Unraveling microbial processes involved in carbon and nitrogen cycling and greenhouse gas emissions in rewetted peatlands by molecular biology

Emilie Gios · Erik Verbruggen · Joachim Audet · Rachel Burns · Klaus Butterbach-Bahl · Mikk Espenberg · Christian Fritz · Stephan Glatzel · Gerald Jurasinski · Tuula Larmola · Ülo Mander · Claudia Nielsen · Andres F. Rodriguez · Clemens Scheer · Dominik Zak · Hanna M. Silvennoinen

Abstract Restoration of drained peatlands through rewetting has recently emerged as a prevailing strategy to mitigate excessive greenhouse gas emissions and re-establish the vital carbon sequestration capacity of peatlands. Rewetting can help to restore vegetation communities and biodiversity, while still allowing for extensive agricultural management such as paludiculture. Belowground processes governing carbon fluxes and greenhouse gas dynamics are mediated by a complex network of microbial communities and processes. Our understanding of this complexity and its multi-factorial controls in rewetted peatlands is limited. Here, we summarize the research regarding the role of soil microbial communities and functions in driving carbon and nutrient cycling in rewetted peatlands including the use of molecular biology techniques in understanding biogeochemical processes linked to greenhouse gas fluxes. We emphasize that rapidly advancing molecular biology approaches, such as high-throughput sequencing, are powerful tools helping to elucidate the dynamics of key biogeochemical processes when combined with isotope tracing and greenhouse gas measuring techniques. Insights gained from the gathered studies can help inform efficient monitoring practices for rewetted

Responsible Editor: Jorgen E. Olesen.

E. Gios (✉) · H. M. Silvennoinen
NINA, Norwegian Institute for Nature Research, PO Box 5685, Torgarden, NO-7485 Trondheim, Norway
e-mail: emilie.gios@nina.no

E. Verbruggen
Plants and Ecosystems Research Group, Department of Biology, University of Antwerp, Universiteitsplein 1, Wilrijk, 2610 Antwerp, Belgium

J. Audet · D. Zak
Department of Ecoscience, Aarhus University, C.F. Møllers Allé, 8000 Aarhus, Denmark

R. Burns
Department of Geosciences and Natural Resource Management, University of Copenhagen, 1350 Copenhagen, Denmark

K. Butterbach-Bahl · C. Scheer
Institute of Meteorology and Climate Research, Atmospheric Environmental Research, Karlsruhe Institute of Technology, 82467 Garmisch-Partenkirchen, Germany

K. Butterbach-Bahl
Department of Agroecology, Pioneer Center for Research in Sustainable Agricultural Futures (Land-CRAFT), Aarhus University, 8000 Aarhus, Denmark

M. Espenberg · Ü. Mander
Department of Geography, Institute of Ecology and Earth Sciences, University of Tartu, 46 St., Vanemuise, 51003 Tartu, Estonia

peatlands and the development of climate-smart restoration and management strategies.

Introduction

Approximately 12% of the world's peatlands have been subject to drainage for the purpose of agriculture, peat extraction, urbanization, or forestry (UNEP 2022). Peatland drainage causes land subsidence, peat loss, greenhouse gas (GHG) emissions and eutrophication of water bodies, leading to a loss of almost all ecological functions (Kreyling et al. 2021). The destruction and subsequent loss of peatland ecosystems and the benefits they provide continues at a rate greater than for any other ecosystem type, including tropical rainforests (Loisel et al. 2021). Through aerobic peat mineralization and increased incidence of fires, drained peatlands emit about 1.9 Gt carbon dioxide (CO_2)-equivalents per year globally (Leifeld and Menichetti 2018), corresponding to 10% of the GHG emissions from agriculture, land-use change and forestry combined (IPCC 2014).

In the last decades, the understanding of biogeochemical processes in rewetted degraded peatlands has increased substantially with new insights gained from monitoring programs accompanying peatland restoration projects. This information has allowed to outline the environmental implications of different restoration measures and to provide guidelines to optimize restoration (Jurasinski et al. 2020; Kreyling et al. 2021). Key driving factors are water table position and source of water (e.g., rainfall, groundwater), while chemical composition of discharging water, physico-chemical soil characteristics, dominant vegetation type and eventually composition of the microbial community are important indicators of ongoing biogeochemical processes (Wen et al. 2018; Walton et al. 2020; Evans et al. 2021). Depending on drainage history and specific characteristics of sites under consideration, such as size, landscape position, soil properties, land use, and the presence of valuable species, different rewetting strategies might be appropriate. For example, topsoil removal or gradual rising of water table in combination with a new form of wetland use called paludiculture (Zak et al. 2018) can be considered. There is no "one-size fits it all" restoration solution; hence, each approach has its own merits and applications (Zak and Mc Innes 2022).

Molecular biology techniques are powerful tools allowing to directly target microbial processes of interest and, thus, contribute to fostering a comprehensive understanding of biodiversity and ecosystem functioning. Gene-targeted approaches can be used to identify taxonomic biodiversity of microorganisms (sequencing barcode regions on e.g., the 16S rRNA and 18S rRNA genes), or to explore the prevalence and changes in specific functions (targeting genes

C. Fritz
Aquatic Ecology and Environmental Biology, Radboud Institute for Biological and Environmental Sciences (RIBES), Radboud University, Heyendaalseweg 135, 6525 AJ Nijmegen, The Netherlands

S. Glatzel
Department of Geography and Regional Research, University of Vienna, Althanstraße 14, 1090 Vienna, Austria

G. Jurasinski
Faculty of Agriculture and Environment, Landscape Ecology and Site Evaluation, University of Rostock, Justus-von-Liebig-Weg 6, 18059 Rostock, Germany

G. Jurasinski
Department of Maritime Systems, Faculty of Interdisciplinary Research, University of Rostock, Albert- Einstein-Straße 3, 18059 Rostock, Germany

T. Larmola
Natural Resources Institute Finland (Luke), 00790 Helsinki, Finland

C. Nielsen · A. F. Rodriguez
Department of Agroecology, Faculty of Technical Sciences, Aarhus University, Blichers Alle 20, 8830 Tjele, Denmark

C. Nielsen
CBIO, Centre for Circular Bioeconomy, Aarhus University, 8830 Tjele, Denmark

D. Zak
Department of Ecohydrology and Biogeochemistry, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Müggelseedamm 301, 12587 Berlin, Germany

coding for enzymes related to biogeochemical cycling for example using quantitative polymerase chain reaction [qPCR]). Metagenomics allows for the analysis of all the genetic material (DNA) present in a sample, providing a comprehensive view of the taxonomic and functional diversity. Transcriptomics provides information about genes that are actively being expressed by microorganisms (by targeting RNA molecules) and avoids inclusion of inactive or even dead genetic material, and enables querying real-time coupling of microbial activity and functional properties.

Here, we provide a comprehensive review of molecular biology methods used to assess microbial functions linked to biodiversity and biogeochemistry in rewetted peatlands. Climate, geology, and legacies from the original pristine state, as well as those from drainage and land use following the drainage form the microbiome of rewetted peatlands. Selection of the restoration method (rewetting/revegetation/paludiculture, see below) will further shape the microbial communities and their functions; this review aims to gather findings from research into how microbial communities respond to this environmental change. We will first summarize the current knowledge on general peat properties affecting the microbiome and its biogeochemical functions in pristine, drained and restored peatlands. We then align the recent literature on GHG emissions and biogeochemical processes, and how these findings link to microbial functions as explored by molecular biology methods. We, thus, provide a tool that helps general readership to get insights in the current stage of knowledge of microbial biogeochemical linkages with peatland rewetting, and ultimately help planning future studies in this field.

Effects of drainage and rewetting on peat biogeochemistry

Peat types and their characteristics are largely controlled by natural hydrology in pristine peatlands. Rainwater-fed bogs (ombrotrophic peatlands) are predominantly lower in nutrients and terminal electron acceptors (TEA) compared to groundwater-fed fens. Compared to drained and rewetted peatlands, both bogs and fens are characterized by lower bulk densities, higher carbon (C) contents per dry weight and lower decomposition status of C compounds

(amongst other parameters, listed in Table S1 with references).

Predominant land use and intensity of use of peatlands vary between geographical regions, and are controlled by climate, culture, as well as general regional socio-economic conditions. While northwest European peatlands are often heavily drained and used for intensive dairy production (de Vos et al. 2010), those in Northern Europe (Sweden, Finland, Estonia, Latvia, Lithuania) are commonly drained to a shallower extent for forestry (UNEP 2022). Mountainous peatlands on the other side are frequently used in traditional ways such as extensive meadows for pasture (e.g., Sjögren 2006; Jenkins and Walker 2022). Abandoned peat extraction areas can be used for agriculture and berry cultivation (Albert et al. 2011), afforestation (Caisse et al. 2008), and bioenergy production, where the last has been shown as an option to mitigate the atmospheric impact in peatland-rich Northern Europe (Hyvönen et al. 2009; Mander et al. 2012; Espenberg et al. 2016). Drainage causes severe peat degradation and thus impairs the ecological functioning of both bogs and fens (Holden et al. 2004). These drastic hydrological changes lead to aeration of the drained peat layers and thus foster the mineralization of organic matter built up within the last several hundred years, therewith turning peatlands into significant sources of C and nutrients. The non-reversible changes of peat soil characteristics following drainage and the consequences for ecosystem functioning are well investigated (see Table S1).

Restoration of degraded peatlands can be carried out with multiple strategies. Rewetting or "blocking ditches" are the most common measures. Degraded peatlands can in addition be revegetated with vegetation typical to local peatlands and the most decomposed, hydrophobic topsoil may be removed to improve the restoration success. When boreal forestry drained peatlands are restored, in addition to rewetting, depending on the pre-drainage tree cover, the tree layer is partially or completely removed to avoid decaying felling residues hampering the peatland's recovery toward its nutrient-poor, pristine conditions and to avoid risk of nutrient leaching (Tolvanen et al. 2020). Peatlands can be either rewetted for nature conservation or paludiculture, where, in the latter, plant biomass is harvested for food, feed, fodder or energy after rewetting (Wichtmann and Schäfer 2007). Different restoration strategies most certainly

lead to different implications to the microbiome and biogeochemical processes, but few studies exist comparing them. Hereinafter, we refer to restored peatlands as a general term where other restoration strategies than rewetting were used (e.g., rewilding, paludiculture, topsoil removal). Rewetting drained organic soils has been proven to be an effective measure to strongly reduce agricultural CO_2 emissions and to revert soil carbon sequestration in the short-term (Cabezas et al. 2014; Nugent et al. 2018). Likewise, the nitrogen sink function can be recovered in the short-term as anaerobic conditions are re-established after only a few days of rewetting (Zak and Gelbrecht 2007; Cabezas et al. 2013). On the other hand, there is evidence that rewetted peatlands become strong emitters of methane (CH_4) (Hahn et al. 2015; Antonievic et al. 2023), nutrients and dissolved organic matter-possibly for decades (Zak and McInnes 2022). Unlike in pristine peatlands, the decomposition of organic matter in rewetted fen peatlands is strongly controlled by the availability of electron acceptors like ferric iron and sulfate, enhanced nutrient availability, circumneutral pH, and lack of polyphenolic substances (Zak et al. 2019). Respiration measurements with different organic substrates from rewetted peatlands suggest that degraded peat without any fresh plant-derived material is relatively inert in terms of decomposition, whereas significant anaerobic production of CO_2 and CH_4 in peat may occur only when enough labile organic matter is available either from root turnover or exudation (Hahn-Schöfl et al. 2011). Elevated nutrient levels in degraded peat soil favor the establishment of fast-growing reed communities and, in case of inundated conditions, the formation of highly active detritus mud layers that function as biogeochemical hotspots for nutrient and CH_4 release (Zak et al. 2018). Overall, revegetation, paludiculture crops and top soil recycling/removal have been shown to be effective measures to reduce the CH_4 emission potential in (re)flooded peatlands (Huth et al. 2020; Boonman et al. 2023; Quadra et al. 2023).

