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# JGR Biogeosciences

## RESEARCH ARTICLE

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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  $\mu\text{mol}$  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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# Peat Particulate Organic Matter Accepts Electrons During In Situ Incubation in the Anoxic Subsurface of Ombrotrophic Bogs

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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  $\mu\text{mol}$  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  $\text{CO}_2$  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 ( $\text{CO}_2$ ) and methane ( $\text{CH}_4$ ) from peat soils. The use of POM as TEA allows for continuous substrate turnover to  $\text{CO}_2$  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<sub>4</sub> 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<sub>4</sub> is oxidized to CO<sub>2</sub> with POM as TEA (Smemo & Yavitt, 2007, 2011). Both pathways have also been hypothesized to substantially lower CH<sub>4</sub> emissions from peatlands and thus are of direct relevance to global atmospheric CH<sub>4</sub> budgets, given that peatlands are a major natural source of CH<sub>4</sub> (Saunio 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<sub>2</sub> and CH<sub>4</sub> (Conrad, 1999). Yet, many of these studies reported CO<sub>2</sub>:CH<sub>4</sub> 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<sub>2</sub> 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<sup>-</sup> (g dry POM)<sup>-1</sup>. 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<sup>3+</sup>-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 Storhultsmossen (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<sub>2</sub> and CH<sub>4</sub> 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<sub>2</sub> (grade 5.0, 99.999%, Linde Gas, Dagsmorsellen, Switzerland) for at least 2 hr prior to transferring them into an anoxic glovebox (pure N<sub>2</sub> atmosphere, <2.3 ppm O<sub>2</sub>, 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<sub>ref</sub>)

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<sub>ref</sub>) from Stockås Torvströ (Torv and Maskinentreorad AB, Mullhyttan, Sweden; Joshi et al., 2021). The POM<sub>ref</sub> 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<sub>ref</sub> 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<sub>ref</sub> material from a single batch. As POM<sub>ref</sub> 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<sub>ref</sub> 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<sub>2</sub>-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<sub>2</sub> and CH<sub>4</sub> from the solution into the headspace of the vials. Upon return to the laboratory, we quantified CO<sub>2</sub> and CH<sub>4</sub> 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 archaeal 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.

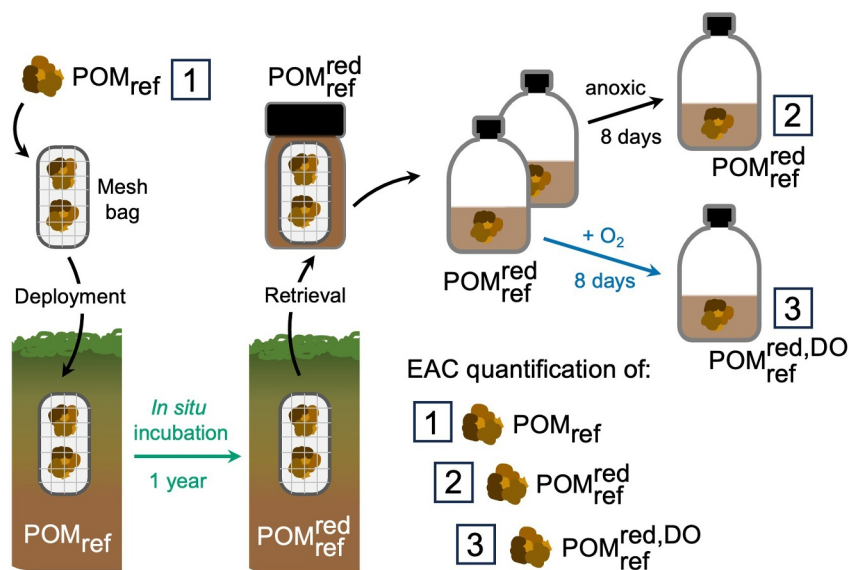
#### 2.3.3. Quantification of Pore-Water CH<sub>4</sub> and CO<sub>2</sub> Concentrations

