UiT The Arctic University of Norway

Faculty of Biosciences, Fisheries and Economics
Department of Arctic and Marine Biology

Impacts of Vegetation and Temperature Changes on Carbon Cycling Microbial Communities in Arctic Wetlands

Kathrin Marina Bender

A dissertation for the degree of Philosophiae Doctor

September 2023

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Cells in the Cold Laboratory

The cover image was created with an open art generating Ai (openart.ai) using the prompt: “Arctic Peatlands Microbes Geese Drawing”.

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Summary

Climate change causes disturbances to soil environments. Such disturbances include shifts in vegetation composition and rising temperatures, which can impact the microbial communities involved in soil carbon cycling. In this thesis, my emphasis was centered on two main aspects: the effects of vegetation changes, caused by herbivory, and the effects of temperature changes on soil organic matter decomposition and greenhouse gas emissions in Arctic wetland soils. We applied a broad range of methods, including detailed vegetation and soil physiochemical descriptions, metagenomics and metatranscriptomics, microbial growth and biomass estimates, and greenhouse gas measurements, to investigate changes in the soils and responses of the microbial communities. By combining these methods, we were able to uncover how vegetation changes can alter the activity of the soil organic matter decomposing microbial community, the soil microbial food web, and the speed of the microbial loop. We were further able describe how microbial physiological adjustments to temperature change can lead to higher methane emissions during autumn cooling compared to spring and summer warming.

This thesis therefore provides valuable new insights into the dynamics of carbon cycling and greenhouse gas emissions in Arctic wetlands, based on the response of the soil microbial communities to vegetation and temperature changes.

List of Papers

Paper 1:

Kathrin M. Bender, Mette M. Svenning, Yuntao Hu, Andreas Richter, Julia Schückel, Bodil Jørgensen, Susanne Liebner, Alexander T. Tveit

Microbial responses to herbivory-induced vegetation changes in a high-Arctic peatland

Polar Biology, DOI: 10.1007/s00300-021-02846-z

Paper 2:

Kathrin M. Bender, Victoria S. Martin, Yngvild Bjørdal, Andreas Richter, Maarten J.J.E. Loonen, Mette M. Svenning, Andrea Söllinger, Alexander T. Tveit

Tundra vegetation changes, in absence of herbivory, are coupled with an altered soil microbial food web and a faster microbial loop

Manuscript research article prepared for submission to Global Change Biology

Paper 3:

Yngvild Bjørdal, Kathrin M. Bender, Victoria S. Martin, Liabo Motleleng, Alena Didriksen, Oliver Schmidt, Torben R. Christensen, Maria Scheel, Tilman Schmider, Andreas Richter, Andrea Söllinger, Alexander T. Tveit

Physiological temperature responses in methanogenic communities control the timing and rates of methanogenesis

Manuscript research article prepared for submission to The ISME Journal

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List of Abbreviations

C	Carbon
CH_4	Methane
CO	Carbon monoxide
CO_2	Carbon dioxide
ER	Ecosystem respiration
GEP	Gross ecosystem photosynthesis
GHG	Greenhouse gas(es)
O_2	Oxygen
OM	Organic matter
N	Nitrogen
NEE	Net ecosystem exchange
NH_3	Ammonia
NO	Nitric oxide
NO_2^-	Nitrite
NO_3^-	Nitrate
N_2O	Nitrous oxide
P	Phosphorus
SIC	Soil inorganic carbon
SO_4^{2-}	Sulfate
SOC	Soil organic carbon
SOM	Soil organic matter

Part I

Thesis

1 Introduction

1.1 Climate change

1.1.1 Facts in 2023

The United Nations defines "Climate change" as long-term shifts in temperature and weather patterns caused primarily by increasing emissions of greenhouse gases (GHG), such as carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O), which are attributed to anthropogenic activities (IPCC, 2023; United Nations, 2023). The concentration of CO_2 in the atmosphere has increased by approximately 50 % since the pre-industrial era (~1750) and is currently at its highest concentration over the past two million years (Fahey et al., 2017; IPCC, 2023). CH_4 emissions have increased by 156 %, and N_2O emissions by 23 %, leading to concentrations higher than in the past 800,000 years (Fahey et al., 2017; IPCC, 2023). Approximately half of this increase has occurred in the past 40 years and was linked to anthropogenic activities. In 2019, the main contributors to anthropogenic GHG emissions were the energy sector (34 %), industry (24 %), agriculture, forestry, and other land use (AFOLU) (22 %), transport (15 %), and buildings (6 %) (IPCC, 2023). While GHG emissions are still increasing, land and ocean sinks have been consistently absorbing around 56 % of the global CO_2 emission per year over the past 60 years (IPCC, 2023).

The accumulation of GHG in the atmosphere has resulted in a wide range of consequences. The most prominent consequence is the increase in global surface temperatures, which has been 1.1 °C higher in 2011 – 2020 compared to 1850 – 1900. This increase in temperature has occurred faster since 1970 than in any other 50-year period over the past 2000 years, with larger temperature increases observed over land (1.59 °C) compared to over oceans (0.88 °C) (IPCC, 2023). Global surface warming has therefore large implications for soil carbon (C) cycling and the microbial communities involved in it.

A more detailed description of terrestrial C cycling and the effect temperature has on the involved microbial communities are provided in chapter **1.2.3** and **1.4.3**, respectively. However, before having a closer look into terrestrial C and soil microorganisms, the more general effects climate change has in the Arctic and its ecological consequences will be introduced.

1.1.2 Climate change in the Arctic

Between 1979 and 2021, the Arctic has experienced a warming rate nearly four times higher than any other region on Earth, a phenomenon known as Arctic amplification (Rantanen et al., 2022). By the end of the 21st century, temperatures in the Arctic are predicted to rise between 3.3 °C to 10 °C, depending on the model (AMAP, 2021). This accelerated warming has profound implications on Arctic environments, including the loss of Arctic sea ice at an annual rate of 3.5 % to 4.1 % since 1979 (AMAP, 2021), the reduction of snow cover in June by 65 % between 1967 and 2012, progressing at a rate of decline of 17.2 % per decade since 1979, the mass losses of ice sheets and glaciers, and the thawing of permafrost (Taylor et al., 2017). The thawing of permafrost and the subsequent release of previously frozen organic matter (OM) has the potential to trigger additional C emissions from soils (Schuur et al., 2015). By 2100, these emissions could range from 2 % to 11 %, resulting in a potential temperature increase of +0.29 (± 0.21 °C) (Taylor et al., 2017). However, through CO_2 uptake, the expected increase in plant growth and primary production in Arctic environments is anticipated to partially counterbalance the increased C emissions induced by permafrost thaw (Taylor et al., 2017).

1.1.3 Examples of ecological consequences of climate change

Climate change has had profound and increasingly irreversible effects on various ecosystems, spanning terrestrial, freshwater, cryosphere, coastal, and open ocean environments (McCarty, 2001; Turner et al., 2020; IPCC, 2023). Animals are directly influenced by climate change in their migration, reproduction (such as changes in breeding dates and accessibility to partners), changes of their habitats, and geographic range and distribution (McCarty, 2001). For instance, the warming of sub-Arctic regions has led to the expansion of the red fox (*Vulpes vulpes*) population, suppressing the Arctic fox (*Vulpes lagopus*) (McCarty, 2001). One of the major concerns arising from climate change is the loss of biodiversity and the unprecedented extinction of animals and plant species due to the loss of habitats (Hallmann et al., 2017; Cavicchioli et al., 2019). The risk of extinction depends on a species' ability to adapt to changes. However, with the increasing frequency and severity of extreme climate changes, this adaptive response becomes increasingly difficult (McCarty, 2001).

Rising temperatures have, further contribute to greater species richness of terrestrial plants in colder regions originally characterized by low species diversity, including Antarctica, the Arctic, and alpine areas. Additionally, elevated spring temperatures have led to longer

growing seasons in the Arctic, resulting in increased vascular plant growth and primary production (Elmendorf et al., 2012; Berner et al., 2015; Keenan and Riley, 2018). However, the trend of Arctic greening may be partly reversed by extreme weather events, that inhibit vegetation growth (Phoenix and Bjerke, 2016; Treharne et al., 2019).

1.2 Terrestrial carbon and climate change

The high soil C content, especially in northern, Arctic, and permafrost regions, makes them particularly important in the context of climate change, as rising temperatures directly affect the C store capacity of soils and thus can lead to substantial C emissions to the atmosphere (Tarnocai et al., 2009).

1.2.1 Terrestrial carbon storage

Soils are important C sinks, storing up to up 2500 gigatons (Gt; 1 Gt = 1 billion metric tons) in the upper 3 m of the soil horizon (Jansson et al., 2021), one quarter of which originates from anthropogenic sources (Cavicchioli et al., 2019). The total soil C pool is comprised of approximately 1550 Gt of soil organic C (SOC) and 950 Gt of soil inorganic C (SIC). This is more than plant biomass (560 Gt), microbial biomass in soil (110 Gt) and atmospheric C (760 Gt) combined (Figure 1) (Jansson et al., 2021).

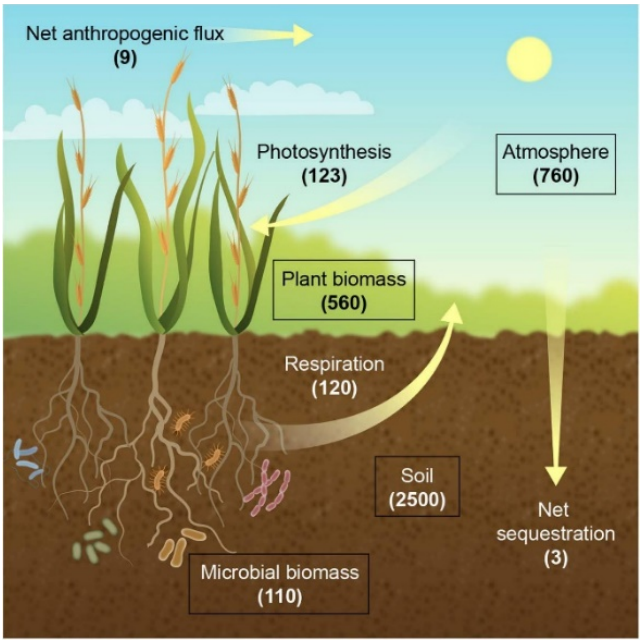

Figure 1: The figure illustrates the different terrestrial (including plant biomass, soil, and microbial biomass) and atmospheric C pools and their fluxes (mainly photosynthesis and respiration). The values of the C pools are given in gigaton (Gt), and the C fluxes in Gt per year (From: Jansson et al., 2021).

Soil C is differently distributed among different soil and vegetation types. Especially important C sinks, however, are Arctic wetland ecosystems, such as peatlands (Table 1).

Table 1: Estimated soil C storage comparing different vegetation and soil types and their respective land surface coverage. The overlap of between the vegetation and soil types causes the estimations to exceed 100%. Emphasis is the low land surface coverage to high C storage ratio of peat soils compared to the other vegetation and soil types.

Vegetation or soil type	Estimated soil C storage	Land surface coverage	References
Grasslands	~ 20 – 34 %	~ 26 – 45 %	Cavicchioli et al., 2019 Jansson and Hofmockel, 2020 Bai and Cotrufo., 2022
Forests	~ 45 %	~ 30 %	Cavicchioli et al., 2019 Poulter et al., 2014
Desert soils	~ 10 %	~ 31 %	Wang et al., 2021 Tchakerian and Pease, 2015
Peat soils	~ 30 – 40 %	~ 3%	Tarnocai et al., 2009 Xu, et al., 2018 Hugelius et al. 2020
Permafrost soils	~ 50 – 68 %	~ 11 %	Tarnocai et al., 2009 Schuur et al., 2015 Obu, 2021

1.2.2 The importance of Arctic peatlands and their role in a changing climate

Peatlands are located mostly in the northern hemisphere, and although covering only ~3 % of (Xu, et al., 2018, Hugelius et al., 2020), they contain 30 – 40 % of the global soil C (Table 1), making them the biggest terrestrial C sinks in surface to C ratio (Tarnocai et al., 2009; Cavicchioli et al., 2019). Arctic peatlands are forming where the underlying permafrost impedes drainage, establishing waterlogged, anoxic conditions (Gorham, 1991). In peatlands, the decomposability of plant litter is restricted by the structure and chemical composition of the plant input material, which is typically decomposition-inhibiting phenolics and recalcitrant polysaccharides of mosses (Ping et al., 2015; Hodgkins et al., 2018). More details about plant litter decomposability are described in chapter **1.3.3**. Additionally, the low oxygen (O_2) availability, low temperatures and often low pH decelerate rates of plant litter decomposition in permafrost affected soils (Limpens et al., 2008; Fenner and Freeman, 2011). Thus, the plant-derived C becomes locked up in the form of peat (Gorham, 1991), leading to the accumulation of enormous amounts of organic C (Limpens et al., 2008; Ping et al., 2015).

During the growing season, the upper peat (0 – 40 cm) is not always saturated with water, supporting a mix of aerobic and anaerobic biological processes. The deeper layers are usually waterlogged and anoxic (Limpens et al., 2008). The major C-fluxes that occur in the active layer of peatlands are: (1) assimilation of CO_2 (primarily through photosynthesis); (2) decomposition of plant material to CO_2 (aerobic) or CH_4 and CO_2 (anaerobic); and (3) oxidation of CH_4 to CO_2 (primarily aerobic) (Gorham, 1991; Wartiainen et al., 2003; Ping et al., 2015). A detailed description the terrestrial C cycle follows in the next chapter.

Peatlands and permafrost affected soils that have acted as C sinks for millennia are now at risk of becoming sources. This transition is driven by the thawing of permafrost (liberating previously stored C available for microbial decomposition), prolonged summer seasons (allowing for longer periods of microbial activity), and increased microbial decomposition rates, induced by higher temperatures (Strack et al., 2008; Jia et al., 2019).

1.2.3 Terrestrial carbon cycle

The terrestrial C cycle describes the balance between atmospheric C fixation and C emissions. Atmospheric C is fixed in the form of OM by autotrophic organisms through photosynthesis, and by photo- and chemoautotrophic microorganisms. Through photosynthesis 123 Gt CO_2 per year are fixed by plants and around the same amount (120 Gt CO_2) is released per year through respiration processes (Figure 1) (Jansson et al., 2021). Heterotrophic respiration by microorganisms and autotrophic respiration by plants are contributing equally to this flux with 60 Gt CO_2 per year each (Jansson et al., 2010; Cavicchioli et al., 2019; Jansson et al., 2021). On a globally scale most soils are oxic and unsaturated, and therefore the majority of C emission from soils occurs in the form of CO_2 (Gougoulias et al., 2014).

Following the fixation of CO_2 through photosynthesis, C becomes accessible to the soil environment via root exudation and through decomposition of plant litter and roots (Jansson et al., 2021). Polysaccharide hydrolysis, the depolymerization of polysaccharides from plant litter into oligo- and monosaccharides, occurs both under oxic and anoxic conditions (Warren, 1996; Tveit et al., 2013). This process is catalyzed by a wide variety of soil microorganisms from many lineages, including fungi and several bacterial phyla, such as *Actinobacteria*, *Verrucomicrobia*, and *Bacteroidetes* (van den Brink and de Vries, 2011; Tveit et al., 2013; Rai et al., 2015). The resulting oligo- and monosaccharides can be further oxidized to CO_2 through aerobic and anaerobic respiration. In aerobic respiration, O_2 acts as the

electron acceptor, while in anaerobic respiration alternative terminal electron acceptors, such as nitrate (NO_3^-) or sulfate (SO_4^{2-}), are used instead of O_2. In the absence of alternative terminal electron acceptors, fermentation of the depolymerized C takes over as the major energy harvesting metabolic process (Kelly et al., 2001; Tveit et al, 2013). Fermentation in soils is attributed to a wide range of microbial lineages, including *Firmicutes*, *Actinobacteria*, and *Bacteroidetes* (Philippot and Hallin, 2005; Wagner et al., 2005; Tveit et al., 2013). Fermentation products (e.g., ethanol, propionate, and butyrate) are degraded by secondary fermentative bacteria to intermediate fermentation products (e.g., hydrogen (H_2), acetate, and formate). Sometimes secondary fermenters and methanogenic archaea (methanogens) establish syntrophic relationships that prove mutually beneficial. This synergy arises when the fermenter benefits from the more favorable thermodynamic conditions created by low product concentrations, owing to the efficient uptake facilitated by the methanogenic partner, while the methanogen benefits from a consistent supply of substrate (Schink, 1997).
Methanogenesis is the final step in the anaerobic degradation of C and is carried out by strictly anaerobic methanogenic archaea (Schink, 1997), ultimately culminating in the production and emission of CH_4 and CO_2 through the utilization of the intermediate fermentation products (Liu and Whitman, 2008; Tveit et al., 2013; Schmidt et al., 2016). The production of CH_4 occurs via three main pathways: aceticlasitic, hydrogenotrophic, and methylotrophic methanogenesis. In aceticlastic methanogenesis, acetate is utilized for CH_4 production. In hydrogenotrophic methanogenesis, CO_2 is reduced using H_2 as electron donors. Additionally, formate, alcohols (e.g. ethanol), or C monoxide (CO) can be used as substrates for CO_2 reduction and methanogenesis (Ferry, 2011; Tveit et al., 2013; Enzmann et al., 2018; Conrad, 2020). The third main pathway of CH_4 production is methylotrophic methanogenesis, which is based on the utilization of methanol or other methylated compounds (Enzmann et al., 2018; Conrad, 2020). In most anoxic environments (e.g., water-saturated soils such as peat, lake sediments, or landfills), CH_4 is mainly produced via either aceticlastic or hydrogenotrophic methanogenesis (Conrad, 2020). Methylotrophic methanogenesis is common in saline environments (Conrad, 2020), but has also been detected in Arctic and temperate peatland soils (Söllinger et al., 2015; Tveit et al., 2015; Söllinger and Urich, 2019).

The net flux of CH_4 depends on the balance between methanogenesis and CH_4 oxidation. In the absence of O_2 in the water-saturated layers of the soils, CH_4 is oxidized by bacteria through nitrite (NO_2^-) reduction and intracellular O_2 production by nitric oxide (NO)

dismutation (Gougoulias et al., 2014, Lyu et al., 2018; Kalyuzhnaya et al., 2019; Rainer et al., 2020). In marine environments, anaerobic CH_4 oxidation by archaea (ANME) is an important process, driven by high concentrations of CH_4 and electron acceptors such as NO_3^- or SO_4^{2-}. However, so far archaeal CH_4 oxidation was not found to be important in freshwater environments or anaerobic soils (Kurth et al., 2019). CH_4 can further be oxidized aerobically in the upper oxic layers of wetland soils or freshwater environments (Rainer et al., 2020).

Terrestrial C cycling is influenced by a variety of factors, including temperature and vegetation changes. How, for example, herbivory and plant litter composition impact substrate quality and availability in soils and how this affect C cycling and C emissions from soils will be introduced in the next chapter.

1.3 Impact of herbivory on vegetation, soil organic matter composition, and carbon emissions

1.3.1 Herbivory in the Arctic

Due to climate warming, snow melt starts earlier in the Arctic, extending and enhancing plant productivity (van der Wal and Stien, 2014; Bjorkman et al., 2015). Changes in food resource availability (bottom-up effects) have the potential to improve reproduction, survival, and ultimately, population sizes of Arctic resident and migratory herbivores (Layton-Matthews et al., 2020). While some Arctic herbivore populations, like the Svalbard reindeer (*Rangifer tarandus platyrhynchus*) or the Barnacle goose (*Branta leucopsis*), have shown positive population trends associated with climate change (Albon et al., 2017; Layton-Matthews et al., 2020), there is no general increasing trend of all Arctic herbivore populations (Post et al., 2009). This can be attributed to top-down controls, for example fluctuations of predator populations like the Arctic fox (*Vulpes lagopus*), which influence the population sizes of small herbivores like lemmings (Angerbjorn et al., 1999) and voles (Ehrich et al., 2017), and the reproductive success of migratory geese (Layton-Matthews et al., 2020).

1.3.2 Arctic breeding geese

Arctic breeding geese are important herbivores (Bazely and Jefferies, 1989; Kuijper et al., 2006; Sjögersten et al., 2011) and contribute to the eutrophication of Arctic ecosystems (Hessen et al., 2017). Flyway geese are migrating to the Arctic in early spring for breeding and moulting. Since the 1950s, population sizes of European Arctic breeding geese have steadily increased, due to changes in agriculture (Fox and Abraham, 2017) and the

implementation of hunting bans (Fox and Madsen, 2017). However, the population sizes of Arctic breeding geese at their breeding grounds vary between species (Goosemap, 2023), but also between different flyway populations of the same species (AEWA, 2021). The European Goose Management International Working Group, has listed three different populations of Barnacle geese in Europe, all of which experienced huge increases in their populations; (1) the Svalbard/South-West Scotland population (Figure 2) increased from 1 350 individuals in 1950 to over 40 000 individuals in 2018, (2) the Greenland/Scotland and Ireland population had 8 277 individuals in 1950 and 72 162 individuals in 2018, and (3) the Russia/Germany and Netherlands population increased from 10 000 individuals in 1950 to 1 200 000 individuals in 2018 (AEWA, 2021). Besides Barnacle geese, the Pink-footed goose (*Anser brachyrhynchus*) also migrates to Svalbard in spring for breeding, coming from their wintering habitats in Belgium and the Netherlands (Figure 2). In 2017, the population of the Pink-footed goose was around 90 000 individuals (Madsen et al., 2017). A third, and less abundant, goose species breeding on Svalbard is the Light-bellied brent goose (*Branta bernicla hrota*), with a population size around 6 000 – 7 300 individuals in 2012 (Goosemap, 2023).

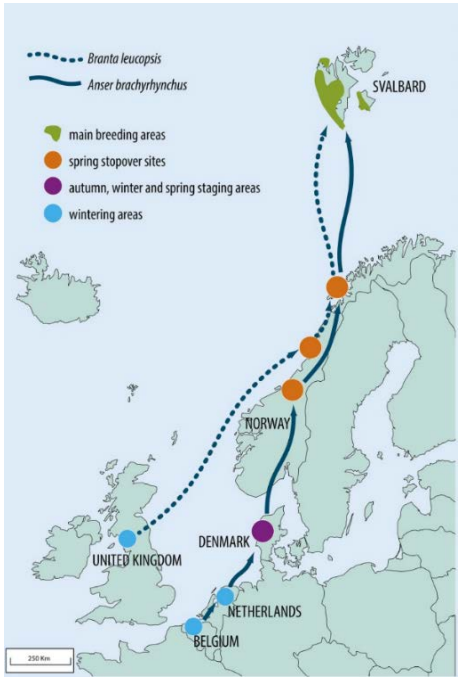

Figure 2. Migration routes of the Svalbard/South-West Scotland Barnacle goose population (*Branta leucopsis*) (blue dotted line) and the Pink-footed geese (*Anser brachyrhynchus*) (blue continuous line). Barnacle geese are wintering in the United Kingdom and South-West Scotland, while the Pink-footed geese stay in Belgium and the Netherlands over winter. Both geese species migrate in spring via Norway to their breeding grounds on Svalbard (From: Hessen et al., 2017).

