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Microbial communities and metabolic networks in Arctic peatlands

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UNIVERSITY OF TROMSØ

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2 Acknowledgements

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3 Thesis abstract

Northern peatlands cover only ~3 % of the global land surface, but store one-third of the world's soil organic carbon (SOC). More than half of this is stored in Arctic peatlands, making it one of the largest SOC storages. Microorganisms are key players in the decomposition of SOC leading to the production of methane (CH_4) and carbon dioxide (CO_2) in these ecosystems and yet, we still have limited knowledge about them. CH_4 in is a very powerful greenhouse gas, with 26 times the effect of CO_2. Thus, its release can have drastic effects on the climate. With climate change, large temperature increases are predicted in the Arctic towards the end of the century (1–6 °C in summer and 2–11 °C in winter). How the microorganisms in Arctic peatlands will respond to this warming, and if it will result in a release of the stored carbon (C) as CH_4 and CO_2 creating a positive feedback to climate change, is currently unknown. In this PhD project we aimed at describing the microbial communities involved in SOC decomposition, CH_4 and CO_2 production in Arctic peatlands in Svalbard (78 °N), and address their sensitivity to temperature increase.

Metagenomics and metatranscriptomics is the study of all microbial genes and transcripts, respectively, in the environment. Using these methods it is possible to describe the composition of microbial communities, their genetic repertoire and gene expression. In this project we have developed and applied metagenomic and metatranscriptomic methods to characterize the *in situ* microbial communities in Arctic peatlands in Svalbard. Further, we exposed the peat to increased temperatures and studied the response of the microbial communities, and the corresponding changes in CH_4 and CO_2 production.

Our results show that the Arctic peatlands inhabit a complex community of microorganisms with members from all three domains of life (Bacteria, Archaea and Eukarya), which cooperate for degradation of SOC to CH_4 and CO_2. Bacteria are the most abundant, while smaller populations of Archaea and Eukarya are present. The different Bacteria have specific roles in the ecosystem. Several bacterial phyla, in particular *Actinobacteria* and *Bacteroidetes* are involved in the decomposition of the complex plant compounds such as cellulose, which make up a substantial fraction of the peat soil. These organisms further oxidize sugars to CO_2 (oxic conditions) or ferment them to fatty acids and alcohols (anoxic conditions). Few microbial groups are specialists in the decomposition of fatty acids and alcohols under anoxic conditions. The last step, CH_4 production, is exclusively carried out by three taxonomic orders of methanogenic Archaea. A highly important microbial function in this Arctic ecosystem is the oxidation of CH_4, generating CO_2. We found that a single species of bacteria, *Methylobacter tundripaludum*, was responsible for this function in the Svalbard peat at the time we performed our studies. This group is primarily active in the top layer of the peat, where it has access to oxygen. However, our results indicate that these organisms might also be active and oxidizing CH_4 in the catotelm of the peat where oxygen is scarce, having a larger impact on CH_4 emissions than previously thought.

In a temperature incubation experiment with anoxic peat soil, we showed that the Arctic microorganisms are well adapted to low temperature, and produce CH_4 much faster at the low temperatures found in Arctic peat than peat microbiotas from temperate environments. However, when we exposed the microbiota to higher temperatures (5–30 °C), it had a drastic effect. We observed that it adapted quickly to these changes, i.e. within weeks, as indicated by a substantial increase in CH_4 production. New functional guilds of microorganisms replaced those that were active at low temperature, resulting in cascade effects throughout the CH_4 producing microbial metabolic network. Predatory eukaryotes became more active, and prevented increased microbial biomass.

Despite this, the result was a substantial increase in CH_4 production, even within the predicted temperature increase for the Arctic.

This study sheds light on the complex microbial communities that are drivers of SOC decomposition leading to the formation of CH_4 and CO_2 in Arctic peatlands, and how these communities are affected by temperature increase.

4 List of papers

Paper I: Organic carbon transformations in high-Arctic peat soils: key functions and microorganisms

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Paper II: Metatranscriptomic analysis of methanogenic Archaea in Arctic peat soils.

Manuscript

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Paper III: Metabolic and trophic interactions modulate methane production by Arctic peat microbiota in response to warming

Manuscript

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5 Abbreviations

SOC: soil organic carbon

Pg: petagrams

Tg: terragrams

mRNA: messenger RNA

rRNA: ribosomal RNA

RNA: ribonucleic acid

DNA: deoxy ribonucleic acid

C: carbon

Pfam: protein family

CH_4: methane

CO_2: carbon dioxide

6 Introduction

6.1 Microorganisms in Earth ecosystems

The biogeochemical cycles on Earth are largely driven by microbially catalyzed redox reactions, determining the fluxes of the major building blocks for life (1). The taxonomic and metabolic diversity of microorganisms is immense. Microbes live in nearly all environments on and within Earth's crust, and can derive energy from a large variety of organic and inorganic substrates. Major redox reactions essential to biogeochemical cycles such as the carbon cycle is catalyzed by a set of key microbial enzymes (1). Thus, microroganisms are responsible for the majority of natural greenhouse gas (GHG) emissions, including CH_4.

The majority of microorganisms are found in oceans, soils, animals, and oceanic and terrestrial sub surfaces. The culture independent studies of microorganisms directly from the environment have yielded insight into the microbial diversity in these ecosystems (2). Metagenomics, the study of all DNA fragments in environmental samples is particularly powerful, yielding knowledge about the genetic composition of the entire microbial community. However, DNA based studies do not provide information about the activities of microorganisms. Metatranscriptomics, the study of expressed genes of a community, can complement metagenomics and provide information on the presumably active community members (3). This has enabled studies of microbial activities in the environment, providing a new understanding of the microbial communities that inhabit the ecosystems on Earth.

Microbial communities are generally complex and include a variety of organisms with different metabolic functions that can be uniquely adapted to their environment. Many of the microorganisms residing in an ecosystem have overlapping niches and therefore not active simultaneously. Thus, an important challenge in the study of microbial ecology is to identify how taxonomic and functional changes in microbial communities and changes in their activities affect the rates of biogeochemical transformations. In the context of climate change and its effect on the C balance between soil, ocean and atmosphere, such changes are of major importance.

6.2 Peatlands and CH_4 emissions

Half of the global wetlands are located at high latitudes above 50 °N (4). Northern wetlands comprise peat soils with high organic matter content and tundra soils. The wetlands are formed where the ground rock or the permafrost impedes draining. Northern wetlands are exposed to low temperatures (<10 °C) most of the year including extended periods of frost.

Northern peatlands cover only ~3 % of the global land surface and store 500 Pg SOC (+/- 100), one-third of the world's SOC (1 petagram (Pg) = 1000 Terragrams (Tg) = 1 billion metric tons) (5-7). This is equivalent to more than half the amount of C in the atmosphere (7). Recent estimates point out that half of this, 277 Pg of SOC, is in Arctic peatlands (8). Northern peatlands have acted as C sinks since the early Holocene (9), accumulating during the postglacial period at an averge net rate of 0.096 Pg/year (7). Most of the C (>270 Pg) accumulated before 7000 years ago, but available data indicate that the rate of sequestration has been higher the last decade than the average over the last 7000 years (5). Peatlands accumulate C because the annual net primary productivity of the vegetation exceeds the annual decomposition. In peatlands, both the net primary productivity (NPP) and decomposition rates are low, but over millennia, the NPP has been greater (10).

Peat soils are generally divided in two sections: the acrotelm and catotelm (11). The acrotelm is above the water table, the density is lower, and the availability of oxygen (O_2) supports a higher

decomposition rate of relatively fresh organic matter. The catotelm is below the water table, the density is higher, and the chemical transformations to more recalcitrant organic matter during the transport of organic matter through the acrotelm combined with the absence of O_2 and low temperatures cause low decomposition rates. The two main types of peatlands (or mire; peat forming wetland) are fens and bogs. The characteristics of fens and bogs are overlapping, and these ecosystems are often described in terms of ecological gradients or a continuum (12). Bogs are generally acidic (pH <6), dominated by sphagnum mosses and nutrient poor with precipitation being the only source of water. Fens have a higher pH (>6), groundwater inflow, often dominated by sedges and grasses and richer in minerals and nutrients.

The vegetation in northern peatlands includes mosses, grasses, sedges, shrubs and trees, depending on the conditions of the site. Changes in plant composition have been shown to be a significant modulator of GHG emissions from peatlands, including CH_4 emissions and net ecosystem CO_2 exchange (13). Warming has already caused changes to the composition and relative abundance of plant species in the Arctic, promoting the growth of shrubs (14). In peatlands, it is expected that warming will lead to an increase in vascular plants at the expense of mosses (15, 16).

Northern peatlands are substantial sources of CH_4, releasing 30-60 Tg per year corresponding to ~6 % of the global CH_4 emissions (17, 18). CH_4 fluxes vary considerably between different peatlands (bogs and fens) and also locally within peatlands (19). CH_4 is a radiatively active trace gas, considered to have a significant effect on the global radiative balance. It is estimated that CH_4 has about 26 times the effect of CO_2 (on a mol to mol basis) in absorbing the infrared radiation from Earth (20). The atmospheric CH_4 concentrations have increased by approximately 150 %, from nearly 700 ppb (parts per billion) to 1775 ppb during a time span of 255 years (1750 to 2005) (21). This represents a much higher rate of increase than that of CO_2, estimated to 36 % increase during the same time period (21).

6.3 Climate change in the Arctic

Northern peatlands are environments characterized by low, but highly variable temperatures in the top layer, while the deeper layers are exposed to continuously low temperatures. These soils are also frozen during extended periods of the year. The C sink function of northern peatlands is labile due to its sensitivity to climate change (22-25).

The mean Earth surface temperature has increased by 0.8 °C since the late 19thth century (26). The trend has continued into the 21st century, the global surface warming trend since 1997 being 0.11-0.12 per decade (27). These changes are similar to the most conservative models of the IPCC 2007. Increases in global mean surface temperature at the end of the 21st century have been estimated at 1 to 3.7 °C depending on the model (26). Contrary to this, the temperatures in Arctic regions have increased at twice the rate of CO_2 over the last 100 years (21). The Arctic is predicted to experience a stronger temperature increase until the end of the century (2.5–10 °C for winter and 1-5 °C for summer, surface air temperatures). However, current and future temperatures are and will be spatially variable (28). Precipitation is predicted to increase in the Arctic regions towards the end of the century (10-50 % in winter and 10-30 % in summer), and also expected to be spatially variable (28).

