




Decoupling of microbial community dynamics and functions in Arctic peat soil exposed to short term warming

Sizhong Yang^{1,2}  | Susanne Liebner^{1,3}  | Mette Marianne Svenning⁴ | Alexander Tøsdal Tveit⁴ 

¹GFZ German Research Center for Geosciences, Section Geomicrobiology, Potsdam, Germany

²Cyrosphere Research Station on the Qinghai-Tibet Plateau, State Key Laboratory of Cryospheric Sciences, Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences, Lanzhou, China

³Institute of Biochemistry and Biology, University of Potsdam, Potsdam, Germany

⁴Department of Arctic and Marine Biology, UiT The Arctic University of Norway, Tromsø, Norway

Correspondence

Sizhong Yang, GFZ German Research Center for Geosciences, Section Geomicrobiology, Telegrafenberg, 14473 Potsdam, Germany.
Email: syang@gfz-potsdam.de

Funding Statement

German Ministry of Education and Research; Helmholtz-Gemeinschaft; Research Council of Norway; Chinese Academy of Sciences; National Key Research and Development Program of China; ERC Marie Curie Grant; UiT The Arctic University of Norway; Tromsø Research Foundation; Open Access funding enabled and organized by Projekt DEAL. WOA Institution: DEUTSCHES GEOFORSCHUNGSZENTRUM POTSDAM Blended DEAL: Projekt DEAL

Abstract

Temperature is an important factor governing microbe-mediated carbon feedback from permafrost soils. The link between taxonomic and functional microbial responses to temperature change remains elusive due to the lack of studies assessing both aspects of microbial ecology. Our previous study reported microbial metabolic and trophic shifts in response to short-term temperature increases in Arctic peat soil, and linked these shifts to higher CH₄ and CO₂ production rates (*Proceedings of the National Academy of Sciences of the United States of America*, 112, E2507–E2516). Here, we studied the taxonomic composition and functional potential of samples from the same experiment. We see that along a high-resolution temperature gradient (1–30°C), microbial communities change discretely, but not continuously or stochastically, in response to rising temperatures. The taxonomic variability may thus in part reflect the varied temperature responses of individual taxa and the competition between these taxa for resources. These taxonomic responses contrast the stable functional potential (metagenomic-based) across all temperatures or the previously observed metabolic or trophic shifts at key temperatures. Furthermore, with rising temperatures we observed a progressive decrease in species diversity (Shannon Index) and increased dispersion of greenhouse gas (GHG) production rates. We conclude that the taxonomic variation is decoupled from both the functional potential of the community and the previously observed temperature-dependent changes in microbial function. However, the reduced diversity at higher temperatures might help explain the higher variability in GHG production at higher temperatures.

KEYWORDS

microbial community, permafrost, species diversity, stochastic, temperature gradient

1 | INTRODUCTION

Northern permafrost soils represent the largest terrestrial organic carbon pool, and a major fraction of this carbon is stored in the near surface (the upper 3 m) that is vulnerable to warming (Hugelius

et al., 2014). The Arctic region is warming at about twice the global average rate, with an increase of ~0.29°C in arctic permafrost soil between 2007 and 2016 (Biskaborn et al., 2019). Disproportionate near-surface warming has made permafrost soil carbon increasingly vulnerable to decomposition into carbon dioxide (CO₂) and methane

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(CH₄) by microorganisms (Schuur et al., 2015). An important concern is whether such microbial feedbacks can in turn accelerate climate change (Mackelprang et al., 2011; Prater et al., 2007; Schuur et al., 2015). The response of microbial communities in arctic soils to global warming has important implications for the permafrost carbon feedback. The critical question centers on how microbial communities will respond to warming and how taxonomic and functional changes can influence GHG emissions. This key information is necessary to predict future carbon fluxes in permafrost soils (Graham et al., 2012).

Warming-induced microbial taxonomic shifts were frequently observed in incubation experiments (Barcenas-Moreno et al., 2009; Chen et al., 2015; Oliverio et al., 2017; Sheik et al., 2011) or temperature gradient analogues represented by latitudinal and altitudinal transects or stratigraphic profiles (Ganzert et al., 2007; Radujković et al., 2018; Wang et al., 2015; Wu et al., 2009). An increasing number of transcriptomic, metabolic, and isotopic experiments suggest that rising temperatures regulate microbial activities and community structures on different time-scales (Frey et al., 2013; Karhu et al., 2014; Mackelprang et al., 2011; Stone et al., 2012; Tveit et al., 2015; Yvon-Durocher et al., 2014). Additionally, in a recent study of multiple sites across north America, some particularly temperature-responsive taxonomic lineages were identified (Oliverio et al., 2017). Arctic peat microbiota can rapidly adapt to increased temperature through taxonomic shifts on the transcriptional level, within functional guilds, to maintain high levels of CH₄ production. For instance, Bacteroidetes replaced Firmicutes for syntrophic propionate oxidation above a threshold temperature of 7°C (Tveit et al., 2015). More broadly, increasing temperatures was shown to result in diversity loss (Garcia et al., 2018). Diversity changes can further influence ecosystem function through selection and complementarity effects (Loreau & Hector, 2001). Hence, changes in community structure and the shift pattern are likely to provide possible linkages between taxonomy and functional potential.

