Science of the Total Environment 956 (2024) 177318

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Effects of burning on vegetation, soil physicochemistry and prokaryotic microbial communities in surface and subsurface peat

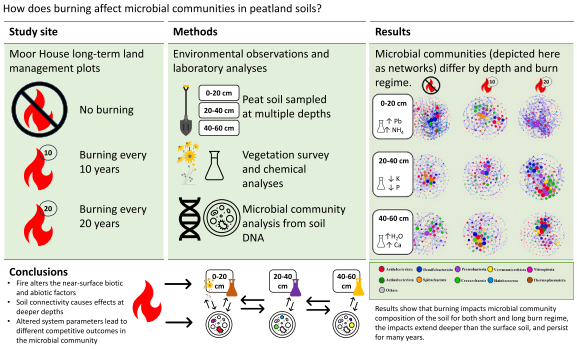
Shaun M. Allingham [a,b,c,*], Samantha J. Drake [b], Andrew Ramsey [d], Chris D. Field [e], Felix C. Nwaishi [f], David R. Elliott [b]

[a] School of Biosciences, The University of Nottingham, Sutton Bonington, Loughborough LE12 5RD, UK
[b] Nature-based Solutions Research Centre, University of Derby, Derby DE22 1GB, UK
[c] NERC Environmental Omics Facility, School of Biosciences, University of Sheffield, Western Bank, Sheffield S10 2TN, UK
[d] Buglife Scotland, Unit 4, Beta Centre, Stirling University, Innovation Park, Stirling FK9 4NF, UK
[e] Department of Natural Sciences, Manchester Metropolitan University, Chester Street, Manchester M1 5GD, UK
[f] Department of Earth and Environmental Sciences, Mount Royal University, 4825 Mt Royal Gate SW, Calgary, AB T3E 6K6, Canada

HIGHLIGHTS

- Prescribed burning altered aboveground vegetation and soil properties altering the structure of prokaryotic communities.
- Co-occurrence networks show positive links in non-burn topsoil and antagonistic links in short-rotation burn treatments.
- Prokaryotic communities show unique indicator species after prescribed burns, helping estimate the site's burning history.
- The study highlights the potential impact on soil biogeochemical processes mediated by prokaryotic communities in peatlands.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Damia Barcelo

Keywords:
Prescribed burning
Peatlands
Biogeochemical cycles
Prokaryotic community
Co-occurrence network
Soil

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 structure. How burning affects prokaryotic community composition across different soil profiles is not well understood. This study explored the effects of prescribed burning on the diversity of prokaryotic 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 non-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 and soil depth. Archaeal community structure was shaped by NH_4^+ and pH in the topsoil; by Pb, moisture and Al in the 20–40 cm profile; and by total N, total C, Al, Ca, Fe and pH in the 40–60 cm profile. Bacterial community structure was shaped by NH_4^+, heather cover, pH and Mg in the topsoil; by Fe, K and Pb in the 20–40 cm profile; and by Al, Ca and Fe in the 40–60 cm

* Corresponding author at: School of Biosciences, The University of Nottingham, Sutton Bonington, Loughborough LE12 5RD, UK.
E-mail address: shaun.allingham@nottingham.ac.uk (S.M. Allingham).

https://doi.org/10.1016/j.scitotenv.2024.177318
Received 17 November 2023; Received in revised form 27 October 2024; Accepted 29 October 2024
Available online 7 November 2024

profile. A co-occurrence network analysis revealed that the topsoil of the non-burn control plots had a 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 20–40 cm profile. The results provide a new insight into the response processes of soil prokaryotic communities to burning in peatland soils, providing valuable knowledge that can support the evaluation and management of ecosystem services in peatlands.

1. Introduction

Peatlands provide a wide range of ecosystem services including sequestration of carbon, providing habitat for 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 of peatlands due to human activities can threaten peatland function which is recognised to be 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 physico-chemical 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, the prevention of wildfires, and the creation of habitat suitable for game birds such as red grouse (*Lagopus lagopus*). Burning regimes play a key role in structuring plant communities and limiting ecological succession (Whitehead et al., 2021), as well as having an impact on above and below-ground C stocks through combustion, and continuous effects on the subsequent 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; Pilkington et al., 2007). Due to the effects on microbial and plant populations, fire can have a long-lasting effect on ecosystem processes. Because soil microbial communities are connected to important soil ecosystem processes, burning may impact the community's ability to withstand future disruptions (Jansson and Hofmockel, 2020). Microbial community responses to fire may therefore influence ecosystem stability and control how an ecosystem changes throughout recovery from a C source to a C sink (Balser et al., 2006). In addition to directly affecting the microbial community and soil environment, fire-induced losses to plant biomass can also have an indirect effect by reducing plant uptake of water and nutrients from the soil, reducing the amount of litter added.

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 (e.g. Andersen et al., 2013; Elliott et al., 2015; Myers et al., 2012; Peltoniemi et al., 2015; Thormann and Rice, 2007). However, many key ecosystem responses occur in the sub-surface soils (Urbanová and Bárta, 2016), which have received much less research attention. 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). As archaea and bacteria play an important role in peatlands, governing soil C cycling, it is important to understand how these communities are affected by land management actions such as prescribed burning throughout different soil profiles.

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). However, 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 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 and 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 and 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 (e.g. 0–10 cm) there is limited knowledge about how these communities interact in the subsoil under prescribed burning. This is because the aim of ‘controlled burns’ is to remove the canopy layer of vegetation without igniting underlying peat. 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 practises on microbial community networks in the topsoil and subsoil.

This research 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) identify key microbial taxa which are indicators or 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 bacterial and archaeal alpha diversity between burn treatments and different soil profiles. It is expected that the diversity of bacteria and archaea will be greater in unburned plots due to the lack of disturbance and greater variety of microsites providing more available niches as a result of the differences in vegetation and the influences on soil parameters mediated by fire; (2) The community composition of archaea and bacteria communities will significantly change across different burn regimes and soil depths due to changes in soil environmental conditions; (3) Environmental characteristics across different burn treatments shape the

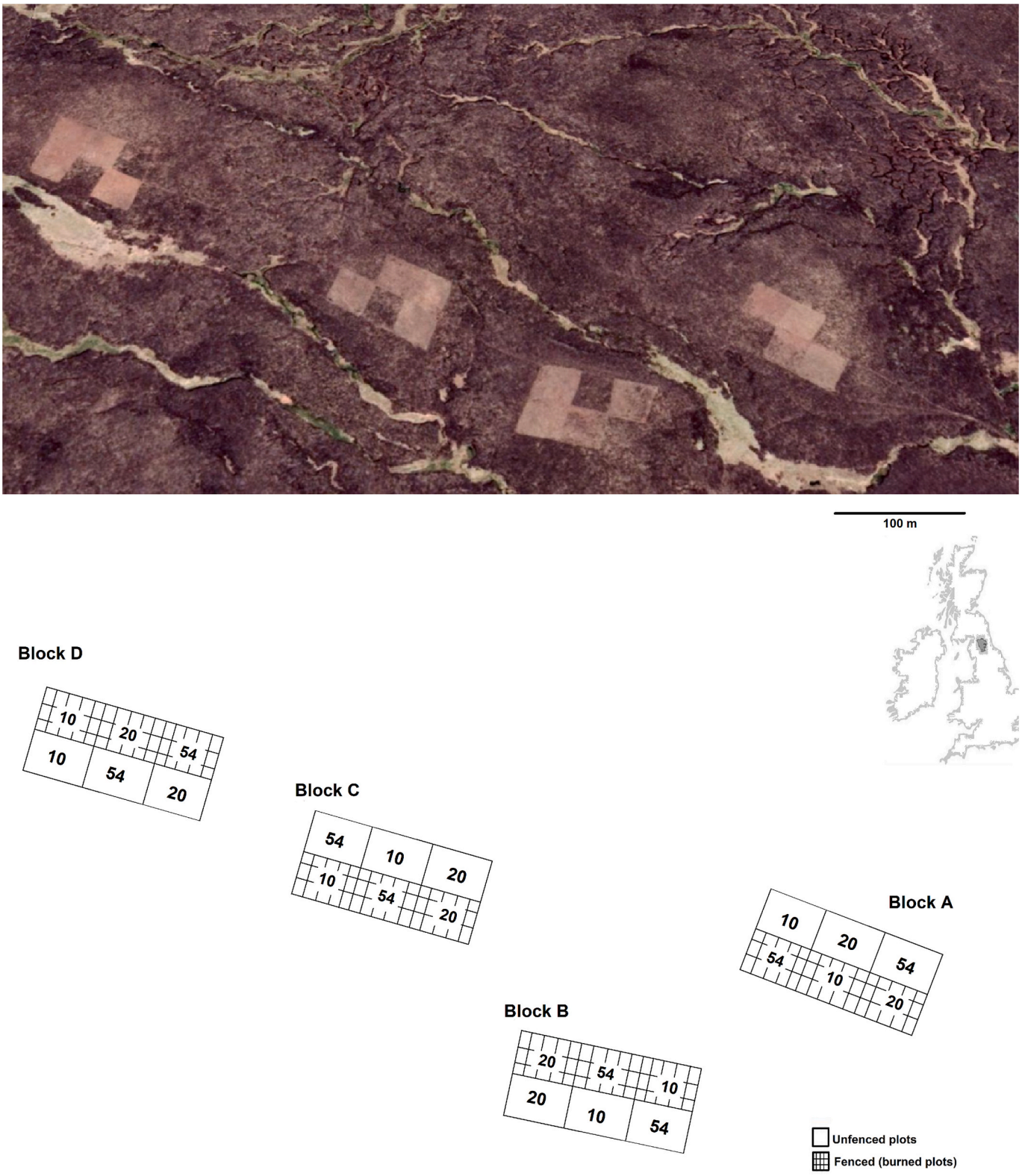


Fig. 1. Map of the Moor house Nature Reserve's experimental plots used for this study (54 = Not burned since 1954), 20 = Long rotation (Burned every 20-years), 10 = Short rotation (Burned every 10-years). All plots used in this study were fenced to exclude grazing.

variation of microbial community structure; (4) 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.

2. Materials and methods

2.1. Study site, experimental design and soil physicochemical measurements

Details of the study site, experimental design and soil physicochemical measurements used in this study were previously described by Allingham et al. (2024). Briefly, the study was conducted in July 2020 at the Hard Hill long-term burning experiment (54°43′N 2°23′W) (Fig. 1). In the North Pennines of the United Kingdom, on a blanket peatland region at Moor House-Upper Teesdale National Nature Reserve (NNR). The long-term monitoring program was started in 1954. The climate is cold and wet with January and July mean temperatures of 0.9 °C and 12.2 °C respectively and an average annual precipitation of 2054 mm (ECN, 25-year means, www.ecn.ac.uk).

The experimental blocks are divided into four blocks, each with six 30 m × 30 m plots. Half of each block (three plots) are fenced to exclude grazing, and three rotational burning treatments are subsequently replicated in both halves - burned every ten years (short-rotation, most intensively burned), burned every twenty years (long-rotation, intermediate burn) and unburned since 1954 (non-burn). All of the different burn and control samples in this study were taken from the fenced (grazing exclusion) plots. Since the experimental plots were set up in 1954, the short rotation plots have been burned seven times, and the long rotation plots four times. These fires have been used to track the responses of plant communities to prescribed burning (e.g. Lee et al., 2013; Milligan et al., 2018; Noble et al., 2018; Noble et al., 2019). The disadvantage of this design is that different treatments have different numbers of fires and intervals between fires. This is because the long rotation and short rotation treatments were both last burned in 2017 (Clutterbuck et al., 2020), the non-burn plots have not been burned since 1954. Thus, burn "regime" rather than burning frequency is most appropriately attributed to treatment effects.

