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Role of Plant-Microbe Interaction for C Dynamic in Northern Peat Bogs

by

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Abstract

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Peatlands in the Northern Hemisphere serve as critical carbon sinks, having sequestered substantial amounts of carbon over millennia. Their long-term carbon storage capacity positions them as key contributors to nature-based solutions for climate change mitigation. Northern peatlands are thus of utmost importance from a global climate system perspective. However, these important C stores are at risk and are susceptible to the impact of enviro-climatic change, with cascading effects on ecosystem processes and functions. C sequestration in peatlands hinges on the delicate imbalance between primary production and decomposition. Microbial communities and associated metabolic processes are possibly fundamental in driving feedback between enviro-climatic change and the peatland C cycle. Microbial communities are important regulators of belowground nutrient cycling processes including C dynamics.

Peatlands are experiencing climate warming and drainage which leads to shifts in vegetation cover and alterations in water table depth, that may drastically effects decomposition rates through change in microbial community composition and metabolic activity. Moreover, other consequences of climate change – e.g. dramatic wildfire – directly affect peatland microbial communities, including the alteration in community composition and diversity, and consequently comprise their C sink function.

In this thesis, I investigated the effects of long-term plant functional type (PFT) removal, specifically ericoids and graminoids, on microbial communities across two microhabitats-lawns and hummocks, characterized by differing water table levels. I show that the fluctuation in water table level across contrasting microbiota modulate the taxonomic composition of prokaryotic communities, while distinct PFTs varying in litter inputs and rhizodeposits significantly influence the abundance of prokaryotes and were microhabitat dependent. I further highlight the potential of plant microbe interaction to examine the relative and interactive impact of plant functional types and microhabitats on peatland decomposition dynamics. The result implies an important role of distinct PFTs, through their impact on microbial mediated metabolic activity, along the lawn-hummock gradient in deriving the process of decomposition. Finally, I demonstrate the abrupt effect of wildfire and post-fire recovery of prokaryotic communities in northern peat bog

at soil different depths, that can help us to reveal that how microbial dynamics contribute to resilience and recovery of these C rich peatland ecosystem following fire disturbance.

Table of Contents

Chapter 4 Response of prokaryotic community to fire severities across peat depth.

Table of Tables

Table of Figures

List of Accompanying Materials

Primary data used in chapter 2 can be found at: https://doi.org/10.5258/SOTON/D3711

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Definitions and Abbreviations

ALA	Alanine aminopeptidase
ANAMMOX	Anaerobic ammonium oxidation
ASV	Amplicon sequence variant
BG	B-Glucosidase
C	carbon
DADA2	Divisive Amplicon Denoising Algorithm 2
DNA	Deoxyribonucleic acid
EEA	extracellular enzymes activity
FDR	False discovery rate
GLS	Generalized Least Square
k	Decomposition rate constant
LDA	linear discriminant analysis
LEFSe	linear discriminant analysis effect size
MH	Microhabitat
NGS	Next Generation Sequences
PCoA	Permutational multivate Analysis of variance
PCR	Polymerase Chain Reaction
PERMANOVA	Permutational multivate Analysis of variance
PHOS	Phosphatase
PFTs	Plant factional types
REML	Restricted Maximum Likelihood Estimnation
S	Stabilization factor
SUL	Sulfatase
TBI	Tea bag index
UK	United Kingdom

Chapter 1 General Introduction

1.1 Importance of peatlands

Peatlands are ecosystems that over millennia have built up an organic soil due to slow decomposition rates that has facilitated the accumulation of a carbon rich peat of at least to 30 cm in depth (Clymo 1996; Turnunen et al. 2002; Joosten & Clarke 2002; Finlayson & Milton 2018; Loisel & Gallego-Sala 2022). On a global scale peatland are extensively distributed but predominantly occupy mid to high boreal latitudes within the northern hemisphere. In total, peatlands occupy an estimated 4 million square kilometres of the Earth's surface (Yu et al. 2010), constituting to approximately 3% of the global land surface (Jackson et al. 2017; Xu et al.2018; Hugelius et al.2020). Notably, peatlands contain up to 30% of the world's soil carbon content (Gorham 1991; Blodau et al. 2002; Limpens et al. 2008; Yu et al. 2012; Leifeld & Menichetti 2018). Since the Last Glacial Maximum (LGM), undisturbed peatlands have been consistently assessed, as small yet continual atmospheric CO_2 sink. Recently, however, the importance of peatlands as carbon vaults has been put forward more often (Temmink et al. 2021). The function as important carbon sinks is attributed to the sustained low levels of carbon (C) mineralization compared to the net primary productivity (NPP) (Roulet 2000; Frolking et al. 2006; Charman et al. 2013 Treat et al. 2019; Qiu et al. 2020). Hence, it has been predicted that the total carbon stock in peatlands may exceed ~1000 Pg C (Nichols & Peteet 2019, 2021).

In recent years, peatlands have been receiving more attention not only because of their contribution to the global carbon budget, but for providing a net cooling effect and helping to mitigate the global climate crisis (Frolking & Roulet 2007; Parish et al. 2007; Antala et al. 2022). The vast organic carbon reservoir within peatlands, accumulated over centuries, underscore their historical role as net carbon sinks. However, the impact of climate change is more pronounced on high latitude regions compared to other biomes that could potentially change the status of CO_2 sink to CO_2 source (Wu & Roulet 2014; IPPC 2021). As a result, such scenarios do not only impede the climate change mitigation but even further fuels climate warming by emission stored peat C to the atmosphere in the form of GHG emission with potentially drastic impacts on current and future world’s climate (Schlesinger & Andrews 2000; Munir et al. 2014 ;Qiu et al. 2022).Given the observed decrease in carbon uptake by peatlands storage and resilience against climate change is an important consideration in stock assessment research studies (Hugelius et al. 2020). Therefore, maintaining C storing capacity of northern peatlands is vital for moderating anthropogenic led atmospheric GHG emissions and lowering further warming (Leifeld & Menichetti 2018).

Peatlands play a crucial global role by serving as carbon storage areas (Joosten et al. 2016; Harris et al. 2022). Moreover, peatlands that remain undrained are anticipated to contribute to water purification and, in specific situations, help in delaying runoff to prevent flooding (Kumar 2012; Ritson et al. 2016). Hence, peatlands have an ecological role by regulating the macroclimate. Peatlands play a crucial role in managing landscape nutrient levels, such as retaining phosphorus (Graham et al. 2005; Koskinen et al. 2017). Additionally, they help regulate hydrologic patterns by buffering downstream streamflow (Holden et al. 2004; Loisel & Gallego-Sala 2022). They sustain high biodiversity and provide a range of cultural ecosystem services, such as recreational opportunities and amenity benefits. They can establish ecological corridors that facilitate movement and provide habitats that are ideal for numerous endemics and/or endangered species of plants and animals (Makila & Saarnisto 2008; Kate et al. 2021) and serve as productive areas for agriculture and horticulture, while playing a vital role in nutrient, sediment, and carbon retention (Rieley et al. 2008; Harenda et al. 2018). Furthermore, peatlands play a crucial role as the sources for rivers, making them known as natural "water towers" (Joosten & Clarke 2002; Gatis et al. 2023). Consequently, it is effective to conserve peatlands to preserve global biodiversity and ensure the retention of water and carbon.

1.2 Northern peatlands and C cycling

Peatlands play a pivotal role in carbon sequestration through the assimilation of atmospheric carbon, incorporation of senesced litter into the soil, and the deposition and turnover processes within the rhizosphere. The primary outputs are driven by the release of carbon dioxide (CO_2) from the peat surface. However, methane (CH_4) emissions and the hydrological leaching of dissolved and particulate carbon compounds can also significantly influence the overall carbon balance of peatland (Davidson & Janssens 2006; Kate & Joosten 2009; Pickard et al. 2022). The imbalance between the uptake of atmospheric CO_2 through plant primary production (photosynthesis) and the decomposition by biotic communities in peatlands leads them to accumulate large amounts of organic C and play a vital role in C sequestration (Blodau et al. 2002; Antala et al. 2022). As compared to other terrestrial ecosystems, both processes are very slow (Gorham 1991; MacDonald 2006), however, even slight alterations in either of these processes have the potential to exert substantial effects on the overarching carbon dynamics.

Decomposition processes in high-latitude northern peatlands are increasingly considered more vulnerable to climate change impacts (Dieleman et al. 2016). In the northern hemisphere, decomposition rates are primarily governed by factors such as low temperatures, acidic pH, specific vegetation types, and oxygen availability due to water-saturated conditions. These factors collectively limit microbial metabolic activity and organic matter decomposition, resulting in the accumulation of recalcitrant phenolic compounds (van Breeman 1995; Moore & Basiliko, 2006; Wang et al. 2021). These constraints on decomposition are intricately regulated by climatic factors, which can be reflected in shifts in vegetation composition or alterations in hydrological regimes. With warming climates and an increase in hot drought events, there is growing concern that these constraints could be lifted, potentially accelerating carbon (C) mineralization through peat aeration. This would release stored C in the form of greenhouse gas (GHG) emissions, potentially creating a positive feedback loop with significant implications for global climate warming (Dise, 2009; Dorrepaal et al. 2009; Ofiti et al. 2022).

Climate warming is more likely to influence plant community composition by growth of the vascular plant community at the expense of mosses in peatlands (Pearson 2013; Dieleman et al. 2015). As a result, alterations in peatland vegetation may influence decomposition process and C dynamics (Ward et al. 2015; Bell et al. 2018). Moreover, it has already been anticipated that alteration in precipitation frequency and greater evapotranspiration will result of climate warming, which may lower the water table level that leads to increase the rate of decomposition with potential drastic impacts on C cycling in peatlands (Fenner et al. 2007; Loisel & Yu 2013; Górecki et al. 2021). Considering the pivotal role of northern peatlands in global biogeochemical processes and their potential susceptibility to climate change, enhancing our understanding of the mechanisms governing the carbon balance, with a specific focus on decomposition for C cycling in these ecosystems makes it an important research priority.

1.2.1 Acrotelm-catotelm model

In northern peatlands, different Earth system models (ESMs) have adopted a dual-layered conceptual framework, known as acrotelm-catotelm dichotomy to understand the hydrological, biogeochemical and ecological processes and play important role in C budget of peatlands. This is also referred to as the diplotelmic model. This framework divides the peat profile into two different layers identified by Ingram (1987): the upper acrotelm (20-30 cm thick) closest to atmospheric oxygen and serving as the zone for living vascular plant roots, higher abundance of labile organic compounds, and periodically aerated. It experiences a fluctuating water table exhibits the highest amount of microbial activity, biological metabolism, decomposition process and nutrient cycling (Lindsay & Immirzi 1996; Junk 1999; Evans et al. 2021). This aerated biological active layer is rich in aerobic heterotrophic bacteria, fungi and mycorrhizal fungi that perform aerobic decomposition due to higher oxygenation and CO_2 as the product of decay of organic matter (Lamit et al. 2021). The lower anoxic layer in the catotelm is characterized by a consistently saturated state, reduced hydraulic conductivity, low temperature that performs biogeochemical processes at lower rates. This layer is typified by anaerobic microbes especially abundant in methanogens (domain Archaea), that significantly reduces metabolic energy, resulting in a dramatic slowdown of decomposition processes (Clymo 1984). The presence of methanogens results in CH_4 as the final product of organic matter decomposition. The boundary between these two layers is defined by depth relative to either water table or a constant value (often set at 0.3 meters below the soil surface) (Pezeshki & Delaune 2012; Spahni et al. 2013; Tian et al. 2023). The mesotelm (metabolically active horizon) is another transition area, between the acrotelm and the deep catotelm, where conditions lie between oxic and anoxic depending upon water table level resulting in shifting in metabolic processes and carbon turnover (Clymo & Bryant 2008) and corresponds to a hotspot of microbial diversity and activity (Lin et al. 2014).

Different processes occur in different layers of peat and that is likely the result of an interplay between different biotic and abiotic interactions. However, the physical and chemical properties of peat can impact the composition of biotic and abiotic communities, thereby indirectly influencing carbon cycling in peatlands (De Deyn et al. 2008; Armstrong et al. 2015). Due to uncertainties about climate change, ecologists are working to better understand the variables influencing microbial C cycling in peatlands (Li et al. 2019).

1.3 Plant and microbe roles in peatland C dynamics

The distribution and composition of below ground communities (microbes) in peatlands may be affected by various abiotic controls, including water table position (Wang et al. 2021) and biotic control comprising distinct plant functional groups (Robroek et al. 2015) which is expected to play a significant role in peatland ecosystem processes in response to climate change by directly controlling C cycling.

1.3.1 Peatland vegetation and microhabitats

There is growing research by ecologists that plant functional traits (PFTs) within northern peatland, have distinct ecophysiological characteristics that can have predictive power on C dynamics by providing beneficial framework to study the belowground ecosystem functions in response to climate change (Ward et al. 2015; Nicholas et al. 2023). Within northern peatlands, the community of vascular plants is composed of two main functional groups: evergreen ericoid shrubs and graminoids. These plants have different physical characteristic in their aboveground biomass, carbon and nutrient chemistry and stoichiometry) (Aerts et al. 2009; Liu & Hu 2020). The primary rates of productivity and decomposition in the peatland ecosystem are influenced by these plant functional types, subsequently shaping the overall C dynamics (Leroy et al. 2017, 2019; Gavazov et al. 2018). Nevertheless, concern is increasing regarding the anticipated impact of a warmer climate on vascular plants with increase in their abundance and production within peatlands. What is more, the physical variations of these plant species in peatlands predominantly control decomposition (Schweitzer et al. 2005; Mao et al. 2018) which is the key regulator of CO_2 and CH_4 emissions to the atmosphere (Blodau 2002; Whitaker et al. 2020). Specifically, climate warming driven spreading of vascular plants at the expense of Sphagnum mosses has been found to accelerate the rate of decomposition, a trend that could potentially lead to the destabilization of peat carbon stocks over extended timescales (Walker et al. 2016; Bell et al. 2018; Laine et al. 2021). Moreover, any little alteration in aboveground dominant vascular plant communities can transform the belowground community structure (Dieleman et al. 2015; Wang et al. 2021), by providing the above- and belowground litter inputs and distinct rhizodeposits with significant control on over all C dynamics (McNamara et al. 2008). Hence it has been concluded that plant composition is fundamental in governing C cycling and C storage within peatland ecosystems. Therefore, the response of peatland to climate change is innately linked to response of their overlying plant community composition.

Northern peat bogs frequently exhibit recognizable spatial variations in microhabitats which differ in their location relative to the water table (Andersen et al. 2011). These microhabitats are categorized as flatter wet lawns (also known as carpets) that are often waterlogged, and elevated hummocks (up to 50cm) (Wang et al. 2018). Wet lawns are positioned near to the water table and the surface tends to fluctuate in conjunction with it (Belyea & Clymo 1998; Rydin & Jeglum 2013). Furthermore, microhabitats within peatlands are frequently characterized by the prevalence of distinct plant species representing distinct functional types (Kvillner & sonesson 1980; Kaštovská et al. 2018). The drier portion of hummocks are primarily colonized by ericaceous shrubs (e.g *Calluna vulgaris*) (Robroek et al. 2015). Ericaceous shrubs are slow growing woody plants with shallow roots (Murphy et al. 2009), mainly found in blanket bog hummocks. They have highly recalcitrant litter and fall branches resulting from a relatively large proportion of secondary chemical compounds (antimicrobial polyphenolic and lignin components) which are known to inhibit/suppress microbial extracellular and intracellular metabolic activity (Lang 2009; Fenner & Freeman 2011; Malhotra et al 2020; Ofiti et al. 2022). Furthermore, shrub litter has low nutrient concentrations, higher C:N ratio and low surface area-to-volume ratio(Ward et al. 2009; Kaštovská et al. 2018) which possibly result to slow down the decomposition process and reduce CO_2 turnover and creating negative feedback to climate change. In addition, ericoids optimize nutrient uptake through symbiotic mycorrhizal associations (Smith & Read 2008; Gavazov et al.

2016). Additionally, these species restrict the photosynthetic capacity of the understory by overshadowing other vascular plant species within peatlands (Myers-Smith et al. 2011).

In contrast, wetter lawns in blanket bogs are mainly dominated by fast growing sedges (known as graminoids) equipped with deep roots, like *Eriophorum angustifolium* (Robroek et al. 2015). The presence of these graminoids is often linked to higher quality and quantity of nutrient rich plant litter and the excretion of root exudates in the form of labile and less aromatic C compounds in surrounding peat (Del Giudice & Lindo 2017; Mastný et al. 2018; Edward et al. 2018; Zeh et al 2019). Due to a shorter life span and lower C:N ratio (Hobbie 1996) these characteristics may facilitate microbial activity and enhance the rate of decomposition and CO_2 turnover and possibly create positive feedback to climate change (Trinder et al. 2008). Most graminoid species, for instance the sedge *Eriphorum vaginatum*, significantly contribute to larger rates of methane emissions from peatlands by supplying labile C substrate to the soil leading to enhance anaerobic decomposition and stimulation of the methanogenic community, thus playing an important role in controlling CH_4 flux from peatlands (Strom et al. 2012; Goud et al. 2018; Lazcano et al. 2020). Their deep roots system is equipped with unique aerenchymous tissues (airy channels in stem and roots) facilitating the transport of oxygen under anoxic conditions, functioning as conduits for CH_4 emission directly from the anoxic catotelm layer to the atmosphere, thereby bypassing the oxic acrotelm layer (Greenup et al. 2000; Goud et al. 2017; Swenson et al. 2019). Despite research studies indicating important recognized impacts of microhabitats and plant functional types on decomposition process in peatlands (Belyea 1996; Zeh et al. 2020), their interactive effects are still uncertain, and additional research requires that both factors are considered concurrently especially when attempting to understand peatland carbon dynamics in the light of future enviro-climatic change.

1.3.2 Microbial communities in peatlands

Northern peatlands support taxonomically and functionally diverse microbial communities including bacteria, eukaryotes, archaea and protists (Andersen et al. 2013; Kitson & Bell. 2020). The most abundant microorganisms in peatlands are prokaryotes which include bacteria and archaea. Bacteria, some of which are characterized by rapid growth, exhibit low carbon-to-nitrogen (C:N) biomass stoichiometry. The microbial community directly decomposes labile carbon or releases extracellular enzymes, mainly hydrolytic enzymes, as part of their metabolic processes into the surrounding environment, facilitating the breakdown and mineralization of various high molecular weight organic compounds i.e cellulose and hemicellulose (Boer et al. 2005; Berg & Laskowski, 2006; Ayres et al. 2006; Xiong et al. 2023). Particularly, prokaryotic communities in soil are characterized as "fast" cyclers due to their ability to rapidly turnover nutrients through metabolic processes and subsequent cell death (Joergensen & Wichern 2008; Strickland & Rousk 2010). Hence, prokaryotes (bacteria and archaea) assume significance in influencing the biogeochemical cycles within peatland ecosystems (Myers et al. 2012; Robinson et al. 2023).

In terms of prokaryotic taxonomic diversity peatlands are dominated by a broad range of bacterial and archaeal phyla that categorized based upon their genetic and morphological traits and have developed physiological and metabolic adaptation to survive in this unique ecosystem with acidic environment and low oxygen availability (Andersen et al. 2013; Robinson et al. 2023). The dominant taxa in northern peatlands are Proteobacteria, Acidobacteria, Actinobacteria, Bacteroidetes, Verrucomicorbia, Firmicutes and Chloroflexi and some archaeal phyla i.e Euryarchaeota and Thaumarchaeota are also reported in peat bogs (Gilbert & Mitchell 2006; Elliot et al. 2015; Urbanova & Barta 2016; Potter et al. 2017). They exhibit diverse metabolic and hydrolytic capabilities that play crucial role in organic matter disintegration, regulate the biogeochemical cycling i.e nutrient cycling and hence over all peatland ecosystem functioning (Garbeva et al. 2004; McGuire et al. 2010; Dedysh 2011). For instance, members of Proteobacteria contains more than 1600 morphologically and physiologically distinct species and play a crucial role in biogeochemical cycling especially C and N cycling. In addition, representatives of Acidobacteria are acidophilic and are well adapted to survive in oligotrophic environmental conditions (Kielak et al. 2016), however Actinobacteria contribute to decomposition of complex organic compounds particularly plant and humic components in peat soil (Lewin et al. 2016). Furthermore, some Verrucomicrobia species are known to be acidophilic methane oxidizers and play fundamental role in C cycling in some extreme environments (van Teeseling et al. 2014). Additionally, Archaea from Euryarchaeota and Thaumarchaeota contribute to carbon and nitrogen cycles (Offre et al. 2013; Li et al. 2022) The activity of microbes participating in biogeochemical cycling depends on the availability of specific substrates and environmental conditions (Andersen et al. 2013). It is proposed that enviro-climatic changes can significantly influence the ecosystem processes, i.e. decomposition dynamics and nutrient cycling in peatlands (Didion et al. 2016) and these modifications are often reflected in the community structure and activity of microbial communities (Zhou et al. 2012) within peatland ecosystems.

Microbial communities within peatland ecosystems have substantially diverse metabolic diversity (Williams & Crawford 1983) and play a crucial role in the ecosystem processes and functions such as biogeochemical cycling, decomposition process, carbon turn over and greenhouse gas (GHG) emissions (Artz 2013; Hopple et al. 2020). However, these communities are sensitive to alteration in level of water table (environmental factor) and plant community changes (biotic) which are two important factors of concern in context of global climate change (Mommer et al. 2018; Tian et al. 2019). These sensitivities are crucial as they have influence on the composition and function of microbial communities. Understanding the variation in microbial dynamics in response to interaction of biotic and abiotic factors is fundamental for predicting and mitigating the potential shifts in carbon cycling patterns within peatlands which are significant reservoir of organic carbon.

In peatland ecosystem, water table level has been demonstrated to be the most influential determinant of variations in microbial community structure as it is directly indicative of the presence or absence of oxygen, which governs the ratio of aerobic to anaerobic microbial processes that results in production of CO_2 and CH_4 (Sundh et al. 1997; Bru et al. 2011; Krohn et al. 2017; Tian et al. 2023). For example, aerobic bacteria are recognized as important and efficient decomposers of chemically recalcitrant components within organic matter situated above the water table, specifically in oxygen-rich (oxic) conditions. Below the water table in conditions of limited oxygen availability (anoxic conditions), anaerobic processes are required for decomposition (Thormann, 2006; Myers et al. 2012; Dom et al. 2021). For instance, hummocks with lower water table level are usually abundant in microbial populations such as aerobic chemoorganoheterotrophs, including methanotrophs, which contribute to regulate C cycling (Kotiaho et al. 2013; Asemaninejad et al. 2019; Perryman et al. 2022). While methanogenic archaea, capable of producing CH_4 (Juottonen et al. 2015), are more typified in lawns with water table depth closer to the peat surface (Robroek et al. 2015; Perryman et al. 2022). However, the response of complex microbial community reaction to hydrologically different microhabitats remain poorly understood and there is lack of research regarding the influence of peatland microenvironments to belowground activities, leading to a significant constraint in our understanding of the role of functional microbial groups in peatland C dynamics.

Vascular plant functional types within peatlands have been shown to influence belowground microbial community structure and metabolic functioning via the living plant biomass, organic litter inputs and rhizodeposits (Robroek et al. 2015).These specific litter inputs and root exudates serve as organic substrate (source of energy) for microbes and have a significant influence on belowground microbial community structure and fuels the microbial enzymatic activity and play fundamental roles in GHG emissions to the atmosphere (De Deyn et al. 2008; Strakova et al. 2011; Wiedermann et al. 2017; Mastny et al. 2021). As an example, anaerobic prokaryotes such as fermenters and syntrophic bacteria predominate in peat with graminoids (Chroňáková et al. 2019). This prevalence is attributed to the rhizodeposition of highly labile low molecular weight carbon substrates, including carbohydrates and other organic compounds, associated with this

PFT. In addition, anaerobic methanogen populations belonging phylogenetically to the domain archaea have been shown to thrive in peat underlying sedge species due to root exudation and oxygen delivery to the rhizosphere through their deep root transport system, which in turn led to substrate for methanogens (Saarinen, 1996; Greenup et al. 2000; Strack et al. 2017; Chen et al. 2023). In contrast to graminoids, ericoid develop symbiotic associations with ericoid mycorrhizal fungi that are associated with faster mineralization of recalcitrant compounds and help in mobilization of nutritive cations, P and N compounds from complex organic substrates (Burke & Cairney 2002; Smith & Read 2008). Moreover, shrubs also host nitrogen fixing bacteria (alpha Proteobacteria) and Acidobacteria that colonize the roots of developed ectomycorrhizosphere (Uroz et al. 2012; Chroňáková et al. 2019).

As a result of these specific associations of peatland PFTs and microbes, any alterations in vegetation at PFT group level bought about by climate change might be used to predict changes in metabolic activity and microbial community structure (Mitchell et al. 2010; Richy et al. 2024). A fundamental understanding of the role of vascular plant functional type composition on the structure and functionality of microbial communities, particularly at the phylogenetic composition and functional diversity, is imperative. This understanding can be employed to determine the specific microbial entities governing biogeochemical cycles, especially carbon cycling, and propelling GHG emissions. Such insights are critical for anticipating the carbon sequestration potential of peatland ecosystems under the context of anticipated warmer and drier climate changes in the future.

1.4 Enviro-climate change and plant-microbe interaction

Peatlands are highly vulnerable to enviro-climate change and to disturbances such as global warming, drainage, nutrient deposition and anthropogenic disturbances (i.e. land use and land-cover change) which can directly affect the ecosystem hydrology, ecology and C balance (Fenner & Freeman, 2011; Olid et al. 2014; Renou-Wilson et al. 2014; Hoyt et al. 2020). Warming as result of climate change is projected to be greatest in northern high latitude regions and can destabilize the C stock and increase the rate of GHG emissions from peat soil to the atmosphere, as result could further accelerate global climate warming. Furthermore, uncertainty in alteration in precipitation amount and frequency, decrease in rainfall and reduced soil moisture due to higher evapotranspiration adds further pressure upon peatland C stores, are more expected consequences of climate change (Davidson & Janssens 2006; Dise, 2009; IPCC, 2013). Water table level draw-down resulting from drier and prolonged warmer conditions in peatlands can alter carbon flux and expose formerly sequestered carbon to aerobic microbial decomposition

(Yavitt et al. 1997; Walker et al. 2016; Zhong et al. 2020), threatening to release the stored deep ancient organic C as CO_2 and CH_4 into the atmosphere.

In addition to direct effects on hydrological gradients, enviro-climatic change can also alter peatland plant community structure, which can trigger a set of biogeochemical reactions and has extended consequences for carbon dynamics (Elmendorf 2012; Kuiper et al. 2014; Jassey et al. 2018). It was reported that vascular vegetation cover is getting more prevalent with increased expansion especially among ericaceous shrubs with an associated shift in microbial community composition under warmer and dryer conditions (Pearson at al. 2013; Dieleman at al. 2015; Bragazza et al. 2015; Malhotra et al. 2020). This can result in the release of more labile organic carbon from plant litter and increase root exudation that accelerates the process of decomposition by (extracellular) enzyme activity (Porazinska et al. 2003; Leroy et al. 2019). Additionally, warming and alteration in plant cover significantly influences microbial community structure and C turnover in ecosystem. In return, microbial communities play a crucial role not only in mediating C sequestration but are also involved in nutrient cycling and releasing essential nutrients as result of decomposition and have intricate feedback on plant productivity and overall ecosystem functioning (Blodau 2002). Hence, there is evidence of a strong relationship between aboveground plants and the belowground microbial community, and this interaction strength can control changes to peatland functioning and play an important role in ecosystem stability and resilience in response to any enviro-climatic change (Morrien et al. 2017; Robroek et al. 2021).

In addition, disruption to plant-microbe interactions can potentially lead to decreased potential of the peatland ecosystem functioning as a C sink. For instance, dominance within different plant community compositions can mediate the C dynamics through changes in microbial composition (Lindo et al. 2013; Robroek et al. 2015). Therefore, to fully understand the long-term response of any ecosystem to global change, it is essential to understand how microbial physiological structure and metabolic diversity contribute to ecosystem processes and functioning i.e. C dynamics are affected and respond to any enviro-climatic change.

1.5 Catastrophic events

Peatlands face various threats and can be subjected to catastrophic events as result of concurrent global enviro-climatic factors and deliberate human activity that can significantly impact their ecosystems. These include drainage-based agriculture, deforestation, drought events and wildfires that can lead to alteration in structure and function of peatlands (Turetsky et al. 2015; Flannigan et al. 2009; Page et al. 2022). Global climate change will lead to rises in air temperature, altered precipitation patterns, reduction in soil moisture and increase in evapotranspiration that will intensify the frequency and severity of climate determined drought events (Kettridge et al. 2013; Helbig et al. 2020). Drought increases the risk of frequent and severe wildfire and poses a significant threat to the ecological integrity and the C retention capability of northern peatlands (Turetsky et al. 2002; Thompson et al. 2019).

Peat fires are an ecologically important catastrophic event, known for their ability to cause substantial and long-term impacts on the physical and ecological structure of peat (Rein et al. 2021). These fires are characterized by smouldering combustion of organic matter, a flameless form of burning, that can smoulder under temperature up to 500 °C high moisture content and low oxygen concentrations (Belcher et al. 2010). Unlike typical fires, smouldering fire can burn for extended periods at deeper depth despite precipitation and result in immediate loss of peat and previously sequestered C (Mack et al. 2011; Davies et al. 2013; Rein, 2013; Turetsky et al. 2015). Along with this, severe wildfires can lead to substantial decrease in plant biomass or result in the entire removal of surface vegetation and initiates the resetting of plant succession (Gilchrist et al. 2004) that can exacerbate peat surface erosion (Benavides-Solorio & MacDonald 2005; Andersen et al. 2024). Furthermore, wildfires can have impacts on the soil microbial community structure and diversity (Andersen et al. 2013). Although peatland C dynamics, biogeochemical cycling and their recovery after wildfire is closely linked to the belowground microbiome, there is limited knowledge about the shift in community composition and abundance because of peatland fire.

Severe wildfire can lead to dramatic transformations to the belowground microbial processes of peatlands. Wildfire can reduce the hydraulic conductivity of peat soils (Martin & Moody 2001; Ackley et al. 2021) and raise peat surface temperatures that increases evapotranspiration rates (Kettridge et al. 2015). This is linked to a reduction in microbial biomass and significantly decreases the ability of northern peatland functions to recover following the wildfire event. Metanalyses (Ficken & Wright 2017), indicated that peat soil microbiome structuring is subject to direct effects of wildfire through change in soil chemistry (i.e. increase in soil pH, lower quality of organic matter) as well as indirect effects through shifts in community composition of aboveground vegetation. Additionally, wildfire most often results in reduction of standing microbial biomass, community diversity and species richness (Brown et al. 2019; Pressler et al. 2019; Qin et al. 2021) and such changes can influence or delay the post-fire vegetation recovery (Ibanez et al. 2021) and thus influence the functioning of the whole ecological landscape.

Furthermore, wildfires result in ephemeral pulses of heavy metal, nitrogen and phosphorus through combustion of vegetation and fire ash can persist for multiple years following fire (Prat et al. 2011; Quigley et al. 2019), which can induce shifts in microbial community composition. For instance, elevated deposition and enrichment of nutrients in peat soil after fire can subsequently result in increased ratios of bacteria to fungi (Barcenas-Morena et al. 2011) and shift peatland towards fast growing prokaryotes from the orders Firmicutes and Proteobacteria. Enhanced microbial activity can facilitate CH_4 and CO_2 emission and cause the system to switch from being net C sink to source with loss of long-term peatland functioning (Waldrop & harden 2008; Kettridge et al. 2015). Perhaps most importantly, recovery of peatland ecosystem functioning is long term process that often-taking decades and is closely coupled with belowground biological processes. However, our knowledge of postfire changes (temporal and spatial variations) in microbial composition and functioning remains limited.

1.6 Research objectives

As described in this chapter, given the vulnerability of northern peatlands to climate change and their crucial role in climate regulation, it is essential to determine how these ecosystems respond to environmental and climatic changes. The community composition and functional diversity of microbial communities and their interactions with plant functional types (distinct PFTs) play crucial roles in controlling C dynamics and essential drivers of biogeochemical cycling that can directly impact ecosystem processes and functioning, which in turn can impact the global climate system. Therefore, understanding how microbial communities and their function respond to enviro-climatic variations could provide much needed insights into the sensitivity and resilience of peatland ecosystems to global change. While there is a substantial amount of literature on carbon balance dynamics and GHG emissions from northern peatlands in relation to factors like plant distribution, water table fluctuations, and climate warming, there is a gap in knowledge regarding the dynamics of microbial communities and their interactions with vegetation and hydrological conditions in context of changing enviro-climatic conditions. Hence, the focus of this thesis will be to fill gaps in our understanding by firstly, delving into how change in plant species (PFTs) alongside water table position relate to belowground microbial community structure and functional diversity. Secondly, how alterations in plant communities impact the microbial metabolic activity and related decomposition dynamics in context of hydrological fluctuations. Thirdly, to examine the depth dependent shift in microbial community structure and functional diversity post fire which offers insight into peatland ecosystem functioning and recovery following wildfire disturbance.

