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IMPACTS OF CONTROLLED REDOX CONDITIONS ON GREENHOUSE GAS DYNAMICS FROM PEAT

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January 2024

Master's thesis
University of Helsinki
Master's programme in Environmental Change and Global Sustainability

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Tiedekunta – Fakultet	**Koulutusohjelma – Utbildningsprogram**	
Bio- ja ympäristötieteellinen tiedekunta	Ympäristönmuutos ja globaali kestävyys	
Tekijä – Författare		
Sofia Marttunen		
Työn nimi – Arbetets titel		
Kontrolloitujen redox-olosuhteiden vaikutus turpeen kasvihuonekaasudynamiikkaan		
Oppiaine/Opintosuunta – Läroämne/Studieinriktning		
Ympäristönmuutos / Maaperä- ja ympäristötiede		
Työn laji – Arbetets art	**Aika – Datum**	**Sivumäärä – Sidoantal**
Maisterin tutkielma	12/2023	60 + 4 liitettä

Tiivistelmä – Referat

Turvemaat ovat merkittäviä hiilen ja typen varastoja tehden niistä potentiaalisia hiilidioksidin (CO_2), metaanin (CH_4) ja dityppioksidin (N_2O) päästölähteitä. Pohjavedenpinnankorkeuden vaihtelu muuttaa turpeen happipitoisuutta vaikuttaen turpeen hapetus-pelkistystilaan eli redox-tilaan, minkä puolestaan tiedetään vaikuttavan biokemiallisiin prosesseihin ja siten kasvihuonekaasupäästöihin (KHK). Tutkimuksen tavoitteena oli arvioida kontrolloitujen anoksisten redox-olosuhteiden ja epäorgaanisten elektroninvastaanottajien (TEA) vaikutusta redox-potentiaaliin (E_h) sekä N_2O-, CH_4- ja CO_2-päästöihin. Tutkimuksessa mitattiin anaerobisena inkubaatiokokeena näiden KHK:en muodostumisnopeudet ja E_h-arvot ajan funktiona ojitetusta (D) ja ojittamattomasta (UD) kolmen ravinteisuustason turpeesta: mesotrofisesta (ME), oligotrofisesta (OL) ja ombrotrofisesta (OM). Redox olosuhteet kontrolloitiin kolmelle tasolle nitraatin (NO_3^-), ferriraudan (Fe^{3+}) ja sulfaatin (SO_4^{2-}) avulla. Lisäksi mittaukset tehtiin käsittelemättömälle (Ctrl) turpeelle.

Turve oli koko inkubaation ajan hapettomassa tilassa (E_h < 300 mV) ja arvot asettuivat TEA:n pelkistymisen mukaiseen järjestykseen vaikkakin ne olivat lähinnä raudan ja mangaanin pelkistymisalueilla johtuen todennäköisesti raudan luontaisesti suuresta määrästä turpeessa. Oletetusti N_2O:n muodostuminen oli suurinta pulloissa, joihin oli lisätty NO_3^-:a, ja N_2O:n muodostuminen oli heikkoa ja loppui ilman lisäystä. CH_4:n muodostuminen heikkeni oletetusti pulloissa, joihin oli lisätty NO_3^-:a tai SO_4^{2-}:a ja SO_4^{2-} hillitsi myös CO_2:n muodostumista, kun NO_3^-:lla ei ollut siihen vaikutusta. Sen sijaan Fe^{3+}:n lisäys lisäsi sekä CO_2:n että CH_4:n muodostumista Ctrl:iin verrattuna ja onkin mahdollista, että metanogeenit osallistuivat Fe^{3+}:n pelkistämiseen. Ctrl pulloissa redox-tila ei laskenut matalimmaksi muihin käsittelyihin nähden, vaikka UD ME turpeessa muodostuikin inkubaation lopussa eniten CH_4:ia. Kaikkien käsittelyiden kohdalla ravinteikkaan turpeen KHK-päästöt olivat suurempia laskevassa järjestyksessä ME > OL > OM. Yleisesti myös UD turpeessa kaasun muodostuminen oli D turvetta suurempaa. Kaikkia KHK:a muodostui eniten E_h-arvon ollessa noin 0 mV ja arvo oli CH_4:in muodostumiselle odotettua korkeampi, mikä johtunee metanogeenien ja raudan linkittymisestä toisiinsa. Pt-elektrodin heikko kyky havaita NO_3^-:a tai happea oli todennäköisin syy NO_3^--pullojen vaihteleviin ja mataliin E_h-arvoihin. Samasta syystä anaerobikaappiin vuotanut happi on todennäköisimmin johtanut vaihteleviin E_h-arvoihin Ctrl-käsitellyssä OM turpeessa.

Tutkimuksen perusteella voidaan todeta, että E_h-arvo on käyttökelpoinen redox-tilaa ja -reaktioita ennustava tekijä, mutta sitä on tarkasteltava yhdessä muiden mittausten, kuten mikrobianalyysin, ravinneanalyysin sekä KHK-mittausten kanssa, jotta voidaan ennustaa redox-prosesseja ja KHK-päästöjä anaerobisessa turvemaassa. Erityisesti raudan rooli CH_4 päästöissä vaatii lisää tutkimusta.

Avainsanat – Nyckelord

turvemaa, redox-potentiaali, kasvihuonekaasut, nitraatti, ferrirauta, sulfaatti

Ohjaajat – Handledare

Markku Koskinen, Mari Pihlatie

Säilytyspaikka – Förvaringsställe

Helsingin yliopiston kirjasto, Viikki

Muita tietoja – Övriga uppgifter

HELSINGIN YLIOPISTO
HELSINGFORS UNIVERSITET
UNIVERSITY OF HELSINKI

Faculty	Degree Programme
Faculty of Biological and Environmental Sciences	Environmental Change and Global Sustainability

Author
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Title
Impacts of controlled redox conditions on greenhouse gas dynamics from peat

Subject/Study track
Environmental Change / Soil and environmental science

Level	Month and year	Number of pages
Master's thesis	12/2023	60 + 4 appendices

Abstract

Peatlands are a significant carbon and nitrogen reservoirs, making them potential sources of carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) emissions. Variations in water table level change the oxygen content of peat, affecting the oxidation-reduction or redox state of the peat, which is known to influence the biochemical processes and thus greenhouse gas (GHG) emissions. The aim of this study was to assess the effect of controlled anoxic redox conditions and inorganic electron acceptors (TEAs) on redox potential (E_h), and N_2O, CH_4, and CO_2 emissions. In this study during an anaerobic incubation experiment, the rates of formation of these GHGs and E_h values as a function of time were measured from drained (D) and undrained (UD) peat of three nutrient levels: mesotrophic (ME), oligotrophic (OL), and ombrotrophic (OM). Redox conditions were controlled to three levels by nitrate (NO_3^-), ferric iron (Fe^{3+}), and sulphate (SO_4^{2-}). In addition, measurements were performed on untreated (Ctrl) peat.

The peat was in an anoxic state throughout the incubation (E_h < 300 mV) and the values were in the order of TEA reduction, even though they were mainly in the iron and manganese reduction zones, probably due to the naturally high iron content of the peat. As expected, N_2O formation was highest in flasks with added NO_3^-, and N_2O formation was weak and ceased without addition. CH_4 formation was reduced in flasks with added NO_3^- or SO_4^{2-}, and SO_4^{2-} addition also inhibited CO_2 formation on which NO_3^- addition had no effect. In contrast, the addition of Fe^{3+} increased both CO_2 and CH_4 formation compared to Ctrl treatment, and it is possible that methanogens were involved in the reduction of Fe^{3+}. In Ctrl flask, the redox state did not decrease to the lowest level compared to the other treatments as expected, but the Ctrl treated UD ME peat had the highest CH_4 formation at the end of incubation. For all treatments, GHG emissions were higher from nutrient-rich peat in the descending order ME > OL > OM. In general, UD peat also had higher gas formation than D peat. All GHGs were formed the most while E_h values were around 0 mV and the value was especially high for CH_4 formation, probably due to the linkage between methanogens and iron. The poor ability of the Pt electrode to detect NO_3^- or oxygen was the most likely reason for the variable and low E_h values of the flasks with NO_3^- addition. For the same reason, oxygen leakage of the anaerobic chamber was most likely responsible for the varying E_h values measured from Ctrl treated OM peat.

This study suggests that E_h measurement is a useful predictor of the redox state and reactions, but it must be considered together with other measurements and analyses such as microbial analysis, nutrient analysis, and GHG measurements to predict redox processes and GHG emissions in anaerobic peatland. In particular, the role of iron on CH_4 emissions requires further research.

Keywords
peatland, redox potential, greenhouse gases, nitrate, ferric iron, sulphate

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Markku Koskinen, Mari Pihlatie

Where deposited
Viikki Campus Library

Additional information

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my supervisors, Postdoctoral researcher Markku Koskinen and Professor Mari Pihlatie for your guidance and support throughout my thesis. Your mentorship has been invaluable, not only from an academic point of view but also in terms of emotional support. Moreover, I want to thank Markku Koskinen for entrusting me with the topic of this thesis and for the opportunity to executing this research from start to finish. In addition to providing me with the resources to carry out the research, you also gave me invaluable advice throughout the thesis. Particularly, your guidance with the R programming language was invaluable and will carry far into the future. I am also grateful to other members of the Department of Soil Science at the University of Helsinki for your help and friendship while implementing the practical experiment. Finally, I would like to thank my family, and especially Aleksi, for your endless support and encouragement. Without you, this thesis would not have been completed.

ABBREVIATIONS

ANAMMOX	anaerobic ammonium oxidation
ANME	anaerobic methanotrophic archaea
AOM	anaerobic oxidation of methane
CH_4	methane
CO_2	carbon dioxide
Ctrl	control
D	drained
DNRA	dissimilatory nitrate reduction to ammonium
E_h	redox potential
Fe^{3+}	ferric iron
G	Gibbs free energy
GHG	greenhouse gas
K	equilibrium constant
ME	mesotrophic
NO_3^-	nitrate
N_2O	nitrous oxide
OL	oligotrophic
OM	ombrotrophic
SO_4^{2-}	sulphate
TEA	terminal electron acceptor
UD	undrained
WTL	water table level

TABLE OF CONTENT

1 INTRODUCTION

Human activities have resulted in a significant increase in the most important anthropogenic atmospheric greenhouse gas (GHG) emissions of carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) since preindustrial times. These GHGs play a crucial role in altering the Earth's radiative properties and are the primary drivers of climate change (Canadell et al., 2021).

Peatlands around the world, especially those in northern regions like boreal and subarctic areas, have proven to play significant role in the global carbon and nitrogen cycles: their capacity for carbon sequestration contributes significantly to the carbon balance of the atmosphere (Yu et al., 2010) as peatlands cover about 3% of the Earth's total land area (Yu et al., 2011) of which boreal peatlands alone store approximately 30% of all soil carbon (Canadell et al., 2021). Until now, peatlands have acted as net sinks of carbon, but with climate change their potential to act as a sources of CO_2 and CH_4 will increase (Limpens et al., 2008). Even though peatlands are usually nitrate-limited and agricultural and natural soils are the largest source of N_2O emissions, of which natural soils account for more than half (Canadell et al., 2021), organic matter contains a significant amount of organic nitrogen. Therefore, there is a great potential for nitrogen mineralisation and large N_2O emissions from peatlands due to climate warming, especially from peatlands with permafrost (Marushchak et al., 2011).

Globally, peatlands have been drained for e.g., forestry, agriculture, and energy production. 9.1 million hectares of Finland's land area are peatlands (about 29%), of which 4.9 million hectares are drained for forestry (LUKE, 2022). Drainage alters the hydrological conditions of peatland by lowering the water table level (WTL), causing leaching of nutrients and thus eutrophication and clouding of water bodies (Nieminen et al., 2010), and affecting GHG emissions (Ojanen et al., 2010).

The WTL fluctuation causes changes in the oxygen content of the peat because the diffusion of oxygen is approximately 10 times slower in water than in air and thus cannot sufficiently replace the oxygen consumed by decomposers. Then again changes in oxygen content affect the oxidation-reduction (redox) state of the peat thus affecting biogchemical processes. Redox potential measurement is a tool to indicate, characterise, and predict these biogeochemical processes and thus environmental emissions. Therefore it is essential to understand redox reactions to identify biogeochemical processes relevant for mitigating climate change (Zhang and Furman, 2021).

1.1 Boreal peatlands

Peatlands are terrestrial areas characterised by a layer of peat, which consists of the remains of autochthonous i.e., locally grown mire vegetation, covering the underlying mineral soil. Partial or total water saturation of peat is a characteristic feature of peatlands. The formation of peat occurs when in water saturated, anoxic conditions the supply of dead plant material exceeds its decomposition rate, resulting in the incomplete decay of vegetation (Laine et al., 2018).

Peatlands are categorized into two primary types based on their water sources. Ombrotrophic peatlands or bogs primarily receive nutrients from precipitation. In contrast, minerotrophic peatlands or fens receive nutrients also from surface and groundwater that has interacted with mineral soil with different amounts of nutrients (Limpens et al., 2008). In addition, minerotrophic peatlands are further subdivided into eutrophic, mesotrophic, and oligotrophic peatlands according to their descending nutrient level, respectively. Thus, the ecohydrological differences between peatlands, i.e. the spatial, temporal, and qualitative variation of incoming water, define the structure and nutrient dynamics of peatland ecosystems (Jeglum and Rydin, 2006).

Water table level (WTL) in peatland varies depending on the water sources of the peatland, evapotranspiration, as well as climatic factors like temperature and precipitation fluctuations (Holden, 2006). WTL serves as a valuable indicator for anticipating various crucial aspects within peatlands including level of water runoff and saturation, reducing or oxidising conditions, greenhouse gas (GHG) emissions, and the decomposition of organic matter (Waddington et al., 2015). Again, GHG emissions from peat depend on the substrate quantity, soil conditions such as temperature, moisture, and oxygen content, as well as climate variations resulting from the geographical location of peatlands (Alm et al., 2007). Though, peatlands function variably as sources and sinks of GHGs. Especially on nutrient-rich peatlands, fluctuations in the WTL and peat oxygen status have a significant impact on carbon dioxide (CO_2), methane (CH_4), (Holden, 2006) and nitrous oxide (N_2O) emissions (Minkkinen et al., 2020).

1.1.1 Forest drainage

In undrained peatlands, WTL is primarily regulated by precipitation and evapotranspiration, but drainage lowers the WTL, which affects several interlinked factors such as peat oxygen content, temperature, stand volume, peat carbon/nitrogen (C/N) ratio, and peatland type (Ojanen et al., 2010). Increasing oxygen content and temperature promote microbial and vegetation respiration but also

contribute to tree growth (Jeglum and Rydin, 2006). Increasing stand volume, in turn, increases nutrient uptake, root respiration, and the amount of litter that accumulates in the soil, but also lowers the WTL (Minkkinen et al., 2007b). The above-mentioned factors reduce the C/N ratio and change the type of peatland, which again affect the runoff nutrient load, microbial activity, vegetation growth, and peat decomposition and thus GHG emissions (Minkkinen et al., 2007a). Peatland CH_4 emissions often decrease, but CO_2 emissions increase compared to undrained peatlands of the same nutrient level. N_2O emissions may increase, especially in ditches (Alm et al., 2007; Minkkinen et al., 2020). Drainage and the resulting increase in erosion and mineralisation of nutrients have also recently been found to cause higher and longer-term nitrogen and phosphorus discharges than expected (Nieminen et al., 2017a).

Moreover, rotation-forestry practices such as clear cutting and ditch maintenance cause the WTL to fluctuate. At first it rises as stand is removed increasing leaching of nutrients and organic matter (Nieminen et al., 2017b) and potentially emissions of CH_4. However, due to ditch maintenance and growing vegetation WTL is lowered, which increases CO_2 emissions (Ojanen et al., 2010). In turn, N_2O emissions are released from ditches, into which nutrients such as nitrogen run off from peatlands (Alm et al., 2007; Minkkinen et al., 2020). GHG emissions and nutrient leaching have noted to be higher from nutrient-rich than from nutrient-poor drained peatlands (Minkkinen et al., 2007a).

Mitigation of the negative effects of drainage has been attempted through, for example, restoration by rewetting the drained areas to improve and secure peatland carbon stocks and biodiversity. Restoration causes at least temporary leaching of nutrients and organic carbon (Koskinen et al., 2011), increases CH_4 emissions (Abdalla et al., 2016), but decreases N_2O emissions probably to similar levels with the original, natural state (Minkkinen et al., 2020). That is why peatlands for restoration should be chosen carefully to minimise emissions.

1.2 Redox chemistry

Oxidation-reduction reactions, or redox reactions, describe an electron transfer reaction that involves both electron donation (oxidation) and electron acceptance (reduction) between two elements or molecules (e.g., James and Brose, 2011). In redox reactions the activity of electrons concerning a specific standard state is considered. The standard state for electron activity is defined with respect to the redox couple formed by hydrogen ions and hydrogen gas. This allows us to treat electrons as

discrete species in equilibrium calculations. Though, it is important to recognize that there are no free electrons in redox reactions, only in theory (Kirk et al. 2004).

