

Greenhouse gas flux over a 50-year post permafrost thaw gradient

Decomposition of soil organic carbon
from the Swedish tussock tundra



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Abstract

As the globe is warming the extent of permafrost drastically decreases in the high latitudes. Upon thaw the stored soil organic carbon (SOC) undergoes rapid decomposition and is partly released as trace gases to the atmosphere. There are few studies that focus exclusively on post permafrost sites even though we estimate that up to 81 % of the present permafrost will thaw before the end of the century. It is of utmost importance to understand how the permafrost will respond to the future warming for us to be able to predict climate change with any precision. This thesis aims to highlight the importance of studying post permafrost regions and to discuss similarities and differences between three tussock tundra communities in northern Sweden. Soil samples were collected from three sites along a 50-year post permafrost gradient located in the Swedish tussock tundra near Abisko. Soil were collected from three soil pits at each site and separated based on which soil horizon they were collected from. The samples were then further divided into four treatment groups (cold aerobic, cold anaerobic, warm aerobic and warm anaerobic) and incubated for 121 days at constant temperatures (5° C and 15 ° C). Gas samples were collected from the headspace of the incubated soil jars and analyzed by gas chromatography (GC). My results provide evidence that GHG flux increases over time in post permafrost tussock tundra sites. The site where permafrost thawed the longest time ago have almost an order of magnitude higher greenhouse gas (GHG) flux compared to the other two sites. Although soil properties were similar the flux was significantly higher in the site where permafrost thawed first. This thesis also provides further evidence that microbial decomposition is most effective in warm aerobic conditions. The GHG flux decreased with both time and depth for all sites and horizons. However, methane (CH₄) flux increased rapidly towards the end of the incubation period in the organic rich A-horizons of the warm temperature treatment. The results can be used as an indicator of the complexity of SOC decomposition. The effect of warming in the high latitudes will likely lead to increased GHG flux. As the thawing process are complex and interacts with several other factors, in reality the GHG flux increases might be offset by other processes not fully disclosed in this thesis. This thesis thoroughly examines how GHG flux responds to experimental warming under fixed conditions.

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Introduction

The Arctic have acted as a carbon (C) sink for centuries to millennia and due to the cold and moist climate, decomposition of SOC have been vastly inhibited (Hobbie, Schimel, Trumbore, & Randerson, 2000; Mikan, Schimel, & Doyle, 2002). D. S. Schimel et al. (2001) suggest that during the period 1980-1990 the northern extratropical carbon sink on average sequestered 2.4 Gt C yr^{-1} (1.5 – 4.3). The northern circumpolar permafrost region alone has been estimated to contain approximately 1672 Pg SOC (Tarnocai et al., 2009). This roughly amounts to half the global SOC pool or twice the atmospheric C pool (Schlesinger & Andrews, 2000). It have been clear for decades that the C stored in the arctic will play a vital role in the global C-cycle (Post, Emanuel, Zinke, & Stangenberger, 1982).

The permafrost extent in the Arctic is declining in response to global warming. As the permafrost thaws ancient SOC becomes increasingly available for microbial degradation. The Intergovernmental Panel on Climate Change (IPCC) estimated in their fifth assessment report (AR5) in 2014 that 37 – 81 %, RCP 2.6 and RCP 8.5 respectively, of all permafrost will have thawed by the end of the century. When permafrost soils diminish, post permafrost soils emerge instead. It is crucial to comprehend the C-dynamics in these substrates if we want to predict the impact that will come with altered conditions. The SOC which are decomposed by microbes will partly be released to the atmosphere and partly contribute to microbial growth. The majority will be respired as carbon dioxide (CO_2) but methane (CH_4) and nitrous oxide (N_2O) will also be released to the atmosphere through different microbial metabolic processes. It is clear that the terrestrial C pool in the arctic will undergo substantial changes as temperatures increase and thus altering the global C-cycle (Koven et al., 2011; E. A. G. Schuur et al., 2015c, 2015b, 2015a).

It has proven to be a difficult task to understand terrestrial Arctic C-dynamics and after decades of research many uncertainties still exist. The major uncertainty has been the connection between biotic and abiotic processes as conditions change (ACIA, 2005; IPCC, 2014). A strong correlation between NPP and soil respiration exists globally (Bond-Lamberty & Thomson, 2010; Hibbard, Law, Reichstein, & Sulzman, 2005; Raich & Tufekciogul, 2000). Increased net primary production (NPP) is favored by both increased temperatures and atmospheric CO_2 concentrations (Ainsworth & Long, 2005; Luo, Hui, & Zhang, 2006). Increasing biomass correlates with increased litterfall and rhizodeposition (Drigo, Kowalchuk, & van Veen, 2008). Le Quéré et al. (2018) suggested that the terrestrial C-sink account for about one fourth of anthropogenic activity. In contrast to the negative feedback of biomass growth the microbial decomposition of SOC is a positive feedback. The gross primary production (GPP) can be described as $\text{NPP} - \text{respiration}$. Studies about net C-flux varies in both direction as both NPP and respiration is expected to increase (Davidson & Janssens, 2006). Edward A. G. Schuur et al. (2009) suggest that the C-balance might vary over time with a net C-uptake following thaw which transits to a C-source after a few decades. Uncertainties in the connection between respiration and GPP is problematic. As the respiration of the biosphere is huge, the current 20 % error margin is approximately equivalent to all anthropogenic emissions (DeLucia, Drake, Thomas, & Gonzalez-Meler, 2007). There is a need to research the controls over SOC decomposition in order to predict this flux more accurately.

Understanding the controls over SOC decomposition is only a small part in a larger system of direct and indirect interactions. However, efforts must be made to further our understanding in this field as it has the capacity to greatly affect the global C-cycle. Nearly all studies are made only on the top 30 – 50 cm of

the soil profile. Many of the incubation studies are focused only on a very short time period. Only decomposition of labile SOC is studied then while the more recalcitrant C-pools are overlooked.

Research questions, excellence and scope of this thesis

This thesis will aim to address the complex nature of SOC decomposition in differing post permafrost tussock tundra communities. More precisely it will try to answer the following questions:

1. Is there a difference in GHG flux in a 50 – year permafrost thaw gradient in the Swedish tussock tundra?
2. How does the GHG flux differ between the soil horizons?
3. What impact does environmental factors have on the decomposition rate?

The scope of this thesis will be limited to the controlled decomposition of SOC under controlled circumstances. I will not aim to address all ecosystem dynamics and what the net outcome of future warming might result in. There are many factors that contribute to the system, such as vegetation response, shift in nutrient balance, hydrology etc. Instead I will focus on the decomposition process.

Background

Global warming

Global warming have increased surface temperatures on average 0.85° C (0.65-1.06° C) from 1880-2012 (Hansen, Ruedy, Sato, & Lo, 2010; Morice, Kennedy, Rayner, & Jones, 2012; Vose et al., 2012). The reason behind global warming is unequivocally human activity (IPCC, 2014). Several different datasets with many climate proxies dating back centuries to millennia clearly reveal the climate shift since the industrial revolution (Mann, Bradley, & Hughes, 1998, 1999). Based on ice-core records, current concentrations of atmospheric greenhouse gases are the highest for an approximated 800 000 years (Loulergue et al., 2008; Lüthi et al., 2008; Schilt et al., 2010). Cook et al. (2013) analyzed 11 944 peer reviewed papers on the topic of anthropogenic global warming and found a 97,1 % scientific consensus. The combustion of fossil fuels has been the primary anthropogenic forcing on the climate system since the industrial revolution. During the period of 1750-2018 atmospheric CO₂ concentrations increased by about 47 % from 278 ppm – 410 ppm (IPCC, 2014). Recent estimations predict that about 555 (±85) PgC were released to the atmosphere as a direct consequence of human activities between 1750-2011. At the same time, methane (CH₄) increased by 150 % from 722 – 1803 ppb, and nitrous oxide (N₂O) by 20 % from 271 – 324.2 ppb. Both CH₄ and N₂O are potent GHGs with a global warming potential (GWP) that is considerably higher than CO₂. The mean residence time (MRT) in the atmosphere differs significantly for these GHGs. The GWP of CH₄ is 25 (25 times more potent than CO₂) and the GWP of N₂O is 298, if you calculate an average over a 100-year period prevalence in the atmosphere. Approximately 50 % of the carbon dioxide that is released to the atmosphere by human activities remains while the rest is returned to carbon sinks. The rate of change is unprecedented for at least 20,000 years. Only during the last 40 years, approximately 50 % of all anthropogenic CO₂ emissions were released. Due to this forcing on the climate system current and new risks associated to climate will be amplified (IPCC, 2014).

Future projections

Future climate projections univocally indicate further warming by the end of the century. The best estimates consist of an aggregate of model projections under varying atmospheric GHG concentrations called representative concentration pathways (RCPs). Models are mathematical representations that calculate the probabilities of a certain outcome based on the current knowledge about physics and the earth systems. The more sophisticated models are based on the Coupled Model Intercomparison Project 5 (CMIP5) ensemble and uses the Hadley Centre Climatic Research Unit Gridded Surface Temperature Data Set 4 (HadCRUT4). Estimates of increased global mean surface temperature from the different RCPs varies between 1° C (± 0.7) – 3.7° C (±1.1), RCP2.6 and RCP8.5 respectively. The Paris Agreement (2015) pushed policymakers to agree on keeping the global warming to less than 2° C by the end of century, relative to preindustrial temperatures. This goal is meant to keep the climate in an equilibrium state and prevent it from catalyzing radical warming amplification. If we were to manage to meet this goal most planetary boundaries will not cross a tipping point (Rockström et al., 2009). The 2° C goal equals a total release of approximately 3650 GtCO₂ eq to the atmosphere. It is only under the RCP 2.6 scenario, which it is likely that this goal can be met. Results from model data implies that climate change will alter the carbon cycle, which will further promote global warming and amplify the rate of change.

There exist several reasons for concern (RFC) due to the occurring and projected changes in climate. IPCC have identified five major RFCs, which can be summarized in the following manner:

1. Unique and threatened systems – Species and cultures that have adapted for a specific niche reacts slowly to change. Systems already under strain will have a hard time coping with only 1° C change. Arctic sea ice and coral reefs are two examples, which will be severely affected by further climate change.
2. Extreme weather events – As temperatures increases the likelihood of extreme weather events will also increase progressively.
3. Distribution of impacts – The impacts of climate change are spatially unevenly distributed. Poorly adapted or prepared communities, ecosystems or regions will be impacted to a greater extent compared to the mean change.
4. Global aggregate impacts – Species and economic losses will add up globally. Severe large-scale loss of biodiversity, ecosystem services and economic loss greatly increases at warming exceeding 3° C.
5. Large scale singular events – The risk of irreversible and catastrophic singular events like thawing of Arctic ice or coral bleaching increases with global warming. There supposedly exists a tipping point of which the system cannot recover. To melt most of the Greenland ice sheet an estimated warming of 1 – 3.5° C (the full process would occur over millennium).

Our understanding of how the carbon cycle will respond to ongoing climate change is imperative to allow for active counter measures. Due to the complexity of the system, our understanding remains limited.

Polar amplification

The Arctic have warmed approximately twice as fast as the global average during the past 50 years, about 0.6 ° C per decade. This phenomenon is referred to as polar amplification. ACIA (2004) have identified five major causes for the increased warming:

1. Ice/albedo feedback loop – As ice melts in the arctic the underlying darker surface is exposed and can absorb more of the incoming sunlight and promote further warming.
2. A greater friction of high latitude GHG goes into warming the surface relative to the tropics where they evaporate.
3. As the spatial extent of the atmosphere is temperature dependent more of the arctic GHG are concentrated to the surface relative to the tropics.
4. As sea ice extent decreases the amount of energy absorbed into the oceans increases. The energy received during the summer is stored and later released during the winter.
5. Atmospheric and oceanic circulation patterns transport heat to the poles and shifts in these circulations further promotes warming.

Our understanding of the processes governing these changes will be imperative for future projections, which are the foundation for policy making (IPCC, 2014). Direct observations have been made to assess the rate of change across the Arctic tundra.

Permafrost temperature is increasing (Chudinova, Frauenfeld, Barry, Zhang, & Sorokovikov, 2006) and was the highest ever recorded in 2016 (dataset extending to 1978). The highest increase of 0.5 ° C where measured in 2017. As a result permafrost is thawing and areas containing permafrost soils decreasing (Christensen, Johansson, Åkerman, & Mastepanov, 2004; Sazonova, 2004). The seasonally thawing active layer that covers the permafrost is also increasing at most monitored sites throughout the Arctic. The tundra and taiga biome have largely adapted to permafrost conditions and as it thaws it will change significantly (Hinzman et al., 2005).

The average sea-ice extent has decreased in both extent and thickness since the 1960's. The multi-year sea ice is also becoming increasingly rare as land and ocean temperatures increase (SWIPA, 2017). Terrestrial snow cover decreased by about 10 % between 1970-2000 (ACIA, 2004). Glaciers are retreating at an increasing pace and the Greenland ice sheet is shrinking (IPCC, 2014).

The growing season is increasing and many feedback processes promote further increase (Black et al., 2000; Euskirchen et al., 2006; Kimball, Keyser, Running, & Saatchi, 2000). A clear expansion of shrubs has occurred in recent years (M. Sturm, Racine, & Tape, 2001). These shrubs outcompete much of the local vegetation (Jägerbrand, Alatalo, Chrimes, & Molau, 2009). The trend for the tree-line is shifting northwards and up to greater altitudes compared to previous decades. The shift in altitude for the tree line in Sweden has been around 0.5 m⁻¹ or 40 m per 1° C increase.