Therefore, the main objectives of this research.

Objective 1 To determine the regulatory role of plant functional types and hydrological conditions on peat microbial community structure and abundance and identify the key microbial groups.

Objective 2 To determine relative and interactive effect of plant community composition and hydrological level impact on belowground decomposition process and microbial metabolic activity, focusing on hydrolytic enzymatic activity.

Objective 3 To investigate the depth dependent microbiome shift post wildfire.

Achieving each of these objectives will allow overarching assessment of the importance of plant-microbe interactions in changing enviro-climatic conditions (Figure 1.1)

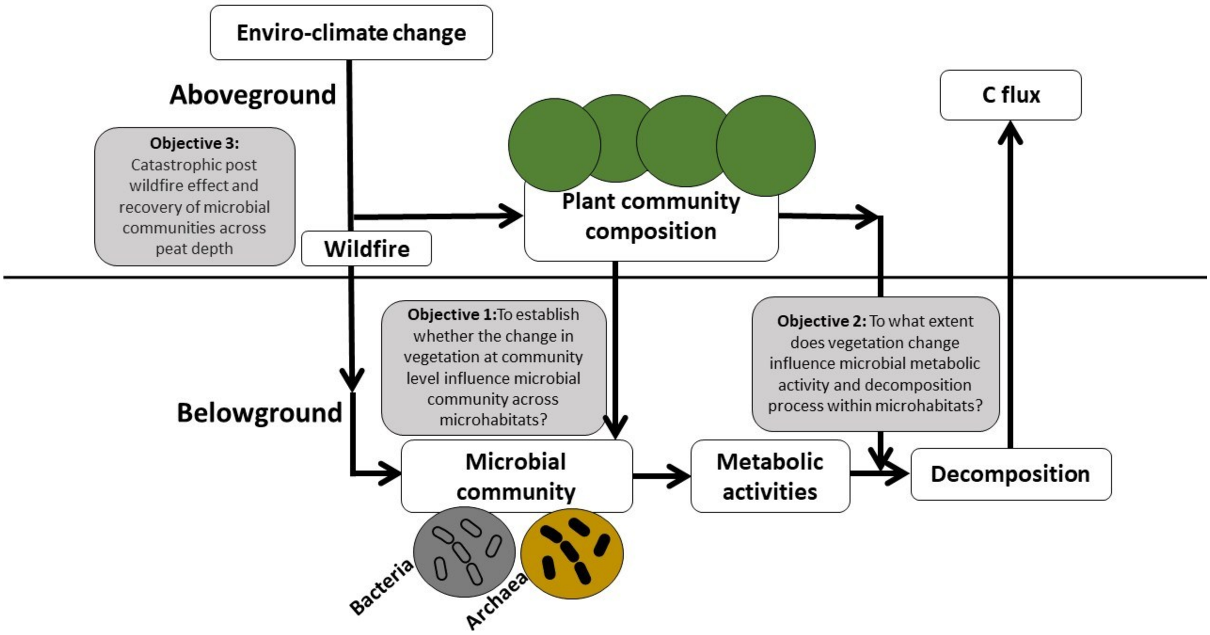

Figure 1.1 A conceptual diagram illustrating the primary objectives of thesis.

The first objective is to understand how relative and interactive effects of vascular plant functional types and microhabitat characteristics collectively shape the diversity of species (measured through the Shannon diversity index) and influence the composition of prokaryotic communities within an ombrotrophic bog ecosystem. By investigating the intricate interplay between these factors, we seek to uncover taxonomic and functional composition of key microbial groups, utilizing advanced molecular techniques to elucidate the genetic signatures of these microorganisms. This research will provide valuable insights into the complex dynamics that govern biodiversity and microbial populations in the unique environment of ombrotrophic bogs.

The research in Objective 2 focuses on unravelling the interaction between vascular plant functional types and microhabitat characteristics, specifically examining their combined impact on initial decomposition through the incubation of standard substrates, and the microbial activity level, with a particular emphasis on evaluating the activity of five extracellular hydrolytic enzymes. By studying these interactions, we aim to gain deeper insights into how plant diversity and microhabitat conditions influence both decomposition dynamics and the enzymatic processes driven by the microbial community with focus on C turnover, shedding light on the intricate relationships between plants and microbes within this ecosystem.

The third objective is to investigate the immediate impact of a wildfire on prokaryotic community composition and post fire recovery of biotic communities temporally at two distinct peat depths – near-surface (0-1cm) and sub-surface (4-5cm) – with a focus on discerning any alterations caused by the fire. Furthermore, the research aims to analyse the subsequent trajectory of prokaryotic community composition and diversity in the peatland post-fire. Through these analyses, we intend to uncover both the immediate responses of prokaryotic communities to the disturbance and the longer-term changes, providing insights into the resilience and adaptation of microbial life within the peatland ecosystem following a wildfire event.

A series of field experiments were conducted to achieve these objectives at study sites located in southern Sweden and Greater Manchester (UK). The thesis includes three chapters which consists of their own research objectives, purpose, and experimental design. These chapters provide insights for the achievement of the objectives.

1.7 Thesis structure and its overview

A paper format has been used to structure this thesis. Chapter 1, the introduction to this thesis, remains unpublished. Chapters 2, 3 and 4 present experimental findings derived from the work conducted during the doctoral program. Each of these chapters is formatted as a scientific journal article. Chapter 3 is published in *Wetlands* and Chapter 2 and 4 are ready for submission to relevant scientific journals. I am the primary author of each of these chapters, and the specific contributions of co-authors are also detailed below. Chapter 5 is a final discussion and is not designed for publication. All references can be found in the respective reference sections of each chapter.

Chapter 2 is the comprehensive study specific plant-microbe connection following long term plant removal experiment (>8 years). To do this, I used the plant removal experiment that is established in Sweden's Store Mosse National Park. It involves four plant removal treatments, including undisturbed control, graminoids removed, ericoids removed, and ericoids plus graminoids removed across microhabitats (lawns and hummocks with different water table position). This provided a unique opportunity to investigate how the presence and absence of specific vascular plant species alongside different hydrological conditions influence microbial community composition (specifically shifts in dominant taxa, changes in prokaryotic diversity, and alteration in prokaryotic communities). This is based on the hypothesis that vascular plant species in peatlands play an important role in ecosystem functioning and processes by shaping the microbial community composition in different microhabitats (Ward et al. 2009; Robroek et al. 2015). This was done by performing amplicon sequencing of 16S rRNA genes to profile bacterial and archaeal communities and provide information on their taxonomic and functional diversity.

In Chapter 3, I analyse the relative and interactive impact of plant functional types and microhabitats on peatland decomposition dynamics conducted in Store Mosse National Park, Sweden. The experiment involved a vascular plant removal experiment nearly a decade established in a Sphagnum-dominated ombrotrophic bog, comprising 80 plots with four PFT removal treatments in both lawn and hummock microhabitats. As it has been previous reported, the pronounced role of plant community composition and variation in microhabitat on decomposition processes (Ward et al. 2015) is mainly driven by microbial metabolic activities (Briones et al. 2022) that can provide insights into carbon cycling in peatland ecosystem. I use the standard litter with the aid of the Tea Bag Index (TBI) method to evaluate the belowground decomposition processes by burying tea bags in the treatment plots across microhabitats over three-month time following established protocols (Keuskamp et al. 2013). Additionally, I assess the microbial metabolic activity by measuring extracellular hydrolytic enzyme activity (ALA, BG, NAG, SUL and PHOS) across wet lawns and dry hummocks in vascular PFT plots.

Within Chapter 4, I examine the immediate effect and recovery of prokaryotic community composition following a wildfire disturbance. The research was conducted in northwest England blanket bog to investigate the impact of wildfire on prokaryotic composition, diversity, and post-fire recovery in peat soil at different depths. I collected the soil samples from areas with varying burn severities (shallow and deep burns) at three different time points (4-, 11- and 14- days) after the fire. The DNA was extracted from these samples and sequenced 16S rRNA using high-throughput Illumina MiSeq technology to identify prokaryotic taxa, changes in community structure and diversity, which can help us evaluate the consequences and potential sensitivity or resilience of peatland ecosystems to fire disturbance.

1.8 Methods for examining microbial communities in peatlands.

Microorganisms were mostly studied using culture-dependent techniques up until mid-1980s. However, it has subsequently been established that these approaches are inadequate for comprehensively describing the immense range of microbial species present in the biosphere. Estimates suggest that merely around 1% of all microorganisms can be grown in laboratory cultures (Staley & Konopka 1985). The traditional culturing method can be time consuming and will not provide comprehensive information about the functionality, biomass, and structure of entire microbial community (White et al. 1993). This approach is biased towards favouring the growth of specific microorganisms that thrive under specific laboratory conditions provided (Rastogi & Sani 2011), due to selective culture conditions.

One of the first cultivation-independent techniques was phospholipid fatty acid (PLFA) analysis, whereby PLFAs of microbes are directly extracted and analysed from environmental soil samples. Therefore, PLFA provided a broader overview of microbial community composition and biomass and has been used in studies of northern peatland ecosystems. However, PLFA analysis can only provide information at macro-taxonomic level and is unable to distinguish the more closely related taxonomic species (Quideau et al. 2016).

Following from PLFA analysis, DNA fingerprinting methods such as terminal restriction fragment length polymorphism analysis (TRFLP) were developed for microbial ecology studies. TRFLP is a cost effective and sensitive technique used to measure microbial diversity using fluorescence and has been frequently used in peatland research studies (Kim et al. 2016). However, this technique has limitations related to presence of multiple copies of marker genes in the same call and is unable to provide species level identification (Woese et al. 1990).

In late 2000s, the development of next-generation sequencing (high through-put sequencing, NGS) significantly advanced the field of microbial ecology by allowing ecologists to sequence microbial genes more easily and cost effectively. Both targeted approaches, such as amplicon sequencing of 16S rRNA genes, and untargeted metagenomics are now commonly used to characterize microbial communities. Metagenomics, which involves directly sequencing DNA directly from environmental sample instead of based on culturable cell characteristics, became a powerful approach for understanding microbial community at functional phylogenetic and taxonomic level (Margulies et al. 2005; Hugenholtz & Tyson 2008). The advent of next-generation sequencing technologies has brought about a transformative impact on studies related to microbial environments, enabling the in-depth studies on microbial diversity and identification of rare, abundant, and unculturable microbes through sequencing of all DNA fragments without need for cloning (Escobar-Zepeda et al. 2015). The high-throughput nature of NGS allows for the generation of massive amounts of sequencing data, advancing the ability to assess microbiota quantitively (diversity) and qualitatively (relative abundance) with unprecedented detail and to characterize and compare microbial communities in response to different variables. Workflows for analysing millions of DNA sequences has been shortened because of improvements in tools made specifically for studying soil microbial communities (Caporaso et al. 2010).

Large projects environmental sequencing projects, such as Earth microbiome Project (Thompson et al. 2017), have been initiated aiming at unveiling the factors structuring microbial composition, physiology, and detailed functional characteristics. Among the various NGS methods at hand, the data produced using Illumina technology, specifically from the MiSeq and HiSeq platforms, currently stands out for producing the highest percentage of error free end-reads (Besser et al. 2018). However, NGS studies of microbial communities still have some potential biases and limitations which are affected by molecular techniques biases or problems including mishandling during DNA extractions, PCR errors, reliability of using accurate Taq polymerase and heterogeneity within 16S rRNA gene copy of microbial samples and sequencing biases (Knief et al. 2014), which can potentially lead to errors in estimation of microbial diversity and their relative abundance.

Rapid progress in high-throughout, high-resolution methods has improved our understanding to directly link microbial communities/functions (phylogenetic and functional diversity) to rate and magnitude of ecosystem processes and functions especially biogeochemical cycling in northern peatlands (Li et al. 2022). It has been suggested that response of ecosystem processes i.e decomposition dynamics to enviro-climatic change will be mediated by shifts in microbial phylogenetic composition and functional diversity. The acknowledgment of both phylogenetic and functional diversity using NGS, along with the recognition of their significance in global biogeochemical cycles has spurred substantial research efforts (Falkowski et al. 2008;) to understand how alteration in microbial communities impact the critical ecosystem processes. For peatland research studies, Chen et al. (2008) was among the first to use high-through-put sequencing to look for metabolically active microbes (methanotrophs) involved in the methane cycle. Moreover, Lin et al. (2012) also used NGS technology to study the role of methanotrophs, methanogens, sulphate reducers and fermenters involved in N cycling within a blanket bog. However, there is still a lack of knowledge about how prokaryotic community composition of peatlands in C cycling using NGS, is influenced by enviro-climatic driven biotic and environmental factors. An integrative approach that links profiling of peatland microbial communities with potential to yield important insights into not only the functioning of peatland ecosystems, but also the general relationship between microbial communities and ecosystem processes.

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Chapter 2 Influence of peatland plant functional types on prokaryotic communities across microhabitat

2.1 Abstract

Peatlands in the northern hemisphere store 3% of Earth's soil carbon. The stability of this carbon depends on enviro-climatic driven alterations in hydrology and plant community composition, and consequential effects on microbial communities. Notably, these microbial communities are important regulators of peatland carbon dynamics, but the impact of the composition of the plant community on the microbial community in the context of peatland microhabitats remains limited. We address this knowledge gap using a long-term plant removal experiment to study the impact of vascular plant functional type (PFT i.e., ericoid and graminoid) across lawn and hummock microhabitats. Ericoids, graminoids, or both ericoids and graminoids were removed within plots on both lawns and hummocks. Our results show that the role of PFT removal on prokaryotic community composition (bacteria and archaea) and diversity was small. However, PFTs affect the relative abundance of prokaryotes, and this influence was microhabitat dependent. Lawns and hummocks comprised a different prokaryotic alpha diversity and community structure. In the lawns, we observed higher alpha diversity indices (observed Amplicon sequence variants, Shannon diversity and Chao1 richness) as compared to hummocks. At the same time relative abundance of both aerobic and anaerobic microbial populations was highest in the lawns, while the communities in the hummocks were typified by aerobic microbes. Our results highlight that the effects of hydrological conditions on microbial community composition was more pronounced than the effect of plant community composition, underlining the importance of direct changes in hydrology when examining peatland C dynamics in response to present and future changes in enviro-climatic scenarios.

2.2 Introduction

Northern peatlands are extremely valuable ecosystems because they store a huge amount of carbon (C) underground. Over the past centuries, these terrestrial ecosystems have accumulated 500-1000 Gt C (Yu, 2012; Nichols & Peteet. 2019), making them one of the most important terrestrial wetland reservoirs for C (Temmink et al. 2022). Moreover, peatlands are characterized by a unique biodiversity (Bridgham et al. 2006; Joosten et al. 2012; Qiu et al. 2020). Yet, peatland ecosystems, and notably their C-sink function, are extremely sensitive to global climate change (Helbig et al. 2022). Hydrological degradation is one of most important factors that plays an important role in modulating decomposition processes and greenhouse gas emissions (specifically CO_2 and CH_4) to the atmosphere (Evans et al. 2021). Moreover, fluctuation in the water table level is known to influence the balance between aerobic and anaerobic decomposition, which are primarily driven by microbial communities (Moore et al. 2007; Ubranova & Barta 2016; Morton & Heinemeyer 2019; Tian et al. 2019). Importantly, microbiota play an important role in biogeochemical processes which are crucial to the functioning of peatlands. For instance, these microbes drive key ecological processes such as carbon turn over, the rate of nutrient cycling, and the decomposition of soil organic matter (Bardgett 2008; Falkowski et al. 2008; Wu et al. 2024). However, of pressing concern is the role of microbes in biogeochemical processes and their sensitivity to both abiotic (environment, climate) and biotic (vegetation) conditions in the context of enviro-climatic changes (Ward et al. 2015; Robroek et al. 2015; Dieleman et al. 2016; Jassey et al. 2018). Environmental conditions (i.e. hydrological regimes) can directly select specific microbial groups and hence influence community composition (Emsens et al. 2020; Tian et al. 2021), as well as indirectly affecting microbial community composition through changes in plant community. Therefore, it is essential to understand how plant community composition influences microbial communities within distinct peatland microhabitats, such as lawns and hummocks, which differ significantly in hydrology. These differences likely affect carbon fluxes (e.g., CO_2 and CH_4 emissions), making it crucial to assess their contributions distinctly to predict how peatland ecosystems will respond to future climate change.

An important ecological characteristic of northern peatlands is the occurrence of natural heterogeneity or microhabitats where water table level and distinct vegetation shape the surface into series of hummock-lawn microtopographies. These refer to elevated or raised hummocks that are dominated with drier shrubby vegetation located above the water table and wet lawns situated or moved along water table which may occasionally experience inundation (Belyea & Clymo 1998; Moser et al. 2007). In addition, microhabitats are well known to impact microbial biomass and metabolic activity (Makiranta et al. 2019; Kaštovská et al. 2018; Sahar et al. 2022).

Apart from being different in their proximity to the water table, hydrological conditions exert control on microbial regulated biogeochemical processes like greenhouse gas (GHG) emission and consumption (Laine 2009; Krohn et al. 2017; Zhong et al.2020). Variations in belowground prokaryotic community composition were observed among microhabitat (Chroňáková et al. 2019; Asemaninejad et al. 2019), is attributed to where at hummock surface, above the water table are dominated by aerobic chemoorganotrophs, methanotrophic archaea and chemoheterotrophs (Asemaninejad et al. 2018). However, wet lawns with fluctuating water table (oxic-anoxic zone) facilitate the condition for facultative anaerobic nitrogen-fixing bacteria, facultative anaerobic chemoorganotrophy, sulphate reducing bacteria and methane oxidizing bacteria (Sundh et al. 1997; Asemaninejad et al. 2018). Consequently, aerobic decomposition and the highest methane consumption take place either at or above the water table within peat layers, whereas the highest methane production primarily occurs in anoxic environments situated below the water table (Moore & Dalva 1997; Kip et al. 2012; Zhang et al. 2021). Hence knowledge of belowground prokaryotic microbial community composition and diversity is important to understand the factors that regulate the biogeochemical processes and GHG emissions under alteration in hydrological conditions, which is necessary to better predict the global C turnover particularly in context of future climate change scenarios.

Across northern peatlands, microhabitats including lawns and hummocks have distinct belowground ecosystem processes, thought to be driven in part by specific types of PFTs. The raised aerated hummocks are dominated by evergreen ericoid shrubs, while lawns are abundant in graminoid plants (Bubier et al. 1993; Jonasson & shaver 1999; Kuiper et al. 2014; Robroek et al. 2015). These vascular PFTs directly impact microbial community composition and metabolic diversity through their distinct nutrient strategies and carbon allocation traits, affecting gas transport and rhizodeposition (Hooper et al. 2000; Ward et al. 2012; Gavazov, 2018; Wang & Moore 2014; Marti et al. 2015). Hence it is well established that there is a direct functional link between plant species (aboveground) and microbial communities (belowground) (Bragazza, 2015) which support ecosystem functioning such as C storage (Morriën et al. 2017). However, these plant-microbe interactions are sensitive to enviro-climatic gradients (Robroek et al. 2021) with consequences for ecosystem processes. Presently, within the context of a drier and warmer climate, peatlands are experiencing rapid transformations, leading to an increase in the prevalence of vascular PFTs such as graminoids and ericoids (Buttler et al. 2015). However, there is still a lack of understanding of the linkage between plant community composition and the belowground microbial community and if these linkages are similar in lawns and hummocks.

In this study, we make use of a long-term plant removal experiment to investigate how changes in PFTs have influenced the composition and diversity of prokaryotic communities within two different microhabitats in peatlands: wet lawns and dry hummocks. Our main objectives were to investigate how vascular plant functional types and microhabitat interactively influence two crucial factors: i) Shannon diversity and ii) the composition of prokaryotic communities within an ombrotrophic bog ecosystem. Our initial hypotheses suggested that different plant functional types and microhabitats would have distinct impacts on bacterial and archaeal communities. We also expected that prokaryotic communities in wet lawns would be less diverse, and more uniform compared to those in dry hummocks. Additionally, we hypothesized that selectively removing graminoids and ericoid plants would decrease diversity and alter prokaryotic community composition.

Overall, this study aimed to provide insights into how different plant types and microenvironments impact the diversity and composition of prokaryotic communities in an ombrotrophic bog ecosystem.

2.3 Methods

2.3.1 Study area and experimental design

We make use of a long-term plant functional type removal experiment that was set up in 2011 in the Store Mosse National Park (57°17054 N, 14°00039 E), the largest natural peatland complex in southern Sweden. The experiment consists of 40 0.5m × 0.5m experimental plots in a *Sphagnum*-dominated peat bog in (Robroek et al. 2015). These plots were equally divided across two microhabitats: lawns (and hummocks (c.f Rydin & Jeglum 2006). Each of these microhabitats exhibited distinct vegetation types and hydrological conditions. The lawns, characterized by wet depressions with the water table situated near the Sphagnum moss surface (approximately 1-3 cm), were primarily dominated by *Eriophorum vaginatum* L., *Trichophorum cespitosum* (L.) Hartm., *Rhynchospora alba* (L.) Vahl., *Vaccinium oxycocccos* L., *Erica tetralix* L. and *Andromeda polifolia* L. In contrast, the hummocks were positioned above the water table (20-50 cm) (Rydin & Jeglum 2006) and consisted of *Eriophorum vaginatum* L, *Erica tetralix V. oxycoccos*, *Calluna vulgaris* (L.) Hull and *A. polifolia*.

In each microhabitat, we implemented four different plant functional group removal treatments. These treatments consisted of an undisturbed control group, the removal of graminoids (- Gram), the removal of ericoids (- Eric), and the removal of both ericoids and graminoids (- Gram / - Eric).

To establish these treatments, we clipped the target vegetation flush to the moss surface. We repeated these manipulations three times every year until sampling in 2019. This period of time also resulted in remaining belowground plant parts, and certainly finer roots, to disappear. We used a randomized blocks design, with all four treatments replicated five times (block).

2.3.2 Prokaryote community composition

To assess the composition of prokaryotic communities in the subsurface, we obtained peat soil samples from the centre of each plot at a depth of 5-10 cm. These samples were promptly placed into sealed plastic bags. Subsequently, the peat samples were preserved at a temperature of 4°C after collection and during transportation to the laboratory. In the laboratory, the samples were then maintained at a maximum temperature of -20°C until they were later processed.

DNA was extracted from a 0.25-gram peat sample following the provided instructions, using the QIAGEN DNeasy Powersoil kit. We evaluated the extracted DNA's quality and concentration by utilizing the QIAGEN QIAxpert and the Qubit fluorometer. Following this, the samples were carefully packed with dry ice and sent via overnight shipping to Novagene Co., Ltd (Cambridge, UK; https://en.novogene.com) for the purpose of DNA amplification and sequencing. The amplification procedure focused on one specific genome region. To target the V4 region of the 16S rRNA gene in prokaryotes, we utilized the 515F/806R primers. These amplicons were then subjected to sequencing on the Illumina paired-end platform, generating reads with a length of 250 base pairs. After the sequencing process, the DADA2 pipeline, developed by Callahan et al. (2016), was employed to remove chimeric reads, assess the sequencing data's quantity and quality, and produce an Amplicon Sequence Variant (ASV) table for the microbial sequences. Taxonomic assignments for the ASVs were made using the SILVA database (https://www.arbsilva.de) for prokaryotic taxa.

2.3.3 Statistical analysis

Analysis of variance (ANOVA) was used to assess the effects of microhabitats (lawns and hummocks) and PFT composition on alpha diversity measures (Chao1, Shannon, and Simpson indices) and the relative abundance of various bacterial and archaeal taxa. The Chao1 index was used to quantify species richness, while diversity and evenness were expressed by the Shannon and Simpson indices (Tom et al. 2003). Principal Coordinate Analysis (PCoA) was employed, using Bray-Curtis distance metrics, to visualize the dissimilarity in community composition between the samples. For the comparison of dissimilarity in the prokaryotic community between vascular PFTs and microhabitats, Permutational Multivariate Analysis of Variance (PERMANOVA) with the Bray-Curtis matrix was applied, as well as alpha and beta diversity analyses, were carried out using the "microeco" and "mecodev" (version 0.2.0) packages in R (version 4.2.). The identification of the most abundant bacterial and archaeal taxa in different PFT treatments and microhabitats was accomplished using Linear Discriminant Analysis Effect Size (LEfSe) as described by Segata et al. (2011). To control for false discoveries, all raw p-values in the LDA were adjusted using the Benjamini-Hochberg False Discovery Rate (FDR) correction method (Benjamini and Hochberg 1995), implemented using the "microeco" package. All these analyses were conducted in R (version 4.2.1) and involved the use of the "phyloseq" (McMurdie et al. 2012), "vegan" (Oksanen et al. 2019), and "microeco" (Liu et al. 2021) packages.

2.4 Results

2.4.1 Sequencing output and general characteristics of prokaryotic community profiles

Amplicon sequencing of the 16S rRNA gene from 40 peat soil samples resulted in a total of 6,129,300 paired-end raw reads, with a mean of 153,233 reads per sample. Quality control was performed using the DADA2 pipeline (version 1.16), which involved several processing steps to ensure data accuracy and reliability. Paired-end reads were first trimmed based on quality scores, with forward reads truncated at base 240 and reverse reads at base 200 to remove low-quality tails.

Quality filtering removed low-confidence sequences, retaining a total of 5,234,585 filtered reads across all samples. Denoising was then performed separately for forward and reverse reads, resulting in 5,012,111 and 5,023,954 denoised reads, respectively. Paired-end reads were subsequently merged, and after chimera removal using DADA2’s consensus method, an average of 76,832 non-chimeric sequences per sample remained for downstream analyses.

A total of 1,757 targeted unique prokaryotic ASVs were recovered. An additional 3,264 ASVs were removed because their taxonomic assignments corresponded to mitochondria or chloroplasts, representing approximately 2.7% of all sequencing reads across the 40 peat samples. The retained ASVs comprised 97.2% of the filtered merged sequences and were affiliated with 309 genera, 200 families, 148 orders, 78 classes, and 41 phyla.

The dominant phyla across all samples, regardless of microhabitat and plant functional type (PFT) community composition, were Proteobacteria, Planctomycetia, Acidobacteria, Verrucomicrobia, and Chlamydiae, accounting for 38.31%, 9.94%, 7.38%, 7.07%, and 4.25% of the reads, respectively. Together, these phyla represented approximately 66% of the 16S rRNA-derived community profiles across all samples. Rarefaction curves indicated that species diversity reached near saturation, and good coverage was achieved, reflecting the sequencing integrity across all peat soil samples.

2.4.2 Most abundant ASVs with PFT treatments and microhabitats

Venn diagrams were used to compare the prokaryotic communities among distinct PFTs in lawn and hummock, based on shared and unique ASVs among the samples (Fig 2.1). The numbers of shared ASVs across the four PFT communities in the wet lawns from control, ericoid removal (– Eric), graminoid removal (– Gram) and absence of vascular PFT (– Gram / – Eric) 1083, while in hummocks were 1377 respectively. Within lawns, total of 580 unique ASVs were found in control, while 722 in ericoid removal (– Eric) ,446 in graminoid removal (– Gram) and 372 in absence of vascular PFT (– Gram / – Eric). However within in hummock, total of 1098 unique ASVs were observed in control, 926 in (– Eric), 794 in (– Gram) and 577 in removal of PFT (Fig 2.1 a,b)

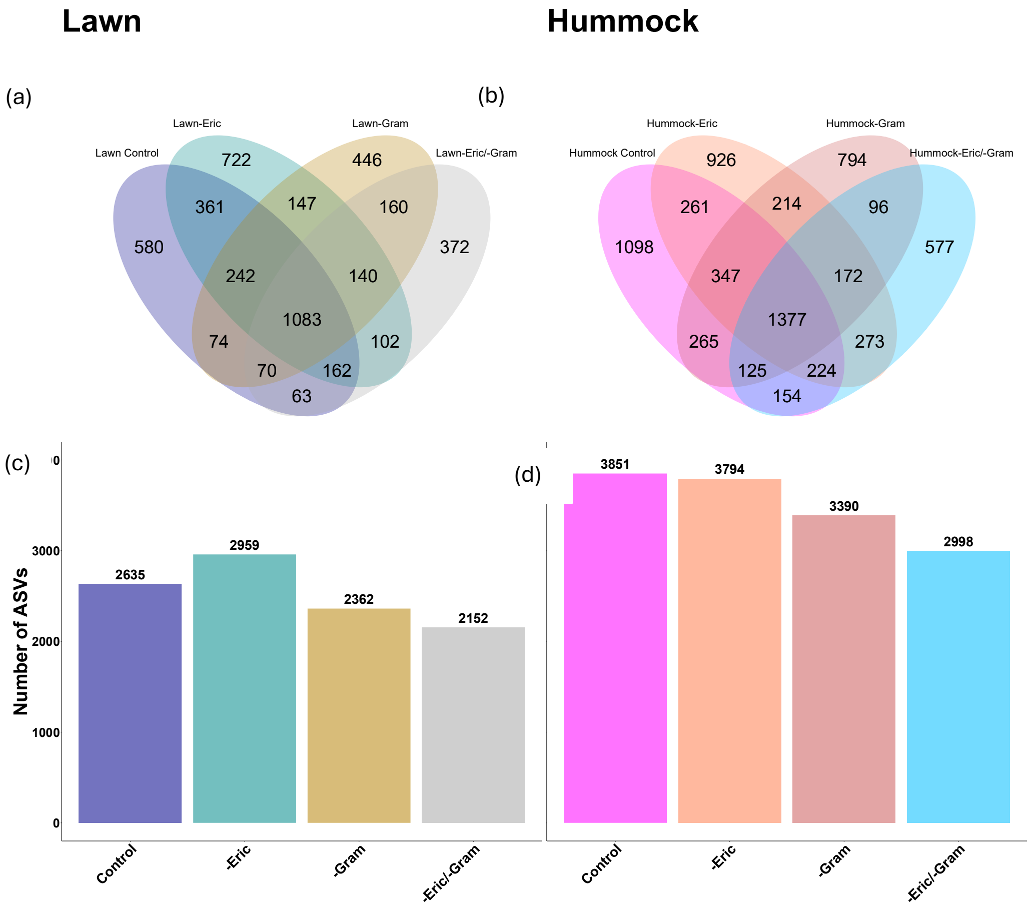

Figure 2.1 Venn diagrams showing numbers of unique and shared prokaryotic ASVs for the (a) lawns and (b) hummocks among vascular plant removal treatments. Control = undisturbed control, – Eric = ericoids removed, – Gram = graminoids removed and – Gram / – Eric = ericoids + graminoids removed. The percentages are relative to the total ASV number observed. Bar plot showing no of ASVs for the (c) lawns and (d) hummock among vascular plant removal treatment.

2.4.3 Prokaryotic richness and diversity

To quantify the relative and interactive effects of microhabitats and vascular PFT community composition on prokaryotic richness, diversity, and evenness, we used the Chao1, Shannon, and Simpson indices. Chao1, which measures richness by accounting for rare taxa, showed higher ASV richness in hummocks than in lawns (Fig. 2.2a, Table 2.1, $F_{1,40} = 15.37$, $P < 0.001$), while PFT removal did not affect richness overall ($F_{3,40} = 2.20$, $P = 0.10$) or differ between microhabitats ($F_{3,40} = 0.02$, $P = 0.99$). Although not significant, richness tended to decrease without graminoids (Fig. 2.2a). Shannon diversity, which captures both richness and evenness, was 18.72% higher in hummocks (Fig. 2.2b, Table 2.1, $F_{1,40} = 11.06$, $P < 0.001$), with no main effect of PFT composition ($F_{3,40} = 1.30$, $P = 0.28$) or interaction with microhabitat ($F_{3,40} = 0.19$, $P = 0.89$). Evenness, assessed by the Simpson index, was not influenced by PFT composition and microhabitat (Fig. 2.2c, Table 2.1, $F_{3,40} = 0.28$, $P = 0.83$). The Shannon diversity index, targeting richness and evenness, was selected as the most robust measure to capture diversity patterns across removal of vascular PFTs and microhabitats in this research study.

