Landscape ecology of microbes in peatlands under different management regimes

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Initialisms, acronyms and abbreviations used in this thesis

16S rRNA- 16S ribosomal RNA

18S rRNA -18S ribosomal RNA

a.s.l- Above sea level

AIC-Akaike Information Criterion

Al- Aluminium

amoA- Ammonia oxidizing archaea

amoB- Ammonia oxidizing bacteria

ANAMMOX- Anaerobic Ammonium Oxidation

AnAOB - anammox, ANaerobic AMMonia Oxidation

ANOVA- Analysis of variance

AOSR- Athabasca Oil Sands Region

ASV- Amplicon sequence variant

B-Boron

CA- Cytoplasmic assimilatory

Ca- Calcium

Cd- Cadmium

CH_4- Methane

chiA- Chitinase

cnorB- Cytochrome bp Nitric Oxide Reductase

CO_2- Carbon dioixide

Cu- Copper

DADA2 - Divisive Amplicon Denoising Algorithm 2

DNA - Deoxyribonucleic acid

DNRA - Dissimilatory nitrate reduction to ammonium

ECN- Environmental change network

EDTA- Ethylenediaminetetraacetic acid

EU- European Union

Fe – Iron

Hao- Hydroxylamine oxidoreductase

HCL- Hydrogen chloride

HNO_3- Nitric acid

HSD-Honest significance test

HSM-Habitat Suitability Modelling

Hzo- Hydrazine Oxidoreductase

IC- Ion chromatography

ICP-OES- Inductively coupled plasma atomic emission spectroscopy

IPCC- The Intergovernmental Panel on Climate Change

ITS- Internal Transcribed Spacers

K- Potassium

KCI - Potassium chloride

M - Mean

mcrA- Methyl coenzyme M reductase

MENA- Molecular Ecological Network Analysis

Mg- Magnesium

Mn- Manganese

MSA- Methanesulfonic acid

mtC- Metric tonnes carbon

N_2 – Dinitrogen

N_2H_4- Hydrazine

N_2O- Nitrous oxide

NapA- Periplasmic nitrate reductase

Nar- Nitate reductase

NarG- Respiratory nitrate reductase subunit alpha

nasA- Assimilatory nitrate reductase catalytic subunit

NGS- Next generation sequencing

NH_2OH- Hydroxylamine

NH_4^+ - Ammonium

NifH – Nitrogenase

Nir- Nitite reductase

NirK- Copper-containing nitrite reductase

NirS- cytochrome *cd* Nitrite reductase

NNR- National nature reserve

NO- Nitric oxide

NO_2^- - Nitrite

NO_2 - Nitrogen dioxide

NO_3^- - Nitrate

nosZ – Nitrous oxide reductase

nrfA- Nitrite reductase (cytochrome; ammonia-forming)

nxr- Nitrite Oxidoreductase

OTU- Operational taxonomic unit

Pb- Lead

PCA- Principal component analysis

PCoA- Principal coordinates analysis

PCR- Polymerase chain reaction

P-Phosphorus

QIIME2- Quantitative insights into microbial ecology 2

qnorB- quinol-oxidizing single-subunit class nitric oxide reductase

qPCR- Quantitative polymerase chain reaction

RDA-Redundancy analysis

RMT- Random matrix theory

RNA- Ribonucleic acid

rpm- rotations per minute

S- Sulphur

SD – Standard deviation

SOM- Soil organic matter

SQI- Soil quality indicators

TIN- Total inorganic nitrogen

TMM- Trimmed mean of M values

UDG- Uracil-DNA glycosylase

UK- United Kingdom

UNESCO - United Nations Educational, Scientific and Cultural Organization

VIF- Variation inflation factor

WEP- Water extractable phosphorus

Units

µl – Microlitre

cm- Centimetre

G cm^{-2} - Grams per Square Centimetre Pressure Unit

km^2- kilometre square

M- Meter

m^2- Metre square

ML- Millilitre

mM-Millimolar

nM- Nanomole

Preface

I can confirm that the presented research and write-up are the researcher's own and that the study proposal and ethical considerations was approved (ETH1819-0101). All fieldwork was undertaken with permission from the landowners. The chapters contained in this thesis have been submitted to, or are being prepared for submission to relevant journals. Beacuse each chapter represents work that has been submitted, or is intended for submission to individual journals, an overlap in some information is unavoidable.

Chapter 3- "Microbial Communities and Biogeochemical Functioning across Peatlands in the Athabasca Oil Sands Region of Canada: Implications for Reclamation and Management" is published as **Allingham, S.M**., Nwaishi, F. C., Andersen, R., Lamit, L. J., & Elliott, D. R. (2022). Microbial Communities and Biogeochemical Functioning across Peatlands in the Athabasca Oil Sands Region of Canada: Implications for Reclamation and Management. *Land Degradation & Development*. **doi.org/10.1002/ldr.4549**

Author contributions: Felix Nwaishi and Roxane Andersen conducted the field work, David Elliott and Louis J Lamit conducted preliminary bioinformatics analysis, I conceptualized the paper, performed all analyses and wrote the manuscript.

Chapter 4- "Effects of a prescribed burning regime on vegetation, soil physicochemistry and prokaryotic microbial communities in surface and subsurface peat" is being prepared for publication as "**Allingham, S.M**., Ramsey, A.D., Drake, S.J., Field, C.D., & Elliott, D.R. Effects of a prescribed burning regime on vegetation, soil physicochemistry and prokaryotic microbial communities in surface and subsurface peat" and is targeted for the journal **'Science of the Total Environment'.**

Contributions: I designed the study with advice from all authors, carried out all fieldwork, bioinformatic and statistical analysis and wrote the manuscript. All authors have assessed the manuscript draft.

Chapter 5 - "Response of soil fungal communities and functional traits to prescribed burning regimes in surface and subsurface peat" is being prepared for publication as "**Allingham, S.M**, Ramsey, A.D, Drake, S.J, Field, C.D., & Elliott, D.R. Response of soil fungal communities and functional traits to prescribed burning regimes in surface and subsurface peat" and is targeted for the journal '**Geoderma'**.

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Chapter 6 - "Changes in microbial populations and nitrogen functional genes in soil profiles of a peatland under different burning regimes" is being prepared as "**Allingham, S.M,** Ramsey, A.D, Drake, S.J, Field, C.D., & Elliott, D.R. Changes in microbial populations and nitrogen functional genes in soil profiles of a peatland under different burning regimes" and is targeted for the journal '**Applied Soil Ecology'.**

Contributions: I designed the study with advice from all authors, carried out all fieldwork, bioinformatic and statistical analysis and wrote the manuscript. All authors have assessed the manuscript draft.

The work has been disseminated through conferences:

Effects of rotational burning regimes on prokaryotic community composition and network stability in surface and subsurface peat- The International Society for Microbial Ecology, Lausanne, Switzerland Upcoming 14-19 August 2022.

Microbial Communities and Biogeochemical Functioning across Peatlands in the Athabasca Oil Sands Region of Canada: Implications for Reclamation and Management. 16th international peatland congress. Estonia. May 2021.

ESRC. Microbial Ecology of a peatland under different burning regimes. Derby, May 2021.

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Abstract

Peatlands are essential ecosystems that play a significant role in the sequestration of carbon, water provisioning and global biodiversity. However, human activities are threatening their ability to sustain important ecosystem services. Soil microbial activity supports ecosystem processes in peatlands, but little is known about the main drivers of microbial community dynamics and their association with ecosystem functioning. Therefore, to better forecast the response of the microbiome to management regimes, a deeper understanding is required. The overall goal of this thesis is to identify the environmental drivers of peatland soil microbial communities and to investigate the effects of land management on community composition, function and resistance to habitat change. The study was based on the analysis of a pre-existing data set on microbial communities regarding land reclamation in Canada and on original data collection and analysis regarding burning regimes at Moor House Nature Reserve, UK. The Canadian data were used to determine how microbial communities and function change along three natural fens and a constructed fen in the Athabasca oil sands region of Alberta and assess the impact of this reclamation practice. The UK research focused on investigating how prescribed burning affects soil properties, microbial community structure and microbial N-cycling using a range of approaches including next-generation sequencing and qPCR. Overall, results show first, total substrate respiration was significantly higher in the constructed fen, yet, the diversity of fungi and prokaryotes was higher in the treed-rich fen and community composition was significantly different between fens. However, prokaryote community composition was similar in the constructed fen to the treed-rich fen showing a resilience of the community to soil transfer. Second, there were changes in archaeal, bacterial and fungal diversity and community composition between burn treatments and soil profiles. Fungal diversity showed a more drastic change across burn treatments throughout the soil profile and there was also a shift in the relative abundance of trophic modes. Co-occurrence network analysis revealed that the non-burn topsoil had a larger and more complex network structure with more positive links than those under rotational burns. Third, *amoA-AOA, amoA-AOB* and *nifH* were higher in the topsoil of the non-burn control while the abundance of *nirK* was higher in plots under short rotation and long rotation regimes. *ChiA* abundance was greater in plots under a short rotation burn regime and decreased with soil depth. This result suggests that microbial N turnover potential is affected by the practice of burning. The changes in microbial communities and function are anticipated to have an impact on important peatland ecosystem services.

Chapter 1. Introduction

1.1: Context of the study and problem statement

Peatlands provide a wide range of ecosystem services, including the sequestration of carbon, biodiversity retention, climate and energy flux regulation, soil erosion control, and land stabilisation (Kløve *et al*., 2017; Lal, 2004; Rosario-Ortz *et al*., 2016; Smith *et al*., 2015;Yang *et al*., 2009). However, degradation, human activity and land mismanagement can threaten the function of peatlands (Evans *et al*., 2014). The soil microbiome is linked to many important soil functions, such as the decomposition of organic matter and biogeochemical cycling (Maier, 2015). Furthermore, microorganisms play critical roles in the promotion of plant growth, and changes in vegetation structure due to their symbiotic relationship (Mendes *et al*., 2015). Peatlands are the most common type of wetland (50-70%), covering approximately 3% of the world's land area (Joosten & Clarke, 2002), with the UK containing 15-19% of ombrotrophic peatland, which has been assigned a priority habitat in the EU and UK Biological Action Plans (Littlewood *et al*., 2010). There is an increasing interest in peatlands due to the wide range of functions and utilisations with regards to the environment (Bonn *et al*., 2016; Kløve *et al*., 2017). However, despite the economic and ecological importance of peatlands, very little is known about how anthropogenic pressures affect microbial communities that are essential to the functioning of the ecosystem. Unfortunately, these fragile ecosystems are under threat from various anthropogenic pressures such as grazing, agriculture, drainage and burning (Page & Baird, 2016). Anthropogenic activity of peatlands is often accompanied by negative effects which include increased levels of greenhouse gas emissions (Veber *et al*., 2018), increased loss of carbon through the decomposition of aerobic peat and reduced carbon sequestration by photosynthesis as well as the loss of biodiversity (Roulet, 2000). Approximately 80% of ecosystem services are connected to soils and thus research into the effects of different land-use as well as restoration should aim not only to recover soil support but restore ecosystem function (Lal, 2001). Thus, the degradation of soil inevitably leads to affecting the ecological function of microorganisms and the important ecosystem services they provide. As a result of these negative effects, it is essential that current research concentrates on developing measures to mitigate these issues through optimal management (Kløve *et al*., 2017).

Plants are an important controlling system of peatlands and plant-microbe interactions are considered to be the driving force for a healthy microbiome and ecosystem functionality (Robroek *et al*., 2015). However, due to environmental constraints such as deep peat, water table variability and low nutrients the potential for plants to grow is limited, making microorganisms a more vital component of the ecosystem (Ritson *et al*., 2021). Research on the links between plants and microbes have been studied intensively (Čapek *et al*., 2018; Kuiper *et al*., 2014; Maslov & Maslova, 2020; Robroek *et al*., 2015; Ward *et al*., 2013) and highlights the importance of plant communities on microbial processes. Management of peatlands centres around re-vegetating bare peat, land rewetting and shifting vegetation assemblages for raising the water table (Murdiyarso *et al*., 2010). Management practices can have a positive impact on the microbial and physiochemical properties of the soil but can often lead to negative impacts on soil quality (Kløve *et al*., 2017). Biological properties such as microbial community structure could prove essential indicators of soil quality as soil productivity is largely determined by microbial activity such as nutrient cycling (Bissett *et al*., 2013). It has been recognised that there is an urgent need for research concerning microbial communities in peatland soil which are an undervalued indicator for land managment (Bonn *et al*., 2016) as their response to environmental change is far more rapid than plants due to their short generation time (Logue *et al*., 2015). By overlooking soil microbial communities, management efforts may fail to restore the major heterotrophs in terrestrial ecosystems and hence affect carbon and nutrient cycling (Nurulita *et al*., 2016). The ecological reality necessitates the development of relevant microbial indicators that are able to assess soil functionality indirectly and are responsive to both management and environmental changes (Paz-Ferreiro & Fu, 2016).

Several studies have explored the composition and activity of soil microbial communities in peatlands as a biometric for soil function (Andersen *et al*., 2006; Artz *et al*., 2009; Chapman *et al*., 2017; Fisk *et al*., 2003; Golovchenko *et al*., 2007; Machado de Lima *et al*., 2021). These studies have highlighted the utility of microbial indicators and links with ecosystem function, particularly in areas undergoing restoration or management. However, there is limited data on the specific distribution of microbial communities in peatlands in relation to land-use. Considering the critical roles microbial organisms play in carbon and nitrogen cycling, determining their responses to management regimes, particularly across the soil profile, is crucial for improving the understanding of peatland management regimes and their

wider effect on microbial communities. Due to the important roles microbial communities play in key ecosystem services such as biogeochemical cycling and the overall biological functioning of the ecosystem, investigating soil microbial community structure and function can improve the ability to predict how peatland ecosystem functions will respond to land management.

1.2: Characteristics and distribution of peatlands

Peat is mostly made up of semi-decomposed plant matter that has gathered at the surface as a result of a lack of decomposition under the conditions of water saturation (Joosten & Clarke, 2002). Peat formation is largely determined by net precipitation, which influences the amount of water available in the landscape, whereas organic matter decomposition is influenced by temperature (Bonn *et al*., 2016). Different interest groups frequently use their own definitions of "peat" and "peatland". For example, Joosten & Clarke (2002) defined peat as "sedentarily accumulated material consisting of at least 30% (dry mass) of dead organic matter," while Burton & Hodgson (1987) defined peat as a soil with at least 50% organic content, which is assessed by measuring the ash remaining after burning. Peatlands are particularly common in cold climates with low temperatures and evaporation, as well as in the tropics with higher precipitation (Charman, 2002). Peatlands are often found in places where the landscape permits water to gather when precipitation and evaporation are low (Fig 1.1). As water logging is easiest on a level surface, peatlands are common in large areas of flatlands such as Canada, Siberia, South East Asia, the Amazon and the Congo (Dargie *et al*., 2017; Ferland & Rochefort, 1997). In parts of the world with an abundant supply of water and limited water loss to the atmosphere, peatlands may also occur on slopes forming blanket bogs (Fraser & Keddy, 2005). Peatland ecosystems are highly diverse and vary from extensive boreal plaudified forests to upland blanket bogs. Overall, peatlands constitute approximately twenty wetland categories in the Ramsar Convention Classification System, over forty habitat types of the EU Habitats Directive and over sixty types of the Endangered Natural Habitats of the Bern Convention.

In natural peatlands, changes in carbon quality and oxygen causes changes in the microbial community and the process of decomposition that are vertically stratified with depth (Andersen *et al*., 2013). The acrotelm (aerobic soil layer) has the highest rate of decomposition, nutrient cycling and microbial metabolism, followed by the mesotelm where

there are fluctuating conditions between anoxic and oxic based on how metabolic activity is impacted by the water table. Finally, litter enters the water-saturated catotelm, where biological material slowly decomposes, primarily by prokaryotes (Tian *et al*., 2022).

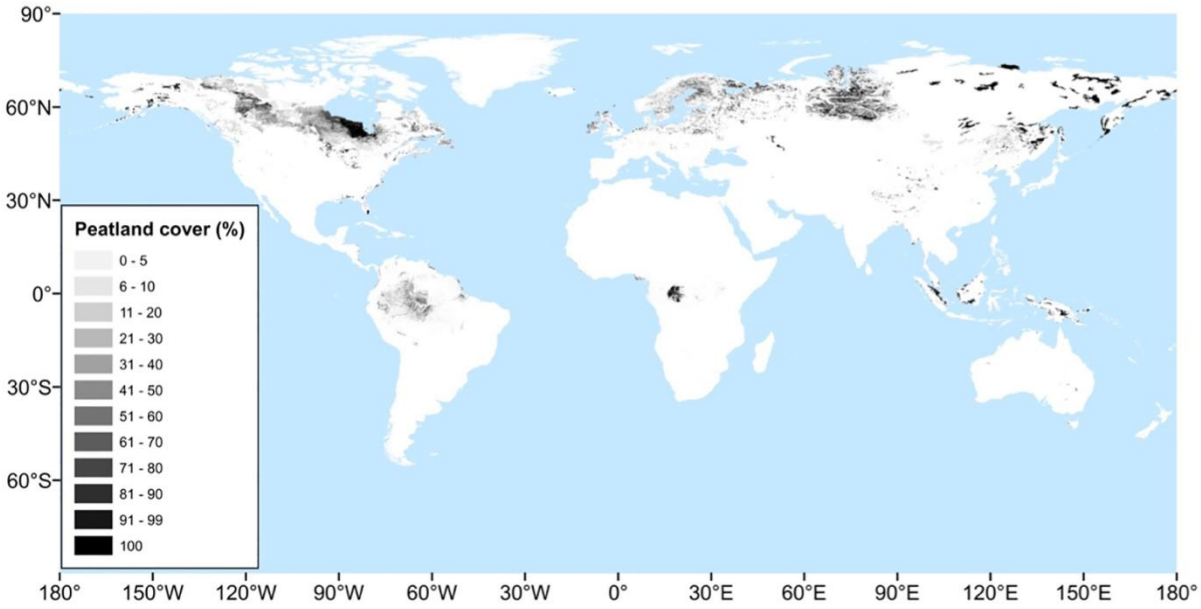

Fig 1.1. Global peatland distribution derived from PEATMAP. The black shading classes indicate the percentage peatland cover (Extracted from Xu *et al*. (2018) with permission, license number '5337561164186').

Globally, approximately 4.23 million km^2 have currently been recorded and peatlands occur in 90% of countries in the world (Xu *et al*., 2018). Natural peatlands are distinguished by a high water table, significant fluctuations in temperature, the absorption of gases, accumulation of organic matter, low oxygen content, limited nutrient availability and high acidity (Crum & Planisek, 1992; Parish *et al*., 2008). Because these conditions limit the amount of space and nutrients available to living organisms, fierce competition for space and nutrients ensues (Minayeva & Sirin, 2012; Ward *et al*., 2009).

Natural peatlands are organised in a unique way in terms of their functionality as well as their structure and is dependent on the relationship between water at various scales and plants in the peatland's immediate vicinity. Locally, excess water encourages the growth of plants while the decomposition of their dead remains is prevented and eventually accumulates as peat (Ward *et al*., 2009). The physical properties of peat allow the retention of water, allowing it to support living organisms even during severe droughts (Moore, 2002). The

waters lateral movement has a significant directional influence, and the movement is influenced by the presence of plants (Strack *et al*., 2006). Peatlands are therefore considered unique in terms of the significance that biodiversity plays in the maintenance of the ecosystem.

1.3: Biodiversity and biodiversity loss

Because of its direct links to ecosystem functioning, biodiversity loss has been widely recognised as a global issue (Loreau, 2010). Microorganisms play essential roles in ecosystems such as nutrient cycling which humans heavily rely on for provisioning (Millennium Ecosystem Assessment, 2005), and changes in ecosystem function have drastic effects on the world's population (Bennett *et al*., 2015; Díaz *et al*., 2006; Pecl *et al*., 2017). This highlights the importance of biodiversity to people. A concentration of biodiversity promotes biomass production, decomposition and recycling, as well as the ecosystem's stability and functionality (Cardinale *et al*., 2012). Impacts on peatland biodiversity at a specific site may not be limited to the level in the spatial hierarchy. The scale of biodiversity loss can be divided into three categories (Minayeva *et al*., 2016) (Table 1.1). Large macroscale activities that are applied at the landscape level and have significant effects at large scales cause the most extensive biodiversity losses. Changes in the connectivity of the landscape, climate and hydrology are a consequence of large-scale spatial impacts. Shrinkage and compaction of peat are hazards associated with intermediate mesoscale activities that affect area variability and therefore biodiversity. These effects are likely to be apparent across a wide range of spatial scales, and they may include changes to peat hydrology and microtopography. Microscale activities have an impact on hydrological factors such as water quality and also impact vegetation cover and microtopography.

Table 1.1. Correlation between spatial level impact and biodiversity losses (modified from Minayeva *et al*., 2016).

Biodiversity losses	**Spatial scale of human activity and impact**		
	Large	**Medium**	**Small**
Biodiversity and adjacent land and catchments			
Mire massif types			
Area and variability of mire types			
Diversity of microform patterns			
Peat composition types			
Present vegetation communities			
Productivity			
Diversity of habitats			
Native species composition			
Alien and invasive species composition			
Structure of populations			
Morphology and forms			
Genotypes			
Strength of relationship between impact			
Strong		Medium	Weak.

Land conversion, habitat fragmentation, harvesting and climate change are some of the negative drivers human activity has on biodiversity and it has been recognised that one of the major causes of biodiversity loss is land-use intensification and mismanagement (Delgado-Baquerizo *et al*., 2016; Newbold *et al*., 2015). Land-use types coexist under various management regimes within the ecosystem along a gradient of land-use intensification. Fertilization, mowing, grazing and burning are examples of land-use intensification. The global effects of land-use intensification were studied in detail by Newbold *et al*. (2015). However, microbial communities were not represented in the study. In general, the response of the soil microbiome under land-use intensification and management regimes is understudied. Microbial ecologists have recently begun to investigate the effects of management regimes on microbial communities using next generation sequencing technology (NGS). These new techniques allow for direct sequencing of metagenomic DNA and RNA in order to gain a better understanding of how microbial communities are structured and function in these environments. NGS techniques have been used to study microbial communities in a variety of environments including peatlands (Elliott *et al*., 2015; Espenberg *et al*., 2018). Utilising this state-of-the-art technology to characterize the microbial

community and the effects of management regimes is critical for future environmental sustainability.

1.4: Greenhouse gas emission and biogeochemical cycle in peatlands

Peatland is considered an important ecosystem for climate moderation and energy fluxes through carbon sequestration and ecosystem stability (Chapman *et al*., 2017; Ward *et al*., 2015). Continuous vegetation production and slow rates of decomposition in waterlogged conditions lead to high amounts of carbon based compounds in the soil (Gorham, 1991), and peatlands store up to one-third of the world's soil carbon (Bradshaw & Warkentin, 2015). Estimation of carbon storage in peatlands varies worldwide. For example, peatlands in the north are estimated to hold up to 1055 Gt (Nichols & Peteet, 2019) peatlands in tropical areas hold up to 152-288 Gt (Ribeiro *et al*., 2021), those in temperate regions can store up to 462 Gt (Alm *et al*., 2007) and those in Subarctic regions hold up to 270-370 Gt (Amendola *et al*., 2018). Peatlands are also important ecosystems for nitrogen cycling (Espenberg *et al*., 2018; Larmola *et al*., 2017). Peatlands store large quantities of nitrogen. Northern peatlands a have accumulated 8–15 Gt (Leifeld & Menichetti, 2018) whereas the N stock in tropical peatlands has not yet been reviewed. Nitrogen and carbon cycles are important for ecology and the economy in terms of climate change and the effects that land-use has on both (Galloway *et al*., 2008; Gruber & Galloway, 2008). Nitrogen and carbon within the soil are essential for soil productivity and have a substantial effect on climate change through emissions which include methane, nitrous oxide and carbon dioxide (Gong *et al*., 2020). Methane has a warming potential 27 times greater than that of carbon dioxide over a 100 year period and nitrous oxide has a warming potential 278 times more potent than that of carbon dioxide over a period of 100 years (IPCC, 2022).

There is a close relationship between carbon and nitrogen cycles in peatlands (Lin *et al*., 2014). The distribution of carbon and nitrogen is governed by biogeochemical cycles, in which soil microbes play an important role. Variation in the spatial distribution and composition of soil nutrients has a significant impact on nitrogen and carbon levels on a global and regional scale (Loisel *et al*., 2014), and recognising the factors that have negative effects on nitrogen and carbon cycling is essential to understanding important processes in peatlands. Several types of Proteobacteria play vital roles in the carbon and nitrogen cycle (Hayatsu *et al*., 2008). The phylum Acidobacteria play key roles in substrate transportation

and nutrient uptake which suggests an adaptation to oligiotrophic conditions, and the phylum Actinobacteria, much like fungi, are largely involved in the decomposition of organic matter (Lewin *et al*., 2016). In addition, members of the phylum Verrucomicrobia have been found to play important roles in carbon cycling within the soil and can make up approximately 20% of a bacterial community (Schimel & Schaeffer, 2012). Euryarchaeota and Crenarchaeota participate in the cycling of sulphur, nitrogen and carbon (Nemergut *et al*., 2005). The activity of microorganisms involved in these processes depends greatly on environmental conditions such as land-use and management practices (Jangid *et al*., 2008).

1.5: The carbon cycle in peatlands

The carbon cycle is a series of processes that occur in the environment to interconvert carbon compounds (Dignac *et al*., 2017) (Fig 1.2). Bacteria, archaea and fungi have been extensively studied in peatlands, since these organisms are known to play significant roles in carbon cycling processes. The carbon balance shifts between ecosystems and carbon-containing greenhouse gases in the atmosphere, such as carbon dioxide and methane, are both closely connected with climate change (Pörtner *et al*., 2022; Reijnders & Huijbregts, 2008). The fixation of carbon by photosynthesis initiates the carbon flux in soils (Billett *et al*., 2010; Flanagan *et al*., 2002). Photosynthetic organisms absorb carbon dioxide and convert it to biomass, albeit some of this acquired carbon dioxide is discharged back into the atmosphere (Yang *et al*., 2008). Some of the carbon is released into the soil by photosynthetic organisms as organic compounds (Paul, 2014). Soil organic matter persists in the soil for long periods of time whereas some of the soil organic matter is mineralized after entering the soil. Processes such as diffusion and transport can restrict access to substrates for microbes therefore microbial communities modulate carbon utilisation (Grayston *et al*., 2004). Some of the carbon is returned into the atmosphere by respiration and organic compounds are synthesized such as extracellular enzymes which are essential for ecosystem functioning (Schimel & Schaeffer, 2012).

Low oxygen content is a natural feature of peatlands (Moore *et al*., 2018). As some organic compounds such as methane are released (Bonaiuti *et al*., 2017) bacteria that control the soil exchange of methane are primarily active in anaerobic and aerobic conditions. All methanogens have so far been found to possess the subunit of methyl-coenzyme M reductase (*mcrA*) gene (Luton *et al*., 2002) the terminal enzyme in methanogenesis that catalyses the reduction of methyl-coenzyme M's methyl group bond to form methane (Luton *et al*., 2002).

The conversion of methane to methanol by the soluble di-iron methane monooxygenase is the first step in the oxidation of methane to carbon dioxide (Banerjee *et al*., 2015). Methane cycling organisms vary across ecosystems and are influenced by environmental variables, anthropogenic activity and climate (Meyer *et al*., 2017). In addition, Fungi depend on soil carbon inputs and actively contribute to the mobilization and stability of carbon and have been functionally categorised according to their capacity for decomposition, with some being resistant polymer degraders while others are able to break down easily degradable compounds (Thormann, 2006). Management regimes may lead to shifts in fungal communities in peatlands and impact those functional guilds with important carbon-degrading enzymatic activities. Despite their abundance, it is still unclear how fungi impact carbon cycling in peatlands.

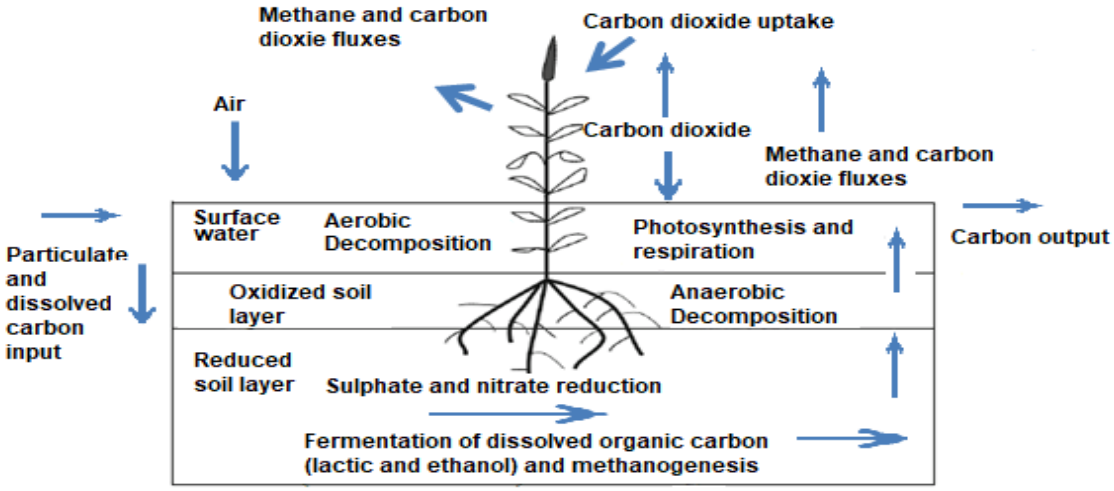

Fig 1.2. The process of the carbon cycling in peatland soil (adapted from Kayranli *et al*., 2010)

Billett *et al*. (2010) estimated that in UK peatlands up to 584.4 Mt C is stored. Table 1.2 shows the distribution of carbon storage in UK peatlands and the percentage of land cover of each type of peatland.

Table 1.2. Estimated carbon storage in UK's peatlands (Natural England, 2010).

Peatland type	mtC	% of total peatland carbon	Land area covered (km)
Blanket bog and upland	138	24	3553
Raised bog	57.5	10	357
Lowland fens and reed beds (deep)	144	25	958
Lowland fens and reed beds (wasted)	186.4	32	1922
Shallow peat soils	58.5	10	5272

1.6: The nitrogen cycle in peatlands

Nitrogen is vital to all organisms as a component of proteins and nucleic acids and is the most abundant compound in the atmosphere (Manahan, 2017). Organisms mediate nitrogen transformation in soils and recently there have been new insights into the role microorganisms play in the nitrogen cycle (Kuypers *et al*., 2018; Manahan, 2017) (Fig 1.3). Microorganisms that oxidise either nitrite or ammonia catalyse aerobic oxidation of ammonia to nitrate or nitrite to ammonium (Könneke *et al*., 2005). Both bacteria and archaea contain the membrane-bound enzyme ammonia monooxygenase (AMO) which is responsible for the biological oxidation of ammonia where the *amoA* gene is encoded by the alpha subunit A (Verhamme *et al*., 2011). The bacterial *amoA* gene is distantly related to the archaeal *amoA* gene and has been broadly used as a molecular marker for environmental studies, particularly in land management (Oton *et al*., 2016; Stahl & de la Torre, 2012). In environmental studies regarding acidic soils such as peatlands, ammonia oxidising archaea are generally more abundant than ammonia oxidising bacteria (Leininger *et al*., 2006). This is due to the high efficiency in anabolism that provides an ecological advantage in these environments (Prosser & Nicol, 2012; Trivedi *et al*., 2019). Thus, this emphasises the importance of ammonia oxidising archaea in the nitrogen cycle and studies in environmental microbiology (Prosser & Nicol, 2012). Complete oxidizers of ammonia were observed in the nitrite oxidizing bacteria *Nitrospira* (Daims *et al*., 2001), a globally distributed group which are present in many environments and is capable of completing oxidation of ammonia to nitrate independently.

Heterotrophic nitrification from diverse fungi and heterotrophic bacteria has also been found to be a nitrogen oxidising process within soils (Francis *et al*., 2007). Furthermore, nitrite dependent anaerobic methane oxidation is the activity where the carbon and nitrogen cycles become intertwined by the anaerobic oxidation of methane (CH_4) with the reduction of NO_2^- to dinitrogen gas (Wu *et al*., 2011). Dinitrogen is produced under anoxic conditions after nitrite becomes reduced to nitric oxide (Ettwig *et al*., 2010). The oxygen that is produced during this process is used to oxidise methane. The one species capable of this (*Candidatus Methylomirabilisoxyfera*) is found in peatlands but very little is known about this organism (Zhu *et al*., 2012). Denitrification is carried out by anaerobic microorganisms and nitrate reductase, nitrite reductase, nitric oxide reductase, and nitrous oxide reductase are examples of the metalloenzymes involved (Smith *et al*., 2015). These organisms adopt oxygen when oxygen is present in the environment (Chen *et al*., 2020). Enzymes catalyse each reductive step in this process (Zumft, 1997). Three forms of nitrate reductases catalyse the conversion of NO_3^- to NO_2^-, each with its own cellular location and metabolic properties: cytoplasmic assimilatory (*CA*), periplasmic dissimilatory (*NAP*) and membrane-bound respiratory (*NAR*) (Moreno-Vivián *et al*., 1999). In general, *NAR* is more widespread among microorganisms, whilst *NAP* is restricted to Gram-negative bacteria (Graf *et al*., 2014). Microorganisms are responsible for only part of this pathway and approximately one-third of microorganisms have *nir*, *nor* and *nosZ* genes (Graf *et al*., 2014; Zumft, 1997) which makes the denitrification process extremely modular. Organisms lacking the *nosZ* genes emit nitrous oxide where others are only capable of reducing nitrous oxide to nitrogen (Jones *et al*., 2008; Putz *et al*., 2016). Previous research has shown that even in aerobic conditions some denitrifying bacteria can retain their ability to reduce nitrogen (Wang *et al*., 2017). In several bacteria, the process is coupled with heterotrophic nitrification (Chen *et al*., 2012; Zhang *et al*., 2017). However, denitrification is the major pathway for nitrite reduction. Nitrogen may be conserved by dissimilatory nitrite reduction where nitrate is transferred to nitrite via ammonium (Robertson *et al*., 2016). Many microorganisms conducting DNRA also produce N_2O depending on environmental conditions. However, the actual contribution of DNRA to N_2O formation remains uncertain (Mania *et al*., 2014).

The *nrfA* gene which codes for the nitrite reductase enzyme *NrfA* is the key step in this process (Smith *et al*., 2007). It is due to this process that nitrogen is more readily available for microbial uptake and is less prone to losses by gaseous compounds.

Microorganisms capable of dissimilatory nitrate reduction to ammonium (DNRA) may release nitrite as a by-product or reduce the nitrite they produced (Rütting *et al*., 2011). This process is expected to be favoured in environments with limited nitrite and organic carbon. Despite many studies on denitrification, the contribution of organisms containing *nrfA* has been studied much less and how they contribute to the retention of nitrogen is relatively unknown (Welsh *et al*., 2014). Although dissimilatory nitrate reduction to ammonium is regarded as a primary process in which nitrogen is conserved in ecosystems (Mania *et al*., 2014). Another mechanism that promotes nitrogen retention in soils is nitrogen fixation (Putz *et al*., 2018). A vast amount of nitrogen is available to a wide variety of bacteria and archaea that act as symbiotes with host plants or fix nitrogen into ammonium (Reed *et al*., 2011). The *nifH* gene which encodes for the reductase subunit of nitrogenase is responsible for this reaction (Smith *et al*., 2015; Zehr *et al*., 2003). The abundance of nitrogen fixing bacteria and archaea may be affected by biotic and abiotic factors within the environment and changes caused by land-use and management (Paul, 2014). Anaerobic ammonia-oxidising bacteria (AnAOB) also oxidise ammonia in peat soils which are anaerobic and use a different pathway to *AOA* and *AOB.* AnAOB (anammox, ANaerobic AMMonia Oxidation) produces dinitrogen gas by using NO_2^- as an electron acceptor (Harhangi *et al*., 2012). It is thought that this is unique to anaerobic ammonium-oxidising metabolism (Harhangi *et al*., 2012). Although these organisms are anaerobic, low oxygen conditions do not always inhibit the process (Hu *et al*., 2011a; Jensen *et al*., 2008). Soil microbes that are able to utilize the nitrogen pathway generally support a greater variety of microbes that are able to make use of different nitrogen pathways (Lam & Kuypers, 2011; Nelson *et al*., 2016). Thus, the quantification of genes involved in the nitrogen cycle can provide an important aid as indicators of nitrogen processes in the soil for sustainable land management.

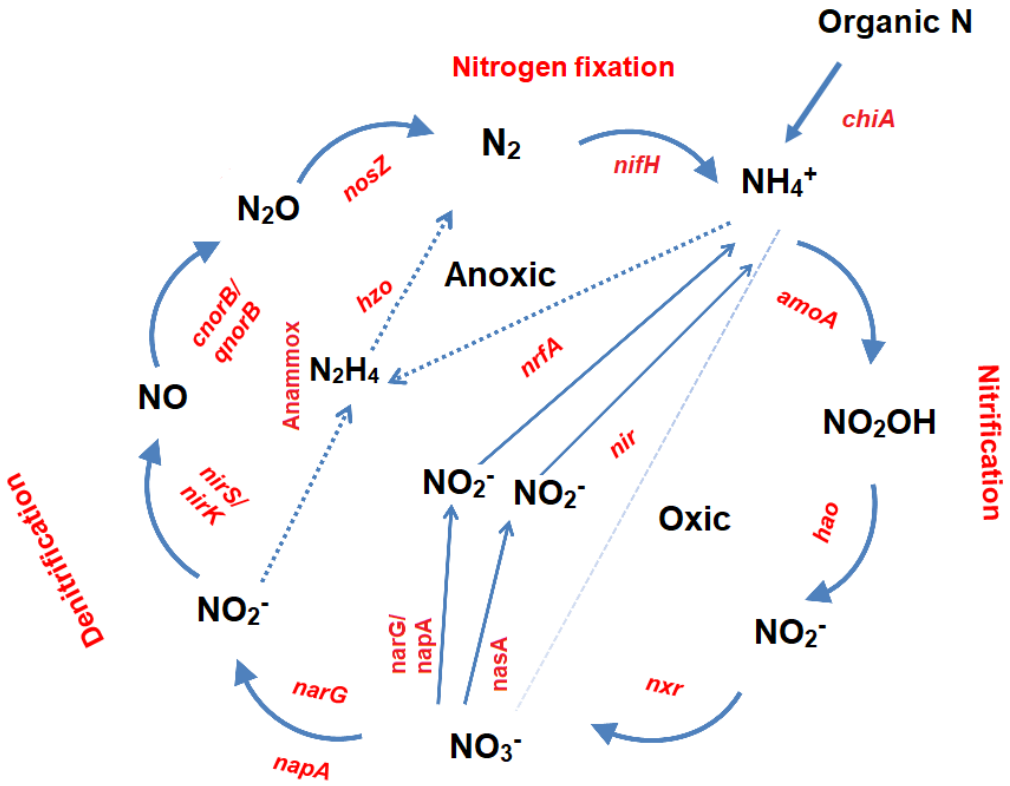

Fig 1.3. Nitrogen cycle and key functional genes involved. Arrows indicate the direction of each reaction. Genes associated with nitrogen-cycling pathways are highlighted in red. The dotted blue line indicates the difference between a high oxygen and low oxygen environment.

1.7: Current management regimes and their impact on the peatland ecosystem

1.7.1: Drainage

Since the 1950s upland peatlands have extensively been drained with the aim to improve bird and livestock populations (Silvan *et al*., 2000). According to Robinson & Armstrong (1988) 100,000 ha of land was being drained annually. Currently, there is little evidence that draining peatlands increases productivity. In regions of peat erosion such as in the UK's Peak District, large areas of gullies function as drainage channels and in some of the most degraded peatlands are the main drainage systems (Evans & Lindsay, 2010).

Drainage leads to a decline in *Sphagnum* which is replaced by grasses and shrubs (Lindsay, 2010). Peat drainage also leads to rapid loss of water and as the water table drops, oxygen penetrates more deeply causing oxidative wastage which converts stored carbon into CO_2 and

other products of dissolved organic carbon (Lindsay, 2010). This is counterbalanced by reductions in layer carbon sequestration by 45-50g cm^{-2} which is no longer transferred to carbon storage. Evans *et al*. (2014) reported oxidative losses of carbon from eroded gullies of >50g cm^{-2}. Wilson *et al*. (2011) reported increases of particle organic carbon in drained catchments. Drains with bare peat can also become vulnerable to wind and rain (Holden *et al*., 2007). Even though lower water tables constrain overland flow, drainage alters peat structure and hydrological flow patterns, making runoff more 'flashy' depending on topography (Holden *et al*., 2006).

1.7.2: Grazing

Sheep are the most common herbivore in the UK uplands. Seventy-one percent of peatlands were stocked at rates that were not sustainable by the 1980's (Holden *et al*., 2007) and this can have significant consequences for the ecosystem and ecosystem services due to changes in vegetation structure, with vascular plant species becoming more dominant (Ward *et al*., 2007) as well as peat erosion initiated by trampling and grazing (Evans & Warburton, 2011). English nature (2001b) has suggested that stocking densities above 1 per ha require careful monitoring. However, even low density can cause soil erosion as a consequence of the decline of important vegetation (Wilson *et al*., 2011). Heavy grazing can lead to the dominance of plant species such as *Molina caerulea, Eriophorum vaginatum*, and important *Sphagnum* species also decline under heavy grazing (Shaw *et al*., 1996). Trampling and tracks left by livestock can cause overland flow generation (Holden *et al*., 2007), resulting in peat erosion causing increased sediment flows into waterways.

1.7.3: forestry

In the UK, government incentives have led to at least 190,000ha of peat being planted with conifers with significant impacts on the ecosystem through associated land preparation (Sloan *et al*., 2019). Afforestation has led to additional soil organic carbon losses through litter formation and carbon acclimating through trees (Hargreaves *et al*., 2003). Data from Morison *et al*. (2010) suggests that CO_2 efflux from soil outweighs the increased rates of sequestration in growing trees. Evans *et al*. (2013) shows that precipitation on forests on peatland release suspended sediments into watercourses and the canopy of these forests increase atmospheric pollutants and enhance ammonium and nitrate leaching from organic soils to surface waters. Draining peatlands before afforestation also alters water quantity regulation (Beheim, 2006).

1.7.4: Impact of prescribed burning on peatlands

Burning is a major driver of ecosystem change in peatlands (Ward *et al*., 2007) and failure to control managed burning can have serious consequences for peatland biodiversity. For the last 100 years, prescribed burning management on small patches of peatland has been used to promote the growth of heather (*Calluna sp*) which is considered beneficial for wildlife (Pearce-Higgins & Grant, 2006) and supports populations of red grouse (*Lagopus lagopus scotica*) (Douglas *et al*., 2015). Prescribed burning is done in strips or patches in rotations which results in a mixture of heather in different developmental stages (Atherden, 1992). Following a rise in the popularity of grouse shooting, heather management and burning regimes were established in 1911 (Lovat, 1911). Currently, Grouse shooting spans approximately 44 counties covering 320,000 ha in England and Wales. Burning has a large influence on the life cycle of heather passing through four development phases throughout this life cycle (Gimingham, 1972). Burning aids in the production of shoots from the mature plants and the germination of new seedlings is carried out before heather reaches the degenerate stage (Gardner *et al*., 1993). However, the role of prescribed fire is currently debateable and highly contentious (Bain *et al*., 2011; Brown *et al*., 2016; Davies *et al*., 2016; Douglas *et al*., 2016; Evans *et al*., 2019; Harper *et al*., 2018; Heinemeyer *et al*., 2019). The central issue is the extent to which the prescribed burning harms the ecosystem services peatlands provide. However, despite the ongoing debate regarding burning, it is still a common management regime (Douglas *et al*., 2015).

Changes associated with burning on peat ecology, peat chemistry, river ecology, and flood risks have been documented (Brown *et al*., 2013). Palmer *et al*. (2013) found that streams draining from burned catchments have higher concentrations of dissolved organic carbon and particulate organic carbon. It has also been shown that prescribed burning causes near-surface macropore blocking which leads to increased surface run-off due to filtration into the peat layer (Holden *et al*., 2014). Areas that have been burned also produce higher drainage water and suspended sediment concentrations which have negative impacts on downstream invertebrate populations (Ramchunder *et al*., 2013).

The effects that burning has on soil chemical, physical and biological properties on peatlands has a consequence for important ecosystem services (Brown *et al*., 2014; Brown *et al*., 2015). It is likely that burned peatlands lose carbon due to fuel consumption (Allen *et al*., 2016).

Soil erosion, as well as an increase in CO_2 and dissolved organic carbon exports has also been shown to be affected after burning (Kinako & Gimingham, 1980; Ward *et al*., 2007; Yallop *et al*., 2010). The consistent net effect of burning on peatland carbon budgets still remains largely unknown (Glaves *et al*., 2013). A lower water table can also result from prescribed burning regimes as a consequence of the decreased heather cover (Clay *et al*., 2009; Worrall *et al*., 2013). For example, Holden *et al*. (2015) found that recently burned plots had a deeper water table, possibly due to higher soil temperature, particularly at the surface (Brown *et al*., 2015). Many of the impacts on plant cover and soil chemistry are likely to interact with the microbial communities, hence an increased understanding of the soil microbiome under burning regimes will aid in clarifying the mechanisms and feedbacks involved (Fig 1.4).

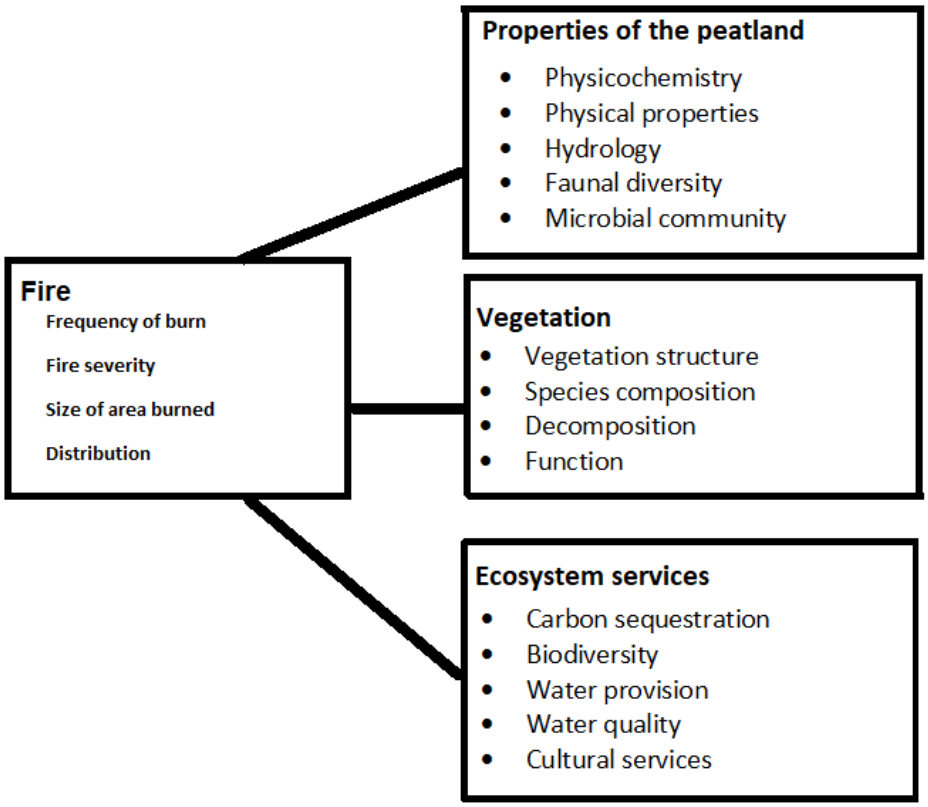

Fig 1.4. Peatland properties and ecosystem services related to prescribed rotational burning.

The prescribed burning plots at the long-term monitoring site of Moor House Nature Reserve has provided a significant amount of data on prescribed burning rotations and the long-term effects it has on plant communities (e.g Lee *et al*., 2013a; Lee *et al*., 2013b; Milligan *et al*., 2018; Noble *et al*., 2018; Noble *et al*., 2019). Several reviews have evaluated how burning affects plant communities and these conclusions mostly agree (Glaves *et al*., 2005; Glaves *et al*., 2013; Harper *et al*., 2018; Shaw *et al*., 1996; Stewart *et al*., 2004; Tucker, 2003; Worrall *et al*., 2010). Currently, the evidence shows that burning on blanket peatlands results in the increase of moss and graminoids such as cotton grass (*Eriophorum vaginatum*) (Ramchunder *et al*., 2013), followed by a replacement of dwarf shrubs. Additional evidence of the effects of burning using microbial communities and microbial function is required to assist in designing policy and management and secure the continued provision of important ecosystem services.

1.8: Peatland reclamation and the restoration of ecosystem functionality

Land reclamation is the process of rebuilding a disturbed area with the goal of restoring the soil, vegetation, and biodiversity to pre-disturbance levels. Land reclamation necessitates several major stages for assessing soil quality and monitoring soil degradation. The first stage entails a pre-disturbance assessment in which suitable zone materials for re-vegetation and geological materials for landscape redesign are excavated and conserved based on soil quality (Audet *et al*., 2015). These soil quality values are encompassed by limits that optimize a particular measure of ecosystem performance, such as plant productivity (Alberta Soil Advisory Committee, 1987).

The process of land reclamation ensures the environmental sustainability of the natural resource industry while maintaining the overall health of the ecosystem (Audet *et al*., 2015). Land reclamation has a critical goal of restoring soil processes in terms of functionality, which is necessary to maintain soil biogeochemical processes, plant productivity, and environmental health, all of which have previously been disrupted by anthropogenic activities (Powter *et al*., 2012). Human-caused degradation is a major concern in peatlands. However, natural degradation can also occur as a result of salinization, drought-induced soil drying or caking, excessive carbon loss, and soil erosion within peatlands (Taufik *et al*., 2022). These processes have a significant impact on the soil's ability to perform specific biogeochemical

functions. As a result, one goal of good soil monitoring for land reclamation operations is to identify and quantify compromised soil capabilities or functionalities and to design mitigation strategies to help restore these to a more normal state. Soil functional restoration is currently justified by using soil quality indicators (SQI) such as soil chemistry, soil enzymes, plant communities and microbial communities that show long-term, stable correlations to specific measures of ecosystem performance such as an increase in plant biomass and diversity (Muñoz-Rojas, 2018).

Soils also have the potential for ecological propagation, such as seedling regeneration and the storage of plant propagules for re-vegetation operations. Furthermore, land reclamation projects necessitate the use of temporary soil for later use in the landscape (Powter *et al*., 2012). As a result, the soil is the most important conserved component of the ecosystem for future use in land reclamation. Although plant diversity is frequently used as a soil assessment indicator in reclamation and rehabilitation practices, it does not provide an accurate assessment of below-ground components such as microbial communities and microbial function, which are critical to ecosystem functionality.

1.9: Monitoring soil health and the impact of land management

The health of soil can be assessed by various techniques to establish its chemical composition, physical attributes and microbial activity (Carter & Gregorich, 2008). Indicators of soil health are associated with their properties in ecosystem processes integrating their chemical, physical and biological qualities (Ferris & Tuomisto, 2015). The most common types of soil indicators are explained in Table 1.3. It is vital that indicators are used together rather than separately in order to gain a better insight into the overall soil health (Zornoza *et al*., 2015).

Table 1.3. Summary of indicators of soil health and quality.

Characteristics	Examples of indicators
Organic matter	Organic matter, total nitrogen and carbon, microbial biomass, activity of soil enzymes and carbohydrates in the soil.
Physical characteristics	Soil density, soil crusting, soil strength and stability.
Chemical characteristics	Plant nutrients, soil pH, soil moisture, hydrology and ion exchange.
Microbial and biological attributes	The population of eukaryotic and prokaryotic organisms.
Visible attributes	Soil erosion and surface water runoff, entry of water into soil, lack of plant growth.

1.9.1: Soil physical and chemical characteristics as indicators of soil health

Indicators are sensitive to anthropogenic changes and are a key to assessing the quality of soil (Zornoza *et al*., 2015). Due to the anthropogenic activity of peatlands the supply of water and oxygen are compromised (Schoenholtz *et al*., 2000). Therefore, the bulk density of the soil is a good physical indicator of soil health that can also be used to monitor the level to which the soil has become compacted. Gas chromatography and infrared detection to monitor the levels of carbon oxides in the soil have proven to be useful methods (Bastviken *et al*., 2015). For example, infrared spectroscopy analyses has proven to be a fast useful analysis of the essential components of soil, such as organic matter and soil moisture, that have an important impact on plant growth (Stenberg *et al*., 2010). The method of infrared spectroscopy has increased in use over the last twenty years despite the cost, as this method requires no chemicals and minimum sampling preparation (Mićić, 2016).

These methods are closely linked and should be used in conjunction with each other. For example, microbial processes are directly influenced by carbon and nutrient supplies and determine the physical and chemical characteristics of the soil (Fierer & Jackson, 2006). The best way to increase the nutrient pool in a sustainable way is to incorporate organic material in the soil (Baldwin, 2009) which provides plant available nutrients. Therefore, it has been suggested that soil organic matter is an essential indicator for soil health because of its vital

link with biological, chemical and physical indicators (Lal, 2016). Soil organic matter also determines a soil's potential to hold water, its susceptibility to degradation by management practices and provides vital nutrients and energy for microbes during the process of mineralisation (Schoonover & Crim, 2015). The acidity of the soil has been recognised as an important indicator for peatlands. For example, low soil acidity is characteristic of peatlands due to high organic matter such as humic acid (Swindles & Roe, 2007). Soil pH provides very important information about the capacity of soil productivity (Zornoza *et al*., 2015). It has also been shown that aggregate stability is stabilized since the flocculating cations such as calcium ions also increase with soil pH, and acidification of soils entails a significant reduction in the nitrification process (Rowley *et al*., 2018). Nutrient concentrations particularly in peatland soils results in decomposition and mineralisation which is directly related to microbial activity (Rousk *et al*., 2009). Other chemical parameters such as carbon, nitrogen, and phosphorus, as well as C:N ratio and total organic carbon, are also critical. Human activities can have a negative impact on soil carbon, and its analysis is a critical indicator of potential issues (Amundson *et al*., 2015). A high C:N ratio indicates that decomposition is reduced due to anaerobic conditions (Farmer *et al*., 2014). The rate of decomposition, on the other hand, can affect nitrogen and phosphorus ratios (Quinton *et al*., 2010).