Despite many previous works documenting the community response to warming, some studies demonstrated slight or insignificant community dynamics as a result of temperature treatment (Barcenas-Moreno et al., 2009; Juottonen et al., 2008; Metje & Frenzel, 2007; Schindlbacher et al., 2011; Selmants et al., 2016). Many of these studies focused generally on samples from different sites or temperature analogues such as natural transects that differ in ecological history or environmental characteristics across sites. With that heterogeneous benchmark, direct comparison of the sample communities can lead to irrelevant correlations. Furthermore, a temperature gradient analogue that contains spatial heterogeneity is usually coaffected by many factors, making it difficult to separate the temperature influence from other influences. In contrast, comparison of the community structure before and after temperature treatment would typically suggest positive temperature dependencies (Frey et al., 2013; Karhu et al., 2014; Mackelprang et al., 2011; Stone et al., 2012; Tveit et al., 2015; Yvon-Durocher et al., 2014). In general, these incubation experiments were conducted for different durations of time at limited sets of temperatures and sometimes covering large temperature intervals. Detailed information remains

limited regarding how samples with high initial homogeneity respond to a wide and fine-resolution temperature gradient.

In our previous work on anoxic microcosms, we integrated metatranscriptomics and targeted metabolomics to reveal clear successive shifts in microbial function and metabolic activities between three temperature windows 3–5°C, 14–16°C and 24–26°C (Tveit et al., 2015). We identified critical temperatures at which microbial acclimatization cause changes in the metabolic bottlenecks of anaerobic carbon-degradation pathways. Our overall conclusion from this work was that the functional dynamics at different levels of the carbon degradation cascade enable fast adjustments of the microbial system resulting in high CH₄ production rates over a wide temperature range (Tveit et al., 2015). However, microbial responses to elevated temperatures at the population (DNA) level remains a missing piece of the puzzle. Based on the inconsistent community responses to temperature seen in recent literature, we propose that elevated temperature will result in community shifts, but that these shifts will be minor. Thus, the community structure shifts may not reflect the functional temperature responses. Knowledge of the magnitude and patterns of both the active layer microbial community dynamics and their function can ultimately provide complementary insights for better understanding and predicting the consequences of soil temperature changes for these important soil ecosystems in the Arctic. Here, we have used DNA extracted from experiment samples harvested for the 2015 study (Tveit et al., 2015) to sequence 16S rRNA genes and *mcrA* genes (encoding the alpha subunit of Methyl coenzyme M reductase, marker gene for methanogens). Combining this new data with reanalyses of metagenomes, metatranscriptomes and methane and CO₂ accumulation data from the 2015 study, we have shown that temperature-driven shifts in the DNA-based microbial community composition are uncoupled from the functional potential of the community. Furthermore, we show that both the community composition and functional potential (metagenomic-based) are uncoupled from the temperature-driven functional shifts described in 2015 (Tveit et al., 2015).

2 | MATERIALS AND METHODS

2.1 | Sampling and incubation

The sample separation, preincubation and temperature gradient experiments have previously been described in detail (Tveit et al., 2015). Here follows a brief description. Samples (peat blocks) were obtained from active layers at 10–20 cm depth of a High Arctic peat soil from Knudsenheia, Svalbard (78°56 N, 11°49 E) in August 2010. The active layer depth was about 40 cm at the study site. The dominant peat-forming species at this site is the moss *Calliergon richardsonii*. The upper 4 cm of the vegetation was removed before peat blocks were placed in plastic boxes and covered with water from the site to prevent oxygen access. After homogenization of peat and water to make a slurry under anoxic conditions, the slurry was distributed in bottles and pre-incubated for 171 days at 4°C to allow the

slurry to equilibrate. The incubations were conducted in the dark to prevent photosynthesis. After the preincubation, the bottles were distributed along a temperature gradient ranging from 1–30°C in single degree steps, with four bottles at each temperature, and incubated for approximately one month (1–10°C for 39 days, 11–20°C for 35 days, and 21–30°C for 26 days). Per temperature, only three of these bottles (a total of 90) are relevant for the current study. Method used for the gas measurements were previously described in detail (Tveit et al., 2015). Here follows a brief summary. CH₄ and CO₂ were measured with gas chromatograph in two of these bottles per temperature, on day zero and at intervals varying between two and four days, until the termination of the experiment. Moles of gas were calculated using the ideal gas law and by comparison with standards of known concentration under defined pressure, temperature, and volume, yielding the masses of the measured gases in the headspace mixture. Rates, corresponding to the difference between the initial and last measurements, divided by the number of days were finally normalized to the dry weight of the soil slurry within each bottle, respectively. Here, we have used samples collected from the one remaining bottle per temperature for DNA extractions and sequencing. These samples were stored at –80°C until being processed for DNA extraction in summer 2015. In this study, we have analysed both these DNA samples and data that was already published in the spring of 2015 (Tveit et al., 2015).

2.2 | DNA extraction, PCR and sequencing

Originally, 30 soil slurry samples, corresponding to 1°C increments from 1–30°C were available from the experiment described above. When we decided to work further on these samples and extract DNA from all temperatures, the samples from 1 and 17°C were not available. Samples from all the remaining 28 temperatures between 1 and 30°C were used for DNA extraction. We extracted the total genomic DNA in technical triplicates for each temperature (three subsamples of soil slurry) by using the protocol described by Tveit et al. (2015). Briefly, a cetrimonium bromide-containing lysis buffer and phenol:chloroform:isoamylalcohol (25:24:1) were added to all peat samples in lysis matrix E tubes (MP Biomedicals) containing silica beads and exposed to 30 s of vigorous shaking in a FastPrep machine (MP Biomedicals) for the extraction of nucleic acids. After PEG precipitation, ethanol washing and dissolution of pellets in nuclease-free water, nucleic acids were treated with DNase or RNase before metatranscriptome and metagenome generation, respectively. Total RNA was amplified using the MessageAmp II-Bacteria Kit (Ambion Life Technologies) following the kit protocol, except that the linear amplification step was carried out for 14 h. Paired-end sequences were generated by the Illumina HighSeq2000 sequencer at the Norwegian Sequencing Centre (University of Oslo, Oslo, Norway). Amplicon libraries were amplified from a template of total nucleic acids. DNA concentrations were double-checked by using a NanoDrop spectrophotometer and Qubit Fluorometric Quantitation (Thermo Fisher Scientific).

