Redox Properties of Peat Particulate Organic Matter: Quantification of Electron Accepting Capacities and Assessment of Electron Transfer Reversibility

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Abstract  Peat particulate organic matter (POM) has been hypothesized to act as a terminal electron acceptor (TEA) for anaerobic microbial respiration in northern peatlands, thereby lowering dissolved methane (CH₄) concentrations in these systems. However, the redox properties of peat POM and an assessment of its redox state in situ remain missing, mainly due to the lack of an accurate analytical approach to quantify the number of electrons that are transferred to/from POM. Here, we first developed a new spectrophotometric method to quantify the number of electrons that are transferrable to peat POM—denoted hereafter as electron accepting capacity (EAC)—by reacting POM with the reduced species of the probe compound 4,4′-bipyridinium-1,1′bis(2-ethylsulfonate). Second, we used this analytical approach to quantify the EAC of an oxidized reference POM material (0.38 ± 0.039 mmol e⁻/g POM) and of redox-preserved POM from three ombrotrophic bogs in Värmland, Sweden (0.17–0.24 mmol e⁻/g POM). These EAC values are substantially higher than previous estimates, likely reflecting kinetic artifacts in previous analytical methods. We further established reversible electron transfer to the oxic reference POM over a cycle of electrochemical reduction and O₂ re-oxidation. Finally, we determined that the EAC of two of the three bog POM samples that were collected anoxically (i.e., redox state-preserved POM) increased by ~0.09 mmol e⁻/g POM upon exposure to air for 8 days, demonstrating that the POM was indeed reduced in situ. Collectively, our results strongly support that POM acts as a sustainable TEA in northern peatlands, which may lower methane release from these systems.

Plain Language Summary  Northern peatlands have accumulated significant amounts of peat organic matter since the last glaciation and now store a significant portion of the total global soil organic carbon. Global warming has triggered interest in understanding organic matter breakdown processes in northern peatland soils which result in the release of stored carbon, particularly in form of the potent warming gas methane (CH₄). Past studies provided evidence that some anaerobic microorganisms respire organic substrates and transfer liberated reducing equivalents (i.e., electrons) to peat particulate organic matter (POM), a respiration pathway that lowers CH₄ concentrations. Herein, we developed a new analytical method based on spectrophotometric measurements to quantify how many electrons POM accepts. Applying this method to peat POM from Swedish peat bogs, we found that all tested POM accept electrons. Moreover, we demonstrated that electron transfer to peat POM was largely reversible when the reduced POM was exposed to air. These findings suggest that peat POM at oxic-anoxic interfaces may be reduced and re-oxidized cyclically, a process that may decrease the amount of CH₄ released from peat soils. The POM redox properties determined herein will help advance a more accurate understanding of organic matter breakdown as well as CH₄ dynamics in northern peatlands.

1. Introduction

Northern peatlands are a major terrestrial carbon pool, having accumulated an estimated 500 ± 100 Pg of carbon—corresponding to approximately 15%–30% of global soil organic carbon—since the end of the last glaciation (Blodau, 2002; Botch et al., 1995; Bridgham et al., 2013; Limpens et al., 2008; Turunen et al., 2002; Yu et al., 2010). In the context of global warming, it is important to understand processes in northern peatlands that result in the release of stored carbon. This is particularly true for processes that release carbon in the form of CH₄ given its high global warming potential (72 times that of carbon dioxide [CO₂] on a 20-year timescale [Forster et al., 2007]), and given that northern peatlands substantially contribute to natural CH₄
emissions to the atmosphere (15%–22% of global natural CH₄ emissions [Bartlett & Harriss, 1993; Saunois et al., 2020]). Methane forms in northern peatlands through anaerobic microbial respiration or fermentation of substrates originating from the breakdown of plant organic matter (Bridgham et al., 2013).

As methanogenesis is a low-energy yielding process, it is expected to be suppressed in the presence of terminal electron acceptors (TEAs) that allow for energetically more favorable anaerobic respiration (Madigan et al., 2018). While concentrations of the canonical inorganic TEAs nitrate, sulfate, ferric iron, and manganese oxides are low in the water-saturated top soils of northern peatlands—particularly of ombrotrophic bogs, a subcategory of peatlands that are hydrologically isolated from their surroundings and receive elemental inputs only through atmospheric deposition (Clymo, 1984; Gorham, 1991; Shotyk, 1988)—a number of recent studies provided several lines of evidence for anaerobic respiration with a previously unrecognized organic TEA which is highly abundant in these systems: peat particulate organic matter (POM) (Bridgham et al., 2013; Broder et al., 2012; Gabriel et al., 2017; Keller & Takagi, 2013; Keller et al., 2009; Yu et al., 2016). First, field measurements as well as laboratory incubation studies reported porewater concentration ratios of CO₂ to CH₄ that far exceeded unity (Amaral & Knowles, 1994; Gabriel et al., 2017; Gao et al., 2019; Keller & Takagi, 2013; Segers & Kengen, 1998; Yavitt et al., 1987). Unity is the theoretically expected concentration ratio at which these gases form through methanogenesis of substrates derived from organic matter with a nominal oxidation state of carbon of zero (Conrad, 1999). The excess amount of measured CO₂ over CH₄ could not be attributed to anaerobic respiration with inorganic TEAs (which were present at insufficient concentrations) and therefore suggested an abundant, previously overlooked TEA (Amaral & Knowles, 1994; Keller & Bridgham, 2007; Segers & Kengen, 1998; Steinmann & Shotyk, 1997). Further, laboratory incubation studies with pre-oxygenated peat POM showed CO₂:CH₄ concentration ratios that were elevated at the onset of the incubations, consistent with anaerobic respiration using POM, but decreased toward unity over time, indicative of a shift to methanogenesis as the extent of POM reduction increased and hence its capacity to accept electrons from anaerobic respiration decreased (Gabriel et al., 2017; Gao et al., 2019; Keller & Takagi, 2013; Yu et al., 2016). Second, several studies demonstrated that POM can accept electrons both from chemical reductants (Keller & Takagi, 2013; Roden et al., 2010) and anaerobic microbial respiration (Gabriel et al., 2017; Roden et al., 2010), in line with previous work that demonstrated anaerobic microbial respiration using dissolved organic matter (DOM) (KLüpfel et al., 2014; Lovley et al., 1996; Scott et al., 1998). Taken together, these studies strongly support the hypothesis that POM acts as TEA and thereby may lower CH₄ concentrations in and CH₄ emissions from northern peatlands (Bridgham et al., 2013; Keller & Bridgham, 2007). Besides directly suppressing methanogenesis by allowing for energetically more favorable anaerobic respiration, POM may also lower CH₄ concentrations by acting as a TEA for anaerobic oxidation of methane (AOM) (Bridgham et al., 2013; Keller & Bridgham, 2007; Smemo & Yavitt, 2007, 2011; Valenzuela et al., 2019, 2017).

Studies linking changes in the redox state of POM to methane concentration dynamics in peatland soils, however, remain scarce, mainly reflecting the analytical challenge of quantifying the exact number of electrons transferred to POM during its reduction. In past studies, the number of electrons transferred was often determined by reacting a reduced POM sample of interest (as well as an unreduced POM sample as a control) with the chemical oxidant ferric nitriloacetate (Fe³⁺-NTA) (Gabriel et al., 2017; Keller & Takagi, 2013; Roden et al., 2010), followed by quantifying Fe²⁺ formation. The excess amount of Fe²⁺ formed upon reaction of reduced POM over unreduced POM was taken as the number of electrons transferred to POM during its reduction. Allowing only a short reaction time of 1 min (Roden et al., 2010) or 5 min (Gabriel et al., 2017; Keller & Takagi, 2013) with POM, this assay yielded values of 0.015–0.093 mmol e⁻/g POM. However, past work showed that electron transfer from reduced DOM to complexed ferric iron is kinetically slow and proceeds over hours to days (Bauer et al., 2007). This would suggest that electron transfer from POM during the assay was also slow and thus incomplete over such a short reaction timescale, resulting in an underestimate of the number of electrons transferred to POM during its reduction.

