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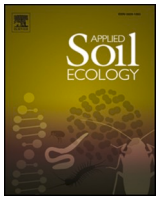

Changes in nitrogen functional genes and microbial populations in soil profiles of a peatland under different burning regimes

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ABSTRACT

Microbes in peatlands provide key ecosystem services and are essential for their role in biogeochemical cycling. Prescribed burning is a common aspect of peatland management but the practice has been criticized for being ecologically damaging due to its effect on the biological, chemical and physical properties of the soil. It is poorly understood how burning affects soil N cycling and previous studies have focused predominantly on the topsoil whilst giving less attention to changes with soil depth. This study investigated the changes of microbial abundance (bacterial 16S rRNA and fungal 18S rRNA) and the abundance of N-cycle functional genes involved in archaeal and bacterial ammonia oxidation (*amoA*-AOA and *amoA*-AOB), denitrification (*nirK* and *nirS*), N fixation (*nifH*) and organic N decomposition (*chiA*) in soil profiles across three burn treatments on a managed peatland landscape (a 'non-burn' since 1954 control, 20 years burn interval, and 10 years burn interval). Our results indicate the abundance of bacterial 16S rRNA and fungal 18 s rRNA was affected by burn treatment, soil depth and their interaction and were greater in the non-burn control plots. The abundances of *amoA-AOA*, *amoA-AOB*, and *nifH* were significantly higher in the topsoil of the non-burn control plots while the abundance of *nirK* was higher in plots subject to short rotation and long rotation burn regimes but also decreased significantly with soil depth. The abundance of *nirS* was not affected by burn treatment or soil depth. *ChiA* abundance was affected by burn treatment, soil depth and their interaction. N-cycle functional gene abundance responded differently to environmental factors associated with prescribed burning and varied with soil depth. These findings suggest that the practice of burning affects microbial N turnover potential and provides an important insight into the soil N-cycling potential of peatlands under different burning regimes.

1. Introduction

Peatlands cover an estimated 4 million km^2 globally and are essential for their biodiversity conservation, hydrological function and climate change mitigation through carbon sequestration (Xu et al., 2018). Prescribed burning has been widely used as a management tool in the UK to decrease the risk of wildfire and maintain peatland vegetation for game bird populations (Simmons, 2003; Yallop et al., 2006). However, burning can influence peatland function particularly due to vegetation change, which modifies below-ground microbial communities (Ciccolini et al., 2016; Evans et al., 2014a, 2014b).

Burning has been a common management regime in the UK since the early 1900s and has been recognised as a major driver for vegetation change (Holden et al., 2015; Milligan et al., 2018) and surface hydraulic conductivity (Holden et al., 2014). As plant communities change there is an indirect effect on decomposer organisms altering ecosystem carbon fluxes (Harte et al., 2015). However, the magnitude of the change depends on the severity and frequency of the burn. Rotational burning is the most common type of burning where vegetation is burned to facilitate the growth of heather (Douglas et al., 2015) and is still a common practice on UK peatlands dominated by *Calluna vulgaris* and managed for grouse shooting (Douglas et al., 2015; Thacker et al., 2000; Yallop et al.,

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2010). The severity of burning varies, but 'cool' burns that remove the vegetation's canopy layer, without igniting the underlying peat or consuming the moss and litter layers, have been recommended (Ashby and Heinemeyer, 2021; Noble et al., 2019). However, there has been debate about the long-term viability of current practices and concern about the potential impact the practice might have on ecosystem function (e.g. Ashby and Heinemeyer, 2021; Harper et al., 2018; IUCN, 2020). However, little is known about how burning affects nitrogen turnover in different soil profiles within peatlands.

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

Many studies on the effects of anthropogenic disturbance on the abundance of microbes and functional genes have focused on the topsoil and ignored the changes of nutrients in relation to soil depth (Espenberg et al., 2018; Fisk et al., 2003; Urbanová and Bárta, 2016). Given the important roles of functional genes in the nitrogen cycle, determining their response to burning, especially across different soil profiles is essential for gaining a process-based understanding of burning as a management strategy on peatlands. This knowledge can help predict how peatlands will respond to land management actions, and thus assist in better decision making processes.

The aim of this research is to assess the influence of burning on microbial abundance and microbially-driven nitrogen turnover in peatlands based on quantification of relevant genes at different depths in the soil profiles including the topsoil (0–20 cm) and the subsoil layers, (20–40 cm, 40–60 cm). The study site is the Moor House nature reserve United Kingdom, which has been managed with three different burning regimes since 1954. The reserve is a flagship Environmental Change Network (ECN) monitoring site with extensive historical and ongoing data collection available across the 74 km^2 site which is characterised by vegetation cover typical of many upland blanket bogs in upland Britain (NVC M19/M20 communities/EUNIS Q1) (Rodwell, 1998).

The following hypotheses were tested. 1) The abundance of bacteria and fungi will decrease with the frequency of burn treatment and be higher in the unburned since 1954 control plots, 2) The abundance of N-cycling genes will vary across burn treatments. It is expected that the non-burn plots will have enhanced microbial nitrogen turnover potential which will be characterised by a higher abundance of functional genes due to the changes in soil environmental factors and vegetation cover caused by burning, 3) There will be vertical differences in N-cycling gene abundance with *AOA* and *AOB* related to energy acquisition *and nifH* and *chiA* genes related to nitrogen acquisition being higher in the topsoil due to higher nutrient content and 4) *nirS* and *nirK* genes involved in denitrification being more abundant in the anoxic subsurface soil characteristic of peatlands.