1.2.1 Redox potential

The intensity of the oxidation-reduction state in a liquid environment, such as soil solution, can be characterised with redox potential (E_h, V) (Zhang and Furman, 2021). E_h value describes the tendency of the system to oxidise or reduce chemicals. When redox potential is high it indicates an oxidising environment and when it's low it indicates a reducing environment (Frohne et al., 2011). Redox potential is also a measure of electron activity in a system (Kirk, 2004).

Theoretical limits for the highest and lowest levels of oxidation and reduction in soil ecosystems are determined by the potential of water to undergo oxidation (to oxygen gas) and reduction (to hydrogen gas). Typically, this potential falls within the range of -1 to 1 V, serving as the upper and lower boundaries for the intensity of oxidation and reduction (Zhang and Furman, 2021).

Redox potential can be derived from electron activity (pe) and redox equilibrium constant (K) by using Gibbs free energy change (ΔG). Gibbs free energy (equations 1 and 2) describes the maximum possible work that a process, in this case an oxidation-reduction reaction, can do at constant pressure and temperature (Strawn et al., 2020). By combining the redox equilibrium constant with Gibbs free energy change, it is possible to derive the Nernst equation and thus redox potential. The following equations 1 to 5 and the derivation of K can be found e.g., from book by James and Brose (2011). Equations 6 to 8 are derived from book by Strawn et al. (2020).

$$\Delta G_r^\circ = -RTlnK \qquad (1)$$

Where, ΔG_r° is Gibbs free energy change under standard conditions (298 K and 1 atm), R is gas law constant (0.00831 kJ K^{-1} mol^{-1}), and T is absolute temperature (298 K).

$$\Delta G_r^\circ = -nFE_h \qquad (2)$$

Where n is number of electrons transferred per reaction and F is Faraday constant (96.5 kJ V^{-1} $equivalent^{-1}$). Both equations 1 and 2 are expressions for Gibbs free energy change from which the Nernst equation (equation 8) is derived:

$$-nFE_h = -RTlnK \quad (3)$$

$$E_h = \frac{RT\ 2.303logK}{nF} = 0.0592\ logK \quad (4)$$

In addition to electrons, protons also move in redox reactions. Oxidation reactions produce protons and reduction reactions consume them. As the number of protons in solution increases, the pH decreases and vice versa. In other words, in addition to pe, the redox potential also depends on pH (Strawn et al. 2020). For one electron, one proton reaction where reduction and oxidation reaction activities are equal, K takes a form:

$$pe + pH = \log K \quad (5)$$

Which, for hydrogen reduction is:

$$pe + pH = 0 \quad (6)$$

Thus, when pH increases one unit, the E_h value decreases by 59 mV (see equations 4, 5, and 6). When pH is 7 production and consumption of protons are in equilibrium, which means that the activities of oxonium and hydroxide ions are equal. In other words, the change in pH and its effect on E_h value should be considered in relation to pH 7:

$$\Delta E_h = 0.0592\ V \times (pH - 7) \quad (7)$$

Where ΔE_h is the change in redox potential caused by the pH change. The change can also be described in relation to the standard electrode potential (E_h^0) (equation 8), which for standard hydrogen electrode is 0.

$$E_h = E_h^0 + \Delta E_h = E_h^0 + 0.0592\ V \times (pH - 7) \quad (8)$$

Thus, the E_h value of the system is driven by the ratio of oxidized and reduced species activity and is directly affected by environmental factors such as temperature and pH value (Frohne et al., 2011).

1.2.2 Redox reactions in peatlands

The specific lower and upper boundaries for redox potential (E_h) in natural systems are primarily determined by the reduction of carbon dioxide (CO_2) to methane (CH_4) and the presence of oxygen (Inglett et al., 2012). In nature, terminal electron acceptors (TEA) are utilized in order of the energy gained from their reduction reaction i.e., the smaller the value of Gibbs free energy change (ΔG) and the higher the estimated E_h value, from highest to lowest $O_2 > NO_3^- > Mn^{4+} > Mn^{3+} > Fe^{3+} > SO_4^{2-} > CO_2$ (table 1). When there are excess electron donors especially hydrogen gas even the redox reactions with the lowest redox potential occur (Achtnich et al., 1995).

Table 1. Terminal electron acceptors (TEA), example of their theoretical reduction half-reactions, and theoretical ranges of redox potential (E_h) for a one electron transfer at pH 7 for soils. Modified from books by Strawn et al. (2020) and James and Brose (2011).

Electron acceptor	Example reaction	E_h (mV)
Oxygen gas (O_2)	$O_2 + 4e^- + 4H^+ = 2H_2O$	+810
Nitrate (NO_3^-)	$NO_3^-(aq) + 2e^- + 2H^+ = NO_2^-(aq) + H_2O$ $2NO_3^-(aq) + 8e^- + 10H^+ = N_2O(g) + H_2O$ $2NO_3^-(aq) + 10e^- + 12H^+ = N_2(g) + 6H_2O$	+750
Manganese (Mn^{4+})	$MnO_2(aq) + 2e^- + 4H^+ = Mn^{2+}(aq) + 2H_2O$	+520
Ferric iron (Fe^{3+})	$Fe(OH)_3 + e^- + 3H^+ = Fe^{2+} + 2H_2O$	+100 to -100
Sulphate (SO_4^{2-})	$SO_4^{2-}(aq) + 8e^- + 8H^+ = S^{2-}(aq) + 4H_2O$	-200
Carbon	$CO_2(g) + 8e^- + 8H^+ = CH_4(g) + 2H_2O$	-240

In theory the primary redox couple in a system can be deduced by examining the E_h values. Nevertheless, in natural environment distinct redox boundaries are rare, as several redox reactions often take place concurrently, influenced by various electron donors and acceptors (Rivett et al., 2008). As a result, the redox reactions in a natural system can never reach a true equilibrium state and the measured E_h value is a mixed potential, mainly affected by the dominant redox couples, instead of the redox potential of a single reaction. Moreover, the measured E_h value is influenced by many factors such as TEAs, temperature, and electron donor availability (Wang et al., 2018). Nevertheless, in theory it is possible to estimate from E_h value the pathway by which organic matter is oxidised and what gases are produced in the process (Hanke et al., 2013).

Reduction reactions other than oxygen reduction occur in oxygen limited environments such as flooded peatlands. Incubation in flooded conditions decreases oxygen concentration and redox

potential, which influences significantly to various biogeochemical processes (Limpens et al., 2008). Based on E_h value, soil can be determined aerobic (> 300 mV) or anaerobic (< 300 mV) (Thompson et al., 2009) and further into oxidising (> 400 mV) and weakly (200 to 400 mV), moderately (-100 to 200 mV), and strongly reducing (< -100 mV) (Inglett et al., 2012). Redox reactions are driven by both biotic and abiotic processes; in natural environments involving organic matter as electron donor primarily biotic processes, which play a crucial role in various soil biogeochemical activities such as soil formation, the mobility and accessibility of nutrients, and the distribution of contaminants. By using different TEAs microbes ensure energy supply in a wide range of conditions (Burgin et al., 2011).

When oxygen begins to run out from peatland, microbes start to utilize first nitrate (NO_3^-) and then manganese as TEA. Nitrogen occurs as several different molecules and oxidation states of which NO^{3-} reduction into nitrogen gas (N_2) produces the most energy (table 1) and is thus preferred by facultative anaerobes as oxygen content decreases (Rivett et al., 2008). The primary sources of nitrogen in peatlands are nitrogen deposition from the atmosphere, nitrogen fixation by bacteria or algae, and the inflow of nitrogen through upland runoff or discharge, whereas the main nitrogen losses from a peat ecosystem are typically attributed to nitrogen exports via runoff or streamflow and the process of denitrification (Limpens et al., 2006). Manganese enters peatlands from the soil mineral fraction with the run-off waters and its concentration in peat is usually low. When reduced, manganese changes from solid to soluble form and is susceptible to leaching since manganese is not as strongly retained in peat as, for example, iron (Wieder and Lang, 1986).

When NO_3^- and manganese are depleted, microbes switch to using ferric iron (Fe^{3+}) as TEA. Iron is the most common redox active metal in soil and the majority of it is found in the mineral matter. Iron ends up in peat soils with run-off water as a result of weathering (Yu et al., 2016), where most of it forms metal complexes with organic matter (Yonebayashi, 2006). Ferrihydrite ($Fe(OH)_3$) is a common solid iron element in wetlands, which reduces into ferrous iron (Fe^{2+}) (table 1). The reduced species, Fe^{2+} is more soluble than Fe^{3+}. The redox potential of Fe^{3+} reduction varies widely from less than -200 mV for solid goethite to more than +700 mV for the organic iron complexes (Strawn et al., 2020).

Again, as Fe^{3+} becomes depleted, microbes switch to using sulphate (SO_4^{2-}) and eventually carbon as TEA. SO_4^{2-} can end up in peatlands through mineralization, acid deposition, and air pollution (Sutton-Grier et al., 2011) most of which is bound to organic matter (Wieder and Lang, 1986). Finally,

inorganic carbon, such as acetate and CO_2, is utilized in the anaerobic mineralization (oxidation) of organic matter as an electron acceptor (Galand et al., 2005).

In peatlands, humic substances (HS) in highly decomposed organic matter can also act as electron acceptors in the anaerobic decomposition of organic matter (Bai et al., 2019). Depending on the study, HS redox potential values have been found to vary between -300 and +300 mV (Aeschbacher et al., 2011; Straub et al., 2001), due to the abundance of different chemical groups of which functional group quinone has been discovered to be the most significant (Ratasuk and Nanny, 2007). The same microbes that can use HS in their redox reactions can also use other electron acceptors or donors (Cervantes et al., 2008).

Furthermore, as noted earlier, only minerotrophic peatlands – mesotrophic and oligotrophic – receive nutrients such as iron and manganese with run-off waters, unlike ombrotrophic peatlands, where NO_3^-, SO_4^{2-}, and C are usually the most important TEAs albeit in low concentrations (Boothroyd et al., 2021). In addition to nutrient level, the diversity and activity of microbial population is also higher in minerotrophic peatlands, which reduces in consequence of drainage, particularly the proportion of methanogens decreases. As a result of drainage, the microbiota of minerotrophic and ombrotrophic peatlands become more similar to each other, especially in the surface peat layer (Urbanová and Bárta, 2016).

1.3 Greenhouse gases as a by-product of redox reactions

The redox potential (E_h) value and greenhouse gas (GHG) emissions are interconnected as the E_h value indicates which redox reaction is dominant at the time of measurement. When E_h value is high, particularly in oxic-anoxic interface (around +300 mV), there is a potential for nitrous oxide (N_2O) emissions as nitrate (NO_3^-) is likely to be utilised as terminal electron acceptor (TEA). Conversely, under anoxic conditions and with low E_h value (below -150 mV), methane (CH_4) may be formed with carbon dioxide (CO_2) and acetate acting as TEA. CO_2 is formed regardless of E_h value but in varying concentrations (Wang et al., 2018). Notably, even under oxic conditions with a very high E_h value, the presence of anoxic microsites with suitable electron donors and acceptors can lead to the formation of N_2O or CH_4 (Zhang and Furman, 2021).

As mentioned earlier, the amount of GHG emissions formed in soil is influenced by several factors such as soil water table level (WTL), biological activity, temperature, nutrients, and pH value

(Vasander and Kettunen, 2006). However, microbial decomposition of organic matter plays an essential role in the production of GHG emissions, especially in the anoxic redox processes where TEAs other than oxygen are utilized (Stein, 2020). Decomposition initiates with the physical breakdown of organic materials, converted into simpler compounds by exoenzymes, enzymes working outside the cell, produced by microorganisms. These compounds microbes can oxidize in microbial respiration by using different TEAs (Sutton-Grier et al., 2011).

Moreover, the quantity and quality of decomposable organic matter has been found to affect GHG emissions more than the concentration of TEAs because the more easily decomposable the organic matter is (such as glucose) and the more abundant it is, the faster the reaction occurs and the more GHGs are produced. Microbes also adapt to utilize the available electron donors and changes in that, for example due to drainage, shifts the microbial population composition, further influencing GHG dynamics (Sutton-Grier et al., 2011).

In other words, GHG emissions are influenced not only by the TEAs but also by the amount of electron donors and the microbiota. The E_h value therefore only indicates which TEAs are most available at the time of measurement and what reactions could potentially occur (Zhang and Furman, 2021).

1.3.1 Carbon dioxide and methane

In peatlands CO_2 is produced by mostly biotic decomposition of organic matter with all TEAs and due to the oxidation of CH_4, whereas CH_4 is formed by methanogens – methanogenic archaea – in methanogenesis through the reduction of carbon, mostly either CO_2 or acetate in anaerobic conditions (E_h < -150 mV) (Candry et al., 2023; Galand et al., 2005). In other words, all other TEA reduction reactions with organic carbon compounds as electron donors produce CO_2 except the reduction of CO_2 (Dettling et al., 2006).

Since CO_2 is formed with all TEAs, its formation is more energy efficient and dominates over CH_4 formation. However, in flooded peatlands, when all the TEAs are consumed, the microbes switch to cooperatively decomposing organic matter to obtain energy, which is very slow process. First, hydrolytic bacteria break down organic matter into e.g., sugars and amino acids. Fermentative microorganisms then convert these substances into short-chain carboxylic acids, hydrogen (H_2) and CO_2. Next, acetogenic bacteria again oxidise the carboxylic acids to produce acetate, H_2, and CO_2

(Candry et al., 2023). Finally, there are two main pathways to CH_4 formation in peatlands, acetoclastic and hydrogenotrophic methanogenesis, in which acetoclastic methanogens convert the produced acetate and hydrogenotrophic methanogens convert the produced CO_2 into CH_4 (Galand et al., 2005).

However, the net CH_4 emissions are affected by how much of the CH_4 produced is oxidised back to CO_2 through oxidation of methane (He et al., 2015). This can be performed by both anaerobic and aerobic methanotrophs (various CH_4 oxidising microbes) utilizing different TEAs, and anaerobic methanotrophic archaea (ANME) in association with, for example, sulphate reducing bacteria or by reducing various TEAs (Stein, 2020). Especially in pristine peatlands, the high diversity of microbes creates the potential to utilize all available electron acceptors (He et al., 2015) and studies have been able to link anaerobic oxidation of methane (AOM) to several different TEAs, including NO_3^- (Candry et al., 2023; Haroon et al., 2013), Fe^{3+} (Cui et al., 2015), SO_4^{2-} (Lozanovska et al., 2016), and HS (Valenzuela et al., 2019).

Microbes also compete for living space and shared electron donors, and the microbes that can use the TEAs with the highest E_h value, i.e. gain most energy from a redox reaction, do best in the competition (e.g., Achtnich et al., 1995). Furthermore, H_2 produced by for example, fermentative bacteria is competed for in anaerobic environments as protons (H^+) are required in all peatland reduction reactions and H_2 is utilized directly as an electron donor by methanogenic, but also NO_3^-, SO_4^{2-}, and some Fe^{3+} reducing bacteria and acetogenic bacteria (Garcia et al., 2000). Therefore, it is assumed that the treatment of peat with TEA will both inhibit the reduction of TEA with a lower E_h value and thus either fully or partially inhibit CH_4 formation (Achtnich et al., 1995).

However, most of the studies of the mitigating effect of especially Fe^{3+} on CH_4 emissions are from other soils and sediments than peatlands (e.g., Jäckel and Schnell, 2000; van Bodegom et al., 2004) and the results for peatlands are uncertain (Smemo and Yavitt, 2007; Smemo and Yavitt, 2011). There are even indications that neither the addition of Fe^{3+} nor the naturally high iron content of the peatland inhibits the CH_4 formation but may even stimulate it (de Jong et al., 2020; Dettling et al., 2006).

Since groundwater contact increases the nutrient content and the proportion of TEAs in the peatland, CH_4 emissions can be expected to be higher in minerotrophic systems compared to ombrotrophic systems (Urbanová et al., 2013). However, as CH_4 formation requires highly reducing conditions, WTL has a great influence on how much CH_4 is formed. As a result of drainage, oxygen is released into peatland and the E_h value rises, which inhibits methanogenesis and increases methane oxidation.

Oxygen is also toxic to methanogens, which means that their contribution decreases because of drainage. In turn, CO_2 emissions increase as organic matter decomposition accelerates (Yrjälä et al., 2011). Thus, over time, drainage changes both the microbial population and the quantity and quality of organic matter. Re-wetting of peatland restores the microbial population relatively quickly to pristine like, but the potential electron donor limitation due to peat decomposition may inhibit the formation of GHG emissions (Urbanová and Bárta, 2020).

The formation of CO_2 and CH_4 emissions in peatland is therefore the sum of several factors, the most important of which are the oxidation state of the peat, the microbial population composition, available TEAs, and the number of electron donors.