The primer set of 341f (5'- CCTACGGGNGGCWGCAG) and 805R (5'-GACTACHVGGGTATCTAATCC) was used to amplify the V3–V4 region of bacterial 16S rRNA gene (Herlemann et al., 2011). The methanogenic population was profiled by *mcrA* gene primer set *mlas/mcrA-rev* (Steinberg & Regan, 2008). For multiplexing sequencing, sample-specific barcodes were tagged both forward and reverse primers at the 5' end. All PCR reactions were performed in triplicate 50 µl reactions containing 1.0 µl DNA template (5–10 ng/µl), 0.1 µM of each primer and 1.0 U Opti Taq DNA Polymerase (Roboklo). The thermal cycle programme consisted of an initial denaturation at 95°C for 3 min, 30 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 45 s, and a final extension at 72°C for 5 min. Afterwards, three parallel PCR products for each sample were pooled and then purified with the MiniElute PCR purification kit (Qiagen). The purified PCR products were equilibrated after quantification with Qubit Fluorometric Quantitation (ThermoFisher). Sequencing was completed by using the Genome Sequencer Illumina MiSeq (300 bp, paired-end) at GATC Biotech (now Eurofins Scientific).

2.3 | Data preprocessing

The data was preprocessed by combining mothur (version 1.39.0; Schloss et al., 2009) and QIIME (version 1.8; Caporaso et al., 2010). The paired-end reads were processed into contigs in mothur by using the `make.contigs` function (parameter `checkorient=t`, `pdiffs=2`, `bdiffs=1`). The output report and groups files from the mothur platform were parsed with a custom python script to discard the poor quality contigs which have minimum overlap length of less than 25, any ambiguous base ("N") or over 5 mismatch bases. Then, the filtered contig sequences were checked to remove chimeras by using `chimera.uchime` function in Mothur. Afterwards, valid sequences were fed to QIIME for OTU clustering and taxonomical assignment at sequence identity of 97% of bacterial 16S rDNA sequence by referring to the database of SILVA138 (released on Dec 16 2019) (Quast et al., 2013). The *mcrA* nucleotide sequences were clustered at 84% similarity by referring to the results and reference database (Yang et al., 2014).

2.4 | Quantitative, statistical data analysis and visualization

The taxonomic composition was collapsed and summarized at different taxonomic levels by the R package `otuSummary` (Yang, 2020). This package was also used to partition the contribution of a group of taxa to the total beta diversity (Bray-Curtis dissimilarity). In this study, the abundances of metatranscriptomic small subunit rRNA (SSU rRNA) and mRNA, which were retrieved from our previous metatranscriptomic data sets (Tveit et al., 2015), were incorporated into the analysis to compare the fluctuation of microbial abundances at DNA and RNA levels. As our previous metatranscriptomic data, including mRNA and SSU rRNA were limited to three representative

temperature windows (3–5°C, 14–16°C and 24–26°C), we compared the 16S rRNA gene data within these temperature sections. The integrated signals should present valuable insights about the varying trend between phylogenetic profiles and functional profiles (SSU rRNA and mRNA). Additionally, the 16S rRNA gene sequencing profiles were used to predict the functional potential of the bacterial communities by using PICRUST2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) (Langille et al., 2013). The functional potential over the temperature sections was compared to the metagenomic and metatranscriptomic profiles which were re-processed with the raw data from our previous work (Tveit et al., 2015). Both metagenomic and metatranscriptomic data, covering three temperature windows (i.e., 3–5°C, 14–16°C and 24–26°C) were processed by using the METAGENOME ATLAS package (Kieser et al., 2020). Within this pipeline, assembly was done using MEGAHIT and functional annotation was performed with eggNOG-mapper. All parameters were set to default. For further details about this pipeline and default parameter settings, please refer to Kieser et al. (2020). The metagenomic, metatranscriptomic and the PICRUST2-predicted data sets were merged by EC numbers (enzyme committee number) for three temperature windows (9 temperature points in total for each EC) and were then compared by using coefficient of variation (also known as relative standard deviation, which takes account of both standard deviation and mean) in R (R Core Team, 2019). Data were visualized with the R environment (R Core Team, 2019). Heatmaps were implemented in the ggplot2 package (Wickham, 2009). For the variation of the major bacterial phyla and methanogenic OTU phylotypes, the bacterial abundances were normalized by the total counts of the corresponding phylum, since the relative abundances vary within different bacterial phyla and large variability makes it difficult to distinguish the differences between color shades.