Changes in temperature directly affects the growth and function of microorganisms and plants, and the depth of the active layer, while precipitation determines the height of the water table, affecting plant growth and the redox conditions of the soil. Thus the projected changes might

have numerous consequences for the SOC composition of northern peat soils,its decomposition and GHG emissions.

6.4 SOC composition in peat soils

6.4.1 Plant fraction of SOC

The majority of the SOC in peat soils stems from mosses and/or vascular plants, primarily grasses and sedges. Plant tissues are made up of structural components in membranes and cell walls and intracellular compounds including storage materials. The intracellular compounds can be grouped into starch, fructans, pigments and proteins, proteins being the most abundant component (29). Cell membranes are composed primarily of lipids. While lipids, proteins and storage compounds such as starch are quickly decomposed, the primary cell wall compounds are decomposed at a slower rate (29), thus making up the majority of SOC. Grass (Fig. 1A) and moss (Fig. 1B) cell walls contain many similar polymers, but there are some notable differences in both composition and structure. The major structural component of these cell walls is cellulose, an easily degradable polysaccharide despite its crystalline properties (85 % is present in this conformation) (30). The cellulose fibers are surrounded by branched hemicelluloses (e.g. xyloglucan, xylan, mannan and arabinoxylan), pectins (e.g homogalacturonan and rhamnogalacturona), lignin (only in vascular plants), other phenolic polymers (mosses) and structural proteins (31, 32). Mosses have been shown to be relatively richer in mannans (hemicellulose), glucuronins and galacturonans (pectin) than vascular plants (33).

6.4.2 Microbial fraction of SOC

The major part of the microbial SOC fraction originates from the cell walls of the microorganisms (29). The microbial cytoplasmic content is easily degradable and makes up a very small fraction of SOC. The cytoplasmic content includes proteins, compatible solutes and storage compounds. The cell membrane consists of lipids and structural proteins. Fungal cell walls are composed of chitin, glucan and cellulose, while bacterial cell walls are composed of peptidoglycan, glycolipids, proteoglycan and glycoproteins. Archaeal cell walls have several different structures, including pseudomurein, other polysaccharide cell walls, and crystalline S-layers composed of proteins. Although little is known about the lifetime of these compounds in soil after the death of microorganisms, it has been shown that large concentrations of easily degradable aminosugars (components of peptidoglycan, glycoproteins and proteoglycan) are released under acid hydrolysis of soil. This indicates that such compounds are stabilized within the recalcitrant cell wall matrix for a long time and degraded together with the overall structure (29). Thus, nitrogen containing compounds can be preserved and remain sources for microbial decomposers in the catotelm.

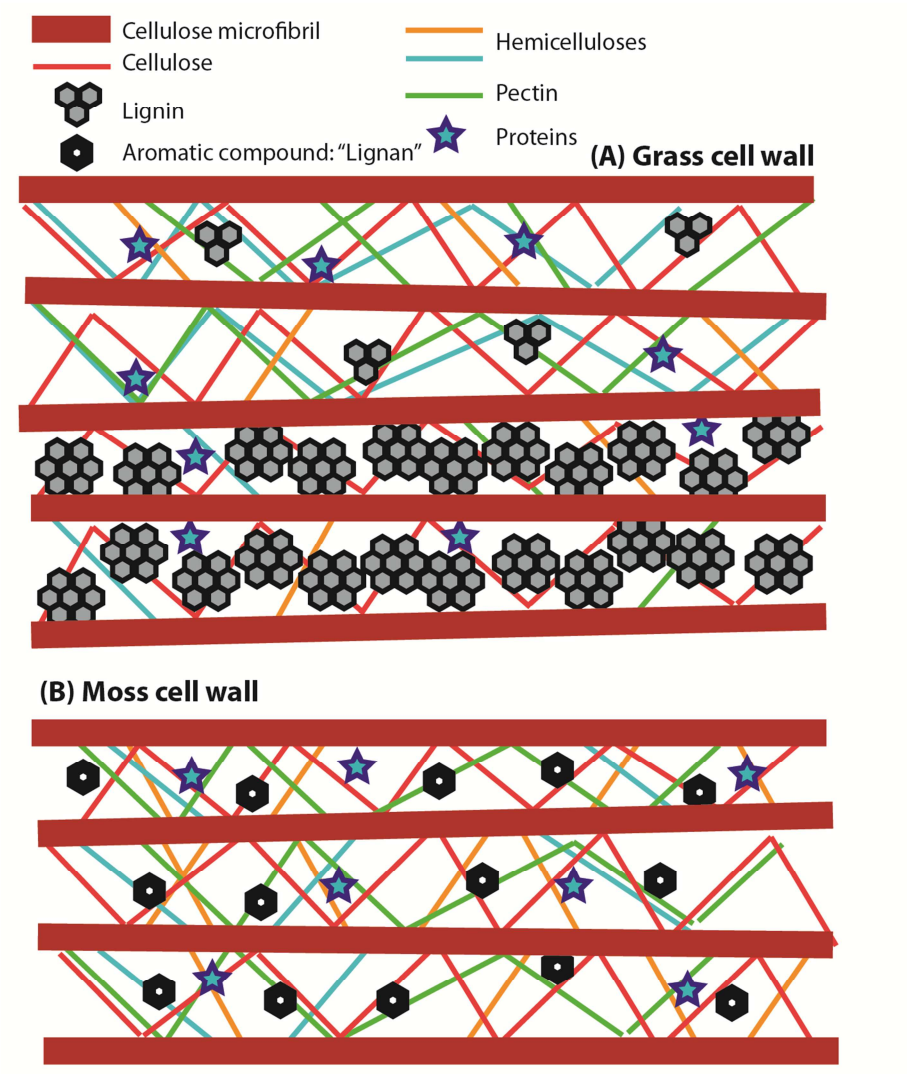

Figure 1: The structure of grass (A) and moss (B) cell walls. Modified from (31).

6.5 Microbial decomposition of SOC

The major players in SOC decomposition are microorganisms of the bacterial, archaeal and eukaryotic domains of life. The microbial communities in peatlands have been reviewed recently (34). These communities are characterized by organisms that have adapted to anoxic conditions and low temperature. Studies suggest that the microbiota in these soils are organized as repeat mosaics, structured by the vegetation, hydrology and redox conditions rather than geographical distance (34). Most known microbial phyla have been identified in peatlands, but some, such as the *Actinobacteria* and Proteobacteria are often dominating (34). These microorganisms participate in a cascade of decomposition steps, eventually resulting in the emission of CO_2 under oxic conditions, and CH_4 and CO_2 under anoxic conditions (Fig. 2). Most of the plant organic matter is polymeric, and the initial step of its decomposition is the hydrolysis of plant polymers, including different polysaccharides and proteins, and the oxidation (aerobic) or reduction (anaerobic) of lignin. Further follows the respiratory mineralization to CO_2 or fermentation followed by methanogenesis. The different decomposition steps are catalyzed by different enzymes, some of which are unique to the respective processes.

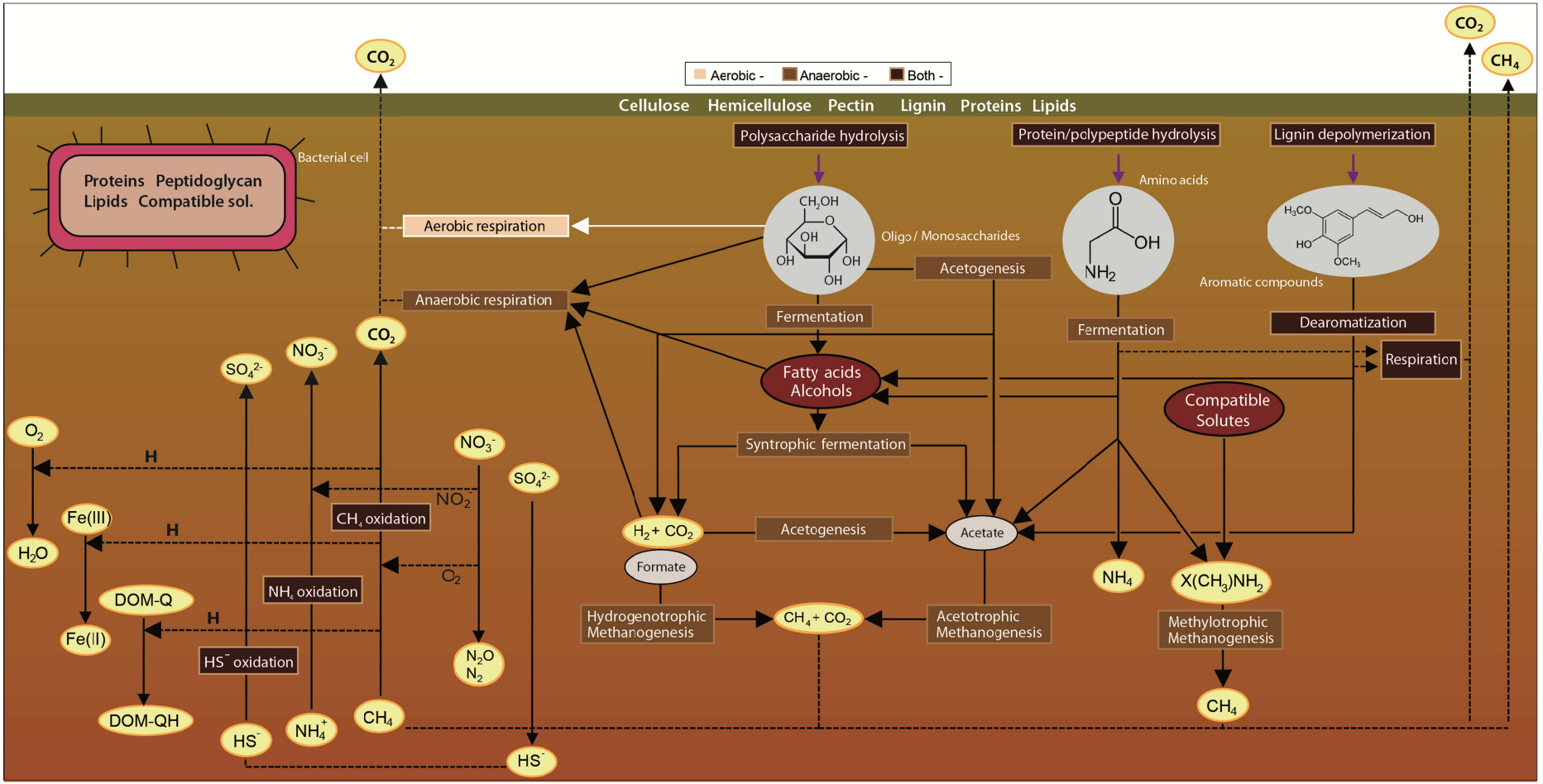
CO2
CH4
Aerobic -
Anaerobic -
Both -
Cellulose Hemicellulose Pectin Lignin Proteins Lipids
Bacterial cell
Proteins Peptidoglycan
Lipids Compatible sol.
Polysaccharide hydrolysis
Protein/polypeptide hydrolysis
Lignin depolymerization
Amino acids
Aerobic respiration
Oligo / Monosaccharides
Acetogenesis
Anaerobic respiration
Fermentation
Fermentation
Aromatic compounds
Dearomatization
Respiration
Fatty acids
Alcohols
Compatible
Solutes
Syntrophic fermentation
NO3-
SO4 2-
O2
H
Fe(III)
H2O
NO2-
CH4 oxidation
H2 + CO2
Acetogenesis
Acetate
Formate
NH4
X(CH3)NH2
DOM-Q
Fe(II)
NH4 oxidation
HS- oxidation
DOM-QH
N2O
N2
Hydrogenotrophic
Methanogenesis
CH4 + CO2
Acetotrophic
Methanogenesis
Methylotrophic
Methanogenesis
CH4
HS-
NH4+