Tussock tundra

The spatial extent of tussock tundra varies between studies but conservative estimates suggest a total cover of about 336,000 km² (Walker et al., 2005), about a third of the pan-arctic tundra. Tussock tundra ecosystems are changing as temperatures increase and permafrost thaws. Many processes contribute to the rapidly changing conditions and it is therefore classified as a vulnerable ecosystem (Ridefelt, Etzelmüller, Boelhouwers, & Jonasson, 2008). The main threat to the tussock tundra is the shrubification where *Betula* and other deciduous shrubs outcompete *Eriophorum* (S. I. F Chapin, Shaver, Giblin, Nadelhoffer, & Laundre, 1995; Molau, 2010). Due to the earlier leaf-setting and increased mineralization shrubs can expand and create a higher canopy compared to lower species thus outcompeting many local species.

Tussock tundra is characterized by cold and moist conditions with the hare's-tail cottongrass (*Eriophorum vaginatum*) as the dominant species. Permafrost is an integral part of the tussock tundra and it cannot form nor persist without it. Tussock tundra typically develop on flat surfaces in the proximity to a lake or water body. The *Eriophorum vaginatum* tussocks grow rapidly with about 75 % of their roots above ground. As much of the biomass is aboveground, they can slightly extend the growing season compared to inter-tussock areas. The tussock form is also great at preserving heat and during summer periods they have been observed to be 6-8° C warmer compared to their surroundings. As their roots are aboveground, they heat up earlier in the season compared to other vegetation and utilize that energy to increase nutrient cycling. The tussocks can raise the mineral soil surface up to 5 cm and keep about 97 % of their biomass in the first 10 cm of the tussocks (I., S. F Chapin, van Cleve, & Chapin, 1979).

Observations have found a crucial positive feedback, which occurs as shrub cover increases in the arctic. The shrubs allow for a buildup of snow as it increases resistance where it was none before. The snow cover creates an insulating effect from the freezing air, which keeps the soil warmer. Increased soil temperature, litterfall and root growth allows for increased active layer depth. All of the above contribute to increased microbial activity and SOC decomposition (Matthew Sturm et al., 2005).

Microbial activity and nutrient cycling

Tectonics and weathering are the fundamental abiotic geochemical processes which drive nutrient cycling. They resupply C, S, and P on a geological timescale, which set the stage for microbial evolution. During the earth's history a series of microbial metabolic pathways developed which have altered chemical processing on our planet. Microbes consist of unicellular organisms, mainly bacteria and fungi. Tectonics and weathering coupled with microbial activity have given us the conditions for life as we

know it today. Microbes are the driving force in most biogeochemical processes where they are active (Falkowski, Fenchel, & Delong, 2008). Abiotic geochemical processes differ from microbial where most energy transfers based on pH chemistry. Microbial biogeochemistry is fundamentally based on six elements – H, C, N, O, S, and P (Schlesinger & Bernhardt, 2013). Microbes however, utilize their energy through a set of thermodynamically constrained redox reactions (Williams, 1997). The use of different energy sources by living organisms changes the balance of compounds in that specific system. Increased nutrient cycling and a decreased dependence on external energy supply have been the result of microbial activity. The global average redox states we observe in nutrient pools today is a result of microbial activity. The rise of photosynthesis and the oxidation of our planet was the fundamental stepping stone for modern biogeochemistry and life on earth (Falkowski & Godfrey, 2008). Autotrophic bacteria are the primary producers and the foundation of food webs. Autotrophs process inorganic compounds like atmospheric CO₂ and use them as energy for biomass growth. The organic biomass is more accessible and can be decomposed by heterotrophs which typically respire most of the C back to the atmosphere (Liang & Balsler, 2011; Trumbore, 2006). Aerobic respiration has the highest energy yield in normal circumstances and is thus the preferred metabolic pathway. In oxygen poor or totally anoxic environments methanogenesis is the primary metabolic pathway. Methanogenesis is a slower process compared to respiration as it is more energy demanding. Recent studies have found that in the intermediate space between oxic and anoxic environments, there is typically an abundance of methane-oxidizing methanotrophs (Gupta et al., 2013; Qiu, Noll, Abraham, Lu, & Conrad, 2008). The nitrogen cycle in soils are dependent on N-fixation by bacteria.

Controls over SOC decomposition

SOC degradation occurs as bacteria exploit the compound as an energy source. There are several factors that affect SOC degradation. Key among them for the tussock tundra communities are temperature, moisture availability and substrate quality (Schlesinger & Bernhardt, 2013). Previous incubation studies have found that decomposition rates increase dependent on temperature (J. P. Schimel & Clein, 1996). Johansson, Berg, and Meentemeyer (1995) found that temperature and evapotranspiration are good indicators for decomposition rates. Edwards (1975) demonstrated that microbial activity increases exponentially with increasing temperature. This have a strong implication that the potential increase in decomposition is greatest at high latitudes. The temperature effect can severely restrict microbial activity in subzero temperatures. Although limited by the cold, microbes are still active and respiration have been measured at -40 C (Panikov, Flanagan, Oechel, Mastepanov, & Christensen, 2006). Microbial reproduction can also be measured already at -10 C (Bakermans, Tsapin, Souza-Egipsy, Gilichinsky, & Neilson, 2003; Rivkina, Friedmann, McKay, & Gilichinsky, 2000) and have been found to persist in soils which have been frozen for thousands to millions of years in a hypometabolic state (Zimov, Schuur, & Chapin, 2006). The temperature sensitivity of subzero soils is generally higher compared to warmer soils and winter respiration is often underestimated or neglected (Mikan et al., 2002).

Substrate quality strongly affects the decomposability of a compound. Carbon chemistry, in particular, affects the lability or recalcitrance of compounds. Fresh litter decomposes quickly compared to most soils. The decomposition rate is normally highest in the topsoil and decreases as the available labile SOC decreases further down in the soil profile (Schaefer, 1990; Swift, Heal, & Anderson, 1979). Although decomposition rates typically decrease with depth, they are not to be neglected as older soils can be thousands of years and have considerable amounts of low activity microbes, which can have a significant net effect on total respiration. Hobbie (1996) found that the substrate quality of fresh litter had larger

effect compared to a 6 C warming effect in Alaskan soils. There is a dominant tendency for recalcitrant compounds to be abundant in soils. As labile compounds are preferred by microbes over more recalcitrant ones the relative abundance of recalcitrant compounds increases over time. Due to humification processes labile compounds can also become recalcitrant. Single events can also rapidly make compounds recalcitrant such as fires. Finally, climate can preserve recalcitrant materials through freezing and flooding which restricts decomposition rates (Hobbie et al., 2000).

The two major inputs of SOC is from (i) above-ground litterfall and DOC along with (ii) rhizodeposition which is the SOC produced below-ground by roots and exudation. The root exudates contain mostly simple molecules like sugars, amino acids, sugar alcohols and organic acids which are known to stimulate microbial growth (priming effect). The simple compounds are easily decomposed (Müller, van der Merwe, Schildknecht, & Visser, 1993), while the plant polymers are structurally more complex containing lignin, cellulose and hemicellulose that decomposes more slowly. Mycorrhizal fungi also decomposes SOC at a relatively high pace and respire it back to the atmosphere (Smith & Read, 2008).

Site description

Three sites were selected along a natural permafrost thaw gradient located near Abisko in northern Sweden (Figure 1. Location of the three field sites and Abisko as a reference site. Data collected from SLU.. The sites were carefully selected in a three-step selection process:

1. Satellite image analysis – Large areas with *Eriophorum* bloom were analyzed near the Abisko area which were known to host a few tussock tundra sites.
2. An aerial survey was carried out by helicopter to confirm the best locations indicated by the satellite data (Molau, 2010).
3. A in-depth field survey for final validation of the areas and optimizing the final selection for field sites (Brinkman, 2006).

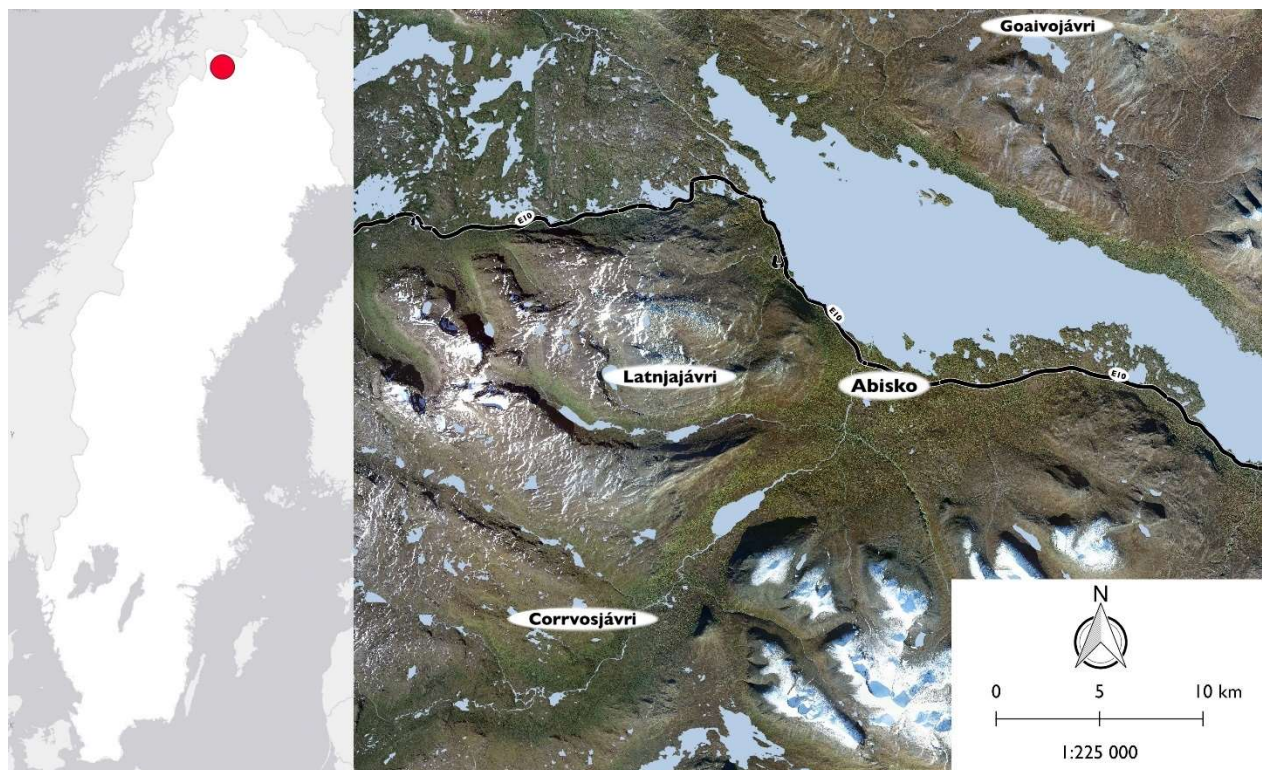


Figure 1. Location of the three field sites and Abisko as a reference site. Data collected from SLU.

The site selection was a major part in the research project with the specific aim to represent a decadal permafrost thaw gradient in the Swedish tussock tundra. All sites are located in glacial U-valleys with close proximity to at least one lake. The ecosystem is defined as post permafrost tussock tundra for all sites (Molau, 2010). The dominating vegetation in the ecosystem is *Eriophorum Vaginatatum*. Waterlogged depressions are frequent at all sites. Soil cores were probed from all sites to characterize the field sites.

The soil can be classified as a fine mineral Haplic Gleysol for all sites (Food and Agriculture Organization of the United Nations, 2006). The soil is easily distinguished by very clear differing layers (Figure 2). These clear soil horizons consist of a thin layer of litter underlain by a humified SOC rich A-horizon. Under that is a weathered B-horizon which represents the former active layer reach prior permafrost loss. Finally, a strongly reduced C-horizon covers the bottom layer which is strongly influenced by groundwater.

Goaivojávri

The most recent shift from permafrost conditions was from the site near lake Goaivojávri, located about 17 km northeast from Abisko (843 m a.s.l., 68°29.6'N, 18°55.6'E). The valley is facing a W-E direction with large open areas. A few peaks exist around the site with altitudes varying from 800-1200 m. The tussock area in the valley is approximated to a maximum of 1 ha. Snow covers the site for most of the year. In 2017 the snow cover lasted until mid-June (Rudner, 2018).



Figure 2. A cross-section from one of the soil pits in Goaivojávri showing the differing soil horizons

Species diversity at Goaivojávri were significantly lower than the other two sites during the field inventory 2006. The bottom layer is dominated by mosses and a significantly lower ratio of lichens compared to Latnjajávri and Corrvosjávri (Brinkman, 2006). The landscape was dominated by *Carex bigelowi* followed by *Eriophorum vaginatum*, *Equisetum arvense*, *Eriophorum angustifolium*, *Poa alpine*, *Salix herbacea*, *Poa arctica*, *Saxifraga foliolosa* and *Cerastium cerastoides* respectively.

Permafrost was confirmed 2006 (Brinkman, 2006) but no permafrost was found during the summer of 2018 during the sampling for this project. The b/c horizon was most shallow at only 4-5 cm depth and the lowest mean thaw depth of 72 cm. The soil temperature was 9.x, slightly warmer than Latnjajávri.

Latnjajávri

Latnjajávri (68°20'N, 18°30'E) is located in the Abisko mountain area 980 m asl, approximately 10 km west from Abisko. The mean temperature is -2.3°C (1993-2001) with a mean annual precipitation of 818 mm (1990-2001). Although temperatures are rising quite rapidly at an average rate of 0.12°C year⁻¹ from 1990-2006 (Björk, Majdi, Klemmedtsson, Lewis-Jonsson, & Molau, 2007). The elevation in the valley ranges from 950 m asl to 1440 m asl. The winds blow predominantly in a westerly direction. The lower part of the valley is covered in snow for most of the year. Snowmelt typically starts at the end of May, but patches of perennial snow remains in a few patches of the valley. Snow accumulation typically starts from late September and onwards (A. A. Beylich & Gintz, 2004).