Belowground microbial processes involved in GHG dynamics

Carbon dioxide

Rewetting of drained organic soils is an effective measure to strongly reduce agricultural CO_2 emissions and to revert soil C sequestration in the short-term (Cabezas et al. 2014; Nugent et al. 2018). A recent meta-analysis by Darusman et al. (2023) showed that rewetting reduced CO_2 emissions by 1.43 ± 0.35 Mg CO_2-C ha^{-1} yr^{-1} on average, but the effects varied depending on climatic zone and nutrient status. Currently, CO_2 emission factors for rewetted peatlands are between -0.34 and -0.55 Mg CO_2-C ha^{-1} yr^{-1} for boreal peatlands, -0.23 and 0.5 Mg CO_2-C ha^{-1} yr^{-1} for temperate peatlands, and 0 Mg CO_2-C ha^{-1} yr^{-1} for tropical peatlands (Wilson et al. 2016). High nutrient concentrations in peat soils, particularly in temperate peatlands, generate larger CO_2 emissions from rewetted sites (Wilson et al. 2016; Hemes et al. 2019; Tiemeyer et al. 2020). Additionally, vegetation can affect emissions by adding fresh plant residues to the soil (Rigney et al. 2018) or by transporting O_2 to the peat through roots (Zhong et al. 2020; Darusman et al. 2023) thereby increasing peat decomposition and CO_2 emissions. These emissions are governed by the dynamics between CO_2 uptake by ecosystems, i.e., photosynthesis by plants and soil autotrophic microorganisms (both photo- and chemoautotrophic), and loss to the atmosphere from ecosystem respiration (Fig. 1). The latter includes both autotrophic respiration from plants and microbial heterotrophic respiration.

While this review largely concentrates knowledge on soil microbial processes, which contribute to the breakdown of soil organic matter and resulting CO_2 emissions, it is important to note that autotrophic respiration by plants constitutes a major component of CO_2 emissions from the ecosystem to the atmosphere. Partitioning between root and soil respiration can shed light on linkages between variable controls of photosynthesis, autotrophic respiration, and soil respiration (including rhizomicrobial respiration carried out by heterotrophs from recent photosynthesis products) (Kuzyakov and Larionova 2005).
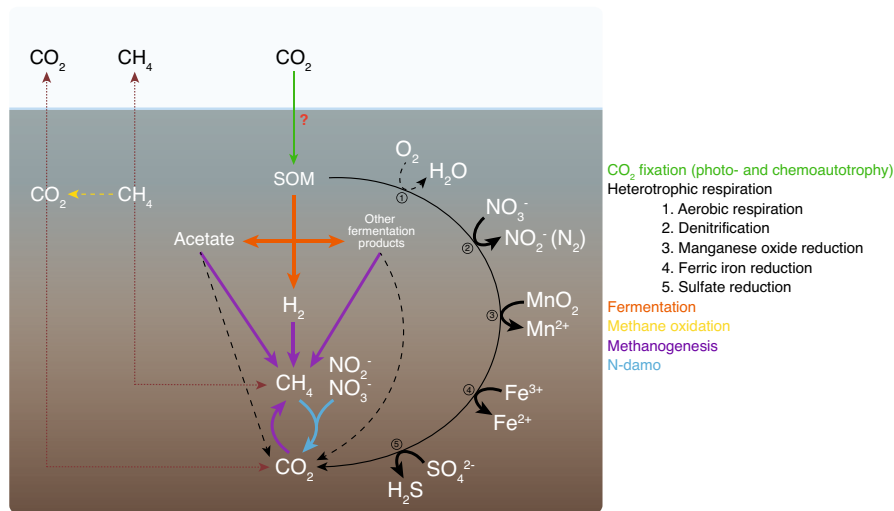
Fig. 1 Soil processes involved in carbon dioxide (CO_2) and methane (CH_4) fluxes and expected effects of peatland rewetting. Dashed lines correspond to pathways expected to decrease their contribution to GHG fluxes after rewetting and thick lines to increase their contribution. Red dotted lines represent gas diffusion pathways in soil for CO_2 and CH_4. Question mark highlights uncertainties regarding the expected effect of rewetting due to lack of information. *N-damo* nitrite-dependent anaerobic methane oxidation, *SOM* soil organic matter

Photosynthesis and chemoautotrophic processes

Microorganisms capable of CO_2 fixation (photoautotrophic prokaryotes and micro-eukaryotes) seem to be ubiquitous in surface soils (Cano-Díaz et al. 2020; Oliverio et al. 2020; Bay et al. 2021). The contribution of phototrophic microorganisms, i.e., direct C uptake through photosynthesis, to C fluxes in peatlands is still largely unknown. Hamard et al. (2021) estimated that these microorganisms are responsible for 10% of C uptake, which roughly equals the magnitude of projected peatland C loss due to climate warming. To the best of our knowledge, no study investigated how the structure and activity of phototropic communities are affected by rewetting to date (Table S2). These organisms can be free-living in the upper few millimeters of soil, and some are associated with *Sphagnum* mosses, where they can be highly abundant (Gilbert et al. 1998; Gilbert and Mitchell 2006; Hamard et al. 2021; Jassey et al. 2013; Tian et al. 2020). We could therefore hypothesize that the recovery of phototrophic microorganisms is dependent on restoration of vegetation, both in terms of plant (e.g., *Sphagnum* mosses) composition, and light penetration to the soil (Davies et al. 2013).

An additional potential C sink in peatlands is represented by dark, non-phototrophic CO_2 fixation. This process, driven by chemoautotrophic microorganisms which convert inorganic carbon to organic carbon, has been shown to occur in a large range of soils, including wetlands (Nowak et al. 2015). While the occurrence of dark fixation of CO_2 by heterotrophic microorganisms in soil is generally accepted, its importance for microbial metabolism and C balance in rewetted peatlands is unknown.

Heterotrophic respiration

Aerobic respiration Oxygen is the most thermodynamically favorable TEA for microbial decomposition of soil organic carbon (SOC) to CO_2. Therefore, aerobic respiration occurs at oxic compartments of the peat, i.e., above water table level, at layers with O_2-saturation in water and in the rhizosphere. Saprotrophic fungi, actinobacteria and methanotrophs are the main aerobic decomposers in peatlands (Dedysh et al. 2006; Thormann 2006; Peltoniemi et al. 2012). Diversity and prevalence of these organisms can be an indicator of decomposition processes. For example, greater richness of saprotrophic fungi was reported under drier conditions, which may stem from both the higher prevalence of oxygen, or high fungal tolerance to drought in diverse soils (Yuste et al. 2011; de Vries

et al. 2012; Barnard et al. 2013; Asemaninejad et al. 2017; Jassey et al. 2018).

Microbial C mineralization is mediated by a suite of intracellular and extracellular enzymes, with the phenol oxidase enzyme considered a key regulator (Freeman et al. 2001). Phenol oxidases degrade phenolic compounds in oxic conditions, enhancing SOC decomposition under drained conditions. In contrast, under anoxic conditions, phenolic compound accumulation limits C mineralization. However, the enzyme latch theory has been disputed as many studies show contradictory results (Laiho 2006; Turetsky et al. 2011; Carter et al. 2012; Wang et al. 2017; Urbanová and Hájek 2021). Other well-documented controls of aerobic respiration include temperature and availability of micro- and macronutrients. A less studied control that may play a significant role in CO_2 release in rewetted peatlands is substrate quality, particularly that of C. In boreal peatlands, litter type appears to be a more important controlling factor of fungal and actinobacterial communities than water table levels (Peltoniemi et al. 2009, 2012; Strakova et al. 2011). The increased content of carboxylic, aromatic, and phenolic compounds in peat due to drainage-induced oxic conditions stimulates fungi to secrete extracellular enzymes for degradation (Peltoniemi et al. 2009).

A handful of studies have investigated aerobic respiration in rewetted peatlands, more specifically the community structure of main microbial decomposers. Fungal abundance (measured through specific membrane fatty acid quantities) was shown to increase after rewetting in the top peat layer but did not reach pristine levels (Groß-Schmölders et al. 2022). Although fungi are main decomposers in the uppermost peat layers due to their competitive advantage over bacteria, they also show higher sensitivity to changes in substrate quality, which may affect SOC decomposition in rewetted peatlands. In coastal peatlands, arbuscular mycorrhiza can be important indicator because in such habitats, plants depend more strongly on mycorrhiza as they need them to avoid salt stress (Dastogeer et al. 2020). Likewise, changes in arbuscular mycorrhiza abundances can indicate temperature change in peatlands because increase in temperature positively contributes to their growth and functions (Wang et al. 2021). Defrenne et al. (2023) demonstrated that drainage significantly changed the dominant type of mycorrhizal association: in

the vicinity of ditches, it abruptly shifted from ericoid mycorrhiza to ectomycorrhiza. Most likely, changes in abundancy ratios of different mycorrhizal types in drained peatlands can indicate peat losses. In addition, bacterial to fungal ratios in the context of decomposition processes have been linked to C storage potentials (measured through ^{13}C incorporation in bulk soil organic matter) in grassland soils (Malik et al. 2016), highlighting the value of combining molecular and biogeochemical techniques to understand C cycling processes and ecosystem functioning. Such an approach could be used in peatland research to assess the status of rewetted peatlands for C cycling. Investigations into aerobic respiration in rewetted peatlands have been carried out using biodiversity analyses (16S and 18S rRNA genes) and other biomarkers, such as membrane fatty acid quantities and enzyme activity assays. In an arctic peatland, metagenomics has also proven to be a useful indicator of changes in functions involved in aerobic respiration (e.g., cytochrome oxidases) across the peat profile (Lipson et al. 2013).

Anaerobic respiration In peat layers where oxygen is depleted, anaerobic respiration is the main process generating CO_2. Anaerobic microorganisms perform complex redox reactions, driving the coupling of elements, and anaerobic respiration is considered to be one of the most flexible and diverse metabolic processes. Different TEAs can be used by the resident microbial community instead of O_2 and their order of use is broadly regulated by differences in the Gibbs free energy of the respective respiration processes and the bioavailability of TEA and electron donors (Fig. 2). The order of preference for TEA based on Gibbs free energy is $NO_3^-/NO_2^- > Mn(IV) > Fe^{3+} >$ sulfate $(SO_4^{2-}) >$ organic substances. It is noticeable that the ability of organic substances to mediate redox processes was described for quinones already in the beginning of the twentieth century (Erdtman 1933). Other microbially driven pathways such as fermentation contribute to CO_2 production in peatlands. Fermentative processes are diverse and occur via the cooperation of different functional microbial groups: primary fermenters hydrolyzing plant polymers and fermenting the monomers (such as sugars). Secondary fermenters then turn the resulting organic acids into acetate, H_2 and CO_2, subsequently feeding methanogenesis (see section below).
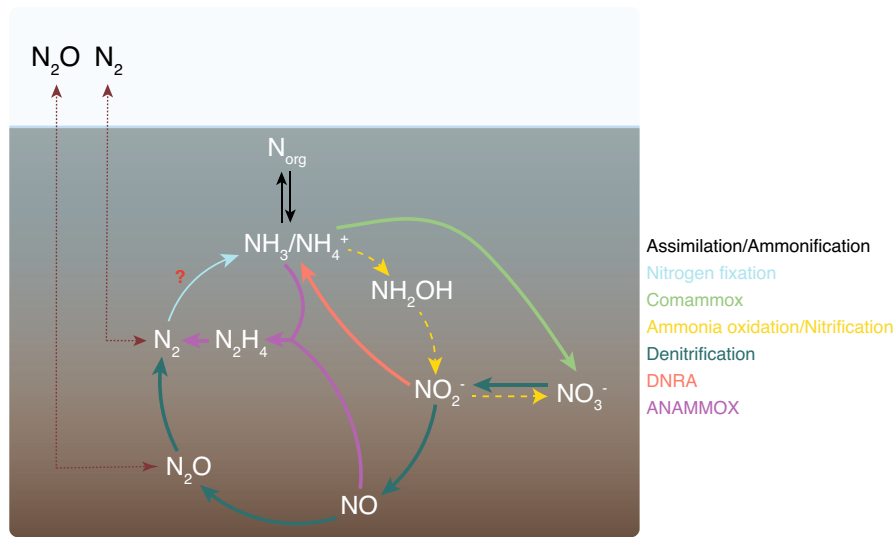
Fig. 2 Soil processes involved in nitrous oxide (N_2O) fluxes and expected effects of peatland rewetting. *Comammox* complete ammonia oxidation; *DNRA* dissimilatory nitrate reduction to ammonium; *ANAMMOX* anaerobic ammonium oxidation. Dashed lines correspond to pathways expected to decrease their contribution to GHG fluxes after rewetting and thick lines to increase their contribution. Dotted lines represent gas fluxes. Questionmark highlights uncertainties regarding the expected effect of rewetting due to lack of information

Nitrite (NO_2^-)/Nitrate (NO_3^-) Canonical denitrification, i.e., the reduction of NO_2^-/NO_3^- via nitric oxide (NO) and nitrous oxide (N_2O) to molecular nitrogen (N_2), contributes to SOC degradation and subsequent CO_2 production, while also having a significant role in N_2O production in peatlands. This process remains poorly investigated for its contribution to CO_2 emissions from rewetted peatlands (Table S2), however denitrification is discussed in more detail in the section below in the context of N_2O dynamics.

