Legacy of peatland erosion shapes microbial communities during recovery

Authors:
Fin Ring-Hrubesh[1,2*] (fin.ring-hrubesh@bristol.ac.uk)
Mike Vreeken[1,2,3] (mike.vreeken@bristol.ac.uk)
Anne Eberle[1,‡] (anne.eberle@gfz.de)
Bradley Welch[4] (brad.welch@beacons-npa.gov.uk)
Paul Sinnadurai[4] (paul.sinnadurai@beacons-npa.gov.uk)
Penny Johnes[2,5] (penny.johnes@bristol.ac.uk)
Robert Griffiths[6] (robert.griffiths@bangor.ac.uk)
Angela Gallego-Sala[7] (a.gallego-sala@exeter.ac.uk)
Richard Pancost[1,2,3] (r.d.pancost@bristol.ac.uk)
Casey Bryce[1,2*] (casey.bryce@bristol.ac.uk)

Affiliations:
1 School of Earth Sciences, University of Bristol, Wills Memorial Building, Queens Road, Bristol BS8 1RJ, UK
2 Cabot Institute for the Environment, Royal Fort House, University of Bristol, BS8 1UH
3 Organic Geochemistry Unit, School of Chemistry, University of Bristol, Cantock's Close, Bristol, BS8 1TS, UK
4 Bannau Brycheiniog National Park Authority, Brecon, Powys, LD3 7HP, UK
5 School of Geographical Sciences, University of Bristol, University Road, Bristol BS8 1SS, UK
6 School of Environmental and Natural Sciences, Bangor University, Bangor, Gwynedd, LL57 2UW, UK
7 Geography, College of Life and Environmental Sciences, University of Exeter, Amory Building, Rennes Drive, Exeter, EX4 4RJ, UK
‡ Present address: GFZ Helmholtz Centre for Geosciences, Telegrafenberg, 14473 Potsdam, Germany
* Corresponding authors

Legacy of peatland erosion shapes microbial communities during recovery

Fin Ring-Hrubesh[1,2*], Mike Vreeken[1,2,3], Anne Eberle[1,‡], Bradley Welch[4], Paul Sinnadurai[4], Penny Johnes[2,5], Robert Griffiths[6], Angela Gallego-Sala[7], Richard Pancost[1,2,3], Casey Bryce[1,2*]

1 School of Earth Sciences, University of Bristol, Wills Memorial Building, Queens Road, Bristol BS8 1RJ, UK

2 Cabot Institute for the Environment, Royal Fort House, University of Bristol, BS8 1UH

3 Organic Geochemistry Unit, School of Chemistry, University of Bristol, Cantock's Close, Bristol, BS8 1TS, UK

4 Bannau Brycheiniog National Park Authority, Brecon, Powys, LD3 7HP, UK

5 School of Geographical Sciences, University of Bristol, University Road, Bristol BS8 1SS, UK

6 School of Environmental and Natural Sciences, Bangor University, Bangor, Gwynedd, LL57 2UW, UK

7 Geography, College of Life and Environmental Sciences, University of Exeter, Amory Building, Rennes Drive, Exeter, EX4 4RJ, UK

‡ Present address: GFZ Helmholtz Centre for Geosciences, Telegrafenberg, 14473 Potsdam, Germany

*** Corresponding authors: Fin Ring-Hrubesh (fin.ring-hrubesh@bristol.ac.uk) and Casey Bryce (casey.bryce@bristol.ac.uk)**

Abstract

Human degradation of peatlands worldwide has turned them into net carbon sources. In upland blanket peatlands, erosion disrupts new plant-derived carbon input and exposes deep peat, putting old carbon at risk of oxidation.

The efficacy of restoration in preventing carbon loss and recovering ecosystem function depends on microbial responses to both water table manipulation and renewed litter input. Yet it is unclear how these factors alter the microbial communities that ultimately control carbon storage and emissions.

We show that microbial community composition in the eroded peatland of Waun Fignen Felen, South Wales, was primarily governed by the bioavailability of organic matter rather than water-table position. Long-term erosion leaves behind a legacy of highly degraded organic matter, unaltered by re-wetting. Where plant litter accumulation is renewed on formerly eroded peat surfaces, the influx of bioavailable organic input supports a distinct microbial community with greater biomass, and evidence of elevated respiration.

1. Introduction

Northern peatlands operate as a large terrestrial carbon store (400–600 GtC; [1-3]), and in the UK peatlands cover 8–12% of the terrestrial surface with ombrotrophic upland bog the dominant peatland type (65% of peatland area)[4]. Peatland surface erosion and vegetation loss are common facets of upland blanket bog degradation[5]. Vegetation loss can be driven by several factors including excessive grazing pressure[6], die-off due to intolerance of atmospheric pollutant and nutrient deposition[7, 8], and fire[9, 10]. As vegetation acts to stabilise the peatland surface, its disappearance can lead to enhanced erosion by surface water flow, wind action and frost-heave[11-13]. A positive feedback can become established where surface instability hinders the re-establishment of vegetation, such that bare peat surfaces persist along with associated carbon losses.

Peatland carbon can be lost as both a direct and indirect result of erosional processes. Organic matter is washed out from the peat profile directly, entering watercourses as particulate organic matter with detrimental consequences for water quality[14], and further decomposing in streams to dissolved organic carbon (DOC) and CO_2[15]. In severely eroded UK blanket peatlands, Pawson *et al.*[16] observed that carbon losses through fluvial export alone total 29–106 MgC km^{-2} yr^{-1}. Stored carbon is also destabilised as an indirect consequence of erosion, where gully formation drives water table drawdown and enhanced mineralisation in the deepened oxic layer[17]. Peatland drying leads to increased CO_2 emissions and a suppression of CH_4 emissions with a net warming effect overall[18, 19]. Although impacts of drying on DOC production and export are reported, there is greater uncertainty over the direction of the response[20-22], with DOC representing a heterogeneous pool of microbial and plant metabolites governed by a wide range of biotic and abiotic processes. To address both direct and indirect carbon loss pathways, restoration of eroded peatlands will typically aim to stabilise peat surfaces and re-establish vegetation, while also implementing re-wetting measures.

Typically, the composition of peatland organic matter follows a depth gradient, with deep peat dominated by less reactive organic compounds, such as lignin and polyphenols, which have persisted over time[23]. While the anaerobic decomposition of these molecular classes does occur, it proceeds slowly, leading to their relative persistence[24]. Conversely, recently deposited near-surface peat will contain more ‘labile’ organics such as polysaccharides which are typically more microbially available[25]. In a system where surface peat has been lost, the contrast in the composition of shallow and deep organic matter could be lessened or even absent[26].

The impact of altering the physico-chemical environment (by concurrent re-wetting and vegetation re-establishment) on microbial communities is uncertain, yet the microbiome response is of major relevance given its central role in the peatland carbon cycle and ecosystem function[27, 28]. While reduced microbial activity has been reported in more mineralised peats in several studies[26, 29, 30], it is unclear to what extent peat macromolecular composition limits carbon degradation in practice, given the generally overriding effect of water table position. Decoupling these drivers is particularly relevant for peatland restoration where water table and vegetation recovery may be superimposed onto a degraded peat matrix. In these contexts, the degree to which peatland microbes can utilise and release modern carbon or carbon from the long-term store is key to the function of the ecosystem as either a carbon sink or source.

We carried out an examination of microbial communities at the upland ombrotrophic bog of Waun Fignen Felen in South Wales. The site serves as a case study, due to the co-location of areas representing long- and short-term regeneration atop erosional surfaces, alongside ongoing surface erosion. We present the geochemical and microbial characterisation of regenerating and degraded peat profiles with the aim of determining the strongest predictors of microbial community composition. We show that peat bulk organic matter composition differed most strongly across depth profiles where peat was actively accumulating. Where long-term recovery had occurred, this surface layer supported a microbial community distinct from that in degraded surface peats exposed by erosion, and separate to the deep peat microbiome. We find that during recovery from erosion, the switch to supply of recent substrate input from vegetation, superimposed onto otherwise low-bioavailability bulk peat can exert an even stronger influence on microbial community composition than water table position.

2. Materials & methods

2.1 Site overview

The study site, Waun Fignen Felen, is an ombrotrophic bog situated on a limestone escarpment south of Fan Hir, South Wales (475 m AOD; 51.8465, –3.7103). The peat stratigraphy in the region of the peatland under investigation was characterised by Smith and Cloutman[31], who describe *Sphagnum* spp. peat forming a raised mound overlying

Phragmites spp. peat deposited during earlier reed-swamp conditions atop late Devensian lake sediments. The region is differentiated from the blanket peat in the surrounding landscape by more severe erosion, greater peat depth and by radial drainage outwards from its centre. In their study, Smith and Cloutman[31] reported conventional radiocarbon dates for five near-surface (<10 cm) samples from across this eroded region, of between 4850 ±70 BP and 3800 ±70 BP (uncalibrated[31, 32]), indicating that erosion had exposed ancient peat.

The presence of ongoing erosion alongside areas representing short- and long-term regeneration within the same peatland complex was a key factor in site selection. As such, we can ascribe the primary differences in geochemistry to the degree of recovery from severe erosion. We examined three areas of the peatland, each deemed to be hydrologically independent, and all situated within the eroded region identified by Smith and Cloutman[31]. Our three sampling sites consisted of: 1) an actively eroding area with a predominantly bare surface; 2) an area which has become re-vegetated following management intervention to block adjacent gullies in 2009; and 3) an area in which vegetation has naturally recolonised a former erosional surface. In the naturally recolonised profile, an abrupt transition between amorphous, dark peat and an upper layer predominantly composed of identifiable plant material marked the inferred former erosional horizon.