1.9.2: The use of soil microorganisms as bioindicators

Soil microorganisms have been recognised as sensitive bioindicators that respond quickly to environmental changes (Andersen *et al*., 2013; Faucon *et al*., 2017; Oliverio *et al*., 2017). Because of the importance of peatlands, the requirements for management to be carried out in a sustainable method have also increased (Chapin lii *et al*., 2000; Madsen, 2011; Vasander & Kettunen, 2006). However, certain land-use types could also bring an environmental risk that includes a potentially negative effect on the soil ecosystem.

The Soil Science Society of America defines soil quality as "the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation" (Karlen *et al*., 1997). In this case, defining soil quality indicators is essential for viable land management (Basak *et al*., 2016; Bier *et al*., 2015; Litchman *et al*., 2015). Microbiological relevant methods are required to assess the potential impacts of management

regimes (Aislabie *et al*., 2013; Marinari *et al*., 2013; Sánchez-Moreno, 2016; Schoenholtz *et al*., 2000). Having to culture many microorganisms from soil samples remains a fundamental drawback in the ability to understand the ecology of microbes in soils under different management regimes (Schloter *et al*., 2018). However, the recent advancements of molecular-based techniques from soil samples have revealed critical information about microbial communities in terms of their community structure and function (Graham *et al*., 2016; Vanwonterghem *et al*., 2014). Molecular techniques such as metagenomics, metaproteomics, and metatranscriptomics are critical for uncovering and distinguishing microbial function and diversity, as well as discerning the dynamic interactions between biotic and abiotic environmental factors (Deng *et al*., 2016; Stępniewska *et al*., 2017; Xia *et al*., 2018). These various methods can be useful in identifying soil microbial indicators for land management. For example, potential microbial indicators are microbial communities, taxa and functional genes that change quickly in response to environmental change (e.g. due to management regimes) and plant stress (e.g. living in inadequate conditions) in an ecological sustainable way, and should be used to support traditional methods such as chemical analysis and vegetation surveys.

1.10: The aim of the current investigation

Using conventional physicochemical and novel microbiological indicators to assess and evaluate the impact of land management on the activity and diversity of soil microbial communities in peatlands.

The objectives of the current investigation include:

1. Determine the differences in microbial community structure, diversity and catabolic activity in natural reference fens compared to a constructed fen in the Athabasca Oil Sands Region of Canada, and discover patterns of microbial communities in these landscapes.
2. Analyse the effects of prescribed burning on the diversity, community composition, and stability of soil microbial communities and their dominant taxa under different burning regimes.

3. Determine the relationship between environmental characteristics and microbial communities under management regimes undergoing reclamation and prescribed burning.
4. Identify the abundance of N-cycling genes and determine the effects of prescribed burning on microbial nitrogen turnover characterized by an increased abundance of N-cycling genes.

Outline of the thesis

A range of methods was used to develop a thorough understanding of changes in microbial abundance and composition caused by land management. First, differences in nutrient dynamics, biogeochemical processes, microbial communities and microbial activity in a constructed fen in a post-mining landscape is compared to the adjacent natural reference fens and the implication for future management is assessed. Second, the effects of prescribed burning on the diversity and composition of microbial communities as well as its impacts on N cycling gene abundance are investigated. The remainder of this thesis consists of the general methods used and the findings of this research, concluding with a discussion chapter that considers the work as a whole and makes recommendations for future research.

Chapter 2 presents general materials and methods, details for methods used in this study that are not exclusive to another chapter.

Chapter 3 is titled "Microbial Communities and Biogeochemical Functioning across Peatlands in the Athabasca Oil Sands Region of Canada: Implications for Reclamation and Management". This chapter reports the findings of a study conducted within the AOSR of Alberta Canada following reclamation efforts of a constructed fen compared to reference analogues. A combination of microbial activity using the Microresp^{TM} and next generation sequencing of microbial communities was used. Importantly, the work detailed in this chapter was performed in collaboration with colleagues from Mount Royal University, Canada.

Chapter 4 is titled "Effects of a prescribed burning regime on vegetation, soil physicochemistry and prokaryotic microbial communities in surface and subsurface peat". This chapter reports the findings of the effects of prescribed burning on vegetation cover, soil physicochemical properties and the community structure of archaea and bacteria. Overall,

this chapter aims to test the hypothesis that bacteria and archaea community structures are affected by different prescribed burn rotations, changing overall microbial network structures.

Chapter 5 is titled “Response of soil fungal communities and functional traits to prescribed burning regimes in surface and subsurface soils”. This chapter aims to determine the effects of prescribed burning on fungal communities and the consequences on important trophic modes, not only across burn treatments but throughout the soil profiles.

Chapter 6 is titled “Changes in microbial populations and nitrogen functional genes in soil profiles of a peatland under different burning regimes”. This chapter evaluates the effects of prescribed burning on the abundance of bacteria, fungi and key nitrogen cycling genes throughout the soil profile using qPCR.

Chapter 7 presents the conclusions. The obtained results are considered in the context of land management as well as to inform future research directions and recommendations for future management practices are considered.

Chapter 2. General materials and methods

This chapter describes the general methodology used in the result chapters. Specific materials and methods related to the study's various objectives are described in detail in separate chapters.

2.1: Description of study sites

2.1.1: Athabasca Oil Sands Region

The study in chapter 3 was conducted in four fen peatlands situated along a hydrologic gradient. These peatlands included three natural fens (poor fen, hypersaline fen and treed-rich fen) and a constructed fen. All the study sites were located within the AOSR near Fort McMurray, northeastern Alberta, Canada (Fig 2.1). The region is dominated by a continental boreal climate characterized by short warm summers and long cold winters, with mean air temperature of 1^{o}C and 418.6 mm precipitation (30 year mean values from Environment Canada, 2015).

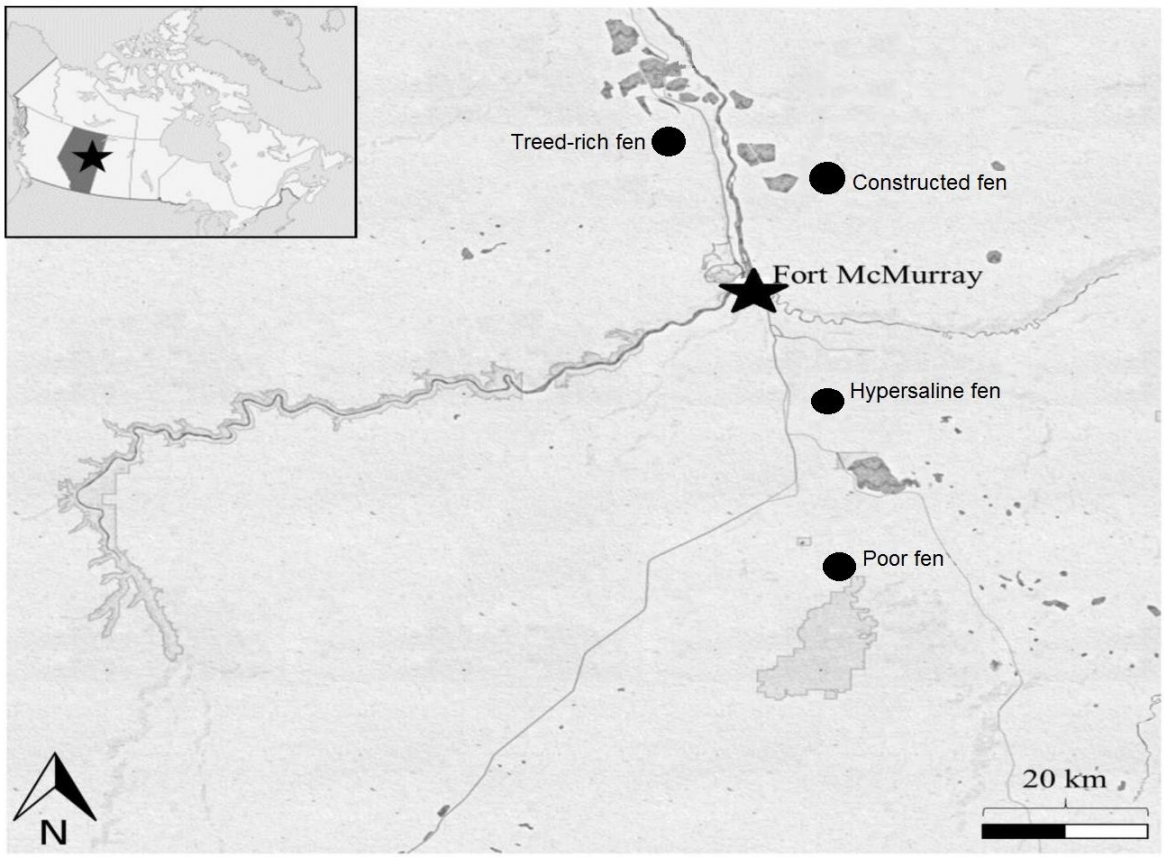

Fig 2.1. Sampling sites within the AOSR near Fort McMurray, northeastern Alberta, Canada.

The constructed fen (Nikanotee fen) is a 3 ha fen watershed that was built between the spring of 2010 and winter of 2013 (56° 56' N, 111° 25' W). Details of the design concept and revegetation approach were described in detail earlier by Daly *et al*. (2012) and Price *et al*. (2010). Briefly, the fen watershed was constructed by placing two meters of dewatered peat over a thin layer (0.5 m) of petroleum coke, which was put in place to enhance the hydrologic connection between the constructed upland aquifer and the fen. Revegetation of the constructed fen was initiated in the spring of 2013 using a strategy that involved transplanting seedlings from a nursery and combining the transplanted seedlings with a living moss layer from a donor fen. Among the vascular plants that were re-introduced in the constructed fen, *Carex aquatilis* and *Juncus balticus* were the dominant vegetation cover in the second growing season, while other peatland species such as *Campyllium stellatum, Tomenthy pnumnitens, Betula glandulosa* and *Triglochin maritima* were sparsely present in some plots.

The poor fen (Pauciflora), is situated on a local topographic peak (Stony Mountain ~740 m above sea level), about 40 km south of Fort McMurray (56° 22' N, 111 14' W). Vegetation cover within the fen is a consequence of the hydrological conditions, with *Sphagnum* spp. (e.g. *S. angustifolium, S. medium* and *S. capillifolium*) as the dominant ground cover vegetation. Also present in abundance are sedges (e.g. *Carex aquatilis, C. pauciflora* and *C. limosa*) and Ericaceous shrubs (*Oxycoccus microcarpus*, *Chamaedaphne calyculata*, and *Andromeda polifolia*), with a discontinuous tree cover including *Betula glandulosa*, stunted *Picea mariana* and some *Larix laricina* (Bocking, 2015; Borkenhagen & Cooper, 2016).

The treed-rich fen (Poplar) is located 20 km north of Fort McMurray, Alberta (56° 56' N, 111° 33' W). This site is the closest in proximity to the constructed fen and shares similar site characteristics with the peatland that was used as the donor fen. The vegetation cover is dominated by *Larix laricina* (larch), *Betula glandulosa* (dwarf birch), *Equisetum fluviatile* (swamp horsetail), *Smilacena trifolia* (three-leaved Solomon's seal), *Carex* spp. and mosses (*Polytrichum* spp., *Tomenthypnum* spp. and *Sphagnum* spp.).

The hypersaline fen (Saline) is located approximately 10 km south-southeast of Fort McMurray, Alberta (56°34′N, 111°16′ W). Detailed descriptions of this site have been previously reported (Phillips *et al*., 2016; Wells & Price, 2015). Briefly, the surface of the site is characterized by a network of ponds, ridges and inter-ridge depressions (lawns), with a northward decline in surface elevation. Peat and vegetation samples for this study were

collected from the ridges and inter-ridge depressions, which had no significant moss cover, but were dominated by salt tolerant species such as *Calamagrostis stricta* (narrow reed grass) and *Hordeum jubatum* (foxtail barley) in the ridges, and *Triglochin maritima* (seaside arrow grass) and *Plantago eriopoda* (redwool plantain) in the inter-ridge depression (Borkenhagen & Cooper, 2016).

2.1.2: Moor House nature reserve, UK

The experiments performed in chapters 4, 5 and 6 were conducted at Moor House - Upper Teesdale National Nature Reserve which covers approximately 3900ha (Marrs *et al*., 1986). The altitude ranges from 290m to 848m a.s.l and the reserve is England's highest national nature reserve, a UNESCO Biosphere Reserve and has been designated as a European Special Protection Area (Price, 2019). Hay meadows, upland grasslands, pastures, deciduous woodlands, blanket peatlands and extensive summits characterise the area.

The reserve's western side is steeper, with more variable soils and vegetation cover. The altitude of the study area ranges from 520m - 710m and is predominantly characterized as having M19 and M20 plant communities (Calluna-Eriophorum vegetation) within the British National Vegetation Classification (Rodwell, 1998).

The mean annual temperature from 1992- 2000 (at 556 m) was 5.8 °C, and 5.1°C from 1953-1978 (Heal & Smith, 1978; Smithson , 1985). This shows the temperature in this region has increased 0.7 °C between 1953- 1978 and 1992 -2000. Over the last decade, the increase in winter temperatures has been significant, increasing by 1.4 - 2°C (Holden & Adamson, 2003).

In 1952, Moor House was designated a national nature reserve after the establishment of a new research station. Universities and other institutions began conducting research in the 1930s at which time blanket peat was heavily eroded. Nicholson (1957) proposed that erosion was caused by poor management practices such as prescribed burning and sheep grazing. It was proposed that the reserve be used as a 'long term monitoring site' investigating moorland management rather than simply preserving biodiversity.

Since its establishment, there has been extensive research on various peatland issues such as climate change, pollution, the processes of blanket bogs and land management (Ward *et al*., 2015). To explore the effects of sheep grazing on soils and vegetation, extensive 'grips' to drain the moor were cut and experimental plots were set-up on a range of vegetation, either

completely excluding sheep or fencing in a high number of animals. As part of the International Biological Programme, the area was extensively researched in the 1960s and 1970s (Joyce *et al*., 2001).

In 1963 and 1969, the Moor House National Nature Reserve was established to safeguard the unique flora and fauna. In January 1992 the UK Environmental Change Network (ECN) includes Moor House NNR as one of their long-term monitoring sites to help analyse and predict environmental changes across the United Kingdom, as well as to produce comparable data sets over time (Burt *et al*., 1998). Moor House has shown how blanket peat moorland that has suffered erosion can be restored through practical conservation management. For example, in 1950, a gently sloping catchment of 4.8ha in area was severely burned and was then named 'Burnt Hill' (Higgitt *et al*., 2001). Since the installation of experimental plots in 1954 the long rotation plots have been burned four times and the short rotation plots seven times, and have been used to monitor the ecological response of plant communities to this fire-frequency gradient (e.g. Lee *et al*., 2013a; Lee *et al*., 2013b; Milligan *et al*., 2018; Noble *et al*., 2018; Noble *et al*., 2019).

2.2: Experimental design and soil sampling at Moor House nature reserve, UK

Soil sampling was conducted in July 2020 for chapters 4, 5 and 6. The experiment is divided into four blocks, each with six 30 m x 30 m plots. Burned plots every ten years (short-rotation, most intensively burned), burned every twenty years (long-rotation, intermediate burn) and unburned since 1954 (non-burn) treatments are used in combination with unfenced grazed treatments (Fig 2.2). The experimental plot's surrounding vegetation has remained unburned for at least 90 years (Noble *et al*., 2019). Each burning regime was arranged into four repeats. In each sampling area, three quadrats (1 m x 1 m) were randomly thrown in each treatment per block (n=36) (Table 2.1). In each quadrat, the mean canopy height and the percent of vegetation cover was estimated. Plants were classified into morphological groups (i.e. heather, graminoid, *Sphagnum* moss, other 'non-Sphagnum' moss and other vascular plants). Next, five soil samples were collected vertically from each quadrat from three commonly selected depth profiles (0-20cm, 20-40cm, 40-60cm) using a 10mm diameter Haglöf Soiltax soil sampler. A sample was taken from each corner of the quadrat and the centre to form a composite sample for chemical analysis and microbial analysis. A total of 12 sets of samples per treatment and per depth was collected (a total of 108 samples). Soil

samples for microbial analysis were brought to the laboratory and stored at -20°C prior to DNA extraction.

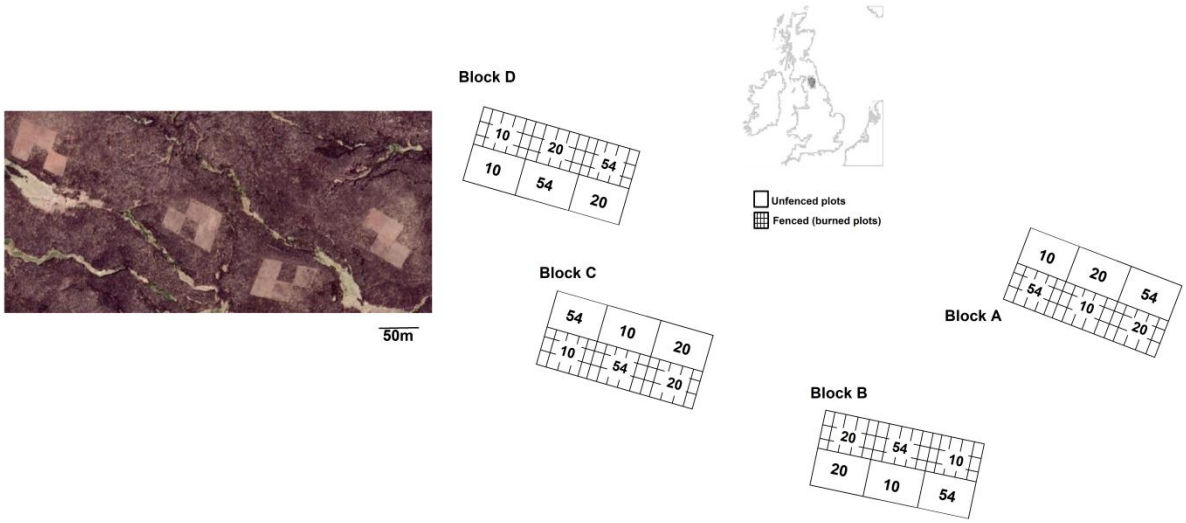

Fig 2.2. Map of the Moor house Nature Reserve's experimental plots used for this study (54 = Not burned since 1954, 20 = Long rotation (Burned every 20-years), 10 = Short rotation (Burned every 10-years).

Table. 2.1. Sampling regime at Moor house burned plots. Total *n* = 108 samples.

Experimental block	**Non-burn (control)**			**Long rotation burn (every 20 years)**			**Short rotation burn (every 10 years)**		
	0-20cm	**20-40cm**	**40-60cm**	**0-20cm**	**20-40cm**	**40-60cm**	**0-20cm**	**20-40cm**	**40-60cm**
BLOCK A	n=3	n=3	n=3	n=3	n=3	n=3	n=3	n=3	n=3
BLOCK B	n=3	n=3	n=3	n=3	n=3	n=3	n=3	n=3	n=3
BLOCK C	n=3	n=3	n=3	n=3	n=3	n=3	n=3	n=3	n=3
BLOCK D	n=3	n=3	n=3	n=3	n=3	n=3	n=3	n=3	n=3

2.3: Physicochemical analysis of Moor House soils

Approximately 15g of soil sample was placed in a heavy-duty aluminium foil tray to determine soil moisture content gravimetrically through the difference in mass between a wet (field condition) sample and an oven dried sample after 105°C for 48 h (Rowell, 2014). Moisture content was determined by the following formula:

Moisture Content (%) = (Wet soil weight – Dry soil weight) / Dry soil weight X 100

Soil pH was determined in a settled slurry that was 2.5g of soil in 10ml of deionised water at a 1:5 ratio and shaken for 1 h before being measured with a Jenway 3510 pH meter. Total C and N concentrations were determined with a vario MACRO cube (Elementar Analysensysteme GmbH, Langenselbold, Germany). Soil was air-dry, sieved (< 2 mm) and weighed between 0.010g - 0.015g and was analysed via the Elementar, which is effective for analysing total C and total N via catalytic combustion and reduction. The combustion oven was set to 950°C and the reduction at 600°C. Sample replicates were run in batches of 20, with Ethylenediaminetetraacetic acid (EDTA) 502-092, soil standards and analytical blanks in-between the 20 samples for quality assurance and control.

2.3.1: Nitrate and ammonium concentration

Estimation of ions including nitrate (NO_3^-) and ammonium (NH_4^+) were measured from 2g air dried peat extracts in 20ml 1% KCl following 30 min orbital shaking at 200 rpm using a dual motion shaker 2D 300. The extracts were then centrifuged for five minutes at 5000 xg using a Hermle z446k centrifuge. The supernatant was filtered through a Whatman No.3 using a 2 μm syringe and diluted (50% v/v) and measured using ion chromatography (Dionex ICS-5000$^+$DC) (Thermofisher, UK). For nitrate, the Dionex DX was equipped with a Thermo IonPac AG18 2x50mm guard column followed by an AS18 2x250mm separation column with an oven temperature of 23°C. A cleaning gradient was eluted with the concentration of the eluent at 18mM KOH injection rising to 50mM at 16min. For ammonium the Dionex ICS-5000$^+$DC was equipped with a Thermo IonPac CG16 3x50mm guard column followed by a CS16 3x250mm separation column at 60°C. Ammonium was then run isocratically with an eluent concentration of 39mM Methanesulfonic acid (MSA). Detection was by suppressed conductivity. Chromatograms were analysed using Chromeleon 7.0 (Thermo Scientific UK).

2.3.2: Analysis of soil extractable elements

The aqua-regia method was used for soil extractable elements as it is safer to use than other extraction methods such as hydrofluoric acid (Zimmermann *et al*., 2020). The aqua-regia solution was made by combining concentrated HCl in a 3:1 (v/v) ratio with 1M HNO_3. A 100 mL glass beaker was then used to weigh 0.5g of air-dried soil from each sample. 5mL of

aqua-regia was then added to the soil samples, swirled, and left in the fume hood for 3 hours at 80°C. After heating, the beakers were then allowed to cool to room temperature for at least an hour and filtered through a Whatman No. 3 filter. The filtrate was diluted to 50 ml with deionsed water and subjected to analyses (Fig 2.3). The resulting diluted samples were analysed for Potassium (K), Calcium (Ca), Magnesium (Mg), Manganese (Mn), Iron (Fe), Aluminium (Al), Lead (Pb), Copper (Cu), Zinc (Zn) and Phosphorus (P). The concentrations were determined using an Icap 6000 SERIES ICP-OES spectrometer (Thermofisher, UK).

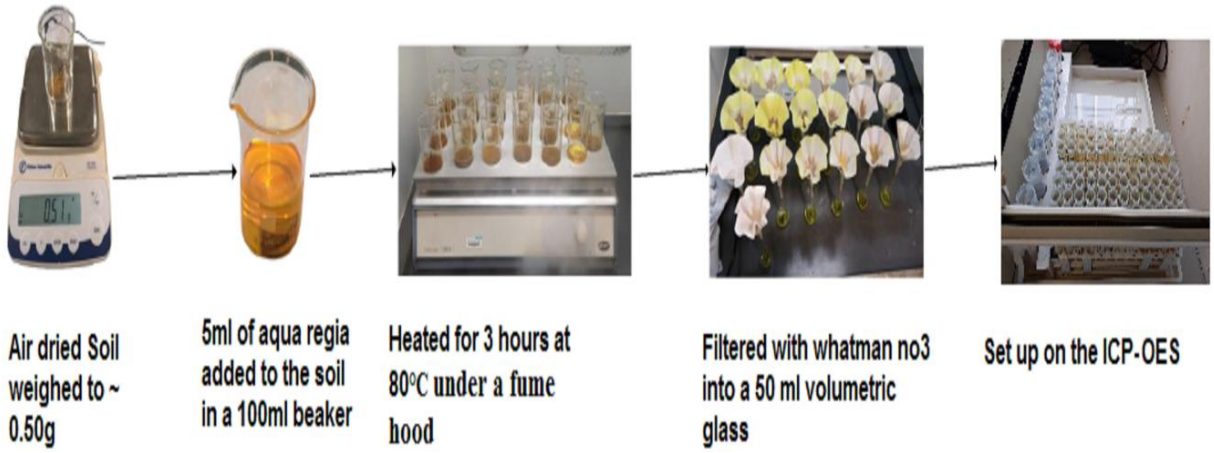

Fig 2.3. Illustration of the aqua-regia digestion method using an ICP-OES spectrometer in this study.

Prior to the analysis, a semi-quantitative solution was prepared by placing approximately 0.5 mL of each sample in a plastic tube, which was then analysed in the ICP-OES for the concentration of each element. Calibration standards were prepared in 50mL volumetric flasks after calculating the required volumes by dilution with 1% (v/v) HNO_3. To eliminate possible instrument errors, all samples and standards were analysed with blank controls.

2.4: DNA extraction of Moor House soils

All chemicals for molecular methods were of analytical grade purchased from Qiagen (Manchester, UK). All glassware, pipettes and tubes were RNase free prior to commencing the molecular work. DNA was extracted using the DNeasy® PowerSoil® kit (Qiagen, Manchester, UK). 0.10g of freeze dried soil was weighed (rather than 0.25g, due to the low bulk density of peat soil) was homogenised and rewetted with 150µl of nuclease-free water. The soil was added to powerbead tubes and gently vortexed to disperse the powerbeads and centrifuged at 10,000 xg for 30 seconds. The 600µl supernatant was removed to a 2ml tube. 200µl of inhibiter removal solution was added and incubated for 4˚C for 5 minutes and then centrifuged 10,000 xg at room temperature. 750µl was transferred to a clean tube and shaken before adding 1.2ml of solution and vortexed for 5 seconds. 650µl of the solution was placed onto a spin filter and centrifuged at 10,000 xg for 1 minute. The flow-through was discarded and an additional 650µl supernatant was added to another spin filter and centrifuged at 10,000 xg. The remaining supernatant was loaded onto another spin filter and centrifuged at 10,000 xg. 500µl of ethanol wash solution was added to the spin filter and centrifuged for 30 seconds at 10,000 xg. A second centrifuge of 10,000 xg for 1 minute was used to discard any remaining solution. 100µl of sterile elution buffer was added to a spin filter and centrifuged at room temperature for 30 seconds at 10,000 xg. The spin filter was discarded and DNA was ready for downstream analysis. The extracted DNA was quantified using a Qubit4 Fluorometer (Invitrogen, UK) and stored at −20°C for subsequent analysis.

Chapter 3. Microbial Communities and Biogeochemical Functioning across Peatlands in the Athabasca Oil Sands Region of Canada: Implications for Reclamation and Management

Abstract

Peatlands play an important role in global biogeochemical cycles and are essential for multiple ecosystem functions. Understanding the environmental drivers of microbial functioning and community structure can provide insights to enable effective and evidence-based management. However, it remains largely unknown how microbial diversity contributes to the functioning of belowground processes. Addressing this gap in knowledge will provide a better understanding of microbial-mediated processes in peatlands that are undergoing restoration or reclamation. This study assessed the changes of microbial community diversity and structure as well as soil function by measuring microbial respiration on a range of substrates from three natural fen types found in the Athabasca Oil Sands region of Alberta, Canada (a poor fen, a hypersaline fen, a treed-rich fen) and a nearby constructed fen undergoing reclamation following open pit mining. Overall substrate induced respiration was significantly higher in the constructed fen. Alpha diversity of fungi and prokaryotes was highest in the treed-rich fen and the composition of microbial communities was significantly different between fens. Both fungal and prokaryotic communities were strongly related to pore water pH and temperature with plant richness also contributing to shape fungal communities. Microbial community structure reflects the underlying differences in soil condition across different fens but plays essential roles in the ecological functions of soil. This present study is the first insight into how soil microbial community structure and activity differ in a constructed fen compared to natural fens in the Athabasca oil sands region and provides a new outlook for the management of peatlands undergoing post-mining reclamation. Future research on peatland reclamation should consider the dynamic interaction between communities and ecosystem functionality for which this study forms a useful baseline.

Keywords: Peatlands; Biogeochemical cycles; Reclamation; Microbial community; Ecosystem functionality

3.1: Introduction

Peatlands are essential ecosystems that play an important part in the Earth's biogeochemical cycles (Carlson *et al*., 2010; Minayeva & Sirin, 2012; Teurlincx *et al*., 2018; Turetsky *et al*., 2002). The role of microbes in the regulation of biogeochemical processes is fundamental to the functioning of peatlands, given the large amount of organic matter they partially-decompose and their role in mediating carbon and nitrogen cycles (Espenberg *et al*., 2018; Zhou *et al*., 2012). The involvement of peatland microbial communities in biogeochemical processes is dependent on their sensitivity to environmental change (Graham *et al*., 2016). For example, microbial community structure has been shown to be driven by a range of environmental factors including pH (Blaser *et al*., 2016; Kaiser *et al*., 2016; Lauber e*t al*., 2009; Ramirez *et al*., 2014; Tedersoo *et al*., 2014; Zhang *et al*., 2013), moisture (Brockett *et al*., 2012; Na *et al*., 2019) and the content of carbon and nitrogen (Kuramae *et al*., 2014; Whitaker *et al*., 2014; Zhang *et al*., 2013). In responding to changes in environmental conditions, microbes mediate the mineralization of organic matter, regulating and releasing nutrients that are essential for ecosystem services such as plant productivity (Kluber *et al*., 2016).

Ecological restoration and reclamation usually focus on restoring the aboveground plant communities and using soil physicochemical parameters as an indicator of soil health (Hata *et al*., 2019). However, above ground surveys do not provide a complete depiction of soil quality particularly in peatlands that are extremely complex with regards to soil physicochemistry (Arias *et al*., 2005). Indeed, studies have only started to highlight the importance of microbial ecology during the monitoring of restoration and management of peatlands in the last decade or so (Andersen *et al*., 2010; Andersen *et al*., 2013; Basiliko *et al*., 2013; Bobuľská *et al*., 2020; Elliott *et al*., 2015; Espenberg *et al*., 2018; Lin *et al*., 2012; Preston & Basiliko, 2016; Wang *et al*., 2020), as well as a bias towards examining above-ground communities. This bias could stem from a notion that microbiota might not require management since microbial communities are everywhere. For example, the Baas-Becking hypothesis states that "everything is everywhere but the environment selects", and thus can adapt to changes in their environment (Baas Becking, 1934). Whilst identification of the environmental controls and patterns of composition of the soil microbial communities is important, process-based understanding of the relationship between environmental conditions

and soil microbiome functioning in peatlands remains a significant challenge (Ritson *et al*., 2021).

Some authors have suggested that similar environmental factors drive soil microbial functioning and microbial community structure (Allison & Martiny, 2008; Martiny *et al*., 2006). The implication of this is that land managers should aim to achieve ecosystem functionality by generating the optimal microbial community structure. The links between functional capabilities and microbial diversity remain unclear as microbial communities may exhibit multiple functional redundancies (Louca *et al*., 2018). This is mainly because microbial taxa are not identified from microbial functional assays that have commonly been used to measure the rate of microbial-mediated processes (Nannipieri *et al*., 2003). Linking together the microbial processes, communities and wider peatland functions is a critical step in the ability to better manage these globally significant carbon stores (Humpenöder *et al*., 2020), but also to support large-scale restoration and reclamation efforts currently taking place across the globe (Rochefort & Andersen, 2017).

This is particularly relevant in the Athabasca Oil Sands Region (AOSR) of Alberta. Part of the pristine boreal forest, which has received much national and international attention due to the intensive anthropogenic alteration and environmental degradation from ongoing mining for bituminous oil sands that are buried beneath the pristine peatlands (Rooney *et al*., 2012). The reclamation efforts involved the relocation of fragmented-mineralized catotelm peat from donor peatland that had been previously drained to a topographically lower point in a reconfigured post-mining landscape, where it can sustain the necessary moisture condition for the establishment of peatland plant communities. Although applicable reclamation guidelines exist for restoring wetland areas in the AOSR (Alberta Environment, 2008b; Chymko, 2000) and biological indices have been developed based on plant communities (Raab & Bayley, 2012), there still remains a lack of appropriate, extensive soil monitoring tools documenting reclamation outcomes in post mining areas. Such soil quality indicators could help improve current practices and provide understanding into the ecological functions of the microbiome during reclamation. At present, there is little knowledge of microbial communities on reclamation sites and also in relation to the surrounding peatlands that these sites aim to emulate.

The specific objectives of this study were (1) determine how prokaryotic and fungal community structure and diversity change along natural reference fens compared to a constructed fen; (2) determine important soil environmental factors responsible for shaping community structure; (3) assess the role of microbial diversity, function, community structure and soil chemistry across a range of different fens. Based on these the following hypotheses were tested: (1) There will be significant differences in the community structure of soil fungi and prokaryotes between different fen types (i.e. the community structure of the constructed fen will be dissimilar from natural sites; (2) Microbial alpha diversity and substrate induced respiration in the constructed fen will be lower compared to natural fens where the range of physiological strategies and niches available will be greater.

3.2: Materials and methods

Details on the study site are given in chapter 3 section 2.1.1.

3.2.1: Sampling procedures

Sampling was conducted in June 2014 at the peak of the growing season. At each site, six sampling plots were selected within the vicinity of existing ecological monitoring plots. Vegetation surveys were conducted within a $1m^2$ quadrat. The total percent cover of every species within the quadrat and directly above (e.g. trees, shrubs) was visually estimated, with species nomenclature following the standard guideline for the region (Moss & Packer, 1983). Shallow peat cores (10 – 20cm) were collected from each plot using a 4cm diameter Russian corer for physicochemical, microbial functional analysis and microbial community analysis. Changes in water table depth over the growing season were determined through existing deep polyvinylchloride (PVC) wells that were installed in all the six monitoring plots in each site for long-term monitoring purposes.

3.2.2: Physicochemical characteristics of the study sites

The von Post humification index (Von Post, 1924) was used to classify the degree of peat humification. Pore water pH and electrical conductivity (EC) were determined *in situ* using a calibrated portable pH-conductivity probe (Oakton 35-Series Testr. Illinois, USA). Soil temperature was measured at a 10cm depth with a portable waterproof temperature probe Peat samples for analysis of extractable nutrients were transported to the laboratory on ice,

and processed within eight hours of collection, using the standard techniques previously described in Nwaishi *et al*. (2015) i.e. nitrate and ammonium extracted using potassium chloride (KCl) and water-extractable phosphorus (WEP). Seasonal dynamics of nutrient cation and anion supply to the rhizosphere was monitored with Plant Root Simulator^{TM} (PRS) probes (Western Ag Innovations Inc., Saskatoon, SK) that were installed to a depth of 15 cm. The procedures used to extract ions from the incubated probes have been previously described by Nwaishi *et al*. (2016).

3.2.3: Characterization of biogeochemical processes

In situ mineralisation experiments using the "buried-bag" method (Eno, 1960) were conducted to determine the rate of nitrogen (N) and phosphorus (P) mineralisation in all the monitoring plots. The incubation commenced towards the end of June (peak of the growing season) and lasted for approximately 30 days. After the incubation period, cores in the buried bags were recovered and returned to the lab in a cooler filled with ice-packs and processed for nitrate (NO_3^-), ammonium (NH_4^+) and WEP (Hart *et al*., 1994). Following the determination of the extractable nutrient concentrations using a Bran Luebbe AA3 AutoAnalyzer, Seal Analytical, Seattle, U.S.A., Methods G-102-93 (NH_4^+), G-109-94 (NO_3^- + NO_2^-) and G-103-93 (WEP), the rates of net nitrification, net ammonification and net P mineralisation (μg g^{-1} dry peat day^{-1}) were estimated using the approach described in Nwaishi *et al*. (2016). The release of carbon dioxide (CO_2) (g m^{2-1} $d^{-1)}$ and methane (CH_4) (mg m^{-2} d^{-1}) by vegetation and microbial processes were quantified *in situ*, using a dynamic closed-chamber technique (Petrone *et al*., 2011). Flux measurements were conducted bi-weekly over the growing season, and average daily seasonal flux was estimated as the mean of the six flux measurements that were taken over the period. Both measurements and estimations of CO_2 and CH_4 fluxes followed the methods described in detail by Munir & Strack (2014), with CH_4 fluxes reported in Bienida *et al*. (2020).

3.2.4: Microbial functional measurements

Microbial catabolic activity was measured on fresh peat samples using the MicroResp^{TM} system (Campbell *et al*., 2003), following a protocol that had been modified for peat, as in Andersen *et al*. (2013) and Nwaishi *et al*. (2016). Five replicated samples were measured from the poor fen, treed-rich fen and hypersaline fen and six replicated samples were measured from the constructed fen.

Substrates that were used to induce microbial respiration included double de-ionized water (as a negative control) and 14 carbon sources that comprised of amino acids (arginine, lysine, alanine, cysteine, γ-aminobutyric acid), carbohydrates (fructose, arabinose, glucose, trehalose), carboxylic acids (oxalic acid, citric acid, malic acid, α-ketoglutarate) and the amino sugar *N*-acetylglucosamine. As well as individual responses for the different carbon (C) sources microbial functional diversity was estimated from the respiration response profiles as catabolic evenness ($E=1/\sum p^{i2}$), which estimates the variation in substrate utilization across the variety of substrates tested (Degens *et al*., 2001; Magurran, 1988) where P^i is the reaction of respiration to individual substrates as a proportion of total respiration (Magurran, 1988).

3.2.5: Microbial community characterization

Microbial community metabarcoding was used to characterize microbial genetic diversity. Peat samples for microbial community characterization were sent frozen to the US Forest Service Northern Research station (Houghton, Michigan) for further processing. DNA extraction and sequencing proceeded as follows. A 50 ml Falcon tube was filled with approximately 10 g of peat from a sample. The mixture was then crushed for two minutes with a modified Mini-Beadbeater-96 (BioSpec Products, Bartlesville, OK, USA) and 20 3.2 mm chrome-steel beads. Using a MoBio PowerSoil® DNA Isolation Kit (MoBio Laboratories; now Qiagen), DNA was extracted from a 0.5 g subsample of the pulverised peat. Following the manufacturer's recommendations, a 30 min incubation period at 65°C was added after adding the C1 lysis buffer and 10 minutes of vortexing. A MoBio PowerClean® Pro DNA Clean-Up Kit was used to clean the extracted DNA, and was then quantified using a Qubit Fluorometer (Invitrogen, Carlsbad, California, USA). Cleaned DNA extracts were then sent to the US Department of Energy Joint Genome Institute (JGI, Walnut Creek, California, USA) for sequencing, following the sample preparation protocol of Caporaso *et al*. (2012). Samples were subjected to polymerase chain reaction (PCR) amplification using primers 515F and 806R (Caporaso *et al*., 2012), which target the prokaryote 16S V4 region, and fITS9 (Ihrmark *et al*., 2012) and ITS4 (White *et al*., 1990), which target the fungal ITS2 region. Primers were fitted with Illumina adaptors and the reverse primer contained 11bp barcode sequences that were unique to each sample. Samples were then pooled into equal molar portions and sequenced on an Illumina MiSeq platform (Illumina, Inc., San Diego, CA) using 2 x 300 bp chemistry. Data are accessible via the JGI portal (http://genome.jgi.doe.gov/).

3.2.6: Bioinformatics

Sequences were processed using QIIME2 v2019.7 (Bolyen *et al*., 2019). First, Illumina adapters and PhiX 174 contamination were removed with BBDuk (sourceforge.net/projects/bbmap/) and PCR primers were removed with Cutadapt 3.0 (Martin, 2011). Prokaryote quality filtering was done by truncating the Forward and reverse V4 sequence reads at 200bp and 150 bp, respectively. Fungal ITS2 sequences were not trimmed to a standard length; however the conserved flanking regions were removed from ITS2 sequences using ITSexpress (Rivers *et al*., 2018). Sequences were merged into ASVs (Amplicon Sequence Variants). Reads were quality filtered (expected error rate = 2), chimeras were removed and reads were joined, all using DADA2 (Callahan *et al*., 2016). Taxonomic assignment was performed with the naive Bayes feature-classifier (Bokulich *et al*., 2018). The taxonomic assignment was performed with the SILVA 138 SSURef NR99 database for prokaryotes (Robeson *et al*., 2021) and UNITE (version 8.3) for fungi (Abarenkov *et al*., 2020; Nilsson *et al*., 2019). ASVs with taxonomic assignments to mitochondria, chloroplast and eukaryote 18S sequences were filtered from the prokaryote dataset and non-fungal eukaryotes were excluded from the ITS2 dataset. ASVs for fungal and prokaryotic communities were rarefied to minimum sampling depths of 10135 and 38554 sequence reads respectively. *Phyloseq* was used for handling import, manipulation, and analysis of microbial community data (McMurdie & Holmes, 2013). Samples that had very low reads, and were therefore considered insufficient for a statistically powerful analysis and a potential source of bias, were removed. This resulted in: poor fen (n=6), hypersaline fen (n=4), treed-rich fen (n=6) and constructed fen (n=6) for fungal community analysis and poor fen (n=6), hypersaline fen (n=6), treed-rich fen (n=6) and constructed fen (n=5) for prokaryote community analysis.

3.2.7: Statistical analysis

All statistical analyses were carried out using R version 4.0.2 software (R Development Core Team, 2020). Significant differences in physicochemical measurements, macro-element concentration, biogeochemical processes and microbial catabolic properties between fens were tested using a Kruskal-Wallis test following the Shapiro-Wilk test and the Bartlett test for normality and homogeneity of variance. Pair-wise differences were then tested by a Dunn's *post-hoc* test. Plant species richness was tested with one-way analysis of variance (ANOVA) followed by Tukey's *post-hoc* (HSD) tests for pair-wise differences. Alpha diversity was calculated to compare microbial community diversity indices including

observed richness, Shannon index and Simpson index using the R package '*Phyloseq'* (McMurdie & Holmes, 2013). One-way analysis of variance followed by Tukey's *post-hoc* (HSD) tests for pair-wise differences was used to test the differences in alpha diversity measures between different fens.

Differences in microbial community composition between fens was visualised with principal-coordinate analysis (PCoA, Gower, 1966) using a Bray-Curtis dissimilarity matrix in the R package '*Vegan*' (Oksanen *et al*., 2013). Permutational Multivariate Analysis of Variance (PERMANOVA) (Anderson, 2001) was conducted to assess the significant difference in community composition between fens. The relationship between environmental variables and microbial community composition and catabolic activity was assessed using redundancy analysis (RDA) using the '*Vegan*' R package. The selection of the 'best' explanatory environmental variables was done by forward selection using the *ordistep* function in '*Vegan*' (Blanchet *et al*., 2008) and was used to select significant environmental variables ($P<0.05$). The variation inflation factor (VIF) was used to check non co-linearity among the explanatory variables ($VIF<10$) as recommended by Montgomery & Peck (1992). The significance of the overall models was determined through ANOVA-like permutation tests (with 999 permutations).

3.3: Results

3.3.1: Soil physicochemistry and environmental variables

Significant differences in soil physicochemistry and environmental conditions were found across the study area. Pore water pH ranged from 3.9 to 7 being highest in the constructed fen and the treed-rich fen, which had similarly high mean values (Table 3.1). Moisture content was also significantly different between different fens ranging from 35% to 97.3%, being highest in the hypersaline fen and constructed fen, and lower in the treed-rich fen and poor fen. Environmental variables such as water table depth were significantly lower in the constructed fen and plant richness was highest in the treed-rich fen and poor fen. Likewise, conductivity (µs cm^{-1}) and soil temperature (°C) varied between different fens and was highest in the hypersaline fen (Table 3.1).

Table 3.1. Water table depth (cm) (distance from the peat surface), plant species richness, soil temperature (°C), pore water pH, moisture (%) and electrical conductivity (µs cm^{-1}) (mean and standard deviation, *n=6*) across the four fen types. Different letters indicate pair-wise significant differences (*P*=<0.05) using Dunn's *post-hoc* test for physicochemical measurements and water table depth and Tukey's HSD *post-hoc* test for plant species richness at a confidence level of 95%.

	Poor fen	**Hypersaline fen**	**Treed- rich fen**	**Constructed fen**
Water table (cm)	0.33±0.47 b	5.33±5.46 b	5.10±1.46 b	11.33±13.79 a
Plant richness	8.5 ±0.54 a	4.5±2.34 b	9±1.67 a	3.16±1.67 b
Soil temperature (°C)	10.08±0.76 b	15.5±0.80 a	5.75±2.33 c	12.23±0.68 b
Pore water pH	4.06±0.134 c	6.52±0.56 b	7.05±0.07 ab	7.26±0.203 a
Moisture (%)	74 ±2.45 b	92±6.45 a	62±12.70 b	88±3.25 a
Conductivity (µs cm^{-1})	33.5±5.05 d	12077.33±156.96 a	231±13.46 c	3368±3.59 b

3.3.2: Nutrient dynamics

Nutrient dynamics varied across different fens. NO_3^- N was higher in the treed-rich fen and poor fen and lower in the constructed and hypersaline fen. The concentrations of extractable NO_3^- were also highest in the poor fen. NH_4^+, TIN, K, Cu and Zn were all higher in the poor fen (Table 3.2). P supply rate was highest in the treed-rich fen and lowest in the hypersaline fen. Ca and S supply rate was significantly higher in the constructed fen. There were no significant differences observed in the supply rate of Fe, B, Pb and Al between fens (Table 3.2).

Table 3.2. Soil nutrient and macro-element concentration (mean and standard deviation, *n=6*) in the four fen types studied. Letters indicate pair-wise significant differences (*P*=<0.05) using Dunn's *post-hoc* test at a confidence level of 95%. WEP = Water Extractable Phosphorus; TIN = Total Inorganic Nitrogen.

	Poor fen	**Hypersaline fen**	**Treed- rich fen**	**Constructed fen**
NO_3^- (µg g^{-1} dry peat day^{-1})	4.43±0.85 a	1.11±0.95 b	3.58±0.29 a	1.28±1.15 b
Extractable NO_3^- (µg g^{-1} dry peat day^{-1})	3.44±1.028 a	2.22±0.44 b	2.04±0.728 b	1.721±0.25 b
NH_4^+ (µg g^{-1} dry peat day^{-1})	9.04±3.58 a	3.7±1.72 b	4.23±1.72 b	1.28±0.28 b
Extractable NH_4^+ (µg g^{-1} dry peat day^{-1})	39.32±17.38 b	85.01±50.62 a	27.78±8.44 b	16.8±6.60 b
WEP (µg g^{-1} dry peat day^{-1})	0.75±0.76 a	2.52±4.73 a	3.10±4.13 a	0.87±0.38 a
TIN (µg g^{-1} dry peat day^{-1})	13.35±2.23 a	4.8±1.33b c	7.81±1.61 b	2.6±1.39 c
N (µg g^{-1} dry peat day^{-1})	42.76±17.72 ab	87.23±50.72 a	29.16±6.19 b	18.52±6.76 b
P (µg g^{-1} dry peat day^{-1})	1.04±0.92 ab	0.5±0.49 b	1.72±0.57 a	1.05±0.407 ab
Ca (µg 10 cm^{-2} incubation $period^{-1}$)	412.7±301.63 c	1196.4±98.38 b	1121.82±369.63 b	2337.33±367.11 a
Mg (µg 10 cm^{-2} incubation $period^{-1}$)	166.90±64.77 b	318.78±24.08 a	178.43±40.49 b	340.5±43.51 a
K (µg 10 cm^{-2} incubation $period^{-1}$)	220.56±154.33 a	15.21±2.03 c	68.30±28.46 b	15.33±4.79 c
Fe (µg 10 cm^{-2} incubation $period^{-1}$)	37.16±15.49 a	28.65±12.78 a	62.31±41.49 a	30.66±11.41 a
Mn (µg 10 cm^{-2} incubation $period^{-1}$)	12.43±9.73 a	2.81±0.81 b	9.72±6.58 a	3±1.41 b
Cu (µg 10 cm^{-2} incubation $period^{-1}$)	0.03±0.01 a	0.03±0.047 a	0.02±0.16 a	0.016±0.037 a
Zn (µg 10 cm^{-2} incubation $period^{-1}$)	1.65±0.68 a	0.23±0.09 b	0.86±0.47 b	0.23±0.14 b
B (µg 10 cm^{-2} incubation $period^{-1}$)	0.82±0.19 a	1.15±0.34 a	0.86±0.149 a	1.35±0.68 a
S (µg 10 cm^{-2} incubation $period^{-1}$)	88.21±109.53 c	30.7±6.23 c	327.74±142.07 b	1237.83±189.20 a
Pb (µg 10 cm^{-2} incubation $period^{-1}$)	0.14±0.07 a	0.16±0.17 a	0.17±0.041 a	0.01±0.037 a
Al (µg 10 cm^{-2} incubation $period^{-1}$)	15±4.9 a	9.45±2.97 a	12.11±0.68 a	17.33±8.43 a
Cd (µg 10 cm^{-2} incubation $period^{-1}$)	0.02±0.024a	0 b	0.02±0.01 a	0 b

3.3.3: Biogeochemical processes

The poor fen exhibited significantly higher net nitrification rates while the hypersaline fen showed negative rates (denitrification). There were also significant negative rates in net ammonification in the hypersaline fen and significant positive rates in the treed-rich fen and constructed fen (Fig 3.1). N-mineralisation ranged from -9.3 to 2.6 (µg g^{-1} dry peat day^{-1}) and exhibited significantly negative rates in the hypersaline fen and positive rates in the treed-rich fen, constructed fen and poor fen (Fig 3.1). P-mineralisation rates overall were very low and not significantly affected by fen type (Fig 3.1).

Significant variation in overall gaseous flux was found between the four study sites. Positive carbon dioxide release was observed in the treed-rich fen and there was a significant negative flux in the poor fen. Methane flux was also affected by fen type with methane release being highest in the poor fen and lowest in the constructed fen (Fig 3.1).

3.3.4: Soil microbial physiological potential across fens

Substrate induced respiration varied across fens (Fig 3.2). Total substrate respiration, measured by the sum of 15 carbon sources, was different between sites (X^2= 6.0 , df = 3, P = 0.02) and ranged from 92.3 µg g^{-1} h^{-1} CO_2-C to 605.1 µg g^{-1} h^{-1} CO_2-C and was highest in the constructed fen (409.2 ± 54.29µg $g^{-1}h^{-1}$ CO_2-C) followed by the treed-rich fen (235.3± 64.26 µg $g^{-1}h^{-1}$ CO_2-C). Lower respiration rates were observed in the hypersaline fen (153.4 ± 12.29 µg $g^{-1}h^{-1}$ CO_2-C), poor fen (197.3 ± 39.5 µg $g^{-1}h^{-1}$ CO_2-C) and treed-rich fen (228.02 ± 64.3 µg $g^{-1}h^{-1}$ CO_2-C) (Fig 3.2). Likewise, catabolic evenness was different between fens being greater in the constructed fen (X^2=10.008, df = 3, P = 0.01).

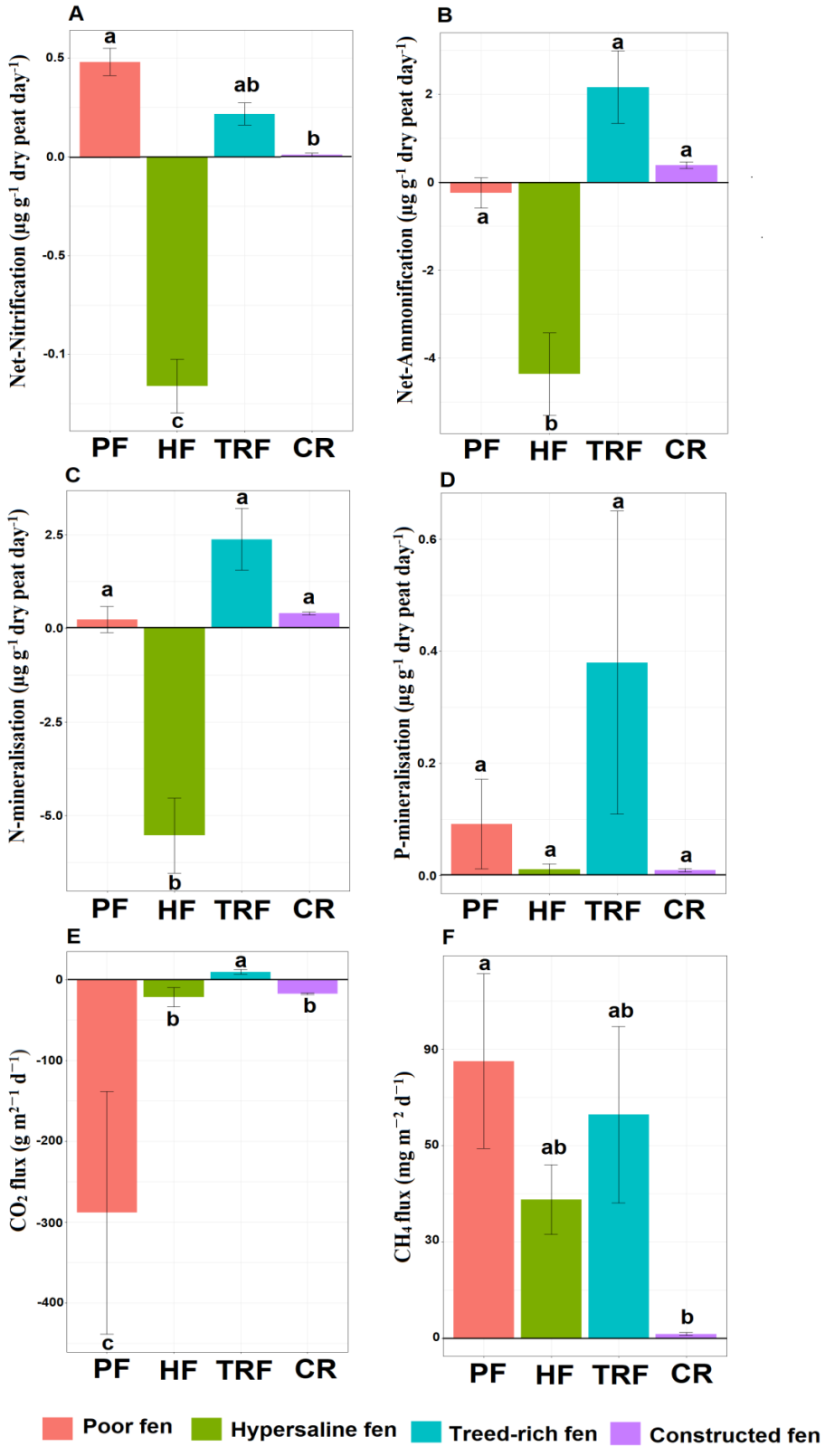

Fig 3.1. Biogeochemical proccesses across four fen types (mean ± standard error, *n* = 6). **(A)** Net nitrification**, (B)** Net ammonification, **(C)** N- minerilisation, **(D)** P- mineralisation**, (E)** CO_2 flux, **(F)** CH_4 flux. Letters indicate significant pair-wise differences (*P*=<0.05) using Dunn's *post-hoc* test at a confidence level of 95%. PF=poor fen, HF=Hypersaline fen, TRF=Treed-rich fen, CR= Constructed fen.