The species diversity was compared with the Shannon index across the temperature gradient, and the coefficient of variation was employed to show the extent of variability in relation to the mean of the population at each temperature. The resultant one-dimensional array was visualized with a scatter plot over temperatures. We also studied to which extent the taxonomic groups at the OTU level varied in relative abundance within three different temperature ranges (1–10°C, 11–20°C and 21–30°C). These temperature ranges were the same as previously applied (Tveit et al., 2015). The main rationale for selecting them were to distinguish common summer soil temperatures (1–10°C), uncommonly observed high range soil temperatures expected to be more frequently encountered in the future (11–20°C) and above the range expected to be observed the high-Arctic (21–30°C). These ranges are based on the available temperature data for sites (Bender et al., 2021; Rainer et al., 2020; Tveit et al., 2013; Westermann et al., 2009) close to the one sampled for the current study. The Bray-Curtis dissimilarity matrix, which was calculated based on the whole taxonomic composition, was used to identify the multivariate homogeneity of group dispersions (variances) at three temperature windows by using the betadisper function from the R package VEGAN (Oksanen

et al., 2019). The dispersions (variances) between groups were tested by permutational ANOVA and classical Tukey's honest significant differences (Tukey HSD) with vegan (Oksanen et al., 2019). In addition, the OTU table were collapsed at the phylum level in the three above-mentioned temperature windows (i.e., 1–10°C, 11–20°C and 21–30°C) which reflect the temperature windows used in our previous study (Tveit et al., 2015) as well as allowing balanced sample sets for robust statistic. A pairwise *t* test was used to compare the relative abundance of top abundant six phyla between these temperature windows using GGPUBR package (v0.4.0) (Kassambara, 2020).

To evaluate the pattern of community responses to increasing temperatures, the null model was used with a null hypothesis stating that such change is a stochastic process based on neutral theory. The observed ecological data were randomly shuffled for multiple times to test whether the observed pattern follows the simulated pattern by chance. If the randomized data resemble the observed data, it suggests that the process of interest follows a stochastic pattern. This analysis was performed with the oecosimu function from R package VEGAN (Oksanen et al., 2019) by using binary matrix (i.e., converting OTU counts to presence-absence matrix). Abundance changes of individual taxa (OTUs) were also examined using Pearson correlation test with temperature in R. A total of 51 OTUs, with zero abundance occurring in less than four samples (i.e., <20% of a total of 28 samples), were fed to correlation test to identify those OTUs with correlation $p < .05$ and absolute values of coefficient $>.6$. An additional check was conducted to see whether the relative abundance of any OTU lineages monotonically changed (increased or decreased) along with the temperature gradient by sequentially comparing the differences of all adjacent numbers. The relative abundance increased monotonically if all the differences were greater than zero, and vice versa. With respect to CH₄ production at different temperatures, a linear model was fitted to evaluate the variability in CH₄ production rates against temperatures; the spread of residuals around the linear model was visualized with a boxplot displaying the distribution of the residuals at different intervals of CH₄ production and temperature using the R environment (R Core Team, 2019).

3 | RESULTS

A total of 2,045,659 nonchimera sequences (min: 12,064, first quartile: 41,604, median: 59,556, third quartile: 102,304, max 154,224) were classified into 900 bacterial OTUs. Across the temperature gradient, the bacterial communities were dominated by members from Actinobacteriota (average \pm sd: 26.18 \pm 6.44%), Firmicutes (18.98 \pm 4.09%), Bacteroidota (16.87 \pm 5.82%), Patescibacteria (13.52 \pm 6.52%), Choloroflexi (7.27 \pm 2.41%) and Proteobacteria (6.87 \pm 2.43%). These top six bacterial phyla together contributed an average of 89.72% to the total abundance, and 81.9% to the total Bray-Curtis dissimilarity (Table S1). At OTU level, six predominating OTUs were identified to have mean relative abundance over 2%

across all samples, which together made up for, on average, 23.42% (min: 17.11%, first quartile: 20.15%, median 22.85%, third quartile: 25.76%, max: 33.47%) of the community. Amongst them, the most abundant, OTU1 (7.81%) and OTU2 (5.9%), were affiliated with Firmicutes and Actinobacteriota, respectively.

Several major OTU phylotypes made up more than 5% of their respective phyla subsets (Figure S1). We found that five, three, five, three, five and six OTUs matched this criterion for Actinobacteriota, Firmicutes, Bacteroidota, Patescibacteria, Chloroflexi and Proteobacteria, respectively. These OTUs varied in abundance with elevated temperature, following each their individual pattern. For example, OTU1 and OTU2 remained abundant over the entire gradient, while other OTUs were much more abundant at one or a few specific temperatures (Figure S1). For methanogenic archaea, a total of 1,746,463 sequences (min: 22,480, first quartile: 34,321, median: 48,368, third quartile: 71,958, max: 169,767) could be assigned to only 16 OTUs. The three most dominant OTUs were affiliated to *Methanosarcina*, *Methanomassiliococcus* and *Methanobacterium*. No methanogen OTUs responded monotonically to temperature and the abundance of several OTUs, including *Methanosarcina* (OTU1), varied substantially across the gradient, and not in synchrony with temperature (Figure S2). Due to the low number of OTUs identified we did not further assess their distribution quantitatively.

With all OTUs, a null model test indicated a very low probability ($p < .01$) that the community shifts observed across the temperature gradient would follow in a series of simulated random communities. Therefore, the response of the community to increasing temperatures could not be categorized as a stochastic pattern. However, no lineages at the OTU level were found to respond monotonically to increasing temperature, despite of two OTUs (out of a total of 900 OTUs) which exhibited positive correlation with temperature (OTU9: $p_{\text{Chloroflexi};g_{\text{Leptolinea}}}$, $p < .05$, $r = .73$, OTU14: $p_{\text{Bacteroidota};g_{\text{WCHB1-32}}}$, $p < .05$, $r = .69$) (Table S2, Figure S3). When the taxonomic composition was compared at higher taxonomic level (phylum), some indications for differences between temperature ranges could be observed (low: 1–10°C, middle: 11–20°C, high: 21–30°C), but most of these differences were insignificant. Only Actinobacteriota and Patescibacteria showed statistically significant differences in relative abundance between temperature ranges, being most abundant in the low and high temperature ranges, respectively (Figure 1). Permutational anova test ($p = .81$) and pairwise comparisons with TukeyHSD (Figure S4) on taxonomic variations at OTU level, did not demonstrate significant differences in community dispersion among the three temperature windows, suggesting that community differences were similar in magnitude across the entire temperature range. Despite the taxonomic variations, which sometimes amounted to more than 20% difference in relative abundance within 10°C difference (see, Actinobacteria, Bacteroidia or Patescibacteria, Figure 1), prediction based on 16S rRNA gene data with PICRUSt2 suggested highly similar predicted functional potential profiles over the entire temperature gradient (Figure 2). In fact, the largest differences in predicted relative abundances of the most abundant functions were much less