In the area of managed restoration, the dominant plants were *Deschampsia flexuosa*, *Hypnum jutlandicum* and *Campylopus introflexus*. In the naturally regenerating area, *Eriophorum angustifolium*, *Sphagnum* species (*capillifolium*, *fimbriatum*, *palustre*) and occasional stands of *Molinia cerulea* were the primary vegetation cover (Supplementary Fig. A). Measured peat depths in the degraded and restoration areas were 3.8–4.3 m, compared to 1.2–2.2 m in the area of natural-regeneration. The shallower peat in the latter area resulting from its position away from the centre of the peatland (basal dates from Smith and Cloutman[31] did not indicate a younger age) and may have been further diminished by a greater extent of historic peat loss in this area which was examined further in this study.

2.2 Water table monitoring and porewater sampling

In each study area water table position was monitored in a centrally located dip-well, while porewater was sampled from three porewater membrane-exchange equilibrator (PWE) samplers each installed 2 m apart. Water table data was collected using pressure transducers calibrated against ambient barometric pressure at the site (HOBO U20L-01). The dip-wells were not anchored to underlying geology, and any vertical shift was recorded and manually re-set on a 6-weekly basis, with a linear correction applied to the intervening data when shifts were observed. As such, water level depths are reported relative to the peat surface. Summary rainfall data at the site (daily totals) were recorded using a ‘tipping-bucket’-type gauge (Ecowitt WH5360).

PWE samplers for the collection of filtered porewater were built according to the specification of Bowes and Hornibrook[33], itself modelled on a sampler design proposed by Hesslein[34]. These samplers consisted of a PVC housing which seals a series of horizontal cells behind a polyether sulfone (PES) membrane (0.2 µm pore size, Pall Life Sciences HT-200). The cells were then connected on either side to tubing, which was routed to the surface while the samplers were installed within the peat. Tubing for water sample collection was connected to the cell base, while the top of each cell was connected to N_2-filled syringes during sampling to maintain an anoxic headspace. After sampling, the wells were refilled with N_2-sparged de-ionised water. Samples were collected from depths of 6, 14, 30 and 50 cm at 6-week intervals between August 2021–October 2022, allowing sufficient time for equilibration between porewater and PWE wells between each sampling event. The samples collected were filtered with 0.2 µm PES syringe filters into N_2-flushed vials and transferred to cold storage at 5°C within 12 hours prior to analysis.

2.3 Porewater geochemical analyses

Porewater pH was recorded within 12 hours using a calibrated probe (Thermo Scientific Orion ROSS Ultra). DOC concentrations were determined within 72 hours using the non-purgeable organic carbon (NPOC) method (Shimadzu TOC-L). Select dissolved inorganic ions were determined by spectrophotometric methods (NH_4^+-N, total NO_3^--N + NO_2^--N, PO_4^{3-}-P, SO_4^{2-}-S using Thermo Scientific Discrete Industrial Analyzer System Reagents for Water/Environmental Samples; total dissolved Fe by the ferrozine method), with only those selected for inclusion in the RDA model presented below. Dissolved CO_2 and CH_4 were determined during 4 of the sampling events (2022: 19th May, 6th July, 26th August, 11th October) using the widely applied headspace-equilibration method[35]. Briefly, immediately after the anoxic sampling of porewater from the equilibrator samplers into a syringe, an equal volume of N_2 gas was drawn from a gas-tight bag before the sample was shaken vigorously with the headspace to equilibrate for 60 seconds. Headspace was then transferred into a vacuum vial (Exetainer) and stored at room temperature until analysis. Concentrations in the headspace samples were determined using gas chromatography (Agilent 7890A), equipped with a flame ionisation detector (FID; for CH_4, and CO_2 after methanisation to CH_4). Dissolved concentrations in the original porewater were determined by application of Henry's Law using water temperatures recorded by the dipwell loggers, with Henry's Law constants and temperature dependence coefficients sourced from the NIST database[36].

2.4 Peat sampling

Peat was sampled in summer 2022 (27th June–1st July), with cores taken using a D-section (Russian type) corer. Within each of the three study areas, a nested sampling strategy was employed with 5 peat sampling locations spaced 20 m apart along a 'W'-shape transect, the central position of which was co-located with the dipwell and porewater sampling (Fig. 1). In the restoration area, each sampling location was sited 2 m away from a blocked gully. Sampling at each position was conducted in triplicate, and all peat was transferred onto dry ice in the field prior to freezer storage at −20°C within 12 hours. Additionally, cores were subsampled in the field using sterile tools to retrieve 4 cm long subsamples, at depths centred on 6, 14, 50, 105 cm, and the peat base, which were transferred into centrifuge tubes for DNA analysis and stored at −80°C on return to the laboratory.

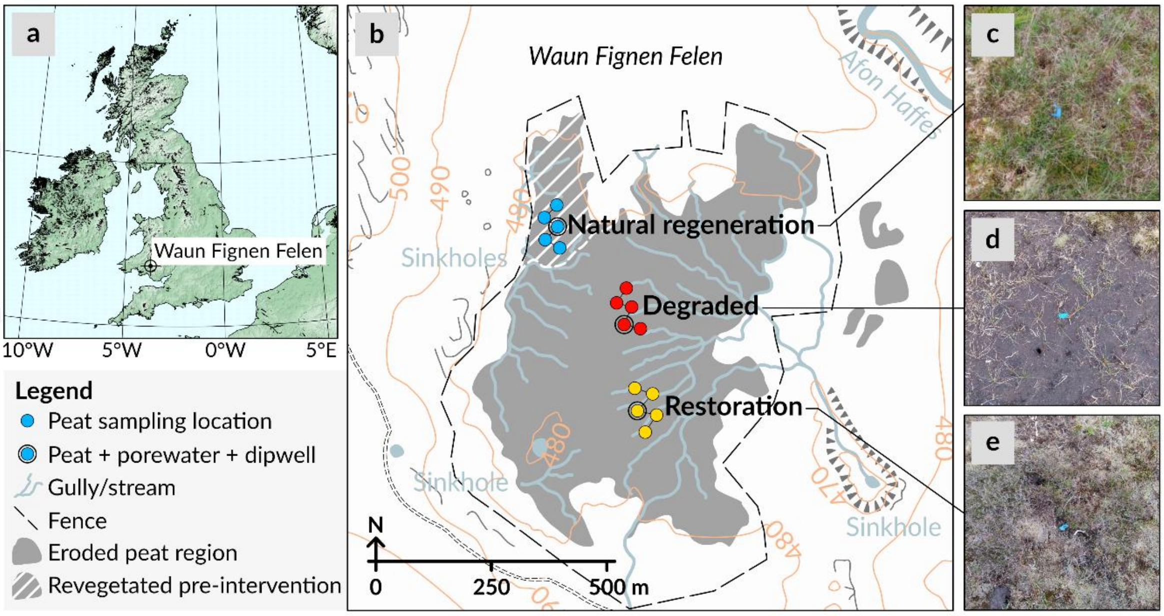

Figure 1 | Site overview and sampling scheme. a Location of Waun Fignen Felen in the British Isles, peatland regions are indicated by black shading[37]. **b** Locations of dipwells, peat and porewater sampling within the fenced study region at Waun Fignen Felen. **c–e** Photographs of the peat surface at the sampling locations; each image covers approximately 1 x 1 m of the ground surface.

2.5 Peat geochemical analyses

The composition of organic macromolecules in the bulk peat was investigated using pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS), at the same depths at which DNA sequencing was carried out. Triplicate samples were analysed, from the central 3 positions in each region (Fig. 1). For this analysis, 0.5±0.1 mg of peat was taken from the freeze-dried, and homogenised subsamples. Peat in furnaced quartz tubes was subjected to an initial thermal desorption step at 300°C for 2 minutes, to remove free lipids that may obscure the lignocellulosic signal. The desorbed peat was subsequently pyrolysed at 610°C for 20 seconds using a Chemical Data System 6200 series pyroprobe with the trap at 50°C. The pyrolysates were released post-pyrolysis by heating to 300°C for 4 minutes, and were transferred at 310°C to a gas chromatograph (GC; Thermo Scientific Trace 1310) fitted with an Rtx-1 column (dimethyl polysiloxane, non-polar, 60 m × 0.32 mm ID, film 0.25 μm). The GC oven was set to heat from 40°C (held initially for 4 min) to 300°C at 40°C min^{-1}, with helium used as the carrier gas. The GC was coupled with a single quadrupole mass spectrometer (Thermo Scientific ISQ7000), with the detector scanning from *m/z* 50-650. Peak identification was carried out in XCalibur (Thermo Scientific) using inhouse libraries and literature. The relative abundances of components were determined from the peak areas from integrated extracted ion chromatograms of pyrolysis product major ions as in Schellekens *et al.*[38], and are reported as a proportion of the sum of all those identified. The compound classes of Schellekens *et al.*[38] were also used to group products, with additional components classed according to Chen *et al.*[39] (2,5-Dimethylfuran) and Nowakowski *et al.*[40] (Desaspidiniol). These fractional abundances enable comparison between the relative abundances of compound classes, which is inherently qualitative[41]. Within the polysaccharides, levoglucosan was used to represent the relative abundance of cellulose as it is the primary pyrolysis product[42]; all other polysaccharide pyrolysis products were grouped to represent the hemicellulose fraction. Lignin was represented functionally through phenol, guaiacol and syringol pyrolysates, while other compound classes identified were the aliphatics (alkane/alkene doublets), N-containing compounds, and aromatics. These additional compound classes affect the relative abundances of the discussed classes, but not the ratios between them.