1.3.2 Nitrous oxide

N_2O emissions are formed in peatland through several abiotic and biotic pathways, with a wide range of E_h values: nitrification ($E_h \approx 400$ mV), hypoxic denitrification ($E_h \approx 200$ mV), anoxic dissimilatory nitrate reduction to ammonium, or DNRA, and anaerobic ammonium oxidation, or ANAMMOX ($E_h < 300$ mV) (Bai et al., 2015; Wang et al., 2018). The most important of these for N_2O formation are microbial nitrification and denitrification (about 70% of production) (Butterbach-Bahl et al., 2013) and in peatlands most of the N_2O formation occurs from denitrification (Pihlatie et al., 2004). The N_2O fluxes are highest when E_h is from +300 to 550 mV and below that the portion of nitrogen gas (N_2) as the end product of denitrification increases (Butterbach-Bahl et al., 2013).

Nitrification is the microbiological oxidation of ammonium or organic nitrogen compounds to nitrite (NO_2^-) or NO_3^-. Complete nitrification, the oxidation of ammonia (NH_3) or ammonium (NH_4^+) to NO_3^-, can occur both aerobically and anaerobically by microbes that conduct nitrification i.e., nitrifiers. In autotrophic nitrification, autotrophic nitrifiers oxidise NH_3 by using copper-containing enzyme that requires oxic conditions. In heterotrophic nitrification either NH_3 or nitrogen containing organic matter is oxidised to NO_3^- mainly under anoxic conditions. Heterotrophic nitrifiers need organic matter as electron donor, which means that the availability of decomposable organic matter limits heterotrophic nitrification (Martikainen, 2022; Regina et al., 1998). Autotrophic nitrification is more important nitrifying process than heterotrophic nitrification, and it is inhibited by high WTL and anoxic conditions (Regina et al., 1998).

N_2O is formed at low oxygen content from the intermediate products of nitrification, hydroxylamine (NH_2OH) and nitroxyl (HNO) or by heterotrophic nitrifier denitrification. One nitrifying microbe can both nitrify and denitrify, or reactions can be done by distinct microbes (Butterbach-Bahl et al., 2013; Martikainen, 2022). In denitrification several different microbes decompose organic matter by reducing nitrate (Philippot et al., 2007). N_2O is produced in incomplete denitrification by reducing NO_3^- to NO_2^-, nitric oxide (NO), and N_2O. In oxygen limited environment, N_2O is further reduced to nitrogen gas (Regina 1998). Nitrifier denitrification is speculated to be the dominant source of N_2O in oxygen limited environment (Wrage-Mönnig et al., 2018).

Other important reactions producing N_2O are anoxic dissimilatory nitrate reduction to ammonium (DNRA) and anaerobic ammonium oxidation (ANAMMOX). In addition to denitrification, the DNRA and ANAMMOX reactions also reduce NO_3^- to NO, N_2O, and N_2. In DNRA reaction N_2O is produced as a byproduct of NO_3^- reduction to NH_4^+ via NH_2OH or NO (Kraft et al., 2011). As for ANAMMOX, contrary to nitrification, NH_4^+ is oxidised in anaerobic conditions by using NO_3^- as TEA instead of oxygen, and contrary to denitrification, NH_4^+ is used as the electron donor instead of organic matter (Bai et al., 2015).

In addition to other factors affecting GHG emissions (E_h, pH, temperature, etc.), especially high NO_3^- and NH_4^+ concentrations and low WTL contribute to N_2O formation (Wang et al., 2018). High WTL and consequent anoxia prevents autotrophic nitrification, so that NO_3^- is not formed for reduction (Leppelt et al., 2014; Regina et al., 1998). On the other hand, denitrification decreases with increasing soil oxygen content as oxygen inhibits nitric oxide reductase (an enzyme, that catalyses the reduction of NO to N_2O) activity. However, there is no denitrification if substrate is not available. In addition, the decomposition rate of the peat, organic matter quality, microbial activity, and plant nutrient uptake affect the availability of electron donors and nitrogen compounds needed for N_2O formation (Roobroeck et al., 2010).

Drainage increases the peat decomposition and nitrification, increasing N_2O emissions (Martikainen et al., 1993). In field studies, N_2O emissions from drained peatlands have been found to be higher than those from pristine peatlands, which is contributed by the lowered WTL and particularly the high nutrient content of the peat (Leppelt et al., 2014; Minkkinen et al., 2020; Regina et al., 1996). Peatlands with high N_2O formation also have the highest formation of hydroxyl radicals (OH) compared to other peatlands. OH-radicals are the most important oxidant in the atmosphere, forming, among other things, ground-level ozone, and oxidising CH_4 to CO_2 (Maljanen et al., 2013).

Organic matter decomposes more slowly under anoxic conditions, which reduces N_2O formation, and N_2O emissions of restored peatlands have been found to return to the levels of pristine peatlands (Minkkinen et al., 2020). However, right after WTL elevation, high amounts of N_2O can be formed as the surface peat NO_3^- is reduced to N_2O (Groffman et al., 2009; Martikainen et al., 1993; Minkkinen et al., 2020). These temporary, abundant N_2O emissions also occur in drained, nutrient-rich peatlands as a result of drainage-rewetting cycles (Groffman et al., 2009; Philippot et al., 2007).

N_2O emissions are highest when at least 70-80 % of pore space is filled with water (Butterbach-Bahl et al., 2013) and both nitrification and denitrification reactions occur simultaneously. More water filled pores (> 80%) is favourable to denitrification, but denitrification may occur even in aerobic peat in anoxic microsites (Regina et al., 1998). In other words, high content of available N and oxic-anoxic interphase, thus redox potential around +300 mV, promotes N_2O emissions (Minkkinen et al., 2020).

1.4 The objectives of the study

Decisions on the use of peatlands must be based on scientific knowledge to protect the climate, water quality, biodiversity, and ensure sustainable utilization of nature. The chemical reaction chains that occur when peatland water table level (WTL) changes are complex and current ecosystem models do not take this into account, making the prediction of changes uncertain. Redox potential (E_h) measurement is little used in ecosystem modelling, even though measured emissions are the end products of redox reactions and measuring E_h values both in the field and laboratory is relatively easy and cheap. In a best-case scenario, E_h value measurement can be used to predict chemical reactions in peat and the resulting emissions in advance, thus increasing the certainty of ecosystem modelling.

This thesis is part of a larger Academy of Finland-funded project (339489) that aims to create a model that predicts the redox processes in anaerobic peat soils. The different phases of the project will investigate how nutrient level and drainage status affect the microflora and its ability to utilise different terminal electron acceptors (TEAs) and how this is reflected in GHG emissions and soluble nutrient concentrations. However, the analysis of peatland microbes and water samples is excluded from this thesis.

The aim of this study is to assess the impact of controlled anoxic redox conditions and role of inorganic TEAs on E_h value and N_2O, CH_4, and CO_2 emissions from peat with different nutrient levels and drainage status with a purpose to investigate weather E_h measurement can in theory be utilized in field conditions to provide information on the TEAs available for redox reactions in peat and to predict GHG emissions.

The aim is to answer the following research questions:

1. How does TEA/nutrient level/drainage status affect N_2O, CH_4, and CO_2 emissions released from peat?
2. How does TEA/nutrient level/drainage status affect the measured E_h values?
3. Can the E_h measurement be used to assess the utilized TEAs and N_2O, CH_4, and CO_2 emissions released from peat?

1.4.1 Hypotheses

1. During anaerobic incubation of peat with added TEAs, N_2O flux is greatest from flasks with added NO_3^- and formation is significantly reduced or eliminated over time without NO_3^- addition to the slurry.
2. During anaerobic incubation of peat, CH_4 flux is suppressed due to NO_3^-, Fe^{3+}, and SO_4^{2-} addition, the most from NO_3^- addition.
3. As incubation continues in Ctrl flasks the redox state decreases causing N_2O flux to decrease or cease and CH_4 flux to increase being the highest compared to other treatments, with methanogenesis being the main redox reaction.
4. During anaerobic incubation of peat with varying nutrient levels, higher N_2O, CO_2, and CH_4 fluxes are observed from nutrient-rich peat due to higher substrate availability, following a descending order of ME > OL > OM peat.
5. In general, gas flux is higher from UD peat throughout the incubation because the microbial population in D peat is adapted to low WTL and oxic conditions, and because the organic matter is in more decomposed form due to drainage.
6. In an anaerobic incubation of peat with added TEAs, the measured E_h values settle in descending order according to the energy released in the reduction of TEA, in the following order $NO_3^- > Fe^{3+} > SO_4^{2-} >$ Ctrl.

2 MATERIAL AND METHODS

2.1 Study site, peat sampling and treatment

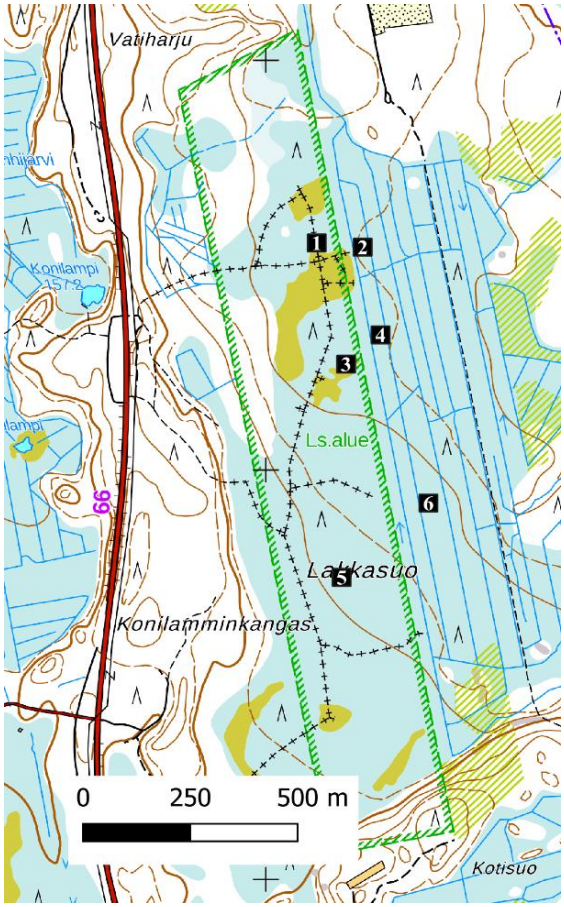

Figure 1. Sampling sites in Lakkasuo, Orivesi. Details in Table 2. Base map downloaded from National Land Survey of Finland 9/2023.

In the experiment, six different peat types were studied. All peat samples were from Lakkasuo, Orivesi, Finland (N 61.79°, E 24.31°). The six sampling sites were from three different nutrient levels, mesotrophic (ME), oligotrophic (OL), and ombrotrophic (OM) each from both drained (D) and undrained (UD) area (Figure 1, Table 2). The D peatland plots used in the study have evolved from UD peatland of similar nutrient level. For example, when tall-sedge pine fen (VSR) was drained it has over time evolved into *Vaccinum vitis-idaea* (Ptkg(II)) type (Laine et al., 2018). The peatlands were drained around 1961 (Martikainen et al., 1993). More details about Lakkasuo in book from Laine et al. (2004).

Sampling took place in May 2022 after the frost was thawed, wherefore the water table levels (WTL) were generally high (Table 2). All peat samples were randomly taken from one depth (5-20 cm) with disinfected equipment and gloved hands. Sampling was done using serrated knife and field shovel by firstly peeling off the living surface layer (about 5 cm) and taking approximately 15 x 15 x 15 cm piece of peat. The samples were tightly wrapped in plastic and transferred in a cooler to the laboratory refrigerator.

Table 2. *Details about the sampling plots used in the experiment. The site number corresponds to the sites in Figure 1.*

Site	Drainage status	Trophic level	WTL (cm)*	Type	Examples of dominant species
1	UD	OL	0	Tall sedge pine fen (VSR)	*Pinus sylvestris* *Carex lasiocarpa* *Sphagnum fallax*
2	D	OL	-15	*Vaccinium vitis-idaea* type (Ptkg(‖))	*Betula pubescens / Pinus sylvestris* *Vaccinium vitis-idaea / V. myrtillus* *Ledum palustre*
3	UD	ME	-5	Herb-rich sedge birch-pine fen (RhSR)	*Picea abies / Pinus sylvestris* *Ledum palustre* *Potentilla palustris*
4	D	ME	-5	*Vaccinum myrtillus* type (Mtkg(‖))	*Picea abies / Pinus sylvestris* *Vaccinum myrtillus / V. vitis-idaea* *Dryopteris carthusiana*
5	UD	OM	-5	*Sphagnum fuscum* pine bog (RaR)	*Pinus sylvestris* *Cladonia stellaris* *Eriophorum vaginatum*
6	D	OM	-5	*Cladonia* type (Jätkg(|))	*Pinus sylvestris* *Cladonia stellaris* *Eriophorum vaginatum*

UD = undrained, D = drained, OL = oligotrophic, ME = mesotrophic, OM = ombrotrophic
*Water table level (WTL) measured at the time of sampling compared to the peat surface.

To determine the water content of each peat sample, subsamples of each in two parallels were dried in an oven (Memmert, Germany) (105 ºC, 1.5 h) and weighed with precision scale (Mettler AE 166, Precisa, Switzerland) before and after drying to determine the dry matter and water contents of the fresh peat samples (Appendix 1, Table 3). The initial dry weights of peat added to each flask (Appendix 2) was calculated by multiplying the mass of peat in the flasks by the dry matter contents of peat samples determined as described above.

2.2 Experimental set-up

The study was carried out in the laboratory as an incubation experiment. First all 500 ml borosilicate glass flasks (VWR) with butyl rubber septa and plastic lids were weighed with precision scale, 180 g of MilliQ water was added to each one, and autoclaved. Redox status of the samples was controlled to four levels by adding MilliQ water enriched with terminal electron acceptors (TEA) ferric iron (Fe^{3+}), nitrate (NO_3^-), or sulphate (SO_4^{2-}) or only MilliQ water (control samples) into each flask. The TEAs were added to the flasks as potassium nitrate (KNO_3), sodium sulphate (Na_2SO_4), and ferric oxyhydroxide (FeOOH). The concentrations of the TEA solutions of the flasks were 10mM KNO_3, 1mM Na_2SO_4, and 10mM FeOOH. These final concentrations were chosen after electron acceptor

experiment by Smemo and Yavitt (2007). Finally each peat type were added to the flasks (peat:water 1:4.5 V:V) (Appendix 2) and the head space was flushed with nitrogen gas (N_2) for 15 minutes. During the experiment, samples were stored in dark refrigerated room in 15 ºC. Each combination of redox state and peat type was repeated in three parallel (*n = 24 treatments as 3 parallels = 72 samples*).

Incubation period was 14 days and gas samples were taken 5 times per period. The pressures of the flasks were measured with a pressure meter (Tensimeter, Soil measurement systems, USA) at the end of each incubation period (Appendix 4), after which water sampling in day 14 started new incubation period, total of five periods (*t = 70 d*). Water samples were taken in an anaerobic chamber (custom built for University of Helsinki) with a nitrogen (N_2) atmosphere and oxygen (O_2) content of 1-5% at the time of sampling (Honeywell BWS-XL-Y, Honeywell Analytics, Great Britain). During water sampling pH and redox potential (E_h) values of the samples were measured (Appendix 3) with pH meter (H170, Hach, USA) and voltmeter (Jensen Instruments, USA). 40 ml water samples were taken from each flask with a Finn pipette and replaced with autoclaved MilliQ water and, if necessary autoclaved TEA solutions and/or concentrated hydrogen chloride (HCl) were added due to change of E_h and/or pH. The amount of solution added depended on the change in the measured value compared to the previous measurement; TEA solutions were added with the Finn pipette so that the measured E_h value remained within the reduction range of the TEA in question (Appendix 3), and HCl solution with the Pasteur pipette so that the pH decreased to approximately the same value as in the previous measurement. The amount of added TEA solution was subtracted from the amount of added MilliQ water.

Gas samples (8 ml) were taken 5 times per round (*n = 5 gas samples over 5 rounds = 25 samples per each 72 flasks = 1800 gas samples*) into 3 ml pre-vacuumed, and helium flushed glass vials. Gas samples were analysed with gas chromatograph (Agilent Technologies 7890B GC System, customised for the University of Helsinki 2013 with D53-Chamstat 01 software, Open LAB CDS ChenStation Edition).

2.3 Measurement methods

2.3.1 Redox potential

The redox potential (E_h) values of the peat slurries were measured with a redox meter (details above). A redox meter requires a voltmeter, a metal electrode that is electron-sensitive and capable of

conducting electrons, and a reference electrode. The voltage or potential difference between electrodes indicates the redox potential and it is derived from the Gibbs free energy (ΔG) of the electrode reaction (cf. chapter 1.2.1). The metal electrode needs to assimilate the electrical potential of the sample without undergoing any chemical reactions or altering the equilibrium of the solution; in simpler terms, it must remain non-reactive or inert. Metals can be considered inert when their stable potential surpasses the redox potential of the sample by more than 100 mV, a characteristic often found in precious metals such as gold and platinum (Galster, 2000). A regular voltmeter with a custom-made platinum (Pt) electrode and silver-silver-chloride (Ag|AgCl) reference electrode filled with 3 M potassium chloride (KCl) electrolyte were used in this study.

