Anaerobic respiration and temperature response along a boreal hydrological transect on a slope from upland forest to peatland

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Key Points:

- We found that the quantity of carbon in the soil sample material was the strongest predictor of measured anaerobic CO_2 production
- Response to temperature treatment varied, indicating that temperature was not a limiting factor to anaerobic CO_2 production in these soils
- Our results emphasize the inclusion of both soil carbon quantity and quality in large scale modeling efforts

Abstract

Climatic warming is predicted to affect high-latitude habitats, such as boreal peatlands, at a larger magnitude than the global average**.** The controls on the breakdown of organic matter in peatlands are complex; it's unclear how climatic warming will affect the stability of the large carbon pool that's currently stored in peatlands. To investigate this, we collected soil cores from three boreal habitats along a hydrological transect (Bog, Intermediate, and Upland Forest) in Finland, and incubated ex-situ for 140 days. Each soil horizon was incubated in three temperatures (0°C, 4°C, 20°C). Here, we found the Intermediate site had the largest CO_2 production considering the entirety of the soil column (per gram dry weight). Statistical analysis found that sample C content was the most indicative independent variable to predict sample CO_2 production. Each soil horizon displayed a different magnitude of response to the temperature incubations (Q_{10}s ranged from 0.60-2.33), and through microbial relative abundance analysis we found that the microbial community structure had significant differences between both habitat and depth of sample origin. Coupling these methods, and the fine scale of the both vertical (soil column horizons) and horizontal (along a hydrological gradient through distinct habitats) transects gives us a novel perspective on the controls of microbial respiration rates. Our results stress that large scale modeling efforts of carbon dynamics should prioritize both soil carbon quantity and quality.

Plain Language Summary

Climate change is affecting northern regions more than other parts of the world. Peatlands in these areas, especially in boreal forests like the studied one in Finland, store a lot of carbon. We were studying three different types of habitats on a slope: a wet bog, an area with scattered trees, and a mature forest. While we know that soil respiration (how fast microbes release carbon) increases with temperature, we are not sure how this works in settings like peatlands. Our goal is to fill this knowledge gap by studying how different habitats affect greenhouse gas production. We incubated soil samples from these habitats at different temperatures for 140 days and analyzed the microbes present. Our findings showed that the amount of carbon in the soil was the biggest factor influencing greenhouse gas production over time. This research helps understanding how carbon is released from soils, which is important for predicting and mitigating climate change effects. Data from this study can be used to contribute information to global soil carbon stock modeling efforts, and improve climate predictions.

1 Introduction

Wetlands play a significant role in the global carbon cycle as substantial carbon sinks (Yu et al., 2010; Bridgham et al., 2006). They contain roughly a third of the world's soil carbon, while only covering 5-8% of the Earth's surface (Mitch and Gosselink 2007). Peatlands are a type of wetlands that can be found globally, but are primarily in high-latitude zones. Of the estimated ~530 Pg of C in peatlands globally, over 80% is stored in northern peatland systems (Hugelius et al., 2020). Peatlands efficiently use carbon from the atmospheric pool and sequester it in the terrestrial carbon pool through the slowed decomposition of vegetative organic matter, enabled by the acidic and waterlogged conditions within the peatland (Clymo 1987). Most

peatlands are found at high-latitudes, and many in the boreal vegetation zone. However, many of the environmental-scale controls contributing to the sustained functionality of high-latitude wetlands, such as water table and vegetation, are predicted to undergo rapid change, with the progression of changing climatic conditions.

On a global scale, temperatures at high latitudes increase more rapidly than those at lower latitudes, generally causing greater disturbance to the seasonal cycle of freeze and thaw that high-latitude peatlands undergo annually (Kirtman et al., 2013; Byun et al., 2021). It remains uncertain how exactly climate warming will affect the carbon flux of northern wetland soils and vegetation, but generally it is agreed that these large carbon sinks have the potential to turn into a significant global net carbon source (Hanson et al 2020; Frolking et al., 2011; IPCC 2023). Peatland organic soil stability is heavily influenced by variables including regional climate, land use in the surrounding area, vegetation and peat chemical composition (Hodgkins et al 2018; Keiluweit et al., 2016; Byun et al., 2021; Clymo and Hayward 1982; Crowther et al., 2016), all factors that are projected to undergo significant change with global climatic warming.

Previous research has generally agreed that peatland substrate responds to increased heat and moisture with increased net greenhouse gas (GHG) production rates, especially in high-latitude environments (Crowther et al., 2016; Grosse et al., 2011). Although, increased net primary productivity from the increase in heat may help balance the net carbon loss. Several ecosystem-scale factors can influence the scale of the net gain and loss of terrestrial carbon - the relative importance of each factor on both local and global scales remain as knowledge gaps in the existing literature and warrants further research (Davidson and Janssens 2006). The temperature response of (sub)arctic soil is generally poorly understood, and hardly follows the textbook knowledge of a temperature reaction rate of Q_{10} equaling 2 (Davidson and Janssens 2006). A commonly used method of estimating carbon turnover and warming potential of soils are laboratory incubations. Laboratory (ex-situ) incubations are able to quantify the stability of organic molecules in soils, while being able to manipulate one dependent variable for each experimental group. They have also shown to preserve the microbial communities, even considering the limitations of a laboratory setting to organic matter C processing, such as the disturbance to the microbial community from field collection and shipment (Wilson et al., 2021). Given the predicted warming climatic trend, the exact quantifications of organic matter (OM) response to temperature is of high interest to further refine climatic warming model predictions. With current earth system models for future climate predictions, the soil carbon-climate feedback is significantly underrepresented as the controls on soil C respiration are yet to be fully understood (Ren et al., 2024).

Ex-situ incubations also allow for the isolation of each soil horizon to be tested individually against temperature changes. In most soils, but especially peat bogs and the surrounding habitats, the water table is an omnipresent determinant of the favorable metabolic processes and can be used at each horizon. Water slows gas exchange within the soil matrix, and in unsaturated conditions the most favorable electron receptor (O_2) is able to diffuse within the soil and spur OM decomposition. Studying soil columns layer-by-layer can show us the exact location of the most vulnerable carbon stocks and better predict the vulnerability of the system as a whole to changing climatic variables. In peatlands, soil horizons are defined by the water table height: the largely aerobic acrotelm (‘top peat’), anaerobic catotelm (‘bottom peat’) and most

recently coined mesotelm (space between the top and bottom peat where the natural fluctuation of the water table periodically submerge the soil (Clymo and Bryant 2008)) compose the peatland soil column (Ingram 1978). These distinctions have been made as the depth group that peatland soils are in govern the metabolic processes available to microbes within the soil matrix and govern the rate at which these processes can happen. In boreal peatlands, these distinct soil layers have been shown to remain interconnected through pore water flow supplying dissolved organic matter interstitially in both vertical and horizontal directions, with distinct molecular moiety differences from each soil horizon source (Chanton et al., 2008; Tfaily et al., 2018). Therefore, the depth from surface, and distance from the water table can be used as an indicator as to the degree to which the organic matter is degraded (Frolking et al., 2010).

Often overlooked in incubation studies is peatland soil's carbon lability in anaerobic environments, despite the availability of oxygen being one of the most important ecosystem scale controls on the microbial respiration pathways. Most studies have focused only on the surface of the soil, the acrotelm, and the largely oxic environment the top of the soil column is adapted for (Schädel et al., 2016, 2020; Kolton et al., 2019; Ren et al., 2024). However, fluctuating precipitation patterns will affect the hydrology of boreal habitats and consequently, the soil moisture (synonymous with ease of which oxygen can circulate within the soil column) is also predicted to undergo regional changes (Ruosteenoja and Jylhä 2021). Additionally, annual freeze-thaw dynamics are predicted to respond to climate change by increasing in intensity (temperature / precipitation extremes) and shortening of the shoulder seasons, affecting the biogeochemical cycles of the soil. Peatlands transitioning from summer to shoulder-season dynamics has been shown to potentially be a significant source of atmospheric C flux (Treat et al., 2018; Song et al., 2017). Here, these regional changes could push the soils to a more anaerobic environment under layers of compacted rain-on-snow cover for extended periods of the year. To better understand the potential metabolic pathways soil microbes use in response shifting ecosystem-scale controls, additional experimental approaches aimed at exploring anaerobic pathways are essential to fill in these knowledge gaps.

