Microbial diversity and activity in thawing permafrost, a case study in Northern Norway

Auteur : Quabron, Gilles
Promoteur(s) : Joaquim-Justo, Celia; 5325
Faculté : Faculté des Sciences
Diplôme : Master en sciences et gestion de l'environnement, à finalité spécialisée en surveillance de l'environnement
Année académique : 2017-2018
URI/URL : http://hdl.handle.net/2268.2/6023

Avertissement à l'attention des usagers :

Tous les documents placés en accès ouvert sur le site le site MatheO sont protégés par le droit d'auteur. Conformément aux principes énoncés par la "Budapest Open Access Initiative" (BOAI, 2002), l'utilisateur du site peut lire, télécharger, copier, transmettre, imprimer, chercher ou faire un lien vers le texte intégral de ces documents, les disséquer pour les indexer, s'en servir de données pour un logiciel, ou s'en servir à toute autre fin légale (ou prévue par la réglementation relative au droit d'auteur). Toute utilisation du document à des fins commerciales est strictement interdite.

Par ailleurs, l'utilisateur s'engage à respecter les droits moraux de l'auteur, principalement le droit à l'intégrité de l'oeuvre et le droit de paternité et ce dans toute utilisation que l'utilisateur entreprend. Ainsi, à titre d'exemple, lorsqu'il reproduira un document par extrait ou dans son intégralité, l'utilisateur citera de manière complète les sources telles que mentionnées ci-dessus. Toute utilisation non explicitement autorisée ci-avant (telle que par exemple, la modification du document ou son résumé) nécessite l'autorisation préalable et expresse des auteurs ou de leurs ayants droit.
Microbial diversity and activity in the active layer of thawing permafrost: a case study in Northern Norway

QUABRON Gilles

Master Thesis submitted for the degree of Master in Environmental Sciences and Management with Professional focus in Environmental Monitoring

Supervisor: Dr. C. Joaquim-Justo
Co-supervisor: Dr. H. Lee
Acknowledgement

First of all, I would like to thank Dr. Hanna Lee for welcoming me at Uni Research and in Bergen in order to complete my Master Thesis. Thank you for giving me this opportunity to gain valuable experience and for making everything possible.

I wish to thank Professor Célia Joaquim-Justo for supervising this thesis and helping me when needed.

I would also like to thank Professor Lise Øvreås for allowing me to work and spend the major part of my stay in the laboratory of Marine Microbiology. Most of the work would not have been possible without you. Thank you for the time spent providing me with informed and useful microbiology advices.

Thanks to Gro Bjerga and Øivind Larsen for helping me with the enzyme assay as well as Dr. Oliver Müller for the precious help with the bioinformatics.

I wish to thank the entirety of the laboratory staff as well as the PhD and Post-doc involved in my work: Hilde and Hilde, Oliver, Alejandro, Jessie, Casper and Pia. Thank you very much for such a nice work atmosphere.

I thank all the people who have, closely or remotely, allowed me to complete this thesis.

Lastly, I would like to show my gratitude to Chloé and my family for their needed support.
Abstract

Permafrost environments, which store large quantities of soil organic carbon, are threatened by climate change-induced thaw that would render frozen organic matter bioavailable for microorganisms leading to an increase in greenhouse gases emissions. Hence understanding the response of permafrost microbial communities to thawing is necessary to evaluate the permafrost carbon feedback to global change. Microbial diversity and activity were studied across two thaw stages (intact and degrading permafrost) at different depths in a palsa found in Northern Norway. This study investigated soil microbial community composition by Illumina Miseq sequencing of the 16S rRNA gene while bacterial, archaeal, methanogens and methanotrophs abundance was assessed by qPCR. Microbial heterotrophic activity was evaluated using enzymatic and functional metabolic assays. Microbial communities' composition and activity were found to differ between the two thaw stages. The intact palsa (IP) presented high richness that decreased with depth while the degrading palsa (DP) exhibited less species-rich communities across depth. Relative abundance of members of the phylum Proteobacteria known to thrive in higher carbon and nutrient availability increased in the DP while members of the phylum AD3, which dominated the deepest part of the IP, almost disappeared in the DP. Bacterial, archaeal, methanogens and methanotrophs were more abundant in the thawing permafrost than in the intact palsa. The DP exhibited high and similar microbial biomass across depths while the IP showed high microbial abundance only in the topsoil layer. In addition, populations of methane-producing microorganisms were found to be strongly positively correlated to methane oxidizers abundance, suggesting a close spatial relationship between these two communities. Heterotrophic prokaryotes found in the DP displayed higher enzymatic and functional metabolic activity across depth than in the IP. Collectively, these results suggest a shift in microbial prokaryotic communities as a result of permafrost thaw characterized by less species-rich populations, by an increasing biomass of greenhouse gases-related microorganisms as well as a higher microbial activity across depth, potentially leading to greater greenhouse gases emissions that would exacerbate the positive feedbacks from permafrost carbon to climate.
# Table of content

1. Introduction ........................................................................................................................................... 1

1.1. Permafrost ............................................................................................................................................. 2

1.1.1. Definition and formation .................................................................................................................. 2

1.1.2. Global distribution ............................................................................................................................. 3

1.1.3. Distribution in Fenno-Scandinavia and Norway ........................................................................... 5

1.1.4. Permafrost carbon pool ..................................................................................................................... 6

1.1.5. Climate change in the Arctic – permafrost and climate ............................................................... 7

1.1.6. Carbon release rate and projecting change ................................................................................. 10

1.2. Permafrost microbiology and its role in C flux ................................................................................. 12

1.2.1. Permafrost as a challenging microbial habitat .............................................................................. 12

1.2.2. Microbial diversity and community composition ....................................................................... 12

1.2.3. Microbial metabolic functions ....................................................................................................... 14

1.3. Objectives ............................................................................................................................................. 16

2. Material and methods ............................................................................................................................. 17

2.1. Site description .................................................................................................................................... 17

2.2. Field sampling ..................................................................................................................................... 19

2.3. Laboratory work flow overview ......................................................................................................... 20

2.4. Soil properties .................................................................................................................................... 20

2.4.1. Soil organic matter content (Loss On Ignition method) and Soil moisture ............................... 20

2.4.2. Soil pH ............................................................................................................................................. 21

2.5. Gene analysis ....................................................................................................................................... 21

2.5.1. Genomic DNA extraction ................................................................................................................ 21

2.5.2. Illumina PCR - Primary PCR .......................................................................................................... 22

2.5.3. Illumina PCR - Nested PCR .......................................................................................................... 23

2.5.4. Products clean-up and pooling ...................................................................................................... 24

2.5.5. Data processing and Bioinformatics ............................................................................................. 24

2.6. Microbial abundance ............................................................................................................................ 25

2.7. Extracellular Enzyme Activity ......................................................................................................... 26

2.8. Microbial community metabolic activity (Biolog EcoPlate assay) .................................................. 29

3. Results and discussion .......................................................................................................................... 32

3.1. Soil properties ..................................................................................................................................... 32
3.2. Gene analysis ........................................................................................................33
  3.2.1. DNA extraction and dual nested PCR ..............................................................33
  3.2.2. Diversity and composition of the microbial communities .........................34
  3.2.3. Microbial abundance ....................................................................................40
3.3. Extracellular β-Glucosidase activity ..................................................................44
3.4. Microbial metabolic activity of heterotrophic communities ..........................46
4. Conclusion and perspective .................................................................................50
5. References ............................................................................................................52
6. Supplementary materials .....................................................................................66
List of Figures

Figure 1: Schematic cross section of a palsa (Liebner et al. 2015). ........................................... 2

Figure 2: Schematic diagram of a permafrost environment depicting the different type of landscapes (glacier, tundra, coast, and sea) combined with cryogenic features differentiated by their thermal regime (Wagner 2008). ......................................................................................... 3

Figure 3: Distribution and classification of permafrost in the Northern hemisphere: including North America and Eurasia (Brown et al. 2002). ......................................................................................... 4

Figure 4: The new permafrost map for Norway Sweden and Finland classified in continuous (blue) discontinuous (purple) and sporadic (pink) permafrost zones. Areas with isolated patches of permafrost in mires are shown in brown (Gisnäs et al. 2017). ......................................................................................... 6

Figure 5: Predicted change in global mean annual air temperature: at 2081-2100 relative to 1986-2005, under Representative Concentration Pathways RCP2.6 and RCP8.5 (IPCC 2014). ......................................................................................... 7

Figure 6: CMIP5 modeled future permafrost area in the Northern Hemisphere. Black line indicates historical data, colored lines indicate predicted areas based on different Representative Concentration Pathways (RCPs); shaded regions show uncertainty (IPCC 2014). ......................................................................................... 8

Figure 7: Permafrost thaw impacts on soil properties and microbial metabolic potential differently at lowland and highland elevations (Mackelprang et al. 2016). ............................................................................. 9

Figure 8: Schematic representation of the key processes controlling the permafrost carbon feedback (Schuur et al. 2015). ......................................................................................... 10

Figure 9: Taxonomic distribution of Bacteria in different permafrost environments based on their 16S rRNA gene classifications. Pie charts represent relative abundances of different phyla in a sample set. (Jansson and Taş 2014). ......................................................................................... 13

Figure 10: Presentation of the study area in Finnmark, Northern Fenno-Scandinavia, Norway and the study site (Iskorás). ......................................................................................... 17

Figure 11: Picture of the palsa mire environment found on the study site. Degrading palsas can be observed with the formation of nearby thermokarst ponds. ............................................................................. 18

Figure 12: Core sampling alongside the structure of a palsa (modified from Polarpedia). .... 19

Figure 13: Diagram summarizing the laboratory workflow realized during this thesis. AL = Active layer, LOI = Loss on ignition, PCR = Polymerase chain reaction, qPCR = Quantitative PCR. ......................................................................................... 20

Figure 14: Diagram summary of the Illumina dual nested PCR amplification of the 16S rRNA gene (Stévenne 2018). ......................................................................................... 22

Figure 15: β-Glucosidase enzymatic reaction from Kumar et al. (2008). β-Glucosidase catalyzes the hydrolysis of cellobiose to release glucose. ......................................................................................... 27
Figure 16: Plate layout of the 96-well black microplates used during the assay of extracellular enzyme activity in our permafrost samples using the fluorometric MUB-linked substrate technique. The eight samples (IP5 to DP35) can be assessed vertically (column 1 to 8) on the same plate. 

Figure 17: Visualization of amplification products (16S rRNA variable V4 region) obtained from the nested PCR. bp = base pairs, + = positive control (from *E. coli* DNA) and - = negative control (reaction mix without DNA).

Figure 18: Alpha diversity values found in all samples (observed number of OTUs or estimated total number of OTUs using Chao1). Bars in Chao1 values represent the confidence interval of 95%.

Figure 19: Relative abundance of the 20 most abundant bacterial and archaeal phyla based on sequencing of 16S rRNA gene.

Figure 20: Constrained Correspondence Analysis (CCA) of microbial community composition computed with Bray-Curtis distances.

Figure 21: Bacterial and archaeal 16S rRNA gene copies quantification along depth profiles of (A) intact palsa, (B) degrading palsa (error bars represent the standard deviation).

Figure 22: (A) mcrA (methanogens) and (B) pmoA (methanotrophs) gene copies number in intact and degrading palsa (error bars represent the standard deviation).

Figure 23: β-Glucosidase potential extracellular enzyme activity (in nmoles of MUB-β-D-G / h. g dry soil) measured across depth and thaw stages after 18 hours of incubation at 5°C (ds=dry soil).

Figure 24: Evolution of Average well color development (AWCD) through time in (A) the intact palsa and in (B) the thawing palsa.

Figure 25: Heterotrophic bacterial functional activity represented by the substrate class averaged well color development (6 substrate classes where Amine=2 substrates, Amino acid=6, Carbohydrate=7, Carboxylic acid=10, Phosphorylated compound=2 and Polymeric substance=4) with thaw stage and depth.
List of Tables

Table 1: List of samples used in this thesis.................................................................19
Table 2: List of forward and reverse barcode sequence combinations used during the nested PCR for each permafrost sample .......................................................................................24
Table 3: Oligonucleotide primers used for quantitative PCR...........................................26
Table 4: List of the carbon substrates found in the EcoPlate assay grouped by substrate classes (Zak et al. 1994; Ernakovich and Wallenstein 2015). .................................................................30
Table 5: Moisture Content (MC), Organic Matter Content (SOM) and pH in soil samples used during this thesis ....................................................................................................................32
Table 6: Concentration (in ng/µl) of extracted genomic DNA from palsa’s active layer samples.........................................................................................................................33
Table 7: Bray-Curtis similarity indices matrix between samples (a score is equal to 0 when two communities are different, while a score of 100 indicates two identical communities) ........................................39
Table 8: Quantitative PCR bacterial and archaeal 16S rRNA, methanogenic mcrA and methanotrophic pmoA gene abundance data at the different sampling depth in the two successional stage of the palsa (IP5-IP35= intact palsa; DP5-DP35=degrading palsa). ds=dry soil.........................................................................................................................66
1. Introduction

Permafrost, defined as subsurface earth materials remaining below 0°C for more than two consecutive years (Schuur et al. 2015), is characteristic of high-latitude and high-altitude regions (Brown et al. 2002). Nearly 50% of the global soil organic carbon (C) is stored in Northern Hemisphere permafrost which covers nearly 16% of the global terrestrial area (Xue et al. 2016). It was estimated that terrestrial soils in the Northern Hemisphere comprise approximately 1.700 Pg (pentagram or billion tons) of organic C (Tarnocai et al. 2009; Schuur et al. 2013) which is nearly twice as much C contained in the atmosphere (Schädel et al. 2016). In these regions, low temperatures and hydrological conditions have prevented active decomposition of soil organic C and thus plants and animals' derived C has accumulated over extended periods (Lee et al. 2012; Xue et al. 2016) making permafrost regions global C sinks (Waldrop et al. 2010).

Due to global warming resulting from anthropogenic activities, temperatures in high-latitude regions have been subjected to a rapid increase of 0.6°C per decade over the last 30 years, faster than in any other region on Earth and two times faster than the global average (IPCC 2014). This temperature augmentation is causing the permafrost to thaw, increasing the depth of the active layer, which thaws in summer and refreezes in winter (Pengerud et al. 2017), thus exposing the previously stored C pool to microbial degradation (Commame et al. 2017).

The aerobic or anaerobic biotransformation, by microbes, of this organic C to greenhouse gases (GHG) such as carbon dioxide (CO$_2$) and methane (CH$_4$) and their transfer to the atmosphere could further increase the rate of future climate change through a positive feedback loop on global climate (Bardgett et al. 2008).

Although microbes are the main actors in this process, their activity and community dynamics in response to thaw have not been investigated until recently (Deng et al. 2015; Xue et al. 2016). A better understanding of permafrost’s microbial activity, diversity, abundance and metabolic capacity are necessary to advance our understanding on the processes involved in order to improve future predictions of permafrost carbon-climate feedback and thus better predict the impacts of organic C decomposition and GHG production on global climate (Mackelprang et al. 2016).

Permafrost areas represent a surface of 23,400 km$^2$ in Fenno-Scandinavia, 56% of which is found in Norway (Gisnås et al. 2017). Some of the most common landforms indicating sporadic permafrost in the northern region of Fenno-Scandinavia are palsas mires (Borge et al. 2017). Palsas are circular or oval mounts with a permafrost core covered by peat (Figure 1) (Fronzek et al. 2006).
Figure 1: Schematic cross section of a palsa (Liebner et al. 2015).

They typically occur in sub-arctic mires at the outer limit of the permafrost zone (Sollid and Sørbel 1998; Seppala 2006). They are vulnerable to climate change and have currently been retreating and experiencing a strong degradation (Farbrot et al. 2013; Olefeldt et al. 2016; Borge et al. 2017; Vorren 2017; Gisnås et al. 2017). They could serve as models to study climate change and permafrost degradation (Fronzek et al. 2006). Therefore, palsa ecosystems are ideal field model systems to study the response of permafrost thaw upon microbial communities (Liebner et al. 2015).

The goal of this thesis is to characterize the bacterial and archaeal diversity, microbial abundance and potential activity in soil samples from Northern Norway, alongside an active layer depth gradient and a permafrost degradation chronosequence.

1.1. Permafrost

1.1.1. Definition and formation

Permafrost is defined as ground (soil or rock including ice or organic material) that remains at or below 0°C for at least two consecutive years (van Everdingen 1998). Permafrost is characterized by a diverse array of environments such as boreal forests, arctic and sub-arctic tundra, Pleistocene ice complexes (yedoma) with depth up to several hundred meters, high altitude mountains and plateaus; and frozen peatlands (including palsas and peat plateaus). These landscapes differ widely in soil composition, soil organic matter (SOM) quality, thermal regimes and hydrology (Graham et al. 2012).