More recently, several studies employed mediated electrochemical reduction (MER) (Aeschbacher et al., 2010) for the determination of (changes in) the redox state of POM (Aeschbacher et al., 2010; Gao et al., 2019; Lau et al., 2015, 2016). In contrast to the Fe³⁺-NTA assay, MER directly quantifies the number of electrons that are accepted by POM (i.e., its “electron accepting capacity”, EAC) when added to an electrochemical cell poised at a constant low reduction potential (E₀) and containing a dissolved redox mediator.
to facilitate electron transfer from the working electrode to POM. The extent of reduction of a given POM sample can then be determined by comparing its EAC to that of a (more) oxidized POM control sample. The longer reaction time (up to 60 min) as well as the low $E_{H^+}$ applied in MER explain why these analyses resulted in substantially higher EAC values of 0.1–0.9 mmol e$^{-}$/g POM (Gao et al., 2019; Lau et al., 2015, 2016) as compared to values from the Fe$^{3+}$-NTA assay. Yet, because the analysis time of POM in MER is constrained to ~60 min, it is conceivable that MER may not detect the redox state of reducible moieties in POM that under go slow (electrochemical) reduction. Additionally, past MER studies of POM may have been susceptible to an artifact that resulted from using cationic electron transfer mediators, which may have sorbed to highly abundant negatively charged carboxylate groups in peat POM (Ishiguro et al., 2007; Richter et al., 2009; Zachara et al., 1986). Such sorption would generate a current response in MER not originating from electron transfer to POM, thereby possibly creating errors in EAC measurements. These points highlight the need for an analytical approach to accurately quantify (changes in) the redox state of POM samples.

The goal of this work was to present a reliable analytical method to accurately quantify the EAC of peat POM samples and to subsequently use this method to quantify changes in the EAC of POM samples that result from redox reactions relevant to POM in northern peatlands, specifically redox cycling between reducing and $O_2$-oxidizing conditions. As a first step, we developed a spectrophotometric assay that quantifies the number of electrons that are transferrable to peat POM samples. We evaluated two viologen probe compounds (1,1'-ethylene-2,2'-bipyridyl (diquat dibromide monohydrate, DQ) and 4,4'-bipyridinium-1,1'-bis(2-ethylsulfonate) (zwitterionic viologen, ZiV) for POM reduction, determined the reaction time required to attain apparent redox equilibrium between the reduced probe and POM, and quantified the EAC values of POM (i.e., 0.17–0.38 mmol e$^{-}$/g POM) of varying redox states from different ombrotrophic bogs in central Sweden. In a second step, we used the analytical method to assess the reversibility of electron transfer to POM over one reduction and $O_2$-reoxidation cycle, and to quantify the extent of POM reduction in situ (defined here as the number of electrons donated from redox-preserved POM samples to dissolved $O_2$). Assessing reversibility and the extent of POM reduction in situ are both critical but heretofore unaddressed POM redox characteristics required to estimate the extent to which POM as TEA can lower CH$_4$ concentrations in and emissions from northern peatlands.

2. Materials and Methods

2.1. Chemicals

Potassium phosphate dibasic trihydrate, potassium phosphate monobasic, and potassium chloride were obtained from Sigma Aldrich (Buchs, Switzerland). The candidate EAC probe compound 1,1’-ethylene-2,2’-bipyridyl (diquat dibromide monohydrate, DQ) was purchased from Supelco (Pennsylvania, USA), whereas probe compound 4, 4’-bipyridinium-1,1’-bis(2-ethylsulfonate) (zwitterionic viologen, ZIV) was synthesized according to established protocols (Bhandari et al., 2010; Gorski et al., 2013). All solutions were prepared in distilled deionized water (resistivity >18.1 MΩ-cm, Merck Milli-Q IQ7000).

2.2. Anoxic Working Conditions

All analyses and sample treatments were conducted in an anoxic glovebox with a pure N$_2$ gas atmosphere (MBraun Unilab 2000; $O_2$ < 2.5 ppm), unless mentioned otherwise. Solutions were sparged with high purity N$_2$ gas (99.999%) for at least 3 h prior to taking them into the glovebox. Glassware, plasticware, chemical powders, and reference POM material used for method development were placed under vacuum overnight in the glovebox antechamber for degassing prior to transfer into the glovebox.

2.3. Peat POM Samples

We used a bulk sample of peat POM provided by Stockås Torvströ (Torv and Maskinentreorad AB, Mullhyt- tan, Sweden) as reference material (referred to as “reference POM”) for the development of the spectrophotometric method and assessment of electron transfer reversibility of POM. In this study, we consider POM to be bulk organic peat material that is free of living plant parts. We follow the broadly accepted definition that POM does not pass through filters with a nominal pore size cutoff of 0.22 μm (while DOM passes
through such filters). This reference POM originates from three *Sphagnum* sp.-dominated, ombrotrophic bogs in the Swedish counties of Värmland and Örebro, and was air-dried and sieved to <2 mm before provision. Based on its treatment (i.e., air-drying, sieving, and mixing), we consider the stock of reference POM in our laboratory to be extensively oxidized and homogeneous with regards to the physicochemical characteristics of the POM. This reference POM material had a gravimetric moisture content of 13.1 ± 0.14% (±standard deviation of six replicates) based on oven drying to a constant mass at 50°C (24 h). We corrected for this moisture content in order to report all numbers of electrons transferred relative to POM dry masses.

To discern the effect of POM particle size on EAC values, we prepared finer reference POM material by milling it using a ball mill (Retsch MM200, 750 rpm for 10 min) with zirconium oxide beads (diameter: 2.8 and 1.4 mm).

In addition to the reference POM, we collected POM samples from three ombrotrophic bogs in Värmland, Sweden: Lungsmossen (LM, N 59°32.969’ E 14°14.313’), Björsmossen (BM, N 59°41.557’ E 14°16.629’), and Storhultsmossen (SM, N 59°34.301’ E 14°07.908’), from the anoxic, water-saturated part of each peat in July 2019. The POM was sampled under anoxic conditions at a depth of 25 cm below the peat surface (corresponding to ~10–15 cm below the water table) directly into glass jars. The POM in the jars was completely water saturated. The jars were closed under the water table and sealed immediately after retrieval from the peat to avoid air intrusion and to thereby ensure that the redox state of the POM was preserved. Details on sampling are provided in the Text S1. We brought these samples under active cooling to the laboratory, where we stored them at 4°C. For analyses, we took one jar filled with peat soil from each site (i.e., a single large sample), transferred it into an anoxic glovebox, opened it, and manually removed clearly distinguishable plant parts (such as roots) from the POM. We refer to this material as peat POM. No size fractionation was performed on these peat POM samples prior to EAC analysis. We prepared POM suspensions of LM, BM, and SM POM for EAC determination. First, we took an aliquot of POM from each jar containing anoxic peat from the field (a single sample), transferred it into a blender (Intertronic Standmixer, 1,000 W), added deionized water in a volumetric ratio of ~3:1 (water:POM), and blended the resulting POM suspension for about 1 min. We determined the exact concentrations of POM in each suspension gravimetrically by drying five technical replicate suspension aliquots of known volumes first in the glovebox for 24 h and then in a drying oven at 50°C for 24 h, followed by determining the weight of the dried POM.

Both the reference POM and POM collected from peat bogs featured high carbon contents and low concentrations of inorganic redox-active elements, consistent with respective values commonly reported for POM from ombrotrophic bogs (Blodau, 2002; Broder et al., 2012; Hornibrook et al., 2000; Moore et al., 2005; Tfaily et al., 2014): 42.65 ± 0.06% C, 0.76 ± 0.04% N, 0.27 ± 0.004% Fe, and 0.10 ± 0.004% S for reference POM, and 33.55 ± 0.36% C, 0.65 ± 0.01% N, 0.07 ± 0.0002% Fe, and 0.23 ± 0.01% S for POM collected from peat bogs (mass%, ±s.d. from triplicate subsamples, Table S2). FTIR spectra of the reference POM and field-collected POM indicated enrichment of aromatic and carboxylic groups relative to polysaccharide groups. The extent of decomposition, based on humification indices and area ratios of assigned wavenumber regions (Text S2, Tables S2–S4), was similar to that in past studies of POM from *Sphagnum* sp.-dominated bogs (Beer et al., 2008; Broder et al., 2012; Klüpfel, 2015; Tfaily et al., 2014). Details of elemental composition analyses, FTIR spectroscopy, and calculation of decomposition indicators from FTIR spectra are given in Text S2.