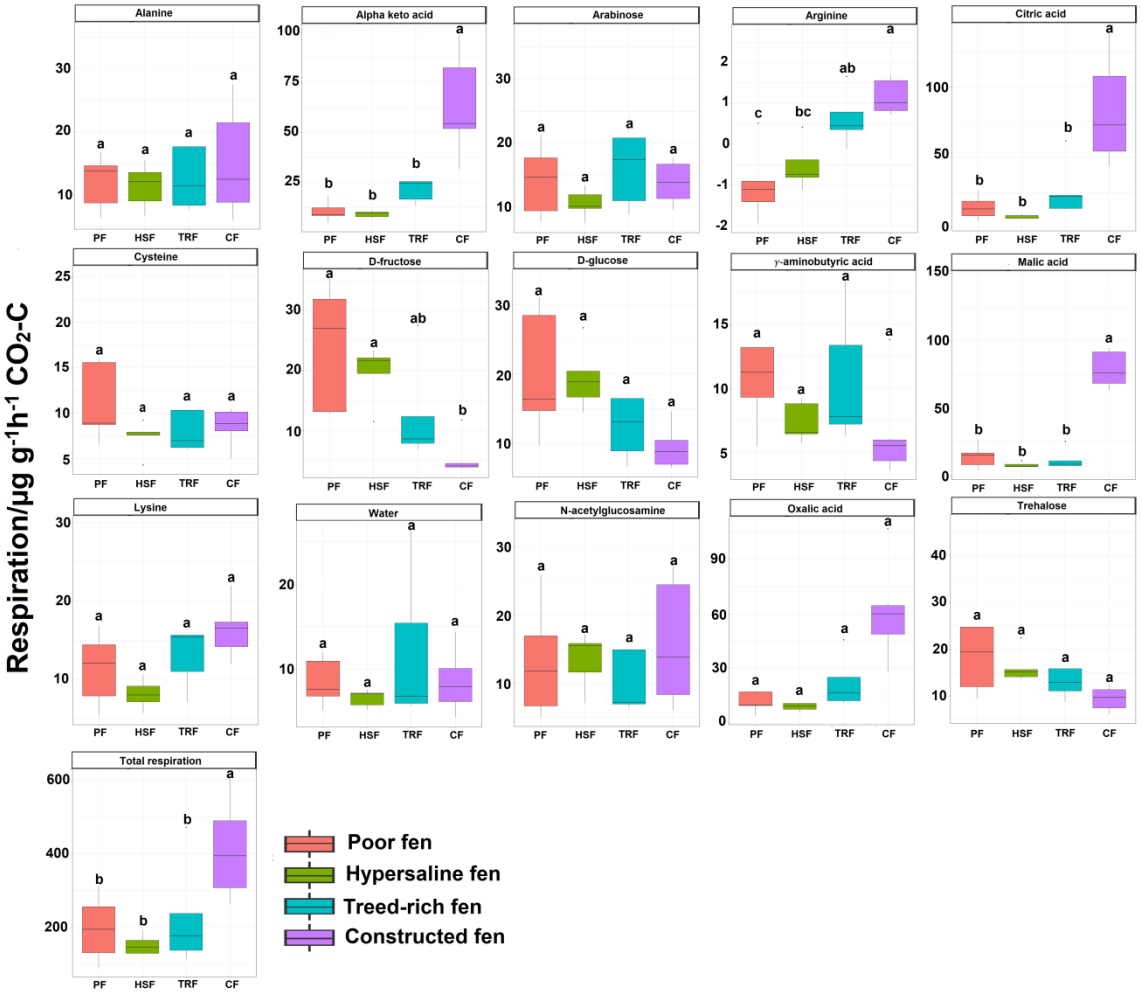

Fig 3.2. Catabolic profiles obtained with MicroResp™ assay in response to the different fens for substrates alanine, α-ketoglutaric acid, arabinose, arginine, citric acid, cystenine. D-fructose, D-glucose, γ-aminobutyric acid, L-malic acid, lysine, water, n-acetylglucosamine, oxalic acid and trehalose are shown per fen type. Total respiration = the sum of all carbon sources. Letters indicate pair-wise significant differences (P=<0.05) using *Dunn's post-hoc* test at a confidence level of 95%. PF=poor fen (n=5), HF=hypersaline fen (n=5), TRF=treed-rich fen (n=5), CR= constructed fen (n=6).

3.3.5: Relationship between environmental factors and multiple substrate induced respiration

Redundancy analysis revealed that a total of 95.3(%) of variance was explained by the model (*P*=<0.05) (Fig 3.3). The RDA for substrate induced respiration showed that pore water pH, plant richness, B, soil temperature, NH_4^+ and K significantly affected microbial activity. The selected model for these variables was significant (*F*=19.63, *P*=0.001). The two-dimensional redundancy plot showed that the RD1 axis explained 87.73(%) of the variance. The second RD axis explained 7.57(%) of the variance (Fig 3.3). The first axis was strongly correlated with pore water pH and NH_4^+, while the second axis was correlated with temperature and plant richness.

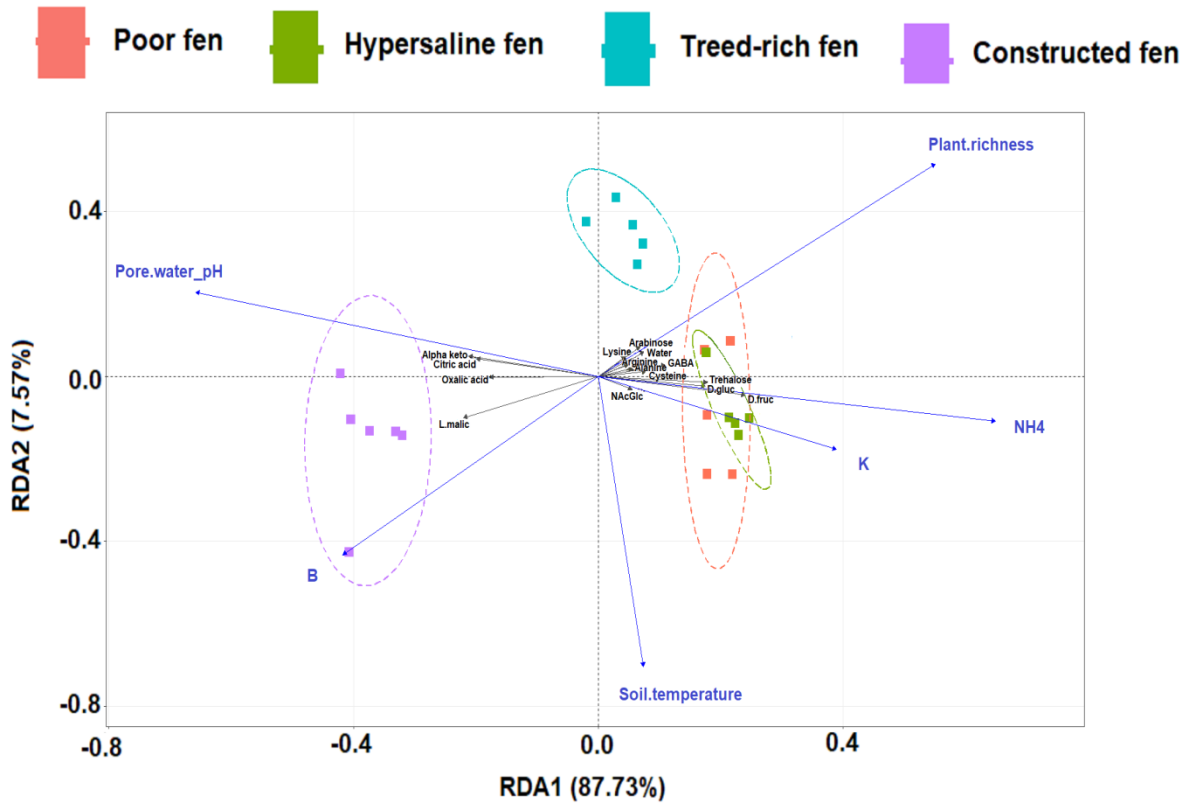

Fig 3.3. Redundancy analysis of catabolic profiles (MicroResp™) on environmental variables shown in the plane of the first two redundancy axes, RD1 and RD2. Carbon substrates used in the MicroResp assay, Alanine, α-ketoglutaric acid, Arabinose, Arginine, Citric acid,Cysteine. D-Fructose, D-Glucose, γ-aminobutyric acid (GABA), L-malic acid, Lysine, n-acetyl glucosamine (NAcGlc), Oxalic acid and Trehalose. Poor fen (*n*=5), Hypersaline fen (*n*=5), Treed-rich fen (*n*=5), Constructed fen (*n*=6).

3.3.6: General characteristics of fungal and prokaryotic community composition

Differences in soil microbial communities were observed between fens. Ascomycota was the predominant fungal phylum across the study and made up 72% of the total fungal communities across all samples being the most abundant phyla in the poor fen (70%), hypersaline fen (81%), treed-rich fen (61%) and constructed fen (80%). Basidiomycota was the second most dominant phylum making up 23% overall and showed an increase in the poor fen (27%) and treed-rich fen (33%) (Fig 3.4A). Proteobacteria was the most abundant prokaryotic phylum making up 36% across all samples and most abundant in the hypersaline fen (30%), treed- rich fen (40%) and constructed fen (47%) but represented a much smaller proportion of the community in the poor fen (18%) where Acidobacteriota represented the most dominant phylum (39%) (Fig 3.4B).

Fig 3.4. Relative abundance of fungi **(A)** poor fen (*n*=6), hypersaline fen (*n*=4), treed-rich fen (*n*=6) and constructed fen (*n*=6) and top ten prokaryotes **(B)** poor fen (*n*=6), hypersaline fen (*n*=6), treed-rich fen (*n*=6) and constructed fen (*n*=5) at phylum level across different fen types.

The diversity patterns of fungi and prokaryotes differed across fens: the treed-rich fen was significantly higher in fungal and prokaryotic diversity compared to the other sites. Whereas the poor fen and hypersaline fen exhibited the lowest fungal diversity (Fig 3.5), the lowest prokaryotic diversity was found in the poor fen. For both fungi and prokaryotes, the diversity measures showed that the treed-rich fen had greater observed diversity compared to the constructed fen (Fig 3.5).

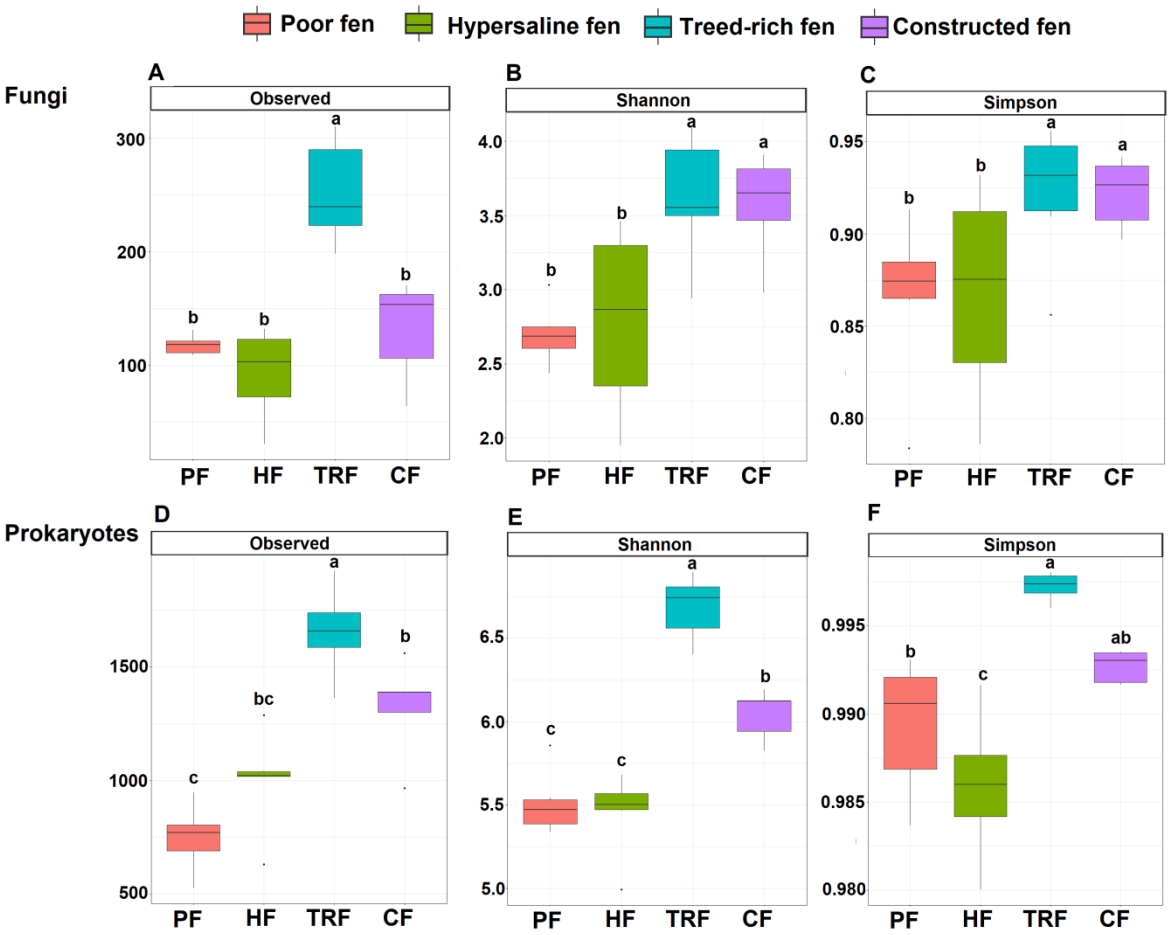

Fig 3.5. Alpha diversity plots between different fen types. Observed species richness **(A),** Shannon index **(B)** and Simpson index **(C)** for soil fungi, PF=Poor fen (*n*=6), HF=Hypersaline fen (*n*=4), TRF=Treed-rich fen (*n*= 6), CR= Constructed fen (*n*=6) and observed species richness **(D),** Shannon index **(E)** and Simpson index **(F)** for prokaryotes, PF=Poor fen (*n*=6), HF=Hypersaline fen (*n*=6), TRF=Treed-rich fen (*n*= 6), CR= Constructed fen (*n*=5). Letters indicate significant pair-wise differences (*P*=<0.05) using Tukey's HSD *post-hoc* test at a confidence level of 95%.

Overall, the composition of fungal communities was significantly different between fens (PERMANOVA, F=2.82, R^2=0.32, P=0.0001). Likewise, the composition for prokaryotic communities was also affected by fen type (PERMANOVA, F= 10.91, R^2=0.63,P = 0.0001). The principal coordinate plots revealed clear differences in fungal and prokaryotic community composition between the different fens. Whilst for fungal communities, all of the sites were well separated on the PCoA plots, the prokaryotic communities show the constructed fen and the treed-rich fen formed a cluster together, indicating that the prokaryotic community structure in the constructed fen is similar to that of the treed-rich fen (Fig 3.6).

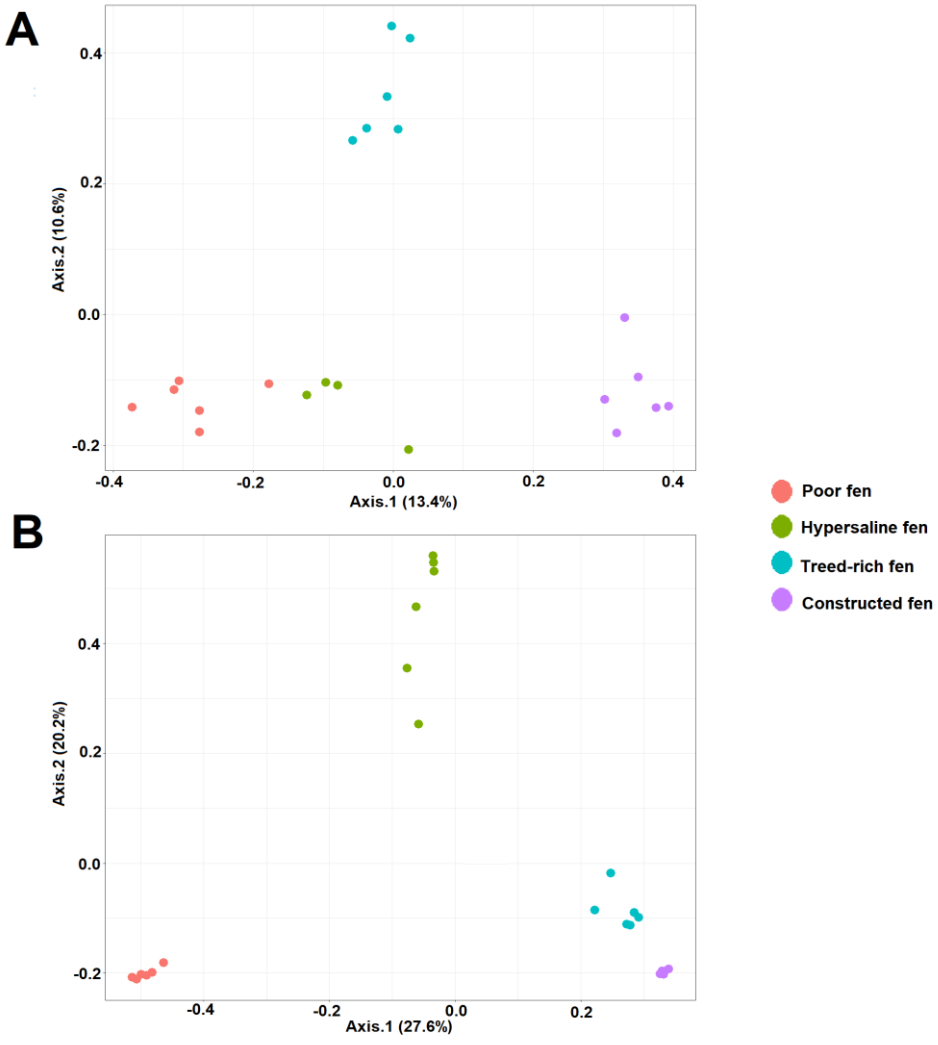

Fig 3.6. Principal coordinate analysis of fungal communities **(A)** poor fen (*n*=6), hypersaline fen (*n*=4), treed-rich fen (*n*=6) and constructed fen (*n*=6) and prokaryotic communities **(B)** poor fen (*n*=6), hypersaline fen (*n*=6), treed-rich fen (*n*=6) and constructed fen (*n*=5) across the different fen types. Different colours indicate four sampling sites including red for poor fen, green for hypersaline fen, blue for treed-rich fen and purple for constructed fen.

3.3.7: Relationship between fungal and prokaryotic communities and environmental factors

The RDA eigenvalues indicated that the axes 1 and 2 accounted for 49.17(%) and 39.67(%) of the overall variance of soil fungal communities respectively, whereas axes 1 and 2 explained 41.33(%) and 30.86(%) of the overall variance for prokaryotic communities respectively. Pore water pH, temperature, and plant richness were the main environmental factors that affected fungal communities. The final RDA model was significant (F=4.24, P=0.001) (Fig 3.7A). Pore water pH, conductivity, temperature, Mg and Pb were all significant in relation to prokaryotic communities. The final RDA model was significant (F=7.773, P=0.001) (Fig 3.7B).

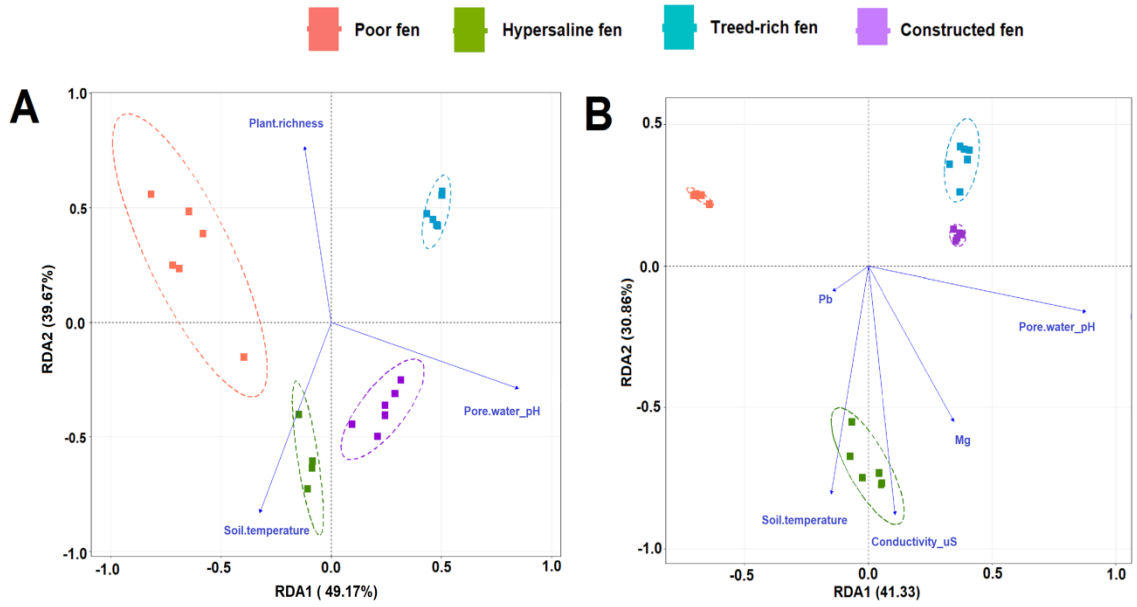

Fig 3.7. Redundancy analysis of fungal communities **(A)** poor fen (n=6), hypersaline fen (n=4), treed-rich fen (n=6) and constructed fen (n=6) and prokaryotic communities **(B)** poor fen (n=6), hypersaline fen (n=6), treed-rich fen (n=6) and constructed fen (n=5). The value of axis 1 and axis 2 are the percentages explained by the corresponding axis. Variables showing co-linearity with one or more variables are excluded from the final model plot. Different colours indicate four sampling sites including red for poor fen, green for hypersaline fen, blue for treed-rich fen and purple for constructed fen. The ASV data were standardized with Hellinger transformation using the *Vegan* package.

3.4: Discussion

Soil microbial communities are essential to soil ecosystem processes through mineralizing soil nutrients and decomposing organic matter. The functions provided by soil microorganisms are essential for the overall health of the soil ecosystem. Therefore, from a management perspective it is vital to understand changes in soil microbial community structure and function under complex environmental conditions. The current study was conducted in four fen peatlands that ranged in vegetation structure and soil chemistry, located within the AOSR. This analysis examined the changes in microbial function and community structure across three natural fens and a constructed fen.

3.4.1: Microbial catabolic activity across fens

The activity and abundance of the microbial communities in relation to management regimes can be used to predict the functional potential of the soil ecosystem. MicroResp™ has been used for determining the soil microbial metabolic activity and is an important indicator of soil quality (Moscatelli *et al*., 2018). The results show the importance of edaphic factors for substrate-induced respiration. Important properties included pore water pH, plant richness, B, temperature, NH_4^+ and K (Fig 3.3). Total respiration was significantly different between the different fens; the number of carbon sources that were utilized was highest in the constructed fen followed by the treed-rich fen, which indicates greater complementarily of carbon use by the relatively diverse microbial communities in these sites. Previous studies have shown that pH is an important controlling factor in the patterns of the use of carbon (Rutgers *et al*., 2016; Yao *et al*., 2011) as the catabolism of carbon sources and associated enzymes are responsive to pH (Tutu & Ciornea, 2011). Here, there were large differences between the lowest and highest pH values across fens. As a result, an unknown amount of the measured CO_2 could have come from CO_2 released from bicarbonate pools in the soil (Martens, 1987). The consumption of carboxylic acids such as citric acid, oxalic acid and malic acid were highest in the constructed fen. Carboxylic acids play vital roles in the mobilization of micronutrients (Clarholm *et al*., 2015) and have the potential to increase the amount of phosphorus that is available to plants, due to their effectiveness in the mobilization of phosphorus (Wu *et al*., 2016). These results may suggest that the other sites have limited availability of the essential mobilizable organic-acid nutrients that are important for the function of citric and oxalic acids (Tsado, 2016). On the other hand, those carboxylic acids excreted by plant roots may

play a vital role in the recovering vegetation structure as carboxylic acids are an essential component for the availability of microbial metabolism and root inputs to soil (Lagomarsino *et al*., 2007).

3.4.2: General characteristics of microbial communities and relationship with environmental variables

A higher alpha diversity for fungi and prokaryotes was observed in the treed-rich fen. This might have resulted from the treed-rich fen hosting higher plant diversity which was a fundamental predictor for higher fungal diversity in this study. Alternatively, systematic differences in soil chemistry were found between the four study areas, and this could also account for the differences in prokaryotic diversity. The findings of this study indicated that Ascomycota were more dominant than Basidiomycota representing 72% of the total ASVs found, and that Proteobacteria were more dominant than Acidobacteriota, Chloroflexi or Actinobacteriota. Ascomycota are known to change according to water logging time and plant diversity (Timling *et al*., 2014). The reclamation process caused vegetation and soil physicochemical changes and hence changed its overall ecology. These prevailing changes can alter populations of particular soil fungal groups (Voříšková *et al*., 2016) (Fig 3.4). This can be shown in the changes in taxa across different fens. For example, in this study the diversity of Basidiomycota was increased in the poor fen and treed-rich fen. It has been found that most saprotrophic fungi in wetlands are from the phylum Basidiomycota (Sterkenburg *et al*., 2015), but they have been shown to respond to habitat heterogeneity in different ways (Tedersoo *et al*., 2016). More research is required to discover how these interacting factors influence fungal communities and to what extent they affect each other, particularly in peatlands.

Proteobacteria was the most dominant phylum in the constructed, treed-rich and hypersaline fens. Litter decomposition is one of the primary functions of Proteobacteria (Huang *et al*., 2016; Mander *et al*., 2012) as well as playing an important part in nutrient cycling (Li *et al*., 2019). For example, Proteobacteria has a close association with the degradation of lignocellulose (Štursová *et al*., 2012; Yoon *et al*., 2010). Previous studies have shown that Proteobacteria is the most common phylum in soil (Chen *et al*., 2013; Kolton *et al*., 2011; Kuffner *et al*., 2012). Thus, despite the differences between the different fens,

Proteobacteria was the most dominant phylum. Previous reports have shown Proteobacteria are common in soil undergoing reclamation (Banning *et al*., 2011; Li *et al*., 2014). This demonstrates that despite environmental limitations such as nutrients, Proteobacteria play a fundamental role in the restoration process. Furthermore, the relative abundance of Bacteroidota was higher in the constructed fen. Generally, Bacteroidota are recognized as copiotrophic, favoring high-nutrient soils (Yang *et al*., 2019). The constructed fen was low in nutrients but high in moisture and pore water pH, which might explain their greater abundance. Interestingly, Acidobacteriota was the most dominant phylum in the poor fen. The top peat layers are commonly dominated with Acidobacteriota which are similar to bogs in vegetation structure and physicochemistry (Ivanova *et al*., 2020) (Fig 3.4).

The recovery of soil microbes after reclamation is not well understood. While microbial compositions varied greatly across different fens along the AOSR. The treed-rich fen and the constructed fen harbor having similar prokaryotic community assemblages indicating the community structure from the donor fen has maintained their community attributes after transfer to the constructed fen. This finding supports the aspect that soil prokaryotic community structure could provide an indicator for the trajectory of ecosystem recovery (Fig 3.6).

Soil microorganisms influence ecological processes by which are affected by changes in the soil environment. Pore water pH and temperature were important predictors for both fungal community and prokaryotic community structure, with plant species richness influencing fungal community structure and conductivity, Mg and Pb influencing prokaryotic community structure. The low abundance of fungi in the constructed fen may be a consequence of the lower plant diversity as it is a well-known driver of fungal community structure (Prober *et al*., 2015). Soil pH in particular has been found to influence the composition of microbial communities (Bahram *et al*., 2018; Fierer & Jackson, 2006; Kaiser *et al*., 2016). This shows the importance of environmental variables on microbial diversity and structure as these communities are sensitive to prevailing conditions. Important environmental parameters may be neglected leading to inappropriate conclusions, potentially leading to landscape mismanagement.

3.4.3: Microbial communities in relation to function

Microbial respiration is widely distributed among different groups of microorganisms and is considered a valuable indicator of microbial activity (Schimel & Weintraub, 2003). In this study, total microbial respiration was significantly higher in the constructed fen despite the presence of higher alpha diversity in the treed-rich fen. This may be a result of the constructed fen having a lower water table as the increased oxygen availability enhances microbial activity in the mineralisation of organic matter (Weldmichael *et al*., 2020). It is also possible that due to the high salinity exposure of the constructed fen from the surrounding landscape, the increased microbial activity could also be an expression of resistance to environmental stress. Previous studies have shown that metabolic activity can increase due to increased soil temperature (Bonnett *et al*., 2006; Kurbatova *et al*., 2013; Li *et al*., 2021; Wang *et al*., 2015). The observed higher soil temperature is likely due to the lack of tree cover in the constructed fen Soil microbial function in a community is likely to be determined by habitat type, which acts as a filter and could explain such a difference between microbial communities and microbial activity. Some groups of bacterial taxa are more sensitive to environmental changes caused by management and are not expected to contribute equally to ecosystem functions (Samaritani *et al*., 2017). Soil edaphic factors play an essential role in determining microbial physiology. Therefore, the function of microbial communities may be hard to transform in environments with insufficient variations in conditions. It is possible that the microbial communities are more responsive to the environmental differences caused by land use than catabolic activity.

In this study, higher respiration was observed for L-malic acid, ketoglutarate and citric acid in the treed-rich fen. The limited availability of nutrients in the constructed fen may cause the microbial community to broaden the capacity to obtain more carbon sources, and this may be a possible explanation for higher catabolic activity in this site. Previous research has shown that reduced activity can often be linked with increased microbial diversity (Becker *et al*., 2012; Huang *et al*., 2018; Jousset *et al*., 2011). The results in this study indicate a direction for further investigation that will increase the understanding of microbial diversity, composition and activity that are related with post-mining reclamation.

3.4.4: Conclusions

This study showed that soil chemistry, nutrient dynamics, biogeochemical processes, and microbial community structure and activity differed significantly between a recently established constructed fen and three contrasting natural fens that were used as "reference" analogues. In this study, the fungal and prokaryotic community structure as well as the microbial function measured through substrate induced respiration displayed differences across the fens that were strongly shaped by edaphic and environmental conditions as well as above-ground vegetation. The relationship between these microbial communities and environmental variables such as pore water pH, temperature and plant richness suggests that the outcome of fen construction and reclamation is likely to depend on how management influences these variables. Therefore, amelioration of these variables should be taken into consideration. In turn, the responses of microbial community structure and environmental conditions are likely to influence key biogeochemical processes and thus ecosystem functions. It has been shown that microbial function can change independently of microbial community structure (Teurlincx *et al*., 2018; Tian *et al*., 2016; Weedon *et al*., 2017). However, a focus on microbial function while ignoring community structure would be inappropriate for management practices, as the realized functioning of the ecosystem could ultimately be constrained by the microbial community structure (e.g. Heijboer *et al*., 2016). Further long-term studies are required to assess the changes in both the functional capacity and microbial community structure across the AOSR.

This study presents a fundamental insight into how soil microbial community structure and functioning differ across constructed and natural fens. Ultimately, our findings provide a valuable baseline for the microbial structure and functions of the constructed fen against which future changes can be measured and linked with ecosystem functioning and stability. Similarly, the trajectory of change in the constructed system can be mapped with our natural reference baseline. Further studies are encouraged to continue to unravel the linkages between microbial community structure and microbial function, to ensure ecosystem sustainability and inform management practices and conservation policies.

Chapter 4. Effects of a prescribed burning regime on vegetation, soil physicochemistry and prokaryotic microbial communities in surface and subsurface peat

Abstract

Prescribed burning is a common management strategy in peatlands that has the potential to affect soil physicochemistry, alter biogeochemical cycles and trigger changes in vegetation type and structure. How rotational burning affects prokaryotic community composition across different soil profiles is not well understood. This study explored the effects of prescribed rotational burning on the diversity of archaeal and bacterial communities in peat soils. Soil samples were collected from Moor House Nature Reserve, UK, a long-term monitoring site initiated in 1954 subject to three burning treatments: Burning at short rotations every 10 years, burning at long rotations every 20 years and a no further burn control. Observed species richness for archaea was highest in the topsoil of the non-burn control plots and highest for bacteria in the topsoil of the non-burn control and plots under a long rotation regime. Community composition was significantly different between different burn treatments as well as different depth profiles. Archaea and bacteria community structure were shaped by different edaphic factors. In particular archaeal community structure was shaped by NH_4^+ and pH in the topsoil and Pb, moisture and Al in the 20-40cm profile, total N, total C, Al, Ca, Fe and pH in the 40-60cm profile while bacterial community structure was shaped by NH_4^+, heather cover %, pH and Mg in the topsoil, Fe, K and Pb in the 20-40cm profile and Al, Ca and Fe in the 40-60cm profile. A co-occurrence network analysis revealed that the topsoil of the non-burn control plots had a larger and more complex network structure with more positive links than those under a rotational burn, but a higher average connectivity with a higher number of negative links was observed in the long rotation 20-40cm soil layer. The spatial variation of archaea and bacteria provides critical information on below-ground soil ecology. The results provide a new understanding of the response processes of soil prokaryotic communities to rotational burning, and offer valuable knowledge supporting the evaluation of management in peatlands.

Keywords: Prescribed burning; Peatlands; Biogeochemical cycles; Prokaryote community; Co-occurrence network.

4.1: Introduction

Peatlands provide a wide range of ecosystem services including sequestration of carbon, harbouring biodiversity and safeguarding drinking water (Lal, 2004; Rosario-Ortiz *et al*., 2016; Smith *et al*., 2015; Yang *et al*., 2009). Soil microbes are essential in sustaining the peatland ecosystem, and degradation due to human activities can threaten peatland function linked to shifts in archaeal and bacterial community structure (Evans *et al*., 2014; Mendes *et al*., 2015). Therefore, archaea and bacteria have been identified as essential indicators for ecological processes in peatlands and it is important to describe the factors that drive the abundance and diversity of these communities (Ritson *et al*., 2021; Wiesmeier *et al*., 2019). Despite soil microbial communities being heterogeneous in time and space it has become widely concluded that site-specific environmental parameters such as soil pH, soil texture, climate conditions, the type of land management and land-use intensity are major drivers of soil microbial community structures (Bauer *et al*., 2017; Kallenbach *et al*., 2016; Thomson *et al*., 2015). On the other hand, it is also recognised that microbes in the soil are ecosystem engineers which alter the physicochemical properties of the soil (Cary *et al*., 2010; Elliott *et al*., 2019; Jones *et al*., 2010), thus the interaction between organisms and the environment is a dynamic one that can be altered by anthropogenic activity.

Prescribed burning is a common management method for peatlands used for land clearance and the prevention of wildfires. Burning regimes plays a key role in structuring plant communities (Whitehead *et al*., 2021), as well as having an impact on above and below-ground C stocks through combustion, and continuous effects on the following ecosystem recovery (Clay *et al*., 2015; Heinemeyer *et al*., 2018; Marrs *et al*., 2019). Prescribed burning can alter critical biotic and abiotic processes and have drastic consequences on vegetation community structure as well as alter soil structure causing nutrient loss through leaching, considerably shifting chemical properties (de Vries *et al*., 2018). In boreal regions, the short-term effects of prescribed burning on cation concentrations have been observed (Brown *et al*., 2014; Fontúrbel *et al*., 2021) which in turn affect microbial community structure. Many studies have focused on microbial community structure in surface peat (Andersen *et al*., 2013; Elliott *et al*., 2015; Myers *et al*., 2012; Peltoniemi *et al*., 2015; Thormann & Rice, 2007). However, many key ecosystem responses occur in the subsoils (Urbanová & Bárta, 2016). Changes in soil condition, as well as water table fluctuation, may drive changes in the depth profiles of archaeal and bacterial communities, which are crucial for regulating peatland biogeochemical cycles (Andersen *et al*., 2013; Lamit *et al*., 2017; Lin *et al*,. 2014;

Peltoniemi *et al*., 2015; Wang *et al*., 2019). Microbial communities in the subsoil also vary greatly and exhibit different characteristics from those in the surface soil (Fritze *et al*., 2000), and community turnover can be under greater influence in deeper soil because of more difficult dispersal, heterogeneous environmental niches and due to the isolation from the surface soil (Du *et al*., 2021). Because archaea and bacteria play an important role in peatlands, governing soil C cycling, it is important to understand how these communities are affected by prescribed burning throughout different soil profiles.

Traditionally, the conservation management of peatlands has focused mainly on above-ground visible communities such as plants (Couwenberg *et al*., 2011; Nishimura *et al*., 2009; Noble *et al*., 2018; Noble *et al*., 2019). To better understand essential processes such as soil health and functioning it is increasingly recognised that it is useful to also take the microbiome into account (Elliott *et al*., 2015; Ritson *et al*., 2021). The recent technological advancements in DNA sequencing has opened up large scale studies concerning the microbial communities (Oulas *et al*., 2015; Tan *et al*., 2015).

The relative balance of community assembly processes determines the structure of microbial communities (Stegen *et al*., 2015). Therefore, in order to understand potential differences in community structure in peatlands, we may gain insight through identifying the community assembly processes that are at work. Microbial community assembly is governed by ecological processes such as selection; thus, microbial interaction should contribute to microbial community assembly by acting as a force of selection (Hunt & Ward, 2015) and its implications on biogeochemical cycling (Morriën *et al*., 2017). Microbial co-occurrence networks have been studied in diverse environments by a growing number of researchers (Agler *et al*., 2016; Shi *et al*., 2016; Wang *et al*., 2016; Wang *et al*., 2018). Networks provide an additional understanding into the organisation of communities, such as demonstrating that a diverse community composition and multiple interactions are critical to the stability of biological communities (Mougi & Kondoh, 2012). In addition, microbial interactions have been highlighted as essential to understanding the changes in microbial community assembly in the face of global climate change (Yuan *et al*., 2021). Since most studies only focus on microbial communities in the topsoil (ie. 0-10cm) there is limited knowledge about how these communities interact in the subsoil under different land management. Network analysis is also useful for identifying keystone species that are essential for maintaining the communities overall structures and functions (Deng *et al*., 2012), yet the effects of burning on microbial

interactions and keystone taxa are unknown. Therefore, it is also vital to consider the indirect impact of prescribed burning practices on microbial community networks in the topsoil and subsoil.

This chapter aims to evaluate the impact of prescribed burning on prokaryotic communities in peat soils and determine (1) how abiotic soil parameters affect below-ground soil communities considering key chemical parameters to assess these impacts, (2) Predict key microbial taxa found to be indicators and keystone species of specific burn treatments, (3) Assess the impact of burning on soil microbial communities at different depth profiles, (4) Determine how co-occurrence network patterns respond to burning regimes. Based on this the following hypotheses were tested:

1. There will be significant changes in archaeal and bacterial alpha diversity between burn treatments and different soil profiles. It is expected that the diversity of archaea and bacteria will be greater in unburned plots due to the lack of disturbance and greater variety of microsites providing more available niches.
2. The structure of archaeal and bacterial communities will significantly change across different burn regimes and soil depths due to changes in soil environmental conditions.
3. Prokaryotic network structure will be more complex and less modular in the non-burned control plots compared to burned plots since unburned plots contain microbial communities and plants that have interacted over a longer period of time.

4.2: Materials and methods

Details of the study site are given in chapter 2 section 2.1.2. The experimental design, vegetation cover and physicochemistry measurements are given in the general methods (chapter 2).

4.2.1: PCR amplification and sequencing

Extracted DNA (chapter 2, section 2.4) was used as a template for PCR reactions and sequencing. The primer pair Bakt_341F (5′-CCTACGGGNGGCWGCAG-3′) and Bakt_805R (5’ GACTACHVGGGTATCTAATCC-3′) was used to amplify the V3 and V4 region of bacterial 16S rRNA gene (Herlemann *et al*., 2011) whereas the V6 and V8 region of the archeal 16S rRNA was amplified using the primer pair A956F (5’-TYAATYGGANTCAACRCC-3’) and A1401R (5’-CRGTGWGTRCAAGGRGCA’3’) (Comeau *et al*., 2011). PCR reactions were performed using a 20µl mixture containing 10µl 2x Qiagen Multiplex PCR master mix, 2µl forward primer, 2µl reverse primer, 5µl of RNase/DNase-free water and 1µl of DNA template. The PCR reactions were conducted in a thermocycler PCR system (MJ Research ptc-225 peltier thermal cycler) using the following program: 3 min of denaturation at 95°C, 35 cycles for 30s at 95°C, 30s for annealing at 55°C, and 45 s for elongation at 72°C, and a final extension at 72°C for 10 min for archaea and 3 min of denaturation at 95°C, 25 cycles of 30 s at 95°C, 30s for annealing at 50°C, and 45s for elongation at 72°C, and a final extension at 72°C for 10 min for bacteria. The presence of a PCR product of the correct size was verified using 1% agarose gel electrophoresis. PCR products were cleaned using Agencourt AMPure XP magnetic beads (Beckman Coulter, Indianapolis, USA), then a secondary PCR was conducted with barcoded Fi5 and Ri7 identifier sequences ligated to each sample. The secondary PCR mixtures (20µl) contained 1µl of Fi5 primer, 1µl Ri7 primer, 8µl of product from PCR 1 and 10µl of Qiagen multiplex master mix. Following the second PCR, a FLUOstar Optima (Promega) was used to measure 2µl of product from each reaction. Based on these results samples were standardised to equal concentrations, pooled into groups of 12 and cleaned using AmPure XP beads (Beckman Coulter, Indianapolis, USA). The Illumina-tagged DNA concentration of each pool was determined using the KAPA Library Quantification Kit on an Applied Biosystems QuantStudio 12K and DNA fragment size was determined using an Agilent 2100 Bioanalyzer (Agilent Technologies Ltd., Stockport, UK). The KAPA Library Quantification Kit and a QUBIT 3.0 with the dsDNA HS test (Invitrogen, UK) was used to quantify the final pools. Libraries were sequenced on an Illumina MiSeq platform at 2 x 250 bp paired-end sequencing (Magoč & Salzberg, 2011) at the Centre for Genomic Research University of Liverpool.

4.2.2: Bioinformatics

Sequences were processed using QIIME2 v2019.7 (Bolyen *et al*., 2019). First, primer sequences were removed using cutadapt v1.9.1 (Martin, 2011). Sequences were then quality filtered and arranged as amplicon sequence variants (ASVs) (expected error rate = 2), chimeras were removed and reads were joined, all using DADA2 (Callahan *et al*., 2016). Before ASV generation, forward and reverse reads were truncated at a length of 234 bp and 226 bp for archaea and a forward truncation length of 0 bp and reverse truncation length of 229 bp for bacteria (appendix 2). The q2-dada2 plugin generates amplicon sequence variants (ASVs) or sequence clusters with 100% similarity instead of the commonly chosen 97% similarity, which estimates the true biological variation within each sample. DADA2 results in fewer erroneous sequences and clusters, as well as a more accurate representation of the true biological variation present (Callahan *et al*., 2016). The taxonomic assignment was performed using the SILVA 138 database (Quast *et al*., 2012). Extrinsic domain ASVs were removed prior to further analysis. Rarefaction curves were generated using the R package "*ampvis2*" (Andersen *et al*., 2018) and rarefaction curves reached asymptote in all cases, indicating that sufficient sequencing depth was achieved (appendix 3). Although the overall results of the analysis using rarefied and unrarefied data were similar, it is thought that rarefied data can overlook the presence of rare species and lead to false positives (McMurdie & Holmes, 2014). Therefore, the analysis was based on unrarefied data. Following quality filtering, three samples of archaea data had very low reads (<1000) which was considered insufficient for a statistically powerful analysis and thus may cause a potential source of bias. As a result, these read-poor samples were removed from further downstream analysis (see appendix 4). Rare microbial taxa were excluded from ordination analyses, leaving only ASVs with a total relative abundance of >0.001, as ordination analysis is sensitive to rare species (Legendre & Gallagher, 2001).

4.2.3: Statistical analysis

All statistical analyses were carried out using R version 4.0.2 software (R Development Core Team, 2020). One-way analysis of variance (ANOVA) was used to evaluate differences caused by burn treatments on vegetation characteristics (vegetation height and percent cover of plant groups), followed by Tukey's *post-hoc* honest significant difference (HSD) for multiple comparison test ($P < 0.05$) after checking for normality and homogeneity of variance

with the Shapiro-Wilk test and the Bartlett test. Analysis to assess the effects of burn treatment, soil depth and their interaction on the measured soil physicochemical parameters were conducted by two-way analysis of variance with Tukey's *post-hoc* test for multiple comparisons following the Shapiro-Wilk test and the Bartlett test for normality and homogeneity of variance respectively. Further, when the interaction was not significant, one-way ANOVA and Tukey's *post-hoc* test for multiple comparisons were used to evaluate differences based on burn treatments within a soil layer, and among the three soil layers within a given burn treatment. Principal Component Analysis (PCA) was then used to explore the differences in soil physiochemical properties between the different burn treatments using the R package *FactoMineR* (Lê *et al*., 2008). Alpha diversity was calculated to compare microbial community diversity between burn treatments and depth, including observed richness, Shannon and Simpson diversity using the R package '*Phyloseq*' (McMurdie & Holmes, 2013). Two-way analysis of variance with Tukey's *post-hoc* test for multiple comparisons was used to test the effects of burn treatment and soil depth on alpha diversity following the Shapiro-Wilk and Bartlett tests for normality and homogeneity of variance, respectively.

The ASV table was normalised for the analysis of community composition (β-diversity) by transforming to proportions using the R package *microbiomeSeq* (Ssekagiri *et al*., 2017). Other normalisation techniques were tested including rarefying, variance-stabilizing transformation and the "trimmed means of M" (TMM) with the R package '*edgeR*' (McCarthy *et al*., 2012; Robinson *et al*., 2010). All methods showed similar results. However, in this study proportions were used as it has been shown to produce more accurate dissimilarities and more efficient at standardizing read depths (McKnight *et al*., 2019). Differences in microbial community composition were visualized using principal coordinates analysis (PCoA, Gower, 1966) based on a Bray-Curtis distance matrix using the R package '*Vegan*' (Oksanen *et al*., 2013). Permutational Multivariate Analysis of Variance (PERMANOVA) (Anderson, 2001) was conducted to assess the significance of different effects (burn treatment and soil depth) using the adonis function in *Vegan* with 999 permutations.

The relationship between environmental variables and prokaryotic communities was assessed using redundancy analysis (RDA) after standardizing the ASV matrix using the Hellinger transformation. The 'best' explanatory environmental variables were chosen with forward selection using the ordistep function (Blanchet *et al*., 2008). Significant environmental

variables, as confirmed by analysis of variance, were retained for the final RDA. The variation inflation factor (VIF) was used to check non co-linearity among the explanatory variables (VIF<10), as recommended by Montgomery & Peck (1992). RDA analysis was performed on topsoil (0-20cm) and subsoil (20-40cm and 40-60cm) separately. Using the function indval in the R package '*labdsv*', indicator species significantly associated with each treatment were determined (Indval values >0.3 and $P< 0.05$ are strong indicators) (Roberts, 2016).

4.2.4: Network analysis

The Molecular Ecological Network Analyses Pipeline was used to conduct the network analysis based on Random Matrix Theory (RMT) (Deng *et al*., 2012). Specifically, for each treatment, only ASVs occurring in >50% of the total samples were used for network computation. Bacteria and archaea data were combined and the original data (ASV table) were classified and uploaded according to the MENA format. The Pearson's correlation of any two ASVs were used to create the correlation matrix, which was then converted into a similarity matrix. Based on random matrix theory, the similarity threshold was automatically identified, and the similarity matrix was converted into an adjoining matrix to the connection strength between ASV nodes. Network properties were calculated based on the determined similarity threshold (network reports in MENA). All of the robust correlations discovered through pairwise ASV abundance comparisons form a correlation network, with one ASV represented by a node and each edge indicating the significance of a correlation between the nodes. To describe network topology, the number of nodes and edges, average path length, harmonic geodesic distance, average connectivity, average clustering coefficient and modularity was calculated. Topological properties used to describe networks and nodes are summarized in Table 4.1.

Table 4.1. Topological indexes used to characterize networks and nodes. Adapted from Deng *et al*. (2012).

Network index	Definition
Number of nodes	The number of individual taxa
Number of links	The number of significant associations between nodes (positive or negative)
R^2 power of law	Quantifies the scale-freeness as the goodness of fit to the node degree distribution
Average connectivity (avgK)	Connection strength between nodes, higher connectivity means a more complex network
Harmonic geodesic distance (HD)	A smaller *HD* means that all the nodes in the network are closer
Average clustering coefficient (avgCC)	Measures the extent of the connection between a node and its neighbour nodes in the network
Average path length	Shortest path between two nodes
Modularity (M)	Tendency of a network to contain sub-clusters of nodes

The topological roles of each node were evaluated by the threshold values of *Zi* and *Pi* (Guimera & Amaral, 2005). Node topologies were categorized by the following: (1) *Zi*> 2.5= highly connected nodes within module hubs; (2) $Z_i > 2.5$ and $P_i > 0.62$ = nodes which are highly connected within the entire network; (3) $P_i > 0.62$= nodes connecting modules, and (4) $Z_i < 2.5$ and $P_i < 0.62$= interconnected nodes within modules (Deng *et al*., 2012; Zhou *et al*., 2011). These topological features indicate the significance of a node as capable of holding communicating nodes together and were used to define keystone species. Module hubs, connectors, and network hubs are ecologically similar to generalists and are classified as keystone taxa (Zhou *et al*., 2011). The networks were visualized using Gephi version 0.9.2 (Bastian, 2017).

4.3: Results

4.3.1: Effects of burn treatment on plant cover and soil properties

Overall vegetation height was significantly different between the three burn treatments (F=30.65, P=0.007) being highest in the non-burn control (M = 47.66cm, SD = 8.55) followed by the long rotation (M = 33.66cm, SD = 5.74) and short rotation (M= 23.75cm, SD = 8.00). The percentage of *Sphagnum* moss cover also showed a significant difference between burn treatments and was highest in plots under a short rotation burn (M = 31.66%,

SD = 20.61) (*F*=5.67, *P*=<0.0001). Other 'non-Sphagnum moss' cover was equally high in the short rotation burn plots (*M* = 11.25%, *SD* = 3.88) and long rotation burn plots (*M* = 14.00%, *SD* = 2.35) and lower in the non-burn control (*M*=3.25, *SD* = 1.95) (*F*=45.99, *P*=<0.0001). Percentage of heather cover was significantly higher in non-burn control plots (69.00% ± 12.00) and lowest in plots under short rotation regimes (*M* = 1.91%, *SD* = 0.96) (*F*=99.48, *P*=<0.0001). Likewise, the cover percentage of other vascular plants was higher in non-burned plots (*M* = 13.91%, *SD* = 10.00) (*F*=17.07, *P*=0.001). Graminoid cover percentage was significantly higher in plots under a short rotation regime (*M* = 53.00%, *SD* = 21.01) and lowest in non-burn control plots (*M* = 3.00%, *SD* = 4.01) (*F*=28.92, *P*=<0.0001) (Fig 4.1).

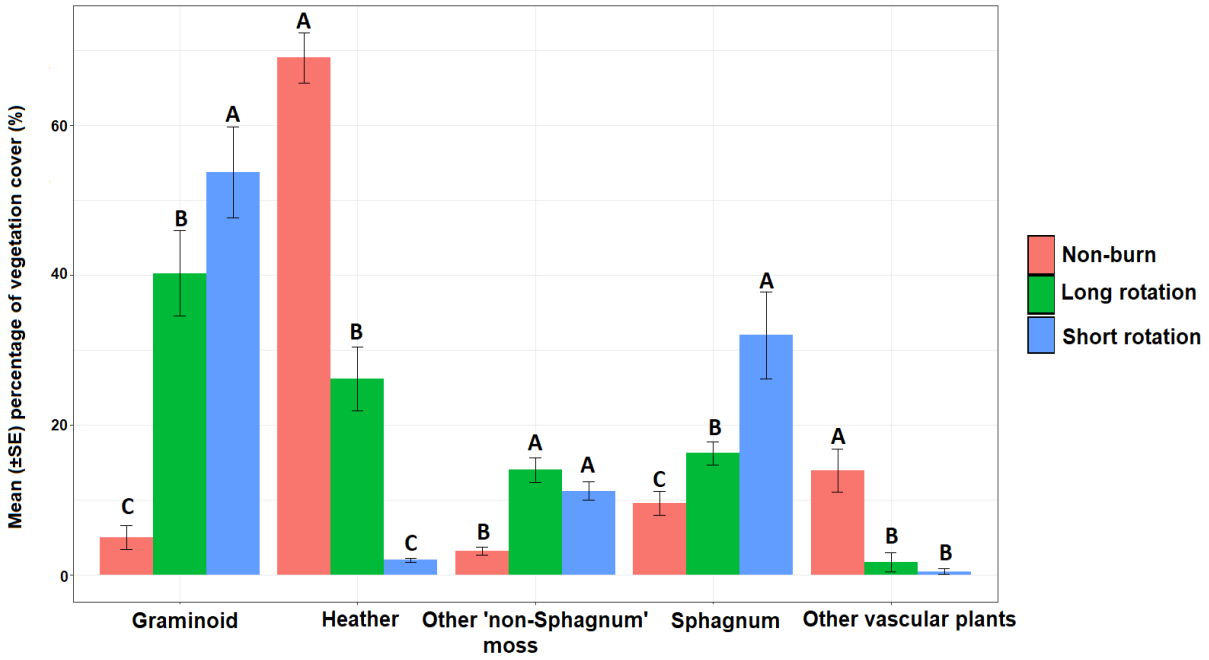

Fig 4.1. Percentage cover of Graminoids, Heather, other 'non-Sphagnum' moss, *Sphagnum* and other vascular plants ($n = 36$). Different letters indicate significant pairwise differences using Tukey's HSD *post-hoc* test at a confidence level of 95% ($P<0.05$).

