Reduced methane emissions in former permafrost soils driven by vegetation and microbial changes following drainage

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Abstract
In Arctic regions, thawing permafrost soils are projected to release 50 to 250 Gt of carbon by 2100. This data is mostly derived from carbon-rich wetlands, although 71% of this carbon pool is stored in faster-thawing mineral soils, where ecosystems close to the outer boundaries of permafrost regions are especially vulnerable. Although extensive data exists from currently thawing sites and short-term thawing experiments, investigations of the long-term changes following final thaw and co-occurring drainage are scarce. Here we show ecosystem changes at two comparable tussock tundra sites with distinct permafrost thaw histories, representing 15 and 25 years of natural drainage, that resulted in a 10-fold decrease in CH$_4$ emissions (3.2 ± 2.2 vs. 0.3 ± 0.4 mg C·CH$_4$·m$^{-2}$·day$^{-1}$), while CO$_2$ emissions were comparable. These data extend the time perspective from earlier studies based on short-term experimental drainage. The overall microbial community structures did not differ significantly between sites, although the drier top soils at the most advanced site led to a loss of methanogens and their syntrophic partners in surface layers while the abundance of methanotrophs remained unchanged. The resulting deeper aeration zones likely increased CH$_4$ oxidation due to the longer residence time of CH$_4$ in the oxidation zone, while the observed loss of aerenchyma plants reduced CH$_4$ diffusion from deeper soil layers directly to the atmosphere. Our findings highlight the importance of including hydrological, vegetation and microbial specific responses when studying long-term effects of climate change on CH$_4$ emissions and underscores the need for data from different soil types and thaw histories.
1 | INTRODUCTION

The northern hemisphere’s permafrost soils store an estimated 1.6 Gt of soil organic carbon (SOC), which is double the amount currently measured in our atmosphere (Hugelius et al., 2014; Schuur et al., 2015). With climate warming, this carbon can become available for microbial decomposition resulting in further carbon dioxide (CO₂) and methane (CH₄) being released to the atmosphere, thus generating a positive climate warming feedback (Biskaborn et al., 2019; Natali et al., 2021; Schuur et al., 2015, 2021). Our current view on the terrestrial Arctic CH₄ budget is strongly biased (Margesin & Collins, 2019; Messan et al., 2020; Stackhouse et al., 2017) towards emission hotspots in peat wetlands (Altshuler et al., 2019; Bäckstrand et al., 2010; Lupascu et al., 2012; Mastepanov et al., 2008; Matveev et al., 2018; Merbold et al., 2009; Moguel et al., 2021; Sachs et al., 2008; Sturtevant et al., 2012; Tom & Chapin, 1993; Van Huissteden et al., 2011; Wagner et al., 2005), while less attention has been given to carbon-poor mineral soils (Emmerton et al., 2014; Jørgensen et al., 2015). These soils, which are subject to much faster thaw rates than peatlands (Westermann et al., 2015), cover ~87% of the region, which corresponds to ~71% of the total estimated SOC within the top 0–3 m (Hugelius et al., 2014; Pérez et al., 2015; Tarnocai et al., 2009). A large proportion of this area is classified as tussock tundra (336,000 km²) which has permafrost as a prerequisite for ecosystem functioning (Molau, 2010; Walker et al., 2005). Therefore, any changes to these environments are expected to have significant impacts on the global CH₄ budget.

Ecosystem soil CH₄ dynamics are controlled by two co-occurring microbiological processes; methanogenesis (the production of CH₄), performed by methanogenic archaea under strictly anoxic conditions in conjunction with fermentative, syntrophic partners; and aerobic methanotrophy (the consumption of CH₄), performed by methanotrophic bacteria when oxygen is available (Nazaries et al., 2013). Methane, once produced in the soil, can be emitted to the atmosphere through molecular diffusion, ebullition and plant-mediated transport by aerenchyma plants (Nielsen et al., 2017; Serrano-Silva et al., 2014). These plants are specialized for flooded areas as O₂ can diffuse through aerenchyma conduits to anoxic root zones, which in turn allows direct CH₄ diffusion to the atmosphere, thus bypassing potential CH₄ oxidation by methanotrophic bacteria. Aerenchyma plants inhabit wetlands, but also intact tussock tundra with permafrost present, where flooded conditions occur frequently due to hampered drainage by the frozen ground (Brix et al., 2001; Greenup et al., 2000; Henneberg et al., 2012; Ström et al., 2003). Tussock forming aerenchyma grasses like the hare’s-tail cottongrass (Eriophorum vaginatum L.) thrive under these conditions as their metabolic activity in spring starts early due to their partly aboveground roots (Chapin et al., 1979).

