$, as compared with the POM reacted at pH 4.54 (*t*-test, $df = 8$, $p = 0.02$, $\alpha = 0.05$) (Figure 3b; corresponding to 63% of the electrons transferred to POM_{ref} during in situ incubation being restored). We note that there was no significant change in the EAC of POM_{ref}^{red} in the anoxic control incubation at pH 6.85 as compared to pH 4.54 (*t*-test, $df = 8$, $p = 0.11$, $\alpha = 0.05$; Text S3 in Supporting Information S1). This finding supports that electron transfer to POM_{ref} during the in situ incubation was more reversible than was apparent from the 8-day reaction of POM_{ref}^{red} with DO at the native peat-soil pH. In fact, we previously demonstrated that a similar POM reference material showed fully reversible electron transfer over an electrochemical reduction and subsequent 8-day DO re-oxidation, both conducted at pH 7 (Joshi et al., 2021). The direct evidence for reversible reduction of electron-accepting moieties in POM is consistent with quinone-hydroquinone type redox chemistry, as previously established for DOM (Aeschbacher et al., 2011, 2012; Klüpfel et al., 2014; Walpen, Getzinger, et al., 2018). As discussed above, our EAC assay does not allow assessment of potential biohydrogenation of double bonds in POM, as recently proposed as respiration pathway to peat DOM (McGivern et al., 2024; Wilson et al., 2017). Future work is needed to establish the relevance of double bond biohydrogenation as a respiration pathway, particularly when involving extracellular POM.

3.4. Electron Transfer to and From POM_{ref} Incubated at a Shallower Depth

In addition to determining POM_{ref} reduction during incubation at ~40 cm depth in the three bogs (see above), we also deployed POM_{ref}-filled mesh bags in BM at an additional depth of ~10 cm below the surface. These incubations served to assess whether POM_{ref} reduction also occurred when incubated only slightly below the oxic-anoxic interface, which was located 5–10 cm below the peat surface. The EAC of the retrieved POM_{ref}^{red} from ~10 cm depth was 315 ± 21 μmol e^- (g dry POM)$^{-1}$ and thus statistically indistinguishable from the EAC of POM_{ref}^{red} retrieved from ~40 cm depth (*t*-test, $df = 8$, $p = 0.40$, $\alpha = 0.05$). After 8 days of re-oxidation of POM_{ref}^{red} from ~10 cm depth by DO, we observed an increase in EAC to 383 ± 18 μmol e^- (g dry POM)$^{-1}$, which was also statistically indistinguishable from the increase observed during re-oxidation of POM_{ref}^{red} collected from ~40 cm depth (*t*-test, $df = 8$, $p = 0.78$, $\alpha = 0.05$). These findings suggest that the reference POM incubated at ~10 cm depth was either never exposed to DO during the incubation, or, if it was intermittently exposed to and oxidized by DO (which may have entered the peat soil through water-table fluctuations (Blodau, 2002; Estop-Aragonés et al., 2012; Gabriel et al., 2017) or from photosynthetically active *Sphagnum*), that it experienced anoxic conditions for a sufficiently long time between DO exposure and sampling to be reduced again to an extent comparable to that at ~40 cm incubation depth. These findings suggest comparable reducing conditions in the peat soil of BM at depths of 10 and 40 cm below the peat surface.

4. Conclusions

We herein demonstrate that an oxidized reference POM material is being reduced in peat soils of three ombrotrophic bogs under in situ anoxic conditions prevailing at ~40 cm depth below the soil surfaces. Exposure of the retrieved material to DO shows partial electron transfer reversibility, likely due to slow reaction of reduced moieties in the POM with DO. These findings therefore largely substantiate that POM acts as a regenerable TEA under in situ conditions in peat soils. Reversible electron transfer to and from POM (i.e., redox cycling) is anticipated to be particularly relevant at the oxic-anoxic interface in peatland soils with alternating anoxic and oxic conditions. While we did not assess the effect of POM as TEA on CO_2 and CH_4 dynamics in the peat soil, given the difficulties of doing so in the field, we note that all three soils in which the POM was reduced were methanogenic. This finding supports that the use of POM as TEA in anaerobic respiration can indeed suppress methanogenesis given that both were observed in the same environment. We note that our findings relied on successfully using POM-filled mesh bags to deploy and retrieve reference POM. We envision that this experimental approach can be used in future work to study the role of POM as TEA in peatland biogeochemistry, for

instance to assess POM reduction kinetics by following EAC decreases over time–also in multi-year incubations–or to assess potential differences in the extent to which POM is reducible across a larger set of peatlands.