Notably, the ecotone between the well-drained Upland Forest and the water saturated bog is included as an Intermediate margin zone. These habitats have been observed to be hotspots for biodiversity, though the hydrology and geochemistry of these zones have largely yet to be explored (Whitfield et al 2009; Korpela 2004; Paradis et al., 2015; Langlois et al., 2015). This knowledge gap in literature is largely hypothesized to be due to the relative difficulty of confidently delineating a habitat with considerable diversity, the relatively small areal coverage of the habitat zone, and geoecology experiments traditionally tending to prefer the homogeneity of larger systems (Fortin et al., 2000). Peatland – forest intermediate habitats, are known to be more sensitive to surrounding disturbances (examples include nearby agriculture development, ditching, or beaver behavior (Johnston and Naiman 1987)) but very little is known about the margin itself, and even less about the potential carbon cycling dynamics (Howie and van Meerveld 2011). Climate change linked alternation (both drought and flood conditions) in the hydrology of high-latitude systems from climate change is actually already detectable (Zhang et al., 2022). However, few studies can be found about the biogeochemical cycles within this habitat; an issue that is mentioned in previous literature but remains to be addressed (Whitfield et al., 2009; Langlois et al., 2015; Dimitrov et al., 2014).

Here, we use a full-factorial experimental soil incubation to determine the temperature response of anaerobic decomposition and microbial and soil quality controls. By studying the Siikaneva peatland and its surrounding habitats, at each soil layer, we aim to pinpoint the most productive microbial communities, and the soil conditions that support them. We identify and refer to the habitat zones here forth as: : *Sphagnum*-dominated Bog, Intermediate, and Upland Forest. This paper quantifies anaerobic CO_2 production, coupled with microbial relative abundance with samples taken along a water-gradient-driven habitat transect from the Bog to Upland Forest discern the response of the soil biogeochemistry cycling to ecosystem scale controls.

The objective of this study was to characterize the microbial response to different temperature treatments, using soils from boreal habitats along a hydrologic gradient, and at each soil horizon, to test effects of environmental, microbial, and soil properties. We hypothesized that we would see positive associations between soil carbon content and CO_2 production, as well as a positive relationship between CO_2 production and the three incubation temperatures. We test our hypotheses by isolating ecosystem-scale controls and quantify the microbial respiration from each soil horizon at each of the three incubation temperatures. The relative differences in production rates in response to laboratory incubation temperature (Q_{10}) directly improve our understanding of how vulnerable these dynamic high-latitude wetland systems will be to the global warming trend.

2 Materials and Methods

Our study site lies within Finland (Suomi). Finland has the highest proportion of peatlands in the world (32% of the total land area) and is unique in the fact that all major bog types can be found within the country. The entirety of the study site is within the Siikaneva peatland and surrounding habitats, in south-western Finland. Siikaneva bog is ombrogenous (acidic, precipitation-fed) wetland preserve surrounded by boreal forest. Here we identify three distinct habitats delineated by vegetation cover and soil types, driven by water table levels. Previous research identifies the Upland Forest, and Bog sites as symbiotic systems but this study aims to also include the Intermediate site that functionally acts as an ecotone between the two, characterized by shade-tolerant and dense understory (Howie and van Meerveld 2011; Korpela et al., 2004; Dimitrov et al., 2014; Ťupek et al., 2008). This ecotone fosters high biodiversity in its shrub-dominant vegetation, in part due to the hydro-topography of the slope carrying nutrient-rich water from the Upland Forest to the soils bordering the Bog. Here, we look at the peatland-to-forest system to better understand the biogeochemical cycles both across the hydrologic gradient and within each habitats soil column.

2.1 Site Description

Siikaneva peatland and the surrounding forested habitat (61.838440 °N, 24.171650 °E) is located in western Finland, within the boreal vegetation zone (Ahti, Hämet-Ahti, & Jalas, 1968). The site was chosen for the combination of the established research infrastructure in this remote habitat. Western Finland experiences daily temperature highs of 0°C in the winters and between 10 and 25°C in the summer growing season (lasting approx. 140 days, the length chosen for this incubation experiment). Annually, the region averaged 4.9°C and 58.2 mm of monthly precipitation in the last ten years from August 2011 to our sampling year of August 2021 (Finnish Meteorological Institute: https://en.ilmatieteenlaitos.fi/, accessed 2023). Soil core

samples were taken along a water gradient from the Upland Forest, along the slope down to the Bog (Fig. 1). Three sites are as follows:

The Bog site had some stunted Scots pines (*Pinus sylvestris*) on the raised peat hummocks, but was otherwise open. Being composed almost homogenously of peat, the soil throughout was typical of a Sphagnofibrist histosol with a high acidity, low bulk density, continuous saturation and more than 75% *Sphagnum* content in the top 90cm of the soil column (Nachtergaele et al., 2001). The *Sphagnum* moss communities varied in their patchwork patterns of hummocks, lawns and hollows. We sampled from a lawn composed of *Sphagnum papillosum, S. magellanicum and S. balticum* and some sedges, including from genus *Eriophorum.* Further vegetation details and descriptions of Siikaneva peatland can be found in Korrensalo et al., (2016, 2017) and Korpela et al 2020. The top of the water table was visible from the boardwalk, and the peat layer has been found to be approximately 2m thick.

Soil found in the Intermediate habitat were histosols, with partially decomposed plant fibers throughout (JRC European Commission, 2010). Although this site was above the water table, the soil was very moist, and spongey to the touch. Previous field campaigns found the water table to be between 25-35cm below surface. In this margin site, the Scots Pines grew further apart and appeared somewhat stunted in growth, with a strong presence of understory shrub plant community, mostly consisting of blueberry (*Vaccinium myrtillus*). The ground was less sloped and was covered by some *Sphagnum*, and some feather mosses (*Hylocomium splenens*).

The Upland Forest site is characterized by a canopy of Scots Pines (*Pinus sylvestris*) growing on haplic podzol soil. The cores here were subdivided into three horizons characteristic of a podzol with an organic, eluvial, and a bottom sandy horizon that decrease in acidity with depth (JRC European Commission, 2010) The sample site had sparse understory, but some blueberry bush (*Vaccinium myrtillus*) and ferns (*Dryopteris dilatata*), punctuated with protruding granite boulders and ground lichens (Cladonia spp.). Open space between trees with direct access to the top of the shade-adapted feather mosses (*Hylocomium splenens*),was abundant. The entirety of the sampling site was at a slight angle towards the Bog.

2.2 Sample Collection

Material was collected during late summer, around peak growing season in mid-August of 2021. Collection of material in Siikaneva was chosen to be near, but far enough removed to not affect the cluster of automatic and manual chamber measurement sites where data collection has been continuously for field flux measurements since 2021.

Before coring, the Eijelkamp peat corer's surface was wiped with ethanol and kimwipes, then air-dried. Air temperature, field notes, and soil temperature were recorded before the start of coring. Samples were taken from surface to bedrock in the Upland Forest and Intermediate site. In the bog, bedrock could be found no shallower than about 2 meters deep.

Four replicate cores each were taken at the bog and Intermediate sites, and six replicates were taken from the Upland Forest site. The additional cores in the forest were taken due to the site having a significantly shallower soil layer before encountering bedrock. In the field, bagged samples were stored in a portable cooler. They were then frozen at -20°C in the dark for storage, until arrival at AWI Potsdam.

2.3 Geochemical laboratory analysis

After transport back to the laboratories, we combined the spatial replicates in each habitat by horizon. Subsamples of each soil horizon were taken for soil descriptive analytics. Results can be seen in Table 1, 2. Samples were freeze-dried, homogenized to a powder, and analyzed in duplicate on the carbon analyzer (soliTOC, Elementar Analysensysteme – AWI Potsdam Carbon and Nitrogen Laboratory) for total organic C. For total nitrogen (N) we used a rapid N exceed (Elementar Analysensysteme, Germany) for generating the data. The pH of samples was taken from each replicate's pore water at the conclusion of the experiment. Bulk density was determined using the weight of the horizon's subsample and the known volume of the Eijelkamp peat corer for each core (n=4 for the Bog and Intermediate habitats, and n=6 for the Upland Forest site).

2.4 Incubation Methods

To begin the incubation, the samples were thawed from -20°C to 4°C and each site's soil horizons were gently homogenized together in anoxic conditions, then separated into pool replicates of each horizon to reduce heterogeneity between the spatial replicate cores. Each sterile 120mL borosilicate vial received approximately 10g wet weight of the homogenate sample with 5mL of autoclaved tap water to create an anaerobic slurry. Samples were capped with sterile rubber septa, and crimped with aluminum seals. Vials remained sealed for the duration of the experiment to maintain constant moisture and the closure of the active microcosm system. Three temperature treatments (0, 4, 20°C) were introduced to the sample material as soon as the vials were capped, and they remained in the temperature incubator, except for brief GC headspace sampling. Blanks were also made that consisted solely of autoclaved tap water were made and ran in parallel, stored at the 4°C temperature incubator.

Sample preparation happened in Don Whitley MACS MG-500 Anaerobic Chamber Workstation with a constantly circulating, oxygen-free (N_2) headspace. Thawed material was gently weighed and placed into its labeled vial with gloved hands inside the Anaerobic Workstation. The samples were then capped and sealed with rubber septa and metal crimps to ensure an airtight seal. The samples were flushed with N_2 within a day after sealing, to ensure an oxygen free headspace. Aliquots of sample from the freshly thawed material was set aside and stored for microbial community composition, C and N analysis, water content measurement, and archive material (Wilson et al., 2021; Corbett et al., 2013; Schädel et al., 2020).