The Latnjajaure Field Station is located in the valley and is part of the International Tundra Experiment (ITEX), which have collected climate data since the late 1990's. The vegetation is diverse compared to similar locations with small ecosystems ranging from heath, meadow and tundra (Björk et al., 2007). Apart from *Eriophorum Vaginatum* a few common species are *V. Vitis-Idaea*, *Carex Bigelowi*, *Salix Herbacea* and *Phylodece Vivipara* (Brinkman, 2006). Shrubs are becoming increasingly dominant in the area as temperature increases and experimental warming plots indicate that this trend will continue (Gray, 2018).

Permafrost in the area was confirmed in 1993 and absent in 2003 but remains not thoroughly investigated. Beylich et al. (2003) suggest that sporadic permafrost might exist up to 80 m below the surface.

Corrvosjávri

The site near lake Corrvosjávri (814 m a.s.l., 68°14.7'N, 18°26.9'E) is the southernmost site included in this study. The tussock tundra here is experiencing a shift where the tundra is no longer tussock forming and intense shrubification occurs. The valley here is facing a NW-SE direction. The total area of tussock tundra is approximated to be larger than the other sites at maximum 10 ha.

The vegetation is dominated by *Salix herbacea* and visibly larger shrubs compared to Latnjajávri. The second most common species *Eriophorum vaginatum* is more uncommon than at the other sites. A few more common species are *Carex bigelowi*, *Eriophorum angustifolium*, *Salix, glauca*, *Salix lanata* and *Polygonum viviparum*.

No permafrost has been documented in Corrvosjávri. The vegetation indicated that permafrost in this area thawed more than 50 years ago. The b/c horizon were diffuse in this site possibly as a result of cryoturbation in the soil. The estimated border between the horizons were approximately at 15 cm depth. Soil temperature were 17°C measured at 5 cm depth, significantly higher than Latnjajávri and Goaivojávri. The thaw depth exceeds the equipment used which could measure a depth up to 105 cm (Brinkman, 2006).

Materials & Methods

Experimental Setup

The experimental set for this report was simple and straightforward (Figure 3). Three sites were selected along a permafrost thaw gradient as mentioned above. At each site three soil pits were dug up and soil samples collected from the different horizons. The soil samples were homogenized and incubated in four treatment groups (1) aerobic 5 °C, (2) anaerobic 5 °C, (3) aerobic 15 °C and (4) anaerobic 15 °C. Additional soil was collected from the soil pits for reference samples of pH, Gravimetric soil water content (GSWC), total carbon content by loss on ignition (LOI) and C:N ratio. Gas samples were collected from the incubated soil jars throughout the incubation where the trace gases CO₂, CH₄ and N₂O were analyzed by gas chromatography (GC). DOC/DON samples were collected from the incubation jars four months into the incubation. The reasoning for this process will be defended in the section below.

Field work

Soil samples were collected from northern Sweden in the late summer months when the active layer is at its maximum. Samples from Latnjajávri and Corrvosjávri were collected in 2016 along with water samples from all lakes. Samples from Goaivojávri were collected in 2018. Triplicates were collected from each site to avoid local anomalies in the acquired soil samples.

At each site three soil pits measuring a minimum of 50 x 50 x 30 cm were dug. Soil samples were collected from the A, B & C horizons. An additional deep sample were collected by drilling as deep as possible in the bottom of the soil pit, with a Hilti soil corer. Reference samples were collected by drilling an additional core in close proximity to the soil pits. All equipment used for excavation and collection of soil samples were sterilized prior to use in the field and between sample collection. The soil was acquired by scraping of soil from the walls of the soil pits. To avoid microenvironmental anomalies soil were scraped from different places in the soil pit. Soil samples were contained in sterile zip-lock plastic bags for transport. Finally, samples were frozen and stored in -30°C freezer at GU, awaiting incubation.

Water samples were collected from the three lakes nearby respective sampling site. These samples were also frozen and stored along with the -30°C soil samples, awaiting incubation.

Preparation

Airtight incubation jars were prepared with a gas tight lid with two valves attached to the lid. Each sample were divided into four treatment groups and incubated at 5°C and 15°C with one aerobic and one anaerobic environment. The average soil temperature at Latnjajávri approximately equals the lower incubation temperature at 5°C.

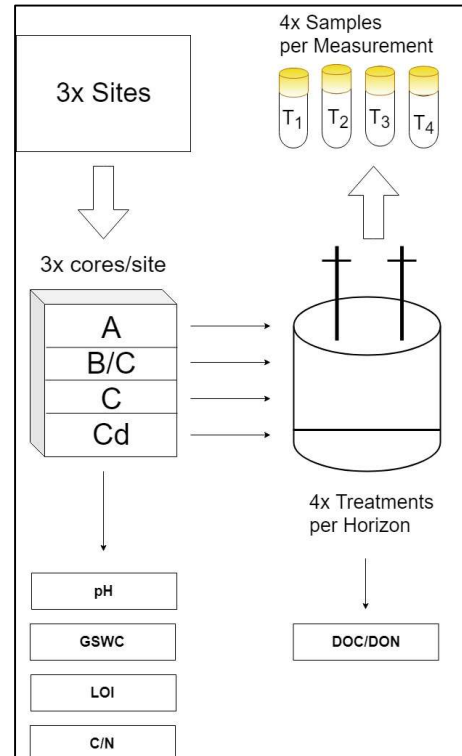


Figure 3. Conceptualized schematic over experimental setup.

The incubation jars which would host aerobic samples were prepared with 100 g sand that had been incinerated for at least six hours at 600°C. A glass fiber filter where placed on top of the sand to separate the sampled soil and the sand and minimize disturbance. The underlying sand were used as a buffer to absorb any excess moisture in the aerobic samples. Thus, prohibiting an anaerobic environment to form in the bottom of the sample.

Frozen soil samples were filtered through an 8 x 8 mm sieve in a -10° C freezing room. All equipment was washed and rinsed with distilled water prior to sampling and between samples. The filtered soils were collected with sterilized spoons and then placed in the incubation jars. The incubation jars were stored in a -5° C incubation freezer until the incubation start.

One week prior to the incubation start the water which would saturate the anaerobic samples were thawed and filtered through Whatman 0.47 mm Ø GF/F filters with a pore size of 0.7 µm. N₂ gas were bubbled through the water for 30 minutes per 1.5 L to induce an anoxic environment. The day prior to the incubation start, the water was added to the incubation jars. 150 ml water from respective lake were added to the soils to cover them completely and create an anoxic environment. Another 25 ml were added to all anaerobic A-horizons before the second measurement period (T₇).

20 ml glass vials were used for the headspace samples. Prior to use, the vials were flushed with N₂ gas at a flowrate of 500 ml/min for 30 seconds. An additional three second of decompression after flushing were added to allow the pressure to equalize in the vials.

Reference data

Reference soil samples were collected to analyze soil water content, SOC, pH, C/N- and DOC/DON ratio. Ceramic beakers were cleaned and incinerated before measurements began. A total of 10 g soil per sample were placed in the ceramic beakers and dried at 70°C for 48 hours to calculate the gravimetric soil water content.

Equation 1

$$\% \text{ Soil Water} = \frac{\text{Weight of wet soil (g)} - \text{Weight of dry soil (g)}}{\text{Weight of dry soil (g)}} \times 100$$

Loss on ignition (LOI) were applied to analyze all of the organic content within the soils. This was done by incinerating the dried soils at 600°C for at least six hours. The soils were then weighed again.

Equation 2

$$\% \text{ LOI} = \frac{(\text{Weight of dry soil (g)} + \text{weight of container (g)}) - \text{weight of incinerated soil (g)}}{(\text{Weight of dry soil (g)} + \text{weight of container (g)}) - \text{Weight of container}} \times 100$$

To account for potential loss of material from the ceramic beaker during this process they were cleaned and weighed a final time.

Five g of soil from each soil sample were collected for pH measurements. 50 ml of distilled water were added to completely cover the soils. Once filled the containers where shook for 30 min on a Heidolph unimax 2010 shaker, and then left to sediment for at least twelve hours. A double decimal pH-meter were used for the measurements. Before measurements the pH-meter were calibrated to pH 4, 5 and 7. Measurements lasted until the pH stopped drifting. The pH meter was cleaned with milli-q water between each measurement and carefully dried with paper. Subsequent the first measurement in the

1:10 soil:water suspension, one ml of a solution of one molar kaliumchloride (KCl₁) were added to each sample. The samples were then shaken for another 30 minutes on the Heidolph unimax 2010 shaker and then left to sediment for at least twelve hours. A final pH measurement was carried out after that by using the same equipment and technique as previously stated.

Dissolved organic carbon- and nitrogen (DOC and DON respectively) were collected from the incubation jars from day 155 into the incubation. Samples containing 10 g soil were kept in 50 ml Fischer Scientific Falcon conical centrifuge tubes. 20 – 25 ml deionized water were added by a VWR Dispensette S. Samples were subsequently mixed about 30 seconds on a VWR vortex mixer followed by 10 minutes on a Heidolph unimax 2010 shaker. To separate the mixture further samples were centrifuged for 2 – 4 minutes in a Thermo Scientific Heraeus Megafuge 16 centrifuge series. The liquid samples were filtered through Whatman 0.47 mm Ø GF/F filters with a pore size of 0.7 µm. Filters were saved in petri dishes and weighed to account for compounds which got stuck in the filter. All samples were stored in a Memmert 750 incubator at -5° C until analyzed.

Incubation

Before the first measurement the incubation jars were flushed with compressed air at ambient GHG concentrations for three minutes per sample and set to time zero concentration. During the sampling period the incubated jars were closed to allow GHG buildup in the headspace. 5 ml gas samples were then collected from the headspace and kept in 20 ml glass vials, to be analyzed by gas chromatography (GC) (Klemedtsson, Klemedtsson, Moldan, & Weslien, 1997). Headspace samples were collected at incubation time intervals (1, 2, 3, 4, 7, 8, 9, 10, 14, 15, 16, 17, monthly from day 21; in total 7-time intervals). During sampling a 5 ml gas tight syringe were used. Just before each sample were collected turbulence were created within the soil jars with the syringe to provide a homogeneous air sample. The same was done when transferring the samples to the glass vials. To account for the dilution a correction was made, and the following equation were used:

Equation 3

$$\frac{(G \times (A_v - (5 \times 10^{-6}))) + (SG \times (5 \times 10^{-6}))}{A_v}$$

Where G accounts for GHG content in the incubation jars. A_v is the air volume in the headspace (which was adjusted when any soil samples were collected from the incubation jars over the course of the incubation time period). SG equals the added standard gas with the known GHG concentration added to the incubation jars.

When the adjustment had been accounted for the respiration rate could be calculated. For each sampling period GHG flux were calculated as a function of changes in GHG concentration over time:

Equation 4

$$\frac{(G \times 10^{-6}) \times (P \times 100) \times A_v \times M}{(R \times (K + S_t)) \times 1000}$$

Where G is the GHG concentration in the incubation jar. P is the ambient air pressure in hPa during the sampling period. Data collected from the rooftop weather station over the lab. In case of missing data,

the P was collected from Älvsborgsbron. A_v is the air volume in the headspace of the incubation jars. M equals the molecular weight in molar mass of the GHG which is calculated at the time. R equals the ideal gas constant (8.314 J K^{-1}). K represents the temperature in Kelvin. Finally, S_t is the soil incubation temperature.

Between measurement periods the valves were left open to prevent GHG buildup in the headspace.

To represent the average flux in $\mu\text{g-C day}^{-1} \text{ g soil}^{-1}$ the following equation were used:

Equation 5

$$\frac{\beta}{S_w \times 1000}$$

Where β is the average respiration rate as calculated above. S_w represent the soil weight.

The temperature sensitivity was calculated as the relationship between the different responses in flux between the two temperature treatments 5°C and 15°C . As no soil were incubated below freezing temperatures no special considerations were made like (Conant et al., 2008) would otherwise suggest. Instead, the following formula were applied to the theoretical framework.

Equation 6

$$Q_{10} = \left(\frac{R_2}{R_1} \right)^{\frac{10^\circ\text{C}}{(T_2 - T_1)}}$$

Where R is the respiration rate and T is the temperature in $^\circ\text{C}$.

Gas Chromatography

Gas Chromatography (GC) were used to analyze GHG flux from the headspace of the soil samples. The model used was an Agilent 7890A GC system connected to an Agilent Technologies 7697A headspace sampler. The GC used in this study analyzed CO_2 , CH_4 and N_2O . GC works by comparing differential partitioning of compounds in a steady state and a mobile state. This was done by moving gases through Hayesep Q stainless steel columns. The steady state is the mixture of gases captured in the glass vials and the mobile phase is represented by a carrier gas which was helium and nitrogen. When the compounds move through the Hayesep Q columns at different speed the compounds separate and cluster in individual types of molecules which can then be measured by specialized detectors. The GC used for this study was equipped with three detectors:

1. Flame ionization detector (FID) – A methanizer-FID were used to analyze the CO_2 and CH_4 content within the gas samples. The FID is calibrated to detect low concentrations of CO_2 . Carbohydrates were burned in a hydrogen-air flame that produces ions which can be collected and measured. As the different molecules arrive to the detector at different times the CH_4 and CO_2 can be analyzed separately. The signal is measured directly.
2. Electron capture detector (ECD) – An ECD were used to analyze the N_2O content within the gas samples. Electrons where absorbed by the compound and measured by a reduction in electric charge in the detector. The signal is measured indirectly based on the loss of charge in the

detector.