Permafrost is covered by an ‘active layer’ which is exposed to seasonal cycles and thus thaws in the summer and refreezes in the winter (Bonnaventure and Lamoureux 2013; Pengerud et al. 2017). This active layer’s thickness varies widely from a few centimeters to several meters (Jansson and Taş 2014). Its depth depends on air temperature, moisture content, vegetation and snow cover (Altshuler et al. 2017) and generally increases going from the High Arctic to the Subarctic (Blaud et al. 2015). In the last few decades, as a result of global warming, the depth of the active layer has been increasing, resulting in a subsequent decline in the areas underlying permafrost (Schuur et al. 2013; Jansson and Taş 2014).
Between the active layer and permafrost, a transition zone (the transient layer) acts as a thermal buffer that oscillates between being seasonally frozen and perennially frozen over decades (Shur et al. 2005). Permafrost environments are characterized by different landforms and geomorphological features (Figure 2) such as palsas, ice-wedges, taliks, cryopegs, massive ground ice, thermokarst lakes, organic matter accumulations, and broken soil horizons (Altshuler et al. 2017).

Figure 2: Schematic diagram of a permafrost environment depicting the different type of landscapes (glacier, tundra, coast, and sea) combined with cryogenic features differentiated by their thermal regime (Wagner 2008).

1.1.2. Global distribution

Permafrost is mostly found in the Northern Hemisphere (Figure 3) and extends throughout the high latitude circumpolar regions (Arctic, sub-Arctic and Antarctic) as well as the high altitude mountains and plateaus (Mongolian and Tibetan plateaus) (Brown et al. 2002).

Permafrost comprises 22% of exposed land surface in the Northern Hemisphere (excluding areas beneath ice sheets) (Tarnocai et al. 2009). Despite the fact that Antarctic permafrost is less studied than Arctic Permafrost, the Antarctic is estimated to contain a large proportion of Earth frozen soil (Altshuler et al. 2017). Permafrost is continuous on the exposed ground of the continent, which represents only 0.35% of the entire continent surface (Campbell and Claridge 2009) but also occurs beneath the ice sheets (IPA 2015).

Besides the poles, permafrost is also found in high altitudes environments such as mountains and plateaus. Considering the Tibetan plateau, a large portion of alpine permafrost is situated in China (Hu et al. 2015). Additionally, the distribution of permafrost in mid and low latitude alpine environments is linked to land surface’s attributes including slope gradient and orientation, vegetation patterns, and snow cover (IPA 2015).
The classification of permafrost regions is traditionally based on geographic continuity of the landscape (IPA 2015). Regions are classified according to the percentage of permafrost that is found in the ground of a specified area (when comparing to unfrozen ground). A region is of continuous permafrost when its ground is underlain by 90 to 100% of permafrost while a region of discontinuous permafrost has permafrost underlying 50 to 90% of the ground. Sporadic permafrost is found in areas where permafrost covers between 10 to 50% of the ground. Isolated permafrost is defined by regions containing remote and patchy occurrences of permafrost (up to 10% of the ground) where terrain without permafrost is most common (IPA 2015; Vonk et al. 2015; Pauly 2017).
Continuous permafrost located in Alaska, Canada and Siberia is found in regions where the mean annual air temperature (MAAT) is below -5°C (Shur and Jorgenson 2007; Tarnocai et al. 2009; Seppälä 2011; Mondav 2014) and has been sequestering C for over 10,000 years (Shur and Jorgenson 2007; Tarnocai et al. 2009; Mondav 2014). Most of the discontinuous, sporadic and isolated permafrost is located in zones with a MAAT ranging from -5 °C up to 2 °C in which insulating characteristics of peat allow the formation of permafrost even when facing positive mean temperatures (Shur and Jorgenson 2007; Sannel and Kuhry 2009; Seppälä 2011). In general, these “modern” frozen peatlands are younger than 8,000 years old. They initially developed as bogs and fens containing woody plants, mosses and sages (Altshuler et al. 2017) and are found in areas that were covered by ice sheets throughout the last glacial maximum (Sannel and Kuhry 2009; Mondav 2014), primarily in the southern Arctic and sub-Arctic regions (Altshuler et al. 2017).

Since they are already approaching the thermal limit for frozen water, some of these discontinuous and sporadic permafrost peatlands (including palsas mires) are especially vulnerable to climate warming in the Arctic and sub-Arctic regions (Seppälä 2011; Fronzek 2013; Mondav 2014; Liebner et al. 2015; Altshuler et al. 2017; Mondav et al. 2017).

1.1.3. Distribution in Fenno-Scandinavia and Norway

Gisnås et al. (2017) recently published a new map presenting the distribution of permafrost across Fenno-Scandinavia (Norway, Sweden and Finland) (Figure 4). This modeled map provides a better detailed and updated repartition of permafrost in these countries than previously available. According to this map, permafrost environments in Fenno-Scandinavia are found in an area of 23,400 km², the majority of which (56%) are found within Norway (Gisnås et al. 2017). Most of the permafrost in Norway is located in alpine landscapes (Gisnås et al. 2013). However, in northern Norway, in particular, permafrost is typically found in mires in which palsas and peat plateaus are located, suggesting a distribution of sporadic permafrost (Borge et al. 2017). In Fenno-Scandinavia, palsas are found prevalently north of the 68th N parallel and from sea level up to 1000 m in altitude (Borge 2015). Palsas are mainly distributed over the continental part of the northernmost County in Norway (Finnmark) with the highest concentration in the inner parts of Finnmarksvidda (Finnmark Plateau) close to the southern border to Finland (Borge et al. 2017).

Palsa distribution usually distinguishes the outer limit for permafrost in a certain region. In northern Fenno-Scandinavia, temperatures of palsas’ core (constituted of permafrost) are therefore relatively warm and close to 0°C (Christiansen et al. 2010). As a result, palsas are vulnerable to climate warming (Johansson et al. 2011). Degradation of sporadic permafrost in palsa mires has already been witnessed in northern Norway (Vorren 2017). Furthermore, in the northernmost county of Norway (Finnmark), Borge et al. (2017) observed a total decrease of 33 to 71 % in the area covered by palsas and peat plateaus during the last 60 years. Therefore, the study of palsas and peat mires in Fenno-Scandinavia can represent a window to the future evolution of larger permafrost areas in the Northern Hemisphere (Borge et al. 2017).
1.1.4. Permafrost carbon pool

Permafrost regions are known to hold large amounts of organic C (van Huissteden and Dolman 2012). While Tarnocai et al. (2009) estimated that the northern permafrost regions were to contain around 1672 Pg of organic C which is equivalent to 50% of the Earth’s soil C and twice as much C stored in the atmosphere; Schuur et al. (2015) reviewed that the known pool of terrestrial permafrost holds approximately between 1330 to 1580 Pg of organic C, 50% of which lie deeper than one meter. Nevertheless, these estimates remain associated with large uncertainties (Hugelius et al. 2014; Parmentier et al. 2017). Almost all of this frozen organic matter rich in carbon is made up of plant materials (leaves, roots and stems) and incompletely degraded plant organic matter (Schaefer et al. 2014). This organic C has accumulated over the years since dead vegetal and animal biomass is only partly decomposed due to low temperatures, short time frame for biological and microbial activity and acidic and/or anoxic conditions (Blaud et al. 2015). In soil, organic C builds up near the surface as plant materials accumulate. Additionally, cryoturbation comes into play (Kaiser et al. 2007). This mechanism allows the transfer of topsoil materials such as freshly buried plant matter to the bottom layer (vertical movements created by freeze-thaw action) and vice versa (Koven et al. 2011; van Huissteden and Dolman 2012). This accumulation of organic matter can last thousands of years leading to the long-term storage of C making permafrost regions global C sinks (Waldrop et al. 2010; Graham et al. 2012; Blaud et al. 2015).
1.1.5. Climate change in the Arctic – permafrost and climate

In the northern high-latitude regions, with a rise of 0.6°C per decade over the last 30 years, mean annual air temperatures (MAAT) have been rapidly increasing (IPCC 2013). The rate of climate warming is faster than in any other regions on Earth and twice faster than the global average (IPCC 2014). While the effects of global warming are already apparent in these regions (Parmentier et al. 2017), models are predicting MAAT to further increase by several °C over much of the Arctic by the end of the 21st according to IPCC’s scenarios (IPCC 2013) (Figure 5). This rapid rise in average temperatures will likely push northern latitude environments beyond their thermal threshold (Mondav 2014).

Figure 5: Predicted change in global mean annual air temperature: at 2081-2100 relative to 1986-2005, under Representative Concentration Pathways RCP2.6 and RCP8.5 (IPCC 2014).

The augmentation in MAAT will lead to alterations in ground thermal conditions and thus to a permafrost warming and thawing (Pengerud et al. 2017) with the deepening of the active layer (Schaefer et al. 2014). As the temperature rise, the active layer would become too deep to completely refreeze in winter (Schaefer et al. 2011). This would therefore render large quantities of previously stored C pool bioavailable to microbial degradation (Schädel et al. 2016; Grosse et al. 2016).

In addition to thaw induced by temperature warming, wildfires, vegetation disruption, local erosion, change in snow thickness and organic matter alteration may speed up permafrost degradation by warming the ground (van Huissteden and Dolman 2012; Parmentier et al. 2017). It was estimated that permafrost area may decrease by 37 to 81 % by the end of the century (IPCC 2013; Pauly 2017) (Figure 6).
Permafrost thaw can also lead to surface soil collapse into the space that was previously filled with ice, resulting in erosion and/or the formation of thermokarst lakes and ponds (Schädel et al. 2016; Olefeldt et al. 2016; Parmentier et al. 2017).

In areas with poor drainage, thaw causes liquid water to increase in permafrost layers and even the active layer can become saturated because of surface inundation (Figure 7). These processes cause faster oxygen depletion in these layers and provoke the development of anaerobic conditions. The rise in temperature and water availability promotes microbial activity and results in carbon organic matter degradation. The thaw of permafrost found at high elevations can generate an increased drainage of the soil moisture, C, and several other nutrients.

This drainage and the depletion of ice might increase soil porosity, thus allowing deep oxygen penetration into soils. Permafrost thaw suggests a strong microbial reaction as genes coding respiratory processes (aerobic and anaerobic) were detected in thawing permafrost soil (Yergeau et al. 2010; Hultman et al. 2015). In contrast, intact permafrost demonstrated reduced metabolic potential for processes such as aerobic and anaerobic respiration, nitrogen assimilation, methane production, and methane oxidation (Mackelprang et al. 2011). It was shown that these same processes were more highly expressed when permafrost is thawing (Hultman et al. 2015).
Therefore, microbial decomposition of soil organic matter can take place either under aerobic conditions in well drained and dry soils resulting in enhanced CO₂ emissions, or under anaerobic conditions in waterlogged soils where C is emitted in the form of CO₂ and CH₄ (methane) (Graham et al. 2012). Gaseous forms of carbon transmitted into to the atmosphere as CO₂ or CH₄ act as greenhouse gases (GHG) with CH₄ being particularly potent because of its greater global warming potential (GWP CH₄=25) and GWP CO₂=1) over a 100 years' time period (IPCC 2014).

Production and emission of these GHG resulting of microbial activity could lead to a positive feedback to global scale climate warming (Bardgett et al. 2008; Mackelprang et al. 2011; Graham et al. 2012; Blaud et al. 2015; Nikrad et al. 2016; Altshuler et al. 2017). The permafrost carbon feedback (PCF) is the amplification of anthropogenic warming due to carbon emissions from thawing permafrost (Figure 8) (Schaefer et al. 2014) increasing the rate of future climate change that would deeply impact all ecosystems as well as human society and economy (Schuur et al. 2013; Hope and Schaefer 2015; González-Eguino and Neumann 2016; Kessler 2017). The PFC could be partially compensated by an enhancement in plant growth due to warmer temperatures, longer growing seasons, higher CO₂ concentrations and increased nutrient released from the decomposing permafrost (Graham et al. 2012; Schuur et al. 2015; Wild et al. 2016; Li et al. 2017). Although plant carbon uptake will remove some CO₂ from the atmosphere, it would only partially compensate for a small part of the losses as permafrost thaw progresses over time (Schaefer et al. 2014; Schuur et al. 2015).
1.1.6. Carbon release rate and projecting change

In order to gain more precise prediction on the fate of permafrost carbon, it is of utmost importance to forecast and estimate the form and the rate of released carbon, which are, in part, controlled by organic carbon decomposability (Wang 2016). Several recent studies have shown, via conceptual model simulation and incubation under laboratory conditions, that a considerable portion of organic carbon is vulnerable to a rapid microbial degradation upon thaw (Mackelprang et al. 2011; Schädel et al. 2014; Xue et al. 2016; Bracho et al. 2016).

Although, initial C release rate is potentially important, it is projected to decrease over time as more labile carbon pools are exhausted (Knoblauch et al. 2013; Schuur et al. 2015). Elberling et al. (2013) ran a 12 years incubation experiment under laboratory conditions using permafrost soils from Greenland and observed a decrease of 50 to 75% of the original carbon pool as a result of microbial decomposition under aerobic conditions. The carbon to nitrogen ratio of the organic matter seems to be one of the controlling factors of carbon decomposability across various soils, where high values lead to more C release (Schädel et al. 2014). Additionally, hydrological conditions and oxygen availability are major factors affecting C release (Lee et al. 2012), especially in regard to the form of C emitted (CO$_2$ or CH$_4$). Lee et al. (2012) showed that, over 500 days of soil incubation, the carbon emitted
under aerobic conditions was four to ten times superior then under anaerobic conditions. After taking into account the greenhouse warming potential (GWP) of CO$_2$ and CH$_4$ emissions, the net warming effect of aerobic incubations was more important than the anaerobic incubations (Lee et al. 2012).

According to several recent studies, despite the greater GWP of CH$_4$ over CO$_2$, one standardized unit of thawed permafrost carbon will have a superior effect on climate over a century if it is thawed and degraded within a dry and aerobic soil in comparison to an equivalent amount of carbon found in a waterlogged anaerobic soil (Lee et al. 2012; Schädel et al. 2014, 2016; Schuur et al. 2015; Treat et al. 2015).

Various ecosystem and Earth system models simulations have been conducted to project the speed and severity that the PCF could represent on global climate change. Simulations under IPCC’s Representative Concentration Pathway 8.5 (RCP 8.5: business as usual and high emissions) scenario showed a potential carbon emission from permafrost region to range from 37 to 175 Pg C by the end of the century with an average over models of 92 ± 17 Pg C (mean ± standard deviation) (Schuur et al. 2015).

Furthermore, the driving force behind global warming would provoke release of GHG in the future that will impact Earth climate for centuries. Models estimated that 59% of total permafrost carbon release will happen after 2100 (Schuur et al. 2015). Even though recent models were improved with the inclusion of physical representation of soil thermodynamics and environmental control such as soil freeze/thaw state and organic carbon decomposability (Koven et al. 2013) they still show a wide range of biogeochemical uncertainties (Schaefer et al. 2014; Schuur et al. 2015). Therefore, further improvements are necessary to better implement physical and biological processes parameters that control permafrost degradation dynamics (Koven et al. 2013; Slater and Lawrence 2013; Schuur et al. 2015).

In that regard, It is necessary to further investigate and understand microbial community diversity and activity dynamics in a transforming environment by way of different molecular biology techniques; such as next generation sequencing, functional genes abundance quantification, enzymatic or metabolic activity assays, proteomics and genomics analysis (Waldrop et al. 2010; Graham et al. 2012; Jansson and Taş 2014; Hultman et al. 2015; Mackelprang et al. 2016; Morgalev et al. 2017). Since palsas found in Northern Norway are already thawing and retreating (Borge et al. 2017), they can be used as a useful model ecosystem for investigating permafrost thaw and subsequent local hydrological gradient’s implication on GHG emissions (Mondav et al. 2017).
1.2. Permafrost microbiology and its role in C flux

1.2.1. Permafrost as a challenging microbial habitat

Microorganisms present in permafrost regions have to cope with several stressful environment factors such as low temperature, osmotic balance and wide amplitude in ionizing radiations (Mondav 2014). It was suggested that a certain evolutionary advantage is given to Bacteria that are active even when facing low temperatures since such Bacteria are able to maintain DNA integrity and prevent amino acid denaturation (Jansson and Taş 2014). They are believed to face such harsh conditions with their combined aptitude to utilize slow metabolic processes, to display long reproduction cycles and to spend most of their energy sustaining molecular machinery (Tuorto et al. 2014). Indeed, microbial survival is hampered by cold temperatures: proteins and enzymes flexibility is decreased and thus they are subject to cold denaturation. Due to high viscosity and low thermal energy, the loss of cell membrane fluidity can disturb the transport of nutrients (Chattopadhyay 2006) and inhibits the replication, transcription and translation of nucleic acids (Nikrad et al. 2016). To face subzero temperatures, microorganisms have evolved to gain special adaptations such as the ability to enter a low metabolically active dormant state, to be able to regulate membrane fluidity or the aptitude to produce cold-adapted proteins, cold-shock proteins or antifreeze and ice-binding proteins (Jansson and Taş 2014).