Figure 2: The main pathways of SOC decomposition, CH_4, nitrogen and sulphur cycling in peat soils. These pathways are also relevant to other wetlands and anoxic ecosystems. The process boxes are color coded to indicate whether they occur under oxic (beige), anoxic (light brown) or both oxic and anoxic conditions (dark brown). The distinction between grey, yellow and red circles is for increased readability, not a classification of compounds.

6.5.1 Enzymes in polymer decomposition

Microbial protein decomposition is initiated by hydrolysis of the peptide bonds of the amino acid chain. Many peptidase families exist, with different substrate specificity, which catalyzes the hydrolysis of peptide bonds (http://merops.sanger.ac.uk/). Microorganisms excrete these into the surrounding environment for hydrolytic decomposition of proteins. Released amino acids are transported into the cell and used for energy conservation and/or assimilation for cell synthesis purposes.

For each type of polysaccharide there are several protein families with different catalytic specificity that catalyze the cleavage into monomers (http://www.cazy.org/) (35). These are named glycoside hydrolases after the hydrolytic (addition of water) reaction they catalyze, cleaving the glycosidic bond between monosaccharides. An overview of the major enzymes involved in polysaccharide hydrolysis and their catalytic activity is given in Fig. 3. In most anaerobic hydrolytic bacteria the enzymes for polysaccharide hydrolysis are organized in complex enzyme systems attached to the outside of the cell walls (30). Aerobic bacteria and some anaerobic bacteria release the enzymes into the surrounding environment. The ability to hydrolyze polysaccharides, e.g. cellulose is widely distributed within the domain Bacteria (36, 37). Due to the complexity of plant cell walls, where the different polymers are intertwined, some cell wall degrading microorganisms have enzyme systems that catalyze both decomposition of different polysaccharides and other polymers (30, 38, 39).

Cellulose is degraded in three sequential steps, exoenzymes cleaving cellulose chains into oligosaccharides, endoenzymes cleaving off mono and di-saccharides at the termini of cellulose chains, and finally, enzymes splitting up oligosaccharides generating monosaccharides (30, 40). Hemicelluloses are often highly branched and composed of many different sugars, and the decomposition requires several sets of enzymes. The debranching enzymes (e.g. Arabinofuranosidases, Rhamnosidases) cleave off monosaccharides from the side-chain, while enzymes equivalent to the three variants of cellulases (e.g. xylanases and mannanases) degrade the core chain (40, 41).

It is recognized that a wide variety of bacteria and fungi have the ability to decompose lignin (42, 43). Most known mechanisms require the availability of O_2 or hydrogen peroxide (H_2O_2), and occurs under oxic conditions (42, 43). Key enzymes for depolymerization of lignin are laccases, peroxidases, catalases and dioxygenases (39, 42-44). In enzymatic combustion of lignin typical for the white rot fungi, enzymatically generated H_2O_2 oxidizes the lignin polymer in a reaction catalyzed by peroxidases (43). While laccases are not able to depolymerize lignin polymers, these enzymes catalyze the oxidation of soluble phenolic compounds, and can also depolymerize lignin in combination with low molecular weight electron transfer agents (e.g. 1-hydroxybenzotriazole) (45). Many studies have addressed the possibility for anaerobic microbial lignin decomposition (39). Anaerobic decomposition of lignin has been proposed to occur via the 4-hydroxyphenylacetate decomposition pathway, catalase/peroxidase, and enzymes of the glutathione biosynthesis pathway, but the mechanism is still not well described (44).

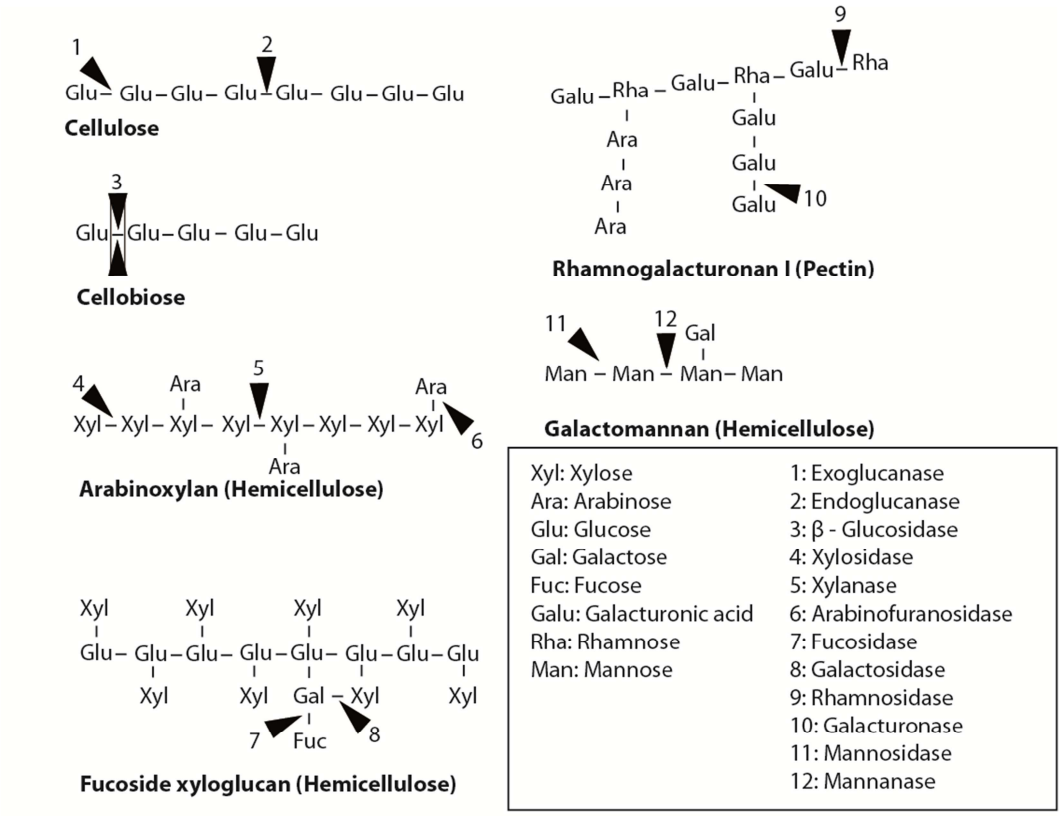

Figure 3: Enzymes catalyzing the hydrolysis of major plant polysaccharides. The arrows indicate the specific bonds that are hydrolyzed by the respective enzymes.

6.5.2 Soil humification

Humification is used to describe the chemical and biochemical changes of plant and microbial litter, and is considered to make it more dense and resistant to microbial decomposition (11, 39, 46). Humification is often noted as one of the major reasons for low decomposition rates in peat soils, along with low temperatures and the lack of O_2. However, the definition of humification ("the process whereby the C of organic residues is transformed and converted to humic substances through biochemical and abiotic processes") is vague and offers no explanation as to how this transformation occurs and what effects it has (47). Humic substances have been summarized to contain aromatic rings, alkyl C chains, simple and polymeric proteinaceous (peptide containing) compounds, lipids and polysaccharides (47). The reason for the presence of relatively easily degradable polysaccharides and peptides might be the folding and aggregation of compounds, creating hydrophobic pockets, making them inaccessible to microbial enzymes (48).

Another explanation is that conformational changes in the compounds render them incompatible with active sites of enzymes (47). For a range of oxic soils, it was shown that the humic substances contained little aromatic C and did not resemble lignin, probably due to complete aerobic oxidation (49). However, it has been shown that phenolic (an aromatic) substances accumulate to high concentrations in anoxic layers of peat soils (24, 50), suggesting incomplete lignin decomposition in these layers. The best-known aerobic decomposition of lignin, by the white rot fungi does not lead to the formation of phenolic compounds (43). Bacterial decomposition, on the other hand, has been shown to produce phenolic compounds (42). The inhibiting effect of phenolic substances on extracellular enzymes has been suggested to be a major reason for the generally low SOC decomposition rates in peat soils (24, 50).

6.5.3 Respiratory processes in peat

In the acrotelm of peat soils, a thin layer from a few centimeters to half a meter thick is primarily oxic (11). Decomposition proceeds at a faster rate in this layer than in the catotelm due to the higher redox potential and energy yield associated with microbial energy metabolism utilizing O_2 as a terminal electron acceptor in respiration. Aerobic decomposition of hexose releases approximately 7 times the amount of energy compared to fermentation (51). This allows a larger microbial population to be sustained (52-54). The decomposition of proteins, lipids and storage compounds such as starch, which are prevalent in fresh litter, is faster because these units are more easily available compared to cell wall structures. Thus it provides the microorganisms with high energy yields compared to the energy demand for cellular maintenance, as well as more nutrients from protein and lipid decomposition.