The disappearance of permafrost marks the onset of an ecosystem transition initiated by changes in hydrology (Zona, 2016). Once the frozen soil layer that acts as a barrier to soil drainage disappears, a number of changes have been shown to occur, such as increased topsoil temperatures and aeration (Kwon et al., 2016) which can lead to a shift in vegetation patterns (Bjorkman et al., 2018) including shrubification (Martin et al., 2016). Drainage experiments, carried out in the peatlands of Siberia and Alaska, have shown that these changes are accompanied by a reduction in CH₄ fluxes (Kwon et al., 2017; Merbold et al., 2009; Sturtevant et al., 2012; Zona et al., 2009) which can be linked to a loss in both methanogenic and methanotrophic microbial communities in the drained soils (Kwon et al., 2017, 2019, 2021). While the overall effect of climate change on drainage and shifts in hydrology is unclear, recent model predictions suggest a long-term drying of surface soil for permafrost regions due to a larger moisture infiltration to deeper soils (Andresen et al., 2020).

Both emissions and uptake of CH₄ are sensitive to temperature changes (Le Mer & Roger, 2001; Segers, 1998), and progression/change of seasons (Arndt et al., 2019; Wagner et al., 2003; Zona et al., 2016), with methanogenesis generally responding faster to warming (Segers, 1998). However, high-affinity methanotrophs, oxidizing CH₄ at low concentrations, have been suggested to be even more sensitive to temperature changes (Oh et al., 2016, 2020). Furthermore, the type of ecosystem also plays a role when it comes to the relationship between temperature and CH₄ emissions, with wet tundra (water table at or above soil surface) being more responsive to changes compared to drier sites (water table below soil surface) where the position of the water table impacts CH₄-cycling processes (Olefeldt et al., 2013). Thus, the sensitivity of these ecosystems to climatic shifts and permafrost thaw makes them particularly vulnerable to climate changes, regarding both temperatures and hydrological conditions (Elberling et al., 2013; Molau, 2010; Ridefelt et al., 2008; Walker et al., 2006).

Although artificial drainage field experiments are conducted and monitoring of permafrost thaw regions is ongoing (Kwon et al., 2021; Merbold et al., 2009; Schuur et al., 2009; Sturtevant et al., 2012; Zona et al., 2009), more data covering a wider range of Arctic sites, including current and post-thaw conditions are required in order to better predict the long-term responses in light of a warming climate (Zona, 2016). To improve our understanding of the long-term effects of permafrost thaw-out on CH₄ emissions, we compared two tussock tundra sites with decadal differences in their complete loss of permafrost. For these upland-mineral soils, we hypothesized that increased drainage, following the loss of permafrost, will alter the ecosystem’s CH₄ production and emission pathways including: changes in plant composition, with a reduction of wetland associated species, and changes in the microbial community, especially a loss of methanogens in drier soils. This would lead to a reduction in CH₄ emissions,
as the amount of CH\textsubscript{4} emitted to the atmosphere ultimately depends on the balance between microbial production and oxidation of CH\textsubscript{4}, in combination with changes in the relative importance of emission pathways (Nazaries et al., 2013).

2 | MATERIALS AND METHODS

2.1 | Field sites

The two tussock tundra sites used in this study are located in the vicinity of Abisko, in sub-arctic Sweden, where the presence of tussock tundra communities are restricted to current or former permafrost grounds (Molau, 2010). The site at lake Latnjajaure (68°21.2′N, 18°29.3′E and 981 m a.s.l.) is located close to the Latnjajaure Field Station (LFS), part of the International Tundra Experiment (ITEX; Beylich, 2021) per year during later years (period 1993 to 2006, Scharn et al., 2021). At this tussock tundra site, the permafrost was last recorded in 1993 and confirmed absent in 2001 (Beylich, 2003; Beylich et al., 2004; Molau, 2010). Although water table depth is not monitored at LFS, the permanently water-filled boulder pits at the site now drain completely over the growing season (Molau, 2010). The second site, at lake Corrvosjávri (68°24.9′N, 18°38.1′E, and 814 m a.s.l.), was first identified as tussock tundra community via LandSat images and helicopter surveillance in 2005, during a pronounced flowering season of Eriophorum vaginatum (Molau, 2010). Here the frozen ground thawed decades ago and the former plant community is now experiencing a transition to a shrub tundra ecosystem, ideal for studying long-term changes following the warming of active layer and thawing of underlying permafrost. Both sites have a mineral soil identified as Haplic Gleysol, topped by a shallow organic layer (1–8 cm), underlain by a brown (ferric-containing) mineral soil, followed by a strongly reduced blackish-grey (ferrous-containing) mineral soil below 12 cm (Molau, 2010).