These microbes have generally been classified as psychrophilic (or even cryophilic), meaning that they are able to grow and multiply in cold environments with temperatures ranging from -10°C to +10°C (Tuorto et al. 2014). In cold Arctic regions, Bacteria have been found to be metabolically active even when facing freezing temperatures in these rather nutrient (C, N) limited environments (Mondav 2014). Panikov et al. (2006) even recorded bacterial activity in permafrost soil with temperatures as low as -17°C. It is suggested that temperature and water availability are most important in determining biomass levels, but also microbial diversity and community structure (Blaud et al. 2015). For example, cultivation of permafrost soil isolates have demonstrated that these microorganisms have ranges for minimum, maximum and optimum temperatures that are specific but overlapping. These temperatures ranges correspond to those used by microbes to survive (mesophilic), cope (psychrotolerant), and thrive (psychrophilic) with progressively colder temperatures (Mondav et al. 2014a). Therefore, these communities are pictured as extremely structured with comprising overlapping niches or partitions regarding temperature specificity (Tuorto et al. 2014).

1.2.2. Microbial diversity and community composition

Until recently, microbial diversity in polar and high altitude regions was believed to be small (Heal 1999) based on the observation that plants and animals diversity decreases with increasing latitude and altitude (Blaud et al. 2015). Although, considering how inhospitable the environmental conditions are, permafrost soils host important microbial community diversity (Figure 9). Microbial diversity found in these regions is almost comparable to what is observed in other environments such as temperate and tropical forests, deserts, prairies and
grasslands (Chu et al. 2010). However, the main challenge to investigate microbes in permafrost soils while using culture-independent techniques is the low microbial biomass and the elevated ice content (Jansson and Taş 2014).

In microbial ecology, the analysis of the 16S Ribosomal ribonucleic acid (rRNA) gene is widely used as a molecular marker due to its ubiquity and evolutionary properties (Case et al. 2007). The sequencing and study of this gene allows the detection and comparison of microbial communities, including a large panel of Bacteria and Archaea, from environmental samples (Gevers et al. 2005).

![Figure 9](image_url)

Figure 9: Taxonomic distribution of Bacteria in different permafrost environments based on their 16S rRNA gene classifications. Pie charts represent relative abundances of different phyla in a sample set. (Jansson and Taş 2014)

While microbial diversity is generally assessed using 16S rRNA gene sequencing; microbial abundance of these ecosystems is regularly measured by microscope cell counts, counting of cells with cultivation on agar plates or quantitative polymerase chain reaction (qPCR) to find the number of rRNA gene copies in a given sample. It was shown via these methods, that the community of microbes living in active layer soils was surprisingly abundant and that microbial abundance tended to decrease with a decline in organic matter (Banerjee et al. 2011) and with depth from surface (Yergeau et al. 2010). The decrease in species richness with depth is also observed in several Arctic soils (Mondav et al. 2017; Müller et al. 2018). The decrease in species with increasing permafrost depth might be linked to an
augmentation in the proportion of psychrotrophic and psychrophilic microbes as suggested by Větrovský and Baldrian (2013).

Using 16s rRNA gene taxonomic classification, the most abundant phyla usually observed in permafrost soils and their active layers are bacterial Acidobacteria, Actinobacteria, Bacteroidetes, Proteobacteria (including Alpha- Gamma- and Beta- Proteobacteria), Verrucomicrobia, Gemmatimonadetes, Planctomycetes, Chloroflexi, Firmicutes (Figure 9); and archaeal Euryarchaeota and Crenarchaeota (Hultman et al. 2015; Blaud et al. 2015; Altshuler et al. 2017).

The relative dominance of members of the phyla Actinobacteria and Proteobacteria might be boosted by the presence of multiple copies of the 16S rRNA operons in their genomes and it might suggest that phyla with fewer copy numbers such as Acidobacteria and Crenarchaeota are more numerous than what is observed using 16S rRNA amplification and sequencing methods (Větrovský and Baldrian 2013). It is also of importance to note that cold temperatures are ideal conditions to preserve extracellular DNA and thus, not all detected sequences necessarily represent active or viable cells (Carini et al. 2016).

1.2.3. Microbial metabolic functions

Some of the most common microbial metabolic functions found in permafrost environments are aerobic and anaerobic heterotrophy, methanogenesis, methane oxidation, nitrification and nitrogen fixation, sulfate and iron reduction and acetogenesis. These are found to be distributed along the active layer and permafrost profile and are mostly controlled by the electron receptors availability (typically oxygen) (Mondav 2014). For example, the metabolic potential for degradation of complex forms of organic C was found to be regularly coded in sequences linked to Bacteroidetes, Verrucomicrobia and Actinobacteria genomes ;but also irregularly in Gammaproteobacteria, Alphaproteobacteria, and Deltaproteobacteria community (Tveit et al. 2013).

Methanogens are frequently found in permafrost and especially in active layer soils (McCalley et al. 2014) in which their abundance is highest close or beneath the water table since they need to enzymatically reduce various C compounds such as CO₂, methylated substrate, formate and acetate under temporary anoxic conditions. In addition, methane oxidation by methanotrophs is critical in mitigating the release of CH₄ from permafrost systems (Mondav et al. 2014b).

1.2.3.1. Microbial degradation of Permafrost carbon

Metagenomics analysis of permafrost soil showed that active layer is rich in genes regulating methanogenesis pathway and methane oxidation, as well as genes controlling the degradation of complex carbohydrate polymers but also genes involved in nitrogen cycling such as nitrite and nitric-oxide reductase (Yergeau et al. 2010; Frank-Fahle et al. 2014). In addition, Yergeau et al. (2010) found the presence of genes coding for several stress responses such as oxidative/cold/sporulation stress, as well as expressed genes involved in polysaccharide and sugar metabolisation. Waldrop et al. (2010) and Taş et al. (2014) also
detected genes coding for C metabolic pathways like cellulose, cellobiose, beta-glucosidase, beta-galactosidase, chitin or lignin degradation.

Incubation induced thaw of permafrost samples showed that thaw caused a decrease in mesophilic and psychrotrophic microorganisms within the community with increasing depth (Yergeau et al. 2010; Mackelprang et al. 2011). This suggests that microbial communities found in permafrost have developed the ability to degrade carbon substrate even when facing cold temperatures.

The microbial degradation of peat organic matter, such as peat found in a palsa, starts with the most bioavailable (labile) C substrate such as plant exudates followed by more complex C source like hemicellulose, cellulose, xylose or starch that can be metabolized by soil microbes including bacterial members of Actinobacteria or Acidobacteria but also by other microorganisms such as Fungi (Pankratov et al. 2011; Mondav 2014). The anaerobic degradation of least bioavailable C (lignin and phenolic compounds) can be carried out by members of the phyla Proteobacteria, Firmicutes, Acidobacteria and Actinobacteria (Mondav et al. 2014a).

In the next step of organic matter degradation, partially oxidized pyruvate can be anaerobically fermented to produce CO$_2$ and other organic compounds byproducts. Fermentative Bacteria are involved in the last stage of reduction if sulphate, nitrate or ferric iron is available (Godin et al. 2012). In an anoxic environment, methanogens can utilize several byproducts like methanol, acetate or CO$_2$ as substrates for energy production through methanogenesis pathways (Wagner and Liebner 2010).

**1.2.3.2. Methanogenesis and CH$_4$ oxidation**

Biogenic methanogenesis is exclusively carried out by obligate anaerobic Archaea belonging to the phylum Euryarchaeota. They are classed according to their metabolic pathway based on utilized substrate (methylotrophic, acetotrophic and hydrogenotrophic) (Blaud et al. 2015). While the most frequent and energetically favorable pathway is hydrogenotrophic, acetotrophic methanogens might be dominant in permafrost environments (Mondav 2014).

Diversity and abundance of methane producing Archaea are generally investigated using the mcrA gene coding for the alpha-subunit of the methyl coenzymes-M reductase which catalyses the terminal step of reduction of methyl group bound coenzyme-M (Wagner and Liebner 2010). This gene is ubiquitously represented in all known methanogens (Barbier et al. 2012).

On the other hand, methanotrophs use CH$_4$ as a source of energy using the methane monoxygenase enzyme by combining oxygen and methane to form formaldehyde. They are distributed in the phyla Proteobacteria (Alpha and Gammaproteobacteria) and Verrucomicrobia. Diversity and abundance of these methane oxidizers is investigated with the pmoA gene coding for the alpha-subunit of the particulate (membrane-bound) methane monoxygenase present in almost all methanotrophs (Blaud et al. 2015).
1.3. Objectives

The main objective of this thesis is to study the microbial diversity and activity along a depth gradient from two soil cores representing two stages of permafrost climate-induced thaw (intact and degrading palsa) in a palsa mire located in Finnmark, Northern Norway in order to gain a better insight into the physiological diversity and activity dynamics of the soil microbial community induced by the current environmental changes found in permafrost-affected palsa mire environments.

More specifically, this thesis aims to:

- Investigate changes in total microbial (archaeal and bacterial) richness, community structure and dynamics with depth and permafrost thaw by using Illumina high-throughput DNA sequencing and quantitative PCR targeting the 16S rRNA genes
- Study the abundance of methane associated functional genes using quantitative PCR in order to evaluate methanogens (mcrA) and methanotrophs (pmoA) populations in the thaw stages soil profiles
- Determine the potential extracellular enzymatic activity of the microbe-produced β-glucosidase involved in the breakdown of plant organic matter
- Assess the microbial metabolic activity and functional potential of the heterotrophic microbial communities using Biolog Ecoplates (containing 31 different carbon sources)
2. Material and methods

2.1. Site description

Figure 10: Presentation of the study area in Finnmark, Northern Fennoscandia, Norway and the study site (Iskorás)

Finnmark is the largest and northernmost County in Norway. It is located approximately between 68° and 71° N and comprises a land surface area of 48,618 km² (Figure 10). Finnmark County is situated within the Arctic Circle and borders to Troms County in the west, Russia in the North-Easternmost part and Finland in the Southwest (Borge 2015). Finnmark’s geomorphology is mainly characterized by alpine mountains in its northwest part, and by the Finnmarksvidda, an undulating peneplain and plateau-like environment with an elevation of around 300-500 m to sea level that is situated in the interior and southern part of the County (Solheim 2016). With an area of more than 22,000 km², the Finnmarksvidda covers almost 50% of the entire Finnmark and is the largest plateau in Norway (Borge 2015). The plateau presents a landscape of mires featuring both peat plateaus and dome palsas. The mires are surrounded by a thick cover of moraine while the bedrock geology consists of mostly old granites and gneisses from the Precambrium era (4.6 billion years to 541 million years ago) (Borge 2015; Solheim 2016). The vegetation on the plateau is sparse, with scrubland, low birch trees or bare mountains. Thousands of wet mires have filled the concavities in between the moraines and ridges (Solheim 2016).

The climate in Finnmark County is affected by the North Atlantic Current. It features a wet and warm maritime climate on the coast and a dry and cold environment on the Finnmarksvidda Plateau. In Norway, the Finnmarksvidda exhibits the lowest mean annual air
temperature in the country (MAAT) with a MAAT comprised between -2.5 to -4 °C. In addition, mean summer temperature ranges from 8 to 10°C and mean winter temperature from -15 to -20 °C (Farbrot et al. 2013). On the plateau, the mean annual precipitations (MAP) are close to 400mm while the mean maximum snow depth in winter is 25–75 cm. In addition, the mean annual number of days with dry snow (MADDS) is between 150 and 200 (Borge et al. 2017). The soil samples used in this study were collected from a site located on the Plateau of Finnmarksvidda. The studied palsa mire is situated 2 km northwest from the Iskoras Mountain ridge (69°20'26.0"N ; 25°17'41.2"E), and about 10 km south of the city of Karasjok, in the easternmost part of Finnmarksvidda (Figure 10). The site has an altitude of 360 m above sea level. Aiming to inspect the microbial diversity and activity changes associated with permafrost thaw, a natural in situ thaw gradient found in sporadic permafrost areas was examined. The mire is in a partially degraded state presenting elevated, drained mounts (palsas) comprised between wet depressions (thermokarst ponds, bogs and fens) representing diverse stages of thaw and dominated by distinguished vegetation (Figure 11).

The permafrost palsas found in at Iskoras site are generally ombrotrophic (rain-fed), aerobic and not in contact with groundwater. The peat carbon found in palsa’s permafrost might be hard for microbes to degrade because of low nitrogen or other limiting nutrients presence, but also because of low temperatures (Davidson and Janssens 2006). Palsas experiencing thaw in Iskoras mire seems to be dominated by bryophytes and lichen. The presence of such vegetation in these palsa might result in less recalcitrant C compound (i.e. lignin). However, carbon-rich litter degradation might still be slow due to higher phenolic content (Freeman et al. 2004). On the other hand, on the edge of the palsa, a minerotrophic (ground-water fed) vegetation settles where warming tend to thaw permafrost provoking subsidence and inundation. This type of vegetation seems to be dominated by graminoids with ensuing change in plant litter. This type of litter is said to have reduced lignin in comparison to palsa peat (Osono et al. 2006).

Figure 11 : Picture of the palsa mire environment found on the study site. Degrading palsas can be observed with the formation of nearby thermokarst ponds
2.2. Field sampling

The sampling was realized on a unique palsa mount. Two cores were probed from the same palsa's active layer (AL). The first core was collected from the top point of the intact palsa while the second one was located on the edge of the palsa, between the top of the palsa and a thermokarst pond (Figure 12). Therefore, the two cores are found alongside a permafrost thaw chronosequence where the first core is intact permafrost/palsa (IP) while the second is from degrading permafrost/palsa (DP). Each core is characterized by a specific hydrological stage, with the first one being dry intact palsa while the second is wetter. Each core was then subsampled into four samples alongside a depth gradient (Table 1) until reaching the permafrost/active layer boundary. Soil samples used in this thesis were collected on the 30th of March 2017 (Table 1). The sampling was carried out using a sterilized soil sampling probe. Samples were transferred into sealable zipper storage plastic bag and stored at -20°C for further analysis. A total of eight frozen soil samples were collected, with four from the first core and four from the second core.

![Figure 12 : Core sampling alongside the structure of a palsa (modified from Polarpedia).](image)

<table>
<thead>
<tr>
<th>Table 1: List of samples used in this thesis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Core N°</strong></td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
2.3. Laboratory work flow overview

During this thesis, several abiotic parameters such as moisture content (MC), soil organic matter content (SOM) and pH were analyzed. On the gene analysis part, extracted DNA from soil samples was processed to investigate the microbial (bacteria and archaea) diversity considering the 16S rRNA gene but also the abundance in copies number of several genes (bacterial and archaeal 16S rRNA, mcrA for methanogens and pmoA for methanotrophs). In addition, studying the potential extracellular enzymatic activity of β-glucosidase and the functional metabolic activity using Biolog Ecoplates allowed us to assess microbial diversity.

Figure 13: Diagram summarizing the laboratory workflow realized during this thesis. AL = Active layer, LOI = Loss on ignition, PCR = Polymerase chain reaction, qPCR = Quantitative PCR

2.4. Soil properties

2.4.1. Soil organic matter content (Loss On Ignition method) and Soil moisture

In order to estimate the soil organic matter content (SOM) in the soil samples, the Loss On Ignition (LOI) method was used, which is a simple method easily implemented in laboratory installation with few required equipment, making it widely used (Santisteban et al. 2004). This method is based on the linear relation between LOI values and organic carbon content (Hoogsteen et al. 2015). LOI rests on a differential thermal analysis: organic matter starts to oxidize at around 200°C and is completely degraded at around 550°C, transforming into CO₂ and ashes (Heiri et al. 2001). In short, moist soil samples were transferred, in triplicates, to previously weighted beakers. Beakers were dried at 105°C and put in a dessicator for 20-30 min before use. Porcelain crucibles containing the soil samples were weighted using an analytical laboratory balance. Samples were dried at 105°C for one hour in an oven, and then put in a dessicator for 20-30 min before being weighted to assess the moisture content.
(MC). Afterward, samples were burned in a muffle oven at 550°C for six hours. Subsequently, crucibles were transferred into a dessicator and weighted to assess the organic matter content. The LOI$_{550}$ is calculated using the following equation:

$$LOI_{550} = \left( \frac{DW_{105} - DW_{550}}{DW_{105}} \right) \times 100$$

- Where LOI$_{550}$ is the Loss on Ignition at 550°C (in %)
- DW$_{105}$ represents the dry weight of the soil sample before ignition at 550°C (in g)
- DW$_{550}$ represents the dry weight of the soil sample after ignition at 550°C (in g)

### 2.4.2. Soil pH

The pH of our permafrost active layer soil samples was measured using a one to five soil water suspension (2 g of soil in 8 ml of distilled water) using a glass electrode connected to a MP220 pH-meter (Mettler Toledo, USA). The suspensions were mixed for 30 min and left to settle for 30 min before measurement.