than one percent. The PICRUSt2-predicted functional profiles were close to the actual functional potential represented by nine shotgun metagenomes available from the previous study (Tveit et al., 2015), and reanalysed here to assess the validity of the PICRUSt2 prediction. The metatranscriptomic profile, on the other hand, had a much higher relative standard deviation (coefficients of variation were 1.78, 1.15, and 1.04 for metatranscriptome, metagenome and PICRUSt2 prediction, respectively). In comparison, the 100 most abundant OTUs had a coefficient of variation of 1.55 within the three temperature windows (Figure S5), more akin to the variability of the metatranscriptomic data and much higher than the variability within the functional potential profiles (i.e., metagenomes and PICRUSt2 predictions).

By comparing different types of fingerprints (16S rRNA genes, SSU rRNA and mRNA), we found that the taxonomic composition was consistent between the 16S rRNA gene- and SSU rRNA transcripts-based results. In contrast, noticeable inconsistency occurred between the DNA based community and the transcriptionally active community, as shown by the rather scattered pattern between the mRNA- and 16S rRNA gene-derived community structures (Figure S6).

Unlike the intricate taxonomic variation on gene and transcript levels, the species diversity analysis exhibited an overall decreasing Shannon index with increasing temperature (Figure 3). The species richness appeared slightly lower at high temperatures, suggesting that richness and not evenness was the driving factor behind the decreasing Shannon index. The CO₂ and CH₄ production rates correlated positively with increasing temperature, indicative of a stable and predictable temperature response. However, we observed higher dispersion in the rates above 15°C, as shown clearly in plots comparing the predicted rates to the residuals (Figure S7).

4 | DISCUSSION

Higher microbial soil organic carbon (SOC) degradation rates in permafrost regions represent a potentially important feedback response to climate change (Schoor et al., 2015). For modelling and prediction, it is necessary to improve our understanding of how climate change can modulate microbial systems. Focus on the influence of single variables such as temperature by minimizing the influence of other factors in experiments is important. This study investigated the responding pattern of microbial communities in high-Arctic anoxic peat soil to a high-resolution temperature gradient and revealed shifts in taxonomy and diversity. The link between community composition and its functionality and functional potential was then assessed.

We saw that the observed temperature responses of individual taxonomic lineages were not unimodal or linear, but rather discrete. This is in line with many previous studies on taxonomic shifts (Barcenas-Moreno et al., 2009; Mackelprang et al., 2011; Oliverio et al., 2017) but it does not match the previously observed switch in metabolic functionality (transcript and metabolite based) occurring