Anaerobic respiration is respiration without O_2 and uses terminal electron acceptors such as nitrate (NO_3^-), sulphate (SO_4^{2-}) and ferric iron (Fe^{3+}). In respiration, energy is generated by electron transport, creating a proton gradient across the cell membrane followed by ATP synthesis driven by proton flux through the ATP-synthase. Energy metabolism via respiration is common in most phyla within all domains of life. The two processes denitrification (reduction of NO_3^- to N_2) and sulphate reduction (reduction of SO_4^2 to H_2S) represent major sinks for nitrogen and sulphur via the release of nitrogen (N_2) and hydrogen sulphide (H_2S) gases. Thus, under anoxic conditions in peatlands and other wetlands, these can be depleted and not responsible for a large part of the SOC mineralization (55). However, in some wetlands, anaerobic respiration might be of considerable importance. Sulphate reduction has been shown to contribute 36-50% of anaerobic C mineralization in wetlands (56). The mechanisms for the regeneration of electron acceptors in wetlands are water flow, water table fluctuations, capillary action and O_2 transport in plants (56). Also, nanowires between the oxidized surface and deeper reduced layers (57) might explain the observed rates of sulphate reduction in wetlands. A complete anoxic sulphur cycle possibly driven by oxidized iron, manganese or organic matter, has been proposed (56). A complete anoxic nitrogen cycle similar to those proposed for the sulphur cycle, supplying the anoxic wetland soils with oxidized nitrogen, might also exist. Anaerobic ammonia oxidation is known, but depends on nitrite (58, 59), which is seldom available at high concentrations in wetlands.

6.5.4 Fermentative processes

Methanogenic conditions, when CH_4 is a product of SOC mineralization, arise when the system is depleted of other electron acceptors. Monomers are then mineralized via fermentation and methanogenesis involving at least four different types of functionally distinct microorganisms, primary and secondary fermenters and two functional groups of methanogens (60).

Primary fermentation is the conversion of sugars to fatty acids, alcohols and/or hydrogen (H_2), energy being generated from substrate level phosphorylation. Major products are propionate, butyrate, ethanol, lactate, acetate, formate, H_2 and CO_2 in addition to a range of other, more unusual products. In primary fermentation, pyruvate is generated from monosaccharides via the glycolysis pathway. Due to the lack of external electron acceptors, reducing equivalents needs to be removed for the regeneration of electron carriers. Thus, pyruvate might be reduced to endogenous compounds such as lactate and propionate, or oxidized to acetyl-CoA, forming acetate, while the reducing equivalents are removed as H_2. Some fermentation pathways are combinations of these reactions. The efficiency of the H_2 and formate utilizing methanogens alter the energetics and thus the pathways of fermentation (51). At low partial H_2 pressures many organisms ferment sugars to

acetate and H_2, while acetate and H_2 production is reduced in favor of fatty acids and ethanol production at higher partial pressures of H_2 (51). Primary fermentation is energetically more favorable than downstream fermentation processes (51), and thus the substrates for fermentation (e.g. hexoses, pentoses), do not accumulate. The ability to carry out primary fermentation is widespread within the domain Bacteria, and some fungi are also able to ferment sugars.

6.5.5 Amino acid fermentation

Amino acid fermentation has received far less attention than the fermentation of sugars, especially in soil ecosystems. However, studies on cultivated microorganisms, rumen, human colon, wastewaters and anoxic lake sediments, document the importance of this process (61-64). Products are primarily ammonia (NH_3), CO_2 and H_2, and acetate, but also propionate, butyrate and valerate and aromatic compounds (63). It has been shown that glycine fermentation leads to the production of monomethylamine in addition to acetate, ammonia and CO_2 (62), possibly via glycine decarboxylation, of the glycine cleavage system.

6.5.6 Cleavage, respiration and fermentation of aromatic compounds

Aerobic decomposition of aromatic compounds consists of two major reactions, hydroxylation by hydroxylating dioxygenases and downstream oxygenolytic ring cleavage of the aromatic ring by oxygenolytic dioxygenases (65, 66). However, most aromatic compounds exist in environments devoid of O_2, such as peat soils, aquatic sediments and oil reservoirs. Anaerobic catabolism of aromatic compounds is based on reductive reactions (67). The different aromatics are degraded via peripheral pathways specific to the different classes of compounds, leading to a few central aromatic intermediates. These are then dearomatized by reduction, electron donors being ATP, ferredoxin or NAD(P)H, and channeled into to the central C metabolism as e.g. acetyl-CoA, pyruvate, acetate or propionate. Energy conservation can be coupled to aerobic respiration or anaerobic respiration with e.g., NO_3^-, SO_4^{2-}, Fe^{3+}. Aromatics can also be degraded by fermentative bacteria, but the complete decomposition via fermentation depends on the utilization of the generated products such as H_2 or formate by syntrophic partners, e.g., methanogens, to be thermodynamically favorable.

6.5.7 Acetogenesis

An acetogen is an organism that can use the acetyl-CoA pathway for the production of acetyl-CoA and from CO_2. Also, energy conservation, and assimilation of CO_2 for cell synthesis occurs via this pathway (68). Most cultivated acetogens are found within the phylum *Firmicutes* (68). Acetate is the major end product of acetogenic metabolism, but some acetogens have been found to produce butyrate and ethanol (68). Acetogenesis from H_2 and CO_2 has received most attention in the study of methanogenic environments. It has been found that acetogens outcompete methanogens for H_2 at temperatures below 15-20 °C in many soils and sediments (69). Although the studies from northern peatlands addressing this are relatively few, these show that acetogens are not outcompeting methanogens for H_2 (70, 71). There might be different reasons for this, e.g., low temperature adapted methanogens that have a higher affinity for H_2 than acetogens (72), and high acetate concentrations might be a thermodynamic constraint for acetogens (71).

6.5.8 Secondary and syntrophic fermentation

Secondary fermentation of alcohols and short chain fatty acids such as propionate and butyrate is a key step in the decomposition of organic matter in anoxic environments. The oxidation of some substrates is known to be exergonic only when the concentrations of the products are kept low. Thus

the oxidation has to be carried out with syntrophic partners, typically methanogenic Archaea, which utilize the products. Transferred products are H_2, formate or acetate (60). In many organisms, propionate oxidation proceeds through the reversible methylmalonyl-CoA pathway (also utilized by some propionate producing primary fermenters, while others use the acryloyl pathway) which share many catalytic steps with the tricarboxylic acid (TCA) pathway. This pathway leads to the formation of acetate. Butyrate oxidation is carried out by β-oxidation of butyrate via crotonyl-CoA and 3-Hydroxybutyryl-CoA to acetate (73). Both pathways involve the reduction of H^+ to H_2 and/or the reduction of CO_2 and H^+ to formate ($HCOO^-$), while energy might be generated by electron transport and associated proton pumps (74). Ethanol can be oxidized in different ways. One known pathway leads to the production of acetate via acetyl-P for ATP generation, and propionate via the methylmalonyl-CoA pathway (75). Alternatively it can be oxidized to acetate, with a methanogenic partner utilizing the generated H_2 (60). Due to the low energetic yield in these metabolisms, and the sometimes dependence on two or more active partners, these steps in decomposition can be particularly sensitive to change. Syntrophic fermentation might therefore act as a rate-limiting step under certain conditions, e.g. low temperature (69, 76, 77) and after disturbance disrupting the syntrophic interactions (69). Cultivated syntrophic bacteria are found within several groups, e.g., *Firmicutes* and the *Deltaproteobacteria* (60).

6.5.9 Methanogenesis

Methanogenesis is the last step of anaerobic decomposition in ecosystems limited in other terminal electron acceptors (e.g., O_2, NO_3^-, Fe^{3+} and SO_4^{2-}). Methanogenesis is carried out by a range of different organisms within the *Euryarchaeota*, a phylum within the domain Archaea (55). There are several different pathways of CH_4 formation, primarily from H_2/CO_2, formate, acetate, methanol and methylamines. Common to all methanogenesis pathways, the methyl transfer to coenzyme M is exergonic and involved in energy generation, while other pathway specific metabolic steps are also involved (78). Methanogenesis from H_2/CO_2 and formate (CO_2 reduction) is carried out by microorganisms within the orders *Methanobacteriales*, *Methanomicrobiales*, *Methanococcales*, while the order *Methanosarcinales* can utilize H_2/CO_2, but not formate (78). Methanogenesis from acetate (acetate disproportionation) is carried out by species within the families *Methanosaetaceae* and *Methanosarcinaceae*, while methanogenesis from methanol and methylamines is carried out by members of the *Methanosarcinaceae* and the recently described order *Methanoplasmatales* (78-80). *Methanosarcinaceae* is the metabolically most versatile of the methanogenic taxa. However, its acetate and H_2 metabolism differs fundamentally from the other taxa; it has a lower affinity for acetate compared to *Methanosaetaceae* (78) and a lower affinity for H_2 compared to other hydrogenotrophic methanogens (those without cytochromes involved in the electron transport) (81). The metabolic properties and ecology of methanogens have been reviewed extensively (55, 78, 82). The ecology of methanogens is also well studied in peatlands (34).

6.5.10 CH_4 oxidation

Due to the low efficiency of energy release from methanogenic decomposition, a lot of the energy remains in the end-product CH_4. Methane oxidizing bacteria (MOB) can harvest this energy via the oxidation of CH_4 to CO_2 shuttling the reducing equivalents generated into aerobic respiration for ATP generation. Thus these organisms act as the biological filter for CH_4 in northern peatlands. The oxidation of CH_4 is primarily considered to be aerobic and is performed by bacteria within the *Alphaproteobacteria*, *Gammaproteobacteria* (83), *Verrucomicrobia* (84) and the candidate phylum NC10 (85). These bacteria oxidize CH_4 for both the conservation of energy and C assimilation, while

some are also able to carry out alternative energy metabolisms (e.g. denitrification, aerobic respiration, methylotrophy) and use alternative C sources (e.g. acetate). A general trend in peat soils is that the proportion of the *Alphaproteobacteria* MOB's seems to increase with decreasing pH (86). Anaerobic CH_4 oxidation is currently known as the reverse methanogenesis by Archaea coupled to sulphate, iron, manganese and nitrate reduction (87-89), and aerobic CH_4 oxidation with O_2 produced intracellularly from nitric oxide (85, 90). Anaerobic CH_4 oxidation has been proposed as a major sink for CH_4 in peatlands, based on stable isotope signature studies (91), but the mechanism and responsible organism(s) are unknown. It has been suggested that oxidized organic matter might be involved in the anaerobic oxidation of CH_4 (92), and anaerobic CH_4 oxidation dependent on oxidized iron has been addressed in several studies e.g. (93-95). Also, the ability of methanotrophs to survive under anoxic conditions with alternative energy metabolism based on fermentation has been shown, although not coupled to CH_4 oxidation (96).