Four repeats of each fenced prescribed burning regime were set up. Three quadrats (1 m × 1 m) were thrown randomly into each treatment per block ($n = 36$). The percentage of vegetation cover in each quadrat was calculated. The plants were divided into groups based on their morphology, such as heather, graminoid, other 'non-Sphagnum' moss and other vascular plants. Next, using a 10 mm diameter Haglöf Soiltax soil sampler, five soil samples were taken vertically from each quadrat across three depth profiles (0–20 cm, 20–40 cm, and 40–60 cm). Samples were collected for chemical and microbiological analyses from the quadrat's corners and the centre. Twelve samples for each treatment and depth (a total of 108 samples) were gathered.

2.2. DNA extraction

DNA was extracted using the DNeasy® PowerSoil® kit (Qiagen, Manchester, UK). Due to the low bulk density of peat soil 0.10 g of freeze-dried soil was weighed (rather than 0.25 g) in consultation with the manufacturer. The soil was homogenised and rewetted with 150 μl of nuclease-free water. The manufacturer's instructions were followed, with the addition of a 30 min incubation period at 65 °C following the addition of the C1 lysis buffer and 10 min of vortexing. The extracted DNA was quantified using a Qubit4 Fluorometer (Invitrogen, UK) and stored at −20 °C for subsequent analysis.

2.3. PCR amplification and sequencing

Extracted DNA 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-V8 region of the archaeal 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 20 μl mixture containing 10 μl 2× Qiagen Multiplex PCR master mix, 2 μl forward primer, 2 μl reverse primer, 5 μl of RNase/DNase-free water and 1 μl 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 30 s at 95 °C, 30 s 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 45 s 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 (20 μl) contained 1 μl of Fi5 primer, 1 μl Ri7 primer, 8 μl of product from PCR 1 and 10 μl of Qiagen multiplex master mix. The following program was used: 95 °C for 15 min followed by 12 cycles of 98 °C for 10 s, 65 °C for 30 s and 72 °C for 30 s. Following the second PCR, a FLUOstar Optima (Promega) was used to measure 2 μl of product from each reaction. Based on these results samples were standardized 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 12 K 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 × 250 bp paired-end sequencing (Magoč and Salzberg, 2011) at the Centre for Genomic Research University of Liverpool. The raw reads were deposited into the National Center of Biotechnology Information (NCBI) Sequence Read Archive (SRA) database under the BioProject accession number PRJNA1002628.

2.4. 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. 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 (Fig. S1), indicating that sufficient sequencing depth was achieved. 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 interpretation (McMurdie and 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 analyses. 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 and Gallagher, 2001).

2.5. Statistical analyses

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) 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 was 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 and 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 is 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 and Peck (1992). RDA analysis was performed on topsoil (0–20 cm) and subsoil (20–40 cm and 40–60 cm) 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).

2.6. 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) was classified and uploaded according to the MENA format. The Pearson's correlation of any two ASVs was 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. The topological roles of each node were evaluated by the threshold values of Z_i and Pi (Guimera and Amaral, 2005). Node topologies were categorized by the following: (1) $Z_i > 2.5$ = 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).

3. Results

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 ($M = 47.66$ cm, $SD = 8.55$) followed by the long rotation ($M = 33.66$ cm, $SD = 5.74$) and short rotation (M = 23.75 cm, $SD = 8.00$) treatments. The percentage of *Sphagnum* moss cover also showed a significant difference between burn treatments and was highest in plots under a short rotation burn ($M = 31.66$ %, $SD = 20.61$) ($F = 5.67$, $P \leq 0.0001$). 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 ($M = 3.25$ %, $SD = 1.95$) ($F = 45.99$, $P \leq 0.0001$). Percentage of heather cover was significantly higher in non-burn control plots (69.00 % ± 12.00) and lowest in plots under short rotation regimes ($M = 1.91$ %, $SD = 0.96$) ($F = 99.48$, $P \leq 0.0001$). 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. 2).

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. S2. The first and second principal components (DIM1 and DIM2) explained 37.2 % and 27.6 % respectively in the 0–20 cm profile, 36.7 % and 22 % in the 20–40 cm profile and 31.8 % and 21.2 % in the 40–60 cm profile. In the 0–20 cm 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,

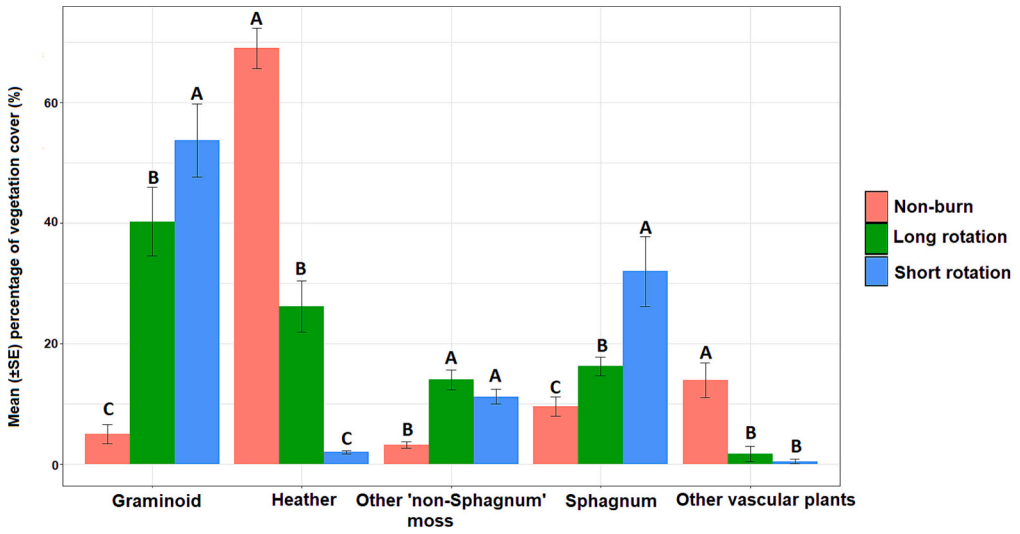


Fig. 2. Percentage cover of graminoids, Heather, other 'non-*Sphagnum*' moss, *Sphagnum* and other vascular plants ($n = 36$). Different letters indicate significant pairwise differences using Tukey's HSD *post-hoc* analysis at a confidence level of 95 % ($P < 0.05$).

NH_4^+, Mn, Total C, Mg, Pb and Cu. In the 20–40 cm profile the first axis correlated with Fe, Ca, Al, P and Cu generally associated with the long rotation regime. In the 20–40 cm 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 40–60 cm 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 (Fig. S2). In PC1, Pb showed a higher loading value (−0.36) while in PC2, Fe showed a higher loading value (−0.42). In the 20–40 cm profile Pb (−0.38) showed a higher loading in PC1 and Fe in PC2 (−0.46) respectively (Table S1). Likewise, in the 40–60 cm profile Fe showed the highest loading value (0.41) in PC1 while in PC2 Al showed the highest loading value (0.36) (Table S1).

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. 3, Table S2). 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. 3, Table S2). The content of K was significantly different in between burn regimes and depth being higher in the topsoil. The content of K was higher in the non-burn control in the 20–40 cm and 40–60 cm depth profiles. Al was significantly different across burn treatments being highest in the long rotation burned plots (Fig. 3, Table S2).

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. The three most dominant archaeal phyla across all samples were Crenarchaeota (36 %), followed by Thermoplasmatota (35 %) and Halobacterota (29 %) (Fig. 4). The phyla, Asgardarchaeota, Euryarchaeota and Micrarchaeota were present in very low abundance representing <1 % across all samples. Acidobacteriota was the most abundant bacterial phylum overall at 48 % followed by Desulfobacterota (22 %) and Proteobacteria (14 %) (Fig. 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 %) (Fig. 4). Acidobacteriota was the most abundant bacterial phylum in the topsoil overall being highest in plots under a long rotation regime (59 %) (Fig. 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 (40–60 cm) 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). 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 40–60 cm profile (Fig. 4). In the 20–40 cm profile, the bacterial phylum Acidobacteriota was the most dominant phylum overall (54 %) followed by Desulfobacterota (27 %) and Proteobacteria (8 %). In the 40–60 cm profile Proteobacteria increased (22 %) in plots under a short rotation regime (Fig. 5).

The relative abundance of Acidobacteriota was higher in the long rotation burned plots across all depth profiles (ANOVA, $P \leq 0.05$) (Fig. 5), whereas Desulfobacterota was higher in the non-burn control across all three depth profiles (ANOVA, $P \leq 0.05$) (Fig. 5). In addition, the relative abundance of Proteobacteria was higher in the short rotation in subsoil profiles (Fig. 5).

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 (Fig. 6; Table S3). Likewise, there was a significant two-way interaction between burn treatment and soil depth on observed, Shannon and Simpson diversity for bacterial communities (Fig. 6; Table S4).

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, $F = 9.27$, $R^2 = 0.154$, $P \leq 0.001$) and soil depth (PERMANOVA, $F = 8.42$, $R^2 = 0.143$, $P \leq 0.001$). The first axis explained 33.5 % of variance and the second axis explained 24.9 % (Fig. 7A). Likewise, there was clear variation in bacterial communities across different burn treatments (PERMANOVA, $F = 7.90$, $R^2 = 0.131$, $P \leq 0.001$) and soil depth (PERMANOVA, $F = 11.17$, $R^2 = 0.176$, $P \leq 0.001$). The first axis explained 36.6 % of variance and the second axis explained 17.6 % of the variance respectively (Fig. 7B).