### 2.4. Selection of Chemical Probe for Spectrophotometric EAC Determination

We tested two viologen compounds, DQ and ZiV, as spectrophotometric probes to determine the EAC values of peat POM. We selected these compounds because they undergo rapid, pH-independent, single electron transfer reactions (Text S3) at low standard reduction potentials (i.e., *E**0** (ZiV/ZiV−) = −0.41 V [Sander et al., 2015] and *E**0** (DQ2+/DQ+) = −0.35 V [Gorski et al., 2012] vs. the standard hydrogen electrode [SHE]). These reduction potentials are expected to be lower than the reduction potential of reducible moieties in POM based on previous reported ranges of reduction potential distributions of reducible moieties in DOM (Aeschbacher et al., 2011; Klüpfel et al., 2014). As a consequence, both reduced ZiV and DQ are expected to extensively (if not completely) reduce electron accepting moieties in peat POM. While DQ has been previously used as electron transfer mediator in MER of POM (Gao et al., 2019), both its oxidized (DQ2+) and reduced (DQ+) species have positive net charges (+2 and +1 respectively, Text S3). It is possible that these cations strongly sorb to negatively charged groups in the POM (Ishiguro et al., 2007; Li et al., 2001; Richter...
et al., 2009; Zachara et al., 1986), possibly resulting in measurement artifacts in MER. In contrast to DQ, ZIV cannot sorb to POM by cation exchange because the oxidized ZIV\(^{0}\) and reduced ZIV\(^{+}\) species are zwitterionic (net uncharged) and anionic, respectively.

### 2.4.1. Determination of Molar Absorption Coefficients of Probe Compounds
We used solution UV-Vis absorption spectroscopy (Mettler-Toledo UV5 Bio spectrophotometer, Greifensee, Switzerland) to determine molar absorption coefficients \(\epsilon\) at wavelengths of maximum absorbance of reduced species ZIV\(^{−}\) (603 nm) and DQ\(^{+}\) (379 nm) \((\epsilon_{\text{ZIV}}(\text{ZIV}^{−})\) and \(\epsilon_{\text{DQ}}(\text{DQ}^{+})\)) analyzing solutions containing different concentration ratios of the corresponding reduced and oxidized species, and of oxidized species ZIV\(^{0}\) (260 nm) and DQ\(^{2+}\) (310 nm) \((\epsilon_{\text{ZIV}}(\text{ZIV}^{0})\) and \(\epsilon_{\text{DQ}}(\text{DQ}^{2+})\)) using solutions containing different concentrations of only the oxidized species. Text S4 provides details on how we electrochemically generated the reduced species. We additionally determined the molar absorption coefficients at the isosbestic points of ZIV (295 nm) and DQ (331 nm) (i.e., the wavelengths at which the molar absorption coefficients are the same for the reduced and oxidized species; \(\epsilon_{\text{ZIV}}(\text{ZIV}^{−}) = \epsilon_{\text{ZIV}}(\text{ZIV}^{0})\) and \(\epsilon_{\text{DQ}}(\text{DQ}^{+}) = \epsilon_{\text{DQ}}(\text{DQ}^{2+})\)). We used the molar absorption coefficients for subsequent quantification of ZIV and DQ species concentrations and thus the extent of electron transfer from the reduced species to POM (detailed below).

### 2.4.2. Assessment of ZIV\(^{0}\) and DQ\(^{2+}\) Sorption to POM
We determined sorption of ZIV\(^{0}\) and DQ\(^{2+}\) to POM in batch reactors in the presence of varying POM concentrations (from no POM controls to 2 g POM/L) and potassium ions (concentrations of 0.016–0.16 M K\(^{+}\), adjusted using KH\(_2\)PO\(_4\) and K\(_2\)HPO\(_4\)), all at pH 7. We assessed sorption only for the oxidized species because they are more positively charged than the corresponding reduced species and thus more likely to sorb to POM. Sorption experiments in solutions containing K\(^{+}\) served to assess potential sorptive competition between DQ\(^{2+}\) and K\(^{+}\) to POM (Droge & Goss, 2012). Sorption experiments were initiated by suspending POM in experimental buffer in triplicate batch reactors and pre-equilibrating POM for 24 h to ensure its hydration. As we used reference POM from a larger stock that was well-mixed, we consider these replicates to be technical. We subsequently added ZIV\(^{0}\) or DQ\(^{2+}\) to an initial aqueous concentration of 40 µM. After 2 h of equilibration, we filtered an aliquot of the reactor suspension (0.22 µm cellulose acetate, BGB) and quantified aqueous ZIV\(^{0}\) or DQ\(^{2+}\) concentrations in the filtrate using UV-Vis spectrophotometry. We included two sets of controls: the first set included ZIV\(^{0}\) or DQ\(^{2+}\) without POM to correct for any losses of ZIV\(^{0}\) and DQ\(^{2+}\) over time (e.g., sorption to batch reactor surfaces, chemical instability). The second set were reaction vials containing only POM and served to correct for background solution absorbance, presumably originating from DOM released from POM.

### 2.4.3. Assessment of Chemical Stability of ZIV\(^{−}\) in Solution
We tested the stability of ZIV\(^{−}\) in POM-free solutions at pH 4 to 7 and at initial reduction potentials \(E_{\text{H}}\) of −0.388 V, −0.409 V, and −0.441 V, which we set by adjusting the initial ZIV\(^{−}\) to ZIV\(^{0}\) concentration ratios. The initial ZIV\(^{−}\) concentration in reactors at all initial \(E_{\text{H}}\) and pH values was the same: 72 ± 3 µM, whereas the total ZIV concentration in reactors at each initial \(E_{\text{H}}\) (across different pH values) was set to: −0.388 V: 247 ± 15 µM, −0.409 V: 145 ± 1 µM, −0.441 V: 90 ± 1 µM. All reactors (treatments in duplicate) contained 0.1 M acetate or phosphate as pH buffer, and 0.1 M KCl as background electrolyte. At 0, 1.75, 5, 7, 20, and 24 h after reactor setup, we withdrew aliquots from the reactors for spectrophotometric quantification of ZIV\(^{−}\) and ZIV\(^{0}\) concentrations.

### 2.5. Determination of EAC Values by Spectrophotometry
The standard approach to quantify the EAC of peat POM involved reacting a suspension of POM with a solution of known concentrations of ZIV\(^{−}\) and ZIV\(^{0}\) (schematically shown in Figure S7). The preparation of the POM suspension depended on the type of experiment. For the development of the EAC assay and to assess electron transfer reversibility to POM, we prepared suspensions by adding air-dried reference POM to a pH-buffered solution (pH 7, 0.1 M phosphate, 0.1 M KCl) and allowed the POM to hydrate for at least 24 h. For the determination of the in situ redox state of POM, we transferred aliquots of either the redox state-preserved POM suspension or the O\(_2\)-exposed POM suspension to a pH-buffered solution (pH 7, 0.1 M phosphate, 0.1 M KCl). In both types of experiments, we then added a solution of known concentrations of ZIV\(^{−}\) and ZIV\(^{0}\) to the POM suspensions.
electrochemically generated ZiV\(^{•−}\) and ZiV\(^0\) to the POM suspensions. The initial \(E_{\text{H}}\) values set by the ZiV\(^0\) to ZiV\(^{•−}\) concentration ratios ranged between \(-0.440\) V and \(-0.431\) V, with absolute initial ZiV\(^{•−}\) concentrations from 270 to 430 \(\mu\)M (range of initial ZiV\(^{•−}\) concentrations chosen to allow for different POM concentrations). We chose these initial \(E_{\text{H}}\) values because they are substantially lower than the reduction potentials previously reported for electron accepting moieties in DOM (Aeschbacher et al., 2011; Klüpfel et al., 2014). As a consequence, we expected extensive, if not complete, reduction of electron accepting moieties in the POM by ZiV\(^{•−}\) under these highly reducing conditions. All analyses were run in triplicate (technical replicates). After predetermined reaction times of POM and ZiV\(^{•−}\), we filtered suspension aliquots (0.22 \(\mu\)m cellulose acetate filters, BGB) and spectrophotometrically determined ZiV\(^{•−}\) and ZiV\(^0\) concentrations in the filtrates. Two sets of triplicate control reactors were set up in parallel. The first set were POM-free control reactors that served to assess changes in ZiV\(^{•−}\) and ZiV\(^0\) concentrations over time unrelated to electron transfer to POM (e.g., small changes due to possible ZiV\(^{•−}\) chemical instability, assessed as described above). The second set of controls contained only POM but no ZiV species and served to correct for background solution absorbance, presumably resulting from DOM released from POM. The number of electrons transferred from ZiV\(^{•−}\) to POM (mmol e\(^−\)/g POM) was calculated based on the following relationship (Equation 1):