### 2.5. Gene analysis

In order to assess the diversity of the microbial communities found in our permafrost palsa’s active layer samples, genomic DNA (gDNA) was extracted from the frozen samples, amplified via Polymerase Chain Reaction (PCR) and sequenced using Illumina MiSeq System.

#### 2.5.1. Genomic DNA extraction

Genomic DNA (gDNA) extractions were achieved using the PowerSoil® DNA Isolation Kit (MoBio Laboratories). This commercial kit is widely used to extract environmental gDNA from soil samples rich in humic acids and also from permafrost soil samples (Waldrop et al. 2010; Frank-Fahle et al. 2014; Deng et al. 2015; Wagner et al. 2017). The kit is based on the patterned technology “Inhibitor Removal Technology” (IRT) that allows the isolation of gDNA while efficiently removing inhibitors of the polymerase chain reaction. In short, under a laminar flow cabinet, approximately 0.50 g of still frozen soil was transferred aseptically into PowerBead tubes using ethanol sterilized knives. Permafrost samples were to be manipulated and put back in the freezer (-20°C) as quickly as possible to prevent them from thawing. Sixty µl of a lysis solution containing sodium dodecyl sulfate (SDS) and other cell membrane disruption agents were added to the tubes. The Lysis solution needed to be warmed at 65°C for 10 min in order to dissolve any potentially precipitated SDS. Tubes were then placed horizontally on a MoBio Vortex Genie 2 and shaken for 10 minutes at maximum speed. Cell lysis is attained via the combined action of the mechanical action of the micro beads and the chemical action of the SDS. Afterwards, PCR inhibitors and non-DNA organic and inorganic contaminants such as proteins, cell debris and humic acids were removed by using the two IRT solutions washing steps. The addition of a high concentration salt solution allowed the specific adsorption of gDNA onto a silica filter. Salt solution and remaining contaminants were removed using several filter washing steps with an ethanol-based solution. Lastly, 100 µl of sterile elution buffer was added to the cleaned silica filter, allowing
selective desorption and elution of gDNA. Obtained eluted DNA was stored at -20°C before further analysis.

The concentration of the extracted DNA was assessed using Qubit™ dsDNA High Sensitivity Assay Kit (ThermoFisher Scientific) in conjuncture with the Qubit Fluorometer 2.0 (ThermoFisher Scientific). The kits include concentrated assay reagents, dilution buffer, and prediluted DNA standards. The UV-absorbance method uses a spectrophotometer to measure the natural DNA absorbance of light at 260 nm. The assay is highly selective for double-stranded DNA (dsDNA) and allows accurate DNA concentration measurement from 10 pg/µl to 100 ng/µl.

2.5.2. Illumina PCR - Primary PCR

In this study, the 16S rRNA variable V4 region was targeted. A dual PCR step approach (Nested PCR) was used to first amplify the 16S rRNA v4 region, leaving an overhang that includes an Illumina adapter and five Ns bases (Caporaso et al. 2011; Mateos-Rivera et al. 2016). Two PCR steps were performed to reduce the variability in the samples and increase its reproducibility (Mateos-Rivera et al. 2016). A diagram summarizing this two-step process is presented in Figure 14.

![Diagram of Illumina dual nested PCR amplification of the 16S rRNA gene](image)

Figure 14 : Diagram summary of the Illumina dual nested PCR amplification of the 16S rRNA gene (Stévenne 2018)

During this first PCR, the forward primer, FAdapter-N5-519F (5’-CTACACTCTTTCCCT ACACGACGCTCTTCCGATCT-NNNNN-CAGCMGCGCGGTAA-3’) and reverse primer, RAdapter-806R (5’-GTGACTGGAGTTCAGACGTGTGCTCTTCGGATCT-GGACTACHVGGGTWTCTAAAT-3’) were used (where W=A/T, V=A/C/G, H=A/C/G and M=A/C). Each sample was amplified in triplicates in order to minimize PCR drift. Per reaction, the PCR mix was composed of 10 µl of HotStart Master Mix (Qiagen), 0.5 µl of 100 % Bovin Serum Albumin (BSA) (ThermoScientific), 1 µl of 10 µM aliquot forward primer FAdapter-N5-
519F, 1 µl of 10 µM aliquot reverse primer RAdapter 806R and 6.5 µl of DNase free water (Qiagen). 1 µl of extracted DNA solution was added to reach a final mix volume per reaction of 20 µl. For positive control, 1 µl of a 10 ng/µl *E. coli* DNA solution was used. The Thermal conditions were as follow: initial denaturation step (95°C for 15 min); followed by 25 cycles of denaturation (95°C for 20 sec), primers annealing (55°C for 30 sec) and extension (72°C for 30 sec), and a final extension of 7 min at 72°C (Müller et al. 2018). All PCR were run on a Veriti 96 wells Thermal Cycler (Applied Biosystems). After PCR amplification, PCR products’ quality was assessed by agarose 1.5 % gel electrophoresis (Seakem LE agarose, LONZA) with the addition of 3 µl of GelRed nucleic acid gel stain 10.000X (Biotium). Samples migrated for 20 minutes at 200 V in a TAE 1X solution. The gel electrophoresis was run on a LKB EPS 500/400 (Pharmacia) with a HE 33 mini Horizontal Submarine Unit (Amersham biosciences). The DNA Loading dye used was the 6X Mass Ruler DNA loading dye (Thermo Scientific) while the DNA ruler was the MassRuler DNA Ladder mix (103 ng/µl) ranging from 80 to 10.000 base pairs (bp) (Thermo Scientific). Final results are visualized using the Molecular Imager ChemiDoc™ XRS (BioRad) with Image Lab ™ Software (BioRad). PCR products were then pooled and purified using the DNA Clean and Concentrator 5 (Zymo research) according to manufacturer’s instructions. After purification, DNA concentration was measured with the Qubit™ dsDNA High Sensitivity Assay Kit (ThermoFisher Scientific) and Qubit Fluorometer 2.0 (ThermoFisher Scientific).

**2.5.3. Illumina PCR - Nested PCR**

The second step consists of a nested PCR realized on the pooled products from the first PCR. During this second amplification, a unique primer combination targets the overhanging Illumina adaptors. Unique sample barcodes and Illumina flow cell P5/P7 adaptors were added to the edges of the amplified sequence of interest. Since there are 8 versions of the forward and 12 of the reverse primer, this second amplification step allows for the tagging and the sequencing of 96 different samples in parallel using the MiSeq v2 chemistry. Thus, each template is distinctively recognizable by its specific combination of its two barcodes. Primers used here are the Fadapter-barcode-FLinker (forward, 5'-AATGATACGGGACACGGAGATCTACAC-XXXXXXXX-ACACTCTTTCCCTACACG) and the RAdapter-barcode-RLinker (reverse, 5'-CAAGCAGAAGACGGCATACGAGATCGGACTTGTGACCTCA-3') where the Xs are replaced by a unique barcode sequence for each sample. The unique barcode sequence combinations used in this thesis are displayed in Table 2. For a 50 µl reaction, the PCR mix was composed of 25 µl of HotStart Master Mix (Qiagen), 15.5 µl of DNase free water (Qiagen), a distinctive combination of 1 µl forward primer (10 M) and 1 µl of reverse primer (10 µM) for each sample. The mix is completed with the addition of 7.5 µl of previously purified PCR products from the first PCR. For positive control, 1 µl of a 10 ng/µl *E. coli* DNA solution was used. The Thermal conditions were as follow: initial denaturation step (95°C for 15 min); followed by 15 cycles of denaturation (95°C for 20 sec), primers annealing (62°C for 30 sec) and extension (72°C for 30 sec), and a final extension of 7 min at 72°C (Müller et al. 2018). PCR products quality was assessed by agarose 1.5 % gel electrophoresis as previously described above.
Table 2: List of forward and reverse barcode sequence combinations used during the nested PCR for each permafrost sample

<table>
<thead>
<tr>
<th>Amplified sample</th>
<th>Unique barcode combination</th>
<th>Barcode sequence combination (5’ – 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IP5</td>
<td>F4/R11</td>
<td>AGAGTAGA/TGCCTCTT</td>
</tr>
<tr>
<td>IP10</td>
<td>F4/R12</td>
<td>AGAGTAGA/TCCCTCTAC</td>
</tr>
<tr>
<td>IP20</td>
<td>F5/R1</td>
<td>GTAAGGAG/TGCCTTTA</td>
</tr>
<tr>
<td>IP35</td>
<td>F5/R2</td>
<td>GTAAGGAG/CTAGTGAC</td>
</tr>
<tr>
<td>DP5</td>
<td>F5/R3</td>
<td>GTAAGGAG/TCTGCGCT</td>
</tr>
<tr>
<td>DP10</td>
<td>F5/R4</td>
<td>GTAAGGAG/GCTCAGGA</td>
</tr>
<tr>
<td>DP20</td>
<td>F5/R5</td>
<td>GTAAGGAG/AGGAGTCC</td>
</tr>
<tr>
<td>DP35</td>
<td>F5/R6</td>
<td>GTAAGGAG/CATGCGCTA</td>
</tr>
</tbody>
</table>

2.5.4. Products clean-up and pooling

The final PCR products were cleaned and purified using AMPure XP Beads (Beckman Coulter). The cleaning process is based on Solid-phase Reversible Immobilization (SPRI) upon paramagnetic beads. The AMPure solution is composed of a buffer containing microscopic magnetic beads to which DNA fragments of 100 bp and larger can bind. A magnet is placed next to the tubes containing the magnetic beads on which the DNA in bound, allowing the aggregation of the beads on the side of the tube. Any undesired organic and non-organic contaminants such as primers, salts, enzymes and nucleotides are discarded during two washes with newly prepared 70% ethanol solution. The remaining ethanol is removed and the DNA is eluted from the beads in 25 μl of Tris buffer 10 mM. The DNA concentration of each sample was measured with the Qubit™ dsDNA High Sensitivity Assay Kit and the Qubit Fluorometer 2.0 (ThermoFisher Scientific). DNA quality was assessed using a Nanodrop One (ThermoFisher Scientific).

All samples were pooled in equimolar concentration (75 ng of DNA per sample) to create the clone Library and sent for sequencing analyses using the Illumina ‘MiSeq sequencing system’ at the Norwegian High-Throughput Sequencing Centre (Oslo, Norway).

2.5.5. Data processing and Bioinformatics

The 16S rRNA gene sequences were processed using the QIIME processing platform (Quantitative Insights into Microbial Ecology) using version 1.9.1. (Caporaso et al. 2010). The sequence similarity threshold of 97% was selected to determine prokaryotic OTUs (Operational Taxonomic Unit) using a de novo unclust OTU clustering method and taxonomy assigned using the Greengenes reference database (DeSantis et al. 2006). Taxonomically identified OTUs (66022 OTUs) were assembled into an OTU table presenting abundances for each sample while excluding singletons and unassigned OTUs.

Microbial species richness, Alpha diversity (Chao1 estimated richness with confidence intervals of 0.95) and Constrained Correspondence Analysis with Bray-Curtis distances were calculated using the package Phyloseq (version 1.22.3) (McMurdie and Holmes 2013) on R and Rstudio software (v. 3.5.1 and 1.0.153 respectively).
Analysis of Beta diversity (microbial community dissimilarity between samples) was completed using the Bray-Curtis dissimilarity index with the software PRIMER6 v6.1.16 (Plymouth, UK). A one-way Analysis of Similarities (ANOSIM) test was carried out on Bray-Curtis similarity matrix to test for significant difference in community structure between the intact and degrading palsa.

2.6. Microbial abundance

Quantitative PCR (qPCR) was used to quantify the abundance of bacterial and archaeal 16S rRNA genes, methanogens (mcrA) and methanotrophs (pmoA) functional gene copies in the DNA extracted from our permafrost soil samples. The abundance of specific gene copies for certain microbial functional groups can be used as an indicator of changes in the microbial biomass of the microbial communities (Zak et al. 2006). All qPCR were performed using a CFX96 Real-Time System C1000 thermal cycler (BioRad) using white Multiplate 96-Wells Unskirted PCR plates with Thin-Wall Polypropylene (BioRad). Resulting data were analyzed using the software CFX Manager 3.1. (BioRad). All primer pairs used for the qPCR quantification are displayed in Table 3.

To quantify the bacterial 16S rRNA gene copies number, 1 µl of each primer of the 10 µM primer pair Bac338F/Eub518R (final primer concentration=0.5 µM) (Fierer and Jackson 2005) were added to 10 µl of the SsoFast EvaGreen Supermix (BioRad), 7 µl of DNAse free water (Qiagen) and 1 µl of 1 ng/µl template DNA to reach a final reaction volume of 20 µl. The qPCR thermocycling conditions were as follows: initial activation for 2 min at 98°C, followed by 40 cycles of amplification (5 s denaturation at 98°C, primers annealing at 56°C for 10 s) (Swan et al. 2010; Mateos-Rivera et al. 2016).

The archaeal 16S rRNA gene copies number was quantified using 1 µl of each primer of the 10 µM primer pair Arch349F/Arch806R (final primer concentration=0.5 µM) (Takai and Horikoshi 2000). Reagents mix and qPCR thermocycling conditions were identical as the one used for the bacterial 16S rRNA gene quantification but with primer annealing temperature set to 51°C.

The abundance of methanogens was estimated by amplifying the mcrA gene using the primer pair mcrAfornew/mcrArevnew (Gagnon et al. 2011). One µl of each primer (10 µM aliquot, final primer concentration=0.5 µM) was added to 10 µl of SsoFast EvaGreen Supermix (BioRad), 6 µl of DNAse free water (Qiagen), 0.5 µl of 0.2% BSA (ThermoScientific) and 2 µl of 1 ng/µl DNA template. . The qPCR thermocycling conditions were as follows: initial activation for 2 min at 98°C, followed by 40 cycles of amplification (30 s denaturation at 98°C, primers annealing at 55°C for 30 s, extension at 72°C for 30 s).

The abundance of methanotrophs was assessed by amplifying the pmoA gene with the primer pair A189F/A682R (Tuomivirta et al. 2009). Reagents mix and qPCR thermocycling conditions were identical as the one used for the methanogen mcrA gene quantification but with only the primer annealing temperature changed to 60°C. For the methanogen and methanotroph assays, at the end of each cycle, the fluorescence was measured at 83°C in order to avoid taking into account primer dimers fluorescence (Steinberg and Regan 2009).
For all qPCR gene quantification assays, fluorescence was assessed after the end of each cycle and a melting curve was obtained from 65°C to 95°C with increments of 0.5°C in order to check for primer dimers and PCR specificity (Barret et al. 2015).

All qPCR standards were created specifically for each gene copies abundance quantification assay through the PCR amplification of the gene fragment of interest (Waldrop et al. 2010; Dhanasekaran et al. 2010; Shahsavari et al. 2016). Specific PCR products were purified using the DNA Clean and Concentrator 5 kit (Zymo research). The concentration of each purified PCR product was measured using Qubit™ dsDNA High Sensitivity Assay Kit and the Qubit Fluorometer 2.0 (ThermoFisher Scientific). Each standard was then diluted to concentrations within the expected range of the samples. For each assay, all standards and samples were run in triplicate.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Targeted gene</th>
<th>Sequence (5’ – 3’ )</th>
<th>Annealing t◦ (◦C)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bac338F</td>
<td>Bacterial 16S rRNA gene</td>
<td>ACTCTACGGAGGCAGCAG</td>
<td>56</td>
<td>(Fierer and Jackson 2005)</td>
</tr>
<tr>
<td>Eub518R</td>
<td>Bacterial 16S rRNA gene</td>
<td>ATTACCAGGCGGTGCTGG</td>
<td>56</td>
<td>(Fierer and Jackson 2005)</td>
</tr>
<tr>
<td>Arch349F</td>
<td>Archaeal 16S rRNA gene</td>
<td>GYGCASCAGKCGMGAAW</td>
<td>51</td>
<td>(Takai and Horikoshi 2000)</td>
</tr>
<tr>
<td>Arch806R</td>
<td>Archaeal 16S rRNA gene</td>
<td>GGACTACVSGGGTATCTAAT</td>
<td>51</td>
<td>(Takai and Horikoshi 2000)</td>
</tr>
<tr>
<td>mcrAfornew</td>
<td>mcrA</td>
<td>GGTGTMGGTTTACCHCARTAYGC</td>
<td>55</td>
<td>(Gagnon et al. 2011)</td>
</tr>
<tr>
<td>mcrArevnew</td>
<td>mcrA</td>
<td>TTCACTNGCRTAGTTHGGRTAGTTT</td>
<td>55</td>
<td>(Gagnon et al. 2011)</td>
</tr>
<tr>
<td>A189F</td>
<td>pmoA</td>
<td>GGNGACTGGGAGTCTTGG</td>
<td>60</td>
<td>(Tuomivirta et al. 2009)</td>
</tr>
<tr>
<td>A682R</td>
<td>pmoA</td>
<td>GAASGCNGAGAGAASGC</td>
<td>60</td>
<td>(Tuomivirta et al. 2009)</td>
</tr>
</tbody>
</table>

2.7. Extracellular Enzyme Activity

Soil microorganisms such as Bacteria and fungi acquire nutrients and carbon compounds through the breakdown of complex organic molecules via the production of certain extracellular enzymes (Burns et al. 2013). These enzymes have the capacity of hydrolyzing polymers into smaller subunits that can be readily absorbed by the cell. Thus, most of the nutrient mineralization and organic degradation occurring in natural systems is caused by these microbial extracellular enzymes (Jackson et al. 2013).