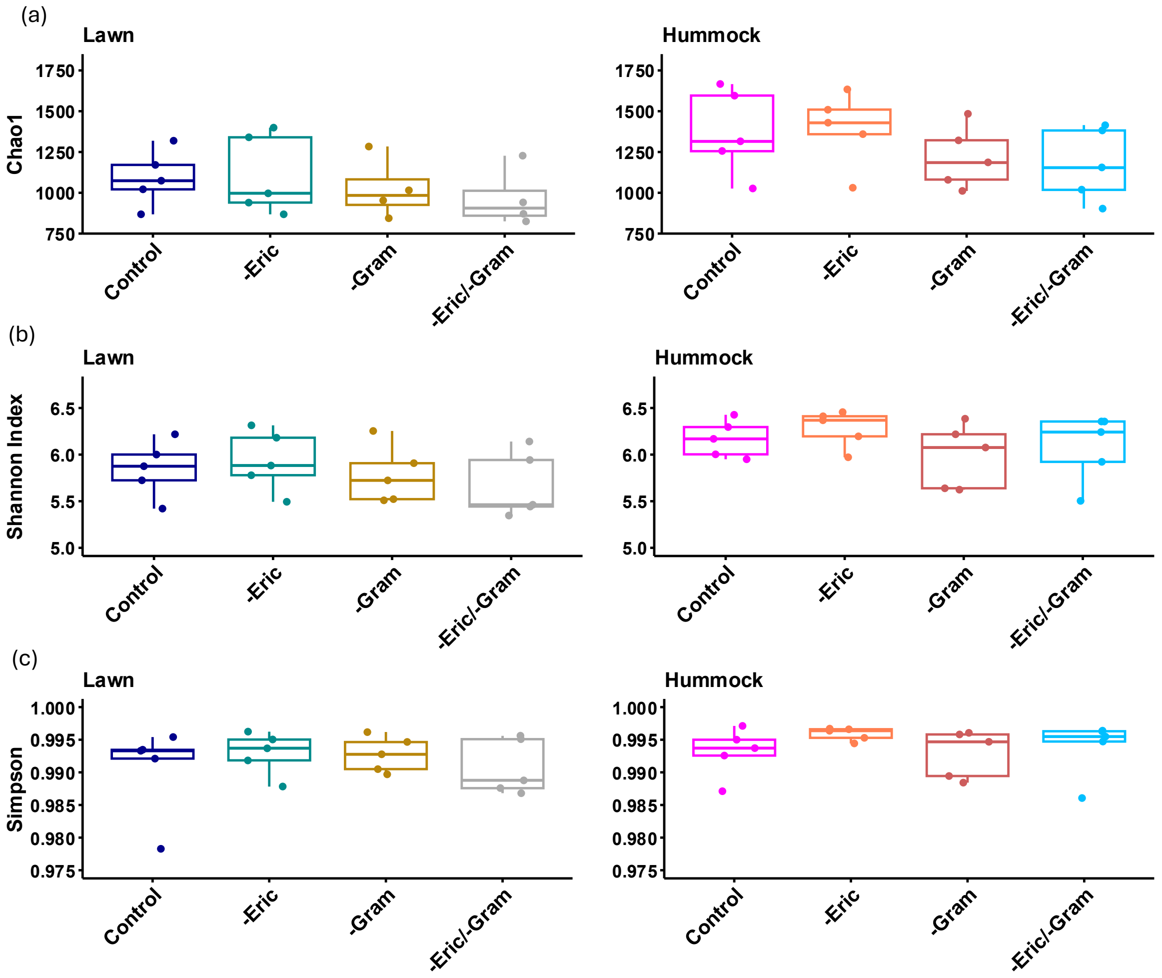

Figure 2.2 - Boxplots of the effects of vascular plant removal treatments and microhabitat (lawns and hummocks) on prokaryotic alpha-diversity (a) Chao1(species richness), (b) Shannon index and (c) Simpson index. The black bar within each box represents the median and the upper and lower bounds of the boxes represent the 1st and 3rd interquartile ranges. Outliers are denoted with circles. (n = 5 per box). Control = undisturbed control, – Gram = graminoids removed, – Eric = ericoids removed, – Gram / – Eric = ericoids + graminoids removed. Outputs for statistics are presented in text.

Table 2.1- Statistical analysis from the ANOVA, testing the influence of vascular plant functional type (PFT) removal treatment and microhabitat (MH) on Shannon diversity, Simpson's evenness, and Chao1 richness. Significant P-values ($P \leq 0.05$) are shown in bold values (n = 5).

Variables		Chao1		Shannon		Simpson	
	Df	F	P	F	P	F	P
PFT	3	2.20	0.10	1.30	0.28	0.77	0.51
MH	1	15.37	**≤ 0.001**	11.06	**≤ 0.001**	2.86	0.10
PFT: MH	3	0.02	0.99	0.19	0.89	0.28	0.83

2.4.4 The relative abundance of archaeal taxa among PFTs within microhabitats

Archaeal communities across distinct vascular PFTs and microhabitats were dominated by Diapherotrites (30.17%), Euryarchaeota (25%), Crenarchaeota (20.68%), and Thaumarchaeota (14.65%) respectively. The vascular PFT removal did not significantly influence the relative abundance of archaeal phyla, however, in context of microhabitat variation, phyla Diapherotrites and Thaumarchaeota were significantly more abundant in hummocks (Fig 2.3,$F_{1,31} = 33.00$, $P < 0.001$: $F_{1,31} = 152.90$, $P < 0.001$) whereas Euryarchaeota and Crenarchaeota preferred lawns (Fig 2.3, $F_{1,31} = 181.84$, $P < 0.001$: $F_{1,31} = 8.24$, $P < 0.007$).

At class level, the Micrarchaeia (30.17%) and Group 1.1c (12.06%) were the most abundant classes in Diapherotrites and Thaumarchaeota phylum respectively and were significantly higher in hummocks(Fig 2.3,$F_{1,31} = 33.00$, $P < 0.001$:$F_{1,31} = 156.14$, $P < 0.001$).Within the phylum Crenarchaeota, class Bathyarchaeia (17.24%) had the highest abundance and was enriched in lawns comparative to hummocks (Fig 2.3,$F_{1,31} = 8.08$, $P < 0.007$)

The methanogenic lineages belonging to the phylum Euryarchaeota and were represented mainly by classes Thermoplasmata (11.20%), Methanomicrobia (6.89%) and Methanobacteria (5.71%). The order Methanomicrobiales (3.44%) (Fig 2.3, $F_{1,31} = 23.97$, $P < 0.001$) and Methanosarcinales (2.5%)(Fig 2.3,$F_{1,31} = 57.13$, $P < 0.001$) were most abundant orders in class Methanomicrobia and order Methanomassiliicocces (3.44%) (Fig 2.3,$F_{1,31} = 9.80$, $P < 0.003$) had greater abundance in class Thermoplasmata and were significantly greater in lawns.

2.4.5 The abundance of bacterial taxa among PFTs within microhabitats

The taxonomic composition of bacterial communities among distinct PFTs in lawn and hummock microhabitat were affiliated to 32 phyla and was dominated mostly by *Proteobacteria* (average 40.82%), Planctomycetes (10.59%), Acidobacteria (7.86%), Verrucomicrobia (7.54%), Chlamydiae (4.53%), Actinobacteria (4.13%), Cyanobacteria (3.71%), Patescibacteria (3.64%), Bacteroidetes (3.64%), Dependentiae (2.87%), Armatimonadetes (2.32%) and Firmicutes (1.48%), accounting for 92.57% of bacterial sequences for each strand. In addition, Chloroflexi (0.51%) and Nitrospirae (0.06%) were present in most of peat samples, at low abundances (<1%).

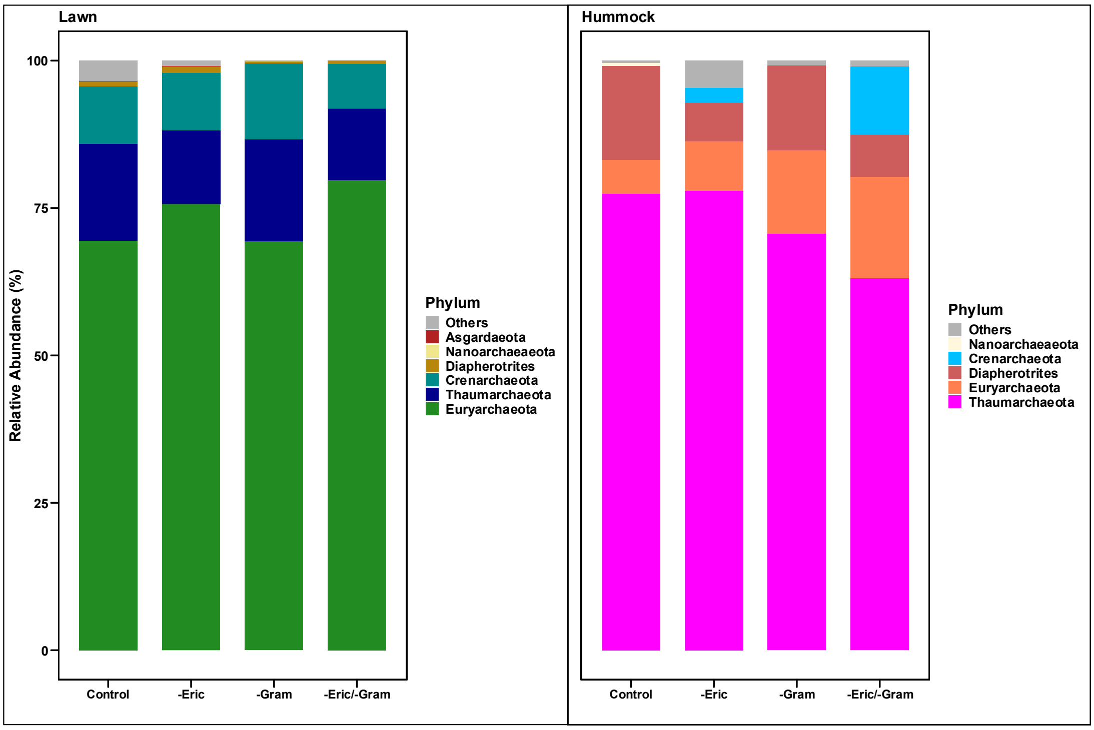

Figure 2.3 - Average relative abundance of top archaeal phyla of four different PFT removal treatments (control, ericoid removed, graminoid removed, both removed) across lawn and hummock. The unidentified phyla were defined as others (n=5)

Among which Planctomycetes, Dependentiae, Chloroflexi, Firmicutes and Nitrospirae were significantly higher in lawn (Fig 2.4,Planctomycetes: $F_{1,32} = 4.44$, Dependentiae: $F_{1,32} = 6.41$, $P = 0.01$,Chloroflexi:$F_{1,32} = 18.24$, $P < 0.001$,Firmicutes:$F_{1,32} = 7.05$, $P = 0.01$,Nitrospirae: $F_{1,32} = 10.84$, $P = 0.002$,) whereas Armatimonadetes was highly abundant in hummocks (Fig 2.4,$F_{1,32} = 4.35$, $P = 0.04$). The removal of vascular PFT removal treatment (–Gram/–Eric) affect significantly increased relative abundance of Verrucomicrobia as compared to control and was microhabitat dependant (Fig 2.4, $F_{1,32} = 2.95$, $P = 0.04$).

At class level, the majority of Proteobacteria, mainly comprised of Alphaproteobacteria (19.44%), Gammaproteobacteria (11.08%) and Deltaproteobacteria (9.01%) classes of total bacterial sequences, while their relative abundance showed no significant differed among PFT removal treatment nor in interaction with microhabitat(Fig 2.4, Alphaproteobacteria:$F_{1,32} = 4.44$, $P=0.61$,Gammaproteobacteria:$F_{1,32} = 1.06$, $P = 0.37$,Deltaproteobacteria:$F_{1,32} = 0.52$, $P = 0.66$).While the relative proportions of relative abundance of classes Holophagae (2.87%) (phylum Acidobacteria) and Acidimicrobiia (2.04%) were significantly higher in lawns(Fig 2.4,Holophagae:$F_{1,32} = 8.31$,$P=0.006$,Acidimicrobiia:$F_{1,32} = 8.96$, $P = 0.005$).However,Actinobacteria (1.17%) and Thermoleophilia (0.87%) were more enriched in hummock microhabitats(Fig2.4,Actinobacteria:$F_{1,32} = 17.32$,$P<0.001$,Thermoleophilia:$F_{1,32} = 21.19$, $P < 0.001$).

At order level, Rickettsiales (5.10%) and Saccharimonadales (1.96%) had significantly higher relative abundances in hummock(Fig 2.4,Rickettsiales:$F_{1,32} = 17.04$,$P<0.001$, Saccharimonadales:$F_{1,32} = 14.40$, $P < 0.001$) whereas, Rhizobiales (4.02%) and Pedosphaerales (3.18%)preferred-lawns(Fig2.4,Rhizobiales:$F_{1,32} = 8.18$,$P=0.007$, Pedosphaerales:$F_{1,32} = 14.40$, $P < 0.001$).The vascular plant removal treatment significantly influences the relative abundances of Myxococcales and Methylacidiphilales and were microhabitat dependant(Fig2.4,Myxococcales:$F_{1,32} = 3.61$, $P = 0.02$,Methyacidiphilales: $F_{1,32} = 3.02$, $P = 0.04$)In lawns, removal of all vascular PFTs (–Gram/–Eric) decreased the relative abundance of Myxococcales while in hummocks increased it (Fig 2.4,$F_{3,32} = 3.61$, $P = 0.02$). However, absence of vascular PFTs treatment significantly increased relative abundance of Methylacidiphilales across microhabitat (Fig 2.4, $F_{3,32} = 3.02$, $P = 0.04$). The relative abundance of Rickettsiales were highest when only ericoids were removed (Fig 2.4, $F_{3,32} = 3.62$, $P = 0.02$)

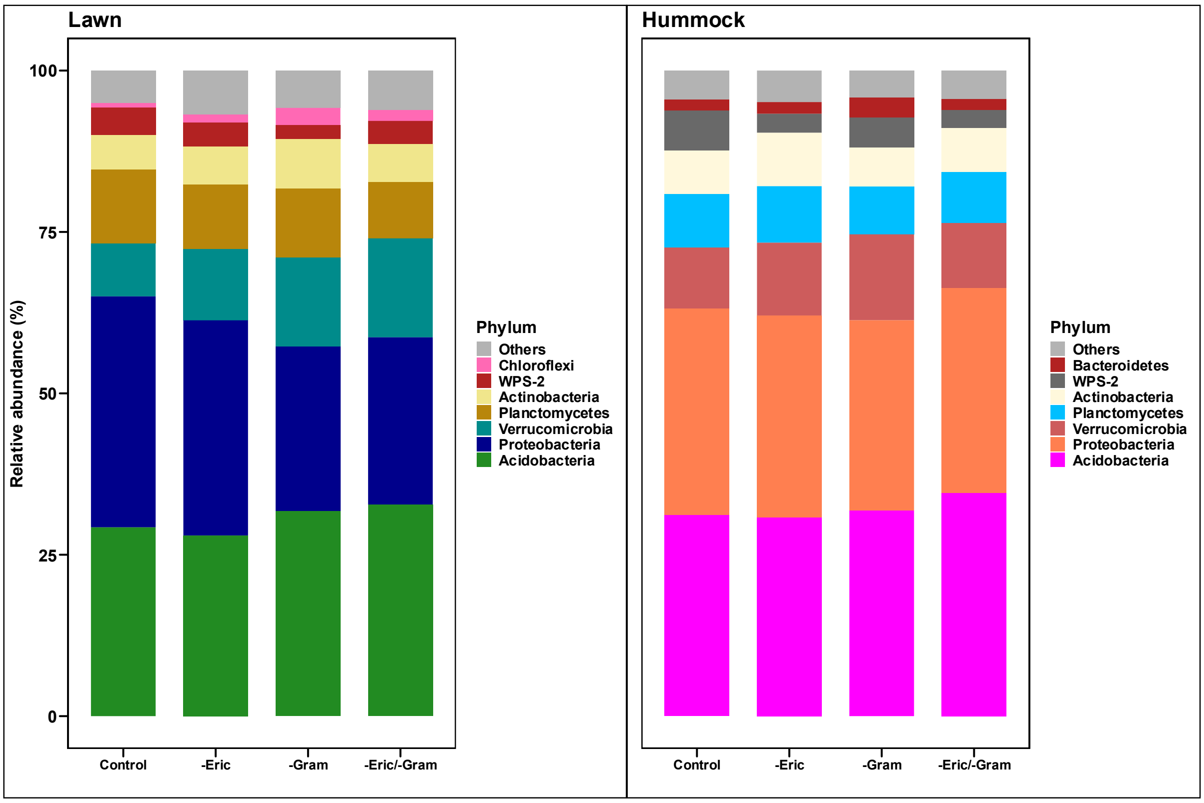

Figure 2.4 - Average relative abundance of top ten bacterial phyla of four different PFT removal treatments (control, ericoid removed, graminoid removed, both removed) across lawn and hummock. The phyla with abundance < 1% were defined as others (n=5)

2.4.6 Comparison of prokaryotic community structure between PFTs and microhabitats

PCoA based on bray-curtis dissimilarities of ASVs indicated that taxonomic and phylogenetic structure of prokaryotic communities did not differ much between the vascular PFT removal treatments. Hence, prokaryotic community composition in the peat soil under different vascular PFT removal treatments were very similar (PERMANOVA; $R^2 = 0.05$, $F_{3,32}= 1.01$, $P = 0.43$; Fig. 2.5). However, microhabitat did, nonetheless, affect the composition of the prokaryotic community (PERMANOVA, $R^2 = 0.25$, $F_{1,32} = 12.80$, $P = 0.001$) in which PC1 and PC2 explained 30.9% and 11.6% of the variation showed substantial differed communities among microhabitats. In general, prokaryotic community structure in lawns appeared to be more homogenous in comparison to hummocks.

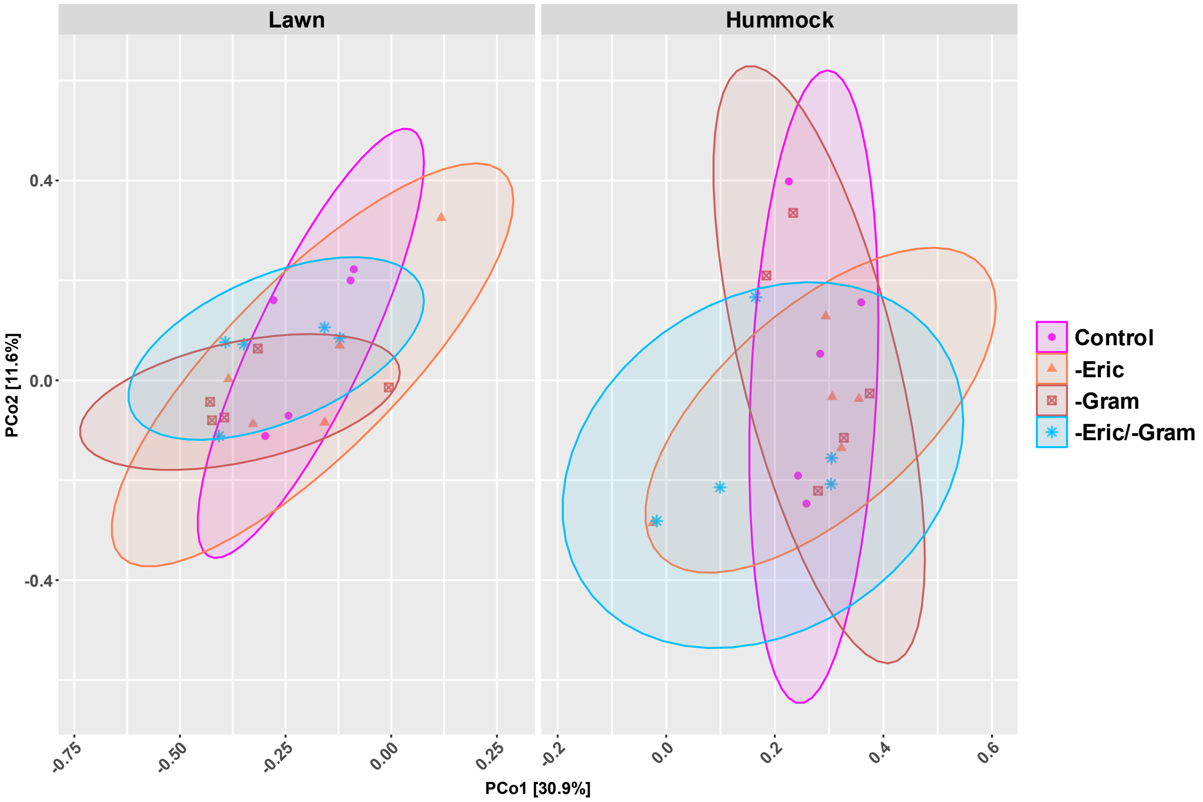

Figure 2.5 - Principal coordinate analysis (PCoA) ordination based on Bray-Curtis algorithm showing the difference in prokaryotic community composition of four different PFT removal treatments (Control = undisturbed control, – Gram = graminoids removed, – Eric = ericoids removed, – Gram / – Eric = ericoids + graminoids removed) across hummock and lawn (n=5). The statistical significance for the differences among microhabitat and PFT treatments were calculated by PERMANOVA. Each data point represents an individual sample. Points closer to each other represent similar microbial communities, while points farther from each other represent dissimilar microbial communities. Color/shape is an indicative of cohort.

Linear discriminant analysis effect size (LEfSe) identified biomarker taxa at various taxonomic levels that had a significant impact (LDA score > 3.0) in differentiating among vascular plant functional type (PFT) treatments in both lawns and hummocks (Fig. 2.6). A greater number of bacterial taxonomic differences were observed among vascular PFT treatments in lawns, with 20 indicator taxa identified, whereas only 2 taxa were detected in hummocks (Fig. 2.6, S 2.3). A total of 5 bacterial taxa were significantly enriched in lawns in the absence of ericoid shrubs (-Eric), with the order *Rhodospirillales* (phylum *Proteobacteria*) showing the largest effect size (LDA score = 4.0). In the absence of graminoids, 4 bacterial taxa were identified as significant by LEfSe, with the order *Pedosphaerales* (phylum *Verrucomicrobia*) having the largest effect size (LDA score = 4.5) among all vascular PFT removal treatments. Additionally, 3 bacterial taxa, including the class *Verrucomicrobiae* (LDA score = 4.5), were significantly more abundant in the absence of vascular PFTs. Within the hummock microhabitat, 2 bacterial taxa were identified as indicators with large effect sizes, with the order subgroup_2 (phylum *Acidobacteria*) being significantly more abundant in the absence of both ericoids and graminoids, showing an LDA score of 3.1 (Fig. 2.7, S2.3).

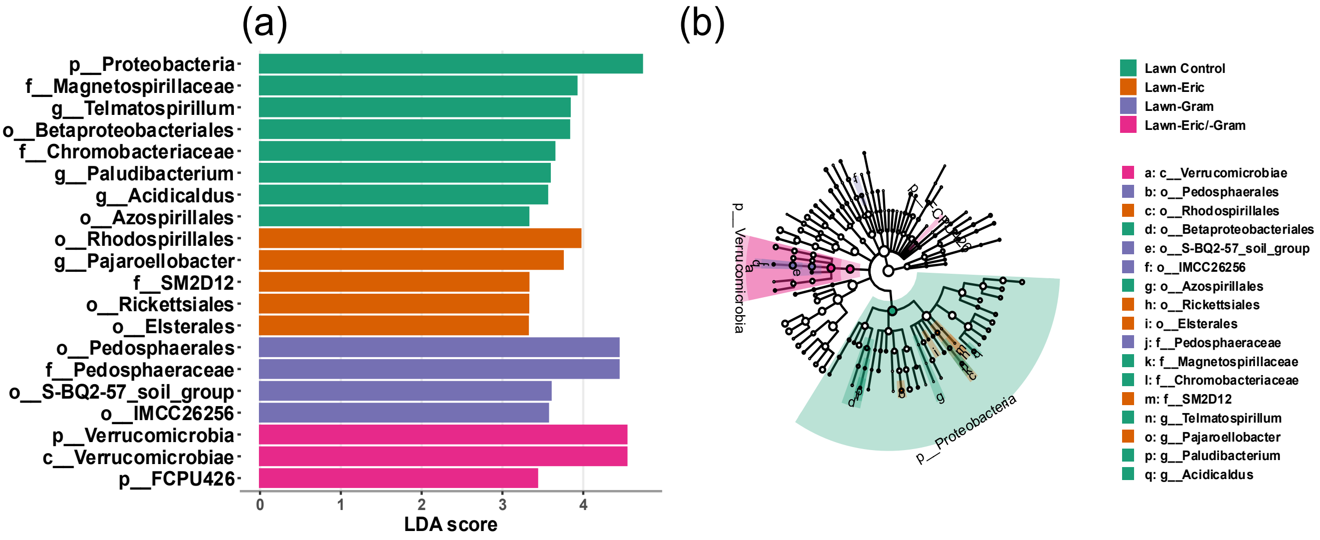

Figure 2.6–(a) LDA histogram showing the indicator prokaryotic groups observed between PFT removal treatments (Control (representing an undisturbed vegetation), - Gram (indicating the removal of graminoid vegetation), - Eric (denoting the removal of ericoid vegetation), and - Gram / - Eric (signifying the removal of both ericoid and graminoid vegetation) in lawn microhabitat and values > 4.0 revealed by linear discriminant analysis effect size (LEfSe) (b) Cladogram indicating the phylogenetic distribution of prokaryotic lineages. The taxon level is abbreviated as p: phylum; c: class; o: order; f: family, and g: genus. White nodes on the cladogram represent instances where there are no statistically significant differences in the composition of bacterial and archaeal taxa among the PFT removal treatments. Conversely, coloured nodes on the cladogram represent statistically significant differences in prokaryotic taxa composition in response to the treatments.

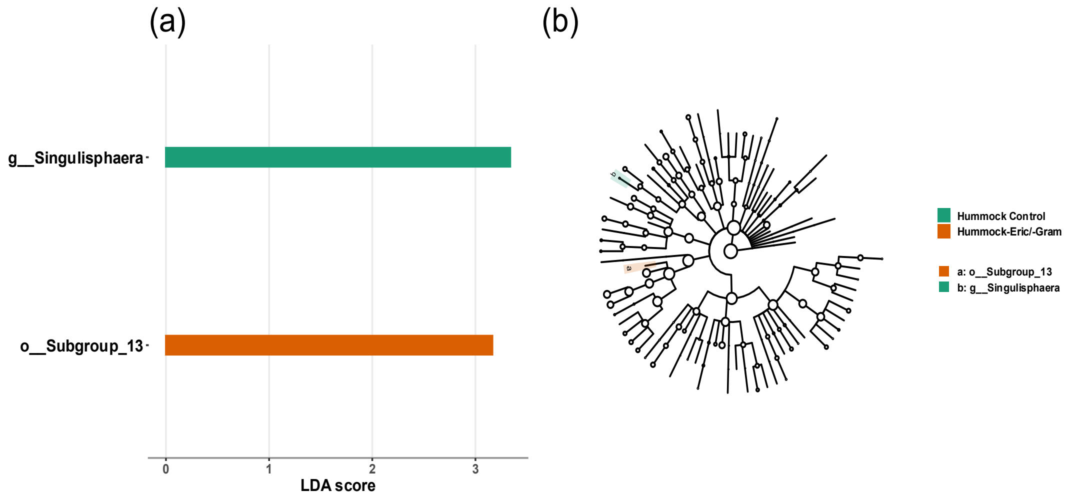

Figure 0.7 – (a) LDA histogram showing the indicator prokaryotic groups observed between PFT removal treatments (Control (representing an undisturbed control group), - Gram (indicating the removal of graminoid vegetation), - Eric (denoting the removal of ericoid vegetation), and - Gram / - Eric (signifying the removal of both ericoid and graminoid vegetation) in hummock microhabitat and values > 3.0 revealed by linear discriminant analysis effect size (LEfSe) (b) Cladogram indicating the phylogenetic distribution of prokaryotic lineages. White nodes on the cladogram represent instances where there are no statistically significant differences in the composition of bacterial and archaeal taxa among the PFT removal treatments. Conversely, coloured nodes on the cladogram represent statistically significant differences in prokaryotic taxa composition in response to the treatments.

2.5 Discussion

Peatlands are known for their vulnerability to effects of changing enviro-climatic conditions, which can induce an alteration in plant community composition and diversity (Robroek et al. 2017; Pinceloup 2020). These changes potentially carry over to significant change in communities and can impact overall ecosystem processes over a longer term. The role of plant functional types on microbial communities and C cycling, mediated by distinct plant litter and rhizosphere inputs are documented in previous peatland studies (Fritz et al. 2011; Ward et al. 2015; Robroek et al. 2015; Palozzi & Lindo 2017). Furthermore, the effect of microhabitats on peat microbial biomass, structure, and activity have also been well studied (Oloo et al. 2016; Heger et al. 2018); however, the impacts of unique PFTs and microhabitats on complex microbial communities, including bacteria and archaea, are less explored. This study focuses on how different PFTs, particularly ericaceous shrubs and graminoid sedges, affect microbial communities in two contrasting microhabitats (lawns and hummocks). Utilizing next-generation sequencing, we found that prokaryotic community composition (Bacteria and Archaea) differs significantly across microhabitats, with a relatively weaker response to PFT change than to spatial variations among microhabitats.

In our study, conducted in a *Sphagnum*-dominated ombrotrophic bog, phylogenetic analysis showed that bacterial community was rich in Proteobacteria (sequences assigned to *α*-, *β*- and *δ*-classes), Planctomycetia, Acidobacteria and Verrucomicrobia, which can thrive in acidic and nutrient poor environments. These results are consistent with previous studies on peatlands (Serkebaeva et al. 2013; Danilova et al. 2016; Ivanova et al. 2016, 2018; Urakawa & Bernhard 2017; Seward et al. 2020). Proteobacteria were the most abundant and are known to play crucial roles in biogeochemical cycles, enhancing soil nutrient availability (Hartman et al. 2007), and are directly linked to increased carbon availability (Fazi et al. 2005). Consequently, they have been shown to play an important role in hydrocarbon degradation within northern peatlands (Chronakova et al. 2019). Elliott et al (2015) reported relatively high proportions of Acidobacteria and Verrucomicrobia in non-vegetated bare peat, hence, this might suggest their adaptability under oligotrophic conditions and an ability to survive in harsh nutrient-deficient and acidic environments. The Verrucomicrobia has been associated with plant-derived polymer degradation (Tveit et al. 2013; Urbanova et al. 2016) while Acidobacteria play important roles in decomposition of cellulose and aromatic compounds (Bardhan et al. 2012). Phylum Planctomycetia, another dominant bacterial group observed in this study, seems to be a slow-acting peat decomposer and capable of degrading heteropolysaccharide compounds in peat soil (Kulichevskaya et al. 2007) with several methanogens (phylum Euryarchaeota) that are members of the domain Archaea were also detected. Among these, Methanomicrobiales and Methanosarcinales are hydrogenotrophs while characterized Methanomassiliicoccales are obligate methylotrophs (Srorkin et al. 2018). The dominance of hydrogenotrophic and methylotrophic methanogenesis is characteristic of boreal acidic peatlands (Metje & Frenzel

2005; Conrad, et al. 2020) and reported previously in acidic and nutrient-poor *Sphagnum*-dominated ombrotrophic peatlands (Juottonen et al. 2005).

The role of vegetation and microhabitat spatial variation on belowground microbial community structure and metabolic activity can be influenced by the effect of established plant–microbe associations (Robroek et al. 2015; Sahar et al. 2022). In contrast to our initial hypothesis about the impact of plant functional type (PFT) removal and microhabitat on microbial diversity and community composition, our findings indicate no direct effect of specific PFTs on the prokaryotic diversity and community structure. Opposite to earlier studies by Robroek et al (2015) and Chroňáková et al (2019), which suggested that different vascular plant communities host distinct microbial communities, our results align with findings by Girkin et al. (2020), and Whitaker et al. (2021), demonstrating that contrasting plant community composition can structure similar microbial communities. De Deyn et al (2008) found that in nutrient-deprived peat habitats with brief growing seasons, plant communities emit less root exudate and produce lower-quality litter, resulting in slower decomposition processes within peat soil. Previous research from same study also documented that change in vegetation species type did not influence the decomposition process (Sahar et al. 2022), implying a potential microbial adaptation mechanism that does not notably impact microbial community composition. Wardle et al. (1999) also reported that microbial communities are often tightly associated with organic carbon, serving as energy source, in contrast to primary production (Moore et al. 2004). This relationship may help stabilize microbial communities against alterations in the structure of plant communities. The hypothesis suggests that the high carbon content in peat soil, acting as a buffer, may explain the observed minimal impact of variations in plant community composition on the below ground biological processes at our study site. Therefore, additional research is needed to validate this hypothesis.

In the present study, spatial variation across hummock and lawns, particularly in water table position, significantly influenced prokaryotic community composition. This microhabitat differentiation is also supported by clustering of microbial community structure established by PCoA analysis. While aligning with findings from previous studies revealing a high spatial heterogeneity of microbial communities among microhabitats (Chroňáková et al. 2019; Asemaninejad et al. 2019), our research extends this understanding to the spatial heterogeneity of prokaryotes specifically between hummocks and lawns. These significant shift in community diversity and composition may be explained by differences in soil physicochemical variables such as distance from the water table, factors related to hydrology and availability of oxygen, as well as proximal gradient of redox conditions (Frenzel & Karofeld 2000; Andersen et al. 2013; Zhong et al. 2017; Suriyavirun et al. 2019; Zhao et al. 2020), as these factors are known to play decisive roles in shaping prokaryotic communities. This differentiation is further evident in the higher alpha diversity (ACE and Shannon indices, ASVs richness) and distinct ASVs in the oxic hummocks compared to anoxic lawns. Hummock located at the surface of peat showed higher diversity, which is consistent with Morales et al. (2006), who also detected higher bacterial diversity near the surface of peat profiles. Therefore, hummocks of peatlands were dominated by aerobic processes and lawns by anaerobic processes (Blodau et al. 2004; Jungkunst et al. 2012). Additionally, hummocks from the same experimental site had higher VPC (vegetation plant cover) (Sahar et al. 2022), which might in turn have increased plant and root biomass (Han 2019; Wang et al. 2019), thereby supporting enriched and more diverse microbial communities. Conversely, lawns near the oxygen interface (oxic-anoxic) zone experience water table fluctuations that expose soil layers to secondary oxic decomposition. This process consumes labile carbon and nutrients while accumulating recalcitrant carbon (Broder et al. 2012) and as a result C flux are lowest in these interface zones (Zhong et al. 2017), which may partially explain the lower bacterial diversity found in lawn microhabitats.