3. Thermal conductivity detector (TCD) – The TCD also measures the CO₂ and CH₄ content within the gas but is not as sensitive as the FID and thus can capture higher concentrations better. The TCD detector works by comparing a change in electric current by differential heat loss in two temperature-controlled chambers. An electric current keeps filament at a steady temperature. As gas flows through the chambers heat is lost dependent on the thermal conductivity of the compound. Carrier gas, CO₂ and CH₄ flows through one chamber and is compared to a second reference chamber which only the carrier gas is moved through. The difference between the heat loss in the chamber is measured and provides a direct signal.

The operating temperature of the GC was 160° C with an oven temperature of 50° C. Temperatures were kept constant with an error margin of 0.01° C. Two reference samples with known gas concentrations were run every 12 samples to account for any potential drift in the GC.

Analyzed sequences which had high std in the reference samples where either excluded from the results or ran again without changing the setup to fully disclose if the error was the GC or the samples.

Statistics

Linear regression was used to determine the accuracy of the GHG flux during the weekly measurements. An accuracy threshold of $R^2 > 0.85$ for CO₂ and $R^2 > 0.75$ CH₄ & N₂O otherwise deemed as unreliable.

To evaluate the validity of the results a few statistical analyses where applied. To test if there was a significant difference in respiration between sites, treatment and soil horizons a three-way ANOVA where used. To further the analysis a post hoc Tukey's HSD test where applied to the dataset.

Results

Data quality

The data used in this report have been controlled as thoroughly as possible to minimize potential errors. Different methods have been used for the varying parts of the data. I fully disclose the shortcomings of my methods and results, as far as my knowledge reaches in the discussion.

- Flux measurements - triplicates with SE ($p < 0.05$), linear regression.
- GC – controlled with two standard gas samples of known trace gas concentrations for every 12 samples to detect potential drift.
- pH – triplicates with SE ($p < 0.05$), measured both pH and pH KCl. pH-meter calibrated before measurements.
- DOC – triplicates with SE ($p < 0.05$), controlled with milli-q, blanks and known TOC concentration and blanks to detect contamination in sampling.
- C/N – triplicates with SE ($p < 0.05$)
- LOI – triplicates with SE ($p < 0.05$), measured beaker weight to correct for further errors
- GSWC – triplicates with SE ($p < 0.05$)

Background data

The SOC measured by LOI were concentrated to the topsoil where the fraction of SOC reached up to 66 % of the dry soil weight (Table 1). The A-horizons from Goaivojávri and Corrvosjávri had the highest relative concentration of SOC of all sites. Topsoil from Latnjajávri contained only about one third SOC compared to Goaivojávri. There was a distinct difference in the SOC content between the horizons were all deeper horizons only contained a few percent of SOC compared to the dry weight of the samples.

Moisture contributed most to the total weight of the topsoil for all sites (Table 1). The gravimetric soil water measurements concluded that the water content decreased with depth. Soil moisture in the A-horizon from Latnjajávri were lower than the other two sites. The B/C- and C – horizons had similar amounts of soil moisture with the largest variation in the B/C – horizon. The highest gravimetric soil water content was found in the soils from Goaivojávri.

The soil temperature was measured during June 2005 (Table 1). Corrvosjávri was significantly higher than the other sites and approximately twice the temperature of Latnjajávri. The temperature decreased with depth for all sites.

Table 1. Soil background data (Gravimetric soil water content, GSWC; loss on ignition, LOI and soil temperature, soil_T). Soil_T data from Brinkman, (2006) with permission. Soil horizon C_D corresponds to the deep samples from the bottom of the soil pit collected with a soil corer.

Site	Horizon	Variable		
		GSWC (%)	LOI (%)	Soil _T (°C)
Goaivojávri	A	78.60 ± 3.01	65.86 ± 16.97	9.55
	B/C	27.46 ± 3.65	5.74 ± 0.36	9.1
	C	18.02 ± 0.53	2.05 ± 0.29	8.6
	C _D	17.82 ± 1.37	2.26 ± 0.16	N/A
Latnjajávri	A	57.78* ± 7.68	23.21* ± 6.59	7.75
	B/C	18.91 ± 0.62	3.24 ± 1.04	6.65
	C	15.26 ± 0.57	2.63 ± 0.12	6
	C _D	19.94 ± 4.54	2.35 ± 1.69	N/A
Corrvosjávri	A	71.78 ± 3.40	55.88 ± 13.49	17
	B/C	14.06* ± 2.23	1.45 ± 1.03	14.4
	C	14.13* ± 0.68	1.78 ± 0.06	12.55
	C _D	16.58* ± 1.59	1.75 ± 0.05	N/A

Mean + SD (n = 3) for GSWC, LOI and C/N. *Where data were missing n = 2. Soil_T measured as mean over June 2005.

The particle size distribution was similar between all sites (Brinkman, 2006). The majority of all particles were smaller than 0.2 mm in all horizons (Table 2). The abundance of coarser particles was highest in the A-horizon.

Table 2. Mean soil particle distribution in the soil horizons from all three sites. Data from Brinkman, (2006) with permission.

Particle size (mm)	Soil horizon		
	A	B/C	C
	Distribution (%)		
> 20 mm	7	2.3	4.7
20 mm > 2 mm	17.1	13.5	11.3
2 mm > 0.2 mm	28.5	24.4	23.4
< 0.2 mm	47.3	59.8	60.6

The pH differed between the sites with a maximum pH variation of 1.8 (Figure 4). The lowest pH measured was a Goaivojávri A-horizon with a pH of 4.9 (single sample in water suspension). The highest pH was 6.7 measured in a C-horizon sample from Corrvosjávri (water suspension). In general, the A-horizon were more acidic. For most samples the pH increased with depth. However, there were a few exceptions as the B/C horizon and C-horizon at both Goaivojávri and Latnjajávri. The mean pH for soil samples from Latnjajávri were lower compared to the other sites.

pH measured in KCl_1 suspension were more acidic. The total variation in pH between samples were approximately 30 % less with the addition of KCl_1 . Corvosjávri A-horizon differed from the rest of the samples where the difference between water and KCl_1 suspension was neglectable. The pH from Latnjávri A-horizon were the lowest measured in KCl_1 suspension. On average, the variation between water- and KCl_1 suspension increased in the deeper soil horizons.

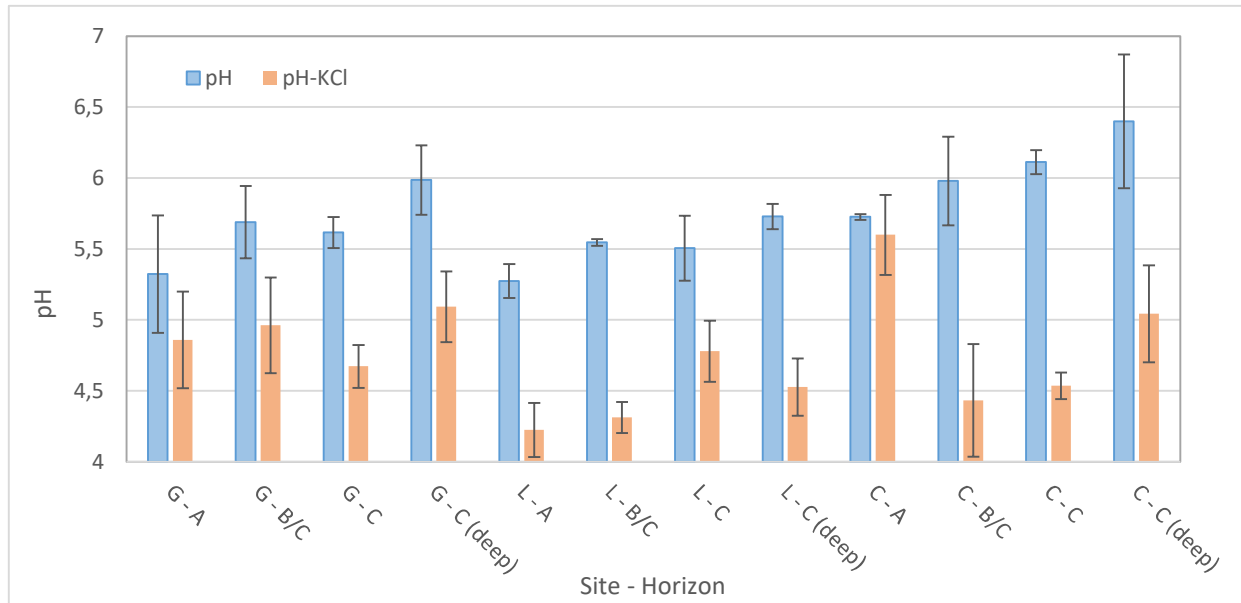


Figure 4. pH measurements showing the mean of three replicates per site and horizon. Mean n and $SE = 3$. G stands for Goaivojávri, L stands for Latnjávri and C stands for Corvosjávri.

The C/N ratio had a range between 8.21 (deep C-horizon from Goaivojávri) and 30.25 (Latnjávri C-horizon) (Table 3). There was a distinct difference in total C and N content between the upper A-horizon and the underlying mineral layers. The N concentration was low in all samples, the median concentration was 0.05 % of the total weight. The highest N content was 2.24 % of the total weight. Variability in $\delta^{13}C$ were low ranging between (-22.59 to -27.34, not shown in table). $\delta^{15}N$ decreased with depth. The only exception was the Latnjávri C-horizon, where the deeper samples had higher $\delta^{15}N$ values compared to the overlying soil.

Table 3. Chemical characteristics of the soil groups derived from isotopic analysis. Variables are shown as mean of several measurements.

Site	Horizon	C_{TOT} (%)	δ¹³C	N_{TOT} (%)	δ¹⁵N	C/N ratio
<i>Goaivojávri</i>	A	33.81 ± 9.32	-26.33 ± 0.31	1.87 ± 0.43	2.25 ± 1.16	17.83 ± 1.12
	B/C	2.08 ± 0.24	-26.79 ± 0.32	0.13 ± 0.01	2.00 ± 0.42	15.93 ± 1.08
	C	0.55 ± 0.13	-25.13 ± 0.71	0.06 ± 0.01	-6.25 ± 0.59	9.76 ± 1.18
	C _D	0.41 ± 0.11	-23.45 ± 1.14	0.05 ± 0.01	-8.31 ± 0.70	8.21 ± 1.30
<i>Latnjajávri</i>	A	13.39 ± 0.61	-25.61 ± 0.19	0.74 ± 0.01	1.39 ± 1.86	18.16 ± 1.18
	B/C	1.65 ± 0.26	-25.85 ± 0.35	0.07 ± 0.01	-5.50 ± 0.53	24.05 ± 2.98
	C	1.18 ± 0.17	-25.30 ± 0.27	0.04 ± 0.01	-12.44 ± 3.16	30.25 ± 2.90
	C _D	1.36 ± 0.37	-25.55 ± 0.47	0.05 ± 0.02	-8.24 ± 3.68	26.56 ± 2.36
<i>Corrvosjávri</i>	A	30.60 ± 7.12	-26.87 ± 0.37	1.76 ± 0.39	-0.62 ± 0.39	17.30 ± 0.33
	B/C	0.62 ± 0.03	-26.13 ± 0.41	0.04 ± 0.00	-8.66 ± 3.86	15.38 ± 0.24
	C	0.35 ± 0.07	-23.78 ± 0.55	0.03 ± 0.01	-13.94 ± 4.82	10.37 ± 1.54
	C _D	0.35 ± 0.15	-24.74 ± 0.49	0.04 ± 0.01	-19.38 ± 5.99	9.16 ± 1.98

Mean + SD n = 3.

The organic A-horizon had on average ten times as high concentration of DOC compared to the underlying soil (Table 4). In the same horizon anaerobic samples had on average 227 % higher concentration of DOC compared to their aerobic counterpart. Goaivojávri and Latnjajávri had higher concentration of DOC in the deeper soil-horizons compared to Corrvosjávri. The higher temperature treatment had on average 9 % less DOC compared to their colder counterpart (A-horizon excluded). DOC concentration in the Goaivojávri soils decreased with depth. In the samples from Latnjajávri the B/C-horizon had lower concentration of DOC compared to the C-horizon. Although, concentrations of DOC were relatively high in the C-horizon compared to the other sites. Several means of controls demonstrated the precision of the measurements (Table 5). There was almost no variability in the milli-q samples. The high concentration TOC₅₀ solution varied about 1.5 % which might suggest there is some additional margin of error for high concentration samples. The blank control samples indicate that the measurements might vary an additional ± 0.40 on average, which equals about 4 % for the average sample.

Table 4. DOC measurements sampled at T_{155} of the incubation.

DOC concentration (mg/L)					
Treatment					
Site	Horizon	5° C aerobic	15° C aerobic	5° C anaerobic	15° C anaerobic
Goaivojávri	A	9.64 ± 0.74	9.5 ± 2.77	38.27 ± 18.95	39.55 ± 10.81
	B/C	4.43 ± 0.28	2.99 ± 0.11	7.33 ± 2.57	7.76 ± 2.94
	C	3.64 ± 0.70	2.15 ± 0.27	3.20 ± 0.44	2.54 ± 0.40
	C _D	1.99 ± 0.18	1.16 ± 0.18	2.40 ± 0.40	1.18 ± 0.41
Latnjajávri	A	9.45 ± 1.94	8.63 ± 2.14	65.33 ± 19.90	52.61 ± 17.38
	B/C	3.76 ± 1.75	3.48 ± 1.62	2.51 ± 0.70	6.06 ± 3.16
	C	6.16 ± 2.98	4.82 ± 2.24	3.38 ± 1.18	4.97 ± 5.08
	C _D	4.29 ± 2.41	3.24 ± 1.48	2.28 ± 0.34	1.96 ± 1.07
Corrvosjávri	A	11.77 ± 1.12	58.56 ± 22.34	29.99 ± 4.85	18.36 ± 7.04
	B/C	1.37 ± 0.22	1.55 ± 0.22	1.81 ± 0.31	1.78 ± 0.34
	C	1.93 ± 0.72	1.43 ± 0.23	1.62 ± 0.14	1.00 ± 0.29
	C _D	1.54 ± 1.66	1.77 ± 1.66	1.72 ± 0.31	0.63 ± 0.16

Mean + SD n = 3.