\[
n_{\text{e}} = \left( [\text{ZiV}_{\text{control}}^{•−}] \times V_{\text{control}} - [\text{ZiV}_{\text{POM}}^{•−}] \times V_{\text{POM}} \right)
\]

where \([\text{ZiV}_{\text{control}}^{•−}]\) and \([\text{ZiV}_{\text{POM}}^{•−}]\) are the concentrations (mM) of ZiV\(^{•−}\) and \(V_{\text{control}}\) and \(V_{\text{POM}}\) are the volumes (L) in the control reactors (no POM) and the reactors containing POM, respectively. To calculate \([\text{ZiV}_{\text{POM}}^{•−}]\), we corrected solution absorbance values measured in the reactors containing ZiV and POM for background solution absorbance values that we determined in ZiV-free controls. We ascribe this absorbance to DOM released from the POM. Additionally, we determined the non-purgeable organic carbon (NPOC) (i.e., DOM concentration) by setting up a separate control experiment in which POM was equilibrated only in buffer (pH 7, 0.1 M phosphate, 0.1 M KCl; triplicate reactors) without added ZiV. After an equilibration time of 24 h, we filtered an aliquot of the reactor suspension (0.22 \(\mu\)m cellulose acetate filters, BGB). The NPOC of the filtrate was as 8.33 ± 0.13 mg C/L (mean ± standard deviation of triplicate reactors), which corresponded to less than 5% of the POM carbon present in the reactors. All analyses were run in triplicate (technical replicates). The EAC of POM (mmol e\(^−\)/g POM) was then calculated by normalizing the number of electrons transferred to the dry mass of POM in the reactor, \(m_{\text{POM}}\) (g) (Equation 2):

\[
\text{EAC} = \frac{n_{\text{e}}}{m_{\text{POM}}}
\]

The EAC is operationally defined as the number of electrons transferred to unit dry mass of POM under the specific analytical conditions (i.e., extent of ZiV reduction and hence initial \(E_{\text{H}}\), solution pH, and reaction time between POM and ZiV\(^{•−}\)).

### 2.5.1. Assessment of Electron Transfer Kinetics From ZiV\(^{•−}\) to POM

We determined the kinetics of electron transfer from ZiV\(^{•−}\) to POM by measuring the decrease in dissolved ZiV\(^{•−}\) concentrations in the presence of POM after 1, 2, 5, 10, 24, and 28 h of equilibration in standard batch reactors that were set up as described above at a POM concentration of 0.49 g/L. We used Equations 1 and 2 to calculate the increase in the apparent EAC of POM over time. Apparent redox equilibrium was attained when ZiV\(^{•−}\) concentrations remained stable over time (i.e., no significant changes in aqueous ZiV\(^{•−}\) concentrations based on a t test, significance level = 0.05). We also tested whether there were any significant changes in total ZiV concentration in the reactors to assess losses of ZiV due to sorption (one-way ANOVA, significance level = 0.05).

### 2.5.2. Assessment of Linearity in Number of Electrons Transferred to Added POM Mass

To establish linearity in the analytical response (i.e., number of electrons transferred) to POM mass analyzed, we set up batch reactors following the standard approach described above but varied the POM concentration (0.22, 0.43, 0.65, and 0.87 g/L). We then quantified the number of electrons transferred to POM after 24 h of reaction between ZiV\(^{−}\) and POM.
2.6. Determination of EAC by Mediated Electrochemical Reduction (MER)

We complemented the spectrophotometric EAC assay (detailed above) by EAC determinations using MER with ZIV as electron transfer mediator for two reasons: (a) to compare the EAC values determined by the two analytical methods and (b) to assess the effect of POM particle size on EAC. Details of the electrochemical cell setup are given in Text S5. Analysis by MER required the use of milled POM as opposed to unmilled POM to ensure that pipette-transfer of POM suspensions to the electrochemical cell was reproducible. The EAC was determined by integrating the reductive current peaks that resulted from POM additions to the cells over time:

\[
EAC = \frac{1}{F} \int I_{\text{MER}} \, dt
\]

where \( I_{\text{MER}} \) (A) is the baseline-corrected reductive current, \( m_{\text{POM}} \) (g) is the mass of POM added and F is the Faraday constant (96,485 C/mol e\(^-\)). Reductive current responses were corrected for baseline currents using a spline function. All analyses were done in Igor Pro (Wavemetrics, v7.08).

2.7. Assessment of Electron Transfer Reversibility to POM

We tested the reversibility of electron transfer to POM by quantifying changes in the EAC of the reference POM that resulted from its electrochemical reduction and subsequent re-oxidation upon exposure to O\(_2\) (schematically shown in Figure S7). We first suspended reference POM in pH buffer (10 g POM/L; pH 7, 0.1 M phosphate, 0.1 M KCl) for 24 h to ensure hydration. We took three aliquots (i.e., three technical replicates) of this POM suspension for later EAC analysis as non-treated POM, and then transferred the rest of the POM suspension into an electrochemical cell similar to that used for MER (Text S5). We poised the cell at a reduction potential \( E_H \) of −0.5 V versus SHE and recorded the reductive currents resulting from electron transfer to POM over time. After 12 h of reduction, the current had decreased to background values <2 μA. To facilitate further POM reduction, we added a small amount of ZIV to the cell to a concentration of 0.19 mM. The number of electrons transferable to the added amount of ZIV corresponded to less than 5% of the EAC of POM in the cell; therefore, POM was the major electron accepting species present in the cell. After ZIV addition, we continued the electrochemical reduction for another 36 h, at which point the current returned to background values. We took three aliquots (i.e., three technical replicates) of the reduced POM suspension from the cell for later EAC analysis. The remainder of the POM suspension was transferred from the cell to a serum bottle, which was removed from the glovebox and stirred for 36 h. Within this time, we opened the serum bottle three times (5 min each to allow air entry) to replenish O\(_2\) in the headspace of the bottle and thereby allow for continuous oxidation of reduced POM by O\(_2\). We then sparged the closed bottle with N\(_2\) for 2 h to remove remaining dissolved and headspace O\(_2\), followed by transferring the bottle back into the glovebox. The sparging time necessary to remove dissolved and headspace O\(_2\) was determined based on independent control experiments (data not shown). Three aliquots (i.e., three technical replicates) were taken from this O\(_2\)-exposed POM suspension for EAC analysis. We used an independent t test to determine whether differences between the measured EACs of non-treated, reduced, and air-exposed POM were statistically significant (significance level = 0.05).