2.2 | Climatic conditions and permafrost

Climate variabilities have been recorded in Abisko since 1913 and reveal a warming event during the late 1930’s and early 1940’s, followed by a colder period (Callaghan et al., 2010). Since the mid-1970’s, the region is experiencing a warming trend exceeding that of the 1930’s to 1940’s, which also influences permafrost conditions for the region (Johansson et al., 2011). Several approaches were used to predict the occurrence of permafrost for this area, and the likelihood of finding permafrost around Latnjajaure is estimated to be <50% (Gisnås et al., 2017; Ridefelt et al., 2008). Corrvosjávri falls outside the predictive area for these local high resolution modelling attempts but is located within the area covered by regional models that estimate the sporadic occurrence of permafrost. Since fine-scale reconstructions of historical permafrost distribution are rare for the Abisko region and surrounding areas (Yang et al., 2012), we used temperature data from LFS and Abisko Scientific Research Station to estimate a time window of permafrost thaw at Corrvosjávri. Environmental lapse rates were established on a monthly basis using daily average temperatures (period 1993–2019) from the automatic weather station at LFS (Björk et al., 2007) and the meteorological observations at Abisko Scientific Research Station (Callaghan et al., 2010). The calculated monthly lapse rates are in line with earlier observations from the region (Table S2) and were used to model the Mean Annual Air Temperatures (MAAT) at both Latnjajaure and Corrvosjávri based on the historical record from Abisko (1913–2019, Figure 1). Furthermore, the MAAT was smoothed using a 5-year running mean according to (Yang et al., 2012) and a polynomial fit was established for the entire period (Callaghan et al., 2010).

Given that permafrost was confirmed present at Latnjajaure 1993, and confirmed absent to a depth of at least 40 m in 2001 (Beylich, 2003; Beylich et al., 2004; Molau, 2010), the final thaw coincides with the period when the long-term MAAT reaches >2°C, a threshold that was previously suggested as the lower limit for sporadic permafrost occurrence in the Scandes (Ødegård et al., 1996). Corrvosjávri passed this threshold during the 1980’s (Figure 1). This results in a final permafrost thaw difference of a minimum of one decade between the two sites. However, different threshold MAATs have been estimated for mountainous regions (Ettzelmüller et al., 2006; Haebler et al., 2011), and local variations in topography, snow cover and aspect, among others (Johansson et al., 2006), may also influence the actual soil temperatures and permafrost conditions. There is a lack of historical data from the two sites, especially Corrvosjávri which lies outside the commonly investigated area around Abisko and LFS. Corrvosjávri might have lost its permafrost earlier than estimated here, and likely had a phase during the 1930’s to 1940’s warm period with less favorable permafrost conditions.

2.3 | Vegetation

The vegetation at both sites was surveyed during mid-July in both 2006 (Molau, 2010) and 2016 using the point-intercept methodology (Molau & Mølgaard, 1996) with 20 point-frame squares (0.25 m\textsuperscript{2} and 25 intercepts each) laid out as two transects (20 m each) in a cross and a 2 m distance between each point-frame location. The 2016 transects were laid out within a couple of meters of the 2006 transects based on photographs. The overall trend in vegetation was analyzed using Non-metric multidimensional scaling (NMDS) with Bray-Curtis distances (Canoco 5 software) where the influence of single occurring plants was down-weighted.