The vascular plant functional community significantly influenced relative abundance of Rickettsiales (Proteobacteria) and Methylacidiphilales (Verrucomirobia). The relative proportion of Rickettsiales were significantly higher under graminoid peat in our study. Some of these species are known to be plant endophytic pathogens (Kunda et al. 2018). Their increased abundance might be due to direct selection by the roots of graminoids or indirectly by better peat aeration due to diffusion of active oxygen provided by well-developed aerenchymatous channels of graminoids (Saarinen 1996). Additionally, Marisa et al. (2020) also reported higher abundance of Rickettsiales in root samples of sedges. The combined removal of ericoid and graminoid plants significantly reduced the relative abundance of members of the order Methylacidiphilales. This was microhabitat dependent, reflecting perhaps the direct impact of vegetation litter inputs and labile root exudate flux as well as microhabitat conditions.

The lawn microhabitat hosted specific bacterial populations differing from hummocks through higher relative abundances of members of the phylum Acidobacteria and Actinobacteria, many of which represented by the class Holophagae and Acidimicrobiia. Some species of Holophagae within the phylum Acidobacteria are known to metabolize aromatic compounds and produce acetate as end product, which is an important substrate for methanogenesis in anoxic environments (Anderson et al. 2013; Asemaninejad et al. 2019). The relative abundance of anerobic phylum such as Planctomycetes, Chloroflexi and Firmicutes appeared to proliferate with higher water table in anoxic lawns. Some members of Planctomycetes (anaerobic chemoorganohetrotrophic autotrophs) are able to perform anammox i.e anaerobic ammonium oxidation to dinitrogen and play important role in nitrogen cycle (Oshiki et al. 2016). Furthermore, a major group of copiotrophic Alphaproteobacteria observed in lawns were the Rhizobiales, which apparently favoured the high-water table. Some species within the families Beijerinckiaceae and Methylocystaceae are methanotrophic and represent keystone taxa in coupling of nitrogen fixation and methane oxidation in nutrient-poor Sphagnum-dominated peatlands (Carvalho et al. 2010; Kolton et al. 2022). Meanwhile, members of Chloroflexi and Firmicutes are typically anaerobes, and more abundant in subsurface peat layers (Tsitko et al. 2014) in which bacterial groups of Chloroflexi are most likely have role in anaerobic decomposition of plant-derived organic matter with an ability to thrive under low nutrient conditions. They have previously been implicated in C cycling in subsurface peat (Zhong et al. 2017). Firmicutes are reported to contribute to the turnover of plant-derived polysaccharide, including cellulose (Dom et al. 2021). Moreover, the lawn microhabitats also possessed a higher abundance of methanogens (Euryarchaeota), which are typically associated with anoxic environments, including three orders of methanogenic archaea: Methanomicrobiales , Methanosarcinales (class Methanomicrobia) and Methanomassiliicoccales (class Thermoplasmata), in which the Methanomicrobiales are hydrogenotrophic, producing CH_4 from H_2 and CO_2, while Methanosarcinales are metabolically more versatile, carrying out hydrogenotrophic, acetoclastic, and methylotrophic methanogenesis whereas Methanomassiliicoccales appear to be obligate methylotrophs. These finding are like previous studies on methanogens in peatlands (Liu & Whitman 2008; Borrel et al. 2014; Suzanna et al. 2020). In addition, lawns with high water content undergo larger water table fluctuation at oxic-anoxic interface, increasing the amount of electron donors and acceptors, thus stimulating redox reactions, which could than increased the abundance of methanogen archaea (Daffonchio et al. 2006). These methanogens are players in methane cycling and can have significant impact on climate change and CO_2 and CH_4 emission in anaerobic ecosystems (Galand et al. 2005). Interestingly, in contrast to wet lawns, drier hummocks were accompanied by peat occupying Methylacidiphilales affiliated with Verucomicrobriae class, are acidophilic heterotrophic and some species are methanotrophic, with not only an ability to utilize plant derived polysaccharide but may also perform methane consumption and hydrogen oxidation as complementary sources of energy in a mixotrophic lifestyle. Some of these microbial groups may mitigate emission of methane in northern peatlands (Carere et al. 2017; Ivanova et al. 2020; Dedysh et al. 2021)

2.6 Conclusion

Peatlands in northern latitudes are experiencing environmental and climatic changes, including warming, drought events, and other anthropogenic disturbances that are driving shifts in plant functional types (PFTs) and alterations in water table levels. These biotic and abiotic changes impact carbon cycling processes in peatlands, particularly through their influence on soil microbiota. In this study, we evaluated the response of soil microbial communities to dominant PFTs and the internal heterogeneity within peatland systems, focusing on microhabitats defined by water table position.

Our results revealed that while microbial community composition and diversity were similar across distinct PFTs in this ombrotrophic bog, the combined influence of PFTs and spatial variation in microhabitats had significant effects on microbial populations. The presence of vascular plants, in particular, interacted with the hydrological conditions of the microhabitats, resulting in distinct shifts in microbial community structure. These effects were most pronounced in relation to water table position, oxygen availability, and redox conditions.

Microhabitat played a central role in shaping prokaryotic diversity and community composition. Lawns supported both aerobic and anaerobic microbial populations, such as chemoorganotrophs, methanotrophs, and methanogens, while hummocks primarily harboured aerobic microorganisms. These findings highlight the critical role of microhabitat variation, in combination with PFT-driven changes, in influencing peatland microbial dynamics. This study emphasizes the need to better understand the linkages between shifts in microbial communities and changes in ecosystem functioning under future climate scenarios. Therefore, investigating how alterations in both PFTs and microhabitat conditions influence nutrient cycling will be essential for predicting changes in belowground ecosystem processes in response to ongoing environmental change.

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2.8 Supplementary materials

Table S 0.1 Mean standard deviations (±SD) values of Chao1, Shannon and Simpson indices for two microhabitats (lawns and hummocks) and plant functional type removal treatments (control, ericoid removed, graminoid removed, both removed) (n=5)

	Lawns				Hummocks			
Parameters	Control	-Eric	-Gram	-Eric-Gram	Control	-Eric	-Gram	-Eric-Gram
Chao1	1090.60 ±168.301	1108.80 ±243.2318	975.80 ±194.670	916.00 ±192.1276	1371.6 ±261.373	1392.6 ±227.196	1216.6 ±189.913	1174.4 ±223.338
Shannon	5.8477 ±0.2995	5.9302 ±0.3263	5.7834 ±0.3096	5.6667 ±0.3516	6.1688 ±0.1988	6.2806 ±0.1990	5.9883 ±0.3438	6.0756 ±0.3656
Simpson	0.9905 ±0.0069	0.9929 ±0.0032	0.9927 ±0.0027	0.9907 ±0.0042	0.9931 ±0.0037	0.9958 ±0.0009	0.9928 ±0.0036	0.9937 ±0.0043

Table S 0.2 Mean standard deviations (±SD) values of microbial taxa for two microhabitats (lawns and hummocks) and plant functional type removal treatments (control, ericoid removed, graminoid removed, both removed) (n=5)

	Lawns				Hummocks			
Parameters	Control	-Eric	-Gram	-Eric-Gram	Control	-Eric	-Gram	-Eric-Gram
Verrucomirobia	8.202	11.070	13.743	15.336	9.390	11.275	13.311	10.067

	±0.873	±1.512	±2.242	±4.0313	±1.862	±2.573	±4.219	±1.901
Dependentiae	0.350	0.557	0.578	0.283	0.252	0.317	0.200	0.307
	±0.108	±0.244	±0.456	±0.083	±0.119	±0.128	±0.137	±0.192
Firmicutes	0.114	0.283	0.296	0.498	0.052	0.058	0.229	0.073
	±0.061	±0.273	±0.250	±0.303	±0.062	±0.020	±0.433	±0.052
Planctomycetes	11.427	9.987	10.691	8.742	8.323	8.748	7.370	7.852
	±4.106	±2.068	±4.618	±3.188	±1.861	±3.573	±2.933	±2.200
Nitrospirae	0.234	0.487	0.423	0.433	0.0200	0.078	0.013	0.149
	±0.133	±0.464	±0.286	±0.635	±0.036	±0.174	±0.025	±0.217
Chloroflexi	0.666	1.195	2.656	1.678	0.138	0.189	0.084	0.157
	±0.677	±2.055	±1.554	±1.226	±0.134	±0.143	±0.121	±0.141
Actinobacteria	5.329	5.816	7.671	5.849	6.713	8.275	6.063	6.875
	±1.587	±2.411	±1.532	±1.944	±1.540	±2.931	±1.221	±2.259
Gammaprotobacteria	0.037	0.016	0.027	0.016	0.083	0.083	0.024	0.074
	±0.027	±0.006	±0.021	±0.019	±0.069	±0.043	±0.016	±0.067
Acidimicrobia	3.579	4.038	5.536	4.449	2.937	3.720	2.453	3.309
	±1.304	±2.236	±1.359	±1.624	±1.146	±1.044	±0.677	±0.971

Thermoleophilia	0.940	1.128	1.103	0.967	1.890	2.219	1.587	2.121
	±0.235	±0.367	±0.650	±0.695	±0.647	±0.639	±0.683	±0.892
Holophagae	0.394	0.689	0.296	0.410	0.002	0.010	0.007	0.063
	±0.408	±1.236	±0.178	±0.155	±0.005	±0.018	±0.014	±0.038
Rickettsiales	0.186	0.265	0.068	0.068	0.278	0.372	0.314	0.238
	±0.089	±0.131	±0.062	±0.048	±0.146	±0.186	±0.065	±0.138
Betaprotobacteriales	1.720	1.933	0.471	0.597	2.031	2.205	2.648	1.661
	±0.437	±1.778	±0.573	±0.625	±0.691	±1.193	±2.578	±0.844
Pedasphaerales	3.400	5.224	8.320	8.894	2.364	3.799	3.993	4.048
	±1.890	±1.749	±2.726	±4.968	±1.273	±0.596	±2.103	±1.357
Methylacidiphilales	1.920	1.909	0.818	1.369	2.800	1.603	3.744	1.227
	±1.176	±1.654	±0.509	±1.165	±1.100	±0.794	±2.588	±0.584
Rhizobiales	6.886	7.063	6.947	7.523	5.476	6.346	4.617	5.581
	±1.692	±1.916	±1.731	±1.052	±1.672	±2.317	±1.475	±2.001
Saccharimonadales	0.134	0.154	0.110	0.099	0.306	0.411	0.216	0.261
	±0.064	±0.103	±0.055	±0.061	±0.178	±0.256	±0.117	±0.190
Myxococcales	2.260	2.642	1.586	1.441	1.935	2.786	1.703	3.381

	±0.603	±0.797	±0.601	±0.504	±0.973	±1.067	±0.597	±1.229

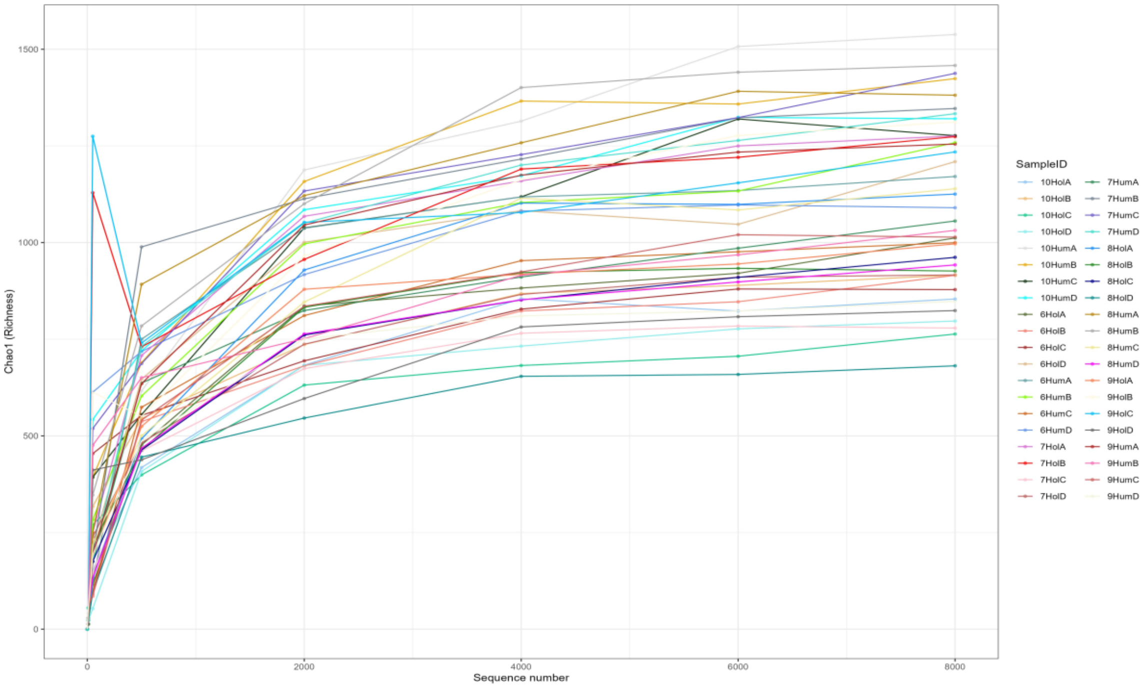

Figure S 0.1 - Richness rarefaction and (b) Shannon index analysis of the different samples

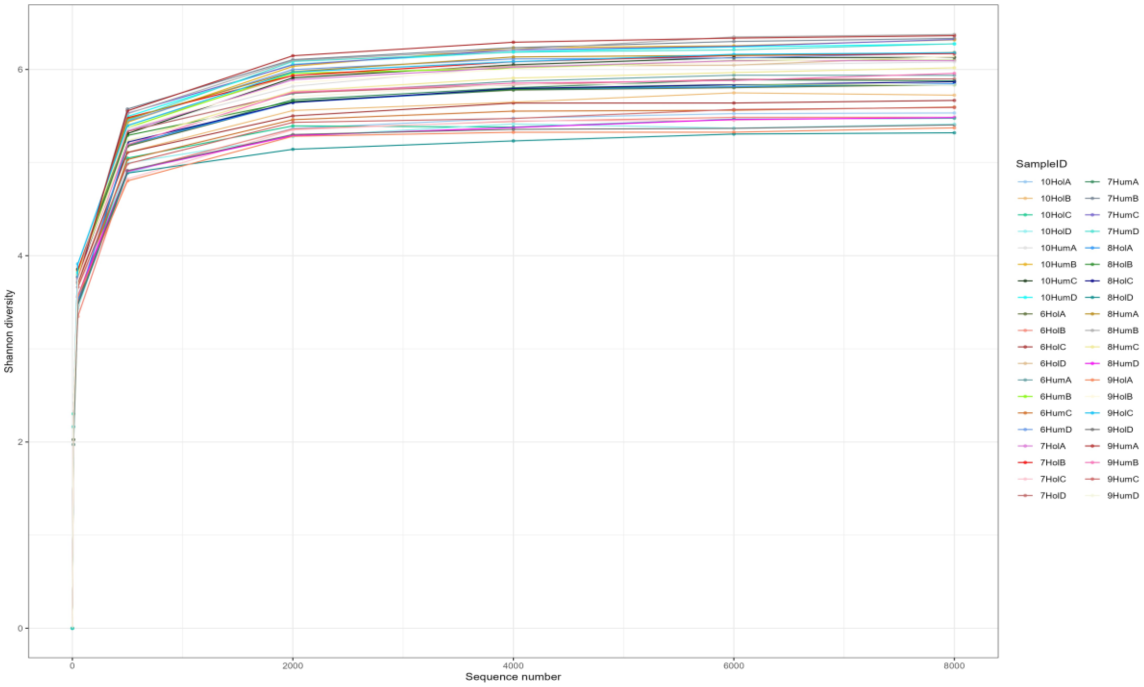

Figure S 0.2 (a) Richness rarefaction and (b) Shannon index analysis of the different samples

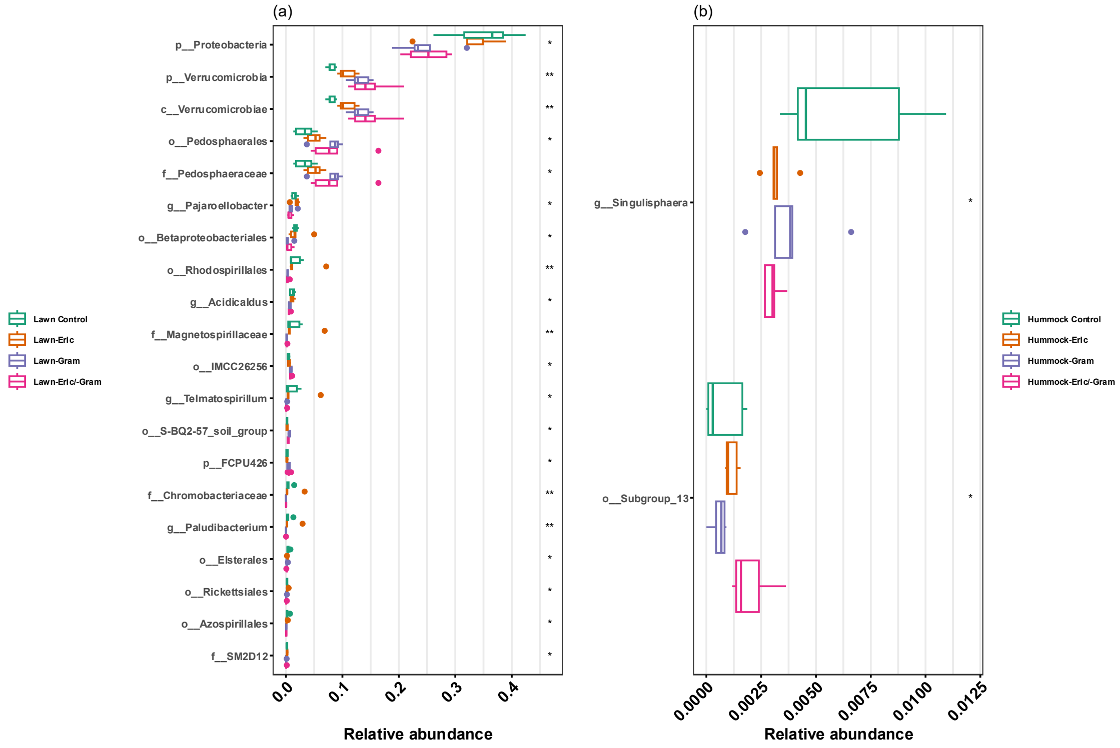

Figure S 2.3 Box plot showing the relative abundance of top 15 indicator prokaryotic groups observed between PFT removal treatments (Control (representing an undisturbed control group), - Gram (indicating the removal of graminoid vegetation), - Eric (denoting the removal of ericoid vegetation), and - Gram / - Eric (signifying the removal of both ericoid and graminoid vegetation) in (a) lawn and (b) hummock microhabitat.

Chapter 3 Peatland plant functional type effects on decomposition factors are non-pervasive, but microhabitat dependent

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Abstract

Ombrotrophic peatlands are important long-term sinks for atmospheric carbon as plant production rates exceed litter decomposition. Changes in plant community composition may alter decomposition rates through alterations in microbial communities and activity. Such plant community driven changes in decomposition rates may however differ between microhabitats. Nevertheless, the microhabitat-context-dependency of plant community composition effects on decomposition remains poorly understood. We used a long-term (>8 years) plant removal experiment to study how vascular PFTs (graminoids and ericoids) influence decomposition processes in wet lawns and hummocks. We employed the Tea Bag Index (TBI) as a general indicator for early litter decomposition and carbon stabilization and assessed the potential activity of five extracellular hydrolytic enzymes as specific indicators for microbial activity. PFT removal had no significant effect on the TBI decomposition rate constant (*k*), nor on the stabilization factor (S). Yet, *k* increased slightly when both PFTs were absent. In the lawns, we observed significantly higher values of *k* and S as compared to hummocks. PFT compostion significanlty influenced four out of five hydrolytic EEAs that can drive decomposition process, however this influence was non-pervasive and depended on microhabitat. In lawns, PFTs removal generally increased enzyme activities, while opposite trends were detected in the hummocks. Our results suggest an important role for vegetation change, through their influence on enzyme activity, along the lawn-hummock gradient in regulating decomposition processes in northern peatlands. Our results highlight it is important to monitor the interplay between plant functional type composition and microhabitat in order to understand decomposition processes in peatlands.

Keywords Decomposition · Extracellular Enzyme Activity · Microhabitat · Peatland · Plant functional types· Tea Bag Index (TBI)

3.1 Introduction

Northern peatlands are important terrestrial carbon (C) stores that for millennia have accumulated non-decomposed plant material as peat (Gallego-Sala et al. 2018) and formed the organic C reservoirs that are currently estimated to hold 600-700Gt of carbon (Yu 2012; Ratcliffe et al. 2021). These values are equivalent to 25-30% of the global soil carbon stock (Gorham 1991). Peatlands are, therefore, key in regulating the global climate and their continued presence is one of the best natural lines of defence against climate change. The peatland C sink function results from the production of decay-resistant plant litter, in combination with low average temperatures and waterlogged conditions that constrain microbial metabolic activity and lead to slow decomposition rates (Yu 2012). Currently, peatlands are undergoing rapid changes in enviro-climatic conditions that puts pressure on the ecological processes supporting their C sink function (Gallego-Sala et al. 2018; Swindles et al. 2019). To anticipate the impact of global change on peatland C dynamics it is essential we understand what drives the decomposition process.

Ombrotrophic bogs often display distinct patterns in microhabitats that differ in their position along the water table: hummocks and wet lawns (sometimes also referred to as carpets). Hummocks are raised mounds where the peatmoss surface is relatively far from the water table. Wet lawns are microhabitats that are situated closer to, and often move with, the water table (Rydin and Jeglum 2013). Moreover, the distance to the water table is known to influence decomposition (Górecki et al. 2021; Wang et al. 2021b) and has been reported to greatly influence the microbial activity (Fisk et al. 2003; Jassey et al. 2018). Apart from being positioned differently along the water table, microhabitats differ in biotic community composition. Moreover, hummocks and wet lawns are each dominated by a distinct community of *Sphagnum* mosses. The vascular plant community is comprised of two functional types, graminoids and ericoids, that differ in their mechanisms for nutrient acquirement (Kaštovská et al. 2018). Although both PFT types are found in both microhabitats, graminoids – which produce relatively high-quality litter – are prevalent in the wetter lawns, where they are associated with faster decomposition rates. Also, many of the graminoids in peatlands possess aerenchyma, sponge-like tissue forming open air canals in stem and roots, that promote the diffusion of oxygen to the roots and activates mineralization in anoxic peat through changes in microbial extracellular enzyme activity (EEA) (Bragazza et al. 2015; Robroek et al. 2015). In contrast, the drier hummocks are dominated by ericaceous shrubs that often form a symbiotic association with ericoid mycorrhizal fungi. These species produce low quality litter, rich in recalcitrant components, which has been linked to inhibition of decomposition processes and associated C loss (Gadgil and Gadgil 1971; Wang et al. 2021a). It is well established that plant functional types can have considerable effects on decomposition processes (Johnson and Damman 1991; Ward et al. 2015; Zeh et al. 2020; Mastný et al. 2021). Moreover, these vascular plant types shape belowground microbial communities and associated EEA (Robroek et al. 2015; Parvin et al. 2018) by providing different quality and quantity of litter (Hobbie 1992; Gartner & Cardon 2004; Handa et al. 2014) and various exudates released from roots (Bais et al. 2006; Kardol et al. 2010; Mastný et al. 2021). These litter deposits and root exudates serve as microbial substrates and stimulate microbial EEA and respiration with subsequent effects on decomposer community composition and consequential C cycling (van der Heijden et al. 2008; De Deyn et al. 2008; Wiedermann et al. 2017).

Decomposition in peatlands is largely driven by soil microbial activity (Fenner et al. 2005; Preston et al. 2012; Briones et al. 2022). In peatlands, there is clear evidence that microbial community composition and activity is strongly dependent on abiotic conditions, including water table depth (Juszczak et al. 2013; Robroek et al. 2015; Jassey et al. 2018; Asemaninejad et al. 2019; Lamit et al. 2021). In addition to directs effect of abiotic condition of decomposition rates, the latter can be affected indirectly through the composition of the plant community (Andersen et al. 2013; Ritson et al. 2021). Relationships between plant and microbial communities, in addition, have been shown to change drastically along enviro-climatic gradients and, often leading to fundamental shift in their interaction dynamics (Robroek et al. 2021). Hence, enviro-climatic change is expected to have unprecedented impact on microbial community composition and activity and can potentially convert peatlands as global carbon sinks to sources of greenhouse gasses (Loisel et al. 2021).

Whether northern peatlands will remain to act as C sinks or become C sources depends on the extent to which peatland plant communities and biological interactions respond to enviro-climatic change. Despite the recognized influence of PFTs and microhabitat on decomposition (Ward et al. 2015), their interactive effects are still unclear. What is more, warmer and drier conditions increase the abundance of graminoids and ericaceous shrubs in peatlands (Walker et al. 2006; Breeuwer et al. 2010; Antala et al. 2022), also belowground (Malhotra et al. 2020). To date, much research has been undertaken on the direct effects of global climate change on carbon dynamics in peatland. How abiotic and biotic changes coalesce to affect peatland processes such as decomposition remains poorly understood. It has been recently highlighted that widespread drying as a result of climate warming can have alarming consequences on the peat C stock (Swindles et al. 2019). Therefore, studying the contribution of PFT on decomposition in the context of microhabitats provide much needed insights in peatland carbon dynamics in the light of a warmer and drier future climate.

Here, we investigate how alterations in vascular plant functional types in an ombrotrophic bog influence the decomposition across two contrasting microhabitats in peatlands: hummocks and wet lawns. This work aims to address two specific objectives: to investigate the relative and interactive effect of vascular plant functional types and microhabitat on i) early decomposition by incubating standard substrates; and more specifically on ii) microbial activity by measuring the activity of five hydrolytic extracellular enzymes. We hypothesized that PFT composition and microhabitat affect decomposition and microbial activity. We postulate lawns to have higher hydrolytic enzyme activity compared to hummocks, which translates into increased decomposition rate but lower levels of stabilization of labile organic compounds. In addition, we expected that removal of graminoids and ericoids decreases microbial activity which leads to a decreased decomposition rate but increases organic C stabilization.

3.1 Materials and Methods

3.1.1 Study area and experimental design

This work has been performed in the Store Mosse National Park (57°17’54 N, 14°00’39 E), the largest peatland complex in the south of Sweden and representative of ombrotrophic peatlands in the nemo-boreal zone. Specifically, in 2011 we established a vascular plant removal experiment in a *Sphagnum*-dominated ombrotrophic bog comprising 80 experimental plots of 0.5 × 0.5 m (c.f. Robroek et al. 2015) that where equally divided over wet lawns (c.f. Rydin & Jeglum 2013; n = 40) and hummocks (n = 40). The bryophyte layer in the wet lawns was dominated by *Sphagnum cuspidatum* Ehrh. ex Hoffm. with sparse cover of *S. balticum* (Russow) C.E.O. Jensen, while the hummocks were largely covered by *S. medium* Limpr. and *S. rubellum* Wilson. The vascular plant cover in the wet lawns consisted of *Eriophorum vaginatum* L.*, Trichophorum cespitosum* (L.) Hartm., *Rhynchospora alba* (L.) Vahl., *Vaccinium oxycocccos* L., *Erica tetralix* L. and *Andromeda polifolia* L. The hummock vascular plant community mainly consisted of *E. vaginatum* L., *Erica tetralix*, *Vaccinium oxycoccos*, *Calluna vulgaris* (L.) Hull and *A. polifolia*. Water tables in the wet lawns were close to (i.e. 1-3 cm below) the *Sphagnum* surface. Water table depth in these microhabitats is relatively stable throughout the year – even in dry periods as the *Sphagnum* surface moves with apparent water table fluctuations. The water table in the hummocks is variable between 20 cm and 35 cm below the *Sphagnum* surface.

In each microhabitat (wet lawn and hummock) four plant functional group removal treatments – undisturbed control, graminoids removed (– Gram), ericoids removed (– Eric), ericoids + graminoids removed (– Gram / – Eric) – were established by selectively clipping aboveground vegetation flush to the *Sphagnum* layer. Regrowth (roots included) was removed at least twice per year since the start of the treatments. The experiment is laid out in a randomized block design, with all treatments replicated ten times within block (4 PFT communities × 2 microhabitats × 10 blocks). This method allowed us to evaluate the influence of plant functional types on below ground ecological *in situ* (Díaz et al. 2003). During the summer of 2019, preceding the installation of standardized litter for this experiment (see below), we estimated the cover (%) for the vascular plant and *Sphagnum* community on a subset – i.e. 40 PFT removal plots (4 treatments x 2 microhabitat x 5 replicates) – using the pinpoint intercept method (Jonasson 1988) with a 100-point frame. At every point, a needle was lowered to the *Sphagnum* surface and all contacts with vascular plants were recorded, specifying taxonomic identity for each hit. Every point ended at the *Sphagnum* layer, resulting in each grid point of the pin-point frame to account for one individual of a certain *Sphagnum* species. Results from these surveys highlight that the PFT removal treatments were successful in creating distinct plant community compositions in the experimental plots (Supplementary Information Fig. S1). Noteworthy is that the natural vascular plant cover, hence the cover in the control plots, was twice as high in the hummocks (66%) as compared to the lawns (31%) ($F_{1,8} = 25.31$, $P < 0.001$), primarily caused by the higher ericoid species abundance in the hummock plots. Consequently, the removal of ericoids or graminoids played out differently for the total vascular plant cover in hummocks and lawns (Fig. S1).

3.1.2 Decomposition rate constant (*k*) and stabilization factor (S)

We used the Tea Bag Index (TBI) method to estimate the role of vascular plant functional types and microhabitat on early decomposition and organic matter stabilization in the peat. The Tea Bag Index (TBI) method employs commercially available green tea (EAN 8722700 05552) and rooibos tea (EAN 8722700 188438), which possess distinct carbon fractions. Green tea, characterized by a high labile carbon content, decomposes more rapidly, whereas rooibos tea, with a greater proportion of recalcitrant carbon, decomposes at a slower rate. This contrast facilitates the evaluation of decomposition rates and carbon stability in soils (Keuskamp et al. 2013). The TBI method is currently widely applied (see also http://www.teatime4science.org) and has been proven to be suitable as a standard methods to study the .influence of environmental drivers on decomposition processes, as the tea and local litters were found to behave comparably (Didion et al. 2016; Macdonald et al. 2018; Duddigan et al. 2020). In July 2019, we buried a pair of tea bags (one green and one rooibos tea bag, accordingly labelled) in all plots. Tea bags were inserted vertically 10 cm apart and at a depth of *c.* 8 cm. The tea bags were recovered in September 2019 after an incubation time of 76 days. After initial air-drying, tea bags were oven-dried (48h at 60°C) in the laboratory, after which, adhered peat and roots were removed. Tea bags were then dried again, and the remaining tea was weighed. Initial weight of both types of tea bags was taken as the average of ten tea bags that were weighed for that purpose only.

We estimated the rate of early decomposition as constant *k* and the stabilization factor S as following Keuskamp et al. (2013). While an estimation of *k* would require a time series, the TBI calculations make use of the contrasting litter quality of green tea and rooibos tea and are based on the two-step decomposition model by Wieder and Lang (1982), which assumes that labile compounds decompose faster than recalcitrant fractions. After two to three months incubation, the faster decomposing green tea will have lost its labile fraction, while in rooibos tea labile compounds are still being consumed. Based on the green tea mass loss S is calculated as:

$$S = 1 - a_g / H_g \qquad \text{(Equation 1)}$$

S (Eq. 1) is defined by the ratio of actual decomposable fraction of green tea litter lost during incubation (a_g) to the expected fraction, namely the hydrolysable fraction (H_g = 0.842 g g^{-1}) (Keuskamp et al. 2013). Hence, high values of S are thought to indicate a larger storage capacity of organic C attributable to local conditions (Fujii et al. 2017; Macdonald et al. 2018). Once S is determined, Eq. 2 can be used to determine the decomposable fraction of rooibos tea (a_r) using the chemically determined hydrolysable fraction of rooibos tea (H_r = 0.552 g g^{-1}) (Keuskamp et al. 2013):

$$a_r = H_r\,(1 - S) \qquad \text{(Equation 2)}$$

Assuming that the weight loss of the recalcitrant litter fraction during the incubation period is negligible (Berg and Meentemeyer 2002), *k* can be calculated as:

$$k = ln\,\frac{\frac{a_r}{W_r - (1 - a_r)}}{t} \qquad \text{(Equation 3)}$$

Where, W_r is the fraction of rooibos tea remaining, t denotes incubation time (days). Thus, the final *k* value is an estimate of the early decomposition rate (day^{-1}).