Headspace CH<sub>4</sub> and CO<sub>2</sub> 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<sub>2</sub> (grade 5.0) as carrier gas and H<sub>2</sub> (5.0, all gases from Linde Gas) as fuel gas for the methanizer detector (a modified flame-ionization detector). In this detector, CH<sub>4</sub> is quantified directly, but CO<sub>2</sub>—to allow its detection and quantification—is first reduced to CH<sub>4</sub> by H<sub>2</sub> on a Ni catalyst operated at 380°C. We then converted the obtained CH<sub>4</sub> and CO<sub>2</sub> concentrations (in μL L<sup>-1</sup>) from GC vials to molar concentrations (μmol L<sup>-1</sup>) using the ideal gas law. Taking the headspace and pore-water sample volumes into account, we report total CO<sub>2</sub> and total CH<sub>4</sub> concentrations present in the peat pore-water samples. More information on CO<sub>2</sub> and CH<sub>4</sub> quantification is provided in Text S2 in Supporting Information S1.

### 2.4. Field Burial Experiment to Assess In Situ Electron Transfer to POM<sub>ref</sub>

#### 2.4.1. Mesh-Bag Preparation and Field Deployment

In the laboratory we transferred 1.5 g of POM<sub>ref</sub> 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<sub>ref</sub> 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<sup>-1</sup> (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<sup>-1</sup> 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<sub>2</sub> 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<sub>ref</sub><sup>red</sup> re-oxidation by DO at pH 7 instead of at native pH. These experiments were conducted only on POM<sub>ref</sub><sup>red</sup> 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<sub>ref</sub><sup>red</sup> 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<sub>ref</sub><sup>red</sup> 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<sup>•-</sup> radical; final concentration 280 μM). The chemical reductant was obtained by bulk electrochemical reduction of oxidized ZiV<sup>0</sup>. The EAC measurements were performed at pH 7 because of optimal stability of ZiV<sup>•-</sup> 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<sup>•-</sup>, we filtered suspension aliquots (1.5 mL) of each triplicate reactor using 0.22 μm cellulose acetate filters (BGB) and quantified the residual ZiV<sup>•-</sup> concentration spectrophotometrically at 603 nm (UV5 Bio, Mettler-Toledo, Switzerland). Two additional sets of triplicate controls were run in parallel to POM/ZiV<sup>•-</sup> triplicates. The first set contained only ZiV<sup>•-</sup> and buffer and was used to determine the extent of slow ZiV<sup>•-</sup> oxidation over 24 hr in the absence of POM. The second set contained only POM and buffer, without ZiV<sup>•-</sup>. 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):

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

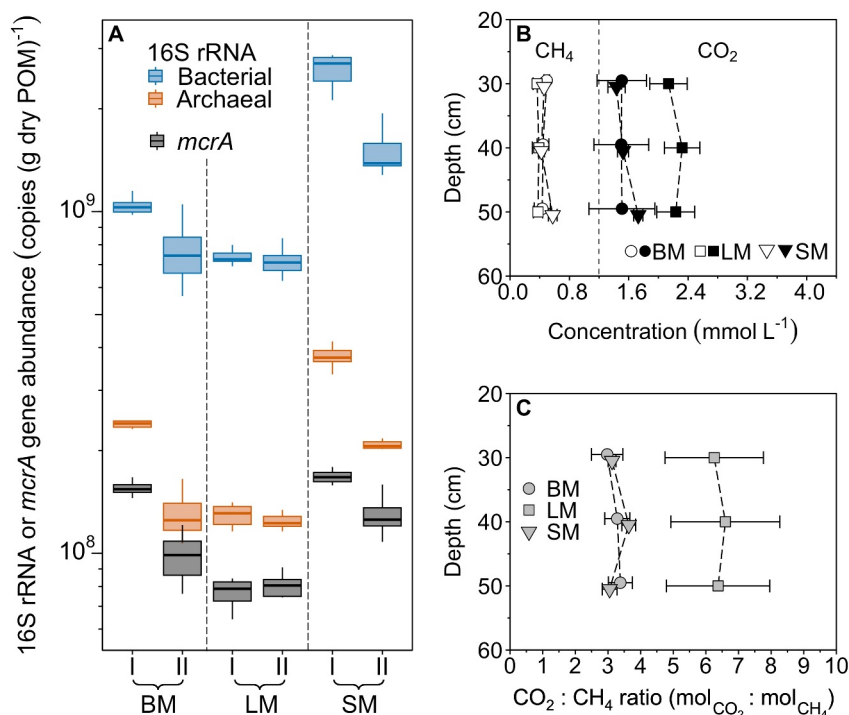
where  $A_{\text{only ZiV}^{\bullet-}}$  (AU) is the average absorbance of ZiV<sup>•-</sup>-containing solutions from the triplicate POM-free controls,  $A_{\text{POM \& ZiV}^{\bullet-}}$  (AU) is the average absorbance of ZiV<sup>•-</sup>-containing solution from the triplicate POM-containing vials,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