\footnote{https://www.polar.se/en/research-support/abisko-scientific-research-station/weather-data/}
2.4 | Flux and environmental measurements

During the growing season of 2016–2018, flux measurements of CH₄ and CO₂ were conducted on a bi-weekly basis at both sites, from early June to the end of August with an additional campaign in early October 2017 (a total of 20 field campaigns), utilizing a closed chamber technique on pre-installed soil collars. Three groups of three collars were installed at each site within the area covered by the vegetation surveys, and gas flux data were averaged for each group (n = 3 per site). Fluxes of CH₄ and CO₂ fluxes were measured using an ultraportable greenhouse gas analyzer (Los Gatos Research) operating at 1 Hz, with a precision of <2 ppm and <300 ppm (respectively) and an operational range of 0–500 and 0–20,000 ppm, respectively. For net exchanges of CH₄ and CO₂, the analyzer was connected to a transparent chamber (r = 9.5 cm, h = 20 cm), while ecosystem respiration was measured in darkness by covering the...
tussock soils were sampled using either an electric drill (r = 1 cm), during the initial frozen conditions, or with a soil auger (r = 1.5 cm). Soil samples were pooled to form one homogenized sample per soil depth for each group of three collars, allowing for comparisons with the flux data ($n = 3$ per site and soil layer). Microbial samples were transferred to sampling tubes with silica gel (both sterilized), samples for chemical analysis were collected in Ziploc bags, and both were stored cold during fieldwork and frozen within one week of sampling. Microbial samples were shipped frozen to the University of Lyon, France, while biochemical samples were shipped to the University of Gothenburg, Sweden.

### 2.6 Soil physical and chemical measurements

Gravimetric soil water content was carried out by drying soil at 70°C for 48 h, and soil organic matter (SOM) was measured as a loss on ignition by heating the dried samples at 550°C for 6 h. Parts of the dried samples were also grinded followed by C and N quantification using Isotope Ratio Mass Spectrometry (HS2022, Sercon Limited). The pH was measured by shaking dry soil with deionized water (ratio: 1:10) for half an hour and pH was then recorded (691 pH Meter, Metrohm, Riverview, Florida, U.S.) after sedimentation overnight. The procedure was repeated with the addition of 1 M KCl to a final concentration of 0.1 M in the solution.

### 2.7 Microbial community analysis

DNA was extracted from 0.25 to 0.5 grams of soil using a protocol that has been successfully applied on various soil types including often difficult to extract clay soils (Griffiths et al., 2000). DNA was subsequently fluorometrically quantified (Qubit, Invitrogen™), diluted to a concentration of 1 ng µl⁻¹ and stored at -20°C until experimental use. Targeted amplicons were generated with a Platinum Taq (Invitrogen™) assay for the PCR step using modified 515f/806r (Walters et al., 2015), MFL/M1r (Luton et al., 2002) and A189f/A650r (McDonald et al., 2008) primer pairs with the Nextera Illumina adapter sequences (5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3′ and 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3′) attached to the 5′ end to amplify the V4 region of the 16s rRNA gene, methyl coenzyme-M reductase subunit A (mcrA) and particulate methane monooxygenase (pMMO) respectively. PCR reactions (25 µl final volume) were set up as recommended in the supplier protocol with 5 to 10 ng of DNA, 0.5 µl of the primer pair (10 µM) and 200 ng of UltraPure BSA (Invitrogen™). The temperature program for the reaction was 3 min at 94°C followed by 30 (16s rRNA), 40 (mcrA) and 40 (pmoA) cycles of 30 s at 94°C, 30 s at 50°C (16s rRNA), and 55°C (mcrA and pmoA) and 60 s at 72°C followed by 10 min at 72°C and subsequent 4°C to stop the reaction. Successful amplifications of the targeted gene amplicons were confirmed on agarose gels and only assays with no amplification in the negative control (ultrapure water used during DNA extraction and library preparation) were further processed. Amplified DNA was cleaned-up using AMPure XP Beads (Beckman Coulter™) and used for the subsequent PCR with Illumina Nextera XT index primers to add barcode sequences to amplicons of each sample followed by another round of bead clean-up. The concentration of resulting libraries of each sample
was measured spectrophotometrically and subsequently combined to 3 equimolar pools, each representing libraries from one targeted gene. The pools were run on a DNA 1000 chip (Agilent 2100 Bioanalyzer) to verify absence of primer dimers and the correct size of the libraries. Molarities of final pools were estimated by quantitative PCR using the QuantiFast SYBR® PCR Kit (Qiagen) with primers targeting the P5 and P7 flanking regions of Illumina Nextera XT libraries with a standard assay and two-step cycling program recommended by the supplier. Dilution series of successfully sequenced libraries were used as standards. Pools were diluted to 4 nM and loaded on a V2 2 × 250 bp flow cell for paired-end sequencing on an Illumina MiSeq platform following the protocol provided by Illumina.