Our work clearly shows that only about one third of the EAC of POM measured in (electro-) chemical reduction assays is microbially accessible in anaerobic respiration under in situ incubation conditions. Therefore, care must be taken when using EAC values determined by assays on oxidized and homogenized POM materials to estimate the effect of this respiration pathway on substrate turnover and CO_2 and CH_4 formation dynamics in peatland soils. Instead, future studies are needed that systematically assess the extent to which native POM is reduced under in situ conditions, for instance by determining the EAC increase resulting from oxidation of native, reduced POM by DO. In such work, extensive re-oxidation periods are needed given that electron transfer from reduced POM to DO is kinetically slow.

Future work is also needed to establish a direct link between the use of POM as TEA and the suppression of methanogenesis, and to experimentally balance the number of electrons transferred to POM (by decrease of EAC) with the number of electrons diverted from methanogenesis to anaerobic microbial respiration. These studies ideally would also provide insight into the relative kinetics of POM reduction (through quantification of EAC decreases over time) and microbial respiration (through determination of excess CO_2 formation over time). Co-occurrence and stoichiometric agreement between the EAC decrease and the excess CO_2 formation would suggest that POM reduction is directly driven by anaerobic respiration. Conversely, faster POM reduction than excess CO_2 formation would indicate fast abiotic reduction of added POM by reduced species present in the peat soil, followed by anaerobic respiration to the electron acceptors formed in this process. In either case, POM would act as TEA that fuels anaerobic respiration.

Based on the herein reported number of electrons transferred to POM during anoxic in situ incubation (i.e., the decrease in EAC from POM_{ref} to POM_{ref}^{red}), we can estimate the extent to which anaerobic respiration to POM competitively suppresses methanogenesis. Conservatively assuming a cumulative yearly water-table fluctuation of 25 cm (Fraser et al., 2001; Treat et al., 2007) and bulk densities of 67 g L^{-1} for a peat soil (Obradović et al., 2023), we calculate that 170 Tg POM accepts electrons per km^2 of a peatland soil. This amount of POM is estimated to accept around 27×10^6 mol e^- km^{-2} yr^{-1}. For suppressing the formation of 1 mol CH_4 through anaerobic respiration instead of methanogenesis (at a substrate NOSC of zero), 8 mol e^- need to be transferred to the POM (Obradović et al., 2023). We therefore estimate that POM reduction can lower CH_4 formation in peatlands by 33×10^5 mol CH_4 km^{-2} per year. This estimate highlights the potential importance of this respiration pathway when compared with reported annual methane emissions of $1–18 \times 10^5$ mol CH_4 km^{-2} for northern peatlands (Blodau, 2002), and 43×10^5 mol CH_4 km^{-2} for peatlands globally (Guth et al., 2022).

Data Availability Statement

The data sets required to analyse the results from this manuscript are provided as Supplementary Information and separate. csv files. These are published at the ETH Research Collection webpage (https://www.research-collection.ethz.ch/; Obradović et al., 2024). All data analyses and plotting were performed in open-access software RStudio (Posit, 2022; Signorell et al., 2017; Wickham, 2016; Wickham & Bryan, 2023; Wickham et al., 2019, 2023; Wilke, 2020).