A PCA showed that soil physicochemistry was distinctly different between burn treatments across all three depth profiles. All of the variables for all of the tested scenarios are presented graphically in Fig 4.2. The first and second principal components (DIM1 and DIM2) explained 37.2% and 27.6% respectively in the 0-20cm profile, 36.7% and 22% in the 20-40cm profile and 31.8% and 21.2% in the 40-60cm profile. In the 0-20cm profile, the first axis correlated highly with the following variables: Total N, NO_3^-, Fe, Ca, P, K and Al. The second axis was positively correlated with moisture and negatively correlated with pH, Zn, NH_4^+, Mn, Total C, Mg, Pb and Cu. In the 20-40cm profile the first axis correlated with Fe, Ca, Al, P and Cu generally associated with the long rotation regime. In the 20-40cm profile, the second axis correlated with Pb, Mg, pH, Zn, Total N, Total C, NH_4^+, NO_3^-, K and moisture associated with the non-burn. The second axis of the 40-60cm was correlated with total N, NO_3^-, pH, Mg and Zn associated with the non-burn control while Cu, Fe, Mn, Ca, P and Al were associated with the long rotation regime (Figs 4.2). In PC1, Pb showed a higher loading value (-0.36) while in PC2, Fe showed a higher loading value (-0.42). In the 20-40cm profile Pb (-0.38) showed a higher loading in PC1 and Fe in PC2 (-0.46) respectively (Table 4.2). Likewise, in the 40-60cm profile Fe showed the highest loading value (0.41) in PC1 while in PC2 Al showed the highest loading value (0.36) (Table 4.2).

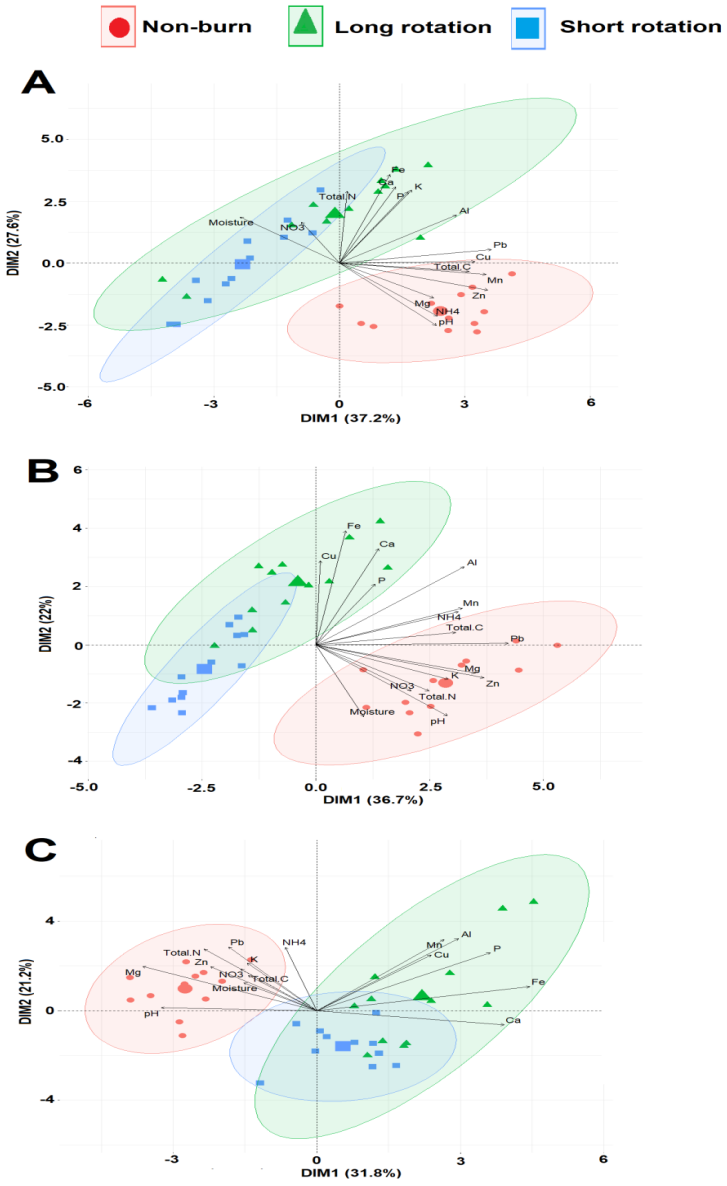

Fig 4.2. PCA biplot of soil physicochemical properties (*n*=12) within tested burn treatments. A= 0-20cm, B=20-40cm, C=40-60cm. Ellipses denote the 95% confidence intervals for the centroids and the separation between soil physicochemistry between different burn treatments respectively. The biplot shows the PCA scores of the explanatory variables as vectors (in black) and samples of each treatment of the first (*x*-axis) and second (*y*-axis) principal components (PCs). Variables on the same side as a sample have an important contribution on it. The dimensions of the vectors show the strength of their contribution to each principal component. Vectors pointing in the same direction show positively correlated variables, vectors pointing in the opposite direction show negatively correlated variables.

Table 4.2. Principal component analysis of soil physicochemical properties across burn treatments in different soil depths (*n*=12). Positive loadings indicate a variable are positively correlated while negative loadings indicate a negative correlation.

	0-20cm		**20-40cm**		**40-60cm**	
	PC1	**PC2**	**PC1**	**PC2**	**PC1**	**PC2**
Moisture	0.239389	-0.21949	-0.10328	0.319771	-0.13755	0.140035
pH	-0.23417	0.295852	-0.2608	0.294043	-0.29279	0.029841
Total N	-0.01903	-0.3415	-0.22963	0.175457	-0.20909	0.311215
Total C	-0.31405	0.039575	-0.28434	-0.04422	-0.11536	0.182601
NO_3^-	0.091284	-0.19513	-0.19797	0.206133	-0.12613	0.223756
NH_4^+	-0.23773	0.25112	-0.28969	-0.12714	-0.0761	0.321803
Mg	-0.22841	0.165664	-0.31957	0.129023	-0.32759	0.233865
Ca	-0.13599	-0.36133	-0.12956	-0.39172	0.359631	-0.08608
Mn	-0.35598	0.054104	-0.29241	-0.17721	0.282255	0.357821
Fe	-0.12248	-0.42088	-0.07386	-0.46201	0.416317	0.106363
Cu	-0.32815	-0.00737	-0.01665	-0.34134	0.228201	0.265659
Zn	-0.35973	0.127658	-0.33555	0.140051	-0.1985	0.226359
P	-0.16604	-0.33809	-0.16024	-0.2226	0.339799	0.288358
Pb	-0.36864	-0.06452	-0.38867	0.001631	-0.1649	0.31822
K	-0.17495	-0.34675	-0.26879	0.155405	-0.13159	0.239129
Al	-0.28368	-0.22771	-0.30478	-0.30839	0.274842	0.361685

Two-way ANOVA showed that burn treatment, soil depth and their interaction had significant effects on soil properties, except for pH, P, K and Al (Fig 4.3, appendix 5, Table A.5.1). Soil pH was significantly higher in the non-burn control and only increased with depth in the long rotation burn treatment. The content of P was significantly different in top soil across treatments being highest in the long rotation plots (Fig 4.3, appendix 5, Table A.5.1). The content of K was significantly different between burn regimes and soil depth being highest in the topsoil. The content of K was highest in the non-burn control in the 20-40cm and 40-60cm depth profiles. Al was significantly different across burn treatments being highest in the long rotation burned plots (Fig 4.3, appendix 5, Table A.5.1).

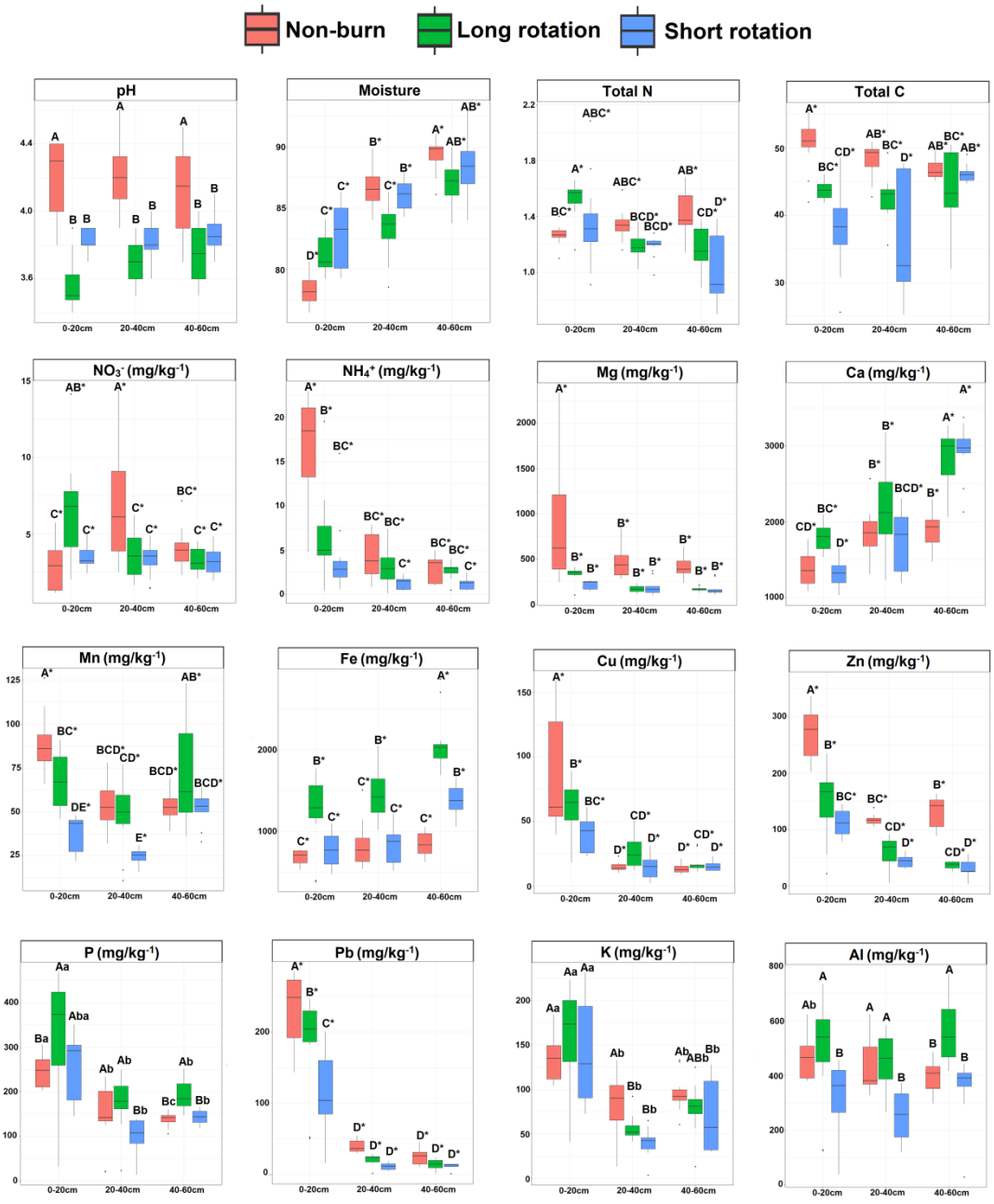

Fig 4.3. Vertical distribution of soil physiocochemical properties under three burn treatments (n=12). Values are expressed as mg kg^{-1} dry soil except for pH, moisture, total N and total C. Different uppercase letters indicate statistically significant differences among the three burn treatments in the same soil layer, different lowercase letters indicate statistically significant differences among the three soil layers across burn treatments and different letters with an asterisk indicate a significant difference among treatments when there was a significant interaction between burn treatment and soil depth at a confidence level of 95% (Tukey's HSD, $P < 0.05$).

4.3.2: General characteristics of archaea and bacteria communities across burn treatments and depth

The relative abundance of different phyla showed clear changes across the three burn treatments and soil depth (Fig 4.4). The three most dominant archaeal phyla across all samples were Crenarchaeota (36%), followed by Thermoplasmatota (35%) and Halobacterota (29%). The phyla, Asgardarchaeota, Euryarchaeota and Micrarchaeota were present in very low abundance representing <1% across all samples. Acidobacteriota was the most abundant bacterial phyla overall at 48% followed by Desulfobacterota (22%) and Proteobacteria (14%) (Fig 4.5). In the topsoil, Crenarchaeota was the most abundant phylum across all burn treatments, with relative abundances of 41% followed by Thermoplasmatota (35%) and Halobacterota (30%). Acidobacteriota was the most abundant bacterial phyla in the topsoil overall being highest in plots under a long rotation regime (59%) (Fig 4.5). In the 20-40 cm profile the abundances of archaeal phyla were; Crenarchaeota (34%), Thermoplasmatota (36%) and Halobacterota (30%). The phylum Thermoplasmatota had the highest abundance (37%) in the lower soil profile (40-60cm) followed by Crenarchaeota (32%) and Halobacterota (30%). Compared to the long rotation burn regime, the relative abundance of Crenarchaeota was higher in plots under short rotation regimes (49%), followed by the non-burn control (42%) in the topsoil (Fig 4.4). However, the relative abundance of Crenarchaeota became lower in plots under a short rotation regime (18%) and higher in the non-burn control (38%) and long rotation burns (36%) in the 40-60cm profile (Fig 4.4). In the 20-40cm profile, the bacterial phylum Acidobacteriota was the most dominant phyla overall (54%) followed by Desulfobacterota (27%) and Proteobacteria (8%). In the 40-60cm profile Proteobacteria increased (22%) in plots under a short rotation regime (Fig 4.5).

The relative abundance of Acidobacteriota was higher in the long rotation burned plots across all depth profiles (ANOVA, P=<0.05) (Fig 4.5), whereas Desulfobacterota was higher in the non-burn control across all three depth profiles (ANOVA, P = <0.05) (Fig 4.5). In addition, the relative abundance of Proteobacteria was higher in the short rotation in subsoil profiles (Fig 4.5).

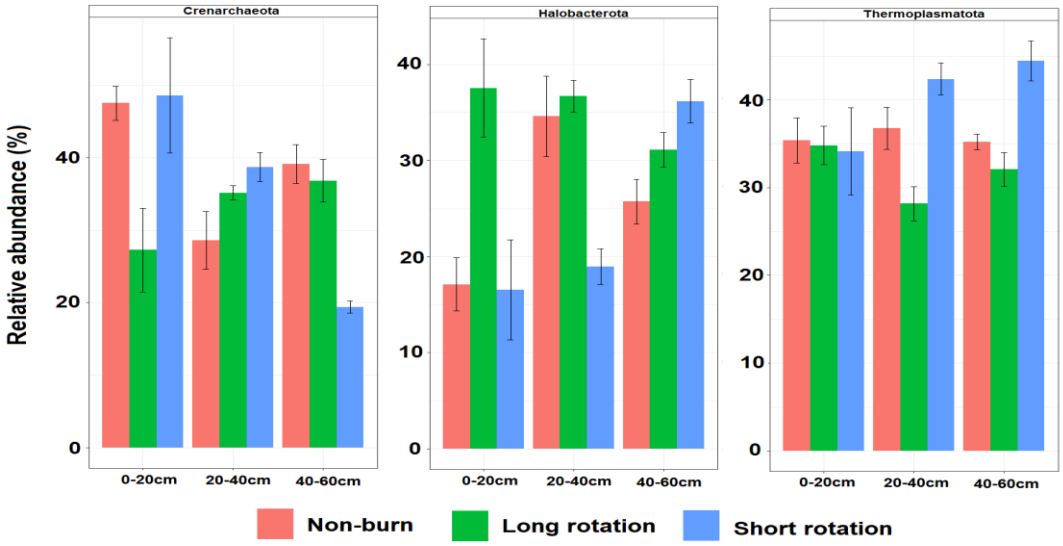

Fig 4.4. Relative abundance of the top 3 archaeal phyla across three different soil depths under three burn treatments. The bars indicate the mean values of each treatment, with the error bars representing the standard error. Non-burn 0-20cm (*n*=11), non-burn 20-40cm (*n*=12), non burn 40-60cm (*n*=12), long rotation 0-20cm (*n*=12), long rotation 20-40cm (*n*=10), long rotation 40-60cm (*n*=12), short rotation 0-20cm (*n*=12), short rotation 20-40cm (*n*=12), short rotation 40-60cm (*n*=11).

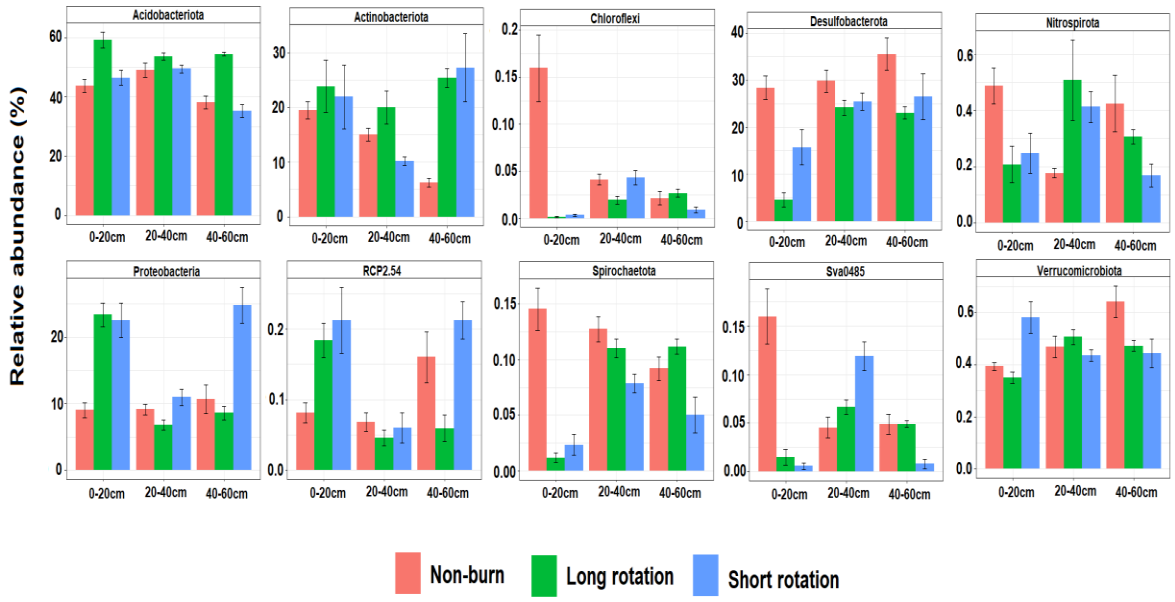

Fig 4.5. Relative abundance of the top 10 bacterial phyla across three different soil depths under three burn treatments. The bars indicate the mean values of each treatment, with the error bars representing the standard error. Non-burn 0-20cm (*n*=12), non-burn 20-40cm (*n*=12), non burn 40-60cm (*n*=12), long rotation 0-20cm (*n*=12), long rotation 20-40cm (*n*=11), long rotation 40-60cm (*n*=12), short rotation 0-20cm (*n*=12), short rotation 20-40cm (*n*=12), short rotation 40-60 (*n*=12).

4.3.3: Archaea and bacteria diversity and community composition

Two-way ANOVA showed that burn treatment, soil depth and their interaction had a significant effect on observed, Shannon and Simpson diversity for archaea communities (Table 4.3; Fig 4.6). Likewise, there was a significant two-way interaction between burn treatment and soil depth on observed, Shannon and Simpson diversity for bacteria communities (Table 4.4; Fig 4.6).

Table 4.3. Two-way ANOVA of archaea alpha diversity indices across three different soil depths under three burn treatments. Result is reported as the mean ± SE. The data in bold indicate archaea diversity that were affected by soil depth, burn treatment and their interaction at a confidence level of 95% ($P < 0.05$). Different letters indicate a significant difference among treatments based on the effect of interactions between burn treatment and soil depth (Tukey's HSD, $P < 0.05$). Non-burn 0-20cm (n=11), non-burn 20-40cm (n=12), non burn 40-60 (n=12), long rotation 0-20cm (n=12), long rotation 20-40cm (n=10), long rotation 40-60cm (n=12), short rotation 0-20cm (n=12), short rotation 20-40cm (n=12), short rotation 40-60cm (n=11).

Burn treatment	**Depth (cm)**	**Observed**	**Shannon**	**Simpson**
Non -burn	**0-20cm**	88.45 ±4.79 A*	2.79 ± 0.05 A*	0.83 ± 0.05 A*
	20-40cm	37.91 ±8.71 C*	1.87 ± 0.08 C*	0.73 ± 0.01 D*
	40-60cm	43.91 ± 2.39 C*	2.37 ± 0.03 AB*	0.82 ±0.07 AB*
Long rotation	**0-20cm**	39.41 ± 7.32 C*	1.96 ± 0.19 BC*	0.73 ± 0.08 CD*
	20-40cm	51.1. ±3.37 BC*	2.06 ± 0.03 BC*	0.78 ± 0.006 ABCD*
	40-60cm	54.08 ± 4.02 BC*	1.96 ± 0.03 BC*	0.76 ± 0.06 ABCD*
Short rotation	**0-20cm**	57.83 ±3.35BC*	2.47 ± 0.18 AB*	0.77 ± 0.04 ABCD*
	20-40cm	71.75 ± 6.15AB*	2.60 ± 0.04 A*	0.82 ± 0.06 ABC*
	40-60cm	57.63 ± 5.41 BC*	1.98 ±0.09 BC*	0.75 ± 0.02 BCD*
Burn treatment		**F=7.19,P=0.001**	**F=9.68,P=<0.01**	F=2.26,P=0.10
Depth (cm)		**F=3.433,P=0.03**	**F=3.90,P=0.02**	F=0.09,P=0.90
Burn treatment* Depth (cm)		**F=18.70,P=<0.01**	**F=9.72,P=0.02**	**F=7.14,P=<0.001**

Table 4.4. Two-way ANOVA of bacteria alpha diversity indices across three different soil depths under three burn treatments. Result is reported as the mean ± SE. The data in bold indicate bacteria diversity that were affected by soil depth, burn treatment and their interaction at a confidence level of 95% ($P < 0.05$). Different letters indicate a significant difference among treatments based on the effect of interactions between burn treatment and soil depth (Tukey's HSD, $P < 0.05$). Non-burn 0-20cm (n=12), non-burn 20-40cm (n=12), non burn 40-60cm (n=12), long rotation 0-20cm (n=12), long rotation 20-40cm (n=11), long rotation 40-60cm (n=12), short rotation 0-20cm (n=12), short rotation 20-40cm (n=12), short rotation 40-60 (n=12).

Burn treatment	**Depth (cm)**	**Observed**	**Shannon**	**Simpson**
Non-burn	**0-20cm**	272.41 ± 19.92 A*	4.06 ± 0.12 AB*	0.94 ± 0.008 AB*
	20-40cm	218.91 ± 20.42 ABC*	3.46 ± 0.10 BC*	0.91 ± 0.01 BC*
	40-60cm	118.58 ± 12.34 D*	3.95 ± 0.09 D*	0.91 ± 0.008 B*
Long rotation	**0-20cm**	246.83 ± 33.03 AB*	4.51 ± 0.15 A*	0.97 ± 0.003 A*
	20-40cm	150 ± 14.25 CD*	3.08 ± 0.07 D*	0.87 ± 0.009 C*
	40-60cm	207 ± 21.67 ABC*	3.56 ± 0.11 BCD*	0.91 ± 0.006 B*
Short rotation	**0-20cm**	169.16 ± 18.19 BCD*	3.86 ± 0.12 BC*	0.94 ± 0.01 AB*
	20-40cm	164.58 ± 11.33 BCD*	3.66 ± 0.07 C*	0.93 ± 0.006 AB*
	40-60cm	155.16 ± 14.07 CD*	3.74 ± 0.14 C*	0.92 ± 0.01 B*
Burn treatment		**F=4.21, P=0.01**	F=0.164,=P=0.84	F=0.85,P=0.479
Depth (cm)		**F=10.13,P=<0.001**	**F=29.44,P=<0.001**	**F=18.027,P=<0.001**
Burn treatment* Depth(cm)		**F=6.02, P=<0.001**	**F=8.872,P=<0.001**	**F=6.93,P=<0.001**

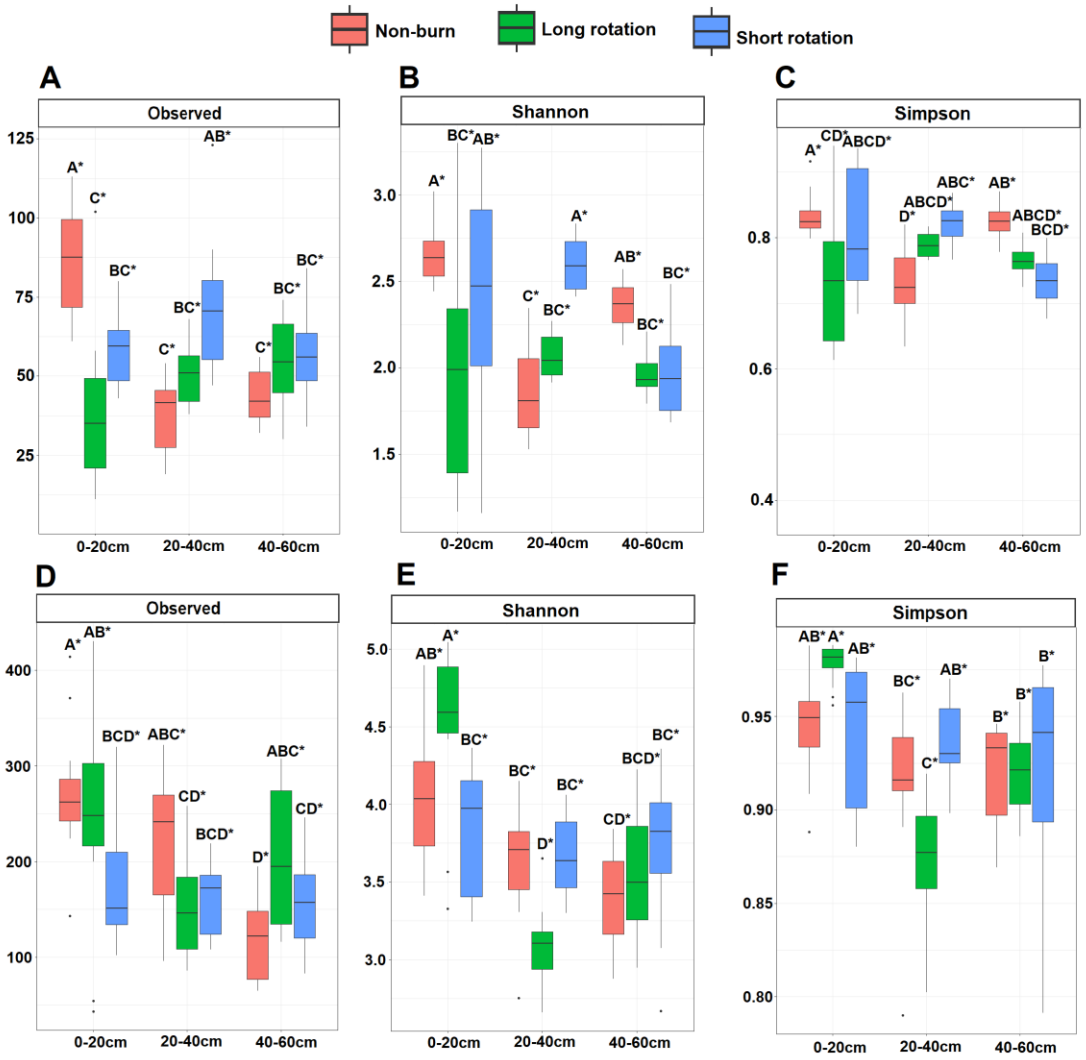

Fig 4.6. Diversity indices for archaea observed richness **(A)**, Shannon index **(B)** and Simpson index **(C).** Non-burn 0-20cm (*n*=11), non-burn 20-40cm (*n*=12), non burn 40-60cm (*n*=12), long rotation 0-20cm (*n*=12), long rotation 20-40cm (*n*=10), long rotation 40-60cm (*n*=12), short rotation 0-20cm (*n*=12), short rotation 20-40cm (*n*=12), short rotation 40-60cm (*n*=11). Bacteria observed richness **(D)**, Shannon index **(E)** and Simpson index **(F).** Non-burn 0-20cm (*n*=12), non-burn 20-40cm (*n*=12), non burn 40-60cm (*n*=12), long rotation 0-20cm (*n*=12), long rotation 20-40cm (*n*=11), long rotation 40-60cm (*n*=12), short rotation 0-20cm (*n*=12), short rotation 20-40cm (*n*=12), short rotation 40-60 (*n*=12) across three different soil depths under three burn treatments. Boxplots with different letters indicate a significant difference among treatments based on a significant interaction between burn treatment and soil depth (Tukey's HSD, $P < 0.05$).

Using the Bray-Curtis dissimilarity, principal coordinate analysis was conducted to illustrate the archaeal and bacterial community variance of samples along the different soil depth gradients in different burn treatments. Overall, community composition of archaeal

communities was significantly different between burn treatment (PERMANOVA, F= 9.27, R^2=0.154, P=<0.001) and soil depth (PERMANOVA, F= 8.42, R^2=0.143, P= <0.001). The first axis explained 33.5% of variance and the second axis explained 24.9% (Fig 4.7A). Likewise, there was clear variation in bacterial communities across different burn treatments (PERMANOVA, F= 7.90, R^2=0.131, P= <0.001) and soil depth (PERMANOVA, F= 11.17, R^2=0.176, P= <0.001). The first axis explained 36.6% of variance and the second axis explained 17.6% of the variance respectively (Fig 4.7B).

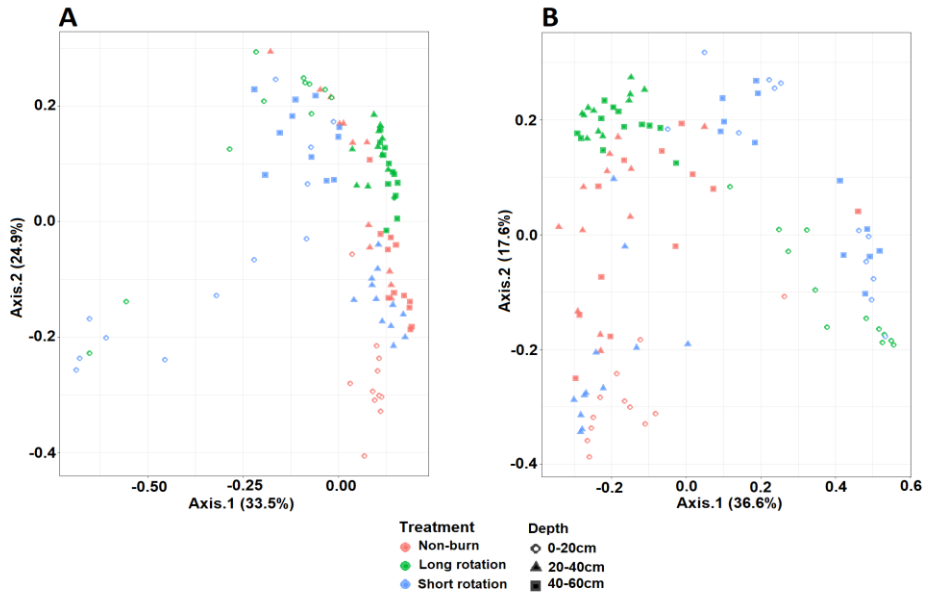

Fig 4.7. Principal coordinates analysis of the archaeal communities **(A).** Non-burn 0-20cm (n=11), non-burn 20-40cm (n=12), non burn 40-60cm (n=12), long rotation 0-20cm (n=12), long rotation 20-40cm (n=10), long rotation 40-60cm (n=12), short rotation 0-20cm (n=12), short rotation 20-40cm (n=12), short rotation 40-60cm (n=11) and bacterial communities **(B).** Non-burn 0-20cm (n=12), non-burn 20-40cm (n=12), non burn 40-60cm (n=12), long rotation 0-20cm (n=12), long rotation 20-40cm (n=11), long rotation 40-60cm (n=12), short rotation 0-20cm (n=12), short rotation 20-40cm (n=12), short rotation 40-60 (n=12) across three different soil depths under three burn treatments. Different colours indicate three burn treatments including red for non-burn, green for long rotation and blue for short rotation. Different shapes indicate different soil depth profiles including circle for 0-20cm, triangle for 20-40cm and square for 40-60cm.

4.3.4: Effects of environmental properties on soil microbial communities

To identify the significant environmental variables influencing archaeal and bacterial community structure, forward selection redundancy analysis (RDA) was used. The results showed that the archaeal and bacterial community structures in the three burn treatments are

different in relation to soil depth. The average importance of each parameter was calculated separately for archaea and bacteria (Fig 4.8). Important variables that influenced archaeal communities were NH_4^+ and pH in the topsoil, Pb, moisture and Al in the 20-40cm profile and total N, total C, Al, Ca, Fe and pH in the 40-60cm profile. All final models were significant (*P*=<0.05) (Fig 4.8 A-C). Important environmental variables that influenced bacterial communities were $NH_4^{+,}$ pH, heather cover % and Mg in the topsoil, Fe, K and Pb in the 20-40cm profile and Al, Ca and Fe in the 40-60cm profile. All final models were significant (*P*=<0.05) (Fig 4.8 D-F).

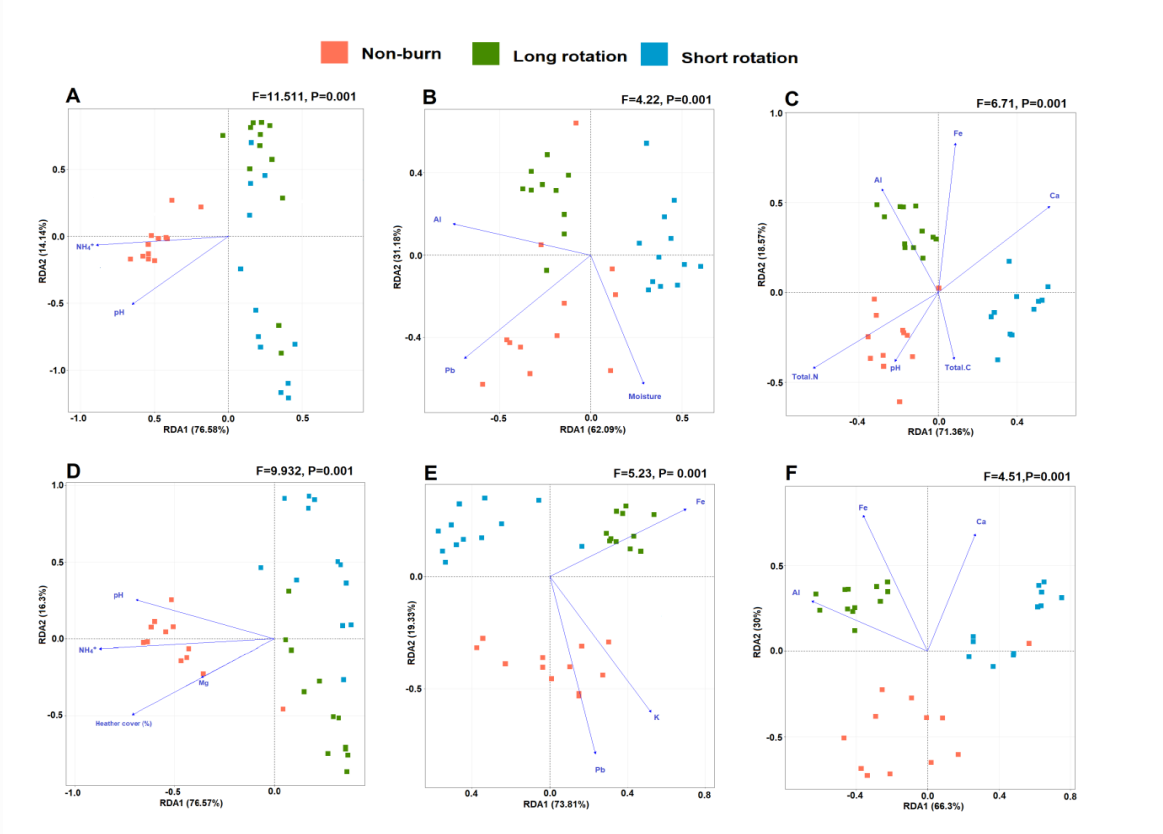

Fig 4.8. RDA ordination plots showing soil related drivers of archaeal communities **A**= topsoil (0-20cm), **B**= subsoil (20-40cm), **C**= subsoil (40-60cm). Non-burn 0-20cm (*n*=11), non-burn 20-40cm (*n*=12), non burn 40-60cm (*n*=12), long rotation 0-20cm (*n*=12), long rotation 20-40cm (*n*=10), long rotation 40-60cm (*n*=12), short rotation 0-20cm (*n*=12), short rotation 20-40cm (*n*=12), short rotation 40-60cm (*n*=11) and bacterial communities **D**= topsoil (0-20cm), **E**= subsoil (20-40cm), **F**= subsoil (40-60cm). Non-burn 0-20cm (*n*=12), non-burn 20-40cm (*n*=12), non burn 40-60cm (*n*=12), long rotation 0-20cm (*n*=12), long rotation 20-40cm (*n*=11), long rotation 40-60cm (*n*=12), short rotation 0-20cm (*n*=12), short rotation 20-40cm (*n*=12), short rotation 40-60 (*n*=12) collected at three different depths under three burn treatments. Only significant variables (*P*=<0.05) are shown. Different colours indicate three sampling treatments. The ASV data were standardized with Hellinger transformation using the *Vegan* package.

4.3.5: Indicator analysis

Archaeal indicator ASVs for each treatment represented seven classes (appendix 7, Table A 7.1). The number of indicators varied with non-burn topsoils having six indicators while there were also six significant indicators in the long rotation subsoil (20-40cm) and short rotation burns contained four indicators. One indicator from the class Methanosarcinia was found in the short rotation 40-60cm profile. No archaea indicators were detected in non-burn subsoils, long rotation topsoil, long rotation 40-60cm profile or short rotation 20-40cm profile (appendix 7, Table A 7.1). Likewise, bacterial indicator ASVs for each burn treatment represented twenty-nine classes (appendix 7, Table A 7.2). Bacterial indicators for each treatment varied widely with the non-burn topsoil having thirty indicators while the long rotation topsoil had eleven indicators and the short rotation topsoil contained three indicators. The subsoil of each burn treatment had fewer indicators overall (appendix 7, Table A 7.2). Alphaproteobacteria, Verrucomicrobiae and Dehalococcoidia were the classes containing the most indicators of non-burn soils (3 indicators of each class). Indicator ASVs for long rotation burns were from classes Acidobacteriae (4 indicators), Acidobacteriae (2 indicators), Alphaproteobacteria (2 indicators) and Bacteroidia (2 indicators). Gammaproteobacteria, Chlamydiae, Polyangia, Syntrophia, Verrucomicrobiae and WPS-2 were all found with 1 indicator. Plots under short rotation regimes had fewer indicators from the classes Bacteroidia and Alphaproteobacteria (appendix 7, Table A7.2).

4.3.6: Network analysis of prokaryotic communities

Individual networks for burn treatments - soil depth combinations were built, their topological parameters measured and were distinctly different across burn treatments (Fig 4.9; Table 4.5). The topsoil of the non-burned control network was more complex than for long rotation and short rotation treatments and was identified by having a greater number of nodes, more links and an increased average connectivity (avgK). However, smaller modularity was found in the topsoil of the short rotation regime (Table 4.5). The long rotation 20-40cm layer had a higher average connectivity but with an increase in negative links (Table 4.5). Multiple measures showed that all empirical networks were different from random networks generated by the randomization procedure, suggesting that the randomly generated networks were distinct from the observed interactions.

Table 4.5. Topological properties of co-occurrence networks obtained from different burning regimes for prokaryotic communities. *Random networks were created by rewiring the links in the empirical network with the same nodes and links. The data was generated from 100 random runs, and the standard deviation from the 100 runs was calculated.

	Network indexes	Non-burn 0-20cm	Non-burn 20-40cm	Non-burn 40-60cm	Long rotation 0-20cm	Long rotation 20-40cm	Long rotation 40-60cm	Short rotation 0-20cm	Short rotation 20-40cm	Short rotation 40-60cm
Emperical networks	**Number of nodes**	171	128	98	141	103	138	104	130	97
	Number of links	497	250	210	377	502	345	237	249	238
	Number of positive links	337	210	133	336	299	256	135	112	120
	Number of negative links	160	40	77	41	203	89	102	137	118
	R^2 power of law	0.843	0.846	0.781	0.860	0.711	0.832	0.763	0.737	0.752
	Average connectivity (avgK)	5.584	3.406	4.287	5.347	9.747	5	4.557	3.830	4.907
	Harmonic geodesic distance (HD)	3.240	3.495	2.954	2.996	2.977	3.435	3.279	3.412	2.896
	Average clustering coefficient (avgCC)	0.760	0.258	0.232	0.304	0.444	0.255	0.211	0.254	0.243
	Average path length	3.917	4.449	3.530	3.665	2.964	4.428	4.088	4.124	3.771
	No of modules	11	17	8	10	7	15	6	9	9
	Modularity (M)	0.657	0.594	0.505	0.659	0.448	0.531	0.566	0.582	0.496
***Random networks**	**Harmonic geodesic distance (HD)**	2.353 ± 0.026	2.985 ± 0.051	2.721 ± 0.030	2.693 ± 0.031	2.111 ± 0.017	2.763 ± 0.039	2.746 ± 0.031	3.080 ± 0.056	2.579 ± 0.040
	Average clustering coefficient (avgCC)	0.079 ± 0.010	0.065 ± 0.011	0.078 ± 0.020	0.085 ± 0.012	0.018 ± 0.014	0.095 ± 0.0014	0.065 ± 0.015	0.053 ± 0.014	0.104 ± 0.015
	Modularity (M)	0.336 ± 0.008	0.456 ± 0.010	0.410 ± 0.011	0.369 ± 0.009	0.226 ± 0.008	0.375 ± 0.008	0.409 ± 0.010	0.476 ± 0.011	0.368 ± 0.010

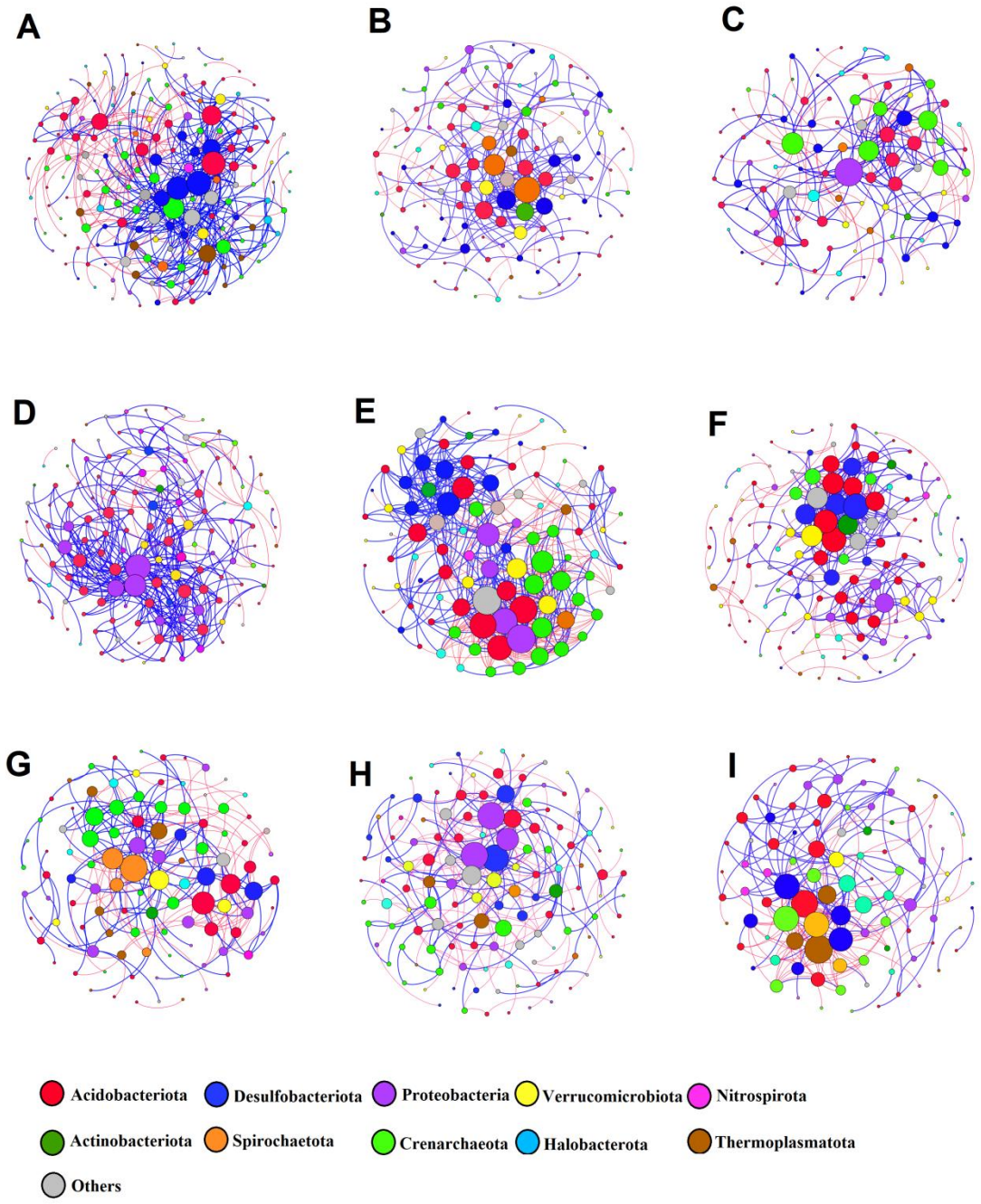

Fig 4.9. Overview of networks under three different burn treatments across three soil depths with node size proportional to node connectivity. Nodes are coloured for different phyla. A red link indicates a negative correlation and a blue link indicates a positive correlation. **(A)** Non-burn 0-20cm, **(B)** Non-burn 20-40cm, **(C)** Non-burn 40-60cm, **(D)** Long rotation 0-20cm, **(E)** Long rotation 20-40cm, **(F)** Long rotation 40-60cm, **(G)** Short rotation 0-20cm, **(H)** Short rotation 20-40cm, **(I)** Short rotation 40-60cm.

4.3.7: Module hubs and connectors

Connectivity within and between modules was used to determine the roles of each node in the networks. Each node could be given one of four ecological roles (peripherals, module hubs, network hubs, or connections). The nodes belonging to connectors, module hubs, or network hubs are critical both within their respective modules and among modules. Generally, the nodes with $P_i > 0.62$ or $Z_i > 2.5$ are recognized as super generalists (Deng *et al*., 2012).

Of the total nodes, peripherals occupied 96% in all networks. An increased number of module hubs and connectors were observed in the non-burn control (Fig 4.10). Various microbial taxa were distributed among module hubs and connectors. Specifically, five module hubs were observed in the non-burn control plots, three in the long rotation plots and six in short rotation burn plots, respectively (Fig 4.10). Compared with the module hubs, more connectors were detected ranging from eighteen in non-burn plots, ten in long rotation burn plots to four under short rotation burn plots (Fig 4.10). Module hubs and connectors were occupied by common taxa such as Acidobacteriota and Desulfobacterota but also rare phyla such as Proteobacteria, Spirochaeota, Sva0485, Verrucomicrobiota and RCP2.54. These rare phyla made up 34% of module hubs and connectors. Although these microbial phyla were relatively low in abundance they were keystone taxa (0.004 - 0.10% in the non-burn vs 0.004 - 0.26% in long rotation burn regimes vs. 0.005% -0.24% in short rotation regimes). Archaeal taxa were also identified as keystone taxa representing 30% of module hubs and connectors (Fig 4.10).

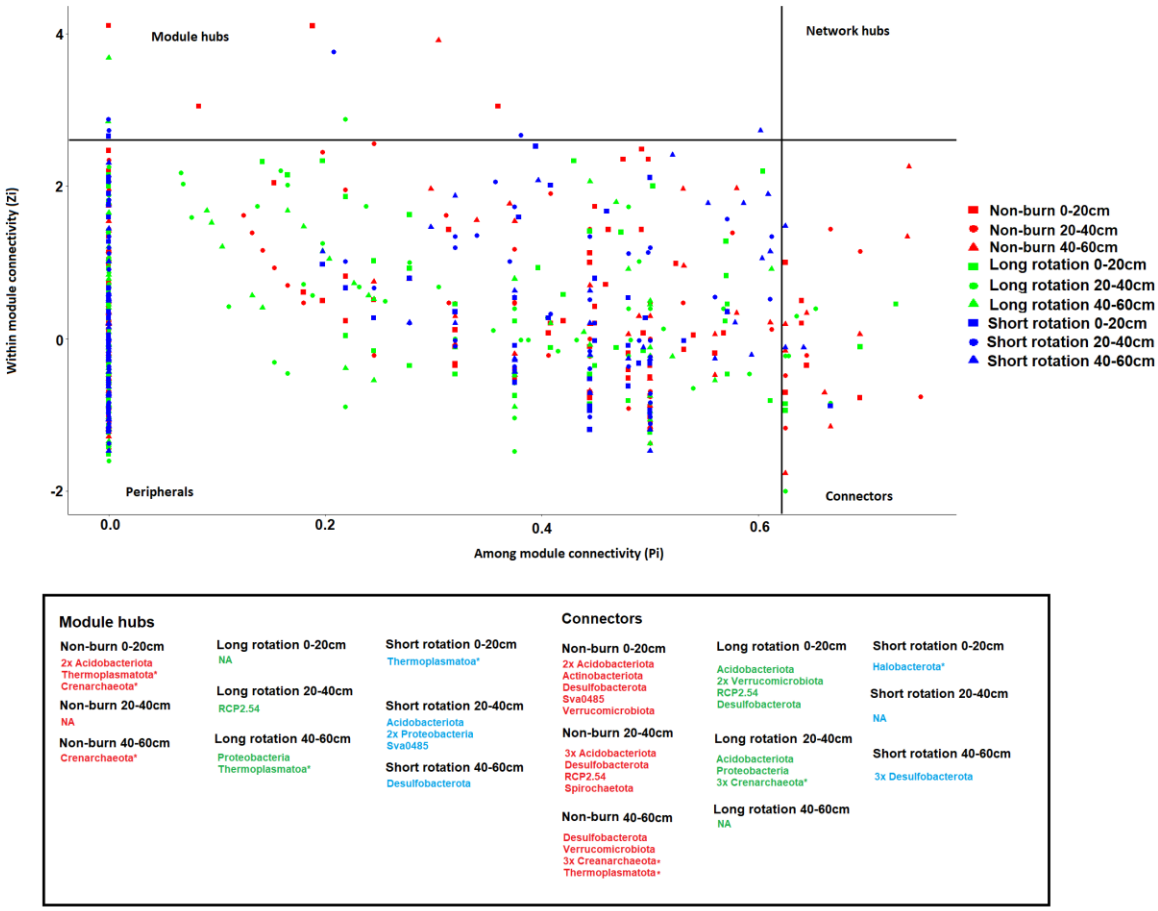

Fig 4.10. Topological roles of soil microbes in nine networks. Phyla marked with an asterisk are Archaea.

4.4: Discussion

This study demonstrates that prescribed burning is a strong driver of plant cover, soil physicochemistry and soil microbial community composition. The diversity of archaea and bacteria was reduced under a short rotation regime. The effects of prescribed burning on vegetation cover, soil physicochemistry and prokaryotic communities are strongly evident in the top soil (0-20cm) and also in the sub soil (20-40cm, 40-60cm) but to a lesser extent. The study found that the effects of prescribed burning on archaeal and bacterial communities are significant and there are associated changes in soil physicochemistry. The microbial community changes observed in relation to burning practice imply a likely impact on soil function not only in the surface but also in deeper soil beneath the rooting zone up to 60cm.

4.4.1: General characteristics of communities across burn treatments

Analysing variations in the relative abundances of microbial taxa can help to understand the functional mechanism of soil biogeochemistry after prescribed burning. In this study, the archaeal phyla Thermoplasmatota was equally abundant across burn treatments in the topsoil and increases under a short rotation regime in the lower soil layers (Fig 4.4). Thermoplasmatota are moderately thermophilic and mesophilic growing in a variety of conditions and have been found to increase with soil depth and are important to contributing to C mineralization (Lin *et al*., 2015). Crenarchaeota was highest under a short rotation regime in the topsoil. The phylum Crenarchaeota appears to have important relationships with plants. Therefore, the observed differences in the relative abundance of Crenarchaeota could be attributed to plant communities and cover associated with different burning rotations (Nicol *et al*., 2003). Halobacterota was generally more prominent in plots under a long rotation regime. Halobacterota are halophilic heterotrophic microbes with a high salt tolerance. This phylum are known to survive in high salt concentration environments (Xiao *et al*., 2021). NO_3^-, Ca, Fe, P, K and Al were all higher in soils under a long rotation regime and serve as readily available nutrient and sources of energy that meet the energy requirements related to the metabolic processes in Halobacterota (Wang *et al*., 2010).

The results indicate that Acidobacteriota, Desulfobacterota and Proteobacteria were the most abundant bacterial phyla accounting for 84% of the total phyla found in the study (Fig 4.5). Acidobacteriota were found to be more abundant in plots under a long rotation burn regime. Acidobacteriota have been described as a late successional phylum and are considered to be oligotrophic (Thomson *et al*., 2010). Low pH in plots under a long rotation regime could explain the higher relative abundance of Acidobacteriota as many representatives this phylum thrives in low pH (Hartman *et al*., 2008). In contrast, soils subjected to short rotation burn regimes had an increase in the relative abundance of Proteobacteria (Fig 4.5). The ability of Proteobacteria to cope with abiotic stress such as desiccation, high temperatures and their fast-growing life strategies is likely the reason for their higher abundance under short rotation burns (Lladó & Baldrian, 2017; Zachow *et al*., 2014). Ecological patterns may not be shared by archaeal and bacterial phyla (Fierer *et al*., 2007). However, the patterns observed in this study are consistent with the available ecological data (Fierer, *et al*., 2007; Lladó & Baldrian, 2017; Zachow *et al*., 2014). Broadly, the abundance of archaeal and bacterial taxa that are able to colonize and take advantage of limited nutrients were higher in plots subject to prescribed burning while non-burn plots are associated with slower growing taxa. For

example, the phylum Acidobacteriota has been reported to be a versatile heterotroph with a K-selected lifestyle (Yao *et al*., 2017). The increase in Acidobacteriota under burning regimes is consistent with their ability to colonize nutrient-limited soils, in which Acidobacteriota contributes to the enhancement of soil nutrients (Yao *et al*., 2017). In addition, indicator taxa were mainly among Acidobacteriota and Proteobacteria in burned soils. For example, Xanthobacteraceae and Acetobacteraceae are among families that were positively affected by fires and are essential in the N cycling process including denitrification and N fixation (Jang *et al*., 2020). These indicator taxa can promote plant growth by fixing nitrogen (Cooper & Scherer, 2012). As a result, taxa within these groups may have a unique adaptation to, or preference for soil conditions.