1.3.3 Vegetation and carbon emissions

The effect of herbivory on tundra vegetation can range from depriving soil C stocks through disturbance and consumption of the plant cover (Sjögersten et al., 2011) to positive effects on the net C stocks through fertilization (Anderson et al., 2018) and removal of easily flammable shrubs, thereby mitigating vegetation destruction by wildfire (Kristensen et al., 2021). Furthermore, herbivory can directly alter the composition of plant communities (Zacheis et al., 2001; Maron and Crone, 2006). For example, herbivory can lead to higher plant productivity through preservation of the dominant plant species while excluding less productive ones (Bardgett et al., 1998; Bardgett and Wardle, 2003, Wardle et al., 2004) or to decreased plant and root biomass due to the depletion of the soil seed bank, by grazing on early plant shoots before they reach the flowering stage (Kuijper et al., 2006; Sjögersten et al., 2011). In Arctic tundra wetlands, herbivory by geese has been shown to negatively affect the moss layer thickness, reducing the insulating effect of mosses and their capacity to retain water (van der Wal et al., 2001; Gornall et al., 2009). Additionally, herbivory can lead to an overall decrease of litter quality by lowering the abundance and biomass of high-quality food plants, such as grasses (Kuijper et al., 2009; Sjögersten et al., 2011). Geese break down plant litter, leaving behind nutrient-rich feces pellets. These pellets contain soluble ammonia (NH_3), which supply N to the soil. This N input may enhance plant litter decomposition processes by alleviating N limitation and favoring more efficient but N-demanding decomposers (Bazely and Jefferies, 1985; Ågren et al., 2001). However, this stands in contrast to a study from 2010 which found that N from feces is mainly taken up by the moss layer, making it largely unavailable for microorganisms (Sjögersten et al., 2010).

The way in which herbivory in Arctic wetlands influences GHG emissions does not follow a clear trend. In a high-Arctic wetland, a herbivory-induced shift in vegetation composition, from vascular plant dominance to bryophyte-dominated vegetation, resulted in the transition from net CO_2 uptake to net emissions of both CO_2 and CH_4 (Sjögersten et al., 2011; Rainer et al., 2020; Foley et al., 2022). However, alterations in vegetation do not consistently result in changes in net C emissions when comparing Arctic wetland soils exposed to herbivory to those protected from it (Petit Bon et al., 2023). Hence, while herbivory by geese influences the vegetation structure and litter quality (as described in the following chapter **1.3.4**), it does not necessarily lead to shifts in GHG emissions. Potential reasons leading to this observation are discussed in chapter **4.3**.

1.3.4 Characterization of soil organic matter

The quality of plant litter can be determined by the number of enzymatic steps required to release a C atom from an organic substance. Consequently, substrates that require more steps are considered more complex and of lower quality (Bosatta and Ågren, 1999). The complexity of plant litter varies between different plant lineages, since the organization and composition of polymeric compounds is distinctive for different types of plant cell walls (Sarkar et al., 2009). Plant cell walls are composed of polysaccharide fibers like cellulose, hemicelluloses (e.g. xylan, xyloglucan, and mannan), and pectins (e.g. homogalacturonan and rhamnogalacturonan), as well as phenolic polymers like lignin, and structural proteins (Sarkar et al., 2009). In Arctic wetlands, the two predominant plant lineages, bryophytes, and vascular plants, are distinctly different in the molecular composition of their cell walls. Bryophyte cell walls are richer in mannans and galacturonans compared to vascular plants, which have higher contents of lignin and xylans (Popper and Fry, 2003; Sarkar et al., 2009). Bryophytes are typically described as decomposition-inhibiting and recalcitrant (Ping et al., 2015; Hodgkins et al., 2018). This is attributed to their high content of polyphenolics (such as lignan), structurally sturdy polysaccharides (including mannan-containing hemicelluloses), and pectins with acidic functional groups (such as rhamnogalacturonan) (Hájek et al., 2011; Roberts et al., 2012). Additionally, the breakdown of bryophyte cell wall components has been shown to release humic acids and lower the pH, further inhibiting microbial decomposition (Hájek et al., 2011; Pipes and Yavitt, 2022). Importantly, although decomposition of bryophyte cell walls may be slower compared to that of vascular plants, decomposition of bryophytes still takes place (Lang et al., 2009).

1.4 Soil microbial responses to environmental and climate changes

In terrestrial environments, microorganisms regulate the turnover of soil organic matter (SOM), making them key players in climate feedback loops (Jansson and Hofmockel, 2020; Naylor et al., 2020; Tiedje et al., 2022). The following chapters will introduce soil microorganisms in the context of climate change (**1.4.1**). Subsequently, the following two chapters will focus on soil C cycling microorganisms in the context of vegetation changes (**1.4.2**), and in the context of temperature changes (**1.4.3**), as the two main investigated factors of change in this thesis.

1.4.1 Soil microorganisms in the context of climate change

Microorganisms play crucial roles in climate change both as producers and consumers of GHG, such as CH_4, CO_2, and N_2O (Singh et al., 2010; Jansson and Hofmockel, 2020; American Society for Microbiology, 2021; Tiedje et al., 2022).

During 3.8 billion years of evolution, microorganisms have adapted to constant changes and have developed resilience. However, the current pace of climate change poses a considerable threat to microorganisms (American Society for Microbiology, 2021; Tiedje et al., 2022). Microorganisms respond to changing conditions through physiological acclimation, adaptation, dormancy, or death, but the ability to adapt to changes depends on the time required for gene regulation, transcription, translation, and mutation (Jansson and Hofmockel, 2020; Naylor et al., 2020). Those climate changes include increased temperatures (**1.4.3**), changes in vegetation (**1.4.2**), soil moisture, elevated atmospheric CO_2 concentrations, increased N deposition, and changes in physiochemical soil properties such as porosity and pH. Each of these factors has the potential to alter microbial communities in their composition, size, diversity, and distribution, both in isolation as well as in combination (Sheik et al., 2011; Maestre et al., 2015; Cavicchioli et al., 2019; Hutchins et al., 2019). Consequently, alterations in microbial communities and the expected loss of microbial diversity have been associated with reduced ecosystem multifunctionality, changing the metabolic potential of an ecosystem (Delgado-Baquerizo et al., 2016; Delgado-Baquerizo et al., 2017; Hutchins et al., 2019; Jansson and Hofmockel, 2020; American Society for Microbiology, 2021). Adding to the complexity of microbial responses to climate change is the fact that different microbial species and strains can react differently to change (Jansson and Hofmockel, 2020; Tveit et al., 2023). The multitude of potentially changing factors associated with climate change and the individual responses of different microbial species, alongside their interactions with each other, highlights the challenges to predict and generalize microbial responses across soil ecosystems (Jansson and Hofmockel, 2020).

1.4.2 Influence of vegetation on carbon cycling microbial communities

Greater diversity of vascular plants can lead to the establishment of different types of microbial communities in microhabitats centered around the roots. This favors more specialized communities that rely on root exudation or specific litter types, that may be unique to the different vascular plant types (Chroňáková et al., 2019; Sokol et al., 2022).

Furthermore, it increases the overall spatial and functional diversity of the soils due to the establishment of more different microhabitats with a more diverse input of plant litter (Sayer et al., 2017; Chroňáková et al., 2019; Sokol et al., 2022). Thereby, shifts in microbial community composition driven by changes in vegetation have the potential to influence soil C cycling by altering the functional potential of the microbial community (Antala et al., 2022).

SOM quality and microbial C decomposition

As mentioned in chapter **1.3.4**, the presence of vascular plants distinctly changes the SOM chemistry towards higher concentrations of labile C compounds in soils, compared to bryophytes (Dieleman et al., 2017). Microorganisms are utilizing SOM as a key energy source (Allison et al., 2010; Blagodatsky et al., 2010), therefore changes in the composition of SOM have the potential to either enhance microbial decomposition through priming with labile C compounds (Blagodatsky et al., 2010; Bengston et al., 2012; Dieleman et al., 2017) or reduce microbial metabolic activity through higher input of recalcitrant C (Straková et al., 2010; Dieleman et al., 2017). Microorganisms require a constant supply of labile C to efficiently degrade the recalcitrant compounds (Fontaine et al., 2007). A more diverse vegetation, composed of vascular plants and bryophytes, can therefore enhance microbial activity, and increase the microbial metabolic potential for C decomposition (Dieleman et al., 2017). Particularly in peat soils, a broadening of microbial metabolic potential can enhance the decomposition of more complex C and is believed to contribute to the breakdown and release of "old" C (Hartley et al., 2012; Walker et al., 2016; Dieleman et al., 2017), thereby leading to increased GHG emissions from soils.

1.4.3 Temperature effects on soil microorganisms

A major concern associated with climate change is the warming-induced acceleration of soil C mineralization rates and the subsequent rise in GHG emissions (Jansson and Hofmockel, 2020). It has been shown that soil warming leads to faster C loss from soil ecosystems (Tveit et al., 2015; Xue et al., 2016; Walker et al., 2018). However, C emissions often decrease after long term soil warming compared to initial warming responses, due to the depletion of more easily degradable labile C compounds (Oechel et al., 2000; Luo et al., 2001; Melillo et al., 2002; Zhou et al., 2012; Frey et al., 2013).

Temperature as control of enzymatic activity

Temperature is an important control of enzyme activity (Wallenstein et al., 2011). Essentially, each enzyme has an optimal temperature range, with reduced activity beyond those limits. Importantly though, in environmental enzyme pools, such as soils, this relationship is more intricate (Daniel et al., 2008) and enzyme activity is controlled also by the physical and chemical conditions (Wallenstein et al., 2011). For instance, the temperature sensitivity of degrading enzymes is influenced by C structure. Labile C compounds exhibit lower temperature sensitivities for enzymatic degradation compared to more recalcitrant compounds. This means that labile compounds can be more easily degraded at lower temperatures compared to more complex C compounds (Bosatta and Ågren, 1999; Knorr et al., 2005; Davidson and Janssens, 2006). Furthermore, even enzymes within the same category can display varying temperature sensitivities across different soil types (Wallenstein et al., 2011). Consequently, static thermodynamic models, such as the Q10 model (which describes the rate change of enzymatic activity with a 10 °C temperature change), are likely over- or underestimating the release of C from soils to the atmosphere in response to warming (Tang and Riley, 2015). This becomes eminent considering that for example the Q10 for soil respiration varies between 1.3 – 3.3 depending on soil type, vegetation cover, and depth (Jiang et al., 2015; Oertel et al., 2016). Additionally, temperature can influence microbial investment into enzyme production (Mairet et al., 2021). This is important because the effect of temperature on the rate of specific process depends not only its impact on catalytic activity but also the number of enzymes involved in catalysis. Therefore, such physiological adjustments can have considerable impact on C cycling.

Temperature effects on C cyling microbial communities

Temperature can also instigate changes in the diversity of soil microbial communities and the abundances of its members (Newsham et al., 2016; Zhou et al., 2016; Guo et al., 2018). Such community alterations can affect soil C cycling (Newsham et al., 2016), as shown in the case of warming-induced increases in fungal abundance corresponding with higher decomposition rates in soils (Mayer et al., 2021). Importantly though, while most microorganisms are sensitive to changes in temperature, the direction of temperature response is not always predictable (Oliverio et al., 2017). Therefore, taxon identity alone is not sufficient to

anticipate microbial responses to temperature change and should be linked to the functional potential of the microbial community (Oliverio et al., 2017; Xue et al., 2016).

Examples of temperatures dependency of different C cycling steps with focus on methanogenesis

In soil C cycling, the different steps in C degradation, which were introduced in chapter **1.2.3**, have different temperature dependencies (Yvon-Durocher et al., 2014). Methanogenesis requires a higher activation Energy (E_a = 106 Kj mol^{-1}), compared to e.g. polysaccharide hydrolysis (E_a = 54 – 125 Kj mol^{-1}) or respiration (E_a = 49 Kj mol^{-1}) (Conrad, 2023). Consequently, methanogens are more rate-restricted relative to other microbial C cycling functional groups at lower temperatures. However, the relatively larger rate increase at higher temperature can allow faster energy harvest, depending on substrate availability (Conrad, 2023).

For example, in an experiment based on anoxic microcosms with Arctic peat soil, a temperature threshold of 7 °C was identified that marked a pathway shift in SOC decomposition, resulting in changes in CH_4 production. At temperatures below 7 °C, propionate and acetate accumulated to much higher concentrations than above 7 °C, suggesting a shift in the ability of the microbial community to convert fermentation intermediates to CH_4 (Tveit et al., 2015). The pathway shifts were linked to alterations in the microbial groups responsible for SOC decomposition leading to CH_4 production, highlighting the intricate nature and complexity of microbial responses to temperature change (Tveit et al., 2015; Xue et al., 2016). Other temperature dependent pathway shifts include which of two main methanogenesis pathways in anoxic soils are dominating. Aceticlastic methanogenesis occurs usually at lower temperatures, and hydrogenotrophic methanogenesis at higher temperatures (Tveit et al., 2015; Conrad, 2020; Conrad, 2023), thereby changing the dependency of methanogens on substrate supply from SOC decomposition.

The temperature dependency of methanogenesis can help explain recent observations of higher CH_4 emission rates from wetlands during autumn cooling (CH_4 hysteresis) relative to spring and summer warming (Chang et al, 2020; Chang et al., 2021). This hysteretic CH_4 emission pattern was suggested to be linked to the accumulation of substrates for methanogenesis and the higher biomass of methanogenic archaea that had increased during

spring and summer warming, leading to the higher rates of CH_4 emissions during autumn (Chang et al, 2020).

This highlights how changing temperatures can influence *in-situ* CH_4 emissions, via changes in substrate supply, microbial community composition, physiological responses, and pathway shifts (Yvon-Durocher et al., 2014; Tveit et al., 2015; Chang et al., 2020)

1.5 Soil microbial food webs

The previous chapters have explained the basis of soil C cycling (**1.2**) and how it may be impacted by climate change (**1.4.1**), vegetation changes (**1.4.2**), and temperature changes (**1.4.3**). This chapter will elaborate on C cycling in the context of microbial trophic interactions.

As reviewed by Fierer (2017) and Thakur and Geisen (2019), abiotic factors such as pH, soil moisture, and substrate availability have traditionally been considered as the primary drivers for soil ecosystem functioning (Fierer, 2017; Thakur and Geisen, 2019). Due to the complexity of soil ecosystems, only recent advances in sequencing (metagenomic and metatranscriptomic approaches) enabled the simultaneous study of bacteria, eukaryotes, archaea, and viruses. This allowed for the inclusion of biotic factors like microbial physiological adjustments (Söllinger et al., 2022) and soil microbial food webs (Thakur and Geisen, 2019; Petters et al., 2021) into comprehensive soil ecosystem studies. We are only starting to understand the impact of trophic interactions in soil C cycling, but there is already consensus that food web interactions play a major role in ecosystem functioning (Trap et al., 2015; Geisen et al., 2018; Thakur and Geisen, 2019; Lucas et al., 2020; Petters et al., 2021).

Keystone taxa of soil microbial food webs

In general, a food web structure describes several trophic interactions among members of an ecosystem in predator-prey relationships and are often used to explain changes in population densities (Berryman,1992). In the study of soil microbial food webs, much focus has been directed towards protists and other eukaryotic micro-predators, such as nematodes and micro-arthropods as keystone taxa (Figure 3) (Trap et al., 2015; Geisen et al., 2018; Thakur and Geisen, 2019; Erktan et al., 2020; Lucas et al., 2020). More recently, prokaryotic predators, such as *Myxococcota* and *Bdellovibrionota*, have gained attention as important participants in soil microbial food webs (Figure 3) (Hungate et al., 2021; Petters et al., 2021), even though

their predatory traits have been known for some time (Stolp and Starr, 1963; Sillman and Casida, 1986; Shimkets, 1990). Additionally, more studies are now published about the soil virome and it is assumed that viruses play an equally important role in soil food webs (Figure 3) and C cycling (Paez-Espino et al., 2016; Emerson et al., 2018; Trubl et al., 2018) as reported for the other keystone taxa.

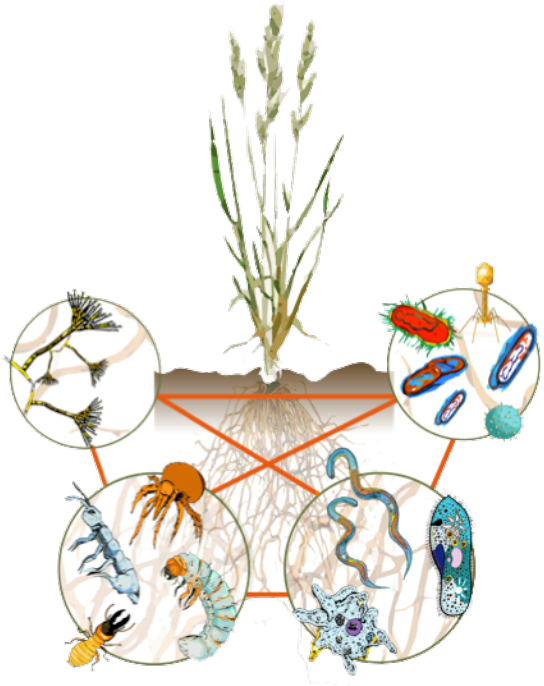

Figure 3: Illustration depicting a microbial food web structure within rhizosphere soil. The diagram comprises four groups: Bacteria and viruses (upper right); nematodes, amoeba, and other protists (lower right); micro-arthropods (lower left); and fungi, including saprophagous and mycorrhizal fungi (upper left). Predation occurs both within and between these groups, highlighting the complex interactions driving nutrient cycling and OM decomposition in the rhizosphere (From: Navarro Lab, 2023).

Ways of microbial predation and its influence on soil C composition

Soil predators exhibit diverse feeding strategies, including both selective or non-selective predation (Thakur and Geisen, 2019; Sokol et al., 2022). Different protist species, for instance, have been observed to act on several trophic levels, preying on other protists, nematodes, fungi, and bacteria (Geisen, 2016; Hünninghaus et al., 2017). Bacterivorous protists, in particular, preferentially feed on easily digestible Gram-negative bacteria (Thakur and Geisen, 2019). Soil nematodes, on the other hand, primarily feed on bacteria or fungi (Neher, 2010; Thakur and Geisen, 2019). Bacterivorous nematodes consume bacteria via

filter-feeding indiscriminately of their taxonomy, while fungi-feeding nematodes have been observed to penetrate fungal hyphae, thereby limiting hyphal growth (Neher, 2010; Thakur and Geisen, 2019). Micro-arthropods, such as collembola or mites, are known for their omnivorous lifestyle (Thakur and Geisen, 2019). While most collembola species primarily feed on fungi (Chahartaghi et al., 2005), some also consume bacteria (Ferlian et al., 2015) and plant material (Potapov et al., 2016). Viruses are commonly host specific (Sokol et al., 2022), but different lineages of viruses can infect a broad range of hosts (Emerson, 2019). Additionally, saprophagous soil animals such as earth worms and isopods as well as fungi are important consumers of decayed OM (Thakur and Geisen, 2019).

The different forms of predation can impact the composition of SOC, as the cause of microbial death can influence the input of microbial necromass into the soil (Sokol et al., 2022). This has important implications for soil C cycling, since microbial necromass is assumed to constitute more than half of the SOC (Liang et al., 2019). For instance, predation by larger protists or nematodes may result in the transfer of necromass to a higher trophic level, making the OM unavailable for other microbial groups, as it becomes sequestered within the predator (Sokol et al., 2022). Bacterial predators, on the other hand, primarily consume the cytoplasm after cell lysis (Pasternak et al., 2014), leading to necromass mainly composed of cell wall compounds and membranes (Sokol et al., 2022). In contrast, viral cell lysis additionally releases the cytoplasm to the environment, leading to a higher OM input, compared to bacterial cell lysis (Jover et al., 2014).

Implications for C cycling

Predation can influence the availability and composition of soil C. Due to the complexity of competitive and trophic microbial interactions, it however remains challenging to predict their response to climate changes (Crowther et al., 2015; Buchkowski et al., 2017), as the roles of microbial trophic interactions in C cycling remain uncertain (Jia et al., 2019).

1.6 Objectives

Arctic wetlands are exposed to numerous environmental changes, including variations in herbivory intensity, shifts in vegetation composition, and fluctuations in temperature. These wetlands store large amounts of organic C, making them highly susceptible to climate change. Whether they continue to act as C sinks or become C sources depends on how they respond to these environmental shifts.

The overall aim of the PhD thesis was to identify mechanisms that underlie microbial responses and therefore control microbial functioning in Arctic wetland soils exposed to changes in vegetation and temperature. Emphasize was laid on microbial community members involved in organic C degradation and CH_4 production.

The main objectives of the 3 papers were:

Paper 1: How does herbivory affect microbial decomposition of organic matter in Arctic peat soils?

Paper 2: How do herbivory-driven shifts in vegetation influence microbial trophic interactions and the microbial loop in Arctic wetlands?

Paper 3: What is the biological basis for CH_4 emission hysteresis (increased CH_4 emissions during autumn cooling) in Arctic wetland soils?

2 Materials and Methods

2.1 Field sites and sampling design (Paper 1 and Paper 2)

The fieldwork for **Paper 1** and **Paper 2** was carried out in the two high-Arctic wetlands Solvatn and Thiisbukta, situated close to the research settlement Ny-Ålesund, Svalbard (78°55´N, 11°56´E) (Figure 4). In the past decade (2013 – 2022) Ny-Ålesund had a mean annual temperature of -2.86 °C, a mean summer (June – August) temperature of 5.03 °C, and a mean annual total precipitation of 544.09 mm. Both wetlands are situated on top of continuous permafrost and are under the influence of herbivory by Barnacle geese (*Branta leucopsis*) and Svalbard reindeer (*Rangifer tarandus platyrhynchus*). Experimental exclosures (areas closed by 0.5 m tall fences) established at different timepoints have been used to study the effects of herbivore exclusion. Generally, herbivore exclusion has led to large changes in the vegetation in both wetlands, characterized by increased vascular plant coverage relative to bryophytes. Samples for **Paper 1** and **Paper 2** were collected inside and outside of the exclosures at the peak of the growing season in early August. In the following, details for both papers are given.