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References

Aeppli, M., Giroud, S., Vranic, S., Voegelin, A., Hofstetter, T. B., & Sander, M. (2022). Thermodynamic controls on rates of iron oxide reduction by extracellular electron shuttles. *Proceedings of the National Academy of Sciences*, *119*(3), e2115629119. https://doi.org/10.1073/pnas.2115629119

Aeschbacher, M., Schwarzenbach, R. P., & Sander, M. (2012). Antioxidant properties of humic substances. *Environmental Science & Technology*, *46*(9), 4916–4925. https://doi.org/10.1021/es300039h

Aeschbacher, M., Vergari, D., Schwarzenbach, R. P., & Sander, M. (2011). Electrochemical analysis of proton and electron transfer equilibria of the reducible moieties in humic acids. *Environmental Science & Technology*, *45*(19), 8385–8394. https://doi.org/10.1021/es201981g

Almquist-Jacobson, H., & Foster, D. R. (1995). Toward an integrated model for raised-bog development: Theory and field evidence. *Ecology*, *76*(8), 2503–2516. https://doi.org/10.2307/2265824

Barreto, C., & Lindo, Z. (2018). Drivers of decomposition and the detrital invertebrate community differ across a hummock-hollow microtopology in Boreal peatlands. *Écoscience*, *25*(1), 39–48. https://doi.org/10.1080/11956860.2017.1412282

Belyea, L. R. (1996). Separating the effects of litter quality and microenvironment on decomposition rates in a patterned peatland. *Oikos*, *77*(3), 529–539. https://doi.org/10.2307/3545942

Björn, L. O., Middleton, B. A., Germ, M., & Gaberščik, A. (2022). Ventilation systems in wetland plant species. *Diversity*, *14*(7), 517–538. https://doi.org/10.3390/d14070517

Blodau, C. (2002). Carbon cycling in peatlands – A review of processes and controls. *Environmental Reviews*, *10*(2), 111–134. https://doi.org/10.1139/a02-004

Boye, K., Noel, V., Tfaily, M. M., Bone, S. E., Williams, K. H., Bargar, J. R., & Fendorf, S. (2017). Thermodynamically controlled preservation of organic carbon in floodplains. *Nature Geoscience*, *10*(6), 415–419. https://doi.org/10.1038/NGEO2940

Conrad, R. (1999). Contribution of hydrogen to methane production and control of hydrogen concentrations in methanogenic soils and sediments. *FEMS Microbiology Ecology*, *28*(3), 193–202. https://doi.org/10.1016/s0168-6496(98)00086-5

Corbett, J. E., Burdige, D. J., Tfaily, M. M., Dial, A. R., Cooper, W. T., Glaser, P. H., & Chanton, J. P. (2013). Surface production fuels deep heterotrophic respiration in northern peatlands. *Global Biogeochemical Cycles*, *27*(4), 1163–1174. https://doi.org/10.1002/2013gb004677

Corbett, J. E., Tfaily, M. M., Burdige, D. J., Cooper, W. T., Glaser, P. H., & Chanton, J. P. (2013). Partitioning pathways of CO_2 production in peatlands with stable carbon isotopes. *Biogeochemistry*, *114*(1–3), 327–340. https://doi.org/10.1007/s10533-012-9813-1

Estop-Aragonés, C., Knorr, K.-H., & Blodau, C. (2012). Controls on in situ oxygen and dissolved inorganic carbon dynamics in peats of a temperate fen. *Journal of Geophysical Research*, *117*(52), 1–14. https://doi.org/10.1029/2011JG001888

Eyer, P. (1991). Effects of superoxide dismutase on the autoxidation of 1,4-hydroquinone. *Chemico-Biological Interactions*, *80*(2), 159–176. https://doi.org/10.1016/0009-2797(91)90022-y

Finér, L., & Laine, J. (2000). The ingrowth bag method in measuring root production on peatland sites. *Scandinavian Journal of Forest Research*, *15*(1), 75–80. https://doi.org/10.1080/02827580050160493

Fraser, C. J. D., Roulet, N. T., & Moore, T. R. (2001). Hydrology and dissolved organic carbon biogeochemistry in an ombrotrophic bog. *Hydrology*, *15*(16), 3151–3166. https://doi.org/10.1002/hyp.322