Metals Manganese and iron are less studied TEAs in the context of peatland rewetting. A small number of studies have explored the role of manganese in the C cycle in general, one referring to Mn playing a role in C decomposition in forest ecosystems (Keiluweit et al. 2015) and another showing associations between Mn and proportions of fungal and microbial communities in an Australian peatland (Birnbaum et al. 2023). Humic and fulvic substances, abundant in peatlands, contain iron and there is increasing evidence for a role of Fe in the C cycle of peatlands both via microbial and abiotic processes. Recent literature highlights both the role of Fe reducing mineralization of SOC in peatlands upon oxia and accelerating its decomposition upon anoxia. Iron oxidation has been suggested to protect SOC in peatlands by increasing the sorption of lignin derivatives and decreasing phenolic oxidase activities ("Iron gate" theory; Wang et al. 2017). Under anoxic conditions, Fe^{2+} additions have been shown to lead to increased SOC decomposition by increasing the phenol oxidative activity most likely due to the production of the hydroxyl radical (OH), which stimulates phenol oxidase and functions as a general oxidant for organic compounds (van Bodegom et al. 2005; Halliwell and Gutteridge 2007; Wen et al. 2019). Fluctuating water levels may lead to repeated redox reactions, where Fe^{2+} is oxidized to Fe^{3+} upon oxic conditions and reduced back to Fe^{2+} as a TEA during high water level—driven anoxia. The levels of iron in peatlands can exhibit significant variability, both within individual peatlands and across different peatland locations. The cycling of iron is intricately connected to the cycling of sulfur and phosphorus. Consequently, microbial processes such as nitrification-denitrification, desulphurization, and other related metabolic processes can display considerable fluctuations in peatland environments (Dollhopf et al. 2005; Zak et al. 2021). Investigating microorganisms involved in iron reactions with typical gene-targeted approaches such as amplicon analyses remains challenging, mainly because of the lack of specific primers

for iron-related functions. However, there are molecular methods available that could help alleviate these limitations (e.g., untargeted sequencing approaches).

Sulfate (SO$_4^{2-}$) Various elements of the C cycle in peatlands can be altered by SO_4^{2-} loading, including primary production, C mineralization and the production and export of DOC (Zak et al. 2021). SO_4^{2-} reducing microorganisms influence C fluxes in peatlands by coupling dissimilatory SO_4^{2-} reduction (SO_4^{2-} to sulfide H$_2$S) with heterotrophic respiration or CO_2 fixation. Dissimilatory SO_4^{2-} reduction is a significant contributor to SOC mineralization in peatlands (up to 36%), depending on sulfur deposition by rain or groundwater (Vile et al. 2003; Blodau et al. 2007; Deppe et al. 2010).

In comparison to other TEAs, more research has been done regarding SO_4^{2-} and anaerobic respiration in pristine peatlands, and microbial processes involved are well characterized. To reduce SO_4^{2-}, some bacteria and archaea encode two key enzymes: the dissimilatory (bi)sulfite (*dsrAB*) and adenosine-5'-phosphosulfate reductases (*apsA*). Specific primers exist for these and dissimilatory SO_4^{2-} reduction is relatively well studied in peatlands, generally. In comparison to other TEAs, dissimilatory SO_4^{2-} reduction has also been explored in rewetted peatlands to some extent. Those studies have shown that O$_2$ concentration and SO_4^{2-} availability are key factors controlling the presence of SO_4^{2-} reducers in rewetted peatlands. Furthermore, sulfate-reducing bacteria are known to tolerate a broad range of temperature and pH conditions although higher rates occur at higher temperature and neutral pH conditions (Neculita et al. 2007; Koschorreck 2008).

Higher proportions of SO_4^{2-} reducers were detected after long-term rewetting compared to drained states (He et al. 2015; Weil et al. 2020) due to restored waterlogged and anaerobic conditions. Genes involved in SO_4^{2-} reduction were stratified across the peat profile based on gene abundance (i.e., lower relative abundance in top layer due to presence of oxygen; Emsens et al. 2020). In addition, increased availability of SO_4^{2-} was suggested to have led to increased CO_2 production from a rewetted coastal peatland that received SO_4^{2-} through inflow of brackish water (Gutekunst et al. 2022). When CO_2 is not produced in aerobic peat decomposition during or from CH$_4$ oxidation, SO_4^{2-} input remains the

strongest CO_2 producer in such ecosystems (Knorr et al. 2008). Independent of the metabolic pathway involved, increased SO_4^{2-} availability might alter the extent of anaerobic C mineralization, i.e., CO_2 and CH$_4$ production. Anaerobic C mineralization rates could increase relative to acetoclastic, hydrogenotrophic, and methylotrophic methanogenesis when microbes can use an electron acceptor with a higher free-energy yield such as SO_4^{2-} (Sutton-Grier et al. 2011; Dean et al. 2018).

By coupling amplicon SIP (stable isotope probing) and 16S rRNA amplicon sequencing, Pester et al. (2010) showed that low abundance microorganisms participate in important biogeochemical cycling functions related to sulfur in peatlands. This may have been missed by typical molecular biology methods such as 16S rRNA amplicon sequencing alone.

General anaerobic microbial communities Succession of redox reaction is governed by thermodynamics, but also by the distribution of TEAs which is stratified with depth as the microbial communities and associated decomposition processes are (Andersen et al. 2013). For example, in both pristine and rewetted peatlands, the anaerobic CO_2 production rate was stratified with depth in both peatland types, being significantly higher in the surface than deeper peat layer (Urbanová and Bárta 2020).

Findings from biodiversity studies employing 16S rRNA amplicon sequencing can serve as indicators to assess the recovery of pristine-like conditions concerning dominant anaerobic microbial communities post-rewetting. A study in rewetted fens showed an increased relative abundance of anaerobic microbial groups compared to pristine peatlands (Weil et al. 2020). However, in another peatland, the anaerobic microbial community did not fully recover, likely related to a not fully restored vegetation cover and low accumulation of new peat 7–16 years after rewetting (Urbanová and Bárta 2020).

In recent years, there has been significant growth in our understanding of the processes influencing soil CO_2 emissions and the biogeochemical and microbial factors that control them. Despite these advances, translating this knowledge into practical applications for predicting CO_2 emissions from rewetted peatlands poses a persistent challenge. Anticipated changes in both plant community succession and microbial communities suggest a potential reduction in the radiative

forcing of rewetted peatlands over time (Wen et al. 2018; Antonijević et al. 2023). Furthermore, many studies on the restoration of peatlands have documented extreme events as flooding or droughts, which may reset microbial successions (Wen et al. 2018).

Methane

CH_4 has taken a central role in endeavours to examine GHG fluxes in rewetted peatlands, primarily owing to its elevated radiative forcing compared to CO_2 and its more substantial contribution to the atmospheric GHG pool than N_2O. Peatland rewetting has been shown to increase CH_4 emissions (Abdalla et al. 2016), with emissions factors defined by the IPCC for rewetted peatlands ranging from 41 to 216 kg CH_4–C ha^{-1} yr^{-1} (IPCC 2014). Although water table is a major driver of CH_4 emissions, peat properties, vegetation type, nutrient availability, climate, land-use, and restoration methods are also important factors influencing CH_4 emissions after rewetting (Le Mer and Roger, 2001; Wilson et al. 2016; Huth et al. 2020; Tiemeyer et al. 2020; Emsens et al. 2021; Zak and McInnes 2022). Furthermore, in general, fens generate larger emissions than bogs (Abdalla et al. 2016).

CH_4 fluxes in peatlands are maintained by a balance of taxonomically diverse aerobic methanotroph and anaerobic methanogen populations, and their respective metabolic activities. Methanogenesis is an anaerobic process in biomass decomposition and occurs where TEAs with higher thermodynamic efficiency are depleted or missing. Substrates for methanogenesis are CO_2, hydrogen (H_2), acetate, and methylated compounds (Fig. 1; Conrad 2020). Methanogens and their activity, commonly measured through *mcrA* (methyl coenzyme M reductase) gene abundance and expression, have consistently been shown to be significantly reduced in drained peatlands (Yrjälä et al. 2011; Urbanová et al. 2013; Urbanová and Bárta 2020). On the other hand, rewetting is expected to increase long-term CH_4 production in peatlands because anoxia favors methanogenesis. However, diverging responses of CH_4-cycling communities to rewetting have been observed (recently reviewed by Kitson and Bell 2020; Table S2). Effects on CH_4 production and consumption patterns differed between peatland types (fens vs. bogs), but also within the same peatland type, where CH_4 and related microbial community dynamics were site-specific.

This is thought to be mainly due to the different types of methanogenesis prevalent in each peatland type (acetoclastic or acetate-dependent vs. hydrogenotrophic or H_2–CO_2-dependent methanogenesis), different controls of respective methanogenic pathways, timescale post-rewetting, and climate.

The variation in responses has been further illustrated in recently published studies (Kitson and Bell 2020). Urbanová and Bárta (2020) showed that methanogenic communities in bogs and spruce mires reached a pristine-like state 7–16 years after rewetting, while resulting CH_4 production rates varied between the peatland types. In this study, the number of methanogens was positively correlated only with pH in rewetted spruce swamps (Urbanová and Bárta 2020), and other parameters such as water level and vegetation only indirectly affected the recovery of methanogenic activity. Rather, the degree of peat decomposition and substrate availability were critical controls of methanogenesis after rewetting, whereby low substrate availability from decomposed peat limited methanogenic activity. Thus, SOC content is thought to be an important control of CH_4 related processes. In addition, the presence of iron oxides led to a rise in CH_4 production in incubations of eutrophic peats upon inundation, likely due to the increased relative abundances of methanogens (de Jong et al. 2020). In coastal rewetted fens, CH_4 production decreased after inflow of brackish water containing sulfate, which stimulates high abundance of sulfate reducers and suppressing methanogens abundance and activity (due to competition for the same substrates such as hydrogen and acetate) but not their abundance (Weil et al. 2020; Gutekunst et al. 2022). pH has also been identified as an important control of methanogenesis in peatlands, as it impacts the chemical status of substrates for methanogenesis (acetate vs. acetic acid).

CH_4 oxidation, acting as biological methane sink in peatlands, is carried out by aerobic methane oxidizing bacteria and anaerobic methanotrophic (ANME) archaea. Aerobic methane oxidation is catalyzed by particulate and soluble methane monooxygenases (pMMO and sMMO are encoded by *pmoA* and *mmoX* genes, respectively), which require molecular oxygen as terminal electron acceptor (Ross and Rosenzweig 2017). Active aerobic methanotrophs are therefore found along the oxic-anoxic boundary in the upper peat layers, at the vascular plant root/

peat interface and associated with *Sphagnum* mosses (Raghoebarsing et al. 2005). Anaerobic methanogens use the reverse reaction of the canonical final step in the methanogenesis pathway (Krüger et al. 2003; Hallam et al. 2004).

Kitson and Bell (2020) reviewed responses of methanotrophs to rewetting and similarly to methanogenic communities, findings varied across studies. Lower abundances of methanotrophs were observed in rewetted compared to pristine fens and bogs. In addition, the recovery of methanotrophic populations was slow compared to the one of methanogens (measured through particulate methane monooxygenase or *pmoA* gene abundance; Putkinen et al. 2018; Wen et al. 2018; Emsens et al. 2020). This could be explained by disturbances to the oxic–anoxic boundary zones following inundation and whether revegetation is part of the restoration efforts. In fact, the recovery of type II methanotrophs was shown to be strongly linked to *Sphagnum* abundance (Putkinen et al. 2018) and plant succession (Urbanová and Bárta 2020). Since the review by Kitson and Bell (2020), recent research reported that in rewetted coastal fens, methanotroph abundances reached pre-drought (i.e., near natural) levels after inflow of brackish water, while low but unaffected levels of methane oxidation were observed (Gutekunst et al. 2022). In addition, anaerobic methane oxidation (from ANME archaea) in NO_2^-/NO_3^- rich boundary layers was suggested to substantially lower methane release in wet peatlands (Zhu et al. 2012; Miller et al. 2019) and rewetted organic soils (Legierse et al. 2023).