Table 5. Control tests to determine the accuracy of the DOC measurements. Milli-q is ultraclean water to measure any contamination in the machine. TOC₅₀ is a solution to determine that the DOC quantity is correct. Blank samples are deionized water which was run as normal samples every 10th sample to determine if there was any contamination in the sampling process.

Controls		
Milli-q	TOC ₅₀	Blank sample
0 ± 0.02	50 ± 0.73	0.40 ± 0.14

Mean + SD n = 12

Flux measurements

CO₂ flux

All soils incubated at 15°C respired significantly more CO₂ compared to the soils incubated at 5 ° C ($p < .000$). On average the warmer treatment respired 139 % more than the cold treatment. The aerobic samples respired approximately twice (92 % more) the amount of the anaerobic samples. The A-horizon differed significantly ($p < .000$) from all the deeper soil horizons. No statistical difference was found between the deeper soil horizons. However, most of the samples had decreased respiration as depth increased. Most of the respiration occurred in the A-horizon. The A-horizon respired more than 11.5 times the amount of CO₂ than the all the deeper layers combined. The flux was highest during the first four measurement (T1 - T21) periods compared to the last (T45 - T118). The fluxed decreased significantly over time ($F(1.94, 6843, 41) = 4,57, p < .012$), with an average decrease of 24 % between the previously stated time intervals. The flux differed between sites. The soils from Corrvosjávri respired significantly more than the other two sites ($p < .000$) on average 3 times that of Goaivojávri and 4.5 that of Latnjajávri. No statistical difference was found between Goaivojávri and Latnjajávri ($p = .518$).

The maximum CO₂ flux was measured in the incubated A-horizons (Figure 5). For the samples incubated at 5° C there was a difference in respiration between sites. Aerobic and anaerobic samples from Corrvosjávri respired on average higher quantities of CO₂ than the other samples. The aerobic samples from Corrvosjávri respired 256 % more CO₂ than the aerobic samples from Goaivojávri. Aerobic samples from Goaivojávri respired 112 % more than samples from Latnjajávri. The highest flux measured in this set was 48.55 µg C day⁻¹ G_{soil}⁻¹ measured the first week from the aerobic soils from Corrvosjávri. The samples from Corrvosjávri had the highest variability and steepest decrease in respiration over time. The respiration in the soil samples from Goaivojávri and Latnjajávri varied to a less degree. All the aerobic samples from respective sites respired more than their anaerobic counterparts.

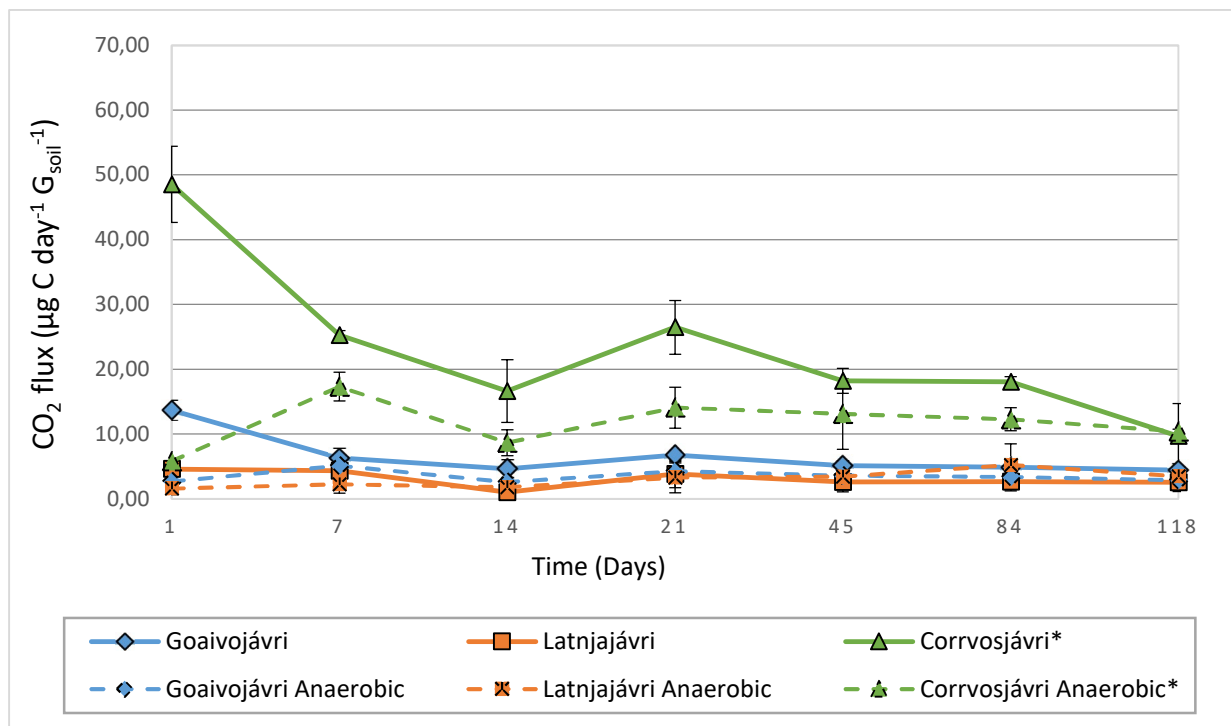


Figure 5 CO₂ respiration measured in µg C day⁻¹ from all A-horizons incubated at 5° C. Each line represents the mean of three samples. All data points represent the flux over one measurement period (four 20 ml gas samples collected in one week, assuming all samples passed significance of R² > 0.85). Standard deviation is displayed with error bars (n = 3).

The highest respiration rates throughout the incubation were measured in the Corrvosjávri A-horizons kept at 15° C (Figure 6). The highest value measured peaked at 160 µg C day⁻¹ G_{soil}⁻¹. The data point was measured during the first measurement period upon thaw. The soils from Corrvosjávri respired four times more than that of Goaivojávri and almost eight times the amount from Latnjajávri. The same site difference as in the 5° C samples could be observed in the warmer incubation. The lowest flux measured was Latnjajávri anaerobic which still would be considered relatively high compared to the other soil

layers and treatments. The lowest measured set had a mean flux of about $7 \mu\text{g C day}^{-1} \text{G}_{\text{soil}}^{-1}$ which is higher than the second highest aerobic measurement from the A-horizons incubated at 5°C .

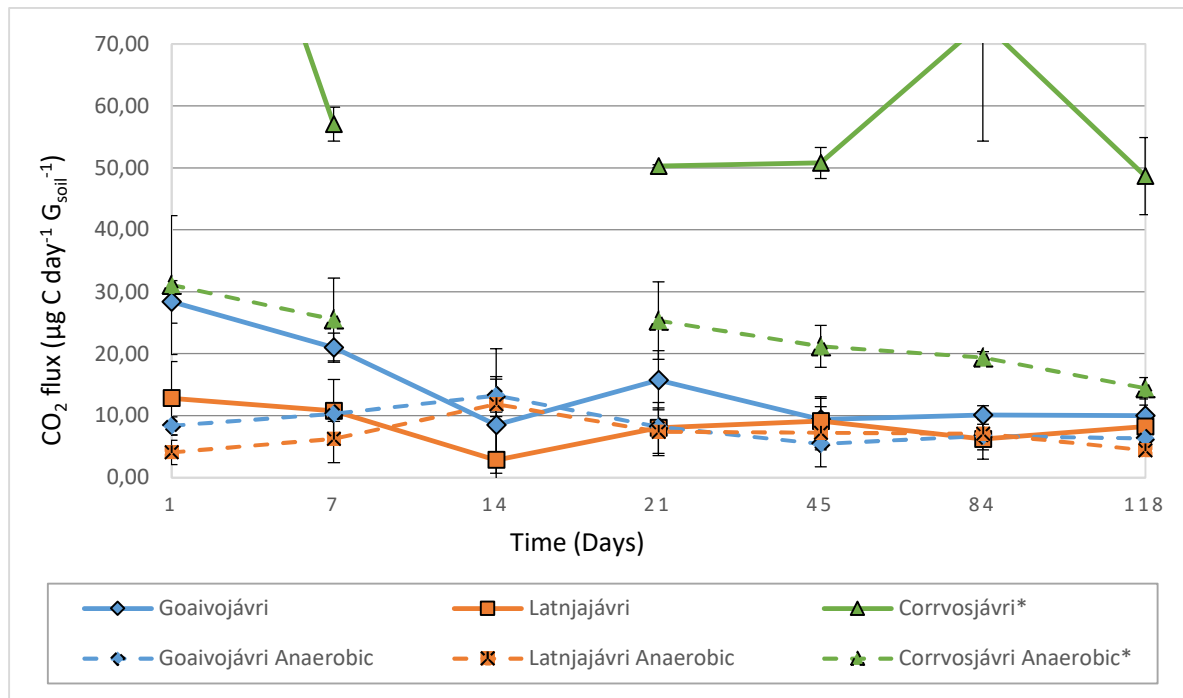


Figure 6. CO₂ respiration measured in $\mu\text{g C day}^{-1}$ from all A-horizons incubated at 15°C . Each line represents the mean of three samples. All data points represent the flux over one measurement period (four 20 ml gas samples collected in one week, assuming all samples passed significance of $R^2 > 0.85$). Standard deviation is displayed with error bars ($n = 3$).

The deeper soil horizons respired less compared to the A-horizon (Figure 7). The variance between measurements, sites and treatment also decreased. The highest CO₂ flux from the B/C-horizon originated from the Goaivojávri samples. Both the aerobic and anaerobic samples from Goaivojávri respired more CO₂ than from the other sites. After the second measurements the anaerobic samples had the highest CO₂ flux. The B/C-horizon from Corrvosjávri and Latnjajávri incubated at 5°C had very similar respiration rates throughout the incubation. The mean CO₂ flux in both aerobic and anaerobic from the two sites varied only $0.05 \mu\text{g C day}^{-1} \text{G}_{\text{soil}}^{-1}$. From the third measurement period onward the anaerobic samples from Goaivojávri respired the highest amounts of CO₂.

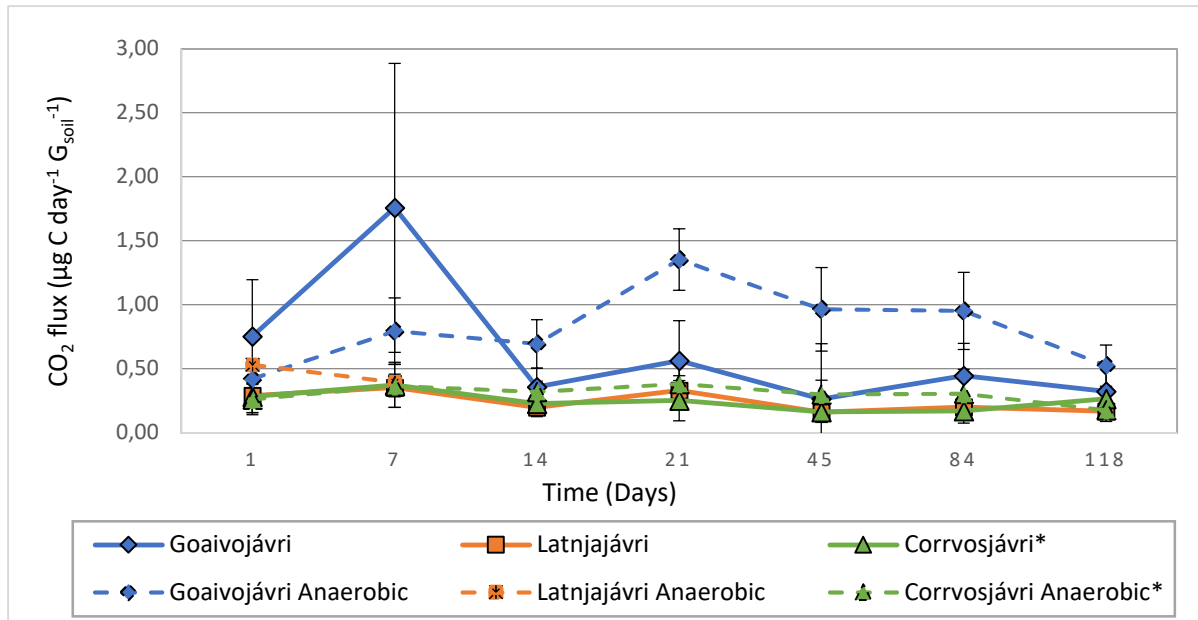


Figure 7. CO₂ respiration measured in µg C day⁻¹ from all B/C-horizons incubated at 5 °C. Each line represents the mean of three samples. All data points represent the flux over one measurement period (four 20 ml gas samples collected in one week, assuming all samples passed significance of R² > 0.85). Standard deviation is displayed with error bars (n = 3).

The warm incubation for the B/C-horizon had higher flux compared to the lower temperature (Figure 8). From the third measurement period onward the anaerobic samples from Goaivojávri and Latnjajávri had the mean highest respiration rates. The aerobic samples from Latnjajávri along with both anaerobic and aerobic samples from Corrvosjávri had low respiration rates throughout the incubation period. Around the third week into the incubation most samples experienced a drastic decrease in respiration. The aerobic samples had a quicker response in the decreased respiration compared to the anaerobic samples.