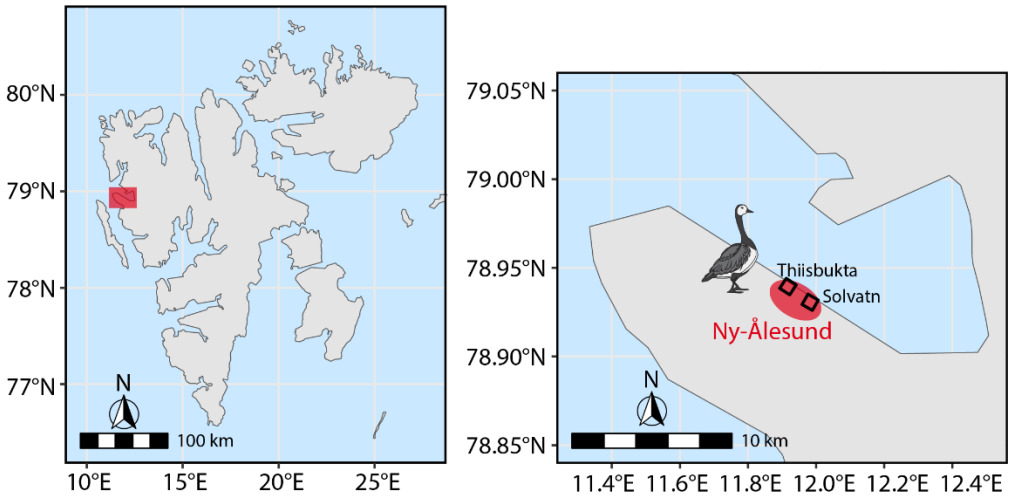

Figure 4: Sampling site location of Paper 1 and Paper 2. The map on the left depicts the Svalbard peninsula, with the Ny-Ålesund area highlighted in red. The map on the right shows the locations of Solvatn wetland (Paper 1) and Thiisbukta wetland (Paper 2) within Ny-Ålesund, where the effect of herbivory by Barnacle geese on the peat soil microbial communities was studied (Disclaimer: Barnacle goose not according to scale).

Paper 1: This study was based on samples from the Solvatn wetland (Figure 4). Experimental exclosures (0.7 x 0.7 m), adjacent to herbivory control plots, had been established in 1998, hence 18 years prior to the sampling campaign in 2016. During these 18 years a different

vegetation, mainly composed of grasses (*Poa arctica*), had formed inside the exclosures. The herbivory exposed vegetation in the control plots was dominated by brown mosses of the family *Amblystegiaceae*. Samples were taken from inside the exclosures and from the herbivory control plots at a depth of 1 – 2 cm after the removal of vegetation.

The following main methods were used to analyze the samples taken for this study (see **Paper 1:** Materials and methods for more details and references):

Vegetation description

We identified the main vascular plants and bryophytes based on their coverage to compare the effect of herbivory and herbivore exclusion on the vegetation composition.

Soil physicochemical parameters

Soil O_2 content, temperature [°C], pH, gravimetric water content [%], and total organic matter content were estimated for the characterization of the peat soils protected from and exposed to herbivory.

Plant polymer profiling

The polysaccharide composition of Arctic peat soils was measured using Comprehensive Microarray Polymer Profiling (CoMPP) and used as an indication for vegetation induced changes of SOM composition comparing the treatments.

Pore-water analysis: Amino acids and sugars

Pore-water amino acids were analyzed via mass spectrometry, and pore-water sugars via high pressure liquid chromatography (HPLC). Similar to the plant polymer profiling, the measurements of amino acids and sugars were used to characterize the soils nutrient contents comparing the treatments.

Enzyme assays

The rates of extracellular enzyme activity, focusing on polysaccharide degrading enzymes, were measured using the GlycoSpot™ technology. Those measurements were used as indications for alterations of degradation processes comparing herbivory to herbivore exclusion.

Total nucleic acids extractions and sequencing

Total DNA and RNA was isolated from the soils using a phenol-chloroform extraction protocol and used for the sequencing of DNA and mRNA. Metagenomes (DNA) and metatranscriptomes (mRNA) were used for the description of the microbial community composition and its functional activity in degradation processes.

Paper 2: This study was based on samples from the Thiisbukta wetland (Figure 4). Experimental exclosures had been established in 2006 and 2016, hence 14 and 4 years prior to the sampling campaign in 2020 (Figure 5). The herbivory control (Hr) and the 14 year exclosures (Ex-14) covered an area of 2 x 2 m. The 4 year exclosures (Ex-4) (1 x 1 m) were established 10 years after Ex-14 and set up within the Hr areas, reducing the size of Hr by the size of Ex-4 (Figure 5). Similarly to **Paper 1**, the exclusion of herbivores in Thiisbukta also led to the establishment of vascular plants dominated by *Poa arctica* and *Equisetium variegatum* within the exclosures. Samples were taken at 1 – 3 cm depth after removal of the vegetation.

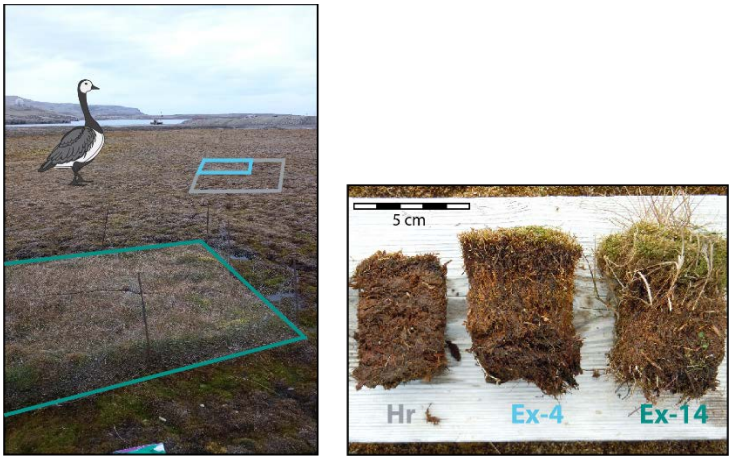

Figure 5: The picture on the left shows an example of the experimental exclosure set-up in Thiisbukta wetland. The area framed in grey depicts the herbivory plot (Hr) that allowed access of herbivores, framed in light blue is the 4-year-old herbivore exclusion plot (Ex-4), and framed in dark green is the 14-year-old herbivore exclusion plot (Ex-14). The picture on the right illustrates a comparison of the top layer peat soils and vegetation between Hr, Ex-4, and Ex-14. (Disclaimer: Barnacle goose not according to scale).

The following main methods were used to analyze the samples taken for this study (see **Paper 2:** Materials and methods for details and references):

Vegetation description

The vegetation composition was identified using the point-intercept method and used for the comparison of the effect of herbivore exclusion for 4 and 14 years compared to herbivory sites.

Root biomass

Root biomass was determined by separating and weighing roots from peat soils of the same volume, comparing the three treatments for the purpose of connecting changes in vegetation to changes in root biomass of the soils.

Soil physicochemical parameters

Soil O_2 content, temperature [°C], pH, and gravimetric water content [%] were measured for the characterization of the peat soils protected from and exposed to herbivory.

Soil carbon (C), nitrogen (N), and phosphorus (P)

Total soil C and N were measured using an elemental analyzer coupled to a continuous-flow isotopic ratio mass spectrometer. Total soil P was photometrically determined using the malachite-green method. Those measurements were used to compare the availability of substrates in the three treatments.

Fingerprinting of soil organic matter (SOM) chemical composition

Pyrolysis-gas chromatography/mass spectrometry was used for characterizing the chemical composition of SOM and to compare the effect of herbivory and exclusion of herbivores on potential alterations in the SOM composition.

Ecosystem respiration (ER)

ER, reflecting the total microbial and root CO_2 production, was measured using gas chromatography (GC). We measured ER to compare the effects of changes in vegetation and microbial community composition between the treatments.

Microbial growth rates

The effect herbivory and herbivore exclusion on microbial growth rates was estimated via $H_2{}^{18}O$ incorporation into DNA.

Microbial biomass

Soil microbial C and N were obtained by the chloroform fumigation extraction method and used as estimates for intact cells comparing herbivory to herbivore exclusion.

Total nucleic acids extractions and sequencing

Total DNA and RNA was isolated from the soils using a phenol-chloroform extraction protocol and used for the sequencing of total RNA. Metatranscriptomes (total RNA) were used for the description of the active microbial community composition and food web structure.

2.2 Experimental design (Paper 3)

Paper 3 was based on temperature incubation experiments of anoxic peat soil microcosms. The samples for the experiments were coming from five different wetlands located around the northern hemisphere focusing on the Arctic; (1) Svalbard - Knudsenheia (78°55´N, 11°56´E), (2) Greenland - Zackenberg (74°28´N, 20°35´W), (3) Norway - Håkøybotn (69°37´N, 18°47´E), (4) Canada - Komakuk beach (69°35´N, 140°11´W), and (5) Germany - Schlöppnerbrunnen (50°07´N, 11°52´E) (Figure 6). The mean annual temperature at the five locations ranged from -8.48 (Greenland) to 9.97 °C (Germany), and the mean annual total precipitation was between 302 (Canada) – 942.47 mm (Norway) in the past decade.

Figure 6: Location of the five wetlands investigated in Paper 3, listed from northernmost to southernmost: (1) Svalbard (78°55´N, 11°56´E), (2) Greenland (74°28´N, 20°35´W), (3) Norway (69°37´N, 18°47´E), (4) Canada (69°35´N, 140°11´W), and (5) Germany (50°07´N, 11°52´E). The samples from the five wetlands were used for anoxic temperature experiments with a focus on CH_4 producing microbial communities.

The samples were taken at -20 – 40 cm depth from the water saturated layer of the peat at all five locations and homogenized with peat water to a slurry in a 1:1 peat to water ratio. Following a two month long pre-incubation phase at 2 °C, the main temperature experiment was carried out (Figure 7). Over a period of 5 weeks the anaerobic peat soil slurry microcosms were incubated at weekly increasing temperatures starting at 2 °C, over 4 °C, 6 °C and 8 °C to a maximum of 10 °C, followed by a temperature decrease back to 2°C over the next 4 weeks (Figure 7).

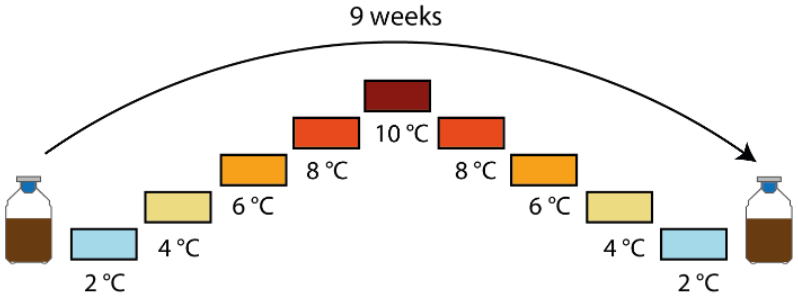

Figure 7: Experimental design of the main temperature incubation experiment. The peat soil samples were incubated under anoxic conditions over the course of 9 weeks. Starting at 2 °C, each temperature was maintained for one week, with weekly increments of 2 °C up to 10 °C, followed by a cooling period back to 2 °C.

The following main methods were used to analyze the samples of this study (see **Paper 3:** Materials and methods for details and references):

Physicochemical parameters: Gravimetric water content [%] and pH were measured to characterize the soils (i.e., the peat soil slurries) from the different locations.

Pore-water analysis: Fermentation intermediates (acetate and propionate)

Acetate and propionate are important intermediates of anaerobic C decomposition to CH_4 and were measured by high pressure liquid chromatography (HPLC). We measured these fermentation intermediates to investigate temperature-induced changes in their concentration and the potential relationship with changes in methanogenesis rates.

CH_4 and CO_2 production rates

CH_4 and CO_2, the end products of anaerobic decomposition of organic C, were measured using gas chromatography (GC) to investigate to effect of temperature on GHG production.

Microbial growth rates

The effect temperature on microbial growth rates was estimated via $H_2{}^{18}O$ incorporation into DNA.

Total nucleic acids extractions Total DNA and RNA was isolated from the soils using a phenol-chloroform extraction protocol. The ratios of RNA to DNA were used for the estimation of changes in cellular content of ribosomes.

3 Summary of Papers

3.1 Paper 1: Microbial responses to herbivory-induced vegetation changes in a high-Arctic peatland

Kathrin M. Bender, Mette M. Svenning, Yuntao Hu, Andreas Richter, Julia Schückel, Bodil Jørgensen, Susanne Liebner, Alexander T. Tveit

Research article published in Polar Biology 2021 (DOI: 10.1007/s00300-021-02846-z)

Herbivory by barnacle geese (*Branta leucopsis*) alters the vegetation composition and reduces ecosystem productivity in high-Arctic wetland, limiting the C sink strength of these ecosystems. Here we investigate how herbivory-induced vegetation changes affect the activities of peat soil microbiota using metagenomics, metatranscriptomics, and targeted metabolomics in a comparison of fenced exclosures and nearby herbivory sites. Our results show that a different vegetation with a high proportion of vascular plants developed due to reduced herbivory, resulting in a larger and more diverse input of polysaccharides to the soil at exclosed study sites. This coincided with higher sugar and amino acid concentrations in the soil at this site as well as the establishment of a more abundant and active microbiota, including saprotrophic fungi with broad substrate ranges, like *Helotiales* (Ascomycota) and *Agaricales* (Basidiomycota). A detailed description of fungal transcriptional profiles revealed higher gene expression for cellulose, hemicellulose, pectin, lignin and chitin degradation at herbivory-exclosed sites. Furthermore, we observed an increase in the number of genes and transcripts for omnivorous eukaryotes such as *Entomobryomorpha* (Arthropoda). In connection to the higher concentrations of polysaccharides, we observed increased potential rates of extracellular hydrolytic enzymes that target plant polysaccharides and transcripts for genes encoding such enzymes, implying that the changes in vegetation led to overall higher decomposition rates. We conclude that in the absence of herbivory, the development of a vascular vegetation alters the soil polysaccharide composition and supports larger and more active populations of fungi and omnivorous eukaryotes, co-occurring with higher potential rates of polymer degradation. This study establishes a fundament for targeting molecular and microbial mechanisms that are key to the interactions between above and below ground biology in high-Arctic wetlands.

3.2 Paper 2: Tundra vegetation changes, in absence of herbivory, are coupled with an altered soil microbial food web and a faster microbial loop

Kathrin M. Bender, Victoria S. Martin, Yngvild Bjørdal, Andreas Richter, Maarten J.J.E. Loonen, Mette M. Svenning, Andrea Söllinger, Alexander T. Tveit

Manuscript research article prepared for submission to Global Change Biology

Increases in geese populations and changes in their migratory routes have large consequences for Arctic tundra soil ecosystems, influencing the vegetation composition and thereby affecting the SOM input from plants. We have investigated the influence of herbivory by barnacle geese on vegetation, SOM quality, microbial activities, and microbial food web composition in a high-Arctic wet tundra. Tundra vegetation was exposed to three treatments: (1) Herbivory (natural state), (2) 4-year exclusion of herbivores, (3) 14-year exclusion of herbivores. These treatments resulted in (1) bryophyte-dominated vegetation, (2) a mix of higher vascular plants and bryophytes, (3) and vascular-plant-dominated vegetation. After 4 and 14 years of herbivore exclusion, we observed increased root biomass, decreased soil pH and moisture, and increased dissolved and total P contents. While dissolved and total soil C did not change with exclusion of herbivores, SOM composition had shifted towards a higher content of N containing compounds and lignin derivates in the 14-year-old exclosures. Microbial growth and ecosystem respiration rates were also higher in the exclosures while the total microbial biomass remained constant, indicating faster microbial turnover and higher activity of SOM decomposition processes. Based on 55 total RNA libraries, the overall relative abundances of prokaryotes, eukaryotes and viruses were similar in the three treatments. However, herbivore exclusion resulted in higher relative abundances of saprotrophic and mycorrhizal fungi and a shift and overall reduction in the relative abundances of multiple bacterial and eukaryotic micro-predators, reflecting a re-structured food web.

We conclude that more than a decade after the changes in aboveground herbivory, the ecosystem has altered into a different state, characterized by changes in vegetation, SOM composition, the soil microbial food web, and a faster microbial biomass turnover. Our results demonstrate the major effect that altered above-ground ecosystem dynamics have on the below-ground microbial interactions that control C cycling in Arctic tundra ecosystems.

3.3 Paper 3: Physiological temperature responses in methanogenic communities control the timing and rates of methanogenesis

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Observations of higher wetland CH_4 emissions during autumn cooling relative to spring warming (CH_4 emission hysteresis) have been reported in recent years, potentially conflicting with the idea that CH_4 emissions are largely controlled by the temperature dependency of methanogenesis. We have investigated the temporal temperature responses of microorganisms involved in CH_4 production, by simulating an Arctic growing season in temperature-ramp time-series laboratory experiments (2 °C – 10 °C – 2 °C), with 2 °C increments per week for nine weeks. Using anoxic wetland soils from high-Arctic Svalbard, we showed that net CH_4 production rates are significantly higher in the second half of the temperature experiment, during cooling. This cooling-induced elevation of net production of CH_4 corresponded with a drop in the concentrations of propionate and acetate. At the same time, the ratio of soil RNA to DNA increased, indicative of larger microbial investment into ribosomes for protein biosynthesis. The highest microbial growth rates were reached after the initiation of cooling, based on ^{18}O-H_2O incorporation into DNA, which corresponded with increasing abundances of methanogenic archaea. We propose that the observed CH4 emission hysteresis resulted from a combination of factors, including the warming-induced increases in methanogenesis, the thermodynamic favorability of aceticlastic methanogenesis, and increased ribosome synthesis in response to cooling to compensate for reduced catalytic activity. . We repeated the experiment with wetland soils from high-Arctic Svalbard, Northern Norway, Greenland, Arctic Canada, and Germany, resulting in three out of five tested soils demonstrating sustained net CH_4 production during cooling. Our study highlights how CH_4 emissions depend both directly on the temperature dependency of enzymatic reactions during warming and indirectly on physiological adjustments to temperature change during cooling. This demonstrated that microbial physiology must be accounted for in predictions of climate change effects on natural GHG emissions.

4 Results and Discussion

The overall goal of this thesis was to examine how Arctic peat soil microbial communities respond to environmental changes, in particular alterations in vegetation and temperature. Climate change is driving shifts in animal populations, vegetation composition and temperature dynamics in Arctic environments. These changes, in turn, can have large effects on microbial communities and their functions, as observed in **Papers 1**, **2**, and **3**.

The papers presented in this thesis are time-series studies, comparing long-term (years to decades) effects of herbivory-induced vegetation changes on microbial community composition and interactions with implications for SOM decomposition (**Paper 1** and **Paper 2**), and short-term (weekly to seasonal) effects of temperature changes on microbial communities and GHG emissions (**Paper 3**).

Paper 1 and **Paper 2** demonstrated how herbivory affects the vegetation in Arctic wetlands. Those changes were leading to the establishment of a different and more active polysaccharide degrading microbial community (**Paper 1**) and to alterations in the microbial food web and the rate of the microbial loop (rates of growth and death) in the peat soils (**Paper 2**). This thesis further illustrates the underlying mechanisms behind the effect of temperature on CH_4 producing microbial communities in Arctic wetlands (**Paper 3)**.

The combination of papers illustrates microbial community and functional responses to changes in vegetation and temperature in Arctic wetlands, providing detailed descriptions of soil ecosystem processes, with a focus on C cycling and GHG emissions.

4.1 Methods for a holistic study approach

In order to obtain a complete picture of C cycling dynamics in Arctic wetlands both the topsoil (**Paper 1** and **Paper 2**) and subsoil (**Paper 3**) layers were investigated. The combination of methods describing vegetation composition, soil physiochemical composition, microbial biomass, growth and activity, and the microbial community composition in **Paper 1** and **Paper 2**, allowed for the simultaneous investigation of multiple aspects of ecosystem functioning in the Arctic wetlands. In **Paper 3**, we combined methods for the description of microbial growth, cellular content of ribosomes, fermentation intermediates, and CH_4 production rates to gain new insights into temperatures dependencies of microbial physiology

and how it affects ecosystem CH_4 emissions. This approach enhanced our comprehension of seasonal patterns in CH_4 emissions from wetlands.

Through the combination of a wide range of methodologies, this work extends our current understanding of microbial C cycling dynamics in Arctic wetlands. The focus on one or a few methods only may prevent new insights and the connection of observations to established theory, because of the complexity of soil ecosystems. Therefore, comprehensive, and interdisciplinary microbe-centric approaches, combining soil and plant science, environmental genomics, and ecosystem modelling, are necessary to fill important gaps in the understanding of microbial ecology and gain deeper insights into the effects of climate change (Singh et al., 2010; Tiedje et al., 2022).

4.2 Herbivory alters the vegetation and peat soil characteristics

4.2.1 Vegetation composition

Herbivory by geese can lead to a reduction in vegetation diversity and the depletion of the soil seed bank (Zacheis et al., 2001; Kuijper et al., 2006), which we also observed in **Paper 1** and **Paper 2**. The two studied wetlands, Solvatn (**Paper 1**) and Thiisbukta (**Paper 2**), were both under the influence of herbivory, mainly by Barnacle geese and occasionally by Svalbard reindeer. Herbivory on both field sites has led to the suppression of vascular plant growth and created a vegetation cover mainly composed of different brown mosses belonging to the family *Amblystegiaceae* with a coverage of 98 – 100 %. Within the experimental exclosures, preventing herbivory, we observed the establishment of vascular plants, dominated by *Poa* grasses and *Equisetum variegatum* (horsetail). After 4 years of herbivore exclusion vascular plants covered 72 % of the ground, and after 14 years of herbivore exclusion the vascular plant coverage had increased to 92 % (**Paper 2**). In the neighboring Solvatn wetland (**Paper 1**), the two dominating vascular plant types after 18 years of herbivore exclusion, were *Poa* grasses and flowering plant *Cardamine pratensis*. These observations align with previous studies by Sjögersten et al. (2011) for the Solvatn wetland and Petit Bon et al. (2023) for the Thiisbukta wetland.

4.2.2 Root biomass

Concurrent with the higher coverage of vascular plants was the higher root biomass content of the peat soils. After 4 years of herbivore exclusion, we observed a 6-fold increase in the root content of the soil, after 14 years the root content was 24-fold higher compared to peat soils exposed to herbivory (**Paper 2**). Our findings align with earlier measurements of root biomass in Thiisbukta wetland, where root biomass was found to be higher after just 4 years of herbivore exclusion compared to the herbivory exposed peat (Sjögersten et al., 2011). We did not quantify root biomass after 18 years of herbivore exclusion (**Paper 1**), however, Sjögersten et al. (2011) found a 12 times higher root content 9 years after establishment of the Solvatn exclosures, indicating a similar trend for this wetland.

4.2.3 Soil chemistry

We measured comparable O_2 levels and temperatures [°C] in the studied peat layers (at 1 – 3 cm depth) in areas protected from and exposed to herbivory in both Solvatn and Thiisbukta wetlands (**Paper 1** and **Paper 2**). The peat soil pH at Thiisbukta wetland significantly decreased from a pH of 7.3 in herbivory controls to a pH of 6.3 after 14 years of herbivore exclusion, while the pH after 4 years of herbivore exclusion was similar to the herbivory control (**Paper 2**). This stands contrary to our observations of the same pH of 7.1 after 18 years of herbivore exclusion and herbivory at the Solvatn wetland (**Paper 1**). Although pH decreased after 14 years of herbivore exclusion in **Paper 2**, the pH in both wetlands was relatively neutral, compared to more acidic *Sphagnum* dominated peat which can have a pH down to 4 (Shotyk, 1988). Therefore, pH is likely not one of the driving factors for alterations of microbial responses in **Paper 1** and **Paper 2.** The gravimetric water content of the peat soils was highest in the herbivory controls compared to the exclosures in both wetlands (**Paper 1** and **Paper 2**), likely due to the high water-holding capacity of bryophytes (Oishi, 2018). The peat soil of the Thiisbukta field site (**Paper 2**) was in general dryer compared to Solvatn (**Paper 1**), at the time of sampling, with a water content of 86.1 H_2O [%] at Thiisbukta and 92.4 H_2O [%] at Solvatn (the two measurements display the water content of the herbivory treatment; i.e., the current natural state).