4.4.2: Diversity and community composition across burn treatments and depth

Burn treatment and soil depth had a significant interactive effect on archaea and bacteria alpha diversity. The observed diversity for archaea was higher in the non-burn topsoil than in soils under prescribed burning, while the observed diversity for bacteria was higher in the topsoil of the non-burn control and under a long rotation regime (Fig 4.6). Microorganisms may be eliminated after a fire (Barreiro *et al*., 2015) and may take several years to return to a pre-burn state. The higher observed diversity may be due to vegetation litter being maintained in non-burned plots influencing the growth of microorganisms. Sun *et al*. (2017) found that residual leaf litter and vegetation positively influences the diversity of the soil microbial communities which also improves soil fertility.

Soil profiles can represent strong environmental gradients under different land management regimes (Chen *et al*., 2021) yet the effects of prescribed burning on archaeal and bacterial communities in relation to different soil profiles is largely unknown. The diversity of archaea and bacteria across soil profiles varied across burn treatments (Fig 4.6). According to these findings, the majority of the effects of prescribed burning are limited to the topsoil (0-20cm) and may extend to the lower profile (20-40cm) in the case of bacteria (Fig 4.6D) which is consistent with the findings that prescribed burning had on soil physicochemistry (Fig 4.2 & 4.3) and may partially be due to the changes in microclimate caused by the effects of vegetation cover (Fig 4.1). Community composition for both archaea and bacteria differed significantly with burn treatment and depth (Fig 4.7 A & B). This is widely in line with the effects wildfire has on soil physicochemistry (Holden *et al*., 2016; Knelman *et al*., 2019; Li *et al*., 2019) and indicates that prokaryotic community assembly is strongly driven by

prescribed burning. These results suggest that the environmental variability caused by prescribed burning across soil profiles can act as a strong environmental filter. The lack of effect of prescribed burning on archaea and bacteria in lower soil profiles suggests that the subsoil has the potential to play an important role in recolonisation. Furthermore, as plants mature, their roots form a link between the topsoil and subsoil. This mechanism is particularly important to non resilient taxa able to recolonise soil under environmental stress.

4.4.3: Effects of environmental factors on archaeal and bacterial diversity

Distinct mechanisms by which prescribed burning influenced archaea and bacteria were distinguished. Edaphic factors such as NH_4^+ and pH influenced archaeal communities in the topsoil and NH_4^+, pH, percent cover of heather, and Mg influenced bacterial communities. Likewise, important factors such as Pb, Moisture, Al, total N, total C, Ca, Fe and pH were important factors for archaeal communities in the subsoil (20-40cm and 40-60cm) and Fe, K, Pb, Al and Ca for bacterial communities in the subsoil (Fig 4.8). This is consistent with global trends indicating that important environmental factors, particularly pH, influence the composition of microbial communities (Bahram *et al*., 2018; Fierer & Jackson, 2006; Kaiser *et al*., 2016; Lauber *et al*., 2009) since pH can mediate other soil nutrients and influence microbial growth (Zhalnina *et al*., 2015). As burning produces hydroxides and oxides it was assumed that pH would be higher in burned plots (Sun *et al*., 2015). However, in this study, higher soil pH was observed in the control non-burn plots. Importantly, prescribed burning influenced changes of soil cations such as Al, Fe and Ca which affected archaeal and bacterial communities (Fig 4.8). Importantly, changes in cations induced by prescribed burning were the main predictors of microbial diversity, particularly in the subsoil. Cations are essential for prokaryotic metabolism (Paul, 2014) and future studies should include these essential nutrients to help understand the impact they have on microbial communities. Moreover, the anaerobic nature of peat soils in deeper horizons will affect the growth of archaea and bacteria. The differences in soil environmental factors at different depths may increase or decrease the relative abundance of archaea and bacteria, resulting in taxonomic differentiation. The RDA analysis shows that some of the same environmental factors were important for both archaea and bacteria. Wei *et al*. (2020) found similar edpahic factors can impact and shape bacteria and archaea communities indicating that archaea and bacteria communities are shaped by important soil environmental factors during land management regimes.

4.4.4: Contrast in microbial co-occurrence networks

Different burning regimes caused vertical changes of soil microbial networks. Different microbial taxa generally prefer different conditions for growth and survival (Chen *et al*., 2019). The main changes in network topological features were accompanied by changes in community composition and overall richness. The increase in negative links observed in plots under short rotation burns suggests an increase in competitive and antagonistic interactions for acquiring substrates or environmental filtering (Jing *et al*., 2015), while positive interactions may be the result of ecological and functional similarity (Hernandez *et al*., 2021) and may indicate that these taxa compete less due to the occupation of specific niche spaces (Wang & Or, 2013). Non-burn soils and the 20-40cm layer under a long rotation regime had the highest average connectedness and a more complex coupling among microbes. The network from the 40-60cm layer under a short rotation regime showed the lowest modularity. How well a network may be separated into modules is determined by its modularity, which may be a consequence of resource partitioning, habitat heterogeneity and specific interactions (Deng *et al*., 2012). Therefore, the lower modularity under a short rotation regime suggests that as a result of the more frequent prescribed burning, the microbial groups that occupy the soil share a common niche.

The fine-scale distribution of microbes can be positively affected by root exudates. Plant roots can help to re-establish microbial networks as ecological succession progresses and associated increases can lead to more positive interactions (Lange *et al*., 2015). The ecological succession can allow plant roots to extend deeper and create conduits for the movement of nutrients through to the subsurface (Clark & Zipper, 2016). It is suggested that an increased microbial network complexity leads to greater stability of the community (Ghoul & Mitri, 2016; Mougi & Kondoh, 2012). Furthermore, it has been demonstrated that compact networks with stronger connections between competitors could improve nutrient transfer when compared to those inhabiting a fragmented space (Morriën *et al*., 2017).

Prescribed burning regimes also changed the distinct keystone taxa within microbial networks favouring Acidobacteriota and Proteobacteria as keystone taxa for different burn regimes increased (Fig 4.10). In soil ecosystems, Proteobacteria is a dominant nitrogen-fixing bacterial phylum (Gaby & Buckley, 2011). However, the prokaryotic community networks of different burn treatments at different depth profiles had different keystone taxa, which further confirms that there was niche differentiation among taxa across both prescribed burning and

soil depths. Keystone species can act as gatekeepers of the ecological functions of microbial communities and have important implications for biogeochemical cycling (Lynch & Neufeld, 2015). These keystone taxa are critical in the management of carbon sources, in which they play an active role (Khodadad *et al*., 2011; Lehmann *et al*., 2011; O'neill *et al*., 2009) and their removal can cause significant changes in microbiome functioning (Herren & McMahon, 2018).

4.4.5: Implications for management

These findings provide critical information for guiding the management and conservation of peatlands in the United Kingdom. Important peat forming cover (*Eriophiorum* spp. and *Sphagnum* spp) was higher in plots under a short rotation burn where heather cover was lowest. It can take between 7-10 years for heather to re-establish (Hobbs, 1984). Cover of important peat-forming species and heather was intermediate under the long rotation regime. The differences in plant cover and soil physicochemistry have important implications for the microbial communities under prevailing management. Efforts to understand the impact of burning on soil microbial dynamics is essential as prescribed fires are still used as a common land management tool. Some conservation groups, prefer the no-burn option to reduce carbon losses (Harper *et al*., 2018; RSPB, 2014; Thompson *et al*., 2016) while risking potential severe wildfires. Given that this site is climatically extreme in England with higher altitude and more rainfall, rotation lengths on other sites may differ in climate and soils (Santana *et al*., 2016). Several processes could explain microbial community stability e.g. the physiological resilience and tolerance to environmental stressors, the growth rate and community properties such as diversity turnover. The resistance to change of microbial communities in management may be linked to an evolution to disturbances.

4.4.6: Conclusions

This study analysed and compared the diversity, community structure and network structure of archaeal and bacterial communities across different prescribed burning regimes throughout peat soil profiles, and highlights the significant influence of environmental dissimilarities caused by prescribed burning on archaeal and bacterial communities. The most complex microbial community networks and positive interactions were found in the non-burn topsoil. It is possible that the lack of disturbance has allowed the community to adapt over time. Moreover, it is likely that with the higher amount of nutrients and resources found in the non-burn plots there is minimal competition. The increase in negative interactions in the short rotation burn treatment suggests an antagonistic and competitive interaction which was concurrent with a decrease in soil nutrients within plots under a short rotation burn regime. Archaea and bacteria both had different indicator species in soils under prescribed burns compared to the control non-burn, showing that site burning history can be estimated from microbial community data.

Loss of microbial diversity may be a consequence if peatlands are burned under a short rotation regime. The results of this study show that surface burning of peatland vegetation alters soil physicochemical properties as well as the prokaryotic microbiome composition across soil profiles. The impact is shown for both short and long burn regime, and extends deeper than the surface soil (Ashby & Heinemeyer, 2021), demonstrating that there is a probable functional impact of burning upon the whole soil. The functional implications of this require further work to fully investigate. Determining how microbes recover over time and their relationship with above-ground plant communities undergoing ecological succession is essential for determining the long-term impacts of burning in peatlands and other ecosystems. This work has provided new insights into the impact of prescribed burning upon the microbial community composition of soils from surface layer down to 60cm. Results show that burning impacts microbial community composition of the soil profile extensively. Prescribed burning is a common procedure but there is a concern that it is environmentally damaging. Understanding the ecological and environmental implications of management practices is essential and further research should pay more attention to the changes in archaeal and bacterial communities.

Chapter 5. Response of soil fungal communities and functional traits to prescribed burning regimes in surface and subsurface peat

Abstract

Prescribed burning of peatlands is a common management practice in the United Kingdom. However, its role in peatland degradation and the loss of key ecosystem services is strongly debated. Therefore understanding the effects of prescribed burning is integral to peatland ecology and effective management. Fungi play important roles in peatland ecosystems such as the decomposition of organic matter, carbon mineralisation, influencing plant growth and assisting in nutrient acquisition. Despite the importance of fungi in the environment few studies have assessed how prescribed burning impacts peatland fungal communities in different soil horizons. This study assessed the impact of prescribed burning on fungal communities using DNA metabarcoding at the Moor House Nature Reserve long-term monitoring site in upland Britain with over 60 years subject to three burning treatments after an initial burn in 1954: burning at long rotations every 20 years, burning at short rotations every 10 years and a no further burn control. The results indicated that prescribed burning had a significant impact on fungal diversity, richness, community composition and structure, including impacts on the surface soil (0–20cm) and subsoils (20-40cm, 40-60cm). Community composition changed across burn treatment and depth and there was a shift in the relative abundance of trophic modes. The study found edaphic factors such as Ca, Mn, other 'non-Sphagnum' moss cover, total C and pH were important in shaping fungal communities in the topsoil, NH_4^+ and moisture in the 20-40cm profile and Fe and Pb in the 40-60cm profile. Although alpha diversity was significantly reduced in plots under a short rotation regime they showed some resilience in plots subject to long rotation burn intervals. This research has brought new, relevant and valuable findings into the impacts of rotational prescribed burning on fungal communities that extend deep into the soil horizons.

Keywords: Prescribed burning; Peatlands; Fungal communities; Metabarcoding; trophic modes

5.1: Introduction

Soil is critical to terrestrial ecosystems because many processes that are essential to the functioning of ecosystems occur in the soil such as providing the structural foundation for vegetation communities (Rillig & Mummey, 2006), carbon and nitrogen cycling (Högberg *et al*., 2001; Kowalchuk & Stephen, 2001) and nutrient acquisition (Sprent, 2001). Soils are frequently disturbed by anthropogenic activities such as prescribed burning which is used to maintain the structure of many terrestrial ecosystems (Fuhlendorf & Engle, 2001). Prescribed burning regimes are a management method utilised in peatland ecosystems throughout the world, including Europe (Davies *et al*., 2022; Hochkirch & Adorf, 2007; Renard *et al*., 2016), North America (Geron & Hays, 2013; Ryan *et al*., 2013) and the tropics (Holden *et al*., 2007) and are frequently used to burn vegetation without affecting the peat underneath, as opposed to wildfires, which can consume surface peat layers. Prescribed burning is commonly practiced in the United Kingdom on patches of up to 4000 m^2 (0.4ha) in rotations of 8–25 years (Noble *et al*., 2018). The canopy vegetation, which is typically dominated by dwarf shrubs such as heather and graminoids on UK peatlands, is burned to produce a variety of vegetation at various stages that are suitable for foraging and nesting for the red grouse (*Lagopus lagopus scotica*). The official guidance encourages against the prescribed burning of peat bogs (Defra, 2007a). In many pyrogenic ecosystems, the dominance of flammable plants such as *Calluna* promotes the spread of wildfire (Beckage *et al*., 2011; Cardoso *et al*., 2018; Davies *et al*., 2016; Staver *et al*., 2011). Low frequency burning could help maintain biotic and abiotic ecosystem components and this type of management regime has been used to help maintain the biodiversity of peatland ecosystems (Peet *et al*., 2018). Burning is still a common management tool in the UK (Douglas *et al*., 2015; Thacker *et al*., 2000; Yallop *et al*., 2006) and previous research has shown that vegetation communities alter due to burning regimes with shorter rotations increasing the dominance of *Sphagnum* moss (Lee *et al*., 2013a; Milligan *et al*., 2018; Noble *et al*., 2018; Whitehead *et al*., 2021).

Fungi are successful soil organisms due to their ability to change forms and show high plasticity in response to unfavourable conditions in the environment (Frąc *et al*., 2018). The diversity and composition of plant communities has a strong influence on fungal populations and in turn the symbiotic relationship affects plant growth through mutualism, nutrient cycling and their effect on nutrient availability (Allison & Treseder, 2011; Read *et al*., 2000; Žifčáková *et al*., 2016). However, little is known about the effects of prescribed burning on peatland fungal communities. In peatlands, fungi are highly specialised, and perform crucial

environmental roles, such as the degradation of complex carbon polymers like hemicelluloses (Thormann, 2006) and the production of methane in aerobic environments (Lenhart *et al*., 2012) which may increase the rate of decomposition under global warming. The loss of organic matter can have an important impact on fungal community structure as well as important functional guilds. For example, mycorrhizal fungi have been identified as determining the growth of individual plants (Smith & Read, 2010), which are essential in determining plant productivity and community structure (Yang *et al*., 2016) and saprotrophic fungi are essential for the decomposition of plant litter and organic matter (Ceci *et al*., 2019).

Fungi are known to be more susceptible than prokaryotes to habitat change but the response of specific ecological guilds may vary (Dooley & Treseder, 2012; Dumontet *et al*., 1996). The majority of studies on the effects of fire on fungal communities have focused on high intensity-wildfires, where burning is much more severe due to deep soil combustion (Cairney & Bastias, 2007; de León *et al*., 2018; Reazin *et al*., 2016; Oliver *et al*., 2015; Whitman *et al*., 2019). Despite the rise of high-intensity wildfires as a result of current climate change, the majority of fires in peatlands are generally low-intensity (Turetsky *et al*., 2015). The effect of burning on fungal communities will differ in managed ecosystems compared to wildfires due to different characteristics such as lower frequency and low intensity. The changes in vegetation and their effects on soil fungi and diversity depends on (1) the ecosystem's productivity, (2) how the changes affect dominant plants and impair productivity and (3) the association of plants and fungi (Hart *et al*., 2005).

Another neglected component of fungal community structure is their vertical distribution and how communities in deeper soil are affected by the above ground management. In peatlands most of the cool burns may be restricted to the top layer due to the moisture content of the lower peat layers. Therefore, the topsoil will be more affected than the subsoil by fire (Certini, 2005). Following fires, the temperature of the soil may rise due to changes in vegetation cover and then be transferred to the deeper soil via conduction, convection and radiation. However, the effects may diminish with depth (Pereira *et al*., 2018; Shakesby, 2011). It remains to be seen if fungal communities colonizing the deeper horizons are different to those in the upper layer or are just a subset of those from the topsoil that have reached the lower horizons due to stochastic vertical dispersion (Li *et al*., 2020; Mujic *et al*., 2016). However, most studies assessing the impacts of wildfire have only focused on communities in the topsoil, with a lack of data available on the effects in lower soil depths. Therefore, understanding the impact that burning management has on fungal community

structure and function, both between management regimes and depth stratification can provide valuable insight into how peatlands are impacted by fire.

Environmental factors such as pH (Högberg *et al*., 2003), nitrogen (Guo *et al*., 2019), soil nutrients (Cassman *et al*., 2016), moisture (Castaño *et al*., 2018) and plant community structure (Ponder *et al*., 2009; Semenova-Nelsen *et al*., 2019) play vital roles in shaping the structure of fungal communities (Goldmann *et al*., 2016; Zhao *et al*., 2019). Fire alters pH by the denaturation of organic acids and alters soil temperature due to changes in canopy cover (Certini, 2005), which may provide a niche for fungi in the short term (Day *et al*., 2019). Land modification through fire can alter the structure of fungal communities and cause changes in the morphology of saprotrophic and symbiotrophic fungi and it may take many years for communities that have been altered by fire to return to their pre-management structure (Dahlberg *et al*., 2001; Greene *et al*., 2010; Treseder *et al*., 2004). When compared to other fungal groups, mycorrhizal fungi have been discovered to be particularly sensitive to fire and suffer a significant decline (Holden *et al*., 2013; Holden *et al*., 2016; Sun *et al*., 2015). Fire affects fungal communities indirectly by modifying plant community composition through its impact on vegetation structure. Changes in vegetation structure caused by fire include changes in concentrations of secondary metabolites and the production of total biomass (Neary *et al*., 1999). Furthermore, because of the importance of fungi and plant interactions, the survival of specific functional guilds could have a significant impact on plant communities and plant growth (Day *et al*., 2019). As a result, the recovery after a fire in key ecosystems may be a reflection of succession in plant and fungal community structure (Day *et al.,* 2019).

In this study, the long-term burning experimental plots were used at Moor House Nature Reserve. Thus, allowing a novel approach to assess the effects of prescribed rotational burning on fungal communities in peatlands. The objectives of this study aim to address (1) if soil fungal communities are affected by prescribed fires throughout the soil profile; (2) if these responses depend on different burning regimes (non-burn, long rotation or short rotation). The following hypotheses were tested. (1) Fungal diversity will change substantially between burn treatments i.e. alpha diversity in the control non-burned plots will be higher than in burned plots; (2) Diversity will significantly decrease with depth due to the anaerobic environment typical of peatlands; (3) Community structure will change significantly between different burning regimes as well as depth with communities in the

non-burn control being dissimilar to those under burn treatments; (4) Burning regimes will alter the relative abundance of functional guilds and trophic modes as burning will favour saprotrophic fungi that have the ability to respond to limited resources post-burning, while symbiotrophic fungi will be more abundant in the non-burned control due to their interaction with less disturbed plant roots. The data will provide evidence that prescribed rotational burning will maintain soil fungal communities that are adapted to fire and are distinct from those inhabiting a non-burned control.

5.2: Materials and methods

Details of the study site are given in chapter 2, section 2.1.2. The experimental design, vegetation cover and physicochemistry measurements are given in the general methods (chapter 2).

5.2.1: PCR amplification and sequencing

Extracted DNA (chapter 2, section 2.4) was used as a template for PCR and sequencing. The extracted DNA was quantified using a Qubit 4 Fluorometer (Invitrogen, UK). Fungal communities were specifically targeted amplifying the ITS regions in the nuclear ribosomal repeat using the primer pair ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-'3) and ITS2R (5'-GCTGCGTTCTTCATCGATGC-3') (Bokulich & Mills, 2013; Hugerth *et al*., 2014). The PCR reactions were carried out in a thermocycler PCR system (MJ Research ptc-225 peltier thermal cycler) using the following program: 3 min of denaturation at 95°C, 35 cycles of 30s at 95°C, 30s for annealing at 53°C, and 45s for elongation at 72°C, and a final extension at 72°C for 10 min (Pauvert *et al*., 2019). The presence of a PCR product was confirmed using 1% agarose gel electrophoresis. PCR products were then cleaned using Agencourt AMPure XP magnetic beads (Beckman Coulter, Indianapolis, USA). Each sample was subjected to a second PCR with barcoded Fi5 and Ri7 identifier sequences. The second PCR mixtures contained 1µl of Fi5 primer, 1µl Ri7 primer, 8µl of product from the first PCR and 10µl of Qiagen multiplex master mix, a total volume of 20µl. Following the second PCR, a FLUOstar Optima (Promega) was used to measure 2µl of product from each reaction. Based on these results, samples were standardised to equal concentrations, pooled into groups of 12 and cleaned using AmPure XP beads (Beckman Coulter, Indianapolis, USA). The Illumina-

tagged DNA concentration of each pool was determined using the KAPA Library Quantification Kit on an Applied Biosystems QuantStudio 12K, and DNA fragment size was determined using an Agilent 2100 Bioanalyzer (Agilent Technologies Ltd., Stockport, UK). The KAPA Library Quantification Kit and a QUBIT 3.0 with the dsDNA HS test (Invitrogen, UK) was used to quantify the final pools. Libraries were sequenced on an Illumina MiSeq platform at 2 x 250 bp paired-end sequencing (Magoč & Salzberg, 2011) at the Centre for Genomic Research University of Liverpool.

5.2.2: Bioinformatics

Bioinformatics analysis was conducted using QIIME2 v2019.7 (Bolyen *et al*., 2019). First, primer sequences were removed using cutadapt v1.9.1 (Martin, 2011). The conserved flanking regions of the ITS1 reads were trimmed using ITSxpress (v 1.8.0) as recommended for amplicon sequencing (Rivers *et al*., 2018). DADA2 was used to filter, dereplicate, detect chimaeras and merge paired-end reads which simultaneously removes chimeras (Callahan *et al*., 2016). The q2-dada2 plugin uses nucleotide quality scores to generate amplicon sequence variants (ASVs), or sequence clusters with 100% similarity which estimates the true biological variation within each sample. The fungal ITS region is highly variable in length and thus was not length trimmed but were quality filtered. Parameters were set with a maxEE score of 2 and truncQ score of 10. The UNITE fungal ITS sequence database (version 8.3) was used as a reference database assigning ASVs to a taxonomic classification (Kõljalg *et al*., 2013). The q2-feature-classifier in QIIME2 (https://github.com/qiime2/q2-feature-classifier) was used to assign taxonomy using a confidence threshold of > 0.70. Singletons and rare taxa (ASVs represented by <5 reads) were discarded as recommended by Lindahl *et al*. (2013). Rarefaction curves were generated using the R package "*ampvis2*" (Andersen *et al*., 2018). Samples were not rarefied to retain important information and avoid false positives despite obtaining similar results from rarefied data (McMurdie & Holmes, 2014) and rarefaction curves reached asymptote in all cases, indicating that sufficient sequencing depth was achieved (appendix 3).

Functional guild analysis was performed using FUNGuild (Nguyen *et al*., 2016) located at https://github.com/UMNFuN/FUNGuild. Using the FUNGuild data, the 'overall' fungal communities were divided into trophic modes: pathotroph, saprotroph, symbiotroph, and multiple trophic modes, i.e. ASVs that are assigned to more than one trophic mode or switch between trophic modes during their life cycle. Fungal sequences that were not assigned a

trophic mode were labelled ‘unknown’. Because ordination analysis is sensitive to rare species, rare microbial taxa were excluded from ordination analyses, leaving only ASVs with a total relative abundance of >0.001 (Legendre & Gallagher, 2001).

5.2.3: Statistical analysis

All statistical analyses were carried out using R version 4.0.2 software (R Development Core Team, 2020). Using the R package '*Phyloseq*,' alpha diversity was calculated to compare community diversity between burn treatments and depth, including observed, Shannon, and Simpson diversity (McMurdie & Holmes, 2013). Two-way analysis of variance with Tukey’s *post-hoc* test for multiple comparisons were used to test the effects of burn treatment, soil depth and their interaction on alpha diversity and the relative abundance of fungal trophic modes following the Shapiro-Wilk and Bartlett tests for normality and homogeneity of variance, respectively. Further, when the interaction was not significant, one-way ANOVA and Tukey’s *post-hoc* test for multiple comparisons were used to evaluate differences based on burn treatments within a soil layer, and among the three soil layers within a given burn treatment.

For analysis of the community composition (β-diversity), the ASV table was normalized by transforming to proportions using the R package *microbiomeSeq* (Ssekagiri *et al*., 2017) as this method is efficient at standardizing read depths (McKnight *et al*., 2019). Similarly to chapter 4, other methods including rarfying, variance stabilizing transformation and the “trimmed means of M” (TMM) method with the R package ‘*edgeR*’ (McCarthy *et al*., 2012; Robinson *et al*., 2010) showed similar results. The variability in community composition across different burn treatments and soil depth was assessed by Bray–Curtis dissimilarity using the R package ‘*Vegan*’ (Oksanen *et al*., 2013) and visualised using principal coordinates analysis (PCoA, Gower, 1966). Permutational Multivariate Analysis of Variance (PERMANOVA) (Anderson, 2001) was conducted to test the significance of community differences between burn treatment and soil depth using the adonis function with 999 permutations in ‘*Vegan*’.

After standardizing the ASV abundance matrix using Hellinger transformation, a redundancy analysis (RDA) was performed on the topsoil (0-20cm) and subsoils (20-40cm and 40-60cm) to visualise differences in fungal community composition between treatments and to test the importance of environmental factors. The 'best' explanatory environmental variables were chosen through forward selection using the ordistep function (Blanchet *et al*., 2008).

Environmental variables confirmed by analysis of variance were retained for the final RDA. The variation inflation factor (VIF) was used to check non-co-linearity among the explanatory variables (VIF<10), as recommended by Montgomery & Peck (1992). Indicator species analysis was performed using the function 'indval' in the R package '*labdsv'* to determine which ASVs were significantly related with each treatment. Indval values >0.3 and *P*<0.05 are considered to be strong indicators (Roberts, 2016).

5.3: Results

5.3.1: General characteristics of fungal communities across burn treatments and depth

Eight fungal phyla were detected across all samples. The phylum Ascomycota showed the highest relative abundance across all samples averaging 83%, with the highest relative abundance in the topsoil in plots under a long rotation regime (93%) and the lowest relative abundance in plots under a short rotation regime (61%). The relative abundance of Ascomycota stayed relatively constant across soil depth (Fig 5.1). The relative abundance of Basidiomycota averaged 13% across all samples being highest in the topsoil in plots under a short rotation regime (38%) and lowest in plots under a long rotation regime that ranged from 10% in the 40-60cm profile and 4% in the 20-40cm profile. The phylum Mortierellomycota was higher in the topsoil of the non-burn control plots compared to plots under long rotation and short rotation burn regimes (Fig 5.1).

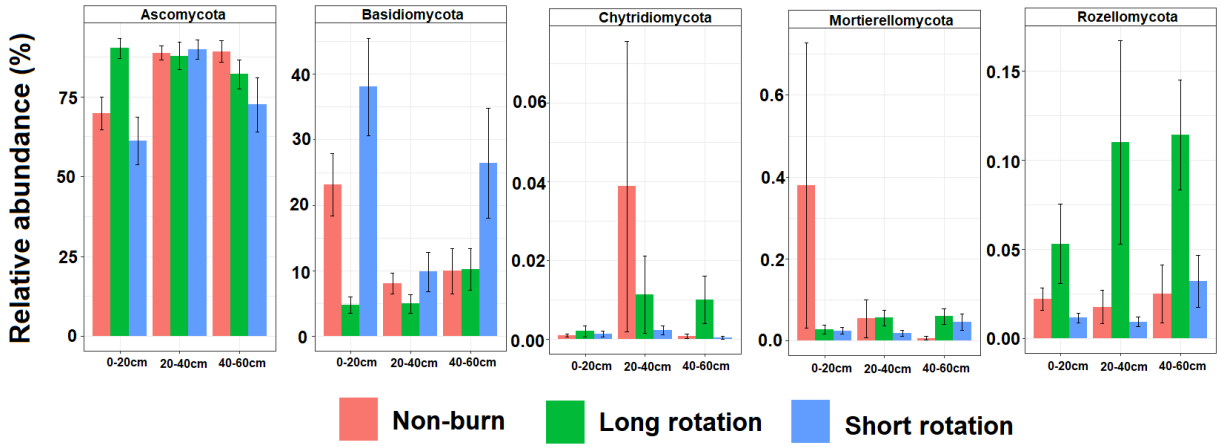

Fig 5.1. Relative abundance of the top five fungal phyla across three different soil depths under three burn treatments. The bars indicate the mean values of each treatment, with the error bars representing the standard error. Non-burn 0-20cm (*n*=12), non-burn 20-40cm (*n*=12), non burn 40-60cm (*n*=9), long rotation 0-20cm (*n*=12), long rotation 20-40cm (*n*=11), long rotation 40-60cm (*n*=12), short rotation 0-20cm (*n*=12), short rotation 20-40cm (*n*=12), short rotation 40-60cm (*n*=12).

Fungal communities were primarily comprised of members of the classes Leotiomycetes which represented a total of 64% in all samples, Agaricomycetess (10%) and Archaeorhizomycetes (6 %) (Fig 5.2). The relative abundance of Leotiomycetes was lowest in the non-burn control across all three depth profiles (Fig 5.2). The abundance of Agaricomycetes generally decreased with depth except for under a long rotation regime (ANOVA, F=10.91, P= <0.0001) and was significantly different across burn treatments (ANOVA, F=7.56 P= 0.008) being highest in plots under a short rotation regime. However, the abundance of Archaeorhizomycetes was greater in non-burn soils than in burned soils (ANOVA, F=9.32, P = <0.0001). Members of the classes Dothieomycetes, Eurotiomycetes, Microbotryomycetes, Mortierellomycetes, Rozellomycotina_cls_Incertae_sedis, Sordariomycetes and Tremellomycetes were found at low relative abundance (Fig 5.2). The classes Dothieomycetes, Microbotryomycetes, Rozellomycotina_cls_Incertae_sedis and Tremellomycetes were all significantly affected by burn treatment (ANOVA, P=<0.05).

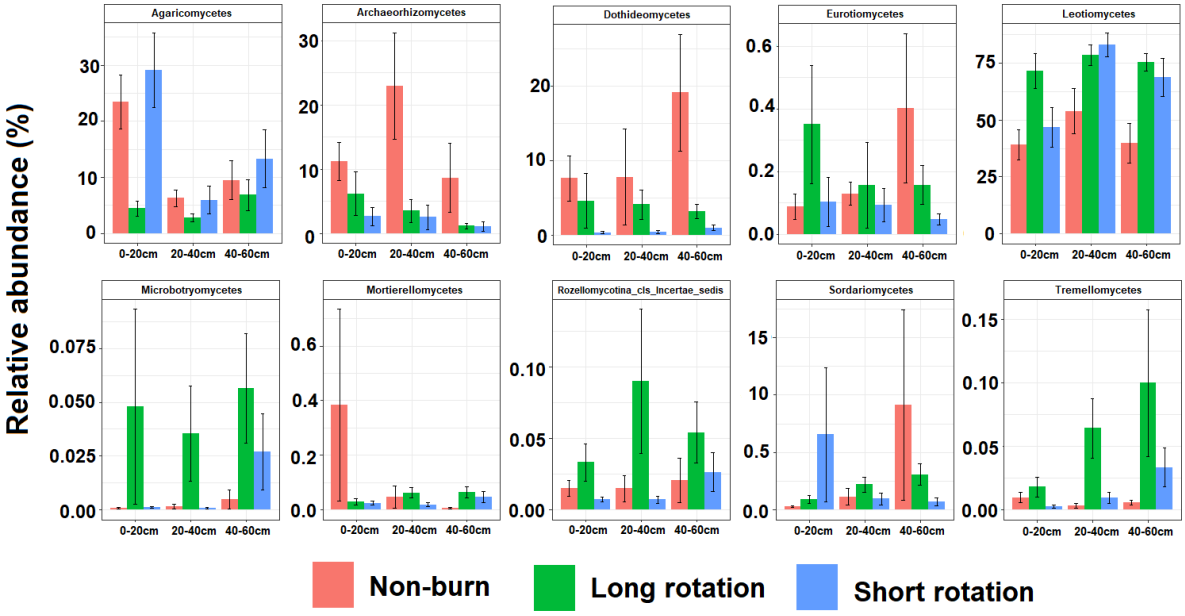

Fig 5.2. Relative abundance of the top ten fungal classes across three different soil depths under three burn treatments. The bars indicate the mean values of each treatment, with the error bars representing the standard error. Non-burn 0-20cm (n=12), non-burn 20-40cm (n=12), non burn 40-60cm (n=9), long rotation 0-20cm (n=12), long rotation 20-40cm (n=11), long rotation 40-60cm (n=12), short rotation 0-20cm (n=12), short rotation 20-40cm (n=12), short rotation 40-60cm (n=12).

5.3.2: Fungal community diversity and community composition across burn treatments and depth

All three diversity measures (observed, Shannon and Simpson) for fungal communities were significantly different between burn treatments and by soil depth. However, their interactive effect was not significant (Table 5.1; Fig 5.3). All three alpha diversity metrics were highest in the topsoil of the non-burn control plots. Observed diversity decreased significantly from the topsoil to the subsoil in all three burn treatments.

Table 5.1. Two-way ANOVA of fungal diversity indices across three different soil depths under three burn treatments. Result is reported as the mean ± SE. The data in bold indicate fungal diversity that were affected by soil depth, burn treatment and their interaction at a confidence level of 95% ($P < 0.05$). Different uppercase letters indicate statistically significant differences among the three burn treatments in the same soil layer and different lowercase letters indicate statistically significant differences among the three soil layers across burn treatments (Tukey's HSD, $P < 0.05$). Non-burn 0-20cm (n=12), non-burn 20-40cm (n=12), non burn 40-60cm (n=9), long rotation 0-20cm (n=12), long rotation 20-40cm (n=11), long rotation 40-60cm (n=12), short rotation 0-20cm (n=12), short rotation 20-40cm (n=12), short rotation 40-60cm (n=12).

Burn treatment	**Depth (cm)**	**Observed**	**Shannon**	**Simpson**
Non-burn	**0-20cm**	88.83 ± 11.53 Aa	2.58 ± 0.17 Aa	0.83 ± 0.03 Aa
	20-40cm	46.00 ± 4.98 Ab	2.15 ± 0.14 Ab	0.75 ± 0.03 Aa
	40-60cm	78.44± 11.01 Aab	2.31 ± 0.14 Ab	0.80 ± 0.02 Aa
Long rotation	**0-20cm**	74.91±8.09 ABa	1.78 ±0.15 Ba	0.65 ± 0.04 Ba
	20-40cm	50.41 ± 5.69 Ab	1.90 ± 0.17 Aa	0.68 ± 0.05 Aa
	40-60cm	56.00 ± 4.98 Ab	2.03 ±0.52 Ba	0.70 ± 0.03 Aa
Short rotation	**0-20cm**	54.00 ± 3.92 Ba	1.91 ± 0.11 Ba	0. 72 ± 0.03 Ba
	20-40cm	35.58 ± 2.92 Bb	1.22 ± 0.22 Bb	0.48 ± 0.07 Bb
	40-60cm	38.72 ± 0.03 Bb	1.64 ± 0.16 Ca	0.63 ± 0.05 Bb
Burn treatment		**F=14.11,P=0.001**	**F=16.42,P=<0.001**	**F=11.70,P=<0.001**
Depth (cm)		**F=16.48,P=<0.001**	**F=4.202,P=0.01**	**F=3.72,P=0.01**
Burn treatment* Depth		F=1.64,P=0.34	F=1.598,P=0.18	F=1.68,P=0.15

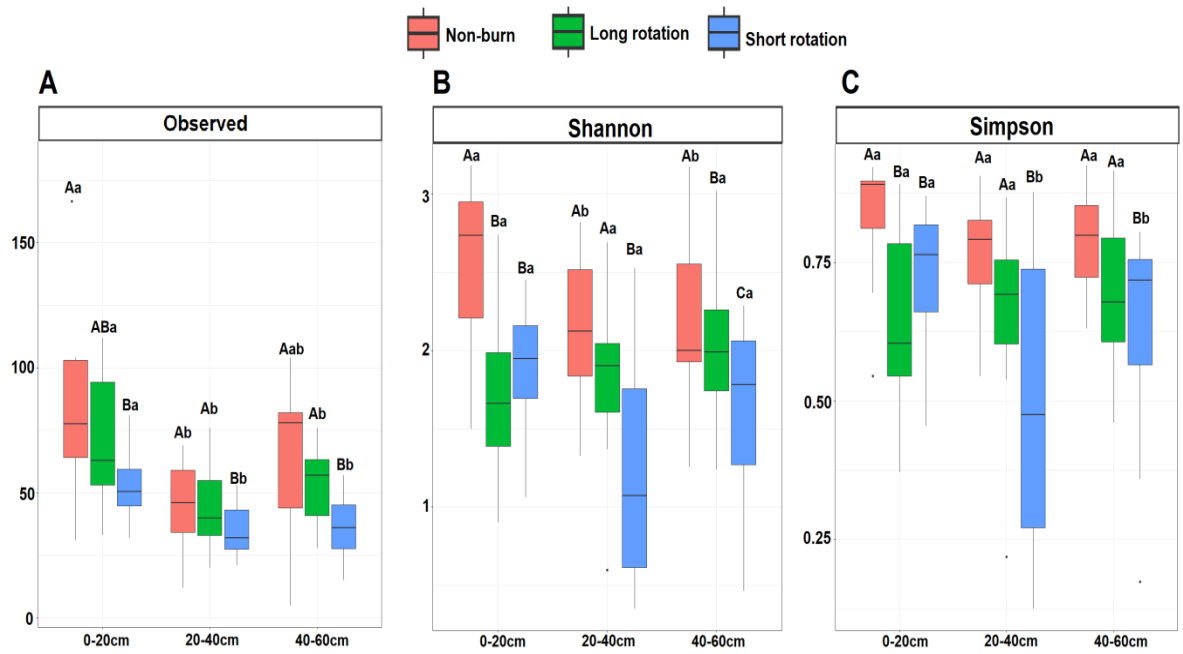

Fig 5.3. Alpha diversity indices of fungal communities across three different soil depths under three burn treatments. Observed richness **(A),** Shannon index **(B)** and Simpson index **(C).** Different uppercase letters indicate statistically significant differences among the three burn treatments in the same soil layer and different lowercase letters indicate statistically significant differences among the three soil layers across burn treatments (Tukey's HSD, $P < 0.05$). Non-burn 0-20cm (n=12), non-burn 20-40cm (n=12), non burn 40-60cm (n=9), long rotation 0-20cm (n=12), long rotation 20-40cm (n=11), long rotation 40-60cm (n=12), short rotation 0-20cm (n=12), short rotation 20-40cm (n=12), short rotation 40-60cm (n=12).

Using the Bray-Curtis dissimilarity, principal coordinate analysis (pCoA) was conducted to illustrate the variance of fungal community structure along the soil depth gradients in different burn treatments. Overall, the pCoA (Fig 5.4) showed that beta diversity of fungal communities was significantly different between burn treatment (PERMANOVA, F= 7.90, R^2=0.131, P=<0.001) and soil depth (PERMANOVA, F= 4.68, R^2=0.085, P= <0.001). In each treatment, samples showed clear separation of plots according to soil depth and burn treatment while samples under the same treatment tend to cluster closer together (Fig 5.4).

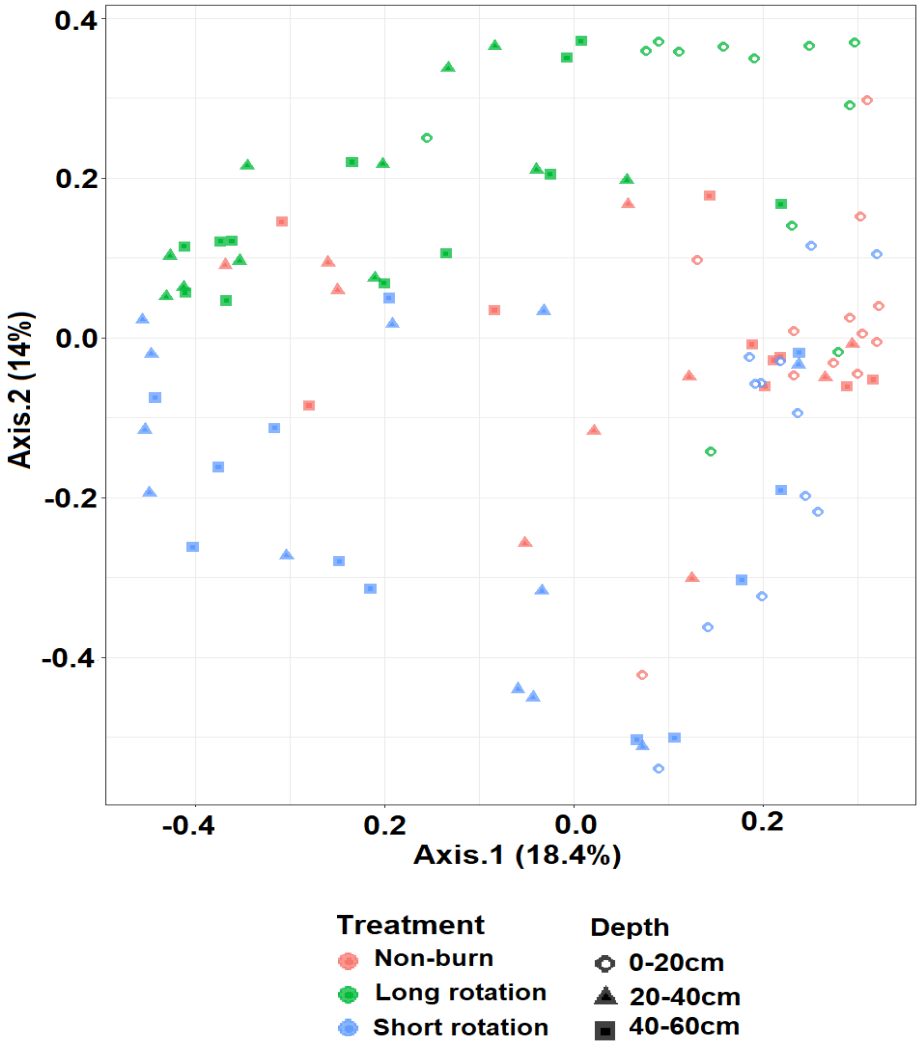

Fig 5.4. Principal coordinate analysis based on the Bray-Curtis dissimilarity of fungal community structure for soil samples collected across three different soil depths under three burn treatments. Different colours indicate three burn treatments including red for non-burn, green for long rotation and blue for short rotation. Different shapes indicate different soil depth profiles including circle for 0-20cm, triangle for 20-40cm and square for 40-60cm. Non-burn 0-20cm (*n*=12), non-burn 20-40cm (*n*=12), non burn 40-60cm (*n*=9), long rotation 0-20cm (*n*=12), long rotation 20-40cm (*n*=11), long rotation 40-60cm (*n*=12), short rotation 0-20cm (*n*=12), short rotation 20-40cm (*n*=12), short rotation 40-60cm (*n*=12).

5.3.3: Environmental factors influencing soil fungal communities

Forward selection redundancy analysis (RDA) was performed to investigate the relationships among environmental variables and fungal community structure. The most significant environmental factors that shaped fungal communities in the topsoil were Ca, Mn, percent of other 'non-Sphagnum' moss cover, total C and pH. NH_4^+ and moisture were the most

important environmental factors in the 20-40cm profile and Fe and Pb in the 40-60cm profile. All final models were significant (ANOVA, *P*=<0.05) (Fig 5.5).

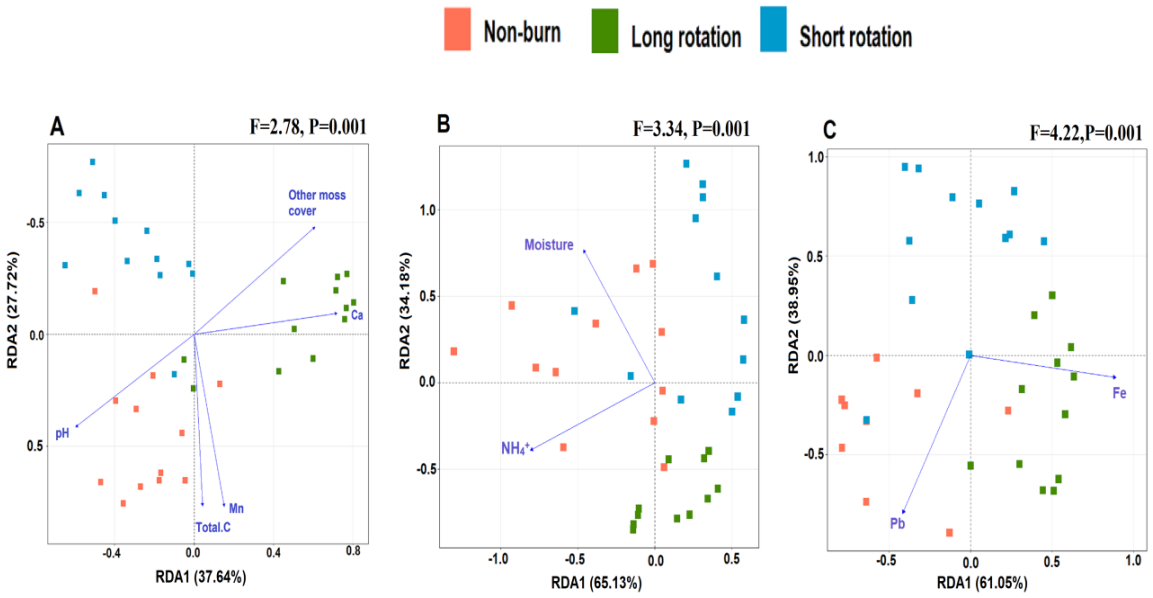

Fig 5.5. RDA Ordination plots showing soil related drivers of fungal communities for soil samples collected at three different depths under three burn treatments. **A**= 0-20cm, **B**= 20-40cm, **C**= 40-60cm. Only significant variables (*P*=<0.05) are shown. Different colours indicate three sampling treatments. The ASV data were standardized with Hellinger transformation using the *Vegan* package. Non-burn 0-20cm (*n*=12), non-burn 20-40cm (*n*=12), non burn 40-60cm (*n*=9), long rotation 0-20cm (*n*=12), long rotation 20-40cm (*n*=11), long rotation 40-60cm (*n*=12), short rotation 0-20cm (*n*=12), short rotation 20-40cm (*n*=12), short rotation 40-60cm (*n*=12).

5.3.4: Functional guilds of fungal communities

FUNGuild classified 40% of the fungal ASVs according to their functional guild and trophic mode. The majority of classified fungi were assigned to the group of saprotrophs (17%); pathotrophs (6.5%); symbiotrophs (5%) and ASVs assigned to multiple trophic modes (11%) with the rest being classified 'unknown'. Trophic modes differed among burn treatments as well as depth (Fig 5.6; Table 5.2). Pathotrophs were significantly affected by burn treatment but not depth. However, there was an increase in the non-burn subsoil (40-60cm) where the relative abundance increased to 14%. Saprotrophic fungi were affected by burn treatment but not depth (Fig 5.6; Table 5.2) as this fungal guild showed a significantly higher relative abundance in the non-burn control across all three depth profiles. Symbiotrophic fungi were affected by burn treatment as well as depth with the relative abundance being highest in the

topsoil of the non-burn control (18%). Surface soils subject to long rotation burning had the highest relative abundance of ASVs with multiple trophic modes (57%) (Fig 5.6; Table 5.2).

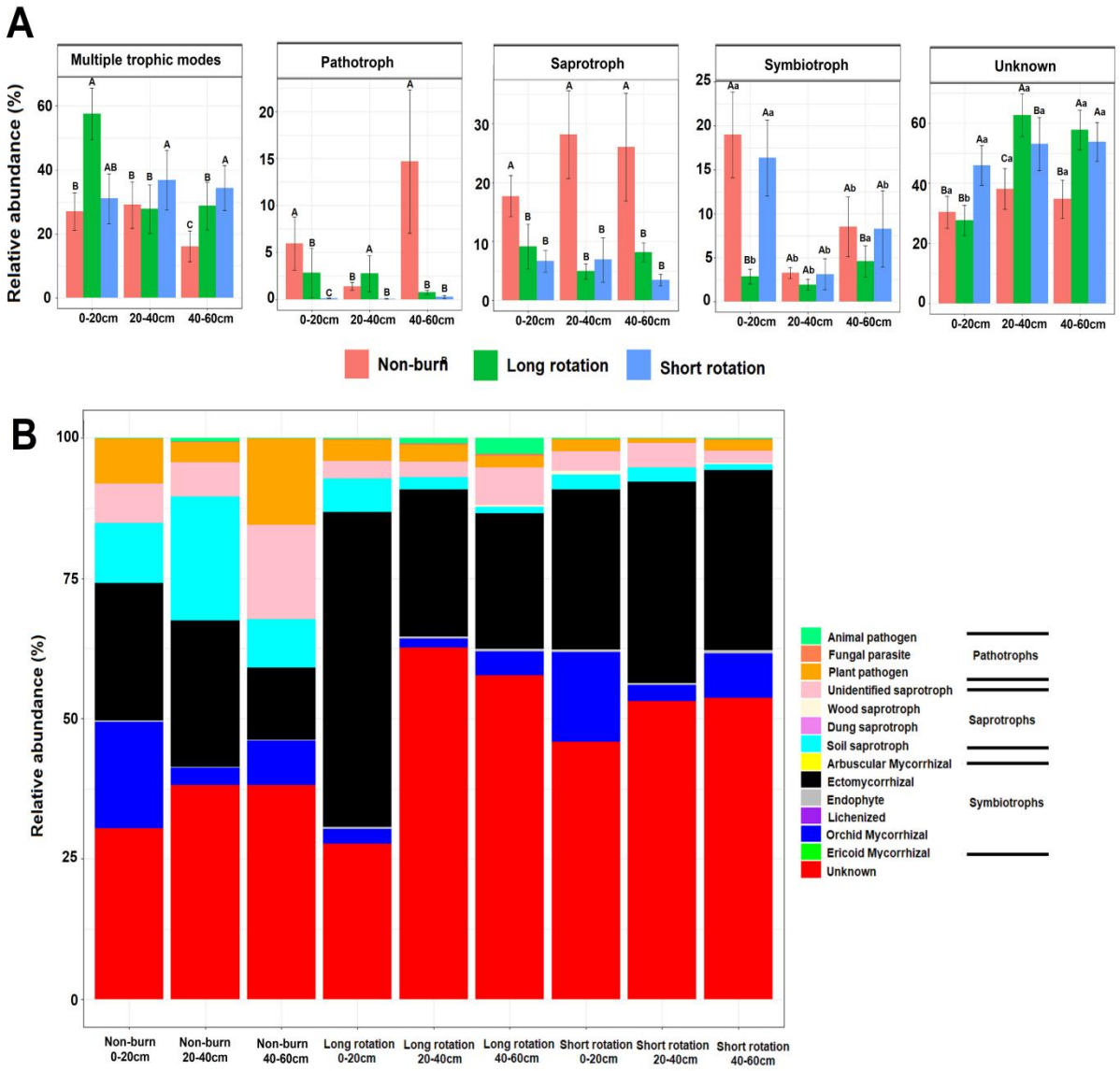

Fig 5.6. Relative abundance of trophic modes **(A)** and functional guilds **(B)** across three different soil depths under three burn treatments. The bars indicate the mean values of each treatment, with the error bars representing the standard error. Different uppercase letters indicate statistically significant differences among the three burn treatments in the same soil layer and different lowercase letters indicate statistically significant differences among the three soil layers across burn treatments (Tukey's HSD, $P < 0.05$). Non-burn 0-20cm (n=12), non-burn 20-40cm (n=12), non burn 40-60cm (n=9), long rotation 0-20cm (n=12), long rotation 20-40cm (n=11), long rotation 40-60cm (n=12), short rotation 0-20cm (n=12), short rotation 20-40cm (n=12), short rotation 40-60cm (n=12).

Table 5.2. Two-way ANOVA of the relative abundance of trophic modes using funGUILD across three different soil depths under three burn treatments. The data in bold indicate trophic modes that were affected by soil depth, burn treatment and their interaction at a confidence level of 95% ($P < 0.05$). Different uppercase letters indicate statistically significant differences among the three burn treatments in the same soil layer and different lowercase letters indicate statistically significant differences among the three soil layers across burn treatments (Tukey's HSD, $P < 0.05$). Non-burn 0-20cm (n=12), non-burn 20-40cm (n=12), non burn 40-60cm (n=9), long rotation 0-20cm (n=12), long rotation 20-40cm (n=11), long rotation 40-60cm (n=12), short rotation 0-20cm (n=12), short rotation 20-40cm (n=12), short rotation 40-60cm (n=12).