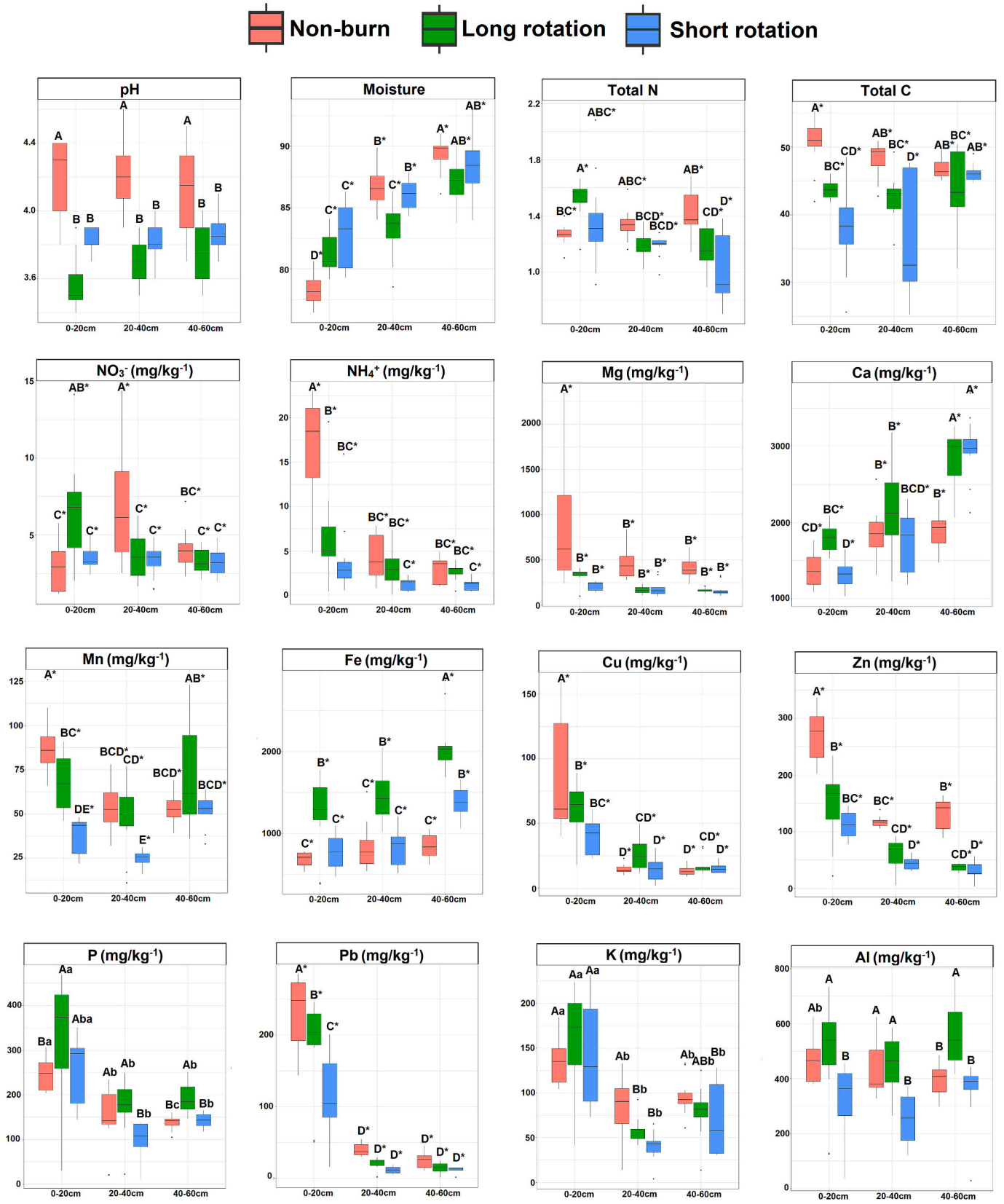


Fig. 3. Two-way ANOVA of soil physicochemical properties across three different soil depths under three burn treatments. Result is reported as the mean ± SE (n = 12). The data in bold indicate soil properties that were affected by soil depth, burn treatment and their interaction at a confidence level 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, $P < 0.05$).

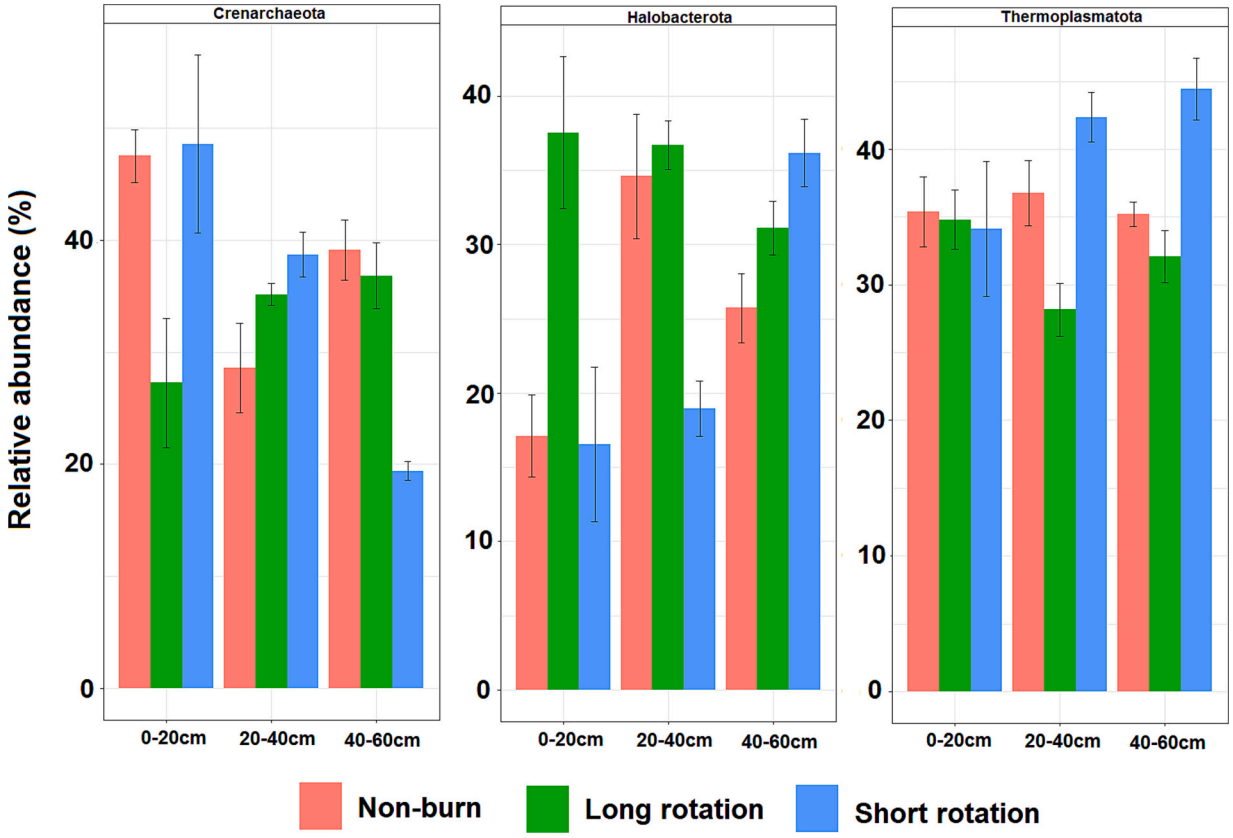


Fig. 4. Relative abundances of the top 3 archaeal phyla across three different soil depths under three burn treatments. The bars indicate the mean values of each treatment, with the error bars representing the standard error. Non-burn 0–20 cm ($n = 11$), non-burn 20–40 cm ($n = 12$), non-burn 40–60 cm ($n = 12$), long rotation 0–20 cm ($n = 12$), long rotation 20–40 cm ($n = 10$), long rotation 40–60 cm ($n = 12$), short rotation 0–20 cm ($n = 12$), short rotation 20–40 cm ($n = 12$), short rotation 40–60 cm ($n = 11$).

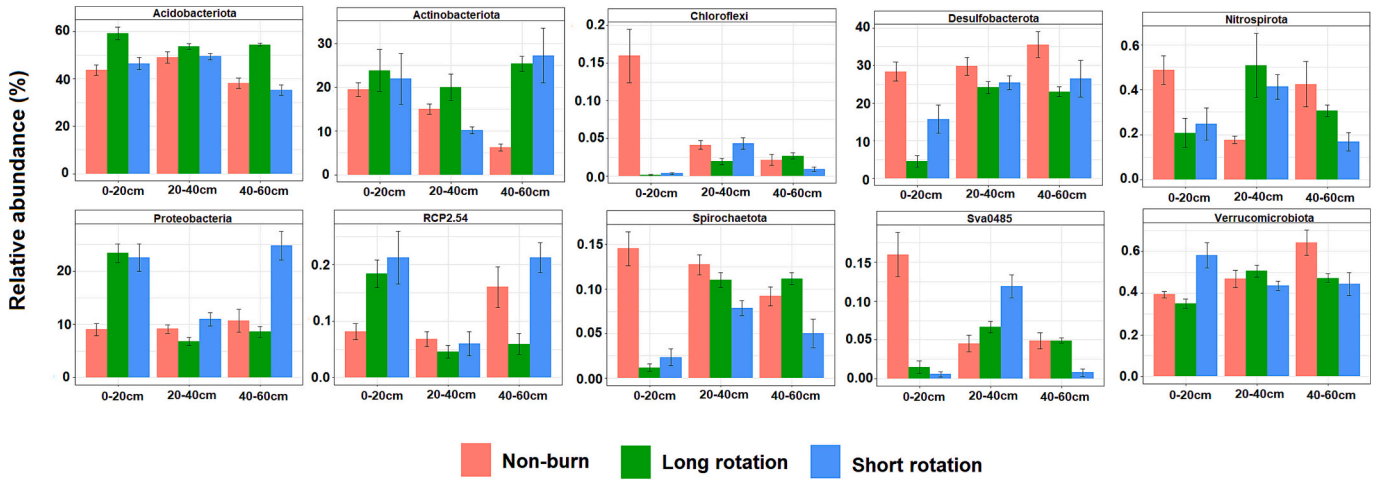


Fig. 5. Relative abundance of the top 10 bacterial phyla across three different soil depths under three burn treatments. The bars indicate the mean values of each treatment, with the error bars representing the standard error. Non-burn 0–20 cm ($n = 12$), non-burn 20–40 cm ($n = 12$), non-burn 40–60 cm ($n = 12$), long rotation 0–20 cm ($n = 12$), long rotation 20–40 cm ($n = 11$), long rotation 40–60 cm ($n = 12$), short rotation 0–20 cm ($n = 12$), short rotation 20–40 cm ($n = 12$), short rotation 40–60 cm ($n = 12$).

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. 8). Important variables that influenced archaeal communities were NH_4^+ and pH in the topsoil, Pb, moisture and Al in the 20–40 cm profile and total N, total C, Al, Ca, Fe and pH in the 40–60 cm profile. All final models were significant ($P \leq 0.05$) (Fig. 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 20–40 cm