While the focus on CH_4 is dominant and processes related to this GHG are well characterized on a genetic and molecular level compared to other processes, uncertainties remain on CH_4 emissions from rewetted peatlands. Findings altogether illustrate the variability in responses of CH_4-cycling communities and functions post-rewetting, which appear to be governed by a variety of factors and are peatland specific (Weil et al. 2023). Relationships between peat depth, decomposition state in relation to availability of substrates for CH_4 related microbial processes still need to be further investigated.

Nitrous oxide

The effects of restoring peatlands on N_2O emissions have yet to be fully understood (expected outcomes of rewetting are shown in Fig. 2). While the IPCC's default methodology assumes that restoring peatlands will reduce emissions to nearly zero (IPCC 2014), a recent meta-analysis of studies showed that soil N_2O emissions from rewetted European peatlands can range from −1.08 to 5.27 kg N_2O–N ha^{-1} yr^{-1} (Lin et al. 2022). In general, restoring peatlands can be considered an effective method for lowering N_2O emissions from drained nutrient-rich peatlands (Lin et al. 2022; Liu et al. 2020; Minkkinen et al. 2020). However, the effects of restoration may vary depending on the type and degradation state of the peatland, land-use history of drained peatland prior to rewetting, and the time since rewetting, as well as the restoration method used. In some cases, rewetted soil may still show high N_2O release rates, especially if fertilized (Kandel et al. 2019, Liu et al. 2019).

The nitrogen cycle is largely performed by microorganisms, and many microbial processes are known to be sources of N_2O (Fig. 2; Kuypers et al. 2018). In water saturated systems, including peatlands, denitrification usually represents the main source of N_2O, favored by O_2 depletion from high water table levels and/or high organic C content (Conrad 1996; Pihlatie et al. 2004; Palmer et al. 2010). Like in pristine peatlands, denitrification is also the main source for N_2O after rewetting as demonstrated in a short-term flooding experiment, due to restoration of anoxic conditions (Masta et al. 2022, 2023). However, depending on the peatland type a large share of N_2O may also have originated from nitrifier denitrification as demonstrated recently for a rewetted coastal peatland (Behrendt and Wrage-Mönnig 2023). The increase in N_2O emissions was correlated to higher gene copy numbers of denitrification genes (*nirK*, *nirS* and *nosZ*) with evidence of incomplete denitrification occurring. These results are in contrast with results of a drought/flooding experiment by Palmer et al. (2016). Authors showed that in situ N_2O emissions were low and fluxes stable during flooding due to higher rates of complete denitrification, which is consistent with other work in both rewetted bogs (Tauchnitz et al. 2015) and freshwater marsh (Yang et al. 2013). This divergence highlights the variability of N-cycling response to peatland rewetting, partially likely due to initial differences in peat nutrient conditions, including external inputs of N by deposition or with freshwater. It has been shown that N_2O production caused by denitrification is mainly controlled

by the availability of NO_3^- in relation to water table levels (Tauchnitz et al. 2015), and that NO_3^- loading causes increased N_2O emissions in rewetted peatlands (Russow et al. 2013), as well as pristine peatlands (Palmer and Horn 2015), and organic soils (Pärn et al. 2018).

To the best of our knowledge, the studies by Masta et al. (2022 and 2023) represent the only ones to date linking N_2O fluxes with ammonia oxidation (by using nitrification gene *amoA* abundances as indicator) in rewetted peatlands (Table S2). There, ammonia oxidation was estimated to be a source for N_2O, secondary to denitrification. Globally, the contribution of nitrifying microorganisms to N_2O fluxes in peatlands is thought to be substantial and underestimated (Siljanen et al. 2019; Bahram et al. 2022). Ammonia oxidizing microorganisms are believed to hold a pivotal role in N_2O emissions because producing both N_2O indirectly (from abiotic transformation of hydroxylamine) and NO_3^- (substrate for denitrification and subsequently more N_2O produced), however their contribution to N_2O fluxes in pristine as well as rewetted peatlands is still understudied. In addition, complete oxidation of ammonium to NO_3^- (comammox; Daims et al. 2015; van Kessel et al. 2015) still needs to be investigated to assess their contribution to N_2O fluxes and their role in many ecosystems (in 't Zandt et al. 2018), not to mention natural or peatlands under restoration. The importance of these processes as a source for N_2O is anticipated to diminish in the initial years. This decline is attributed to the fact that the ammonium pool, resulting from prior mineralization processes under drained conditions, does not undergo replenishment in rewetted conditions. Furthermore, nitrate is expected to disappear at an accelerated rate, typically within a few weeks to months, as outlined by Zak et al. (2010).

Higher N_2O emissions after rewetting were also correlated with increased DNRA (dissimilatory nitrate reduction to ammonium; Espenberg et al. 2018; Jahangir et al. 2020; Masta et al. 2022). *nrfA* (cytochrome c nitrite reductase) gene copies were positively correlated with N_2O emissions both in pristine and rewetted peatlands, further highlighting the contribution of this process to N_2O fluxes. While typically a process considered conserving N in the ecosystem, DNRA can also represent a source of N_2O as a byproduct of the reduction of NO_3^- to ammonia. This process is favored in competition with denitrifiers when NO_3^- concentrations are low, the latter being most likely related to the inherent peat chemical composition prior to rewetting, as also discussed above for denitrification.

Complete heterotrophic denitrification is the main biological process known as a sink for N_2O, through the last step of denitrification performed by the nitrous oxide reductase Nos (encoded by *nosZ* clade I and *nosZ* clade II genes; Hallin et al. 2018). While controls of this process are critical to consider for mitigating N_2O emissions from peatlands, the effect of rewetting on N_2O-related genes and enzymes remains poorly studied. The absolute requirement of Nos for copper for activity, as well as the absence of any parallel pathways that can reduce N_2O, account for the critical role of this element in the success of the final step of denitrification (Richardson et al. 2009). Additionally, pH has been shown to represent an important factor for *nosZ* enzyme maturation (Bakken et al. 2012), which is essential to consider as water pH can be impacted by rewetting (Lundin et al. 2017). In a study by Masta et al. (2022), flooding peat led to a concurrent increase in *nosZ* activity and N_2O emissions. In this study, the ratio of *nosZ* to *nirS* and *nirK* gene proportions indicated incomplete denitrification, possibly explaining high N_2O emissions. The in situ flooding-drying experiments in a drained peatland forest (Masta et al. 2023) revealed that denitrification dominated the small emission of N_2O under flooded conditions, possibly reduced by complete denitrification (increased *nosZ* genes abundance), whereas drained peat emitted significantly more N_2O. In the last case, ammonia oxidation was the main N_2O source which was indicated by elevated abundance of bacterial, archaeal and comammox *amoA* genes. This has also been suggested in restored agricultural wetlands (Kasak et al. 2021). While nosZI-type denitrifiers play an important role in controlling N_2O and N_2 gas fluxes in both natural and rewetted peatlands, it should be noted that slightly more than half of clade II nosZ organisms are apparently non-denitrifying N_2O reducers and therefore have the potential to be a sink without contributing to N_2O release (Hallin et al. 2018; Espenberg et al. 2018). Recently, the novel species *Flavobacterium azooxidireducens* sp. nov. was isolated from a *Phragmites* litter decomposition experiment which was able to consume significant amounts of N_2O under anaerobic conditions (Behrendt et al. 2022). The Nos enzyme is also

used in n-damo pathway (nitrite dependent anaerobic methane oxidation), where NO_2^- / NO_3^- is reduced to N_2 and CH_4 is anaerobically oxidized to CO_2 (Raghoebarsing et al. 2006). The effect of rewetting on microorganisms possessing n-damo capacities has not been investigated in rewetted peatlands to date.

The vast majority of studies investigating N_2O related processes in rewetted peatlands have used gene-targeted approaches, mainly qPCR, focusing on known key N-cycling functions. However, soil microbial communities are generally highly diverse and contain massive unknown taxonomic and functional diversity (see paragraph below). There are few studies about natural and restored peatlands synthesizing different methods like metagenomics, qPCR, N_2O and N_2 emissions (Espenberg et al. 2018; Bahram et al. 2022) or qPCR, isotopes and N_2O emissions (Masta et al. 2022, 2023). The use of untargeted molecular approaches such as metagenomics and -transcriptomics would help (i) explore the unknown microbial diversity, (ii) detangle the complex networks of processes behind GHG emissions in peatlands. Recent examples of new genes/processes discovered using these molecular techniques and involved in biogeochemical cycles include the identification of comammox (Daims et al. 2015) and *nosZ* clade II (Sanford et al. 2012). Moreover, in the study by Palmer et al. (2016), rewetting-driven fluctuations in water table/ oxygen content resulted in impacts on microbial activity (of denitrifiers) rather than community composition. The metabolic flexibility of most denitrifiers, together with high functional redundancy in soil microbial communities highlight the need to look at key gene expression rather than taxonomic biodiversity to better understand N_2O-related processes in rewetted peatlands (see section below on molecular methods).

While the rewetting of drained peatlands can stimulate microbial nitrogen cycling processes and lead to associated N2O production, as mentioned earlier, a swift decline is anticipated once ammonium and nitrate are depleted and not replenished by contaminated ground or surface water. This needs to be considered when developing viable management options to reduce N_2O emissions from drained peatlands. However, the magnitude of N-cycling and changes in the N_2O production to consumption balance (i.e., the N_2O product ratio of denitrification) is strongly affected by peat nutrient conditions, availability of

NO_3^- and other electron acceptors (e.g., Fe, SO_4^{2-}) and water table depth and its fluctuations (de Jong et al. 2020). Many environmental factors that govern N_2O fluxes at a large scale in pristine peatlands (e.g., soil C/N ratio, temperature, pH, peat type, climate zones, and vegetation cover; Martikainen et al. 1993; Repo et al. 2009; Shi et al. 2021; Yao et al. 2022) haven't been addressed in rewetted peatlands. Site dependences of microbial N cycling responses most likely explain why some studies observed a net N_2O uptake by rewetted peatlands (Berendt et al. 2023, Ye and Horwath 2016). Still, the underlying controls over N_2O consumption and the capacity of rewetted peat soils to act as potential sink for atmospheric N_2O are poorly understood.

Potentials of molecular biology tools in peatland research

Measuring GHG fluxes and their controls on the field and at large scale involves tedious and expensive work and GHG are mainly end- or by-products of biotic processes. Due to the complex network of GHG production and consumption processes, it is challenging to trace GHG emissions to discrete processes. Alternatives for assessing restoration success, or prior to restoration to predict its impact on microbial functions and GHG dynamics, could include a broader use of molecular methods and the development of a wider suite of microbial molecular biomarkers (Fig. 3). This is especially relevant because of the variation in peatland response to rewetting in terms of GHG emissions highlighted in this review.