Burn treatment	Depth (cm)	Pathotroph	Saprotroph	Symbiotroph	Multiple trophic modes	Unknown
Non-burn	**0-20cm**	0.05 ± 0.02 A	0.17 ± 0.03 A	0.18 ± 0.04 Aa	0.26 ± 0.05 B	0.30 ± 0.05 Ba
	20-40cm	0.01 ± 0.0001 B	0.28 ± 0.07 A	0.03 ± 0.007 Ab	0.29 ± 0.07 B	0.38 ± 0.06 Ca
	40-60cm	0.14 ± 0.007 A	0.26 ± 0.08 A	0.08 ± 0.003 Ab	0.16 ± 0.04 C	0.34 ± 0.06 Ba
Long rotation	**0-20cm**	0.02 ± 0.0002 B	0.09± 0.03 B	0.02 ± 0.008 Bb	0.57 ± 0.08 A	0.27 ± 0.04 Bb
	20-40cm	0.02 ± 0.0002 A	0.049 ± 0.01 B	0.01 ± 0.006 Ab	0.27 ± 0.07 B	0.62 ± 0.07 Aa
	40-60cm	0.0007 ± 0.0001 C	0.06 ± 0.01 B	0.04 ± 0.001 Ba	0.28 ± 0.07 B	0.57 ± 0.06 Aa
Short rotation	**0-20cm**	0.001 ±0.0002 C	0.06 ± 0.003 B	0.16 ± 0.004 Aa	0.31 ± 0.07 AB	0.45 ± 0.06 Aa
	20-40cm	0.007 ± 0.0003 B	0.03 ± 0.01 B	0.03 ± 0.01 Ab	0.36 ± 0.009 A	0.53 ± 0.08 Ba
	40-60cm	0.002 ±0.001 B	0.08 ± 0.04 B	0.08 ± 0.04 Ab	0.34 ± 0.07 A	0.53 ± 0.04 Aa
Burn treatment		**F=5.05 ,P=0.008**	**F=14.80,P=<0.001**	**F=4.91,P=0.001**	**F=2.80,P=0.04**	**F=5.48,P=0.005**
Depth (cm)		F=1.36,P=0.25	F=0.197,P=0.082	**F=8.47,P=0.001**	F=2.02P=0.013	**F=5.42P=0.005**
Burn treatment *Depth		F=2.26,P=0.06	F=0.87,P=0.42	F=1.97,P=0.10	F=2.10, P=0.08	F=1.71, P=0.15

5.3.5: Indicator species

Fungal indicators for each burn treatment represented ten classes (Indval >0.3, P=<0.05). The number of indicators for each treatment varied widely with the non-burn topsoil having nine indicators the subsoil 20-40cm profile having one indicator belonging to the class Archaeorhizomycetales and the 40-60cm profile having four indicators belonging to the classes Eurotiomycetes, Leotiomycetes and Dothideomycetes while the topsoil of the long rotation regime had six indicators belonging to the classes Leotiomycetes, Eurotiomycetes, Microbotryomycetes and Agaricomycetes. The subsoil (20-40cm and 40-60cm) had seven indicators belonging to the classes Rozellomycotina_cls_Incertae_sedis, Tremellomycetes Malasseziomycetes, Microbotryomycetes, Dothideomycetes, Sordariomycetes and Eurotiomycetes. Only one indicator was detected in plots under a short rotation regime belonging to the class Agaricomycetes (appendix 8, Table 8.1).

5.4: Discussion

This study aimed to characterise the effects of different prescribed burning regimes on soil fungal communities in an upland peatland. The long term monitoring site at Moor House Nature Reserve has been in place since 1954 and provides a unique opportunity to evaluate how prescribed burning regimes affect fungal communities. The significant changes observed in the diversity and community structure of fungal communities across prescribed burn treatment and the effects that extended into the deeper soil horizons provide a critical insight into a component of soil ecology that is crucial to the recovery of fungal communities, and therefore the ecosystem following prescribed burning.

5.4.1: Soil fungal community characteristics

Community composition varied across burn treatments and soil depth. The most abundant phyla found in this study were Ascomycota (Fig 5.1). Previous studies from peatland ecosystems have revealed the dominance and importance of this group (Juan-Ovejero *et al*., 2020; Thormann & Rice, 2007; Zhang *et al*., 2017). With average relative abundances of 46% and 40%, respectively, Ascomycota and Basidiomycota dominate the fungal communities in peatlands (Thormann & Rice, 2007). The large classes Leotiomycetes and Eurotomycetes were found in this study and have been widely studied (Asemaninejad *et al*., 2017; Ekanayaka *et al*., 2019; Geiser *et al*., 2006). The class Leotiomycetes has important capabilities in lignocellulose degradation and important root associated fungi (Vrålstad *et al*., 2002) and is frequently observed in nutrient poor soils (Asemaninejad *et al*., 2017). The phylum Ascomycota is associated with burning and has been known to increase in abundance after a fire (Ammitzboll *et al*., 2021; Robinson *et al*., 2008). Interestingly, Ascomycota was abundant in all treatments including the subsoil showing that this phylum may play a role in recolonizing areas in the subsoil. However, it showed a slight decline in plots under a short rotation regime (Fig 5.1). Ascomycota have been known to dominate areas cleared for regeneration, becoming less dominant over time (Yan *et al*., 2018). In contrast, Basidiomycota increased in plots under a short rotation regime with the majority being assigned multiple trophic modes. The relative abundance of saprotrophs was greatly reduced in plots under long rotation and short rotation regimes while the relative abundance symbiotrophs were relatively high in the non-burn control and short rotation regime (Fig 5.6; Table 5.2). It was expected that the relative abundance of saprotrophs would increase after fire as saprotrophs play a role in ecological succession and are part of an enrichment process

after disturbance (Alem *et al*., 2020). However, the increase in the non-burn control could possibly be due to the increased input of vegetation that serve as new substrates and contain fungal saprotrophs (Boddy *et al*., 2007). Saprotrophs have been shown to play an important role in carbon cycling in peat bogs (Rice *et al*., 2006). Thus, the increase in the relative abundance of saprotrophic fungi in the non-burn control may enhance the activity of carbon-degrading enzymes and drastically increase the decomposition of organic matter in peatlands. There was an increase in the abundance of fungi with multiple trophic modes in plots under a long rotation regime in the topsoil and short rotation regime in the 20-40cm and 40-60cm profiles. Therefore, these findings may have important implications for maintaining the diversity of functional guilds in peatlands. Furthermore, the co-occurrence of these fungi has numerous benefits, such as exchanging water via mycorrhizal hyphal networks as well as nutrients (Brundrett, 2002; Brundrett, 2004). Thus, the investigation of the fungal community diversity and structure in this study provides an insight into conserving functional guilds and trophic modes in peatlands under prescribed burning regimes.

Indicator taxa in plots subject to prescribed burning possessed characteristics that aid in the resistance of high temperatures and environmental conditions following fire. Indicator species were represented by fungal taxa that are known to be thermo-tolerant and rapid post-fire colonizers such as Helotiales and Agaricales (Cutler *et al*., 2017; Salo *et al*., 2019). In burned plots, fires removed much of the above ground plant biomass and canopy leaving the soil exposed to warmer temperatures, which likely favoured taxa which can tolerate higher temperatures.

5.4.2: Fungal richness, diversity and community composition

Soil fungi are essential to nutrient cycling, yet little is known about how fungi respond to prescribed fire in peatlands and how the effects extend into deeper soil profiles. The results indicate that fungal richness and diversity were affected by burning across soil profiles. There was higher observed richness in the control non-burn plots and significantly lower observed richness in plots under short rotation burns (Table 5.1; Fig 5.3). In contrast, long rotation burns had only a small reduction in observed species richness (Table 5.1; Fig 5.3). It is possible that ecological succession may create new conditions owing to differences in rotation and severity of burning. Prescribed burning most likely eliminates fungal species that cannot withstand the higher temperatures and reduces species composition to those that can

survive through fire-resistant spores. Furthermore, soil physiochemical and vegetation changes are likely to select for species that best compete under conditions altered by burning (Cairney & Bastias, 2007; Hart *et al*., 2005). As fungi are related to the composition and productivity of plants, reduced species are likely to contribute to a decrease in ecosystem processes (Juan-Ovejero *et al*., 2020).

Principal coordinate analysis showed that fungal community composition was strongly affected by burn treatments and soil depth (Fig 5.4). This is in agreement with the effects of wildfire in forests, possibly due to the similar effects wildfire has on soil physicochemistry in other ecosystems (Ammitzboll *et al*., 2021; Holden *et al*., 2016). This suggests that fungal community composition is strongly driven by the practice of burning and underpins the need to evaluate the effects of this management regime on fungi within peatlands across multiple sites, and further research is required such as sampling immediately after burning to determine which fungal taxa are tolerant to higher temperatures.

5.4.3: Fungal diversity in soil horizons.

Previous studies have assessed the effects of wildfire on soil fungi in other ecosystems at the topsoil but the effects below 20cm are unstudied (Alem *et al*., 2020; Ammitzboll *et al*., 2021; Holden *et al*., 2013). Observed richness decreased below the topsoil (Table 5.1;Fig 5.3). The decrease in fungal richness with depth may in part be due to lower nutrients and the anaerobic nature of peat soils (Koretsky *et al*., 2006). Fungi are known to be sensitive to anoxic conditions and have limited hyphal capacity (Hiiesalu *et al*., 2017). It is surprising that prescribed burning affected soil fungi across all depth profiles as peat soil is generally wet below 20cm and can be effective at delaying fire penetration. As burning can have a negative effect on plant root biomass (Tufekcioglu *et al*., 2010) and roots can extend far into the deeper soil layers this may also explain the impact of burning practices in deeper soil due to the close relationship fungi have with plant roots. It is also important to note that fungi do not recolonise as fast as prokaryotes and are less resilient during land disturbance as the recovery of fungi and prokaryotes from environmental stress such as burning is differentially governed by the physiological responses of plants (Bardgett *et al*., 2013) for example, by reducing the transfer of recently plant-assimilated C to prokaryotes but not to fungi (Fuchslueger *et al*., 2014). Hence, the effects of burning on fungi may persist for a longer period of time (Zhou *et al*., 2019). Overall, these findings indicate the presence of distinct niches along the soil

profile containing specific fungal communities. However, little is known about the relative abundance of fungal taxa that varied significantly across the soil profile, and more research is required to investigate the role and function of these fungal taxa in peatlands.

5.4.4: Environmental factors influencing fungal communities.

RDA analysis showed that Ca, Mn, other 'non-Sphagnum' moss cover, total C and pH, were among the most important factors driving community composition in the topsoil, NH_4^+ and moisture in the 20-40cm profile and Fe and Pb in the 40-60cm profile respectively (Fig 5.5). Soil fungi are strongly influenced by edaphic factors in the environment (Vyas & Gupta, 2014). The majority of studies investigating the impact of management regimes on fungal communities focus on SOM and pH, with less attention towards available cations. Importantly, it was found that changes in nutrient cations such as Ca, Al, Fe, and Pb were the main environmental factors shaping fungal communities. Several reviews have shown how fire affects soil physicochemistry (Augustine & McNaughton, 1998; Certini, 2005; González-Pérez e*t al*., 2004; Hrelja *et al*., 2020; McSherry & Ritchie, 2013; Zhou *et al*., 2017). Fungi are more impacted by climatic conditions than archaea and bacteria and they are known to follow similar environmental niche trends as plants (Bahram *et al*., 2018). Furthermore, fungi rely heavily on plants for resources as they are heterotrophs (Antunes & Koyama, 2017). For example, host metabolites for symbiotrophs and plant litter quality are important factors for saprotrophs. However, correlations must be carefully investigated as it is difficult to establish the relationships between nutrient cycling and microbial activity.

5.4.5: Conclusions

Soil fungi play significant roles in the environment. For example, they have a vital contribution to the growth of plants, litter decomposition and nutrient cycles (Ritz & Young, 2004). Therefore, their effective management is essential for ecosystem health. This study highlights the significant impact of prescribed burning on soil fungal communities throughout the soil profile. Short rotation burns were found to have a significant negative impact on fungal richness and diversity across all depth profiles, while greater observed richness was found in the non-burn control, followed by a long rotation regime. Ca, Mn, other 'non-Sphagnum' moss cover, total C and pH were the most important environmental drivers in the topsoil, NH_4^+ and moisture were the most important in the 20-40cm profile and Fe and Pb were most important in the 40-60cm profile. Prescribed burning on peatlands is a highly contentious issue and there is a lack of research on the diversity of fungi across pristine peatlands, and studies from tropical peatlands are particularly scarce. Categorizing the effects of this management regime on fungal diversity and ecological guilds in peatlands across the world is an important issue that will aid in the preservation of biodiversity within the soil and the high carbon storing capacity of peatlands. Because functional traits are increasingly being used to study the responses of fungi to land management in terrestrial ecosystems, they are an appropriate tool for linking biogeochemical processes to functional traits (Talbot *et al*., 2015; Treseder & Lennon, 2015; Wang *et al*., 2019), and their utilisation in peatlands necessitates additional urgent research. This study has enhanced the understanding of how prescribed fire disturbance in a peatland impacts the soil fungal communities across soil horizons that are important in ecosystem functioning, which has broader implications for fire management and restoration of northern peatlands.

Chapter 6. Changes in microbial populations and nitrogen functional genes in soil profiles of a peatland under different burning regimes

Abstract

Microbes in peatlands are important for providing key ecosystem services and are essential for their role in biogeochemical cycling. Prescribed burning is a common aspect of peatland management but the practice has been criticized for being ecologically damaging due to its effect on the biological, chemical and physical properties of the soil. It is poorly understood how the impact of prescribed burning effects soil N cycling and previous studies on microbial analysis have focused on the topsoil ignoring the changes in nutrient accumulation with soil depth. This study investigated the changes of microbial abundance (bacterial 16S rRNA and fungal 18S rRNA) and the abundance of N functioning genes involved in archaeal and bacterial ammonia oxidation (*amoA*-AOA and *amoA*-AOB), denitrification (*nirK* and *nirS*), N fixation (*nifH*) and organic N decomposition (*chiA*) in soil profiles across three burn treatments (no burn, long rotation every 20 years and short rotation every 10 years). The abundance of bacterial 16S rRNA was greater in the non-burn control plots and fungal 18S rRNA was greater in non-burn control plots and plots subject to a long rotation burn regime. The abundances of *amoA-AOA*, *amoA-AOB*, and *nifH* were significantly higher in the topsoil of the non-burn control plots while the abundance of *nirK* was higher in plots subject to short rotation and long rotation burn regimes and decreased significantly with soil depth. The abundance of *nirS* was not affected by burn treatment or soil depth. *ChiA* abundance was greater in plots under a short rotation burn regime and decreased with soil depth. N functioning gene abundance responded differently to environmental factors associated with prescribed burning and varied with soil depth. These findings suggest that the practice of burning affects microbial N turnover potential and provides an important insight into the soil N-cycling potential of peatlands under different burning regimes.

Keywords: Peatlands; Biogeochemical cycling; Prescribed burning; N cycling; N functioning genes.

6.1: Introduction

Peatlands cover an estimated 4 million km^2 globally and are essential for their biodiversity, hydrological function and role in mitigating climate change (Xu *et al*., 2018). Prescribed burning has been widely used as a management tool in the UK to decrease the risk of wildfire and maintain peatland vegetation for game bird populations where the land is too poor for agricultural use (Simmons, 2003; Yallop *et al*., 2006). However, burning can influence peatland function particularly due to vegetation change (Ciccolini *et al*., 2016; Evans *et al*., 2014).

Land-use represents a globally important driver for ecosystem change with implications for biogeochemical functioning. Changes in the environment have an impact on the amount of residual vegetation that is returned to the soil, which is the primary source of soil carbon storage (Arneth *et al*., 2017; Lai *et al*., 2016). For the most part, northern peatlands have remained relatively unaffected by direct human activity except in areas of high human density (Joosten, 2009c). Burning has been a common management regime in the UK since the early 1900s and has been recognised as a major driver for vegetation change (Holden *et al*., 2015; Milligan *et al*., 2018) and surface hydraulic conductivity (Holden *et al*., 2014). As plant communities change there is an indirect effect on decomposer organisms altering ecosystem carbon fluxes (Harte *et al*., 2015). However, the change depends on the severity and frequency of the burn. Rotational burning is the most common type of burning where vegetation is burned to facilitate the growth of heather (Douglas *et al*., 2015) and is still a common practice in peatlands in the UK (Douglas *et al*., 2015; Thacker *et al*., 2000; Yallop *et al*., 2010). The severity of burning varies, but 'cool' burns that remove the vegetation's canopy layer, without igniting the underlying peat or consuming the moss and litter layer, have been recommended (Ashby & Heinemeyer, 2021; Noble *et al*., 2019). As the use of burning to manage peatlands in the UK is still common, there has been debate about the long-term viability of current practices and concern about the potential impact the practice might have on ecosystem function (e.g. Ashby & Heinemeyer, 2021; Harper *et al*., 2018; IUCN, 2020). However, little is known about how burning affects nitrogen turnover in different soil profiles within peatlands.

Nitrogen is a critical factor in peatland management as a principle limiting factor for microbial function as well as plant productivity (Blodau & Zajac, 2015; Levy-Booth *et al*., 2014). Nitrogen dynamics in these environments are primarily driven by N-cycling microbes and are closely linked to soil function and atmospheric nitrogen processes (Shukla *et al*., 2021). Availability of nutrients such as carbon and nitrogen also affects microbial composition and diversity (Berthrong *et al*., 2013). Soil N-cycling genes have been investigated using molecular markers in previous studies, including studies on grasslands (Song *et al*., 2019), agricultural land (Li *et al*., 2018), forests (Tang *et al*., 2018) and tropical peatlands (Espenberg *et al*., 2018; Nurulita *et al*., 2016). N-cycling genes code for enzyme subunits that are important to nitrogen cycling. Bio-available nitrogen in the soil is predominantly fixed from the atmosphere by the Nitrogenase enzyme encoded by the *nifH* gene and organic matter decomposition is linked to the *chiA* gene. N fixation for atmospheric nitrogen and nitrogen organic matter decomposition both depend on the amount of organic matter available (Pajares & Bohannan, 2016; Song *et al*., 2019). The *amoA* gene is involved in the initial stage of autotrophic nitrification, where ammonia-oxidizing archaea and bacteria oxidise ammonia to hydroxylamine to obtain energy and fix carbon (Che *et al*., 2018). *NirK* and *nirS* genes encode for nitrite reductases which participate in the conversion of nitrite to nitric oxide during denitrification and completing the N cycle. Thus, due to the centrality of nitrogen in the ecosystem, all key stages of the soil nitrogen cycle must be considered in order to gain a thorough understanding of nitrogen transformations in soils under land management.

Many studies on the effects of anthropogenic disturbance on the abundance of microbes and functional genes have focused on the topsoil and ignored the changes of nutrients in relation to soil depth (Espenberg *et al*., 2018; Fisk *et al*., 2003; Urbanová & Bárta, 2016). Given the important roles of functional genes in the nitrogen cycle, determining their response to burning, especially across different soil profiles is essential for gaining an insight of the processes of burning as a management strategy on peatlands. This knowledge can help predict how peatlands will react to anthropogenic disturbances.

Knowledge of the N cycle in the subsoil is critical for researching the effects of anthropogenic activity, but it is limited in comparison to surface soils and varies across ecosystems. For example, the relative abundances of *nirS*, was higher in the surface soil of tropical forest soils at the Luquillo Critical Zone Observatory in northeast Puerto Rico (Stone *et al*., 2015) and three paddy soils in China (Wang *et al*., 2017). However, there were no

changes in the abundances of denitrifying genes *nirK* and *nirS* across different soil profiles in an estuarine ecosystem (Lee & Kang, 2016). There is a scarcity of information on microbial activity associated with nitrogen cycling in the subsoil in peatlands under prescribed burning regimes. Therefore, it is critical to understand the intensity of nitrogen fluxes in peatlands under prescribed rotational burning.

The aim of this chapter is to assess the influence of prescribed burning on microbial abundance and soil microbial nitrogen turnover, and how it relates to plant cover and soil physicochemistry, by investigating the changes in the abundances of bacteria, fungi and N-cycling genes within different soil profiles (0–20cm, 20–40cm, 40–60cm) across different burn treatments at Moor House nature reserve United Kingdom. The reserve is a flagship Environmental Change Network (ECN) monitoring site with extensive historical and ongoing data collection available, including different management practices in use across the 74 km^2 site and is characterised by vegetation cover typical of many moorlands in upland Britain (M19/M20 communities) (Rodwell, 1998).

The following hypotheses were tested. 1) The abundance of bacteria and fungi will decrease with the frequency of burn treatment and be higher in non-burn control plots; 2) There will be changes in the abundance of N-cycling genes across burn treatments. It is expected that no further burning will enhance microbial nitrogen turnover potential which will be characterized by a higher abundance of functional genes due to changes in soil nutrient content and vegetation cover caused by burning; 3) *AOA, AOB, nifH* and *chiA* genes related to nitrogen acquisition will be higher in the topsoil due to an increase in nutrient content compared to that in the lower soil layers; 4) *nirS* and *nirK* genes, involved in denitrification, will be more abundant in the anoxic subsurface soil characteristic of peatlands.

6.2: Materials and methods

Details of the study site are given in chapter 2, section 2.1.2. The experimental design, vegetation cover and physicochemistry measurements are given in the general methods (chapter 2).

6.2.1: Preparation of standards for qPCR

Extracted DNA (chapter 2, section 2.4) was used for a template to measure gene targets. Quantitative PCR (qPCR) assays were used to quantify the total bacterial communities (16S rRNA gene), total fungal communities (18S rRNA gene), as well as functional genes (*AOA-amoA, AOB-amoA, nirS, nirK, nifH and chiA*) using primer pairs summarised in Table 6.1.

Table 6.1. PCR primers used for the amplification of microbial populations and functional gene targets. Note: S=C/G; K=G/T; Y=C/T; R=A/G; W=A/T; B= C/G/T; D=A/G/T; N= Any base.

Target gene	Primer	Primer sequence (5'-3')	Annealing temperature (°C)	Function	Reference
Bacteria 16s rRNA gene	Eub338	ACTCCTACGGGAGGCAGCAG	55°C	Bacterial population	Fierer *et al*. (2005)
	Eub518	ATTACCGCGGCTGCTGG			
Fungi 18s rRNA gene	nu-SSU-0817	TTAGCATGGAATAATRRAATAGGA	56°C	Fungal population	Borneman & Hartin (2000)
	nu-SSU-1196	TCTGGACCTGGTGAGTTTCC			
AOA amoA Archaea Ammonia monooxygenase	Arch-amoAF	STAATGGTCTGGCTTAGACG	53°C	Ammonia oxidising archaea	Francis *et al*. (2005)
	Arch-amoAR	GCGGCCATCCATCTGTATGT			
AOB amoA Bacteria Ammonia monooxygenase	amoA-1F	GGGGTTTCTACTGGTGGT	57°C	Ammonia oxidising bacteria	Rotthauwe *et al*. (1997)
	amoA-2R	CCCCTCKGSAAAGCCTTCTTC			
NirS Cytochrome cd1 nitrite reductase	nirS-cd3aF	GTSAACGTSAAGGARACSGG	57°C	Denitrification	Throbäck *et al*. (2004)
	nirS-R3cd	GASTTCGGRTGSGTCTTGA			
NirK Copper-containing nitrite reductase	nirk-FlaCu	ATCATGGTSCTGCCGCG	56°C	Denitrification	Hallin & Lindgren (1999)
	nirk-R3Cu	GCCTCGATCAGRTTGTGGTT			
NifH Nitrogenase	nifH-F	AAAGGYGGWATCGGYAARTCCACCAC	60°C	Nitrogen fixation	Rösch *et al*. (2002)
	nifH-R	TTGTTSGCSGCRTACATSGCCATCAT			
chiA Chitinase	GA1F	CGTCGACATCGACTGGGARTDBCC	57°C	Organic N decomposition	Williamson *et al*. (2000)
	GA1R	ACGCCGGTCCAGCCNCKNCCRTA			

Standard curves were generated using serially diluted custom-made gBlocks® (Integrated DNA Technology, Leuven, Belgium) designed based on *Pseudomonas denitrificans*, accession number- MK085084.1 (bacterial 16S rRNA gene), *Aspergillus niger*, accession number- MZ330851.1 (Fungal 18S rRNA gene), Uncultured archaeon clone, accession number- MW937510.1 (*AOA-amoA*), *Nitrosomonadales bacterium*, accession number- MN061768.1 (*AOB-amoA*), *Pseudomonas stutzeri*, accession number- LR134482.1 (*nirS), Achromobacter cycloclastes*, accession number- AF114787.1 (*nirK*), *Uncultured Sinorhizobium* sp. clone *(nifH),* accession number- KC445685.1 *(nifH*) and *Burkholderia gladioli* chitinase gene accession number- CP068050.1 (*chiA*) (appendix 9).

To generate a stock standard, Gblock synthetic oligonucleotides require only one resuspension, whereas linear PCR plasmids require PCR amplification, separation from the gel, gel excision, purification and quantification. There is a potential contamination risk, not only within the lab but also for the standard. The preparation of a gblock fragment is cheap, fast and simple with less risk of contamination. The gblocks were resuspended in 50µl TE buffer and incubated at 50°C in a water bath for 30 minutes to ensure the synthetic oligonucleotides were properly diluted; the concentration was then measured using a Qubit 4 Fluorometer (Invitrogen, UK). Gene copy number of the standard was calculated using the equation:

$$\frac{\text{Standard concentration ng/µl} \times 6.023 \times 10^{23}\ \text{(copies/mol)}}{\text{Lenth of amplicon (bp)} \times 1 \times 10^{9} \times 660}$$

To assess the quality of the qPCR assay, pre-optimisation trails of the standards were performed. The standards derived from the linear regression of the standard dilution Ct values and the gene copy number were evaluated to ensure high efficiency ($E = (10^{1/\text{slope}} - 1) \times 100$). This ensured that PCR efficiencies ranged between 90 and 110%.

6.2.2: qPCR

Duplicate sample replicates were run in parallel for each gene target on a StepOne Plus thermocycler (Applied Biosystems). qPCR reaction mixtures contained 5 µL of 2 x PowerUp SYBR Green master mix (Applied Biosystems), 0.4 µL of forward and reverse primer at a final concentration of 400 nM, 1 µL of DNA template and 3.2 µL of RNase/DNase-free water to a final volume of 10 µL, following methods from Thompson *et al*. (2020).

A UDG activation step was used for the conduction of the qPCR at 2 min at 50°C, an initiation step at 95 °C for 2 min, followed by 40 cycles of denaturing at 95 °C for 15 s, annealing at 55 °C (bacterial 16S), at 56 °C (fungal 18S and *nirK*), at 53°C *(AOA- amoA*) at 60°C (*nifH*) or at 57 °C (*AOB- amoA , nirS and chiA*) for 15 s, followed by elongation at 72 °C for 60 s.

All unknown samples in the qPCR assays were amplified in parallel with a triplicate serial dilution (10^1–10^8 gene copies per reaction) of gBlock standards. For each gene, high amplification efficiency was achieved with R^2s ranging from 0.993 to 0.998 and standard curve slopes ranging from −3.2837 to −3.5576 by testing serial dilutions of DNA extracts in order to decrease the inhibition of amplification (Thompson *et al*., 2020) (appendix 10). No template controls were run in triplicate and no signal was observed. Amplicon specificity was confirmed with a melt curve analysis which consisted of 95°C for 15 seconds, 60°C for 1 minute and 95°C for 15 seconds with a continuous ramp increment. The unit of abundance of targets is log copies g^{-1} dry soil.

6.2.3: Statistical analysis

All statistical analysis was carried out using R version 4.0.2 software (R Development Core Team, 2020). Two-way analysis of variance followed by Tukey's honest significant difference (HSD) for multiple comparisons with a $P = 0.05$ grouping baseline was used to test the effects of burn treatment, soil depth and their interaction on the abundance of bacteria, fungi and N-cycling genes, this followed the Shapiro-Wilk test for normality and Bartlett test for homogeneity of variance. Further, when the interaction was not significant, one-way ANOVA and Tukey's *post-hoc* test for multiple comparisons were used to evaluate differences based on burn treatments within a soil layer, and among the three soil layers within a given burn treatment. In this study, the abundance of total nitrogen functioning genes

was calculated by the sum of the abundance of genes detected (Song *et al*., 2019). Pearson's correlation analysis was used to determine the significant negative or positive correlations between the abundances of bacteria, fungal and N-cycling genes and environmental variables. The correlation coefficients were calculated and plotted using the R package '*corrplot*'. The probability level $P < 0.05$ was considered to be statistically significant. Correlation analysis was performed on top soil (0-20cm) and subsoil (20-40cm, 40-60cm). In this study, any sample below the detection limit was considered as a zero.

6.3: Results

6.3.1: Abundance of Bacteria and Fungi

The bacterial 16S rRNA copy numbers from all treatments ranged from 5.46 x 10^7 - 2.17 x 10^{10} copies g^{-1} dry soil, and were more abundant than the fungal 18S rRNA copy numbers which ranged from 6.19 x 10^4 - 9.23 x 10^8 copies g^{-1} dry soil. The abundance of bacterial 16S rRNA gene copy number ranged from 1.95 x 10^8 - 2.17 x 10^{10} copies g^{-1} dry soil in the non-burn control plots, from 5.46 x 10^7 - 5.22 x 10^9 copies g^{-1} dry soil in plots under a long rotation regime and 1.27 x 10^8 - 5.14 x 10^9 copies g^{-1} dry soil in plots under a short rotation regime. There was a significant two-way interaction between burn treatment and soil depth on bacterial abundance (Fig 6.1A; Table 6.2). Bacterial abundance was greater in the topsoil of the non-burn control and decreased from the topsoil to the 20-40cm profile.

The abundance of fungal 18S rRNA gene copy number ranged from 1.68 x 10^5 - 9.23 x 10^8 copies g^{-1} dry soil in non-burn control plots, 1.22 x 10^5 - 3.34 x 10^8 copies g^{-1} dry soil in plots under a long rotation regime and 6.19 x 10^4 - 4.14 x 10^8 - copies g^{-1} dry soil in plots under a short rotation regime. There was a significant two-way interaction between burn treatment and soil depth on fungal abundance (Fig 6.1B; Table 6.2). The abundance of fungi was greater in the topsoil of the non-burn control and long rotation regime compared to treatments subject to a short rotation burn.

The ratio of 16S rRNA and 18S rRNA ranged from 1.22–1.25 in the topsoil to 1.34–1.55 in the 40–60cm profile. The variation in the ratio of bacteria to fungi suggested that the abundance of the fungal 18S rRNA genes declined more than 16S rRNA genes in subsoils, according to changes in 16S rRNA and 18S rRNA gene abundances with soil depth (Fig 6.1C).

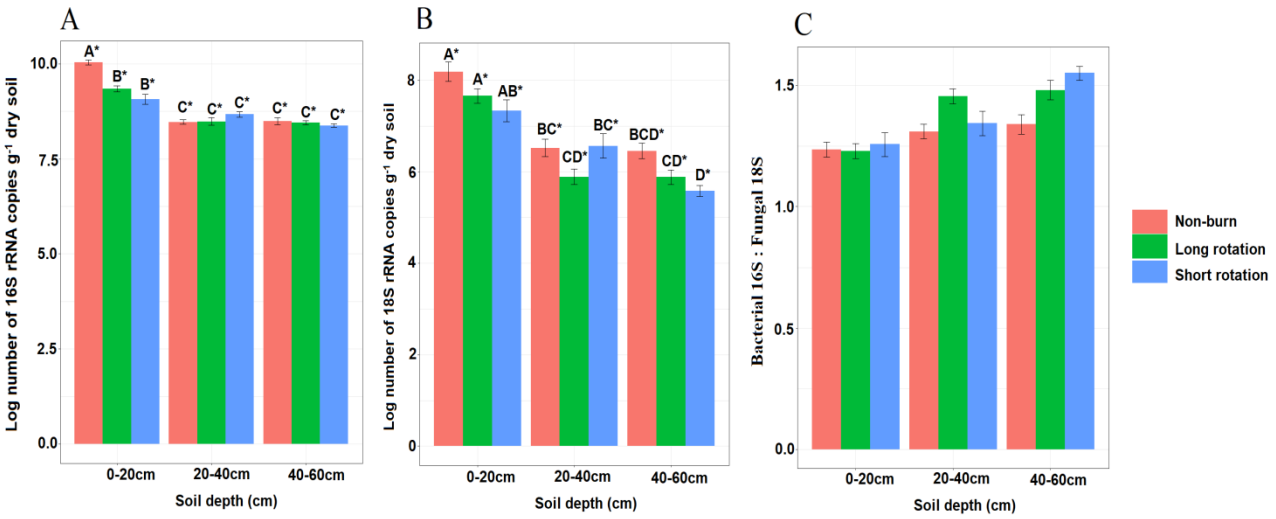

Fig 6.1. The abundances of bacterial 16S rRNA (**A**), fungal 18S rRNA (**B**) and the ratios of bacterial 16S rRNA and fungal 18S rRNA copy numbers (**C**) across three different soil depths under three burn treatments (n=12). The bars indicate the mean values of each treatment, with the error bars representing the standard error. Different letters indicate a significant difference among treatments based on a significant interaction between burn treatment and soil depth (Tukey's HSD, $P < 0.05$).

Table 6.2. Two-way ANOVA of the abundances (log copies^{-1} g dry soil) of bacterial 16S rRNA and fungal 18S rRNA across three different soil depths under three burn treatments. Result is reported as the mean ± SE (n=12). The data in bold indicate that abundance was affected by burn treatment, soil depth and their interaction at a confidence level of 95% ($P < 0.05$). Different letters indicate a significant difference among treatments based on a significant interaction between burn treatment and soil depth (Tukey's HSD, $P < 0.05$).

Treatment	**Depth (cm)**	**Bacteria**	**Fungi**
Non-burn	**0-20cm**	10.03±0.06 A*	8.18±0.21 A*
	20-40cm	8.46±0.06 C*	6.31±0.19 BC*
	40-60cm	8.49±0.09 C*	6.45±0.17 BCD*
Long rotation	**0-20cm**	9.34±0.08 B*	7.65±0.16 A*
	20-40cm	8.43±0.10 C*	5.88±0.17 CD*
	40-60cm	8.45±0.05 C*	5.86±0.16 CD*
Short rotation	**0-20cm**	9.06±0.13 B*	7.34±0.24 AB*
	20-40cm	8.66±0.07 C*	6.53±0.27 BC*
	40-60cm	8.36±0.05 C*	5.58±0.12 D*
Burn treatment		**F=2.64, P=0.05**	**F=8.33 P=<0.001**
Depth (cm)		**F=25.89, P=<0.001**	**F= 66.33,P=<0.001**
Burn treatment*Depth		**F= 3.25,P=0.01**	**F=2.58, P=0.04**

6.3.2: N-cycling functional genes

6.3.2.1: Nitrification

Archaeal *amoA* gene abundance was below the detection limit for 17% of samples. Detected *AOA amoA* gene copy number abundance ranged from 8.36 x 10^4 - 9.39 x 10^7 copies g^{-1} dry soil in non-burn control plots, 2.18 x 10^4- 8.71 x 10^6 copies g^{-1} dry soil in plots under a long rotation regime and 2.88 x 10^4 - 9.88 x 10^7 copies g^{-1} dry soil in plots under a short rotation regime. The abundance of *AOA amoA* was significantly different between burn treatments being highest in the non-burn control (P<0.05) (Fig 6.2A; Table 6.3). There was a decrease in abundance with depth in the non-burn control as an 18% decrease was observed between 0-20cm and 20-40cm profiles (Fig 6.2A; Table 6.3).

Bacterial *amoA* gene abundance was below the detection limit for 11% of samples. Detected *AOB amoA* gene copy number abundance ranged from 1.27 x 10^5 - 9.34 x 10^7 copies g^{-1} dry soil in the non-burned plots, 1.23 x 10^5 - 9.60 x 10^7copies g^{-1} dry soil in plots under a long rotation regime and 1.08 x 10^5 - 1.34 x 10^7 copies g^{-1} dry soil in plots under a short rotation regime. The abundance of *AOB amoA* was significantly different between burn treatments being highest in the non-burn control (P<0.05) (Fig 6.2B; Table 6.3). *AOB amoA* decreased between the 0-20cm soil profile and the 20-40cm in the non-burn control 19% but increased from the 0-20cm to 20-40cm in plots under a long rotation burn regime 5% (Fig 6.2B; Table 6.3).

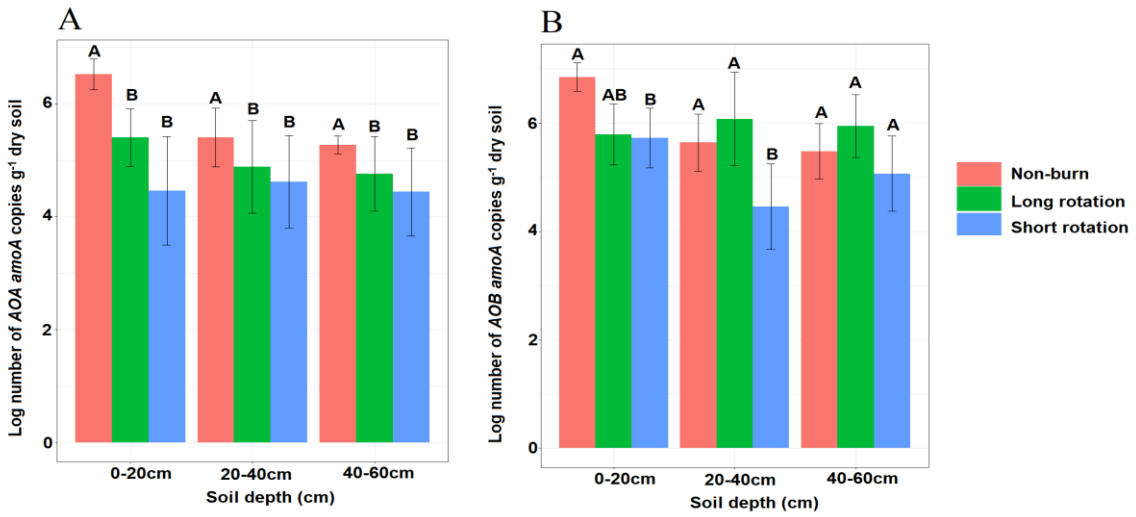

Fig 6.2 The abundances of ammonia oxidising archaea (*AOA amoA*) (**A**) and ammonia oxidising bacteria (*AOB amoA*) (**B**) across three different soil depths under three burn treatments (*n*=12). The bars indicate the mean values of each treatment, with the error bars representing the standard error. Different uppercase letters indicate statistically significant differences among the three burn treatments in the same soil layer (Tukey's HSD, $P < 0.05$).

6.3.2.2: Denitrification

The *nirS* gene copy number abundance ranged from 4.64×10^4 - 7.47×10^7 copies g^{-1} dry soil in the non-burned control, 1.06×10^5 - 1.47×10^7 copies g^{-1} dry soil in plots under a long rotation regime and 1.21×10^5 - 6.39×10^6 copies g^{-1} in plots under a short rotation regime. *NirS* was not significantly affected by burn treatment or soil depth (Fig 6.3A; Table 6.3). There was a 16% decrease in abundance from the 0-20cm to 20-40cm profile in the non-burn control (Fig 6.3A; Table 6.3). The *nirK* gene copy number ranged from 1.16×10^6 – 2.30×10^7 copies g^{-1} dry soil in the non-burn control plots, 1.06×10^5 - 1.25×10^8 copies g^{-1} dry soil in plots under a long rotation regime and 8.06×10^5 - 7.90×10^8 copies g^{-1} dry soil in plots under a short rotation regime. There was a significant difference between the abundance of *nirK* and burn treatment as well as soil depth being highest in the topsoil of the short rotation regime (Fig 6.3B; Table 6.3). The abundance of *nirK* decreased between the topsoil and intermediate profile in all three burn treatments. There was a 9% decrease from the 0-20cm - 40-60cm in the non-burn control, 11% decrease in the plots under a long rotation burn and a 16% decrease plots under a short rotation burn (Fig 6.3B; Table 6.3).

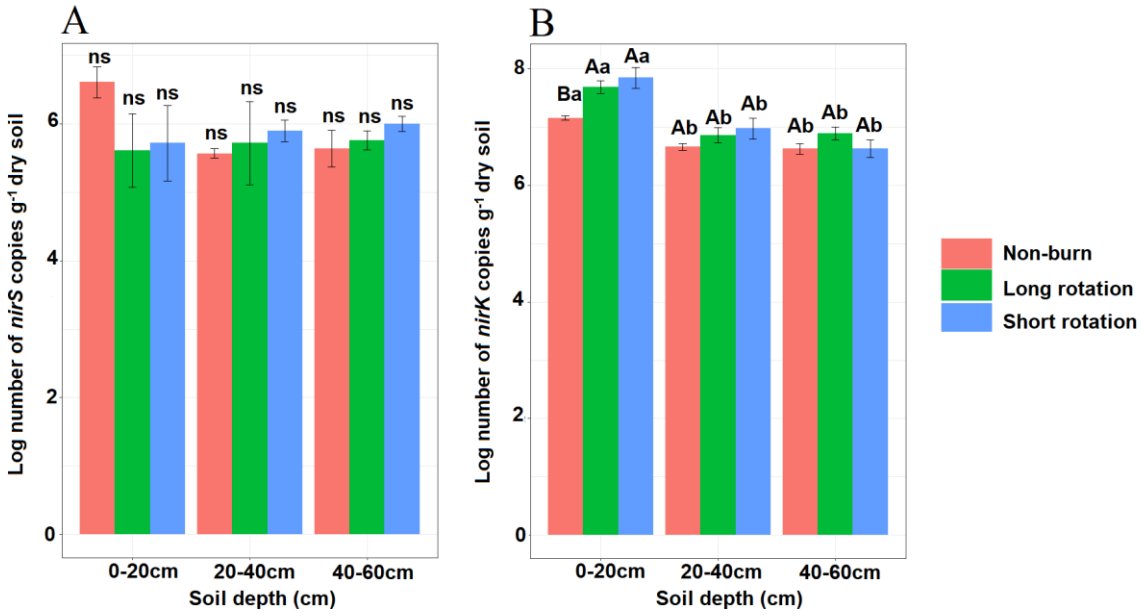

Fig 6.3. The abundances of *nirS* (**A**) and *nirK* (**B**) across three different soil depths under three burn treatments (*n*=12). The bars indicate the mean values of each treatment, with the error bars representing the standard error. Different uppercase letters indicate statistically significant differences among the three burn treatments in the same soil layer and different lowercase letters indicate statistically significant differences among the three soil layers across burn treatments (Tukey's HSD, $P < 0.05$) 'ns' = not significant.

6.3.2.3: Nitrogen fixation and N decomposition

The *nifH* gene copy number abundance ranged from 1.08 x 10^5 - 9.64x 10^8 copies g^{-1} dry soil in non-burned plots, 8.05 x 10^4 - 1.44 x 10^8 copies g^{-1} dry soil in plots under a long rotation regime and 7.42 x 10^4 - 6.75 x 10^7 copies g^{-1} dry soil in plots under a short rotation regime (Fig 6.4A, Table 6.3). There was a significant difference in abundance between different burn treatments and with soil depth ($P < 0.05$). The abundance of *nifH* was significantly higher in the topsoil compared to the subsoil as a decrease of 24% in abundance was observed in the non-burn control, 18% in the plots under a long rotation burn and 14% in plots under a short rotation burn between the 0-20cm and 20-40cm soil profiles respectively. The *nifH* copy number was significantly higher in the non-burned control in surface soils (Fig 6.4A; Table 6.3). *ChiA* copy numbers ranged from 3.25 x 10^5 - 2.60 x 10^7 copies $-g^{-1}$ dry soil in the non-burn control, 1.70 x 10^5 - 1.52 x 10^7 copies g^{-1} dry soil in plots under a long rotation regime and 3.25 x 10^5 - 8.54x 10^7 copies g^{-1} dry soil in plots under a short rotation regime. There was a significant two-way interaction between burn treatment and soil depth on *chiA* abundance (Fig 6.4B; Table 6.3). *ChiA* abundance was greater in the topsoil of plots under a

short rotation burn regime and decreased with depth as a 12% decrease in abundance was observed in the non-burn control, 14% in plots under long rotation burns and 18% in plots under short rotation burns between the 0-20cm and 20-40cm soil profiles respectively (Fig 6.4B; Table 6.3).

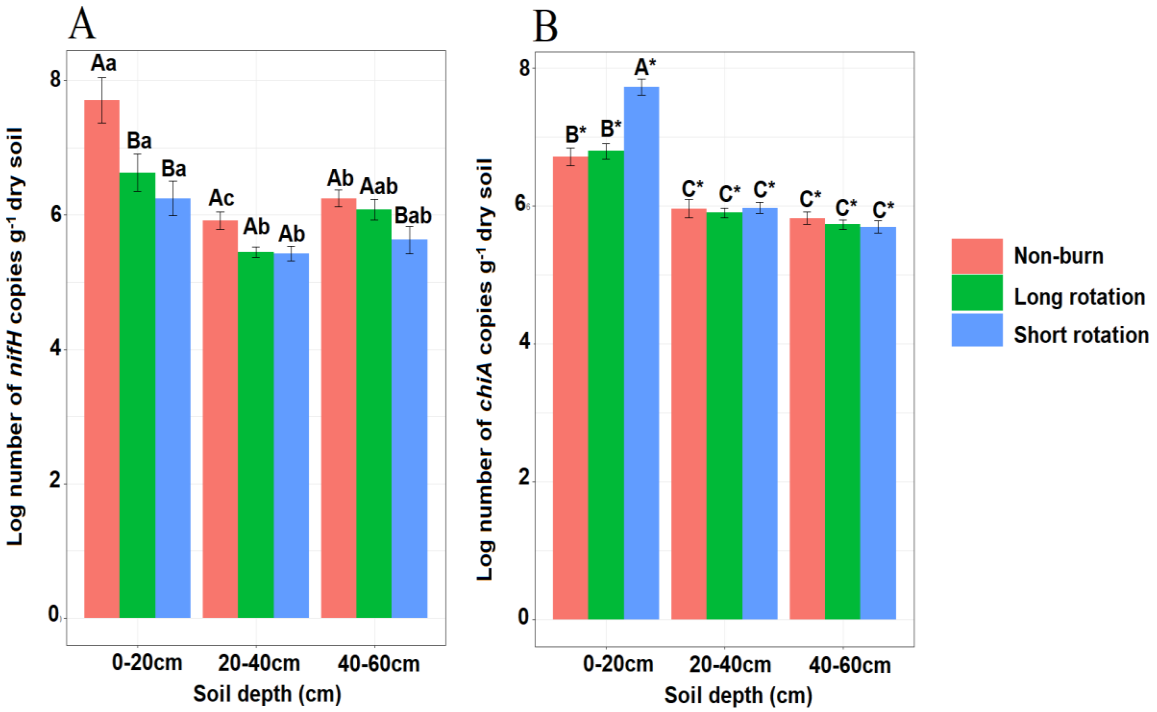

Fig 6.4. The abundances of *nifH* (**A**) and *chiA* (**B**) across three different soil depths under three burn treatments (*n*=12). The bars indicate the mean values of each treatment, with the error bars representing the standard error. Different uppercase letters indicate statistically significant differences among the three burn treatments in the same soil layer, different lowercase letters indicate statistically significant differences among the three soil layers across burn treatments and different letters with an asterisk indicate a significant difference among treatments based on a significant interaction between burn treatment and soil depth (Tukey's HSD, $P < 0.05$).

Table 6.3. Two-way ANOVA of the abundances (log copies^{-1} g dry soil) of N-cycling genes across three different soil depths under three burn treatments. The data in bold indicate N-cycling genes that were affected by soil depth, burn treatment and their interaction at a confidence level of 95% ($P < 0.05$). Different uppercase letters indicate statistically significant differences among the three burn treatments in the same soil layer, different lowercase letters indicate statistically significant differences among the three soil layers across burn treatments and different letters with an asterisk indicate a significant difference among treatments based on a significant interaction between burn treatment and soil depth (Tukey's HSD, $P < 0.05$). Total NFG = total nitrogen functioning genes, 'ns'= not significant.

Treatment	Depth (cm)	AOA	AOB	NirS	NirK	NifH	ChiA	Total NFGs
Non-burn	**0-20cm**	6.52±0.27 A	6.88±0.26 A	6.61±0.23 ns	7.16±0.04 Ba	7.71±0.34 Aa	6.71±0.13 B*	41.61±0.48Aa
	20-40cm	5.40±0.52 A	5.64±0.53 B	5.57±0.07 ns	6.66±0.06 Ab	5.92±0.13 Ac	5.96±0.13 C*	35.17±0.79 Bb
	40-60cm	5.72±0.16 A	5.48±0.51 A	5.64±0.27 ns	6.63±0.09 Ab	6.25±0.20 Ab	5.82±0.09 C*	35.57±0.70 Bb
Long rotation	**0-20cm**	5.40±0.51 B	5.79±0.56 AB	5.61±0.54 ns	7.69±0.11 Aa	6.63±0.39 Ba	6.79±0.11 B*	37.93±1.18 Ba
	20-40cm	4.68±0.82 B	6.08±0.83 A	5.72±0.17 ns	6.88±0.12 Ab	5.45±.0.10 Ab	5.90±0.07 C*	34.73±1.28 Ab
	40-60cm	4.76±0.64 B	5.95±0.58 A	5.78±0.14 ns	6.89±0.10 Ab	6.08±0.15 Aab	5.73±0.07 C*	35.22±1.05 Ab
Short rotation	**0-20cm**	4.46±0.96 B	5.73±0.53 B	5.72±0.55 ns	7.85±0.18 Aa	6.25±0.26 Ba	7.27±0.12 A*	36.30±1.53 Ba
	20-40cm	4.62±0.85 B	4.62±0.79 B	5.90±0.16 ns	6.98±0.18 Ab	5.43±0.11 Ab	5.97±0.08 C*	33.38±1.53 Ba
	40-60cm	4.44±0.78 B	5.07±0.69 A	6.00±0.11 ns	6.63±0.15 Ab	5.63±0.20 Bab	5.69±0.009 C*	33.49±0.96 Bb
Burn treatment		**F=3.32,P=0.04**	**F=2.83, P=0.04**	F= 0.48,P=0.61	**F=6.42, P=0.002**	**F=13.13 ,P=<0.001**	F=2.21 ,P=0.14	**F=5.46,P=0.005**
Depth (cm)		F=6.62, P=0.54	F=0.81 ,P=0.44	F=0.52, P=0.59	**F= 37.16,P=<0.001**	**F=28.85,= P=<0.001**	**F=105.97, P=<0.001**	**F=15.71,P=<0.001**
Burn treatment* Depth		F= 0.25,P=0.90	F=0.92, P=0.61	F= 1.74,P=0.53	F=1.97 ,P=0.10	F=2.07 ,P=0.08	**F=3.25, P=0.01**	F=0.93,P=0.44

6.3.3: Relationships between microbial abundance, functional gene abundance and environmental parameters

The correlation between environmental parameters and the abundances of bacterial 16S rRNA gene, fungal 18S rRNA gene and N-cycling functional genes were compared using Pearson's correlation coefficients. Soil pH, total C, NH_4^+, and heather cover were positively correlated with bacterial 16s rRNA gene abundance in the topsoil and pH and NO_3^- were positively correlated in the subsoil. Likewise, pH, total C and NH_4^+ was correlated with fungal 18s rRNA gene abundance in the topsoil while pH, total N, Mg, Zn and Pb were significantly correlated in the subsoil (Fig 6.5B) *AOA*, *AOB* and *nifH* gene abundances were significantly correlated with pH, total C, NH_4^+, Mg, Mn, Cu, Zn, Pb and heather cover in the topsoil (Fig 6.5A). Furthermore, *nirK* was positively correlated with moisture, total N, NO_3^-, graminoid cover, *Sphagnum* cover and other moss cover (Fig 6.5A).

In the subsoil there was a positive correlation between *AOA* abundance and total N and NO_3^- while *AOB* was positively correlated with NH_4^+, Al and Cu. There was a positive correlation between *nirS* abundance and moisture while *nirK* was positively correlated with total N, total

C and NH_4^+ and negatively correlated with Mg (Fig 6.5B). *NifH* was positively correlated with moisture, total C and NH_4^+, while *chiA* was positively correlated with pH and Mg (Fig 6.5B).

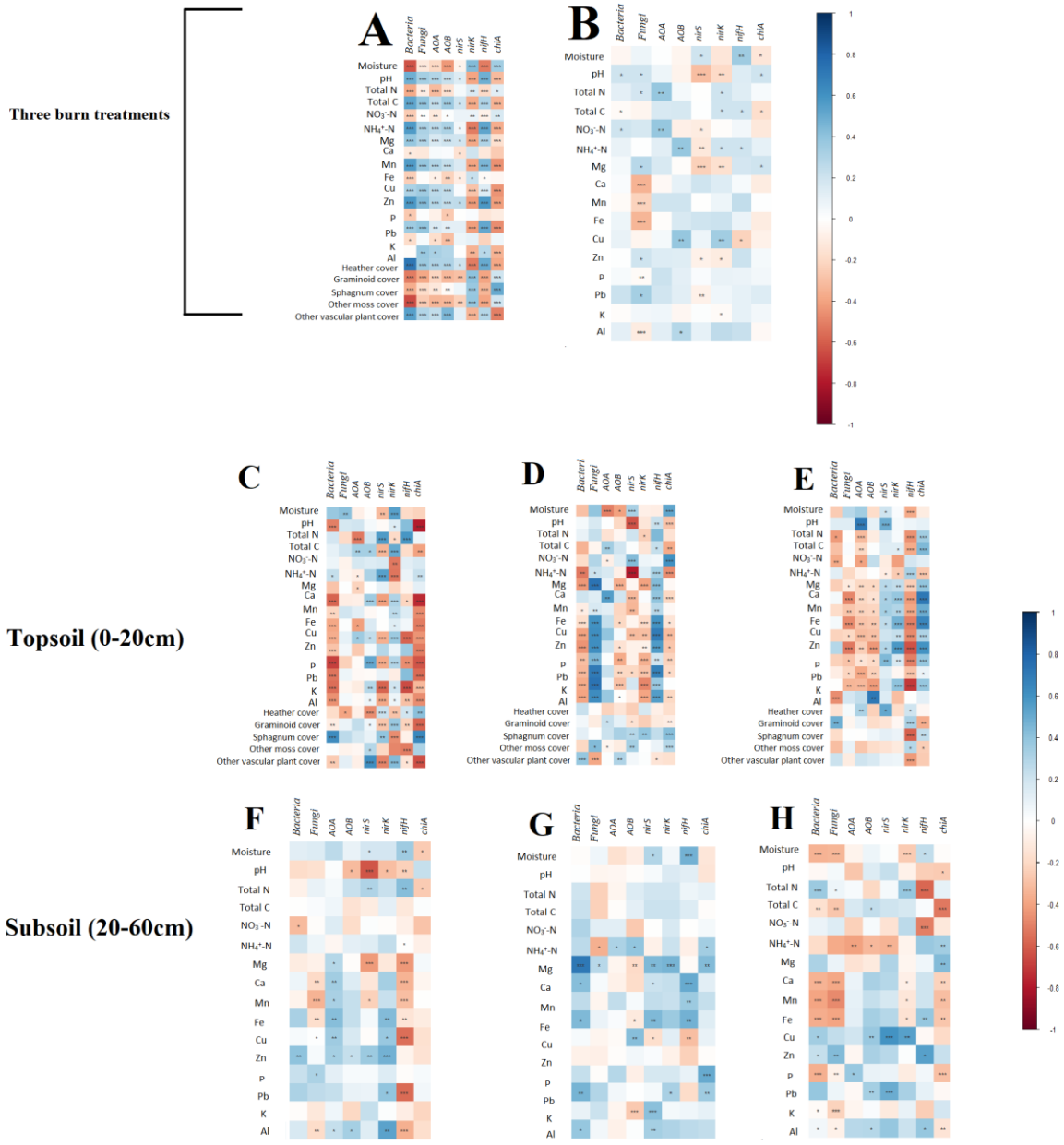

Fig 6.5. Correlogram representing Pearson's correlation coefficient between environmental parameters and abundances of bacterial populations, fungal populations and N-cycling genes. (**A**) Topsoil (0-20cm), (**B**) Subsoil (20-60cm), (**C**) Non-burn topsoil, (**D**) Long rotation topsoil, (**E**) Short rotation topsoil, (**F**) Non-burn subsoil, (**G**) Long rotation subsoil, (**H**) Short rotation subsoil. The correlation relationships ranging from negative to positive are indicated by the intensity of colour changing from red to blue. **P* < 0.05, ***P* < 0.01, ****P*<0.001.