Fourier-transform infrared spectrometry (FTIR) was conducted complementarily on the same samples to analyse the quality of the peat organic matter. FTIR spectra were obtained by averaging 16 scans of the 4000 to 600 cm^{-1} region (4 cm^{-1} resolution, 1 cm^{-1} data interval; PerkinElmer Spectrum Two). Freeze-dried and homogenized samples were placed on an ATR crystal with pressure applied to attain even coverage and contact. An automatic background correction was applied to all samples using the instrument software. Relevant bands were subsequently identified and processed using the method set out by Hodgkins *et al.*[43] to provide relative abundances for aromatic and carbohydrate fractions, which they calibrated using wet chemistry methods to provide semi-quantitative %-aromatic and %-carbohydrate values. Additionally, the total carbon and nitrogen content of the pyrolysis/FTIR subsamples was determined using an elemental analyser calibrated with reference standard NC Soil 341506 (EA; Elementar Vario MACRO Cube), with C:N ratios computed using these mass fractions. Cores taken at the porewater sampling locations were subsampled at 10 cm intervals for determination of peat bulk density profiles by drying a known volume of sample at 105°C.

2.6 DNA extraction and sequencing

Peat subsamples for DNA extraction were stored at –80°C and were handled in a laminar flow hood, with a homogenised sample extracted across the whole 4 cm depth interval by scouring a groove along the frozen sample with a fine spatula. This scoured peat material (*ca.* 500 mg) was processed using DNeasy PowerSoil Pro extractions kits (QIAGEN). Extract concentrations were quantified using a fluorescence assay (ThermoFisher Qubit 2), and DNA concentrations were corrected for mass of raw peat to provide total soil microbial DNA, used here as a proxy for biomass.

PCR amplification, library preparation and sequencing were carried out commercially by Novogene (Cambridge, UK). The V4 region of the 16S rRNA gene was targeted using the Earth Microbiome Project (EMP)[44] primer pair 515F (5’-GTGCCAGCMGCCGCGGTAA-3’); 806R (5’-GGACTACHVGGGTWTCTAAT-3’), before PCR products of the correct size were selected by gel electrophoresis. These primers were selected as they were designed as universal primers to target both bacteria and archaea and have been widely applied[45]. An equal amount of PCR products was pooled from each sample and end-repaired, A-tailed and further ligated with Illumina adapters. Libraries were then sequenced on an Illumina MiSeq platform to generate 250 bp paired-end raw reads.

After sequencing, the DADA2 algorithm[46] was used to distinguish amplicon sequence variants (ASVs) and their relative abundance within each sample, using standard settings for trimming and quality filtering. Assignment of taxonomy was carried out using the SILVA database (v.138.1)[47]. ASVs which were assigned to chloroplasts and mitochondria were removed, and the sequence tables were randomly rarefied to an even size using the phyloseq R package[48].

2.7 Statistical analysis

To determine the key environmental controls on the microbial community, redundancy analysis (RDA) was applied across the depths for which highly replicated sequencing data, as well as porewater samples were retrieved (6, 14, 50 cm). Porewater (annual mean) and peat geochemical parameters were used as explanatory variables while greenhouse gases were excluded as they were considered equally likely to be response variables. To avoid overrepresentation of multivariate geochemistry datasets, dimensionality reduction was carried out whereby the summary ratios of polysaccharides:lignin and hemicellulose:cellulose were used to represent the bulk organic geochemistry (as determined by Py-GC-MS), rather than each compound or compound class; while ammonium and oxides of nitrogen were grouped as 'dissolved inorganic N'. To determine which combination of variables explained the most variance in microbial community composition (ASV counts), step-wise removal of the remaining variables took place according to their multicollinearity. This was assessed by the VIF scores (Variance Inflation Factors) of each variable when used as RDA constraints, and redundant variables were removed until those remaining had low VIF scores (VIF < 5)[49]. Subsequently, forward and backward stepwise selection of variables was performed using the ordistep function in the vegan R package[50], whereby iterative addition and removal of variables was conducted based on Akaike's Information Criterion (AIC). The final RDA thus retains only those variables that significantly contributed to the explained variation in the ASV abundance data and is plotted with type 2 scaling to show the effect of explanatory variables.

3. Results & Discussion

3.1 Water table regimes align with regeneration status

Over the two-year monitoring period, water tables were consistently highest in the natural regeneration site, intermediate in the managed restoration site and lowest in the degraded site (Fig. 2). This trend, aligning with the degradation status, was magnified by more rapid drops in water table in the degraded and restoration areas during periods of low precipitation (Supplementary Fig. B). None of the site water tables were extremely deep, with mean water table in the degraded peat at 12.9 ±10.1 cm below the surface, compared to 9.7 ±8.9 cm in the restoration profile, and 5.5 ±7.2 cm in the naturally regenerating area (± standard deviations). Across the monitoring period Waun Fignen Felen received >2400 mm of annual rainfall. This is considerably above the minimum typically associated with upland blanket peatland (1600 mm[51]). The high water tables across the whole site are likely supported by this high annual precipitation as well as the relatively flat topography of the eroded site, conferring lower rates of runoff.

3.2 Extent of recovery evident in geochemical profiles

There was evidence for significant surface peat loss at all three study areas within the peatland, with recent accumulation visibly apparent in the top 3–5 cm of the restoration profiles and the upper 5–15 cm in the natural regeneration area. Despite this, the bulk density of surface peat was comparable across the three conditions. The shallower peat base in the natural regeneration site was evident in the bulk density profiles (rapid increase associated with the peat base; Fig. 2), and elevated bulk density values were seen in this profile between 40–110 cm relative to the degraded and restoration areas.

Natural regeneration was associated with a surface layer that has the highest C:N ratio. In the degraded and restoration areas, lower C:N ratios at 6, 14 and 30 cm corresponded with the absent or thin accumulating layers in these profiles, suggesting that shallow peat in these areas is more decomposed than in the natural regeneration region, as differential loss of C during decomposition has been reported widely[52, 53]. Beneath the natural regeneration surface layer, there was a transition to amorphous peat, which was reflected in the steep decline in C:N ratios in this profile compared to consistent values downcore in the other areas (Fig. 2).

While the peat profile in the naturally regenerating area may have been somewhat shallower due to its location further from the centre of the raised peat dome, the differences in peat physical properties also indicate that prior erosion may have been more advanced in this area, having exposed older deep peat before the onset of natural recovery. Regeneration atop erosional horizons, has been described in other UK peatlands [54, 55], with Milner *et al.*[55] setting out the case whereby exposure of an erosion-resistant layer allows the system to transition to a new stable state in which renewed peat accumulation can proceed. At Waun Fignen Felen, exposure of peat layer with a higher bulk density and lower hydraulic conductivity (Supplementary Fig. C) may have facilitated a comparable transition.

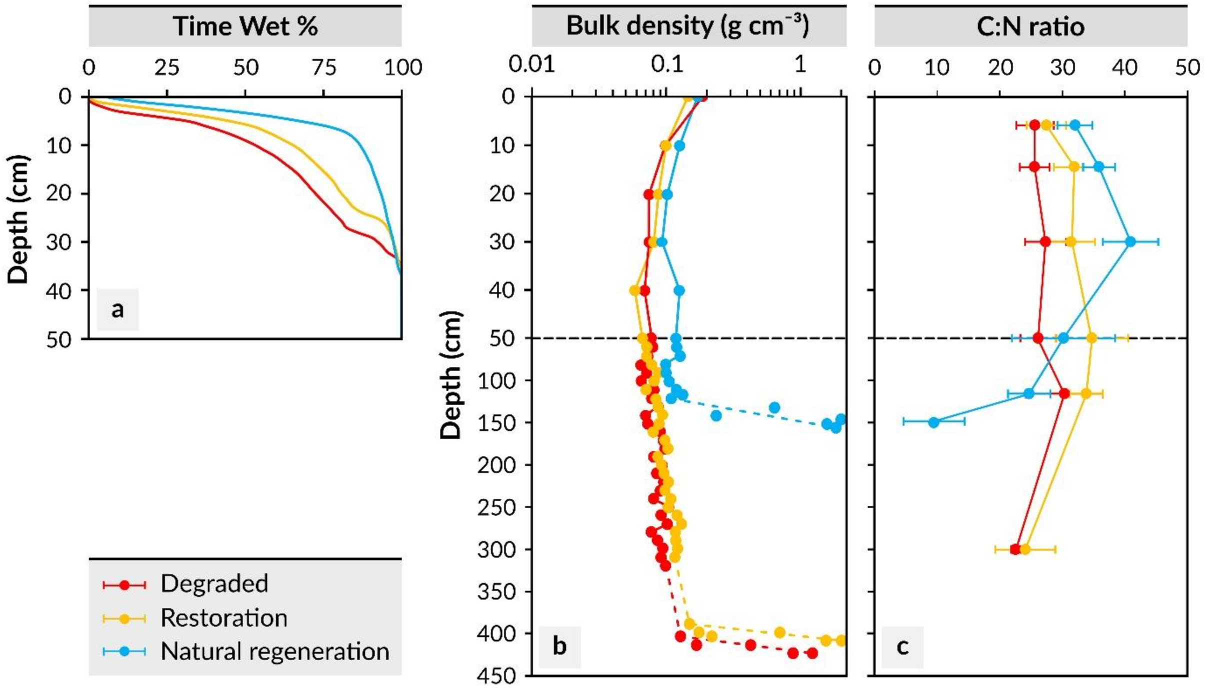

Figure 2 | Summary of peat properties across the three conditions. a The proportion of time for which peat depths were below the water table during the two week period prior to core sampling, below 50 cm peat was permanently inundated during the entire monitoring period. **b** The peat bulk density (dry mass/*in situ* volume) at 10 cm intervals, presented along a log-scale due to steep change at peat base. Values were derived from the core taken at the porewater sampling location. A solid line is used to connect datapoints; near the peat base a dashed line is used to approximate the trend. **c** The carbon-to-nitrogen ratio of bulk peat across all cores, with error bars representing the standard deviation. For depths 6, 14, and 50 cm n=15 (3 replicate cores at 5 locations); for depths 30, 105 and 147/300 cm n=3 (3 locations). Note change in y-axis scale at 50 cm.