Many plant biomarkers have been established based on detection of compounds via GC/MS (e.g., lignin, polysaccharides, N compounds, etc.) to assess peat chemical composition and plant composition in natural and drained peatlands (reviewed in bogs by Klein et al. 2022). Fewer biomarkers for microbial transformations exist. Studies focusing on drained or rewetted bogs and fens employed methods such as PLFA (phospholipid-derived fatty acids) analyses for estimation of total biomass and broad changes in community composition (Xu et al. 2021; Groß-Schmölders et al. 2021, 2022). However, this type of analysis only targets specific taxonomic groups of organisms, or broad groups (e.g., all bacteria). Additionally, enzyme activity assays have been used
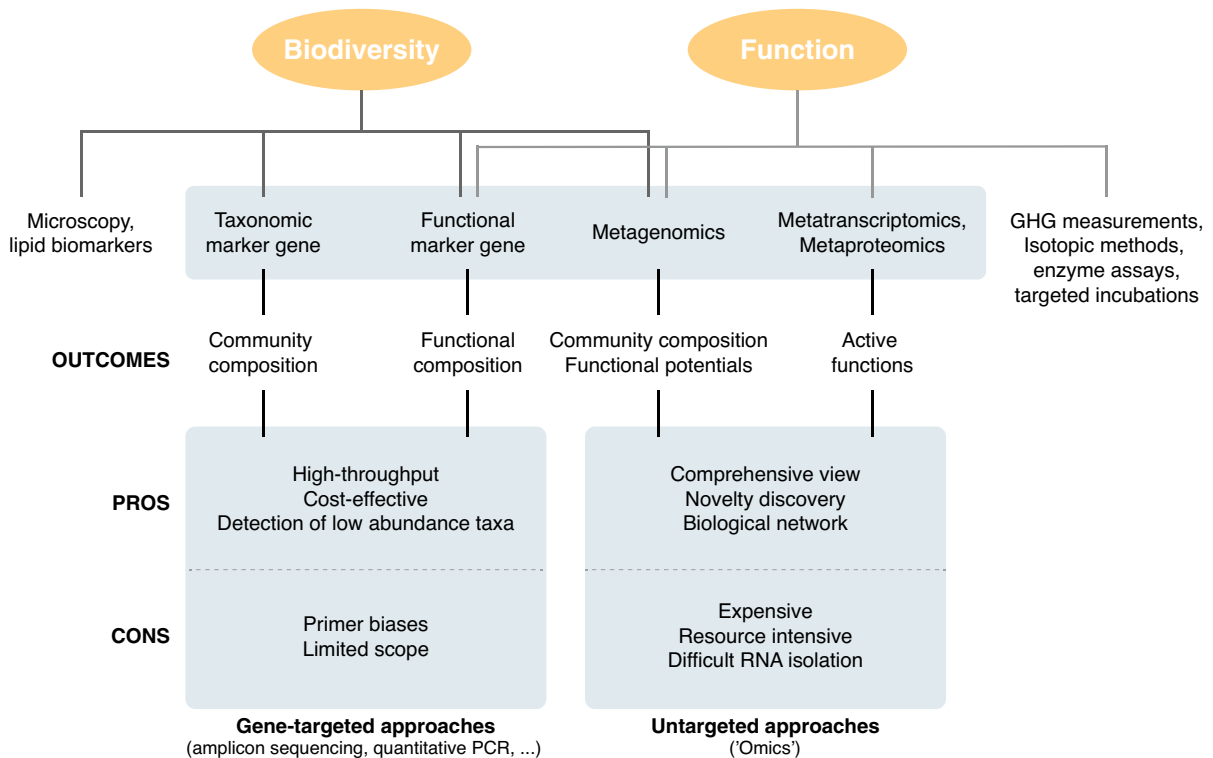
Fig. 3 Overview of molecular biology methods and their respective "Pros and Cons" in investigating microbial diversity and biogeochemical processes. Emphasis is placed on exploring microbial functions linked to greenhouse gas (GHG) emissions in rewetted peatlands. (*PCR* polymerase chain reation)

to assess microbial activity in peat, but they have to date mostly been applied to near-natural or drained peatlands (Xu et al. 2021; Xue et al. 2021). Molecular tools such as high-throughput sequencing can contribute greatly to understanding peat microbial taxonomic and functional diversity. Whether utilizing DNA- or RNA-based methods, whether employing targeted or untargeted approaches, integrating these tools into peatland research holds promise for evaluating restoration status and ecosystem functioning. These methodologies have demonstrated efficiency in investigating ecosystem functioning in diverse environments, underscoring their applicability and value in peatland studies.

Commonly used 16S rRNA gene surveys focus on characterizing taxonomy and community structure rather than function. However, gene abundance and activity better represent peatland processes than species presence/absence for several reasons: (i) DNA-based analyses are not representative of active microbial communities due to the presence of legacy DNA.

DNA from dormant or dead cells leads to biases as cell mortality/dormancy and subsequently legacy DNA might increase when physicochemical conditions change drastically and/or reoccurring environmental stresses (e.g., drought/rewetting cycles). (ii) High functional redundancy exists in soil microbial communities (Chen et al. 2022). Moreover, gene-targeted methods currently used in peatland research limit investigations exploring the so-called 'microbial dark matter' (i.e., the enormous diversity of yet-uncultivated microorganisms), that most likely play important roles in biogeochemical cycles. There is still a huge pool of unknown taxonomic diversity and microbial functions in soils, including peat, that remains to be assessed and that represents potential reservoirs of functions impacting nutrient recycling and GHG fluxes directly (catalytic enzymes) or indirectly (gene regulators).

While RNA-based analyses have become popular to generate information of active members and functions in microbial communities and thus

describe ecosystem processes, they are chiefly lacking in peatland research. This is mainly due to the difficulty of recovering RNA from peat, because of high humic acid content inhibiting traditional nucleic acid extraction protocols and downstream analyses such as PCR amplification. No metatranscriptomic studies assessing changes in active functions after rewetting and compare to pristine peatlands were found in our literature search, but some have been carried out in natural peatlands (Lin et al. 2014; Hausmann et al. 2019). Additionally, other metatranscriptomic analyses have focused so far on *Sphagnum* associated microbiomes (Ivanova et al. 2018; Stough et al. 2018; Dedysh and Ivanova 2019; Carrell et al. 2022a, b; Kolton et al. 2022), arctic peatlands (Tveit et al. 2013, 2014, 2015; Belova et al. 2018; Dedysh and Ivanova 2019; Ziegelhofer and Kujala 2021; Bender et al. 2021) and specific microbial groups such as protists (Geisen et al. 2015) and Planctomycetes (Ravin et al. 2018; Ivanova et al. 2018; Dedysh and Ivanova 2019). Additionally, in cases where peat chemical composition cannot be measured, metatranscriptomics could prove helpful as the function of active genes can provide information to some extent regarding what substrate is being used (with the caveat that information in databases used to functionally annotate genes are limited).

Conclusion and future directions

Up to now, precisely predicting trajectories of changes in the net C and GHG balance of rewetted peatlands remains difficult. That is largely due missing tools for rapid assessments of changes in microbial processes and communities in response to water status changes. The utilisation of rapidly advancing technologies, such as high-throughput sequencing, is poised to enhance our comprehension of soil microbial diversity, as well as the physiological abilities and roles of individual taxa in rewetted peatlands. Eventually, this information on microbial ecology can be used for narrowing down future outcomes of a particular rewetted peatland in terms of GHG dynamics.

To achieve this vision, further research is needed on:

a. Changes in microbial community structure and function across peat depth, vegetation types and degree of degradation, in combination with multi-dimensional (spatial and temporal) assessments of GHG production, consumption and emission dynamics; more emphasis should be placed on the indicator values of microbial and fungal communities to detect changes in environmental factors in peatlands.

b. Processes starting from substrate concentrations towards active microbial functions, up to GHG dynamics in rewetted peatlands using e.g., isotope tracing approaches.

c. Combining different methods like qPCR, metagenomics, metatranscriptomics, isotopes and GHG analyses to study and validate microbial functions and community.

A more harmonized approach, linking similar molecular biology methods with biogeochemistry on rewetted peatlands with variable characteristics, including time before/after restoration and measurement methods and intensity, would facilitate calibrating this rapidly evolving research tool as a bioindicator for restoration outcomes.

Data availability There are no original data associated with this paper.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

References

Abdalla M, Hastings A, Truu J et al (2016) Emissions of methane from northern peatlands: a review of management impacts and implications for future management options. Ecol Evol 6:7080–7102. https://doi.org/10.1002/ece3.2469

Albert T, Karp K, Starast M, Moor U, Paal T (2011) Effect of fertilization on the lowbush blueberry productivity and fruit composition in peat soil. J Plant Nutr 34:1489–1496. https://doi.org/10.1080/01904167.2011.585205

Andersen R, Chapman SJ, Artz RRE (2013) Microbial communities in natural and disturbed peatlands: a review. Soil Biol Biochem 57:979–994. https://doi.org/10.1016/j.soilbio.2012.10.003

Antonijević D, Hoffmann M, Prochnow A et al (2023) The unexpected long period of elevated CH_4 emissions from an inundated Fen meadow ended only with the occurrence of cattail (*Typha latifolia*). Glob Chang Biol 00:1–14. https://doi.org/10.1111/gcb.16713

Asemaninejad A, Thorn RG, Lindo Z (2017) Vertical distribution of fungi in hollows and hummocks of boreal peatlands. Fungal Ecol 27:59–68. https://doi.org/10.1016/j.funeco.2017.02.002

Bahram M, Espenberg M, Pärn J et al (2022) Structure and function of the soil microbiome underlying N_2O emissions from global wetlands. Nat Commun 13:1430. https://doi.org/10.1038/s41467-022-29161-3

Bakken LR, Bergaust L, Liu B, Frostegård Å (2012) Regulation of denitrification at the cellular level: a clue to the understanding of N_2O emissions from soils. Philos Trans R Soc B Biol Sci 367:1226–1234. https://doi.org/10.1098/rstb.2011.0321

Barnard RL, Osborne CA, Firestone MK (2013) Responses of soil bacterial and fungal communities to extreme desiccation and rewetting. ISME J 7:2229–2241. https://doi.org/10.1038/ismej.2013.104

Bay SK, Waite DW, Dong X et al (2021) Chemosynthetic and photosynthetic bacteria contribute differentially to primary production across a steep desert aridity gradient. ISME J 15:3339–3356. https://doi.org/10.1038/s41396-021-01001-0

Behrendt U, Spanner T, Augustin J et al (2022) Consumption of N_2O by *Flavobacterium azooxidireducens* sp. nov. isolated from decomposing leaf litter of *Phragmites australis* (Cav). Microorganisms 10:2304. https://doi.org/10.3390/microorganisms10112304

Belova SE, Ravin NV, Pankratov TA et al (2018) Hydrolytic capabilities as a key to environmental success: chitinolytic and cellulolytic *Acidobacteria* from acidic sub-arctic soils and boreal peatlands. Front Microbiol. https://doi.org/10.3389/fmicb.2018.02775

Bender KM, Svenning MM, Hu Y et al (2021) Microbial responses to herbivory-induced vegetation changes in a high-arctic peatland. Polar Biol 44:899–911. https://doi.org/10.1007/s00300-021-02846-z

Berendt J, Wrage-Mönnig N (2023) Denitrification is not necessarily the main source of N_2O from rewetted fens. J Soil Sci Plant Nutr. https://doi.org/10.1007/s42729-023-01291-7

Berendt J, Jurasinski G, Wrage-Mönnig N (2023) Influence of rewetting on N_2O emissions in three different Fen types. Nutr Cycl Agroecosyst 125:277–293. https://doi.org/10.1007/s10705-022-10244-y

Birnbaum C, Wood J, Lilleskov E et al (2023) Degradation reduces microbial richness and alters microbial functions in an Australian peatland. Microb Ecol 85:875–891. https://doi.org/10.1007/s00248-022-02071-z

Blodau C, Mayer B, Peiffer S, Moore TR (2007) Support for an anaerobic sulfur cycle in two Canadian peatland soils. J Geophys Res 112:G02004. https://doi.org/10.1029/2006JG000364

Boonman CC, Heuts TS, Vroom RJ, Geurts JJ, Fritz C (2023) Wetland plant development overrides nitrogen effects on initial methane emissions after peat rewetting. Aquat Bot 184:103598. https://doi.org/10.1016/j.aquabot.2022.103598

Cabezas A, Gelbrecht J, Zak D (2013) The effect of rewetting drained fens with nitrate-polluted water on dissolved organic carbon and phosphorus release. Ecol Eng 53:79–88. https://doi.org/https://doi.org/10.1016/j.ecoleng.2012.12.016

Cabezas A, Pallasch M, Schoenfelder I, Gelbrecht J, Zak D (2014) Carbon, nitrogen, and phosphorus accumulation in novel ecosystems: shallow lakes in degraded Fen areas. Ecol Eng 66:63–71. https://doi.org/10.1016/j.ecoleng.2013.10.037

Caisse G, Boudreau S, Munson AD, Rochefort L (2008) Fertiliser addition is important for tree growth on cutover peatlands in eastern Canada. Mires Peat 3:1–15

Cano-Díaz C, Maestre FT, Eldridge DJ et al (2020) Contrasting environmental preferences of photosynthetic and non-photosynthetic soil cyanobacteria across the globe. Glob Ecol Biogeogr 29:2025–2038. https://doi.org/10.1111/geb.13173

Carrell AA, Lawrence TJ, Cabugao KGM et al (2022a) Habitat-adapted microbial communities mediate *Sphagnum* Peatmoss resilience to warming. New Phytol 234:2111–2125. https://doi.org/10.1111/nph.18072