Redox measurements are relatively easy to perform, and it has been possible to apply the measurement to water samples of pure chemicals with good results. In natural environments, biogeochemical reactions are complex, and many different elements are present in the soil solution. The Pt electrode reacts to redox couples, which rapidly reach electrochemical equilibrium, as the potential between the electrodes is measured when the reaction is in equilibrium. However, redox reactions containing carbon, sulphur, and nitrogen are slow to equilibrate, making E_h measurement difficult for solutions containing multiple elements. Indeed, Fe^{3+}/Fe^{2+} is the only redox couple with a fast reaction and high ionic activity (Christensen et al., 2000). In addition, the Pt electrode does not detect O_2 or NO_3^- very well due to lack of electroactivity, but is instead sensitive to high iron concentrations (Appelo and Postma, 2004).

2.3.2 Gas chromatography

Gas chromatography (GC) is a technique used to separate and identify chemical components in a sample, such as greenhouse gases (GHGs). The GC instrument used in this study (details above) uses inert helium as a carrier gas to move the sample through the system. First, the sample is introduced into the GC by an automatic sampler. Inside the instrument is a long, narrow tube called the analytical column, which is lined with a solid phase called the stationary phase. This column is heated to separate the components. The separation of gases is based on the interaction of the components with the analytical column, for which they have different affinities. As a result, different gases move through the tube at different speeds. As the sample leaves the column, it is detected by a specialised detector for each gas, which generates a signal. This signal is recorded by a computer program to produce a chromatogram (Turner, 2022).

The fact that the analysis is automated as much as possible reduces errors, but nevertheless, GC may leak during analysis or suffer other interferences. Several factors, such as column and injection temperatures, injection speed, flow rate of the standard gas, and sample volume, affect GC analysis and its results (Barwick, 1999). For error detection, a standard gas with known properties is analysed before, between and after the actual samples. By comparison with the standard gas, inaccuracies can either be corrected or the results excluded from the final analysis.

2.4 Calculations

Gas chromatograph (GC) gives the concentration of elements as parts per million (ppm). First the results of the GC analysis were corrected by drift-correction by Simojoki (2022) using standard samples. Because the total amounts from GC analysis aren't exactly one million, the amounts of gas samples were therefore corrected by using equation 9.

$$Corrected\ amount = \frac{Gas\ amount \times 1\ 000\ 000}{Total\ amount} \quad (9)$$

A linear model of gas concentrations per round as a function of time was then constructed, which allowed individual errors in measurement or analysis to be excluded from the final analysis.

The flask pressures were measured to correct gas content of the sample (8ml) to the amount of gas in the head space of the flask (equation 11). The pressure meter (details above) gives the difference in pressure (mbar) between flask and atmosphere (Appendix 4). The pressure values were therefore corrected to match the internal pressure of the flasks by using Boyle's law assuming a constant temperature (equation 10). The amount of gas removed from the flasks during gas sampling was considered by adding the volume of the sample to the volume of the head space of the flask.

$$p_2 = p_a + \frac{p_1 \times V_1}{V_1 + V_s} \quad (10)$$

Of which p_1 *= measured pressure (Pa),* p_2 *= corrected pressure (Pa),* p_a *= ambient pressure (Pa),* V_1 *= head space volume (*m^3*),* and V_s *= sample volume (*m^3*).* After that the amount of gas in the sample was changed from parts per million to decimals by dividing with the total volume (10^6), and then adjusted to represent the amount of gas in moles in the head space of the flask by multiplying with the total amount of gas (mol) in the head space calculated from the ideal gas law (equation 11).

$$n = \frac{pV}{Rt} \quad (11)$$

Of which *n = amount of substance (mol), p = corrected pressure (Pa), V = head space volume (m^3), R = the molar gas constant (Pa m^3 (mol K)$^{-1}$),* and *t = temperature (K)*. The gas flux was calculated by making a linear model of gas production as amount of substance per unit time and dividing the slope with initial weight of the peat. The mass flux was obtained by multiplying the molar flux by the molar mass of the considered gas.

The redox potential (E_h) values were calibrated to the standard hydrogen electrode by adding 200 mV (the correction factor for Ag|AgCl 3M KCl reference electrode at 20 °C) to the measured value. To account for the different pH levels measured during incubation, E_h values were adjusted as the corresponding values at pH 7 using the inverse relationship between E_h and pH (equation 8 in chapter 1.2.1) which follows the Nernst equation (Strawn et al. 2020). This adjustment allowed for a standardized comparison of E_h values across different pH levels of peat.

2.5 Statistical analysis

In scientific research, either a 5% or 1% significance level is commonly used. If a result falls within the 5% significance level, i.e., the p-value describing the probability of an incorrect conclusion is less than 0.05, it means that the result is valid with 95% confidence, but also that the probability of error is 5%. When comparing to the data, this means that in 5 out of 100 random samples the null hypothesis would be incorrectly rejected i.e., a type 1 error or false positive result is obtained (Storch and Zwiers, 1999). In this study the level of significance was set at $p < 0.05$.

Modelling can, at its best, be used to understand, predict, and analyse the behaviour of complex systems. A linear model describes a continuous response variable or dependent variable as a function of one or more predictor variables or independent variables. Linear regression, on the other hand, is the statistical method used to create a linear model (Walker, 2018).

In this study the statistical analysis was done with R-Studio (R version 4.3.0). The gas flux or redox potential (E_h) in relation to peat characteristics and the added terminal electron acceptor (TEA) was analysed by creating linear mixed effect models with the R package “lme4” (Bates et al., 2015). A linear mixed effects model allows a broader range of data to be observed than a simpler linear model,

considering both fixed and random effects. A fixed effect means that no relationship is assumed between the variables under consideration. In this study, a model was run with gas flux or E_h as the dependent variable, which was influenced by independent variables, i.e., added TEA, peat nutrient levels, and peat drainage status, which are not influenced by each other. They were therefore selected as fixed variables. Random effects, on the other hand, mean that each level of a variable can be thought of as a random variable in the underlying process (Midway, 2022). In this study, each flask ID comprised a grouping variable that was completely independent of the other factors and was therefore selected as a random variable.

Linear models, such as ANOVA (Analysis of Variance), are used to estimate differences in means between levels. When ANOVA indicates that the null hypothesis can be rejected, it means that there are statistically significant differences in the means of at least one pair of variables. However, ANOVA does not directly tell us between which pairs of variables the differences are, how large they are, nor their direction. To find out more precisely which pairs of variables differ and by how much, post hoc analyses such as the multiple comparison test (MCT) are needed to make more precise comparisons between means. This allows a deeper understanding of the nature and significance of differences in multivariate data (Midway et al., 2020).

There are several different tests for pairwise comparisons, of which Tukey's HSD (honestly significant difference) test is considered the best when all pairwise comparisons are made, and confidence intervals are desired, or the sample size is not very large (Midway et al., 2020). MCT with Tukey’s HSD test was thus used in this study to estimate significant differences between variables by using the R package “multcomp” (Hothorn et al., 2008).

3 RESULTS

Based on the dry matter analysis of peat, most of the peat weight added to the flasks was water, up to about 94%. In all undrained (UD) peat samples, the pH value was higher than in drained (D) peat samples of the same nutrient level (Table 3).

Table 3. *Peat sample characteristics without TEA addition. The pH values are from the control treatments after the first week of incubation. Water contents are means of two parallels and pH are means of three parallels with standard deviation (SD).*

Drainage status	Trophic level	Water content (%)	pH (SD)
UD	OL	92.88	5.81 (0.17)
D	OL	83.63	3.84 (0.25)
UD	ME	90.87	5.51 (0.27)
D	ME	85.74	4.00 (0.09)
UD	OM	93.45	4.08 (0.26)
D	OM	94.34	4.04 (0.09)

UD = undrained, D = drained, OL = oligotrophic, ME = mesotrophic, OM = ombrotrophic

3.1 N_2O flux

Of all terminal electron acceptors (TEA), only nitrate (NO_3^-) had a highly statistically significant impact on nitrous oxide (N_2O) flux compared to other added TEAs and the control treatment (Ctrl). In all flasks with added NO_3^-, greater amount of N_2O was produced compared to the flasks with other added TEAs or Ctrl treatment. Also, trophic level had a statistically significant influence on the N_2O flux between ombrotrophic (OM) and mesotrophic (ME) samples, with the ME samples showing higher N_2O fluxes than the OM samples.

Drainage did not have a statistically significant impact on N_2O flux (Table 4), but in general N_2O production was lower in D peat, the difference being greatest between ME peats. The effect of both nutrient level and drainage on N_2O flux is mainly seen in flasks with added NO_3^- (Figure 2). Regardless of the peat type, almost no N_2O was produced in Ctrl flasks, although minor quantities were consumed (Figure 2, Table 4).

Table 4. *The difference in means, standard errors (SE), and statistical significances of N_2O fluxes between terminal electron acceptors, drainage statuses, and trophic levels by using a multiple pairwise comparisons with Tukey HSD procedure.*

	Linear hypotheses $a - b = 0$	N_2O flux difference (SE) (µg/g/h)	sig.
TEA	NO – Fe	0.8909 (0.18)	< 0.0001
	NO – SO	0.8901 (0.18)	< 0.0001
	NO – C	0.8913 (0.18)	< 0.0001
	Fe – SO	-0.0009 (0.18)	1
	Fe – C	0.0004 (0.18)	1
	SO – C	0.0012 (0.18)	1
Drainage	UD – D	0.1289 (0.13)	< 1
Trophic	ME – OM	0.5115 (0.16)	< 0.01
	ME – OL	0.4193 (0.16)	0.062
	OL – OM	0.0922 (0.16)	< 1

NO = nitrate, Fe = ferric iron, SO = sulphate, C = control
UD = undrained, D = drained
ME = mesotrophic, OL = oligotrophic, OM = ombrotrophic
Statistically significant $p < 0.05$

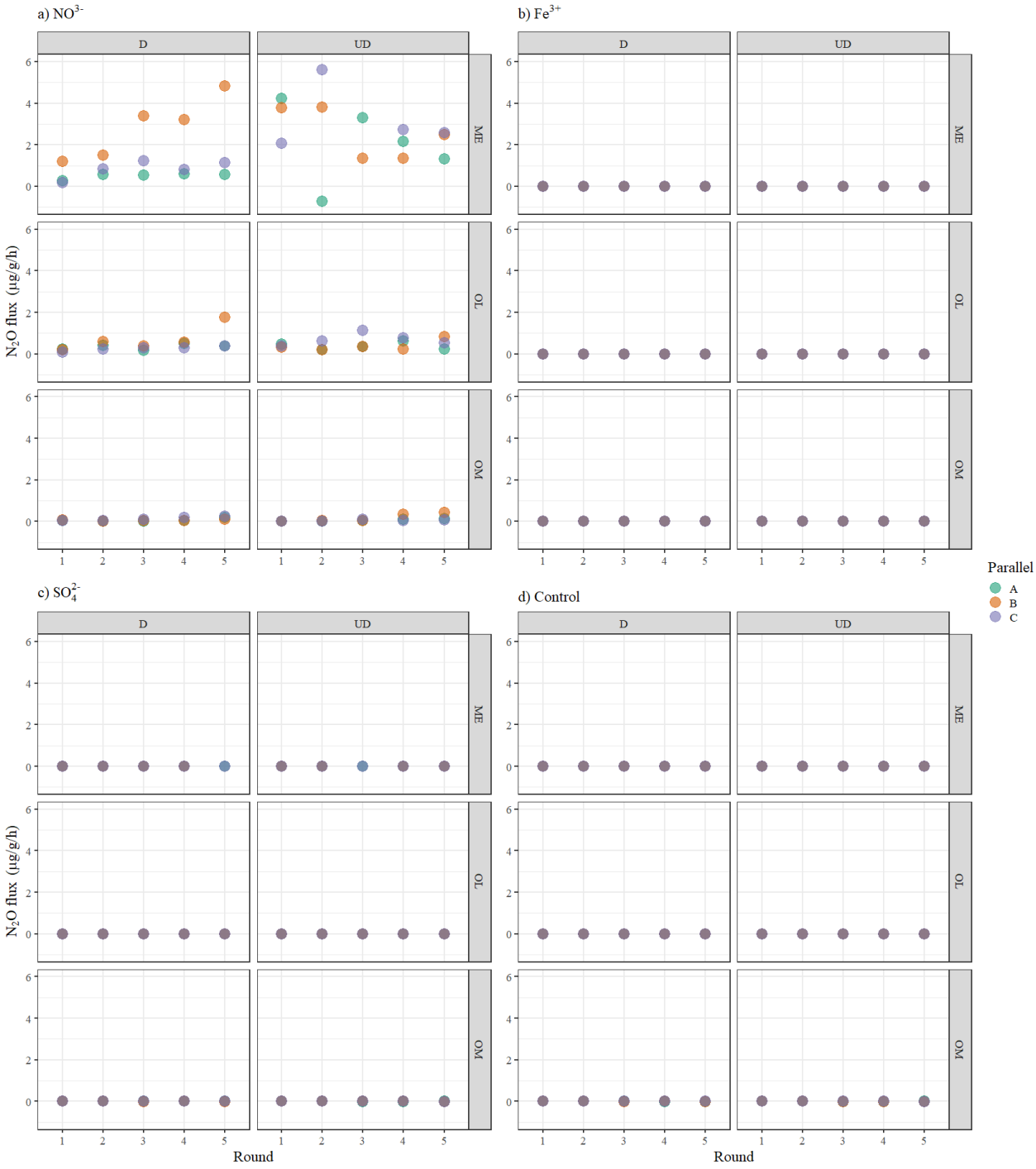

Figure 2. N_2O flux (per gram of peat dry weight) from flasks with different peat types and added TEAs. One round is 14 days. Added terminal electron acceptors are NO_3^- = nitrate, Fe^{3+} = ferric iron, SO_4^{2-} = sulphate. Peat types are classified to UD = undrained or D = drained and ME = mesotrophic, OL = oligotrophic, or OM = ombrotrophic.

3.2 CH_4 flux

NO_3^- addition had a statistically significant impact on methane (CH_4) flux but only when compared to ferric iron (Fe^{3+}) addition, resulting in reduced CH_4 production in flasks with added NO_3^-. None of the TEA additions had a statistically significant impact on CH_4 flux compared to the Ctrl treatment (Table 5).

Additionally, CH_4 was produced increasingly in all flasks with UD ME and UD OL peat during the experiment (Figure 3) and drainage had a highly statistically significant impact on CH_4 flux, leading to greater gas production in UD peat. Concerning nutrient levels, ME peat produced significantly more CH_4 than OM peat. No statistical significance was observed when compared to oligotrophic (OL) peat (Table 5). Moreover, the impact of drainage on CH_4 flux was highest between ME peats and lowest between OM peats (Figure 3).