Peat macromolecular composition, as assessed using Py-GC-MS and FTIR, also reflected the recovery status, driven by a combination of historical factors, including burial depth and erosion (Fig. 3). In shallow samples the central role of fresh plant-derived input was apparent, with a greater proportion of labile, polysaccharide-derived compounds present in the vegetated areas. Most polysaccharides decompose preferentially to lignin, such that all of the peat in the study area, except that in the shallow peat of re-vegetated sites, has become polysaccharide-depleted; this occurs throughout the depth profile at the degraded site where fresh vegetation is absent. In addition, the polysaccharide pool within shallow samples from vegetated profiles contained a greater relative contribution from hemicellulose-derived pyrolysis products than cellulose (Fig. 3), indicating fresh accumulation of plant material. By contrast, markers of lignin and other phenolics (and aromatic signals in FTIR, representing approximately the same pool) were depleted in the shallow peat, especially at the two revegetated sites. All these observations are consistent with the contemporary regrowth of fresh vegetation at the natural regeneration site.

With increased depth, the proportion of pyrolysates associated with lignin, as well as the aromatic FTIR signal, increased. This is expected with downcore degradation of peat organic matter, but we suggest that this trend primarily reflects the switch from fresh vegetation to more strongly decomposed peat below the erosion horizon. This would explain the lack of a downcore change at the degraded bare surface sites, where vegetation recovery is absent or limited. The increase in lignin/aromatic components with depth at the natural regeneration sites was particularly steep, so much so that their proportions were greater than at the degraded and restored sites (similar to C:N profiles). Given that all three sampling locations were sited in the basin set out by Smith and Cloutman[31], with comparable dates of peat initiation; the shallower peat depth now present in the natural regeneration area; and greater inferred erosional loss; we suggest that the high aromatic content reflects older peat exposed in that area, potentially with different, more lignin-rich vegetation inputs (greater contribution from vascular plants with depth; for example in Biester *et al.*[56]).

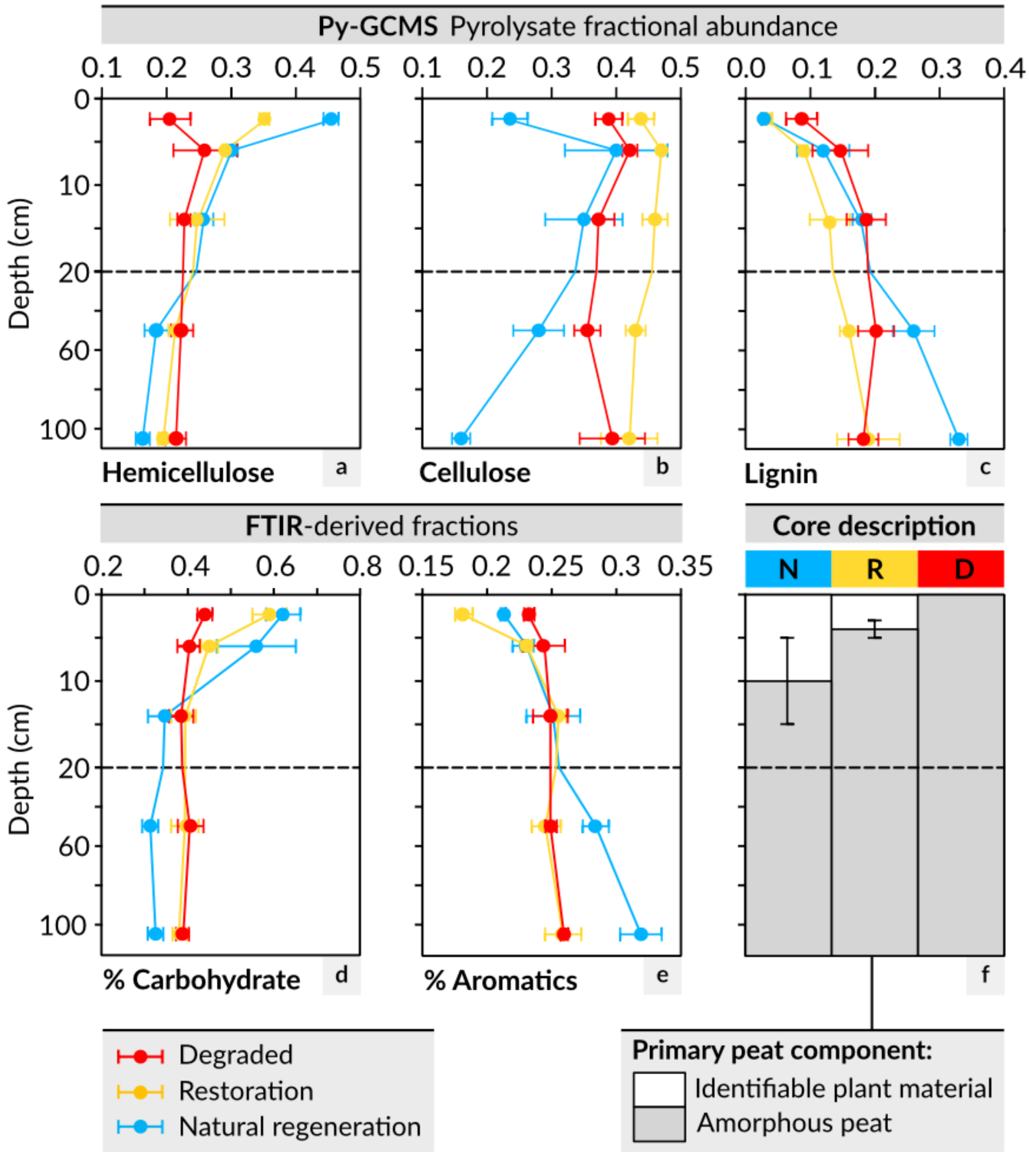

Figure 3 | Peat organic matter composition. A comparison of relative abundances of organic macromolecular groups in bulk peat as derived by Py-GC-MS (**a**–**c**) and FTIR (**d**–**e**) from triplicate samples collected in summer 2022. Note change in y-axis scale at 20 cm. Error bars for Py-GC-MS and FTIR-derived fractions represent the standard deviation between samples. **f** An overview of the position of the erosional horizon within profiles, with error bars indicating the observed range in position.

Porewater concentrations of dissolved organic carbon (DOC) were notably lower in the degraded area than either of the re-vegetated areas (Fig. 4). Studies on DOC release after peatland restoration report highly contrasting outcomes, and when reviewing available data Evans *et al.*[20] found only limited support for any decrease associated with restoration, and Darusman *et al.*[57] show no consistent effect. In cases such as ours where apparent DOC production is instead lower in degraded sites than under recovery, several explanations have been suggested: Comparing eroded and intact peatland sites in the Pennines, Evans *et al.*[58] attributed lower DOC export from eroded sites to either lower 'lability' or minimal interaction between water and the peat matrix. Elsewhere a recovery of plant productivity has been reported to cause increased porewater DOC[59], while Pinsonneault *et al.*[60] demonstrate higher water extraction of DOC from fresh *Sphagnum* and vascular plant material than from litter or peat material. Bernard-Jannin *et al.*[22] report that DOC export did not decrease under restoration, instead being sustained by a supply of more recent organic matter at the peat surface. We found that where highly aromatic macropolymers comprised a greater proportion of the organic substrate, this was associated with lower accumulation of dissolved organic compounds in porewater. In the naturally regenerating area, where porewater DOC was highest, there was a temporally consistent peak at a depth of 14–20 cm (Fig. 4). The peak in DOC concentrations in the near-surface, and association between higher concentrations and areas with vegetated peat surfaces, suggest that it originates from modern plant matter through litter or root exudates. This would represent a more accessible pool of potential substrates for microbial metabolism than DOC derived from the bulk peat organic matter[60].

Correspondingly, dissolved CO_2 was elevated in the region of natural regeneration throughout the downcore profile, despite the higher water table in this profile, whereas CO_2 concentrations were low under enhanced degradation, pointing to the lower availability of the remaining organic matter. Our interpretation that elevated CO_2 under the re-vegetated scenario predominantly results from the cycling of modern plant-derived carbon, would imply that this is not detrimental to the long-term carbon store. Yet this would need to be confirmed by further investigation of the peatland carbon balance, particularly as priming of microbial communities by labile carbon input has been proposed to enhance peat decomposition[61-63]. Our combined data suggest that re-vegetation may act as an overriding control on CO_2 production, with restoration adding labile inputs that are superimposed onto low bioavailability bulk peat in formerly-eroded peatlands. No similar effect was observed with regard to methane concentrations, which were similar between the conditions (Supplementary Fig. D).