Carrell AA, Velickovic D, Lawrence TJ et al (2022b) Novel metabolic interactions and environmental conditions mediate the boreal peatmoss-cyanobacteria mutualism. ISME J 16:1074–1085. https://doi.org/10.1038/s41396-021-01136-0

Carter MS, Larsen KS, Emmett B et al (2012) Synthesizing greenhouse gas fluxes across nine European peatlands and shrublands—responses to climatic and environmental changes. Biogeosciences 9:3739–3755. https://doi.org/10.5194/bg-9-3739-2012

Chen H, Ma K, Lu C et al (2022) Functional redundancy in soil microbial community based on metagenomics across the globe. Front Microbiol. https://doi.org/10.3389/fmicb.2022.878978

Conrad R (1996) Soil microorganisms as controllers of atmospheric trace gases (H_2, CO, CH_4, OCS, N_2O, and NO). Microbiol Rev 60:609–640. https://doi.org/10.1128/mr.60.4.609-640.1996

Conrad R (2020) Importance of hydrogenotrophic, aceticlastic and methylotrophic methanogenesis for methane production in terrestrial, aquatic and other anoxic environments: a mini review. Pedosphere 30:25–39. https://doi.org/10.1016/S1002-0160(18)60052-9

Daims H, Lebedeva EV, Pjevac P et al (2015) Complete nitrification by Nitrospira bacteria. Nature 528:504–509. https://doi.org/10.1038/nature16461

Darusman T, Murdiyarso D, Anas I (2023) Effect of rewetting degraded peatlands on carbon fluxes: a meta-analysis. Mitigation Adapt Strategies Global Change 28:10. https://doi.org/10.1007/s11027-023-10046-9

Dastogeer KMG, Zahan MI, Tahjib-Ul-Arif M, Akter MA, Okazaki S (2020) Plant salinity tolerance conferred by arbuscular mycorrhizal fungi and associated mechanisms: a meta-analysis. Front Plant Sci 11:588550. https://doi.org/10.3389/fpls.2020.588550

Davies LO, Schäfer H, Marshall S et al (2013) Light structures phototroph, bacterial and fungal communities at the soil surface. PLoS ONE 8:e69048. https://doi.org/10.1371/journal.pone.0069048

de Jong AEE, Guererro-Cruz S, van Diggelen JMH et al (2020) Changes in microbial community composition, activity, and greenhouse gas production upon inundation of drained iron-rich peat soils. Soil Biol Biochem 149:107862. https://doi.org/10.1016/J.SOILBIO.2020.107862

de Vos JA, van Bakel PJT, Hoving IE, Smidt RA (2010) Raising surface water levels in peat areas with dairy farming: upscaling hydrological, agronomical and economic effects from farm-scale to local scale. Agric Water Manage 97:1887–1897. https://doi.org/10.1016/j.agwat.2010.06.017

de Vries FT, Bloem J, Quirk H et al (2012) Extensive management promotes plant and microbial nitrogen retention in temperate grassland. PLoS ONE 7:e51201. https://doi.org/10.1371/journal.pone.0051201

Dean JF, Middelburg JJ, Rockmann T et al (2018) Methane feedbacks to the global climate system in a warmer world. Rev Geophys 56:207–250. https://doi.org/10.1002/2017RG000559

Dedysh SN, Ivanova AA (2019) Planctomycetes in boreal and subarctic wetlands: diversity patterns and potential ecological functions. FEMS Microbiol Ecol 95:fiy227. https://doi.org/10.1093/femsec/fiy227

Dedysh SN, Pankratov TA, Belova SE et al (2006) Phylogenetic analysis and in situ identification of *Bacteria* community composition in an acidic *Sphagnum* peat bog. Appl Environ Microbiol 72:2110–2117. https://doi.org/10.1128/AEM.72.3.2110-2117.2006

Defrenne CE, Moore JAM, Tucker CL et al (2023) Peat loss collocates with a threshold in plant–mycorrhizal associations in drained peatlands encroached by trees. New Phytol 240:412–425. https://doi.org/10.1111/nph.18954

Deppe M, McKnight DM, Blodau C (2010) Effects of short-term drying and irrigation on electron flow in mesocosms of a northern bog and an alpine Fen. Environ Sci Technol 44:80–86. https://doi.org/10.1021/es901669z

Dollhopf SL, Hyun JH, Smith AC, Adams HJ, O'Brien S, Kostka JE (2005) Quantification of ammonia-oxidizing bacteria and factors controlling nitrification in salt marsh sediments. Appl Environ Microbiol 71:240–246. https://doi.org/10.1128/AEM.71.1.240-246.2005

Emsens WJ, van Diggelen R, Aggenbach CJS et al (2020) Recovery of Fen peatland microbiomes and predicted functional profiles after rewetting. ISME J 14:1701–1712. https://doi.org/10.1038/s41396-020-0639-x

Emsens W, Verbruggen E, Shenk P et al (2021) Degradation legacy and current water levels as predictors of carbon emissions from two Fen sites. Mires and Peat 27:1–15

Erdtman HGH (1933) Studies on the formation of complex oxidation and condensation products of phenols. A contribution to the investigation of the origin and nature of humic acid. Part I. studies of the reactivity of simple monocyclic quinones. Proc Roy Soc A 143:177–191. https://doi.org/10.1098/rspa.1933.0212

Espenberg M, Truu M, Truu J et al (2016) Impact of reed canary grass cultivation and mineral fertilisation on the microbial abundance and genetic potential for methane production in residual peat of an abandoned peat extraction area. PLoS ONE 11:e0163864. https://doi.org/10.1371/journal.pone.0163864

Espenberg M, Truu M, Mander Ü et al (2018) Differences in microbial community structure and nitrogen cycling in natural and drained tropical peatland soils. Sci Rep 8:4742. https://doi.org/10.1038/s41598-018-23032-y

Evans CD, Peacock M, Baird AJ et al (2021) Overriding water table control on managed peatland greenhouse gas emissions. Nature 593:548–552. https://doi.org/10.1038/s41586-021-03523-1

Freeman C, Ostle N, Kang H (2001) An enzymic 'latch' on a global carbon store-shortage of oxygen locks up

carbon in peatlands by restraining a single enzyme. Nature 409:149. https://doi.org/10.1038/35051650

Geisen S, Tveit AT, Clark IM et al (2015) Metatranscriptomic census of active protists in soils. ISME J 9:2178–2190. https://doi.org/10.1038/ismej.2015.30

Gilbert D, Mitchell EAD (2006) Microbial diversity in *Sphagnum* peatlands. In: Martini IP, Matinez Cortizas A, Chesworth W (eds) Peatlands: basin evolution and depository of records on global environmental and climatic changes. Elsevier, Amsterdam, pp 287–318

Gilbert D, Amblard C, Bourdier G, Francez AJ (1998) The microbial loop at the surface of a peatland: structure, function, and impact of nutrient input. Microb Ecol 35:83–93. https://doi.org/10.1007/s002489900062

Groß-Schmölders M, Klein K, Birkholz A et al (2021) Rewetting and drainage of nutrient-poor peatlands indicated by specific bacterial membrane fatty acids and a repeated sampling of stable isotopes (δ15N, δ13C). Front Environ Sci 9:378. https://doi.org/10.3389/fenvs.2021.730106

Groß-Schmölders M, Klein K, Emsens WJ et al (2022) Stable isotopes (δ13C, δ15N) and biomarkers as indicators of the hydrological regime of fens in a European east–west transect. Sci Total Environ 838:156603. https://doi.org/10.1016/j.scitotenv.2022.156603

Gutekunst CN, Liebner S, Jenner AK et al (2022) Effects of brackish water inflow on methane-cycling microbial communities in a freshwater rewetted coastal Fen. Biogeosciences 19:3625–3648. https://doi.org/10.5194/BG-19-3625-2022

Hahn J, Köhler S, Glatzel S, Jurasinski G (2015) Methane exchange in a coastal Fen in the first year after flooding-a systems shift. PLoS ONE 10:e0140657. https://doi.org/10.1371/journal.pone.0140657

Hahn-Schöfl M, Zak D, Minke M et al (2011) Organic sediment formed during inundation of a degraded Fen grassland emits large fluxes of CH_4 and CO_2. Biogeosciences 8:1539–1550. https://doi.org/10.5194/bg-8-1539-2011

Hallam SJ, Putnam N, Preston CM et al (2004) Reverse methanogenesis: testing the hypothesis with environmental genomics. Science 305:1457–1462. https://doi.org/10.1126/science.1100025

Hallin S, Philippot L, Löffler FE, Sanford RA, Jones CM (2018) Genomics and ecology of novel N_2O-reducing microorganisms. Trends Microbiol 26:43–55. https://doi.org/10.1016/j.tim.2017.07.003

Halliwell B, Gutteridge JMC (2007) Free radicals in biology and medicine, 4th edn. Oxford University Press, New York

Hamard S, Küttim M, Céréghino R, Jassey VEJ (2021) Peatland microhabitat heterogeneity drives phototrophic microbe distribution and photosynthetic activity. Environ Microbiol 23:6811–6827. https://doi.org/10.1111/1462-2920.15779

Hausmann B, Pelikan C, Rattei T et al (2019) Long-term transcriptional activity at zero growth of a cosmopolitan rare biosphere member. mBio 10:e02189. https://doi.org/10.1128/mBio.02189-18

He S, Malfatti SA, McFarland JW et al (2015) Patterns in wetland microbial community composition and functional gene repertoire associated with methane emissions. mBio 6:e00066–e00015. https://doi.org/10.1128/mBio.00066-15

Hemes KS, Chamberlain SD, Eichelmann E et al (2019) Assessing the carbon and climate benefit of restoring degraded agricultural peat soils to managed wetlands. Agric for Meteorol 268:202–214. https://doi.org/10.1016/j.agrformet.2019.01.017

Holden J, Chapman PJ, Labadz JC (2004) Artificial drainage of peatlands: hydrological and hydrochemical process and wetland restoration. Prog Phys Geogr 28:95–123. https://doi.org/10.1191/0309133304pp403ra

Huth V, Günther A, Bartel A et al (2020) Topsoil removal reduced in-situ methane emissions in a temperate rewetted bog grassland by a hundredfold. Sci Total Environ 721:137763. https://doi.org/10.1016/j.scitotenv.2020.137763

Huth V, Günther A, Bartel A et al (2022) The climate benefits of topsoil removal and *Sphagnum* introduction in raised bog restoration. Restor Ecol 30:e13490. https://doi.org/10.1111/REC.13490

Hyvönen NP, Huttunen JT, Shurpali NJ et al (2009) Fluxes of nitrous oxide and methane on an abandoned peat extraction site: effect of reed canary grass cultivation. Bioresour Technol 100:4723–4730. https://doi.org/10.1016/j.biortech.2009.04.043

in' t Zandt MH, de Jong AE, Slomp CP, Jetten MS (2018) The hunt for the most-wanted chemolithoautotrophic spook-microbes. FEMS Microbiol Ecol 94:fiy064. https://doi.org/10.1093/femsec/fiy064

IPCC, Pachauri RK, Meyer LA (2014) Climate change 2014: synthesis report. Contribution of Working groups I, II and III to the fifth assessment report of the intergovernmental panel on climate change. Core writing team. IPCC, Geneva

Ivanova AA, Wegner C-E, Kim Y et al (2018) Metatranscriptomics reveals the hydrolytic potential of peat-inhabiting *Planctomycetes*. Antonie Van Leeuwenhoek 111:801–809. https://doi.org/10.1007/s10482-017-0973-9

Jahangir MMR, Fenton O, Carolan R et al (2020) Application of ^{15}N tracing for estimating nitrogen cycle processes in soils of a constructed wetland. Water Res 183:116062. https://doi.org/10.1016/j.watres.2020.116062

Jassey VEJ, Chiapusio G, Binet P et al (2013) Above- and belowground linkages in *Sphagnum* peatland: climate warming affects plant-microbial interactions. Glob Chang Biol 19:811–823. https://doi.org/10.1111/gcb.12075

Jassey VEJ, Reczuga MK, Zielińska M et al (2018) Tipping point in plant–fungal interactions under severe drought causes abrupt rise in peatland ecosystem respiration. Glob Chang Biol 24:972–986. https://doi.org/10.1111/gcb.13928