Table 5. *The difference in means, standard errors (SE), and statistical significances of CH_4 fluxes between terminal electron acceptors, drainage statuses, and trophic levels by using a multiple pairwise comparisons with Tukey HSD procedure.*

	Linear hypotheses $a - b = 0$	CH_4 flux difference (SE) (µg/g/h)	sig.
TEA	NO – Fe	-0.1176 (0.039)	< 0.05
	NO – SO	-0.0384 (0.039)	< 1
	NO – C	-0.1061 (0.039)	0.056
	Fe – SO	0.0792 (0.039)	< 0.5
	Fe – C	0.0115 (0.039)	< 1
	SO – C	-0.0677 (0.039)	< 0.5
Drainage	UD – D	0.1165 (0.028)	< 0.001
Trophic	ME – OM	0.1233 (0.034)	< 0.005
	ME – OL	0.0576 (0.034)	0.5
	OL – OM	0.0657 (0.034)	< 0.5

NO = nitrate, Fe = ferric iron, SO = sulphate, C = control
UD = undrained, D = drained
ME = mesotrophic, OL = oligotrophic, OM = ombrotrophic
Statistically significant $p < 0.05$

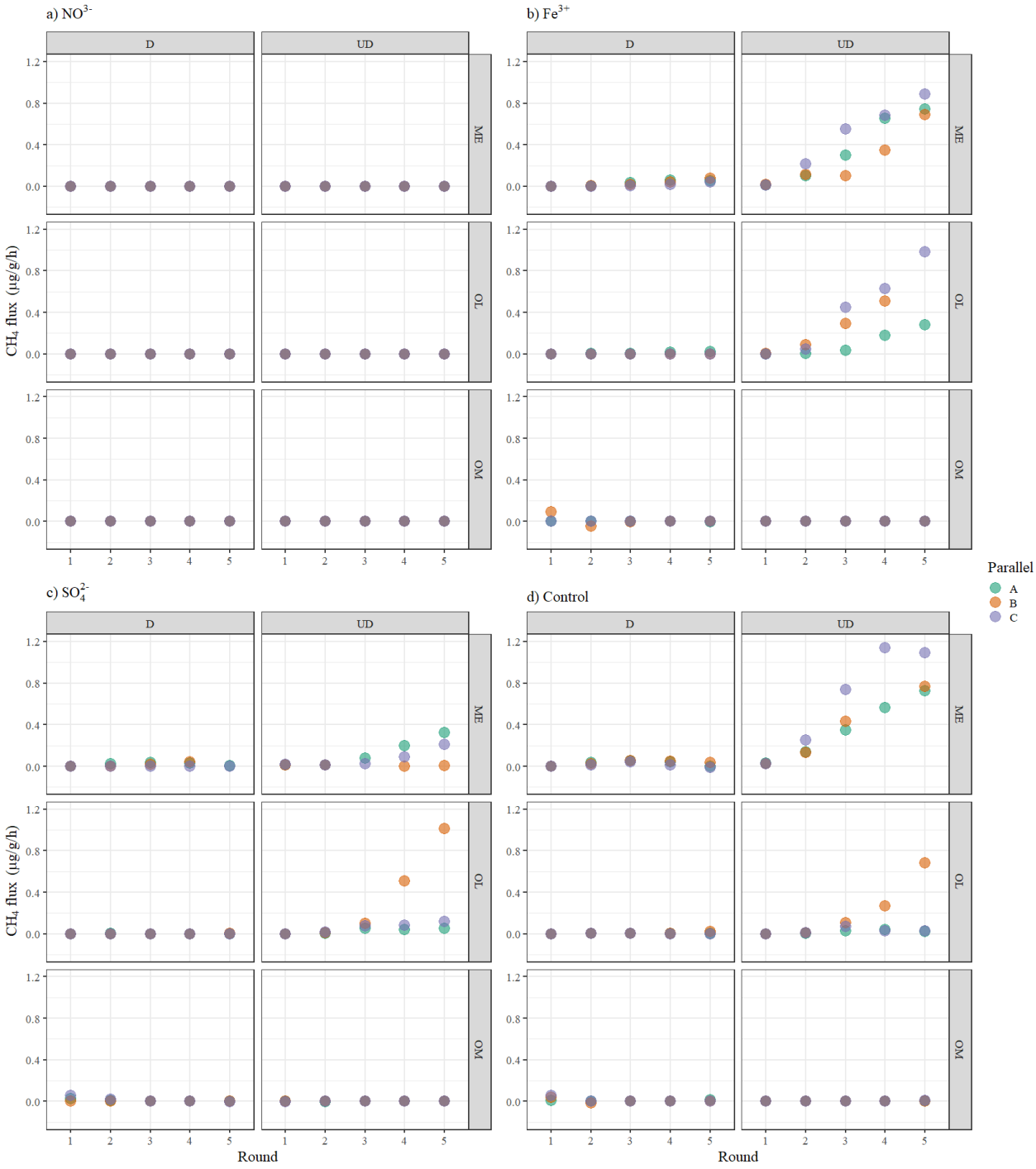

Figure 3. CH_4 flux (per gram of peat dry weight) from flasks with different peat types and added TEAs. One round is 14 days. Added terminal electron acceptors are NO_3^- = nitrate, Fe^{3+} = ferric iron, SO_4^{2-} = sulphate. Peat types are classified to UD = undrained or D = drained and ME = mesotrophic, OL = oligotrophic, or OM = ombrotrophic.

3.3 CO_2 flux

The addition of NO_3^- reduced the carbon dioxide (CO_2) flux compared to the other treatments but the difference was statistically significant only in relation to Fe^{3+} addition. CO_2 flux was therefore highest in flasks with added Fe^{3+} but the difference was not statistically significant compared to sulphate (SO_4^{2-}) addition or Ctrl treatment (Table 6).

Drainage had a highly statistically significant impact on CO_2 flux, leading to more gas production from UD peat (Table 6). The effect of drainage was smallest when comparing OM peats (Figure 4). There was no statistically significant difference in CO_2 flux between minerotrophic peat types (ME and OL), but the difference was highly statistically significant when compared to OM peat. In both cases, less CO_2 was produced in OM peat (Table 6). The differences between trophic levels were noticeable for all treatments (Figure 4).

Table 6. *The difference in means, standard errors (SE), and statistical significances of CO_2 fluxes between terminal electron acceptors, drainage statuses, and trophic levels by using a multiple pairwise comparisons with Tukey HSD procedure.*

	Linear hypotheses $a - b = 0$	CO_2 flux difference (SE) (µg/g/h)	sig.
TEA	NO – Fe	-0.6729 (0.23)	< 0.05
	NO – SO	-0.4706 (0.23)	< 0.5
	NO – C	-0.0656 (0.23)	1
	Fe – SO	0.2023 (0.23)	< 1
	Fe – C	0.6072 (0.23)	< 0.1
	SO – C	0.4050 (0.23)	< 0.5
Drainage	UD – D	1.4952 (0.17)	< 0.001
Trophic	ME – OM	1.1484 (0.20)	< 0.001
	ME – OL	0.3358 (0.20)	< 1
	OL – OM	0.8125 (0.20)	< 0.001

NO = nitrate, Fe = ferric iron, SO = sulphate, C = control
UD = undrained, D = drained
ME = mesotrophic, OL = oligotrophic, OM = ombrotrophic
Statistically significant $p < 0.05$

CO_2 was abundantly produced in all Ctrl flasks regardless of the peat type, with notably higher production in UD ME and UD OL peat (Figure 4). None of the TEAs had a statistically significant impact on CO_2 flux compared to the Ctrl treatment (Table 6). Throughout the experiment, there was a decrease in CO_2 flux in all flasks across all peat types, TEA additions, and Ctrl treatment as can be seen from Figure 4.

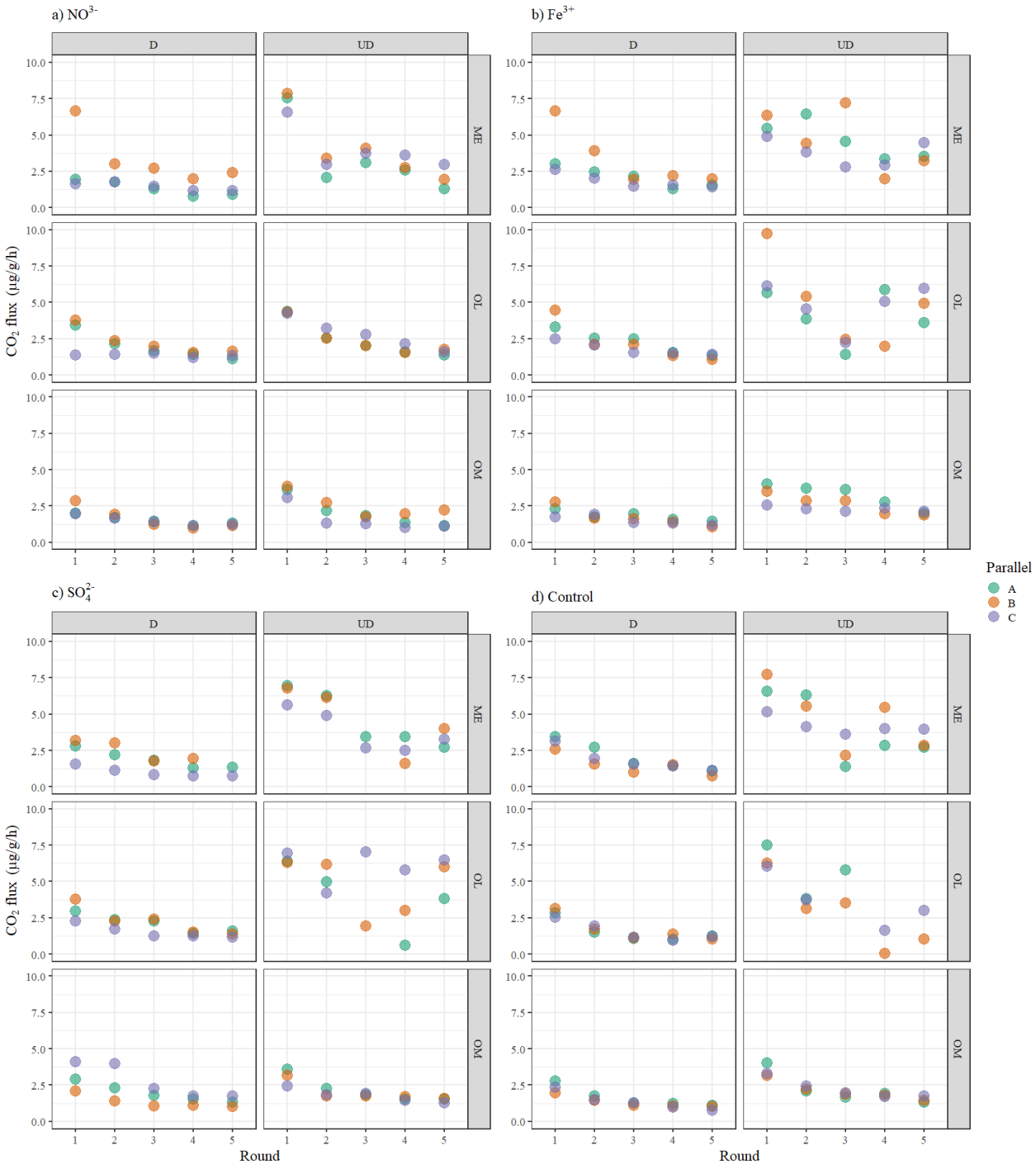

Figure 4. CO_2 flux (per gram of peat dry weight) from flasks with different peat types and added TEAs. One round is 14 days. Added terminal electron acceptors are NO_3^- = nitrate, Fe^{3+} = ferric iron, SO_4^{2-} = sulphate. Peat types are classified to UD = undrained or D = drained and ME = mesotrophic, OL = oligotrophic, or OM = ombrotrophic.

3.4 E_h values

There was a highly statistically significant difference in the measured redox potentials (E_h) among all added TEAs so, that values measured in the NO_3^- flasks were higher than those in the Fe^{3+} or SO_4^{2-} flasks, and the values in the Fe^{3+} flasks were higher than those in the SO_4^{2-} flasks. Also, the measured E_h values from flasks with added Fe^{3+} and SO_4^{2-} were highly statistically significantly smaller compared to Ctrl treatment (Table 7).

There was no statistically significant difference between measured E_h values from OM and OL peat, whereas the difference was highly statistically significant when compared to ME peat. In both cases, the E_h value measured from ME peat was lower. Drainage did not have a statistically significant impact on the E_h value but the E_h values in UD peat were marginally higher (Table 7).

Table 7. *The difference in means, standard errors (SE), and statistical significances of E_h values between terminal electron acceptors, drainage statuses, and trophic levels by using a multiple pairwise comparisons with Tukey HSD procedure.*

	Linear hypotheses $a - b = 0$	E_h value difference (SE) (mV)	sig.
TEA	NO – Fe	95.34 (14.23)	< 0.0001
	NO – SO	147.59 (14.29)	< 0.0001
	NO – C	15.95 (14.23)	< 1
	Fe – SO	52.25 (14.26)	< 0.005
	Fe – C	-79.39 (14.19)	< 0.0001
	SO – C	-131.64 (14.26)	< 0.0001
Drainage	UD – D	14.29 (10.07)	< 1
Trophic	ME – OM	-55.88 (12.36)	< 0.0001
	ME – OL	-50.18 (12.36)	< 0.0005
	OL – OM	-5.70 (12.29)	< 1

NO = nitrate, Fe = ferric iron, SO = sulphate, C = control
UD = undrained, D = drained
ME = mesotrophic, OL = oligotrophic, OM = ombrotrophic
Statistically significant $p < 0.05$

The measured E_h values deviated notably from the estimated E_h values, especially in the flasks with added NO_3^-, where measured values were more than three times lower than the estimated E_h value for NO_3^- reduction. Moreover, the E_h values measured from the SO_4^{2-} flasks were approximately 30 % higher than the highest estimated value for SO_4^{2-} reduction. The E_h values in the Ctrl flasks, on average, fell within the range of estimated E_h values of Fe^{3+} reduction (Table 8) but were closest to E_h values measured from NO_3^- flasks (Table 7 and 8). Only some parallel values measured on first

three incubation rounds from UD OM peat with added NO_3^- were higher than estimated E_h value +250 mV (Figure 5).

Table 8. *The most common terminal electron acceptors (TEA), means of redox potentials (E_h) measured per added TEA during the experiment, and the estimated redox potentials of the most common TEAs. Estimated values from Inglett et al. (2012).*

TEA	E_h variation range (mV) a / b ($mean$)	Estimated E_h value (mV)
Nitrate NO_3^-	38.13 / 136.51 (80.24)	250
*Manganese ion Mn^{4+}		225
Ferric iron Fe^{3+}	-46.78 / 7.97 (-15.14)	-100 to +100
Sulphate SO_4^{2-}	-125.32 / -2.15 (-66.55)	-200 to -100
*Carbon dioxide CO_2		< -200
Control (no added TEA)	6.53 / 121.64 (63.67)	

*Not added to the flasks
Variation range means the min and max E_h values of the parallel means over the experiment from flasks with the same TEA.

All E_h values varied between -200 to +300 mV but most of the values were between -100 to +200 mV. E_h values varied also between parallel samples during incubation, especially in NO_3^- and Ctrl flasks, but remained relatively constant in flasks with D peat, and towards the end of incubation also in UD peat, with Fe^{3+} or SO_4^{2-} addition. In these flasks, the values stabilised from round 3 onwards. In almost all Ctrl flasks E_h decreased relatively steadily as incubation continued (Figure 5).

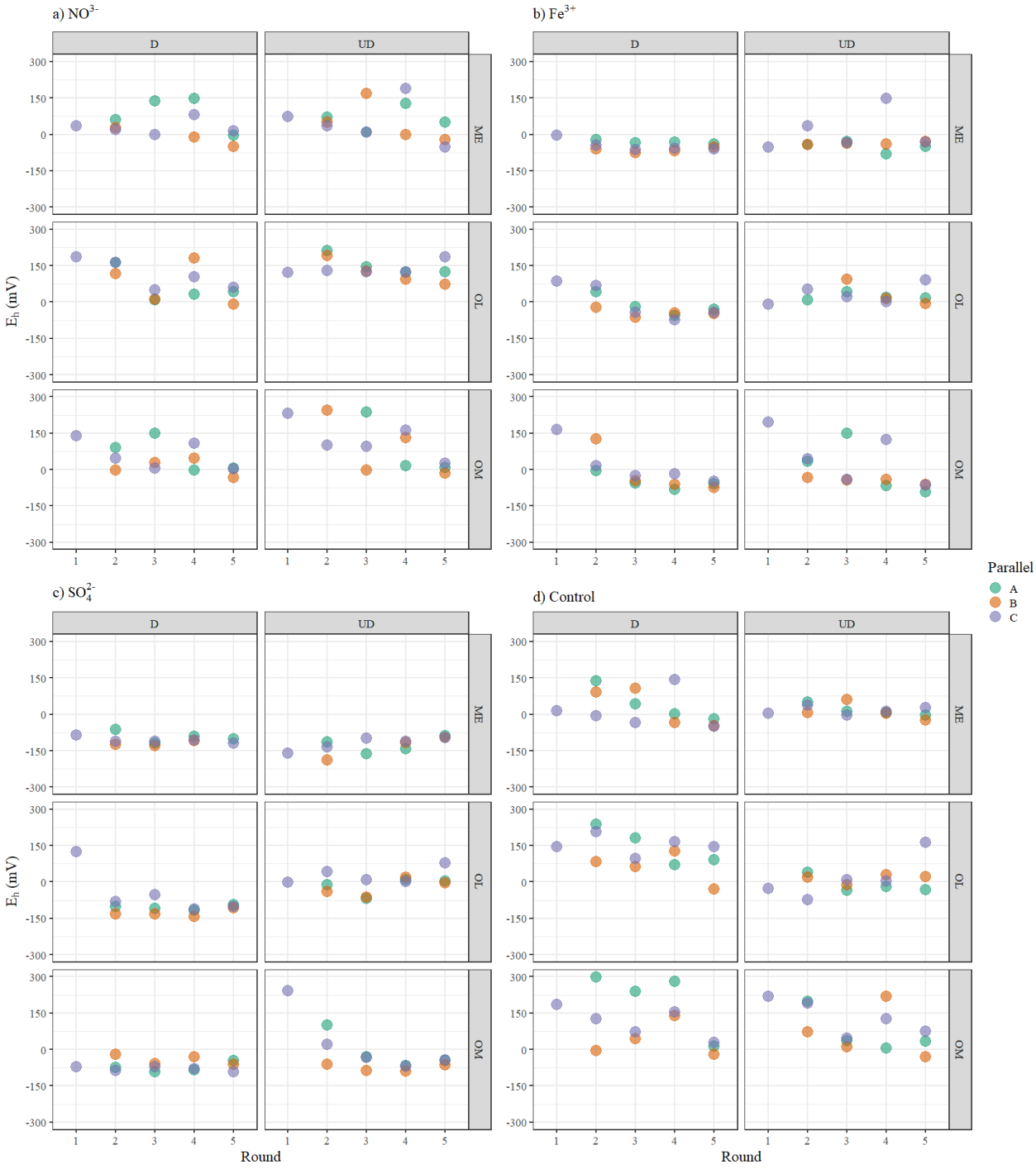

Figure 5. Measured E_h values per round from flasks with different peat types and added TEAs. One round is 14 days. Added terminal electron acceptors are NO_3^- = nitrate, Fe^{3+} = ferric iron, SO_4^{2-} = sulphate. Peat types are classified to UD = undrained or D = drained and ME = mesotrophic, OL = oligotrophic, or OM = ombrotrophic.