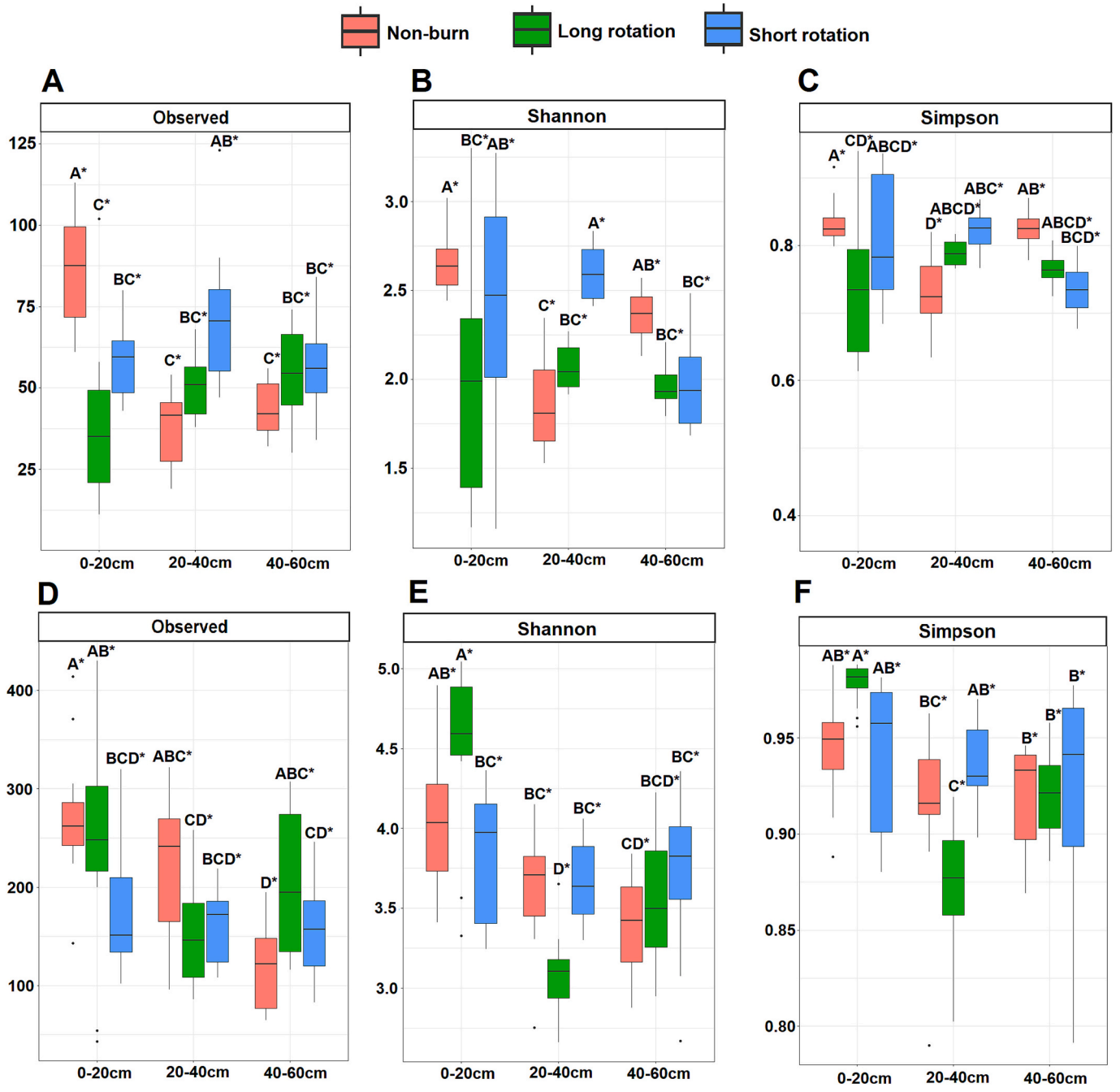


Fig. 6. Diversity indices for archaea observed richness **(A)**, Shannon index **(B)** and Simpson index **(C)**. Non-burn 0–20 cm ($n = 11$), non-burn 20–40 cm ($n = 12$), non-burn 40–60 cm ($n = 12$), long rotation 0–20 cm ($n = 12$), long rotation 20–40 cm ($n = 10$), long rotation 40–60 cm ($n = 12$), short rotation 0–20 cm ($n = 12$), short rotation 20–40 cm ($n = 12$), short rotation 40–60 cm ($n = 11$). Bacteria observed richness **(D)**, Shannon index **(E)** and Simpson index **(F)**. Non-burn 0–20 cm ($n = 12$), non-burn 20–40 cm ($n = 12$), non-burn 40–60 cm ($n = 12$), long rotation 0–20 cm ($n = 12$), long rotation 20–40 cm ($n = 11$), long rotation 40–60 cm ($n = 12$), short rotation 0–20 cm ($n = 12$), short rotation 20–40 cm ($n = 12$), short rotation 40–60 cm ($n = 12$) 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, $P < 0.05$).

profile and Al, Ca and Fe in the 40–60 cm profile. All final models were significant ($P \leq 0.05$) (Fig. 8 D–F).

3.5. Indicator analysis

Archaeal indicators for each treatment represented seven classes (Table S5). The number of indicators varied widely across burn treatment and soil depth with non-burn topsoils having six indicators while there were also six significant indicators in the long rotation subsoil (20–40 cm) and short rotation burns contained four indicators. One indicator from the class Methanosarcina was found in the short rotation 40–60 cm profile. No archaeal indicators were detected in non-burn subsoils, long rotation topsoil, long rotation 40–60 cm profile or short rotation 20–40 cm profile (Table S5). Likewise, bacterial indicators for each burn treatment represented twenty-nine classes (Table S6). 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

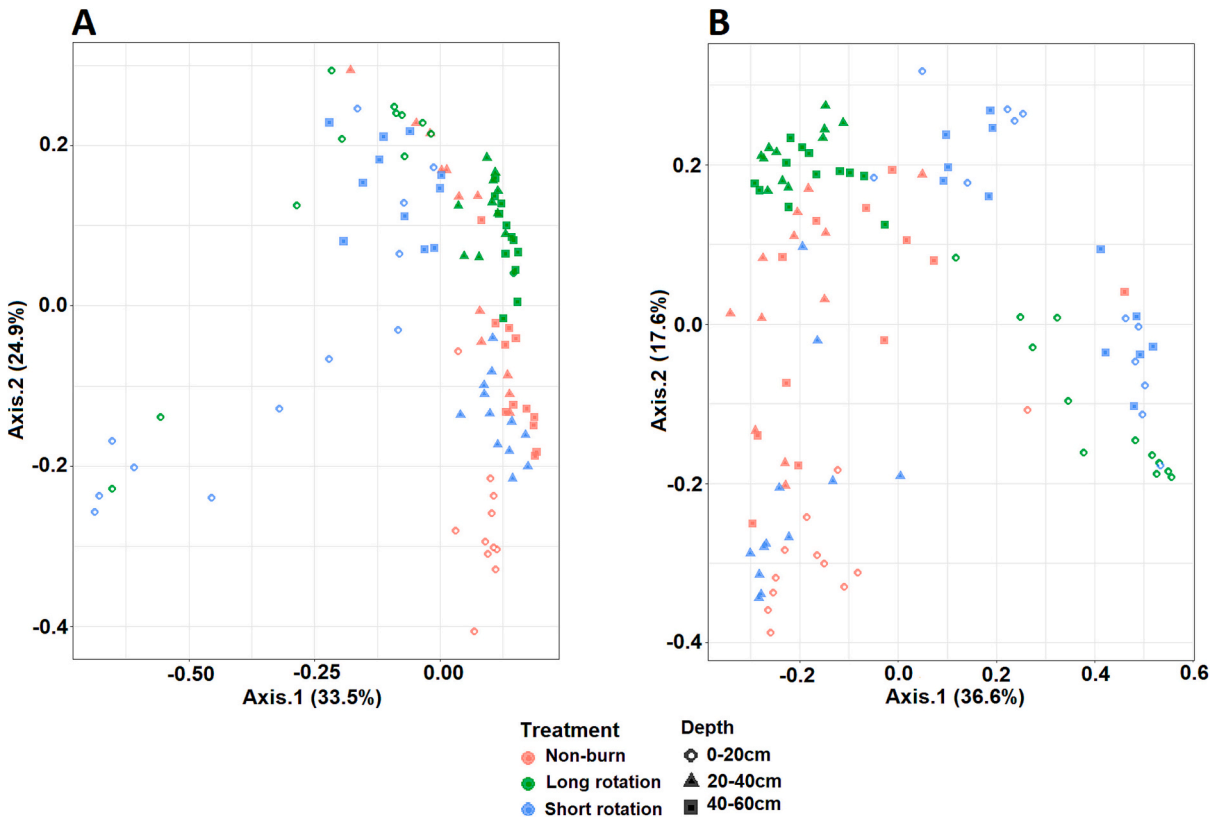


Fig. 7. Principal coordinates analysis of the archaeal communities **(A)**. Non-burn 0–20 cm ($n = 11$), non-burn 20–40 cm ($n = 12$), non-burn 40–60 cm ($n = 12$), long rotation 0–20 cm ($n = 12$), long rotation 20–40 cm ($n = 10$), long rotation 40–60 cm ($n = 12$), short rotation 0–20 cm ($n = 12$), short rotation 20–40 cm ($n = 12$), short rotation 40–60 cm ($n = 11$) and bacterial communities **(B)**. Non-burn 0–20 cm ($n = 12$), non-burn 20–40 cm ($n = 12$), non-burn 40–60 cm ($n = 12$), long rotation 0–20 cm ($n = 12$), long rotation 20–40 cm ($n = 11$), long rotation 40–60 cm ($n = 12$), short rotation 0–20 cm ($n = 12$), short rotation 20–40 cm ($n = 12$), short rotation 40–60 cm ($n = 12$) 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 0–20 cm, triangle for 20–40 cm and square for 40–60 cm.

indicators. The subsoil of each burn treatment had fewer indicator species overall (Table S6). 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 species. Plots under a short rotation regime had fewer indicator species from classes Bacteroidia and Alphaproteobacteria (Table S6).

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. 9; Table 1). 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 1). The long rotation 20–40 cm layer had a higher average connectivity but with more negative links (Table 1). 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.

3.7. Module hubs and connectors

Connectivity within and between modules was used to infer potential roles of each node in the networks. One of four topological roles (peripherals, module hubs, network hubs or connectors) could be assigned to each node. 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 recognised 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. S3). 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. S3). 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.S3). 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. S3).