Friedrich, M. W. (2005). Methyl-coenzyme M reductase genes: Unique functional markers for methanogenic and anaerobic methane-oxidizing archaea. *Methods in Enzymology*, 428–442. https://doi.org/10.1016/s0076-6879(05)97026-2

Gabriel, K. N., Medvedeff, C. A., & Keller, J. K. (2017). Microbial organic matter reduction in a peatland soil: The importance of water-table level. *BIOS*, *88*(1), 39–45. https://doi.org/10.1893/BIOS-D-16-00002.1

Gao, C., Sander, M., Agethen, S., & Knorr, K.-H. (2019). Electron accepting capacity of dissolved and particulate organic matter control CO_2 and CH_4 formation in peat soils. *Geochimica et Cosmochimica Acta*, *245*, 266–277. https://doi.org/10.1016/j.gca.2018.11.004

Górecki, K., Rastogi, A., Strozecki, M., Gabka, M., Lamentowicz, M., Lucow, D., et al. (2021). Water table depth, experimental warming, and reduced precipitation impact on litter decomposition in a temperate Sphagnum-peatland. *Science of the Total Environment*, *771*, 145452. https://doi.org/10.1016/j.scitotenv.2021.145452

Gorski, C. A., Klüpfel, L., Voegelin, A., Sander, M., & Hofstetter, T. B. (2012). Redox properties of structural Fe in clay minerals. 2. Electrochemical and spectroscopic characterization of electron transfer irreversibility in ferruginous smectite, SWa-1. *Environmental Science & Technology*, *46*(17), 9369–9377. https://doi.org/10.1021/es302014u

Guth, P., Gao, C., & Knorr, K.-H. (2022). Electron accepting capacities of a wide variety of peat materials from around the globe similarly explain CO_2 and CH_4 formation. *Journal of Geophysical Research: Biogeosciences*, *37*, 1–20. https://doi.org/10.1029/2022GB007459

Inglett, K. S., Inglett, P. W., Reddy, K. R., & Osborne, T. Z. (2012). Temperature sensitivity of greenhouse gas production in wetland soils of different vegetation. *Biogeochemistry*, *108*(1–3), 77–90. https://doi.org/10.1007/s10533-011-9573-3

Joshi, P., Schroth, M. H., & Sander, M. (2021). Redox properties of peat particulate organic matter: Quantification of electron accepting capacities and assessment of electron transfer reversibility. *Journal of Geophysical Research: Biogeosciences*, *126*(8), 1–18. https://doi.org/10.1029/2021JG006329

Keller, J. K., Bridgham, S. D., Takagi, K. K., Zalman, C. A., Rush, J. E., Anderson, C., et al. (2023). Microbial organic matter reduction regulates methane and carbon dioxide production across an ombrotrophic-minerotrophic peatland gradient. *Soil Biology and Biochemistry*, *182*, 109045. https://doi.org/10.1016/j.soilbio.2023.109045

Keller, J. K., & Takagi, K. K. (2013). Solid-phase organic matter reduction regulates anaerobic decomposition in bog soil. *Ecosphere*, *4*(5), 1–12. https://doi.org/10.1890/ES12-00382.1

Keller, J. K., Weisenhorn, P. B., & Megonigal, J. P. (2009). Humic acids as electron acceptors in wetland decomposition. *Soil Biology and Biochemistry*, *41*(7), 1518–1522. https://doi.org/10.1016/j.soilbio.2009.04.008

Klüpfel, L., Piepenbrock, A., Kappler, A., & Sander, M. (2014). Humic substances as fully regenerable electron acceptors in recurrently anoxic environments. *Nature Geoscience*, *7*(3), 195–200. https://doi.org/10.1038/ngeo2084

LaRowe, D. E., & van Cappellen, P. (2011). Degradation of natural organic matter: A thermodynamic analysis. *Geochimica et Cosmochimica Acta*, *75*(8), 2030–2042. https://doi.org/10.1016/j.gca.2011.01.020

Leifeld, J., Klein, K., & Wüst-Galley, C. (2020). Soil organic matter stoichiometry as indicator for peatland degradation. *Scientific Reports*, *10*(1), 7634–7642. https://doi.org/10.1038/s41598-020-64275-y