An equilibration period (25 days) in the experimental temperature to allowed the sample to adjust to the temperature treatment and microbially exhaust any oxygen or other terminal electron receptors that may have been introduced during setup (Wilson et al., 2021). The equilibration temperatures were the same as those for the full 140 day run of the experiment: 0, 4, and 20°C. After an initial first week of sampling on days 0, 1, 3, and 7 the samples were measured once per week, then regularly after the first month. The vials were flushed as needed once the headspace reached 1,000ppm CO_2 to represent field conditions over the 140-day sampling period. The headspace gas was analyzed with a Agilent Technologies 7890A GC System starting from the initial measurement. The same GC system was used throughout, with the same settings (column temperature was kept at 50°C, and helium was the carrier gas in the GC System). Before each vial was sampled, it was sterilized with ethanol and enflamed to ensure the headspace and sample maintained a sterile environment.

The GC System's output is given in units of ppmv of the injected headspace sampled. Production rate of CO_2 was determined by the difference in GHG concentration in vial headspace from one measurement, to the next and divided by the difference in days between measurement. We began by taking the GC System output (ppmv) and applied the Ideal Gas Law correction. Then, we corrected for the volume of the sample with the added water, from the total 120mL vial headspace volume. This corrected value then is also used to subtract the previous measurements flush residual, when the previous vial had measured $CO_2 \geq$ 1,000 ppm in the headspace of the vial. On "flush" days, the sample was measured, flushed with N_2, and measured again within the same hour. With the values corrected for the flush residual, the values were then converted from per unit vial, and then to per gram dry weight of sample inside each vial. Henry's Law was applied as autoclaved water had been added to the sample to ensure anaerobic conditions in the headspace. The aqueous CO_2 is accounted for in the values used. Values were then normalized to gram dry weight of sample, and to per gram soil carbon. The cumulative production was calculated by summing the difference between measurements, and normalizing to the gram dry weight of sample, and to gram soil carbon (Robertson et al., 1999). Triplicate blanks with only autoclaved water were measured on each day of sampling, and the average of the three blank replicates difference between measurements was used as a benchmark of the minimum detected flux. Upon the conclusion of the incubation, samples were sacrificed, pH measured, baked, and weighed for the dry weight of each individual vial. The measurement of each day is reported in units of μg CO_2-C g DW^{-1} d^{-1} (μg C/gW/d) and μg CO_2-C gC^{-1} d^{-1} (μg C/gC/d). While CH_4 was measured, the cumulative production was not significant and not further discussed.

Analysis of each soil horizon's temperature coefficient (known henceforth as the Q_{10}), was calculated. The Q_{10} is a standardized parameter used frequently in literature describing soil respiration activity as it relates to temperature differences (Hamdi et al., 2013). Q_{10}s represent the rates with a ten-degree temperature difference, here we fit an exponential equation from our data as our temperature differences are not ten (Equation 1, 2 from Hamdi et al., 2013).

$$(1)\ SR = Ae^{kT}$$
$$(2)\ Q_{10} = e^{10k}$$

Where Equation 1 uses the rate of soil respiration (*SR)* with incubation temperature (*T*) with *A* and *k* as fitted parameters to calculate the Q_{10} value in Equation 2.

2.5 Microbial Community Structure Analysis

Here, we began by opening the sealed bags in the anaerobic headspace in sequence by habitat and soil horizon. The samples were gently mixed together to homogenize the 4-6 spatial replicate cores with gloved hands and sterilized lab tools. Care was taken to remove roots, leaf litter from surface, and rocks from deeper soil horizons.

Aliquots for microbial community structure analysis were stored in Eppendorf vials and kept frozen at -30°C during the week that all horizons were gathered, then -80°C until analysis.

2.5.1 DNA extraction, PCR and sequencing

Total nucleic acids were extracted in duplicates using the PowerSoil-Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Amplicon libraries were prepared by using barcoded primer pair sets (Uni515-F[5'-GTGTGYCAGCMGCCGCGGTAA-3'] / Uni806-R[5'-CCGGACTACNVGGGTWTCTAAT-3']) targeting the V3-V4 hypervariable regions of the 16S rRNA, with duplicates for each sample. PCR reactions (50 µL) contained 10× Pol Buffer C (Roboklon GmbH, Berlin, Germany), 25 mM $MgCl_2$, 0.2 mM dNTP mix (ThermoFisher Scientific), 0.5 mM each primer (TIB Molbiol, Berlin, Germany) and 1.25 U of Optitaq Polymerase (Roboklon, Berlin, Germany). The PCR program included an initial denaturation step at 95 °C for 7 min, followed by 33 cycles at 95 °C for 15 s, annealing at 60 °C for 30 s, extension at 72 °C for 30 s and a final extension step at 72 °C for 5 min. After purification with the Agencourt AMPure XP kit (Beckman Coulter, Switzerland), the recovered PCR products were equilibrated into comparable equal amounts before pooling together with positive and negative controls. For the positive controls, we utilized a commercially available mock community (ZymoBIOMICS Microbial Community DNA Standard II; Zymo Research Europe, Freiburg, Germany). As for the negative controls, they consisted of the DNA extraction buffer and the PCR buffer. Sequencing was run in paired-end mode (2 × 300 bp) on Illumina MiSeq platform by Eurofins Scientific (Konstanz, Germany).

2.5.2 Data processing and analysis

DNA raw sequences were processed by a custom workflow. Demultiplexing was performed using Cutadapt v3.4 (http://dx.doi.org/10.14806/ej.17.1.200). The demultiplexed sequencing raw data was upload to the ENA (European Nucleotide Archive) with the project accession number PRJEB72044 (https://www.ebi.ac.uk/ena/browser/view/PRJEB72044). The resulting sequences were subjected to DADA2 v1.20 (Callahan et al. 2016), including filtering, dereplication, chimera detection, sequence merging, and the identification of amplicon sequence variants (ASVs). Taxonomy of ASVs was assigned by referring to the SILVA138 database (Quast et al., 2013).

Statistical analysis of the incubation gas production and geochemical data was performed using R packages "tidyverse" (Wickham et al., 2019), "dplyr" (Wickham et al., 2023), and "stats*"* (R version 4.1.2). The incubation data set was uploaded up the PANGAEA research data repository (https://doi.pangaea.de/10.1594/PANGAEA.964303). Data normality was testing using both a Shapiro-Wilk normality test and a QQ plot, using the R functions "shapiro.test" and "qqnorm*"* included in the base package "stats*"*. Data was not found to be normally distributed, thus the Kruskall-Wallis test was used to evaluate the significance in differences of CO_2 production of each soil horizon groups using the R function "kruskall.wallis" (Venables et al., 2002). Key parameters (included in the analysis was: total organic carbon (%), total carbon (%), total nitrogen (%), water content (%), pH, *pmoA* cell copies (Suppl. Fig. S8), *mcrA* cell copies (Suppl. Fig. S9), bulk density (g/cm^3), temperature of incubation (°C)) on measured CO_2 production variance were determined with Akaike Information Criterion (AIC). Using linear models, the multiple regression analysis were made using a backward-selection, with the "stepAIC" function in the "MASS" package (Venables et al., 2002). The same parameters were used in a principal component analysis (PCA) using the "vegan" package (Oksanen et al., 2022).

383 Temperature sensitivity was determined from the calculation of the Q_{10} value, as described by
384 Hamdi et al., 2013. Individual outlier measurements were removed on basis of visual inspection
385 of measurements of incubation timeseries, four measurements were removed (of the total 605
386 headspace measurements) and determined to be from user error. A bubble plot was generated to
387 visualize the microbial community composition at family level using ggplot2 package (v 3.4.2).
388 The community data were collapsed at family level using the 'otuCollap' function of R package
389 otuSummary (Yang, 2020).