3.1.3 Hydrolytic enzyme activity

The activity of the decomposer community has a large influence on the decomposition of peat material (Preston et al. 2012). Therefore, we measured the activity of five hydrolytic enzymes (Table 3.1) in the rooting zone (0-15 cm) of 40 plots (4 treatments x 2 microhabitats x 5 replicates) following Jassey et al. (2011). In brief, 3 g homogenized fresh peat was added to 50 mL 0.1 M $CaCl_2$ solution with 0.05 % Tween 80 and 20 g polyvinylpolypyrrolidone and shaking at room temperature on shaker for 90 mins with 150 rpm. The mixture was centrifuged at 10,000 rpm for 5 mins at 4°C and the supernatant was filtered using Whatman GF/C of 1.2 µm. Next, the filtrate was poured into a cellulose dialysis tube of 10-12kDa molecular mass and then concentrated using polyethylene glycol. The concentrated solution was added to 10mL of phosphate buffer (pH 5.6) and divided into two equal aliquots. One aliquot – active enzyme extract – was stored at 4℃ overnight, while the other aliquot – inactivated enzyme extract – was boiled for 3 h at 90℃. For each sample, four technical-replicates assay wells (using opaque 96-well micro-plates) received 38 µl of enzyme extract and 250 µl of substrate (Table 3.1). As a control, the same procedure was followed but with 38 µl inactivated enzyme extract. Incubation was performed in the dark at 25℃ for 3h, after which the reactions were halted with 1 µl 0.5 M NaOH. Fluorescence intensity was measured spectrophotometrically at 365 nm excitation wavelength and 450 nm emission wavelength (BMG LABTECH Omega multidetector plate reader). Potential activity of hydrolytic enzymes was expressed as nmol of MUF/MUC (4-methylumbelliferyl (MUF)/ 4-methylumbelliferyl-β-D-cellobioside (MUC) released per gram of dry soil per hour (nmol g^{-1} h^{-1}).

Table 3.1 Description of the peat extracellular enzymes, the substrates labelled with fluorophore methylcoumarin (MUC) or methylumbelliferone (MUB) used for the hydrolytic enzyme activity measurements.

Enzyme	Abbr.	Substrate	Hydrolysis type	Targets
Alanine-aminopeptidase	ALA	L-Alanine7-amido-4-MUC	N-acquisition	Oligopeptides
β-glucosidase	BG	β-D-glucoside-4-MUC	C-acquisition	Cellulose, starch and disaccharides
β-glucosaminidase	NAG	N-acetyl-β-D-glucosaminide-4-MUB	N-acquisition	Chitin
Acid phosphomonoesterase	PHOS	Phosphate-4-MUC	P-acquisition	Organic phosphorus
Sulfatase	SUL	Sulphate-4-MUB	S-acquisition	Organic sulphur

3.1.4 Data analysis

Difference in vascular plant cover between wet lawns and hummocks was assessed by fitting a linear model with generalized least squares (gls) on the data from the control plots, using microhabitat as a fixed factor, and after testing for block effects. The addition of block as a random factor was not significant ($P > 0.05$) in any of the models and therefore not included in downstream models. The effects of the PFT removal treatment, microhabitat and their interaction on the early decomposition rate (*k*), labile carbon stabilization (S), and the activity of five hydrolytic enzymes (ALA, BG, NAG, PHOS, SUL) were tested by fitting gls models. Heterogeneity across PFT removal treatment and microhabitat in the *k* data was accounted for by using a VarComb variance structure in the model. All models were fitted with restricted maximum likelihood estimation (REML) and following the protocols outlined in (Zuur et al. (2009). Residuals of the final model were analyzed for normality and homogeneity, with a Kolmogorov-Smirnov test and Levene's test. All statistical analyses and visualizations were performed in the R software environment for statistical computing and graphics (version 4.1.2.)

3.2 Results

3.2.1 Decomposition rate constant and stabilization factor

Mass loss of the two types of litter (green tea and rooibos tea) differed between the wet lawn and hummock microhabitats ($P < 0.05$). The green tea in the hummocks lost 71.6% ± 0.04 (mean ± SD) which was higher ($F_{1,72} = 30.59$, $P < 0.001$) than the 64.1% ± 0.07 mass loss in the lawns. As expected, the mass loss from the rooibos tea bags was lower, but not significantly different ($F_{1,72} = 3.42$, $P = 0.068$), in the wet lawns (20.1% ± 0.03) as compared to the hummocks (21.4% ±0.03). We found no effect of PFT removal treatments on the mass loss of green tea ($F_{3,72} = 0.99$, $P = 0.403$) or rooibos tea ($F_{3,72} = 0.35$, $P = 0.786$).

The decomposition rate constant *k* was higher in lawns as compared to *k*-values in the hummock microhabitats ($F_{1,72} = 4.55$, $P = 0.036$). PFT removal did not influence *k*, neither as an overall effect ($F_{3,72} = 1.77$, $P = 0.160$) nor in interaction with microhabitat ($F_{3,72} = 0.24$, $P = 0.864$). Despite the non-significant PFT treatment results, *k* appeared to increase with the combined removal of graminoids and ericoids (Fig. 1; – Gram / – Eric). The stabilization factor S, that is the potential of the labile fraction of the green tea litter to become stabilized, was higher in lawns than hummocks ($F_{1,72} = 30.59$, $P < 0.001$). However, no effect of PFT removal on S was observed, neither as overall effect ($F_{3,71}$ = 0.99, P = 0.403) nor in interaction with microhabitat ($F_{3,72}$ = 0.79, P = 0.501). Nevertheless, S tended to slightly increase in the absence of vascular plants (– Gram / – Eric).

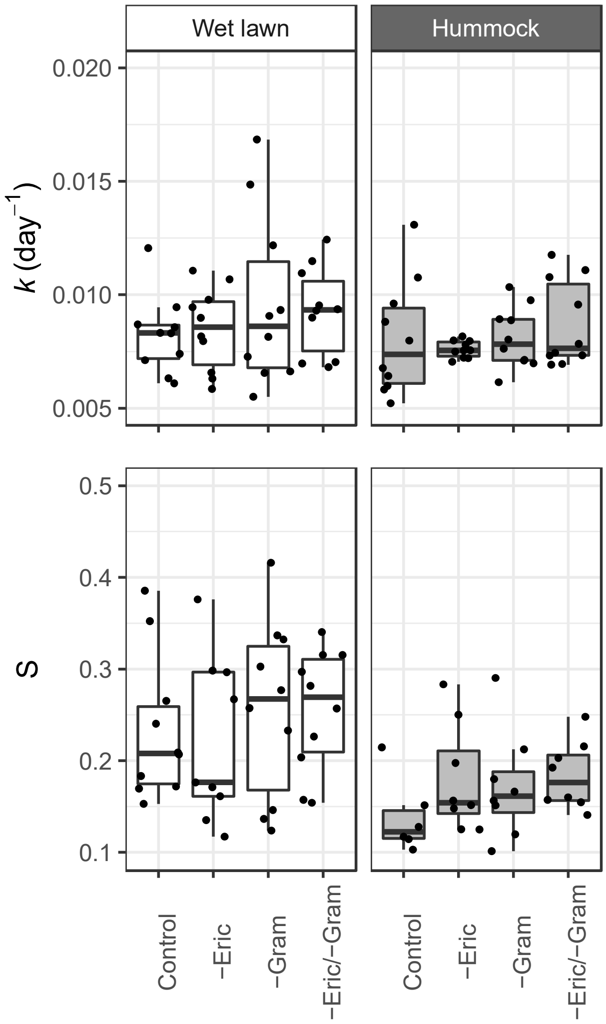

Figure 3.1 Boxplots of the effects of vascular plant removal treatments and microhabitat (white panels = wet lawns, grey panels = hummocks) on decomposition rate constant (*k* [d^{-1}]) and organic matter stabilization factor (S), derived from mass loss data according to equations 1-3 (n = 10). Whiskers in each box plot represent mean± SE. Control = undisturbed control, – Gram = graminoids removed, – Eric = ericoids removed, – Gram / – Eric = ericoids + graminoids removed. Outputs for statistics are presented in text.

3.2.2 Hydrolytic enzymatic activity

To assess the relative and interactive effect of microhabitats and vascular PFT treatments on belowground enzyme activities, five hydrolytic EEA were measured. The hydrolytic enzyme activity of alanine-aminopeptidase (ALA), β-glucosidase (BG), and acid phosphomonoesterase (PHOS) not to be affected by microhabitat. On the other hand, β-glucosaminidase (NAG) activity was higher in lawns compared to hummocks, while sulfatase (SUL) activity was highest in the hummocks (Table 3.2, Fig. 3.2, Table S1). PFT removal treatment did not affect ALA activity, but the activities of the other enzymes did vary significantly between PFT removal treatments and were microhabitat dependent ($P < 0.05$, Table 3.2, Fig. 3.2). In lawns, the removal of all vascular PFTs (– Gram / – Eric) resulted in an increase in BG (22%), NAG (13%), PHOS (77%) and SUL (26%) activities compared to the control, while in the hummocks this resulted in a decrease in activities of BG (50%), NAG (46%), PHOS (30%) and SUL (48%) (Table S1). NAG and SUL activity in the lawns were lowest when only graminoids were removed (Fig. 3.2).

Table 3.2 Statistical analysis from the ANOVA, testing the influence of vascular plant functional type (PFT) removal treatment and microhabitat (MH) on the hydrolytic enzymes alanine-aminopeptidase (ALA), β-glucosidase (BG), β-glucosaminidase (NAG), acid phosphomonoestesterase (PHOS) and sulfatase (SUL). Significant *P-values* ($P \le 0.05$) are shown in bold values (n = 5).

Variables		ALA		BG		NAG		PHOS		SUL	
	d.f.	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
PFT	3	0.02	1.00	16.13	≤ **0.001**	210.3	≤ **0.001**	16.50	< **0.001**	13.54	≤ **0.001**
MH	1	0.90	0.35	2.88	0.10	1159.8	≤ **0.001**	0.00	0.97	148.42	≤ **0.001**
PFT: MH	3	1.03	0.39	84.53	≤ **0.001**	355.3	≤ **0.001**	69.85	< **0.001**	45.32	≤ **0.001**

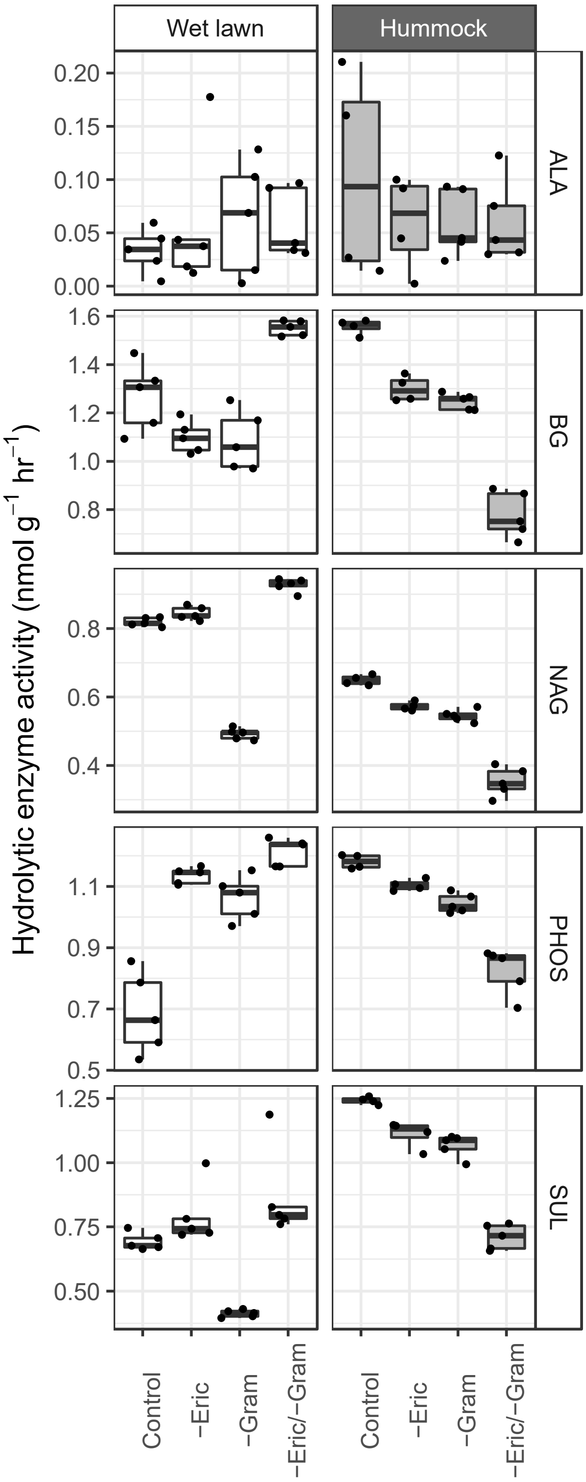

Figure 3.2 Boxplots of the effects of vascular plant removal treatments and microhabitat (white panels = wet lawns, grey panels = hummocks) on the hydrolase activity of the enzymes alanine-aminopeptidase (ALA), β-glucosidase (BG), β-glucosaminidase (NAG), acid phosphomonoesterase (PHOS) and sulfatase (SUL) (n = 5). Whiskers in each box plot represent mean± SE. Outputs for statistic are presented in Table 3.2.

3.3 Discussion

Peatland ecosystems face changes in enviro-climatic conditions that may evoke shifts in the vegetation. While knowledge on the role of plant functional types (PFTs) on peatland processes is mounting (Ward et al. 2009; Lang et al. 2009; Rupp et al. 2019; Chroňáková et al. 2019), their influence in the context the microtopography in peatlands is less well understood. Here, we studied how plant functional types (graminoids and ericaceous shrubs) influence decomposition in two contrasting microhabitats (wet lawns and hummocks). Our results demonstrate that PFTs greatly influence microbial metabolic processes (i.e. hydrolytic enzyme activity), and that this effect is microhabitat dependent. Despite these effects of PFTs on potential process rates, this result was not mirrored in standardized indices for early decomposition (decomposition rate constant *k* and stabilization factor S), which only differed between lawns and hummocks.

3.3.1 Effects on decomposition rate constant and stabilization factor

The lack of response of indices *k* and S to the plant removal treatments goes against our expectations. Yet, the values for *k* (0.008 – 0.010 day^{-1}) and S (0.118 – 0.256) we found fall in the range reported by other studies (Keuskamp et al. 2013; Macdonald et al. 2018; Górecki et al. 2021). Previous studies have documented the role of the plant community composition as well as spatial variation in microhabitats on belowground decomposition processes (Dorrepaal 2007; Laiho 2006; Mäkilä et al. 2018; Ward et al. 2010, 2015). Also, previous research outputs concluded that vegetation composition was the main driver for decomposition processes and C flux in peatlands (Basiliko et al. 2012; Linkosalmi et al. 2015). Shifts in the microbial community in response to the PFT removal treatments found earlier at the same experimental site (Robroek et al. 2015) make microbial adaptation a likely mechanism. While the responses of the enzymatic activity support this (as discussed below), our TBI results do not. Djukic et al. (2018) propose that microbial influence on decomposition may only become apparent in later stages of decomposition, during which more specialized microbes are responsible for the break-down of the recalcitrant compounds. This argument is supported by observations of Lin et al. (2020) who point out that microbial driven differences in decomposition, home-field advantage specifically, vary with incubation time and are stronger at later decomposition stages. Additionally, the TBI method relies on a standard substrate which is foreign in most ecosystems. While several studies conclude that the TBI method is suitable for replacing local litter for detecting responses to general decomposition drivers, such as temperature and precipitation (Didion et al. 2016; MacDonald et al. 2018;Duddigan et al. 2020), it may fail to pick-up local adaptation of the microbial community to specific litter inputs.

In our study, we found a significant influence of hummock-wet lawn microhabitat on the decomposition rate constant and stabilization factor. According to Keuskamp et al. (2013) and Fanin et al. (2020), *k* indicates early decomposition rates while S shows the stabilizing effect of the environment on the labile fraction of the litter. Both *k* and S values were significantly higher in lawns compared to hummocks. The combination of a higher *k* and S in lawns could indicate that high mass loss rates of green and rooibos tea bags fast coincide with incomplete break-down, during early decomposition and a higher potential for labile carbon to become stabilized within the ecosystem. Temperature and soil moisture are known to promote decomposition of green tea and rooibos tea (Fanin et al. 2020). At the experimental site, lawns are warmer than hummocks (Robroek et al. 2014), and the water table is closer to the surface. Earlier decomposition studies in peatlands recorded highest decomposition rates of litter placed in or just above the zone with fluctuating water levels (Belyea 1996), which explains the higher *k* values found in the lawns. Moreover, a higher proportion of fungal biomass to bacterial biomass in hummocks (Robroek et al. 2014) may result in a more complete break-down of litter and explain the higher S values, indicative of incomplete break-down, found in lawns. Further research on the decomposer community and chemistry during decomposition would be needed to confirm this.

3.3.2 Effects on extracellular enzyme activity

The role of the vegetation on belowground microbial community structure and metabolic processes can result from established plant–microbe associations (Robroek et al. 2015). In addition, there are several mechanisms, such as rhizodeposition and rhizosphere oxygenation, which can directly influence ecosystem functioning (Wardle et al. 2004). Interestingly, in line with our hypothesis, altering vascular plant functional composition significantly influenced belowground potential EEAs in contrasting microhabitats for four out of five hydrolytic enzymes. Previous findings from the same experiment demonstrated that removal of PFT was associated with distinct microbial community composition in different microhabitats (Robroek et al. 2015). Moreover, Basiliko et al. (2013) and Matulich and Martiny (2015) link a change in microbial community composition to a shift in the activity of EEAs. The observed changes in EEAs under different PFT removal treatments in wet lawn-hummock microhabitats may thus have been mediated by shifts in microbial community composition.

The influence of vascular PFTs on hydrolase activity showed opposite effects in the two microhabitats. In hummocks, the removal of PFTs decreased hydrolytic enzyme activity; however, in lawns PFT removal increased it, reflecting perhaps the direct effect of plant litter and rhizosphere inputs (or absence thereof). Earlier observations demonstrated lower overall potential microbial activity in hummocks than in lawns, while PFT removal treatment effects were only observed in hummocks (Robroek et al. 2016). It was suggested by these authors that the higher vascular plant cover in hummocks may have resulted in a higher dependency of the microbial community on plant-derived substrates. Indeed, this may play a role in our observations as vascular plant cover in hummocks are twice as high as compared to lawns, with a more pronounced influence on belowground hydrolytic enzyme activity in hummocks. As hummock's vascular plant cover enhanced the hydrolase activity of four out of five enzymes, this shows that microbial EEAs were greatly influenced by vegetation inputs (labile rhizosphere inputs) as well as distinct microhabitats. It has been shown already that aerobic microbial respiration is faster as compared to anaerobic microbial respiration, that requires a higher degree of microbial metabolic processes (potential EEAs) to fuel this aerobic respiration in oxic hummocks (Freeman et al. 2001; Blodau et al. 2004; Jungkunst et al. 2012). In lawns, the removal of all vascular PFTs generally resulted in an increase in EEA. In other words, the presence of vascular plants seems to restrict belowground potential microbial EEAs. Previous research has shown that in hummocks with aerobic conditions, rhizosphere PFT inputs are essential source of substrate and metabolic energy for hydrolytic enzyme activities (Dieleman et al. 2017) . However, in lawns, microbial activity is largely restricted by adverse environmental conditions (Fisk et al. 2003) The combined removal of graminoids and ericoids plants increased hydrolase activity, which may be caused by the absence of shrub-derived phenolics (Wang et al. 2021b). Interestingly, the removal of graminoids alone had a larger negative effect on hydrolytic enzyme activity in the wet lawns, particularly in NAG and SUL activities. As wet lawns are mostly dominated by the graminoid *Eriophorum vaginatum*, which possess aerenchymatic tissue (open air canals in stem and roots), this promotes the diffusion of oxygen to deep roots (Greenup et al. 2000). Absence of graminoids may therefore decrease microbial metabolism due to reduced peat oxygenation, this being more pronounced in the lawn microhabitat.

We found clear differences between microhabitats in the activities of hydrolytic enzymes, which is consistent with other wetland studies (Parvin et al. 2018; Minick et al. 2019). Two out of five EEAs (NAG and SUL) showed significant difference in activities between hummock and lawns. Previous research has reported that drier hummocks had higher activity of NAG compared to wet lawns (Wang et al. 2021b). In addition, Xu et al. (2021) reported that NAG activity was significantly higher in the aerated zone of drained peat as compared to activities in the water-saturated zone. Contradicting these studies, we found that NAG activity was greater in lawn microhabitats, which are closer to the water table. This may be explained by as lawns being extraordinarily dry during the warm summer of 2019 which might improve the peat aeration (increased oxygen diffusion) by decreasing water table level, resulting in enhanced NAG activity, as microbial necromass is rapidly mineralized by the extant microbial community under dry conditions. Furthermore, lawns are usually abundant in aerenchymous graminoids that transport oxygen into deep peat, which might enhance the metabolic efficiency of rhizosphere microbial communities. In contrast, we also observed that SUL activity was higher in hummocks than in lawns. It has been reported that sulphatase activity was stimulated due to enhanced nutrient mineralization upon lowering the water table drawdown. Furthermore, the vascular plant cover in hummocks was more than twice as high as that in lawns, which is likely reflected belowground and, may in-turn have increased hydrolytic activity. In addition, drier hummocks are usually nutrient poor environments due to dominance of recalcitrant shrubs. In order to meet the nutrient demands, soil microbes might produce more hydrolytic enzymes towards internal cues of nutrition stoichiometry (Allison and Vitousek 2004).

3.4 Conclusions

In response to global climate warming, vascular plant cover is expected to increase (Elmendorf et al. 2012). We highlight that the role of plant functional type composition is important for belowground decomposition processes through their impact on enzyme activity along with microhabitats. Our results indicate that vascular plants control microbial activity in peat with specific roles of plant functional types varying between lawns and hummocks. Moreover, microhabitat controls over the decomposition process were more pronounced as compared to that of the vegetation. This shows that carbon turn-over in peatland ecosystems is vulnerable to changes in aboveground plant communities and hydrological conditions. Our results emphasize the need to re-focus on carbon dynamics of peatland ecosystem in the light of climate change, and particularity the role of changes in the plant community composition therein.

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Supplementary Information (SI)

Table S 3.1 Mean (± SD) for decomposition rate constant k, stabilization factor S and five hydrolytic enzymes (nmol g−1 h−1) for two microhabitats (lawns and hummocks) and different plant functional type removal treatments (control (C), ericoid removal (-Eric), graminoid removal (-Gram), ericoid and graminoid removal (-E/-G).

Microhabitat		Lawn				Hummock			
Treatment		C	-Eric	-Gram	-E/-G	C	-Eric	-Gram	-E/-G
k (d^{-1})	Mean	0.0082	0.0085	0.0096	0.0093	0.0080	0.0076	0.0081	0.0087
	SD	(0.002)	(0.002)	(0.004)	(0.002)	(0.003)	(0.000)	(0.001)	(0.002)
S	Mean	0.2337	0.2098	0.2561	0.2548	0.1176	0.1609	0.1558	0.1628
	SD	(0.079)	(0.093)	(0.097)	(0.067)	(0.041)	(0.065)	(0.062)	(0.055)
ALA	Mean	0.0333	0.0579	0.0635	0.0589	0.1029	0.0597	0.0590	0.0606
	SD	(0.021)	(0.068)	(0.054)	(0.033)	(0.097)	(0.045)	(0.031)	(0.039)
BG	Mean	1.2677	1.0991	1.0858	1.5509	1.5563	1.2996	1.2474	0.7778
	SD	(0.142)	(0.066)	(0.123)	(0.031)	(0.031)	(0.053)	(0.033)	(0.095)
NAG	Mean	0.8189	0.8442	0.4922	0.9269	0.6492	0.5727	0.5452	0.3522
	SD	(0.013)	(0.019)	(0.016)	(0.020)	(0.014)	(0.013)	(0.018)	(0.042)
PHO	Mean	0.6862	1.1352	1.0627	1.213	1.1809	1.1035	1.0443	0.8230
	SD	(0.134)	(0.026)	(0.073)	(0.045)	(0.023)	(0.018)	(0.031)	(0.076)
SUL	Mean	0.6931	0.7939	0.4130	0.8709	1.2419	1.1107	1.0660	0.7113
	SD	(0.034)	(0.116)	(0.014)	(0.179)	(0.014)	(0.053)	(0.044)	(0.049)

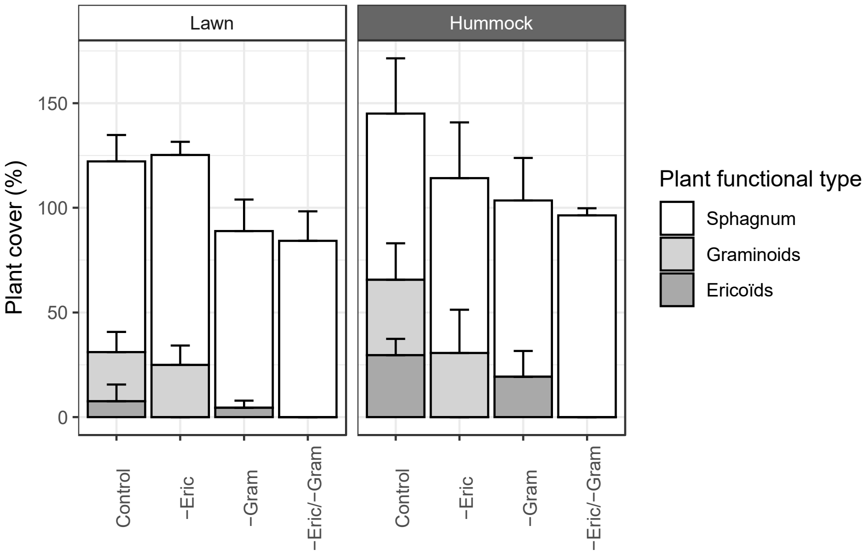


Figure S 3.1 Peat moss (Sphagnum spp.) and vascular plant cover (% + st. dev.) of different plant functional types in four plant removal treatments in two microhabitats (lawns and hummock). Percentages of other species (*Rubus chamaemorus*, *Pinus sylvestris*, *Drosera rotundifolia*, *D. anglica*, and *Betula nana*) are not plotted as their cover did never exceed 1.5% in all treatment plots.

Chapter 4 Response of prokaryotic community to fire severities across peat depth.

4.1 Abstract

Northern peatlands represent a large amount of sequestered carbon (C). However, peatland carbon pools are extremely vulnerable to climate change, including wildfires, which can reshape the plant and microbial communities, with long term impacts on ecosystem processes and functioning. The intensity of wildfire under rapid changes in enviro-climatic conditions is likely to increase in peat bogs with massive effects on ecosystem recovery, soil microbiota and associated biogeochemical processes. Though various studies have documented the altered wildfire regimes on aboveground vegetation, less attention has been paid to immediate effects and recovery of microbial community structure and diversity in peatlands despite their central role in controlling belowground C and deriving nutrient cycling in peatlands. Using 16S rRNA amplicon sequencing, this study examined prokaryotic diversity, community composition and population abundances across depth (near surface and subsurface) under shallow burn and deep burn severity over time post fire in Stalybridge moor, Northwest United Kingdom (UK). The results indicated the prokaryotic alpha diversity was constant across peat depth. Under shallow burn severity, prokaryotic diversity was found to increase at near surface and deep surface over time while the opposite trend was observed with deep burn severity in comparison to control (unburned treatment). Time since shallow burn severity was the primary driver of prokaryotic community composition at near-surface soil depth while deep burn severity affected community composition at the sub-surface soil depth. Hence, the findings demonstrate that the effect of wildfire on bacterial and archaeal community structure can last up to at least 14 months, which indicates that the significant influence of wildfire on prokaryotic communities. Therefore, monitoring long-term belowground microbial communities is crucial for understanding the restoration and maintenance of peatlands.

4.2 Introduction

Natural peatlands are major terrestrial for carbon storage that has accumulated during the Holocene. Currently, it is estimated that northern peatland ecosystems store approximately around 500-1000 GtC of carbon (Turunen et al. 2002; Yu 2012; Nichols & Peteet 2019), largely in the form of partially decomposed plant litter. Due to their capacity to sequester large amounts of carbon, peatlands are a natural line of protection against global warming. The carbon storing function of peatlands is however fragile and are becoming susceptible to wildfires. An overreaching concern is that unlike drier habitats, projected periods of increased summer temperatures, and associated drought events increase the risk of wildfire (Turetsky et al. 2015). This is especially the case in many upland peatlands in the United Kingdom, as historically they have been ditched and drained for agriculture, making them increasingly susceptible for wildfire. The effects of these wildfire events are complex. Furthermore, the effects on the C store can be immediate, as peat deposits are combusted (Turetsky et al. 2011; Turetsky et al. 2015; Davies et al. 2016), and long-lasting through to eradication of aboveground plant communities and shifts in belowground microbial communities (Heisler et al. 2004; Campbell et al. 2008).

Wildfires can burn at high temperatures and severely affect the functioning of peatlands. First immediately apparent, fire eradicates the plant community and leaves the peat barren, resulting in the loss of the capacity to sequester atmospheric carbon. Additionally, the loss of vegetation enhances the risk of carbon runoff via erosion (Ramchunder et al. 2009). Wildfire also alters the physical structure of the soil, which affects processes such as hydrology and nutrient cycling. Furthermore, fire induced disturbance also changes the diversity and phylogenetic abundance of microbes (Hart et al. 2005). These shifts in microflora have significant impact on biogeochemical processes and degrade the ecosystem services, functions, and possibly shift the ecosystem to another state (Ward et al. 2012). Therefore, it is important to understand the potential ecological effects of fire which are critical for the succession, stability, functions, dynamics, and restoration of ecosystems (Pressler et al. 2019), particularly in northern peatlands.

Microbial communities are of paramount importance in peatlands, where they significantly contribute to carbon dynamics and various biogeochemical processes. These microbes directly contribute to decomposition of organic matter and availability of nutrients, which is an important driver for plant species diversity and productivity (Kardol et al. 2010). Furthermore, plant diversity has been recognized to underline the robustness of ecosystems and their ability to cope with changes in climatic and environmental conditions (Isbell et al. 2017). More recently such a role has also been shown for diversity in the microbial community (Wagg et al. 2021). While the impact of fire and the subsequent recovery of plant populations, along with the resulting changes in carbon dynamics, are relatively well understood, recent research by Shepherd et al. (2021) underlines propagule availability as a driver of post-wildfire vascular plant recovery. Post-fire recovery of the plant community has been regarded as key for the recovery of the carbon sink function of peatlands, but the post-fire peatland plant communities do not always turn back to their old selves (Kettridge et al. 2015). Surprisingly, the effects of fire on belowground communities, another key driver for peatland carbon processes, remains largely overlooked. While transient fires are reported not to affect microbial communities (Wanner & Xylander 2003), high-intensity or repeated fires are known to have significant effects on their size and composition (Belova et al. 2014; Qin et al. 2017). To date, the implications of peat fires on belowground communities in the immediate period after the burn remains unknown.

Previous research has documented that the impact of heat on microbial community composition and diversity is most intense in the near-surface soil horizon where microbes are abundant (Neary et al. 1999). Hart et al. (2005) and Wang et al. (2016) have reported that longer burning periods during fire can markedly reduce microbial biomass and influence community structure and diversity. Microbial communities differ in their sensitivity to heat induced fire (Vigo & Building 2009). For example, the heat-resistant capacity and post-fire recovery rate of prokaryotic community are higher than eukaryotes (Mabuha et al. 2003). This may be influenced by higher pH and an increased dissolved organic matter production in pore water resulting from fire, which supports bacterial growth and recolonization (Rousk & Bååth 2007; Xiong et al. 2014). For instance, bacteria tend to be highly resistant to heat which result in increase in bacterial relative abundance and functional redundancy instantly after intense fire (Inen 1995) and recover more rapidly than fungi after exposure to heat induced fire (Mabuhay et al. 2003). Nevertheless, research on recovery on prokaryotic community composition and diversity as a function of time since disturbance of wildfire in boreal peatlands are comparatively rare. Therefore, it is of critical importance to understand the effect of variations in fire severity on bacterial community for predicting the carbon accretion in fire disturbed peatlands.

Although numerous research studies have investigated the response of above and belowground microbial communities to climate change and associated disturbances such as fire (Andersen et al. 2006; Park 2008), they have mostly focused on fire impacts on microbial biomass and activity. Hence, there still is a significant gap in our understanding of the response and alteration of soil microbial community composition and diversity in response to fires in peatland ecosystems

(Otsuka 2008; Comer 2019). The aim of this research study was to observe the response of prokaryotic structure, diversity at different times from fire disturbance in a peatland ecosystem by 16S rRNA sequencing. The influence of different fire severities (deep burn, shallow burn) on communities at two different peat soil depths were assessed.