Microbial enzymes such as cellobiohydrolase (CBH) and β-glucosidase are major actors in the breakdown of plant material such as cellulose. The β-1,4 linked glucose polymer, cellulose, can be enzymatically hydrolyzed into glucose by these enzymes to provide a usable carbon substrate for microbial assimilation (Mann et al. 2014). During this process, CBHs are degrading cellulose to cellobiose, then, β-glucosidases hydrolyze cellobiose to free glucose molecules (Yeoman et al. 2010). Thus β-Glucosidase is a key enzyme catalysing the final step of cellulose depolymerization, and can be used as an indicator of cellulase activity (Song et al. 2018).
One of the first methods to assess microbial extracellular enzyme activity in environmental samples was the use of p-nitrophenyl (pNP) linked substrates (Sinsabaugh 2010). This technique is based on the detection of p-nitrophenol (colored product) which is released when the substrate is degraded by the appropriate enzyme. Thus, the p-nitrophenol can be evaluated by quantifying its colorimetric absorbance at around 400-410 nm (Jackson et al. 2013). However, colorimetric methods for studying soil enzymes tend to be relatively insensitive when measuring samples with low enzyme activity (e.g. permafrost and peatland) (Fenner et al. 2005). Furthermore, interferences can be caused by highly colored phenolic compounds which dominates the peatland dissolved organic carbon and cause background absorbance.

Previous pNP colorimetric enzymatic assays were carried out on our samples but without clear success because of the limitations cited above. Therefore, the use of fluorometric methods could be used to alleviate this problem (Freeman et al. 1995). With the adoption of microplates (typically 96 wells), the fluorometric methods have become more common. This is because they facilitate the rapid measurement of large numbers of samples and are considered more sensitive than the colorimetric methods (Burns et al. 2013). In acidic peatland soils, the potential activity of hydrolytic enzymes, such as 1,4-β-glucosidase, can be determined using methylumbelliferone or methylumbelliferyl (MUB/MUF) linked substrates (Preston et al. 2012; Gittel et al. 2014; Romanowicz et al. 2015). The released end product (4-methylumbelliferone) is fluorescent and can be detected using a fluorometer with an excitation/emission wavelength at around 360/460 nm (Jackson et al. 2013).

The potential microbial enzymatic activity involved in the extracellular decomposition of organic molecules was assayed on our soil samples. In this study, I investigated the fate of the enzyme β-glucosidase (EC 3.2.1.21) which is a glycoside hydrolase involved in the breakdown of the β-1,4 glucosidic bonds of cellobiose to produce glucose monomers (Jeng et al. 2011).

β-glucosidase potential activity was determined using fluorescent MUB linked substrate with the general approach developed by Sinsabaugh et al. (1997) and a slightly modified method demonstrated by Jackson et al. (2013) and Bell et al. (2013) that have been previously used on soil and sediment samples (Millar et al. 2015; Rüa et al. 2015; Rietl et al. 2016).
In short, approximately 0.5 g of frozen soil was aseptically transferred into 50 ml Falcon tube containing 25 ml of autoclaved 50 mM Sodium acetate anhydrous buffer (CH$_3$COONa or NaAc) at pH 5. Glass beads (0.5 cm of diameter) were added to the tubes before vortexing for 1 min. Samples were sonicated for 3 min in order to further disaggregate the soil particles using a sonicator (Transsonic 460, Elma). Afterward, soil mixture was homogenized for 30 min on a horizontal shaker. Buffer was added to reach a total volume of 50 ml.

In the meantime, the substrate solution, a 200 μM solution of 4-Methylumbelliferyl β-D-glucopyranoside or MUB-β-D-G (M3633, Sigma Aldrich) is prepared in 50 mM NaAc buffer in sterile 15 ml centrifuge tubes wrapped in aluminum foil to protect it from light and store in refrigerator before use. Substrate can be kept stable for around one week if stored at 5°C.

The 4-Methylumbelliferone (MUB) (M1381, Sigma Aldrich) standard solution was prepared as a 100 μM solution of dissolved 4-MUB in 50 mM NaAc buffer in sterile 15 ml centrifuge tubes wrapped in aluminum foil. Immediately prior to use, the 100 μM stock solution is diluted 1/10 into sterile 50 mM NaAc buffer to make a working solution of 10 μM for the enzyme assays. Assays were conducted using black 96 wells microplates (Thermo Scientific) with experimental set up outlined in Figure 16.

![Figure 16](image)

Figure 16: Plate layout of the 96-well black microplates used during the assay of extracellular enzyme activity in our permafrost samples using the fluorometric MUB-linked substrate technique. The eight samples (IP5 to DP35) can be assessed vertically (column 1 to 8) on the same plate.

In the layout depicted in Figure 16, “Samples” corresponds to each soil suspension with 4-MUB-β-D-G substrate. “Samples controls” contain each soil suspension and NaAc buffer. They are used to measure the background fluorescence of the soil suspension with buffer. “Quench standards” are made up of each soil suspension and a standard amount of the fluorescent tag standard (MUB) and are used to assess the quenching effect of the soil on MUB fluorescence. “Spiked controls” are used as a positive control for each sample to
validate the enzyme activity as they contain soil suspension, MUB-β-D-G substrate and a given volume of 7.7 U/ml solution of pure β-Glucosidase from almonds active at pH 5 (REF:49290, Sigma Aldrich). “Substrate controls” are used to assess the background fluorescence of the MUB-β-D-G substrate. “Standards” are composed of soil suspension and different dilutions of the MUB standard stock solution. All reagents and soil sample solutions depicted in Figure 16 were transferred into the plate using an 8-channel pipette with 200 µl wide orifice tips.

Two different plates were prepared: one with fluorescence reading at t=0h to give a referential fluorescence background for further plates readings; and one a t=18h (fluorescence reading after 18 hours). The microplates were incubated at 5°C for 18 hours on a horizontal shaker at 300 rpm in the dark.

Just before measuring the fluorescence, 40 µl of 0.1 M NaOH pH 10 was added to each well to increase the pH as MUB fluorescence intensity is pH dependent, with the highest fluorescence signal around pH 10 (Dick et al. 2018). Fluorescence was read using a Hidex Sense Microplate Reader 425-301 (Turku, Finland) with excitation/emission wavelength at 355/460 nm. Final extracellular potential activity was calculated in nmoles of MUB consumed per hour and per gram of dry soil.

2.8. Microbial community metabolic activity (Biolog EcoPlate assay)

The Biolog EcoPlate (Biolog Inc., Hayward, CA) contains 31 different carbon substrates and water (blank control) in triplicate in a 96 well microplate (Table 4). Each well contains lyophilized carbon substrate containing tetrazolium dye which change color when reduced by NADH (induced by the respiration of the cells in the community). Thus, the formation of purple color indicates that the microbes can metabolize and degrade the carbon source (Garland and Mills 1991). Fungi do not respond to the EcoPlate assay, because they cannot reduce the tetrazolium dye included in the substrate (Dobranic and Zak 1999; Ernakovich and Wallenstein 2015). Certain microorganisms can consume some of the compounds directly, such as amino acids, while others, such as glycogen, cyclodextrin, and Tween, require breakdown by extracellular enzymes. The degradation of low molecular weight compounds was found to be correlated with their breakdown in-situ, illustrating that EcoPlates are ecologically relevant (Glanville et al. 2012). One of the first methods to analyze Biolog plates was to read the colorimetric absorbance of the wells at a single time point and utilize the absorbance data to assess the substrate utilization profiles or community level physiological profiles (CLPP). Nevertheless, measurement results are sensitive to several parameters such as the length of the assay (E. Lindstrom et al. 1998; Konopka et al. 1998) and the inoculum density (Garland and Mills 1991; Garland 1996; E. Lindstrom et al. 1998; Konopka et al. 1998; Garland et al. 2001). Consequently, one alternative methods for the colorimetric evaluation of community metabolic activity has been proposed and this method investigate the substrate utilization profiles at a common average well color development (AWCD) (Garland 1996; Garland et al. 2001). For such a method, a time series of data is required. Thus, it allows the comparison of microbial community substrate utilization without the bias of the assay time (Ernakovich and Wallenstein 2015).
Inoculum density was normalized for each sample using the quantitative PCR data obtained from the DNA. Thus, a standardized amount based on the quantity of bacterial 16S rRNA gene copies number was inoculated in each well.

### Table 4: List of the carbon substrates found in the EcoPlate assay grouped by substrate classes (Zak et al. 1994; Ernakovich and Wallenstein 2015).

<table>
<thead>
<tr>
<th>Substrates class</th>
<th>Substrate name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amines</td>
<td>Phenylethylamine</td>
</tr>
<tr>
<td>Amino acid</td>
<td>Putrescine</td>
</tr>
<tr>
<td>Amino acid</td>
<td>Glycyl-L-Glutamic acid</td>
</tr>
<tr>
<td>Amino acid</td>
<td>L-Arginine</td>
</tr>
<tr>
<td>Amino acid</td>
<td>L-Asparagine</td>
</tr>
<tr>
<td>Amino acid</td>
<td>L-Phenylalanine</td>
</tr>
<tr>
<td>Amino acid</td>
<td>L-Serine</td>
</tr>
<tr>
<td>Amino acid</td>
<td>L-Threonine</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>D-Cellobiose (i)</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>D-Mannitol</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>D-Xylose</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>i-Erythritol</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>N-Acetyl-D-Glucosamine</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>α-D-Lactose</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>β-Methyl-D-Glucoside</td>
</tr>
<tr>
<td>Carboxylic acid</td>
<td>2-Hydroxy benzoic acid</td>
</tr>
<tr>
<td>Carboxylic acid</td>
<td>4-Hydroxy benzoic acid</td>
</tr>
<tr>
<td>Carboxylic acid</td>
<td>D-Galactonic acid γ-lactone</td>
</tr>
<tr>
<td>Carboxylic acid</td>
<td>D-Galacturonic acid</td>
</tr>
<tr>
<td>Carboxylic acid</td>
<td>D-Glucosaminic acid</td>
</tr>
<tr>
<td>Carboxylic acid</td>
<td>D-Malic acid</td>
</tr>
<tr>
<td>Carboxylic acid</td>
<td>Itaconic acid</td>
</tr>
<tr>
<td>Carboxylic acid</td>
<td>Pyruvic acid methyl ester</td>
</tr>
<tr>
<td>Carboxylic acid</td>
<td>α-Ketobutyric acid</td>
</tr>
<tr>
<td>Carboxylic acid</td>
<td>γ-Hydroxybutyric acid</td>
</tr>
<tr>
<td>Phosphorylated chemicals</td>
<td>D,L-α-Glycerol phosphate</td>
</tr>
<tr>
<td>Phosphorylated chemicals</td>
<td>Glucose-L-phosphate</td>
</tr>
<tr>
<td>Polymers</td>
<td>Glycogen</td>
</tr>
<tr>
<td>Polymers</td>
<td>Tween 40</td>
</tr>
<tr>
<td>Polymers</td>
<td>Tween 80</td>
</tr>
<tr>
<td>Polymers</td>
<td>α-Cyclodextrin</td>
</tr>
</tbody>
</table>

The Biolog Ecoplates were used for colorimetric evaluation of heterotrophic community metabolic activity of our active layer soil samples by calculating the AWCD through time as it has been done on permafrost soil samples in other studies (Oelbermann et al. 2008; Wagner et al. 2009; Ernakovich and Wallenstein 2015; Goordial et al. 2017; La Ferla et al. 2017; Morgalev et al. 2017).

Biolog EcoPlates were prepared as described in Goordial et al. (2017). In short, 1 g of each soil sample (dry weight equivalent) was weighted into a sterile centrifuge tube containing 9 ml of autoclaved NaCl 0.9 % solution. Tubes were vortexed at maximum speed for 30 min.
using the MoBio Vortex Genie 2 (MoBio) and then centrifuged for 2 min at 2000 rpm (Beckman-Coulter, Allegra 21 R centrifuge) in order to decrease the amount of soil particles in the supernatant and allowing to separate the Bacteria from the mineral and organic debris (Morgalev et al. 2017).

For each sample, the supernatant was diluted in sterile NaCl 0.9 % solution to reach a standardized number of bacterial 16S rRNA gene copies number \(1.00 \times 10^8\) gene copies/ml. A total of 140 µl of the diluted supernatant was transferred into each well of the EcoPlates using a multichannel pipette. Plates were sealed using Parafilm and incubated at 10°C for 28 days. The temperature of 10°C was selected because a first test of the assay was conducted at 5°C but color development was too slow to increase in a reasonable time frame. The colorimetric absorbance was measured at 595 nm at the beginning of the incubation and every other day with a time step of 24 hours using an EnSpire Multilabel Reader 2300 (PerkinElmer). Derived from the recorded color development of each plate in triplicate, the AWCDs of each plate were calculated daily (Morgalev et al. 2017).

ACWDs were calculated using a slightly modified version described by Choi and Dobbs (1999): 

\[
AWCD = \sum \frac{(C - R)}{n}
\]

Where C is the color production within each well, measured by optical density absorbance at 595 nm (OD595), R is the background absorbance value of the measured well at the beginning of the incubation, and n is the number of substrate (here n=31). Negative OD595 values calculated by subtracting C by R were corrected to zero. Mean AWCD (of triplicate wells) where plotted over time for each sample (Choi and Dobbs 1999).

Substrate usage of each substrate class (Table 4) was calculated by averaging the OD595 measured for all members of the same class (Amine, n=2; Amino acid, n=6; Carbohydrate, n=7; Carboxylic acid, n=10; Phosphorylated chemical, n=2 and Polymers, n=4).
3. Results and discussion

3.1. Soil properties

The moisture content (MC) was found to be significantly greater in the core sampled in the active layer (AL) of the degrading palsa (DP) in comparison to the core sampled in the AL of the intact part of the palsa (IP) (ANOVA, p<0.05). This trend could be observed on the field as the degradation of the palsa is leading to the formation of wet miré where the non-degraded palsa once stood. Thus the water table was closer to the samples from the 2nd core than the one from the 1st core. Upon visual inspection, frozen soil samples coming from the 2nd core exhibited a higher content of ice and were therefore harder to work with while frozen. Furthermore, the seasonally thawed peat layer of palsas is known to be a well drained environment (Mondav et al. 2017).

No significant correlation between pH values, MC and soil organic matter content (SOM) with depth could be demonstrated. Nevertheless, the lowest values of SOM tended to be found in the deepest layer of the soil while the highest values were found in the top 20 centimeters of the active layer.