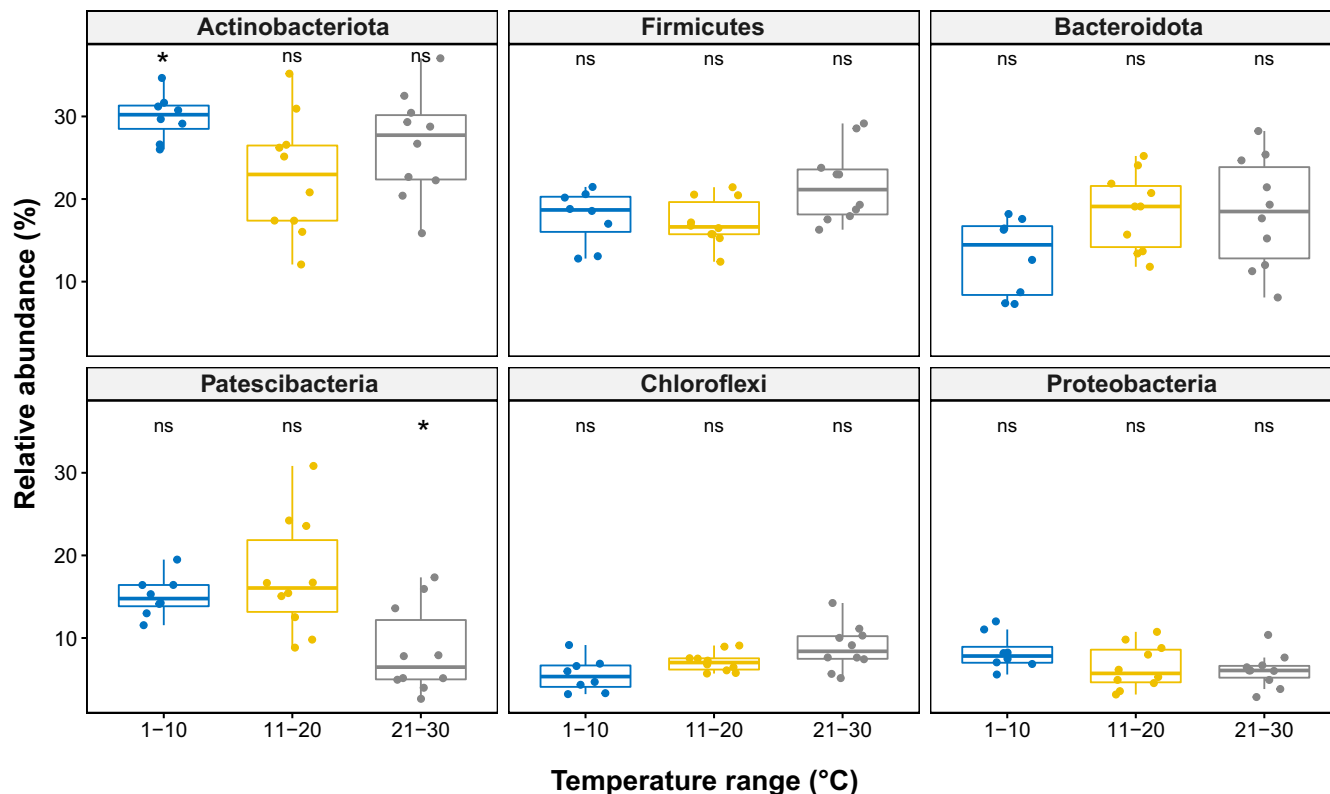


FIGURE 1 The relative abundance of top six abundant phyla at different temperature windows. The temperature windows followed and expanded our previous work (Tveit et al., 2015). All OTUs affiliated to the six phyla were collapsed by phylum level. The numbers of observations for each temperature window were 9, 9 and 10, respectively. ns, not significant. The phyla for which the relative abundances were significantly different ($p < .05$) from one temperature window to the two other were marked with an asterisk above the corresponding box

between temperatures above and below the threshold temperature of 7°C (Tveit et al., 2015). The discrete response of individual lineages to temperature may be associated with the thermal fitness of individual species which determines the performance (traits) of species under changing thermal regimes (Garcia et al., 2018; Limberger et al., 2014) and generally declines much more rapidly after the optimal temperature (Angilletta et al., 2003; Huey et al., 2012). Alternatively, these discrete shifts might be signs of bottle-specific selection processes. After temperature change, substrate flows change (Tveit et al., 2015), leading to a situation where functional guild members must compete for their common substrate under a different set of circumstances. We suspect that during this competition, only a certain number of strains carry the properties to win and become the dominant member. This might help to explain the large community differences that seem to occur irrespective of temperature. Our results show that the community shifts are not random, although they appear random. The reason for this might be that while a large number of theoretical possibilities for community change exist, only a restricted fraction of these are realistic due to biological and physical constraints. The abundance shifts of the dominant phyla Firmicutes and Bacteroidota, although insignificant, may indicate subtle differences in suitable temperature ranges. Each taxonomic group constitutes various lineages with different preferences, possibly including different optimum temperatures for growth, and

are thus likely to represent portfolios of thermal fitness, substrate affinities and other properties. These adaptations can further mediate ecological dynamics and ecosystem functioning (Norberg et al., 2001). Many members of Firmicutes and Bacteroidota are typical carbon fermenters (Fischbach & Sonnenburg, 2011; Huang et al., 2018), and different functional performances of Bacteroidota and Firmicutes have been observed at low temperatures 3–5°C compared to higher temperatures (14–16°C and 24–26°C) (Tveit et al., 2015). The shift in taxonomic composition at higher taxonomic ranks (Figure 1) may thus indicate possible exchanges or replacements of important ecosystem players, which can affect carbon degradation pathways and rates, contributing to the increasing GHG production rates (Figure S7). In addition, rising temperatures can indirectly regulate microbial community structure through modulating multiple habitat elements such as soil water content, nutrient pools, plant root properties and plant-microbe interaction (Classen et al., 2015). A recent study even suggested that indirect effects can prevail over direct impacts of temperature on soil microbial community structure (Deltedesco et al., 2020).

It should be noted that the taxonomic composition does not necessarily represent the active population as shown in the mRNA-derived data. Decoupling of microbial community composition and functioning under warming have been frequently observed (Frey et al., 2013; Karhu et al., 2014; Mackelprang et al., 2011). Communities