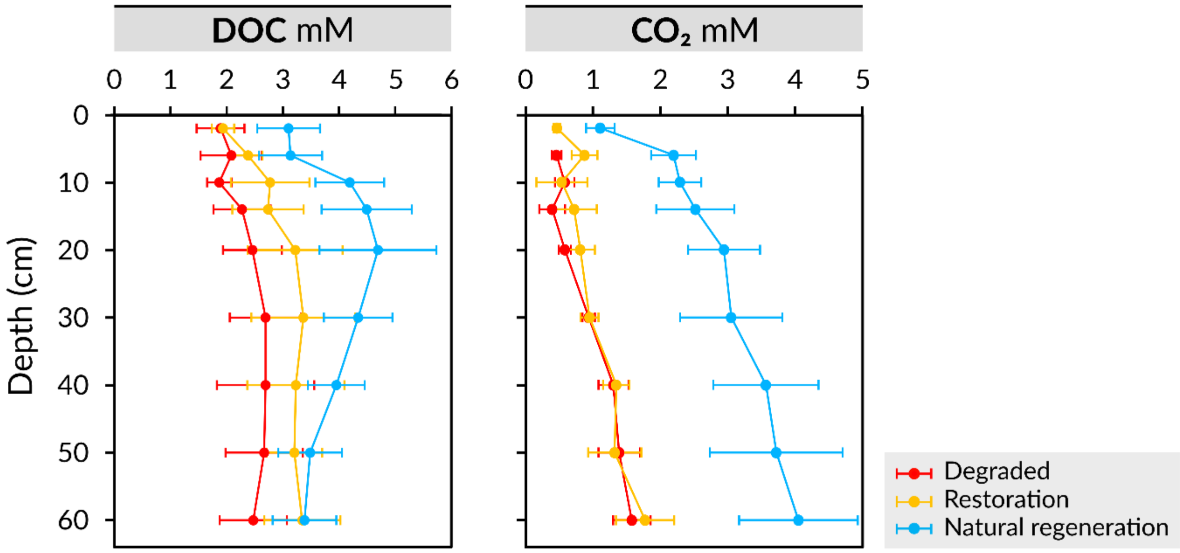

Figure 4 | Depth profiles of dissolved organic carbon and carbon dioxide in porewaters at Waun Fignen Felen. Points represent mean values with standard deviation shown by bars. Dissolved organic carbon was sampled on 12 occasions between August 2021–October 2022, CO_2 samples were collected on 4 occasions between May–October 2022.

3.3 Microbial community composition is vertically stratified

In the upper part of the profiles (6 and 14 cm), the natural regeneration area hosted more microbial biomass, as assessed by extractable DNA, than either the bare or restoration peat profiles (Fig. 5). In shallow peat, the microbial community composition in the natural regeneration locations was distinct from that in the degraded and restored areas (Fig. 5), with a markedly greater abundance of members of the order *Acidobacteriales* and family *Xanthobacteraceae*, and lower abundances of Group 1.1c *Thaumarchaeota* and the *Sideroxydans* and *Spirochaeta*.

Acidobacterial sequences are frequently among the most abundant in peatlands, with *Acidobacteriales* one of two orders which are dominant in acidic bogs globally (alongside *Bryobacterales*, which were less abundant here)[28, 64, 65]. A dominance of *Acidobacteriales* in the surface litter layer and subsequent decline in deeper peat has also been reported by Golovchenko *et al.*[66] in pine swamp peatland. The order includes both aerobic and anaerobic acidophilic chemoorganoheterotrophs, known to degrade a wide range of mono-, di-, and polysaccharides (Dedysh and Oren, 2020). The other taxon which was more abundant in the natural regeneration area, was the *Xanthobacteraceae*, elsewhere found to be common among the acrotelm microbiome[66-68]. These are chemoorganoheterotrophs with some strains capable of sulfur oxidation and others able to fix nitrogen[69]. By contrast, the near-surface of the managed-restoration and degraded areas was enriched in the genera *Sideroxydans* and *Candidatus Nitrosotalea* (Fig. 5), both of which are known chemolithotrophs. These differences in the community composition, as well as apparent biomass, correspond with the finding of greater resource-availability in the naturally regenerating area due to a greater abundance of labile organics relative to the peat everywhere else at the site.

The composition of the microbial community began to converge in samples retrieved from below 50 cm and clustered closely in deeper samples, where peat composition and conditions were similar across the three conditions (Fig. 6). This was accompanied by a decline in apparent biomass in all the sites, relative to the upper profile, with similar low yields of microbial DNA in all three locations below 50 cm (<0.2 ng/mg peat; Fig. 5). The deep-peat community was enriched in members of the genus *Spirochaeta*, which contains obligate and facultative anaerobes involved in saccharolytic fermentation[70]. Also more abundant were the *Thaumarchaeota*, of which members described to date have been chemolithoautotrophic with some additionally capable of utilising simple organics heterotrophically[71]. In the deepest samples, proximal to the peat base, an increase in *Bathyarchaeota* abundance was observed. Archaea from this phylum could be key degraders of organic matter in peatlands particularly at depth, given their similar dominance in deep marine cores[72] and the diverse strategies for carbon assimilation that have been attributed to them, ranging from lignin metabolism to methanogenic CO_2 uptake[73, 74]. These findings support the inference that the lower rates of heterotrophic microbial activity observed in deep and highly decomposed peats[30, 75, 76], reflect the lower bioavailability of remaining substrate.

Many of the taxa associated with deep peat also dominated in the shallow degraded and restoration sites, and as a result the vertical change in community abundance was weaker in those profiles (Fig. 5). At these depths, peat was permanently inundated at all locations, although the organic matter composition diverged among them (Fig. 3), primarily due to the relatively elevated content of aromatic polymeric material at depth in the natural regeneration profile.

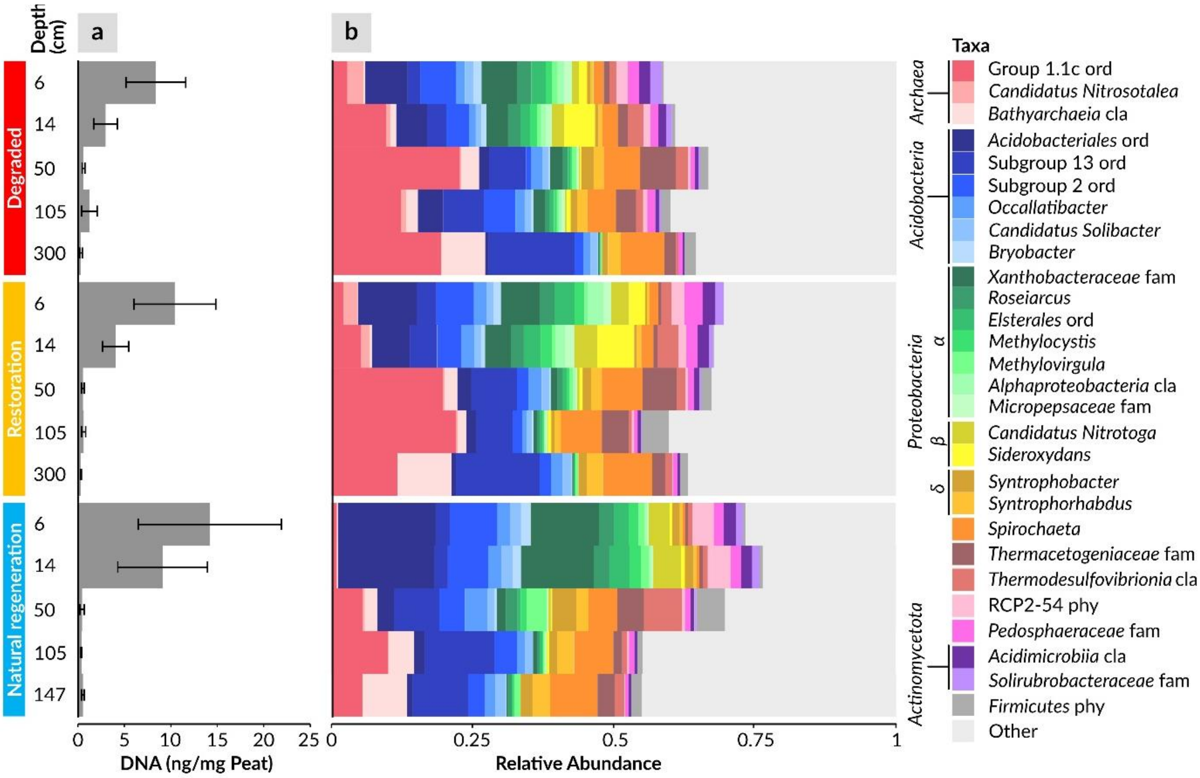

Figure 5 | a Microbial biomass across depths at Waun Fignen Felen. Extracted DNA per g of peat is used as a proxy for biomass, error bars represent standard deviation between samples. **b Microbial community composition.** Mean relative abundance of the most abundant 28 taxa across all samples with taxa ordered by phylum. Where identification at the genus-level was not possible the rank presented is indicated by abbreviation.

3.4 Organic matter quality shapes microbial community structure

To assess the relevance of the geochemical parameters measured in this study on the observed community composition, redundancy analysis (RDA) was employed after selection of the strongest explanatory variables as detailed previously. To assess the role of water table position, the proportion of time for which each sampling depth was below the measured water level ('time wet') was calculated over the preceding 2 weeks. Water level position was not parameterised directly as this is both highly intercorrelated with other depth-related parameters (including lignin proportion, dissolved ammonium and pH), and is less closely related to oxygen availability, the mechanism by which wetting is primarily expected to impact microbes. There was greater difference for time wet among the three sites than was derived from the dip to water level.