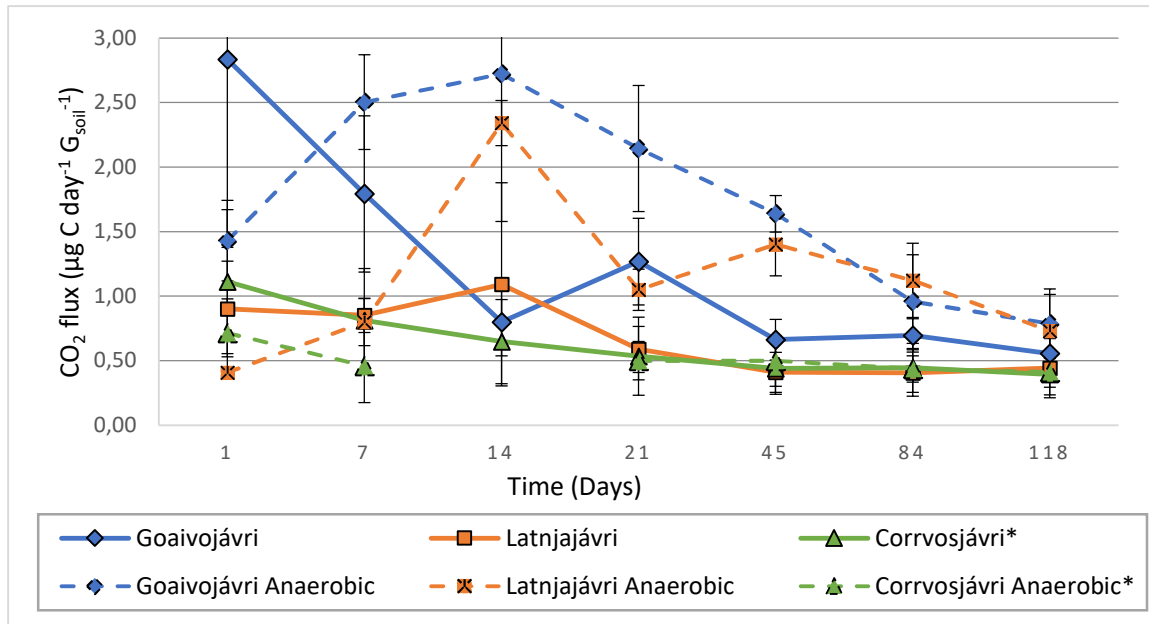


Figure 8. CO₂ respiration measured in $\mu\text{g C day}^{-1}$ from all B/C-horizons incubated at 15 °C. Each line represents the mean of three samples. All data points represent the flux over one measurement period (four 20 ml gas samples collected in one week, assuming all samples passed significance of $R^2 > 0.85$). Standard deviation is displayed with error bars ($n = 3$). C 5 °C

The CO₂ flux from the C-horizon were low throughout the incubation with the lowest variation between samples (Figure 9 and Figure 10). The respiration rates were highest in a reversed permafrost thaw gradient where the most recently thawed site Goaivojávri respired most, and Corrvosjávri respired least. Anaerobic samples respired on average only slightly less compared to the aerobic samples. From the second measurement onward the anaerobic samples from Latnjajávri respired more than the aerobic samples. The anaerobic samples had a noticeable peak in respiration when they had been incubated for 45 days. Deep soil samples collected from the bottom of the soil pit had a slightly lower mean respiration compared to the overlying soil from the same horizon.

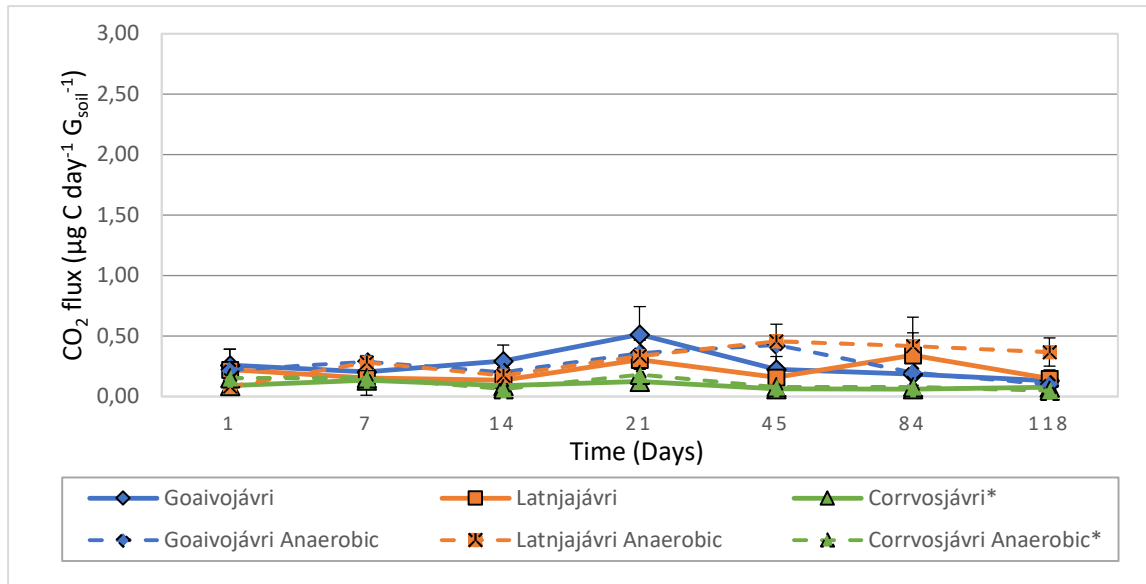


Figure 9. CO₂ respiration measured in µg C day⁻¹ from all C-horizons incubated at 5° C. Each line represents the mean of three samples. All data points represent the flux over one measurement period (four 20 ml gas samples collected in one week, assuming all samples passed significance of R² > 0.85). Standard deviation is displayed with error bars (n = 3).

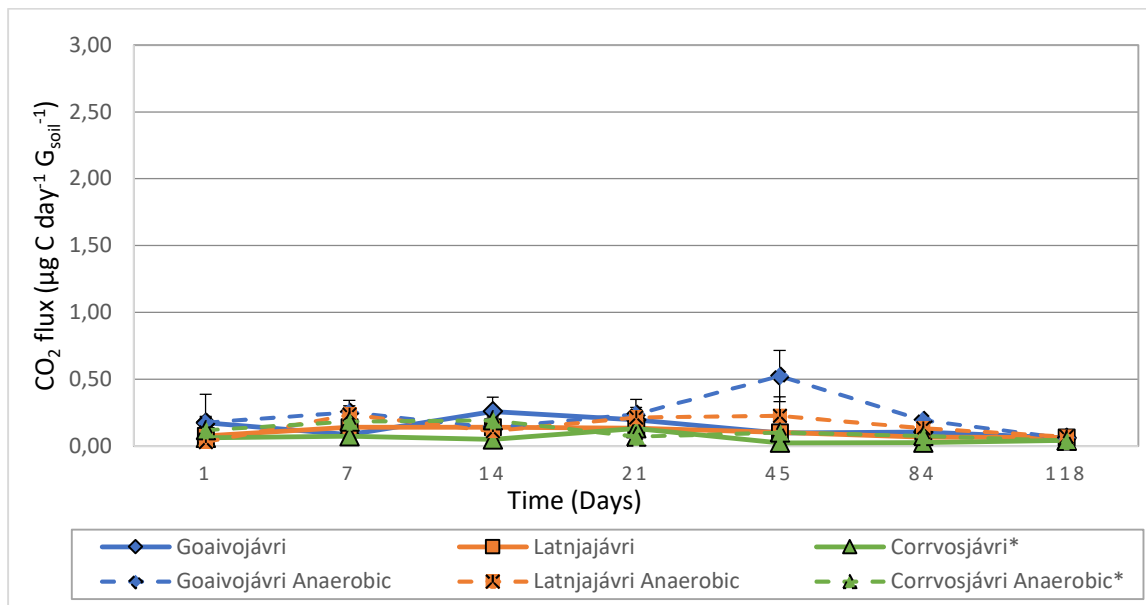


Figure 10. CO₂ respiration measured in µg C day⁻¹ from all deep C-horizons collected with a soil corer and incubated at 5° C. Each line represents the mean of three samples. All data points represent the flux over one measurement period (four 20 ml gas samples collected in one week, assuming all samples passed significance of R² > 0.85). Standard deviation is displayed with error bars (n = 3).

The warm incubation treatment of the C-horizon respired on average 128 % more CO₂ than the cold treatment (Figure 11 and Figure 12). In the C-horizon the highest respiration rates originated from anaerobic treatments. The flux measured from Goaivojávri was the highest following the same pattern as the B/C-horizon. The lowest and least variable respiration was measured in the Corrvosjávri soils. The respiration from the soils from Latnjajávri was slightly lower than soils from Goaivojávri, which holds true for the majority of measurements.

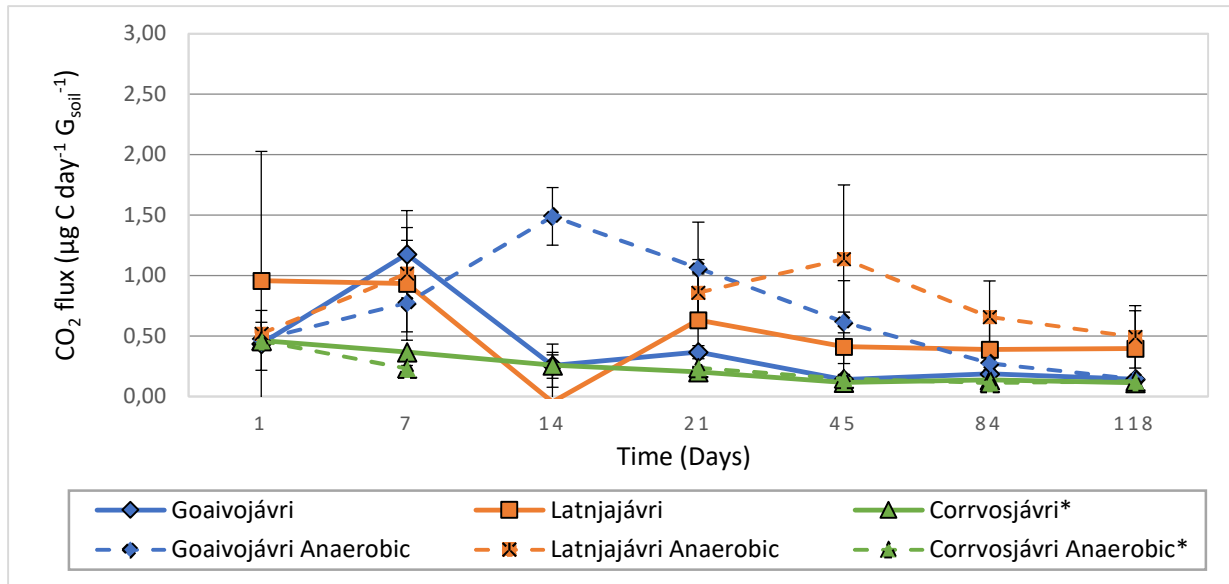


Figure 11. CO₂ respiration measured in µg C day⁻¹ from all C-horizons incubated at 15° C. Each line represents the mean of three samples. All data points represent the flux over one measurement period (four 20 ml gas samples collected in one week, assuming all samples passed significance of R² > 0.85). Standard deviation is displayed with error bars (n = 3).

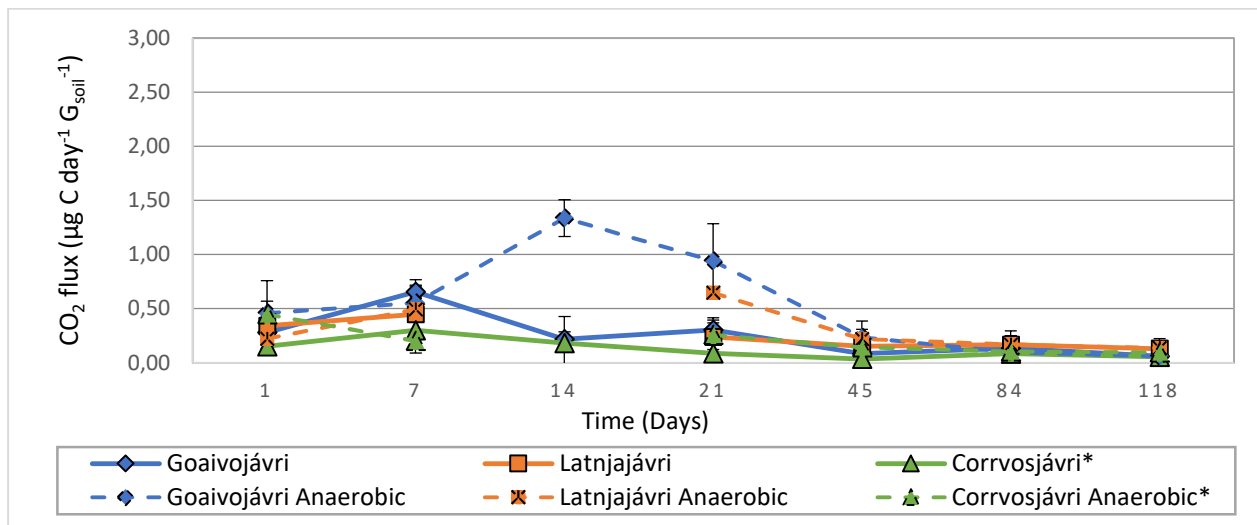


Figure 12. CO₂ respiration measured in µg C day⁻¹ from all deep C-horizons collected with a soil corer and incubated at 15° C. Each line represents the mean of three samples. All data points represent the flux over one measurement period (four 20 ml gas samples collected in one week, assuming all samples passed significance of R² > 0.85). Standard deviation is displayed with error bars (n = 3).