390

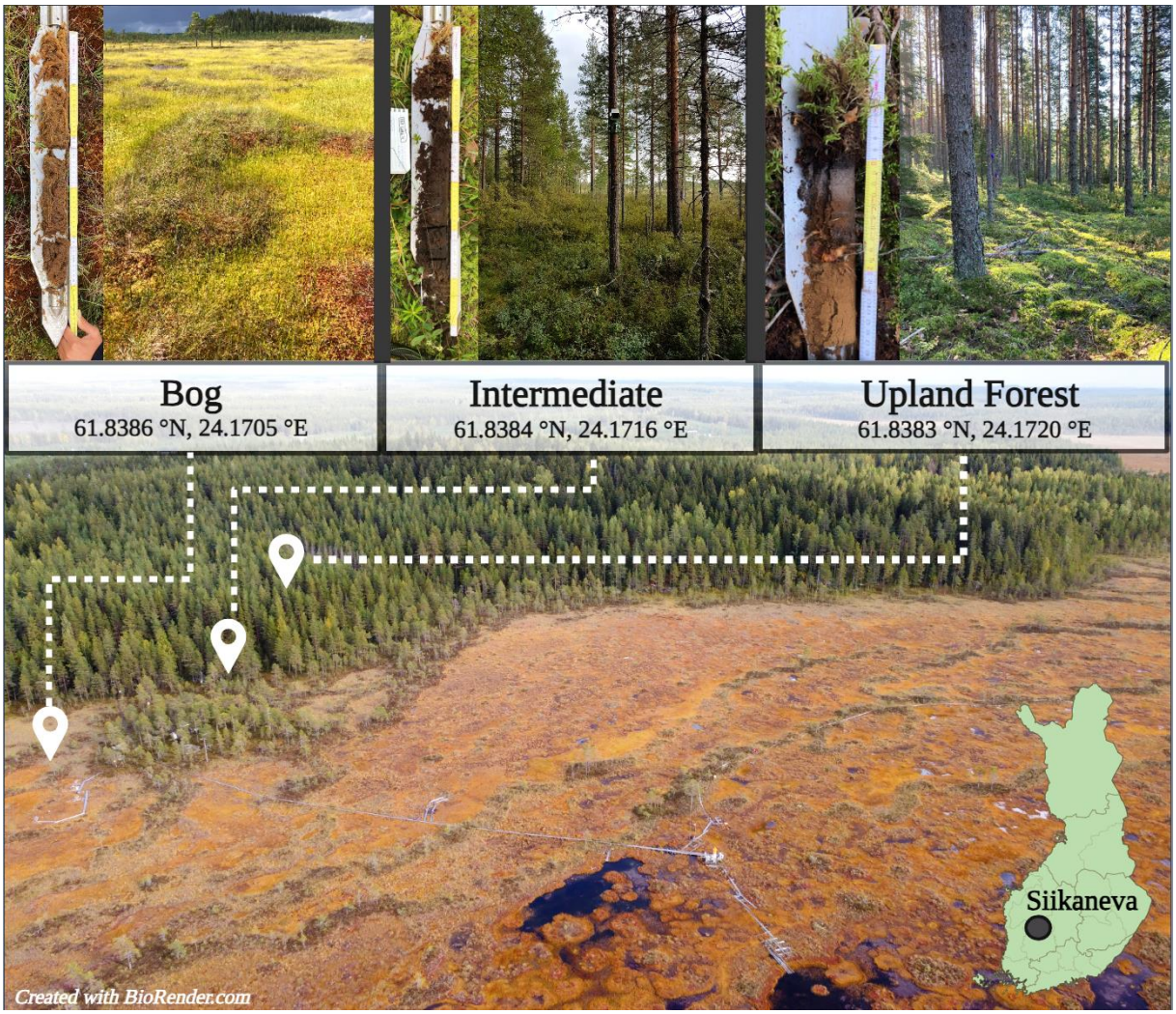

Figure 1: Sample site map. Our three sites represent three different habitats in the Siikaneva peatland and surrounding habitats (Western Finland). A boardwalk (visible in aerial view) transects the bog and provides infrastructure for other ongoing studies (such as continuous autochamber flux measurements and field meteorological stations). Inlaid, shows researchers' view of each field site, with image of cores directly to the left of their respective habitat. Drone image taken in August 2022 by T. Rettelbach and L. Golde

391

392 # 3 Results

393 ## 3.1 Soil description

394

Table 1

Soil Horizon Descriptions and Sampling Depth

Habitat	Surface Vegetation	Soil Class*	Bulk Density (g/cm^3)	Top of Horizon (cm)	Bottom of Horizon (cm)	Average Depth of Horizon (cm)	Soil Horizon Depth Group
Bog	*Sphagnum* moss lawn including: *Sphagnum papillosum, S. magellanicum* and *S. balticum*, some assorted sedges including from genus *Eriphorum*	O	0.03	0.0	30.0	15.0	Top
		O	0.09	30.0	50.0	40.0	Bottom
Intermediate	Scots pine (*Pinus sylvestris*), ground lichens (Cladonia spp.), blueberry shrub (*Vaccinium myrtillus*), feather mosses (*Hylocomium splenens*), some *Sphagnum* mosses	O	0.02	0.0	15.0	7.0	Top
		O	0.04	15.0	35.0	25.0	Middle
		O/M	0.18	35.0	50.0	42.5	Bottom
Upland Forest	Scots pine (*Pinus sylvestris*), ground lichens (Cladonia spp.), ferns (*Dryopteris dilatata*), feather mosses (*Hylocomium splenens*), glacial erratic boulders (granite rock)	O	0.03	0.0	15.0	7.5	Top
		M	0.19	15.0	25.0	20.0	Middle
		M	0.52	25.0	43.0	34.0	Bottom

Note: Photos of cores, surface vegetation can be seen in Fig. 1

*Soil Class denotes the soil being Organic or Mineral, with mineral being less than 20% C

395

396 Properties of soil samples collected from Siikaneva peatland and surrounding habitats
397 during August 2021 are shown here, in Table 1, 2. In each habitat, the factors considered reflect
398 a diversity of soil types of both organic and mineral compositions. However, some overall trends
399 can be seen. The highest amount of moisture in the soil (the volumetric water content) can be
400 found on the top of the soil column, even in the water saturated bog site. The Upland Forest and
401 Intermediate site had a water table was below the maximum coring depth of 50 cm and had soil
402 moisture contents ranging from 16% to 96%. Siikaneva peatland had most recently seen water
403 from the last observed rain on July 29th, 30th 2021 (10 days prior to soil core sampling). The
404 peatland is not fed from any known groundwater source or adjoining waterbody. Sample source

material differences could be clearly seen from above the vegetation, and when looking at data by soil horizons (Table 1, 2).

Table 2

Soil Horizon Geochemical Properties

Habitat	Soil Horizon Depth Group	Soil Description	pH	Water Content %	TOC %	TN %	C:N
Bog	Top	Whole *Sphagnum* strands, suspended in bog water in floating mats, some sedges	4.10 ± 0.00	96.10 ± 4.21	44.05 ± 0.51	0.90 ± 0.01	49.38 ± 0.80
	Bottom	Partially decomposed *Sphagnum* moss. Medium brown in color	3.90 ± 0.00	96.61 ± 0.45	45.41 ± 0.18	1.51 ± 0.03	30.27 ± 0.61
Intermediate	Top	Mixture of mosses and moist organics	3.83 ± 0.20	93.77 ± 1.25	46.15 ± 0.13	1.43 ± 0.28	32.31 ± 6.33
	Middle	Homogenous, moist coffee-brown organics	3.90 ± 0.28	87.39 ± 1.80	46.02 ± 0.19	1.74 ± 0.14	26.46 ± 2.13
	Bottom	Black-colored organic layer, some grey mineral	4.53 ± 0.27	65.05 ± 3.92	16.54 ± 0.24	0.80 ±0.06	20.66 ± 1.57
Upland Forest	Top	Mixture of mosses and moist organics	3.46 ± 0.08	62.89 ± 8.00	35.98 ± 0.32	2.73 ± 0.05	13.20 ± 0.27
	Middle	Grey podzol mineral layer	3.77 ± 0.16	37.47 ± 11.52	5.44 ± 3.41	0.16 ± 0.00	33.57 ± 21.04
	Bottom	Tan-colored clay with frequent woody root intrusions (larger pieces removed before incubations)	4.47 ± 0.15	12.98 ± 2.29	2.43 ± 0.04	0.10 ± 0.01	25.44 ± 2.58

Note: n = 6 for pH and Water Content, n=2 for TOC and TN

Soil pH and bulk density increased with depth across all habitats, except the Bog (Table 2). pH ranged from 3.5 to 4.5 and was lowest in the surface Intermediate and Upland Forest samples. Bulk density was higher in mineral soils (mostly found in the Upland Forest and Intermediate site) and lowest in the organic soils of the Bog and topsoil of the other two habitats.

The gradient patterns in pH and bulk density by soil horizon from all habitats support greater levels of decompositions and compression from the top layer to the deeper soil layers. In the bottom layers of the Intermediate and Forest site were a mix of O/M and mineral soil, with soil TOC contents < 20%.

Organic soils had similar TOC content, ranging from 35.98 to 46.15 %. Forest-Top had over 30% more N than the horizon with the next most layer (2.7% vs. 1.7%). The mean nitrogen content in the Intermediate site was higher than the other sites (1.3%, 1.2%, and 1.0% in the Intermediate, bog, and Upland Forest, respectively). The amount of nitrogen largely drove the C:N ratio, and varied from site to site. The largest C:N ratio was in bog top soil horizon. We found the lowest C:N values in the forest-top (13.20) and Intermediate-bottom layers (20.66), as seen in Table 2.