The aim of this work is to address two main hypotheses:

1. The prokaryotic community composition and abundance will vary distinctly among peat soil depth (near surface and sub-surface). It is expected that prokaryotic communities in topsoil will show higher diversity.
2. The variation in depth-dependent prokaryotic communities along a time gradient post-fire will exhibit a distinct response to fire severities, with a significantly stronger effect observed in deep burn compared to shallow burn severity.

The research focuses on a poorly explored aspect of post-fire disturbances: the response and recovery of microbiome communities within ecosystems. These microbial communities, including bacteria and archaea, play essential roles in ecosystem functioning. By studying how they adapt after a fire, our findings have significant implications for ecological restoration efforts. We can potentially harness these microorganisms to promote vegetation growth, and mitigate invasive species in fire-affected areas, offering more effective and sustainable restoration strategies. This knowledge opens innovative and sustainable solutions for preserving natural environments in an era of increasing fire risks and climate change.

4.3 Material and Methods

4.3.1 Site Description:

This research took place within the Stalybridge estate, situated in the Saddleworth moors above the eastern outskirts of Greater Manchester. This region is in the Dark Peak area of the Peak District National Park in Northwest England, with geographic coordinates at 53°32'36"N and 1°57'21"W (as shown in Figure 1.1-A). The climate in this area is characterized as semi-humid and cold continental, with an average annual temperature of 8.2 °C. The mean annual rainfall for this location is 1160 mm per year (Fick & Hijmans 2017). The flora in the blanket moorland of this area is primarily composed of a limited number of species, including heather (Calluna vulgaris), cotton grass (Eriophorum species, primarily *Eriophorum vaginatum* L. and *Eriophorum angustifolium*), and various types of moss, such as *Sphagnum, Pleurozium schreberi*, and *Hypnum jutlandicum*.

These plants thrive on the underlying dry peat. The dark peat in this blanket bog, approximately 9,000 years old, has been historically drained for agriculture, reducing water retention, and resulting in extensive surface erosion, which has left large areas of bare peat exposed. On June 24, 2018, a large-scale peat wildfire ignited and persisted for approximately three weeks across Saddleworth Moor in Northwest England, (BBC, 20180. At its peak, this moorland fire expanded to cover an area of about 7 square miles (18 km^2), marking it as one of the largest recorded wildfires in the UK, particularly close to a residential population (Figure 1.1-A) (Graham et al. 2020).

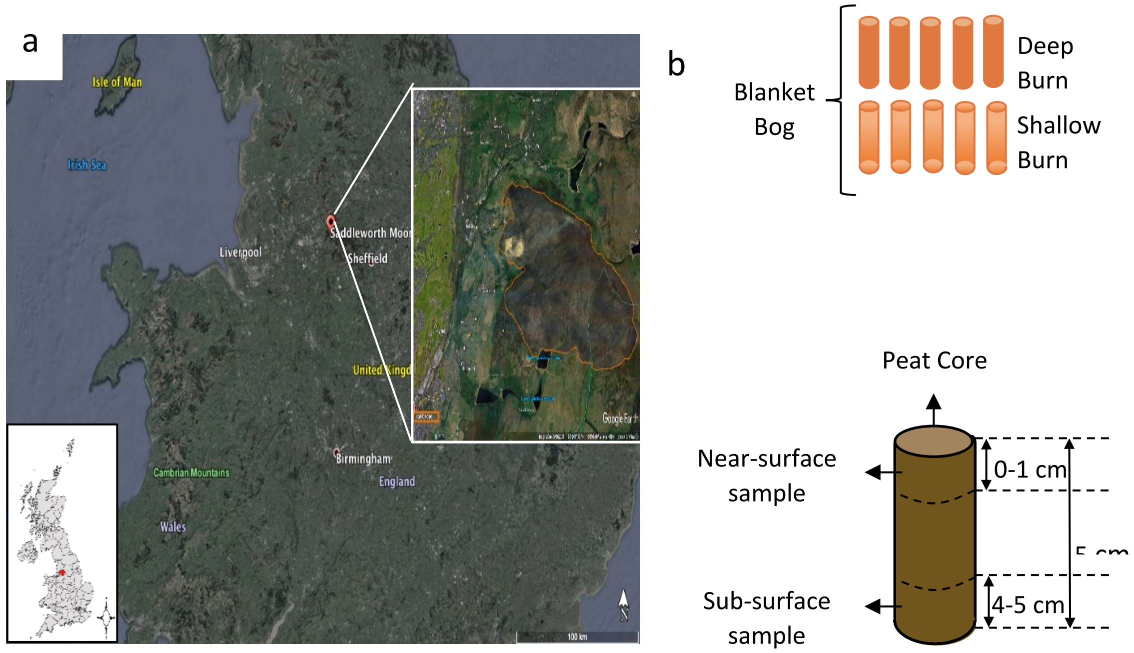


Figure 4.1Study region of Stalybridge Moor located near Peak District National Park, Manchester, United Kingdom with orange boundary burnt area indicating the region of fire-affected Stalybridge Moor. (b) Overview of the experiment showing the different peat cores collected for different treatment as well as depth. (Source: Google Earth)

4.3.2 Experimental Design

The burnt moorland area was divided into various sections based on the fire severity. The unburned control area was adjacent to the burned location and thus the composition of the plant and microbial communities were assumed to represent pre-fire condition. Peat soil samples were gathered at three different time points: 4 months (October 11, 2018), 11 months (May 6, 2019), and 14 months (August 15, 2019) after the fire. We identified 10 plots, each measuring 10 meters by 10 meters, equally distributed between two burn severity levels: deep burn and shallow burn. The determination of burn severity was made by visually assessing the remaining vegetation and ash layer, complemented by burn severity maps of the impacted areas. To investigate the impact of the wildfire on prokaryotic composition and diversity at different soil depths, we collected peat samples from three random locations within each 10m x 10m plot, at near-surface (0-1 cm) and sub-surface (4-5 cm) depths. The three subsamples per depth were merged to form one composite sample per plot. In total, this gave 60 merged peat samples, with 30 samples from the near surface and 30 samples from the subsurface soil, at three distinct time points following the fire.

4.3.3 Nucleic Acid Extraction and Illumina Sequencing

Two soil samples, one from the uppermost surface layer (0-1cm) and another from the subsurface layer (4-5cm), were extracted from peat cores and stored at -80°C for DNA isolation. Prior to the extraction process, these samples were taken out of the freezer and submerged in liquid nitrogen. Subsequently, they were manually fragmented using a mortar and pestle. After achieving homogenization, total DNA was extracted from 0.25g of peat soil using the DNAeasy PowerLyzer Power Soil DNA extraction kit from QIAGEN (Germantown, MD), following the provided protocol. The DNA's quality and concentration were evaluated using a spectrophotometer (QIAxpert System). For the sequencing of 16S rRNA genes and the initial quality control checks, the procedures outlined in the Earth Microbiome Project (http://www.earthmicrobiome.org/) were followed. To amplify the V4 region of the 16S rRNA gene for both bacterial and archaeal sequencing, primer pairs 515f (5’ GTGCCAGCMGCCGCGGTAA-3’) and 806r (5’GGACTACHVGGGTWTCTAAT-3’) were employed (Lauber et al. 2009; Bates et al. 2011). The sequencing itself was carried out on an Illumina MiSeq system in rapid run mode, generating 300 bp paired end reads.

4.3.4 Processing of Illumina Sequencing Data

After eliminating low-quality reads as part of the Earth Microbiome Project's preprocessing, the demultiplexed raw data files for all samples were imported into R. Raw data files containing paired forward and reverse reads underwent trimming, which involved removing primers, and untrimmed sequences were discarded using Cutadapt v1.14 (v1.9.1) (Saeidipour & Bakhshi 2013). During the demultiplexing process, any sequences with low-quality bases were also discarded. The resulting filtered and trimmed files were then subjected to analysis using the Divisive Amplicon Denoising Algorithm (DADA2) bioinformatic pipeline within the R (version 4.2.1) statistical computing system (Callahan et al. 2016). This pipeline encompassed steps such as dereplication, denoising, and merging of reads. In the context of the DADA2 software, additional quality filtering was performed by calculating error rates and subsequently removing reads with errors from the de-replicated dataset. An initial sequence table was generated, followed by the identification and removal of chimeras using the remove Bimera Denovo function from the DADA package (Edgar et al. 2011). Lastly, the taxonomic classification of bacterial and archaeal sequences was accomplished by aligning them against the Silva database using DADA2's native RDP Bayesian classifier (Quast et al. 2012). Ultimately, the prokaryotic sequences that had undergone quality filtering were grouped into amplicon sequence variants (ASVs), creating a high-resolution version table.

4.3.5 Statistical analysis

All analyses were completed in R (version 4.3.1) (https://www.r-project.org/). Analyses included functions in the R packages phyloseq (Biocmanager) (McMurdie et al.,2012), vegan (Oksanen et al. 2019), Tidyverse (2.0.0), microeco (version 1.3.0) (CRAN -(r-project.org) (Liu et al. 2021). We used linear mixed effects models to determine the response of prokaryote community (at phylum level) to time and burn severity along the peat depth. Shannon, Simpson, Chao1 and prokaryotic abundances as a function of time (months) since fire, burn severity (shallow/deep) along depth and their two-way interaction were included. Plot was included as a random intercept in each model to account for repeated measurements. Mixed effect models were fit with lmerTest package (Bates et al. 2014). Marginal R^2 (Rm^2) and conditional R^2 (Rc^2) were calculated for each best model by the function 'r.squaredGLMM' in MUMIN and lmer4 packages. Marginal R^2 (Rm^2) represents the variance explained by fixed effects, whereas conditional R^2 (Rc^2) represents the variance explained by both fixed and random effects. To compare prokaryote community composition (beta diversity) across the period of initial recovery and the varying burn severities, the principal coordinate analysis (PCoA) ordination plots using Bray-Curtis dissimilarities were constructed. Statistically significant variation of clusters of burn severity treatments and months since fire were tested for the different peat depths (near surface and sub surface) using a stratified permutational multivariate analysis of variance (PERMANOVA) (permutations = 999). Lastly, the indicator prokaryotic taxa in different burn severities across peat surface along time gradient were identified via linear discriminant analysis effect size (LEfSe) as described by Segata et al. (2011). To control for false discoveries, all raw p-values in the LDA were adjusted using the Benjamini-Hochberg False Discovery Rate (FDR) correction method (Benjamini & Hochberg 1995), implemented using the "microeco" package.

4.4 Results

4.4.1 Amplicon Sequencing and Bioinformatic processing

Total genomic DNA was extracted from peat soil samples collected under both control and post-fire conditions across different depths and time points. Amplicon sequencing of the 16S rRNA gene was performed using the Illumina MiSeq platform, generating 5,074,219 raw paired-end reads, with a mean of 71,468 reads per sample.

Initial quality inspection was conducted using the DADA2 pipeline (version 1.16). Based on the quality profiles, reads were truncated at base 240 for forward reads and base 160 for reverse reads to remove low-quality regions. Reads containing ambiguous nucleotides (maxN=0) or exceeding an expected error threshold (maxEE=2) were filtered out. After quality filtering, approximately 3,768,908 high-quality reads remained for downstream analysis.

Following filtering, dereplication was performed to collapse identical reads, and error rates were learned directly from the dataset. Denoising was applied separately to forward and reverse reads, yielding averages of 3,542,497 and 3,571,694 denoised reads, respectively. Merging of paired-end reads resulted in a substantial reduction in read counts due to non-overlapping regions or poor-quality overlaps, with an average of 1,922 merged reads per sample.

Subsequent chimera removal using DADA2's consensus method further refined the dataset, resulting in an average of 10,651 non-chimeric reads per sample for downstream taxonomic analysis. The average merged read length was approximately 400 bp.

From the final dataset, a total of 969 unique amplicon sequence variants (ASVs) were inferred. Taxonomic classification was assigned using a naïve Bayes classifier trained on the SILVA 99%

reference database. The ASVs were affiliated with 345 genera, 193 families, 132 orders, 68 classes, and 35 phyla.

The microbial community structure was dominated by Proteobacteria (31.36%), Planctomycetota (10.22%), Acidobacteriota (8.49%), Verrucomicrobiota (7.14%), Actinobacteriota (6.71%), Patescibacteria (6.68%), and Bacteroidota (6.43%), which together accounted for more than 77% of the total reads across all samples, regardless of treatment, depth, or time point. Less abundant phyla included Chlamydiota (3.60%), Armatimonadota (2.28%), Dependentiae (2.24%), Cyanobacteriota (1.99%), and WPS-2 (1.00%). Archaeal taxa such as Euryarchaeota (0.49%) and Thaumarchaeota (0.44%) were detected primarily in burned peat soil. Additionally, 20 other rare phyla (<1% relative abundance) were identified, indicating the presence of a diverse rare biosphere within the peatland microbial communities.

4.4.2 Prokaryotic community composition

Fire changed the proportion of some dominant bacterial phyla in the near surface soil and sub-surface soil (Fig 4.2 a,b). The near surface soil showed significantly higher relative abundances of Proteobacteria, Bacteroidetes and Patescibacteria, but significantly lower relative abundance of Thaumarchaeota after shallow and deep burn severities as time passed as compare to control (unburned) (Fig 4.2 a,b; Table 4.1, S4.2,3).

As time progressed, wildfire induced significant shifts in the relative abundance of four dominant bacterial phyla Proteobacteria, Acidobacteria, Verrucomicrobia, Bacteroidetes, and Planctomycetes across burned and unburned peat soil depths, as indicated by LMMs (Fig. 4.2a; Table 4.1). In near-surface soil following shallow burns, the relative abundance of Acidobacteria steadily increased from 4- to 11- and 14-months post-fire, whereas Bacteroidetes and Proteobacteria exhibited a consistent decline over the same period. Verrucomicrobia and Planctomycetes showed a significant increase between 4 and 11 months but declined again by 14 months. In sub-surface soils exposed to shallow burns, Proteobacteria, Acidobacteria and Planctomycetes significantly decreased at 14-months, while Verrucomicrobia and Bacteroidetes increased as compared to control (pre-fire peat). Under deep burn severity in subsurface layers, Acidobacteria, Verrucomicrobia, and Planctomycetes abundances declined sharply from 4- to 11- months, before recovering by 14- months, whereas Bacteroidetes continued a persistent decline throughout time horizons in comparison to unburned treatment.

We performed linear discriminant analysis effect size to identify the prokaryotic taxa associated with burn severities along the time gradient within both peat depths (near-surface and deep-surface) (Fig 4.7,4.8). In response to shallow burn severity, 49 and 43 prokaryotic taxa were significantly abundant as time passed across peat soil depths with significant response detected at 14 months in sub-surface peat (LDA threshold of >3.0) (Fig 4.7 a,b,c and d, S4.1). However, for deep burn severity, 51 and 50 taxonomic groups were considerably enriched at different time periods (4, 11 and 14-month) in near-surface and sub-surface, with the strongest effect detected at post 4 months in subsurface peat (LDA threshold of >3.0) (Fig4.8 a,b,c and d, S4.2). These prokaryotic taxa could be considered as the most abundant biomarkers.

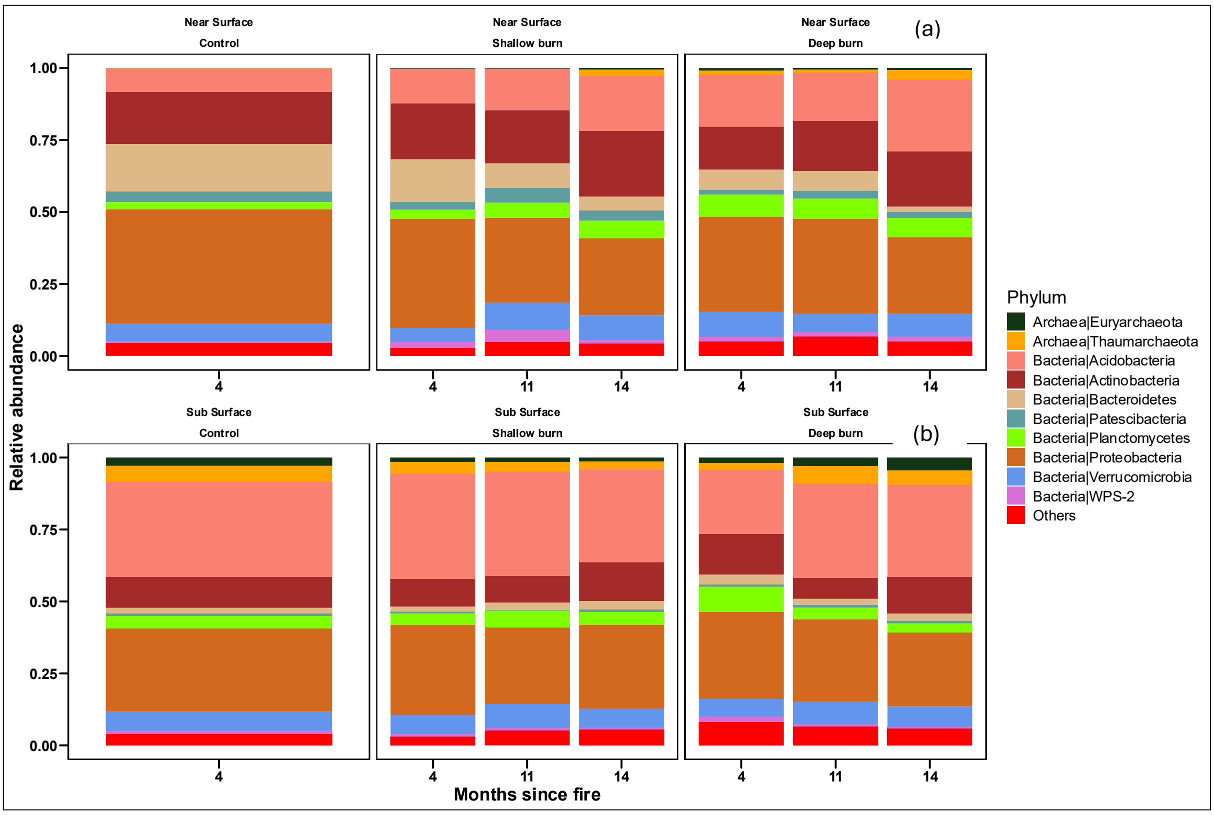


Figure 4.2 Bar plot showing percentage relative abundance of top ten prokaryotic phyla of near-surface (a) and sub-surface soil horizons (b) in control, shallow and deep burn sites post fire 4, 11 and 14 months. Others indicate rare and unidentified phyla.

Table 4.1 Result of linear mixed-effects model for effect of time (months), control (unburned) and fire burn severity (shallow/deep) on top 10 relative abundances of prokaryotic communities across peat depth (n=53)

Variable		Depth	Time	Treatment	Depth: Time	Depth: Treatment	Time: Treatment	Depth: Time: Treatment
	Df	1	1	2	1	2	1	1
Proteobacteria	F	9.70	18.31	3.32	2.94	3.32	2.513	0.984
	P	**0.009**	**<0.002**	0.493	0.09	0.330	0.745	**0.057**
Acidobacteria	F	161.29	10.09	5.02	0.63	13.70	4.56	6.40

	P	**<0.001**	**0.002**	0.08	0.42	**<0.0001**	**0.001**	**0.03**
Actinobacteria	F	44.35	1.75	0.30	1.19	1.70	0.71	2.06
	P	0.11	0.20	0.91	0.27	0.10	0.10	0.20
Bacteroidetes	F	95.33	20.87	18.36	20.72	19.66	1.27	5.33
	P	**<0.001**	**<0.001**	0.07	**<0.001**	**<0.001**	0.43	**<0.001**
Verrucomicrobia	F	0.85	2.82	0.73	0.18	0.38	2.23	4.34
	P	0.92	0.07	0.32	0.66	0.08	0.67	**0.06**

Variable		Depth	Time	Treatment	Depth: Time	Depth: Treatment	Time: Treatment	Depth: Time: Treatment
	Df	1	1	2	1	2	1	1
Planctomyctes	F	0.35	0.69	6.14	4.46	1.39	10.37	0.62
	P	0.10	0.16	**<0.003**	**0.03**	0.92	**0.005**	**0.02**
Patescibacteria	F	70.26	1.61	3.32	1.42	5.36	0.29	0.06
	P	**0.03**	0.18	0.20	0.23	0.25	0.83	0.47
WPS-2	F	6.11	1.49	1.59	1.29	4.58	0.56	1.08
	P	0.84	0.21	0.18	0.25	0.13	0.20	0.31

FCPU426	F	3.82	27.19	5.52	1.38	2.89	1.18	0.06
	P	**0.05**	**<0.001**	**0.001**	0.63	0.06	0.31	0.41
Thaumarchaeota	F	21.11	2.53	0.86	0.43	1.85	2.04	2.21
	P	**0.02**	0.06	0.16	0.51	0.17	**0.02**	0.18

Table 4.2 Variation among shannon diversity, simpson and chao1 along with top ten relative abundances of bacterial and archaeal phylum. Rm^2: variance explained by fixed effects and Rc^2: variance explained my fixed and random effects. n=63

Variables	**Rm^2**	**Rc^2**
Shannon diversity	0.453	0.631
Simpson	0.431	0.631
Chao1	0.512	0.541
Proteobacteria	0.439	0.614
Acidobacteria	0.780	0.795
Actinobacteria	0.465	0.465
Bacteroidetes	0.779	0.779
Verrucomicrobia	0.169	0.377
Planctomycetes	0.339	0.626
Patescibacteria	0.594	0.594
WPS-2	0.269	0.269
FCPU426	0.448	0.554
Thaumarchaeota	0.351	0.527

4.4.3 Prokaryotic Diversity and Richness

Prokaryotic alpha diversity was calculated based on ASV abundance. Phylogenetic diversity, measured using Shannon and Simpson indices, was assessed for both bacterial and archaeal sequences (Fig. 4.3; 4.4), along with phylotype richness estimated using the Chao1 index (Fig. 4.5a,b). According to the linear mixed-effects model, there were no significant differences in prokaryotic diversity (Shannon and Simpson indices) or richness (Chao1) across peat soil depths; however, diversity and richness appeared to be higher in near-surface soils compared to sub-surface soils (Shannon: P = 0.513; Simpson: P = 0.280; Chao1: P = 0.671) (Table 4.3).

Despite the non-significant effect of soil depth, fire severity (shallow vs. deep) significantly influenced Shannon diversity, Simpson diversity, and Chao1 richness at different time points (Shannon: P < 0.0001; Simpson: P < 0.0001; Chao1: P = 0.005). Although the effect of burn treatment across soil depths was not statistically significant at each time point, shallow-burn fire tended to increase prokaryotic phylotype richness (Chao1) and phylogenetic diversity (Shannon) in both peat layers, with the highest diversity and richness observed 14 months post-fire (Table 4.3) compared to the unburned control. In contrast, deep-burn severity appeared to reduce Shannon and Simpson diversity indices at 14 months, although Chao1 richness showed an increasing trend across peat depths compared to the unburned treatment.

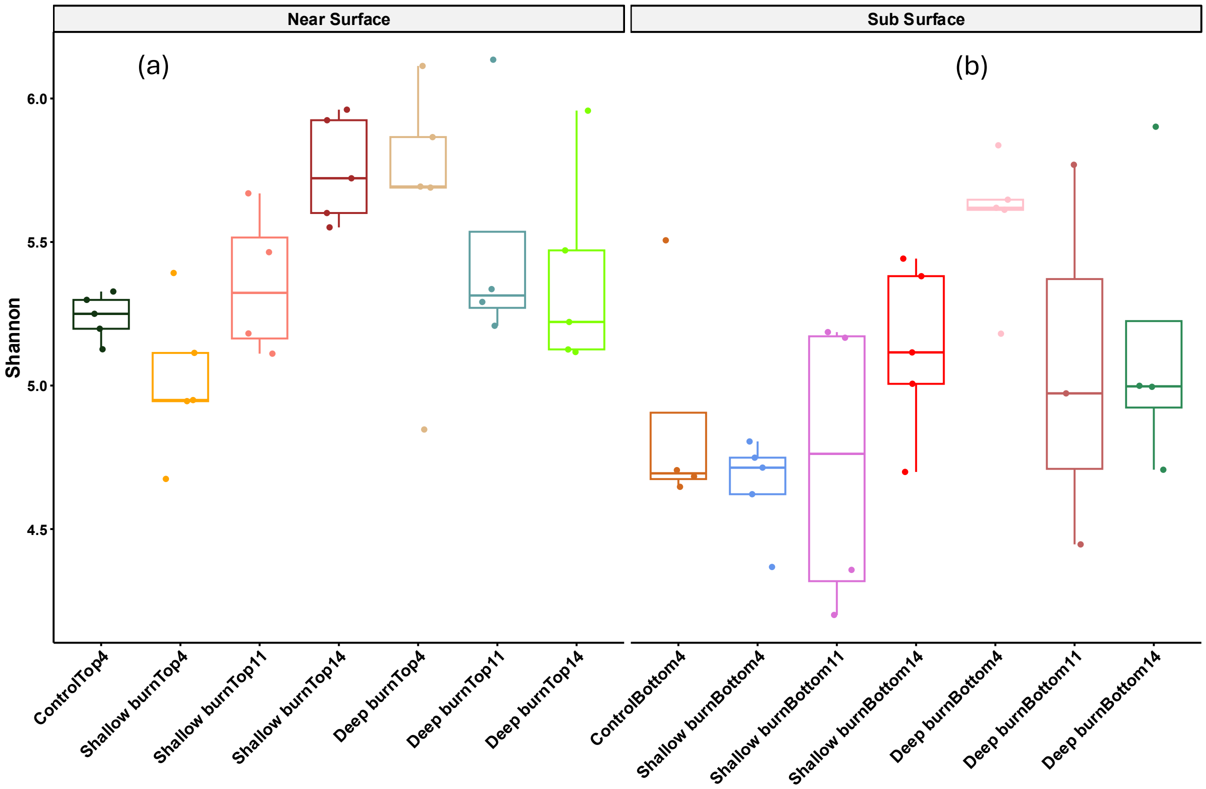


Figure 4.3 Prokaryotic Shannon diversity of near-surface (a) and sub-surface soil horizons (b) in shallow and deep burn sites at 4 , 11 and 14-months post fire.

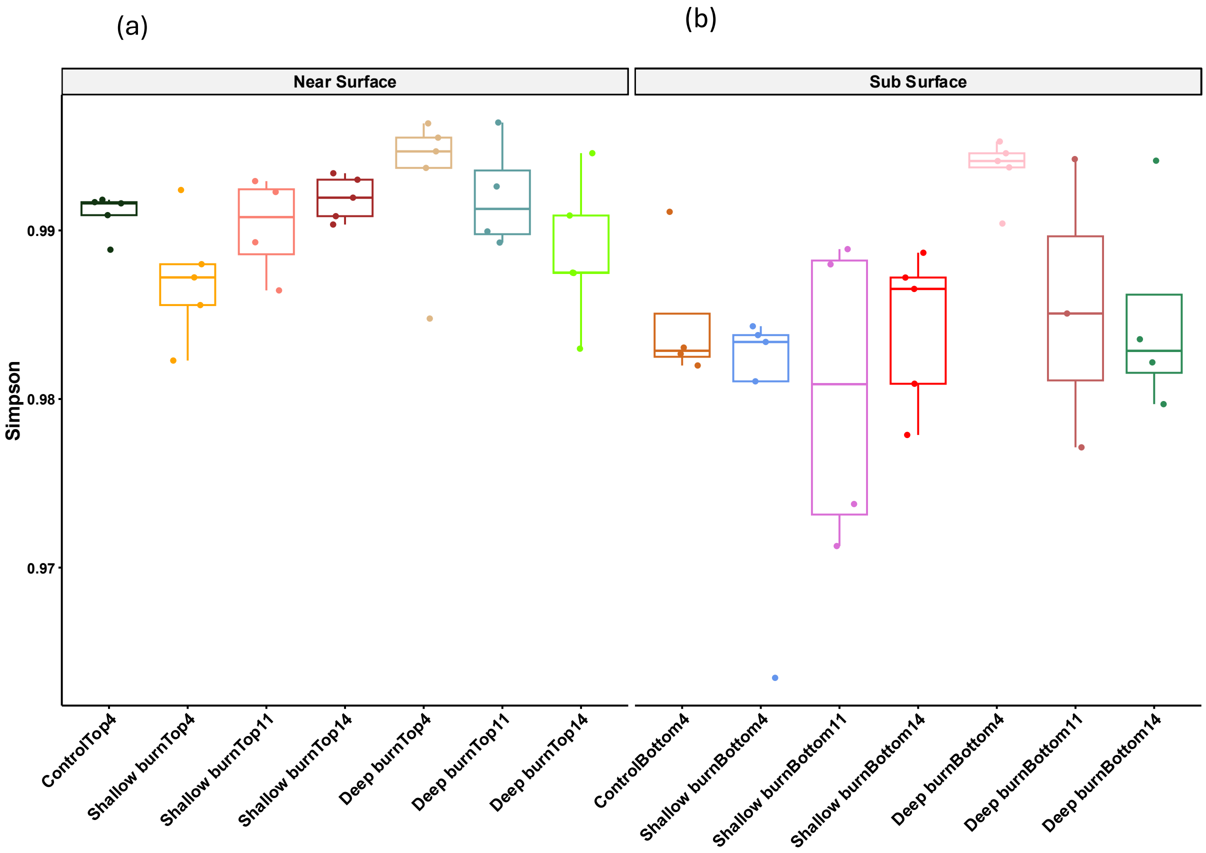


Figure 4.4 Prokaryotic Simpson index of near-surface (a) and sub-surface soil horizons (b) in shallow and deep burn sites at 4 , 11 and 14 months post fire.

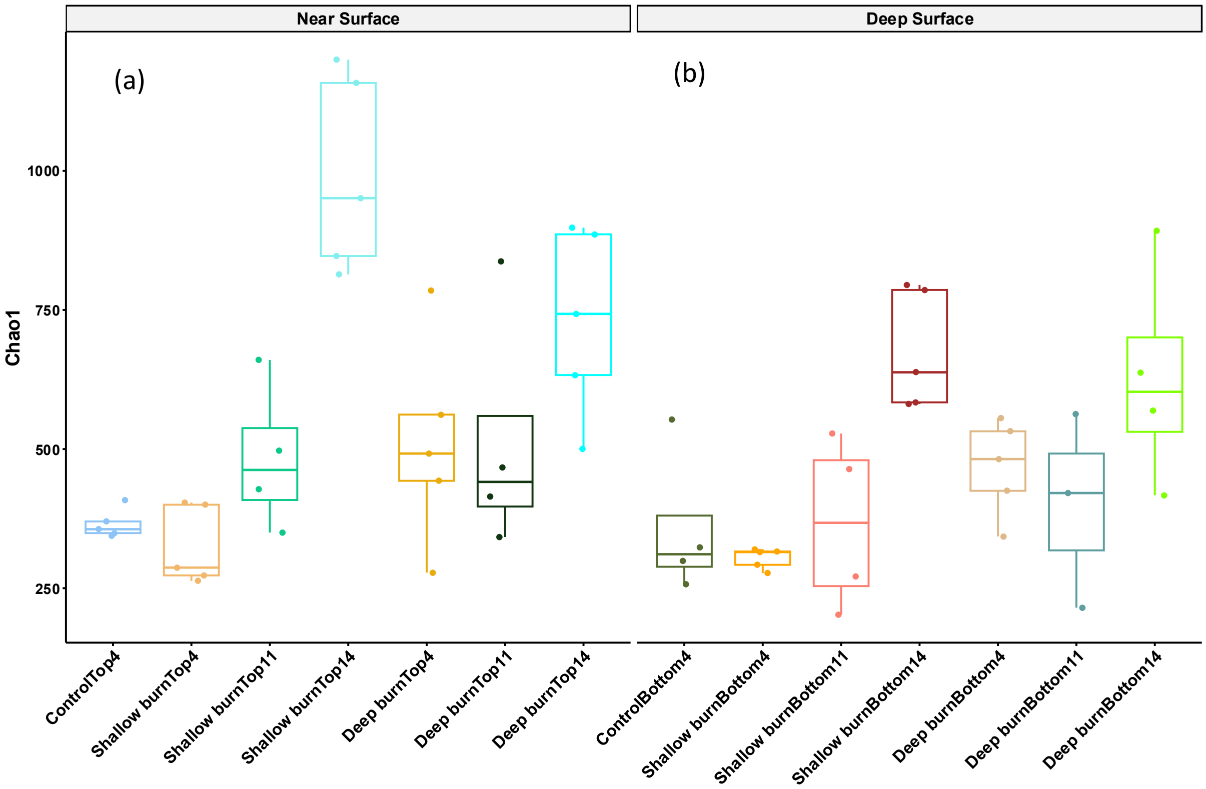


Figure 4.5 Chao index of prokaryotic amplicon sequence variants (ASVs) in near-surface (a) and sub-surface soil horizons (b) in shallow and deep burn severity at post fire 4, 11 and 14- months.