6.5.11 The peat soil microbial loop

Grazing, on bacteria, mainly by predatory protists, nematodes and predatory bacteria, has a major impact on the dynamics of soil microbial communities and nutrient availability (97, 98). It shapes the microbial communities and limits the accumulation of microbial biomass. In forest soil ecosystems protists have been shown to make up a large fraction of the eukaryotic community (99). Also in peatlands, the abundance and diversity of protists is substantial (100). Grazing selects for bacteria with high growth rates, thus speeding up the microbial loop and releasing more nutrients into the soil (98). In addition to NH_3, nitrogen might be released as amino acids (101), triggering pathways for microbial decomposition that results in the production of methylamines, and further NH_3, CO_2 and CH_4 (See sections "fermentation of amino acids" and "methanogenesis" above).

6.5.12 Temperature effects on peat microbiota

Temperature is a measure of the kinetic energy that can be transferred between atoms or molecules, and is related to their microscopic motion. When the temperature increases, the level of energy (molecular motion or speed) that can be transferred between atoms and molecules increases (102). Chemical reactions occur when atoms or molecules collide. Thus, as the molecular speed increases, the probability of a collision between two molecules at any given point in time increase.

Under northern and particularly Arctic conditions, microorganisms adapt to low temperatures. Adaptation to low temperature includes an amino acid composition that ensures protein conformations better suited for performance at low kinetic energy, and enzyme regulation compensating for the low activity by producing more enzymes (103). Also, the membrane composition of microorganisms is different, ensuring sufficient fluidity of the membrane (103). This allows the metabolism of cold adapted microorganisms to be more efficient at low temperature (103). However, since many cold adapted microorganisms are psychrotrophs (alt. Eurypsychrophiles) and not truly psychrophilic, their activity increases with increasing temperatures, above that experienced in Arctic peat soils (104).

Modeling of the temperature dependence of microbial community metabolism in aquatic ecosystems has indicated that organisms are more or less competitive for substrates along a temperature gradient due to their thermal adaptation, resulting in different relative contribution to community metabolism from its members with temperature change (105). This is in agreement with studies on soil ecosystems finding that thermal adaptation is the switch from cold adapted taxa to warm adapted taxa (106). Many studies have indicated that biomass decreases with increasing temperature, primarily due to increased maintenance energy demand (106, 107). However, energy

spilling has been suggested as an alternative explanation, but the mechanisms of reduced growth remain unexplained (106).

In order to predict temperature related changes in mineralization rates in peat soils, it is necessary to model the relationship. According to the Arrhenius law, the rate of chemical reactions can be modeled as a linear relationship of the natural logarithm of the rate against the reciprocal of the absolute temperature. Within the thermal range of enzyme function for any given enzyme, the temperature dependence of the catalyzed reaction is assumed to follow the Arrhenius law as long as substrate concentrations are high, corresponding to a Q_{10} of 2–3 (108). Attempts to model microbial soil respiration (109) and microbial growth (110), have shown that these processes violate the assumptions of the Arrhenius law, and alternative models have been proposed to describe the temperature dependence of respiration and growth (109, 110).

6.5.13 The effect of hydrology and SOC composition on peat microbiota

Under drought, the effects of GHG emissions on radiative forcing will be primarily due to CO_2, from aerobic decomposition and increased aerobic CH_4 oxidation. Although aerobic decomposition in general has a higher rate than anaerobic decomposition, the radiative forcing effect of CO_2 is less severe, and, increased plant growth in drier soils might balance the C budget (111). Climate changes are expected to cause an increase in abundance of vascular plants with higher lignin content (15, 16). This might contribute to reduced decomposition rates in oxic soils (111), but, the effect in anoxic soils can be more drastic. Incomplete anaerobic lignin decomposition might lead to the formation of phenols, while anaerobic decomposition of phenols and other aromatics can be slow due to low energy yields (67). Thus the introduction of more lignified plant litter under anoxic conditions might promote mechanisms that limit the rate of SOC decomposition; slow decomposition of recalcitrant polymers and monomers and phenolic inhibition of microbial enzymes.

The combination of drought and rewetting, however, might enhance the overall contribution to radiative forcing (24). Drought will enhance the removal of enzyme inhibiting phenolic compounds by aerobic phenol oxidation, while the rewetting enhances CH_4 production in a less inhibiting anoxic environment.

6.5.14 Metagenomics and metatranscriptomics

The study of microbial metabolic networks in peat soil and their response to environmental change necessitates a holistic, but at the same time detailed overview of the present pathways for energy, C and nutrient metabolism, which can be inferred from the presence of genes and transcripts of key enzymes in SOC degradation. Metagenomics and metatranscriptomics are the analyses of microbial DNA and RNA that is extracted directly from communities in environmental samples, and enables a survey of the composition and activities of the different microorganisms present in a specific environment. This includes genes and protein coding transcripts (mRNA), SSU (small subunit) and LSU (large subunit) rRNA. Sequencing is preferably carried out using high-throughput sequencing technology such as 454 pyrosequencing or Illumina dye sequencing. 454 pyrosequencing has the major advantage that it generates long sequence reads 300–800bp. Illumina on the other hand, and in particular the Illumina HighSeq, has the advantage that it generates a higher number of sequences at lower cost, but the sequences are shorter; 100–200bp. With metagenomics and metatranscriptomics, it is possible to study the genomes and gene expression of uncultivated microorganisms in their natural habitat without the need for cultivation. Homology to genes encoding enzymes with known functions can be used as a criterion to infer the function of enzymes encoded by the environmental sequences. A technical advantage of these non-targeted methods is

that neither primers nor probes are needed so there is no need to anticipate the importance of genes beforehand. Studies based on high-throughput sequencing have however some limitations; dependence on database coverage of the true genetic diversity; inaccuracy of methods for functional and taxonomic annotation; for some environments such as soils, enzyme inhibiting compounds that prevent the processing of extracted DNA and RNA for metatranscriptome and metagenome sequencing; comparably poor functional annotation of short sequences.

However, the application of these methods can provide new knowledge about the microbiota and the functions encoded therein. In particular, when integrated with complementary methods such as mineralization rates, metabolic profiling and enzyme assays to test the hypotheses derived from metagenomics and metatranscriptomics. They are particularly powerful for the study of complex microbial networks, where the information stored in public databases can be applied to unravel the structure and composition of microbial communities, their genetic potential, and the environmental regulation of microbial activities and microbial interactions that determine the biogeochemical fluxes of ecosystems.

The enzymes catalyzing the major reactions of polysaccharide hydrolysis are shown in Fig. 3. For each function, one or more protein families (Pfam) that catalyze the respective reaction have been identified. Conserved structural domains have been identified for many protein families, including those that catalyze polysaccharide hydrolysis. Homology to unknown DNA or RNA sequences can be inferred by comparison to the conserved domains of known Pfam's (http://pfam.sanger.ac.uk/) using hidden markov models (HMM) (Program: HMMER; http://hmmer.janelia.org/). The rationale for basing the functional annotation on conserved protein domains is that it is more accurate (112). Sequences that encode different hydrolytic enzymes are often very similar since they share the same core function, making it difficult to differentiate them by sequence similarity searches, e.g., with BLAST (113).

However, BLAST methods are preferred for the identification of homologues to sequences encoding enzymes for downstream metabolism such as fermentation and methanogenesis. These sequences are most often easily differentiated since they encode fundamentally different enzymes, thus the width of sequence databases compatible with BLAST is preferred over the accuracy of the HMM's. Many databases are available for comparison using BLAST. Characterized enzymes and their encoding genes are structured in global metabolic networks such as the Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.genome.jp/kegg/). The major metabolic pathways leading to CH_4 and CO_2 formation under anoxic conditions, with associated enzyme category numbers, gene or enzyme names for each metabolic reaction are shown in Fig. 4.

To decrease the rate of false positive predictions in annotation of protein coding genes and transcripts, on should compare the sequences to several sequence databases using different methods, and accept only those annotations that are common to all methods. In addition, one can set as criteria that the best matching sequences in the database of choice, within a user-defined statistical threshold, needs to share the same annotated function, or it is degraded to the lowest common functional category in a hierarchical tree data representation of metabolic functions (114). This principle is frequently applied when assigning a taxonomic identity to unknown sequences using the evolutionary tree of life as a backbone for hierarchical clustering and is known as lowest common ancestor concept (LCA) (115).