2.8. Determination of the In Situ Redox State of POM

We assessed the extent of POM reduction in three Swedish ombrotrophic bogs by quantifying changes in the EAC of POM samples collected from these bogs before and after exposure to O\(_2\) (schematically shown in Figure S7). We therefore quantified the number of electrons that were transferable from the POM collected under anoxic conditions to dissolved O\(_2\) as oxidant. From each suspension of blended POM (detailed in the section Peat POM samples above), we transferred two aliquots (35 mL) into serum bottles. One bottle remained in the glovebox and served as redox state-preserved (non-treated) POM. The second bottle was exposed to O\(_2\) by uncapping the bottle (allowing air entry) for 5 min each day for a total of 8 days under continuous stirring. We chose a reaction time of 8 days between POM and O\(_2\) because initial experiments had suggested that oxidation of the POM by O\(_2\) was slow and proceeded over multiple days. Slow oxidation kinetics possibly reflected spin restrictions on the oxidation of hydroquinone groups in POM by O\(_2\) (Jiang et al., 2015; Roginsky & Barsukova, 2000). At the end of 8 days,
we sparged the closed serum bottle with N$_2$ for 2 h to remove remaining dissolved and headspace O$_2$, and then transferred the bottle back into the glovebox. Three aliquots (i.e., three technical replicates) were taken from both non-treated and O$_2$-exposed POM suspensions for spectrophotometric EAC determination. To ensure that sample handling and sparging with N$_2$ did not cause changes in EAC, we prepared control POM suspensions in serum bottles that were first taken out of the glovebox, not opened to air, sparged with N$_2$, and then transferred back into the glovebox. We then measured the EAC of these control POM suspensions. For POM from each bog we used an independent t test to determine if differences between the EACs of non-treated POM and O$_2$-exposed POM were significant (significance level = 0.05).

3. Results and Discussion

3.1. Selection of Probe Compound to Reduce POM

3.1.1. Determination of Molar Absorption Coefficients of Probe Compound Redox Species

The absorbance spectra of the two probe compounds ZiV and DQ at varying concentration ratios of reduced to oxidized species at a constant total ZiV concentration of 100 µM (a) and reduced (DQ$^+$) and oxidized (DQ$^{2+}$) species at a constant total DQ concentration of 50 µM (b). Color gradients depict varying concentration ratios of reduced to oxidized species with darker colors corresponding to higher ratios, expressed as the fraction (in terms of %) of total probe compound that is present as reduced species. Insets: (i) Linear relationships between the absorbance at wavelengths of maximum absorbance of the reduced species ($\lambda_{\text{Amax,ZiV}^-}$: 603 nm, $\lambda_{\text{Amax,DQ}^+}$: 379 nm) and the concentrations of reduced species ZiV$^-$ (a) and DQ$^+$ (b). The slopes of these relationships correspond to the molar absorption coefficients: $\epsilon_{603}(\text{ZiV}^-) = 14,740 \pm 52 \ M^{-1} \ cm^{-1}$ and $\epsilon_{379}(\text{DQ}^+) = 29,699 \pm 145 \ M^{-1} \ cm^{-1}$ (Figures 1a and 1b). (ii) Linear relationships between the absorbance at wavelengths of maximum absorbance of the oxidized species ($\lambda_{\text{Amax,ZiV}^0}$: 260 nm, $\lambda_{\text{Amax,DQ}^{2+}}$: 310 nm) and concentrations of the oxidized species ZiV$^0$ (a) and DQ$^{2+}$ (b). Note that these data are collected from independently prepared dilution series of solutions containing only either ZiV$^0$ or DQ$^{2+}$. (iii) Solution absorbance measured at the isosbestic point wavelengths of $\lambda_{\text{iso,ZiV}} = 295 \ nm$ and $\lambda_{\text{iso,DQ}} = 331 \ nm$ (denoted by * in the Figure) as a function of the concentrations of ZiV$^-$ (a) and DQ$^+$ (b).

Figure 1. Determination of the molar absorption coefficients ($\epsilon$) of reduced and oxidized species of probe compounds ZiV (a) and DQ (b) used for determination of electron accepting capacity (EAC). UV-Vis absorption spectra of solutions containing different concentration ratios of reduced (ZiV$^-$) and oxidized (ZiV$^0$) species at a constant total ZiV concentration of 100 µM (a) and reduced (DQ$^+$) and oxidized (DQ$^{2+}$) species at a constant total DQ concentration of 50 µM (b). Color gradients depict varying concentration ratios of reduced to oxidized species with darker colors corresponding to higher ratios, expressed as the fraction (in terms of %) of total probe compound that is present as reduced species.
and not from the absorbance spectra of the mixtures shown in Figures 1a and 1b because ZiV •− and DQ •+ also absorb light at 260 and 310 nm, respectively. For all subsequent calculations of dissolved ZiV 0 and DQ 2+ concentrations based on solution absorbance measurements at 260 and 310 nm, we corrected for the absorbances of ZiV •− (ε 260 (ZiV •−) = 779 ± 51 M⁻¹ cm⁻¹) and DQ •+ (ε 310 (DQ •+) = 5,909 ± 90 M⁻¹ cm⁻¹) at these wavelengths. Of the four species (ZiV •−, ZiV 0, DQ •+, and DQ 2+), ε values have been previously published only for reduced ZiV •− (ε 603 (ZiV •−) = 15,050 ± 100 M⁻¹ cm⁻¹ [Walpen et al., 2018]) and oxidized DQ 2+ (ε 310 (DQ 2+) = 17,500 M⁻¹ cm⁻¹ [EFSA, 2015]) and are in good agreement with ε 603 (ZiV •−) and ε 310 (DQ 2+) determined herein. The ε values of ZiV and DQ at their isosbestic points at 295 and 331 nm were ε 295 (ZiV •−) = ε 295 (ZiV 0) = 3,042 ± 40 M⁻¹ cm⁻¹ and ε 331 (DQ •+) = ε 310 (DQ 2+) = 2,351 ± 85 M⁻¹ cm⁻¹ (Figures 1a and 1b, insets ii). Absorbance measurements at these wavelengths subsequently allowed us to readily quantify the total concentration of dissolved probe compounds.

3.1.2. Assessment of ZiV 0 and DQ 2+ Sorption to POM

Using ZiV or DQ for the quantification of EAC requires that changes in the aqueous concentrations of the respective reduced and oxidized species in the presence of POM result from electron transfer to POM, while other processes that may affect the redox speciation of the probe, such as sorption of the oxidized or the reduced species to POM, are excluded. Given the high abundance of negatively charged carboxylate groups in peat POM (Schnitzer & Desjardins, 1965; Vinci et al., 2020) and the high affinity of organo-cations to such groups (Ishiguro et al., 2007; Li et al., 2001; Zachara et al., 1986), we expected stronger sorption of the oxidized species ZiV 0 and DQ 2+ to POM as compared to their reduced counterparts, which were more negatively and less positively charged, respectively. We note that DQ 2+ has a net positive charge of +2, while ZiV 0 is zwitterionic (i.e., two positive and two negative charges on the bipyridyl nitrogen and the sulfonate groups, respectively [Text S3]). Figure 2a shows the results of batch sorption experiments in which we varied the concentration of reference peat POM (0.5 g/L to 2 g/L) in batch reactors. As POM concentration increased, the concentration of aqueous DQ 2+ decreased whereas the concentration of aqueous ZiV 0 was unaffected. Concentrations of both aqueous DQ 2+ and ZiV 0 in POM-free batch reactors were in good agreement with the nominally added concentrations of 40 µM (Figure 2a). These findings demonstrate that DQ 2+ sorbed extensively to POM (13–16 µmol DQ 2+/g POM), consistent with expectation.

To confirm that sorption of DQ 2+ to POM resulted from cation binding to negatively charged moieties in POM, we conducted separate sorption experiments in the presence of varying concentrations of dissolved K+ as sorptive competitor (Droge & Goss, 2012). Consistent with cation binding, sorption of DQ 2+ to POM decreased, as inferred from increasing aqueous DQ 2+ concentrations, with increasing dissolved K+ concentrations.
concentration (from 16 to 160 mM K⁺; Figure 2b). The extent of DQ²⁺ sorption (66-13 μmol DQ²⁺/g POM) is smaller than previous estimates of carboxylate contents of organic soils (300–1,100 μeq[-COOH]/g soil [Schnitzer & Desjardins, 1965]). The suppression of DQ²⁺ sorption in the presence of K⁺ suggests that such sorption may have played only a small role in past EAC measurements by MER, which were conducted in the presence of high K⁺ and Na⁺ concentrations (Gao et al., 2019; Lau et al., 2015, 2016).

In contrast to DQ²⁺, ZIV⁰ concentrations did not decrease in the presence of POM and were unaffected by varying dissolved K⁺ concentrations, implying that ZIV⁰ did not sorb to POM to measurable extents. Based on these results, we chose ZIV as opposed to DQ as redox probe compound for all subsequent spectrophotometric analyses of EAC of POM.