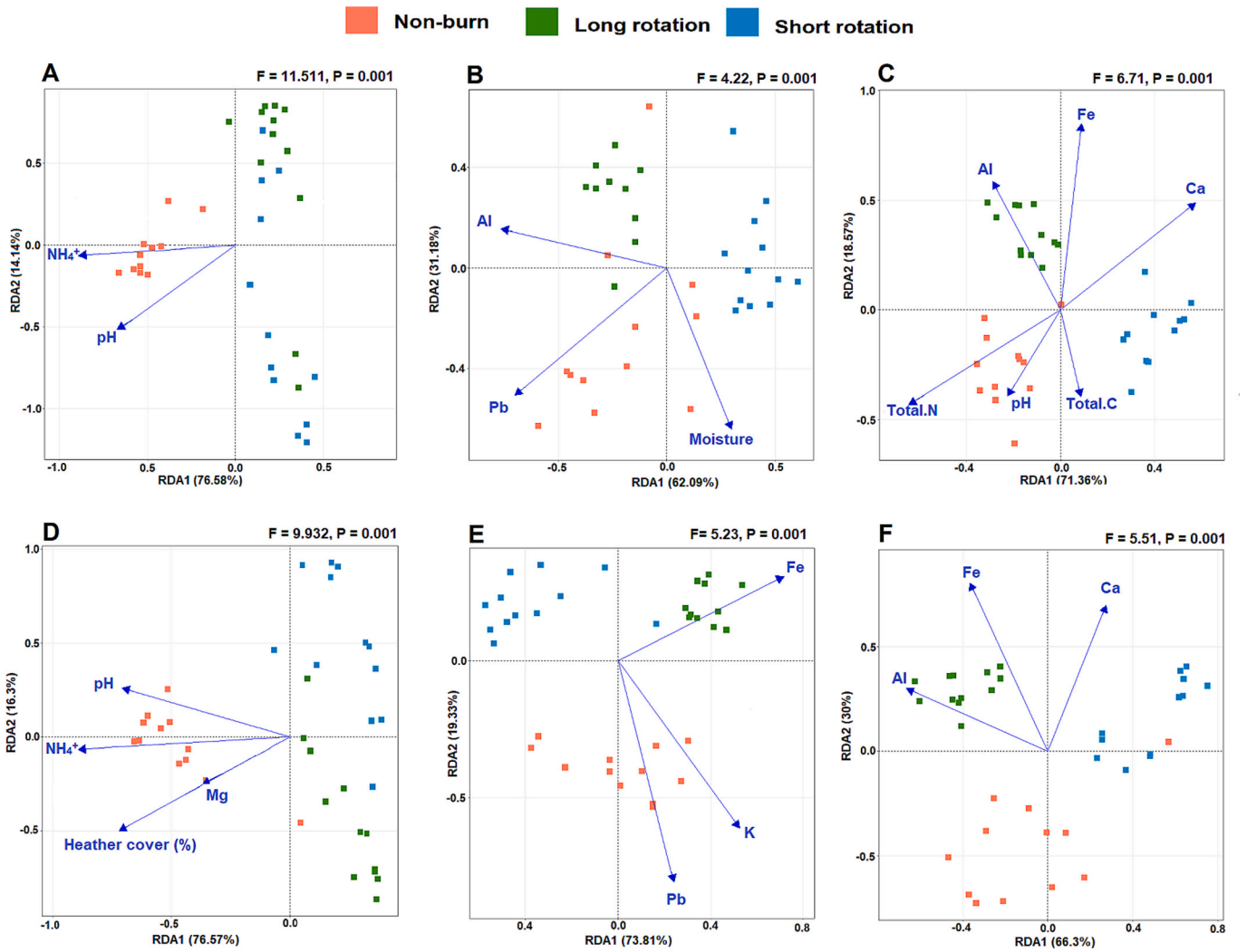


Fig. 8. RDA ordination plots showing soil related drivers of archaeal communities **A** = topsoil (0–20 cm), **B** = subsoil (20–40 cm), **C** = subsoil (40–60 cm). Non-burn 0–20 cm ($n = 11$), non-burn 20–40 cm ($n = 12$), non-burn 40–60 cm ($n = 12$), long rotation 0–20 cm ($n = 12$), long rotation 20–40 cm ($n = 10$), long rotation 40–60 cm ($n = 12$), short rotation 0–20 cm ($n = 12$), short rotation 20–40 cm ($n = 12$), short rotation 40–60 cm ($n = 11$) and bacterial communities **D** = topsoil (0–20 cm), **E** = subsoil (20–40 cm), **F** = subsoil (40–60 cm). Non-burn 0–20 cm ($n = 12$), non-burn 20–40 cm ($n = 12$), non-burn 40–60 cm ($n = 12$), long rotation 0–20 cm ($n = 12$), long rotation 20–40 cm ($n = 11$), long rotation 40–60 cm ($n = 12$), short rotation 0–20 cm ($n = 12$), short rotation 20–40 cm ($n = 12$), short rotation 40–60 cm ($n = 12$) collected at three different depths under three burn treatments. Only significant variables ($P \leq 0.05$) are shown. Different colours indicate three sampling treatments. The ASV data were standardized with Hellinger transformation using the *Vegan* package.

4. Discussion

This study demonstrates that prescribed burning is a strong driver of plant cover percentage, soil physicochemistry and soil microbial community composition. The diversity of archaea and bacteria was reduced under a short rotation burn regime. The effects of prescribed burning on vegetation cover, soil physicochemistry and prokaryotic communities are strongly evident in the top soil (0–20 cm) and also in the sub soil (20–40 cm, 40–60 cm) but to a lesser extent. The study found that the effects of prescribed burning on archaeal and bacterial communities are significant and that there are associated changes in soil physicochemistry.

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). Thermoplasmatota are moderately thermophilic and mesophilic, growing in a variety of conditions with increased abundance in deeper soils, and are important contributors to C mineralization (Lin et al., 2015). Crenarchaeota were 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 is 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. 5). Acidobacteriota were found to be more abundant in plots under a long rotation burn regime.

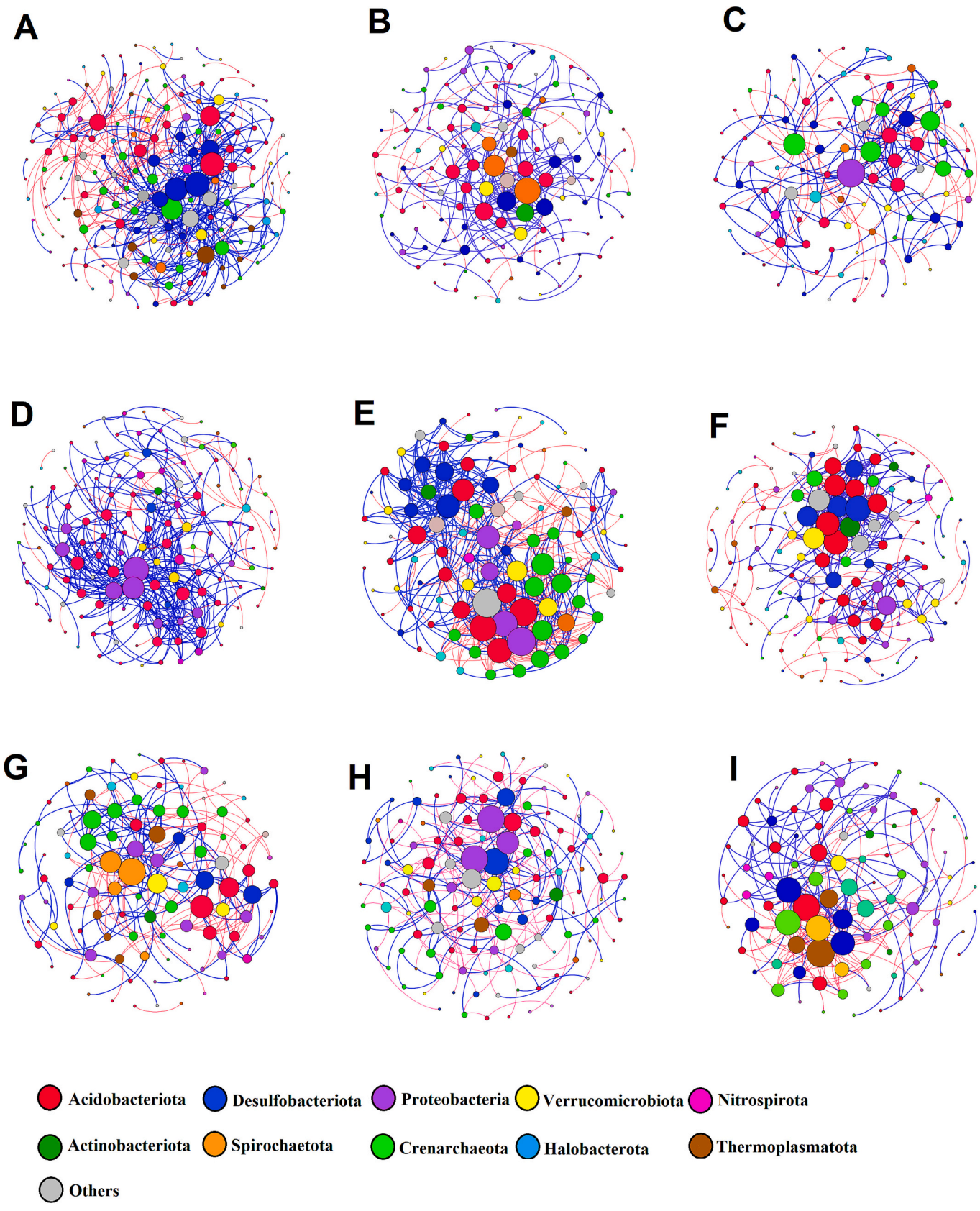


Fig. 9. Overview of networks under three different burn treatments across three soil depths with node size proportional to node connectivity. Nodes are coloured for different phyla. A red link indicates a negative correlation and a blue link indicates a positive correlation. **(A)** Non-burn 0–20 cm, **(B)** Non-burn 20–40 cm, **(C)** Non-burn 40–60 cm, **(D)** Long rotation 0–20 cm, **(E)** Long rotation 20–40 cm, **(F)** Long rotation 40–60 cm, **(G)** Short rotation 0–20 cm, **(H)** Short rotation 20–40 cm, **(I)** Short rotation 40–60 cm.

Table 1
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 0–20 cm	Non-burn 20–40 cm	Non-burn 40–60 cm	Long rotation 0–20 cm	Long rotation 20–40 cm	Long rotation 40–60 cm	Short rotation 0–20 cm	Short rotation 20–40 cm	Short rotation 40–60 cm
Empirical 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

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 the phylum thrive 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. 5). The ability of Proteobacteria to cope with abiotic stress such as desiccation and their fast-growing life strategies is likely the reason for their higher abundance under short rotation burns (Lladó and 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 and Baldrian, 2017; Zachow et al., 2014). Broadly, prescribed burning appears to have increased the abundance of certain archaeal and bacterial taxa that are able to colonize and take advantage of limited nutrients while non-burn plots are associated with slower growing taxa. For example, the phylum Acidobacteria 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 and Scherer, 2012). As a result, taxa within these groups may have a unique adaptation to, or preference for soil conditions.

4.2. Diversity and community composition across burn treatments and depth

In line with the first hypothesis, alpha diversity was affected by burning regime and 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. 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. 6). According to these findings, the majority of the effects of prescribed burning are limited to the topsoil (0–20 cm) and may extend to the lower profile (20–40 cm) in the case of bacteria (Fig. 6D) which is consistent with the findings that prescribed burning had on soil physicochemistry (Fig. S2 & Fig. 3) and may partially be due to the changes in microclimate caused by the effects of vegetation cover (Fig. 2). Community composition for both archaea and bacteria differed significantly with burn treatment and depth (Fig. 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.

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 (20–40 cm and 40–60 cm) and Fe, K, Pb, Al and Ca for bacterial communities in the subsoil (Fig. 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 and Jackson, 2006; Kaiser et al., 2016) 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 elements such as Al, Fe and Ca which affected archaeal and bacterial communities (Fig. 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 that similar edaphic factors impact bacteria and archaea supporting the third hypothesis that archaeal and bacterial communities will be shaped by soil environmental factors.

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., 2021). The main changes in network 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 and Or, 2013). Non-burn soils and the 20–40 cm layer under a long rotation regime had the highest average connectedness and a more complex coupling among microbes. The network from the 40–60 cm profile under a short rotation regime showed the lowest modularity. Modularity indicates how well a network can be subdivided into modules, 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 and Zipper, 2016). It is suggested that an increased microbial network complexity leads to greater stability of the community (Ghoul and Mitri, 2016; Mougi and 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. S3). In soil ecosystems, Proteobacteria is a dominant nitrogen-fixing bacterial phylum (Gaby and 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 and 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 and McMahon, 2018).

4.5. Limitations of the study

This study was based on samples taken from the Hard Hill long-term burning experiment at a single time point in 2020. The Hard Hill experiment did not record certain useful data such as burn severity, fuel load and moisture, however the experiment does represent the common practice for UK heather burning. Sampling at a single time point does not capture the full temporal progression of ecological recovery and successional changes over time, for instance in the burn treatments we observed higher levels of *Sphagnum* moss, but this may not be a stable condition. As the plots recover from burning and the heather (*Calluna vulgaris*) canopy closes over time, it is likely that *Sphagnum* abundance will decrease due to shading and competition; the balance between these species will be determined by the hydrology at the site, with *Sphagnum* preferring wetter sites and *Calluna* drier sites. Furthermore, our study compares plots at different stages of heather development: the burned plots are in a 'building' phase, characterized by a tighter canopy, early successional stages with acrocarpous bryophytes and greater open ground, whereas the unburned plots are in a more 'mature' or 'degenerate' phase, where canopy cover is greater, and vegetation communities including pleurocarpous bryophytes reflect later successional stages. These differences in canopy openness and vegetation structure inherently influence microbial diversity and ecosystem function. Long-term monitoring is necessary to fully understand the trajectory of these ecological changes and their effects on both vegetation and microbial communities over time.