During incubation, N_2O flux was minimal without added NO_3^-, and despite the addition, the E_h values where low compared to estimated E_h value, variating on average between +40 and +140 mV (Table 8). E_h value was around 0 mV when N_2O was produced the most in UD ME peat on third round of incubation, and even though E_h value was above +200 mV, no N_2O flux occurred (Figure 6).

Figure 6. N_2O flux (per gram of peat dry weight) as a function of redox potential (E_h). Added terminal electron acceptors are NO_3^- = nitrate, Fe^{3+} = ferric iron, SO_4^{2-} = sulphate. Peat types are classified to UD = undrained or D = drained and ME = mesotrophic, OL = oligotrophic, or OM = ombrotrophic.

Conversely, NO_3^- addition restrained the CH_4 flux. CH_4 was primarily produced in the Ctrl, Fe^{3+}, and SO_4^{2-} flasks, with the E_h value ranging between approximately -100 and +100 mV, falling within the estimated range of Fe^{3+} reduction (Table 8). The highest CH_4 fluxes occurred from UD ME peat at E_h around 0 mV and the lowest E_h values were measured with no effect of CH_4 fluxes (Figure 7).

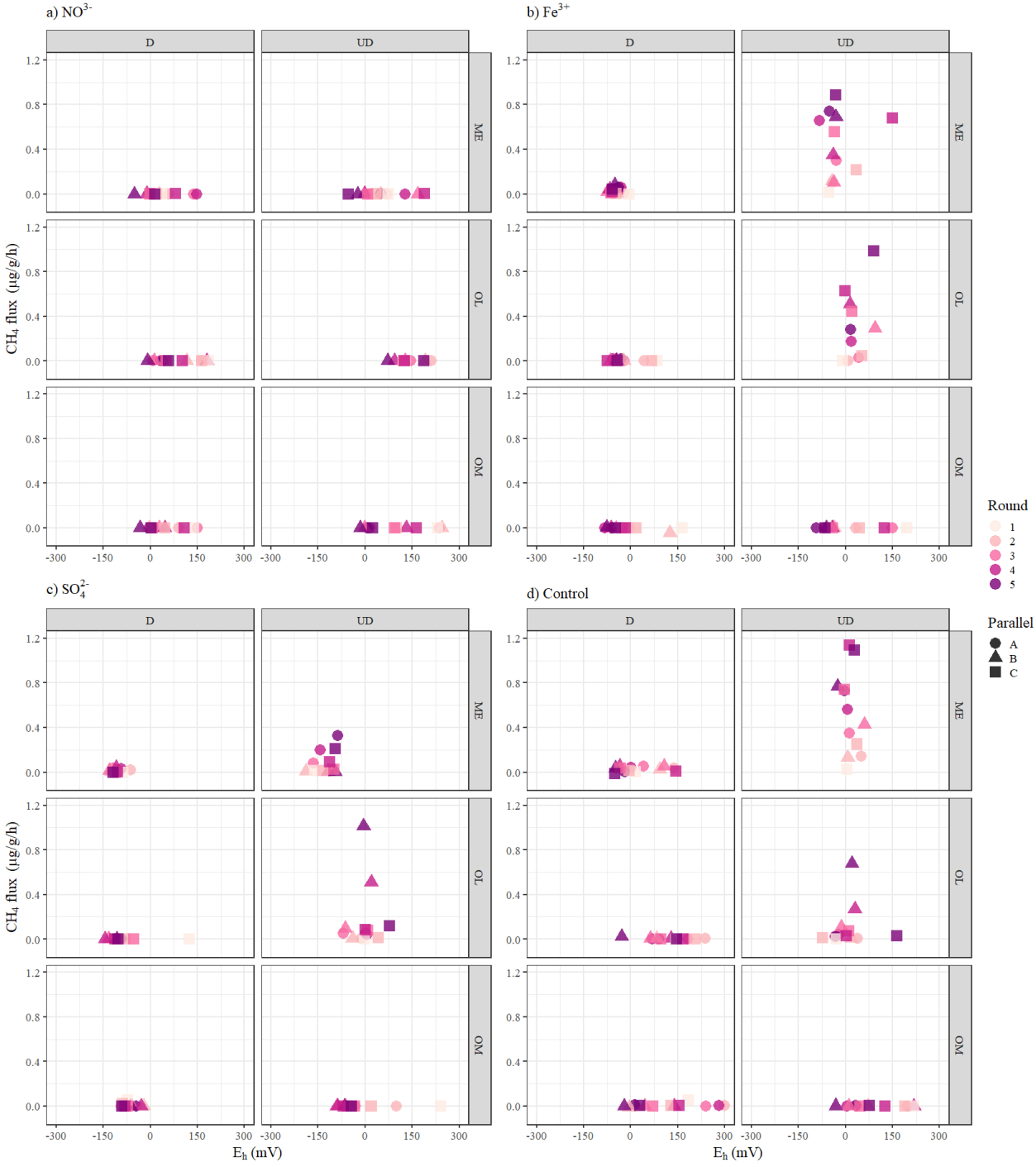

Figure 7. CH_4 flux (per grams of peat dry weight) as a function of E_h. Added terminal electron acceptors are NO_3^- = nitrate, Fe^{3+} = ferric iron, SO_4^{2-} = sulphate. Peat types are classified to UD = undrained or D = drained and ME = mesotrophic, OL = oligotrophic, or OM = ombrotrophic.

CO_2 flux was also highest from UD ME peat at the Fe^{3+} reduction zone with the E_h value ranging between approximately -100 and +100 mV. Especially in OM peat E_h values varied widely, but this did not seem to influence CO_2 fluxes.

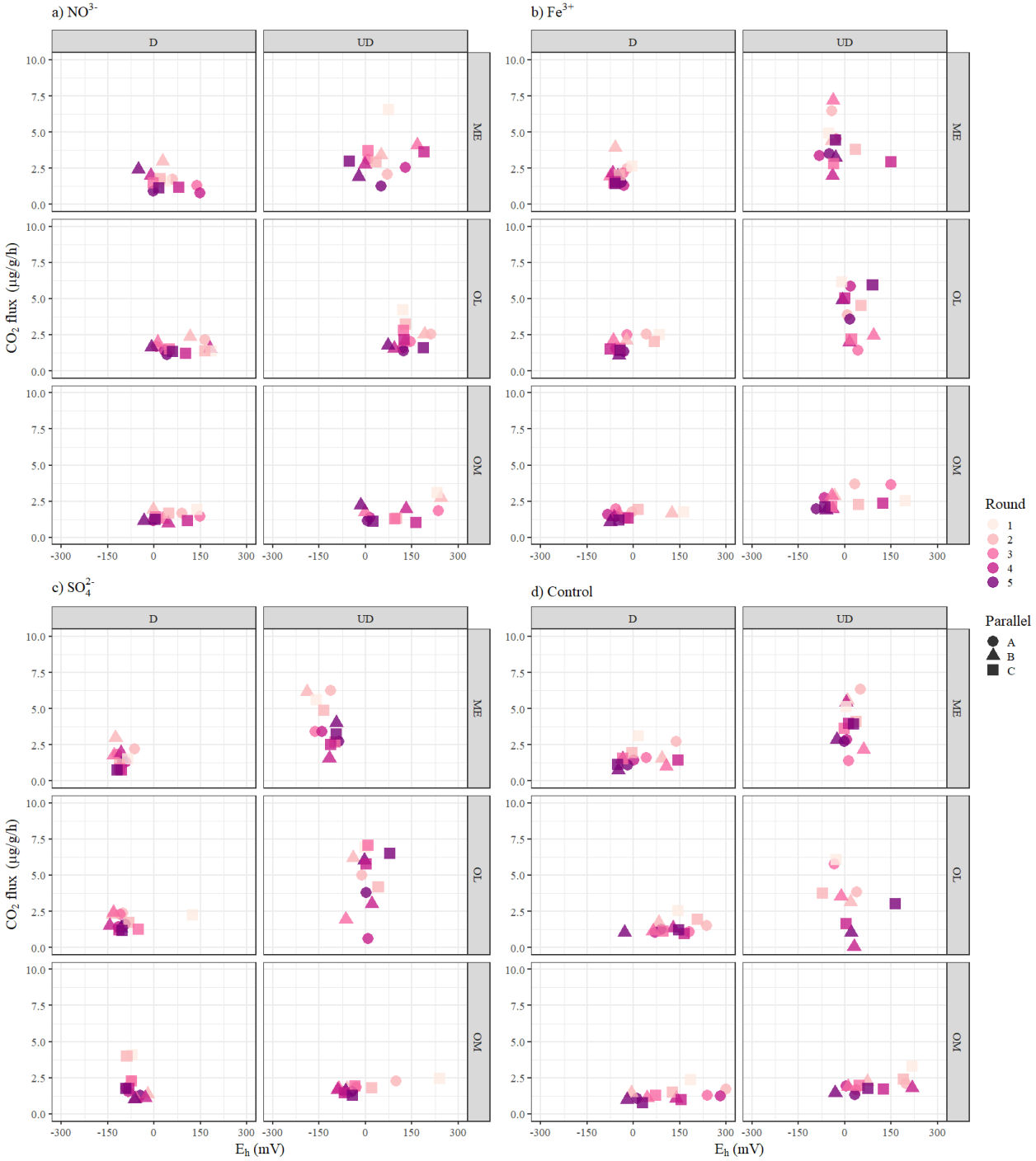

Figure 8. CO_2 flux (per grams of peat dry weight) as a function of redox potential (E_h). Added terminal electron acceptors are NO_3^- = nitrate, Fe^{3+} = ferric iron, SO_4^{2-} = sulphate. Peat types are classified to UD = undrained or D = drained and ME = mesotrophic, OL = oligotrophic, or OM = ombrotrophic.

3.5 CH_4/CO_2 production ratio

From all experiments, only in Ctrl treated flasks containing UD ME peat the CH_4/CO_2 production ratio reached approximately 80 % at the end of incubation, indicating that methanogenesis was the

predominant redox reaction. The addition of Fe^{3+} and SO_4^{2-} increased the production ratio to 30-60 % in flasks with UD ME and UD OL peat, more with added Fe^{3+}. The addition of any TEA caused almost no change in the production ratio in flasks with D peat or UD OM peat. In the flask with added NO_3^-, the production ratio remained close to zero throughout the incubation (Figure 9).

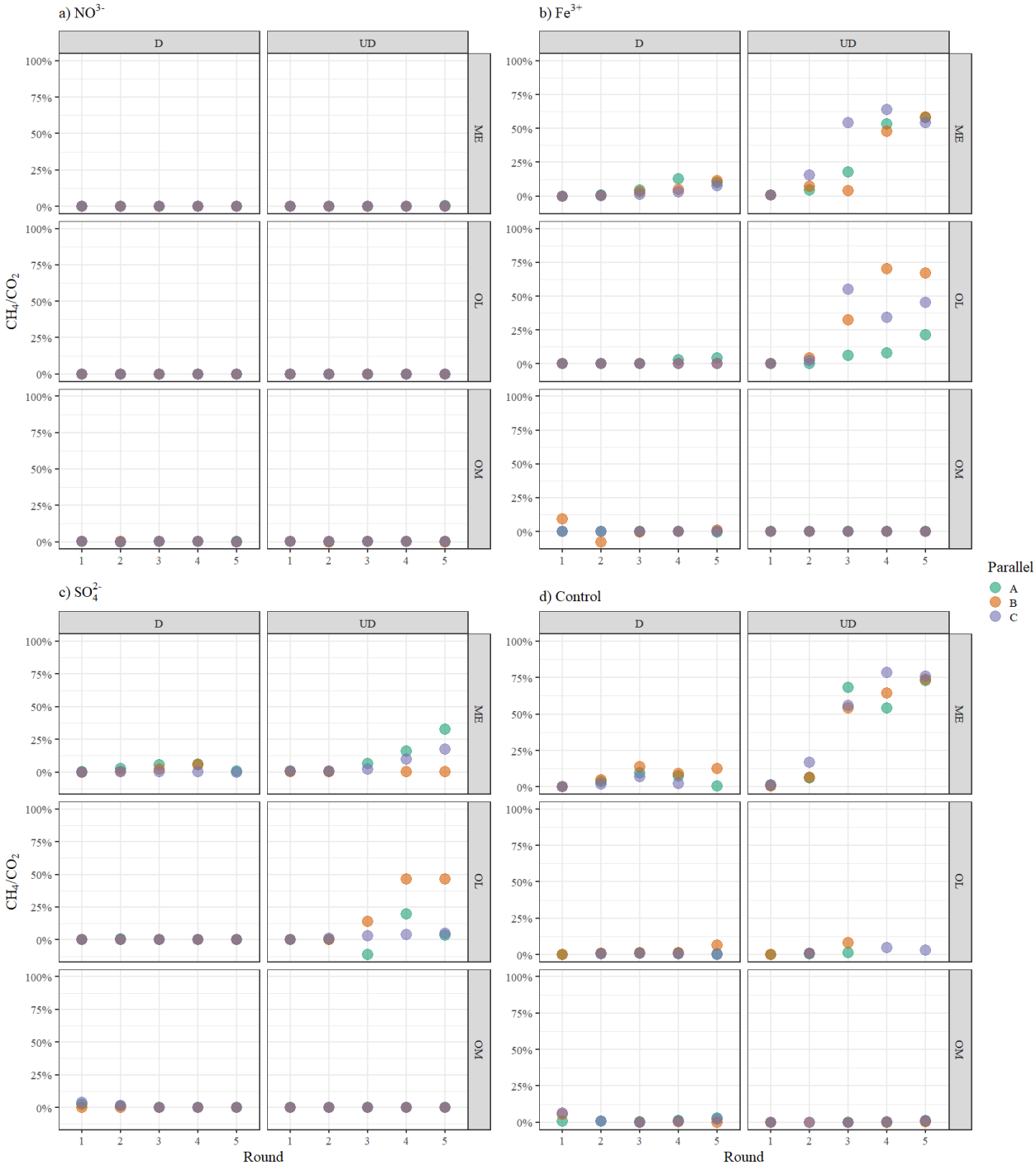

Figure 9. CH_4/CO_2 production ratio (mol g^- h^- / mol g^- h^- × 100 %) per round from flasks with different peat types and added TEAs. One round is 14 days. Added terminal electron acceptors are NO_3^- = nitrate, Fe^{3+} = ferric iron, SO_4^{2-} = sulphate. Peat types are classified to UD = undrained or D = drained and ME = mesotrophic, OL = oligotrophic, or OM = ombrotrophic.

4 DISCUSSION

The aim of this experiment was to assess the impact of controlled anoxic redox conditions and the role of inorganic terminal electron acceptors (TEA) on redox potential (E_h) and produced nitrous oxide (N_2O), methane (CH_4), and carbon dioxide (CO_2) emissions from peat types with different nutrient levels and drainage status. The study was carried out in the laboratory as an anaerobic incubation experiment on six different peat types, both drained (D) and undrained (UD) mesotrophic (ME), oligotrophic (OL), and ombrotrophic (OM) peat. Samples were incubated for 10 weeks under different redox conditions controlled by addition of TEAs nitrate (NO_3^-), ferric iron (Fe^{3+}), or sulphate (SO_4^{2-}). In addition, the experiment was performed on untreated peat (Ctrl). During the experiment N_2O, CH_4, and CO_2 fluxes and E_h values were measured from the samples and their correlation with peat type and added TEA was evaluated using a mixed effect model and pairwise comparison.

4.1 The impact of peat type on pH value

The pH values measured from the peat samples were lower in D peat compared to UD peat of the same nutrient level and all pH values in the D samples were very close to the UD OM peat. This was probably due to the fact, that drainage increases peat decomposition, especially in minerotrophic (ME and OL) peatlands, which releases hydrogen and lowers pH causing D peatlands start to resemble UD OM peatlands (Urbanová and Bárta, 2016).

On the other hand, according to the Lakkasuo book (Laine et al., 2004, 86), the pH values measured near the sampling sites were about 1-2.5 pH units higher than the pH values of the peat samples, probably due to the increased water flow as a result of the spring. The differences were greatest in UD ME and UD OL peats, where pH of the samples were above 5.5, compared to the sampling sites where pH values were about 3. Water content was also highest in peat samples with the highest pH and correspondingly lowest in those with the lowest pH value.

4.2 The impact of added TEA, nutrient levels, and drainage status on GHG emissions

As hypothesized (Hypothesis 1), N_2O was formed mainly in flasks with added NO_3^-, the most in ME peat, which means that in both D and UD ME peatlands there is readiness for denitrification in anaerobic conditions if NO_3^- is available, and N_2O emissions are likely. However, according to study by Liimatainen et al. (2018) N_2O emissions are linked to high content of phosphorus and copper and lower concentrations of them can limit emissions even if NO_3^- is available.