### 3.1.3. Assessment of Chemical Stability of ZIV⁻⁻ in Solution

The use of ZIV to quantify the EAC of POM requires that both ZIV⁻⁻ and ZIV⁰ are stable in solution in the absence of POM. However, given the low standard reduction potential of ZIV: \( E_{\text{H}}^{0} (\text{ZIV}^{0}/\text{ZIV}^{-}) = -0.41 \text{ V} \) (Sander et al., 2015), the stability of ZIV⁻⁻ may be compromised in acidic solutions due to electron transfer to \( \text{H}^{+} \) to form \( \text{H}_{2} \) (Ebbesen, 1984). We therefore assessed the chemical stability of ZIV⁻⁻ over 24 h in solutions at pH values of 4–7 and at varying initial reduction potentials \( (E_{\text{H}}) \) by adapting the concentration ratios of ZIV⁻⁻ to ZIV⁰. At all tested pH and initial \( E_{\text{H}} \) conditions, ZIV⁻⁻ concentrations decreased while concentrations of ZIV⁰ increased (Figures S3–S6). Constant absorbance values at the isosbestic point of ZIV implied that these changes resulted from the oxidation of ZIV⁻⁻ to ZIV⁰. Decreases in ZIV⁻⁻ concentrations increased with decreasing pH and \( E_{\text{H}} \) values (i.e., more reducing conditions), consistent with ZIV⁻⁻ reducing \( \text{H}^{+} \) in solution.

Based on these pH stability data, we decided to perform EAC analyses with ZIV⁻⁻ at pH 7, at which ZIV⁻⁻ was comparatively stable (10%–14% of the ZIV⁻⁻ was oxidized after 24 h over the reduction potential range of \( E_{\text{H}} = -0.388 \text{ V to } -0.441 \text{ V} \). While calculating the number of electrons transferred to POM, we accounted for the small decreases in ZIV⁻⁻ concentrations due to \( \text{H}^{+} \) reduction in POM-containing reactors by determining decreases in ZIV⁻⁻ concentrations in POM-free controls. However, because the \( E_{\text{H}} \) values in the POM-free controls remained more negative than in reactors containing POM (i.e., less ZIV⁻⁻ was oxidized to ZIV⁰ in the controls), we expect that we slightly overestimated proton reduction by ZIV⁰ in the POM-containing reactors. As a consequence, we may have slightly underestimated the EAC of POM (by up to 5%, expanded discussion in Text S5).

We also recognize that pH 7 used in EAC quantification is considerably higher than the natural pH of ~4 in ombrotrophic bogs (Shotyk, 1988). Assuming that electron transfer to reducible moieties in POM is coupled to an equimolar proton transfer (e.g., quinones typically undergo 2e⁻ and 2H⁺ reductions at circumneutral pH), a lower reduction potential \( E_{\text{H}} \) is therefore required to reduce the POM at pH 7 as compared to pH 4 (according to the Nernst equation, a decrease of ~59 mV per pH (25°C) [Gamage et al., 1991; Warren et al., 2010]). Therefore, we set the initial reduction potential in the EAC analyses to very low values \( (E_{\text{H}}) = -0.41 \text{ V} \). These \( E_{\text{H}} \) values are lower than the reported range of \( E_{\text{H}} \) values for reducible moieties in DOM (Aeschbacher et al., 2011; Klüpfel et al., 2014). We expected that the low \( E_{\text{H}} \) values led to the reduction of most, if not all, reducible moieties in the POM. Because POM reduction was extensive if not exhaustive, we expect that variations in the pH in the EAC assay led to minor changes in the determined EAC values. A more extensive discussion of the possible effects of solution pH on measured EAC values of POM as well as on electron transfer from reduced POM to oxygen is given in the supporting information (Text S6).

### 3.2. Electron Accepting Capacity (EAC) of the Reference POM by Analytical Spectrophotometry

#### 3.2.1. Kinetics of Electron Transfer From ZIV⁻⁻ to POM

We determined the time required to attain apparent redox equilibrium between ZIV⁻⁻ and reducible moieties in POM by monitoring the decreases in concentration of aqueous ZIV⁻⁻ in the presence of POM over time. The ZIV⁻⁻ concentration decreased rapidly over the first 2 h of its addition to POM, followed by a slower, continuous decrease over the next 26 h of equilibration (Figure 3a). The spectrophotometric analysis showed that ZIV⁻⁻ concentration decreases resulted in equal increases in ZIV⁰ concentration at all sampling
Figure 3. Concentration changes of ZIV\textsuperscript{−}(blue squares) and ZIV\textsuperscript{0}(black circles) in reactors with (a) and without (b) particulate organic matter (reference POM) over time. Markers denote mean values and error bars denote standard deviation of triplicate reactors. Changes in the number of electrons transferred from ZIV\textsuperscript{−} to POM over time (c), calculated from the changes in concentration of ZIV\textsuperscript{−} in reactors containing POM (a), after correction for the small decrease in ZIV\textsuperscript{−} concentrations in control reactors without POM (b). Markers denote mean values and error bars denote standard deviation. Linear relationship between the number of electrons transferred to POM and POM concentration in the reactors (d). Markers denote means and error bars denote standard deviations of triplicate reactors. The solid line denotes the linear fit to the data, and dashed lines denote 95% confidence intervals. The slope of the fitted correlation line corresponds to the electron accepting capacity (EAC) of POM. Baseline-corrected reductive current responses during mediated electrochemical reduction (MER) of milled reference POM using ZIV as the electron transfer mediator (e). Current peaks are labeled with the respective POM masses added to the MER cell. Linear relationship between the number of electrons transferred to POM and the mass of POM added (f). Electrons transferred were quantified by integrating baseline-corrected reductive current peaks using Equation 3. The solid line denotes the linear fit to the data, and dashed lines denote the 95% confidence intervals. The slope of the linear regression fit corresponds to the EAC of milled POM determined by MER.
times, consistent with ZIV$^{\cdot-}$ reducing POM and being oxidized to ZIV$^0$. In comparison, there was only a small decrease in ZIV$^{\cdot-}$ concentration in control reactors without POM (Figure 3b), presumably reflecting slow H$^+$ reduction by ZIV$^{\cdot-}$ at this pH to form ZIV$^0$, as discussed above (Section 3.1.3). Further, the total ZIV concentration did not change significantly over the course of experiment in both the POM and POM-free control reactors (one-way ANOVA test; F(5, 38) = 1.816, p = 0.137), implying that there were no sorptive losses of ZIV to POM or reactor surfaces during the assay.

In reactors with POM, ZIV$^{\cdot-}$ and ZIV$^0$ concentrations did not change significantly between 24 and 28 h of equilibration (t test; t(2) = -0.028, p = 0.98), indicating attainment of apparent redox equilibrium between ZIV and POM over 24 h of reaction. We therefore used 24 h as the reaction time for all subsequent EAC determinations. The decrease in ZIV$^{\cdot-}$ concentration in the presence of reference POM (corrected for smaller concentration decreases in POM-free controls) after 24 h corresponded to an EAC of 0.38 ± 0.065 mmol e$^-$/g POM (Figure 3c).

The reaction time of 24 h to attain apparent redox equilibrium between ZIV$^{\cdot-}$ and POM is significantly longer than the previously reported time of 60 min to attain apparent redox equilibrium of viologen reductants with DOM (Aeschbacher et al., 2010, 2011; Klüpfel et al., 2014; Walpen et al., 2018). These differences between POM and DOM likely reflect that reducible moieties in POM are less accessible to ZIV$^{\cdot-}$ than those in DOM, given that the POM particles were µm-sized (Figure S2) as opposed to DOM which are nm-sized assemblies (Armanious et al., 2014; Duval et al., 2005). While diffusion rates of ZIV in POM are unknown, past work focusing on diffusive equilibration during sorption of low-molecular-weight organic compounds with comparable chemical structures (such as pyridine and paraquat) to soil particles and activated carbon reported equilibration times of 6–40 h (Amondham et al., 2006; Benoit et al., 2008; Mohan et al., 2004), in good agreement with the timescale of redox equilibration observed in this work. We also considered an alternate explanation for slow electron transfer to POM: an increase in the reduction potential ($E_r^0$) of the ZIV$^0$/ZIV$^{\cdot-}$ redox couple over the course of the reaction, and hence a decrease in the thermodynamic driving force for POM reduction. However, we consider this explanation less likely because of the low initial reduction potential of $E_{iH}^0$ (ZIV$^0$/ZIV$^{\cdot-}$) = −0.437 V in EAC assays, which is expected to be below the reduction potentials of reducible moieties in POM, as inferred from past DOM work (Aeschbacher et al., 2011; Klüpfel et al., 2014). In addition, the initial $E_{iH}$ was about 25 mV below the standard reduction potential ($E_{r}^0$(ZIV$^{\cdot-}$/ZIV$^0$) = −0.41 V) of ZIV, thereby ensuring effective redox buffering around the $E_{iH}$. As a result, the $E_{iH}$ increases resulting from the oxidation of some ZIV$^{\cdot-}$ to ZIV$^0$ upon the reaction with POM was small (~40 mV), with an $E_{iH}$ (ZIV$^{\cdot-}$/ZIV$^0$) = −0.396 V at the end of 28 h.