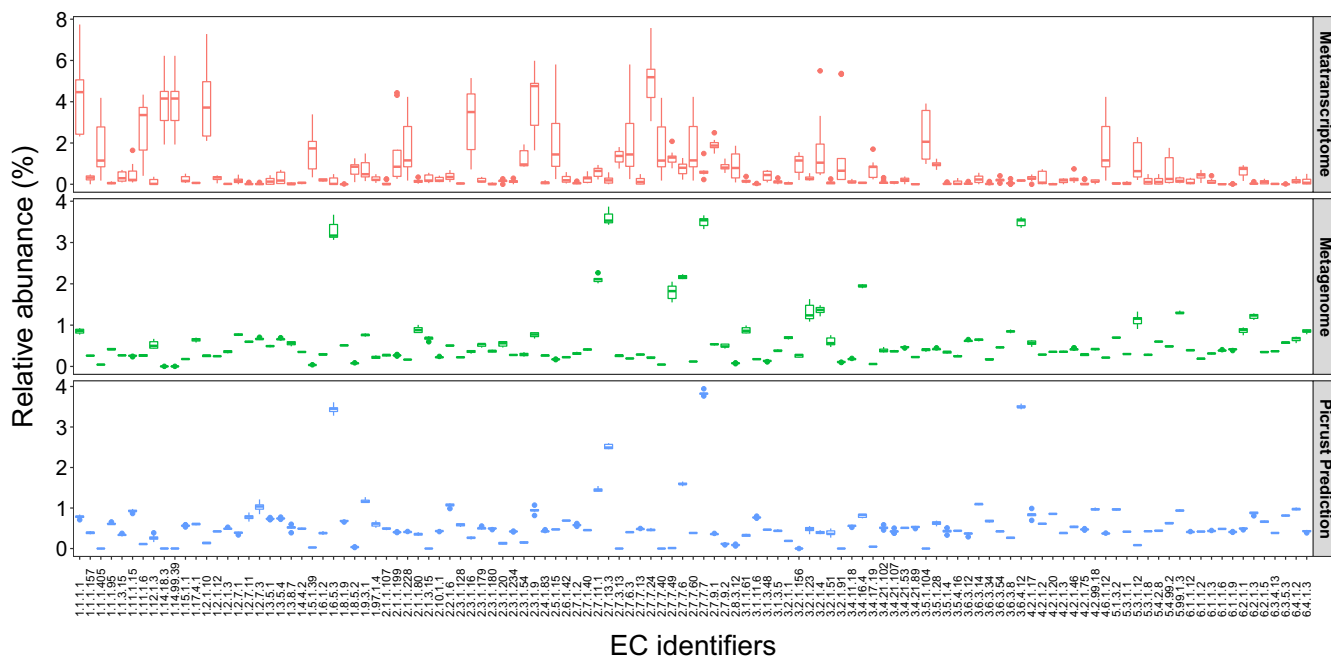


FIGURE 2 Boxplot showing the functional profiles of metatranscriptome, metagenome, as well as the PICRUSt2-predicted functional potential for the most abundant functions with mean relative abundance $>0.25\%$. Here, each box displays the distribution of relative abundance of each EC item over nine temperatures ($3\text{--}5^\circ\text{C}$, $14\text{--}16^\circ\text{C}$ and $24\text{--}26^\circ\text{C}$). The functional potential prediction was performed by PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) based on 16S rRNA gene profiling results. The functional profiles from metagenomes and metatranscriptomes were reprocessed from previously generated datasets (Tveit et al., 2015). As we targeted the general signature of the functional potential, the rare lineages with total counts <10 across all samples were excluded from this analysis. The x-axis was placed according to the EC (enzyme committee numbers)

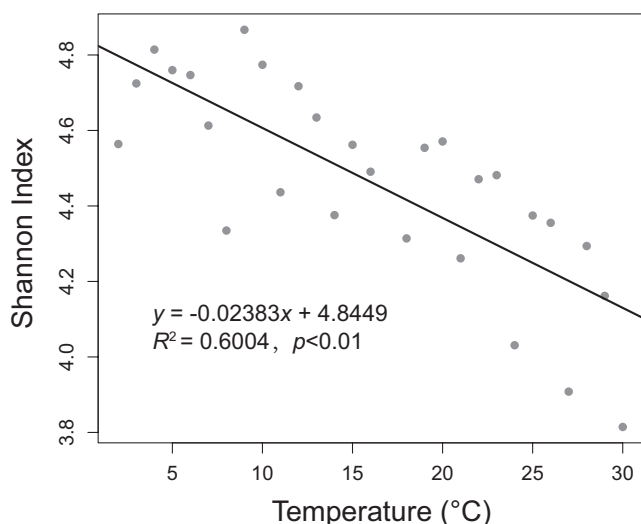


FIGURE 3 Changes of diversity in terms of Shannon index along temperature gradient

typically exhibit high functional redundancy as multiple coexisting, taxonomically distinct organisms can perform the same metabolic function (Louca et al., 2018). For example, comparisons of gene pools for SOC degradation from geologically distant Arctic, temperate and subtropical soils revealed similar metabolic potentials (Tveit et al., 2013). Even disparate phylogenetic responses to warming may

still have similar functional consequences (Mackelprang et al., 2011). Consistent with previous studies, the predicted functional potential using our 16S marker gene data, and supported by our metagenomics analyses, implied relatively consistent functional potential at different temperatures (Figure 2). In our opinion this reflects a disconnection of functional potential and taxonomy with increasing temperatures, where the stability of functional guilds contrasts the variable taxonomy, both of these observations contrasting the previously observed functional shifts based on metatranscriptomics and targeted metabolomics (Tveit et al., 2015). We acknowledge that our prediction of the functional potential from 16S rRNA genes is not entirely equivalent to metagenomic profiles, but we could show that the approach is suitable for our environmental samples as shotgun metagenomes generated from nine of the same homogenized samples agreed with the PICRUSt2 prediction. While the difference in functional potential and taxonomic variation suggest that microbial communities are functionally redundant, even across large temperature gradients, we consider that the clearest manifestation of a decoupling between taxonomy and function was the contrast between the discrete taxonomic changes and the linear temperature-dependence of GHG production (Figure 2 and Figure S7). Thus, we would propose that under changing thermal regimes, temperature may mediate the traits that influence the performance of different species, but taxonomic variations appear to be a poor predictor for functional performance.