Finally, we calculated the differences in EAC (i.e., ΔEAC) for each POM<sub>ref</sub> and POM<sub>ref</sub><sup>red</sup> 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<sub>ref</sub> Incubations

We characterized the peat soils at the sites that we selected for deployment and incubation of the POM<sub>ref</sub>. The characterization included determining the genetic potential of the microbial community for methanogenesis, as well as the quantification of pore-water CO<sub>2</sub> and CH<sub>4</sub> concentrations, both as indicators for in situ



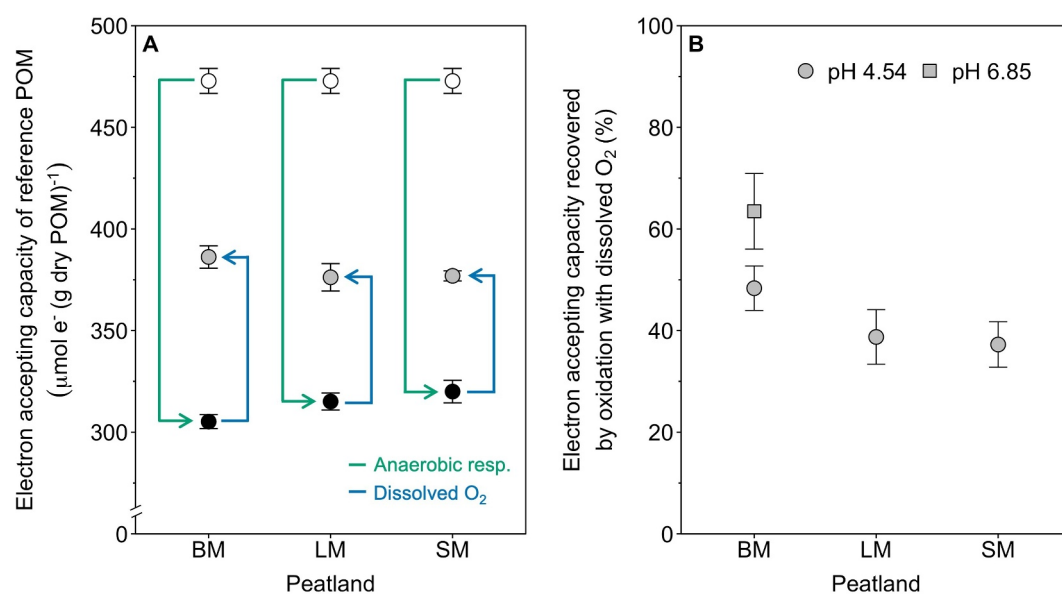
**Figure 2.** Characterization of peat soils in the three selected ombrotrophic bogs Björsmossen (BM), Lungsmissen (LM), and Storhultsmossen (SM) for in situ incubations of reference peat particulate organic matter (POM<sub>ref</sub>). (a) Gene copy numbers of the archeal and bacterial 16S rRNA genes, 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<sub>2</sub>) and dissolved methane (CH<sub>4</sub>), and (c) the resulting molar CO<sub>2</sub>:CH<sub>4</sub> 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<sub>ref</sub>, we aimed to deploy samples in methanogenic systems given that anaerobic respiration to POM has been implicated in the decrease of pore-water CH<sub>4</sub> 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  $2.6 \times 10^9$  copies (g dry POM)<sup>-1</sup>) outnumbered the archeal 16S rRNA gene copies 6-fold on average. The ratio of *mcrA* gene copies to archeal 16S rRNA gene copies was  $0.62 \pm 0.1$ . 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 ( $6 \times 10^8$  *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<sub>2</sub> and CH<sub>4</sub> in the pore water of BM, LM and SM for depths of 30, 40 and 50 cm (Figure 2b). Neither dissolved CO<sub>2</sub> nor CH<sub>4</sub> concentrations showed an apparent depth dependence. The concentrations were 1.4–2.3 mM CO<sub>2</sub> and 0.36–0.58 mM CH<sub>4</sub>, which translated to a narrow range of CO<sub>2</sub>:CH<sub>4</sub> molar ratios of approximately 3–6.5 mol CO<sub>2</sub>: mol CH<sub>4</sub> (Figure 2c). The presence of pore-water dissolved CH<sub>4</sub> 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<sub>2</sub> and CH<sub>4</sub> were 19–31 and 3–4 times lower than the water solubilities of CO<sub>2</sub> (44.73 mM) and CH<sub>4</sub> (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  $POM_{ref}^{red}$  (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  $473 \pm 6 \mu\text{mol e}^- (\text{g dry POM})^{-1}$  ( $n = 4$ ) (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  $\mu\text{mol e}^- (\text{g dry POM})^{-1}$  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  $473 \pm 6 \mu\text{mol e}^- (\text{g dry POM})^{-1}$  to values of  $305 \pm 3$ ,  $315 \pm 4$  and  $320 \pm 6 \mu\text{mol e}^- (\text{g dry POM})^{-1}$ , respectively (Figure 3a; *t*-test, degrees of freedom ( $df$ ) = 7,  $p \ll 0.05$ , significance level ( $\alpha$ ) = 0.05). This EAC decrease indicates that approximately 150–170  $\mu\text{mol e}^- (\text{g dry POM})^{-1}$  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).