### 3.2.2. Linearity in the Number of Electrons Transferred With POM Mass

We ran separate batch experiments to establish linearity in the response of the spectrophotometric assay to POM mass analyzed after 24 h of redox equilibration. The number of electrons transferred from ZiV to POM increased linearly with POM suspension concentration in the reactors (Figure 3d). The slope of the linear fit to the data yielded an EAC of 0.38 ± 0.039 mmol e$^-$/g POM. This EAC value was in good agreement with the EAC value for the same reference POM material determined in the kinetic experiment above (EAC = 0.38 ± 0.065 mmol e$^-$/g POM). The EAC values could be primarily attributed to the POM in the assay reactors and not to DOM released from the POM, based on the comparatively low concentration of DOM released (i.e., 8.33 ± 0.13 mg C/L) in separate control experiments (see Section 2.5 above). Assuming EAC values of DOM of 1.06–1.94 mmol e$^-$/g C (Walpen et al., 2018), the DOM in the assay reactors accounted for 4.8%–8.8% of the total measured EAC, demonstrating that POM was the major electron-accepting phase in our reactors (complete calculations are given in Text S7).

The EAC value of POM measured here (0.38 ± 0.039 mmol e$^-$/g POM) falls within the range of EAC values of bog POM determined by MER in previous work (0.2–0.7 mmole e$^-$/g C [Gao et al., 2019]). The EAC values of the reference POM is, however, approximately 4 to 20-fold higher than previously determined EAC for POM of 0.015–0.093 mmol e$^-$/g POM based on the Fe$^{3+}$-NTA assay (Gabriel et al., 2017; Keller & Takagi, 2013; Roden et al., 2010). We ascribe this large difference in the apparent redox capacities of the POM to two methodological differences between the studies. First, as detailed in the introduction, the Fe$^{3+}$-NTA assay typically uses only short reaction times with the POM (1–5 min), which we expect to be insufficiently long to attain redox equilibrium and hence to lead to a significant underestimation of true EAC values.
Second, the EAC values of POM determined by the Fe$^{3+}$-NTA method reflect the difference in the redox states of a microbially or chemically pre-reduced POM and an untreated control POM. Fewer electrons are expected to be transferred to POM if conditions during microbial and possibly chemical pre-reduction in studies using the Fe$^{3+}$-NTA assay were less reducing than the highly reducing conditions in our spectrophotometric assay. The ZIV$^{\text{-}}$ used herein may transfer electrons also to reducible moieties in POM with low $E_{\text{H}}$, which may not be (readily) reducible by microbial respiration or milder chemical reductants. We deliberately chose such a low $E_{\text{H}}$ herein to ensure rapid and extensive POM reduction in the spectrophotometric assay, but acknowledge that only a fraction of the EAC may be (microbially) accessible in situ (see Section 3.4 below on in situ redox states of POM).

The EAC for the reference POM determined herein needs to be considered an operationally defined value that reflects the solution pH and the initial $E_{\text{H}}$ during EAC analysis, the ZIV-POM reaction time, as well as the POM physical state (e.g., hydration state and particle size distribution). Reference POM material was pre-dried and sieved to <2 mm prior to analysis in order to remove large plant pieces and to increase the homogeneity of the material. We expected that any additional treatment that decreases the average particle size of the reference POM, such as milling, would lead to faster redox equilibration between the ZIV$^{\text{-}}$ and POM as well as the possible exposure of additional reducible moieties in POM that were inaccessible to the ZIV$^{\text{-}}$ in the 2 mm-sieved material. To assess this possibility, we also determined the EAC of milled reference POM material with a size distribution shifted to smaller values (Figure S2) using the spectrophotometric method developed here. The EAC of milled POM was 0.51 ± 0.05 mmol e$^{-}$/g POM and therefore almost 1.5 times higher than EAC values of the non-milled reference POM (0.38 ± 0.039 mmol e$^{-}$/g POM), consistent with our expectation.

We additionally analyzed milled reference POM by MER to compare EAC values obtained by MER and the spectrophotometric assay. The addition of increasing amounts of milled reference POM to the electrochemical cell resulted in increasing reductive current peaks (Figure 3e), demonstrating electron transfer from the working electrode via ZIV$^{\text{-}}$ to POM. While reductive currents returned to baseline values within approximately 40 min of POM addition, we expect that electron transfer continued beyond 40 min, but at rates too low to be detected by MER. Integration of reductive current peaks according to Equation 3 showed that the number of electrons transferred to POM increased linearly with increasing added POM mass (Figure 3f), resulting in an EAC of 0.19 ± 0.02 mmol e$^{-}$/g POM. This value was significantly larger than the estimated EAC value for non-milled reference POM of 0.13 ± 0.02 mmol e$^{-}$/g POM determined by the spectrophotometric assay over the same reaction time of 40 min (estimated by interpolating the kinetic data in Figure 3e), but smaller than the EAC of milled POM after 24 h of reaction determined spectrophotometrically (0.51 ± 0.05 mmol e$^{-}$/g POM). These findings highlight that EAC values are operationally defined and thus the importance of reporting ZIV$^{\text{-}}$-POM equilibration times as well as information about the particle size distribution of POM samples in any discussion of POM EAC values. When analyzing different POM samples, care should be taken to ensure that the assay conditions (reaction time between the chemical reductant and POM as well as pH and reduction potential $E_{\text{H}}$) are identical. For a given sample (or set of samples with similar or identical characteristics such as particle size), the EAC assay developed here is well suited to quantify changes in the redox state that may occur due to processes such as microbial reduction or oxidation by O$_2$. We provide two example cases below.

### 3.3. Reversibility of Electron Transfer to POM

Electron transfer reversibility to/from POM upon oxidation is a key assumption underpinning the hypothesis that POM acts as a sustainable TEA for anaerobic microbial respiration and thereby lowers CH$_4$ concentrations in northern peatlands. Without electron transfer reversibility, the capacity of POM to accept electrons would decrease over time and ultimately become depleted when all reducible moieties in the POM accepted electrons from anaerobic microbial respiration, resulting in methanogenic conditions. We herein established electron transfer reversibility to POM by quantifying changes in the EAC of the reference POM that resulted from its electrochemical reduction and subsequent exposure to dissolved O$_2$. We chose this oxidant because it is likely the most relevant at anoxic-oxic interfaces in the soil profile where POM is exposed to alternating anoxic, reducing conditions under water saturation and oxic, oxidizing conditions when O$_2$ enters the peat soil (Clymo, 1984; Ingram, 1978).
The electrochemical reduction of POM over 36 h resulted in a pronounced decrease in its EAC from $0.34 \pm 0.01$ mmol e$^-$/g POM for the non-treated POM to $0.10 \pm 0.003$ mmol e$^-$/g POM following electrochemical POM reduction (Figure 4). Subsequent exposure of reduced POM to air resulted in a pronounced increase in EAC to $0.38 \pm 0.002$ mmol e$^-$/g POM. This increase demonstrates that reduced POM was effectively re-oxidized by O$_2$ and, therefore, electron transfer to reference POM was reversible over a reduction and O$_2$ oxidation cycle. In fact, the EAC value after re-oxidation was slightly but significantly higher than that of the non-treated POM prior to electrochemical reduction ($t$ test; $t(2) = -4.59$, $p = 0.019$), suggesting that non-treated POM contained some reduced moieties that were oxidizable by dissolved O$_2$ at pH 7. The demonstrated reversibility of electron transfer to POM substantiates the role of peatland POM as a regenerable TEA, and thus its long-term effects on methane dynamics in peat soils.