4.2.4 Soil organic matter composition and concentration

The observed shifts in vegetation composition corresponded with changes in the SOM composition. We found overall higher SOM contents in peat soils from herbivore exclosures compared to herbivory controls in the Solvatn wetland. This difference was attributed to

higher concentrations of monosaccharides, polysaccharides, and amino acids, which reflected the changes in vegetation towards higher abundance of vascular plants (**Paper 1**). We further found higher contents of lignin derivates within the exclosures in Thiisbukta wetland (**Paper 2**). Lignin and some of the measured polysaccharides found in higher concentrations, do not occur in mosses and are likely originating from the altered plant litter input (Sarkar et al., 2009) reflecting the changes in vegetation.

Despite the observed changes in SOM composition, total soil C content was comparable in herbivore exclosures and herbivory controls (**Paper 2**). In the herbivore exclosures, we found slightly higher, yet not significantly different, total N and total P contents (**Paper 2**). This observation corresponds to previous descriptions of the same wetland (Petit Bon et al., 2023). Considering the slightly elevated N concentrations in the herbivore exclosures, it appears that the observed input of feces in herbivory controls did not lead to increases in N concentrations. A possible explanation is that additive N from feces is mainly taken up by mosses (Sjögersten et al., 2010), where it remains locked due to the rather slow decomposition rates of mosses (Hájek et al., 2011). Additionally, geese are likely removing more N from the system through grazing on vascular plants than the input of N through feces could compensate for, resulting in the lower N contents at herbivory sites. The elevated concentrations of P as observed in herbivore exclosures may be attributed to the presence of a deeper-reaching root systems, which can access lower P pools and transport them to the upper soil layers.

4.3 Herbivory alters peat soil microorganisms and processes

4.3.1 Microbial growth and biomass turnover

In connection with the increases of vascular plant biomass in herbivore exclosures, we observed trends (p-value: < 0.1) of elevated mass-specific microbial growth rates after 14 years of herbivore exclusion leading to significantly (p-value: < 0.05) shorter DNA turnover times. This faster DNA turnover reflects a combination of faster growth and death, the difference to the mass-specific microbial growth rates being whether the ^{18}O isotope incorporation from H_2O into DNA is normalized by the microbial biomass estimates or by DNA content (**Paper 2**). As such, the different p-values are due to the variation in the dataset used to normalize the data and not the rate of isotope incorporation. The higher growth rates were likely driven by the observed system change after 14 years of herbivore exclusion, including the shift in vegetation, higher root exudation, and an altered SOM composition.

Concurrently, microbial biomass, based on the measurement of microbial C and N from intact cells, remained unchanged when comparing herbivory to herbivore exclusion (**Paper 2**). A similar observation was previously reported for the Thiisbukta and Solvatn wetlands (Sjögersten et al., 2011). The combination of accelerated growth rates and stable biomass in the 14-year-old herbivore exclosures therefore suggests a faster microbial loop, where the microbial community despite growing faster, does not increase in size.

We further found significantly lower DNA and RNA concentrations in peat soils after 14 years of herbivore exclusion in Thiisbukta wetland (**Paper 2**). The lower DNA concentrations might result from the observed shift in the microbial community towards higher relative abundances of fungi (based on total RNA). In general, bacteria have a higher DNA to C ratio compared to fungi (Neidhardt et al., 1990; Yamada and Sgarbieri, 2005; Feijó Delgado et al., 2013). A shift towards higher abundances of fungi could therefore lead to lower amounts of DNA relative to microbial C. Alternatively, given that we detected shorter DNA turnover times, indicated by a combination of faster isotope incorporation into DNA and a smaller DNA pool, the lower DNA concentration in herbivore exclosures may be attributed to a more rapid consumption of extracellular DNA, compared to herbivory. Additionally, there might be fewer microbial cells in the peat soils from herbivore exclosures, resulting in the lower DNA concentrations.

In **Paper 1,** we detected higher concentrations of DNA after 18 years of herbivore exclusion compared to herbivory, while RNA concentrations were similar under both treatments. These observations therefore imply somewhat different effects of herbivore exclusion and altered vegetation between the two study sites. However, the different number of replicates analyzed, and the time of sampling could have contributed to those variations.

4.3.2 Ecosystem respiration

In **Paper 2,** ecosystem respiration (ER), which is the net CO_2 production rate [µmol CO_2 g^{-1} DW soil $day(d)^{-1}$], was determined. We found significantly higher ER in peat soils after 14 years of herbivore exclusion compared to both the 4-year-old exclosures (1.2 times higher) and the herbivory control plots (1.4 times higher). Petit Bon et al. (2023) described a similar relationship between ER and the length of herbivore exclusion at Thiisbukta wetland. Their study further reported a 3 times greater gross ecosystem photosynthesis (GEP) after 15 years of herbivore exclusion compared to herbivory exposure. Importantly though, while both ER

and GEP were higher under a vegetation dominated by vascular plants (herbivore exclosures) compared to bryophytes (herbivory controls), neither condition resulted in the release of CO_2 into the atmosphere (Petit Bon et al., 2023). ER was not measured in **Paper 1**, but Sjögersten et al. (2011) reported similar trends of ER and GEP from Solvatn wetland comparing herbivory controls to herbivore exclosures of 4 years. However, the net ecosystem exchange (NEE) of CO_2 was slightly positive in herbivory controls in Solvatn wetland, and negative following herbivore exclusion (Sjögersten et al., 2011). These observations suggest slightly varying responses to herbivory and changes in vegetation at the two neighboring wetlands, highlighting that even seemingly similar vegetation changes can result in slightly different ecosystem responses. It is possible that the more easily degradable plant litter and root exudates originating from vascular plants is causing the increase of ER in herbivore exclosures at both field sites, through priming effects on the microbial community that accelerate the mineralization of organic C (Blagodatsky et al., 2010; Bengston et al., 2012; Dieleman et al., 2017). This illustrates that the inclusion of below-ground microbial responses can help explain differences in ecosystem processes that cannot be understood based on vegetation changes alone.

4.3.3 Microbial decomposition of soil organic matter

Consistent with the alterations in SOM composition attributed to the changes in vegetation, we observed higher potential enzymatic activities and higher relative abundances of transcripts for the decomposition of the most common plant polymers in the peat soils of herbivore exclosures (**Paper 1**). Higher availabilities of C substrates in rhizosphere soils have been suggested to fuel microbial investments into production of extracellular polymer degrading enzymes (Zak and Kling, 2006; Wallenstein and Weintraub, 2008; Nuccio et al., 2020), supporting our observation in **Paper 1**.

Microbial transcription and enzymatic activities for polymer decomposition were found to be highest for cellulose degradation, followed by hemicellulose, pectin, and lignin, and were always higher inside the herbivore exclosures compared to herbivory exposure (**Paper 1**). The higher transcriptional activities for polysaccharide degradation were assigned primarily to *Actinobacteriota* and *Proteobacteria,* accounting for 42.20 % of all transcripts for polysaccharide degradation in herbivore exclosures and for 34.65 % when exposed to herbivory. Additionally, *Ascomycota* and *Basidiomycota* contributed to polysaccharide degradation, accounting for 16.88 % of the transcripts for polysaccharide degradation in

herbivore exclosures and for 1.08 % when exposed to herbivory. Therefore, we observed a shift in the polysaccharide degrading microbial community towards higher involvement of fungi and slightly higher bacterial activities in herbivore exclosures. This increase might be linked to the combination of higher concentrations of polysaccharides, presumably stemming from root exudates and plant litter, but also from the change towards a more complex mix of polymers (Edgecombe, 1938; Thormann, 2006; Broeckling et al., 2008; Koranda et al., 2014). Lignin degradation was mainly assigned to *Proteobacteria* in both herbivore exclosures and herbivory controls, accounting for 28.15 % and 33.03 % of transcripts for lignin degradation, respectively, while fungi were seemingly not involved in the degradation of lignin compounds (**Paper 1**). Hence, even though total lignin degradation was higher (based on the total number of transcripts) in herbivore exclosures, the same core community was responsible for the degradation of lignin in the peat soils in herbivore exclosures and herbivory controls (**Paper 1**).

The observed higher decomposition rates in the peat soils in herbivore exclosures in Solvatn wetland (**Paper 1**) are likely contributing to the observed higher ER under the same treatment in Thiisbukta wetland (**Paper 2**), as the first steps in SOM degradation, polysaccharide hydrolysis and the subsequent oxidation of monosaccharides, can result in the release of CO_2 (Tveit et al., 2013). Given the similar changes in vegetation and soil chemistry comparing Solvatn and Thiisbukta wetlands, combined with the higher ER in Thiisbukta wetland, we therefore assume, that similar decomposition processes might also be present in Thiisbukta wetland.

Additionally, we noted an increase in the relative abundances of omnivores, specifically the springtail collembola. The investigation of the degradation profile of one family within collembola, *Entomobryomorpha*, revealed its capability to depolymerize bacterial cell walls as well as plant and fungal polymers (**Paper 1**). While microbial omnivores, such as collembola, are well known for their involvement in SOM decomposition (Thakur and Geisen, 2019), our study adds more in-depth insights about their range of substrate usage and participation in decomposition processes.

4.3.4 Microbial food webs and the microbial loop

After 14 years of herbivore exclusion, we observed faster microbial growth rates while the microbial biomass remained stable in peat soils exposed to and protected from herbivory. This suggested the establishment of a faster microbial loop, with accelerated microbial death and turnover rates, resulting in a new ecosystem state, as discussed in chapter **4.3.1** (**Paper 2**). Although microbial mortality can have various causes, such as stress events (Camenzind et al., 2023) or exposure to antibiotics (Kohanski et al., 2010), in **Paper 2**, we focused on alterations in microbial predation patters and viral lysis as a potential cause for the faster microbial death and turnover rates.

The relative abundances of prokaryotes and eukaryotes, based on DNA sequences (16s rRNA gene and 18s rRNA gene) in **Paper 1** and RNA sequences (16s rRNA and 18s rRNA) in **Paper 2**, were similar comparing herbivory to herbivore exclusion, with about 80 % of all reads assigned to prokaryotes and ~ 20 % assigned to eukaryotes.

Following the 14 years of herbivore exclusion, we observed a decrease in the relative abundance of predatory prokaryotic and eukaryotic phyla and families. This decline was attributed to potentially predatory families belonging to the two bacterial phyla, *Bdellovibrionota* and *Myxococcota*, as well as several potential predatory eukaryotic families, belonging to the phyla *Choanoflagellida, Cercozoa, Ciliophora, Euglenida,* and *Lobosa*. Despite the general decrease in relative abundance of potential predatory microorganisms, we found that a few predatory families were increasing in relative abundance (**Paper 2**). This and the faster DNA turnover rate suggested a change in the food web dynamic towards a smaller, but more potent, and possibly more specialized predatory community. Additionally, we observed higher relative abundances of viruses within herbivore exclosures. This increase was mainly attributed to an increase in fungi infecting viruses, suggesting that the overall increase of the fungi kingdom was responsible for the increase of viruses. We therefore concluded that viruses may be contributors to the increasing rate of the microbial loop, but not the main driving factor.

Consequently, even with fewer members, the microbial predatory community, and viruses in herbivore exclosures appeared more potent by maintaining the microbial biomass and DNA concentrations at the same or lower levels, respectively, despite strong indications for increasing microbial growth and DNA turnover (**Paper 2**).

The higher root biomass observed in the soils within herbivore exclosures (**Paper 2**), could potentially create rhizosphere hotspots of microbial growth and biomass turnover, due to the higher localized input of easily degradable nutrients (Kuzyakov, 2010). Therefore, predatory microorganisms and viruses may have easier access to higher concentrations of their prey within these hotspots. This could further explain, how a select group of microbial predators come to dominate and lead to a faster microbial loop within the herbivore exclosures.

Broader relevance

Trophic interactions play major roles in ecosystem functioning (Trap et al., 2016; Geisen et al., 2018; Thakur and Geisen, 2019; Lucas et al., 2020; Petters et al., 2021). Changes in the vegetation have been identified as a driver of alterations in trophic interactions, leading to faster biomass cycling and higher rates of organic C flow (Sokol et al., 2022, Wang et al., 2022), as also observed in **Paper 1** and **Paper 2**. However, the exact mechanisms by which microbial food webs shape and impact soil C cycling remain uncertain (Jia et al., 2019).

Currently, global scale C models often overlook microbial dynamics in their predictions, relying on the comparison of the flow of C from one large pool (like SOM) to another (like GHG emissions) (Sokol et al., 2022). The complexity of microbial ecology and soil ecosystems is challenging for the incorporation of those fine-scale processes into large-scale global C models (Crowther et al., 2015; Buchkowski et al., 2017). Nevertheless, the inclusion of microbial ecology into global scale C models is essential for enhancing our understanding of SOM cycling and predicting future GHG emissions.

Therefore, in order to fully comprehend the role of microbial food webs for soil C cycling, more comprehensive studies are needed. Through the combination of a broad range of methods, we were able to connect different levels of ecosystem functioning, by identifying the underlying microbial drivers (**Paper 1** and **Paper 2**). The large shifts in vegetation, microbial decomposition, growth and biomass, and food web interactions, resulted in two very different ecosystems states, with however seemingly very similar ER. The findings presented in **Paper 1** and **Paper 2** represent valuable contributions to advancing our understanding of this complex field.

4.4 Seasonal temperature changes influences greenhouse gas fluxes in Arctic wetlands

4.4.1 Mechanisms behind seasonal methane hysteresis

We wanted to investigate the impact of seasonal temperature changes (specifically, spring warming compared to autumn cooling) on GHG production in Arctic wetlands (**Paper 3**). For this purpose, we conducted several anoxic temperature ramp experiments, incubating peat soils from 2 °C to 10 °C, in weekly increments of 2 °C (thereby simulating an Arctic spring), followed by a cooling period from to highest temperature at 10 °C back to 2 °C (simulating an Arctic autumn).

In the first experiment, using high-Arctic peat soils from Svalbard, we observed significantly higher net CH_4 production rates during cooling, compared to the same temperatures during warming. Similar hysteretic patterns of seasonal CH_4 emissions have been documented in previous *in-situ* studies from sub-Arctic wetlands (Chang et al., 2020; Chang et al., 2021). This suggested that our experimental observations were consistent with patterns observed in natural settings. In their studies from 2020 and 2021, Chang et al. suggested that the basis for the CH_4 emission hysteresis was an accumulation of methanogenic archaea and methanogenic substrates, such as acetate, during spring and summer, leading to the higher rates of net CH_4 production during autumn (Chang et al., 2020; Chang et al., 2021). Contrarily, in our experiment, the concentrations of acetate remained stable during warming, and started depleting during cooling after reaching the temperature maximum of 10 °C. This suggested two things: (1) during warming the upstream processes leading to production of acetate were still able to balance the consumption of acetate during methanogenesis, but this balance shifted after reaching 10 °C; (2) reaching 10 °C triggered a response of the aceticlastic methanogens, enabling them to use the available acetate faster.

Different temperature dependencies

In comparison to upstream C degradation processes, such as hydrolysis and respiration, aceticlastic methanogenesis has a higher temperature dependence, requiring an activation energy (Ea) of 106 Kj mol $^{-1}$, while hydrolysis requires an Ea between 54 – 125 Kj mol $^{-1}$ and respiration an Ea of 49 Kj mol $^{-1}$ (Conrad, 2023). This could explain the higher rates of methanogenesis after reaching 10 °C, as methanogens were able to gain more energy at this maximum temperature in the experiment relative to the other processes.

Further, Yvon-Durocher et al. (2014) showed that the temperature dependence of methanogenesis is similar in natural settings compared to methanogens in pure cultures. We therefore assume that the methanogens in our anoxic incubations follow similar temperature dependencies.

Increasing microbial growth rates

Shortly before the onset of acetate depletion, we observed increasing microbial growth rates at 8 °C during warming, with the highest rates at the start of the cooling period at 8 °C. This coincided with an increase of the *mcrA* gene (marker gene for anaerobic CH_4 cycling, commonly used as a biomarker for methanogenic archaea), suggesting that methanogenic archaea were among the actively growing microorganisms. We therefore assumed that methanogenic archaea were more abundant at the start of the cooling compared to warming. This suggested that the hysteretic CH_4 emissions during cooling were likely triggered by the increased consumption of acetate by the more abundant methanogenic archaea. This finding aligns with the observations of higher *in-situ* methanogenic biomass at the beginning of autumn, which has been identified as one of the main factors leading to higher autumn CH_4 emissions (Chang et al., 2020; Chang et al., 2021).

Increases of cellular content of ribosomes

We further observed increasing ratios of RNA to DNA at the onset of cooling, indicating an accumulation of ribosomes in microbial cells during this phase. Temperature changes have previously been related to alterations in the investment of microorganisms into ribosomes for protein biosynthesis, resulting in different rRNA contents of the cells (Söllinger et al., 2022; Tveit et al., 2023), suggesting that similar mechanisms might be occurring in our study.
We hypothesize that the larger methanogenic population, coupled with their capacity to extract more energy from acetate at higher temperatures compared to other processes, allowed for an increased investment into ribosomes during subsequent cooling. As a result, the presumed increase in ribosome content within methanogenic cells could drive the depletion of acetate by compensating for lower protein synthesis rates during cooling.

Potential feedback loop leading to CH_4 hysteresis

The observed CH_4 hysteresis pattern in our experiment appears to be the result of a potential feedback loop. At higher temperatures of 10 °C, aceticlastic methanogenesis gains more energy from one acetate molecule compared to the lower temperatures (Conrad, 2023). This may have allowed for increased investments by methanogens into growth and protein production, leading to the depletion of acetate, and thereby to the higher net CH_4 production during cooling.

Remaining questions

The energy yield gained from methanogenesis decreases relatively faster during cooling compared to other C degrading processes. Therefore, while we are able to explain the basis to CH_4 hysteresis in our experiment, it does not provide an explanation as to why the upstream processes could not balance the consumption of acetate during cooling. However, we hypothesize that the accumulation of methanogen biomass and ribosomes leads to a relatively faster rate during the cooling phase. Over time, as the acetate pool depletes, the energy limitation of the methanogens results in reduced cellular ribosome concentrations, ultimately restoring the balance between upstream decomposition and methanogenesis. To test this hypothesis, methanogens and methanogen ribosomes need to be quantified throughout the time series experiments.

4.4.2 Broader relevance

To investigate the relevance of the mechanisms described above in wetlands from different locations, we repeated the temperature experiment using wetland soils from Svalbard, Norway, Greenland, Canada, and Germany. These experiments revealed varying patterns of CH_4 production rates during cooling compared to the initial experiment, as described in the previous chapter. Wetlands soils from Svalbard, Norway, and Greenland, consistently exhibited higher net CH_4 production rates during cooling, with some variations in the strength of the hysteretic pattern. In the wetland soils from Canada and Germany we did not observe higher net CH_4 production rates during cooling compared to warming. The relatively warmer in-situ temperatures of the wetland soils from Germany compared to our experiment temperatures, which were designed to simulate an Arctic growing season, might explain why we did not observe CH_4 emission hysteresis in the German wetland soils.

The variations in the strength of hysteretic patterns observed across different locations underscore the complexity of CH_4 emissions in response to changing temperatures and substrate availability. The question arises, whether similar mechanisms underlie hysteresis when it occurs, and whether the absence of hysteresis is due to different temperature thresholds that trigger it.

The findings of **Paper 3** therefore highlight how CH_4 emissions from Arctic wetlands, are influenced both directly by the temperature dependence of enzymatic reactions and indirectly by physiological adaptations of microorganisms to changing temperatures. This emphasizes the importance in understanding these nuances to accurately account for wetland microbial processes and their implications for GHG emissions in a changing climate.

5 Conclusion and Outlook

Herbivory has led to the suppression of vascular plant growth in two high-Arctic wetlands. The comparison of soils exposed to herbivory and soils protected from herbivory revealed subsequent changes in the soils SOM composition, decomposition rates of SOM, microbial growth, and the microbial food web. Although microbial growth rates were higher in herbivore excluded soils, microbial biomass remained stable in both conditions, suggesting an acceleration of the microbial loop and microbial turnover times. The acceleration of the microbial loop was driven by changes in microbial predation, as indicated by the alterations of the microbial food web. The overall higher rates of soil C flow in herbivory excluded soils, as indicated by the faster SOM decomposition rates and microbial turnover times, were leading to higher ER rates. Despite those observed large scale responses to herbivory, other studies of the same sites have shown that no net CO_2 emissions were detected under both treatments, likely due to the higher CO_2 uptake through photosynthesis in herbivore exclosures. In conclusion, both herbivory-exposed and herbivory-protected wetlands maintained their C stability, albeit through different underlying mechanisms as reflected in two very different ecosystem states. The microbial food web appeared to act as a stabilizing system, capable of balancing increased growth rates and C emissions when SOM input changed.
This dynamic raises questions regarding the stabilizing capability of microbial food webs amidst additional climate change stressors, such as higher temperatures or droughts. It is possible that one of the treatments, either herbivory or herbivore exclusion, may have a stronger capacity to mitigate the effect of such stressors, while the other could prove more vulnerable, transitioning into a CO_2 source. Future work should investigate how both treatments respond to additional environmental stressors to gain better understanding of how C cycling microorganisms might react.

Based on the insights gained from **Paper 3**, we further conclude that the hysteretic CH_4 emission pattern, as observed in the anoxic incubations of high-Arctic wetland soils during the simulated autumn cooling from 10 °C to 2 °C, was facilitated by the increased consumption of acetate. This increase was driven by the combination of kinetic and thermodynamic favorability of methanogenesis at higher temperatures relative to other C degrading processes, and the increase in abundances of methanogenic archaea and higher cellular content of ribosomes.

The intensity of the hysteretic response showed variation among wetland soils from different locations and was absent in some cases, indicating that location-specific factors are influencing the microbial physiological response to temperature. Despite this variability, this study provides valuable new insights into the fundamental mechanisms behind CH_4 hysteresis and the importance of temporal acclimation of microbial physiology to temperature change generally. Consequently, our findings enhance our ability to make more accurate predictions regarding future wetland CH_4 emissions.

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Part II

Publications

Paper 1

Polar Biology
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ORIGINAL PAPER

Microbial responses to herbivory-induced vegetation changes in a high-Arctic peatland

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Abstract

Herbivory by barnacle geese (*Branta leucopsis*) alters the vegetation cover and reduces ecosystem productivity in high-Arctic peatlands, limiting the carbon sink strength of these ecosystems. Here we investigate how herbivory-induced vegetation changes affect the activities of peat soil microbiota using metagenomics, metatranscriptomics and targeted metabolomics in a comparison of fenced exclosures and nearby grazed sites. Our results show that a different vegetation with a high proportion of vascular plants developed due to reduced herbivory, resulting in a larger and more diverse input of polysaccharides to the soil at exclosed study sites. This coincided with higher sugar and amino acid concentrations in the soil at this site as well as the establishment of a more abundant and active microbiota, including saprotrophic fungi with broad substrate ranges, like *Helotiales* (*Ascomycota*) and *Agaricales* (*Basidiomycota*). A detailed description of fungal transcriptional profiles revealed higher gene expression for cellulose, hemicellulose, pectin, lignin and chitin degradation at herbivory-exclosed sites. Furthermore, we observed an increase in the number of genes and transcripts for predatory eukaryotes such as Entomobryomorpha (Arthropoda). We conclude that in the absence of herbivory, the development of a vascular vegetation alters the soil polysaccharide composition and supports larger and more active populations of fungi and predatory eukaryotes.