The RDA (Fig. 6a) reflected the convergence of the community at depth, with the greatest spread in relation to factor 1. The contrasting communities in the shallow peat were also represented in the RDA with minimal overlap, primarily explained by factor 2. This clustering was the same as that present in non-constrained ordination using NMDS (non-metric multidimensional scaling; Fig. 6b), indicating that the environmental factors included could account for much of this difference. After sequential removal of explanatory variables, the 6 primary environmental factors included in the redundancy analysis (RDA) were the hemicellulose : cellulose ratio, dissolved inorganic N content, lignin : polysaccharide ratio, DOC concentration, pH and proportion of time below the water table (Fig. 6a). The proportion of time below the water table, accounted for less variance than either of the variables relating to peat organic geochemistry. The shallow community under natural regeneration clustered separately along the RDA2 axis, associated most strongly with differences in the hemicellulose : cellulose ratio and DOC concentration. At 50 cm the communities of the three states clustered more closely and were differentiated from the shallow peat community by a spread along the RDA1 axis. This depth-related spread was strongly associated with dissolved inorganic N and the ratio of lignin : polysaccharides. Depth trends in dissolved inorganic N across the site were predominantly derived from elevated ammonium and not oxidised N species (nitrate and nitrite; Supplementary Fig. E) at depth. As a result of the strong depth trend, dissolved inorganic N was the strongest predictor of variance identified (Table 1), but was of limited relevance to the treatment (between-state) effect observed in shallow peat. In unconstrained ordination (NMDS), clustering according to degradation history was also observed, and where the same environmental factors are fitted, a comparable relationship with community composition can be seen (Fig. 6b).

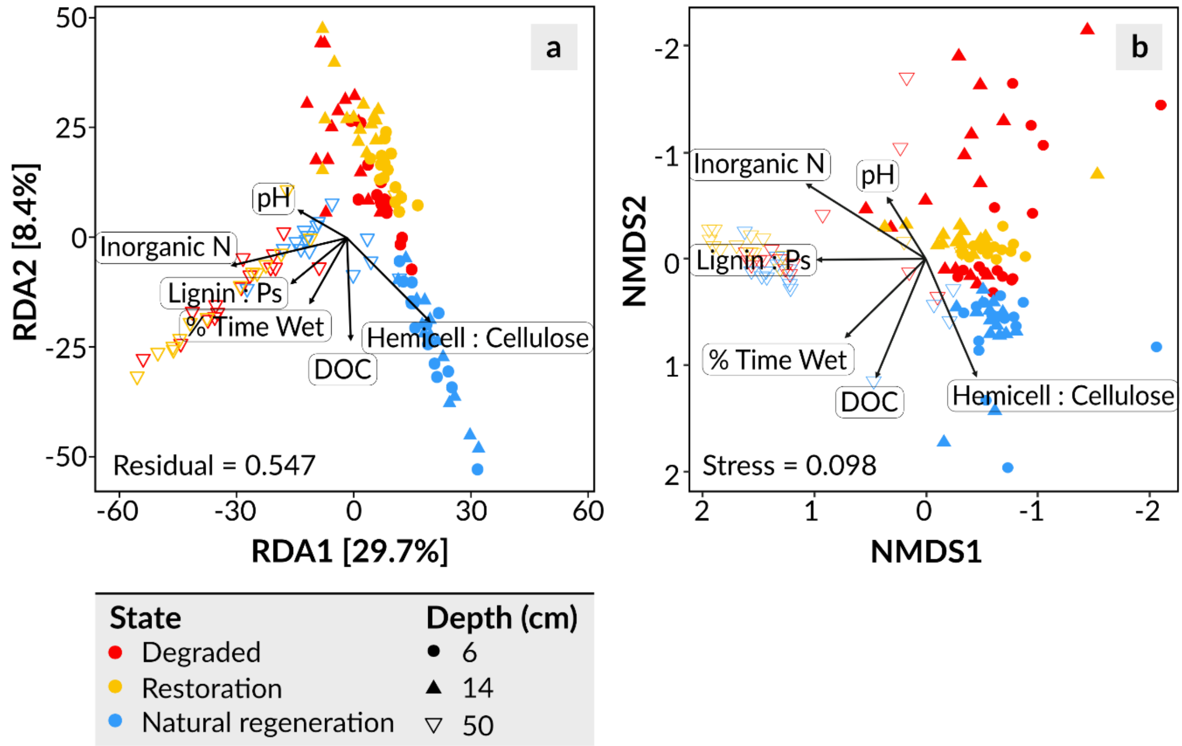

Figure 6 | Geochemical drivers of the microbial community. a Redundancy analysis (RDA) with geochemical parameters that contributed significantly to the explained variation in the ASV abundance.
b Non-metric multidimensional scaling based on distance matrices computed from ASV abundances using the Bray-Curtis method, the same environmental variables selected for the RDA are fitted to the unconstrained ordination using the envfit function in the vegan R package.

Table 1 | Results of ANOVA from redundancy analysis (RDA). Assessment of variables which significantly influenced community composition. All parameters are statistically significant at the $p<0.05$ threshold.

Parameter	Variance (%)	F-statistic	p-Value
Dissolved inorganic N	12.6	23.7	0.001
Hemicellulose : Cellulose	8.1	15.2	0.001
Lignin : Polysaccharide	4.1	7.6	0.002
DOC	3.8	7.2	0.001
pH	1.8	3.3	0.021
Time wet %	1.7	3.2	0.014

The time that peat layers were below the water table did not emerge as a strong driver (Table 1), although this might have been expected given the dominant role of water table in regulating oxygen availability and thus microbial processes[19, 77, 78]. Here, the depth trends in the composition of the microbial community were largely decoupled from the water table differences. If water table was to act as the primary control, the more frequent and deeper drying of the upper profiles in the degraded and restoration areas would be expected to support a stronger vertical gradient in prokaryote community composition. Instead, the natural-regeneration profiles displayed a more marked contrast between the communities of shallow and deep peat despite more stable inundation throughout the profile, with the shallow to deep contrast instead corresponding to the boundary of accumulating and old peat.

Redundancy analysis indicated that in shallow peat, differences between the community composition (spread along RDA2; Fig. 6a), were most strongly explained by the hemicellulose : cellulose ratio, an indicator of a higher supply of relatively fresh plant substrate. Elsewhere the enzyme activity of peatland decomposer communities has been shown to be more sensitive to the quality of surface litter than to water table drawdown[79]. Here we found that community composition followed a similar trend, though in our case, the comparison was between an absence of litter in the degraded area, a thin layer of vegetation under recent restoration (3–5 cm) and thicker layer (5–15 cm) in the area of natural regeneration. Other studies have similarly found bare peat to host to a distinct community relative to long-term vegetated comparisons, and to support lower levels of microbial biomass and activity[80, 81]. We suggest that the higher lability of surface material may sustain the higher microbial biomass observed in the upper profile under natural regeneration, while CO_2 concentrations of more than double those observed in the degraded and restoration areas were another indication of greater microbial respiration. Again, microbial growth in the degraded peat is apparently restricted by factors other than water table position, differences in which were small at the site.

Past mineralisation likely caused the enhanced relative stability of the deep and erosionally exposed peat, with the residual organic matter being less reactive due to the greater proportion of aromatic compounds. Lower respiration and decomposition rates[26, 30, 75], enzyme activity[76], reduced microbial biomass[29, 82] have been attributed to poor substrate quality in more highly decomposed peats, due to a higher aromatic content. Here we find the tight clustering of the deep peat microbiome (Fig. 6) to be strongly aligned with geochemical parameters (dissolved inorganic N content, and lignin : polysaccharide ratio), rather than the water table. Furthermore, in those profiles without or with only a thin regenerated layer, dissolved CO_2 was lower than observed under natural regeneration, which may reflect a lower basal rate of respiration where the peat substrate is of limited availability. Although we examined dissolved CO_2 concentration and not emission, such findings have consequences for the stability and continued release of peat carbon after restoration, and therefore our interpretation of restoration success. They suggest that in systems where past carbon loss was extensive while water tables remain high, the rate of microbial carbon utilisation would be limited as a consequence of altered organic matter quality. It should be noted that this is not evidence of a barrier to future degradation, or a resilience of peatlands to complete remineralisation, as proposed priming effects[61-63], or more extreme drainage than in the peatland studied here could still have the potential to lift the constraints on degradation of otherwise 'recalcitrant' compounds[83].

4. Conclusions

This study investigated differential recovery on former erosional horizons, a widespread feature of UK upland peatlands, by examining the site at Waun Fignen Felen, South Wales. We aimed to identify differences in the composition and availability of peat substrates and determine key drivers of the microbial community composition. We were particularly interested in the competing roles of water table position and vegetation recovery where both processes have occurred in an eroded setting. In this study, differences in water table position were consistent with their degradation history, yet play only a limited role in shaping the microbiome. Instead, the renewal of plant litter input at the peat surface was the predominant control on microbial community composition, with communities in the recovering layer expected to utilise this more bioavailable recent organic matter. Given the stratification of the microbial community with fresh peat accumulation, longer-term recovery (decadal timescales in this study) or transplant of plant communities may be required for microbiome shifts to occur on formerly eroded peat.

Acknowledgements

This work was funded by a Natural Environment Research Council (NERC) PhD studentship at FRESH Centre for Doctoral Training in Freshwater Biosciences and Sustainability (GW4+ FRESH CDT) awarded to F. Ring-Hrubesh [NE/R011524/1]. C. Bryce and F. Ring-Hrubesh are grateful for support from a Royal Society Research Grant [RGS/R2/202221] and the University of Bristol/Research England Policy Support Fund. M. Vreeken and A. Eberle were funded through PhD studentships at the Great Western Alliance Doctoral Training Partnership (NERC GW4+ DTP2) [NE/S007504/1]. We thank NERC for partial funding of the National Environmental Isotope Facility (NEIF; NE/V003917/1) and the project Climate, Energy and

Carbon in Ancient Earth Systems, awarded to R. D. Pancost, selected by the ERC and funded by UKRI [EP/X023214/1].