Lindberg, R. D., & Runnells, D. D. (1984). Ground water redox reactions: An analysis of equilibrium state applied to EH measurements and geochemical modeling. *Science*, *225*(4665), 925–927. https://doi.org/10.1126/science.225.4665.925

Lindsay, R. (2016). Peatland (mire types): Based on origin and behavior of water, peat genesis, landscape position, and climate. In C. Finlayson, G. Milton, R. Prentice, & N. Davidson (Eds.), *The wetland book*. Springer. https://doi.org/10.1007/978-94-007-6173-5_279-1

Lovley, D. R., Coates, J. D., Blunt-Harris, E. L., Phillips, E. J. P., & Woodward, J. C. (1996). Humic substances as electron acceptors for microbial respiration. *Nature*, *382*(6590), 445–448. https://doi.org/10.1038/382445a0

Lovley, D. R., Fraga, J. L., Blunt-Harris, E. L., Hayes, L. A., Phillips, E. J., & Coates, J. D. (1998). Humic substances as a mediator for microbially catalyzed metal reduction. *Acta Hydrochimica et Hydrobiologica*, *26*(3), 152–157. https://doi.org/10.1002/(sici)1521-401x(199805)26:3<152::aid-aheh152>3.0.co;2-d

Madigan, M. T., Martinko, J. M., Stahl, D. A., & Clark, D. P. (2012). *Brock biology of microorganisms*. Pearson Education.

Martí, M., Juottonen, H., Robroek, B. J. M., Yrjälä, K., Danielsson, Å., Lindgren, P. E., & Svensson, B. H. (2015). Nitrogen and methanogen community composition within and among three Sphagnum dominated peatlands in Scandinavia. *Soil Biology and Biochemistry*, *81*, 204–211. https://doi.org/10.1016/j.soilbio.2014.11.016

McGivern, B. B., Cronin, D. R., Ellenbogen, J. B., Borton, M. A., Knutson, E. L., Freire-Zapata, V., et al. (2024). Microbial polyphenol metabolism is part of the thawing permafrost carbon cycle. *Nature Microbiology*, *9*(6), 1454–1466. https://doi.org/10.1038/s41564-024-01691-0

Morris, J. C., & Stumm, W. (1967). Redox equilibria and measurements of potentials in the aquatic environment. *Advances in Chemistry*, 270–285. https://doi.org/10.1021/ba-1967-0067.ch013

Obradović, N., Joshi, P., Arn, S., Aeppli, M., Schroth, M. H., & Sander, M. (2023). Reoxidation of reduced peat organic matter by dissolved oxygen: Combined Laboratory Column-Breakthrough Experiments and in-field push-pull tests. *Journal of Geophysical Research: Biogeosciences*, *128*(11). https://doi.org/10.1029/2023jg007640

Obradović, N., Schmitz, R. A., Haffter, F., Meier, D., Lever, M., Schroth, M. H., & Sander, M. (2024). Peat particulate organic matter accepts electrons during *in situ* incubation in the anoxic subsurface of ombrotrophic bogs [Dataset]. https://doi.org/10.3929/ethz-b-000690096

Posit team. (2022). RStudio: Integrated development environment for R [Software]. *Posit Software, BC, Boston, MA. (Version 2022.12.0.353, USA)*. http://www.posit.co/

Roden, E. E., Kappler, A., Bauer, I., Jiang, J., Paul, A., Stoesser, R., et al. (2010). Extracellular electron transfer through microbial reduction of solid-phase humic substances. *Nature Geoscience*, *3*(6), 417–421. https://doi.org/10.1038/ngeo870

Roginsky, V., & Barsukova, T. (2000). Kinetics of oxidation of hydroquinones by molecular oxygen. Effect of superoxide dismutase. *Journal of the Chemical Society, Perkin Transactions*, *2*(7), 1575–1582. https://doi.org/10.1039/b000538j

Rush, E. J., Zalman, A. C., Woerndle, G., Hanna, L. E., Bridgham, D. S., & Keller, K. J. (2021). Warming promotes the use of organic matter as an electron acceptor in a peatland. *Geoderma*, *401*, 115303–115310. https://doi.org/10.1016/j.geoderma.2021.115303

Rydin, H., Jeglum, J. K., & Hooijer, A. (2013). *The biology of peatlands*. Oxford University Press.