### 2.8 Sequence processing

Forward and reverse read files for all sequenced genes of each sample were merged using the **iu-merge-pairs** command from the Illumina-Utils v2.6 libraries using the—enfore-Q30-check flag to ensure quality filtering over the entire read length before merging (Eren et al., 2013). Successfully merged amplicons of the V4 region of the 16S rRNA gene were filtered for chimeric sequences and further annotated with the RDP classifier (Edgar et al., 2011; Wang et al., 2007). Taxonomic annotation of the sequenced marker genes was performed by placing sequenced amplicons in a reference tree using GraftM (version v0.13.1; https://github.com/geronimp/graftM—released under GNU General Public License v3+) (Boyd et al., 2018). The graftM package including reference sequences for mcrA gene was downloaded from the repository at GitHub. For pmoA annotation, a multiple sequence alignment calculated with MAFFT (version v7.475) (Katoh et al., 2002) of 7809 sequences downloaded from the pmoA gene reference database at the GFZ Potsdam (Yang et al., 2016) was used to create a graftM package. All further processing and analysis on annotation tables were carried out using the R-package *phyloseq* (McMurdie & Holmes, 2013; Core R Team, 2019). Significances of differences of means between sample groups were estimated by the *ggpubr* package. Differential abundance analysis on the 16S rRNA gene dataset was performed using the DESeq2 package. Diversity estimates were calculated with the R-packages *vegan* and *betadisper*.

### 2.9 Marker gene quantification of methane cyclers

Primer pairs 341F/S34R (Watanabe et al., 2001), MLF/MLr (Luton et al., 2002) and A189f/A650r (McDonald et al., 2008) targeting the 16S rRNA, mcrA and pmoA gene respectively were used to estimate gene copy abundances in DNA samples by quantitative PCR (QuantifiFast SYBR® PCR Kit; Qiagen). For standards, amplicons of the respective genes were amplified from a soil sample, inserted in a pGEM-T vector (Promega) and cloned in chemically competent *E.coli* cells (InvivogenTM) spread out on selective LB-agar. PCR with M13 primers flanking the insert site on the vector on successfully cloned colonies was sent for Sanger sequencing to confirm correct sequences of the targeted genes (Eurofins Genomics). M13 amplicons of correct sequences were quantified (n=3) by a Qubit assay (InvivogenTM) for estimation of standard copy numbers. Gene quantification assays contained 1–5 ng of DNA, 0.5, 1.4 and 1.4 µM of respective primers for 16S rRNA, mcrA and pmoA genes, 200 ng of UltraPure BSA (InvivogenTM), 10 µL of the 2x master mix andultrapure water (final volume 20 µL). A two-step cycling program with 5 min at 95°C followed by 30, 35 and 35 cycles (16S rRNA, mcrA and pmoA, respectively) of 5 sec at 95°C and 30 s at 60°C finished by a melting curve (60–98°C; 1°C /min increase) was performed in a Corbett Rotor-Gene 6000 real-time PCR cycler. Each run included standards of the respective targeted gene in a range of 10^2–10^7 copies/reaction as well as non-template controls to check for contamination. Runs with no amplification in the no-template-control and values >0.98 and between 0.9 and 1.1 for R^2 and efficiency from the standard curves were used for further analysis and plotting in R.

### 3 RESULTS

#### 3.1 CH_4, CO_2 emissions and soil geochemistry

Measured CH_4 emissions at the study sites with decadal differences in their permafrost history decreased 10-fold. Latnjajaure (loss of permafrost between 1993 and 2001) showed emissions of 3.9 ± 3.2 mg C-CH_4 m^-2 day^-1 (growing seasons 2016–2018), while Corvosjärv (estimated loss of permafrost in the 1980s based on MAAT records) showed 0.2 ± 0.3 mg C-CH_4 m^-2 day^-1 (growing seasons 2016–2018, Figure 1), with similar magnitude for the seasonal flux (Table S1). Based on the three years of monitoring data, our two sites had comparable surface soil temperatures, ecosystem respiration, and gross primary productivity, but deeper soil temperatures and net ecosystem uptake rates were significantly higher at the site with longer post permafrost progression (Figure 2). Furthermore, higher pH was measured at both top and deeper soil layers in Corvosjärv (Figure S1). Soil water content (SWC), soil organic matter (SOM) and total carbon were found to be significantly higher in Latnjajaure topsoils, while in deeper layers only total carbon and nitrogen (and resulting C/N ratios) were found to be higher at this site (Figure S1).