The authors thank the technical staff in the Schools of Earth Sciences and Geography at the University of Bristol (Fotis Sgouridis, Harry Li-Kam-Tin, Monica Huerta-Lopez, Ioanna Petropoulou); and the wardens of the Bannau Brycheiniog National Park Authority who supported the research fieldwork (Steffan Edwards, Wyn Morgan).

Author contributions

Fin Ring-Hrubesh: conceptualization, methodology, investigation, data curation, formal analysis, visualization, writing – original draft preparation.

Casey Bryce: conceptualization, funding acquisition, methodology, investigation, data analysis, supervision, writing – original draft preparation

Mike Vreeken: methodology, data curation, formal analysis, investigation, writing – review & editing

Anne Eberle: conceptualization, methodology, investigation, writing – review & editing

Rich Pancost: conceptualization, funding acquisition, methodology, data analysis, supervision, writing – review & editing

Angela Gallego-Sala: conceptualization, funding acquisition, methodology, supervision, writing – review & editing

Robert Griffiths: conceptualization, funding acquisition, methodology, data analysis, supervision, writing – review & editing

Bradley Welch: conceptualization, funding acquisition, investigation, supervision, writing – review & editing

Penny Johnes: conceptualization, funding acquisition, methodology, writing – review & editing

Paul Sinnadurai: conceptualization, writing – review & editing

All authors contributed substantially to the article and approved the submitted version.

Competing interests

The authors declare no competing interests.

Data availability

All data displayed in figures 2–5a, and raw geochemical datasets are available at Zenodo (https://doi.org/10.5281/zenodo.17368979). Raw sequencing data have been deposited at NCBI in the Sequence Read Archive (SRA) under BioProject accession number PRJNA1346434 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1346434).

References

1. Yu, Z., Loisel, J., Brosseau, D.P., Beilman, D.W., and Hunt, S.J. Global peatland dynamics since the Last Glacial Maximum. *Geophysical Research Letters*. **37**:L13402 (2010).
2. Yu, Z.C. Northern peatland carbon stocks and dynamics: a review. *Biogeosciences*. **9**:4071-4085 (2012).
3. Hugelius, G.*, et al.* Large stocks of peatland carbon and nitrogen are vulnerable to permafrost thaw. *Proceedings of the National Academy of Sciences*. **117**:20438-20446 (2020).
4. Evans, C.*, et al.* Implementation of an Emissions Inventory for UK Peatlands. *Report to the Department for Business, Energy and Industrial Strategy, Centre for Ecology and Hydrology, Bangor*. (2017).
5. Evans, M. and Warburton, J. Fluvial Processes and Peat Erosion in *Geomorphology of Upland Peat* (eds., M. Evans and J. Warburton) 76-103 (Royal Geographical Society, 2007).
6. Parry, L.E., Holden, J., and Chapman, P.J. Restoration of blanket peatlands. *Journal of Environmental Management*. **133**:193-205 (2014).
7. Smart, S.M.*, et al.* Impacts of pollution and climate change on ombrotrophic Sphagnum species in the UK: analysis of uncertainties in two empirical niche models. *Climate Research*. **45**:163-176 (2010).
8. Larmola, T.*, et al.* Vegetation feedbacks of nutrient addition lead to a weaker carbon sink in an ombrotrophic bog. *Global Change Biology*. **19**:3729-3739 (2013).
9. Yeloff, D.E., Labadz, J., and Hunt, C. Causes of degradation and erosion of a blanket mire in the southern Pennines, UK. *Mires and Peat*. **1**:04 (2006).
10. Worrall, F.*, et al.* Carbon fluxes from eroding peatlands – the carbon benefit of revegetation following wildfire. *Earth Surface Processes and Landforms*. **36**:1487-1498 (2011).
11. Shuttleworth, E.L., Evans, M.G., Hutchinson, S.M., and Rothwell, J.J. Peatland restoration: controls on sediment production and reductions in carbon and pollutant export. *Earth Surface Processes and Landforms*. **40**:459-472 (2015).
12. Foulds, S.A. and Warburton, J. Significance of wind-driven rain (wind-splash) in the erosion of blanket peat. *Geomorphology*. **83**:183-192 (2007).
13. Groeneveld, E.V.G. and Rochefort, L. Polytrichum Strictum as a Solution to Frost Heaving in Disturbed Ecosystems: A Case Study with Milled Peatlands. *Restoration Ecology*. **13**:74-82 (2005).
14. Aspray, K.L., Holden, J., Ledger, M.E., Mainstone, C.P., and Brown, L.E. Organic sediment pulses impact rivers across multiple levels of ecological organization. *Ecohydrology*. **10**:e1855 (2017).

15. Goulsbra, C.S., Evans, M.G., and Allott, T.E.H. Rates of CO2 efflux and changes in DOC concentration resulting from the addition of POC to the fluvial system in peatlands. *Aquatic Sciences*. **78**:477-489 (2016).
16. Pawson, R.R., Evans, M.G., and Allott, T.E.H.A. Fluvial carbon flux from headwater peatland streams: significance of particulate carbon flux. *Earth Surface Processes and Landforms*. **37**:1203-1212 (2012).
17. Evans, M. and Lindsay, J. The impact of gully erosion on carbon sequestration in blanket peatlands. *Climate Research*. **45**:31-41 (2010).
18. Huang, Y., *et al.* Tradeoff of CO2 and CH4 emissions from global peatlands under water-table drawdown. *Nature Climate Change*. **11**:618-622 (2021).
19. Evans, C.D., *et al.* Overriding water table control on managed peatland greenhouse gas emissions. *Nature*. **593**:548-552 (2021).
20. Evans, C.D., Renou-Wilson, F., and Strack, M. The role of waterborne carbon in the greenhouse gas balance of drained and re-wetted peatlands. *Aquatic Sciences*. **78**:573-590 (2016).
21. Ritson, J.P., *et al.* The effect of drought on dissolved organic carbon (DOC) release from peatland soil and vegetation sources. *Biogeosciences*. **14**:2891-2902 (2017).
22. Bernard-Jannin, L., *et al.* Hydrological control of dissolved organic carbon dynamics in a rehabilitated Sphagnum-dominated peatland: a water-table based modelling approach. *Hydrology and Earth System Sciences*. **22**:4907-4920 (2018).
23. Tfaily, M.M., *et al.* Vertical Stratification of Peat Pore Water Dissolved Organic Matter Composition in a Peat Bog in Northern Minnesota. *Journal of Geophysical Research: Biogeosciences*. **123**:479-494 (2018).
24. Verbeke, B.A., *et al.* Latitude, Elevation, and Mean Annual Temperature Predict Peat Organic Matter Chemistry at a Global Scale. *Global Biogeochemical Cycles*. **36**:e2021GB007057 (2022).
25. Artz, R.R.E., Chapman, S.J., and Campbell, C.D. Substrate utilisation profiles of microbial communities in peat are depth dependent and correlate with whole soil FTIR profiles. *Soil Biology and Biochemistry*. **38**:2958-2962 (2006).
26. Leifeld, J., Steffens, M., and Galego-Sala, A. Sensitivity of peatland carbon loss to organic matter quality. *Geophysical Research Letters*. **39**:14704 (2012).
27. Robinson, C.H., *et al.* Aspects of microbial communities in peatland carbon cycling under changing climate and land use pressures. *Mires and Peat*. **29: 2** (2023).
28. Andersen, R., Chapman, S.J., and Artz, R.R.E. Microbial communities in natural and disturbed peatlands: A review. *Soil Biology and Biochemistry*. **57**:979-994 (2013).
29. Könönen, M., *et al.* Deforested and drained tropical peatland sites show poorer peat substrate quality and lower microbial biomass and activity than unmanaged swamp forest. *Soil Biology and Biochemistry*. **123**:229-241 (2018).
30. Schimmel, H., Braun, M., Subke, J.-A., Amelung, W., and Bol, R. Carbon stability in a Scottish lowland raised bog: potential legacy effects of historical land use and implications for global change. *Soil Biology and Biochemistry*. **154**:108124 (2021).
31. Smith, A.G. and Cloutman, E.W. Reconstruction of Holocene vegetation history in three dimensions at Waun-Fignen-Felen, an upland site in South Wales. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*. **322**:159-219 (1988).
32. Dresser, Q. University College Cardiff radiocarbon dates I. *Radiocarbon*. **27** (1985).
33. Bowes, H.L. and Hornibrook, E.R.C. Emission of highly 13C-depleted methane from an upland blanket mire. *Geophysical Research Letters*. **33**:04401 (2006).
34. Hesslein, R.H. An in situ sampler for close interval pore water studies1. *Limnology and Oceanography*. **21**:912-914 (1976).
35. Kling, G.W., Kipphut, G.W., and Miller, M.C. The flux of CO2 and CH4 from lakes and rivers in arctic Alaska. *Hydrobiologia*. **240**:23-36 (1992).
36. NIST, NIST chemistry web book. 2023, National Institute of Standards and Technology, Gaithersburg, USA.
37. Xu, J., Morris, P.J., Liu, J., and Holden, J. PEATMAP: Refining estimates of global peatland distribution based on a meta-analysis. University of Leeds. [Dataset]. (2017).