Keywords Arctic peat soils · Predation · Saprotrophic fungi · Metagenomics · Metatranscriptomics · Vascular plants · Herbivory

Introduction

Arctic terrestrial peatlands store 30–40% of the world`s soil organic carbon (SOC) (Tarnocai et al. 2009), the fate of which is determined by the balance between plant growth, herbivory and microbial decomposition (Ping et al. 2015). Herbivory has a large impact on the composition of plant communities (Zacheis et al. 2001; Maron and Crone 2006), which in turn affects the quality of litter input to the soil (Bardgett and Wardle 2003; Wardle et al. 2004; Van der Heijden et al. 2008; Fivez et al. 2014). Plant cell wall polymers, such as cellulose, hemicelluloses, pectins and lignin, are the major constituents of photosynthetically fixed organic carbon in peatlands (Chesworth et al. 2008; Gilbert 2010) and the cell walls of different plant lineages have characteristic macromolecular organization and polymer composition (Sarkar et al. 2009). Thus, the vegetation is assumed to determine the SOC composition and its decomposability (Davidson and Janssens 2006; Ping et al. 2015).

Our knowledge of soil ecosystem functioning is largely based on studies that focus on one or a few components of these complex ecosystems, but in order to thoroughly understand processes like decomposition it is important to study the microbial food web structures and linkages (Crotty et al. 2014). Fungi degrade detritus organic matter, consisting of a variety of plant polysaccharides and lignin (Thormann 2006). Bacterial communities in soils are known to be involved in

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numerous activities including degradation of detritus polymers (Tveit et al. 2013), microbial necromass (Müller et al. 2018) and active predation (Davidov et al. 2006; Morgan et al. 2010). Groups of non-fungal eukaryotes are involved in both predation and degradation of microbial and plant polymers (Crotty et al. 2011). The numbers of omnivorous eukaryotes such as Collembola (orders: Entomobryomorpha, Poduromorpha, Symphypleona) have been shown to positively and negatively correlate with microbial biomasses (Sabais et al. 2011; Thakur et al. 2015), making it difficult to reconstruct the linkages of microbial food webs based on abundances of taxa alone. Geisen et al. (2016) described different groups of protists and arthropods, so far believed to be bacteriovorous, as facultative mycophagous, refining our understanding of protists and arthropods as key players that control the biomass of both bacteria and fungi in soil food webs.

In a productive ecosystem, herbivory can lead to higher productivity by maintaining the dominant plant species while excluding less productive species (Bardgett et al. 1998; Bardgett and Wardle 2003). In less productive ecosystems, herbivory can lead to decreased plant and root biomass like shown for a high-Arctic peatland, Svalbard (Sjögersten et al. 2011) or in the coastal marshes of Hudson Bay, Canada (Jefferies et al. 2006). The effect of increased plant species richness and productivity resulted in larger microbial biomasses and rates of respiration (Zak et al. 2003; Zak and Kling 2006), and an overall increase in the density and diversity of microorganisms (Eisenhauer et al. 2013). In western Svalbard, increased peatland herbivory by barnacle geese (*Branta leucopsis*) has led to a suppression of vascular plant growth and dominance of mosses within the family Amblystegiaceae (brown mosses) (Kuijper et al. 2006), possibly changing these peatlands from carbon sources into carbon sinks (Sjögersten et al. 2011).

Here we compared peat protected from grazing for 18 years (exclosure; experimental condition) with grazed peat (grazed sites; natural condition), studying the differences in soil, soil polysaccharide composition, microbial community composition and microbial activities directed at polysaccharide decomposition, targeting all three domains of life. For this, we have sequenced eight metagenomes and eight metatranscriptomes, and performed targeted metabolomics, antibody staining of polysaccharides and extracellular enzyme assays in addition to describing the peat soil and the vegetation.

Materials and methods

Study site and sampling

The fieldwork was carried out in the high-Arctic peatland Solvatn, situated close to the research settlement Ny-Ålesund, Svalbard (78° 55′ N, 11° 56′ E), in August 2016. In the Solvatn peatland, experimental exclosures had been maintained since 1998 by 0.5-m tall fences prohibiting Barnacle geese grazing from areas of 0.7 × 0.7 m (Sjögersten et al. 2011) (Fig. 1). A total of four replicates from the grazed areas and four replicates for the exclosed areas were collected for DNA and RNA extractions, enzyme assays and plant polymer profiling while 12 replicates from each condition were collected for pore water amino acid and sugar measurements. Samples were collected at 1–2 cm depth and immediately frozen in liquid nitrogen or processed for pore water extraction and filtration; 400 µl of pore water was filtered with Whatman™ Mini-UniPrep™ G2 Syringeless Filters (GE Healthcare, Buckinghamshire, UK). The O_2 [%] and temperature T [°C] of the sites were measured at four different depths using an optical O_2 sensor and thermometer (Fibox 4, PreSens Precision Sensing GmbH, Regensburg, Germany) (Table S1—online resource 2). The water [%] and total organic matter (TOM) contents were estimated as described in Tveit et al. (2013) (Table S2—online resource

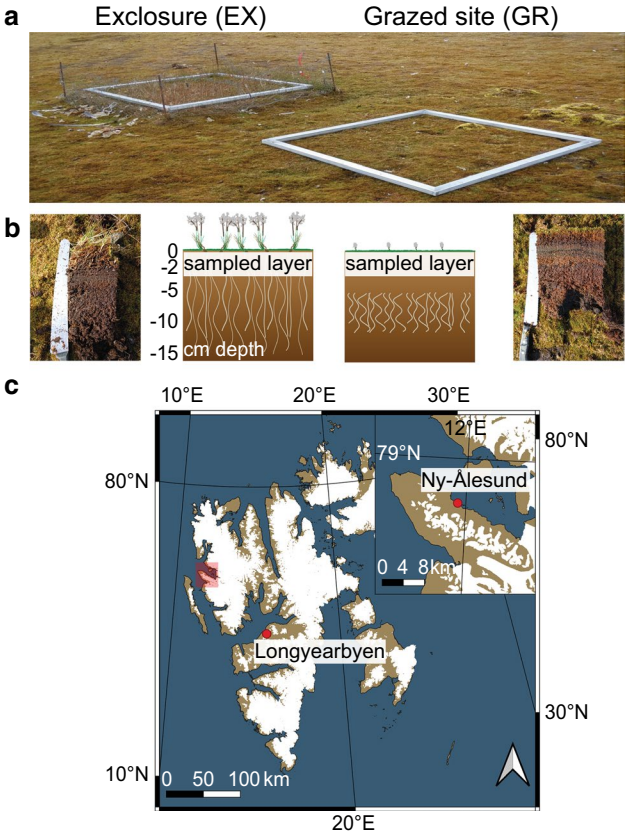


Fig. 1 Sampling sites and sample collection. **a** In the picture one of the exclosures, EX1, and grazed sites, GR1, are shown, displaying the different vegetation. **b** The top peat layer at 1–2 cm below the living vegetation was sampled as indicated by the boxes across the peat profile figures. **c** Location of the Solvatn peatland, within the settlement Ny-Ålesund, Svalbard

2). The pH was measured in pore water using a portable field pH meter (Multi 350i, WTW, Weilheim, Germany). Plant material was collected for plant species characterizations.

Analysis of pore water amino acids and sugars

Free amino acids in the pore water samples were analyzed using a Thermo UPLC system consisting of an Accucore HILIC column (150 mm × 2.1 mm, 2.6 µm particle size) coupled to an Orbitrap Exactive mass spectrometer. The separation was carried out using a gradient from 95% eluent B (acetonitrile, 0.1% v/v formic acid) to 40% eluent A (water, 0.1% v/v formic acid) (Hu et al. 2017). The mass resolution was set to 50.000 and the injection volume was 25 µL. Sugar concentrations in pore water were measured with an HPLC (Dionex ICS-5000) using a Thermo CarboPac column (0.4 mm × 150 mm; pre-column 0.4 mm × 35 mm) and an electrochemical detector. The eluent was 3 mM KOH and samples were processed at 30 °C at a flow rate of 9 µl min^{-1}, with an injection volume of 40 µL. All of the peaks were integrated by Xcalibur 2.2.

Comprehensive microarray polymer profiling (CoMPP)

The polysaccharide composition of Arctic peat soils and of three different plant types were measured using Comprehensive Microarray Polymer Profiling (CoMPP) at the University of Copenhagen as described in Moller et al. (2008). For further details, see supplementary materials and methods section 1 (online resource 1).

Enzyme assays

Polysaccharide degradation enzyme assays were carried out using the GlycoSpot™ technology (Copenhagen/Denmark). The substrates used were xylan (beechwood), arabinoxylan (wheat), 2HE-cellulose (synthetic), arabinan (sugar beet), pectic galactan (lupin), galactomannan (carob), xyloglucan (tamarind) and rhamnogalacturonan (soy bean). For further details, see supplementary materials and methods section 2 (online resource 1).

Nucleic acids extraction and rRNA depletion

Nucleic acids were extracted and quantified as described previously (Urich et al. 2008; Tveit et al. 2013). Total RNA samples were processed with the Ribo-Zero Magnetic Kit for Bacteria from Illumina (San Diego, CA/USA) to remove 16 and 23S rRNA molecules and enrich the mRNA fraction of the metatranscriptome. For further details, see supplementary materials and methods section 3 (online resource 1).

Sequencing and sequence preprocessing

Sequencing was performed at the "Norwegian High Throughput Sequencing Centre" NSC Oslo, Norway (http://www.sequencing.uio.no). DNA samples were prepared for sequencing with the TruSeq Nano DNA Library Prep Kit (Illumina, San Diego, CA/USA), with an input mass of 100 ng DNA. RNA samples were prepared with the TruSeq Stranded mRNA Library Prep Kit (Illumina, San Diego, CA/USA) with random primers and an input mass of 10 ng RNA. Single reads were sequenced using the HiSeq 4000 with a read length of 150 bp, resulting in approximately 38–55 Mio reads per library. Trimmomatic (Bolger et al. 2014) was used for an initial quality filtering of the sequences to remove low quality reads. Sequences were further processed with SortMeRNA v. 2.0 to separate reads into SSU rRNA, LSU rRNA and non-rRNA (Kopylova et al. 2012).

Analysis of SSU rRNA coding reads

Blastn searches against the SILVA SSU reference database (v. 128) were performed to taxonomically classify the SSU rRNA gene reads (-evalue 10^{-1}—num_alignments 50—num_descriptions 50). The Blast outputs were analyzed using MEGAN (Huson et al. 2011) v. 6.13.1 (parameters: min bit score 100.0; top percent 2.0; min support percent 0.01, 25 best hits) as described previously (Söllinger et al. 2018).

Taxonomic and functional annotation of mRNA and mRNA coding genes

Randomly selected subsamples of 5 million nucleotide reads from the non-RNA datasets were taxonomically and functionally classified using NCBI nr (as of March 2017) and KEGG (v. 81.0) databases, respectively. NCBI nr was used for taxonomic annotation with DIAMOND v. 0.9.17 (Buchfink et al. 2015) applying an e-value threshold of 10^{-3}. The output was uploaded in MEGAN v. 6.13.1 (parameters: min score 50.0; top percent 2.0; min support percent 0.01, 25 best hits), as described previously (Söllinger et al. 2018). Reads that had been taxonomically classified using the NCBI nr database were used as queries in a blastx search against the KEGG database with e-value threshold 10^{-10} as described previously (Tveit et al. 2015).

Functional and taxonomic annotation of CAZyme encoding genes and transcripts

Randomly selected subsamples of 5 million nucleotide reads from each of the DNA and non-rRNA datasets (for non-rRNA the same subsets were used as above) were translated into open reading frames (ORFs) of 30 amino acids or longer

by the program Open Reading Frame (ORF) finder (Wheeler et al. 2003). The ORFs were screened for Protein families using the Pfam (protein family) database (v. 31) (Finn et al. 2014) and HMMsearch, a tool within the hidden markov models (HMMs) package (v 3.1b2) (Finn et al. 2015). All database hits with e-values below a threshold of 10^{-4} were counted. The resulting Pfam annotations were screened for CAZymes using Pfam models of previously identified CAZymes (Tveit et al. 2015; Söllinger et al. 2018) including starch, cellulose, hemicellulose, pectin and lignin degrading enzymes. Translated reads assigned to the selected CAZymes were extracted, followed by blastp against the NCBI nr database with an e-value threshold of 10^{-1} to obtain taxonomic information (as of March 2017) and analyzed using MEGAN (parameters: min score 50; top percent 2.0; min support percent 0.01; 25 best hits). In order to provide more depth in the analysis of the eukaryotic taxa, the full datasets were taxonomically annotated and reads assigned to *Helotiales*, *Agaricales* and Entomobryomorpha were extracted and functionally annotated as described above.

Statistical analyses and data visualization

Significance testing was performed with the Wilcoxon rank sum test using the R package (R Core Team 2014). The results from statistical tests are summarized in Table S3 (online resource 2). Correspondence analyses (Greenacre 2017) were performed as described previously (Tveit et al. 2015). The R packages ggplot2 and heatmap3 were used for plotting. Spearman correlation analysis was performed, using the function cor() in R. The geographical map of Svalbard was created using QGIS (v. 3.18.0), the base map was provided by the Norwegian Polar Institute (https://geodata.npolar.no).

Results

The effects of herbivore grazing on peat vegetation and soil O_2 availability

The exclosures (EX) were established 18 years prior to sampling, preventing the access of herbivores to the vegetation (Fig. 1a). During this time, a vascular plant community dominated by *Poa arctica* (up to 90% coverage) and *Cardamine pratensis* (up to 20% coverage) had developed. The vascular plants *Ranunculus hyperborus, Saxifraga cernua* and *Saxifraga cespitosa* were also present. Eight moss species within the brown moss family Amblystegiaceae were identified, making up the entire moss community within the exclosures (Table 1). At the grazed sites (GR), a total of seven moss species dominated the vegetation (up to 100% coverage), while two vascular plant species were detected (*P. arctica* and *R. hyperborus*) (Table 1).

Due to the higher coverage of vascular plants in the exclosures, these sites contained much higher root densities, previously quantified by Sjögersten et al. (2011) to be 12 times higher. The O_2 [%] within the first two centimeters of peat was ~ 20% in both the exclosures and grazed sites. The O_2 concentration decreased with depth, being ~ 19–20% at 5 cm depth within the exclosures and 13–16% in the grazed sites (Table S1—online resource 2). We observed surface temperatures in the range of 10.4–10.8 °C (EX) and 8.3–14 °C (GR), while at 10 cm soil depth the temperature was approximately 5–6 °C in both sites (Table S1—online resource 2). The water content of the peat soils was lower in the exclosures (mean: 84.5%) compared to the grazed sites (mean: 92.4%) (Table S2—online resource 2). The total soil organic matter percentage of the fresh weight was higher in the exclosures (mean EX: 13.0%; mean GR: 6.5%) (Table S2—online resource 2). The pH of the upper soil layer in both the exclosures and grazed sites was between 7.0 and 7.1.

Soil polysaccharide, sugar and amino acid content vary between exclosures and grazed sites

To identify the relationship between vegetation and soil polysaccharide composition we mapped the polysaccharide content in three different plant types—*P. arctica*, *S. cespitosa* and a mixture of Amblystegiaceae mosses. The same polysaccharide identification was done for the peat soil from the exclosures and grazed sites. A correspondence analysis confirmed that all three plant polysaccharide profiles were distinctly different from one another (Figures S1a and S1b—online resource 3). *Saxifraga cespitosa* was richer in the pectins homogalacturonan, rhamnogalacturonan and arabinan as well as cellulose and the hemicelluloses xyloglucan, mixed linkage glucans (MLG) and xylan. *Poa arctica* was richer in glucan, arabinoxylan and the glycoprotein AGP (arabinogalactan protein), while the mosses were richer in mannan-containing hemicelluloses, homogalacturonan (HG) with an intermediate degree of methyl esterification (DE) and galactan (Table S4—online resource 2).

Correspondingly we found that cellulose, glucan, xyloglucan, arabinoxylan and rhamnogalacturonan were more abundant in the soils from the exclosures while homogalacturonan was equally abundant at both sites. For arabinan and mannan-containing hemicelluloses, only some tested antibodies had significantly higher abundances in the exclosures (Table 2 and Figure S1c and S1d—online resource 3).

The pore water concentrations of sugars, including glucose, fructose, mannose, xylose and galactose, were significantly higher in the peat soils from the exclosures (Fig. 2a). Glucose was the most abundant sugar in the pore water of

Table 1 Vegetation description of the Solvatn peatland

	Exclosure 1	Exclosure 2	Grazed 1	Grazed 2
Vascular plants				
Poa arctica	90	15	15	< 1
Cardamine pratensis	< 1	20		
Ranunculus hyperboreus	< 5	1		< 1
Saxifraga cernua		5		
Saxifraga cespitosa		1		
Mosses				
Sanonia type	30	50	40	40
Plagiomnium type	5	10	1	
Polytrichum type	1			
Pohlia/Bryum type	3	4	5	5
Mnium type	10	5	2	
Aulacomnium palustre	1	1		1
Calliergon richardsonii	40	25	50	40
Paludella squarrosa	10	5	2	
Lichens				
Cetraria islandica	1	3		
Stereocaulon				< 1
Mushroom	< 1			
Bare ground/dead mosses				14

Comparison of the coverage (%) of vascular plants, mosses and lichens at exclosed and grazed sites. The coverage is estimated at different heights. Thus, the vascular plants that are growing above the mosses can have a 100% coverage while the mosses growing below the vascular plants in the same site can simultaneously reach a 100% coverage

the exclosures (mean: 40.3 µM) and the grazed sites (mean: 9.9 µM), followed by fructose (EX mean: 18.3 µM and grazed site mean: 5.4 µM). There was less mannose, xylose and galactose in the peat, but the concentrations were always higher in the exclosures (mean EX: mannose 5.8 µM, xylose 2.2 µM, galactose 0.4 µM) than the grazed sites (mean GR: mannose 0.8 µM, xylose b.d., galactose 0.02 µM).

We also observed significantly higher concentrations of a broad range of amino acids in the exclosures (Fig. 2b and Table S3—online resource 2). Aspartic acid and alanine had the highest concentrations with mean values of 10.5 and 13.2 µM, respectively, in the exclosures, while the concentrations in the grazed sites were much lower (0.9 and 2.3 µM). The amino acids glycine, leucine, proline, serine and threonine ranged from 4.0 to 6.8 µM in the exclosures and 0.6 to 1.3 µM in the grazed sites. Only glutamic acid was found at lower concentrations in the exclosures (mean EX: 0.9 µM) compared to the grazed sites (mean GR: 2.0 µM). The other amino acids were below 1.8 µM (mean values) but always at higher concentrations in the exclosures than the grazed sites.

Microbial activities in the peat soil

To study the link between the vegetation, soil chemistry and the composition and activity of the microbial community we extracted total nucleic acids for the analysis of DNA and RNA from four replicate samples of the upper 2 cm oxic layer of peat soil in the exclosures and four from the grazed sites. As depicted in Fig. 2c we observed higher DNA amounts in the exclosures than the grazed sites (mean: EX 92.5 µg DNA gDW peat^{-1}, GR 58.3 µg DNA gDW peat^{-1}), while the amount of RNA per gram dry weight was equally high at the two sites (mean: EX 71.2 µg RNA gDW peat^{-1}, GR 69.6 µg RNA gDW peat^{-1}) (Fig. 2c and Table S5—online resource 2). Next, we investigated the potential enzyme activities for decomposition of some of the most common plant polysaccharides—cellulose, mannan, xyloglucan, xylan, arabinoxylan, galactan, arabinan and rhamnogalacturonan (Sarkar et al. 2009). This confirmed that the potential for polysaccharide degradation was significantly higher in the exclosures than the grazed sites (Fig. 2d and Table S3—online resource 2).