5. 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. 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, and these furthermore differed by depth 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 at least to a depth of 50 cm. The impact is shown for both short and long burn regime, and extends deeper than the surface soil (Ashby and Heinemeyer, 2021), demonstrating that there is a probable

functional impact of burning not only in the surface but also in deeper soil beneath the rooting zone. 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 60 cm. Results show that burning impacts microbial community composition of the soil profile extensively and that these impacts may persist for many years.

CRediT authorship contribution statement

Shaun M. Allingham: Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Samantha J. Drake:** Writing – review & editing, Supervision, Methodology. **Andrew Ramsey:** Writing – review & editing, Supervision, Methodology. **Chris D. Field:** Writing – review & editing, Supervision, Methodology. **Felix C. Nwaishi:** Writing – review & editing. **David R. Elliott:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Special thanks to Natural England for granting access to the Moor House Nature Reserve plots and Martyn Harvey for his assistance in collecting samples in the field. This project was supported by the NERC Environmental Omics Facility, funded by the UK Natural Environment Research Council grant NEOF1272 and the University of Derby Environmental Sustainability Research Centre. We would like to thank the NERC Environmental Omics facility for providing computational resources. We would like to thank David Mckendry for supporting work in the lab at Manchester Metropolitan University, Dr. Gavin Horsburgh for his support in the Omics lab and Dr Kathryn Maher for computational assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.177318.

Data availability

Data will be made available on request.

References

Agler, M.T., Ruhe, J., Kroll, S., Morhenn, C., Kim, S.T., Weigel, D., Kemen, E.M., 2016. Microbial hub taxa link host and abiotic factors to plant microbiome variation. PLoS Biol. 14 (1), e1002352. https://doi.org/10.1371/journal.pbio.1002352.

Allingham, S.M., Drake, S.J., Ramsey, A., Field, C.D., Nwaishi, F.C., Elliott, D.R., 2024. Changes in nitrogen functional genes and microbial populations in soil profiles of a peatland under different burning regimes. Appl. Soil Ecol. 200, 105426. https://doi.org/10.1016/j.apsoil.2024.105426.

Andersen, K.S., Kirkegaard, R.H., Karst, S.M., Albertsen, M., 2018. ampvis2: an R package to analyse and visualise 16S rRNA amplicon data. BioRxiv, 299537. https://doi.org/10.1101/299537.

Andersen, R., Chapman, S.J., Artz, R.R.E., 2013. Microbial communities in natural and disturbed peatlands: a review. Soil Biol. Biochem. 57, 979–994. https://doi.org/10.1016/j.soilbio.2012.10.003.

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 26 (1), 32–46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x.

Ashby, M.A., Heinemeyer, A., 2021. A critical review of the IUCN UK Peatland Programme's "Burning and Peatlands" position statement. Wetlands 41 (5), 1–22. https://doi.org/10.1007/s13157-021-01400-1.

Bahram, M., Hildebrand, F., Forslund, S.K., Anderson, J.L., Soudzilovskaia, N.A., Bodegom, P.M., Bengtsoon-Palme, J., Anslan, S., Coelho, L.P., Harend, H., Huerta-Cepas, J., Medema, M.H., Maltz, M.R., Mundra, S., Olsson, P.A., Pent, M., Põlme, S., Sunagawa, S., Ryberg, M., Tedersoo, L., Bork, P., 2018. Structure and function of the global topsoil microbiome. Nature 560 (7717), 233–237. https://doi.org/10.1038/s41586-018-0386-6.

Balser, T.C., McMahon, K.D., Bart, D., Bronson, D., Coyle, D.R., Craig, N., Flores-Mangual, M.L., Forshay, K., Jones, S.E., Kent, A.E., Shade, A.L., 2006. Bridging the gap between micro-and macro-scale perspectives on the role of microbial communities in global change ecology. Plant Soil 289, 59–70. https://doi.org/10.1007/s11104-006-9104-5.

Barreiro, A., Fontúrbel, M.T., Lombao, A., Martín, A., Vega, J.A., Fernández, C., Carballas, T., Díaz-Raviña, M., 2015. Using phospholipid fatty acid and community level physiological profiling techniques to characterize soil microbial communities following an experimental fire and different stabilization treatments. Catena 135, 419–429. https://doi.org/10.1016/j.catena.2014.07.011.

Bastian, M., 2017. Gephi (Version 0.9. 2)[Software].

Bauer, E., Zimmermann, J., Baldini, F., Thiele, I., Kaleta, C., 2017. BacArena: individual-based metabolic modeling of heterogeneous microbes in complex communities. PLoS Comput. Biol. 13 (5), e1005544. https://doi.org/10.1371/journal.pcbi.1005544.

Blanchet, F.G., Legendre, P., Borcard, D., 2008. Forward selection of explanatory variables. Ecol 89 (9), 2623–2632. https://doi.org/10.1890/07-0986.1.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37 (8), 852–857. https://doi.org/10.1038/s41587-019-0209-9.

Brown, L.E., Holden, J., Palmer, S.M., 2014. Effects of Moorland Burning on the Ecohydrology of River Basins. Key Findings From the EMBER Project. University of Leeds.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13 (7), 581–583. https://doi.org/10.1038/nmeth.3869.

Cary, S.C., McDonald, I.R., Barrett, J.E., Cowan, D.A., 2010. On the rocks: the microbiology of Antarctic Dry Valley soils. Nat. Rev. Microbiol. 8 (2), 129–138. https://doi.org/10.1038/nrmicro2281.

Chen, X., Zhang, Z., Han, X., Hao, X., Lu, X., Yan, J., Biswas, A., Dunfield, K., Zou, W., 2021. Impacts of land-use changes on the variability of microbiomes in soil profiles. J. Sci. Food Agric. 101 (12), 5056–5066. https://doi.org/10.1002/jsfa.11150.

Clark, E.V., Zipper, C.E., 2016. Vegetation influences near-surface hydrological characteristics on a surface coal mine in eastern USA. Catena 139, 241–249. https://doi.org/10.1016/j.catena.2016.01.004.

Clay, G.D., Worrall, F., Aebischer, N.J., 2015. Carbon stocks and carbon fluxes from a 10-year prescribed burning chronosequence on a UK blanket peat. Soil Use Manag. 31 (1). https://doi.org/10.1111/sum.12164, 39-5.

Clutterbuck, B., Lindsay, R., Chico, G., Clough, J., 2020. Hard Hill Experimental Plots on Moor House–Upper Teesdale National Nature Reserve-A Review of the Experimental Set Up (NECR321).

Comeau, A.M., Li, W.K., Tremblay, J.É., Carmack, E.C., Lovejoy, C., 2011. Arctic Ocean microbial community structure before and after the 2007 record sea ice minimum. PLoS One 6 (11), e27492. https://doi.org/10.1371/journal.pone.0027492.

Cooper, J.E., Scherer, H.W., 2012. Nitrogen fixation. In: Marschner, P. (Ed.), Marschner's Mineral Nutrition of Higher Plants, Third edition. Academic Press, San Diego, pp. 389–408.

Couwenberg, J., Thiele, A., Tanneberger, F., Augustin, J., Bărisch, S., Dubovik, D., Liashchynskaya, N., Michaelis, D., Minke, M., Skuratovich, A., Joosten, H., 2011. Assessing greenhouse gas emissions from peatlands using vegetation as a proxy. Hydrobiologia 674 (1), 67–89. https://doi.org/10.1007/s10750-011-0729-x.

de Vries, F.T., Griffiths, R.I., Bailey, M., Craig, H., Girlanda, M., Gweon, H.S., Hallin, S., Kaisermann, A., Keith, A.M., Kretzschmar, M., Lemanceau, P., 2018. Soil bacterial networks are less stable under drought than fungal networks. Nat. Commun. 9 (1), 3033. https://doi.org/10.1038/s41467-018-05516-7.

Deng, Y., Jiang, Y.H., Yang, Y., He, Z., Luo, F., Zhou, J., 2012. Molecular ecological network analyses. BMC Bioinf. 13 (1), 113. https://doi.org/10.1186/1471-2105-13-113.

Du, X., Deng, Y., Li, S., Escalas, A., Feng, K., He, Q., Wang, Z., Wu, Y., Wang, D., Peng, X., Wang, S., 2021. Steeper spatial scaling patterns of subsoil microbiota are shaped by deterministic assembly process. Mol. Ecol. 30 (4), 1072–1085. https://doi.org/10.1111/mec.15777.

Elliott, D.R., Caporn, S.J., Nwaishi, F., Nilsson, R.H., Sen, R., 2015. Bacterial and fungal communities in a degraded ombrotrophic peatland undergoing natural and managed re-vegetation. PLoS One 10 (5), e0124726. https://doi.org/10.1371/journal.pone.0124726.

Elliott, D.R., Thomas, A.D., Strong, C.L., Bullard, J., 2019. Surface stability in drylands is influenced by dispersal strategy of soil bacteria. Eur. J. Vasc. Endovasc. Surg. 124 (11), 3403–3418. https://doi.org/10.1029/2018JG004932.

Evans, C.D., Bonn, A., Holden, J., Reed, M.S., Evans, M.G., Worrall, F., Couwenberg, J., Parnell, M., 2014. Relationships between anthropogenic pressures and ecosystem functions in UK blanket bogs: linking process understanding to ecosystem service valuation. Ecosyst. Serv. 9, 5–19. https://doi.org/10.1016/j.ecoser.2014.06.013.

Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. PNAS 103 (3), 626–631. https://doi.org/10.1073/pnas.0507535103.
Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil bacteria. Ecology 88 (6), 1354–1364. https://doi.org/10.1890/05-1839.
Fontúrbel, T., Carrera, N., Vega, J.A., Fernández, C., 2021. The effect of repeated prescribed burning on soil properties: a review. Forests 12 (6), 767. https://doi.org/10.3390/f12060767.
Fritze, H., Pietikäinen, J., Pennanen, T., 2000. Distribution of microbial biomass and phospholipid fatty acids in Podzol profiles under coniferous forest. Eur. J. Soil Sci. 51 (4), 565–573. https://doi.org/10.1111/j.1365-2389.2000.00346.x.
Gaby, J.C., Buckley, D.H., 2011. A global census of nitrogenase diversity. Environ. Microbiol. 13 (7), 1790–1799. https://doi.org/10.1111/j.1462-2920.2011.02488.x.
Ghoul, M., Mitri, S., 2016. The ecology and evolution of microbial competition. Trends Microbiol. 24 (10), 833–845. https://doi.org/10.1016/j.tim.2016.06.011.
Gower, J.C., 1966. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53 (3–4), 325–338. https://doi.org/10.1093/biomet/53.3-4.325.
Guimera, R., Amaral, L.A.N., 2005. Functional cartography of complex metabolic networks. Nature 433 (7028), 895–900. https://doi.org/10.1038/nature03288.
Hartman, W.H., Richardson, C.J., Vilgalys, R., Bruland, G.L., 2008. Environmental and anthropogenic controls over bacterial communities in wetland soils. PNAS 105 (46), 17842–17847. https://doi.org/10.1073/pnas.080825410.
Heinemeyer, A., Asena, Q., Burn, W.L., Jones, A.L., 2018. Peatland carbon stocks and burn history: blanket bog peat core evidence highlights charcoal impacts on peat physical properties and long-term carbon storage. Geo: Geogr. Environ. 5 (2), e00063. https://doi.org/10.1002/geo2.63.
Herlemann, D.P., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J.J., Andersson, A.F., 2011. Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. ISME J. 5 (10), 1571–1579. https://doi.org/10.1038/ismej.2011.41.
Hernandez, D.J., David, A.S., Menges, E.S., Searcy, C.A., Afkhami, M.E., 2021. Environmental stress destabilizes microbial networks. ISME J. 15 (6), 1722–1734. https://doi.org/10.1038/s41396-020-00882-x.
Herren, C.M., McMahon, K.D., 2018. Keystone taxa predict compositional change in microbial communities. Environ. Microbiol. 20 (6), 2207–2217. https://doi.org/10.1111/1462-2920.14257.
Holden, S.R., Rogers, B.M., Treseder, K.K., Randerson, J.T., 2016. Fire severity influences the response of soil microbes to a boreal forest fire. Environ. Res. Lett. 11, 035004. https://doi.org/10.1088/1748-9326/11/3/035004/meta.
Hunt, D.E., Ward, C.S., 2015. A network-based approach to disturbance transmission through microbial interactions. Front. Microbiol. 6, 1182. https://doi.org/10.3389/fmcb.2015.01182.
Jang, S.W., Yoou, M.H., Hong, W.J., Kim, Y.J., Lee, E.J., Jung, K.H., 2020. Re-analysis of 16S amplicon sequencing data reveals soil microbial population shifts in rice fields under drought condition. Rice 13 (1), 1–7. https://doi.org/10.1186/s12284-020-00403-6.
Jansson, J.K., Hofmockel, K.S., 2020. Soil microbiomes and climate change. Nat. Rev. Microbiol. 18 (1), 35–46. https://doi.org/10.1038/s41579-019-0265-7.
Jing, X., Sanders, N.J., Shi, Y., Chu, H., Classen, A.T., Zhao, K., Chen, L., Shi, Y., Jiang, Y., He, J.S., 2015. The links between ecosystem multifunctionality and above-and belowground biodiversity are mediated by climate. Nat. Commun. 6 (1), 1–8. https://doi.org/10.1038/ncomms9159.
Jones, C.G., Gutiérrez, J.L., Byers, J.E., Crooks, J.A., Lambrinos, J.G., Talley, T.S., 2010. A framework for understanding physical ecosystem engineering by organisms. Oikos 119 (12), 1862–1869. https://doi.org/10.1111/j.1600-0706.2010.18782.x.
Kaiser, K., Wemheuer, B., Korolkow, V., Wemheuer, F., Nacke, H., Schöning, I., Schrumpf, M., Daniel, R., 2016. Driving forces of soil bacterial community structure, diversity, and function in temperate grasslands and forests. Sci. Rep. 6 (1), 1–12. https://doi.org/10.1038/srep33696 (2016).
Kallenbach, C.M., Frey, S.D., Grandy, A.S., 2016. Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. Nat. Commun. 7, 13630. https://doi.org/10.1038/ncomms13630 (2016).
Khodadad, C.L., Zimmerman, A.R., Green, S.J., Uthandi, S., Foster, J.S., 2011. Taxa-specific changes in soil microbial community composition induced by pyrogenic carbon amendments. Soil Biol. Biochem. 43 (2), 385–392. https://doi.org/10.1016/j.soilbio.2010.11.005.
Knelman, J.E., Schmidt, S.K., Garayburu-Caruso, V., Kumar, S., Graham, E.B., 2019. Multiple, compounding disturbances in a forest ecosystem: fire increases susceptibility of soil edaphic properties, bacterial community structure, and function to change with extreme precipitation event. Soil Syst. 3 (2), 40. https://doi.org/10.3390/soilsystems3020040.
Lal, R., 2004. Soil carbon sequestration impacts on global climate change and food security. Science 304 (5677), 1623–1627. https://doi.org/10.1126/science.1097396.
Lamit, L.J., Romanowicz, K.J., Potvin, L.R., Rivers, A.R., Singh, K., Lennon, J.T., Tringe, S.G., Kane, E.S., Lilleskov, E.A., 2017. Patterns and drivers of fungal community depth stratification in Sphagnum peat. FEMS Microbiol. Ecol. 93 (7). https://doi.org/10.1093/femsec/fix082.
Lange, M., Eisenhauer, N., Sierra, C.A., Bessler, H., Engels, C., Griffiths, R.I., Mellado-Vázquez, P.G., Malik, A.A., Roy, J., Scheu, S., Steinbeiss, S., 2015. Plant diversity increases soil microbial activity and soil carbon storage. Nat. Commun. 6 (1), 1–8. https://doi.org/10.1038/ncomms7707 (2015).
Lê, S., Josse, J., Husson, F., 2008. FactoMineR: an R package for multivariate analysis. J. Stat. Softw. 25, 1–18. https://doi.org/10.18637/jss.v025.i01.
Lee, H., Alday, J.G., Rose, R.J., O'Reilly, J., Marrs, R.H., 2013. Long-term effects of rotational prescribed burning and low-intensity sheep grazing on blanket-bog plant communities. J. Appl. Ecol. 50 (3), 625–635. https://doi.org/10.1111/1365-2664.12078.
Legendre, P., Gallagher, E.D., 2001. Ecologically meaningful transformations for ordination of species data. Oecologia 129 (2). https://doi.org/10.1007/s004420100716, 271-28.
Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C., Crowley, D., 2011. Biochar effects on soil biota–a review. Soil Biol. Biochem. 43 (9), 1812–1836. https://doi.org/10.1016/j.soilbio.2011.04.022.
Li, W., Niu, S., Liu, X., Wang, J., 2019. Short-term response of the soil bacterial community to differing wildfire severity in Pinus tabulaeformis stands. Sci. Rep. 9 (1), 1–10. https://doi.org/10.1038/s41598-019-38541-7.
Lin, X., Tfaily, M.M., Green, S.J., Steinweg, J.M., Chanton, P., Imvittaya, A., Chanton, J. P., Cooper, W., Schadt, C., Kostka, J.E., 2014. Microbial metabolic potential for carbon degradation and nutrient (nitrogen and phosphorus) acquisition in an ombrotrophic peatland. Appl. Environ. Microbiol. 80 (11), 3531–3540. https://doi.org/10.1128/AEM.00206-14.
Lin, X., Handley, K.M., Gilbert, J.A., Kostka, J.E., 2015. Metabolic potential of fatty acid oxidation and anaerobic respiration by abundant members of Thaumarchaeota and Thermoplasmata in deep anoxic peat. ISME J. 9 (12), 2740–2744. https://doi.org/10.1038/ismej.2015.77.
Lladó, S., Baldrian, P., 2017. Community-level physiological profiling analyses show potential to identify the copiotrophic bacteria present in soil environments. PLoS One 12 (2), e0171638. https://doi.org/10.1371/journal.pone.0171638.
Lynch, M.D., Neufeld, J.D., 2015. Ecology and exploration of the rare biosphere. Nat. Rev. Microbiol. 13 (4), 217–229. https://doi.org/10.1038/nrmicro3400.
Magoč, T., Salzberg, S.L., 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinform 27 (21), 2957–2963. https://doi.org/10.1093/bioinformatics/btr507.
Marrs, R.H., Marsland, E.L., Lingard, R., Appleby, P.G., Piliposyan, G.T., Rose, R.J., O'Reilly, J., Milligan, G., Allen, K.A., Alday, J.G., Santana, V., 2019. Experimental evidence for sustained carbon sequestration in fire-managed, peat moorlands. Nat. Geosci. 12 (2), 108–112. https://doi.org/10.1038/s41561-018-0266-6.
Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet J. 17 (1), 10–12. https://doi.org/10.14806/ej.17.1.200.
McCarthy, D.J., Chen, Y., Smyth, G.K., 2012. Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. Nucleic Acids Res. 40 (10), 4288–4297. https://doi.org/10.1093/nar/gks042.
McKnight, D.T., Huerlimann, R., Bower, D.S., Schwarzkopf, L., Alford, R.A., Zenger, K.R., 2019. Methods for normalizing microbiome data: an ecological perspective. Methods Ecol. Evol. 10 (3), 389–400. https://doi.org/10.1111/2041-210X.13115.
McMurdie, P.J., Holmes, S., 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One *8* (4), e61217. https://doi.org/10.1371/journal.pone.0061217.
McMurdie, P.J., Holmes, S., 2014. Waste not, want not: why rarefying microbiome data is inadmissible. PLoS Comput. Biol. 10 (4), e1003531. https://doi.org/10.1371/journal.pcbi.1003531.
Mendes, L.W., Tsai, S.M., Navarrete, A.A., De Hollander, M., van Veen, J.A., Kuramae, E. E., 2015. Soil-borne microbiome: linking diversity to function. Microb. Ecol. 70 (1), 255–265. https://doi.org/10.1007/s00248-014-0559-2.
Milligan, G., Rose, R.J., O'Reilly, J., Marrs, R.H., 2018. Effects of rotational prescribed burning and sheep grazing on moorland plant communities: results from a 60-year intervention experiment. Land Degrad. Dev. 29 (5), 1397–1412. https://doi.org/10.1002/ldr.2953.
Montgomery, D.C., Peck, E.A., 1992. Introduction to Linear Regression Analysis. Wiley, New York.
Morriën, E., Hannula, S.E., Snoek, L.B., Helmsing, N.R., Zweers, H., De Hollander, M., Soto, R.L., Bouffaud, M.L., Buée, M., Dimmers, W., Duyts, H., 2017. Soil networks become more connected and take up more carbon as nature restoration progresses. Nat. Commun. 8 (1), 1–10. https://doi.org/10.1038/ncomms14349.
Mougi, A., Kondoh, M., 2012. Diversity of interaction types and ecological community stability. Science 337 (6092), 349–351. https://doi.org/10.1126/science.1220529.
Myers, B., Webster, K.L., Mclaughlin, J.W., Basiliko, N., 2012. Microbial activity across a boreal peatland nutrient gradient: the role of fungi and bacteria. Wetl. Ecol. Manag. 20 (2), 77–88. https://doi.org/10.1007/s11273-011-9242-2.
Nicol, G.W., Glover, L.A., Prosser, J.I., 2003. The impact of grassland management on archaeal community structure in upland pasture rhizosphere soil. Environ. Microbiol. 5 (3), 152–162. https://doi.org/10.1046/j.1462-2920.2003.00399.x.
Nishimura, A., Tsuyuzaki, S., Haraguchi, A., 2009. A chronosequence approach for detecting revegetation patterns after Sphagnum-peat mining, northern Japan. Ecol. Res. 24 (2), 237–246. https://doi.org/10.1007/s11284-008-0499-8.
Noble, A., O'Reilly, J., Glaves, D.J., Crowle, A., Palmer, S.M., Holden, J., 2018. Impacts of prescribed burning on Sphagnum mosses in a long-term peatland field experiment. PLoS One 13 (11), e0206320. https://doi.org/10.1371/journal.pone.0206320.
Noble, A., Palmer, S.M., Glaves, D.J., Crowle, A., Holden, J., 2019. Peatland vegetation change and establishment of re-introduced Sphagnum moss after prescribed burning. Biodivers. Conserv. 28 (4), 939–952. https://doi.org/10.1007/s10531-019-01703-0.
Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., Oksanen, M.J., 2013. Package 'vegan'. Community Ecology Package, Version, 2, 9, pp. 1–295.
O'neill, B., Grossman, J., Tsai, M., Gomes, J.E., Lehmann, J., Peterson, J., Neves, E., Thies, J.E., 2009. Bacterial community composition in Brazilian Anthrosols and adjacent soils characterized using culturing and molecular identification. Microb. Ecol. 58 (1), 23–35. https://doi.org/10.1007/s00248-009-9515-y.
Oulas, A., Pavloudi, C., Polymenakou, P., Pavlopoulos, G.A., Papanikolaou, N., Kotoulas, G., Arvanitidis, C., Iliopoulos, L., 2015. Metagenomics: tools and insights