6.4: Discussion

Key steps in N-cycling can be significantly affected by human activity such as management regimes. N-cycling genes play a crucial role in the cycling of soil nutrients and plant productivity (Xie *et al*., 2014). This chapter focused on the effects of different prescribed burning regimes on the abundance of bacteria and fungi as well as important soil N-cycling genes at different soil depths. The results show that the abundance of bacteria, fungi and N-cycling genes varied significantly with burning regime as well as soil depth. The impacts of different burning regimes on soil conditions throughout different soil depths, and subsequently on the abundance of bacteria, fungi and N-cycling genes serves as an indicator for the sustainability of peatlands.

6.4.1: Changes in the abundance of bacteria and fungi across different burn treatments and depths

There was a significant two-way interaction between burn treatment and soil depth on bacterial and fungal abundance with bacteria abundance being greater in the topsoil of the non-burn control and the abundance of fungi being greater in the topsoil of the non-burn control and long rotation regime. Both bacteria and fungi were more abundant in the topsoil than in the subsoil (Fig 6.1). A decrease in microbial abundance with depth has been observed in previous studies (Blume *et al*., 2002; Fierer *et al*., 2003; Kramer *et al*., 2013; Wang *et al*., 2017). The topsoil is likely to have more favourable environmental conditions such as nutrient resources that favour the growth of bacteria and fungi. Although the abundance of bacteria and fungi decreased with soil depth, the abundance of fungi decreased more than bacteria. This is in line with previous research on community structure, which found that soil depth impacted fungal communities more than bacterial communities (Liu *et al*., 2016; Wang *et al*., 2017) and is likely to be due to the anaerobic soil at the lower depths in peatlands. Fungi are recognised as essential decomposers of complex carbon polymers in these habitats. Therefore, they play an essential role in nutrient cycling (Myers *et al*., 2012). Despite fungi isolated and identified from peatlands being mostly aerobic (Andersen *et al*., 2013), fungi isolated from anaerobic lower peat layers exhibit a tolerance to these conditions (Thormann & Rice, 2007). Previous research in Canada (Day *et al*., 2019), China (Yang *et al*., 2020), the boreal forests of United States (Holden *et al*., 2016) and Australian shrublands (Munoz-Rojas *et al*., 2016) have found a reduction of fungal abundance and diversity one-year following wildfires. Research on how long it takes for fungal abundance to recover following fire effects has shown a range of conclusions. For example, Munoz-Rojas *et al*.

(2016) report that fungal abundance reaches pre-burn levels in 5 years and Holden *et al*. (2013) report 12 to 24 years, respectively, while Yang *et al*. (2020) show that reduced fungal abundance persists 126 years after a wildfire. Clearly, further study is needed to determine the long-term effects of prescribed burning on fungal abundance.

6.4.2: Changes in functional gene abundance across burn regimes

This study determined the absolute abundance of nitrogen cycling genes to evaluate N turnover in a peatland under prescribed burning regimes. It is important to note that the abundance of nitrogen functioning genes can vary in the genome across the population of microbes and not all genes may be detected (Song *et al*., 2019). Nevertheless, this approach can be an important indicator for biological nitrogen turnover (Nelson *et al*., 2015; Wang *et al*., 2014) and has been reported as having a close relationship with N processing rates in order to assess N turnover in ecosystems (Bu *et al*., 2020; Song *et al*., 2019; Tang *et al*., 2018; Wang *et al*., 2014).

In line with the second hypothesis, the abundance of N-cycling genes varied across burn treatments and total NFGs were higher in the non-burn topsoil. The non-burned control plots have not been burned since 1954 and provide a context to ecosystem recovery through the succession of vegetation. The marker genes *AOA amoA* and *AOB amoA* have an essential role in the transformation of energy and is often used to study nitrification in ecosystems (Norton *et al*., 2002). The results indicate that *AOA* and *AOB* was sensitive to environmental change as both were more abundant in the topsoil of the non-burn control (Fig 6.2; Table 6.3) where NH_4^+ was significantly correlated with *AOA* and *AOB* overall (Fig 6.5A). *AOA amoA* genes have been discovered in a variety of environments, including ocean sediments (Francis *et al*., 2007) and soils (Di *et al*., 2010; Leininger *et al*., 2006). *AOB amoA* genes have been found to be more abundant than *AOA amoA* in some soils (Di *et al*., 2010). In this study, *AOB* was found to be more abundant than *AOA* despite previous studies showing *AOA amoA* to be more dominant in wetland type environments (Baolan *et al*., 2012; Chen *et al*., 2008). One possible explanation for this is that *AOB* are more likely to prefer higher NH_4^+ than *AOA* (Dong *et al*., 2020; Liu *et al*., 2011). Therefore, higher NH_4^+ content could increase the competitiveness of *AOB* within this environment. Plant species richness, root biomass, and total C have been found to be strongly related to the *AOA* and *AOB* abundance (Boyle-Yarwood *et al*., 2008; Rasche *et al*., 2011; Rooney *et al*., 2010; Szukics *et al*., 2010; Szukics

et al., 2012; Zeglin *et al*., 2011). *AOA* and *AOB* are slow growing which could explain their relatively low abundance compared with other functional genes in this study (Prosser & Nicol, 2008).

Denitrification is critical to the nitrogen cycle and involves four reaction steps that convert nitrate to nitrous oxide or dinitrogen gas (Hayatsu *et al*., 2008). The results indicate that the dentrification gene *nirK* varied across burn treatments being greater in the topsoil of the short rotation and long rotation plots (Fig 6.3B; Table 6.3). There was no significant difference between the abundance of *nirS* and burn treatment or soil depth (Fig 6.3A; Table 6.3) indicating that microorganisms harbouring the '*nir*' gene select for different habitats when soil is affected by anthropogenic activity (Levy-Booth *et al*., 2014). Previous studies indicate that *nirS* and *nirK* also change their respective behaviour in relation to land-use (Bu *et al*., 2020; Li *et al*., 2018). However, in this study *nirK* was more abundant overall. This is in agreement with Li *et al*. (2018) where *nirK* was more abundant in every stage of restoration indicating that *nirK* is a good indicator for denitrification. *NirS* has been identified to be more resilient during land-use changes (Chen *et al*., 2010) but in this study *nirK* was more resilient to prescribed burning. The differences in abundance may be due to differences in the soil environment. For example, soil moisture has been identified as a major driver for *nirK* abundance as moisture primarily regulates denitrification (Klemedtsson *et al*., 1988). Therefore, the change in soil moisture in burned rotations can explain the relatively high abundance of *nirK*.

The activity of the *nifH* gene is related to the ability of nitrogen-fixing archaea and bacteria to fix N_2 (Bürgmann *et al*., 2003). Prescribed burning could have lasting impacts on N-cycling microorganisms, as the organic material is affected. In this study, *nifH* abundance correlated with total C overall (Fig 6.5A). Previous research has found a positive correlation between the *nifH* gene and carbon (Kennedy & Egger, 2010; Levy-Booth & Winder , 2010; Morales *et al*., 2010) as diazotrophs are dependent on carbon as N fixation requires a large amount of adenosine triphosphate (ATP) (Chen *et al*., 2010). In line with the third hypothesis, the *nifH* gene was most abundant in the topsoil of the non-burn control (Fig 6.4A; Table 6.3). There was a positive relationship between the *nifH* gene and heather cover (%) (Fig 6.5A) suggesting that N fixation was aligned with decomposing plant material and previous studies have shown that N fixation is achieved with a symbiotic relationship with plant roots (Hayden *et al*., 2010). Because bulk soils were sampled in this study, the *nifH* genes detected were expected to be primarily from N-fixing bacteria that are free-living. There was a

negative correlation between *nifH* gene abundance and total N overall (Fig 6.5A) and particularly in soils subject to short rotation burns (Fig 6.5 E & H), suggesting that short rotation burning could inhibit dinitrogenase reductase and lead to a reduction in the biological capacity for nitrogen fixation.

Plots under a short burn rotation regime harboured a higher abundance of the *chiA* gene (Fig 6.4B; Table 6.3). There was a positive correlation with moisture, graminoid cover %, *Sphagnum* cover % and other 'non-Sphagnum' moss cover %, but a negative correlation with NH_4^+ (Fig 6.5A). These results show that these microorganisms select for different habitats when soil is affected by anthropogenic activity. However, it is also important to acknowledge that chitin degradation is commonly associated with fungi as well as bacteria (Talbot & Treseder, 2010), hence care must be taken when assessing the true biological potential of these genes.

6.4.3: Changes in functional gene abundance across soil profiles

The distribution of microorganisms across different soil profiles is tightly linked with environmental factors and soil properties (Bu *et al*., 2020; Castellano-Hinojosa *et al*., 2018). Microorganisms are not as active in subsoils as important resources decrease with depth (Li *et al*., 2018; Stone *et al*., 2015). In this study, the abundance of *AOA* was higher in the topsoil and showed a general decrease in the subsoil. However, in plots under a short rotation regime, *AOA* was higher in abundance in the 20-40cm soil profile. A similar trend was found with *AOB* where the highest abundance was found in the non-burn topsoil but there was an increase in the subsoil of plots under a long rotation regime. Here *AOA* and *AOB* both correlated with NH_4^+, which is an important nutrient overall as previous studies have shown that increased *AOB* abundance is a result of a high amount of NH_4^+ (Tian *et al*., 2014; Zhang *et al*., 2019). *AOB* are considered to be copiotrophic and abundant in soils with higher nutrients whereas *AOA* are less restricted due to their smaller cell size (Kim *et al*., 2012; Martens-Habbena *et al*., 2009). The results show that *AOA* and *AOB* can inhabit different habitats in the soil profile and environmental factors affect the relative abundance of these genes.

In this study, *nirS* showed a general increase with soil depth in plots under long rotation and short rotation burn regimes but decreased in the non-burn control, while *nirK* decreased across soil profiles suggesting *nirS*-harboring denitrifiers thrived in deeper soils under burn regimes. This result is not consistent with a previous study where *nirS* and *nirK* were more

abundant in the topsoil of paddy soils (Wang *et al*., 2017). This variation could be attributed to changes in the soil environment (Levy-Booth *et al*., 2014). This is supported by Tang *et al*. (2016) and Bu *et al*. (2020) who showed that *nirS* and *nirK* can differ across soil profiles as a result of differing environmental factors. There were significant changes in soil properties across soil profiles in this study (see chapter 4) which could be an important factor determining the abundance and distribution of these denitrifying genes.

The inputs of N are dependent on biological N fixation which is processed by nitrogen-fixing bacteria and archaea (Zehr, 2011). In this study the *nifH* gene significantly declined from the topsoil to subsoil across all treatments. This is consistent with previous research that the fixation of N_2 occurs mainly at the topsoil (Bu *et al*., 2020; Li *et al*., 2018; Song *et al*., 2019; Wang *et al*., 2017). A higher abundance of heterotrophic decomposers with higher nitrogen requirements could explain the higher abundance in surface soils.

Organic nitrogen obtained from the detritus of soil microorganisms is a critical substrate for microbes carrying the *chiA* gene. Here, *chiA* showed a decrease with soil depth in all treatments. The results here are in contrast to Li *et al*. (2018) where *chiA* increased with depth in forest soils. The cause of different responses may be due to specific environmental conditions. This study emphasises the specificity of a peatland under different burning regimes and the response of NFGs are considered unique.

6.4.4: Conclusions

Previous research has shown that soil physicochemical properties and vegetation cover play a vital role in determining the distribution of nitrogen cycling genes (Bu *et al*., 2020; Castellano-Hinojosa *et al*., 2018; Song *et al*., 2019). NFGs have been previously used as indicators to investigate nitrogen turnover. The long term cessation of burning increased the abundance of bacteria, fungi and improved microbial N turnover potential categorised by an increase in the abundance of N-cycling genes. The abundance of *nirK, nifH* and *chiA* showed a vertical reduction from the topsoil to the subsoil showing these genes have a different habitat preference and a varied demand for nutrients and other environmental factors. Contrasting correlation results among soil properties in the topsoil and subsoil indicate a distinct selective environment represented by depth and should be further explored to assess their roles in N-cycling for applications in sustainable land management. Further research on peatlands under burning regimes is needed to confirm this conclusion. Further research could integrate transcription and the metabolic labelling of microorganisms with stable heavy

nitrogen isotopes (^{15}N). However, the use of N-cycling genes can provide useful information about N turnover in peatlands under different management regimes. The results provide new insight into the effects of prescribed burning on the abundance of bacteria, fungi and soil N-cycling genes in a peatland.

Chapter 7. Conclusions

Ecosystem functions in the terrestrial environment are strongly linked to the soil (Delgado-Baquerizo *et al*., 2017) and extraordinary diversity of soil microbial communities across different environments has been revealed due to advancements in molecular methods in the last decade. However, despite peatlands experiencing rapid land-use change through management regimes, there have been comparatively few studies regarding how land management in peatlands affects below-ground communities and activity. Anthropogenic disturbance by land-use can have great impact on microbial communities and associated biogeochemical cycles on which they have a strong influence (Teurlincx *et al*., 2018). Due to the increase in anthropogenic pressures there is a loss of ecosystem services and functionality globally (Lal, 2014). Because of the complexity of soil and ecological interactions within the soil ecosystem, a number of ecological assessments must be evaluated for the purpose of better land management. Therefore, a dataset must integrate different soil components such as vegetation, physicochemistry and microbiology.

The aim of this thesis was to better understand the environmental factors and biotic interactions influencing the soil microbial communities in peatlands under two significant but poorly understood management regimes. First, this study aimed to obtain a thorough insight into the quality of soil post-reclamation in the Athabasca region of Canada. The aim was to assess the potential recovery of a constructed fen and make recommendations on best practices and decision making by investigating the richness, community structure and catabolic activity of microbial communities within the Athabasca oil sands region under reclamation. The data were analysed based on two assumptions: (1) There will be significant differences in the community structure of soil fungi and prokaryotes between different fen types (i.e. the community structure of the constructed fen will be dissimilar from natural sites; (2) Microbial alpha diversity and substrate induced respiration in the constructed fen will be lower compared to natural fens where the range of physiological strategies and niches available will be greater.

Second, the project sought to evaluate the impacts of prescribed burning on microbial communities - a highly debated and contentious management regime used across peatlands. The data was obtained through high throughput sequencing and the determination of the absolute abundance of bacteria, fungi and nitrogen cycling genes using qPCR. The following hypotheses were tested:

- There will be significant changes in alpha diversity between burn treatments and different soil profiles.
- The structure of communities will significantly change across different burn regimes and soil depths due to changes in soil environmental conditions.
- Prokaryotic network structure will be more complex and less modular in the control non-burned plots compared to burn regimes since unburned plots contain microbial communities and plants that have interacted over a longer period of time.
- Burning regimes will alter fungal functional guilds and trophic modes as burning will favour saprotrophic fungi that are able to take advantage of soil nutrients post-burning, while symbiotrophic fungi will be higher in the control non-burned plots.

- The abundance of bacteria and fungi will decrease with the frequency of burn treatment and be higher in non-burn control plots;

- There will be changes in the abundance of N-cycling genes across burn treatments. It is expected that no further burning will enhance microbial nitrogen turnover potential which will be characterized by a higher abundance of functional genes due to changes in soil nutrient content and vegetation cover.

- *AOA, AOB, nifH* and *chiA* genes related to nitrogen acquisition will be higher in the topsoil due to an increase in nutrient content compared to that in the lower soil layers while *nirS* and *nirK* genes, involved in denitrification, will be more abundant in the anoxic subsurface soil characteristic of peatlands.

In addition to the conclusions and discussion in previous chapters, this final chapter seeks to summarise the project conclusions, discuss the potential implications and suggest further research.

7.1.1: Microbial Communities and Biogeochemical Functioning across Peatlands in the Athabasca Oil Sands Region of Canada: Implication for Reclamation and Management

Chapter 3 investigated the community structure, diversity and microbial metabolic activity across a range of natural peatlands and a constructed peatland in the Athabasca oil sands region of Canada. The community composition and alpha diversity of both fungi and prokaryotes showed clear site-specific differences and both fungi and prokaryotes responded to different environmental factors. However, the community structure of the constructed fen was most similar to the treed-rich fen for prokaryotic communities suggesting some recovery of the community within the constructed fen after soil transfer. Microbial catabolic activity was higher in the constructed fen despite prokaryotic diversity being higher in the treed-rich fen. One possible reason for this is functional redundancy of the communities. Another possible explanation is the constructed fen having a lower water table, increasing oxygen availability and the higher salinity in the constructed fen as a consequence of the surrounding landscape enhancing microbial activity (Weldmichael *et al*., 2020). However, future long-term studies are required to confirm the mechanism leading to the results of this study.

Microbes serve as excellent indicators of ecosystem recovery during reclamation (Shao *et al*., 2019). The responsiveness of microbes to changes with soil physicochemistry and changes in vegetation demonstrates that studies must be combined with these physicochemical properties and vegetation cover.

In this study the MicroResp^{TM} assay was used to measure the microbial functional diversity in the soil using a multiple carbon-source substrate-induced respiration system. The technique is based on the utilization of select carbon sources by organisms that are exclusively heterotrophic (Thiele-Bruhn *et al*., 2020). Future research should incorporate the analysis of soil functional genes with qPCR as a supplement to using the MicroResp^{TM}, which would allow for the measurement of specific soil functions. In the context of soils under reclamation, qPCR analysis of genes related to plant growth and biogeochemical cycling (e.g. *phoN* and *phoD* for phosphorus cycling , *amoA, nirS, nirK* and *nifH* for nitrogen cycling and *mcrA* and *pmoA* for measuring methanogenesis and mathanotrophs) can be used as an indicator for important microbial processes (Thiele-Bruhn *et al*., 2020).

7.1.2: Recommendations for best reclamation practice

The end goal of peatland reclamation and restoration is to create a self-sustaining peat accumulating wetland. Peat substrate should help with hydrology and restrict the movement of unwanted solutes, as well as aid in the maintenance of soil moisture for plants and the restoration of essential microbial communities and activity. Reclaimed peatlands that have been chemically altered after being mixed with mineral subsoil peat may not be capable of providing optimal hydrological functions (Pouliot *et al*., 2012). Nwaishi *et al*. (2015) also claims that modifications to salvaged peat could impact its ability to support ecohydrological functions in a constructed peatland. Farooq (2011) concluded that reclamation depends on adequate hydrological properties of the peat. The water table can influence peat through expansion and peat properties also influence water table height. It is essential that the conditions are conducive to storing and transporting ground water in similar ways to those in natural peatlands (Biagi *et al*., 2021).

Maintaining the integrity of peat by not mixing with topsoil may be beneficial for reclamation. In addition, placing maintained layers that can be rewetted and then moving it as soon as possible to the reclaimed peatland may also be beneficial. For example, rewetting and transplantation from natural peatlands to degraded sites has been attempted with positive results (Farooq, 2011) and shows that this retains soil moisture that allows plants to survive and grow, even through periods of drought.

The inclusion of microbial inocula could be beneficial in the future as restoration should aim to restore species and activity rather than just the growth of the vegetation. Previous research has shown that microbial inocula could promote the establishment of plant communities (Kumaresan *et al*., 2017; Wubs *et al*., 2016) suggesting that the manipulation of the soil microbial community is a powerful tool in land reclamation.

Reconstructed ecosystems have different soil properties and microbial community composition when compared with natural peatlands, and long-term studies should be done to address the different compositional and functional attributes at different temporal scales (e.g. across several seasons) to reveal any shifts in microbial community composition and activity. Plant communities have been used as an indicator for government and industry to assess the health of constructed wetlands in the AOSR due to their ease of sampling and tolerance to disturbance (Gadzała-Kopciuch *et al*., 2004; Raab & Bayley, 2012), and a nutrient profile index has also been developed for the AOSR (Hogberg *et al*., 2020). Reclamation directly

affects the soil microbial community and plant communities respond to the belowground functionality. Biological indicators that include microbial biodiversity and physicochemical parameters such as nutrients in the soil can provide more reliable conclusions about the success of soil reclamation.

7.2: Effects of a prescribed burning regime on vegetation, soil physicochemistry and prokaryotic microbial communities in surface and subsurface peat

The high diversity of microbial communities in the soil presents a major challenge in explaining the spatial and temporal patterns across environments. The percentage of land cover as well as connections with other terrestrial ecosystems makes identifying local and regional scale drivers across peatlands difficult (Limpens *et al*., 2008). Management regimes in terrestrial ecosystems increase the dissimilarity of microbial communities (Yu *et al*., 2019) and the complexity of principle controls on microbes in peatlands is still poorly characterized. Chapter 4 aimed to characterize the effects of different burning regimes on archaeal and bacterial communities across different soil profiles. The results show that observed species richness for archaea was higher in the topsoil of the non-burn control plots and highest for bacteria in the topsoil of non-burn and long rotation regimes. Community composition was also significantly different between different burn treatments as well as different depth profiles. These findings contribute to the understudied field of archaea and bacteria ecology in a peatland under prescribed burning regimes and raise new questions about how microorganisms influence the efficacy of management regimes.

Archaea and bacteria abundance may have important implications for the success of burn regimes as the recovery of the microbiome depends on the severity of the burn and how deep into the soil profile the burn penetrates. Fire changes the environment by charring organic matter and producing charcoal (Köster *et al*., 2021) and microbial communities may respond to burning by shifting through the soil profile if the surrounding temperature is outside their optimum range. In this study, the results show that archaea and bacteria were less affected by burning in the deeper soils suggesting that for archaea and bacteria the lower soil profiles have the potential to play an important role in recolonization.

It has been shown that wildfires change the soil microbiome and it can take several years for the community to recover to pre-fire level (Pérez-Valera *et al*., 2020). However, there has

been a scarcity of research into the effects of prescribed fires in peatlands. Post-fire recovery enhances the amount of organic matter in the soil, essentially restoring archaea and bacteria, thus natural succession is an important driver of the recovery of prokaryotic communities (Köster *et al*., 2021).

Archaea and Bacteria modulate several biogeochemical processes that promote ecosystem productivity (Delgado-Baquerizo *et al*., 2016). Interactions between archaea and bacteria are important in determining the respective roles of the groups, and analysing these interactions with network analysis adds to a better understanding of their ecology. A wide range of positive and negative interactions occur in the environment. As a result, bacteria and archaea that co-occur are likely to exhibit negative or positive interactions (Faust *et al*., 2015). Individuals form positive relationships or compete during ecological processes such as the decomposition of substrates or the cycling of nutrients, as represented by positive and negative links. Furthermore, changes in the availability of resources such as nutrients may cause or change the way microorganisms interact. The results show that network connectivity was lower under short rotation burns suggesting a lower degree of robustness. The lack of herbaceous plants and the lower nutrients under this burn regime may be the reason for this result. Under a short rotation regime many organisms may not recover. The number of negative links under a short rotation regime suggests a strong competition between microorganisms. Microbes will compete when resources are limited (Faust *et al*., 2015) and are more likely to coexist under high resource conditions. It would be valuable to connect soil processes to microbial co-occurrence networks for studying microbial niche partitioning and inferring the physiology of archaea and bacteria that co-occur with known specific ecological functions (Williams *et al*., 2014).

Since the networks were reconstructed using a correlation-based approach with taxa occurrence and abundance data, it is important to be cautious when interpreting the mechanisms underlying these networks. Theoretically, microbial co-occurrence patterns could be driven mainly by three ecological processes; dispersal limitation, biotic interactions and environmental filtering where microorganisms do not interact directly despite a preference for similar environmental conditions (Levy & Borenstein, 2013). Although caution needs to be used when interpreting correlation based networks, the networks from this project highlight the unique characteristics of different burn regimes in driving the co-occurrence of soil microorganisms and is a useful first step in the investigation of potential associations within soil microbial communities.

7.3: Response of soil fungal communities and functional traits to prescribed burning regimes in surface and subsurface peat

Investigations into the effect of prescribed burning on fungal alpha diversity, community composition and community structure revealed that (1) alpha diversity was affected by prescribed burning across different soil profiles, (2) there was significant community differentiation across prescribed burn treatments as well as soil profiles and (3) there were changes in functional traits across burn treatments as well as soil profiles with saprotrophs being more abundant in the non-burned control, and treatments under a long rotation burn regime harboured fungi with multiple trophic modes.

Alpha diversity was negatively affected even at the deepest soil profile under different burn regimes. This is an important finding as prescribed burning is not expected to penetrate deep into the soil in peatlands as the heat applied to the surface soil is only evident down to a few centimetres due to the low thermal conductivity of the lower soil profiles (Mallik *et al*., 1984). One possibility for the lower diversity across burn regimes at lower soil depth is that the roots from woody plants such as heather are more distributed in areas that have not been burned and extend deeper into the soil allowing root associated fungi to be distributed throughout the soil profile. It is also due to the thermal tolerance and mortality of plant hosts whose roots die after fire. Burning has a significant impact in determining the degree to which communities suffer, and fungi are known to be more sensitive to anthropogenic activity and management than prokaryotes as the growth rate of fungi is lower and have been shown to decline more drastically (Sun *et al*., 2015; Zhou *et al*., 2020). The results in this study also show that fungal community composition was significantly affected across burn treatments and across different depth profiles indicating that prescribed burning is a significant driver of the changes of fungal community structure, both in the surface and subsoils.

7.4: Changes in microbial populations and nitrogen functional genes in soil profiles of a peatland under different burning regimes

Chapter 6 characterized the abundance of bacteria, fungi and nitrogen cycling genes across Moor House Nature Reserve's long-term prescribed burning experiment. As vegetation structure changes due to land-use, there is a decreased root capture of water and nutrients

from the topsoil (Querejeta *et al*., 2021) which could have an impact on the abundance of bacteria, fungi and N-cycling genes. This study examined the abundance of key marker genes including the bacterial 16S rRNA, the fungal 18S rRNA and the abundance of key nitrogen cycling genes *AOA amoA*, *AOB amoB*, nitrogenase *nifH*, cytochrome cd1 nitrite reductase *nirS*, copper-containing nitrite reductase *nirK* and bacterial chitinase *chiA* quantified by qPCR, and changes associated with prescribed burning.

There were clear changes in the abundance of bacteria, fungi and the bacteria : fungi ratios across burn treatments and soil profiles, with the abundance of bacteria being greater in the non-burn control and fungi being greater in the non-burn control and plots subject to long rotation burns. Bacteria : fungi ratios can be affected by management and play important roles in biogeochemical cycling as well as food web stability (Engelhardt *et al*., 2018), and determine the proportionate change in fungi compared to bacteria under management regimes. There were also clear changes in the abundance of N-cycling genes. The abundances of *AOA*, *AOB* and *nifH* were higher in non-burn control plots and *nirK* was higher in plots under short rotation and long rotation burn regimes, while *nirS* was not affected by burn treatment or soil depth. The abundance of *ChiA* was greater in plots under a short rotation burn regime and decreased with soil depth. The abundance of N-cycling genes also varied widely across soil profiles suggesting that soil depth plays an important role in the distribution of N-cycling microbes under prevailing management regimes. For example, the *nifH* gene was significantly more abundant in the top soil suggesting N fixation occurs mainly in the topsoil and was effected by prescribed burning, possibly as a consequence of decreased nutrients and organic matter. These findings provide a valuable insight into the effects of prescribed burning on microbial N turnover in peatlands.

7.5: Recommendations for traditional managed burning

Moving forward, prescribed burning regimes must place importance on developing an ecological approach where specific prescriptions for the use of burning are defined and the trade-offs are clearly quantified. Burning is an important tool for maintaining habitat heterogeneity and plant community structure that benefits biodiversity. For example, burning can reinvigorate *Sphagnum* in wet peatlands where other vegetation may cover it leading to an increase in feather moss. *Sphagnum* moss may recover after an initial burn (Noble *et al.*, 2019) as many species are resilient and are able to recover rapidly. In this study, plots treated

under a short rotation burning regime had the highest cover of *Sphagnum* moss. This is in a agreement with Lee *et al*. (2013a) and Milligan *et al*. (2018) who found *Sphagnum* moss was more abundant in short rotation burn plots than in adjacent plots. However, larger-scale correlative studies have shown *Sphagnum* moss to be negatively associated with burned and grazed locations (Noble *et al*., 2018). In all cases, disentangling the effects of fire on peatlands remains challenging, and it is essential to use other metrics as well as plant community structure as indicators of ecosystem health following management. For example, peatlands are unusual because of a number of features that limit the ability of vascular plants to have a strong influence on the ecosystem (e.g. waterlogged conditions and low nutrient availability). Hence, from a functional perspective, microbial communities are relatively more significant in peatlands and should be a key component in the decisions regarding land management.

There has been a growing interest in the use of managed burning as a tool in mitigating wildfires in peatlands which is of particular concern due to climate change and the flammability of woody plants such as heather. The high risk in some peatlands suggests that burning should be an important consideration for land management as wildfires can cause significant damage to large areas impacting plant communities, water supply and carbon storage as well as the belowground microbial communities and therefore having a potential negative impact on the ecosystem function as a whole.

The current recommendations are simplistic, and all peatlands should not be managed in a uniform way. It is important to maintain a diversity of fire return intervals that produces a mosaic of landscapes comprising of heather at different stages that will benefit biodiversity as a whole and not severely impact microbial communities. Traditional management displays a somewhat limited variability in tactics for burning. In line with the results of this project and current literature important recommendations are considered. The frequency of burning should be a function of site productivity. Based on the results of this project, it is recommended that burn intervals should be >10 years as a short rotation burn interval has a negative impact on microbial communities across depth profiles which likely has a negative impact on microbial function, and it may take a long time for prokaryotic and especially fungal communities to recover from fires (Meng *et al*., 2021). However, all peatlands should demonstrate site heterogeneity to ensure plant and wildlife diversity. Therefore, it is important that burns are well distributed.

It is also important that managers plan where a fire should be extinguished so suitable fire breaks should be incorporated. Wet lines and cut fire breaks may be used where these are created immediately before the fire. Managers should also seek to achieve low severity that avoids prolonged burning and to minimize the consumption of moss. Severe burns may have an impact on plant communities, microbial communities, soil erosion and carbon dynamics and thus it is important to concentrate burning on areas where vigorous vegetation regeneration is likely.

The debate to use fire will likely continue and it is essential that the use of fire is driven by robust data on the ecology of fire. Ultimately, fire should continue to play an important role in peatland management as peatlands are fire adapted systems where the ecosystem composition and diversity are a function of decades of fire use. Hence, fire will be a component whether it is used as a management tool or not. It is also worth noting that current burning has evolved in response to a narrow set of management goals related to livestock production and game bird populations.

In the future, managers and policy makers should debate the trade-offs between various environmental effects of prescribed burning more effectively where microbial communities play an essential role.

7.6: Future research opportunities

The findings from this work show the importance of using microbial communities as indicators to gain a broad picture of land management regimes. Given the majority of taxa remain uncharacterized and the high diversity of microbial communities, genomic approaches can be extremely valuable (Solden *et al.*, 2016). However, there still remains a lack of information regarding the genomic mechanisms underlying soil processes. Incorporating the potential function of taxa and ecological response models obtained from genomic data would allow for a broad picture of how microbial communities and functions respond to environmental change. Microbial abundance can be predicted by soil and other environmental conditions and these environmental factors can also drive important ecosystem functions (Rillig *et al.*, 2019). It is necessary to address the question of how the changes of functional genes translate to changes in the function of the soil. For example, Jansson & Hofmockel (2020) described the term “metaphenome” as the product of expressed functions encoded

in microbial genomes. This is particularly important in the future research of peatlands due to the role peatlands play in climate change. Future research should study the physiological responses of microbes to prescribed burning and other common management regimes and changes in microbial taxa and function using a combination of metabarcoding and metatranscriptomics. Although metagenomic data provides a useful insight into the expected functional potential, more research is needed to discover how well changes in soil processes can be predicted using changes in functional gene content. The anaerobic soils of peatlands allow for the preservation of biological material and detected genes in soils may contain the DNA from dead organisms or relic DNA that are not making significant contributions to soil functioning, and organisms that are active may not be transcribed (Carini *et al*., 2016; Nannipieri *et al*., 2020). Soil metaproteomics offers another potential technique to use in the future research of peatlands under management regimes such as burning as the results obtained from proteins and nucleic acids have shown a strong correlation at the phylogenetic level (Starke *et al*., 2019). For example, do the changes in vegetation and physicochemistry associated with burning regimes influence the protein reserves and what are the implications for peatland ecosystem services and function? And does protein diversity reflect resilience of soil communities? Because proteins provide phylogenetic and functional information, the functionality of microbes should be analysed taking peatland ecosystem services into account (Hettich *et al*., 2013; Starke *et al*., 2019; Von Bergen *et al*., 2013). Soil metaproteomics also avoids the problem of relic DNA as the humic substances in soil will allow extracellular proteins to remain active (Burns *et al*., 2013). The further development of resources that forecast changes in soil microbial communities and function, as well as testing the significance of community and functional change in delivering soil ecosystem services in peatlands, is critical. Future research should address the extent to which genomic change can constitute actual functional change by utilising more advanced measures of ecosystem processes in soil.

Measuring microbial functionality, or the types of mechanisms that microbial communities carry out is often used to assess soil quality (e.g. decomposition) and can be of great value and should be used in conjunction with taxonomic information (Wood *et al*., 2015). Future studies on the effects of prescribed burning in peatlands should employ catabolic profiling using the MicroResp^{TM} to study the functional diversity of the microbial community and identify how the utilization of carbon substrates changes between the different burning practices (see chapter 3 regarding this method). The advantage of this method is that it relies

on the analysis of the microbial community active in the process of decomposition, an important functional consideration in the management of the ecosystem. In addition, another research area that requires attention is the methane oxidizing capacity of peat soils under prescribed burning. For example, understanding the mechanisms underlying variations in CH_4 sink capacity across different burning regimes may benefit from establishing linkages between oxidation of CH_4 and active methanotrophs, and determine if burning impacts their abundance in the soil.

7.7: Limitations of the project

With the ongoing change in climate, wildfires are projected to increase in frequency and intensity in peatlands (Kelly *et al*., 2018; Turetsky *et al*., 2015), which are generally more severe than the rotational burns used for management, causing significant changes to vegetation structure and soil processes. Many processes after wildfire may have similar impacts to those as prescribed burns (Alcañiz *et al*., 2018). Anthropogenic activities including wildfires have caused many changes to the UK's peatlands at some point. There are also other important anthropogenic activites such as drainage, grazing and pollution across many UK peatlands (Harper *et al*., 2018). Moor House Nature Reserve's long-term monitoring site has been a valuable experimental reserve since its implementation. However, a variety of factors such as the weather condition and the vegetation type of a specific site can influence the characteristics of burn intensity and severity. Changes in vegetation post-burn will also vary due to temperature and rainfall patterns which vary across locations (Milligan *et al*., 2018). Another limitation of this study is the lack of functional measurement on the active community using a method such as the MicroResp^{TM} technique. This was originally planned in this study to gain an insight into the functional alterations of microbes across burn treatments but was hindered due to the Covid-19 pandemic causing time constraints. This study was done under the short term. Therefore, future long term monitoring of microbial communities and function under burning regimes is encouraged. However, in spite of the limitations, this project is unique in evaluating the effects of prescribed burning rotations on peatland microbial communities.

7.8: Concluding remarks

Peatlands provide a wide range of ecosystem services beyond their involvement in the carbon cycle and are exceptionally productive. Because of the activity of soil microbial communities, soil plays a significant role in all processes on Earth. Since soil microorganisms have an impact on the key stages in biogeochemical cycles, conservation and enhancement of the soil ecosystem promote the ability of the soil to function properly and the diversity of microorganisms shapes how the soil ecosystem responds to changes. Microbial diversity evidently influences multiple ecosystem processes. Thus, it is crucial to investigate how land management impacts the structure of soil microbial communities and their spatio-temporal dynamics. This study investigated the effects of two different management regimes on vegetation, physicochemistry and microbial diversity. This project found that that the physicochemical characteristics of the soils and vegetation had significant influences on microbial diversity and community structure. Characterizing soil abiotic factors can shed light on how they affect microbial communities and the degree to which these communities change. The functional activity and community composition of soil microbes are associated with changes related to soil nutrients and plant species richness in fens across the Athabasca oil sands region indicating that microbial communities and activity are important indicators for the trajectory of future peatland reclamation projects. As observed in microbial activity, disparity in the makeup of microbial populations inevitably influences ecological processes. Likewise, prescribed fire regimes significantly impacted the diversity and community composition of microbial communities, the network structure of prokaryotic communities and the abundance of N-cycling genes. Biogeochemical processes can be linked to the variability of microbial community structures and this has significant implications on various ecosystem processes and the fertility of soils. More research on the effects of peatland management on soil microbial communities and function is required since soil microbial communities regulate biogeochemical cycles and neglecting them could result in a loss of biodiversity and negative effects on biogeochemical cycling, particularly C cycling.

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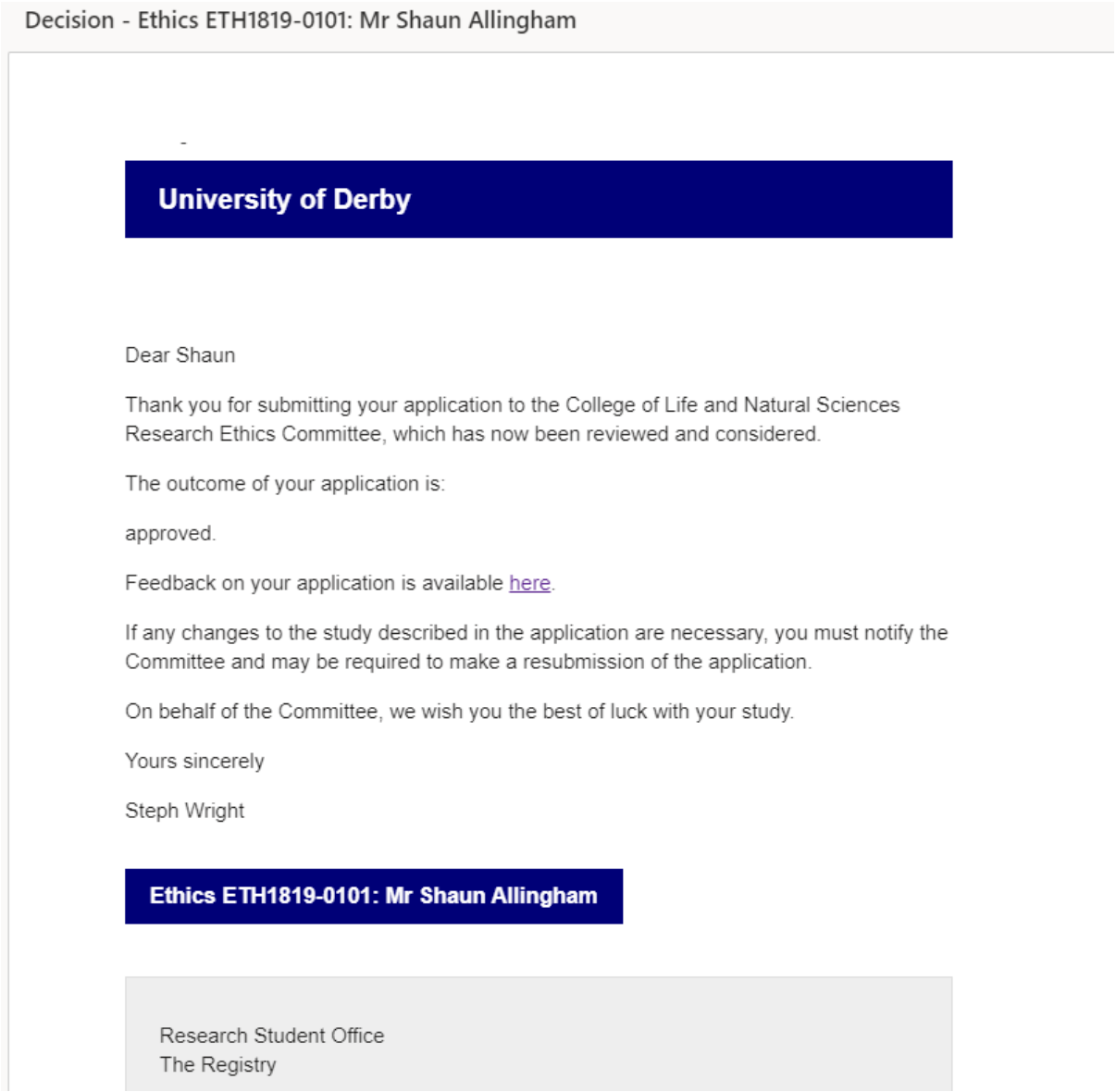
Appendix 1. Ethical approval



Decision - Ethics ETH1819-0101: Mr Shaun Allingham

-

University of Derby

Dear Shaun

Thank you for submitting your application to the College of Life and Natural Sciences Research Ethics Committee, which has now been reviewed and considered.

The outcome of your application is:

approved.

Feedback on your application is available here.

If any changes to the study described in the application are necessary, you must notify the Committee and may be required to make a resubmission of the application.

On behalf of the Committee, we wish you the best of luck with your study.

Yours sincerely

Steph Wright

Ethics ETH1819-0101: Mr Shaun Allingham

Research Student Office
The Registry

Fig A 1.1. Proof of ethical approval.

Appendix 2. Quality plots produced from QIIME2

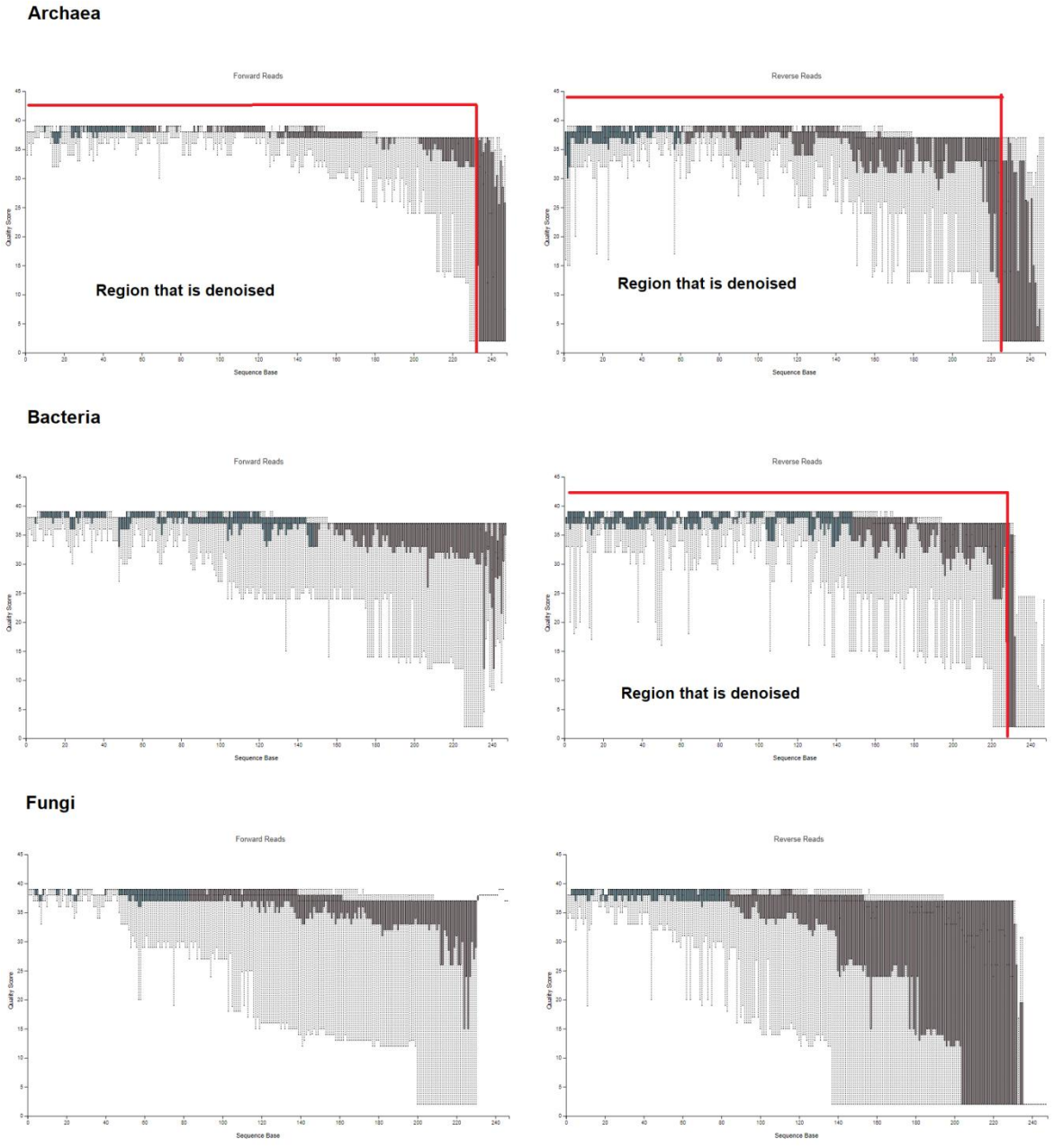


Fig A 2.1. Quality score box plots sampled from paired-end reads on a 2 x 250 bp Miseq Illumina. Red lines indicate the point of truncation for denoising with DADA2. Fungal reads were trimmed by quality scores due to the high read variability.

Appendix 3. Rarefaction curves generated to assess sufficient read depth

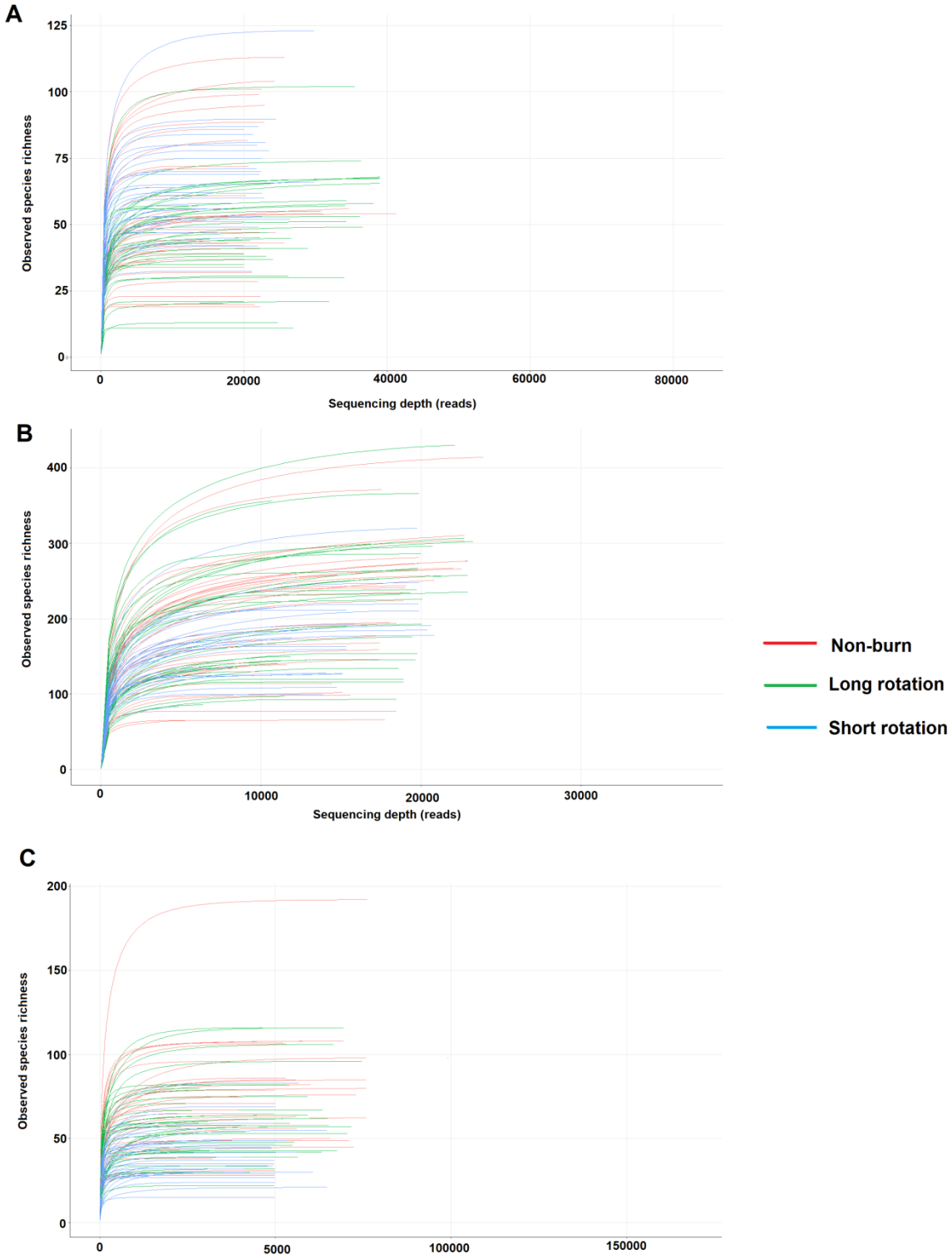


Fig A 3.1. Rarefaction curves based on observed species richness **(A)** Archaea, **(B)** Bacteria and **(C)** Fungi.

Appendix 4. DADA2 denoising outputs

Data shows sample-id, input- Number of sequence pairs the sample started with prior to the denoising steps. **Filtered-** Number of sequence pairs retained after user specified filtering. **Denoised-** Number of filtered sequence pairs that were successfully denoised. **Merged**: Number of filtered and denoised paired sequences that were successfully merged (forward and reverse read merged into one sequence). **Non-chimeric**: Number of filtered, denoised, and merged sequences retained after chimera removal. This is the final number of sequences per sample.