In order to study the microbial communities and their patterns of gene transcription, we removed ribosomes from the total RNA by ribodepletion and sequenced the remaining RNA as well as the total DNA from four replicates collected in the exclosures, and four in the grazed sites, giving eight metatranscriptomic and eight metagenomic libraries in total. Each of the 16 libraries contained ~ 38–55 million sequence reads with a length of ~ 150 bp. The ribodepleted RNA libraries contained 48–83% non-rRNA sequences, ~ 30%

Table 2 Polysaccharide composition of the peat soils

Polysaccharide (antibody ID)	Exclosure Mean value ± sd ($n = 4$)	Grazing Mean value ± sd ($n = 4$)
Cellulose (mAb CBM3a)	1.50E−03 ± 1.63E−04	5.70E−04 ± 1.58E−04*
(1 → 3)-β-D-glucan (mAb BS-400–2)	2.58E−04 ± 3.12E−05	1.15E−04 ± 3.72E−05*
(1 → 3)(1 → 4)-β-D-glucan (mAb BS-400–3)	5.61E−03 ± 2.12E−03	8.20E−04 ± 4.19E−04*
Xyloglucan (mAb LM15)	2.60E−03 ± 7.33E−04	1.38E−04 ± 9.97E−05*
Xyloglucan (mAb LM24)	2.40E−04 ± 2.09E−05	7.89E−05 ± 1.89E−05*
Xyloglucan (mAb LM25)	2.58E−03 ± 2.48E−04	5.29E−04 ± 2.63E−04*
(1 → 4)-β-D-xylan (mAb LM10)	1.09E−03 ± 3.02E−04	4.50E−04 ± 2.06E−04*
(1 → 4)-β-D-xylan/arabinoxylan (mAb LM11)	2.45E−03 ± 1.18E−03	3.25E−04 ± 2.16E−04*
(1 → 4)-β-D-(galacto)mannan (mAb BS-400–4)	3.40E−03 ± 9.71E−04	1.15E−04 ± 5.08E−04
(1 → 4)-β-D-(galacto)(gluco)mannan (mAb LM21)	2.96E−03 ± 6.60E−04	1.18E−03 ± 2.78E−04*
(1 → 4)-β-D-(gluco)mannan (mAb LM22)	7.80E−04 ± 1.44E−04	4.22E−04 ± 1.88E−04
Non-acetylated xylosyl residues (mAb LM23)	1.27E−03 ± 4.54E−04	3.74E−04 ± 7.14E−05*
Anti callose/MLG like binding (mAb JIM6)	4.90E−04 ± 1.24E−04	2.61E−04 ± 2.28E−04
Arabinogalactan protein (mAb LM2)	1.49E−03 ± 8.74E−04	1.08E−04 ± 4.54E−05*
Homogalacturonan (Low DE) (mAb JIM5)	2.11E−04 ± 1.48E−04	2.46E−04 ± 9.68E−05
Homogalacturonan (Intermediate DE) (mAb LM7)	1.86E−04 ± 1.17E−04	4.36E−05 ± 1.39E−05
Homogalacturonan (High DE) (mAb JIM7)	8.48E−04 ± 6.13E−04	2.42E−04 ± 8.83E−05
Homogalacturonan (mAb LM18 MUC2)	3.94E−04 ± 4.41E−04	3.53E−04 ± 1.21E−04
Homogalacturonan (mAb LM19 XGA2)	4.73E−04 ± 6.01E−04	3.49E−04 ± 1.35E−04
Xylogalacturan (mAb LM8)	6.08E−05 ± 1.60E−05	2.69E−05 ± 7.94E−06
(1 → 4)-β-D-galactan (mAb LM5)	2.16E−03 ± 1.02E−03	5.37E−04 ± 3.65E−04
(1 → 5)-α-L-arabinan (mAb LM6)	1.07E−03 ± 2.58E−04	3.00E−04 ± 7.65E−05
(1 → 5)-α-L-arabinan (mAb LM13)	1.66E−05 ± 6.75E−06	3.52E−06 ± 1.95E−06*
(1 → 5)-α-L-arabinan (mAb LM16)	4.88E−05 ± 2.69E−05	2.11E−05 ± 1.01E−05
Rhamnogalacturonan (mAb INRA-RU1)	2.26E−03 ± 3.82E−04	5.94E−04 ± 2.53E−04*
Rhamnogalacturonan (mAb INRA-RU2)	8.94E−04 ± 3.74E−04	1.13E−04 ± 6.08E−05*

The table lists the mean values (±—the standard deviations) of binding signals (for dry weight soil; gDW) for polysaccharides in the peat soils. The mean values are derived from four replicates from the exclosures and four replicates from the grazed sites. The polysaccharide composition of the soil matrix was determined using Comprehensive Microarray Polymer Profiling (CoMPP). In cases where there are several antibodies for the same polysaccharide, these are antibodies with different binding properties, e.g., for different numbers of backbone repeats. All polysaccharides marked with an '*' show a significant difference of the grazed and the exclosed. For statistical testing the Wilcoxon rank sum test was used with R (v3.6.1), values can be found in Table S3

mAb monoclonal antibody, *MLG* mixed linkage glycan, *DE* degree of methyl esterification

of which could be taxonomically classified and 10–15% of which could be functionally classified (Table S6—online resource 2). The microbial community composition based on taxonomic annotation of mRNA was overall similar in the exclosures and grazed sites, but notable differences were also observed (Figs. 3 and S2—online resource 3). Bacterial genes and transcripts comprised approximately 90% of total mRNA and 92% of total rRNA genes at exclosed sites, compared to approximately 96% of total mRNA and 95% of total rRNA genes at grazed sites. Correspondingly, the abundances of reads assigned to eukaryotic kingdoms (fungi, Protista and Metazoa) were particularly high in the exclosures relative to the grazed sites (Figs. 3 and S3—online resource 3). However, the 16S rRNA gene abundance and transcriptional activity were dominated by *Actinobacteria*, followed by *Alpha-*, *Beta-*, *Delta-* and *Gamma-proteobacteria*, *Chloroflexi*, *Acidobacteria*, *Verrucomicrobia* and *Bacteriodetes* in both grazed sites and exclosures. There were also considerable numbers of reads assigned to Ciliophora, fungi and Nematoda (Figs. 3 and S2—online resource 3). Overall, the most abundant taxa (SSU rRNA gene abundance) were the transcriptionally (mRNA abundance) most active ($r = 0.86$–0.94; only the taxa displayed in the boxes considered: Fig. 3a and b).

To identify which microbial taxa were responsible for polymer degradation we extracted from the metagenomes and metatranscriptomes genes and transcripts encoding enzymes for polysaccharide and lignin degradation (Table S7—online

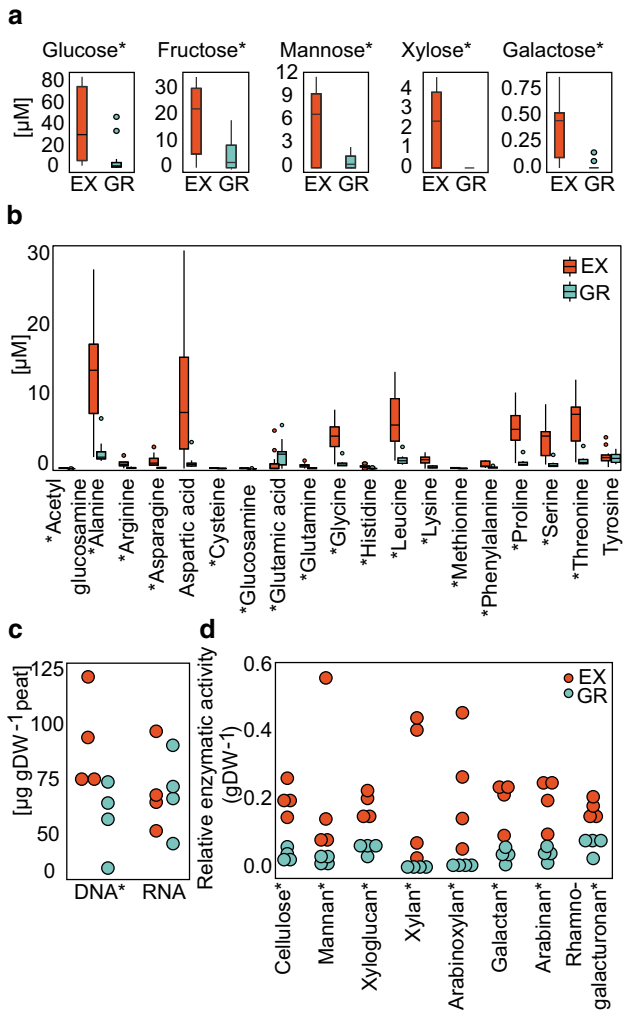
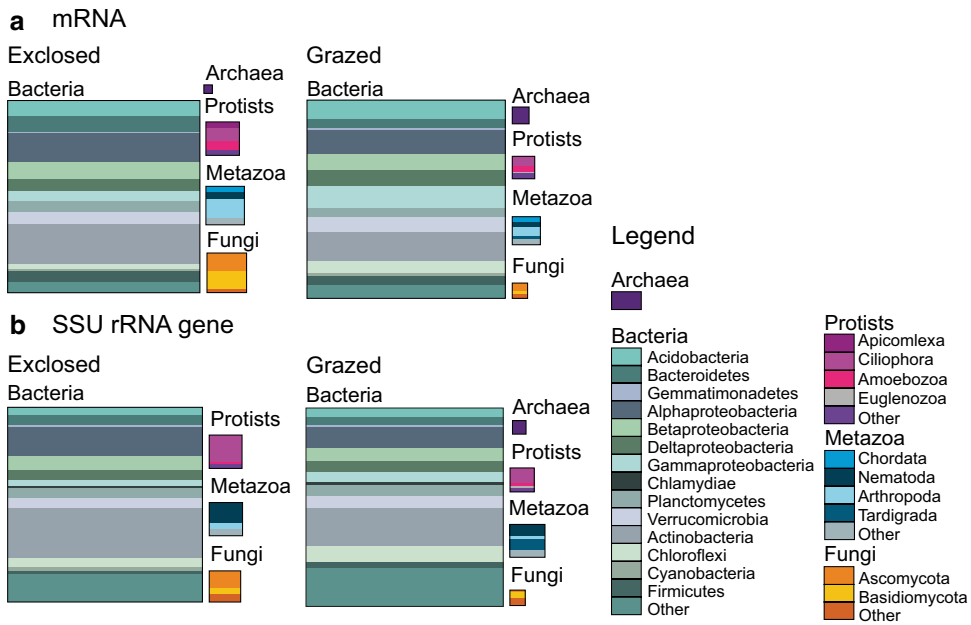


Fig. 2 Sugar and amino acid concentrations, masses of nucleic acids and polysaccharide degrading enzyme rates in peat soil samples exclosures (red) and grazed sites (blue). **a** Pore water concentrations [µM] of the sugars glucose, fructose, mannose, xylose and galactose. **b** Pore water concentrations [µM] of amino acids. **c** Masses of DNA and RNA in micrograms per gram of dry peat soil [µg gDW^{-1} peat]. **d** Enzymatic potential for polysaccharide degradation on eight polysaccharide substrates. Individual dots show the signal strength relative to the sum of measured signal strength for each substrate. The values thus indicate the potential enzyme activity in the eight samples relative to each other. The rates were normalized by dry weights (DW). All compounds marked with an '*' show a significant difference of the grazed and the exclosed. For statistical testing the Wilcoxon rank sum test was used with R (v3.6.1), values can be found in Table S3

resource 2). In the exclosures, the microbial transcription for cellulose degradation was highest (0.40%), followed by hemicellulose (0.36%), pectin (0.24%) and lignin (0.16%) (Fig. 4a). In the grazed sites, the transcription for hemicellulose degradation was highest (0.31%), followed by cellulose (0.21%), pectin (0.07%) and lignin (0.04%). Corresponding to the higher abundance of fungi (Figs. 3 and S3—online resource 3), a larger fraction of the genes and transcripts for polymer decomposition were assigned to fungi in the exclosures (18.95%) than the grazed sites (1.38%) (Fig. 4a). The majority of these transcripts were assigned to the fungal phyla *Basidiomycota* (6.32% EX & 0.60% GR) and *Ascomycota* (10.56% EX & 0.48% GR). *Actinobacteria* (30.92% EX & 20.14% GR), *Proteobacteria* (11.28% EX & 14.51% GR) and *Bacteriodetes* (8.46% EX & 13.82% GR) were the most transcriptionally active bacterial polysaccharide degraders, while the majority of transcripts for lignin degradation were assigned to *Proteobacteria* (28.15% EX & 33.03% GR) (Fig. 4b). The taxonomic distribution of genes for polymer degradation was similar at exclosed and grazed sites, with the exception that the transcript to gene ratio was much higher for fungal than bacterial taxa (Fig. 4b).

Taxa that had different transcriptional activities in the exclosures and grazed sites were identified by correspondence analysis (Figure S4a—online resource 3). The transcriptional profiles from the exclosures were separated from the grazed sites along the first axis, explaining 42.7% of the inertia. By their contribution to the first axis inertia we identified the major eukaryotic and prokaryotic orders with different transcriptional activities in the exclosures and grazed sites. Out of the 20 taxa contributing most to inertia in each direction (Figure S4b—online resource 3; and Table S8—online resource 2), 15 taxa had higher numbers of transcripts in either the exclosures or the grazed sites, and an average relative abundance of mRNA transcripts above 0.5%. Among these, three eukaryotic (*Agaricales*, *Helotiales* and Entomobryomorpha) and seven bacterial orders (*Lactobacillales, Chitinophagales, Burkholderiales, Sphingobacteriales, Nakamurellales, Corynebacteriales, Micrococcales*) were more active in the exclosures, whereas five bacterial orders (*Methylococcales*, *Anaerolineales*, *Solibacterales*, *Thiotrichales*, *Desulfobacterales*) were more active in the grazed sites. These 15 orders accounted for 7 to 18% of the total number of microbial mRNA transcripts. We then compared the transcriptional activity and relative abundance of SSU rRNA genes for these 15 taxa (Figure S4b—online resource 3), finding similar patterns in the SSU rRNA genes and the mRNA for some of the taxa. Particularly interesting were the much higher relative abundances of the three eukaryotic orders in the exclosures: *Agaricales* was on average 7.7-fold (SSU rRNA genes) and 71.4-fold (mRNA) higher, *Helotiales* was 29.8-fold (SSU rRNA genes) and 46.9-fold (mRNA) higher, while Entomobryomorpha was 20.4-fold (SSU rRNA genes) and 11.8-fold (mRNA) more abundant and transcriptionally active (Fig. 5).

The number of polymer degradation transcripts assigned to *Helotiales* (of fungal transcripts: 21.5% EX, 4.0% GR) and *Agaricales* (of fungal transcripts: 37.6% EX, 4.3% GR) show that these are the major fungal polysaccharide degraders in the exclosures. Their profiles



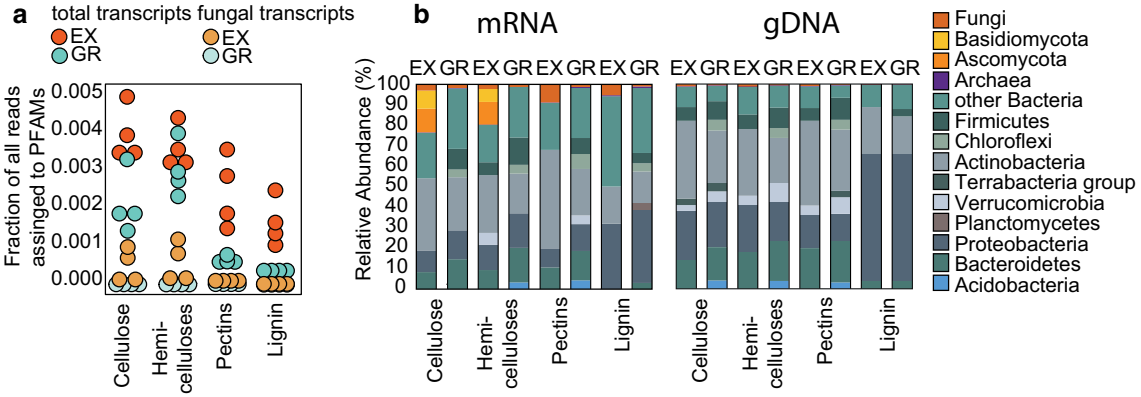


Fig. 3 Microbial community composition **a** based on mRNA sequences and therefore representing the transcriptionally active microbial community. **b** Based on SSU rRNA gene sequences reflecting the potential microbial community in peat soil from exclosures and grazed sites. The size of the boxes reflect the relative abundances of taxa. Taxonomy profiles are displayed at phylum-level (class-level for *Proteobacteria*) and are generated by averaging data sets from four replicates

Fig. 4 Genes and transcripts for polysaccharide and lignin degradation in prokaryotes and eukaryotes. **a** Number of transcripts for cellulose, hemicellulose, pectins and lignin degradation relative to the total number of reads assigned to a function for all microorganisms (left) and fungi (right). **b** Taxonomic assignment of metatranscriptomic (mRNA) and metagenomic (gDNA) sequences encoding enzymes involved in polysaccharide and lignin degradation. The functional annotation is at phylum-level, comparing exclosures (EX) and grazed (GR) sites

included transcripts for cellulose, xylans, glucans, mannans, pectins, lignin, chitin and bacterial cell walls (Fig. 5a and Table S9—online resource 2). *Helotiales* expressed genes for a broader range of substrates than *Agaricales* (Table S9—online resource 2), but for both fungal orders the relative abundance of transcripts for most polymers were higher in the exclosures (Fig. 5a). Similarly, the transcriptional activity of the arthropod order Entomobryomorpha was much higher in the exclosures (Fig. 5b). It had a narrower substrate range than the fungi, lacking transcripts for xylan, pectin and lignin decomposition (Table S9—online resource 2). However, Entomobryomorpha transcripts for bacterial cell wall, chitin, cellulose and oligosaccharide degradation were present, and consistently more abundant in the exclosures than the grazed sites (Fig. 5b). Finally we assessed whether the above patterns of transcription for polymer degradation were reflected in the transcription for central metabolisms in these three orders. We functionally annotated the *Agaricales*, *Helotiales* and Entomobryomorpha transcripts using KEGG (Kyoto encyclopedia of genes and genomes). This revealed that the transcript abundances for central metabolisms were highest in the exclosures for all three taxa (Figure S5—online resource 3), but with considerable variation between samples, especially for the fungi.

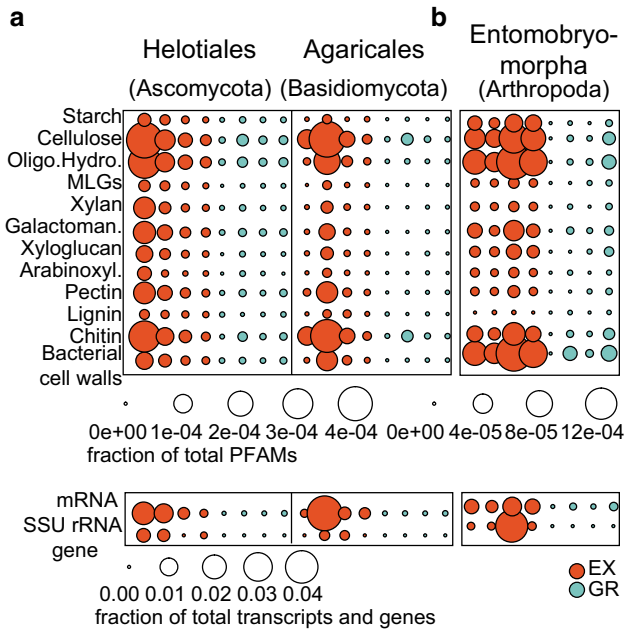


Fig. 5 Transcriptional profiles for polymer degradation by *Helotiales, Agaricales* and Entomobryomorpha. **a** Functional profile of the two fungal orders *Helotiales* and *Agaricales* displaying relative abundances of transcripts in exclosures (red) and grazed sites (blue) for enzymes involved in polymer degradation as described in Table S9. The fraction of total transcripts (mRNA) and rRNA genes assigned to these two fungal orders are shown in the lower box. **b** Functional profile of the order Entomobryomorpha showing its transcription for polymer degradation in the replicates of peat soils from the exclosures (red) and grazed sites (blue). In the lower square, the fraction of total transcripts (mRNA) and rRNA genes assigned to Entomobryomorpha is displayed. Oligo.Hydro—oligosaccharide hydrolysis; MLGs—mixed linkage glycans; Galactoman.—galactomannan; Arabinoxyl.—arabinoxylan

Discussion

In this study we have compared the microbial communities in soils below high-Arctic peat vegetation exposed to grazing and soils below peat vegetation protected from grazing for 18 years. The investigation of both the above- and belowground biology has allowed us to study the relationship between the vegetation and the soil microorganisms. Our comparison of the two soil treatments demonstrated that the removal of herbivores and altered vegetation leads to a different soil polysaccharide composition, lower potential extracellular enzymatic activities and a different microbial community with lower abundances and activities of fungi and putative predatory eukaryotes.

Reduced herbivore grazing alters the vegetation, soil structure and soil composition

We observed that a different vegetation with an 8 times larger coverage of vascular plants had established after 18 years of protection inside the exclosures. This shift, primarily caused by increased abundance of *P. arctica*, was already observed some years earlier as evidenced by a 12 times larger root biomass and 28 times larger vascular plant biomass in the exclosures than the grazed sites (Sjögersten et al. 2011). The vascular vegetation was associated with reduced soil water contents and higher oxygen levels in deeper layers, presumably due to the increased root formation. Similar observations were made in a study of a temperate peatland, where lower water table heights were observed in areas with greater vascular plant biomass (Murphy et al. 2009). The lowering of the water table and the diffusion of gases through the roots of vascular plants might be the reasons for the higher oxygen availabilities (Colmer 2003). However, at the surface (top ~ 0–2 cm) sampled for our molecular and chemical analyses, the oxygen levels were the same in exclosures and grazed sites, suggesting that oxygen limitation is mostly relevant in deeper layers.

The increased input of organic matter from *P. arctica* and other vascular plants to the soil inside the exclosures can explain the higher organic matter content and abundance of polysaccharides compared to the grazed sites. Previously, geese grazing and grubbing were found to reduce carbon stocks in Arctic wetlands from Svalbard and Canada (Van der Wal 2006; Speed et al. 2010; Sjögersten et al. 2011). In particular, we identified higher soil content of xyloglucan, arabinoxylan and pectins in the exclosures, matching the higher concentrations of monosaccharides. However, determining the exact origin of the monosaccharides in soils is challenging, as they might be root exudates (Bertin et al. 2003; Bais et al. 2006), polysaccharide hydrolysis products (Tveit et al. 2013) or both. The release of low-molecular weight compounds like sugars through root exudation varies e.g. with the plant type, its age and environment (Uren 2000; Bertin et al. 2003; Bais et al. 2006; McNear 2013). Monosaccharide stocks also depend on the kinetics of their usage, for which we do not have estimates. Nevertheless, the occurrence of more roots, more polysaccharides and higher potential rates of extracellular polysaccharide degrading enzymes suggests a combination of root exudation and polysaccharide hydrolysis as sources for the monosaccharides.

Increased input from vascular plants supports a more abundant and active microbiota

The higher availability of carbohydrates fueled a more abundant and active microbiota in the exclosures, judging by the amount of DNA per gram of dry soil. Although not a direct measure of total microbial biomass or the number of cells, nucleic acids provide good estimates for relative differences in the size of the microbiota between samples with similar properties for nucleic acids extraction (Tveit et al. 2015; Söllinger et al. 2018). Considering the higher

organic matter content in the exclosures, the more abundant microbiota might be the result of higher substrate availability. This could explain the higher rates for extracellular polysaccharide degradation in the exclosures as more organisms generally would produce more enzymes. We also observed a considerably higher relative abundance of transcripts for extracellular polymer degrading enzymes in the exclosures, supporting the view that increased substrate availabilities leads not only to increased number of microorganisms but also to larger investments into extracellular polymer decomposing enzymes by microorganisms (Zak and Kling 2006; Wallenstein and Weintraub 2008). The altered organic matter quality may also be the reason for the increased relative abundance of fungi as they are believed to be specialists for the decomposition of, e.g., lignocellulose (Baldrian 2008). The effect of substrate quality on microbial activities was also seen in the higher potential enzyme rates for degradation of multiple different polysaccharides, in the exclosures, matching the higher abundances of these polysaccharides in the exclosures.

Altered structure and activity of the microbial communities

In line with the considerable changes in vegetation and soil chemistry we observed two-fold higher eukaryote to prokaryote gene ratios and five-fold higher transcript ratios in the exclosures than the grazed sites. A handful of taxa were instrumental to this shift; saprotrophic fungi of the orders *Helotiales* (*Ascomycota*) and *Agaricales* (*Basidiomycota*), and microbial predators and plant litter consumers of the order Entomobryomorpha (Arthropoda). In previous studies, fungal abundances were shown to be positively correlating with root exudation (Broeckling et al. 2008) and more generally the availabilities of easily degradable compounds like sugars (Edgecombe 1938; Thormann 2006) or increased nitrogen availability (Koranda et al. 2014). Increased monosaccharide concentrations could also be products of fungal degradation of polysaccharides. Thus, in our case, it is not possible to conclude on the causality between monosaccharide concentrations and fungal abundances. Nevertheless, *Helotiales* and *Agaricales* have very broad substrate ranges that includes cellulose and all major hemicelluloses and might thus have benefitted from the altered substrate quality and contributed to a variety of carbon decomposition activities once established in the exclosures.

Geisen et al. (2016) found that many protists and arthropods are facultative mycophagous, making them key players in soil microbial food webs. Hence, the higher fungal abundances in the exclosures might have supported the increase in protists and metazoan taxa abundances.