#### 3.2 Vegetation cover

Plant cover for forbs, graminoids, aerenchyma plants, deciduous shrubs and evergreen shrubs was recorded in 2006 and 2016 at the two study sites (Figure 3), deviating in their species composition but indicating similar development trajectories through time (Figure 4). A significant loss of aerenchyma plants was observed during this time at both sites (Figure 3). While still present at Latnjajaure in
2016, aerenchymatous plants had almost completely disappeared at Corrvosjávri, with only a few datapoints confirming their presence at low abundance cover (<10%). At Corrvosjávri, graminoids and deciduous shrubs also showed a significant decrease during this timeframe (Figure 3).

3.3 Soil microbial communities

The overall bacterial and archaeal community structure, based on sequenced 16S rRNA amplicons, was comparable between the two sites in both top and deeper soil layers (Figure 2 and Figure S2).

Methanogenic communities, based on sequenced mcrA amplicons, were found to be similar at both sites and soil depth based on relative abundance with Methanobacterium as the dominating genus (between 55% and 68% in relative abundance) followed by Methanosarcina (Figure 5). However, the absolute abundance of mcrA gene copies estimated by qPCR showed a near-complete absence of methanogens in the topsoil of Corrvosjávri (9.6 × 10^2 copies g_dry soil^{-1}; detected in one out of 18 replicates), while the topsoil at Latnjajaure held similar abundances (mean of 3.5 × 10^4 copies g_dry soil^{-1}; detected in 9 out of 24 replicates) as the deep layers of both sites (mean of 6.2 × 10^4 and 7.7 × 10^4 copies g_dry soil^{-1}; detected in 22 out of 24 and 11 out of 18 replicates at Latnjajaure and Corrvosjávri, respectively) where no significant difference in mcrA abundance was observed (Figure 6).

There were also no significant differences between the methanotrophic communities of both sites based on sequenced and quantified pmoA genes (Figure 5). Relative abundances showed a similar community structure dominated by sequences annotated to the upland soil cluster alpha (USC–alpha; between 76% and 96% in relative abundance) followed by the genus Methylocystis. Abundance
estimates of pmoA gene copies showed no significant difference between the two sites (Figure 6).

Genera known to act as syntrophic partners in methanogenesis were extracted from total sequenced bacterial 16S rRNA gene amplicons showing presence of Geobacter, Smithella, Desulfovibrio and Pelotomaculum related sequences among others (Figure 5). When absolute abundances of these taxa were estimated with data from quantitative PCR on bacterial 16S rRNA genes, a drop in abundance for syntrophic partners in the topsoil of Corrvosjávri, similar to the results of the mcrA gene abundance, was observed (Figure 5). Following these results, a differential abundance analysis on the total 1150 identified bacterial and archaeal taxa was used to identify those who significantly differ in abundance between the two studied sites. This resulted in a list of 16 facultative or obligate anaerobic taxa (threshold log₂ fold change = 2) with significantly higher abundances at Latnjajaure, of which 9 have been shown to act as syntrophic organisms in methanogenesis and 3 were methanogens (Table S2). In contrast, nine taxa found to be higher abundant in Corrvosjávri were linked to aerobic lifestyles. The identified differences in community structure are in line with the qPCR results showing lower methanogen and syntrophic organism
abundances in topsoil of Corrvojävri compared to Latnjajaure, as described above.