Sander, M., Hofstetter, T. B., & Gorski, C. A. (2015). Electrochemical analyses of redox-active iron minerals: A review of nonmediated and mediated approaches. *Environmental Science & Technology*, *49*(10), 5862–5878. https://doi.org/10.1021/acs.est.5b00006

Saunois, M., Bousquet, P., Poulter, B., Peregon, A., Ciais, P., Canadell, J. G., et al. (2016). The global methane budget 2000–2012. *Earth System Science Data*, *8*(2), 697–751. https://doi.org/10.5194/essd-8-697-2016

Scheller, S., Goenrich, M., Boecher, R., Thauer, R. K., & Jaun, B. (2010). The key nickel enzyme of methanogenesis catalyses the anaerobic oxidation of methane. *Nature*, *465*(7298), 606–608. https://doi.org/10.1038/nature09015

Segers, R., & Kengen, S. W. M. (1998). Methane production as a function of anaerobic carbon mineralization: A process model. *Soil Biology and Biochemistry*, *30*(8–9), 1107–1117. https://doi.org/10.1016/s0038-0717(97)00198-3

Shima, S., & Thauer, R. K. (2005). Methyl-coenzyme M reductase and the anaerobic oxidation of methane in methanotrophic archaea. *Current Opinion in Microbiology*, *8*(6), 643–648. https://doi.org/10.1016/j.mib.2005.10.002

Signorell, A., Aho, K., Alfons, A., Anderegg, N., Aragon, T., Arachchige, C., et al. (2017). DescTools: Tools for descriptive statistics [Software]. *R package version 0.99.23*. https://github.com/AndriSignorell/DescTools/

Smemo, K. A., & Yavitt, J. B. (2007). Evidence for anaerobic CH_4 oxidation in freshwater peatlands. *Geomicrobiology Journal*, *24*(7–8), 583–597. https://doi.org/10.1080/01490450701672083

Smemo, K. A., & Yavitt, J. B. (2011). Anaerobic oxidation of methane: An underappreciated aspect of methane cycling in peatland ecosystems? *Biogeosciences*, *8*(3), 779–793. https://doi.org/10.5194/bg-8-779-2011

Speight, J. G., & Lange, N. A. (2017). *Lange's handbook of chemistry*. McGraw-Hill Education.

Straub, K., Benz, M., & Schink, B. (2001). Iron metabolism in anoxic environments at near neutral pH. *FEMS Microbiology Ecology*, *34*(3), 181–186. https://doi.org/10.1016/s0168-6496(00)00088-x

Stumm, W. (1984). Interpretation and measurement of redox intensity in Natural Waters. *Schweizerische Zeitschrift für Hydrologie*, *46*(2), 291–296. https://doi.org/10.1007/bf02538067

Teickner, H., Gao, C., & Knorr, K.-H. (2022). Electrochemical properties of peat particulate organic matter on a global scale: Relation to peat chemistry and degree of decomposition. *Global Biogeochemical Cycles*, *36*(2), e2021GB007160. https://doi.org/10.1029/2021GB007160

Thauer, R. K. (2019). Methyl (alkyl)-coenzyme M reductases: Nickel F-430-containing enzymes involved in anaerobic methane formation and in anaerobic oxidation of methane or of short chain alkanes. *Biochemistry*, *58*(52), 5198–5220. https://doi.org/10.1021/acs.biochem.9b00164

Treat, C. C., Bubier, J. L., Varner, R. K., & Crill, P. M. (2007). Timescale dependence of environmental and plant-mediated controls on CH_4 flux in a temperate fen. *Journal of Geophysical Research*, *112*(G1), G01014. https://doi.org/10.1029/2006JG000210