for analyzing next-generation sequencing data derived from biodiversity studies. Bioinf. Biol. Insights 9. https://doi.org/10.4137/BBI.S12462 pp.BBI-S12462.
Paul, E. (Ed.), 2014. Soil Microbiology, Ecology and Biochemistry. Academic press.
Peltoniemi, K., Laiho, R., Juottonen, H., Kiikkilä, O., Mäkiranta, P., Minkkinen, K., Pennanen, T., Penttilä, T., Sarjala, T., Tuittila, E.S., Tuomivirta, T., 2015. Microbial ecology in a future climate: effects of temperature and moisture on microbial communities of two boreal fens. FEMS Microbiol. Ecol. 91 (7). https://doi.org/10.1093/femsec/fiv062.
Pilkington, M.G., Caporn, S.J., Carroll, J.A., Cresswell, N., Phoenix, G.K., Lee, J.A., Emmit, B.A., Sparks, T., 2007. Impacts of burning and increased nitrogen deposition on nitrogen pools and leaching in an upland moor. J. Ecol. 95 (6), 1195–1207.
Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, F., Glöckner, F.O., 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41 (D1), D590–D596. https://doi.org/10.1093/nar/gks1219.
R Development Core Team, 2020. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL. https://www.R-project.org/.
Ritson, J.P., Alderson, D.M., Robinson, C.H., Burkitt, A.E., Heinemeyer, A., Stimson, A. G., Gallego-Sala, A., Harris, A., Quillet, A., Malik, A.A., Cole, B., Robroek, B.J.M., Heppell, C.M., Rivett, D.W., Chandler, D.M., Elliott, D.R., Shuttleworth, E.L., Lilleskov, E., Cox, F., Clay, G.D., Diack, I., Rowson, J., Pratscher, J., Lloyd, J.R., Walker, J.S., Belyea, L.R., Dumont, M.G., Longden, M., Bell, N.G.A., Artz, R.R.E., Bardgett, R.D., Griffiths, R.L., Andersen, R., Chadburn, S.E., Hutchinson, S.M., Page, S.E., Thom, T., Burn, W., Evans, M.G., 2021. Towards a microbial process-based understanding of the resilience of peatland ecosystem service provisioning – a research agenda. Sci. Total Environ. 759, 143467. https://doi.org/10.1016/j.scitotenv.2020.143467.
Roberts, D.W., 2016. Labdsv: Ordination and Multivariate Analysis for Ecology. R package version 1.7–0, p. 2015.
Robinson, M.D., McCarthy, D.J., Smyth, G.K., 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinf 26 (1), 139–140. https://doi.org/10.1093/bioinformatics/btp616.
Rosario-Ortiz, F., Rose, J., Speight, V., Von Gunten, U., Schnoor, J., 2016. How do you like your tap water? Science 351 (6276), 912–914. https://doi.org/10.1126/science.aaf0953.
Shi, S., Nuccio, E.E., Shi, Z.J., He, Z., Zhou, J., Firestone, M.K., 2016. The interconnected rhizosphere: high network complexity dominates rhizosphere assemblages. Ecol. Lett. 19 (8), 926–936. https://doi.org/10.1111/ele.12630.
Smith, C.J., McKew, B.A., Coggan, A., Whitby, C., 2015. Primers: functional genes for nitrogen-cycling microbes in oil reservoirs. In: Hydrocarbon and Lipid Microbiology Protocols. Springer, Berlin, Heidelberg, pp. 207–241.
Ssekagiri, A.T., Sloan, W., Ijaz, U.Z., 2017. August. microbiomeSeq: an R package for analysis of microbial communities in an environmental context. In: ISCB Africa ASBCB Conference, Kumasi, Ghana. https://github.com/umerijaz/microbiomeSeq, Vol. 10.
Stegen, J.C., Lin, X., Fredrickson, J.K., Konopka, A.E., 2015. Estimating and mapping ecological processes influencing microbial community assembly. Front. Microbiol. 6, 370. https://doi.org/10.3389/fmicb.2015.00370.
Sun, H., Santalahti, M., Pumpanen, J., Köster, K., Berninger, F., Raffaello, T., Jumpponen, A., Asiegbu, F.O., Heinonsalo, J., 2015. Fungal community shifts in structure and function across a boreal forest fire chronosequence. Appl. Environ. Microbiol. 81 (22), 7869–7880. https://doi.org/10.3389/fmicb.2015.00370.
Sun, H., Wang, Q.X., Liu, N., Li, L., Zhang, C.G., Liu, Z.B., Zhang, Y.Y., 2017. Effects of different leaf litters on the physicochemical properties and bacterial communities in Panax ginseng-growing soil. Appl. Soil Ecol. 111, 17–24. https://doi.org/10.1016/j.apsoil.2016.11.008.
Tan, B., Ng, C.M., Nshimyimana, J.P., Loh, L.L., Gin, K.Y.H., Thompson, J.R., 2015. Next-generation sequencing (NGS) for assessment of microbial water quality: current progress, challenges, and future opportunities. Front. Microbiol. 6, 1027. https://doi.org/10.3389/fmicb.2015.01027.
Thomson, B.C., Ostle, N., McNamara, N., Bailey, M.J., Whiteley, A.S., Griffiths, R.I., 2010. Vegetation affects the relative abundances of dominant soil bacterial taxa and soil respiration rates in an upland grassland soil. Microb. Ecol. 59 (2), 335–343. https://doi.org/10.1007/s00248-009-9575-z.
Thomson, B.C., Tisserant, E., Plassart, P., Uroz, S., Griffiths, R.I., Hannula, S.E., Buée, M., Mougel, C., Ranjard, L., Van Veen, J.A., Martin, F., 2015. Soil conditions and land use intensification effects on soil microbial communities across a range of European field sites. Soil Biol. Biochem. 88, 403–413. https://doi.org/10.1016/j.soilbio.2015.06.012.
Thormann, M.N., Rice, A.V., 2007. Fungi from peatlands. Fungal Divers. 24 (2415), 299.
Urbanová, Z., Bárta, J., 2016. Effects of long-term drainage on microbial community composition vary between peatland types. Soil Biol. Biochem. 92, 16–26. https://doi.org/10.1016/j.soilbio.2015.09.017.
Wang, G., Or, D., 2013. Hydration dynamics promote bacterial coexistence on rough surfaces. ISME J. 7 (2), 395–404. https://doi.org/10.1038/ismej.2012.115.
Wang, J.T., Egidi, E., Li, J., Singh, B.K., 2019. Linking microbial diversity with ecosystem functioning through a trait framework. J. Biosci. 44 (5), 1–3. https://doi.org/10.1007/s12038-019-9928-9.
Wang, K.R., Zhang, S.J., Li, S.H., Sang, X.X., Bai, L.H., 2010. Osmotolerance property and mechanism of a moderately halophilic bacterium halomonas sp. NY-011. Chin. J. Appl. Environ. Biol. 16 (2), 256–260. https://doi.org/10.3724/SP.J.1145.2010.00256.
Wang, S., Wang, X., Han, X., Deng, Y., 2018. Higher precipitation strengthens the microbial interactions in semi-arid grassland soils. Glob. Ecol. Biogeogr. 27 (5), 570–580. https://doi.org/10.1111/geb.12718.
Wang, Y., Zhang, R., Zheng, Q., Deng, Y., Van Nostrand, J.D., Zhou, J., Jiao, N., 2016. Bacterioplankton community resilience to ocean acidification: evidence from microbial network analysis. ICES J. Mar. Sci. 73 (3), 865–875. https://doi.org/10.1093/icesjms/fsv187.
Wei, G., Li, M., Shi, W., Tian, R., Chang, C., Wang, Z., Wang, N., Zhao, G., Gao, Z., 2020. Similar drivers but different effects lead to distinct ecological patterns of soil bacterial and archaeal communities. Soil Biol. Biochem. 144, 107759. https://doi.org/10.1016/j.soilbio.2020.107759.
Whitehead, S., Weald, H., Baines, D., 2021. Post-burning responses by vegetation on blanket bog peatland sites on a Scottish grouse moor. Ecol. Indic. 123, 107336. https://doi.org/10.1016/j.ecolind.2021.107336.
Wiesmeier, M., Urbanski, L., Hobley, E., Lang, B., von Lützow, M., Marin-Spiotta, E., van Wesemael, B., Rabot, E., Ließ, M., Garcia-Franco, N., Wollschläger, U., 2019. Soil organic carbon storage as a key function of soils-a review of drivers and indicators at various scales. Geoderma 333, 149–162. https://doi.org/10.1016/j.geoderma.2018.07.026.
Xiao, F., Li, Y., Li, G., He, Y., Lv, X., Zhuang, L., Pu, X., 2021. High throughput sequencing-based analysis of the soil bacterial community structure and functions of Tamarix shrubs in the lower reaches of the Tarim River. PeerJ 9, e12105. https://doi.org/10.7717/peerj.12105.
Yang, J., Kloepper, J.W., Ryu, C.M., 2009. Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci. 14 (1), 1–4. https://doi.org/10.1016/j.tplants.2008.10.004.
Yao, F., Yang, S., Wang, Z., Wang, X., Ye, J., Wang, X., DeBruyn, J.M., Feng, X., Jiang, Y., Li, H., 2017. Microbial taxa distribution is associated with ecological trophic cascades along an elevation gradient. Front. Microbiol. 8, 2071. https://doi.org/10.3389/fmicb.2017.02071.
Yuan, M.M., Guo, X., Wu, L., Zhang, Y.A., Xiao, N., Ning, D., Shi, Z., Zhou, X., Wu, L., Yang, Y., Tiedje, J.M., 2021. Climate warming enhances microbial network complexity and stability. Nat. Clim. Chang. 11 (4), 343–348. https://doi.org/10.1038/s41558-021-00989-9.
Zachow, C., Müller, H., Tilcher, R., Berg, G., 2014. Differences between the rhizosphere microbiome of Beta vulgaris ssp. maritima—ancestor of all beet crops—and modern sugar beets. Front. Microbiol. 5, 415. https://doi.org/10.3389/fmicb.2014.00415.
Zhalnina, K., Dias, R., de Quadros, P.D., Davis-Richardson, A., Camargo, F.A., Clark, I.M., McGrath, S.P., Hirsch, P.R., Triplett, E.W., 2015. Soil pH determines microbial diversity and composition in the park grass experiment. Microb. Ecol. 69 (2), 395–406. https://doi.org/10.1007/s00248-014-0530-2.
Zhou, J., Deng, Y., Luo, F., He, Z., Yang, Y., 2011. Phylogenetic molecular ecological network of soil microbial communities in response to elevated CO_2. MBio 2 (4). https://doi.org/10.1128/mBio.00122-11.