In other flasks, possible NO_3^- originally present in peat was reduced and the potential production ceased as incubation continued because NO_3^- formation via nitrification under anoxic conditions is weak (Leppelt et al., 2014; Pihlatie et al., 2004), and in some flasks N_2O production shifted to minor consumption. At the time the study was conducted, the order in which the flasks were opened in the anaerobic chamber varied, so the flasks with high greenhouse gas (GHG) content may have affected the results of flasks with low GHG content. This is probably the reason why some negative N_2O fluxes were measured from flasks without added NO_3^-.

Methane flux was suppressed due to addition of NO_3^- and SO_4^{2-}, more due to NO_3^- addition as hypothesized (H2), supporting previous studies (e.g., de Jong et al., 2020). There are several potential reasons for inhibition of CH_4 formation due to addition of NO_3^- and SO_4^{2-}: the toxicity of NO_3^- and denitrification intermediates, suppression of methanogenesis due to competition with SO_4^{2-} and NO_3^- reducing bacteria especially in the topsoil, and/or increased anaerobic oxidation of methane (AOM) (Dettling et al., 2006; Eriksson et al., 2010).

Unexpectedly addition of Fe^{3+} did not inhibit the CH_4 flux but even stimulated it from UD ME peat and especially from UD OL peat compared to control (Ctrl) treatment. There are several studies from peatlands that got results where Fe^{3+} addition didn't inhibit, or stimulated CH_4 emissions (de Jong et al., 2020; Dettling et al., 2006; Smemo and Yavitt, 2007). According to Metje and Frenzel (2005), methanogens participate in Fe^{3+} reduction. They noticed that CH_4 production and Fe^{3+} reduction decreased in almost same proportion with the addition of a methanogenesis inhibiting solution, with no effect on the activity of Fe^{3+} reducing bacteria.

However, drainage and nutrient deficit had the greatest decreasing effect on CH_4 flux probably due to poor readiness of microbiota for utilizing terminal electron acceptors (TEAs) or minor electron donor availability. The lack of electron donors is supported, for example, by several studies showing that the addition of glucose increases CH_4 emissions (Dettling et al., 2006; Sutton-Grier et al., 2011; Urbanová and Bárta, 2016). However, there is also evidence that CH_4 production and emissions in D peat returns to pristine like as time goes by and microbe population adapts to the change (Urbanová et al., 2013). The samples in the study were from surface peat, so D peat has probably less methanogens as they disappear from oxic conditions. In contrast, low nutrient peat may have sufficient microbial diversity to produce CH_4 but lacks the necessary electron donors.

The assumption that "as incubation continues in Ctrl flasks the redox state decreases causing N_2O flux to decrease or cease and CH_4 flux to increase being the highest compared to other treatments, with methanogenesis being the main redox reaction" (H3) applied directly only to Ctrl flasks with UD ME peat. In UD ME peat with Ctrl treatment there was a marginally low N_2O formation in the first round of incubation, and the CH_4/CO_2 production ratio was about 80 % at the end of incubation, i.e., almost the same amounts of CH_4 and CO_2 (nmol g^{-1} h^{-1}) were formed. There was no similar change in other Ctrl flasks. Likewise, only UD ME and UD OL peat showed changes in CH_4/CO_2 production ratio with added TEAs Fe^{3+} and SO_4^{2-}, with 30-60 % ratio at the end of incubation, with higher ratios in Fe^{3+} flasks.

All gas fluxes in anaerobic conditions were highest from nutrient rich peat in the order ME > OL > OM (H4) and from UD peat (H5) as expected, supporting previous studies (Maljanen et al., 2010). Moreover, in OM peat, D or UD, with or without TEA treatments, no N_2O or CH_4 was formed. CO_2 flux was highest in the beginning of the incubation from all the flasks, as expected and fell steadily throughout the period, since as peat decomposes it is more difficult to utilize causing decreasing CO_2 emissions. Drainage and nutrient scarcity (ombrotrophy) had the greatest mitigating effect on CO_2 flux. Notably, only Fe^{3+} addition stimulated CO_2 flux when compared to Ctrl treatment, when NO_3^- addition did not stimulate CO_2 flux above Ctrl at all, and above Fe^{3+} only in the first round of incubation from ME peat. Lozanovska et al. (2016) got similar results and they explained the suppressing effect of NO_3^- on CO_2 emissions by NO_3^- involving in microbial metabolism instead of decomposition processes. The addition of SO_4^{2-}, on the other hand, suppressed CO_2 flux compared to the other treatments, especially in the beginning of incubation, which was probably due to SO_4^{2-} reducing bacteria displacing methanogens (Achtnich et al., 1995).

4.3 The impact of added TEA, nutrient levels, and drainage status on E_h values

All measured redox potential (E_h) values were below +300 mV, meaning that the peat was in an anaerobic state and conditions were reducing (Thompson et al., 2009), as desired. Most of the values fell in the range -100 to +200 mV and only the flasks with added SO_4^{2-} gave some values with E_h < -100 mV i.e., peat was either moderately or strongly reducing, respectively (Inglett et al., 2012). As hypothesised, measured E_h values were in order of the amount of energy obtained from the TEA reduction reaction (H6) and the differences were statistically significant. However, E_h values measured from the Ctrl flasks were in the Fe^{3+} reduction area and most comparable with the E_h values measured from flasks with added NO_3^- rather than being the lowest as expected.

Most of the measured E_h values, regardless of the added TEA, fell within the reduction area of iron and manganese. The dispersion between parallel samples was lowest in flasks with added Fe^{3+} or SO_4^{2-} especially in D peat and the dispersion decreased with each round. Moreover, also the measurements from SO_4^{2-} flasks were partly in the iron reduction area. Despite the addition of NO_3^-, E_h values decreased during incubation, and most of them were as well in the iron reduction area. However, in the Ctrl flask, the results were most clearly decreasing. Drainage had no statistically significant impact on the E_h value though values measured from UD peat were slightly higher. ME on the other hand decreased E_h by about -50mV. Compared to other variables the addition of SO_4^{2-} or Fe^{3+}, and mesotrophy had the greatest decreasing effect on E_h value.

In general, it can be concluded that all GHGs were systematically formed most when E_h value was around 0 mV, which was particularly surprising for CH_4. However, there is evidence that CH_4 has been formed even when E_h is around +200 mV (Szafranek-Nakonieczna and Stpniewska, 2015), although the assumption is that it is only formed when E_h is around -150 mV (Inglett et al., 2012). Moreover, as noted earlier, the addition of Fe^{3+} to the peat did not inhibit CH_4 formation, on the contrary, and the iron content of the peat is presumably naturally high, thus forcing all E_h values close the iron reduction area without inhibiting the CH_4 flux. Other reason is that the platinum (Pt) electrode of the E_h meter does not detect NO_3^- or oxygen very well.

On the other hand, the consistency of the E_h values measured from flasks with added Fe^{3+} and SO_4^{2-} is probably due to good ability of the Pt electrode to detect Fe^{3+} in particular, and the dispersion was further decreased as excess TEAs were utilized as incubation continued. On the other hand, the high dispersion of the E_h values measured from NO_3^- and Ctrl flasks is probably either due to the inability of the Pt electrode to detect NO_3^- or the 1-5% oxygen content of the incubation chamber (the oxygen content of the peat slurry is unknown), which has most likely disturbed the values measured especially from Ctrl flasks with D peats and UD OM peat by increasing dispersion. Moreover, the effect of both drainage and trophic level on the E_h value is marginally small when assessed visually, and the statistically significant difference of the measured E_h values from ME peat is probably due to the smaller dispersion of the values compared to other trophic levels (Figure 5), which again is due to higher nutrient concentrations.

Even though studies have found that organic matter is an even more prevalent electron acceptor than NO_3^- and Fe^{3+} (e.g., Gao et al., 2019), this study did not find clear evidence of this with iron clearly

controlling the measurements. On the other hand, the increasing effect of Fe^{3+} on CH_4 and CO_2 emissions was unexpected, and it is not known what role organic matter plays in this as the increasing effects of Fe^{3+} on GHG emissions have been observed specifically in organic soils (de Jong et al., 2020).

4.4 Sources of error

The results of a reliable study are based on high quality data, which in the case of this study consists of E_h and pH measurements, gas sample analysis, and dry matter analysis of the peat samples. To minimize sampling variability, three parallel experiments were conducted for each experimental setup and all sampling and measurements were performed by the same person. The collected peat samples were already in anaerobic conditions at the sampling site because the soil was cold after the winter and the WTL was close to the soil surface. Temperature was the main change to which the samples had time to adapt before the experiment started. The peat samples were also exposed to oxygen, but the exposure was relatively short. During the sampling and experiment preparation phase, the exposure of peat samples to oxygen could have affected the results, especially during the first incubation round. This was minimised by taking large peat samples, wrapping them tightly in plastic wrap and storing them in a cool, dark place until the start of the experiment. In addition, the samples of the experiment were collected from inside a larger peat clod, where they were most likely anaerobic, and transferred directly to water, which was finally well bubbled with nitrogen gas. Moreover, all steps were performed with disinfected instruments and hands, and the used water was autoclaved MilliQ water to prevent microbial contamination.

Despite the creation of a nitrogen atmosphere, the anaerobic chamber where E_h and pH measurements were made had leaks, allowing atmospheric oxygen in the chamber. This was monitored using an oxygen meter. At its lowest point, the oxygen concentration in the chamber was around 1%, but during sampling it increased to a maximum of 5%. Sampling took about three hours in total and the more oxygen exposure the peat samples received the later flasks were opened. Flasks were opened in a random order and the order of opening was not recorded. On the other hand, the randomness of the sampling did not cause a systematic error in certain samples. Moreover, it was observed only afterwards to what extent the gases released from flasks with different nutrient levels affected the results of the other flasks, especially with N_2O. The most significant the effect was probably on E_h values measured from flasks with OM peat. In addition, it took a long time for the redox meter to stabilise, and it gave very variable results. This was most likely due to the poor sensitivity of the Pt

electrode to NO_3^- and oxygen. The E_h meter was also not calibrated before or between measurements, relying instead on controlled redox conditions to tell if the results were incorrect.

Possible sources of error in gas sampling were possible leakage of the vials, which would have resulted in less gas in the analytical results than in reality as the vials had to be pressurised before analysis (filling them with helium gas to allow gas chromatograph (GC) to analyse the sample). Vials with a clear vacuum failure, i.e. not absorbing the sample gas in, were written down and replaced by other vials. If it was not possible to replace the vial, we excluded the results from the final analysis. Moreover, during the GC analysis, the order of vials was realised to control from nutrient poor to nutrient rich and according to different TEAs only from second analysing round onwards. From one round of GC analysis, the standard samples were completely forgotten, as the vials were not filled with standard gas and GC analysed only the empty vials. The results of this round were compared to other results of the same flask and the differences were sufficiently small.

In the analysis phase of the results, a linear model of the different gas fluxes as a function of time was created and obvious errors were removed. Temperatures, pressures, gas sampling, and pH values were acknowledged in the calculations and the results were observed as amounts of substance when comparing to each other or as masses. The calculations, modelling, and statistical analysis were based on generally accepted methods and the final results were checked several times.

5 CONCLUSIONS

This thesis acquainted the reader on the influence of peat biogeochemical properties on greenhouse gas emissions and redox potential (E_h). This study is the first of its kind and we could not find any similar study concerning the impacts of added terminal electron acceptors on both greenhouse gas emissions and redox potential, not to mention in combination with different nutrient levels and drainage status. Overall, the results of this study highlight the influence of peatland nutrient levels, minerotrophy, oligotrophy, and ombrotrophy, drainage status and microbial resource competition on the formation of the greenhouse gases nitrous oxide, methane, and carbon dioxide, as well as the role of external electron acceptor additions in peatland ecosystems.

The microbial community originally in peat are the main cause of differences of gas production in samples with the same terminal electron acceptors and electron donors. This study supports previous findings that both pristine and drained mesotrophic and possibly oligotrophic peatlands are potential sources of nitrous oxide, as microbes have the capacity to use nitrate if it is available. Especially in ditches where nutrients are leached, nitrous oxide emissions can reach very high levels. On the other hand, pristine minerotrophic peatlands particularly are a source of methane emissions. This study also indicates that the high iron content of peat may even contribute to methane formation in anoxic, nutrient rich peatlands, and only a significant addition of nitrate inhibited it.

Based on this study, it can be concluded that the E_h value indicates the redox state, potential for redox reaction, and thus, the capacity to produce gases, but if the microbiota is not able to utilize terminal electron acceptors or the organic matter is too difficult to decompose, as is likely to be the case with drained peat and undrained ombrotrophic peat in this study, no gases will be produced. Therefore, the redox potential measurement and E_h value should be considered alongside other measurements, such as microbial analysis, nutrient/chemical analysis, and gas concentrations to understand and predict the redox processes and greenhouse gas emissions in anaerobic soils.

The results of water samples, microbial analysis, or peat analysis were not considered in this study, so the nutrient content, microbial population, or utilization rate of the added terminal electron acceptors in the samples under consideration is not known. However, the measurements have been taken and can be examined in the future. By examining these, it will be possible to discern more precisely the causes of the different gas formation rates and the effect on E_h value. Excluding these was a conscious decision, as the thesis would have been overly extended. In addition, more research

is needed, particularly on the role of iron in methane emissions and the ability of methanogens to reduce iron compounds as part of methane production, to better understand its effects in peatlands.

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APPENDICES

Appendix 1. Dry matter analysis of different peat types

Peat masses before and after drying, and results of dry matter analysis.

Table 9. Initial and final weights and dry matter and water contents of peat for dry matter analysis.

Peat type	m_1 (g)	m_2 (g)	Dry matter content m-%	Parallel mean	Water content m-%	Parallel mean
UD OL 1	2.46	0.17	6.91		93.09	
UD OL 2	2.32	0.17	7.33	7.12	92.67	92.88
D OL 1	2.07	0.36	17.39		82.61	
D OL 2	2.41	0.37	15.35	16.37	84.65	83.63
UD OM 1	2.28	0.16	7.02		92.98	
UD OM 2	2.3	0.14	6.09	6.55	93.91	93.45
D OM 1	2.18	0.11	5.05		94.95	
D OM 2	2.07	0.13	6.28	5.66	93.72	94.34
UD ME 1	2.21	0.2	9.05		90.95	
UD ME 2	2.39	0.22	9.21	9.13	90.79	90.87
D ME 1	2.21	0.31	14.03		85.97	
D ME 2	2.14	0.31	14.49	14.26	85.51	85.74

Peat type tells the trophic level (OL = oligotrophic, OM = ombrotrophic, ME = mesotrophic), and the drainage status (UD = undrained, D = drained) of the peat.

Appendix 2. Sample dry matter content

Weights of peat samples added to each flask and their dry matter contents as percentages and dry weights as grams. Dry matter content represents the peat type in question (Appendix 1).

Table 10. The weights of the peat samples measured to the flasks, their dry matter contents, and the resulting dry weights of the peat samples. All weights are means of three parallel samples and dry matter contents are means of two parallel samples with standard deviations (SD).

Flask ID	m peat (SD) (g)	Dry matter content (SD) (%)	Dry weight (SD) (g)
NO OL UD	36.58 (1.62)	7.12 (0.29)	2.604 (0.12)
Fe OL UD	34.50 (2.11)	7.12 (0.29)	2.456 (0.15)
SO OL UD	33.78 (0.84)	7.12 (0.29)	2.405 (0.06)
C OL UD	36.28 (2.06)	7.12 (0.29)	2.583 (0.15)
NO OL D	33.38 (3.74)	16.37 (1.44)	5.465 (0.61)
Fe OL D	32.63 (2.96)	16.37 (1.44)	5.343 (0.48)
SO OL D	31.89 (3.84)	16.37 (1.44)	5.221 (0.63)
C OL D	33.90 (4.39)	16.37 (1.44)	5.551 (0.72)
NO OM UD	27.95 (4.89)	6.55 (0.66)	1.831 (0.32)
Fe OM UD	28.82 (4.37)	6.55 (0.66)	1.888 (0.29)
SO OM UD	28.33 (4.86)	6.55 (0.66)	1.856 (0.32)
C OM UD	26.65 (2.55)	6.55 (0.66)	1.746 (0.17)
NO OM D	32.04 (4.51)	5.66 (0.87)	1.815 (0.26)
Fe OM D	32.18 (6.05)	5.66 (0.87)	1.823 (0.34)
SO OM D	33.04 (3.46)	5.66 (0.87)	1.871 (0.20)
C OM D	32.44 (4.99)	5.66 (0.87)	1.837 (0.28)
NO ME UD	35.14 (1.19)	9.13 (0.11)	3.208 (0.11)
Fe ME UD	34.65 (1.77)	9.13 (0.11)	3.163 (0.16)
SO ME UD	35.50 (1.65)	9.13 (0.11)	3.240 (0.15)
C ME UD	34.08 (2.85)	9.13 (0.11)	3.111 (0.26)
NO ME D	35.62 (3.45)	14.26 (0.32)	5.078 (0.49)
Fe ME D	34.23 (1.55)	14.26 (0.32)	4.880 (0.22)
SO ME D	36.31 (1.66)	14.26 (0.32)	5.176 (0.24)
C ME D	34.85 (1.63)	14.26 (0.32)	4.968 (0.23)

Flask ID tells which TEA is added to the flasks (NO = nitrate, Fe = ferric iron, SO = sulphate, C = control), the trophic level (OL = oligotrophic, OM = ombrotrophic, ME = mesotrophic), and the drainage status (UD = undrained, D = drained) of the peat.