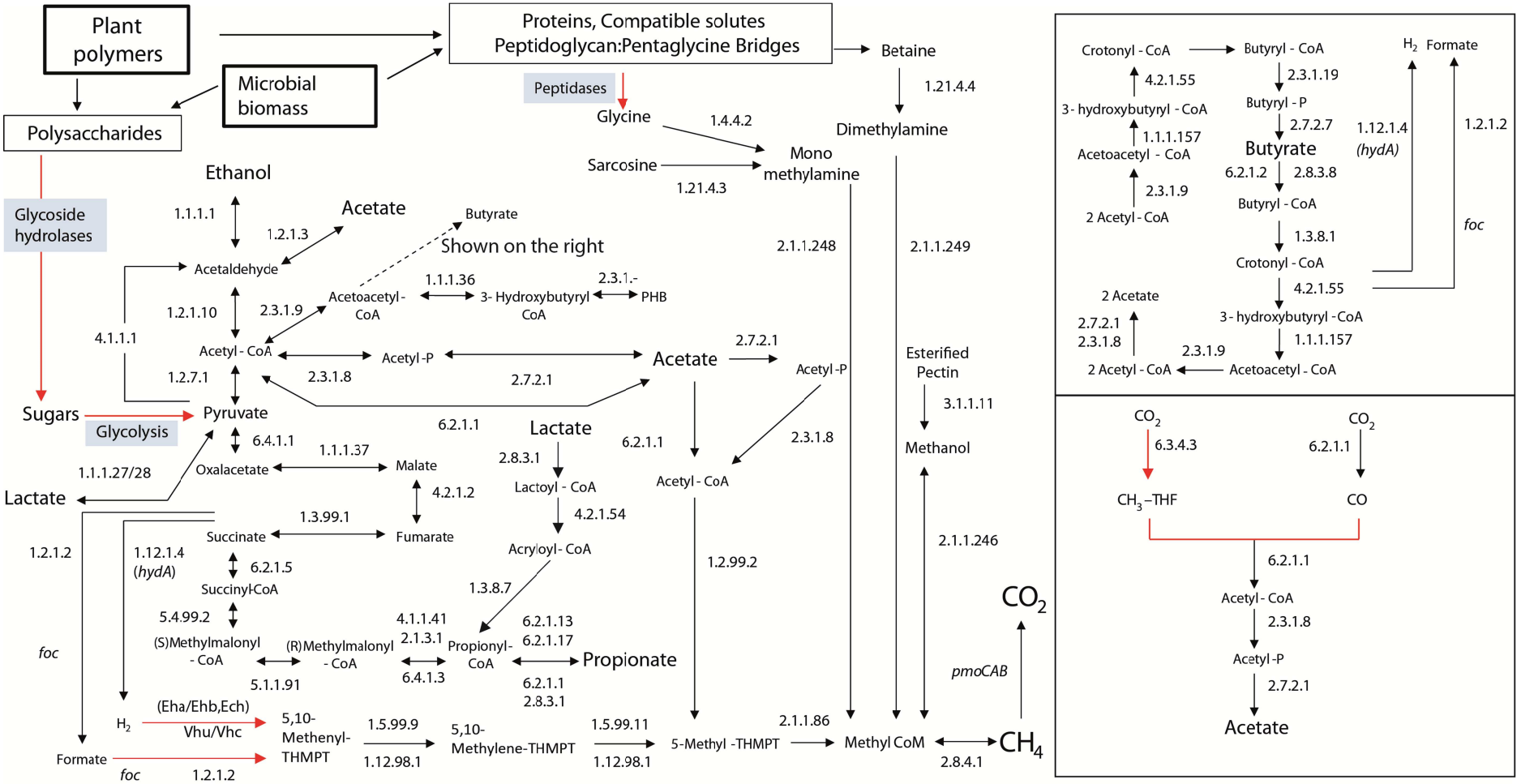
Plant polymers
Microbial biomass
Polysaccharides
Glycoside hydrolases
Sugars
Glycolysis
Proteins, Compatible solutes
Peptidoglycan:Pentaglycine Bridges
Peptidases
Betaine
Glycine
Sarcosine
Dimethylamine
Mono methylamine
1.21.4.4
1.4.4.2
1.21.4.3
Ethanol
Acetate
Butyrate
Shown on the right
Acetaldehyde
Acetoacetyl - CoA
3- Hydroxybutyryl CoA
PHB
Acetyl - CoA
Acetyl -P
Acetate
Pyruvate
Lactate
Oxalacetate
Malate
Fumarate
Succinate
Succinyl-CoA
Lactoyl - CoA
Acryloyl- CoA
Acetyl - CoA
Propionyl- CoA
Propionate
(S)Methylmalonyl - CoA
(R)Methylmalonyl - CoA
Esterified Pectin
Methanol
Methyl CoM
5-Methyl -THMPT
5,10-Methenyl-THMPT
5,10-Methylene-THMPT
Formate
H2
(Eha/Ehb,Ech)
Vhu/Vhc
foc
pmoCAB
CO2
CH4
2.1.1.248
2.1.1.249
2.1.1.246
2.1.1.86
1.1.1.1
1.2.1.3
1.2.1.10
2.3.1.9
1.1.1.36
2.3.1.-
4.1.1.1
1.2.7.1
2.3.1.8
2.7.2.1
6.2.1.1
6.4.1.1
1.1.1.37
4.2.1.2
1.1.1.27/28
1.3.99.1
1.2.1.2
1.12.1.4 (hydA)
6.2.1.5
5.4.99.2
5.1.1.91
4.1.1.41
2.1.3.1
6.4.1.3
6.2.1.13
6.2.1.17
6.2.1.1
2.8.3.1
1.3.8.7
2.8.3.1
4.2.1.54
1.2.99.2
3.1.1.11
2.3.1.8
1.5.99.9
1.12.98.1
1.5.99.11
1.12.98.1
2.8.4.1
Crotonyl - CoA
Butyryl - CoA
3- hydroxybutyryl - CoA
Butyryl - P
Butyrate
Acetoacetyl - CoA
2 Acetyl - CoA
2 Acetate
3- hydroxybutyryl -CoA
4.2.1.55
2.3.1.19
2.7.2.7
6.2.1.2
2.8.3.8
1.3.8.1
1.1.1.157
2.3.1.9
2.7.2.1
2.3.1.8
H2 Formate
1.12.1.4 (hydA)
1.2.1.2
foc
CO2
6.3.4.3
6.2.1.1
CH3 –THF
CO
Acetyl - CoA
2.3.1.8
Acetyl -P
2.7.2.1
Acetate

Figure 4: Major functions in the downstream decomposition of SOC in anoxic peat, including secondary fermentations, acetogenesis and methanogenesis. Included functions are those necessary to distinguish between metabolic pathways. Top right: Crotonyl-CoA pathway for butyrate production and oxidation. Lower right: Last steps of all pathways of acetogenesis. For secondary fermentations, complete or almost complete pathways are shown due to the substantial overlap of enzymatic reactions between different pathways. Red arrows: incomplete pathways. Blue boxes: collection of protein families. Numbers: Enzyme categories (E.C.). Abbrevations; THMPT: tetrahydromethanopterin (THS(sarcina)PT within Methanosarcinales); CoA: coenzyme A; CoM: coenzyme M. Enzyme names; Eha/Ehb and Ech: energy–converting hydrogenase; Vhu/Vhc: F_{420}-nonreducing hydrogenase. Abbreviations in italic: gene names; *foc:* formate channel; *pmoCAB*: particulate methane monoxygenase subunits c, a and b.

6.6 Aims

There are a number of gaps in our understanding of microbial function in peat soils. Major pathways of C and nutrient cycling are not understood (See Fig. 2 for overview of pathways). In particular, the knowledge about anaerobic oxidation of CH_4, ammonium/ammonia (NH_4^+/NH_3) and H_2S is incomplete. Although several mechanisms have been proposed (Fig. 2), the importance of these in northern peatlands is not yet known. Anaerobic decomposition of lignin is known, but the mechanisms are largely unexplained. Although sources for methanol and methylamines have been described, the production of, and methanogenesis from these substances are understudied aspects in peat microbiology. Also, the structure and impact of the microbial food web has been little addressed in these ecosystems. The linking of microbial function and taxonomy is a major challenge in the study of how microbial networks operate in peat and other soil ecosystems, and currently we do not know what the majority of organisms in these ecosystems are doing.

The response of microorganisms in northern peatlands to environmental change, including temperature, hydrology and SOC composition is not clear. Particularly, the effect on terminal mineralization from changes in the microbiota is not understood. Predictive models can be generated based on knowledge of the relationship between environmental change and GHG fluxes. However, the extrapolation of these associations into the near or distant future disregards the possibility that changes to the microbiota might alter the quantity of the effect from environmental change on GHG fluxes. This highlights the need for studies on the microbiota in northern peatlands, important ecosystems in Earth's C cycle.

This PhD thesis in the project entitled; microbial genomes and community gene expression in high-Arctic terrestrial ecosystems; was initiated to address these gaps in our knowledge. The project aimed to describe the microbial community, its functional potential for SOC decomposition, and its response to increased temperature in Arctic peat soils. Model peatland sites in the high Arctic Svalbard were selected based on previous studies. The first objective was to establish laboratory and bioinformatics methods for metagenomic and metatranscriptomic analysis of the microbiota in these peatlands. Further, the objective was to perform a detailed *in situ* descriptive study of the active microbial community and its functional potential for SOC decomposition, combining metagenomics and metatranscriptomics. Finally, the objective was to study the response of the Arctic peat microbiota to increased temperatures combining metagenomics, metatranscriptomics, potential enzyme activities, metabolic profiling and mineralization rates.

We wanted, by favoring analysis depth over environmental coverage, to establish the chosen peatlands as model systems for studying fundamental aspects of Arctic microbial communities in high organic soils and their response to climate change. Peat soils and soils in general are heterogeneous, and differences within a region might be as large as those found between different

regions. However, the issues we have addressed are not restricted to the specific site of study, but can be relevant for a broad range of northern peatlands as well as other wetland ecosystems.

Our hypotheses were:

- The pristine Arctic peat soil ecosystem inhabits a complex microbial community with members from all three domains of life, and contains functionally and taxonomically unique microorganisms.
- The community structure and function will change substantially as a result of increased temperature.
- Functional guilds with key roles in CH_4 production are sensitive to temperature changes; important for this process is substrate availability, which depends on fermenting microorganisms sensitive to temperature changes.

6.7 Field sites

Svalbard is a group of islands located between 74–81°N and 10–35°E, with a total land area of 62,700 km^2. Spitsbergen is the largest island with an area of 39,000 km^2. Svalbard has midnight sun from April to August, and polar night from October to February. Annual mean surface air temperature in Svalbard is between -4 and -7 °C. July is the warmest month (4–6 °C) (116). The annual mean temperature in Svalbard has increased by 2.6 °C over the last century, while the summer temperature has increased by 1 °C (117). The annual precipitation is 190–525mm (116). Svalbard has continuous permafrost. The topsoil temperature fluctuates to a large extent with the air temperature, due to limited vegetation and thin snow cover. The depth of the active layer (thawed during summer) is between 35 and 150cm (118). Svalbard has an active plant growth season of 60–70 days, during which soil temperatures are mostly well below 10 °C, only sporadically reaching higher values in the vegetation layer (119, 120). Two Ny-Ålesund peatlands were used in this study. The site Solvatn has been studied with regard to methanotroph and methanogen communities during the last two decades (119-125), while the site Knudsenheia was identified during a field survey in 2007 (ARCFAC project). Based on this, these were chosen as the field sites for the project. Both peats are located in the vicinity of the research settlement Ny Ålesund. Solvatn is situated on a marine terrace right next to the settlement, while Knudsenheia is located approximately 5 km northwest of Solvatn. The active layers of Solvatn and Knudsenheia are slightly acidic (~pH6), have a water content of ~70–90%, an organic matter content of 40–90% and is characterized by a moss cover dominated by *Calliergon richardsonii*. The mosses are interspersed by grasses (*Dupontia pelligera)* which are heavily suppressed by grazing Barnacle geese. The location of Ny Ålesund, pictures of the peatlands Solvatn and Knudsenheia, including peat structure, are shown in Fig. 5.

Figure 5: Illustration of the model peatland sites Knudsenheia and Solvatn, Svalbard peninsula**.** The model sites are located close to the Ny-Ålesund research station north-west of the Spitsbergen island. The peat surface and structure are illustrated with sampling in the field and a schematic vertical gradient.