SOM was found to be homogenous across all samples. SOM values were high across all samples, showing that all AL samples are mainly formed of organic matter with only a few percentage of mineral material. This is coherent with data retrieved from a palsa mire studied in Finnish Lapland (Kujala et al. 2008). Upon visual inspection, the organic matter found in the 2nd core consisted mainly of coarse plant litter while organic matter in the 1st core was formed by totally degraded plant biomass excepted for the AL’s top sample (IP5). In IP5 sample, similar amount of plant debris as the ones found in the 2nd core could be observed.

pH values of all sample were acidic and similar to what has been already reported in other acidic palsa mire environment (Kujala et al. 2008; Seppälä 2011; Johansson et al. 2011; Palmer and Horn 2012; Liebner et al. 2015). pH values were also found to be homogenous across all samples without any significant correlation with other variables.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Depth (cm)</th>
<th>Core</th>
<th>MC (%) ±-SD</th>
<th>SOM (%)±-SD</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>IP5</td>
<td>5</td>
<td>1</td>
<td>81.57% ±1.95%</td>
<td>96.17% ±0.50%</td>
<td>4.39</td>
</tr>
<tr>
<td>IP10</td>
<td>10</td>
<td>1</td>
<td>73.26% ±2.05%</td>
<td>98.33% ±0.36%</td>
<td>4.38</td>
</tr>
<tr>
<td>IP20</td>
<td>20</td>
<td>1</td>
<td>80.88% ±1.00%</td>
<td>97.59% ±0.79%</td>
<td>4.09</td>
</tr>
<tr>
<td>IP35</td>
<td>35</td>
<td>1</td>
<td>77.25% ±1.58%</td>
<td>94.87% ±1.05%</td>
<td>4.05</td>
</tr>
<tr>
<td>DP5</td>
<td>5</td>
<td>2</td>
<td>91.02% ±1.62%</td>
<td>98.34% ±0.46%</td>
<td>3.95</td>
</tr>
<tr>
<td>DP10</td>
<td>10</td>
<td>2</td>
<td>91.51% ±0.61%</td>
<td>98.58% ±0.40%</td>
<td>4.34</td>
</tr>
<tr>
<td>DP20</td>
<td>20</td>
<td>2</td>
<td>89.66% ±0.95%</td>
<td>96.60% ±0.65%</td>
<td>4.18</td>
</tr>
<tr>
<td>DP35</td>
<td>35</td>
<td>2</td>
<td>83.46% ±1.21%</td>
<td>93.14% ±1.47%</td>
<td>4.23</td>
</tr>
</tbody>
</table>
3.2. Gene analysis

3.2.1. DNA extraction and dual nested PCR

To perform the different gene analysis, gDNA needs to be successfully extracted from the soil. gDNA was extracted from our eight frozen soil samples and concentrations ranged from 13.4 (sample IP5) from top layer of intact palsa) to 5.24 ng/µl (sample IP35) from deep layer of intact palsa). The extracted gDNA could not be visualized on gel agarose electrophoresis since gDNA concentrations were too low. Concentration of extracted gDNA from the first core significantly correlated with depth (Pearson’s r = -0.95, p<0.05) suggesting a decrease in biomass with depth.

Table 6: Concentration (in ng/µl) of extracted genomic DNA from palsa’s active layer samples

<table>
<thead>
<tr>
<th>Sample name</th>
<th>gDNA concentration (ng/µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IP5</td>
<td>13.4</td>
</tr>
<tr>
<td>IP10</td>
<td>10.1</td>
</tr>
<tr>
<td>IP20</td>
<td>8.56</td>
</tr>
<tr>
<td>IP35</td>
<td>5.24</td>
</tr>
<tr>
<td>DP5</td>
<td>6.3</td>
</tr>
<tr>
<td>DP10</td>
<td>10</td>
</tr>
<tr>
<td>DP20</td>
<td>7.5</td>
</tr>
<tr>
<td>DP35</td>
<td>10.6</td>
</tr>
</tbody>
</table>

Nevertheless, the primary PCR of the 16S rRNA variable V4 region successfully amplified the targeted sequence of interest in all samples (sample amplification in triplicates). The secondary PCR (nested PCR) allowed the amplification of the sequence of interest while adding specific barcodes to each template and sequencing adapters. As seen in Figure 17, the subsequently amplified templates from the nested PCR are therefore longer than gDNA amplified from the primary PCR (+340 bp in comparison to +290 bp). A gel agarose electrophoresis was necessary to verify the proper amplification, length and quality of the targeted sequence in order for the samples to be sent to the Norwegian High-Throughput Sequencing Centre in Oslo.

Figure 17: Visualization of amplification products (16S rRNA variable V4 region) obtained from the nested PCR. bp = base pairs, + = positive control (from E.coli DNA) and − = negative control (reaction mix without DNA)
3.2.2. Diversity and composition of the microbial communities

3.2.2.1. Microbial species richness

A total of 66022 different operational taxonomic units (OTUs) were retrieved from the sequencing data across all eight samples. IP5 showed the highest number of observed prokaryotic species with 21691 while IP35 showed the lowest with 10018 (Figure 18). In the intact palsa, the observed number of OTUs significantly decreases with depth (Pearson’s $r = -0.96$, $p<0.05$), while species richness tend to be homogenous in the samples from the degrading palsa. In contrast with the intact palsa, the top layer of the DP (DP5) showed the lowest number of observed OTUs (with 11462) while samples DP10, DP20 and DP35 showed comparable numbers (13096, 12856 and 13224 respectively).

To study the alpha diversity (species richness), in addition to the observed number of OTUs, the Chao1 index (Chao 1984) with 95% confidence intervals (CI) was selected as a metric for species richness estimation. This index is highly recommended when analyzing microbial alpha diversity since it estimates the total number of species by taking into account the importance of rare OTUs (Wang et al. 2018).
Obtained results show an estimated total chao’s OTUs richness which is approximately three times as high as the number of observed OTUs, and that in each sample. This suggests that a large proportion of rare prokaryotic species were not retrieved or represented in the extracted gDNA. While the number of estimated OTUs is much larger, the values’ trend is similar to what is seen for the observed richness. In the intact palsa’s AL, the top layer sample estimated total richness is significantly higher than in every other sample, while the layer close to the permafrost boundaries (IP35) comprised the lowest significant estimated total richness. In the thawing palsa’s AL, the top layer (DP5) exhibits significantly lower estimated total richness than the ones from the other samples found in the same core. The highest estimated total richness from the DP was found at 10 cm depth. There was no significant difference in microbial species richness when comparing the diversity between the IP’s AL and the AL from the DP. This lack of significant difference contrasts with findings found in another study assessing the microbial diversity in the active layer of palsas along a thawing chronosequence in a Swedish palsa mire located 285 km southwest of our study site (Mondav et al. 2017). Mondav et al. (2017) found that microbial richness in a partially degrading palsa (bog) was lower than in the intact palsa. The bog richness had even the lowest microbial richness of all three stages studied (intact palsa stage, thawing palsa stage and completely degraded palsa stage). In addition, they observed that depth of sample throughout the active layer was related to a decrease in alpha diversity metrics in their intact palsa site. This is in consistency with our results. However, in contrast to what was found in our thawing palsa site they witnessed a decrease in richness with depth in their bog site.

Müller et al. (2018) found that microbial richness was higher in the active layer of continuous permafrost located in Svalbard archipelago and that it decreased significantly with depth down to the palsa layer. Similar observations have been reported across other permafrost sites in the Arctic region (Steven et al. 2008; Mackelprang et al. 2011; Deng et al. 2015) as well as in the Tibetan Plateau (Chen et al. 2017).

It was suggested that the distinction in microbial richness with depth could be attributed to the difference in environmental conditions between the two distinct layers. Indeed, the active layer is exposed to larger environmental fluctuations while the permafrost layer features coercive factors such as temperature, low oxygen concentration, small nutrient availability and low thermal energy (Jansson and Taş 2014). Changes in ecological conditions with depth create a selection filter leading to a potential decrease in microbial diversity along depth profile, since the survival of many taxa is likely to diminish in deeper soil environment (Tripathi et al. 2018). Therefore, only few psychrotolerant and psychrophilic species of microbes adapted to such difficult environment might survive close to the permafrost layer (Jansson and Taş 2014).
**3.2.2.2. Community composition**

Relative abundance of prokaryotic phyla based on sequencing of 16S rRNA gene (Figure 19) is dominated by bacteria. The most abundant bacterial phylum across all samples was *Acidobacteria* (29.52% on average) followed by *Proteobacteria* (22.28%), *Actinobacteria* (12.51%), *Planctomycetes* (11%), *Verrucomicrobia* (8.20%), WPS-2 (5.23%), AD3 (4.21%) and *Bacteroidetes* (1.21%). All these phyla are found ubiquitously through depth and thaw stage. Several studies of permafrost and its active layer from other Arctic and sub-Arctic regions have described a high abundance and dominance of Bacteria belonging to the phyla of *Acidobacteria*, *Proteobacteria*, *Actinobacteria*, *Planctomycetes* and *Verrucomicrobia* (Yergeau et al. 2010; Mackelprang et al. 2011; Frank-Fahle et al. 2014; Deng et al. 2015; Hultman et al. 2015; Schostag et al. 2015; Kim et al. 2016; Mondav et al. 2017; Tripathi et al. 2018; Taş et al. 2018; Monteux et al. 2018; Müller et al. 2018).

![Figure 19: Relative abundance of the 20 most abundant bacterial and archaeal phyla based on sequencing of 16S rRNA gene](image-url)
Archaea were generally underrepresented with a maximum relative abundance of 2.31% and OTUs were almost exclusively represented by Crenarchaeota (classes MBGA and Thaumarchaeota) and Euryarchaeota (classes Methanobacteria and Thermoplasmata). Relative abundance of methane producing Methanobacteria was extremely low in the entire microbial community but it seems that they were more abundant in the degrading palsa, suggesting an increase of methane producing Archaea with palsa thaw.

The ubiquitous distribution of the main bacterial phyla across the palsa thawing stages might result from local dispersal mechanisms such as palsa dome runoff transporting microbes into lower levels (Mondav et al. 2014a) or aerial dispersal (Bowers et al. 2011).

All these phyla, (Alpha-, Beta-, Gamma-) Proteobacteria, Actinobacteria, Acidobacteria, Planctomycetes and Verrucomicrobia, are recognized to be involved in the C cycle throughout organic matter degradation and/or fermentation processes (Goldfarb et al. 2011; Tveit et al. 2013; Ganzert et al. 2014; Tsitko et al. 2014; Deng et al. 2015; Schostag et al. 2015).

Acidobacteria is the most abundant phylum in almost all samples. Their relative abundance is highest in 6 out of 8 of our samples. They were also found to dominate the bacterial communities of Finnish Arctic tundra (Männistö et al. 2013) and in the upper horizon of a permafrost thaw gradient in Alaska (Deng et al. 2015). Although Acidobacteria are acidophilic (Kielak et al. 2016) and are the most abundant microorganism in almost all samples, we could not observe any significant correlation between relative abundance and depth, nor with pH, this is in accordance with the study by Schostag et al. (2015). However, in the IP, relative abundance drops dramatically in the 35 cm layer (from 30.28% to 14.76%), suggesting a further decrease in deeper layer. Nevertheless, Acidobacteria relative abundance tends to increase with depth in the degrading palsa, especially at the 20 cm layer where the phylum constitutes 56.67% of the community. Abundance of members of the phylum Acidobacteria tends to be higher in the DP than in the IP. Since members of the phylum Acidobacteria are known to degrade plant-derived carbohydrates (Schostag et al. 2015) but also complex polymeric substrate (Ganzert et al. 2014), they could play a significant role in the decomposition of high molecular compounds in cold terrestrial habitat (Ganzert et al. 2014).

Proteobacteria is the dominant phylum in only one sample (DP5) and its relative abundance values are rather stable in the different depths. However, Proteobacteria abundance is higher in the DP than in the IP (ANOVA, p<0.05). Throughout depth and thaw stage, Proteobacteria phylum is mainly represented by Alphaproteobacteria, which constitutes more than 50% of the population, whereas Gammaproteobacteria make up as much as 35%. Betaproteobacteria represents less than 4% of the Proteobacteria community. High relative abundance of Alphaproteobacteria and Gammaproteobacteria in the upper AL is suggested to be correlated with higher C and nutrient availability (Kim et al. 2016; Müller et al. 2018).

Class of Alphaproteobacteria is mostly constituted of members of the Rhizobiales Order which are heterotrophic and rhizosphere symbiotic Bacteria with the ability to fix atmospheric nitrogen (Ganzert et al. 2014), this could indicate a significant role of our microbial community in the nitrogen cycle in this cold environment. On the other hand, the
**Gammaproteobacteria** was dominated by the *Xantomonadales* Order and the *Sinobacteraceae* Family in particular. Members of this Family are known to have the ability to degrade and use lignin carbon as a source for food (Goldfarb et al. 2011).

The higher relative abundance of sample dominant *Acidobacteria* and *Proteobacteria* in the DP than in the IP might be explained by the fact that the DP harbored more vegetation and showed higher amount of coarse plant litter with depth than in the IP. In addition, an increase in *Acidobacteria* and *Proteobacteria* might be the results of an increase in bioavailability in carbon substrate and nutrients, thus, these phyla could be potentially used as a proxy to indicate permafrost degradation and increase in microbial activity.

Bacterial methanotrophs such as members of the Order *Methylococcales* (*Gammaproteobacteria*) were found to be present with a small relative abundance (0.33%) in the deepest layer of the degrading palsa, while members of the nitrogen fixing acidophile Order *Methylacidiphilae* (Brewer et al. 2017) were present heterogeneously throughout all samples with a relative abundance close to 1%. This suggest the presence of a bacterial methanotrophs populations in all samples, however, qPCR data might give higher resolution on the population dynamics with depth and thaw.

Within the *Actinobacteria* phylum, *Solirubrobacterales* and *Actinomycetales* were the most abundant Orders. *Actinomycetales* were dominant in the top layer of the IP and throughout all layers in the DP, whereas the relative abundance of *Solirubrobacterales* increased with depth in the IP. This dominance of *Solirubrobacterales* with depth might presumably due to their aptitude to survive in cold and scarce nutrient availability conditions (Jansson and Taş 2014; Taş et al. 2018). This ubiquitous dominance of *Actinomycetales* might suggest the presence of more favorable conditions in the DP with higher temperatures and carbon substrate availability.

*Planctomycetes* phylum is here dominated by the *Planctomycetia* Class mainly comprising members of the uncharacterized Gemmatales Order which were found to be facultative heterotrophic anaerobes with the ability to hydrolyze sugars and organic acids (Tsitko et al. 2014). *Planctomycetes* second highest relative abundance is attributed to the Phycisphaerae Class which was found to dominate sub-Arctic tundra environment (Ivanova et al. 2016). Similarly as for *Acidobacteria*, *Planctomycetes* relative abundance decreases dramatically after reaching the IP 35 cm layer. This could be, in part, explained by the considerable increase in relative abundance of the phylum AD3 with depth (Pearson’s r = 0.95, p<0.05).

Indeed, AD3 phylum is almost non present in the degrading palsa, whereas in the intact palsa, its relative abundance starts at 0.29% on the top layer and then turns into the most abundant phylum in the bottom layer (22.53%). In the same way, relative abundance of the Gemmatimonadetes phyla significantly correlated with depth in the IP (Pearson’s r = 0.96, p<0.05). Similar observations for AD3 and Gemmatimonadetes were made across different permafrost affected environments (Deng et al. 2015; Kim et al. 2016; Wu et al. 2017, 2018; Müller et al. 2018). It is still not clear which factors are initiating their increase, as these phyla are still uncharacterized. While dry soils have been associated with elevated abundance of both phyla (DeBruyn et al. 2011; Ji et al. 2017), AD3 population were linked to low carbon
concentrations (Taş et al. 2014; Müller et al. 2018). Thus the almost non-presence of AD3 in the DP could mean that they were not competitive with other phyla such as Proteobacteria or Acidobacteria which are known to thrive in higher available C substrate environments, suggesting that an increase in Proteobacteria or Acidobacteria abundance followed by a decrease in AD3 might represent the first step of a heterotrophic microbial community shift in a thawing palsa environment.

The relative abundance of still uncharacterized WPS-2 phylum was significantly superior in the intact palsa than in the degrading palsa (Student’s t-test, p<0.05). this could be explained by the drier conditions of the IP as WPS-2 members were identified in dry surface soil of Antarctica (Ji et al. 2017). The decrease in abundance of photosynthetic bacterial Cyanobacteria with depth might be explained by the reduction in sunlight exposure in deeper layers of the soil (Tripathi et al. 2018).

| Table 7 : Bray-Curtis similarity indices matrix between samples (a score is equal to 0 when two communities are different, while a score of 100 indicates two identical communities) |
|---------|---------|---------|---------|---------|---------|---------|---------|---------|
|         | IP5     | IP10    | IP20    | IP35    | DP5     | DP10    | DP20    | DP35    |
| IP5     | 47.99   | 62.14   |         |         |         |         |         |         |
| IP10    | 48.12   | 62.14   |         |         |         |         |         |         |
| IP20    | 25.29   | 33.71   | 40.1    |         |         |         |         |         |
| DP5     | 48.23   | 34.61   | 32.97   | 21.25   |         |         |         |         |
| DP10    | 50.35   | 37.51   | 39.00   | 23.80   | 64.51   |         |         |         |
| DP20    | 31.52   | 35.25   | 35.63   | 28.23   | 31.86   | 31.63   |         |         |
| DP35    | 44.48   | 37.29   | 40.44   | 24.20   | 45.03   | 51.86   | 34.31   |         |

Bray-Curtis similarity indices between samples (Table 7) showed that, especially in the IP (IP5 to IP35), the difference in microbial communities tended to increase with depth. However, no such trend is observed in the degrading palsa (DP5 to DP35), suggesting a more homogenous community across depth with land surface subsidence under permafrost thaw. This difference in microbial communities’ trend in the two thaw stages could be partially supported by the pattern observed in microbial richness, where richness tended to be important in the top layer of the IP and to decrease with depth, while DP’s richness was rather homogenous.