38. Schellekens, J., *et al.* Influence of source vegetation and redox conditions on lignin-based decomposition proxies in graminoid-dominated ombrotrophic peat (Penido Vello, NW Spain). *Geoderma*. **237-238**:270-282 (2015).
39. Chen, H., *et al.* Integration of an automated identification-quantification pipeline and statistical techniques for pyrolysis GC/MS tracking of the molecular fingerprints of natural organic matter. *Journal of Analytical and Applied Pyrolysis*. **134**:371-380 (2018).
40. Nowakowski, D.J., Woodbridge, C.R., and Jones, J.M. Phosphorus catalysis in the pyrolysis behaviour of biomass. *Journal of Analytical and Applied Pyrolysis*. **83**:197-204 (2008).
41. Klein, K., Gross-Schmölders, M., De la Rosa, J.M., Alewell, C., and Leifeld, J. Investigating the influence of instrumental parameters and chemical composition on pyrolysis efficiency of peat. *Communications in Soil Science and Plant Analysis*. **51**:1572-1581 (2020).
42. Pouwels, A.D., Eijkel, G.B., and Boon, J.J. Curie-point pyrolysis high-resolution gas chromatography–mass spectrometry of microcrystalline cellulose. *Journal of Analytical and Applied Pyrolysis*. **14**:237–280 (1989).
43. Hodgkins, S.B., *et al.* Tropical peatland carbon storage linked to global latitudinal trends in peat recalcitrance. *Nature Communications*. **9**:3640 (2018).
44. Caporaso, J.G., *et al.* Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences*. **108**:4516-4522 (2011).
45. Thompson, L.R., *et al.* A communal catalogue reveals Earth's multiscale microbial diversity. *Nature*. **551**:457-463 (2017).
46. Callahan, B.J., *et al.* DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods*. **13**:581-3 (2016).
47. Quast, C., *et al.* The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*. **41**:D590-D596 (2012).
48. McMurdie, P.J. and Holmes, S. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLOS ONE*. **8**:e61217 (2013).
49. James, G., Witten, D., Hastie, T., and Tibshirani, R. *An Introduction to Statistical Learning* (Springer New York, 2013).
50. Oksanen, J., *et al.* vegan: Community Ecology Package. *v 2.6-6.1. R package. Available: https://CRAN.R-project.org/package=vegan*. (2024).
51. Rodwell, J.S. *British plant communities: Volume 2, Mires and Heaths* (Cambridge University Press, 1998).
52. Wang, M., Moore, T.R., Talbot, J., and Riley, J.L. The stoichiometry of carbon and nutrients in peat formation. *Global Biogeochemical Cycles*. **29**:113-121 (2015).
53. Loisel, J., *et al.* A database and synthesis of northern peatland soil properties and Holocene carbon and nitrogen accumulation. *The Holocene*. **24**:1028-1042 (2014).
54. Crowe, S., Evans, M., and Allott, T. Geomorphological controls on the re-vegetation of erosion gullies in blanket peat: implications for bog restoration. *Mires & Peat*. **3** (2008).
55. Milner, A.M., *et al.* A regime shift from erosion to carbon accumulation in a temperate northern peatland. *Journal of Ecology*. **109**:125-138 (2021).
56. Biester, H., Knorr, K.H., Schellekens, J., Basler, A., and Hermanns, Y.M. Comparison of different methods to determine the degree of peat decomposition in peat bogs. *Biogeosciences*. **11**:2691-2707 (2014).
57. Darusman, T., Murdiyarso, D., Impron, and Anas, I. Effect of rewetting degraded peatlands on carbon fluxes: a meta-analysis. *Mitigation and Adaptation Strategies for Global Change*. **28**:10 (2023).
58. Evans, M.G., *et al.* Carbon Loss Pathways in Degraded Peatlands: New Insights From Radiocarbon Measurements of Peatland Waters. *Journal of Geophysical Research: Biogeosciences*. **127** (2022).
59. Strack, M., Zuback, Y., Mccarter, C., and Price, J. Changes in dissolved organic carbon quality in soils and discharge 10 years after peatland restoration. *Journal of Hydrology*. **527**:345-354 (2015).

60. Pinsonneault, A.J., Moore, T.R., Roulet, N.T., and Lapierre, J.-F. Biodegradability of Vegetation-Derived Dissolved Organic Carbon in a Cool Temperate Ombrotrophic Bog. *Ecosystems*. **19**:1023-1036 (2016).
61. Hamer, U. and Marschner, B. Priming effects of sugars, amino acids, organic acids and catechol on the mineralization of lignin and peat. *Journal of Plant Nutrition and Soil Science*. **165**:261-268 (2002).
62. Basiliko, N., Stewart, H., Roulet, N.T., and Moore, T.R. Do Root Exudates Enhance Peat Decomposition? *Geomicrobiology Journal*. **29**:374-378 (2012).
63. Jilling, A., Keiluweit, M., Gutknecht, J.L.M., and Grandy, A.S. Priming mechanisms providing plants and microbes access to mineral-associated organic matter. *Soil Biology and Biochemistry*. **158**:108265 (2021).
64. Ivanova, A.A.*, et al.* Closely Located but Totally Distinct: Highly Contrasting Prokaryotic Diversity Patterns in Raised Bogs and Eutrophic Fens. *Microorganisms*. **8**:484 (2020).
65. Dedysh, S.N.*, et al.* Acidobacteria in Fens: Phylogenetic Diversity and Genome Analysis of the Key Representatives. *Microbiology*. **91**:662-670 (2022).
66. Golovchenko, A.V.*, et al.* Diversity and Functional Potential of Prokaryotic Communities in Depth Profile of Boreo-Nemoral Minerotrophic Pine Swamp (European Russia). *Forests*. **14**:2313 (2023).
67. Zubov, I., Shpanov, D., Ponomareva, T., and Aksenov, A. Bog bacterial community: data from north-western Russia. *Biodivers Data J*. **12**:e118448 (2024).
68. Carroll, R., Jeffries, T.C., and Reynolds, J.K. Sulfur Regulation on Microbial Biodiversity in a Montane Peatland. *European Journal of Soil Science*. **76**:e70045 (2025).
69. Oren, A. The Family Xanthobacteraceae in *The Prokaryotes: Alphaproteobacteria and Betaproteobacteria* (eds., E. Rosenberg, E.F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson) 709-726 (Springer Berlin Heidelberg, 2014).
70. Leschine, S., Paster, B.J., and Canale-Parola, E. Free-Living Saccharolytic Spirochetes: The Genus Spirochaeta in *The Prokaryotes: Volume 7: Proteobacteria: Delta, Epsilon Subclass* (eds., M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer, and E. Stackebrandt) 195-210 (Springer New York, 2006).
71. Aylward, F.O. and Santoro, A.E. Heterotrophic Thaumarchaea with Small Genomes Are Widespread in the Dark Ocean. *mSystems*. **5** (2020).
72. Biddle, J.F.*, et al.* Heterotrophic Archaea dominate sedimentary subsurface ecosystems off Peru. *Proceedings of the National Academy of Sciences*. **103**:3846-3851 (2006).
73. Evans, P.N.*, et al.* Methane metabolism in the archaeal phylum Bathyarchaeota revealed by genome-centric metagenomics. *Science*. **350**:434-438 (2015).
74. Yu, T.*, et al.* Growth of sedimentary Bathyarchaeota on lignin as an energy source. *Proceedings of the National Academy of Sciences*. **115**:6022-6027 (2018).
75. Jayasekara, C., Leigh, C., Shimeta, J., Silvester, E., and Grover, S. Organic matter decomposition in mountain peatlands: effects of substrate quality and peatland degradation. *Plant and Soil*. **506**:639-654 (2025).
76. Preston, M.D., Smemo, K.A., McLaughlin, J.W., and Basiliko, N. Peatland microbial communities and decomposition processes in the James Bay Lowlands, Canada. *Frontiers in Microbiology*. **3**:70 (2012).
77. Russow, R.*, et al.* Nitrate turnover in a peat soil under drained and rewetted conditions: results from a [15N] nitrate–bromide double-tracer study. *Isotopes in Environmental and Health Studies*. **49**:438-453 (2013).
78. Wiedermann, M.M., Kane, E.S., Potvin, L.R., and Lilleskov, E.A. Interactive plant functional group and water table effects on decomposition and extracellular enzyme activity in Sphagnum peatlands. *Soil Biology and Biochemistry*. **108**:1-8 (2017).
79. Straková, P.*, et al.* Litter type affects the activity of aerobic decomposers in a boreal peatland more than site nutrient and water table regimes. *Biogeosciences*. **8**:2741-2755 (2011).
80. Elliott, D.R., Caporn, S.J.M., Nwaishi, F., Nilsson, R.H., and Sen, R. Bacterial and Fungal Communities in a Degraded Ombrotrophic Peatland Undergoing Natural and Managed Re-Vegetation. *PLOS ONE*. **10**:e0124726 (2015).

81. Andersen, R., Wells, C., Macrae, M., and Price, J. Nutrient mineralisation and microbial functional diversity in a restored bog approach natural conditions 10 years post restoration. *Soil Biology and Biochemistry*. **64**:37-47 (2013).
82. Mpamah, P.A., Taipale, S., Rissanen, A.J., Biasi, C., and Nykänen, H.K. The impact of long-term water level draw-down on microbial biomass: A comparative study from two peatland sites with different nutrient status. *European Journal of Soil Biology*. **80**:59-68 (2017).
83. Chen, X., *et al.* Drainage-Driven Loss of Carbon Sequestration of a Temperate Peatland in Northeast China. *Ecosystems*. **27**:207-221 (2024).