Cumulative CO₂ respiration

The samples incubated at 15° C had a higher cumulative respiration compared to the samples incubated at 5° C (**Fel! Hittar inte referenskälla.**). The highest cumulative respiration was measured from the aerobic samples from Corrvosjávri incubated at 15° C. After about four months into the incubation the cumulative respiration was about 3.2 times higher than the second highest treatment. Day 38 into the incubation it had respired an average of 1400 µg C – CO₂ g soil⁻¹, as much CO₂ as the second highest aerobic treatment respired in four months. The majority of the respiration from all sites originated from

the A-horizon. The 5° C aerobic treatment from Latnjajávri respired only 51 % that of Goaivojávri, and 27 % of Corvosjávri respectively. In the warmer treatment the total variation increased between the sites. The relative difference between Goaivojávri and Latnjajávri decreased.

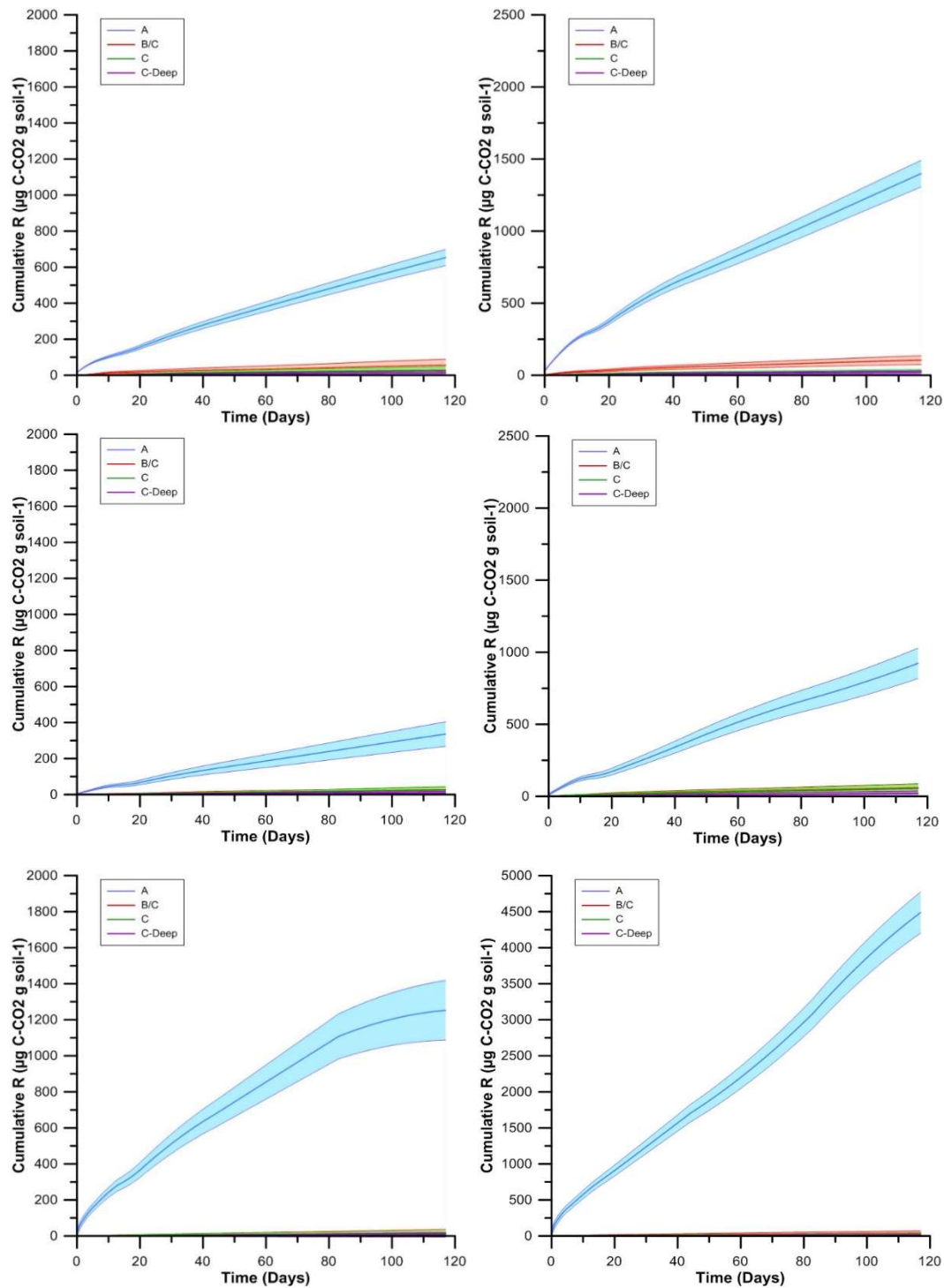


Figure 13 Cumulative CO₂ respiration from the aerobic treatment, grouped in sites. To the left surrounded by blue outline is the results of samples incubated at 5° C. To the right outlined by red is the results from the samples incubated at 15° C. The sites are the following, top to bottom: Goaivojávri, Latnjajávri and Corvosjávri respectively. Each line represents the average cumulative respiration for that soil horizons. Standard deviation is highlighted above and below the mean.

Cumulative anaerobic

The cumulative respiration for the anaerobic samples from Goaivójavri and Corrvosjavri remained lower than the aerobic throughout the incubation (Figure 14). The anaerobic samples from Latnjajávri had higher cumulative respiration in the 5° C treatment, the 15° C treatment remained slightly below their aerobic counterparts. The Corrvosjavri soils incubated at 15° C had the highest cumulative respiration for the anaerobic samples. However, the maximum respiration is only about one third that of the aerobic counterpart.

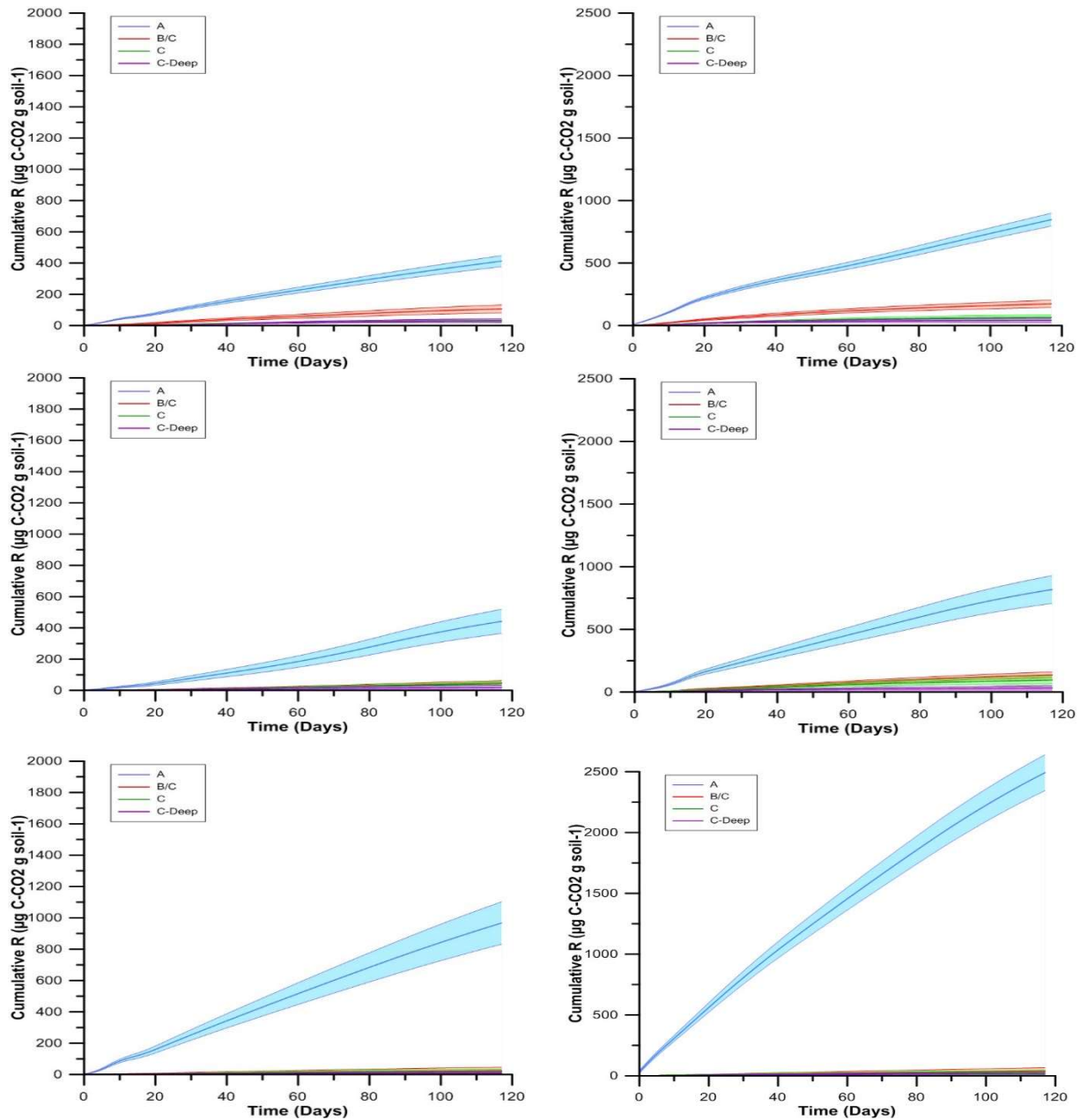


Figure 14. Cumulative CO₂ respiration from the anaerobic treatment, grouped in sites. To the left surrounded by blue outline is the results of samples incubated at 5° C. To the right outlined by red is the results from the samples incubated at 15° C. The sites are the following, top to bottom: Goaivójavri, Latnjajávri and Corrvosjavri respectively. Each line represents the average cumulative respiration for that soil horizons. Standard deviation is highlighted above and below the mean.

CH₄ flux

There was low or no methane flux for most samples throughout the incubation period. However, the anaerobic A-horizon developed a few consistent samples which developed consistent methane flux (Figure 15 and Figure 16). For the deeper soil horizons, the quantity and frequency of methane flux was inadequate. There was no methane flux in any aerobic sample. The highest measured methane flux was from the Corrvosjávri A-horizon incubated at 15° C. The earliest measured CH₄ flux were a sample from Goaivojávri which started a consistent flux from the second measurement period. Most of the samples which had a methane flux developed it between T₂₁ and T₄₅.

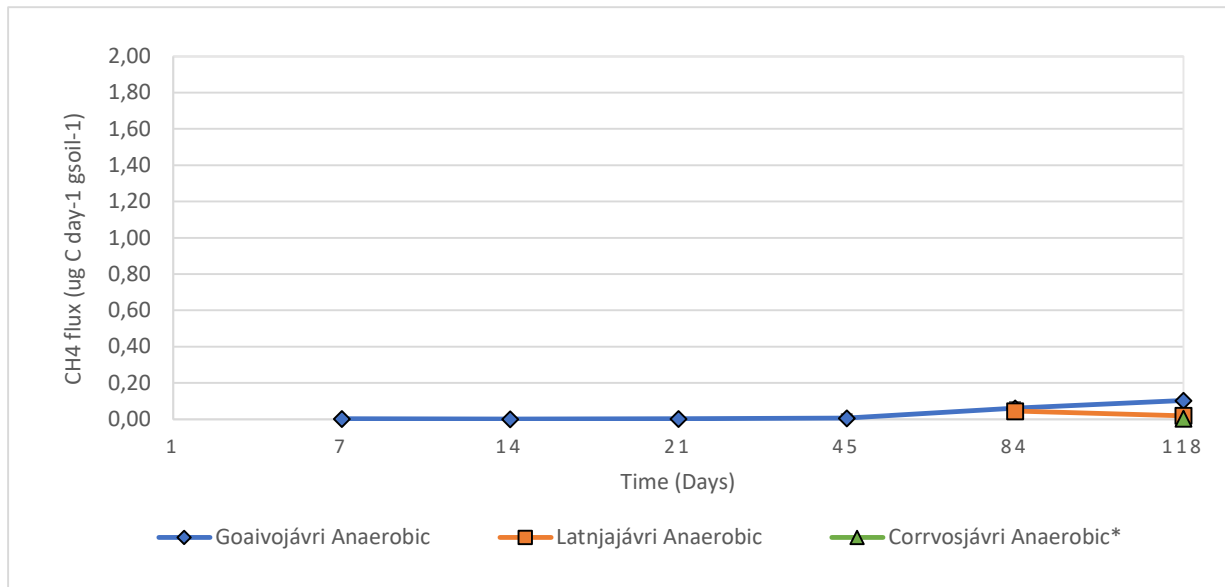


Figure 15. CH₄ flux measured in µg C day⁻¹ from all A-horizons incubated at 5° C. Each line represents the mean of three samples. All data points represent the flux over one measurement period (four 20 ml gas samples collected in one week, assuming all samples passed significance of $R^2 > 0.75$). Standard deviation is displayed with error bars ($n = 3$).