Unlike the intricate taxonomic variation, rising temperature resulted in reduced species diversity, and increased GHG production rates (Figure 3 and Figure S7). Previously we have shown that the temperature dependence of CO₂ and CH₄ production rates (Figure S7) follow the square root model and not the Arrhenius equation, probably due to a combination of different temperature responses in multiple enzyme-catalyzed reactions and changes in the microbiota along the temperature gradient (Tveit et al., 2015). In line with our observations, diversity loss with increasing temperature was also observed in other studies (Garcia et al., 2018; MacLean et al., 2019; Parain et al., 2019). Biodiversity change can influence ecosystem function through selection effect and complementarity effect, that is, a dominance of species with particular traits that affect ecosystem processes while resource partitioning or positive interactions lead to increased total resource use (Loreau & Hector, 2001). At lower temperatures, more diverse communities have a greater probability of including species with broad-spectrum thermal traits that help maintain function. Conversely, higher temperatures tend to favour those lineages that can tolerate higher temperatures and use resources at the relatively fast rates driven by higher temperatures. At higher temperatures, inter-species complementarity could partially compensate the influence of biodiversity loss on ecosystem function (Garcia et al., 2018). Owing to the above factors, increasing temperature is likely to support the fittest individuals at the cost of a decline in diversity and functional stability. This change may be a possible reason for the higher variability in GHG production rates above 15°C (Figure S7). In addition, our previous meta-omics study revealed higher abundances of predatory Cercozoa (unicellular eukaryotes) with increasing temperature (Tveit et al., 2015). Predation may disproportionally scale the diversity through discriminant attacks on specific groups and limit the total biomass of prokaryotic population as shown by the reduced mass of DNA extract per gram of soil at higher temperatures (Figure S8). Nevertheless, the response of the microbial community to increasing temperature is not a random process. Instead, the community have reacted to the increasing temperatures, resulting in predictable system functionality although more variable GHG production rates.

This study attempted to focus on the impact of changing temperatures on microbial communities in a closed system without any nutrient amendments, simplifying the process compared to natural ecosystems. In addition to direct effects, temperature can indirectly regulate microbial GHG production through influencing for example, nutrient availability, soil moisture and plant root exudates (Barcenas-Moreno et al., 2009; Frindte et al., 2019; Heinze et al., 2017). Further interaction among these factors could additionally complicate the response patterns under natural conditions. All samples in our experiment originated from one homogenous batch of soil slurry material to minimize the system heterogeneity, then split into multiple bottles prior to incubation. Although this design did not reach an ideal homogeneous condition, in part due to preincubation in separate bottles, the simplicity of our approach attempted to highlight the effects of temperature without becoming

entangled in complex interactions of many variables. In fact, the preincubation itself may have facilitated parts of our approach, as it allowed for sufficient variation between bottles to enable a thorough evaluation of the link between temperature, the microbial community, functional potential and functional shifts under boundary conditions.

5 | CONCLUSIONS

We have previously shown how the temperature-induced effects of substrate availability cause taxonomic shifts within functional guilds and functional changes within taxa, leading to increased GHG production in Arctic peat soil (Tveit et al., 2015). The follow-up study presented here utilizes samples from the same experiment to demonstrate how broader, nonrandom, taxonomic responses to rising temperatures are not linked to the functional potential or any of the previously observed functional shifts. Such taxonomic changes clearly illustrated the flexibility and functional redundancy of the microbial community, suggesting that this feature is involved in sustaining the metabolic potential of the community under change. The microbial communities also responded to increasing temperature by decreasing species diversity (Shannon index) matching a clearly reduced functional stability above 15°C. Thus, our observations also indicate that microbial community changes in Arctic peat soil can affect its functionality through a reduced species diversity that correlates with a higher variability in CH₄ and CO₂ production rates.

ACKNOWLEDGEMENTS

This study was supported by the Helmholtz Gemeinschaft (HGF) through funding for SL's Helmholtz Young Investigators Group (VH-NG-919). SY and SL were supported by the German Ministry of Education and Research as part of the projects CarboPerm (grant no. 03G0836A, 03G0836D), and KoPf (grant no. 03F0764A, 03F0764F). SY also acknowledges support from Chinese Academy of Sciences and National Key Research and Development Program of China (2020YFA0608501). MMS and SL were supported by the ArcBiont project funded by Helmholtz Foundation and UiT The Arctic University of Norway. ATT was supported by the Research Council of Norway FRIPRO Mobility Grant Project Time and Energy 251027/RU, cofounded by ERC under Marie Curie Grant 606895, and Tromsø Research Foundation starting grant project Cells in the Cold 16_SG_ATT.

AUTHOR CONTRIBUTIONS

Alexander Tøsdal Tveit, Mette Marianne Svenning, Susanne Liebner and Sizhong Yang designed the research. Sizhong Yang, Susanne Liebner, Alexander Tøsdal Tveit performed and analysed the research. Sizhong Yang, Susanne Liebner, Mette Marianne Svenning and Alexander Tøsdal Tveit interpreted the results and wrote the paper. All authors contributed to the discussions and reviewed the manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

DNA amplicon sequences have been deposited to the European Nucleotide Archive (ENA) (<https://www.ebi.ac.uk/ena>) under accession number PRJEB40939 (ERS5241115-ERS5241170). In addition, the metagenomic and metatranscriptomic data were retrieved from NCBI SRA database by SRP014474. The metatranscriptomic raw data were downloaded by accession numbers of SRR1958837, SRR1958842, SRR1958847, SRR1958852, SRR1958857, SRR1958863, SRR1958867, SRR1958872 and SRR1958877. The metagenomic raw data were retrieved by accession numbers of SRR1958836, SRR1958840, SRR1958845, SRR1958851, SRR1958855, SRR1958859, SRR1958865, SRR1958869 and SRR1958874.

ORCID

Sizhong Yang  <https://orcid.org/0000-0001-8417-593X>

Susanne Liebner  <https://orcid.org/0000-0002-9389-7093>

Alexander Tøsdal Tveit  <https://orcid.org/0000-0002-4163-3416>

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Yang, S., Liebner, S., Svenning, M. M., & Tveit, A. T. (2021). Decoupling of microbial community dynamics and functions in Arctic peat soil exposed to short term warming. *Molecular Ecology*, 00, 1–11. <https://doi.org/10.1111/mec.16118>