Communities showing the highest similarity indices are spatially contiguous (i.e. DP5-DP10 with 64.51 and IP10-IP20 with 62.14) while the lowest similarity indices are found between the two cores (i.e. IP35-DP5 and IP35-DP10). Interestingly, microbial community in IP5 is more similar to the top soil samples of the DP (DP5 and DP10) than the underlying samples (IP10 and IP20) from the IP, suggesting that top layers communities are similar across thaw stages as illustrated in Figure 20.
This observation is supported by the fact that the one-way Analysis of Similarities (ANOSIM) test that was carried out on Bray-Curtis similarity matrix showed a significant difference in community structure between the intact and degrading palsa (Global R=0.323, p<0.05), suggesting a structural response to ecosystem disturbance (Sannel and Kuhry 2011). This is illustrated in Figure 20, where samples can be grouped and discriminated according to the thaw stage. It was previously suggested by Mondav et al. (2014) that variation in microbial richness and community composition was linked to variation in water table supporting the fact that site inundation, generated by permafrost thaw causing soil subsidence, is a mechanistic driver of community structure and function in the degrading part of the palsa (Mondav et al. 2014a). However, in our case, specific factors causing this difference in community structure could not be significantly identified.

### 3.2.3. Microbial abundance

To the extent of our published literature research, this study is the first to investigate the copies number of several genes by quantitative PCR in the permafrost AL through a depth gradient while comparing intact and thawing permafrost.

The number of 16S rRNA operons per genome of the investigated bacterial and archaeal community was estimated using the Ribosomal RNA database v 5.4 (https://rrndb.umms.med.umich.edu/) elaborated by Stoddard et al. (2015). Taking into account the five more abundant Phyla per Kingdom (Bacteria or Archaea), the mean number of ribosomal RNA operons was estimated at 2.22 in Bacteria and 1 for Archaea. On the other hand, the number of pmoA functional gene copies per genome in a single methanotroph cell...
is set at 2 (Stolyar et al. 2001; Kolb et al. 2003) while the number of mcrA copies per genome per cell is known to be 1 (Alvarado et al. 2014).

Obtained bacterial and archaeal 16S rRNA, mcrA and pmoA gene abundance values (Figure 21, Figure 22 and Table 8 in supplementary materials) were higher (10 to 100 times) than what has been already recorded in the AL of a Swedish palsa mire (Liebner et al. 2015) and in the AL of Canadian high Arctic permafrost (Frank-Fahle et al. 2014).

Bacterial and archaeal 16S rRNA gene abundances (Figure 21 and Table 8) showed that bacterial gene copy numbers were 100 to 1000 times higher than the archaeal copy numbers, with $10^8$–$10^{11}$ copies per g of dry soil (g ds$^{-1}$) for Bacteria versus $10^7$–$10^8$ copies (g ds$^{-1}$) for archaea. Obtained results indicate that members of the archaeal group tend to follow the same pattern as Bacteria when comparing values from the same core. In addition, bacterial and archaeal abundance are significantly higher in the thawing palsa than in the intact one (ANOVA, p<0.05), suggesting that microbial biomass is superior in a thawing environment resulting in more GHG emissions.

Considering the bacterial 16S rRNA gene copies number, IP’s bacterial abundance is significantly higher in the top layer than in any other deeper layer of the same core (Student’s t-test, p<0.05). Bacterial abundance dramatically decreases when reaching the 10 cm layer with a further decrease when reaching 20 cm but then slightly increases at 35 cm (Figure 21). However, in the DP, bacterial abundance values tend to stay homogenous across depth with only a slight increase at the 10 and 20 cm layers.

The abundance of Archaea follows the same pattern as that of Bacteria. A decrease by a factor of ten in gene copies is seen when going from 5 cm to 10 cm depth layer in the IP. This dramatic decrease is followed by similar values for 20 and 35 cm depth layers. However, in the DP, archaeal abundances are comparable across layers.

![Figure 21](image-url)
High abundance in bacterial and archaeal 16S rRNA copies number in surface layer compared to deeper layer, such as what is seen in the samples from the intact palsa (Figure 21), has already been reported in the Canadian High Arctic (Yergeau et al. 2010; Frank-Fahle et al. 2014) and Alaskan continuous permafrost (Tripathi et al. 2018). It was suggested by Frank-Fahle et al. (2014) and Wagner et al. (2005) that, even though organic carbon is largely accumulated in permafrost soils, a diminution in bioavailability of carbon with increasing depth can create a substrate limitation close to the permafrost boundaries. Thus, the decline in copies of quantified genes with depth might be linked with the decreasing of carbon to nitrogen (C:N) ratio with active layer depth (Wagner et al. 2005).

Knowing that the mcrBDCGA operon (containing the mcrA subunit) is almost uniquely found in Archaea, and considering that all known genomes of the methanogenic Archaea encode one copy of the mcrBDCGA operon (Alvarado et al. 2014), these assumptions imply that archaeal methanogens dominate the archaeal populations in our samples, as this has already been observed in a palsa mire (Liebner et al. 2015). In our samples, methanogens supposedly represent between 9.41 to 46.18% of the total archaeal population.

In addition to the archaeal and bacterial abundance, a significant difference in mcrA (methanogens) and pmoA (methanotrophs) gene copies abundance was observed between the intact and the degrading palsas (ANOVA, p<0.05), with mcrA and pmoA copies being more abundant in the DP than in the IP (Figure 22), suggesting a greater CH₄ production and oxidation in the DP. This comfort the fact that palsa thaw initiates a fast development of intense CH₄ dynamics (Liebner et al. 2015). However, our results contrast with findings from Liebner et al. (2015) where they could not demonstrate significant differences in mcrA and pmoA gene copies abundance between a palsa and a thermokarst pond.

![Figure 22: (A) mcrA (methanogens) and (B) pmoA (methanotrophs) gene copies number in intact and degrading palsa (error bars represent the standard deviation)](image)

In the degrading palsa, mcrA abundance is significantly higher than pmoA’s (ANOVA, p<0.05). This may suppose that CH₄ production rate could be superior to its oxidation rate. However depth specific methane production and oxidation rates measurements are necessary to confirm such a statement.
mcrA and pmoA gene copies number were found to be strongly positively correlated to one another (Pearson's $r=0.85$, $p<0.05$). This suggests that methanogens communities are spatially located close to methanotrophs populations. Such identical observations were made in peat bogs from Whales (Freitag et al. 2010) and Japan (Akiyama et al. 2011). Thus, this could illustrate the close spatial proximity of CH$_4$ production and oxidation in the studied AL. This is supported by results from Barbier et al. (2012) showing that methane oxidation rates in the AL of Arctic wet tundra correlated to depths at which the highest methane production occurred. Singleton et al. (2018) also found, in a palsa mire, that the highest abundance of methanotrophs happened in the region of the peat profile where there was maximum dissolved methane.

Methane producing microorganisms (only archaea) are obligate anaerobes while methanotrophs (mostly bacteria) are usually aerobes (Singleton et al. 2018). Therefore, soil oxic/anoxic condition and soil organic matter content (SOM) are important factors controlling the methane-related C cycle in mire habitats (Akiyama et al. 2011). While moisture content (MC) was positively correlated with mcrA and pmoA copies number (Pearson’s $r=0.73$, $p<0.05$ and Pearson’s $r=0.79$, $p<0.05$ respectively), SOM was not. Since archaeal methanogens are obligate aerobes, high MC found in the DP might explain why mcrA gene copies number are higher in the DP than in the IP. This is supported by observations from Hoj et al. (2006) in which they could only detect methanogenic community in Arctic soil that was wet during most of the growing season. High MC and anoxic conditions should inhibits the development of methanotrophs as they require the presence of oxygen as an electron acceptor for methane oxidation (Conrad 2007). Therefore, pmoA abundance should not supposed be correlated with MC. However, there is increasing evidence that bacterial methanotrophs are micro-aerobic and are even present in environment with low oxygen concentration (Crevecoeur et al. 2017). In addition, methane can also be oxidized by anaerobic methanotrophic Archaea (Knittel and Boetius 2009; Martineau et al. 2010). Fluctuations in the water table might create complex micro to macro level niches for aerobic and anaerobic microbe’s development (Mondav 2014), thus enabling the presence of methanotrophs populations.

It is well known that extracellular DNA from dead microorganisms can persist in soil (Nielsen et al. 2007; Levy-Booth et al. 2007), especially in cold permafrost environments (Willerslev et al. 2004) where DNA can be stored for thousands of years. It is generally assumed that microbial DNA recovered from environmental samples mostly represents intact cells. However Carini et al. (2016) found that almost 40% of microbial DNA was extracellular or altered cells. As a result, this relic DNA might affect molecular analysis such as qPCR or environmental DNA sequencing (Carini et al. 2016). In order to alleviate such technical difficulties and to distinguish living cells from the dead several methods are described in the review by Emerson et al. (2017).
3.3. Extracellular β-Glucosidase activity

Averaged Beta-Glucosidase activity obtained after 18h of incubation at 5°C ranged between being almost null with 0.18±0.38 (all values are expressed in nmoles of MUB-β-D-G / h. g ds) in the deepest layer of the intact palsa to reach 9.02±0.98 in sample IP5 (Figure 23). Highest values of potential extracellular enzymatic activity were found in the degrading palsa, with 15.37±4.39 on the top layer and 13.12±2.91 at 20 cm soil depth. The range of measured beta-glucosidase activity was slightly lower to what is observed in sites with mild climates (Sinsabaugh et al. 2008)

Our obtained values are complicated to compare to results from other studies focused on permafrost environment as numerous different methods and units are used throughout the literature. This lack of quantitative comparisons highlights the need for a standardized fluorometric enzymatic assay in soil (Dick et al. 2018).

Nevertheless, beta-glucosidase activity values were found to be lower than those obtained by Lee et al. (2012) and Waldrop et al. (2010) in Alaskan permafrost. However, incubation temperatures used in their studies were superior to our (15°C and room temperature respectively in comparison to 5°C), resulting in greater enzymatic activity. Lower beta-glucosidase activity values obtained by Wallenstein et al. (2009) in an Arctic tundra and by La Ferla et al. (2017) in Antarctic permafrost soil were assessed with a protocol similar to ours (same temperature and incubation time).

![Figure 23: β-Glucosidase potential extracellular enzyme activity (in nmoles of MUB-β-D-G / h. g dry soil) measured across depth and thaw stages after 18 hours of incubation at 5°C (ds=dry soil).](image-url)
In the intact palsa, no significant correlation was found between beta-glucosidase activity, and qPCR data, soil characteristics or phyla relative abundance. Still, there was a positive correlation between the enzymatic activity with depth and microbial richness (Pearson’s r=0.99, p<0.05 ; r=0.95 , p<0.05 respectively). More elevated temperature and less recalcitrance of readily degradable carbon source generally found on the upper horizons of the soil might explain the observed higher microbial diversity and enzymatic activity (Wallenstein et al. 2009; Waldrop et al. 2010; Turner et al. 2017; Song et al. 2018). On the other hand, in the degrading palsa, the enzymatic activity showed no correlation with any parameters. However, many unmeasured factors might affect the enzymatic activity such as vegetation cover, nitrogen and carbon form or availability, oxygen availability, C/N ratio, and etc (Coolen et al. 2011; Gittel et al. 2014; Schnecker et al. 2015; Čapek et al. 2015).

In the IP, enzymatic activity tend to decrease with depth as Beta-Glucosidase activity in sample IP5 is significantly higher than IP20 and IP35 (Student’s t-test, p<0.05). The activity in IP35 is statistically smaller than in any other sample (Student’s t-test, p<0.05). A decrease in enzymatic activity with depth was also observed by La Ferla et al. (2017) in permafrost from the Antarctic and by Taş et al. (2014) in a boreal forest.

In the DP, in contrast to the trend in decline observed in the IP, potential extracellular beta-Glucosidase activity decreases significantly between the 5 and 10 cm layers (Student’s t-test, p<0.05) , but then increases significantly (Student’s t-test, p<0.05) between the 10 and 20 cm layer to values comparables as in DP5. Finally, enzymatic activity significantly decreases (Student’s t-test, p<0.05) between the 20 and 35 cm layers to reach an activity similar as in DP10. These results seem to highlight a more homogenous trend in enzymatic activity with depth in the degrading palsa.

When comparing the Beta-Glucosidase activity between the two thaw stages, a significant difference is observed, which means that the DP palsa exhibits higher Beta-Glucosidase activity than the IP (ANOVA, p<0.05). This illustrates the fact that permafrost thaw allows an increase in microbial extracellular enzymatic activity on a broader scale and that the deeper layers of the soil are more active when thawing is present, thus increase GHG emissions (Schuur et al. 2015). However, extracellular enzyme activity can be interpreted both as an indicator of microbial activity and of substrate bioavailability. Beta-Glucosidase activity increases in the thawing part of the palsa, suggesting that less substrate was bioavailable in the colder intact palsa, and that microbes synthesize more enzymes as permafrost thaws. However, this trend could also appear because the microbial activity is limited by physiological and environmental constraints (other than access to plant debris) in colder soils (Wallenstein et al. 2009).

It is still important to note that enzymes pools are measured as potential activity, as the concentration of enzymes effectively present in the soil cannot be measured (thus specific activity is not measurable) and that enzymes kinetics cannot be assessed. Substrates used in our protocol are simple chemicals and thus, are not reflecting the real substrate complexity in situ. In addition, enzyme activity was measured at a single temperature, often not representative of typical soil conditions (Wallenstein and Weintraub 2008).
Furthermore, there exist several other extracellular enzymes important in soil processes (Mann et al. 2014), such as phenol oxydase and peroxydase (degradation of lignin), alkaline phosphatase (releases ester-bound phosphate), leucine aminopeptidase (degradation of proteins into amino acids), α-glucosidase (release glucose from soluble saccharides) or glucosaminidase (degradation of chitin), but they could not be investigated during this thesis.

3.4. Microbial metabolic activity of heterotrophic communities

![Graph showing AWCD values over time](image)

Figure 24: Evolution of Average well color development (AWCD) through time in (A) the intact palsa and in (B) the thawing palsa

After incubation, measured AWCD values in the 5, 10 and 20 cm layers were lower to those observed by Morgalev et al. (2017) at similar depth. However, AWCD found in the bottom layer was similar to what Morgalev et al. witnessed at 35 cm deep into Siberian permafrost peat soil. This might be explained by the higher incubation temperature used in their study. Indeed, they incubated their Biolog Ecoplates at 20°C whereas I used 10°C.

After 27 days of incubation, with 0.45 ± 0.01, the highest AWCD value was present in the IP5 sample from the intact palsa (Figure 24). This is surprising as I would have expected the thawing palsa to harbor superior values as this thaw stage seems to be more active than the intact palsa. Furthermore, I could not discern a significant difference in AWCD between the IP and the DP. This contrast with the results obtained from the beta-glucosidase assay. However, during this Ecoplate assay, the metabolic functions upon many different C sources were investigated in contrast to only one during the beta-glucosidase experiment.

There seems to be a general pattern of decrease in the ACWD with depth with the lowest values always being present in the bottom layer, similarly to what was observed in the enzyme activity assay. This is supported by the fact that a positive correlation is present in the intact palsa between AWCD values with beta-glucosidase activity and microbial richness (Pearson’s r=0.98, p<0.05 and Pearson’s r=0.97, p<0.05 respectively). Furthermore, this decrease in microbial activity was accompanied by a decrease in the number of classes of substrates metabolized (Figure 25).
The number of utilized substrates is limited near the permafrost table in comparison with the top layers. Indeed, all substrate classes are metabolized in the 5 cm layer while they are not when reaching the deepest layers. This suggests that a lesser microbial richness might reflect a lesser functional metabolism diversity and thus a lesser metabolic and extracellular enzymatic activity. Indeed, similar observations were made in Svalbard and in Northwest Canadian Arctic where the metabolic potential of soil microorganisms tended to lessen with depth toward the active layer/permafrost table boundaries (Tveit et al. 2013; Frank-Fahle et al. 2014). Thus, communities of microorganisms found near the permafrost table seem to be less functionally competent in degrading diverse types of C substrates in comparison to microorganisms from the top of the active layer. It was suggested that this phenomenon might be the result of a lower taxonomic diversity with depth or a decrease in chemical complexity/substrate source toward the permafrost boundary (Tveit et al. 2013; Frank-Fahle et al. 2014; Morgalev et al. 2017). This is supported by results from Gundelwein et al. (2007) where they found that soil organic matter in the upper layers of a tundra soil contained more than two third of intact and labile light-carbon fraction, which presents a broad range of organic carbon substrates. On the contrary, this labile and light-carbon fraction is mostly degraded and therefore less biodegradable and bioavailable near the permafrost table (Wagner et al. 2009).