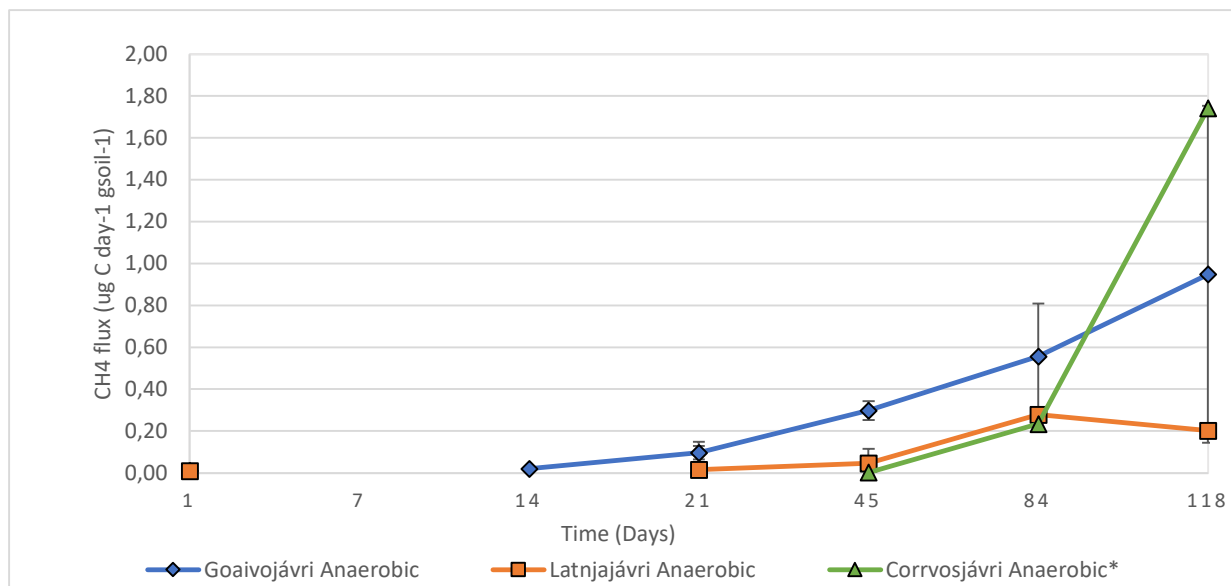


Figure 16. CH₄ flux measured in µg C day⁻¹ from all A-horizons incubated at 15° C. Each line represents the mean of three samples. All data points represent the flux over one measurement period (four 20 ml gas samples collected in one week, assuming all samples passed significance of R² > 0.75). Standard deviation is displayed with error bars (n = 3).

N₂O flux

The flux from N₂O was extremely low and sporadic in nature. Therefore, the results will be neglected in this report.

Temperature sensitivity

Soil samples from Latnjajávri were the most temperature sensitive (Table 6). Both the aerobic and anaerobic treatment responded stronger than the soils from the two other sites, except for the deep C-horizon from Goaivojávri which in the anaerobic treatment had a stronger temperature response. The Q₁₀ did not vary particularly much between sites nor horizons. The least variability in Q₁₀ between soil horizons were from Corrvosjávri (± 0.39).

Table 6. Temperature sensitivity for all soil samples and treatments. Represented Q₁₀ values equal the mean value throughout the incubation.

Q ₁₀				
Aerobic				
Soil horizon				
Site	A	B/C	C	C _D
Goaivojávri	2.28	2.25	1.79	2.25
Latnjajávri	2.82	3.00	2.92	2.62
Corrvosjávri	2.49*	2.54	2.64	2.31
Anaerobic				
Soil horizon				
Site	A	B/C	C	C _D
Goaivojávri	2.55	2.32	2.81	3.04
Latnjajávri	2.68	4.09	2.92	2.88
Corrvosjávri	2.48*	2.25	2.49	2.56

Discussion

The results from this report concludes that there is an observable difference in trace gas flux between different tussock tundra communities along a permafrost thaw gradient. This report found that the site where permafrost thawed the longest time ago had a significantly higher CO₂ flux compared to the other two sites. The high respiration in Corrvosjávri A-horizon is interesting as the high C-content alone cannot explain the activity, because Goaivojávri A-horizon contained higher amounts of C available for decomposition. I therefore suggest that the environmental factors have allowed microbes to adapt to new conditions in which they have become more effective in decomposing the available SOC compared to newly thawed sites. However, this difference could only be observed between Corrvosjávri and the two other sites. No significant difference in GHG flux was detected between Goaivojávri and Latnjajávri. It is possible that it could take some decades of permafrost thaw before a major change in microbial composition occurs and decomposition increases significantly. This is in accordance with the findings of Edward A. G. Schuur et al. (2009). The A-horizon from Latnjajávri had a lower GHG flux most likely due to the fact that it contained considerably less SOC. GHG flux from Latnjajávri where surprisingly close to that of Goaivojávri even though it contained only 38.8 % SOC compared to Goaivojávri. There is a possibility that Goaivojávri and Latnjajávri where similar in GHG flux because the microbial community in Latnjajávri where more developed since the permafrost thawed there before Goaivojávri. The physical properties restricted microbial growth in the incubated soils from Latnjajávri. The low SOC content was the primary restriction as there was less labile SOC available for decomposition. It would be surprising to find it had the same levels of respiration. If the SOC content would be the same between Latnjajávri and Goaivojávri the respiration would probably have been higher in Latnjajávri. There could also be a difference in the quality of the SOC available for decomposition where Goaivojávri soils might contain more labile SOC compared to Latnjajávri. Current research suggests that it would be probable that more labile SOC should be present in the most newly thawed site because it would have been preserved (Schlesinger & Bernhardt, 2013) for the longest time and the first source to be decomposed as the opportunity for decomposition arises. SOC content and quality is also more directly associated to the response in respiration (Conant et al., 2008; Davidson & Janssens, 2006; Uhlířová, Šantrůčková, & Davidov, 2007). It could also be that it simply takes longer time to develop a different microbial community and that certain conditions must have existed for a longer time period. This could have occurred in Corrvosjávri but not yet at Latnjajávri but at a certain threshold value the conditions arise for more rapid decomposition of available SOC. Differing vegetation cover might provide the soils with differing sources of litter which in turn affect the available SOC and thus decomposition.

Respiration decreased over the incubation time period. As microbes decompose the available labile SOC first the increasingly more recalcitrant SOC will be left. This result was expected and contribute further support for existing research. The most pronounced example of this was observed in the 15° C aerobic treatment. It was no surprise that the effect was clearest in the warm aerobic treatment as the thermodynamic reactions would favor the decomposition rate, particularly in that treatment group (Schlesinger & Bernhardt, 2013). The warm aerobic conditions allow for rapid microbial growth, which makes the comparisons between sites and depth all the more interesting to analyze. The majority of the measured GHG flux originated from the A-horizon. Mean respiration from A-horizon samples exceeded 20 times the amounts of the mean respiration from the B/C-horizon samples, which in turn respired more than the C-horizon. A trend in decreasing respiration with depth was observed for all treatments and sites. The least difference was observed in the deeper soil horizons and could be explained by the lack of labile SOC to begin with. As the deep mineral soil rarely get fresh input of SOC only the low-

quality recalcitrant C remains, which are less accessible and more difficult for microbes to decompose. Similar effect would be expected in the incubated organic soils if just enough time were given for microbes to deplete all the easily accessible labile SOC. The energy yield is lower for microbes which decompose low quality recalcitrant SOC, and therefore the GHG flux would decrease with time and depth.

Overall the aerobic treatments had a higher GHG flux, but the increasing methanogenesis observed in the last measurements were considerable. Since the GWP in methane is so high even low fluxes can have a serious impact. The difference in methanogenic bacteria between sites might have an impact in the pace and temporal variety of the SOC decomposition. If the methanogenesis were to increase further throughout the incubation the anaerobic samples would have higher GHG flux than the aerobic samples. The rapid response in methanogenesis from Goaivojávri was likely due to the wet field conditions. As it is the coldest site with permafrost thaw most recently it would have more reason to naturally develop higher amounts of methanogens. The snowmelt there is also later than other sites leaving it totally saturated for long periods of time. Under more suitable conditions the methanogens from Corrvosjávri were more effective at decomposing SOC as indicated by the 15° C incubation. Methanogenesis could only be observed in the organic topsoil. It is possible that there will be an increase in methane flux from all the anaerobic samples as the incubation time increases. Since methanogenesis is a low yield metabolic pathway it is likely that it takes time to develop in the deeper soil horizons (Schlesinger & Bernhardt, 2013). In real life it is much tougher for anaerobic conditions that stimulate methanogenic development to develop. That is because the surface layer would have to be saturated for several months. Methanogens are likely to develop in areas like the depressions found over our field sites but those constitute only a miniscule part of the landscape. Mostly the topography is complex where the conditions shift between anaerobic and aerobic over the course of the seasons. In the shifting areas methanogens are unlikely to be successful as it is a low yield metabolic pathway.

The Q_{10} data varied less than expected. Commonly the more recalcitrant and slower systems with low C content and high MRT have higher Q_{10} values compared to the topsoil (Zhou, Xu, Zhou, & Luo, 2018). It is possible and even likely that this effect will be more clearly observed as the soils are incubated for longer time periods. In this study, measurements were only collected for about four months. The soils in this study are all more sensitive to shifts in climate compared to the global average of Q_{10} for soil which is 1.5 (Bond-Lamberty & Thomson, 2010; Conant et al., 2008). The average Q_{10} used for permafrost modeling is 2 in the CMIP4 model (Friedlingstein et al., 2006) and our results come closer to that. Compared to other soils from the tundra the Q_{10} response was low. Waldrop et al. (2010) had active layer soils that had Q_{10} values of 7.5 and (Nadelhoffer, Giblin, Shaver, & Laundre, 1991) had Q_{10} values between 1 and 3.1. Biasi et al. (2005) mean Q_{10} values of 2.6 in organic and mineral horizons of arctic soils. As our incubation only lasted the length of about one growing season it is difficult to draw any conclusions from this. It will be interesting to see if the Q_{10} increases as the incubation time progresses in the future.

It was interesting to see the difference in respiration between Corrvosjávri and Goaivojávri as they did not differ much in available SOC in the A-horizon. If it was a question solely of which site had the most available SOC for decomposition Goaivojávri would have respired more. Instead it respired less than a third compared to the Corrvosjávri topsoil. Available C and GSWC had the strongest correlation with total GHG flux ($R^2 > .87$, $p < .05$). DOC correlated well with most samples, and particularly well with the anaerobic samples. The cause is likely easily explained by that when much DOC exists in a sample there

are very easily degradable fuel for microbes to utilize and so respiration is high, and vice versa. The poorest correlation was from the pH samples which on average had a R^2 value of 0.15 ($p < .05$).

Incubation time series become more interesting to analyze the longer they are incubated. As the soils experience initial thaw, they behave erratic and tend to release large portions of GHG. This initial release of GHG is linked to both to thawing processes and SOC decomposition. Following thaw the change in flux becomes interesting as the function of the differing carbon pools become observable where a difference between the active, slow and passive emerges (Trumbore, 2006). The incubation time for this report could document the active pool well but only start to analyze the slow processes in the deeper soil horizons. The development of microbes well suited to the different treatments also depends on time. As mentioned earlier the methanogens would likely become more active as the incubation proceeded which might skew the results in a different direction in the future. These possibilities are extremely important to keep in mind as we research the GHG flux. If we were to translate our results to the real world it differs whether it is aerobic or anaerobic conditions that have the highest GWP.

No direct measurement of microbial communities was made throughout this study and therefore it is impossible to decide if the comparison between different sample measure the same microbial activity. However, this is no concern for this report as the focus have been on the total GHG flux under a set treatment. Although it might have been interesting to study how microbes might differ between samples and develop over time, it would be of little addition to this report.

There were some technical issues with the equipment used for this study. The GC drifted during the analysis of T₇₋₁₄. The loss of data points during these measurements were higher compared to other time periods. The method used for data quality control was strict so even though there were some complications I do not believe it would affect the results in any major way. The results provided might be somewhat underestimated as fluxes tend to be higher early in the incubation. There were also a quite large data-series in total, so some loss does not affect the outcome of the final calculations in any major way.

This incubation study was particularly disruptive for the microbes as soil samples for time of flight (TOF) measurements were gathered during the end of every measurement period. Attempts to minimize disturbances was made by isolating the sampling to a small part of each sample and being careful during the sampling. It was inevitable to cause no disturbance and difficult to extent to which degree samples were disturbed as no control group for this existed in this experimental setup. Except for the practical technicalities of this procedure some additional changes to the calculations were made. I subtracted the removed weight and added the additional volume to compensate for the removed TOF sample.

The DOC samples were filtered and pumped through a mechanical system. This system was not entirely exempt from contamination and cleaned by hand. Minor contamination accumulated through the process of filtering and a detectable difference was noted in the control samples. This is somewhat problematic as the deeper soil-horizons contain low quantities of DOC. As to which extent this may affect the results were clearly stated in the results section. The DOC should be critically evaluated with modest interpretation. In this report DOC were mainly used to evaluate relations to GHG flux and not to be the focus of the report. I would argue that it still provides some additional support for this report and therefore is still better included rather than ignored.

Conclusion

Soil samples were collected from three sites in the Swedish tussock tundra along a 50-year permafrost thaw gradient. Three soil pits were dug at every site and soil samples were collected and divided into A-, B/C- and C-horizons. A total of 144 soil samples were incubated for 121 days in four treatment groups (1) 5° C aerobic, (2) 5° C anaerobic, (3) 15° C aerobic and (4) 15° C anaerobic. Throughout the incubation gas samples were collected from the headspace of the soil jars. During the first month gas samples were collected weekly and thereafter monthly. GC was used to analyze GHG content and a three-way ANOVA with a Tukey HSD post hoc analysis was applied to find statistical interactions and differences.

An increased rate of SOC decomposition was confirmed in the site were permafrost thawed first. No statistical difference was found between the recently thawed and the site were the permafrost thawed about 20 years ago. Most of the decomposition occurred as respiration in the A-horizon where the organic content was highest. The 15° C aerobic treatment had the highest GHG flux and the 5° C anaerobic treatment had the lowest flux. The flux decreased with depth for all treatments, with only a few exceptions. The organic A-horizon differed significantly from the underlying mineral soils. Methanogens were active at all sites with a particularly quick response at Goavojávri. Methanogens in the warm anaerobic treatment contributed considerably to the total GHG flux in the top layer of the soils, with an increasing contribution to the total over time. The aerobic treatment was temperature sensitive where there was a trend matching the permafrost thaw gradient. The newly thawed soil was the least temperature sensitive and the longer the soils had been permafrost free the more temperature sensitive they were.

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