3.2 Cumulative CO_2 production across the sites

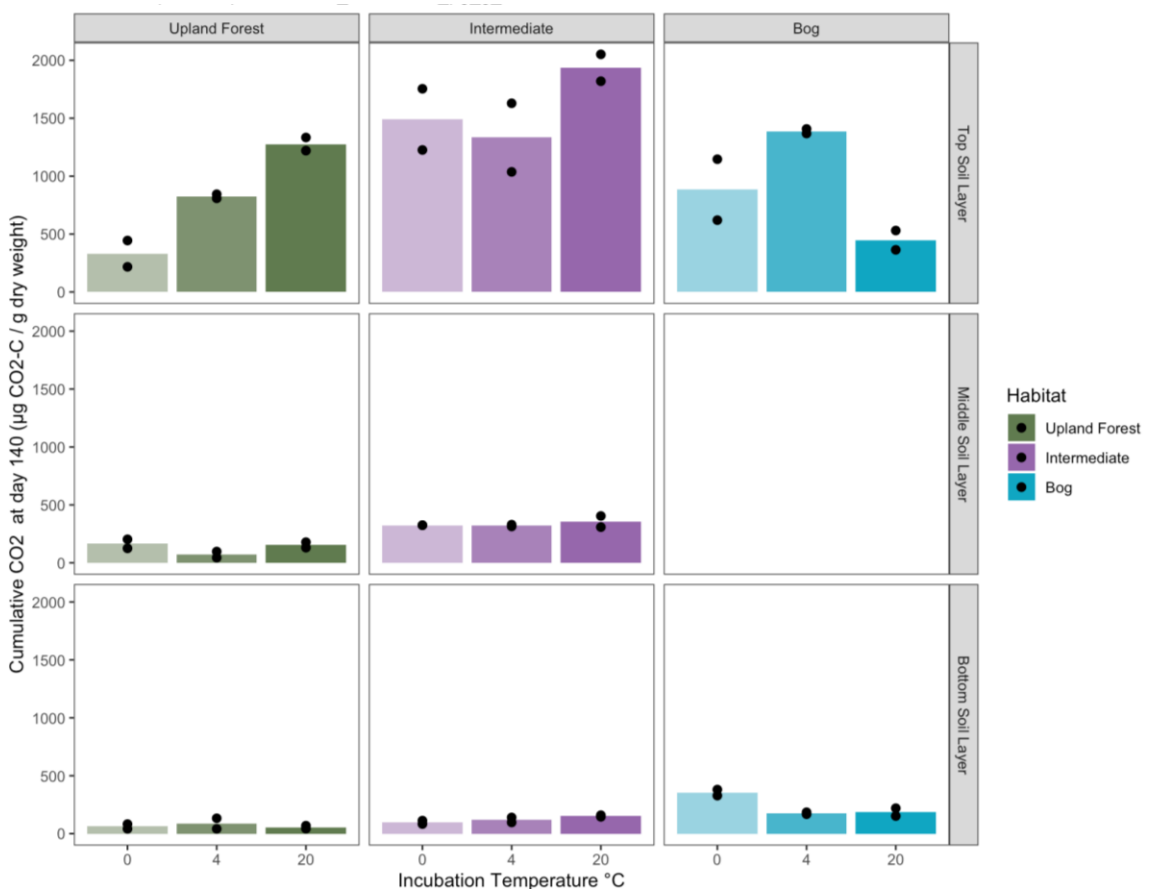

Figure 2: Displayed is cumulative CO_2-C production from the incubation of Siikaneva peatland and surrounding habitat soil cores when normalized to gram dry weight of sample. Samples were taken along a hydrological transect, from the well-drained Upland Forest to the completely saturated Bog habitat (denoted by bar color). Intensity of color corresponds to the temperature that samples were incubated at for the duration of the 140 day experiment (also denoted on the x-axis: 0, 4, 20°C). The bars represent the average between the two replicates per treatment group, with the individuals represented as dots.

Across sample groups, cumulative values of CO_2 production measured to day 140 ranged from an average of 1936 ± 160 µg CO_2-C g^{-1} DW (from the Intermediate-Top site, averaged replicates) to a 55.8 ±18.5 µg CO_2-C g^{-1} DW from Upland Forest-Bottom samples (Fig. 2). Generally, the habitat that produced the most CO_2 per gram dry weight was the Intermediate site, followed by the bog, and forest site respectively. Within the Intermediate site, the top (0-15cm) layer was significantly more productive in terms of CO_2 production over the course of the 140 days of incubation ($\chi^2 = 15.16$, d$f = 2$, $p = 0.0005$), producing 137% more CO_2 than in the middle layer and 171% more than the bottom layer within the habitat. The Upland Forest habitat also showed significant separations in CO_2 production by depth, but to a lesser degree (per µg CO_2-C g^{-1} DW, Kruskal-Wallis ($\chi^2 = 13.05$, d$f = 2$, $p = 0.001$). The bog site's top and bottom layers were found to also be significantly different, but to a lesser extent than the preceding habitats ($\chi^2 = 7.41$, d$f = 1$, $p = 0.006$).

When considering production per gram Carbon in the source material, the Upland Forest site was the most productive, followed by the Intermediate then Bog (Suppl. Fig. 10). Normalized to gram Carbon, the Upland Forest site produced CO_2 at each soil horizon at a rate that was comparable to the top soil horizons (the most productive layer). This suggests the low values seen of % C in the Upland Forest site has selected for a microbiome of adaptive organisms that are able to fully utilize the limited C available. The Upland Forest site was the only site that's relationship between soil horizon and CO_2 productivity changed when assessing production normalized to the percentage of carbon in the sample. When the sample was normalized to percentage of carbon, the Upland Forest did not display significant differences between soil horizons ($\chi^2 = 0.22$, d$f = 2$, $p = 0.89$).

3.2.1. Bog Habitat Incubation Results

Across both depth groups' (top 0-30cm and bottom 30-50cm) averaged laboratory replicates, the cumulative CO_2 produced ranged from 176 ± 12.1 to 1390 ± 27.9 µg CO_2-C g^{-1} DW with an overall mean of 573 µg CO_2-C g^{-1} DW (Fig. 2). The most CO_2 was produced in the top 30 cm incubated at 4°C (1390 ± 27.9 µg CO_2-C g^{-1} DW). This group also had the most cumulative CO_2 production when normalized by gram C (3150 ± 63.3 µg CO_2-C g^{-1} C; Suppl. Fig. 10). The least CO_2 production occurred in the bottom 30-50 cm. The bottom 20cm of the bog were unresponsive to the temperature treatments, and the 4°C treatment was the lowest producing group of the bottom depth (176 ± 12.2 µg CO_2-C g^{-1} DW; 388 ± 26.8 µg CO_2-C g^{-1} C). Of the independent variables, the Total Organic Carbon content and the temperature were found to explain 73.7% of the variance (adjusted $R^2 = 0.64$), using at backwards stepwise regression model (stepAIC: ($F_{2, 8} = 7.46$, $p < 0.05$) (Fig. 2)).

3.2.2. Intermediate Habitat Incubation Results

The cumulative CO_2 produced over 140 days from the Intermediate habitat ranged from 97.8 ± 20.8 to 1940 ± 164 (µg CO_2-C g^{-1} DW) between averaged replicates (Fig. 2). The most CO_2-C was produced in the top soil horizon at 20°C, and the least amount of CO_2 production came from the bottom horizon of the soil column, in the treatment group incubated at 0°C. Samples, when normalized by gram C maintained the same superlatives of production groups (Suppl. Fig. 10). By gram C the samples showed a wide spread of cumulative production values,

with values ranging from as low as 592 ± 126 µg CO_2-C g^{-1} C to the highest production 4190 ± 355 µg CO_2-C g^{-1} C, and an average CO_2 production of 1640 µg CO_2-C g^{-1} C (Suppl. Fig. 10). When the independent variables were analyzed using a backwards stepwise regression, total organic carbon, and Carbon % both were highly significant predictors of cumulative CO_2 produced, as well as Temperature groups (4°C and 20°C) explaining 93.0% of the variance (adjusted $R^2 = 0.91$; stepAIC ($F_{3, 13} = 43.30$, $p < 0.01$) (Fig. 2)).

3.2.3. Upland Forest Habitat Incubation Results

The cumulative CO_2 produced over 140 days from the Upland Forest samples habitat ranged from 55.8 ± 18.5 to 1280 ± 80.3 µg CO_2-C g^{-1} DW. The most cumulative CO_2 was produced in the Forest-Top soil horizon, in the 20°C incubation temperature group (1280 ± 80.3 µg CO_2-C g^{-1} DW; Fig. 2). The 20°C Forest-bottom soil horizon was seen to have the least CO_2 produced (55.8 ± 18.5 µg CO_2-C g^{-1} DW; Fig. 2). While the differences between temperature treatments were not significant between soil horizon depth groups, this was the only habitat's bottom horizon that measured the least amount of CO_2 produced in the 20°C incubation temperature group. When the cumulative CO_2-C values are normalized by sample material carbon, the quality of the C can be compared. Here, the values measured from a minimum of 918 ± 446 to a maximum of 3580 ± 220 µg CO_2-C g^{-1} C, and an average cumulative CO_2-C value of 2485 µg CO_2-C g^{-1} C. Of note, the Forest-Middle and Forest-Bottom have comparable cumulative CO_2-C production to the Forest-Top layer when normalized to C, which was not the case when normalized only to production per gram dry weight of sample material (Fig. 2; Suppl. Fig. 10). When the independent variables were analyzed using a backwards stepwise regression, % C and the temperature groups (4°C and 20°C), pH, and field conditions water content were highly significant predictors of cumulative CO_2 produced, explaining 91.8% of the variance ($F_{5, 12} = 26.93$, $p < 0.01$); adjusted $R^2 = 0.88$)(Fig. 2)).