Jenkins V, Walker J (2022) Maintaining, enhancing and restoring the peatlands of Wales: unearthing the challenges of law and sustainable land management. J Envir Law 34:163–193. https://doi.org/10.1093/jel/eqab042

Jurasinski G, Ahmad S, Anadon-Rosell A et al (2020) From understanding to sustainable use of peatlands: the WETSCAPES approach. Soil Syst 4:14. https://doi.org/10.3390/soilsystems4010014

Kandel TP, Karki S, Elsgaard L et al (2019) Fertilizer-induced fluxes dominate annual N_2O emissions from a nitrogen-rich temperate Fen rewetted for paludiculture. Nutr Cycl Agroecosyst 115:57–67. https://doi.org/10.1007/s10705-019-10012-5

Kasak K, Espenberg M, Anthony TL et al (2021) Restoring wetlands on intensive agricultural lands modifies nitrogen cycling microbial communities and reduces N_2O production potential. J Environ Manage 299:113562. https://doi.org/10.1016/j.jenvman.2021.113562

Keiluweit M, Nico PS, Harmon ME et al (2015) Long-term litter decomposition controlled by manganese redox cycling. Proc Natl Acad Sci 112:E5253–E5260. https://doi.org/10.1073/pnas.1508945112

Kitson E, Bell NGA (2020) The response of microbial communities to peatland drainage and rewetting. Rev Front Microbiol 11:2647. https://doi.org/10.3389/fmicb.2020.582812

Klein K, Schellekens J, Groβ-Schmölders M et al (2022) Characterizing ecosystem-driven chemical composition differences in natural and drained finnish bogs using pyrolysis-GC/MS. Org Geochem 165:104351. https://doi.org/10.1016/j.orggeochem.2021.104351

Knorr K-H, Glaser B, Blodau C (2008) Fluxes and ^{13}C isotopic composition of dissolved carbon and pathways of methanogenesis in a Fen soil exposed to experimental drought. Biogeosciences 5:1457–1473. https://doi.org/10.5194/bg-5-1457-2008

Kolton M, Weston DJ, Mayali X et al (2022) Defining the *Sphagnum* core microbiome across the north American continent reveals a central role for diazotrophic methanotrophs in the nitrogen and carbon cycles of boreal peatland ecosystems. mBio 13:e03714–e03721. https://doi.org/10.1128/mbio.03714-21

Koschorreck M (2008) Microbial sulphate reduction at a low pH. FEMS Microbiol Ecol 64:329–342. https://doi.org/10.1111/j.1574-6941.2008.00482.x

Kreyling J, Tanneberger F, Jansen F et al (2021) Rewetting does not return drained Fen peatlands to their old selves. Nat Commun 12:5693. https://doi.org/10.1038/s41467-021-25619-y

Krüger M, Meyerdierks A, Glöckner FO et al (2003) A conspicuous nickel protein in microbial mats that oxidize methane anaerobically. Nature 426:878–881. https://doi.org/10.1038/nature02207

Kuypers MMM, Marchant HK, Kartal B (2018) The microbial nitrogen-cycling network. Nat Rev Microbiol 16:263–276. https://doi.org/10.1038/nrmicro.2018.9

Kuzyakov Y, Larionova AA (2005) Root and rhizomicrobial respiration: a review of approaches to estimate respiration by autotrophic and heterotrophic organisms in soil. J Soil Sci Plant Nutr 168:503–520. https://doi.org/10.1002/jpln.200421703

Laiho R (2006) Decomposition in peatlands: reconciling seemingly contrasting results on the impacts of lowered water levels. Soil Biol Biochem 38:2011–2024. https://doi.org/10.1016/j.soilbio.2006.02.017

Legierse A, Struik Q, Smith G et al (2023) Nitrate-dependent anaerobic methane oxidation (N-DAMO) as a bioremediation strategy for waters affected by agricultural runoff.

FEMS Microbiol Lett 370:fnad041. https://doi.org/10.1093/femsle/fnad041

Leifeld J, Menichetti L (2018) The underappreciated potential of peatlands in global climate change mitigation strategies. Nat Commun 9:1071. https://doi.org/10.1038/s41467-018-03406-6

Le Mer J, Roger P (2001) Production, oxidation, emission and consumption of methane by soils: a review. Eur J Soil Biol 37:25–50. https://doi.org/10.1016/S1164-5563(01)01067-6

Lin X, Tfaily MM, Steinweg JM et al (2014) Microbial community stratification linked to utilization of carbohydrates and phosphorus limitation in a Boreal Peatland at Marcell experimental Forest, Minnesota, USA. Appl Environ Microbiol 80:3518–3530. https://doi.org/10.1128/AEM.00205-214

Lin F, Zuo H, Ma X, Ma L (2022) Comprehensive assessment of nitrous oxide emissions and mitigation potentials across European peatlands. Environ Poll 301:119041. https://doi.org/10.1016/j.envpol.2022.119041

Lipson DA, Haggerty JM, Srinivas A et al (2013) Metagenomic insights into anaerobic metabolism along an Arctic peat soil profile. PLoS ONE 8:e64659. https://doi.org/10.1371/JOURNAL.PONE.0064659

Liu H, Zak D, Rezanezhad F, Lennartz B (2019) Soil degradation determines release of nitrous oxide and dissolved organic carbon from peatlands. Environ Res Lett 14:094009. https://doi.org/10.1088/1748-9326/ab3947

Liu H, Wrage-Mönnig N, Lennartz B (2020) Rewetting strategies to reduce nitrous oxide emissions from European peatlands. Commun Earth Environ 1:17. https://doi.org/10.1038/s43247-020-00017-2

Loisel J, Gallego-Sala AV, Amesbury MJ et al (2021) Expert assessment of future vulnerability of the global peatland carbon sink. Nat Clim Chang 11:70–77. https://doi.org/10.1038/s41558-020-00944-0

Lundin L, Nilsson T, Jordan S et al (2017) Impacts of rewetting on peat, hydrology and water chemical composition over 15 years in two finished peat extraction areas in Sweden. Wetl Ecol Manag 25:405–419. https://doi.org/10.1007/s11273-016-9524-9

Malik AA, Chowdhury S, Schlager V et al (2016) Soil fungal:bacterial ratios are linked to altered carbon cycling. Front Microbiol 7:1247. https://doi.org/10.3389/fmicb.2016.01247

Mander Ü, Järveoja J, Maddison M et al (2012) Reed canary grass cultivation mitigates greenhouse gas emissions from abandoned peat extraction areas. Glob Change Biol Bioenergy 4:462–474. https://doi.org/10.1111/j.1757-1707.2011.01138.x

Martikainen PJ, Nykänen H, Crill P, Silvola J (1993) Effect of a lowered water table on nitrous oxide fluxes from northern peatlands. Nature 366:51–53. https://doi.org/10.1038/366051a0

Masta M, Espenberg M, Gadegaonkar SS et al (2022) Integrated isotope and microbiome analysis indicates dominance of denitrification in N_2O production after rewetting of drained Fen peat. Biogeochemistry 161:119–136. https://doi.org/10.1007/s10533-022-00971-3

Masta M, Espenberg M, Kuusemets L et al (2023) ^{15}N tracers and microbial analyses reveal in situ N_2O sources

in contrasting water regimes on drained peatland forest. Pedosphere. In press. https://doi.org/10.1016/j.pedsph.2023.06.006

Miller KE, Lai CT, Dahlgren RA, Lipson DA (2019) Anaerobic methane oxidation in high-arctic alaskan peatlands as a significant control on net CH_4 fluxes. Soil sys 3:7. https://doi.org/10.3390/soilsystems3010007

Minkkinen K, Ojanen P, Koskinen M, Penttilä T (2020) Nitrous oxide emissions of undrained, forestry-drained, and rewetted boreal peatlands. For Ecol Manage 478:118494. https://doi.org/10.1016/j.foreco.2020.118494

Neculita C, Zagury G, Bussière B (2007) Passive treatment of acid mine drainage in bioreactors using sulfate-reducing bacteria. J Environ Qual 36:1–16. https://doi.org/10.2134/jeq2006.0066

Nowak ME, Beulig F, von Fischer J et al (2015) Autotrophic fixation of geogenic $CO2$ by microorganisms contributes to soil organic matter formation and alters isotope signatures in a wetland mofette. Biogeosciences 12:7169–7183. https://doi.org/10.5194/bg-12-7169-2015

Nugent KA, Strachan IB, Strack M, Roulet NT, Rochefort L (2018) Multi-year net ecosystem carbon balance of a restored peatland reveals a return to carbon sink. Glob Change Biol 24:5751–5768. https://doi.org/10.1111/gcb.14449

Oliverio AM, Geisen S, Delgado-Baquerizo M et al (2020) The global-scale distributions of soil protists and their contributions to belowground systems. Sci Adv 6:eaax8787. https://doi.org/10.1126/sciadv.aax8787

Palmer K, Horn MA (2015) Denitrification activity of a remarkably diverse Fen denitrifier community in finnish lapland is N-oxide limited. PLoS ONE 10:e0123123. https://doi.org/10.1371/journal.pone.0123123

Palmer K, Drake HL, Horn MA (2010) Association of novel and highly diverse acid-tolerant denitrifiers with N_2O fluxes of an acidic Fen. Appl Environ Microbiol 76:1125–1134. https://doi.org/10.1128/AEM.02256-09

Palmer K, Köpp J, Gebauer G, Horn MA (2016) Drying-rewetting and flooding impact denitrifier activity rather than community structure in a moderately acidic Fen. Front Microbiol 7:727. https://doi.org/10.3389/fmicb.2016.00727

Pärn J, Verhoeven JTA, Butterbach-Bahl K et al (2018) Nitrogen-rich organic soils under warm well-drained conditions are global nitrous oxide emission hotspots. Nat Commun 9:1135. https://doi.org/10.1038/s41467-018-03540-1

Peltoniemi K, Fritze H, Laiho R (2009) Response of fungal and actinobacterial communities to water-level drawdown in boreal peatland sites. Soil Biol Biochem 41:1902–1914. https://doi.org/10.1016/j.soilbio.2009.06.018

Peltoniemi K, Straková P, Fritze H et al (2012) How water-level drawdown modifies litter-decomposing fungal and actinobacterial communities in boreal peatlands. Soil Biol Biochem 51:20–34. https://doi.org/10.1016/j.soilbio.2012.04.013

Pester M, Bittner N, Deevong P et al (2010) A 'rare biosphere' microorganism contributes to sulfate reduction in a peatland. ISME J 2010 4:1591–1602. https://doi.org/10.1038/ismej.2010.75

Pihlatie M, Syväsalo E, Simojoki A et al (2004) Contribution of nitrification and denitrification to N_2O production in peat, clay and loamy sand soils under different soil moisture conditions. Nutr Cycl Agroecosyst 70:135–141. https://doi.org/10.1023/B:FRES.0000048475.81211.3c

Putkinen A, Tuittila ES, Siljanen HMP et al (2018) Recovery of methane turnover and the associated microbial communities in restored cutover peatlands is strongly linked with increasing *Sphagnum* abundance. Soil Biol Biochem 116:110–119. https://doi.org/10.1016/J.SOILBIO.2017.10.005

Quadra GR, Boonman CCF, Vroom RJ et al (2023) Removing 10 cm of degraded peat mitigates unwanted effects of peatland rewetting: a mesocosm study. Biogeochemistry 136:65–84. https://doi.org/10.1007/s10533-022-01007-6

Raghoebarsing AA, Alfons JP, Smolders AJP et al (2005) Methanotrophic symbionts provide carbon for photosynthesis in peat bogs. Nature 436:1153–1156. https://doi.org/10.1038/nature03802

Raghoebarsing AA, Pol A, Van De Pas-Schoonen KT et al (2006) A microbial consortium couples anaerobic methane oxidation to denitrification. Nature 440:918–921. https://doi.org/10.1038/nature04617

Ravin NV, Rakitin AL, Ivanova AA et al (2018) Genome analysis of *Fimbriiglobus ruber* SP5^T, a planctomycete with confirmed chitinolytic capability. Appl Environ Microbiol 84:1–12. https://doi.org/10.1128/AEM.02645-17

Repo ME, Susiluoto S, Lind SE et al (2009) Large N_2O emissions from cryoturbated peat soil in tundra. Nat Geosci 2:189–192. https://doi.org/10.1038/ngeo434