Overall, the vascular vegetation seems to sustain a more complex food web of prokaryotic and fungal detritivores that are food sources for predatory eukaryotes. Whether this is what sustains the larger amino acid pools observed in the exclosures is unclear, but if the transcriptional activity and relative abundance of putative predators reflects predation rates, it may, as bacterial and fungal cells consist of 10–70% protein (Ritala et al. 2017). Our results suggest that Entomobryomorpha are able to degrade bacterial cell walls but also plant and fungal polymers. By regulating microbial turnover, Entomobryomorpha have previously been shown to enhance nutrient mineralization rates and increase nitrogen availability in soils (Cragg and Bardgett 2001; Thakur et al. 2015). Additionally, amino acids may be released through root exudations (Canarini et al. 2019), offering an alternative explanation for the amino acid pools. The origin of the nitrogen needed to support a larger microbial biomass and amino acid pool cannot be directly identified with our data. However, Solheim et al. (1996) showed that nitrogen fixation in Svalbard soils protected from grazing is limited compared to grazed soils where it was supported by epiphytic cyanobacteria. This indicates that nitrogen is lost from grazed systems and regained through fixation, while in the absence of herbivores the microbiota are able to recycle the nitrogen in decaying plants, supporting larger plant and microbial biomasses, and pools of free amino acids.

Low molecular weight nitrogen sources, like amino acids, are important for several microbial processes, hence fueling the microbial community in soils (Schimel and Weintraub 2003; Jones et al. 2004), but also the vegetation (Jones et al. 2004, 2005; Sauheitl et al. 2009). Thus, in the absence of herbivory, feedback effects between an increasingly active microbial community and vegetation might have accelerated the establishment of a new ecosystem state. An important future task will be to identify the fluxes of amino acids, sugars and other metabolites between plants and microorganisms during this ecosystem transition from a grazed to non-grazed state.

Conclusion

The establishment of a larger vascular plant biomass in the absence of herbivores led to higher soil concentrations of polysaccharides, monosaccharides and amino acids. We found that this corresponded with larger and more active populations of saprotrophic fungi and putative predatory eukaryotes. This study establishes a fundament for targeting molecular and microbial mechanisms that control the interactions between above and below ground biology in high-Arctic peatlands.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00300-021-02846-z.

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Author contributions ATT, KMB and MMS conceived the study. KMB and ATT performed fieldwork. KMB, YH, JS and BJ performed experiments. AR contributed pore water analyses. KMB and ATT analyzed the data. KMB and ATT wrote the manuscript with input from all authors.

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Data availability Metagenomes and metatranscriptomes have been deposited in the Sequence Read Archive (SRA) database (BioProject Accession: PRJNA170725, https://www.ncbi.nlm.nih.gov/sra/PRJNA170725). For details about run accession numbers, see supplementary data availability (online resource 1).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Research involving human and animal participants The research was performed without involving human participants or animals subjects. We declare informed consent.

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Supplementary figures:

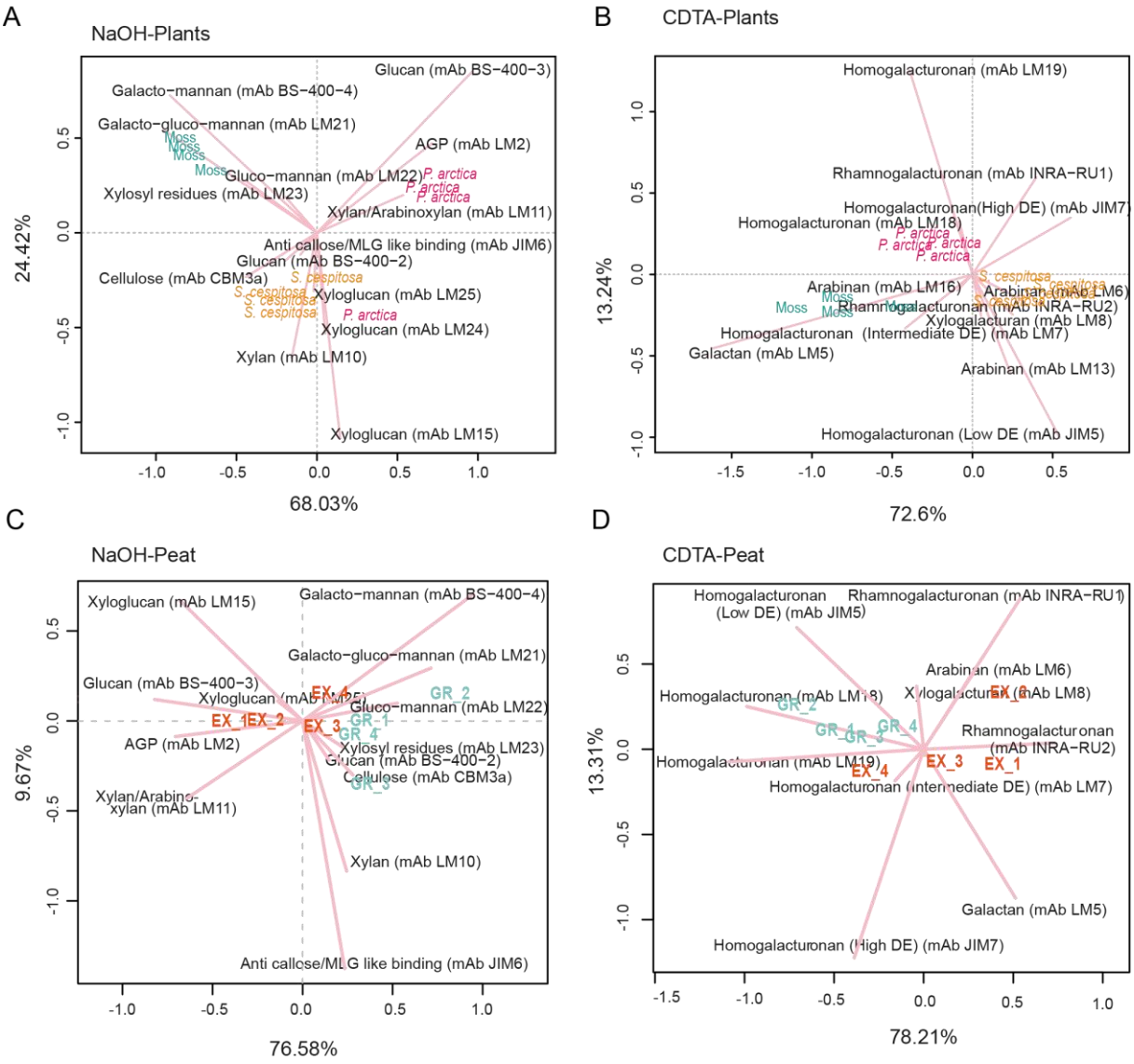

Figure S1: Correspondence Analysis of polysaccharide binding signals in *Poa arctica*, *Saxifraga cespitosa* and a moss mix. A) NaOH extraction (cellulose, hemicelluloses and glycoproteins) of the three plants. B) CDTA extraction (pectins) of the three plants. **Correspondence Analysis of polysaccharide binding signals in exclosed and grazed sites.** C) NaOH extraction (cellulose, hemicelluloses and glycoproteins) of the two sites. D) CDTA extraction (pectins) of the two sites.

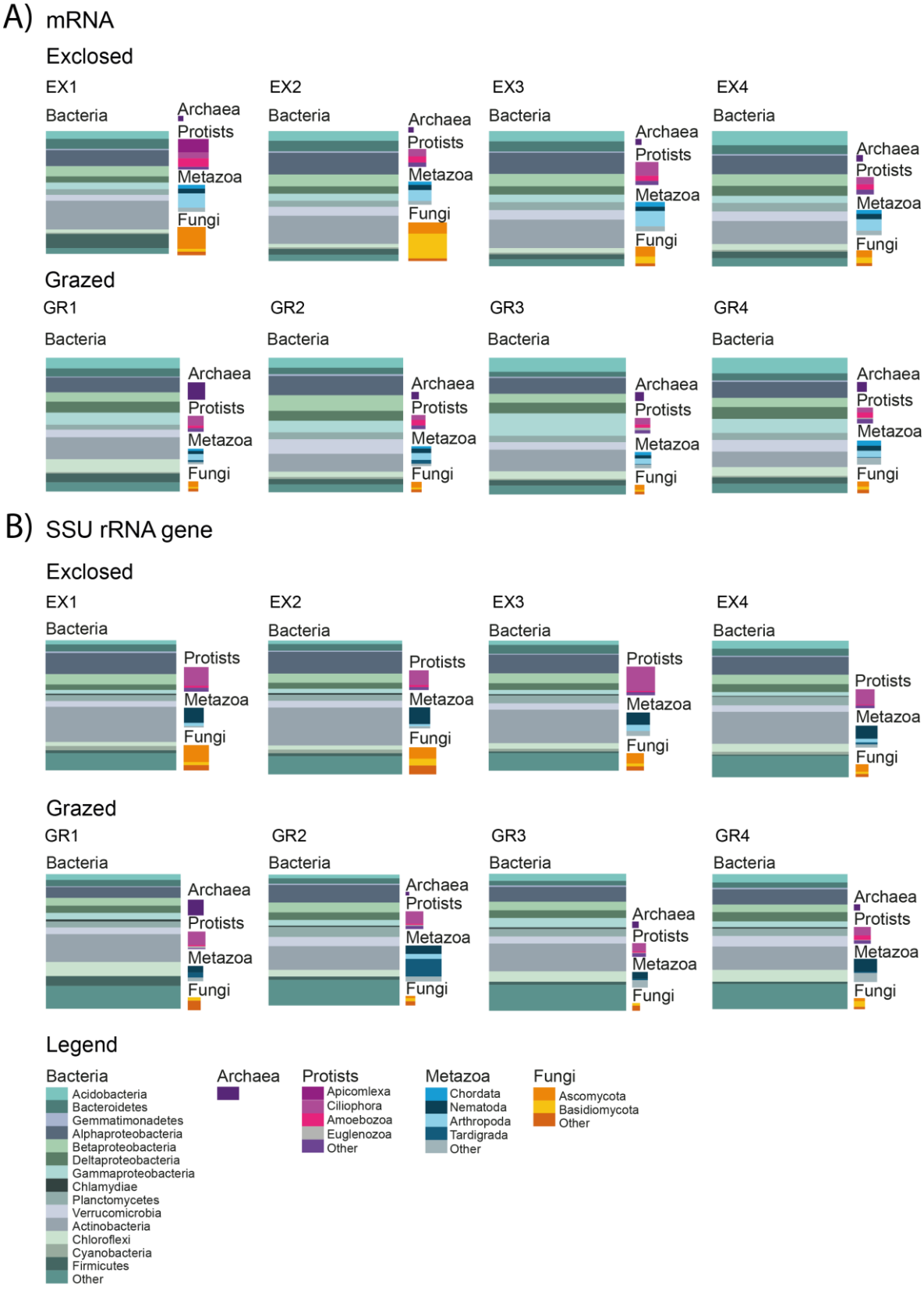

Figure S2: Microbial community composition A) based on mRNA sequences and therefore representing the transcriptionally active microbial community. B) based on SSU rRNA gene sequences reflecting the potential microbial community in peat soil from exclosures and grazed sites. The size of the boxes reflect the relative abundances of taxa. Taxonomy profiles are displayed at phylum-level (class-level for *Proteobacteria*) and show each replicate separately.

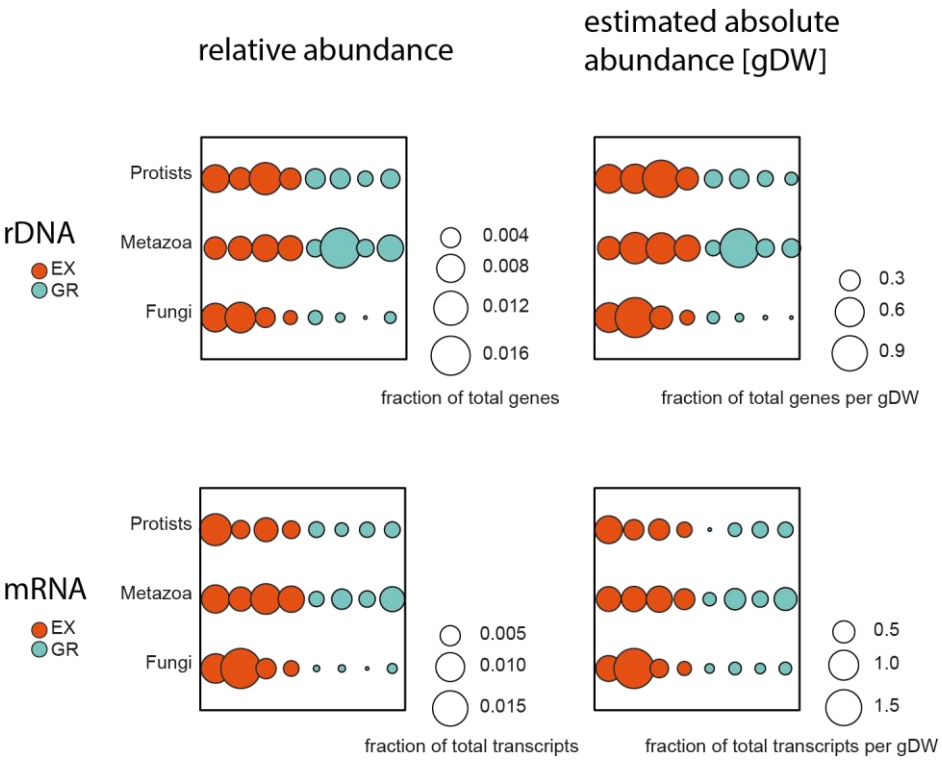

Figure S3: Abundances of eukaryotic kingdoms in the grazed sites and exclosures. Fraction of eukaryotic reads belonging to fungi, Protista and Metazoa for the mRNA and SSU rRNA gene datasets as relative abundances and relative abundances normalized by DNA and RNA masses per gram of dry soil (gDW). The size of each circle represents the fraction of reads in the total number of genes or transcripts.

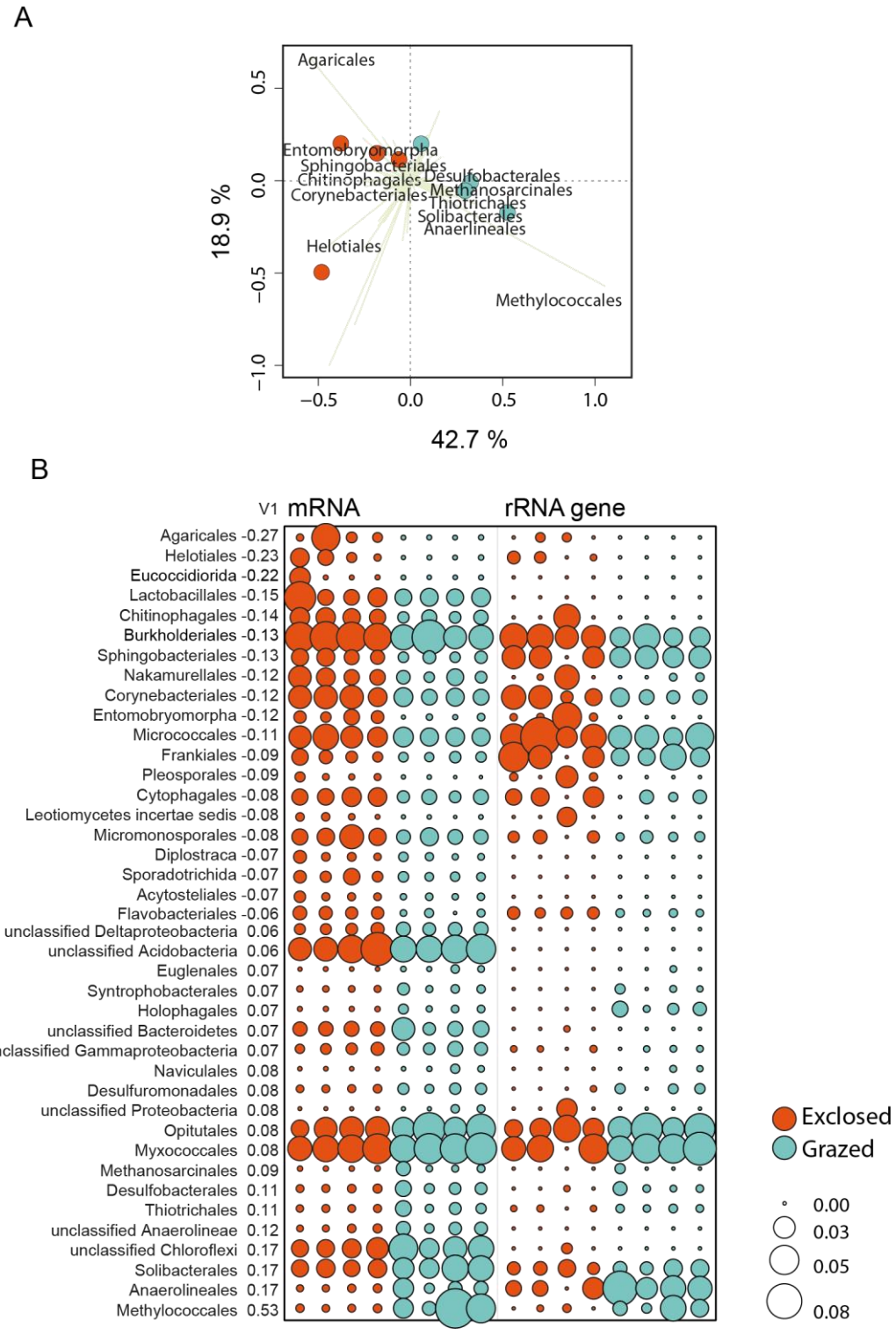

Figure S4: Extraction of the microbial orders that have the largest differences in transcriptional activity between the exclosures and grazed sites. A) Correspondence analysis based on mRNA counts at order level taxonomy. B) Number of mRNA transcripts and rRNA genes assigned to the 20 taxa with the largest differences in transcriptional activity between exclosures and grazed sites along the first axis in both directions from A).

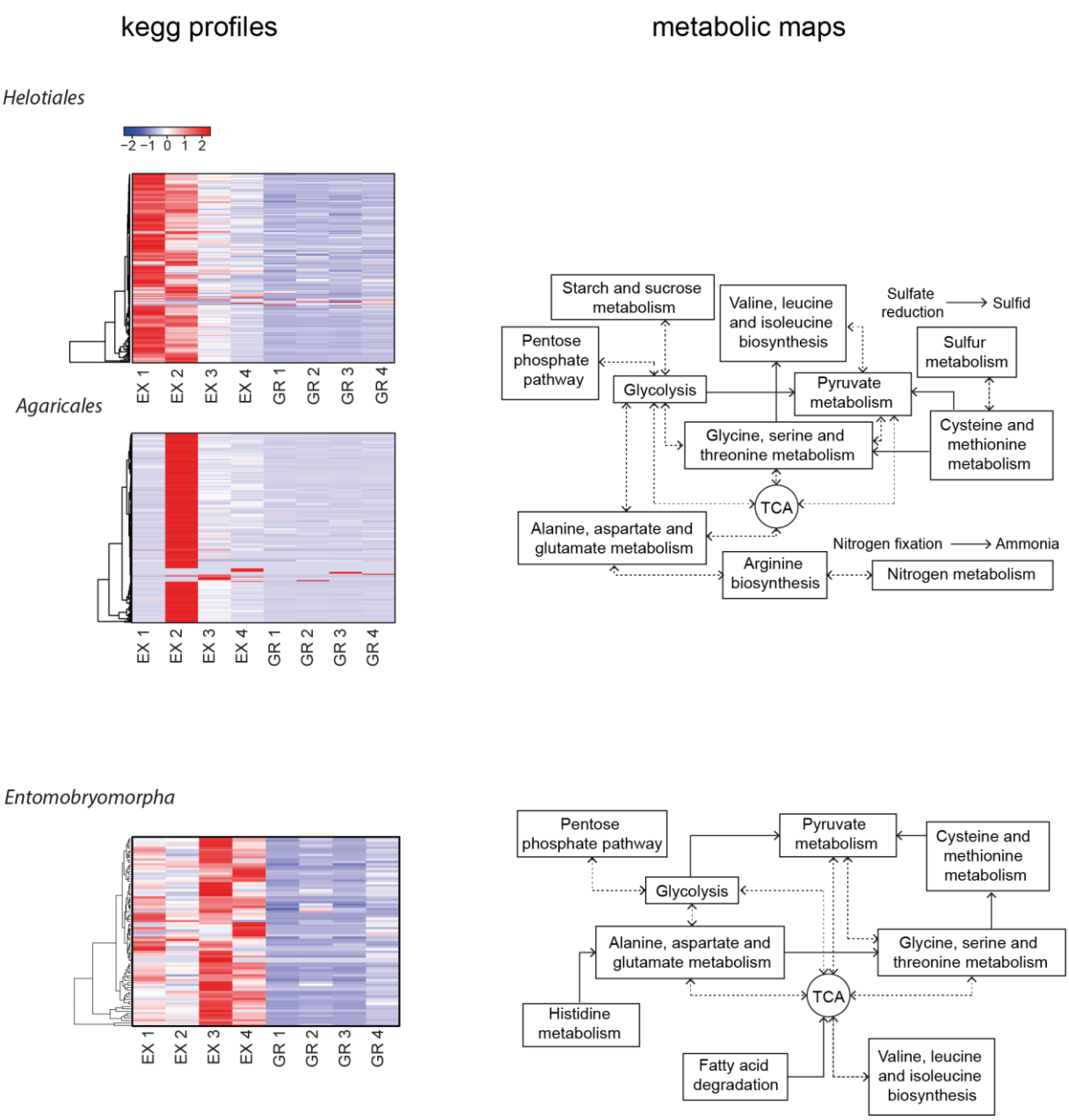

Figure S5: Kegg profiles of the central metabolism in the eukaryotic orders *Helotiales*, *Agaricales* and Entomobryomorpha. Each line in the heatmap corresponds to a k number, which represents one molecular function. The central metabolism map to the right of each heatmap show the categories of central metabolism functions included in the heatmap. Overall, the figure shows that the transcription of genes for the central metabolism enzymes is higher for all three taxa in the exclosures.

Supplementary tables:

Table S1: Comparison of air saturation [O2 %] and temperature [T °C] for the exclosed and grazed sites at four different depths.

	O_2 [%]				T [°C]				measured
	- 2 cm	-5 cm	-10 cm	-15 cm	0 cm	-5 cm	-10 cm	-15 cm	
Exclosure 1	20.0	18.9	13.2	6.9	10.4	6.5	4.9	3.6	01.08.2016
Exclosure 2	20.3	19.9	16.4	-	10.8	8.8	5.4	-	02.08.2016
Grazed 1	20.1	16.4	4.0	3.0	8.3	7.1	4.9	3.4	01.08.2016
Grazed 2	19.9	13.2	0.4	1.6	14	10	6.9	4.9	02.08.2016

Table S2: Water content and total organic matter (TOM) content of the peat soils from the exclosed and grazed sites.

Sample ID	Water content [%]	TOM [%]
EX1	83.5	14.2
EX2	85.3	12.4
EX3	85.9	11.3
EX4	83.2	14.0
GR1	94.7	4.7
GR2	92.8	6.5
GR3	90.5	7.7
GR4	91.6	7.2

Table S3: Statistical testing of results presented in figure 2 (sugars, amino acids, microbial biomass and enzymatic activity) and table 3 (polysaccharides), comparing the exclosed and grazed sites. The Wilcoxon rank sum test were used with R (v3.6.1).