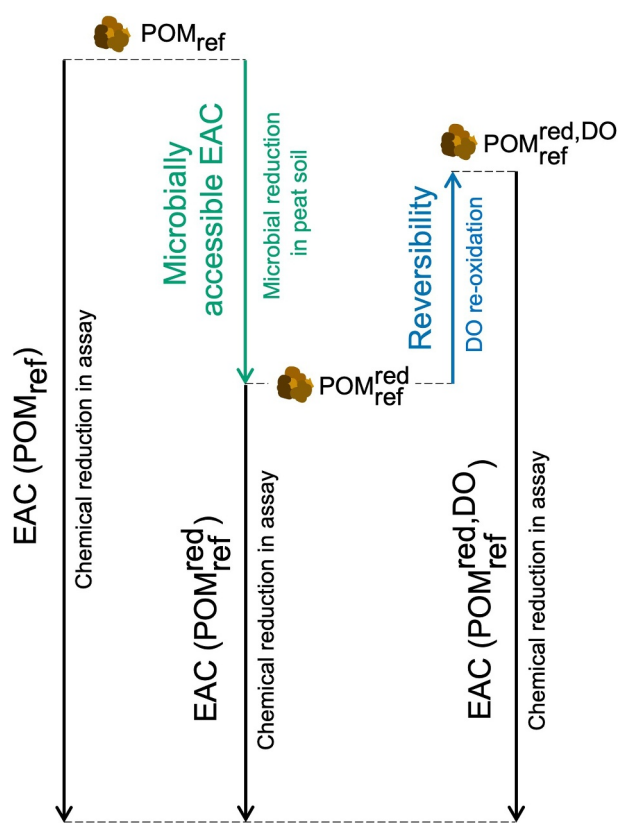
The finding that  $POM_{ref}^{red}$  exhibited non-zero EAC values implies that it still contained redox-active moieties that are chemically reducible in the EAC assay and, therefore, that in situ incubation resulted in only partial reduction of  $POM_{ref}$ . We cannot completely rule out that incomplete 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  $POM_{ref}^{red}$  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  $386 \pm 6$ ,  $376 \pm 7$  and  $377 \pm 3$   $\mu\text{mol e}^- (\text{g dry POM})^{-1}$ , 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.,  $POM_{ref}^{red}$ ) 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,  $POM_{ref}^{red,DO}$ . The difference between EAC( $POM_{ref}$ ) and EAC ( $POM_{ref}^{red}$ ) 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 ( $POM_{ref}^{red,DO}$ ) and EAC ( $POM_{ref}^{red}$ ) 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  $POM_{ref}^{red}$  with DO therefore removed between 57 and 81  $\mu\text{mol e}^- (\text{g dry POM})^{-1}$  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  $90 \pm 40 \mu\text{mol e}^- (\text{g dry POM})^{-1}$  (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  $POM_{ref}^{red}$  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  $POM_{ref}^{red,DO}$  as compared to  $POM_{ref}^{red}$  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  $POM_{ref}^{red,DO}$  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  $POM_{ref}^{red}$  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  $POM_{ref}^{red}$  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  $\text{POM}_{\text{ref}}^{\text{red}}$  retrieved from BM with DO for 8 days at a solution pH of 6.85 (Figures 3a and 3b). We chose to test only  $\text{POM}_{\text{ref}}^{\text{red}}$  from BM given that incubations in all three bogs resulted in comparable POM reduction extents. Consistent with the above kinetic explanation, reaction of  $\text{POM}_{\text{ref}}^{\text{red}}$  with DO at the higher pH resulted in a significantly larger EAC increase of the POM of  $106 \pm 12 \mu\text{mol e}^- (\text{g dry POM})^{-1}$ , 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  $\text{POM}_{\text{ref}}$  during in situ incubation being restored). We note that there was no significant change in the EAC of  $\text{POM}_{\text{ref}}^{\text{red}}$  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  $\text{POM}_{\text{ref}}$  during the in situ incubation was more reversible than was apparent from the 8-day reaction of  $\text{POM}_{\text{ref}}^{\text{red}}$  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 $\text{POM}_{\text{ref}}$ Incubated at a Shallower Depth