On the other hand, in the degrading/thawing palsa, AWCD values exhibit a strong positive correlation with the organic matter content (SOM) (Pearson’s r=0.99, p<0.05) but also with the moisture content (MC) (Pearson’s r=0.99, p<0.05). Permafrost thaw, and thus increase in MC, is rendering organic matter more bioavailable to microbial degradation and consequently metabolic activity tend to increase.

This could highlight a difference in microbial metabolic activity with thaw stage. Activity in the intact palsa seems to be linked to the functional metabolism diversity and richness of the highly stratified community whereas when thaw occurs, activity seems to be associated with a more homogenous community of heterotrophs thriving in higher readily bioavailable organic matter. This hypothesis is illustrated in Figure 24, where AWCD of IP samples decreases with depth and with potential microbial functional diversity while AWCD of DP samples exhibits rather homogenous metabolic activity towards the 20 cm layer.

Near surface layer of the DP and IP generally showed the highest activity (beta-glucosidase and AWCD) but also the highest microbial abundance (qPCR). This might be due to the fact that the top layer is the major location for plant root exudates which is mainly formed of labile C compounds that are known to favor carbon rich dwelling microorganisms like Alpha- and Gammaproteobacteria (Deng et al. 2015; Morgalev et al. 2017).

Alternatively, microbial communities found in the lowest part of the soil were less active but bacterial and archaeal 16S gene abundance was similar to what is seen in the 10 and 20 cm layers. This low metabolism activity but similar microbial abundance might be explained by the presence of recalcitrant substrate and of dormant microbes unable to efficiently process the present organic substrates (Morgalev et al. 2017).
Carbohydrates, carboxylic acids, sugars, peptides and amino-acids are common components of the organic carbon pool found in Arctic soils facing alternating freeze-thaw cycle (Foster et al. 2016). Their role was found to be central in microbial metabolism in Arctic tundra soils (Boddy et al. 2008).

Across all samples, the most metabolized class of substrates used by Bacteria were carbohydrates and polymeric substances. Analogous observations were made in Siberian permafrost affected soils from Siberia (Wagner et al. 2009; Morgalev et al. 2017) and Antarctica (La Ferla et al. 2017). Nevertheless, low degradation of carbohydrates by heterotrophic Bacteria in the surface layers of Antarctic soil (Kenarova et al. 2013) contrasts with our results, suggesting that palsa peat soil presents superior amount of carbohydrates usable for microbial growth.

In the class Carbohydrates, the C substrates mostly used were plant biomass-related such as D-xylose (derived from plant hemicelluloses), D-mannitol (widely distributed soluble carbohydrate secreted and produced by plants) and D-cellulose (results from the hydrolysis of cellulose); or arthropods/fungi/bacteria biomass-related such as N-acetyl D-glucosamine and glucosaminic acid involved in the monomeric unit of the polymer chitin (arthropod exoskeleton and fungi cell wall) and peptidoglycan (bacterial cell wall). For the polymeric substances, Tween 40 and 80 (polysorbates) as well as glycogen were predominantly metabolized. The degradation of Tween 40 and Tween 80, which are produced antifreeze substances generally present in frozen soil nutrient pool, suggests that they are extensively metabolized by Bacteria in such environments (La Ferla et al. 2017).

The intensity of classed C substrate averaged AWCD changed with depth and thaw stage as illustrated in Figure 25. A lag phase of 7 or more days was observed in every sample with the longest lag phases found in the 20 and 35 cm depth layers.

In the DP, after 27 days of incubation, carbohydrates seemed to be the most used class of substrate in the 5-10-20 cm layers but is overtaken by polymeric substances in the deepest layer. This could be explained by the higher amount of vegetation and plant coarse litter in the top soil of the DP. On the contrary, in the intact palsa, substrate metabolization is dominated by polymeric substances over carbohydrates (except for the 20 cm layer where amino acids degradation dominates). In addition, heterotrophic Bacteria degrading amino acids substrates were mostly active in the upper 0 to 20 cm layer. Indeed, in the IP and DP, amino acid utilization significantly decreases with depth (Pearson’s r=0.98, p<0.05). This diminution of amino acids consumption by Bacteria found near the deepest part of the active layer is consistent with results from Alaskan tundra soil where the soil close to the permafrost table no longer presented peptides and protein rich substances as a source of nitrogen for the microbial communities (Coolen et al. 2011). Such observations suggest a potential increase in the carbon to nitrogen (C:N) ratio with depth as vegetation roots and leaf litter are supposed to contribute prominently to the organic matter on the top soil (especially in the DP), thus less N available in deeper layers could mean less microbe adapted to utilizing it. Therefore microbes tend to degrade the more complex and recalcitrant forms of C substrates, particularly when facing low N availability (Oelbermann et al. 2008).
Figure 25: Heterotrophic bacterial functional activity represented by the substrate class averaged well color development (6 substrate classes where Amine=2 substrates, Amino acid=6, Carbohydrate=7, Carboxylic acid=10, Phosphorylated compound=2 and Polymeric substance=4) with thaw stage and depth.
4. Conclusion and perspective

Permafrost environments, which store nearly 50% of the known soil organic carbon, are facing global climate change-induced thaw. This thawing of permafrost will render previously frozen organic matter bioavailable for soil microorganisms, leading to faster degradation of this carbon pool and an increase in greenhouse gases emissions. Investigating the response of permafrost microbial community diversity and activity to climate warming is crucial for understanding the permafrost carbon feedback with increasing temperatures. Here I investigated changes in diversity and activity of microbial communities found in different soil depths within the active layer of two soil cores collected at two distinctively different permafrost thaw stages (intact and thawing permafrost) in a palsa mire in Finnmark, Northern Norway.

The high-throughput Illumina MiSeq sequencing of bacterial and archaeal 16S rRNA allowed to investigate potential changes in microbial community species richness and structure with depth in the intact (IP) and the degrading part of a palsa (DP). The intact palsa exhibited the three most species-rich soil layers but this richness decreased with depth, while species richness values were found to be similar across the degrading permafrost soil depth profile. Sequencing analysis of the 16S rRNA showed that Bacteria dominated the microbial community with the bacterial phyla Acidobacteria, Proteobacteria, Planctomycetes, Actinobacteria and Verrucomicrobia, known to be involved in C cycling, were found to be dominant in all samples. The relative abundance of Proteobacteria phylum was higher in the DP than in the IP. As this phylum was dominated by heterotrophic members of the orders Alpha- and Gammaproteobacteria known to thrive in higher C and nutrient rich environments, this might suggest an increase in C substrates and nutrients bioavailability in the DP. Interestingly, relative abundance of the poorly characterized phylum AD3 increased with depth in the IP while being almost null in the DP, suggesting that an increase in Proteobacteria abundance followed by a decrease in AD3 might represent the first step of a heterotrophic microbial community shift in a thawing palsa environment.

The two thaw stages were characterized by a significant difference in microbial community structure, with a change in community along the depth profile in the intact palsa, while the degrading palsa exhibited similar microbial populations across depth meaning that microbial communities underwent a shift with permafrost thaw.

Microbial abundance assessed by qPCR showed that Bacteria dominated the prokaryotic population. Bacteria, Archaea, methanogens and methanotrophs were more abundant in the thawing than in the intact palsa. Microbial populations tended to follow the same pattern within the same thaw stage, with the intact palsa exhibiting high abundance in the top layer followed by a drop in the underlying layers, while the degrading palsa was characterized by high and similar microbial abundance along depth suggesting an increase in microbial biomass along the soil profile with permafrost thaw. In addition, methanogens communities seemed to be spatially located near to methanotrophs populations, suggesting a strong relationship between these two actors involved in methane cycle.
Heterotrophic microbes were found to be more active in the DP as they exhibited higher enzymatic and functional metabolic activity on a broader depth scale than microbes found in the IP. They preferably metabolized carbohydrates and polymeric substances. In the intact palsa, higher enzymatic and functional metabolic activities could be linked to higher microbial richness. On the other hand, in the DP, higher microbial functional metabolic activity was correlated with higher organic matter content. Therefore, heterotrophic microbial activity in the IP might be linked to the microbial richness of a highly functionally diverse community while the activity in the DP seems to be associated with a less species-rich community of heterotrophs thriving in higher bioavailable organic matter.

In order to further deepen our knowledge of the microbial dynamics involved in the studied environment under different thaw conditions, it would be interesting to investigate thawing permafrost on a deeper depth soil profile as well as in other sites found along the permafrost thaw chronosequence (e.g. completely thawed palsa). In addition, a higher number of soil samples in each thaw stages and soil depths might give us an enhanced and more representative insight into the prokaryotic activity and diversity. New gene analysis methods such as metagenomics could improve our understanding of the microbial metabolic functions present in the soil’s communities, while metatranscriptomics and metaproteomics might be used to comprehend which genes are actively expressed and which proteins are presently produced in the microbial population. Furthermore, an increase in the number of investigated biogeochemical parameters (biotic and abiotic) is essential to gain an improved picture on the environmental parameters driving the microbial communities’ dynamics (e.g. vegetation cover, nitrogen and carbon forms and availability, in situ temperature, oxygen availability, water table levels, C/N ratio, etc). In the near future, results from the ongoing Uni Climate FEEDBACK project, measuring several in-situ GHG production and fluxes along the same study sites’ palsa thaw chronosequence might be linked to the results gained during this thesis, thus providing a broader picture on the microbial processes involved in permafrost thaw.

In conclusion, this thesis showed that microbial prokaryotic communities shifted as a result of permafrost thaw with the increased presence of certain bacterial phyla linked to C bioavailability, an increase in bacterial, archaeal, methanogens and methanotrophs biomass as well as a higher enzymatic and functional metabolic activity directed towards plant-derived carbohydrates and polymeric substances. Thus suggesting greater greenhouse gases emissions that would exacerbate the positive feedbacks from permafrost carbon to climate.
5. References


Borge AF (2015) Development and distribution of palsas in Finnmark, Northern Norway, for the period 1950s to 2010s. University of Oslo


Davidson EA, Janssens IA (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. doi: 10.1038/nature04514


Mondav R (2014) Microbial dynamics in a thawing world: Microbial ecology of a permafrost active layer. The University of Queensland


Solheim I (2016) Transient thermal modeling of palsa distribution in Northern Norway, Finnmark and the importance of estimating local factors controlling palsa development. University of Oslo


gene abundance in bacteria and archaea and a new foundation for future development.

Stolyar S, Franke M, Lidstrom ME (2001) Expression of individual copies of Methylococcus

Respond Differently to Environmental Gradients in Anoxic Sediments of a California
Hypersaline Lake, the Salton Sea. Appl Environ Microbiol 76:757–768. doi:
10.1128/AEM.02409-09

Takai K, Horikoshi K (2000) Rapid detection and quantification of members of the archaeal
community by quantitative PCR using fluorogenic probes. Appl Environ Microbiol

circumpolar permafrost region. Global Biogeochem Cycles 23:n/a-n/a. doi:
10.1029/2008GB003327

microbial communities and metagenomes in an upland Alaskan boreal forest. ISME J
8:1904–1919. doi: 10.1038/ismej.2014.36


production from anoxic soil incubations. Glob Chang Biol 21:2787–2803. doi:
10.1111/gcb.12875

along depth profiles of Alaskan soil cores. Sci Rep 8:1–11. doi: 10.1038/s41598-017-18777-x

Tsitko I, Lusa M, Lehto J, et al (2014) The Variation of Microbial Communities in a Depth
Profile of an Acidic, Nutrient-Poor Boreal Bog in Southwestern Finland. Open J Ecol
4:832–859. doi: 10.4236/oje.2014.413071

Tuomivirta TT, Yrjälä K, Fritze H (2009) Quantitative PCR of pmoA using a novel reverse
primer correlates with potential methane oxidation in Finnish fen. Res Microbiol

temperatures in permafrost. ISME J 8:139–149. doi: 10.1038/ismej.2013.140

Depth Profiles Over 120,000 Years of Ecosystem Development. Front Microbiol 8:874.

Tveit A, Schwacke R, Svenning MM, Urich T (2013) Organic carbon transformations in high-
Arctic peat soils: key functions and microorganisms. ISME J 7:299–311. doi:
10.1038/ismej.2012.99
van Everdingen RO (1998) Multi-language Glossary of Permafrost and Related Ground-ice Terms in Chinese, English, French, German ... Arctic Inst. of North America University of Calgary


6. Supplementary materials

Table 8: Quantitative PCR bacterial and archaeal 16S rRNA, methanogenic mcrA and methanotrophic pmoA gene abundance data at the different sampling depth in the two successional stage of the palsa (IP5-IP35=intact palsa; DP5-DP35=degrading palsa). ds=dry soil.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Depth (cm)</th>
<th>Bacteria 16S rRNA gene copies (g ds⁻¹) (+ SD)</th>
<th>Archaea 16S rRNA gene copies (g ds⁻¹) (+ SD)</th>
<th>mcrA (methanogens) gene copies (g ds⁻¹) (+ SD)</th>
<th>pmoA (methanotrophs) gene copies (g ds⁻¹) (+ SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IP5</td>
<td>5</td>
<td>9.12E+10 + 6.28E+09 2.66E+08 + 1.74E+07 7.74E+07 + 5.07E+06 1.61E+07 + 2.21E+06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IP10</td>
<td>10</td>
<td>9.94E+09 + 6.25E+08 1.79E+07 + 2.64E+06 8.26E+06 + 8.24E+05 1.70E+06 + 1.48E+05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IP20</td>
<td>20</td>
<td>3.68E+09 + 3.11E+08 2.08E+07 + 9.90E+05 1.96E+06 + 3.49E+05 9.74E+05 + 1.45E+05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IP35</td>
<td>35</td>
<td>8.78E+09 + 5.43E+08 2.03E+07 + 1.52E+06 4.88E+06 + 6.72E+05 2.08E+06 + 1.28E+05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DP5</td>
<td>5</td>
<td>7.74E+10 + 4.44E+09 3.34E+08 + 1.68E+07 5.84E+07 + 3.29E+06 3.32E+07 + 2.67E+06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DP10</td>
<td>10</td>
<td>8.55E+10 + 2.52E+09 3.07E+08 + 6.11E+06 7.55E+07 + 1.09E+07 3.31E+07 + 4.40E+06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DP20</td>
<td>20</td>
<td>1.06E+11 + 5.75E+09 3.48E+08 + 6.58E+06 1.23E+08 + 7.98E+06 4.06E+07 + 3.85E+06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DP35</td>
<td>35</td>
<td>7.41E+10 + 6.84E+09 2.42E+08 + 1.77E+07 7.22E+07 + 4.89E+06 4.54E+07 + 7.05E+06</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Abstract

Permafrost environments, which store large quantities of soil organic carbon, are threatened by climate change-induced thaw that would render frozen organic matter bioavailable for microorganisms leading to an increase in greenhouse gases emissions. Hence understanding the response of permafrost microbial communities to thawing is necessary to evaluate the permafrost carbon feedback to global change. Microbial diversity and activity were studied across two thaw stages (intact and degrading permafrost) at different depths in a palsa found in Northern Norway. This study investigated soil microbial community composition by Illumina Miseq sequencing of the 16S rRNA gene while bacterial, archaeal, methanogens and methanotrophs abundance was assessed by qPCR. Microbial heterotrophic activity was evaluated using enzymatic and functional metabolic assays. Microbial communities’ composition and activity were found to differ between the two thaw stages. The intact palsa (IP) presented high richness that decreased with depth while the degrading palsa (DP) exhibited less species-rich communities across depth. Relative abundance of members of the phylum Proteobacteria known to thrive in higher carbon and nutrient availability increased in the DP while members of the phylum AD3, which dominated the deepest part of the IP, almost disappeared in the DP. Bacterial, archaeal, methanogens and methanotrophs were more abundant in the thawing permafrost than in the intact palsa. The DP exhibited high and similar microbial biomass across depths while the IP showed high microbial abundance only in the topsoil layer. In addition, populations of methane-producing microorganisms were found to be strongly positively correlated to methane oxidizers abundance, suggesting a close spatial relationship between these two communities. Heterotrophic prokaryotes found in the DP displayed higher enzymatic and functional metabolic activity across depth than in the IP. Collectively, these results suggest a shift in microbial prokaryotic communities as a result of permafrost thaw characterized by less species-rich populations, by an increasing biomass of greenhouse gases-related microorganisms as well as a higher microbial activity across depth, potentially leading to greater greenhouse gases emissions that would exacerbate the positive feedbacks from permafrost carbon to climate.

Host institutions: Uni research AS / Uni research Climate / Bjerkness center for climate research / University of Bergen, Department of Microbiology – Bergen, Norway