Walpen, N., Getzinger, G. J., Schroth, M. H., & Sander, M. (2018). Electron-Donating phenolic and electron-accepting quinone moieties in peat dissolved organic matter: Quantities and redox transformations in the context of peat biogeochemistry. *Environmental Science & Technology*, *52*(9), 5236–5245. https://doi.org/10.1021/acs.est.8b00594

Walpen, N., Lau, M. P., Fiskal, A., Getzinger, G. J., Meyer, S. A., Nelson, T. F., et al. (2018). Oxidation of reduced peat particulate organic matter by dissolved oxygen: Quantification of apparent rate constants in the field. *Environmental Science & Technology*, *52*(19), 11151–11160. https://doi.org/10.1021/acs.est.8b03419

Wickham, H. (2016). ggplot2: Elegant graphics for data analysis [Software]. https://ggplot2.tidyverse.org

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L. D., François, R., et al. (2019). Welcome to the tidyverse [Software]. *Journal of Open Source Software*, *4*(43), 1686. https://doi.org/10.21105/joss.01686

Wickham, H., & Bryan, J. (2023). readxl: Read excel files [Software]. *GitHub*. https://github.com/tidyverse/readxl

Wickham, H., François, R., Henry, L., Müller, K., & Vaughan, D. (2023). dplyr: A grammar of data manipulation [Software]. *GitHub*. https://github.com/tidyverse/dplyr

Wilke, C. (2020). cowplot: Streamlined plot theme and plot annotations for 'ggplot2' [Software]. *R package version 1.1.1*. https://CRAN.R-project.org/package=cowplot

Wilson, R. M., Tfaily, M. M., Rich, V. I., Keller, J. K., Bridgham, S. D., Zalman, C. M., et al. (2017). Hydrogenation of organic matter as terminal electron sink sustains high CO_2:CH_4 production ratios during anaerobic decomposition. *Organic Geochemistry*, *112*, 22–32. https://doi.org/10.1016/j.orggeochem.2017.06.011

Yavitt, J. B., Lang, G. E., & Wieder, R. K. (1987). Control of carbon mineralization to CH_4 and CO_2 in anaerobic, sphagnum-derived peat from Big Run Bog, West Virginia. *Biogeochemistry*, *4*(2), 141–157. https://doi.org/10.1007/bf02180152

Yu, Z.-G., Göttlicher, J., Steininger, R., & Knorr, K.-H. (2016). Organic sulfur and organic matter redox processes contribute to electron flow in anoxic incubations of peat. *Environmental Chemistry*, *13*(5), 816–825. https://doi.org/10.1071/en15091

References From the Supporting Information

Cadillo-Quiroz, H., Bräuer, S., Yashiro, E., Sun, C., Yavitt, J., & Zinder, S. (2006). Vertical profiles of methanogenesis and methanogens in two contrasting acidic peatlands in central New York State, USA. *Environmental Microbiology*, *8*, 1428–1440. https://doi.org/10.1111/j.1462-2920.2006.01036.x

Lever, M. A., Torti, A., Eickenbusch, P., Michaud, A. B., Šantl-Temkiv, T., & Jørgensen, B. B. (2015). A modular method for the extraction of DNA and RNA, and the separation of DNA pools from diverse environmental sample types. *Frontiers in Microbiology*, *6*, 476. https://doi.org/10.3389/fmicb.2015.00476

Ohkuma, M., & Kudo, T. (1998). Phylogenetic analysis of the symbiotic intestinal microflora of the termite *Cryptotermes domesticus*. *FEMS Microbiology Letters*, *164*(2), 389–395. https://doi.org/10.1111/j.1574-6968.1998.tb13114.x

Steinberg, L. M., & Regan, J. M. (2008). Phylogenetic comparison of the methanogenic communities from an acidic, oligotrophic fen and an anaerobic digester treating municipal wastewater sludge. *Applied and Environmental Microbiology*, *74*(21), 6663–6671. https://doi.org/10.1128/AEM.00553-08

Yu, Y., Lee, C., Kim, J., & Hwang, S. (2005). Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. *Biotechnology and Bioengineering*, *89*(6), 670–679. https://doi.org/10.1002/bit.20347