		Wilcoxon rank sum test	
Sugars			
	Glucose	0.0023	*
	Mannose	0.047	*
	Xylose	0.0011	*
	Galactose	0.0006	*
	Fructose	0.0455	*
Amino acids			
	AcetylGlucosamine	0.01	*
	Phenylalanine	0.0145	*
	Tyrosine	0.8428	
	Leucine	0.0018	*
	Methionine	0.0173	*
	Cysteine	0.0173	*
	GlutamicAcid	0.0483	*
	Alanine	0.0023	*
	Threonine	0.0014	*
	Serine	0.0112	*
	AsparticAcid	0.0529	
	Glycine	0.0009	*
	Proline	0.0112	*
	Asparagine	0.0002	*
	Glutamine	0.0068	*
	Glucosamine	0.002	*
	Histidine	0.01	*
	Arginine	0.0145	*
	Lysine	0.0121	*
Microbial biomass			
	DNA [gDW]	0.0286	*
	RNA [gDW]	0.4857	
Enzymes			
	Cellulose	0.029	*
	Mannan	0.029	*
	Xyloglucan	0.029	*
	Xylan	0.029	*
	Arabinoxylan	0.029	*
	Galactan	0.029	*
	Arabinan	0.029	*
	Rhamnogalacturonan	0.029	*
Polysaccharides			
	mAb		
(1→3)-β-D-glucan	BS-400-2	0.02857	*

(1→3)(1→4)-β-D-glucan	BS-400-3	0.02857	*
(1→4)-β-D-(galacto)mannan	BS-400-4	0.05714	
Cellulose	CBM3a	0.02857	*
Rhamnogalacturonan_1	INRA-RU1	0.02857	*
Rhamnogalacturonan_2	INRA-RU2	0.02857	*
HG (Low DE)	JIM5	0.6857	
Anti callose/MLG like binding	JIM6	0.2	
HG (High DE)	JIM7	0.3429	
(1→4)-β-D-xylan	LM10	0.02857	*
(1→4)-β-D-xylan/arabinoxylan	LM11	0.02857	*
(1→5)-α-L-arabinan_2	LM13	0.02857	*
Xyloglucan_1	LM15	0.02857	*
(1→5)-α-L-arabinan_3	LM16	0.1143	
HG_1	LM18 (MUC2)	0.8857	
HG_2	LM19 (XGA2)	0.8857	
AGP	LM2	0.02857	*
(1→4)-β-D-(galacto)(gluco)mannan	LM21	0.02857	*
(1→4)-β-D-(gluco)mannan	LM22	0.05714	
Non-acetylated xylosyl residues	LM23	0.02857	*
Xyloglucan_2	LM24	0.02857	*
Xyloglucan_3	LM25	0.02857	*
(1→4)-β-D-galactan	LM5	0.05714	
(1→5)-α-L-arabinan_1	LM6	0.02857	*
HG (Intermediate DE)	LM7	0.02857	*
Xylogalacturan	LM8	0.02857	*

Table S4: Polysaccharide composition of two vascular plants and a mix of the mosses growing in the Solvatn peatland. The table list the mean values (+- the standard deviations) of binding signals for each tested polysaccharide in the plants from four replicates from the exclosures and four replicates from the grazed sites. The polysaccharide composition of the soil matrix was determined using Comprehensive Microarray Polymer Profiling (CoMPP) as described in the materials and methods.

	Polysaccharide	Antibody ID	*Poa arctica* mean value		sd	*Saxifraga cespitosa* mean value		sd	mosses mean value		sd
1	(1→3)-β-D-glucan	BS-400-2	0.0005	+-	0.0003	0.0023	+-	0.0003	0.0012	+-	0.0007
2	(1→3)(1→4)-β-D-glucan	BS-400-3	0.0382	+-	0.0186	0.013	+-	0.0037	0.0058	+-	0.0003
3	(1→4)-β-D-(galacto)mannan	BS-400-4	0.0004	+-	0.0003	0.0068	+-	0.0012	0.0123	+-	0.0017
4	Arabinogalactan protein	LM2	0.0123	+-	0.005	0.0011	+-	0.0008	0.0007	+-	0.0006
5	(1→4)-β-D-galactan	LM5	0.0112	+-	0.0013	0.0118	+-	0.0015	0.0126	+-	0.0004
6	(1→5)-α-L-arabinan_1	LM6	0.0014	+-	0.0006	0.0062	+-	0.0002	0.0004	+-	0.0002
7	Homogalacturonan (Intermediate DE)	LM7	0.0004	+-	0.0001	0.0005	+-	0.0002	0.0007	+-	0.0001
8	Xylogalacturan	LM8	0.0003	+-	0.0003	0.0013	+-	0.0002	0.0002	+-	0.0001
9	(1→4)-β-D-xylan	LM10	0.0037	+-	0.0015	0.0103	+-	0.0014	0.0024	+-	0.0004
10	(1→4)-β-D-xylan/arabinoxylan	LM11	0.0156	+-	0.0081	0.0096	+-	0.0018	0.002	+-	0.0004
11	(1→5)-α-L-arabinan_2	LM13	0.0004	+-	0.0003	0.0036	+-	0.0015	0.0003	+-	0.0001
12	Xyloglucan_1	LM15	0.0184	+-	0.0014	0.0325	+-	0.0043	0.0037	+-	0.001
13	(1→5)-α-L-arabinan_3	LM16	0.0001	+-	0	0.0003	+-	0.0001	0.0001	+-	0
14	Homogalacturonan_1	LM18 (MUC2)	0.0066	+-	0.0018	0.0131	+-	0.0018	0.0029	+-	0.0006
15	Homogalacturonan_2	LM19 (XGA2)	0.0104	+-	0.002	0.0117	+-	0.0019	0.0035	+-	0.0007
16	(1→4)-β-D-(galacto)(gluco)mannan	LM21	0.0018	+-	0.0012	0.0116	+-	0.0013	0.0159	+-	0.0007
17	(1→4)-β-D-(gluco)mannan	LM22	0	+-	0.0001	0.002	+-	0.0006	0.003	+-	0.0004
18	Non-acetylated xylosyl residues	LM23	0.0004	+-	0.0002	0.0007	+-	0.0002	0.0011	+-	0.0002
19	Xyloglucan_2	LM24	0.0038	+-	0.0017	0.0063	+-	0.0007	0.0008	+-	0.0002
20	Xyloglucan_3	LM25	0.0171	+-	0.0065	0.0261	+-	0.0022	0.009	+-	0.0001
21	Homogalacturonan (Low DE)	JIM5	0.0065	+-	0.0024	0.0338	+-	0.0015	0.0041	+-	0.0015
22	Anti callose/MLG like binding	JIM6	0.0002	+-	0.0002	0.0011	+-	0.0003	0.0004	+-	0.0002
23	Homogalacturonan (High DE)	JIM7	0.0152	+-	0.0027	0.0447	+-	0.0035	0.0033	+-	0.0008
24	Rhamnogalacturonan_1	INRA-RU1	0.0045	+-	0.0021	0.0106	+-	0.001	0.0002	+-	0.0001
25	Rhamnogalacturonan_2	INRA-RU2	0.004	+-	0.0017	0.0122	+-	0.0018	0.0021	+-	0.0034
26	Cellulose	CBM3a	0.0034	+-	0.0018	0.0151	+-	0.0044	0.0072	+-	0.0011

Table S5: DNA and RNA extractions. The table shows masses of DNA and RNA, the percentage of plant DNA and RNA in the samples and the masses of microbial DNA and RNA [µg/g soil].

	EX1	EX2	EX3	EX4	GR1	GR2	GR3	GR4
g soil/sample [dw]	0.11	0.08	0.09	0.09	0.04	0.06	0.07	0.08
ug DNA/g soil [dw]	82.53	132.23	109.17	84.14	58.05	66.31	74.82	35.72
%plant_DNA [dw]	8.88	7.14	13.06	8.46	0.58	1.49	0.39	0.49
ug microbial DNA/g soil [dw]	75.2	122.79	94.91	77.02	57.71	65.32	74.52	35.54
ug RNA/g soil [dw]	90.95	117.98	75.59	62.06	46.98	92.57	67.67	73.39
%plant_mRNA [dw]	24.3	16.91	13.34	15.37	0.56	1	0.53	0.9
ug microbial RNA/g soil [dw]	68.85	98.03	65.51	52.52	46.71	91.64	67.31	72.73

Table S6: Sequence read processing.

Sample	Label	Molecule	PE input read pairs	After trimmomatic	rRNA	non-rRNA	fraction non-rRNA	Assigned taxonomy Diamond NCBI nr	subsample	Assigned taxonomy Diamond NCBI nr	assigned pfam - HMMER
1	1-SV1EX1_S7	DNA	40110589	39549509	32907	39516602	0.999		5000000		857484
2	2-SV1EX2_S8	DNA	39211438	38786159	32549	38753610	0.999		5000000		908511
3	3-SV2EX1_S1	DNA	41387588	40887012	32234	40854778	0.999		5000000		922797
4	4-SV2EX2_S2	DNA	38868695	38444822	27553	38417269	0.999		5000000		902805
5	5-SV1GR1_S3	DNA	46640253	45177386	45941	45131445	0.999		5000000		928532
6	6-SV1GR2_S4	DNA	47843485	47324098	32082	47292016	0.999		5000000		933670
7	7-SV2GR1_S5	DNA	47539855	46909364	29765	46879599	0.999		5000000		932655
8	8-SV2GR1_S6	DNA	52234340	51672485	32981	51639504	0.999		5000000		928690
9	9-SV1EX1_S10	RNA	44840112	44556971	22720940	21836031	0.49	7756628	5000000	1706160	586026
10	10-SV1EX2_S11	RNA	45916642	45336765	23189787	22146979	0.488	8560199	5000000	1817805	674879
11	11-SV2EX1_S12	RNA	53742180	53371652	25019438	28352214	0.531	10374434	5000000	1685816	624831
12	12-SV2EX2_S13	RNA	54049261	53646056	18623860	35022196	0.653	10922592	5000000	1600519	582726
13	13-SV1GR1_S14	RNA	52320760	52016247	87954335	43220815	0.831	8483536	5000000	1264058	457988
14	14-SV1GR2_S15	RNA	39681563	39443065	11535736	27907330	0.708	7888546	5000000	1335876	501529
15	15-SV2GR1_S16	RNA	45171463	44848274	12397684	32450591	0.724	9342801	5000000	1358142	502498
16	16-SV2GR2_S9	RNA	54813817	54388451	22157802	32230650	0.593	9309721	5000000	1342685	500670

Table S7: Plant cell wall polymer degrading enzymes that were used for the functional annotation of the main figures 4 and 5 and the supplement figure S6. Adjusted from Rytioja *et al.*(1).

	Substrate	Enzyme Activity	CAZyme family	EC no
Hemicelluloses	Cellulose	exo-1,3-1,4-glucanase	GH_3	EC 3.2.1.-
		endo-β-1,4-glucanase	GH_5, _6, _7, _8, _9, _44, _45, _48	EC 3.2.1.4
		endo-β-1,3(4)-glucanase	GH_9, _16	EC 3.2.1.6
		α-glucan lyase	GH_31	EC 4.2.2.13
		endo-β-1,3-glucanase	GH_16, _81	EC 3.2.1.39
		exo-β-1,4-glucanase	GH_1, _5, _9	EC 3.2.1.74
		reducing end-acting cellobiohydrolase	GH_7, _48	EC 3.2.1.176
	Mixed-linkage glucan	endo-β-1,3-glucanase	GH_16, _81	EC 3.2.1.39
	Xylan	xylan endotransglycosylase	GH_10	EC 2.4.2.-
		endo-β-1,4-xylanase	GH_5, _8, _10, _11, _30, _43	EC 3.2.1.8
		endo-β-1,3-xylanase	GH_10, _11, _26	EC 3.2.1.32
		reducing-end-xylose releasing exo-oligoxylanase	GH_8	EC 3.2.1.156
	Galactomannan	mannan transglycosylase	GH_5	EC 2.4.1.-
		galactan galactosyltransferase	GH_27	EC 2.4.1.-
		β-1,3-mannanase	GH_5	EC 3.2.1.-
		mannobiose-producing exo-β-mannanase	GH_26	EC 3.2.1.-
		[inverting] exo-α-1,5-L-arabinanase	GH_43	EC 3.2.1.-
		endo-β-N-acetylglucosaminidase	GH_18	EC 3.2.1.96
		exo-β-1,4-mannobiohydrolase	GH_26	EC 3.2.1.100
		α-1,6-mannanase	GH_76	EC 3.2.1.101
		endo-β-1,6-galactanase	GH_5, _30	EC 3.2.1.164
		mannosylglycerate hydrolase	GH_38, _63	EC 3.2.1.170
	Xyloglucan	[inverting] exo-α-1,5-L-arabinanase	GH_43	EC 3.2.1.-
		isoprimeverose-producing oligoxyloglucan hydrolase	GH_3	EC 3.2.1.120
		xyloglucanase	GH_5, _9, _16, _44	EC 3.2.1.151
		xyloglucan:xyloglucosyltransferase	GH_16	EC 2.4.1.207
	Arabinoxylan	arabinoxylan-specific endo-β-1,4-xylanase	GH_5	EC 3.2.1.-
		[inverting] exo-α-1,5-L-arabinanase	GH_43	EC 3.2.1.-
		glucuronoarabinoxylan endo-β-1,4-xylanase	GH_30	EC 3.2.1.136
	Pectin	galactan galactosyltransferase	GH_27	EC 2.4.1.-
		endo-xylogalacturonan hydrolase	GH_28	EC 3.2.1.-
		[inverting] exo-α-1,5-L-arabinanase	GH_43	EC 3.2.1.-
		polygalacturonase	GH_28	EC 3.2.1.15

	invertase	GH_32	EC 3.2.1.26
	α-galacturonase	GH_4, _28	EC 3.2.1.67
	endo-β-1,3-galactanase	GH_53	EC 3.2.1.89
	3-O-α-glucopyranosyl-L-rhamnose phosphorylase	GH_43	EC 3.2.1.99
	rhamnogalacturonase	GH_28	EC 3.2.1.171
	rhamnogalacturonan α-1,2-galacturonohydrolase	GH_28	EC 3.2.1.173
	rhamnogalacturonan α-L-rhamnohydrolase	GH_78	EC 3.2.1.174
	β-porphyranase	GH_16	EC 3.2.1.178
	endo-β-1,3-galactanase	GH_16	EC 3.2.1.181
	3-O-α-glucopyranosyl-L-rhamnose phosphorylase	GH_65	EC 2.4.1.282
		Pfam accession no	
Lignin	Peroxidase	PF00141.18	
	Dioxygenase_C	PF00775.16	
	Cu-oxidase_4	PF02578.10	

Table S8: Microbial taxa for which the largest differences in transcription between exclosures and grazed sites were found. Shown are the twenty taxa (order level) that contribute the most to the inertia represented by the x-axis in figure S4. Negative values indicate that the highest numbers of transcripts were found in the libraries from the exclosures. Positive values indicate the opposite.

	V1
Agaricales	-0.27
Helotiales	-0.23
Eucoccidiorida	-0.22
Lactobacillales	-0.15
Chitinophagales	-0.14
Burkholderiales	-0.13
Sphingobacteriales	-0.13
Nakamurellales	-0.12
Corynebacteriales	-0.12
Entomobryomorpha	-0.12
Micrococcales	-0.11
Frankiales	-0.09
Pleosporales	-0.09
Cytophagales	-0.08
Leotiomycetes incertae sedis	-0.08
Micromonosporales	-0.08
Diplostraca	-0.07
Sporadotrichida	-0.07
Acytosteliales	-0.07
Flavobacteriales	-0.06
unclassified Deltaproteobacteria	0.06
unclassified Acidobacteria	0.06
Euglenales	0.07
Syntrophobacterales	0.07
Holophagales	0.07
unclassified Bacteroidetes	0.07
unclassified Gammaproteobacteria	0.07
Naviculales	0.08
Desulfuromonadales	0.08
unclassified Proteobacteria	0.08
Opitutales	0.08
Myxococcales	0.08
Methanosarcinales	0.09
Desulfobacterales	0.11
Thiotrichales	0.11
unclassified Anaerolineae	0.12
unclassified Chloroflexi	0.17
Solibacterales	0.17
Anaerolineales	0.17
Methylococcales	0.53

Table S9: Expression of genes for polymer degradation by the detritivorous fungi *Helotiales* and *Agaricales* and the predator and omnivore Entomorbyomorpha. Presence of a coloured circle indicate that transcripts for the respective pfam domain, represented by a Pfam accession number, is assigned to the corresponding taxon. Several circles per line indicate that the Pfam domain is known to be part of genes for decomposition of more than one polymer.

	Cellulose	Galacto-mannan	MLG	Xylo-glucan	Xylan	Arabino-xylan	Pectins	Lignin	Chitin	Bact. cell walls
PF01055										
PF01915										
PF00232										
PF04616										
PF00141										
PF01532										
PF00933										
PF00722										
PF02156										
PF03311										
PF00728										
PF01476										
PF00775										
PF07745										
PF02837										
PF06964										
PF01183										
PF00295										
PF03639										
PF02015										
PF00704										
PF00251										
PF00703										
PF00150										
PF02836										
PF01301										
PF09206										
PF03663										
PF01341										
PF00457										
PF00840										
PF12891										
PF00759										
PF03632										
PF07748										
PF01074										
PF02055										
sum	9-10-5	6-3-5	2-1-2	3-4-3	5-3-1	2-1-1	7-5-2	2-1-0	9-6-4	6-5-7
									Total sum PFAMs	120

References

1. Rytioja J, Hildén K, Yuzon J, Hatakka A, de Vries RP, Mäkelä MR. Plant-Polysaccharide-Degrading Enzymes from Basidiomycetes. Microbiol Mol Biol Rev. 2014;78(4):614–49.

Supplementary Data availability

Metagenomes and metatranscriptomes have been deposited in the Sequence Read Archive (SRA) database (Run Accession nos.: SRR13614422, SRR13614421, SRR13614414, SRR13614413, SRR13614412, SRR13614411, SRR13614410, SRR13614409, SRR13614408, SRR13614407, SRR13614420, SRR13614419, SRR13614418, SRR13614417, SRR13614416, SRR13614415).

Supplementary materials and methods

Section 1: **Comprehensive microarray polymer profiling (CoMPP)**

The polysaccharide composition of Arctic peat soils were measured using Comprehensive Microarray Polymer Profiling (CoMPP) at the University of Copenhagen as described in Moller *et al.* (1). To optimize the method for best possible signal strength for peat samples some adjustments were made to the development solution. The optimized recipe of the development solution is as follows: 10 mL of alkaline phosphatase buffer (100 mM NaCl, 5 mM $MgCl_2$, 100 mM diethanolamine, pH 9.5) with 82.5 µL of BCIP (20 mg/mL) (5-bromo-4-chloro-3-indolylphosphate) and 66 µL of NBT (50 mg/mL) (nitro blue tetrazolium). The strength of a binding signal was used as an indication for the amount of a polysaccharide in a sample. Since all samples were processed the same way, the differences in the binding signals indicate the differences in the amount of polysaccharides per gram of dry weight of the different samples. In theory, hemicelluloses should be in the NaOH extract and pectins in the CDTA extract. However, some of the pectins showed higher binding signals in the NaOH extract. This might be due to covalent attachments of pectin side chains with hemicelluloses (2). Whenever pectins were showing binding signals in both extractions, the strongest binding signal was used for the evaluation. Stronger binding signals of pectins in the NaOH extraction were found for the antibodies LM5, LM6, INRA-RU1 and INRA-RU2. The same method was used to investigate the polysaccharide composition of the three plant samples (*Poa arctica, Saxifraga cespitosa* and a moss mix). The reason for selecting

these two vascular species is that they are both among the most common vascular plants in the Arctic vegetation of Svalbard and that *P. arctica* was the by far most abundant vascular plant in the studied site. A moss mix was chosen since the moss vegetation consisted of an even mix of many different species. An overview of the antibodies used for polysaccharide detection is given in table 2 and table S4.

Section 2: **Enzyme Assays**

Polysaccharide degradation enzyme assays were carried out using the GlycoSpot™ technology (Copenhagen/Denmark). The ground peat samples were tested on eight different blue stained Chromogenic Polymer Hydrogel (CPH) substrates distributed in eight 96 well plates according to the kits instructions (3). The tested substrates were xylan (beechwood), arabinoxylan (wheat), 2HE-cellulose (synthetic), arabinan (sugar beet), pectic galactan (lupin), galactomannan (carob), xyloglucan (tamarind) and rhamnogalacturonan (soy bean). Each sample was diluted in a ratio of 0.2 g soil/ml reaction buffer (100 mM sodium phosphate buffer, pH 6.0). During pipetting the sample solutions were stirred in a glass beaker on a magnetic plate to gain best possible homogenization and equal distribution of the samples in the reaction buffer. Finally, 150 µL of each sample solution (in triplicates) was added to a well in a 96 well plate. The plates were sealed and shaken at 10 °C for 44.5 h to achieve a sufficient signal strength from the enzymes. After incubation, the plates were centrifuged for 10 min at 2700 x g and the absorbance of the supernatants was measured at 595 nm (blue CPH substrate) using a plate reader (SpectraMax M5, Molecular Devices, Sunnyvale, USA).

Section 3: **Nucleic acids extraction and rRNA depletion**

Nucleic acids were extracted from eight samples. Referring to table S5, we used four replicates from the fenced exclosures and four replicates from the grazed sites. Prior to extraction, 5

grams of soil from each replicate was ground in liquid nitrogen. From each of these ground samples, three nucleic acid extractions were performed as described previously (4,5). The three extracts were pooled prior to further processing.

To purify DNA from the total nucleic acids, the samples were treated with RNase A/T1 (Thermo Fisher Scientific, Waltham, MA/USA) for 30 min at 37°C to digest the RNA. The remaining DNA was recovered with a phenol:chloroform:isoamylalcohol and chloroform:isoamylalcohol extraction to remove the RNase enzyme. For the purification of RNA, DNA was digested using the Promega DNAase kit (Promega, Madison, WI/USA) and the remaining RNA purified with the Megaclear™ Kit (Thermo Fisher Scientific, Waltham, MA/USA) according to the manufacturers' protocol. The absence of DNA in the RNA preparations was verified by PCR assays targeting bacterial SSU rRNA genes. The total RNA samples were processed with the Ribo-Zero Magnetic Kit for Bacteria from Illumina (San Diego, CA/USA) to remove 16 and 23S rRNA molecules and enrich the mRNA fraction of the metatranscriptome. Nucleic acids were quantified (ng/µL) using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Madison, WI/USA). The quality of DNA extracts were evaluated on a 1 % agarose gel. The quality of the RNA extracts were evaluated by automated gel electrophoresis using a standard sensitivity chip (Experion™, BioRad, Hercules, CA/USA).

In order to quantify the microbial DNA and RNA in the total DNA and RNA, we substracted the fraction of DNA and RNA corresponding to the proportion of plant sequences (as judged from the abundance of the 18s rRNA genes or plant mRNA transcripts) from the total DNA or RNA per gram of dry soil.

References

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Paper 2

Paper 3