Table 4.3Result of linear mixed-effects model for effect of time (months) and fire burn severity (shallow/deep) on alpha diversity and ASVs richness across peat depth

Variables		Shannon		Simpson		Chao1	
	Df	**F**	**P**	**F**	**P**	**F**	**P**
Depth	1	0.348	0.513	0.953	0.280	0.167	0.671
Time	1	0.855	0.349	0.762	0.377	33.361	**<0.0001**
Treatment	1	29.639	**<0.0001**	25.328	**<0.0001**	7.993	**0.015**

Depth: Time	1	2.073	0.159	1.471	0.234	2.596	0.116
Depth: Treatment	1	0.406	0.630	3.103	0.145	0.276	0.808
Time: Treatment	1	25.594	**0.001**	22.228	**<0.0001**	8.708	0.148
Depth:Time:Treatment	1	0.002	0.351	1.260	0.261	8.745	0.155

4.4.4 Prokaryotic Community Structure

Principal coordinate analysis (PCoA) ordination showed that prokaryotic community composition differed significantly between near-surface and sub-surface peat layers (R^2 = 0.208, P = 0.001) (Table 4.4). PERMANOVA analysis based on Bray-Curtis dissimilarities revealed that bacterial and archaeal communities varied over time following both deep and shallow burn severities, with a marginally significant effect observed (R^2 = 0.059, P = 0.055) compared to unburned controls. Additionally, temporal changes in community composition across soil depths and fire severities were significant (Fig. 4.6), indicating a combined effect of depth and burn severity on prokaryotic community structure (R^2 = 0.059, P = 0.055).

In shallow burn areas, the PCoA ordination revealed significant variation in prokaryotic communities across the post-fire time gradient and soil depth, with the most pronounced shift observed at 14 months post-fire in near-surface peat compared to unburned controls (R^2 = 0.108, P = 0.028). In contrast, in deep burn areas, PERMANOVA of the PCoA ordination indicated significant differences in prokaryotic community composition across peat depths relative to unburned samples (R^2 = 0.280, P = 0.001), while no significant temporal change was detected in these communities over time post-fire (R^2 = 1.682, P = 0.096).

Table 4.4 Permutational multivariate analysis of variance (PERMANOVA) of prokaryotic community composition across peat depth, burn severities at different time (n=19)

Variables	**Df**	**R^2**	**F**	**p**
Treatment	2	0.104	1.430	0.083
Depth	1	0.208	5.692	**0.001**
Time	1	0.072	1.971	**0.024**
Treatment: Depth	2	0.106	1.461	0.068
Treatment: Time	1	0.059	1.629	**0.055**
Depth: Time	1	0.043	1.202	0.208
Tretment:Time:Depth	2	0.083	1.140	0.274

Table 4.5 Permutational multivariate analysis of variance (PERMANOVA) of shallow burn along with control of prokaryotic community composition across peat depth at different time (n=13)

Variables	**Df**	**R^2**	**F**	**p**
Time	1	0.101	2.010	**0.032**
Depth	1	0.253	2.144	**0.001**
Time: Depth	1	0.108	2.144	**0.028**

Table 4.6 Per mutational multivariate analysis of variance (PERMANOVA) of deep burn of prokaryotic community composition across peat depth at different time (n=11)

Variables	**Df**	**R^2**	**F**	**p**
Time	1	0.106	1.851	0.024
Depth	1	0.280	4.897	**0.001**
Time: Depth	1	0.096	1.682	0.089

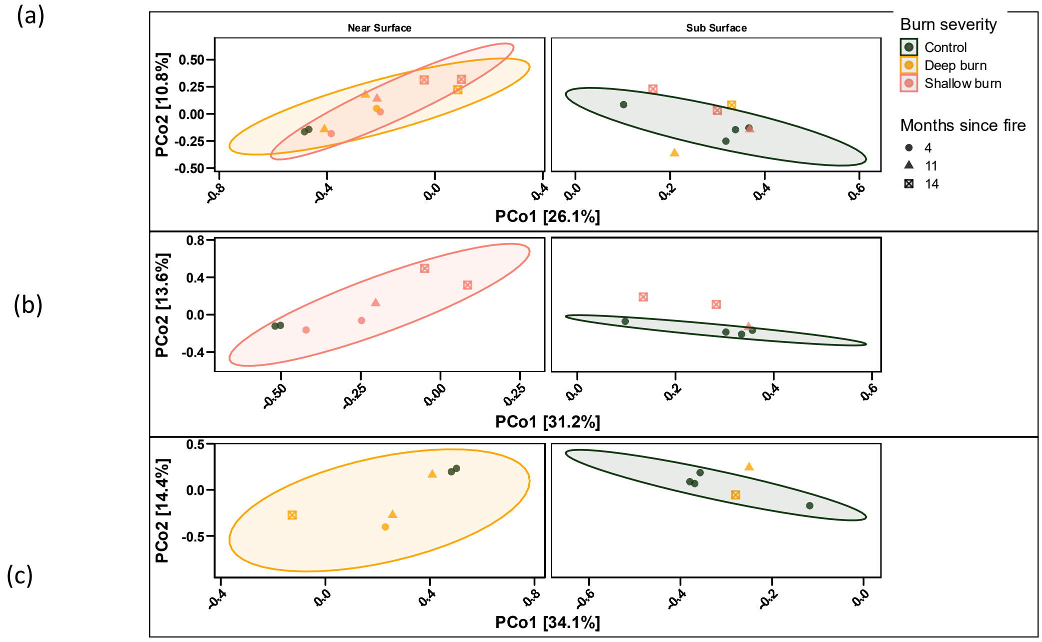
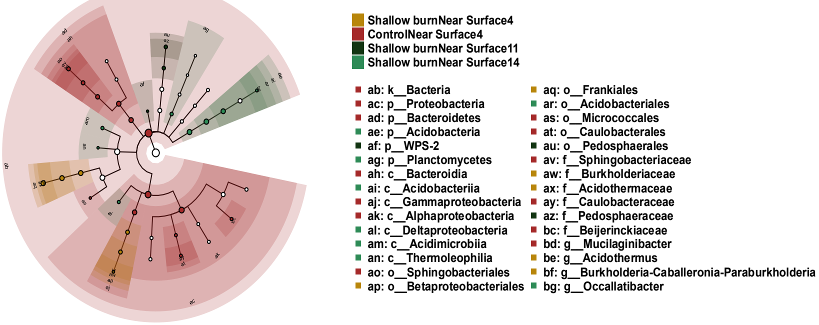
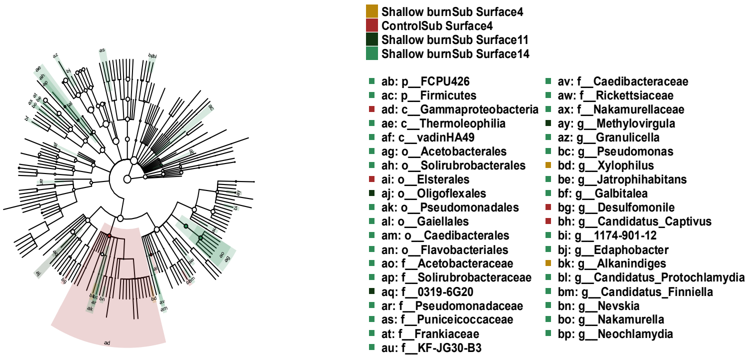


Figure 4.6 Principal coordinate analysis (PCoA) ordination of shallow and deep burn severity together based on Bray-Curtis algorithm showing the difference in prokaryotic community composition among near-surface and sub-surface peat soil at 4, 11 and 14 months post fire. (b) and (c) show PCoA ordination of shallow burn severity and deep burn severity separately. The significance for the differences among depth, time and burn treatments were calculated by PERMANOVA. Each data point represents an individual sample. Points closer to each other represent similar microbial communities, while points farther from each other represent dissimilar microbial communities.

(a)


🟨 Shallow burnNear Surface4
🟥 ControlNear Surface4
⬛ Shallow burnNear Surface11
🟩 Shallow burnNear Surface14
🟥 ab: k__Bacteria
🟥 ac: p__Proteobacteria
🟥 ad: p__Bacteroidetes
🟩 ae: p__Acidobacteria
⬛ af: p__WPS-2
🟩 ag: p__Planctomycetes
🟥 ah: c__Bacteroidia
🟩 ai: c__Acidobacteriia
🟥 aj: c__Gammaproteobacteria
🟥 ak: c__Alphaproteobacteria
🟩 al: c__Deltaproteobacteria
🟩 am: c__Acidimicrobiia
🟩 an: c__Thermoleophilia
🟥 ao: o__Sphingobacteriales
🟨 ap: o__Betaproteobacteriales
🟨 aq: o__Frankiales
🟩 ar: o__Acidobacteriales
🟥 as: o__Micrococcales
🟥 at: o__Caulobacterales
⬛ au: o__Pedosphaerales
🟥 av: f__Sphingobacteriaceae
🟨 aw: f__Burkholderiaceae
🟨 ax: f__Acidothermaceae
🟥 ay: f__Caulobacteraceae
⬛ az: f__Pedosphaeraceae
🟥 bc: f__Beijerinckiaceae
🟥 bd: g__Mucilaginibacter
🟨 be: g__Acidothermus
🟨 bf: g__Burkholderia-Caballeronia-Paraburkholderia
🟩 bg: g__Occallatibacter

(b)


🟨 Shallow burnSub Surface4
🟥 ControlSub Surface4
⬛ Shallow burnSub Surface11
🟩 Shallow burnSub Surface14
🟩 ab: p__FCPU426
🟩 ac: p__Firmicutes
🟥 ad: c__Gammaproteobacteria
🟩 ae: c__Thermoleophilia
🟩 af: c__vadinHA49
🟩 ag: o__Acetobacterales
🟩 ah: o__Solirubrobacterales
🟥 ai: o__Elsterales
⬛ aj: o__Oligoflexales
🟩 ak: o__Pseudomonadales
🟩 al: o__Gaiellales
🟩 am: o__Caedibacterales
🟩 an: o__Flavobacteriales
🟩 ao: f__Acetobacteraceae
🟩 ap: f__Solirubrobacteraceae
⬛ aq: f__0319-6G20
🟩 ar: f__Pseudomonadaceae
🟩 as: f__Puniceicoccaceae
🟩 at: f__Frankiaceae
🟩 au: f__KF-JG30-B3
🟩 av: f__Caedibacteraceae
🟩 aw: f__Rickettsiaceae
🟩 ax: f__Nakamurellaceae
⬛ ay: g__Methylovirgula
🟩 az: g__Granulicella
🟩 bc: g__Pseudomonas
🟨 bd: g__Xylophilus
🟩 be: g__Jatrophihabitans
🟩 bf: g__Galbitalea
🟥 bg: g__Desulfomonile
🟥 bh: g__Candidatus_Captivus
🟩 bi: g__1174-901-12
🟩 bj: g__Edaphobacter
🟨 bk: g__Alkanindiges
🟩 bl: g__Candidatus_Protochlamydia
🟩 bm: g__Candidatus_Finniella
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🟩 bp: g__Neochlamydia

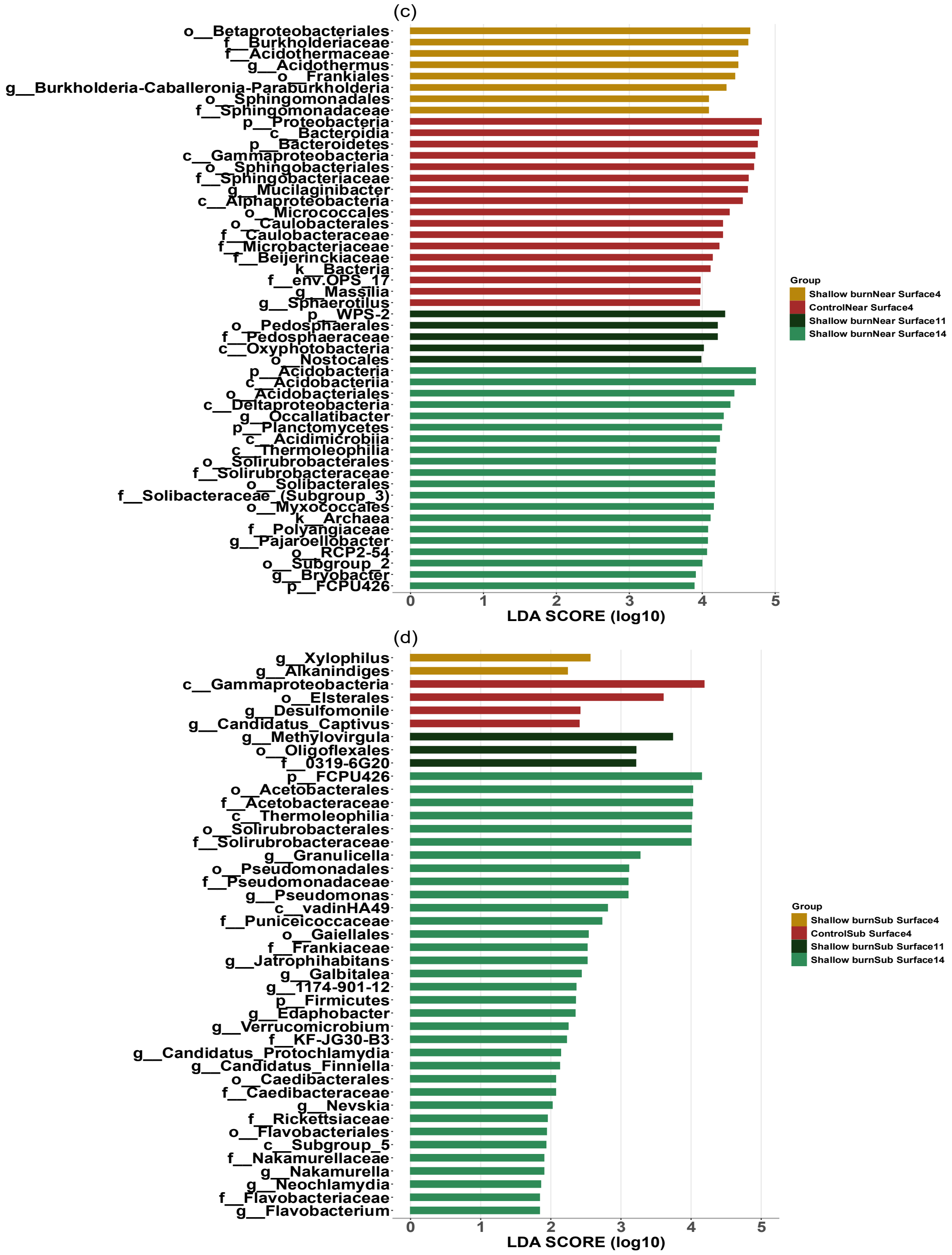
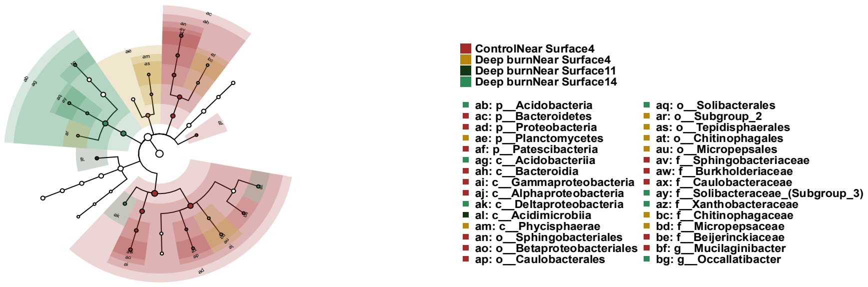
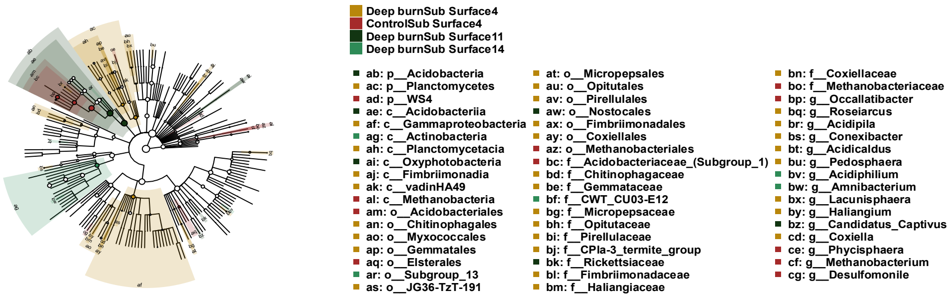


Figure 4.7 The LEfSe analysis illustrates biomarker taxa distributions across peat depths under control and shallow burn treatments. Panels (a) and (b) present cladograms for near-surface and sub-surface soils, respectively, showing the phylogenetic distribution of taxa across different time horizons. In both cladograms, dot sizes are proportional to each taxon's relative abundance, and white nodes indicate taxa with no statistically significant variation. The taxonomic hierarchy spans from phylum (p) to species (s). Panels (c) and (d) display LDA score histograms for near-surface and sub-surface soils, respectively, highlighting the taxa that are most differentially abundant across time (LDA > 3.5 for near-surface and LDA > 1.5 for sub-surface; $P < 0.05$.

(a)


ControlNear Surface4
Deep burnNear Surface4
Deep burnNear Surface11
Deep burnNear Surface14
ab: p__Acidobacteria
ac: p__Bacteroidetes
ad: p__Proteobacteria
ae: p__Planctomycetes
af: p__Patescibacteria
ag: c__Acidobacteriia
ah: c__Bacteroidia
ai: c__Gammaproteobacteria
aj: c__Alphaproteobacteria
ak: c__Deltaproteobacteria
al: c__Acidimicrobiia
am: c__Phycisphaerae
an: o__Sphingobacteriales
ao: o__Betaproteobacteriales
ap: o__Caulobacterales
aq: o__Solibacterales
ar: o__Subgroup_2
as: o__Tepidisphaerales
at: o__Chitinophagales
au: o__Micropepsales
av: f__Sphingobacteriaceae
aw: f__Burkholderiaceae
ax: f__Caulobacteraceae
ay: f__Solibacteraceae_(Subgroup_3)
az: f__Xanthobacteraceae
bc: f__Chitinophagaceae
bd: f__Micropepsaceae
be: f__Beijerinckiaceae
bf: g__Mucilaginibacter
bg: g__Occallatibacter

(b)


Deep burnSub Surface4
ControlSub Surface4
Deep burnSub Surface11
Deep burnSub Surface14
ab: p__Acidobacteria
ac: p__Planctomycetes
ad: p__WS4
ae: c__Acidobacteriia
af: c__Gammaproteobacteria
ag: c__Actinobacteria
ah: c__Planctomycetacia
ai: c__Oxyphotobacteria
aj: c__Fimbriimonadia
ak: c__vadinHA49
al: c__Methanobacteria
am: o__Acidobacteriales
an: o__Chitinophagales
ao: o__Myxococcales
ap: o__Gemmatales
aq: o__Elsterales
ar: o__Subgroup_13
as: o__JG36-TzT-191
at: o__Micropepsales
au: o__Opitutales
av: o__Pirellulales
aw: o__Nostocales
ax: o__Fimbriimonadales
ay: o__Coxiellales
az: o__Methanobacteriales
bc: f__Acidobacteriaceae_(Subgroup_1)
bd: f__Chitinophagaceae
be: f__Gemmataceae
bf: f__CWT_CU03-E12
bg: f__Micropepsaceae
bh: f__Opitutaceae
bi: f__Pirellulaceae
bj: f__CPla-3_termite_group
bk: f__Rickettsiaceae
bl: f__Fimbriimonadaceae
bm: f__Haliangiaceae
bn: f__Coxiellaceae
bo: f__Methanobacteriaceae
bp: g__Occallatibacter
bq: g__Roseiarcus
br: g__Acidipila
bs: g__Conexibacter
bt: g__Acidicaldus
bu: g__Pedosphaera
bv: g__Acidiphilium
bw: g__Amnibacterium
bx: g__Lacunisphaera
by: g__Haliangium
bz: g__Candidatus_Captivus
cd: g__Coxiella
ce: g__Phycisphaera
cf: g__Methanobacterium
cg: g__Desulfomonile

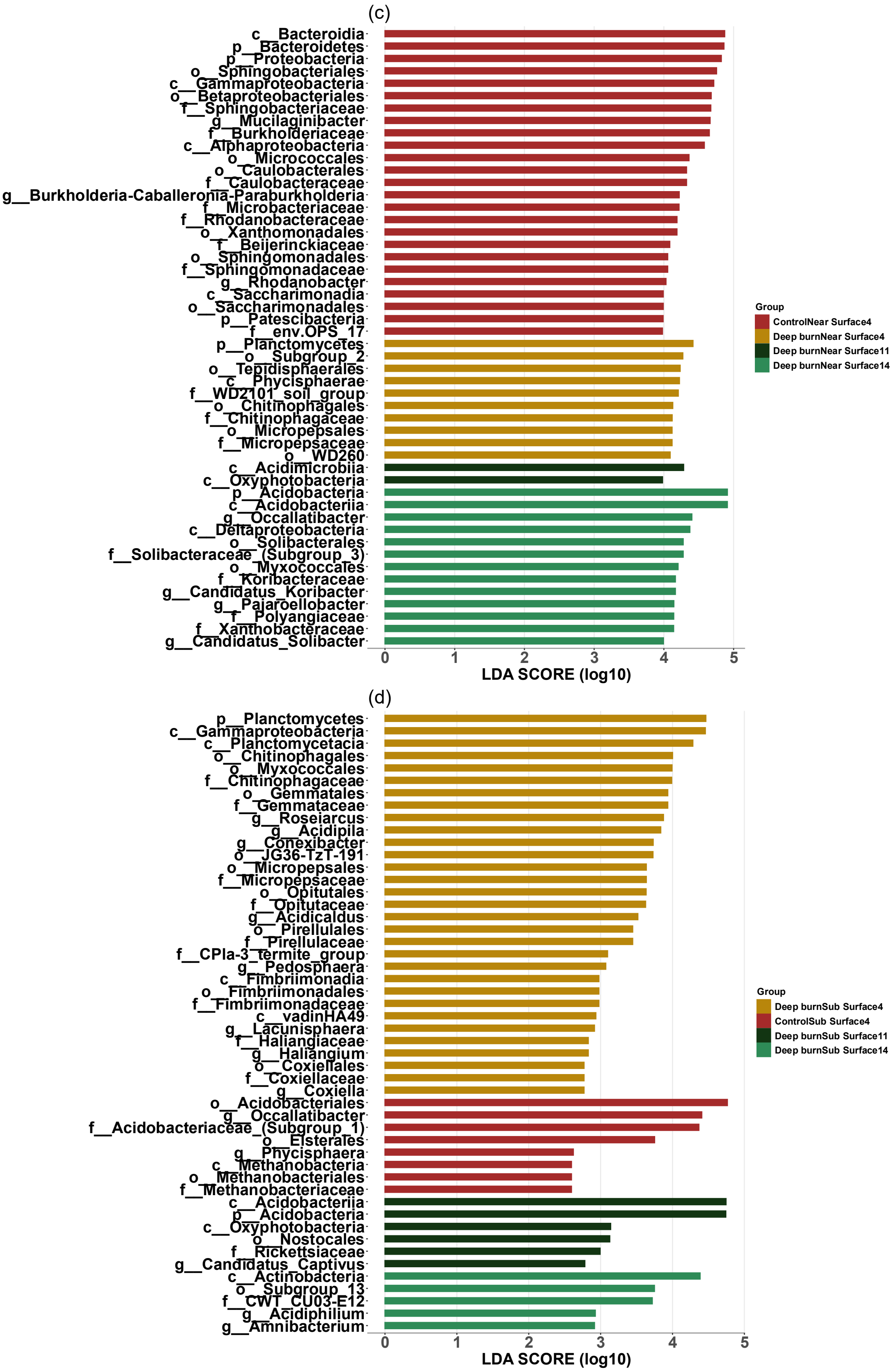


Figure 4.8 The LEfSe analysis shows biomarker taxa distributions across peat depths under control and deep burn treatments using cladograms and LDA histograms. The cladograms (a and b) illustrate the phylogenetic distribution of taxa in near-surface and sub-surface soils, respectively, across different time horizons. Dot sizes are proportional to each taxon's abundance, and white nodes indicate taxa with no statistically significant variation. The

taxonomic hierarchy is represented from phylum (p) to species (s). The LDA score histograms (c and d) highlight taxa that are most differentially abundant over time in near-surface and sub-surface soils, respectively, with thresholds of LDA > 4.0 ($P < 0.05$) for near-surface soil and LDA > 2.5 ($P < 0.05$) for sub-surface soil.

Discussion

Fire causes many ecological disturbances (Bond & Keeley 2005) and are one of the main concerns for global climate change. Wildfire is expected to happen more often because of warmer, drier climate patterns (Harden et al. 2000; Dioumaeva et al. 2003) and cause shifting in aboveground species composition, boosting carbon cycling and declines in bacterial biomass (Certini 2005; Wang & Kemball 2005). Soil microbial communities are essential for terrestrial ecosystem recovery and stability (Morriën et al. 2017). Although the impact of fire on microbial community structure is widely studied yet how prokaryotic community composition and diversity are affected by fire and how they change over time following fire in peatlands still needs to be explored. However, the impact of fire disturbance on peat soil properties introduces diversity, potentially enhancing bacterial richness by influencing the competitive dynamics among bacteria for resources (Artz et al. 2009). Bacterial phyla including Proteobacteria, Planctomycetia, Acidobacteria, Verrucomicrobia and Actinobacteria were the key dominant phyla in Stalybridge peat soil, consistent to results from burned soil of mesotrophic peatland (Belova et al. 2014). Furthermore, archaeal phyla including Euryarchaeota and Thaumarchaeota were detected in very low abundance, like the observations from soil collected in burned Qinghai peatlands (Li et al. 2022).

There were no statistically significant disparities in α-diversity, as assessed through Shannon, Simpson, and Chao1 indices, between the two peat soil layers within regions impacted by varying degrees of fire severity. This aligns with the findings of a prior study by Li et al. (2019), which also noted the absence of variation in prokaryotic sequences with respect to soil depth in fire affected sites. This phenomenon is likely attributed to the significant organic horizon consumption during fire disturbance, leading to the deposition of fire driven substantial ash and nutrient release, as demonstrated by Hammill and Bradstock (2006) and Sawyer et al. (2018). As a result, these mechanisms foster the homogenization of abiotic soil chemical characteristics and supports the microbial activity persistently throughout soil profiles. Consequently, at the intra-site level, comparable nutrient concentrations can support microbial diversity throughout various soil depths. However, our findings demonstrate that fire led to an augmentation in α-diversity within the shallow burn treatments at both peat depths, with significant increases observed at 4-, 11-, and 14- months post-fire. This aligns with Shen et al. (2013), who similarly documented a notable enhancement in bacterial α-diversity in the surface soil following burning treatments. The increase in microbial diversity in reaction to wildfire could be attributed to the increased accumulation of fire-tolerant species following fire disturbance, as observed in burned pine areas of North America, leading to greater microbial diversity in burned areas compared to non-burning control treatments (Crandall & Platt 2012). Previous research has noted that microbial diversity tends to exhibit a gradual increase and recovery following wildfire disturbances (Ferrenberg et al. 2013; Shen et al. 2016). In contrast, Shannon diversity exhibited an unexpected decline over time in deep burn treatments at both soil depths. This corresponds with the findings of Scott et al. (2013), who documented a significant decrease in soil bacterial α-diversity 4 and 16 weeks following a severe wildfire. A possible explanation is that soil bacterial diversity could potentially rebound within a single growing season due to rapid division and colonization processes (Xiang 2014). In summary, these results illustrate that fire had a profound and time-dependent effect on prokaryotic diversity.

In this study, fire severity had a pronounced and lasting effect on peat microbial communities over time. Previous research has shown that seasonal variation can strongly influence belowground microbial abundance and composition (Monson et al. 2006; Schmidt et al. 2007). Similarly, we observed significant shifts in prokaryotic community structure across the 4-, 11-, and 14-month post-fire sampling points, which coincided with autumn, spring, and summer, respectively. While these changes are primarily interpreted in relation to time since fire, it is important to recognise that post-fire timing and seasonal variation are inherently linked in this study. As such, some of the differences we observed may also reflect seasonal dynamics rather than recovery alone. Future studies would benefit from sampling across consistent seasonal windows or incorporating seasonal replicates to better distinguish fire effects from seasonal variation.

Shifts in prokaryotic community composition with time following shallow burn severity were notably more pronounced in the near-surface peat layer, potentially attributed to the rapid progression of bacterial secondary succession in post disturbance land reported by Scott (2013), which might significantly cause heterogeneity in our study in prokaryotic community structure at 14 months postburn. The deep burn severity had a substantial impact on sub-surface soil prokaryotic communities across the time gradient. This effect can be attributed to high severity fires, which remove aboveground plant communities and can result in significant alterations in microbial community structure (Tian et al. 2021). Whitman et al. (2019) also proposed that an escalation in burn severity leads to increased dissimilarity in microbial community composition in boreal forests.

A classification of bacterial groups as copiotrophic and oligotrophic based on nutrition and growth has been proposed (Fierer et al. 2007). Accordingly, Acidobacteria and Verrucomicrobia are considered oligotrophic (Dion & Nautiyal 2008), while Proteobacteria and Thaumarchaeota are typically copiotrophic (Smit et al. 2001). Our study demonstrated that wildfire significantly changed the relative abundance of these phyla along the time gradient and across peat layers. Despite a decrease in Proteobacteria following a burn event, it consistently constituted the most prevalent bacterial phylum, encompassing 26% to 37% of total sequences throughout all fire stages, irrespective of deep or shallow burn severity. Notably, Proteobacteria, characterized as Gram-negative bacteria, represents one of the most widely distributed soil bacterial lineages. Following wildfire, we observed a significantly reduced presence of Proteobacteria in the subsoil compared to the topsoil and decreased with respect to time. Current knowledge shows that some members of the Proteobacteria employ an “r-strategy" for growth characterized by fast growth rates, and a strong capacity for environmental adaptation (Mickan et al. 2017). Proteobacteria are also associated with the rhizosphere of plant (Wang et al. 2014). The deep burn fire severity can kill aboveground vegetation and destroy plant roots, which could be a significant factor explaining the different in relative abundance of Proteobacteria between near-surface and subsurface soil. However, the constant presence of dominant Proteobacteria after fire disturbance can enhance ecological resilience, signifying carbon-rich soil conditions (Fierer et al. 2007). To validate this hypothesis, further analyses focusing on their ecological functions would be required. For instance, in line with the findings of most previous studies, Acidobacteria exhibited a noteworthy decline in the top-surface soil following a wildfire event (Sun et al. 2016). Nonetheless, an elevated relative prevalence of Acidobacteria was observed in the subsurface soil, potentially attributable to the oligotrophic environmental conditions (Dion & Nautiyal 2008). In addition, we also observed that as time went on, Acidobacteria abundance persistently increased following deep burn fire. This might be due to postfire disturbance, increased soil pH, and a decrease in the amount of organic matter and nutrients, which provide competition for available carbon resources leading to an increase in Acidobacteria abundance (Bardhan et al. 2012; Meng 2021). It has been documented (Fierer et al. 2007) that Acidobacteria are typically "k-stratigets (Kapazitätsgrenze-strategistis)" and able to thrive in nutrient poor and stable environments and have highly sensitive to changing or disturbed conditions (Mickan et al. 2018). The increased in relative abundance of Acidobacteria observed in this study may result in a change in overall C storage capacity of the ecosystem (Pan et al. 2011).

This decline in Acidobacteria and Verrucomicrobia populations in near surface peat and their increase in subsurface due to altered soil conditions may influence carbon storage. This suggests an adaptive capability for these bacteria to flourish in challenging, nutrient-deficient conditions, indicative of a preference for oligotrophic habitats. Elliott et al. (2015) also identified a notable prevalence of Acidobacteria and Verrucomicrobia in unvegetated peat environments. Thaumarchaeota were also found to be higher in subsurface as compared to the upper peat layer. Our results are in line with the findings of Lin et al. (2014) who reported that peat soils exhibit a heightened presence of Thaumarchaeota constituting up to 60% of the microbial community in subsurface soil. Overall, wildfires show a significant effect on microbial community composition across peat depths that are persistent over time. However, further research is needed to uncover their ecological roles and interactions with the abiotic factors in relation with C cycling to comprehensively grasp the impact of frequent and intense disturbances on microbial communities. This study contributes to understanding the long-term microbial responses to wildfires in peatlands.

4.5 Conclusion

This study demonstrates that the temporal duration since a fire event is a significant determinant of most of the variance in prokaryotic community composition and structure. Fire severities induced a significant shift in prokaryotic diversity with time and eliminated the depth distribution. However, fire severity provoked significant impact community structural, and composition shifts in community composition across depth over 14 months. In the present study we have only three data points (4-, 11- and 14- months) and recovery of microbial community after fire is continuous and dynamic process. Collectively, these findings indicate that soil bacteria within peatlands possess a notable ability to rapidly regenerate following fire, potentially contributing to the preservation of carbon stability. However, longer term monitoring of microbial community composition after fire is essential. This study will improve our understanding and ability to predict the consequences on ecosystem function and C dynamics of peatland fire, which are critical in context of climate change mitigation and peatland conservation.