4 | DISCUSSION

4.1 | Impact of drainage on CH$_4$ emissions

Here we show how long-term ecosystem change alters CH$_4$ cycling using a space for time approach (Blois et al., 2013) on two field sites (space) with decadal differences (time) in the permafrost thaw histories (Figure 1). The two sites represent differential natural drainage regimes and can be used to test the long-term effects of climate change-induced shifts in hydrology in natural tundra soils (Molau, 2010). Measured CH$_4$ emissions decreased 10-fold with the longer absence of permafrost. These fluxes are allocated in the lower end of earlier observations from permafrost tussock tundra ecosystem, typically ranging from slight uptakes (~1.5 to 0 mg C–CH$_4$ m$^{-2}$ day$^{-1}$, e.g. Blanc-Betes et al., 2016; Kwon et al., 2017; Whalen & Reeburgh, 1990), to low (0 to 100 mg C–CH$_4$ m$^{-2}$ day$^{-1}$, e.g. (Oberbauer et al., 1998; Sturtevant et al., 2012; Torn & Chapin, 1993; Zona et al., 2009)), to high and extreme (100 to >1000 mg C–CH$_4$ m$^{-2}$ day$^{-1}$, e.g. Christensen et al., 2000; Corradi et al., 2005; Kwon et al., 2017; Merbold et al., 2009; Mastepanov et al., 2008) emissions, determined by water saturation level, carbon content, plant composition and climatic conditions. Reduction in CH$_4$ emissions, similar to ours, have also been observed in experimentally drained sites (Kwon et al., 2017) and were attributed to a shift in vegetation and a loss in both methanotrophic and methanogenic communities in topsoils. Our results on soil temperature and CO$_2$ emissions contrast findings from experimental drainage studies in organic-rich flood plains (Kwon et al., 2016, 2021; Merbold et al., 2009), that show warmer topsoils, reduced CO$_2$ emissions, higher respiration and decreased gross primary production following drainage. In these studies, the increase in topsoil temperatures was attributed to a loss in heat capacity and thermal conductivity, which also may explain the contrasting CO$_2$ fluxes (Kwon et al., 2016). In mineral soils, like ours, where the mineral layer is overlaid by a shallow organic surface layer, heat transfer differs due to the contrasting soil profiles properties. In addition, drainage was shown to occur over time in the topsoil (Figure S1) but did not affect water content significantly in the deeper soil mineral layers. Although the deeper soil layers at Corrvojävri had higher temperatures, they did not contribute to significantly higher respiration. The generally observed lower microbial abundance in deeper soil layers at both sites (Figure S2) supports such a scenario with respiration rates dominated by surface soils. In addition, the overall bacterial and archaeal communities were not significantly different between sites (Figure 2 and Figure S2), indicating similar respiratory capacities.

4.2 | Impact of vegetation shifts on CH$_4$ emissions

The observed loss of aerenchyma plants at both sites is likely due to changes in the topsoil water conditions (Figure 3 and Figure S1). This trend is in line with previous long-term observations at Latnjajaure (1995–2016, Figure S3) (Molau 2012; Scharn et al., 2021, 2021). Aerenchyma plants have a competitive advantage in flooded soil due to their ability to transport O$_2$ from the atmosphere to anoxic root zones, an advantage which is lost once the soil is drained (Iversen et al., 2015). Therefore, the observed higher water content in the Latnjajaure topsoil might still support aerenchyma plants, while the dryer topsoil at Corrvojävri led to a near to complete loss of these plants over the same period. Furthermore, the increased net ecosystem uptake rates observed at Corrvojävri (Figure 2) are likely linked to a change in vegetation cover (Mekonnen et al., 2018) between the two sites (Figure 4), with an increased occurrence of deciduous shrubs and forbs at this site compared to Latnjajaure (Figure 3).

4.3 | Impact of microbial community composition on CH$_4$ emissions

Despite the differences in observed CH$_4$ emissions between Latnjajaure and Corrvojävri, the overall structure of both
methanogenic and methanotrophic communities was similar at both sites and soil layers (Figure 5). This contrasts to experiments conducted in the laboratory, where strong shifts in the microbial community were reported when permafrost was thawed in controlled conditions (Coolen & Orsi, 2015; Wei et al., 2018; Yang et al., 2017). Such experiments are generally conducted over shorter time scales and are difficult to compare to direct measurements in our field sites, as field sites have slowly progressed towards non-permafrost conditions over the past 40–50 years. This included natural variabilities in climate conditions and soil water content over seasons and years, smoothening any abrupt changes and thereby leading to the development and maintenance of comparable microbial communities. It is likely that the significant changes in community composition, similar to observations in laboratory studies, occurred just after permafrost thaw and drainage, which we were unable to capture in this study.

Methanotrophs belonging to the USC-alpha dominated the CH$_4$ oxidizing community, a clade ubiquitous in soil describing high-affinity methanotrophs able to metabolize CH$_4$ at atmospheric concentrations (Holmes et al., 1999; Kolb, 2009; Lau et al., 2015). Abundances of the CH$_4$ oxidizing community were found in similar levels in both sites and soil layers, while CH$_4$ producing microbes were lost from topsoil with ongoing time after permafrost disappearance. Altogether, the results on the CH$_4$ transforming microbiome suggest that while the overall potential for CH$_4$ oxidation is unaffected over time, CH$_4$ production in drier surface soil layers cannot be maintained over time due to a reduction of the methanogenic community.