Table A 4.1. DADA2 denoising stats for archaea**.** Samples highlighted yellow was not included in the analysis due to very low reads.

sample-id	input	filtered	denoised	merged	non-chimeric
#q2:types	numeric	numeric	numeric	numeric	numeric
Non burn 0-20-1	49816	45507	44894	39752	22664
Non burn 0-20-2	52463	48614	47895	41212	22123
Non burn 0-20-3	42728	39176	38683	35837	22929
Non burn 0-20-4	31161	28975	28547	25780	15775
Non burn 0-20-5	44783	41072	40626	36709	22495
Non burn 0-20-6	43973	40846	40371	37153	22260
Non burn 0-20-7	24838	23032	22719	20905	13353
Non burn 0-20-8	39874	36713	36291	33678	22173
Non burn 0-20-9	43315	40035	39643	36819	23047
Non burn 0-20-10	208	180	163	140	138
Non burn 0-20-11	30301	28093	27585	22203	9527
Non burn 0-20-12	22969	21117	20581	16408	9964
Non burn 20-40-1	70272	61805	61507	55890	41393
Non burn 20-40-2	34742	31553	31305	29267	25737
Non burn 20-40-3	12490	11131	10978	9701	6483
Non burn 20-40-4	15080	13614	13449	12032	9034
Non burn 20-40-5	30563	27319	27100	24524	17904
Non burn 20-40-6	13750	12351	12250	10893	7294
Non burn 20-40-7	14431	13145	13006	11557	7556
Non burn 20-40-8	16263	14663	14535	13159	9924
Non burn 20-40-9	22118	20119	19843	18191	13576
Non burn 20-40-10	51694	46408	46122	42107	31117
Non burn 20-40-11	35267	31976	31772	29319	23769
Non burn 20-40-12	36233	32320	32141	30066	25932
Non burn 40-60-1	57455	52726	52353	47471	34692
Non burn 40-60-2	41979	38963	38639	34830	24474
Non burn 40-60-3	14716	13576	13309	11665	7372
Non burn 40-60-4	14685	13739	13494	11834	7492

Non burn 40-60-5	35839	32990	32680	29131	18903
Non burn 40-60-6	19861	18522	18322	16376	11117
Non burn 40-60-7	16219	15129	14961	13234	9072
Non burn 40-60-8	14414	13333	13070	11599	7750
Non burn 40-60-9	13444	12550	12416	10978	6687
Non burn 40-60-10	26914	24939	24647	22288	14868
Non burn 40-60-11	22361	20666	20465	18011	11477
Non burn 40-60-12	27943	25675	25471	22883	16706
Long rotation 0-20-1	12907	11742	11502	10366	8203
Long rotation 0-20-2	14027	12918	12668	11427	8839
Long rotation 0-20-3	21041	19021	18715	16073	10510
Long rotation 0-20-4	21876	20056	19873	18073	12687
Long rotation 0-20-5	56367	51442	50976	46335	35594
Long rotation 0-20-6	14391	12979	12623	9658	5534
Long rotation 0-20-7	6991	6468	6324	5819	4891
Long rotation 0-20-8	8510	7808	7708	7159	5652
Long rotation 0-20-9	14687	13527	13277	12243	9450
Long rotation 0-20-10	15086	13630	13340	10076	6611
Long rotation 0-20-11	20801	19089	18824	17608	13577
Long rotation 0-20-12	15270	13739	13415	11804	8786
Long rotation 20-40-1	57477	52870	52640	49271	36631
Long rotation 20-40-2	46484	43329	43022	40721	29014
Long rotation 20-40-3	37322	34488	34301	32177	23170
Long rotation 20-40-4	36673	34394	34164	31928	22321
Long rotation 20-40-5	48872	45040	44846	41971	30810
Long rotation 20-40-6	51833	48366	48087	45299	34171
Long rotation 20-40-7	55506	51723	51433	48189	36213
Long rotation 20-40-8	65	47	32	9	9
Long rotation 20-40-9	82318	76760	76335	70960	51597
Long rotation 20-40-10	80768	74812	74414	69946	53504
Long rotation 20-40-12	24787	23029	22868	21242	15339
Long rotation 40-60-1	129108	118593	118251	109288	79183
Long rotation 40-60-2	58851	54503	54143	50434	34368
Long rotation 40-60-3	25186	23168	22992	21404	14351
Long rotation 40-60-4	61477	57461	57138	53577	38174
Long rotation 40-60-5	128603	118230	117782	109593	82828
Long rotation 40-60-6	58383	54299	53939	50073	34335
Long rotation 40-60-7	37559	34996	34832	32685	24149
Long rotation 40-60-8	34637	32060	31809	29630	20906
Long rotation 40-60-9	67050	62305	61778	56630	36377
Long rotation 40-60-10	53146	49048	48654	43824	28542
Long rotation 40-60-11	40601	37632	37371	34677	23466
Long rotation 40-60-12	45554	41760	41459	38408	26975
Short rotation 0-20-1	17077	15234	14873	11504	7917

Short rotation 0-20-2	26446	23799	23381	18126	9170
Short rotation 0-20-3	15508	14028	13776	11813	9213
Short rotation 0-20-4	16794	15278	14975	12313	8831
Short rotation 0-20-5	24669	22158	21868	19359	14878
Short rotation 0-20-6	24674	22939	22645	20185	16322
Short rotation 0-20-7	18671	16585	16365	13657	9070
Short rotation 0-20-8	33216	29881	29397	24123	14281
Short rotation 0-20-9	33436	30283	29712	23331	13870
Short rotation 0-20-10	17284	15691	15202	12172	9337
Short rotation 0-20-11	14181	12903	12682	11286	8847
Short rotation 0-20-12	17675	15937	15658	13688	11197
Short rotation 20-40-1	33541	30232	29777	26463	16051
Short rotation 20-40-2	21711	19921	19572	17217	11224
Short rotation 20-40-3	17669	15995	15814	13833	9201
Short rotation 20-40-4	15858	14580	14330	12350	8661
Short rotation 20-40-5	44411	40377	40035	36285	24764
Short rotation 20-40-6	36231	33290	32973	29651	19181
Short rotation 20-40-7	20001	18456	18145	16248	10914
Short rotation 20-40-8	17616	15973	15769	13697	10203
Short rotation 20-40-9	31750	29295	29039	26145	17299
Short rotation 20-40-10	59653	54496	53872	47510	29880
Short rotation 20-40-11	33656	30915	30551	26964	17069
Short rotation 20-40-12	24001	21732	21200	17812	10507
Short rotation 40-60-1	39488	34680	34373	30184	21696
Short rotation 40-60-2	51434	46205	45854	41064	29862
Short rotation 40-60-3	26353	23382	22808	19327	13926
Short rotation 40-60-4	42	34	14	0	0
Short rotation 40-60-5	41463	36862	36383	32450	22595
Short rotation 40-60-6	28404	25593	25393	23282	17698
Short rotation 40-60-7	15165	13628	13426	12004	9842
Short rotation 40-60-8	27247	24178	23851	21383	16717
Short rotation 40-60-9	28666	25785	25485	22815	17875
Short rotation 40-60-10	44964	39839	39300	34131	23789
Short rotation 40-60-11	37687	33760	33295	28890	21699
Short rotation 40-60-12	44261	39281	38711	35336	26548

Table A 4.2. DADA2 denoising stats for Bacteria**.**

sample-id	input	filtered	denoised	merged	non-chimeric
#q2:types	numeric	numeric	numeric	numeric	numeric
Non burn 0-20-1	47527	34671	33044	22689	14793
Non burn 0-20-2	54372	40553	39306	31537	26825
Non burn 0-20-3	47562	34035	32849	26197	21463
Non burn 0-20-4	19801	14290	13277	8728	7099
Non burn 0-20-5	38425	28416	26880	18221	13425
Non burn 0-20-6	62714	46250	45125	37655	30620
Non burn 0-20-7	45403	33407	32385	25563	19711
Non burn 0-20-8	44847	31846	30127	20636	15484
Non burn 0-20-9	49771	37011	35882	29017	23863
Non burn 0-20-10	58183	42910	41031	29529	24439
Non burn 0-20-11	50727	36908	34813	22476	17878
Non burn 0-20-12	29249	20969	18667	7347	5623
Non burn 20-40-1	67989	47797	46374	36209	25809
Non burn 20-40-2	42027	30131	29438	25246	20895
Non burn 20-40-3	15073	10921	10090	6857	4450
Non burn 20-40-4	22784	16073	15332	11406	9436
Non burn 20-40-5	53113	38563	37126	28837	22343
Non burn 20-40-6	49639	36765	35636	28773	24077
Non burn 20-40-7	66978	48660	47945	43149	39802
Non burn 20-40-8	37511	26100	24969	18355	14787
Non burn 20-40-9	33536	24859	24046	18972	14408
Non burn 20-40-10	36568	26218	24650	16874	13762
Non burn 20-40-11	51466	36675	35557	29570	25534
Non burn 20-40-12	25535	17021	16132	11939	9700
Non burn 40-60-1	67431	37693	36809	30669	21953
Non burn 40-60-2	11177	7292	6468	3648	2562
Non burn 40-60-3	21734	12451	11945	9114	7197
Non burn 40-60-4	22875	13476	12731	8952	7928
Non burn 40-60-5	39349	24407	23275	16070	10976
Non burn 40-60-6	48174	29617	28751	23750	19586
Non burn 40-60-7	13783	8636	8089	5759	4757
Non burn 40-60-8	30655	17546	16818	12946	10684
Non burn 40-60-9	36981	22382	21663	17303	14123
Non burn 40-60-10	23257	14001	13235	9941	7687
Non burn 40-60-11	36478	22300	21542	16482	13783
Non burn 40-60-12	14860	8377	7833	5120	4435
Long rotation 0-20-1	58560	40461	37750	19417	10918
Long rotation 0-20-2	50113	35927	34010	20699	9783
Long rotation 0-20-3	38398	26206	24437	14144	10606
Long rotation 0-20-4	40409	27436	25572	13964	9259
Long rotation 0-20-5	4076	2795	2568	1696	1458

Long rotation 0-20-6	30612	22415	19429	7495	6032
Long rotation 0-20-7	28631	20765	19053	8999	5498
Long rotation 0-20-8	47166	32050	30151	18434	10690
Long rotation 0-20-9	4243	2968	2597	1301	1048
Long rotation 0-20-10	85641	58481	55691	34508	22630
Long rotation 0-20-11	53183	35072	33174	20802	15325
Long rotation 0-20-12	64460	42270	39802	23496	17434
Long rotation 20-40-1	54462	38479	37237	29676	19714
Long rotation 20-40-2	28452	20388	19820	16464	12268
Long rotation 20-40-3	33343	23128	22251	17679	13571
Long rotation 20-40-4	32241	21831	21056	17113	12819
Long rotation 20-40-5	48600	36134	35046	26628	18173
Long rotation 20-40-6	19478	14326	13616	9769	7201
Long rotation 20-40-7	28032	20855	20156	16055	12384
Long rotation 20-40-8	47293	33484	32671	27328	21249
Long rotation 20-40-9	38813	28021	27082	21841	15844
Long rotation 20-40-10	30063	20728	19921	15507	10539
Long rotation 20-40-12	51700	36437	35463	29666	24967
Long rotation 40-60-1	70275	48319	46832	36650	24844
Long rotation 40-60-2	28947	20179	19229	14331	10606
Long rotation 40-60-3	44791	29456	28617	23963	17828
Long rotation 40-60-4	38036	25887	24775	19782	15444
Long rotation 40-60-5	73026	52480	50675	38061	25642
Long rotation 40-60-6	65878	46523	45553	38748	31866
Long rotation 40-60-7	60795	44033	42843	34517	27869
Long rotation 40-60-8	27780	18760	17908	13532	10615
Long rotation 40-60-9	56352	40963	39876	32657	26362
Long rotation 40-60-10	61326	43305	41763	31350	22830
Long rotation 40-60-11	40924	27407	26602	21861	15926
Long rotation 40-60-12	28116	18317	17514	13220	9816
Short rotation 0-20-1	32082	23070	21309	10981	5612
Short rotation 0-20-2	23711	17824	16690	10491	6443
Short rotation 0-20-3	20718	15056	13877	7798	3777
Short rotation 0-20-4	28095	19595	17775	9467	7445
Short rotation 0-20-5	15348	11231	10103	5029	2390
Short rotation 0-20-6	15946	11990	10774	5064	2370
Short rotation 0-20-7	26836	20149	19091	12345	8621
Short rotation 0-20-8	59616	41980	40273	27847	20788
Short rotation 0-20-9	33379	25077	23910	15313	9381
Short rotation 0-20-10	30298	22070	20711	13054	6496
Short rotation 0-20-11	47385	33856	32640	23925	18663
Short rotation 0-20-12	23277	16415	14915	7137	3687
Short rotation 20-40-1	37081	24257	23258	17424	13106
Short rotation 20-40-2	22565	15376	14690	11081	8956

Short rotation 20-40-3	51012	33561	32591	25944	20353
Short rotation 20-40-4	16596	11002	10137	6395	5231
Short rotation 20-40-5	32585	22123	21196	15654	12306
Short rotation 20-40-6	39419	27010	26189	21072	15811
Short rotation 20-40-7	20219	13688	13064	9831	8598
Short rotation 20-40-8	18716	12296	11416	7402	5775
Short rotation 20-40-9	36592	25689	24816	19540	16007
Short rotation 20-40-10	38409	25472	24440	17722	13359
Short rotation 20-40-11	42037	27239	26100	19049	15555
Short rotation 20-40-12	20822	13413	12421	7754	6831
Short rotation 40-60-1	57149	34817	32972	22912	17165
Short rotation 40-60-2	18297	11637	10980	7402	6085
Short rotation 40-60-3	30621	18565	17402	11121	8666
Short rotation 40-60-4	27589	17337	16225	11380	9909
Short rotation 40-60-5	46669	30518	29294	21893	17069
Short rotation 40-60-6	34333	22498	21511	15902	13318
Short rotation 40-60-7	20972	13700	12929	7889	5336
Short rotation 40-60-8	19449	11948	11121	6623	4903
Short rotation 40-60-9	36946	23640	22319	14459	11165
Short rotation 40-60-10	12437	7835	6705	2844	2238
Short rotation 40-60-11	23020	14827	13243	6335	4318
Short rotation 40-60-12	19963	11401	10704	7409	6273

Table A 4.2. DADA2 denoising stats for Fungi**.** Samples highlighted yellow was not included in the analysis due to very low reads.

sample-id	input	filtered	denoised	merged	non-chimeric
#q2:types	numeric	numeric	numeric	numeric	numeric
Non burn 0-20-1	100606	86671	86223	83993	74179
Non burn 0-20-2	75523	65780	65479	61742	56406
Non burn 0-20-3	108633	96342	95379	87707	79531
Non burn 0-20-4	24110	20741	20420	19176	18623
Non burn 0-20-5	117747	100276	99578	96164	93568
Non burn 0-20-6	49964	43717	43334	41687	39131
Non burn 0-20-7	52671	45428	45057	41499	39693
Non burn 0-20-8	75770	64578	64112	51630	46766
Non burn 0-20-9	69517	60138	59746	56738	45869
Non burn 0-20-10	30588	26715	26409	24514	20839
Non burn 0-20-11	75862	64554	63975	55585	48423
Non burn 0-20-12	57903	49266	48758	44441	41276
Non burn 20-40-1	135153	116300	115815	85377	84589
Non burn 20-40-2	2763	2418	2326	2131	1915
Non burn 20-40-3	97448	84951	84653	82666	82605
Non burn 20-40-4	11212	10099	9970	9304	9242
Non burn 20-40-5	94190	80735	80269	69511	66967
Non burn 20-40-6	42404	37169	37003	36140	34315
Non burn 20-40-7	18926	15245	15039	13895	12399
Non burn 20-40-8	80245	69117	68824	62378	56250
Non burn 20-40-9	91353	79040	78761	75552	75173
Non burn 20-40-10	44997	39041	38715	34710	30762
Non burn 20-40-11	56526	48946	48649	41669	40061
Non burn 20-40-12	42860	36762	36523	30232	28725
Non burn 40-60-1	250511	222496	221635	213963	203338
Non burn 40-60-2	100511	88081	87376	84138	77817
Non burn 40-60-3	368	288	195	102	102
Non burn 40-60-4	78371	65006	64374	61380	56044
Non burn 40-60-5	131082	116958	116337	112185	99899
Non burn 40-60-6	9730	8723	8563	8204	7324
Non burn 40-60-7	397	300	223	141	141
Non burn 40-60-8	161239	143743	143143	138654	124961
Non burn 40-60-9	136	86	44	18	18
Non burn 40-60-10	45874	39506	39025	36335	30359
Non burn 40-60-11	125680	112621	112015	107182	96677
Non burn 40-60-12	92572	81161	80526	75792	60731
Long rotation 0-20-1	88051	76851	76243	72922	67522
Long rotation 0-20-2	96310	85896	85586	83025	73109
Long rotation 0-20-3	65246	57060	56550	48250	42409
Long rotation 0-20-4	12916	11630	11407	11092	9965

Long rotation 0-20-5	115420	97431	96661	90356	82675
Long rotation 0-20-6	64964	57664	57300	51465	47894
Long rotation 0-20-7	45439	40588	40275	38731	36785
Long rotation 0-20-8	81224	71021	70761	63815	61449
Long rotation 0-20-9	98904	87217	86874	84671	79856
Long rotation 0-20-10	60473	53338	53057	51556	46511
Long rotation 0-20-11	72703	64493	64047	60581	55902
Long rotation 0-20-12	77645	68684	68306	65088	53014
Long rotation 20-40-1	64517	56719	56394	55363	54642
Long rotation 20-40-2	88522	77681	77374	76203	75404
Long rotation 20-40-3	42990	39304	39099	38491	36705
Long rotation 20-40-4	101286	94697	94553	92739	91393
Long rotation 20-40-5	56263	49937	49578	47528	40714
Long rotation 20-40-6	86410	77734	77348	74276	62798
Long rotation 20-40-7	55211	49210	49007	47651	47470
Long rotation 20-40-8	77510	68619	68412	67212	66455
Long rotation 20-40-9	90743	80404	80129	78278	76627
Long rotation 20-40-10	65689	59061	58948	58505	57718
Long rotation 20-40-12	32039	27592	27371	25856	23897
Long rotation 40-60-1	79519	70554	70263	69251	68643
Long rotation 40-60-2	59896	53791	53598	52538	48733
Long rotation 40-60-3	108902	100538	100320	99405	98538
Long rotation 40-60-4	36841	32245	32023	30568	29621
Long rotation 40-60-5	93438	83305	82791	79527	71284
Long rotation 40-60-6	81815	73163	72907	72173	71359
Long rotation 40-60-7	37141	32467	32158	31253	30537
Long rotation 40-60-8	53515	47250	47012	45942	44735
Long rotation 40-60-9	74233	65267	64951	55103	54768
Long rotation 40-60-10	34049	30230	30086	29190	28197
Long rotation 40-60-11	79502	72361	72119	71360	70951
Long rotation 40-60-12	102444	89773	89398	87222	86511
Short rotation 0-20-1	56991	49395	48861	41778	37611
Short rotation 0-20-2	61959	53158	52754	46265	43532
Short rotation 0-20-3	20530	17935	17695	15380	14082
Short rotation 0-20-4	43437	31969	31675	18066	16530
Short rotation 0-20-5	86656	75358	74911	68758	65366
Short rotation 0-20-6	62289	40893	40474	37390	35049
Short rotation 0-20-7	24624	21478	21218	18873	17456
Short rotation 0-20-8	59410	51399	50958	41887	34714
Short rotation 0-20-9	47705	41553	41157	38686	34028
Short rotation 0-20-10	50778	44458	44056	39688	35421
Short rotation 0-20-11	41066	36829	36646	35291	33011
Short rotation 0-20-12	39425	34033	33641	28907	24433
Short rotation 20-40-1	104271	93457	93102	86066	84694

Short rotation 20-40-2	77386	69092	68938	66892	65682
Short rotation 20-40-3	32779	29080	28956	28319	27291
Short rotation 20-40-4	39747	35470	35117	32315	31879
Short rotation 20-40-5	70030	61522	61306	58141	56770
Short rotation 20-40-6	48632	42861	42723	41886	40894
Short rotation 20-40-7	49359	43778	43660	41288	40894
Short rotation 20-40-8	49895	43956	43828	42398	41427
Short rotation 20-40-9	70575	62625	62455	61574	61043
Short rotation 20-40-10	34391	30466	30243	29137	27793
Short rotation 20-40-11	23054	20119	19869	15651	15242
Short rotation 20-40-12	22079	19186	19026	18296	17535
Short rotation 40-60-1	25441	22248	22041	18070	17671
Short rotation 40-60-2	28324	24969	24844	22395	22259
Short rotation 40-60-3	19246	16972	16860	15527	15467
Short rotation 40-60-4	13022	11648	11508	10553	10452
Short rotation 40-60-5	23522	20560	20389	19609	18527
Short rotation 40-60-6	23516	20846	20659	20261	19793
Short rotation 40-60-7	24510	21712	21542	18895	18788
Short rotation 40-60-8	17461	15451	15233	14767	14502
Short rotation 40-60-9	21993	19386	19261	18027	17806
Short rotation 40-60-10	20000	17783	17605	17239	15285
Short rotation 40-60-11	12722	11236	11102	9169	8959
Short rotation 40-60-12	10011	8648	8440	7527	7253

Appendix 5. Two-way ANOVA of soil properties across three soil depths in three burn treatments

Table A. 5.1. Two-way ANOVA of soil physiocochemical properties across three different soil depths under three burn treatments. Result is reported as the mean ± SE (n = 12). The data in bold indicate soil properties that were affected by soil depth, burn treatment and their interaction at a confidence level of 95% ($P < 0.05$). Different uppercase letters indicate statistically significant differences among the three burn treatments in the same soil layer, different lowercase letters indicate statistically significant differences among the three soil layers across burn treatments and different letters with an asterisk indicate a significant difference among treatments based on a significant interaction between burn treatment and soil depth (Tukey's HSD, $P < 0.05$).

	Soil depth (cm)	Non-burn	Long rotation	Short rotation	Burn treatment	Depth (cm)	Burn treatment*Depth
pH	0-20cm	4.1±0.06 **A**	3.56±0.04 **B**	3.81±0.0.02 **B**	**F=75.603,P=<0.001**	F=1.135,P=.0.32	F=1.979,P=0.10
	20-40cm	4.21±0.0.06 **A**	3.7±0.03 **B**	3.80±0.03 **B**			
	40-60cm	4.11±0.0.7 **A**	3.75±0.0.04 **B**	3.86±0.0.03 **B**			
Moisture (%)	0-20cm	78.22±0.38 **D***	81.30±0.49 **C***	82.89±0.79 **C***	**F=9.709,P=<0.001**	**F=137.98,P=<0.001**	**F=12.35,P=<0.001**
	20-40cm	86.75±0.55**B***	83.31±0.56 **C***	86.03±0.36 **B***			
	40-60cm	89.27±0.38**A***	86.96±0.53 **AB***	88.41±0.71 **AB***			
Total N (%)	0-20cm	1.26±0.02 **BC***	1.52±0.03 **A***	1.36±0.09 **ABC***	**F=6.25,P=<0.001**	**F=12.93,P=<0.001**	**F=9.10,P=<0.001**
	20-40cm	1.34±0.03 **ABC**	1.18±0.03 **BCD***	1.19±0.02 **BCD***			
	40-60cm	1.41±0.04 **AB***	1.15±0.04 **CD***	1.01±0.02 **D***			
Total C (%)	0-20cm	50.50±1.06 **A***	43.65±0.37 **BC***	37.87±1.72 **CD***	**F=26.33,P=<0.001**	F=2.929,P=0.058	**F=4.78,P=0.01**
	20-40cm	48.14±0.07 **AB***	42.61±0.79 **BC***	36.09±2.46 **D***			
	40-60cm	46.74±0.38 **AB***	43.34±1.74 **BC***	46.11±0.32 **AB***			
NO_3^- (mg/kg^{-1})	0-20cm	2.88±0.46 **C***	6.45±0.96 **AB***	3.51±0.24 **C***	**F=4.194,P=<0.001**	F**=2.887,P=0.05**	**F=9.499P=<0.001**
	20-40cm	6.89±1.09 **A***	3.59±0.42 **C***	3.34±0.33 **C***			
	40-60cm	4.04±0.36 **BC***	3.31±.0.24 **C***	3.18±0.25 C*			
NH_4^+ (mg/kg^{-1})	0-20cm	16.88±1.62 **A***	6.86±1.39 **B***	4.05±1.29 **BC***	**F=32.44,P=<0.001**	**F=53.33,P=<0.001**	**F=13.35,P=<0.0001**
	20-40cm	4.24±0.70 **BC***	2.96±0.51 **BC***	1.31±0.19 **C***			
	40-60cm	3.04±0.44 **BC***	2.69±0.24**BC***	1.19±0.17 **C***			
Mg (mg/kg^{-1})	0-20cm	916.4±215.08 **A***	316.33±30.09 **B***	211.46±14.22 **B***	**F=27.86,P=<0.001**	**F=8.606,P=<0.001**	**F=3.08,P=0.01**
	20-40cm	457.6±48.77 **B***	167.84±9.26 **B***	182.93±24.79 **B***			
	40-60cm	414.76±32.73 **B***	163.69±6.88 **B***	167.69±21.00 **B***			
Ca (mg/kg^{-1})	0-20cm	1368.7±66.35 **CD***	1782.00±54.32 **BC***	1320.9±53.727 **D***	**F=24.05,P=<0.001**	**F=86.21,P=<0.001**	**F=10.90,P=<0.001**
	20-40cm	1842.5±95.47 **B***	2194.5±136.34 **B***	1754.6±116.72 **BCD***			
	40-60cm	1889.8±67.03 **B***	2823.5±113.64 **A***	2967.1±117.22 **A***			
Mn (mg/kg^{-1})	0-20cm	89.83±4.83 **A***	67.89±4.58 **BC***	37.5±2.97 **DE***	**F=32.37,P=<0.001**	**F=21.05,P=<0.001**	**F=9.17,P=<0.001**
	20-40cm	52.67±4.16 **BCD***	47.92±4.50 **CD***	24.83±1.33 **E***			
	40-60cm	52.92±2.43 **BCD***	71.25±8.29 **AB***	52.08±2.56 **BCD***			
Fe (mg/kg^{-1})	0-20cm	681.51±26.23 **C***	1236.93±130.10 **B***	777.45±63.75 **C***	**F=74.49,P=<0.001**	**F=38.49,P=<0.001**	**F=4.76,P=<0.001**
	20-40cm	830.03±79.28 **C***	1480.23±81.21 **B***	824.47±68.569 **C***			
	40-60cm	840.16±38.95 **C***	2079.39±103.41 **A***	1395.4±56.09 **B***			
Cu (mg/kg^{-1})	0-20cm	87.75±12.63 **A***	59.5±6.58 **B***	39.42±4.06 **BC***	**F=7.025,P=<0.001**	**F=50.48,P=<0.001**	**F=4.76,P=0.01**
	20-40cm	14.75±1.03 **D***	26.50±3.15 **CD***	15.00±2.72 **D***			
	40-60cm	13.50±1.13 **D***	17.50±1.94 **CD***	15.17±1.05 **D***			
Zn (mg/kg^{-1})	0-20cm	271.55±13.70 **A***	145.37±19.15 **B***	111.89±6.70 **BC***	**F=40.96,P=<0.001**	**F=41.97,P=<0.001**	**F=1.37,P=<0.01**
	20-40cm	117.25±2.67 **BC***	60.66±7.13 **CD***	44.06±3.33 **D***			
	40-60cm	131.64±7.68 **B***	63.27±7.20 **CD***	31.38±4.21 **D***			
P (mg/kg^{-1})	0-20cm	245.61±10.96 **Ba**	316.81±43.22 **Aa**	260.19±21.11 **ABa**	**F=8.38,P=<0.001**	**F=42.13,P=<0.001**	F=0.728,P=0.57
	20-40cm	146.09±19.90 **Ab**	174.68±14.26 **Ab**	101.53±10.57 **Bb**			
	40-60cm	138.3±4.88 **Bc**	193.14±9.50 **Ab**	144.16±4.37 **Bb**			
Pb (mg/kg^{-1})	0-20cm	232.78±14.00 **A***	1.874±19.22 B*	118.85±15.73 **C***	**F=20.70,P=<0.001**	**F=296.265,P=<0.001**	**F=8.40,P=<0.001**
	20-40cm	39.79±2.54 **D***	21.06±1.79 **D***	11.49±1.49 **D***			
	40-60cm	25.96±3.29 **D***	13.56±2.35 D*	13.27±1.07 **D***			
K (mg/kg^{-1})	0-20cm	134.84±7.18 **Aa**	156.25±17.56**Aa**	140.1±16.87 **Aa**	**F=3.68,P=0.009**	**F=50.85,P=<0.001**	F=2.31,P=0.08
	20-40cm	86.43±9.17 **Ab**	56.21±3.31 **Bb**	40.68±4.57 **Bb**			
	40-60cm	96.07±5.84**Ab**	78.54±7.78 **ABb**	67.48±11.33 **Bb**			
Al (mg/kg^{-1})	0-20cm	463.09±23.35 **AB**	495.99±56.51**A**	330.26±35.21**B**	**F=26.03,P=<0.001**	F=2.71,P=0.07	F=1.89,P=0.11
	20-40cm	431.56±30.53 **A**	457.01±23.76 **A**	249.53±25.26 **B**			
	40-60cm	398.56±18.47 **B**	561.57±33.22 **A**	355.58±31.67 **B**			

Appendix 6. Statistsics from the forward selection RDA analyses used in this study

Table A 6.1. Summary of the results from the ordistep forward selection RDA used in chapter 3 for the MicroResp^{TM} data and environmental variables.

Variable	AIC	F value	P value
Pore water pH	-75.620	6.98	0.01
Plant richness	-73.775	4.79	0.01
B	-101.941	3.61	0.01
Soil temperature	-99.892	2.62	0.01
NH_4^+	-102.94	2.45	0.01
K	-103.90	2.31	0.04

Table A 6.2. Summary of the results from the ordistep forward selection RDA used in chapter 3 for fungal and prokaryote communities and environmental variables.

Variable	AIC	F value	P value
Fungi			
Pore water pH	-6.155	4.15	0.005
Soil temperature	-8.351	3.99	0.005
Plant richness	-7.513	2.64	0.005
Prokaryotes			
Pore water pH	-9.766	7.91	0.005
Conductivity	-15.277	7.72	0.005
Soil temperature	-27.187	3.02	0.005
Pb	-23.648	1.19	0.02
Mg	-23.797	1.56	0.04

Table A 6.3. Summary of the results from the ordistep forward selection RDA used in chapters 4 and 5.

Variable	AIC	F value	P value
Archaea 0-20cm			
NH_4^+	-34.396	16.64	0.005
pH	-29.703	2.26	0.005
Archaea 20-40cm			
Pb	-68.129	5.20	0.005
Moisture	-70.528	4.29	0.005
Al	-70.988	2.29	0.001
Archaea 40-60cm			
Total N	-70.133	10.229	0.005
Total C	-72.545	4.295	0.005
Al	-74.899	4.10	0.005
Ca	-80.172	6.92	0.005
Fe	-81.305	2.71	0.1
pH	-81.895	2.15	0.01
Bacteria 0-20cm			
NH_4^+	-33.598	16.14	0.001
pH	-34.396	9.14	0.001
Heather cover (%)	-28.881	2.84	0.001
Mg	-23.741	2.79	0.002
Bacteria 20-40cm			
Fe	-55/148	6.50	0.005
K	-58.168	5.41	0.005
Pb	-59.203	2.37	0.005
Bacteria 40-60cm			
Al	-36.192	4.48	0.005
Ca	-39.468	5.20	0.005
Fe	-40.430	2.74	0.005
Fungi 0-20cm			
Ca	-11.857	3.17	0.005
Mn	-12.579	2.99	0.005
Other moss cover %	-12.862	2.56	0.005
Total C	-12.697	2.69	0.003
pH	-12.425	2.16	0.001
Fungi 20-40cm			
NH_4^+	-23.114	3.78	0.005
Mositure	-23.974	2.17	0.01
Fungi 40-60cm			
Fe	-15.864	3.78	0.005
Pb	-23.978	2.17	0.01

Appendix 7. Indicator analysis for archaea and bacteria in three burn treatments across different soil depths

Table A 7.1. Treatments associated with archaeal indicator species of highest abundance ($P < 0.05$) associated with burn treatments and soil profiles.

Treatment	Class	Order	Family	Genus	Species	Indicator value	P value
Non-Burn 0-20cm	Bathyarchaeia	Bathyarchaeia	Bathyarchaeia	Bathyarchaeia	Uncultured_ euryarchaeote	0.6523	0.0001
	Nitrososphaeria	SCGC_AB_179_E04	SCGC_AB_179_E04	SCGC_AB_179_E04	Uncultured_ archaeon	0.4309	0.0001
	Thermoplasmata	Methanomassiliicoccales	Methanomassiliicoccaceae	uncultured	Uncultured_ archaeon	0.428	0.0001
	Methanomicrobia	Methanomicrobiales	Methanomicrobiaceae	uncultured	Uncultured_ archaeon	0.4213	0.0001
	Methanomicrobia	Methanomicrobiales	Rice_Cluster_II	Rice_Cluster_II	Uncultured_ methanogenic	0.3465	0.0008
	Thermoplasmata	A10	A10	A10	Uncultured_ Thermoplasmatales	0.3217	0.0023
Non- burn 20-40cm	NA	NA	NA	NA	NA	NA	NA
Non-burn 40-60cm	NA	NA	NA	NA	NA	NA	NA
Long rotation 0-20cm	NA	NA	NA	NA	NA	NA	NA
Long Rotation 20-40cm	Methanomicrobia	Methanomicrobiales	Methanoregulaceae	Methanoregula		0.3502	0.001
	Methanomicrobia	Methanomicrobiales	Methanoregulaceae	Methanoregula	Uncultured_archaeon	0.3387	0.0005
	Thermoplasmata	Methanomassiliicoccales	Methanomassiliicoccaceae	uncultured	Metagenome	0.3366	0.0032
	Bathyarchaeia	Bathyarchaeia	Bathyarchaeia	Bathyarchaeia	Uncultured_archaeon	0.3234	0.0001
	Methanomethylicia	Methanomethyliales	Methanomethyliaceae	Candidatus_ Methanomethylicus	Unidentified	0.508	0.001
	Nitrososphaeria	Nitrosotaleales	Nitrosotaleaceae	Candidatus_ Nitrosotalea	Unidentified	0.6716	0.0002
Long Rotation 40-60	NA	NA	NA	NA	NA	NA	NA
Short rotation 0-20cm	Nitrososphaeria	Group_1.1c	Group_1.1c	Group_1.1c	Uncultured_archaeon	0.529	0.0001
	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanobacterium		0.4725	0.0002
	Thermoplasmata	Uncultured	Uncultured	Uncultured		0.3279	0.0027
	Thermoplasmata	Uncultured	Uncultured	Uncultured	Mine_drainage	0.3774	0.0031
Short rotation 20-40cm	NA	NA	NA	NA	NA	NA	NA
Short rotation 40-60cm	Methanosarcinia	Methanosarciniales	Methanosarcinaceae	Methanosarcina	Uncultured_bacterium	0.3961	0.0229

Table A 7.2. Treatments associated with bacterial indicator species of highest abundance ($P < 0.05$) associated with burn treatments and soil profiles.

Treatment	Class	Order	Family	Genus	Species	Indicator value	P value
Non- burn 0-20cm	Dehalococcoidia	MSBL5	MSBL5	MSBL5	Uncultured _bacterium	0.8291	0.0001
	KD4-96	KD4-96	KD4-96	KD4-96	Uncultured _Chloroflexi	0.7715	0.0001
	SAR324_clade (Marine_group_B)	SAR324_clade (Marine_group_B)	SAR324_clade (Marine_group_B)	SAR324_clade. (Marine_group_B.	Uncultured _bacterium	0.7664	0.0001
	Kryptonia	Kryptoniales	BSV26	BSV26	Uncultured _bacterium	0.7186	0.0001
	Subgroup_18	Subgroup_18	Subgroup_18	Subgroup_18	uncultured_ bacterium	0.6537	0.0001
	Dehalococcoidia	GIF9	GIF9	GIF9	Uncultured _bacterium	0.6379	0.0001
	Fibrobacteria	Fibrobacterales	B122	B122	Uncultured _bacterium	0.5843	0.0001
	SJA-28	SJA-28	SJA-28	SJA-28	Uncultured _bacterium	0.5684	0.0001
	Sva0485	Sva0485	Sva0485	Sva0485		0.5383	0.0001
	Methylomirabilia	Methylomirabilales	Methylomirabilaceae	Sh765B-TzT-35	Uncultured bacterium	0.5339	0.0001
	Verrucomicrobiae	Opitutales	Opitutaceae	Lacunisphaera	Verrucomicrobia _bacterium	0.5281	0.0001
	Desulfobaccia	Desulfobaccales	Desulfobaccaceae	Desulfobacca	Uncultured _bacterium	0.4831	0.0001
	Endomicrobia	Endomicrobiales	Endomicrobiaceae	Endomicrobium	Uncultured _bacterium	0.4167	0.0001
	Dehalococcoidia	GIF3	GIF3	GIF3	Uncultured _bacterium	0.4167	0.0001
	Acidobacteriae	Acidobacteriae	Acidobacteriae	Paludibaculum	Uncultured _Acidobacteriaceae	0.3911	0.0001
	Spirochaetia	Spirochaetales	Spirochaetaceae	Spirochaeta	Spirochaeta _sp	0.3872	0.0001
	Dehalococcoidia	RBG-13-46-9	RBG-13-46-9	RBG-13-46-9	Uncultured _bacterium	0.3865	0.0009
	Gammaproteobacteria	Burkholderiales	Gallionellaceae	Gallionella	Uncultured_ bacterium	0.3722	0.0039
	Acidobacteriae	Subgroup_13	Subgroup_13	Subgroup_13		0.3602	0.0001
	Syntrophorhabdia	Syntrophorhabdales	Syntrophorhabdaceae	Syntrophorhabdus	Uncultured_ bacterium	0.3559	0.0004
	Kapabacteria	Kapabacteriales	Kapabacteriales	Kapabacteriales		0.334	0.0002
	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	ADurb.Bin063-1		0.3189	0.0003
	Pla4_lineage	Pla4_lineage	Pla4_lineage	Pla4_lineage	Uncultured _bacterium	0.3685	0.0065
	Gammaproteobacteria	Coxiellales	Coxiellaceae	Coxiella	uncultured_ Coxiellaceae	0.3627	0.006
	Alphaproteobacteria	Rhizobiales	Rhodomicrobiaceae	Rhodomicrobium		0.3441	0.03
	Parcubacteria	Parcubacteria	Parcubacteria	Parcubacteria	Uncultured_ bacterium	0.3396	0.0036
	Verrucomicrobiae	Chthoniobacterales	Terrimicrobiaceae	FukuN18_freshwater_group	Uncultured _bacterium	0.3391	0.0038
	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Roseiarcus		0.3292	0.0476
	Phycisphaerae	Tepidisphaerales	WD2101_soil_group	WD2101_soil_group	Uncultured _bacterium	0.3133	0.0293
	Alphaproteobacteria	Rhodospirillales	Magnetospirillaceae	Magnetospirillaceae	uncultured_ bacterium	0.3045	0.0342
Non-burn 20-40cm	AD3	AD3	AD3	AD3	uncultured_ Chloroflexi	0.3771	0.0082
Non -burn 40-60cm	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylocella	Methylocella_ palustris	0.3586	0.0024
Long rotation 0-20cm	Acidobacteriae	Bryobacterales	Bryobacteraceae	Bryobacter		0.5685	0.0001

	Acidobacteriae	Acidobacteriales	Koribacteraceae	Candidatus_Koribacter	Uncultured _Acidobacteriaceae	0.4469	0.0001
	Bacteroidia	Chitinophagales	Chitinophagaceae	Puia		0.4213	0.0002
	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Pseudolabrys		0.4138	0.0002
	WPS-2	WPS-2	WPS-2	WPS-2	Uncultured _bacterium	0.3977	0.0002
	Gammaproteobacteria	WD260	WD260	WD260	Uncultured_ bacterium	0.3778	0.0001
	Actinobacteria	Frankiales	Acidothermaceae	Acidothermus		0.3312	0.0126
	Polyangia	Polyangiales	Polyangiaceae	Pajaroellobacter	Uncultured _bacterium	0.3132	0.0021
	Acidobacteriae	Solibacterales	Solibacteraceae	Candidatus_Solibacter		0.393	0.0006
	BD7-11	BD7-11	BD7-11	BD7-11	Uncultured _bacterium	0.35	0.0025
	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium		0.3464	0.0104
Long rotation 20-40cm	Anaerolineae					0.482	0.001
Long rotation 40-60cm	Syntrophia	Syntrophales	Smithellaceae	Smithella		0.3735	0.0342
	Acidobacteriae	Subgroup_2	Subgroup_2	Subgroup_2	Uncultured_ bacterium	0.3538	0.0001
	Chlamydiae	Chlamydiales	cvE6	cvE6	Uncultured _bacterium	0.3129	0.0001
	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylocystis	uncultured_ Methylocystis	0.3111	0.0019
	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	Ellin516	Uncultured_ Verrucomicrobia	0.3974	0.0157
	Bacteroidia	Bacteroidales	SB-5	SB-5		0.3419	0.0006
Short rotation 0-20cm	Bacteroidia	Sphingobacteriales	CWT_CU03-E12	CWT_CU03-E12	Uncultured _bacterium	0.3882	0.0318
	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Rhodoplanes		0.3838	0.0057
	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	Acidicaldus	Uncultured_ bacterium	0.3354	0.0206
Short rotation 20-40cm	NA	NA	NA	NA	NA	NA	NA
Short rotation 40-60cm	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Rhodoblastus		0.613	0.0001
	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Uncultured		0.573	0.0001

Appendix 8. Indicator analysis for fungi in three burn treatments across different soil depths

Table A 8.1. Treatments associated with fungal indicators of highest abundance ($P < 0.05$) associated with burn treatments and different depths.

Treatment	Class	Order	Family	Genus	Species	Indicator value	P value
Non burn 0-20cm	Dothideomycetes	Venturiales	Venturiaceae	Venturia	unidentified	0.6759	0.0001
	Agaricomycetes	Sebacinales	Serendipitaceae	Serendipita	unidentified	0.4545	0.0021
	Archaeorhizomycetes	Archaeorhizomycetales	Archaeorhizomycetaceae	Archaeorhizomyces	unidentified	0.4034	0.013
	Dothideomycetes	Capnodiales	Unidentified	Unidentified	unidentified	0.3882	0.0009
	Leotiomycetes	Helotiales	Unidentified	Unidentified	unidentified	0.3799	0.0093
	Agaricomycetes	Agaricales	Entolomataceae	Entoloma	Entoloma_ cetratum	0.3669	0.0053
	Leotiomycetes	Unidentified	Unidentified	Unidentified	unidentified	0.3627	0.0034
	Sordariomycetes	Sordariales	Lasiosphaeriaceae	Unidentified	unidentified	0.3452	0.0135
	Leotiomycetes	Helotiales	Leotiaceae	Flagellospora	Flagellospora_ saccata	0.3832	0.0191
Non-burn 20-40cm	Archaeorhizomycetes	Archaeorhizomycetales	Archaeorhizomycetaceae	Unidentified	unidentified	0.3103	0.0299
Non burn 40-60cm	Eurotiomycetes	Chaetothyriales	Unidentified	Unidentified	unidentified	0.4797	0.0374
	Leotiomycetes	Helotiales	Helotiaceae	Gremmeniella	Gremmeniella_ laricina	0.3654	0.0106
	Dothideomycetes	Pleosporales	Massarinaceae	Neottiosporina	unidentified	0.3502	0.0169
	Dothideomycetes	Venturiales	Venturiaceae	Venturia	unidentified	0.3067	0.0458
Long rotation 0-20cm	Leotiomycetes	Helotiales	Hyaloscyphaceae	Hyaloscypha	unidentified	0.5917	0.0007
	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	unidentified	unidentified	0.5432	0.0017
	Leotiomycetes	Helotiales	Helotiaceae	Meliniomyces	unidentified	0.378	0.0003
	Microbotryomycetes	unidentified	unidentified	unidentified	unidentified	0.3145	0.0105
	Agaricomycetes	Agaricales	Tricholomataceae	Mycena	unidentified	0.3934	0.0032
	Leotiomycetes	Helotiales	Vibrisseaceae	Unidentified	unidentified	0.3684	0.0111
Long rotation 20-40cm	Rozellomycotina _cls_Incertae_sedis	Branch03	unidentified	unidentified	unidentified	0.3797	0.0457
	Tremellomycetes	Filobasidiales	Piskurozymaceae	Piskurozyma		0.316	0.0165
Long rotation 40-60cm	Malasseziomycetes	Malasseziales	Malasseziaceae	Malassezia	unidentified	0.4924	0.0036
	Microbotryomycetes	Sporidiobolales	Sporidiobolaceae	Rhodotorula	Rhodotorula_ toruloides	0.4045	0.0002
	Sordariomycetes	Coniochaetales	Coniochaetaceae	Coniochaeta	Coniochaeta_ taeniospora	0.3487	0.0081
	Dothideomycetes	Capnodiales	Teratosphaeriaceae	Devriesia	Devriesia_ tardicrescens	0.3478	0.0429
	Eurotiomycetes	Eurotiales	Aspergillaceae	Aspergillus	Aspergillus_ nidulans	0.3097	0.0278
Short rotation 0-20cm	Agaricomycetes	Agaricales				0.5976	0.0006
Short rotation 20-60cm	NA	NA	NA	NA	NA	NA	NA
Short rotation 40-60cm	NA	NA	NA	NA	NA	NA	NA

Appendix 9. Sequences used to produce standard curves

Table A 9.1. Sequences used for Gblock standards in this study. Yellow highlights indicate the forward and reverse compliment primers. The red and blue sequences at the 3' and 5' end are randomly generated sequences with 50:50 GC content.

Target	Accession number	GBlock Sequence (5'-3')
Bacteria 16S rRNA gene	**MK085084.1**	TgcatgatctacgtgcgtcacatgcagtacACTCCTACGGGAGGCAGCAG TGGGGAATATTGGACAATGGGGGAAACCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTT CGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGTTGTTGGCTAATACCCAGCAATTT TGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAAT cactagctcagattcagtagaccgctgttg
Fungi 18S rRNA gene	**MZ330851.1**	tgcatgatctacgtgcgtcacatgcagtacTTAGCATGGAATAATAGAATAGGA CGTGCGGTTCTATTTTGTTGGTTTCTAGGACCGCCGTAATGATTAATAGGGATAG TCGGGGGCGTCAGTATTCAGCTGTCAGAGGTGAAATTCTTGGATTTGCTGAAGAC TAACTACTGCGAAAGCATTCGCCAAGGATGTTTTCATTAATCAGGGAACGAAAGT TAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTATGCCGA CTAGGGATCGGACGGTGTTTCTATTATGACCCGTTCGGCACCTTACGAGAAATCA AAGTTTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGAAATTG ACGGAAGGGCACCACCAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGG GGAAACTCACCAGGTCCAGAcactagctcagattcagtagaccgctgttg
AOA amoA Archaea Ammonia monooxygenase	**MW937510.1**	tgcatgatctacgtgcgtcacatgcagtacGTAATGGTCTGGCTTAGACG ATGTACGCACTACTTATTCATAGTAGTCGTTGCAGTCAACTCAAC CCTGCTTACAATCAACGCAGGAGACTACATCTTCTACACTGACTG GGCATGGACTTCATTTGTCGTGTTCTCAATATCACAGACATTGAT GTTAGTCGTAGGTGCAACCTACTATCTAACATTCACTGGAGTTCC AGGAACCGCAACATACTACGCGCTTATTATGACCGTGTATACATG GGTCGCAAAAGGCGCTTGGTTTGCACTCGGTTACCCATATGACTT CATTGTTACACCAGTTTGGTTGCCATCAGCAATGTTGCTTGATCT GGCATACTGGGCGACAAAGAAGAACAAGCACTCACTGATACTCTT CGGTGGTGTACTGTGTGGAATGTCACTGCCATTGTTCAACATGGT CAATCTAATTACCGTGGCTGATCCATTGGAGACTGCATTCAAATA TCCAAGACCAACTTTGCCTCCATACATGACTCCTATAGAACCCCA AGTGGGCAAATTCTATAACAGTCCAGTTGCACTCGGTGCAGGCGC AGGTGCTGTATTAGCATGTACCTTCGCCGCTCTCGGATGTAAGCT GAACACATGGACATACAGATGGATGGCCGC cactagctcagattcagtagaccgctgttg
AOB amoA Bacteria Ammonia monooxygenase	**MN061768.1**	tgcatgatctacgtgcgtcacatgcagtacGGGGTTTCTACTGGTGGT CACACTACCCAATTAACTTTGTGACTCCATCCATCATG CTCCCAGGTGCATTGATGCTGGATATCACCCTGTATCT GACACGTAACTGGCTGGTAACCGCATTGGTTGGTGGT GGATTCTTCGGTTTATTCTTCTATCCAGGCAACTGGG TAATTTTTGGACCAACCCACTTGCCAGTTGTTGTTGA AGGCGTATTGCTATCAATGGCTGACTACATGGGGCAC CTCTACATCCGTACAGGTACACCGGAATATGTAC GCTTGATTGAGCAAGGTTCATTGCGTACCTTTGGTG GTCACACCACAGTGATTGCTGCATTCTTTGCAGCGT TCGTATCCATGCTGATGTTTGTTGTTTGGTGGTACC TAGGCAAAGTTTACTGTACAGCTTTCTTCTACGTTA AAGGTAAGAGAGGCCATATTGTGAAAAGAGACGACGT TACAGCGCTTGGTGAAGAAGGCTTTGCAGAGGGG cactagctcagattcagtagaccgctgttg
NirS Cytochrome cd1 nitrite reductase	**LR134482.1**	tgcatgatctacgtgcgtcacatgcagtacGTCAACGTGAAGGAAACCGG GCAGATCATCCTGGTCGACTACACCGATCTGAAGAACCTCAAGACCACCA CCATCGAGTCGGCCAAGTTCCTCCATGACGGCGGCTGGGACTACTCCAAG CGCTACTTCATGGTTGCTGCCAACGCCTCGAACAAGGTCGCTGCGGTCGA TACCAAGACCGGCAAGCTGGCCGCATTGGTCGACACCGCGAAGATCCCGC ACCCGGGTCGCGGCGCCAACTTCATCCATCCGCAGTTCGGCCCGGTCTGG ACCACCGGTCACCTTGGCGATGACGTGGTCTCGCTGATCTCCACCGCTTC CGACGATCCGAAGTACGCCAAGTACAAGGAGCACAACTGGAAGGTGGTGC AGGAACTGAAGATGCCGGGTGCCGGCAACCTGTTCGTCAAGACCCATCCGAAGTC cactagctcagattcagtagaccgctgttg
NirK Copper-containing nitrite reductase	**AF114787.1**	tgcatgatctacgtgcgtcacatgcagtacATCATGGTGCTGCCGCGC GACGGACTGAAGGACGAGAAGGGCCAGCCGCTGACG TACGACAAGATCTACTATGTCGGCGAGCAGGACTTCT ACGTGCCGAAGGACGAGGCCGGGAACTACAAGAAGT

		ACGAAACCCCCGGCGAAGCCTATGAAGATGCTGTCA AGGCGATGCGCACGCTGACCCCGACCCACATCGTCT TCAACGGTGCGGTCGGCGCGCTGACCGGCGACCATG CTTTGACTGCGGCCGTGGGCGAGCGTGTGCTCGTCG TCCATTCGCAGGCCAACCGCGATACGCGGCCGCACC TGATCGGCGGGCATGGTGACTATGTCTGGGCGACCG GCAAGTTCCGCAACCCGCCGGATCTCGACCAGGAAA CCTGGCTCATTCCGGGCGGAACCGCGGGCGCTGCCT TCTACACCTTCCGCCAGCCGGGTGTGTACGCCTACGT CAACCACAACCTGATCGAGGCcactagctcagattcagtagaccgctgttg
NifH Nitrogenase	**KC445685.1**	tgcatgatctacgtgcgtcacatgcagtacAAAGGCGGAATCGGTAAGTCCAC CACGTCCCAAAACACCCTGGCAGCTTTGTCTGACCTGGGTCAAAAAATCTTGAT CGTCGGATGCGATCCCAAAGCTGACTCCACACGTCTGATTTTGCACGCAAAGGC ACAGGACACGATTCTGTCTCTCGCTGCTGAAGCCGGTTCTGTGGAAGATCTGGA ACTCGAAGACGTGATGAAGATTGGTTACAAAAACATCCGTTGCGTCGAATCCGG TGGCCCAGAGCCAGGCGTTGGTTGTGCTGGCCGCGGTGTGATCACCTCGATCAA CTTCCTGGAAGAAAACGGCGCTTATGACGGCGTGGACTACGTGTCTTACGACGT GTTGGGTGACGTGGTGTGTGGCGGCTTCGCCATGCCCATCCGCGAAAACAAGGC GCAAGAAATCTACATCGTCATGTCTGGCGAAATGATGGCCATGTATGCCGCGAACAA cactagctcagattcagtagaccgctgttg
chiA Chitinase	**CP068050.1**	tgcatgatctacgtgcgtcacatgcagtacCGTCGACATCGACTGGGAATTCCCC GGCGGCAAGGGCGCGAATGCCTCGCTCGGCGATCCGCTCAAGGACGG CCCGCTCTACGTGACGCTGATGAAGGAGCTGCGCCAAATGCTCGACC GGCTGTCCCGCGACACCGGCAAGTCCTACCAGCTGACGTCCGCGATC GGCTCCGGCGACGACAAGATCGCCGTGGTCGACTACCGGGAGGCGTC GAAGTACATGGACTACATCTTCGACATGAGCTACGACTTTTACGGCG CCTGGAGCATGAGCGACCTCGGCCACCAGACGGCGCTCAACGCCCCG GCCTGGCGTCCGGACACGGCCTACACCACGGCCAATTCGGTGAAGGC GCTGCTCGCCCAGGGCGTGAAGCCGGGCAAGATCGTGGTGGGCGCGG CCATGTACGGGCGCGGCTGGACCGGCGTcactagctcagattcagtagaccgctgttg

Appendix 10. Standard curves

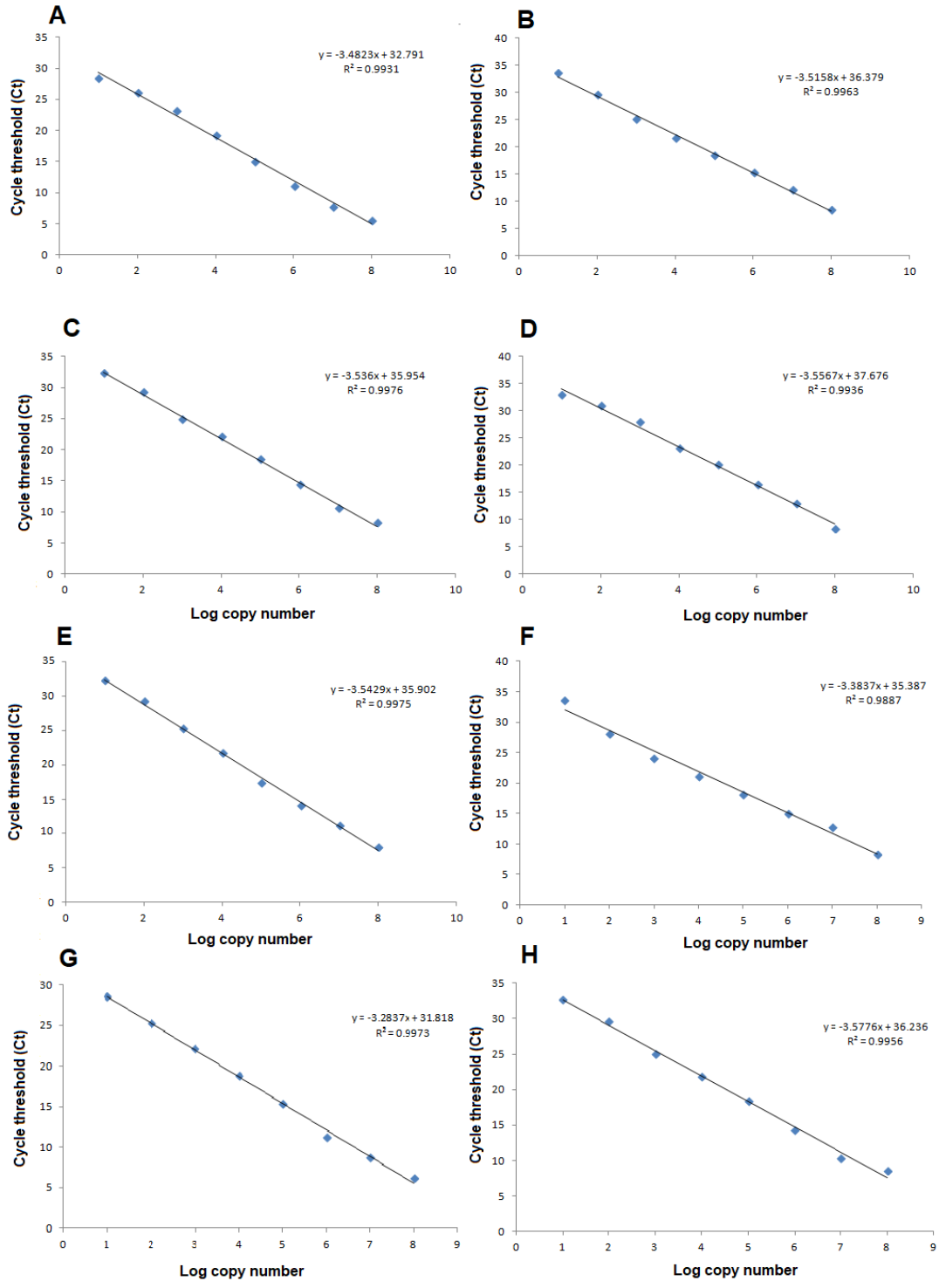


Fig A 10.1. Standard curves generated from the Gblocks for measuring the abundance of bacteria, fungi and N cycling genes. **(A)** Bacterial 16S, **(B)** Fungal 18S, **(C)** *AOA- amoA*, **(D)** *AOB- amoA*, **(E)** *nirS*, **(F)** *nirK*, **(G)**, *nifH*, **(H)** *chiA*.