3.3 Respiration rates incubation temperature response

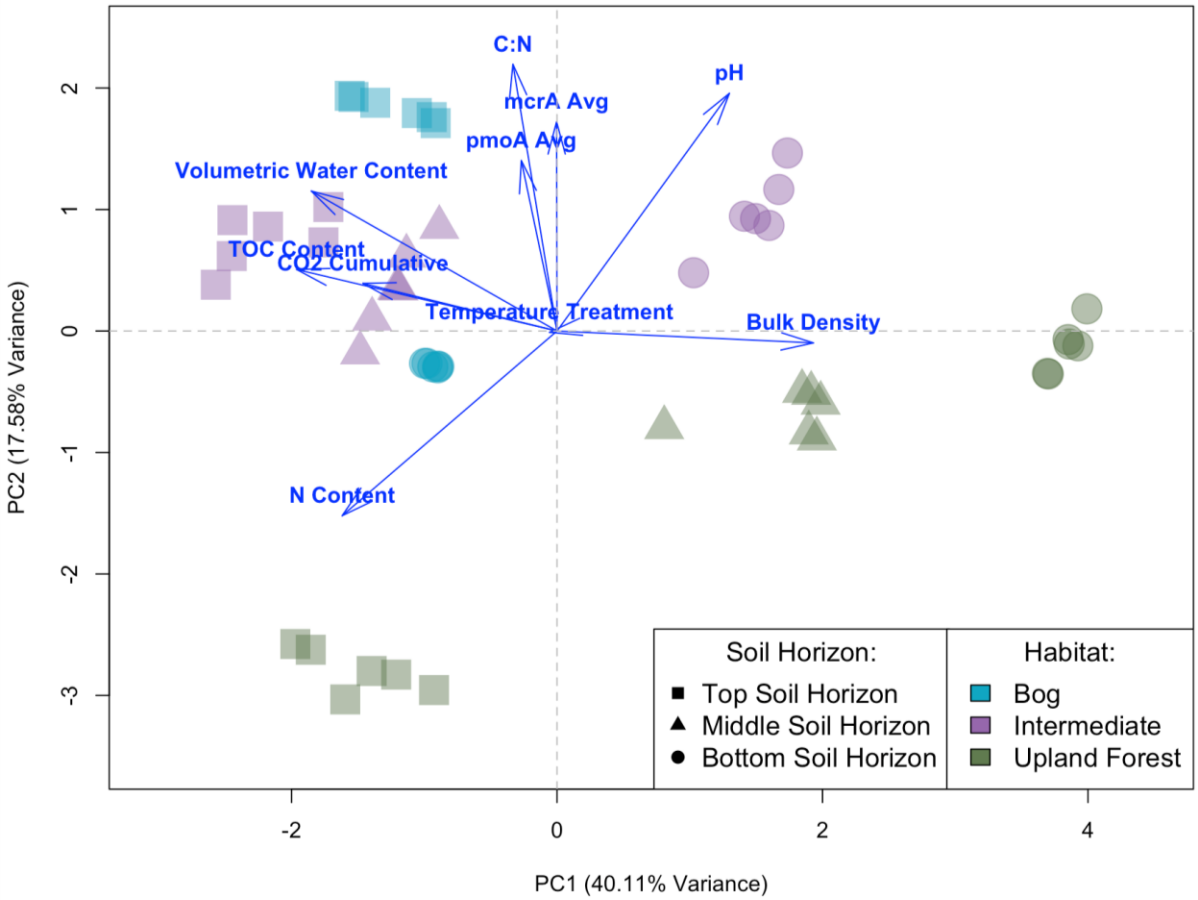

Figure 3. Biplot of the PCA with explanatory vectors. Each sample is represented as one data point, with color representing the habitat and shape representing the soil horizon.

Overall, the effect of the three incubation temperature treatments were less influential to the cumulative CO_2-C measured than hypothesized, with variable results from each habitat group (Fig. 3). In the principal component analysis, the temperature treatments were the shortest vector, and explained very little variance in the data. PC1 explained 40% of the variance and was negatively correlated with samples water content, nitrogen content and carbon content. The cumulative CO_2-C per gram dry weight was tightly correlated with carbon content of sample. The strongest positive correlation with PC1 was the sample bulk density, and the combination of these factors clearly separated out the soil horizons along this axis. PC2 explained 18% of the variance and was most strongly positively correlated with pH and negatively with N content.

In the scope of the full 140 days, the Q_{10} values remained relatively low (0.6 – 2.33), with the top layers showing the largest values (For more on Q_{10} data, See Supplemental Material Table 1, Discussion).

The Bog was the only habitat that had the most CO_2 production at 4°C, and not the 20°C incubation temperature, in agreement with other anaerobic Sphagnum-dominated samples from boreal-latitude incubation experiments (Kolton et al., 2019). When looking at temperature

responses by depth groups in the bog, the bottom 30-50cm had the most CO_2 production in the 0°C incubation (354 ± 36.9 CO_2-C per g^{-1} DW), which was also true when normalized to gram C (780 ± 81.3 CO_2-C g^{-1} C). Within the temperature treatment, the Bog-Top (0-30cm) and Bog-Bottom (30-50cm) of the 4°C temperature group varied (see Fig. 2, 3), with the Bog-Top layer producing 1390 ± 27.9 and the bottom layer producing 176 ± 12.2 µg CO_2-C g^{-1} DW by the end of the incubation at measurement day 140.

Within the Intermediate habitat, the 20°C incubation temperature group produced the most cumulative CO_2 measured. In the 20°C top, middle and bottom soil horizon we measured 1940 ± 164, 356 ± 68, and 152 ± 9.84 µg CO_2-C g^{-1} DW, respectively. The Intermediate's top 15 cm, middle 15-35cm, and bottom 35-50cm in 4°C varied significantly, with the top, middle and bottom having produced 1330 ± 418, 322 ± 10.8, and 118 ± 30.4 cumulative µg CO_2-C g^{-1} DW by the end of the incubation at measurement day 140. The lowest overall cumulative values came from the 0°C group, particularly Intermediate-Bottom samples. Here the samples averaged 97.8 ± 20.9 µg CO_2-C g^{-1} DW, or normalized to the carbon content, 592 ± 126 µg CO2-C g^{-1} C.

The Upland Forest samples had a strong positive correlation with the temperature treatment of the incubations, but this was primarily observed in the Forest-Top 15cm. The Forest-Middle 15-25 and the Forest-Bottom 25-43cm did not show a visible relationship between incubation temperature and CO_2 production (Fig. 2), which was the case for the other two habitats lower soil horizons. Between temperature groups, the samples incubated at 20°C were 52% more productive than the samples incubated at 4°C in terms of CO_2 produced per gram dry weight (all depth groups summed). The 20°C samples produced 168% more cumulative CO_2-C than the 0°C samples (all depth groups summed) considering the same metrics.

For all habitats, temperature strongly influenced the measurement point at which the largest flux was found ($\chi^2 = 7.80$, d$f = 2$, $p = 0.02$), as well as the TOC % from sample measured at the start of the incubation ($\chi^2 = 25.71$, d$f = 7$, $p = 0.0006$). However, the length of the lag times (time from incubation Day 0 to day of measured maximum production rate) weren't predictive of cumulative CO_2-C by day 140. Higher temperatures resulted in the maximum flux being closer to Day 0, and lower temperatures delayed the peak CO_2 production to as late as measurement day 71.