6.8 Project proceedings

Initially, an *in situ* descriptive study of the active microbial community and its functional potential for SOC decomposition was carried out combining metagenomics and metatranscriptomics (paper I).

Some problems arose during this study which needed to be addressed; we were not able to generate data from the deeper layers of the peat soils due to enzymatic inhibition, and RNA sequencing using 454 pyrosequencing provided insufficient depth for the analysis of mRNA. These issues were solved in the second study, generating high throughput metatranscriptomic data for the quantitative analyses of mRNA in different peat layers using Illumina technology (Paper II).

Finally, a study investigating the response of the Arctic peat microbiota to altered temperatures, drawing on the knowledge generated in the two first studies was carried out (Paper III). In this study, metagenomics and metatranscriptomics were combined with targeted metabolic profiling, hydrolytic enzyme assays and terminal process measurements.

7 Discussion of main findings

7.1 Challenges and solutions for peat soil metatranscriptomics

A major limitation for the utilization of metatranscriptomics in the study of active soil microorganisms is the short half-lives of mRNA (126), and variations in half-lives between species and different genes (3). Thus, changes in the soil conditions upon sample retrieval might cause a change in the transcript patterns. In our studies (paper I, II, and III), changes in transcript patterns during sampling were counteracted by flash-freezing the samples in liquid nitrogen as quickly as possible after sampling. This was particularly challenging during fieldwork (paper I and II). A field laboratory was set up in Ny Ålesund, however, the samples needed transportation from the sites to the laboratory. To accommodate this, large peat blocks were transported in closed plastic bags under cooling. The edges of the blocks were trimmed and subsampling and processing was performed on the inner sections not contaminated by oxygen under nitrogen atmosphere. Directly after subsampling the samples were flash-frozen in liquid nitrogen.

A general problem for DNA and RNA based analyses of soil microbes is the co-extraction of enzyme inhibiting compounds (3). In the anoxic part of peat soils, inhibition is particularly problematic due to the accumulation of phenolic compounds (50). Thus, we were not able to generate metatranscriptomes from the deeper peat layers in the first study (paper I). We circumvented this problem by dilution of the RNA to lower inhibitor concentrations, and subsequent poly-A tailing and reverse transcription, and DNA dependent linear RNA amplification, generating large amounts of high quality RNA (paper II and III). Due to the low fraction of mRNA in metatranscriptomes (1-5%), we attempted to enrich the mRNA by removal of rRNA. The application of mRNA enrichment increased the mRNA fraction of the metatranscriptomes to 40 % of total RNA (paper II). This is as efficient as or more efficient than achieved in other complex ecosystems (127, 128). However, due to a skewing effect on the relative distribution of transcripts from the mRNA enrichment, a non-enrichment protocol was preferred.

High-throughput sequencing data from two different sequencing platforms were analysed in this project; 454 titanium pyrosequencing data (paper I) and Illumina HighSeq paired end (PE) data (Paper II and III). At the time of sequencing with the Illumina platform, artificial replication of cDNA fragments during sequencing was not considered an issue (129), and no dereplication was performed. However, during analysis we observed that there was 100–1000 fold difference in the relative abundances of some mRNAs between biological replicates (Paper III), indicating artificial replication; a major problem for quantitative analysis if not handled. This has also been observed in viral metagenomes generated with Illumina technology (130). We decided to remove all but one sequence in pools of identical sequences to remove these presumably artificial replicates. In consequence, the proportion of naturally occurring replicate sequences removed will by chance be equal for all samples, thus allowing quantitative analysis and cross sample comparison.

7.2 The microbial community and its metabolic function

We described the active microbial community and the overall functional potential in Arctic peat soils in Svalbard by metatranscriptomic and metagenomic (paper I). The linking of taxonomy and function has been proposed as one of the major challenges in microbial ecology (131). Several methods exist that enables it. Among these are correlations of microbial community profiles and geochemical data (132), DNA-SIP (133) and RNA-SIP (134) and the taxonomic annotation of environmental protein coding genes and transcripts (Paper I and III). The major advantage of taxonomic annotation is that it can be applied without adding labeled substrates. Metagenomic studies had been carried out in other Arctic soils prior to our study (135, 136); however these did not address the active microbial communities. Our study showed that the Arctic peat soils harbored a diverse active bacterial community dominated by *Actinobacteria* and *Deltaproteobacteria*, a large protist population and a very small archaeal population in the upper layers of the peat soil. By taxonomic assignment of protein coding genes and transcripts we identified the putative roles of microbial phyla in important functions such as hydrolysis, respiration and fermentation. From the taxonomic assignment of mRNA and 16S rRNA it was shown that nearly all active methanotrophs were closely related to *Methylobacter tundripaludum*. The microbial communities in anoxic microcosm incubations of the Arctic peat soil were different from the upper peat layers (paper III). Anoxic conditions favored *Firmicutes* and *Bacteroidetes* and *Actinobacteria*. By the taxonomic annotation of mRNA, these groups were found to be the major players in key steps of anaerobic SOC decomposition, e.g., hydrolysis of polysaccharides and secondary fermentation.

With the generated short sequence length it can be difficult to taxonomically assign protein coding gene and transcript sequences correctly, but also due to reference database biases and horizontal gene transfer. Therefore, we restricted most taxonomic assignments of protein coding genes and transcripts to phylum level taxonomy (paper I, II and III). However, correlation of mRNA relative abundances across the temperature gradient allowed us to extend our analyses to subpopulations within some phyla (paper III). Furthermore, the simultaneously obtained community composition data based on the SSU rRNA, the gold-standard phylogenetic marker of prokaryotes, enabled to some extend the verification of protein gene taxonomic assignments. For example, the majority of SSU rRNA assigned to methanotrophs was closely related to *M. tundripaludum* (paper I and III), which was isolated from Solvatn (122). Thus we could assign the mRNA binned to *Methylococcales* further to genomes, most sequences being similar to the genome of *M. tundripaludum*.

7.3 A broad functional potential for plant polymer decomposition

The metagenomic data suggest that the genetic potential for polysaccharide hydrolysis and decomposition of lignin in Arctic peat soils were very similar to soils in e.g., sub-tropical forest and grasslands, despite differences in the plant composition of the ecosystems (paper I). The quantitative relationship between plant polymers in different soils can vary due to differences in the plant community and their cell walls (31). This indicates that indigenous microorganisms in Arctic peat soils have the potential to adapt to changes in plant communities and SOC composition.

The genetic potential for phenol oxidases in all layers of the Arctic peat soils suggested that drought events could lead to decomposition of enzyme inhibiting phenolic compounds (paper I). This in turn might lead to increased extracellular decomposition of plant polymers, and increased mineralization rates, as suggested previously (24). Interestingly, the metatranscriptomic analysis of the deeper layers of the Arctic peat soils showed high relative abundance of transcripts for aerobic

decomposition of lignin relative to the upper layers (paper II). This indicates that decomposition of lignin occur under anoxic conditions in the Arctic peat soils, involving the same enzymes as aerobic decomposition, as previously suggested (44). However, the presence of transcripts and even the functional protein gives no proof of respective processes occurring. In addition, the inhibition problems experienced during the generation of metatranscriptomes (paper I) suggests that the anaerobic decomposition is not efficient and lead to the formation of phenols.

Although the genetic potential for polymer decomposition in the different peat layers in Svalbard was essentially the same (paper I), there were substantial differences in the relative abundance of transcripts for different hydrolases (paper II). In particular, the relative abundance of transcripts for cellulases decreased with depth, while those encoding enzymes that catalyze the hydrolysis of highly branched hemicelluloses increased with depth. This suggests that cellulose is being depleted, and that the microbial resource allocation is directed towards utilization of remaining substrates. However, this needs further investigations.

7.4 Anaerobic CH_4 oxidation in Arctic peat soils

The genome of *Methylobacter tundripaludum* was shown to contain nitrate and nitrite reductase operons, indicating an ability to carry out partial denitrification (137) (paper I). It has been shown that *Methylomirabilis oxyfera* has the ability to couple denitrification to aerobic CH_4 oxidation under anoxic conditions (85). Based on this, we proposed that the dominating methanotrophs related to *M. tundripaludum,* could be able to oxidize CH_4 under anoxic conditions, similarly to *Methylomirabilis oxyfera*, or alternatively respire nitrate and nitrite under O_2 deficiency (paper I). Later we found that there was a high relative abundance of protein coding genes and transcripts most similar to the genome of *M. tundripaludum* under strictly anoxic conditions (paper III). In addition, there were large gaps in the mass balances of the temperature experiment, which could be explained by anaerobic CH_4 oxidation (paper III). However, since no nitrate or nitrite was measured, some other, yet to be discovered, mechanism must explain the observations.

At least, these findings imply that *M. tundripaludum* has a metabolic state under anoxic conditions which allows it to sustain a “high alert” cellular condition characterized by remarkably high levels of pMMO transcripts. At most, the findings imply that anaerobic oxidation is a major sink for CH_4 in these soils, and that this very important environmental function is carried out primarily by *M. tundripaludum*.

7.5 Temperature response of functional guilds

A remarkable effect of temperature change was the taxonomic switches within functional guilds and the metabolic switches within taxa (paper III). Hall et al. (105) proposed that organisms in aquatic ecosystems physiologically adapt to be more or less competitive for substrates across a thermal gradient, which results in changes of relative contribution to community metabolism. In agreement with this, previous studies in soil ecosystems have showed that thermal adaptation is the switch from cold-adapted taxa to warm-adapted taxa (106). Our results indicate that similar mechanisms are triggered in the Arctic peat soils under warming, suggesting that the high diversity (i.e. richness) of the microbiota enables its functional flexibility at different temperatures. All observed effects were in the terminal steps of anaerobic decomposition: syntrophic oxidation of fatty acids and methanogenesis. This indicates that the temperature effect, which was primarily thermokinetic in upstream metabolism, becomes systemic (changes in pathways and taxa) in downstream and terminal metabolism.

The methanogenic community in the temperature experiment microcosms (paper III) was different from that *in situ*, at the time of sampling (paper II). However, these are two states of the same system and thus the observed functional plasticity should apply equally to both. Thus, we believe that taxonomic switches within functional guilds would occur under warming also *in situ*. The observed mechanisms could be relevant to a broad range of anoxic low temperature ecosystems, and also over environmental gradients other than temperature.