In addition to determining  $\text{POM}_{\text{ref}}$  reduction during incubation at  $\sim 40$  cm depth in the three bogs (see above), we also deployed  $\text{POM}_{\text{ref}}$ -filled mesh bags in BM at an additional depth of  $\sim 10$  cm below the surface. These incubations served to assess whether  $\text{POM}_{\text{ref}}$  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  $\text{POM}_{\text{ref}}^{\text{red}}$  from  $\sim 10$  cm depth was  $315 \pm 21 \mu\text{mol e}^- (\text{g dry POM})^{-1}$  and thus statistically indistinguishable from the EAC of  $\text{POM}_{\text{ref}}^{\text{red}}$  retrieved from  $\sim 40$  cm depth ( $t$ -test,  $df = 8$ ,  $p = 0.40$ ,  $\alpha = 0.05$ ). After 8 days of re-oxidation of  $\text{POM}_{\text{ref}}^{\text{red}}$  from  $\sim 10$  cm depth by DO, we observed an increase in EAC to  $383 \pm 18 \mu\text{mol e}^- (\text{g dry POM})^{-1}$ , which was also statistically indistinguishable from the increase observed during re-oxidation of  $\text{POM}_{\text{ref}}^{\text{red}}$  collected from  $\sim 40$  cm depth ( $t$ -test,  $df = 8$ ,  $p = 0.78$ ,  $\alpha = 0.05$ ). These findings suggest that the reference POM incubated at  $\sim 10$  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-Aragónés 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  $\sim 40$  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  $\sim 40$  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  $\text{CO}_2$  and  $\text{CH}_4$  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<sub>2</sub> and CH<sub>4</sub> 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<sub>2</sub> formation over time). Co-occurrence and stoichiometric agreement between the EAC decrease and the excess CO<sub>2</sub> formation would suggest that POM reduction is directly driven by anaerobic respiration. Conversely, faster POM reduction than excess CO<sub>2</sub> 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<sub>ref</sub> to POM<sub>ref</sub><sup>red</sup>), 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<sup>-1</sup> for a peat soil (Obradović et al., 2023), we calculate that 170 Tg POM accepts electrons per km<sup>2</sup> of a peatland soil. This amount of POM is estimated to accept around 27 × 10<sup>6</sup> mol e<sup>-</sup> km<sup>-2</sup> yr<sup>-1</sup>. For suppressing the formation of 1 mol CH<sub>4</sub> through anaerobic respiration instead of methanogenesis (at a substrate NOSC of zero), 8 mol e<sup>-</sup> need to be transferred to the POM (Obradović et al., 2023). We therefore estimate that POM reduction can lower CH<sub>4</sub> formation in peatlands by 33 × 10<sup>5</sup> mol CH<sub>4</sub> km<sup>-2</sup> per year. This estimate highlights the potential importance of this respiration pathway when compared with reported annual methane emissions of 1–18 × 10<sup>5</sup> mol CH<sub>4</sub> km<sup>-2</sup> for northern peatlands (Blodau, 2002), and 43 × 10<sup>5</sup> mol CH<sub>4</sub> km<sup>-2</sup> 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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