3.4 Microbial data

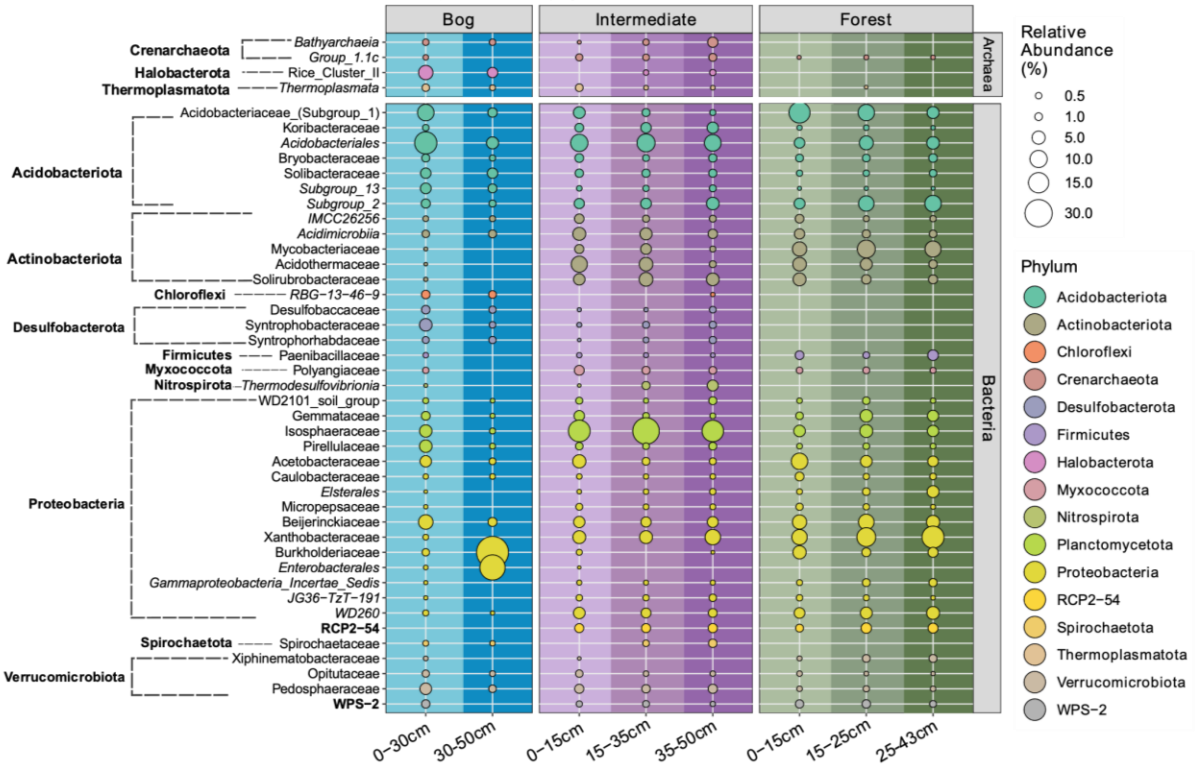

Figure 4. Relative abundances of the microbial community structure in the Siikaneva peatland and surrounding habitats. Shown are those making up more than 0.25% of the total sequences. Bubbles presence represents taxonomic groups, and the diameter represents the percentage of abundance. Color used for emphasis of functional groups, habitat, and soil horizon of origin.

In our study site, the three habitats each support a microbial community that reflects the defined differences of each habitat's soil geochemistry. This data underscores the heterogeneity of the three habitats, as they move from the well-drained Upland Forest, to the waterlogged Bog site.

In the bog site, we see a distinct microbial community, reflective of highly acidic, extremophile habitat typical of *Sphagnum* moss mires. In this site we found the community was dominated by *Acidobacteria* and *Protobacteria*. *Acidobacteria* is present in up to one third of all 16S rDNA sequences from *Sphagnum* moss bogs (Dedysh et al., 2006; Pankrotov et al., 2008) and describes a phylum of bacteria that can be found in many rather oligotrophic soil habitats but remains poorly understood taxonomically and functionally. The bog site showed the lowest alpha diversity of all sites (Shannon index : 584 for the Top soil horizon, 129 for the Bottom soil horizon). The bog site is the only site where a substantial abundance of methanogenic archaea (Rice Cluster II) was detected.

The Intermediate site had consistent microbial community structure throughout the soil column. This was the only habitat that had few differences in bacteria and archaea phyla by depth. Here, the soil community is represented by a diverse community of soil microbes, most

notably from the Phylum *Actinobacteria* and *Proteobacteria* (Family: *Isosphaeraceae*). In the Intermediate site, we saw that the alpha diversity was highest in the top 15cm depth group, and interestingly this was also the highest value of all soil horizons. The alpha diversity was relatively similar between the lower two soil horizons (Shannon index: 879, 631, 698 respectively).

Generally, we measured a wider distribution of microbial abundance, and diversity on the phylum level present in the Upland Forest site (Fig. 4). In this site, no group was measured at more than a 10% relative abundance. Notably, the presence of Archaea was no larger than 0.5% in any of the Upland Forest soil horizon depth groups and methanogens were not detected. The Upland Forest site had the highest measure of alpha diversity, when considering the soil column as a whole. Similar to the other two habitats, the highest measure of diversity was in the top layer (Shannon index : 828, 704, 777 respectively).

4 Discussion

4.1 Soil properties and potential anaerobic decomposition

Our results from the 140 days of ex-situ incubation revealed that the soils carbon content was the largest determinant of anerobic CO_2 production. We had hypothesized that we would observe a positive relationship between the initial soil carbon content and CO_2 produced, as well as a positive response to the incubation temperature treatments within each horizon to CO_2 production. This hypothesis saw a partial realization as the soil carbon was the strongest predictor of the measured CO_2 (Fig. 3). Both the Intermediate-Top and Upland Forest-Top had high soil C content, when compared to the other soil horizons (Table 2). Both sites were also characterized by a high abundance of *Actinobacteria* (Fig. 4), organisms that are suggested to cope well in environments where substrate concentrations are high (Ho et al., 2017). Although, the relationship with temperature and CO_2 produced varied with each horizon. We found that the Intermediate-Top at 20°C produced the most cumulative CO_2 per gram dry weight at the end of the 140 day incubation (1936 µg CO_2-C g^{-1} DW). The high productivity from the Intermediate site was unsurprising, on account of its microbiome likely being primed for dynamic temperature changes in both aerobic and anaerobic environments (Ťupek et al., 2008; Langlois et al., 2015). Although the Intermediate habitat was above the water table when we collected samples from this site, we observed high water content and know that this habitat seasonally is covered with snow and is occasionally inundated with rainwater.

The transect study design lends itself to the natural inclusion of variable soil C between habitats and soil horizons. Of all the soil properties, we found that TOC was most strongly correlated with CO_2 production (Fig. 3). Soil C can be influenced by a number of factors such as degree of OM decomposition, decomposition pathways, and parent vegetation. (Clymo and Hayward 1982). Organic substrate quality and quantity has been known to be a significant influence on decomposition rates, but the exact relationship of soil C and decomposition is only starting to be more fully understood (Reichstein et al., 2005; Wetterstedt et al., 2010). For example, soil cores from the bog habitat had relatively high TOC content but low CO_2 production (Fig. 3). The dominant vegetation of the Bog (*Sphagnum* mosses) is known to have high C content, mostly in the form of carbohydrates. However, carbon sampled from the dissolved organic carbon of *Sphagnum* extracts in bogs have been found to have a relatively low

nominal oxidation state of the carbon, suggesting the sample is in an oxidation state unfavorable to be an electron donor to the terminal electron acceptors within the soil matrix, and thus be unfavorable to decomposition processes on a chemical level (Wilson et al., 2022). This low energetic potential of the molecular compounds within the *Sphagnum*-sourced OM is confounding, but can be explained by the Bog soils' saturation, low pH, and nutrient-poor environment that is unfavorable to decomposition processes (LaRowe and van Cappellen 2011; Wilson et al., 2022). Introduction of terminal electron acceptors to soils experimentally (NO_3^-, SO_4^{2-}) have been found to increase the soil dissolved organic carbon's nominal oxidation state of carbon and stimulate decomposition, resulting in increased respiration of CO_2 (Naughton et al., 2021).

Furthermore, another indicator that the *Sphagnum* peat has low or inhibited energetic potential is the higher abundance of *Acidobacteria* in the Bog site. *Acidobacteria* are known to be oligotrophs able to compete in environments where resources are limited (Fierer et al., 2007). Additionally, the Bog habitat showed substantial abundance of methanogenic archaea indicating a lack of alternative electron acceptors other than those serving methanogenesis. Thus, anaerobic CO_2 production in this site may have been largely driven through methane production but not so much through thermodynamically more favorable processes like denitrification.

The Upland Forest site was the most productive when considering the CO_2 production in term of CO_2 per gram C in the sample source material (Suppl. Fig. 10). These findings show the microbial community was able to metabolize the limited C at an equal, if not larger rate than the other horizons, a tribute to the adaptability of the microbial community to metabolize the limited C in their environment. The aerobic nature of the upland forest soils and prevalence of roots throughout the soil column all could contribute to the high utilization of available soil carbon. Roots are known to stimulate the soil microbial community, and the presence of roots here could also contribute to the Upland Forest having the highest Shannon index of the three habitats (Moore et al., 2015). Plant roots are also known to increase rates of soil organic matter decomposition. In a study 10km away of our projects study site (the SMEAR II Hyytiälä Station: 61.7667∘N, 24.2833∘E) researchers aimed to see the role plant roots played in the balance of decomposition and organic matter formation in the Upland Forest soils. Adamczyk et al., (2019) processed the soil, placed in mesh bags of different sizes, and monitored over three years to assess root and fungal penetration, enabling subsequent enzyme and DNA analyses along with nitrogen quantification. They found that the presence of roots increases organic matter decomposition, while also increasing the nitrogen pool in the soil, which is significant for the extremely N limited podzol soils.