Appendix 3. Redox potentials and pH values

Redox potentials and pH values measured during incubation in each round in parallel means. In addition, if terminal electron acceptor was added to the flasks, the amount of it is mentioned. The measurements were done before the potential TEA addition.

Table 11. The pH values and pH corrected E_h values of the flasks per round and their mean values during experiment. All pH and E_h values are means of three parallel samples (except for the first round, where the E_h value is from one parallel) with standard deviations (SD). If TEA solution was added to the flasks, the amount is mentioned as mean of three parallel samples per round.

Flask ID	Round	pH (SD)	Mean pH (SD)	E_h (SD) (mV)	Mean E_h (SD) (mV)	Added TEAs* (ml)
C D ME	1	4.00 (0.09)		14.95		
	2	4.09 (0.10)		75.31 (73.48)		
	3	3.96 (0.06)		38.11 (70.20)		
	4	4.03 (0.10)		36.44 (94.60)		
	5	3.97 (0.19)	4.01 (0.05)	-38.77 (17.88)	25.21 (41.84)	
C UD ME	1	5.51 (0.27)		5.06		
	2	4.86 (0.12)		32.07 (22.19)		
	3	4.52 (0.07)		23.48 (32.58)		
	4	4.51 (0.06)		8.29 (3.79)		
	5	4.52 (0.07)	4.78 (0.43)	0.35 (26.74)	13.85 (13.37)	
C D OL	1	3.84 (0.25)		144.40		
	2	4.18 (0.42)		175.48 (81.84)		
	3	4.03 (0.70)		113.30 (61.13)		
	4	3.88 (0.28)		121.12 (48.50)		
	5	4.05 (0.08)	4.00 (0.14)	69.09 (89.23)	124.68 (39.38)	
C UD OL	1	5.81 (0.17)		-28.16		
	2	6.18 (0.34)		-5.24 (59.12)		
	3	5.72 (0.54)		-12.19 (21.83)		
	4	6.28 (0.43)		4.38 (25.10)		
	5	6.25 (0.36)	6.05 (0.26)	50.75 (100.85)	1.91 (29.77)	
C D OM	1	4.04 (0.09)		184.77		
	2	3.64 (0.13)		140.29 (153.08)		
	3	3.67 (0.03)		118.33 (105.44)		
	4	3.68 (0.17)		190.98 (78.28)		
	5	3.59 (0.11)	3.72 (0.18)	6.95 (24.99)	128.26 (74.31)	
C UD OM	1	4.08 (0.26)		217.61		
	2	4.18 (0.20)		153.62 (69.99)		
	3	3.76 (0.50)		31.31 (18.32)		
	4	3.89 (0.26)		116.31 (107.30)		
	5	3.91 (0.12)	3.96 (0.17)	26.02 (53.74)	108.98 (81.79)	
Fe D ME	1	4.12 (0.13)		-4.66		3.3
	2	4.36 (0.23)		-41.76 (19.63)		6.7
	3	4.17 (0.27)		-56.97 (21.05)		6.7
	4	4.42 (0.17)		-52.42 (19.06)		6.7

	5	4.20 (0.09)	4.25 (0.13)	-50.00 (10.09)	-41.16 (21.14)	
Fe UD ME	1	5.73 (0.34)		-52.60		3.3
	2	4.77 (0.78)		-16.57 (43.68)		
	3	4.88 (0.41)		-33.61 (4.90)		
	4	4.61 (0.31)		9.19 (122.89)		6.7
	5	4.79 (0.30)	4.96 (0.44)	-36.86 (11.26)	-26.09 (23.51)	
Fe D OL	1	3.93 (0.19)		85.07		3.3
	2	4.16 (0.47)		29.30 (46.12)		
	3	4.33 (0.21)		-42.33 (22.10)		3.3
	4	4.03 (0.38)		-58.37 (15.03)		6.7
	5	4.17 (0.16)	4.13 (0.15)	-40.30 (8.33)	-5.33 (60.78)	
Fe UD OL	1	5.72 (0.66)		-8.69		3.3
	2	6.09 (0.19)		-83.69 (199.95)		
	3	5.66 (0.42)		52.61 (37.17)		6.7
	4	5.80 (0.01)		11.06 (10.08)		
	5	5.73 (0.04)	5.80 (0.17)	33.21 (50.70)	0.90 (52.60)	
Fe D OM	1	3.88 (0.09)		163.61		3.3
	2	3.87 (0.15)		45.53 (69.66)		
	3	3.98 (0.05)		-42.98 (15.43)		
	4	3.74 (0.45)		-53.81 (33.65)		10.0
	5	3.35 (0.23)	3.77 (0.25)	-60.35 (14.13)	10.40 (95.77)	
Fe UD OM	1	3.87 (0.03)		196.15		3.3
	2	4.22 (0.10)		14.12 (42.36)		
	3	3.53 (0.75)		22.13 (110.84)		3.3
	4	3.42 (0.25)		5.64 (102.91)		3.3
	5	3.73 (0.26)	3.75 (0.31)	-72.73 (17.58)	33.06 (98.77)	
NO D ME	1	4.05 (0.06)		35.82		3.3
	2	4.06 (0.35)		35.34 (21.41)		13.3
	3	4.31 (0.04)		68.20 (98.80)		6.7
	4	4.25 (0.41)		72.55 (79.35)		3.3
	5	3.93 (0.38)	4.12 (0.16)	-12.80 (34.09)	39.82 (34.21)	
NO UD ME	1	5.94 (0.25)		74.43		3.3
	2	5.09 (0.22)		51.51 (18.71)		33.3
	3	4.30 (0.26)		62.17 (91.70)		6.7
	4	4.72 (0.22)		105.28 (97.69)		6.7
	5	4.13 (0.70)	4.83 (0.72)	-7.86 (53.12)	57.11 (41.53)	
NO D OL	1	3.77 (0.32)		184.96		3.3
	2	3.95 (0.17)		148.05 (27.29)		20.0
	3	4.00 (0.15)		23.20 (23.07)		6.7
	4	4.04 (0.11)		105.56 (73.84)		
	5	4.27 (0.12)	4.01 (0.18)	30.60 (34.73)	98.47 (71.17)	
NO UD OL	1	4.96 (0.60)		121.60		3.3
	2	5.41 (0.25)		177.66 (42.86)		24.0
	3	5.57 (0.31)		132.10 (11.89)		
	4	5.79 (0.11)		113.61 (17.38)		
	5	5.89 (0.12)	5.52 (0.36)	127.65 (57.36)	134.52 (25.09)	

NO D OM	1	3.26 (0.06)		138.75		3.3
	2	3.22 (0.02)		45.12 (46.64)		28.3
	3	3.15 (0.06)		61.38 (76.94)		3.3
	4	3.24 (0.06)		50.02 (55.28)		6.7
	5	3.36 (0.05)	3.25 (0.08)	-7.90 (20.98)	57.47 (52.68)	
NO UD OM	1	3.35 (0.12)		231.39		3.3
	2	3.35 (0.01)		219.79 (109.43)		16.7
	3	3.66 (0.53)		109.80 (119.71)		
	4	3.49 (0.12)		102.91 (78.33)		3.3
	5	3.71 (0.22)	3.51 (0.17)	5.89 (20.15)	133.96 (93.29)	
SO D ME	1	4.18 (0.15)		-85.64		3.3
	2	4.16 (0.15)		-99.23 (32.89)		
	3	3.90 (0.33)		-119.76 (9.97)		
	4	4.09 (0.17)		-101.69 (8.53)		
	5	4.20 (0.07)	4.11 (0.12)	-86.67 (41.20)	-98.60 (13.86)	
SO UD ME	1	5.24 (0.41)		-159.31		3.3
	2	5.41 (0.38)		-145.28 (39.00)		13.3
	3	4.72 (0.52)		-129.72 (32.92)		
	4	4.79 (0.14)		-123.53 (15.90)		
	5	4.93 (0.05)	5.02 (0.30)	-92.33 (4.50)	-130.03 (25.27)	
SO D OL	1	4.07 (0.37)		123.92		3.3
	2	4.00 (0.10)		-105.53 (26.25)		
	3	4.45 (0.51)		-97.31 (41.07)		
	4	4.02 (0.27)		-124.15 (16.85)		
	5	4.27 (0.05)	4.16 (0.19)	-101.07 (7.02)	-60.83 (103.79)	
SO UD OL	1	5.57 (1.12)		-0.71		3.3
	2	6.18 (0.28)		-3.38 (41.42)		1.7
	3	5.68 (0.56)		-41.21 (43.86)		
	4	6.09 (0.25)		9.64 (9.27)		
	5	5.91 (0.15)	5.89 (0.26)	25.89 (46.33)	-1.95 (24.77)	
SO D OM	1	3.47 (0.05)		-71.91		3.3
	2	3.48 (0.14)		-60.82 (35.13)		
	3	3.45 (0.11)		-74.45 (16.42)		
	4	3.47 (0.12)		-64.74 (30.34)		
	5	3.51 (0.10)	3.48 (0.02)	-66.11 (23.45)	-67.60 (5.52)	
SO UD OM	1	3.52 (0.16)		240.83		3.3
	2	3.53 (0.06)		19.47 (80.04)		
	3	3.77 (0.17)		-50.37 (31.82)		
	4	3.55 (0.07)		-75.22 (12.31)		
	5	3.63 (0.11)	3.60 (0.11)	-50.30 (11.78)	16.88 (130.08)	

Flask ID tells which TEA is added to the flasks (NO = nitrate, Fe = ferric iron, SO = sulphate, C = control), the trophic level (OL = oligotrophic, OM = ombrotrophic, ME = mesotrophic), and the drainage status (UD = undrained, D = drained) of the peat.
* The added TEA solution always corresponds to the flask ID

Appendix 4. Pressures

The pressure differences with respect to ambient pressure measured at the end of each round as parallel means of each TEA + peat type.

Table 12. Pressure differences compared to atmospheric pressure of the flasks per round and their mean values during experiment. All values are means of three parallel samples with standard deviations (SD).

Flask ID	Round	Pressure (SD) (mbar)	Mean per experiment (SD) (mbar)
C D ME	1	-52.67 (16.20)	
C D ME	2	-79.00 (35.08)	
C D ME	3	-88.67 (12.74)	
C D ME	4	-92.00 (10.15)	
C D ME	5	-105.33 (15.39)	-83.53 (19.66)
C UD ME	1	-85.00 (14.18)	
C UD ME	2	-91.00 (3.00)	
C UD ME	3	-92.67 (11.93)	
C UD ME	4	-66.33 (10.41)	
C UD ME	5	-82.67 (11.06)	-83.53 (10.46)
C D OL	1	-56.00 (32.70)	
C D OL	2	-64.00 (42.25)	
C D OL	3	-108.67 (15.72)	
C D OL	4	-62.33 (16.50)	
C D OL	5	-69.00 (16.29)	-72.00 (21.02)
C UD OL	1	-74.33 (11.15)	
C UD OL	2	-76.00 (12.86)	
C UD OL	3	-77.00 (29.09)	
C UD OL	4	-74.67 (13.45)	
C UD OL	5	-68.33 (29.14)	-74.07 (3.38)
C D OM	1	-77.33 (29.14)	
C D OM	2	-51.00 (27.97)	
C D OM	3	-57.00 (32.60)	
C D OM	4	-101.33 (26.85)	
C D OM	5	-74.67 (9.50)	-72.27 (19.76)
C UD OM	1	-73.00 (34.51)	
C UD OM	2	-47.00 (19.40)	
C UD OM	3	-73.67 (15.04)	
C UD OM	4	-67.33 (27.32)	
C UD OM	5	-75.00 (22.14)	-67.20 (11.67)
Fe D ME	1	-52.33 (35.12)	
Fe D ME	2	-66.00 (42.03)	
Fe D ME	3	-104.67 (11.93)	
Fe D ME	4	-99.33 (61.65)	
Fe D ME	5	-93.67 (7.64)	-83.20 (22.80)

Fe UD ME	1	-79.33 (22.14)	
Fe UD ME	2	-71.00 (14.05)	
Fe UD ME	3	-67.33 (28.59)	
Fe UD ME	4	-89.33 (5.00)	
Fe UD ME	5	-39.00 (34.77)	-69.20 (18.89)
Fe D OL	1	-68.33 (54.15)	
Fe D OL	2	-87.00 (14.57)	
Fe D OL	3	-79.33 (39.66)	
Fe D OL	4	-98.00 (17.01)	
Fe D OL	5	-74.67 (50.48)	-81.47 (11.48)
Fe UD OL	1	-59.67 (48.64)	
Fe UD OL	2	-47.33 (54.67)	
Fe UD OL	3	-89.67 (24.25)	
Fe UD OL	4	-36.67 (28.58)	
Fe UD OL	5	-22.67 (50.27)	-51.20 (25.45)
Fe D OM	1	-73.67 (37.24)	
Fe D OM	2	-75.67 (22.14)	
Fe D OM	3	-104.33 (20.43)	
Fe D OM	4	-102.67 (19.35)	
Fe D OM	5	-105.00 (51.39)	-92.27 (16.10)
Fe UD OM	1	-75.67 (7.23)	
Fe UD OM	2	-83.67 (9.61)	
Fe UD OM	3	-79.00 (34.21)	
Fe UD OM	4	-74.33 (42.46)	
Fe UD OM	5	-56.67 (60.08)	-73.87 (10.27)
NO D ME	1	-63.33 (7.51)	
NO D ME	2	-73.33 (36.59)	
NO D ME	3	-94.00 (13.65)	
NO D ME	4	-90.67 (44.43)	
NO D ME	5	-63.33 (17.04)	-76.93 (14.69)
NO UD ME	1	-64.67 (11.50)	
NO UD ME	2	-67.67 (9.87)	
NO UD ME	3	-66.33 (18.19)	
NO UD ME	4	-65.00 (2.89)	
NO UD ME	5	-68.33 (35.12)	-66.40 (1.61)
NO D OL	1	-54.67 (33.98)	
NO D OL	2	-55.00 (57.17)	
NO D OL	3	-64.33 (42.34)	
NO D OL	4	-39.33 (16.65)	
NO D OL	5	-15.00 (51.03)	-45.67 (19.35)
NO UD OL	1	-32.00 (46.18)	
NO UD OL	2	-38.00 (38.80)	
NO UD OL	3	-35.33 (36.43)	
NO UD OL	4	-56.00 (25.24)	
NO UD OL	5	-44.00 (44.64)	-41.07 (9.44)
NO D OM	1	-86.33 (7.02)	

NO D OM	2	-45.67 (29.09)	
NO D OM	3	-50.33 (42.25)	
NO D OM	4	-52.67 (26.85)	
NO D OM	5	-19.00 (19.00)	-50.80 (24.00)
NO UD OM	1	-26.00 (24.79)	
NO UD OM	2	-30.67 (7.09)	
NO UD OM	3	-66.00 (3.06)	
NO UD OM	4	-48.33 (28.75)	
NO UD OM	5	-62.67 (27.75)	-46.73 (18.14)
SO D ME	1	-79.00 (5.86)	
SO D ME	2	-56.33 (37.58)	
SO D ME	3	-61.67 (7.81)	
SO D ME	4	-85.33 (17.56)	
SO D ME	5	-75.33 (6.66)	-71.53 (12.13)
SO UD ME	1	-78.00 (11.85)	
SO UD ME	2	-80.67 (27.68)	
SO UD ME	3	-89.00 (50.71)	
SO UD ME	4	-90.33 (19.40)	
SO UD ME	5	-69.67 (39.00)	-81.53 (8.47)
SO DO OL	1	-79.00 (5.69)	
SO DO OL	2	-73.33 (27.21)	
SO DO OL	3	-81.00 (41.10)	
SO DO OL	4	-58.33 (7.77)	
SO DO OL	5	-44.33 (48.91)	-67.20 (15.56)
SO UD OL	1	-44.33 (19.67)	
SO UD OL	2	-40.00 (46.68)	
SO UD OL	3	-93.33 (39.04)	
SO UD OL	4	-62.00 (36.75)	
SO UD OL	5	-34.00 (41.02)	-54.73 (23.97)
SO D OM	1	-77.67 (44.50)	
SO D OM	2	-90.33 (42.34)	
SO D OM	3	-97.67 (41.59)	
SO D OM	4	-74.67 (9.02)	
SO D OM	5	-94.00 (51.69)	-86.87 (10.16)
SO UD OM	1	-82.00 (6.03)	
SO UD OM	2	-57.33 (49.10)	
SO UD OM	3	-73.00 (37.47)	
SO UD OM	4	-64.00 (11.27)	
SO UD OM	5	-45.33 (54.04)	-64.33 (14.12)

Flask ID tells which TEA is added to the flasks (NO = nitrate, Fe = ferric iron, SO = sulphate, C = control), the trophic level (OL = oligotrophic, OM = ombrotrophic, ME = mesotrophic), and the drainage status (UD = undrained, D = drained) of the peat.