3.4. In Situ Redox State of POM

The EAC values above were determined for the reference POM material, which we expect to have undergone extensive air oxidation during sampling, processing, and storage. To assess the extent of POM reduction in situ (and variations in the redox state between POM samples), we collected anoxic POM samples from three Swedish bogs (LM, BM, and SM) 15–20 cm below the oxic-anoxic transition zone. We then used the spectrophotometric method to assess changes in EAC that resulted from exposing this redox-preserved (i.e., non-treated) POM to dissolved O$_2$.

The EAC values of the redox-preserved POM from the three bogs were $0.18 \pm 0.026$, $0.24 \pm 0.034$, and $0.17 \pm 0.028$ mmol e$^-$/g POM for LM, BM, and SM, respectively (Figure 5). Upon exposure to air, the EAC of POM from LM and BM significantly increased to $0.26 \pm 0.029$ and $0.33 \pm 0.04$ mmol e$^-$/g POM, respectively (Figure 5, Table S5). The increase in EAC ($0.09 \pm 0.04$ mmol e$^-$/g POM) demonstrates that the POM samples were in a reduced state in situ relative to oxic conditions, consistent with POM acting as TEA in anaerobic microbial respiration. We note that the increase in EAC upon exposure to O$_2$ was too large to be explained by reduction of the iron present in POM ($0.07 \pm 0.0002$ wt%, corresponding to $0.013$ mmol Fe/g POM). While the sulfur content of POM was higher ($0.23 \pm 0.01$ wt%, corresponding to $0.071$ mmol S/g POM) and could theoretically be sufficient to explain the increase in EAC, we consider this scenario unlikely because the majority of sulfur in the solid phase in peatlands is expected to be organically bound (Novák & Wieder, 1992; Wieder & Lang, 1986, 1988), and sulfate reduction has been observed to be negligible in organic-rich peat soils (Yu et al., 2016). While exposure of POM from SM to air also resulted in a small increase in EAC to $0.19 \pm 0.026$ mmol e$^-$/g POM (Figure 5), the difference in EAC between the redox-preserved and air exposed POM was statistically insignificant (Table S5). Therefore, the POM sample collected from SM was not in a reduced state relative to oxic conditions. The smaller increase in EAC of SM POM compared to LM and BM POM may reflect differences in their degrees of decomposition. This explanation is supported by qualitative observations of more discernible plant fragments in SM peat samples, suggesting that POM from SM was less decomposed relative to LM and BM POM. However, the decomposition indicators derived from FTIR measurements are comparable for POM from LM, BM, and SM (Table S4), suggesting that these indicators are not sufficiently sensitive to capture differences in the contents of redox-active moieties in POM. The EAC values of re-oxidized POM samples ranged from $0.19$ to $0.33$ mmol e$^-$/g POM and therefore were slightly lower than the EAC of
the reference POM (0.38 ± 0.065 mmol e⁻/g POM), presumably reflecting differences in both the chemical composition as well as the physical states (particle sizes) of these samples.

Redox-preserved POM samples from the three bogs had appreciable EAC values. In addition, the increase in EAC of 0.09 ± 0.04 mmol e⁻/g POM upon exposing the redox-preserved POM from LM and BM to O₂ corresponded to about 30% of the EAC values of the re-oxidized POM samples. Taken together, these two findings imply that only a fraction of the POM moieties that accept electrons in the spectrophotometric EAC assay was reduced in situ. These results suggest that microorganisms in bogs are capable of using only a subset of the reducible moieties in POM as electron acceptors in anaerobic respiration. This subset may be composed of reducible moieties that have comparatively high reduction potentials and are readily accessible as TEA for anaerobic microbial respiration. This explanation is supported by earlier work with DOM, which showed that microbial respiration to DOM ceased at reduction potentials of approximately $E_{H^+} = -$0.2 V, while the same DOM accepted additional electrons by electrochemical reduction between $E_{H^+} = -$0.2 V to approximately $-$0.3 V (Aeschbacher et al., 2010, 2011; Klüpfel et al., 2014). While increasing the reduction potential to higher values in the spectrophotometric assay would result in EAC values potentially more closely matching the EAC that is microbially accessible, we deliberately chose to work at low $E_{H^+}$ of $-0.440$ to $-0.431$ V to ensure a high thermodynamic driving force for electron transfer to reducible moieties in POM and thus facile electron transfer to POM in the spectrophotometric analyses. Future efforts should aim at elucidating which reducible moieties in POM can be used as TEA for microbial respiration, and whether this subset of the total reducible moieties is quantitatively detected even at higher reduction potentials in the spectrophotometric assay.

4. Conclusions

In this study, we developed a new spectrophotometric method to quantify the number of electrons that can be transferred to POM using a negatively charged, one electron-reduced viologen reductant, ZiV•⁻. In contrast to previously used viologen reductants, we established that neither the reduced nor oxidized species of ZiV sorbed to POM, thereby allowing for an accurate quantification of electrons transferred to POM from the oxidation of dissolved ZiV•⁻ to ZiV⁰. We demonstrated attainment of apparent redox equilibrium between ZiV and POM within 24 h of reaction and linearity in the spectrophotometric assay response (number of electrons transferred to POM) with added POM mass.

Using this method, we quantified EAC values of a reference POM material and three field POM samples (0.19 ± 0.026 to 0.38 ± 0.039 mmol e⁻/g POM), which were significantly higher than those reported by previous studies for other POM samples. However, not all of these reducible moieties may be microbially accessible in situ. Normalized to carbon content, the EAC values of POM ranged from 0.48 ± 0.07 to 0.95 ± 0.09 mmol e⁻/g C and therefore ~50% of the previously measured EAC range for DOM (1–2 mmol e⁻/g C) (Aeschbacher et al., 2010; Walpen et al., 2018). Yet, we expect POM to be a far more important TEA than DOM in these systems, given that most organic carbon in bogs is present as POM (>98.5%) (Gorham, 1991). We showed that electron transfer to POM is reversible based on the changes in EAC values of the reference POM material over a cycle of electrochemical reduction and subsequent exposure to dissolved O₂. Additionally, we demonstrated that exposure of redox-preserved POM samples (collected under anoxic conditions) from three ombrotrophic bogs to dissolved O₂ removed 0.09 ± 0.04 mmol e⁻/g POM from two of the field POM samples.

While the EAC assay allows quantifying changes in the redox state of POM during biogeochemically relevant redox processes (e.g., during POM oxidation by O₂, as shown herein, or during microbial POM reduction), we note that the EAC remains an operationally defined quantity because it is dependent on the measurement conditions (i.e., applied $E_{H^+}$, suspension pH, as well as reaction time between the reductant ZiV•⁻ and the POM) as well as on the physical state of the POM sample (e.g., particle size distribution). For this reason, care must be taken when comparing EAC values of POM samples that differ in their physical state and decomposition extents (e.g., collected from different peatlands or from different locations in a given peat). In these cases, it is critical to ensure that all samples are analyzed under the same measurement conditions. Furthermore, we recommend that future work is directed toward coupling EAC values of POM
samples to their chemical and spectral properties in order to link POM redox properties to POM chemical composition.

The EAC estimates presented here coupled with the reversibility of electron transfer and evidence of reduced POM in the field strongly support the hypothesis that POM acts as a long-term TEA in peatlands. Based on the results presented here, we roughly estimate that using POM as TEA may suppress methane on the order of 20–25 μmol CH₄/g POM in ombrotrophic bogs. The spectrophotometric EAC assay developed herein lays the foundation to directly link changes in the redox state of POM to methane concentration dynamics.

Conflict of Interest

The authors declare no conflicts of interest relevant to this study.

Data Availability Statement

Datasets collected in this study are accessible through the ETH Research Collection (https://doi.org/10.3929/ethz-b-000477356).

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