7.6 Temperature thresholds and rate-limiting steps in SOC decomposition

The rate-limiting step for CH_4 production from polysaccharides is defined as the step with the lowest rate. Since polysaccharides make up a major fraction of SOC in peat soils, identifying the rate limiting step and its sensitivity to temperature is important in order to understand how CH_4 production is regulated by temperature. The accumulation of propionate and acetate indicated that terminal processes were rate limiting for CH_4 production below 7 °C in the peat soil microcosms (paper III). At temperatures above 7 °C, propionate was depleted, while the concentration of acetate decreased, suggesting that more efficient acetate utilization allowed a more efficient propionate oxidation. The removal of the terminal bottleneck above 7 °C resulted in hydrolysis of polysaccharides becoming the rate-limiting step for CH_4 production at higher temperature.

Terminal processes might also be rate limiting for CH_4 production *in situ,* considering that this is in the range of Arctic summer soil temperatures and that high concentrations of fermentation intermediates were measured *in situ* (paper I). Thus temperature increases in Arctic might lead to a balanced and more efficient anaerobic SOC decomposition with less accumulating fermentation intermediates. Also, knowing that syntrophic fermentation might be limiting *in situ*, it is important to identify the effect of other conditions on this specific step to be able to predict changes in CH_4 production rates in Arctic peatlands. However, it remains to be shown if the metabolisms identified here are also rate limiting in other Arctic peat soils with different characteristics.

7.7 Accelerated peat soil microbial loop triggers shifts in methanogenesis pathways

The most pronounced temperature-related taxonomic shift was a tenfold increase in SSU rRNA of the predatory protist phylum Cercozoa, indicating increased predation of protists on prokaryotes (paper III). This was supported by a constant microbial biomass across the temperature gradient despite increased activity and substrate turnover (paper III). The increase in Cercozoa correlated with the increased relative abundance of transcripts for methanogenesis from methylamine. Methylamine originates from anaerobic decomposition of many plant and microbial cell constituents such as proteins, lipids and bacterial cell walls via the intermediates glycine, sarcosine and glycine-betaine (61, 62). Correspondingly, our results indicated that glycine, sarcosine and glycine –betaine were the major intermediates in methylamine production. This indicates that increased temperature lead to an accelerated microbial loop driven by predation of protists on prokaryotes, resulting in CH_4 production from methylamines (paper III). The large proportion of protists in the top layers of the peat *in situ* suggests that the grazing pressure might be high even at low temperatures under oxic conditions (paper I). However, in the deeper layers *in situ*, the relative abundance of protists and transcripts for methanogenesis from methylamines were low (paper II), suggesting a low grazing pressure at low temperature under anoxic conditions. However, our results suggests that Arctic warming will lead to increased predation, preventing increases in microbial biomass, but leading to

increased CH_4 production from methylamines. A putative effect of microbial loop acceleration is an increased pool of available NH_4^+/NH_3, particularly important to nitrogen limited anoxic Arctic soils.

7.8 A model for the temperature dependence of the CH_4 production rate

The CH_4 production in Arctic peat soil was high at low temperature compared to soils and sediments from sub-Arctic and temperate regions (paper III), indicating low temperature adaptation. The CH_4 production rate increased with increasing temperature, showing that the microbiota can quickly adapt to higher temperatures.

The Ratkowsky model (110) provided the best description of the temperature dependence of the CH_4 production rate in the Arctic peat soil (paper III). The Arrhenius equation adequately describes the temperature dependence of enzymatic reactions within a thermal range where the activation energy does not change (109). Modeling the CH_4 production rate with the Arrhenius equation indicated that the activation energy for CH_4 production changed continuously with temperature, thus violating this assumption of the model. This shows that the relationship between temperature and CH_4 production is not a simple relationship, as previously shown for the temperature dependence of microbial growth (110), but a result of many independent reactions, and, as shown above, a remarkable flexibility of the microbial community in its ability to adapt to changing conditions.

8 Conclusions

By the end of this century, summer temperatures in the Arctic are predicted to increase by 1–6 °C, with substantial spatial and temporal variations (28). Precipitation is predicted to increase in the same period (10–30% in summer and 10–50% in winter) (28). This will result in a warmer and wetter Arctic summer, while winter precipitation increases the extent of spring floods. The plant community is expected to be affected by the climatic changes, with an increase in shrubs and trees relative to grasses and mosses; however, this will depend on grazing and the hydrology of the soil, drier soil favoring shrubs and trees over mosses. The response of the Arctic peat microbiota to these changes is, however, poorly understood, although the microbiota determines the current and future CH_4 emissions.

In this project we have generated new and modified methods for metagenomic and metatranscriptomic analyses, and addressed the function of microorganisms in the Arctic peat soil as a biological unit. We have integrated these cutting edge molecular tools with classical methods in experimental soil microbiology to test the hypotheses generated from the meta-omics analyses. This has brought the research towards ecosystems biology, and has enabled the contribution of new knowledge about the microbiota in Arctic peat soils, and its response to temperature increase.

Our studies have shown that the functional potential for SOC decomposition in Arctic peat soil is broad. The microbiota has the potential to meet changes in SOC composition and changes in hydrology, e.g., affecting the decomposition of enzyme inhibiting phenolic compounds. The microbiota is able to respond rapidly to increased temperature, thus quickly reaching a high activity, indicating that the microbiota can cope with frequent and extreme changes in temperature. Our results indicated that this flexibility was due to subpopulations within the different functional guilds that had unique responses to environmental change.

Protist grazers became more abundant at higher temperatures, correlating with changes in the transcripts for methanogenesis from methylamines, indicating that Arctic warming can lead to an

accelerated microbial loop due to protist predation on prokaryotes and possibly a shift in methanogenic pathways. Syntrophic fermentation was rate limiting at temperatures below 7°C, as indicated by the high and negatively correlated concentrations of propionate and acetate. In a warming Arctic, the constraints on syntrophic fermentation might be relieved, leading to more efficient CH_4 production. The overall CH_4 emissions from Arctic peatlands depend on the efficiency of aerobic and possibly also of anaerobic CH_4 oxidation. These processes are carried out by a small group of methanotrophs in the peat soils in Svalbard and possibly other Arctic soils. The sensitivity of this group to climate change will be important in determining the rates and magnitudes of CH_4 emissions in a warming Arctic.

The hypotheses set for the project have been addressed and new knowledge about the microbiota in high-Arctic peatland ecosystem has been generated. However, as one question is answered, two new questions arise. Thus, we are left with more questions and identified challenges than we started out with. Below, some of the major ones are put forward.

9 Future perspectives

The interaction between substrate availability and microbial activity should be further explored. Identification of polymers in different peat layers can reveal if microbial gene expression reflects the overall changes in SOC composition or if microbial decomposition is directed towards specific components in the soil. The analysis of SOC composition needs to identify polymeric structures at the resolution of Pfam substrate specificity. New methods applying microarray technology (microarray polymer profiling), used for plant cell wall characterization (*139*) allow for such analyses. Further studies should also link gene expression to the potential activity of the corresponding enzymes, and the actual rate of decomposition in the peat layers. This would make it possible to design experiments that specifically target the climate sensitivity of the microbial decomposition of the major polymers in peat layers. Also, it would provide information about another important issue; whether higher mineralization rates over time will lead to decreased rates due to depletion of accessible C pools.

Future studies should also aim to identify the mechanisms underlying syntrophy and in particular the negative correlations of propionate and acetate concentrations at low temperatures (see paper III). This can be done by time series monitoring of anoxic microcosms above and below the temperature threshold, combined with fatty acid and CH_4 measurements and either metagenomics/ metatranscriptomics or RT qPCR targeting the propionate oxidizer and the hydrogenotrophic and acetoclastic methanogen. Also, calculating the Gibbs free energy (ΔG) for these reactions for different temperatures and different concentrations of substrates and products can reveal whether the phenomenon can be explained by thermodynamic constraints. Identifying the mechanism(s) can help to explain, under which conditions *in situ*, syntrophic fermentation becomes a limiting step for CH_4 formation.

Stable isotope probing (SIP) with $^{13}CH_4$ could provide the experimental framework for testing the hypothesis that anaerobic CH_4 oxidation is a major sink for CH_4 in Arctic peatlands and that *M. tundripaludum* is the dominant methanotroph. Analysis of labeled CO_2, if any, could reveal the extent of anaerobic CH_4 oxidation, while the analysis of labeled mRNA could identify the mechanisms involved, further enabling the cultivation of *M. tundripaludum* under anoxic conditions.

The microbial food web appears to be important in the Arctic ecosystem and could be investigated further with stable isotope labeled glycine, sarcosine, glycine-betaine or bacteria and

measurement of labeled methylamines and CH_4. This could reveal whether there is a link between the microbial loop and the methane cycle. The additional analysis of labeled mRNA could indicate which organisms are actively involved and identify the pathways through which these compounds are metabolized. Investigating in more detail the effect of grazing on these pathways and CH_4 production is however challenging. One strategy could be to isolate the predator, and analyze the chemical composition of its excrements when fed bacterial biomass. However, to quantify its effect on the bacterial biomass and the CH_4 cycle in microcosms at different temperatures, one could inhibit the protist with specific toxins towards the 28S rRNA (*140*), and analyze the microbial biomass, CH_4 production and transcripts for the degradation of glycine, sarcosine, glycine-betaine and methylamines in comparison to control microcosms.

Efficient analysis pipelines for the large amounts of data generated is needed for future research in microbial ecology. In particular, it is important to assemble environment specific metabolic network models where all enzymatic steps in metabolic pathways important to biogeochemical cycles are represented. With combined functional and taxonomic annotation, the goal is to provide a pipeline, which generates taxa-specific metabolic profiles represented by matrices of gene and transcript counts. Further, when analyzing data from environmental gradients, e.g., temperature gradient, statistical correlation of transcript or gene abundances can be used to identify environmental regulation of genes and gene expression by pattern correlation, and identify complete metabolic pathways in taxa sensitive to environmental change. This task is a major undertaking, since the models must be built, sequence databases curated, and different sequence analysis methods needs to be implemented together with statistical and graphical software.

The network analysis should be applied to other environmental gradients such as hydrology and SOC composition to help deduce the link between biogeochemical cycles and the microbiota. However, in time this needs to be expanded to multifactor analyses (e.g. simultaneous changes in hydrology and temperature). Finally, these data might be implemented in ecosystem models, where the quantitative changes in microbial communities and their activity act as both a response to change and a predictor for feedbacks to CH_4 and CO_2 fluxes. Such models can be tested by the monitoring of ecosystems *in situ*, where the effect of annual changes on the microbial community and the resulting effect on GHG fluxes are measured.

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Paper I

Paper II

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Paper III

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