4.4 | Conceptual framework

The marked decrease of potential CH$_4$ producing organisms in the topsoil decades after permafrost thaw suggests that conditions had become unfavorable for methanogenesis in the Corrvošjávri surface layer, which has also previously been observed in drying experiments (Kwon et al., 2017, 2021). Methanogenesis occurs in strict anoxic conditions, for example in water-saturated soils such as swamps, fens, deeper soil layers, and anoxic micro aggregates in the soil (Bengtson et al., 2012; Le Mer & Roger, 2001; Serrano-Silva et al., 2014; Watanabe et al., 2007). Increased O$_2$ levels and availability of other electron acceptors, which provide a higher redox potential than CO$_2$, have been shown to inhibit CH$_4$ production (Dalal et al., 2008). A decrease in soil water content in the topsoil (Figure S1), was measured across our thaw gradient, pointing towards increased drainage, or potentially a combination of drainage and increased evapotranspiration (Bring et al., 2016). Drier topsoil also facilitates O$_2$ diffusion from the atmosphere into deeper soil layers.

**FIGURE 7** Schematic overview of ecosystem changes that influence CH$_4$ emissions in Tussock tundra soils with an estimated decade difference in permafrost disappearance. Arrows in the graph indicate increase (upward arrow), decrease (downward arrow) and no change (horizontal arrow) in parameters. With progressing time, the water table drops and changes the overall hydrology, reduces water saturation and increases oxygen availability in the topsoil layers. These conditions are unfavorable for methanogenesis, leading to a decrease in the abundance of methanogens and their syntrophic partners. Aerenchyma plants that mediate CH$_4$ diffusion from deeper soil through their spongy tissue, are replaced by deeper rooting shrubs found in dryer ecosystems. The change in plant species composition and dryer surface soils leads to reduced transport of CH$_4$ by ebullition and through plants, thus increasing the residence time of CH$_4$ within the soil profile. This enhances the fraction of CH$_4$ consumed by methanotrophs in the oxic zone.
that consequently inhibits methanogenic activity (Figure 7). In addition, a lower water table favours shrubs over aerenchyma plants (Kwon et al., 2016), thereby reducing plant-mediated CH$_4$ transport (Figure 7). Increased root biomass (Björk et al., 2007) might further impact drainage by the creation of macropores and increase evapotranspiration potential (Angers & Caron, 1998; Fan et al., 2017). As methanogenesis is a syntrophic process depending on fermenting bacteria that provide precursors for CH$_4$ formation by the breakdown of complex organic matter, we hypothesized that a shift in members of this community would also have occurred. Results from a differential abundance analysis on the total microbial community supports such a scenario as almost all taxa found to be significantly higher abundant in Latejirage were found to be linked to methane production (Table S2). This is striking, as the observed decrease in emitted CH$_4$ is linked to a change of a very small fraction of the microbiome (16 out of 1150 taxa), while the remaining microbial community seems to follow the same progression pattern at both sites and does not change anymore with ongoing time. Taken together, our data suggest that anaerobic processes become restricted to deeper soil layers over time in former permafrost mineral soils (Figure 7).

5 | CONCLUSION

In conclusion, the observed changes in soil properties, plant cover and microbial communities will reduce CH$_4$ emissions in former permafrost soils by: (a) restricting CH$_4$ production in surface soil as a result of increased oxygen availability in drained and dryer surface soils, (b) reducing plant-mediated CH$_4$ transport (diffusion) via aerenchyma tissue as a result of unfavorable growing conditions for sedges and rushes and the introduction of taller shrubs, and (c) limiting CH$_4$ emissions from deeper soil horizons due to increased oxidation by methanotrophs as a result of longer CH$_4$ diffusion time through the aerated surface soil.

The mechanistic explanation provided here highlights the importance of accurately estimating soil drainage conditions and plant-mediated effects on surface soil processes when assessing climate feedbacks from the Arctic. Areas with a topographic background for drainage would thus reduce their CH$_4$ emissions to almost zero over decadal scales after final permafrost thaw, which is in line with previous findings (Bansal et al., 2016; Kwon et al., 2021; Whalen & Reeburgh, 1990). This is to date not accounted for in global climate models. Our results highlight the need for more long-term, field-based approaches when it comes to climate-driven changes in drainage conditions not the least in relation to permafrost-affected ecosystems.

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AUTHOR CONTRIBUTIONS

C.K. and C.L. conducted analysis and wrote the manuscript. M.R., A.P., S.D. obtained field data and/or conducted analysis. B.E., L.K. and R.G.B. contributed to research design and data interpretation. M.P.B. designed the research, obtained field data, conducted analysis and wrote the manuscript. All authors discussed the study results and reviewed the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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