Richardson D, Felgate H, Watmough N et al (2009) Mitigating release of the potent greenhouse gas N_2O from the nitrogen cycle—could enzymic regulation hold the key? Trends Biotechnol 27:388–397. https://doi.org/10.1016/j.tibtech.2009.03.009

Rigney C, Wilson D, Renou-Wilson F et al (2018) Greenhouse gas emissions from two rewetted peatlands previously managed for forestry. Mires Peat 21:1–23

Ross MO, Rosenzweig AC (2017) A tale of two methane monooxygenases. J Biol Inorg Chem 22:307–319. https://doi.org/10.1007/S00775-016-1419-Y

Russow R, Tauchnitz N, Spott O et al (2013) Nitrate turnover in a peat soil under drained and rewetted conditions: results from a [^{15}N]nitrate–bromide double-tracer study. Isot Environ Health Stud 49:438–453. https://doi.org/10.1080/10256016.2013.831089

Sanford RA, Wagner DD, Wu Q et al (2012) Unexpected nondenitrifier nitrous oxide reductase gene diversity and abundance in soils. Proc Natl Acad Sci 109:19709–19714. https://doi.org/10.1073/pnas.1211238109

Shi Y, Wang J, Ao Y et al (2021) Responses of soil N_2O emissions and their abiotic and biotic drivers to altered rainfall regimes and co-occurring wet N deposition in a semi-arid grassland. Glob Chang Biol 27:4894–4908. https://doi.org/10.1111/GCB.15792

Siljanen HMP, Alves RJE, Ronkainen JG et al (2019) Archaeal nitrification is a key driver of high nitrous oxide emissions from Arctic peatlands. Soil Biol Biochem 137:107539. https://doi.org/10.1016/j.soilbio.2019.107539

Sjögren P (2006) The development of pasture woodland in the southwest Swiss Jura Mountains over 2000 years, based on three adjacent peat profiles. Holocene 16:210–223. https://doi.org/10.1191/0959683606hl921rp

Stough JMA, Kolton M, Kostka JE et al (2018) Diversity of active viral infections within the *Sphagnum* microbiome. Appl Environ Microbiol 84:e01124–e01118. https://doi.org/10.1128/AEM.01124-18

Strakova P, Niemi RM, Freeman C et al (2011) Litter type affects the activity of aerobic decomposers in a boreal peatland more than site nutrient and water table regimes. Biogeosciences 8:2741–2755. https://doi.org/10.5194/BG-8-2741-2011

Sutton-Grier AE, Keller JK, Koch R, Gilmour C, Megonigal JP (2011) Electron donors and acceptors influence anaerobic soil organic matter mineralization in tidal marshes. Soil Biol Biochem 43:1576–1583. https://doi.org/10.1016/j.soilbio.2011.04.008

Tauchnitz N, Spott O, Russow R et al (2015) Release of nitrous oxide and dinitrogen from a transition bog under drained and rewetted conditions due to denitrification: results from a [^{15}N]nitrate–bromide double-tracer study. Isot Environ Health Stud 51:300–321. https://doi.org/10.1080/10256016.2015.1011634

Thormann MN (2006) Diversity and function of fungi in peatlands: a carbon cycling perspective. Can J Soil Sci 86:281–293. https://doi.org/10.4141/S05-082

Tian W, Xiang X, Ma L et al (2020) Rare species shift the structure of bacterial communities across *Sphagnum* compartments in a subalpine peatland. Front Microbiol 10:3138. https://doi.org/10.3389/fmicb.2019.03138

Tiemeyer B, Freibauer A, Borraz EA et al (2020) A new methodology for organic soils in national greenhouse gas inventories: data synthesis, derivation and application. Ecol Indic 109:105838. https://doi.org/10.1016/j.ecolind.2019.105838

Tolvanen A, Tarvainen O, Laine AM (2020) Soil and water nutrients in stem-only and whole-tree harvest treatments in restored boreal peatlands. Restor Ecol 28:1357–1364. https://doi.org/10.1111/rec.13261

Turetsky MR, Donahue WF, Benscoter BW (2011) Experimental drying intensifies burning and carbon losses in a northern peatland. Nat Commun 2:514. https://doi.org/10.1038/ncomms1523

Tveit A, Schwacke R, Svenning MM, Urich T (2013) Organic carbon transformations in high-Arctic peat soils: key functions and microorganisms. ISME J 7:299–311. https://doi.org/10.1038/ismej.2012.99

Tveit AT, Urich T, Svenning MM (2014) Metatranscriptomic analysis of Arctic peat soil microbiota. Appl Environ Microbiol 80:5761–5772. https://doi.org/10.1128/AEM.01030-14

Tveit AT, Urich T, Frenzel P, Svenning MM (2015) Metabolic and trophic interactions modulate methane production by Arctic peat microbiota in response to warming. Proc Natl Acad Sci USA 112:E2507–E2516. https://doi.org/10.1073/pnas.1420797112

UNEP (2022) Global peatlands assessment—The State of the World's peatlands: evidence for action toward the conservation, restoration, and sustainable management

of peatlands. Main report. Global Peatlands Initiative. United Nations Environment Programme, Nairobi

Urbanová Z, Bárta J (2020) Recovery of methanogenic community and its activity in long-term drained peatlands after rewetting. Ecol Eng 150:105852. https://doi.org/10.1016/J.ECOLENG.2020.105852

Urbanová Z, Hájek T (2021) Revisiting the concept of 'enzymic latch' on carbon in peatlands. Sci Total Environ 779:146384. https://doi.org/10.1016/j.scitotenv.2021.146384

Urbanová Z, Bárta J, Picek T (2013) Methane emissions and methanogenic archaea on pristine, drained and restored mountain peatlands, Central Europe. Ecosystems 16:664–677. https://doi.org/10.1007/s10021-013-9637-4

van Bodegom PM, Broekman R, Van Dijk J et al (2005) Ferrous iron stimulates phenol oxidase activity and organic matter decomposition in waterlogged wetlands. Biogeochemistry 76:69–83. https://doi.org/10.1007/s10533-005-2053-x

van Kessel M, Speth D, Albertsen M et al (2015) Complete nitrification by a single microorganism. Nature 528:555–559. https://doi.org/10.1038/nature16459

Vile MA, Bridgham SD, Wieder RK, Novák M (2003) Atmospheric sulfur deposition alters pathways of gaseous carbon production in peatlands. Global Biogeochem Cycles 17:1058. https://doi.org/10.1029/2002gb001966

Walton CR, Zak D, Audet J et al (2020) Wetland buffer zones for nitrogen and phosphorus retention: impacts of soil type, hydrology and vegetation. Sci Total Envir 727:138709. https://doi.org/10.1016/j.scitotenv.2020.138709

Wang Y, Wang H, He JS, Feng X (2017) Iron-mediated soil carbon response to water-table decline in an alpine wetland. Nat Commun 81:1–9. https://doi.org/10.1038/ncomms15972

Wang HT, Weil M, Dumack K et al (2021) Eukaryotic rather than prokaryotic microbiomes change over seasons in rewetted Fen peatlands. FEMS Microbiol Ecol 97:fiab121. https://doi.org/10.1093/femsec/fiab121

Weil M, Wang H, Bengtsson M et al (2020) Long-term rewetting of three formerly drained peatlands drives congruent compositional changes in pro- and eukaryotic soil microbiomes through environmental filtering. Microorganisms 8:550. https://doi.org/10.3390/microorganisms8040550

Weil M, Wang H, Zak D, Urich T (2023) Spatial and temporal niche separation of methanomassiliicoccales phylotypes in temperate fens. FEMS Microbiol Ecol. https://doi.org/10.1093/femsec/fiad049

Wen X, Unger V, Jurasinski G et al (2018) Predominance of methanogens over methanotrophs in rewetted fens characterized by high methane emissions. Biogeosciences 15:6519–6536. https://doi.org/10.5194/BG-15-6519-2018

Wen Y, Zang H, Ma Q et al (2019) Is the enzyme latch or iron gate the key to protecting soil organic carbon in peatlands? Geoderma 349:107–113. https://doi.org/10.1016/j.geoderma.2019.04.023

Wichtmann W, Schäfer A (2007) Alternative management options for degraded fens—utilization of biomass from rewetted peatlands. In: Okruszko T, Maltby E,

Szatylowicz J et al (eds) Wetlands: Monitoring, Modelling and Management. Taylor, London, pp 273–279

Wilson D, Blain D, Couwenberg J et al (2016) Greenhouse gas emission factors associated with rewetting of organic soils. Mires Peat 17:1–28

Xu Z, Wang S, Wang Z et al (2021) Effect of drainage on microbial enzyme activities and communities dependent on depth in peatland soil. Biogeochemistry 155:323–341. https://doi.org/10.1007/s10533-021-00828-1

Xue D, Liu T, Chen H et al (2021) Fungi are more sensitive than bacteria to drainage in the peatlands of the Zoige Plateau. Ecol Indic 124:107367. https://doi.org/10.1016/j.ecolind.2021.107367

Yang J, Liu J, Hu X et al (2013) Effect of water table level on CO_2, CH_4 and N_2O emissions in a freshwater marsh of Northeast China. Soil Biol Biochem 61:52–60. https://doi.org/10.1016/j.soilbio.2013.02.009

Yao Z, Yan G, Ma L et al (2022) Soil C/N ratio is the dominant control of annual N2O fluxes from organic soils of natural and semi-natural ecosystems. Agric For Meteorol 327:109198. https://doi.org/10.1016/j.agrformet.2022.109198

Ye R, Horwath WR (2016) Nitrous oxide uptake in rewetted wetlands with contrasting soil organic carbon contents. Soil Biol Biochem 100:110–117. https://doi.org/10.1016/j.soilbio.2016.06.009

Yrjälä K, Tuomivirta T, Juottonen H et al (2011) CH_4 production and oxidation processes in a boreal Fen ecosystem after long-term water table drawdown. Glob Chang Biol 17:1311–1320. https://doi.org/10.1111/j.1365-2486.2010.02290.x

Yuste JC, Peñuelas J, Estiarte M et al (2011) Drought-resistant fungi control soil organic matter decomposition and its response to temperature. Glob Chang Biol 17:1475–1486. https://doi.org/10.1111/j.1365-2486.2010.02300.x

Zak D, Gelbrecht J (2007) The mobilisation of phosphorus, organic carbon and ammonium in the initial stage of Fen rewetting (a case study from NE Germany). Biogeochemistry 85:141–151. https://doi.org/10.1007/s10533-007-9122-2

Zak D, McInnes RJ (2022) A call for refining the peatland restoration strategy in Europe. J Appl Ecol 59:2698–2704. https://doi.org/10.1111/1365-2664.14261

Zak D, Wagner C, Payer B, Augustin J, Gelbrecht J (2010) Phosphorus mobilization in rewetted fens: the effect of altered peat properties and implications for their restoration. Ecol Appl 20:1336–1349. https://doi.org/10.1890/08-2053.1

Zak D, Goldhammer T, Cabezas A et al (2018) Top soil removal reduces water pollution from phosphorus and dissolved organic matter and lowers methane emissions from rewetted peatlands. J Appl Ecol 55:311–320. https://doi.org/10.1111/1365-2664.12931

Zak D, Roth C, Unger V et al (2019) Unraveling the importance of polyphenols for microbial carbon mineralization in rewetted riparian peatlands. Front Environ Sci. https://doi.org/10.3389/fenvs.2019.00147

Zak D, Hupfer M, Cabezas A et al (2021) Sulphate in freshwater ecosystems: a review of sources, biogeochemical cycles, ecotoxicological effects and bioremediation. Earth Sci Rev 212:103446. https://doi.org/10.1016/j.earscirev.2020.103446

Zhong Y, Jiang M, Middleton BA (2020) Effects of water level alteration on carbon cycling in peatlands. Ecosyst Health Sustain 6:1806113. https://doi.org/10.1080/20964129.2020.1806113

Zhu B, van Dijk G, Fritz C et al (2012) Anaerobic oxidization of methane in a minerotrophic peatland: enrichment of nitrite-dependent methane-oxidizing bacteria. Appl Environ Microbiol 78:8657–8665. https://doi.org/10.1128/AEM.02102-12

Ziegelhofer A, Kujala K (2021) Assessing the diversity and metabolic potential of psychrotolerant arsenic-metabolizing microorganisms from a subarctic peatland used for treatment of mining-affected waters by culture-dependent and -independent techniques. Front Microbiol 12:1777. https://doi.org/10.3389/fmicb.2021.648412