4.6 References

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4.7 Supplementary Information

Table S 4.1 Means± standard deviation of prokaryotic Shannon, Simpson and Chao1 across peat depth ,control and burn severities at different time.

	Variables	**Shannon**	**Simpson**	**Chao1**
Near-surface burn	Control 4 months	5.240±0.080	0.990±0.001	365.4±25.744
	Shallow burn 4 months	5.015±0.263	0.987±0.003	325.4±70.475
	Shallow burn 11 months	5.356±0.258	0.990±0.002	483.75±131.955
	Shallow burn 14 months	5.751±0.185	0.991±0.001	994±176.911
	Deep burn 4 months	5.641±0.476	0.993±0.004	512±185.031
	Deep burn 11 months	5.492±0.431	0.992±0.003	515.25±220.542
	Deep burn 14 months	5.378±0.353	0.988±0.004	732±169.571
Sub-surface burn	Control 4 months	4.885±0.414	0.984±0.004	358±132.830
	Shallow burn 4 months	4.651±0.171	0.979±0.008	304±18.668
	Shallow burn 11 months	4.727 ±0.522	0.980±0.009	366.25±154.760
	Shallow burn 14 months	5.128±0.300	0.984±0.004	676.8±106.290

	Deep burn 4 months	5.579±0.241	0.993±0.001	467.6±85.891
	Deep burn 11 months	5.062 ±0.665	0.985±0.008	399.666±174.978
	Deep burn 14 months	5.150 ±0.518	0.984±0.006	628.75±198.138

Table S 4.2 Mean± standard deviation of top ten significant prokaryotic taxa across peat depth and burn severities at different time.

	Variables	**Proteobacteria**	**Acidobacteria**	**Actinobacteria**	**Bacteroidetes**	**Verrucomicrobia**
	Control 4 months	39.801±3.782	8.182±2.561	18.069±3.554	16.486±4.079	6.188±2.594
Near-surface burn	Shallow burn 4 months	37.784±2.529	12.015±4.322	19.411±2.945	14.796±3.026	4.985±3.001
	Shallow burn 11 months	29.308±3.144	14.358±4.8001	18.429±4.108	8.676±3.530	9.269±4.248
	Shallow burn 14 months	26.708±3.347	19.043±3.328	22.747±4.646	4.934±2.249	8.664±2.106
	Deep burn 4 months	31.265±5.531	36.382±4.068	9.394±2.633	1.940±0.734	6.626±1.231
	Deep burn 11 months	26.444±6.073	36.168±3.675	9.249±4.868	2.730±1.810	8.498±0.979

	Deep burn 14 months	28.911±4.491	32.341±5.246	13.509±6.395	2.928±1.034	6.700±0.983
	Control 4 months	28.902±6.925	32.847±5.027	10.739±3.468	1.846±0.417	6.632±1.466
Sub-surface burn	Shallow burn 4 months	32.896±5.479	18.115±4.054	14.884±2.450	7.005±2.780	8.836±2.812
	Shallow burn 11 months	32.686±5.611	16.687±8.496	17.240±3.530	6.984±5.059	6.598±0.980
	Shallow burn 14 months	26.511±5.656	24.861±4.355	19.086±7.417	2.057±1.030	8.130±3.165
	Deep burn 4 months	30.135±3.232	22.145±2.819	14.161±3.169	3.325±1.112	5.991±2.138
	Deep burn 11 months	28.584±2.585	32.823±7.171	7.245±2.727	2.116±1.251	7.883±1.210
	Deep burn 14 months	25.628±1.586	32.120±5.256	12.554±3.804	2.759±0.520	7.114±1.565

Table S 4.3Means± standard deviation of prokaryotic taxa across peat depth and burn severities at different time.

	Variables	**Planctomycetes**	**Patescibacteria**	**WPS-2**	**FCPU426**	**Thaumarchaeota**
	Control 4 months	2.480±0.888	3.707±1.103	0.479±0.205	0.024±0.042	0.065±0.082

Near-surface burn	Shallow burn 4 months	3.213±1.242	2.783±1.996	1.926±1.311	0.003±0.007	0.140±0.153
	Shallow burn 11 months	5.395±2.291	5.039±0.490	4.345±1.980	1.113±1.214	0.161±0.139
	Shallow burn 14 months	6.107±2.273	3.546±1.870	1.170±0.727	1.543±0.654	2.187±2.580
	Deep burn 4 months	4.063±1.324	0.562±0.349	0.875±0.345	0.530±0.382	4.203±2.797
	Deep burn 11 months	5.780±2.250	0.244±0.204	0.745±0.397	3.233±1.403	3.236±2.333
	Deep burn 14 months	4.514±1.811	0.824±0.398	0.676±0.383	2.753±1.415	2.706±1.119
	Control 4 months	4.323±1.307	0.974±0.858	1.197±1.192	0.345±0.283	5.793±4.161
Sub-surface burn	Shallow burn 4 months	7.783±2.241	1.671±0.428	1.438±0.944	0.790±1.603	1.334±2.517
	Shallow burn 11 months	7.126±5.372	2.745±1.108	1.420±0.404	1.017±1.536	1.193±1.362
	Shallow burn 14 months	6.627±5.836	2.072±1.261	1.504±1.333	1.942±1.229	3.353±3.906
	Deep burn 4 months	8.865±1.672	0.873±0.309	2.031±0.771	0.384±0.199	2.405±1.974
	Deep burn 11 months	3.990±1.937	0.988±0.771	0.748±0.345	0.620±0.926	6.028±4.430

	Deep burn 14 months	3.222±0.295	0.773±0.521	0.623±0.509	2.258±1.080	4.953±2.248

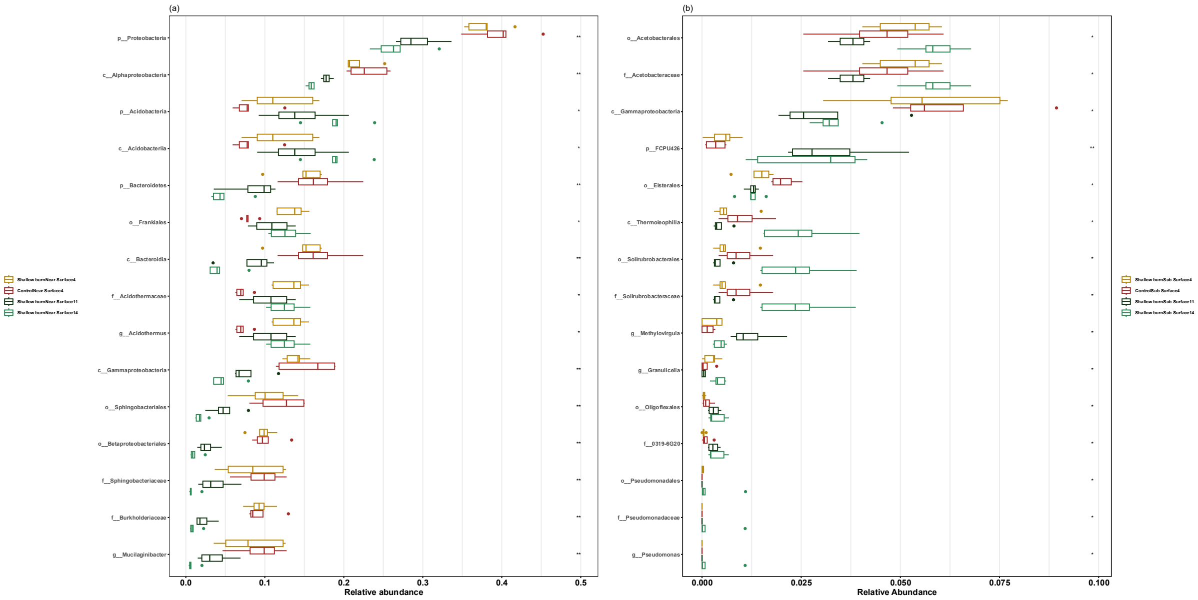

Figure S4.1Box plots showing percentage relative abundance of top 15 indicator prokaryotic phyla (bacterial and archaeal) of shallow burn (a) near-surface (b) sub-surface peat soil of control, 4- ,11- and 14-months across peat depths.

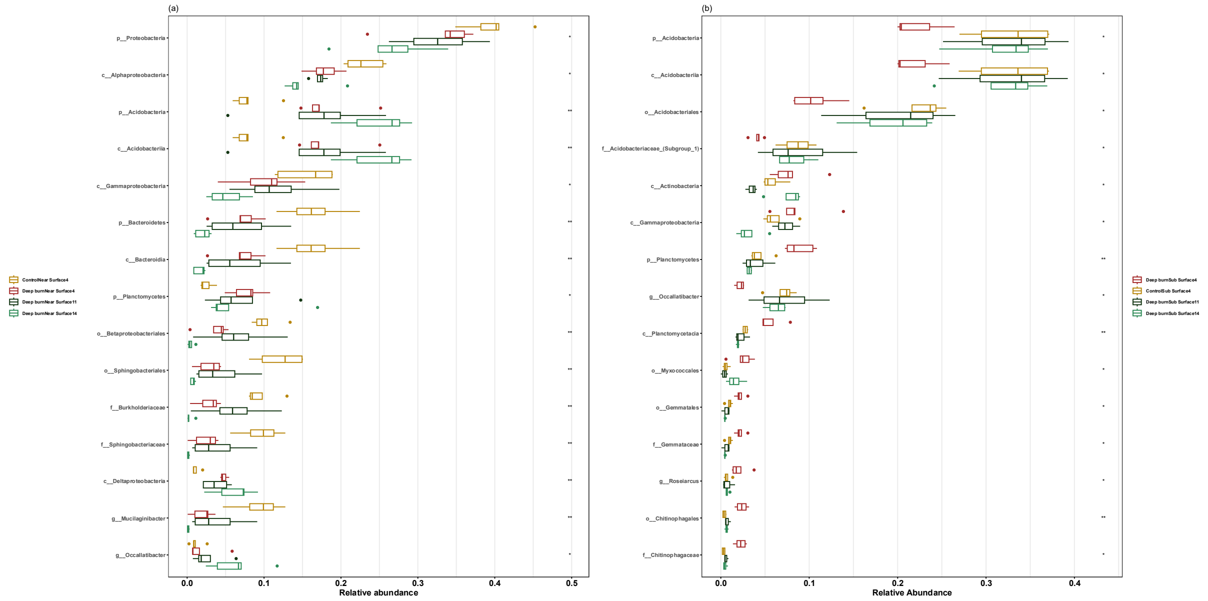

Figure S4.2 Box plots showing percentage relative abundance of top 15 indicator prokaryotic phyla (bacterial and archaeal) of deep burn (a) near-surface (b) sub-surface peat soil of control, 4- ,11- and 14-months across peat depths.

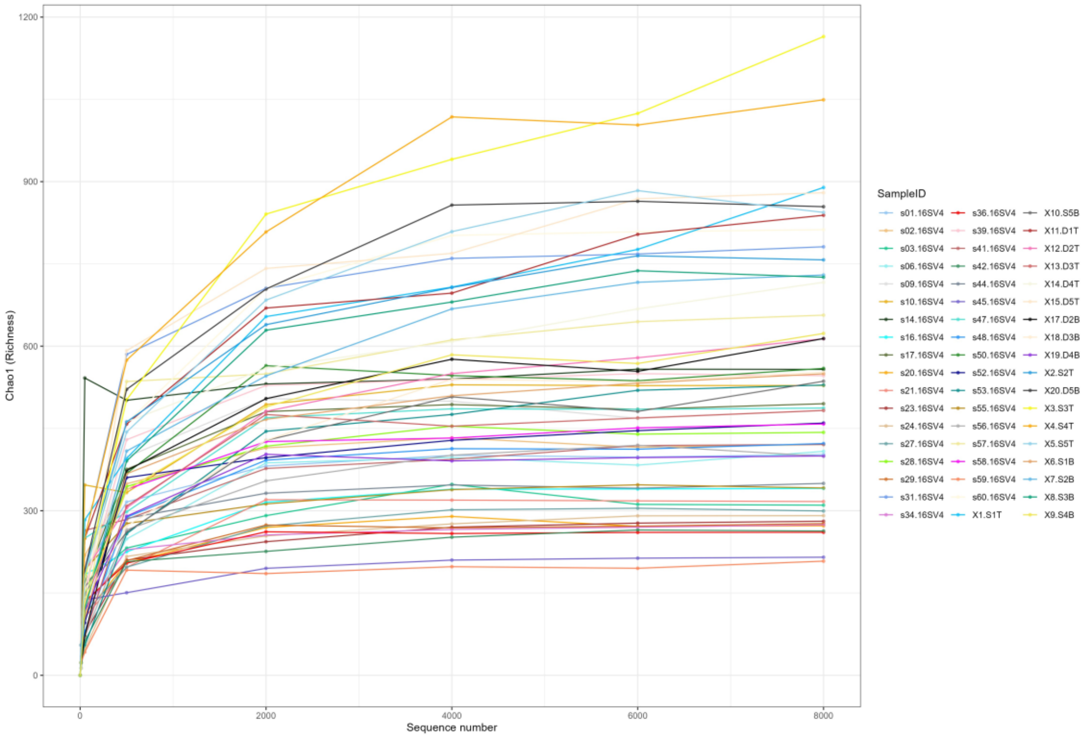

Figure S 4.3 (a) Richness rarefaction of the different samples

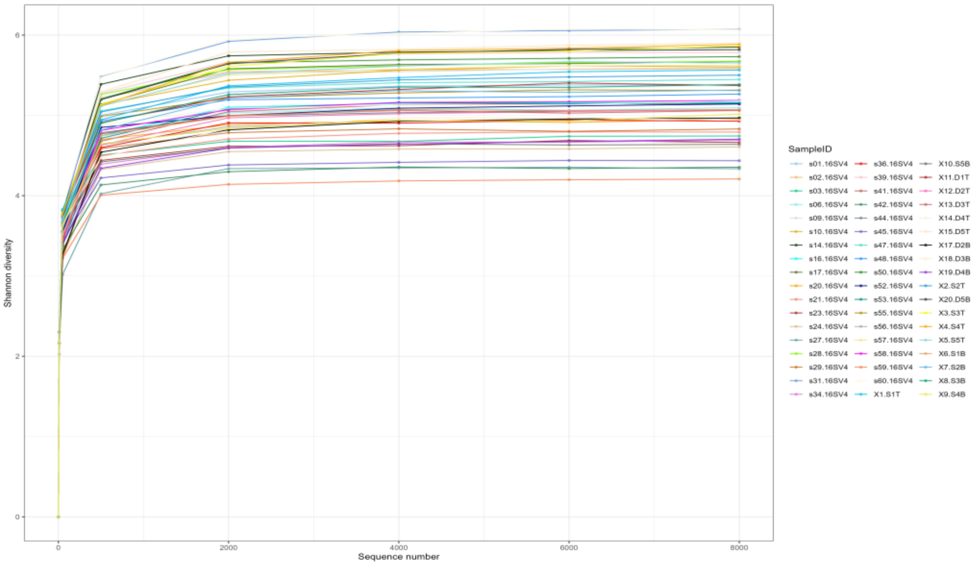

Figure S 4.2 (b) Shannon index analysis of the different samples

Chapter 5 General Discussion

Northern peatlands have accumulated substantial carbon (C) stocks due to their unique environmental conditions, including acidic and anoxic environments, and the specific composition of their vegetation. These factors have made peatlands a vital global carbon reservoir, playing a key role in regulating the carbon cycle and climate. However, these ecosystems are highly sensitive to current and future environmental and climatic changes, which could alter their capacity to store carbon. Such changes may weaken their role as carbon sinks or potentially convert them into net carbon sources (Dise 2009; Leifeld et al. 2019).

There is a strong functional link between aboveground plant communities and belowground microbial communities that mediate carbon cycling in peatlands (Lindo et al. 2013; Ivanova et al. 2020). Despite this, significant knowledge gaps remain regarding the vulnerability of microbially-driven carbon processes in peatlands and how plant community composition, along with abiotic factors like water table depth, influence ecosystem functional processes (Limpens et al. 2008; Turetsky et al. 2014). Understanding how Sphagnum-dominated ombrotrophic bogs respond to environmental and climatic changes is critical for predicting the future dynamics of carbon cycling in these ecosystems.

Given the vulnerability of peatlands to changing environmental and climatic conditions, and their critical role as global climate regulators, there is a growing need to better understand their overall carbon (C) balance. Microbial community dynamics, closely linked to plant community composition, are essential in driving nutrient cycling, C sequestration, and overall ecosystem functioning. While substantial research exists on microbial-driven C cycling processes and related greenhouse gas (GHG) emissions from northern peatlands in response to climate warming, nutrient deposition, and land-use change, there is still limited knowledge about microbial community composition and its interaction with plants across environmental gradients. Understanding the complexities of plant-microbe interactions under changing global climate conditions is crucial to gaining insights into the sensitivity and resilience of these ecosystems. Such knowledge will enhance predictions of how peatlands may respond to future environmental changes, particularly in terms of their capacity to store carbon and maintain ecosystem stability.

In my doctoral research, I investigated the functional relationship between plant and microbial communities in peatlands, focusing on how shifts in plant communities and microhabitats affect belowground processes. Specifically, I explored the long-term impacts of plant community shifts, > 8 years of vascular plant removal, on peatland ecosystem functioning. This approach allowed me to use plant-microbe interactions to study belowground processes such as decomposition dynamics across different microhabitats. My research aimed to test the overarching hypothesis that peatland plant functional types (PFTs) and their interactions with environmental and climatic factors such as changes in ecohydrological regimes can explain variations in microbial-driven carbon (C) cycling processes and biological properties within peatlands.

Additionally, I examined the ability of microbial communities to recover after abrupt disturbances, such as wildfires, addressing a critical knowledge gap about the recovery of biotic communities at both taxonomic and functional levels post-disturbance. In this discussion, I will highlight the key findings related to the effects of environmental and climatic changes, specifically long-term disturbances (vascular plant removal) and abrupt disturbances (wildfire), on microbial community composition and associated processes, including metabolic activity and decomposition dynamics in northern peatlands.

5.1 Plant microbe interaction across hydrological gradient

In Chapter 2, I examined the linkages between aboveground plant communities and belowground microbial communities across hydrological gradients in contrasting peatland microhabitats, specifically lawns and hummocks. These microhabitats represent distinct ecohydrological niches within peatlands, with hummocks generally drier and lawns typically more waterlogged. My results showed that the relative abundance of microbial populations was primarily influenced by microhabitat type, with notable differences in microbial community structure between lawns and hummocks. Using amplicon sequencing, I observed that certain microbial taxa, such as Rickettsiales (Proteobacteria) and Methylacidiphilales (Verrucomicrobia), displayed significant shifts in relative abundance depending on the presence or absence of specific PFTs. This finding extends earlier work by Limpens et al. (2008) and Turetsky et al. (2014), which highlighted the influence of PFTs on microbial community structure, by illustrating that hydrological differences also shape these microbial dynamics.

A key observation was the increase in Rickettsiales in graminoid-dominated plots, suggesting that graminoid species may play a more prominent role in carbon cycling compared to other dominant PFTs, such as ericoids. This observation aligns with findings by Richardson et al. (2014), who noted the significant impact of graminoids on GHG emissions. In this study, the selective increase in Rickettsiales under graminoid cover implies that these microbial groups may be involved in the decomposition and assimilation of carbon compounds derived from graminoid plants, particularly root-derived labile carbon. These findings underscore the role of graminoids in shaping belowground microbial communities and influencing C fluxes within peatlands, indicating their potential as bioindicators of plant-driven carbon cycling processes.

While these findings support the broader evidence that plant community composition can drive shifts in microbial community composition in peatlands, a key question remains about the specific mechanisms behind this relationship. It is unclear whether microbial community composition is shaped more by differences in litter quality (e.g., recalcitrant vs. labile carbon) or by root exudates produced by different vascular plant species. My research findings suggest that plant-derived labile carbon, particularly from graminoid species, may play a critical role in selecting specific microbial taxa. This hypothesis provides a basis for future studies to investigate the mechanistic underpinnings of plant-microbe interactions in peatlands.

Additionally, the differential responses of microbial taxa to plant removal treatments across hydrologically distinct microhabitats indicate that these microbes may be key indicators of plant-driven carbon cycling processes in northern peatlands. For instance, microbial communities in lawns, which are characterized by more water-saturated conditions, may have a different functional capacity for carbon processing compared to those in drier hummocks. These findings align with research by Kaštovská et al. (2018) and Chroňáková et al. (2019), who documented the influence of hydrological gradients on microbial community composition. My results suggest that prolonged exposure to water table heterogeneity along the lawn-hummock gradient likely contributed to the introduction and establishment of unique prokaryotic taxa, resulting in distinct microbial assemblages across microhabitats.

This chapter’s findings support the synthesis by Andersen et al. (2013), who emphasized the importance of plant community composition and hydrological regimes in shaping microbial communities within disturbed peatlands. Furthermore, the role of PFTs as regulators of biological properties in the context of altered hydrological gradients supports the idea that the composition of aboveground plant communities can be a predictor of belowground microbial community composition and diversity, influencing nutrient cycling and carbon (C) fluxes (Harrison et al. 2010). In light of these findings, distinct PFTs and water table depth may serve as integrative proxies for microbial-driven processes that support peatland C cycling, as highlighted by Clark et al. (2010).

5.2 Role of Plant functional types and ecohydrological gradient in decomposition dynamics

Building on the results from Chapter 2, Chapter 3 extended the investigation by examining the influence of PFTs on microbial metabolic activity, specifically extracellular enzymatic activities (EEAs), and decomposition processes across two contrasting hydrological microenvironments (lawns and hummocks) in a blanket bog. Through a long-term plant removal experiment, I observed that vegetation composition was a major factor driving microbial metabolic activity and influencing decomposition dynamics in these microhabitats, which differ in hydrological conditions. In particular, vascular PFTs had a more pronounced effect on EEAs, especially hydrolases, compared to the legacy effects of long-term vegetation removal on decomposition.

These findings support the work of Matulich and Martiny (2015), who documented a direct link between shifts in microbial community composition and EEA activity. The observed changes in EEAs across lawn-hummock microhabitats likely reflect underlying shifts in microbial community composition, as presented in Chapter 2. Additionally, water table gradients significantly influenced early-stage decomposition dynamics, as indicated by the decomposition rate constant (k) and stabilization factor (S) based on the TBI protocol (Keuskamp et al. 2013). Lawns, which exist at the oxic-anoxic interface, exhibited more pronounced decomposition dynamics, suggesting that they may contribute to higher carbon sequestration potential and play a critical role in carbon cycling (Hoyos-Santillan et al. 2019).

The results of this chapter add to the evidence that PFTs strongly influence potential EEAs, serving as proxies for microbial metabolic activity. My findings show that PFTs significantly impact metabolic activity, particularly hydrolytic enzymes, and that this effect is microhabitat dependent. In lawns, microbial activity appeared less reliant on plant-derived labile carbon, leading to increased hydrolase activity in the absence of vascular PFTs. In contrast, the removal of vascular PFTs in hummocks reduced hydrolytic activity, suggesting that climate warming, which is expected to promote the growth of vascular plants over mosses, may enhance rhizosphere carbon inputs and alter ecosystem functions by changing carbon flow in peatlands (Jassey et al. 2013; Robroek et al. 2016).

5.3 Response and recovery of microbial communities following wildfire

Among the various ways in which environ-climatic changes can affect belowground biological properties, wildfire is one of the most extreme natural disturbances in peatlands (Mataix-Solera et al., 2009; Dooley & Treseder, 2012). As climate warming intensifies and droughts lengthen, fire regimes are shifting, with long-lasting effects on carbon (C) dynamics in these ecosystems (Turetsky et al., 2015). Chapter 4 focused on assessing the direct impacts of wildfire, particularly shallow and deep burn severities, on microbial communities across peat depths. The results reveal that microbial diversity remained relatively stable across peat layers. However, shallow burn severity led to an increase in alpha diversity over time, whereas deep burn severity caused a decline in biodiversity, suggesting distinct recovery trajectories depending on burn severity.

To understand these findings, I applied the copiotroph-oligotroph theory, which classifies microbial groups based on their ecological strategies (Fierer et al., 2007). My results show that wildfires influence microbial communities, with copiotrophic Proteobacteria remaining dominant post-fire, suggesting resilience and adaptability that likely support sustained ecosystem functioning. Proteobacteria, known for their fast growth and preference for carbon-rich environments, may help maintain peatland carbon cycling by rapidly utilizing post-fire organic matter inputs. In contrast, oligotrophic Acidobacteria, which thrive in low-carbon environments and tend to grow more slowly, exhibited a different response, potentially leading to shifts in long-term carbon sequestration and organic matter turnover (Pan et al., 2011).

These shifts in microbial composition have significant implications for carbon stocks in peatlands following wildfire disturbances. The resilience of copiotrophic microbes such as Proteobacteria could stabilize ecosystem functions and carbon cycling in the immediate aftermath of fire, but the reduced presence of oligotrophic taxa like Acidobacteria may alter the peatland's capacity for long-term carbon storage. These findings highlight the importance of incorporating microbial dynamics into carbon cycling models for peatlands, particularly given the anticipated increase in wildfire events with climate change. Including microbial responses in these models could improve the accuracy of long-term predictions related to climate-carbon feedback in peatland ecosystems (Ritson et al., 2021), providing a more comprehensive understanding of peatland resilience and function under increasing environmental pressures.

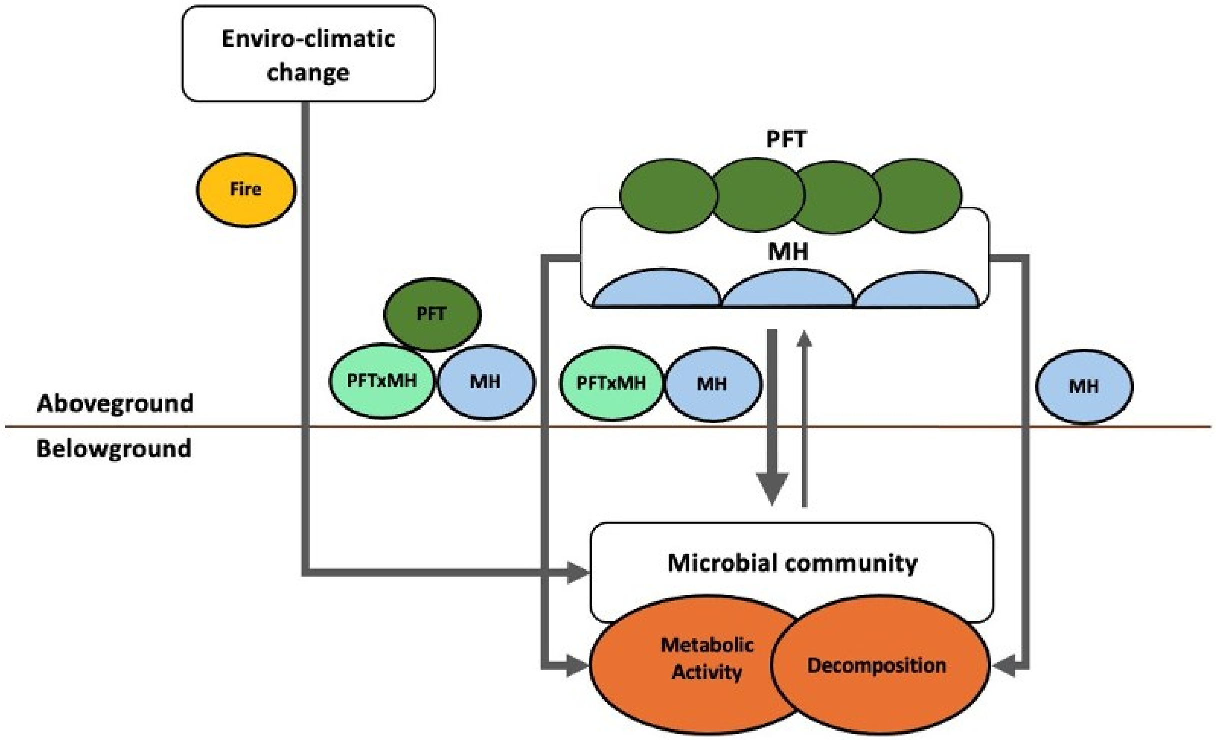

Figure 5.1 A schematic overview of the overall outcomes of this research thesis. The relative and interactive effects of plant functional type (PFT), microhabitat (MH), PFTx MH and fire that were found to have an impact on microbial communities are shown in circles. A wider arrow indicates a direct and clearer observed effect.

5.4 Conclusion

The primary objective of this research was to enhance our understanding of how peatland microbial communities respond to climate-driven changes in vegetation composition and hydrological regimes, as well as to assess their sensitivity or resilience to wildfire (Figure 5.1). The findings reveal that the interactive effects of plant community composition and microhabitat exert a stronger influence on microbial communities than individual plant functional type (PFT) variations alone. Chapter 2 demonstrated that bacterial group abundances were influenced by plant community composition, underscoring the combined role of PFTs and hydrological fluctuations across different microhabitats in shaping microbial communities. Spatial variability in microbial composition, particularly among bacterial and archaeal groups, was also observed, likely driven by peat biogeochemical factors such as redox status and oxygen availability. These findings highlight the critical role of hydrological feedback when evaluating the impact of current and future climate-driven changes on peatland plant-microbe interactions.

The research also emphasizes the importance of plant-microbe interactions in regulating decomposition dynamics, as demonstrated through long-term plant removal experiments across varying microhabitats. Changes in vegetation, coupled with soil moisture gradients, modulated microbial extracellular enzymatic activities (EEAs), which in turn influenced belowground decomposition processes. This has important implications for carbon dynamics in peatlands, suggesting that both vegetation shifts and hydrological properties must be considered when predicting ecosystem responses to environmental changes.

In addition to the effects of climate-driven shifts in plant community composition on microbial communities and metabolic activity across the lawn-hummock gradient (Chapters 2 and 3), wildfire exacerbated by climate warming and prolonged drought also directly influenced prokaryotic community composition (Chapter 4). Prokaryotic communities exhibited significant temporal and depth-dependent changes in response to wildfire, indicating that they can be either sensitive or resilient to such disturbances. These shifts have potential consequences for ecosystem processes like carbon sequestration, further emphasizing the importance of considering microbial dynamics in the context of climate change. Collectively, these findings underscore the complex interactions between vegetation, hydrology, and microbial communities in peatland ecosystems and highlight the need to integrate microbial processes into future models of carbon cycling under changing environmental conditions.

5.5 Recommendations for future work

The findings from this thesis provide valuable insights into plant-microbe interactions across microtopographic gradients in peatlands, as well as the response and recovery of microbial communities after wildfires. Based on these results, several key areas for further research have been identified, each building on the knowledge gained from this work.

1. **Functional validation of microbial communities using multi-omics approaches:**

   A crucial next step is to extend the analysis beyond community composition and explore the functional potential of microbial communities. By employing techniques such as transcriptomics and metabolomics, future research can link community shifts to functional processes, such as carbon and nutrient cycling. This approach will help to understand how microbial community dynamics directly influence ecosystem function, providing critical insights into the resilience of peatlands under environmental changes (Singleton et al. 2018; Wilson et al. 2021).

2. **Incorporating eukaryotic communities into plant-microbe interaction studies:**

   While this thesis primarily focused on prokaryotic communities, the roles of fungi and protists are equally vital, particularly in decomposition and nutrient cycling. Given their importance in peatland ecosystems, future work should prioritize the inclusion of these eukaryotic groups to create a more complete picture of microbial contributions to biogeochemical processes (Juan-Ovejero et al. 2020).

3. **Extending decomposition studies with long-term monitoring:**

   The short-term decomposition study using the TBI method provided valuable insights, but future research should aim for longer incubation periods to capture the full adaptation of microbial communities to organic matter breakdown. By extending the monitoring over multiple seasonal cycles, we can better understand decomposition dynamics and how they fluctuate in response to environmental variables (Prescott 2010). This is particularly important for predicting long-term carbon storage in peatlands.

4. **Integrating soil physicochemical parameters in post-wildfire studies:**

   Understanding how wildfire affects microbial communities requires an in-depth analysis of the altered soil environment. Future studies should incorporate physicochemical soil properties such as pH, moisture content, and nutrient stoichiometry to better model how these abiotic factors influence microbial recovery and carbon dynamics (Bandla et al. 2024). This combined approach could provide more comprehensive models of peatland carbon cycling under fire disturbances.

5. **Exploring post-wildfire vegetation succession and its impact on microbial communities:**

   The recovery of plant communities after a wildfire plays a key role in shaping microbial dynamics. Investigating the succession of vegetation and its effects on microbial community composition and function will help clarify the feedback mechanisms between plants and soil microbes (Hart et al. 2005). This could also inform management strategies aimed at promoting ecosystem recovery and resilience following wildfires.

Focusing on these priority areas will allow future research to build on the insights from this thesis and deepen our understanding of the complex interactions between plant communities, microbial dynamics, and ecosystem processes in peatlands.

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