Similarly, surface samples in the Intermediate and Bog sites also showed higher anaerobic CO_2 production than deeper layers (Fig. 2). Though few studies have been done incubating Upland Forest and Intermediate equivalent habitats in anaerobic settings, the limited consensus from incubations show that these sites regularly experience thermal and hydrologically driven environmental change, and that they respond accordingly with large variations in microbial respiration patterns (Hartshorn et al., 2003; Ťupek et al 2008; Oelbermann and Schiff 2008; Wickland and Neff 2008). When the amount of CO_2 produced across all the sample sites was normalized to gram soil C, the quantity of C no longer shapes the data but rather the quality (biolability).

The soil moisture, and bulk density of the samples also had a strong positive correlation to CO_2 production. Soil horizons nearest to the surface (in the "top" depth group) had the highest CO_2 production and generally had the lowest bulk density, most TOC and as much, if not higher water contents than any other layer in its respective column (Fig. 2; Table 2). That the surface samples with the lower bulk density and high porosity had the most respiration activity was surprising in the sense that these would be the layers most exposed to oxygen in nature. However, one possible explanation is that samples at the top layer of the soil column are known to host more diverse and responsive microbial communities than horizons that are adapted to more the more stable and cooled anaerobic conditions of the lower depths. We observed the most CO_2 produced (per gram dry weight) at the Intermediate top site, and the least in the lower mineral soil layers of the forest site (per gram dry weight). Here, we see that the combination of above-mentioned trend of low bulk density, high field soil moisture and (most relevantly) TOC is highest in the Intermediate site top layer. In carbon flux measurements at the nearby Lakkasuo mire (Lakkasuo: 61.800∘N, 24.317∘E), researchers set up a similar hydrologic gradient from Upland Forest to bog and measured CO_2 fluxes from *in situ* chamber measurements (Ťupek et al 2008). They report that CO_2 was found to be largely influenced by the openness of the forest canopy, a feature that varies markedly in the dynamic conditions of the Intermediate site. The Bog site also has the parameters that point to high potential CO_2 production (high VWC, high TOC, low BD) but the acidity of the waterlogged moss and the recalcitrant and confounding nature of the dominant vegetation (*Sphagnum* moss) is widely known to limit decomposition and C cycling processes (Clymo and Hayward 1982).

In general, our results indicated that the influence that pH may have played was likely obfuscated by the larger vector of influence that sample C composition had on CO_2 production, (Fig. 3; Table 2). We also measured the nitrogen content of each horizon and found both the highest and the lowest content of nitrogen (%) in the Upland Forest cores. Depth had a negative correlation with nitrogen content in this habitat, with most of the nitrogen being contained in the top organic layer. Nitrogen in boreal forest soils and peatlands is a limiting factor for primary production and the nitrogen in these soils tend to be competitively recycled by the biota (Aerts et al., 1992; Wickland and Neff 2008; Kuhry and Vitt 1996). Vegetation in boreal forests and peatlands have been shown to have extraordinary adaptations on a cellular level to navigate N limitation, such as the feather moss (*Hylocomium splenens* – notably, the same species found in the Upland Forest and Intermediate site in this study) releasing chemo-attractants to targeted strains of N_2 fixing cyanobacteria when the moss is under N-limitation stress, forming a symbiotic relationship between the organisms (Bay et al., 2013). The ratio of carbon to nitrogen (C:N) found in each soil horizon was highest on the top of the soil horizon, due to the most fresh plant input, and decreased with depth/maturity, as found in previous literature where increasingly anaerobic conditions result in increasing loss of C (Janssen 1996; Kuhry and Vitt 1996).

4.2 Temperature Response

The results from this incubation series demonstrate how each soil horizon responds to temperature change according to its biogeochemistry. Generally, warmer temperatures produce more soil respiration products (Fang et al., 2005, 2006; Knorr et al., 2005; Davidson and Janssens 2006), but in this study, this relationship is obscured (Fig. 3). In our results, there was no definitive trend across soil horizons to temperature influence, and some groups showed no statistical or visible response to temperature (Fig. 2). In general, samples in the 20°C group were

most productive, at the top of the soil column. The top of the soil column in the boreal zone experiences large temperature fluctuations throughout the year. Temperatures of 20°C and above are regular summertime daily highs in this region. The top soil samples followed the expected response to temperature (having a higher incubation temperature treatment resulted in an increase of cumulative CO_2) which may indicate that the C availability there is of higher quality. The exception to this were the Bog samples, which showed little response to temperature, though produced more CO_2 at the 4°C in the Bog-Top and at 0°C in the Bog-Bottom (Fig. 2). The Bog's high TOC but minimal response to temperature could also indicate its higher composition of more recalcitrant forms of C, as each form of soil carbon likely has different interactions with the biotic environment at different temperatures. Additionally, diversity in the structure of the SOM molecules and environmental inhibition of enzyme activity are examples of factors that could diminish the decomposition processes sensitivity to temperature changes in anaerobic environments, as mentioned above (Davidson and Janssens 2006) in addition to the presence of *Sphagnum* and its associated complex compounds (Wilson et al., 2022). The low temperature sensitivity in the Bog could also suggest that microbial community was not as well adapted for higher temperatures in the Bog as the well-drained sites were. Across all plots, samples from the lower depth groups showed little temperature response (Fig. 2). In general, these result show that temperature is not likely the limiting factor for anaerobic CO_2 production within the 140 days of incubation in this experiment. The sample Q_{10}s range from 0.6-2.33 and are within the range of previous high latitude wetland studies that report anaerobic incubations measuring CO_2 produce Q_{10} values of 0.67-4.10 (n=219; Treat et al., 2015). In a global synthesis of available incubation studies, the global mean of soil Q_{10}s was found to be 2.04±1.09 (n=494; Hamdi et al., 2013), though most of these studies incubate material for less than a month and do not include (or have a much shorter) equilibration period than this studies 25-day incubation equilibration.

While it is unexpected that we saw insignificant CH_4 production, the processes underlying methane production are sensitive to change and are influenced by a variety of inputs. In this study we postulate this lack of measured CH_4 is due to the resident methanogen community being unable to re-acclimatize after frozen transport, despite standard procedure being used, and a 25 day equilibration period before the start of the 140 days incubation. The incubations lacking sufficient soil nutrients, incubation moisture, pre-existing community of methanogenic organisms, and insufficient length of incubation time can all be likely ruled out from careful consideration undertaken from selection of lab analysis and methods.

Despite the knowledge that climatic warming is amplified near the global poles, there is still a significant knowledge gap on how these C-rich, high-latitude soil biogeochemical cycles will respond to warming. Here, we contribute with data from a laboratory incubation that shows varying response across the landscape, driven by soil properties, *Sphagnum* mosses, and microbial communities, giving a novel perspective that highlights the intricacies of both carbon quality and quantity on anaerobic CO_2 production.

5 Conclusion

In conclusion, our findings underscore the significant role of initial soil carbon content as the primary predictor of anaerobic-produced CO_2. Samples incubated at the highest temperature (20°C) generally produced the most CO_2; this is especially true for soil horizons at the top of the soil column. However, the temperature response varied across habitats and soil horizons,

indicating the nuanced effects of climate change-induced alterations such as shifts in precipitation patterns, temperature regimes, and shoulder season intensity on the carbon cycle within boreal ecosystems . Therefore, these results highlight the complexity of ecosystem-scale controls on carbon cycling in the boreal zone and emphasize the need for comprehensive inclusion of multiple factors in earth system modeling to accurately capture future carbon dynamics.

This study introduces a novel perspective of the biogeochemical cycling of boreal peatlands, particularly in the Intermediate ecotone between peatlands and forests. Future studies could enhance our ecosystem scale modeling efforts by the explicit inclusion of the Intermediate habitat in peatland studies, and the assessment of additional environmental factors (e.g. nutrient limitation). Climatic warming introduces a high uncertainty of future habitat conditions and several knowledge gaps on soil carbon stability. Given the potential for boreal peatlands and surrounding ecosystems to transition from carbon sinks to sources under changing environmental conditions, integrating soil carbon quantity and quality, especially regarding *Sphagnum* mosses as shown in this study, into global climate models should be on high priority, and calls for more exploration of the highly productive and dynamic Intermediate peatland-forest habitat.

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Open Research

The geochemical, and gas (CO_2, CH_4) production data used in this study of Siikaneva peatland and the surrounding habitats are available at PANGAEA research data repository

780 (https://doi.pangaea.de/10.1594/PANGAEA.964303; (Baysinger et al., 2023)). The
781 demultiplexed sequencing raw data of the microbial DNA was upload to the ENA (European
782 Nucleotide Archive) with the project accession number PRJEB72044
783 (https://www.ebi.ac.uk/ena/browser/view/PRJEB72044).

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