2. Materials and methods

2.1. Study site

The experiment was carried out at the Hard Hill long term burning experiment (54°43′N 2°23′W, Fig. 1) in July 2020. The area is a *Calluna vulgaris-Eriophorum vaginatum* blanket mire, a form of blanket bog typical of the English Pennines that has undergone variable degrees of modification due to management practises such as grazing and burning. Over the course of a 33-month research, the water table depth at the site was measured using dipwells and ranged from 0 (peat surface) to 671 mm (Clay et al., 2009). The experiment was established in 1954 on an area of blanket peatland at Moor House-Upper Teesdale National Nature Reserve (NNR) in the North Pennines, UK. Since the installation of experimental plots in 1954 the short rotation plots have been burned seven times and the long rotation plots four times and have been used to monitor the ecological response of plant communities to this burn-severity gradient (e.g. Lee et al., 2013a; Lee et al., 2013b; Milligan et al., 2018; Noble et al., 2018; Noble et al., 2019). The drawback of this design is that the treatments vary in the number of fires and the amount of time that has passed since the last fire. This is because the long rotation and short rotation treatments were both last burned in 2017, while the non-burn plots have not been burned since 1954 (Clutterbuck et al., 2020). Therefore treatment effects are most accurately attributed to burn 'regime' rather than the frequency of burning. The climate is cold and wet with average temperatures between 0.9 °C and 12.2 °C between January and July respectively with an average annual precipitation of 2054 mm (Rennie et al., 2020).

2.2. Experimental design and soil sampling

Soil sampling was conducted in July 2020. The experiment is divided into four blocks, each with six 30 m × 30 m plots. The experimental treatments are unburned since 1954 (non-burn), short-rotation burning (10 year interval), and long-rotation burning (20 year interval) (Fig. 1). The surrounding vegetation outside of experimental plots has remained unburned for at least 90 years (Noble et al., 2019). Each burning regime was arranged into four repeats. In each sampling area, three quadrats (1 m × 1 m) were randomly thrown in each treatment per block ($n = 36$). In each quadrat, the percent of vegetation cover was recorded. Plants were grouped by their morphology (i.e. heather, graminoid, *Sphagnum* moss, other 'non-*Sphagnum*' moss and other vascular plants). Next, using a 10

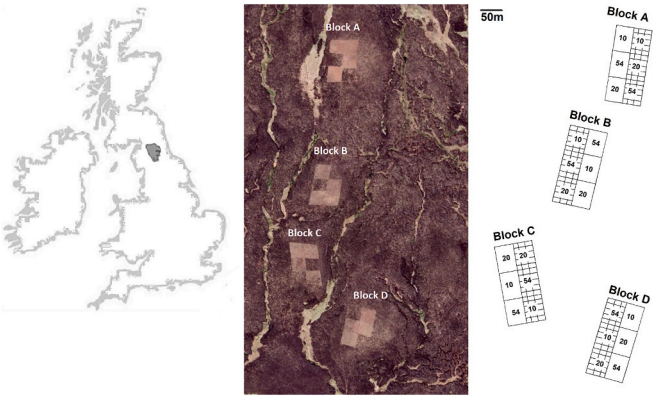

Fig. 1. Location of Moor house Nature Reserve and arrangement of experimental plots used for this study (54 = Not burned since 1954, 20 = Long rotation (Burned every 20-years), 10 = Short rotation (Burned every 10-years).

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 from four corners of the quadrat and the centre for chemical and microbial analyses. A total of 108 samples, 12 samples for each treatment and depth were collected. Soil samples for microbial analysis were brought to the laboratory and stored at −20 °C prior to DNA extraction. The soil characteristics of sampling sites are shown in Table 1.

2.3. Soil physicochemical analysis

A Jenway 3510 pH metre was used to measure the pH of fresh peat, which was weighed at 2.5 g, combined with deionized water at a ratio of 1:5, and agitated for one hour. Soil moisture content was determined gravimetrically following oven drying at 105 °C for 48 h (Rowell, 2014). An elemental analyzer (vario MACRO cube, Elementar Analysensysteme GmbH, Langenselbold, Germany) was used to measure the concentrations of nitrogen and carbon by dry combustion at a temperature of 450 °C. Ion chromatography (Dionex ICS-5000 + DC, IonPacCG16 276 guard column) (Thermofisher, UK) was used to measure ammonium (NH_4 + -N) and nitrate (NO_3-N) from 2 g air-dried peat extracts in 20 ml 1 M KCl after 30 min orbital shaking at 200 rpm (Stuart SSL1, Bibby Scientific Ltd., UK) and Whatman No. 3 and 0.2 µm syringe filtration and dilution (50 % *v*/v). For elemental extraction, a 50 ml conical flask was filled with 0.5 g of air-dried soil, amended with 5 ml of concentrated nitric acid (HNO_3), heated for three hours at 80 °C, and filtered through a Whatman No. 3 filter. The filtrate was diluted to 50 ml with deionsed water and subjected to analyses of magnesium, calcium, manganese, iron, copper, zinc, phosphorus, lead and aluminium using an Icap 6000 SERIES ICP-AES spectrometer (Thermofisher, UK).

2.4. 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 instead of the normally recommended 0.25 g wet soil suggested by the kit instructions. This method adjustment was done after consultation with the manufacturer. The soil was homogenised and rewetted with 150 µl of nuclease-free water. The rest of 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.

2.5. Preparation of standards for qPCR

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

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

To generate a stock standard, gblock synthetic oligonucleotides require only one resuspension, whereas linear PCR plasmids require PCR amplification, gel excision, purification and quantification. There is a

Table 1
Soil properties at different soil depths under three burn treatments.

	Soil depth (cm)	Non-burn	Long rotation	Short rotation
pH	0-20 cm	4.1 ± 0.06	3.56 ± 0.04	3.81 ± 0.0.02
	20-40 cm	4.21 ± 0.0.06	3.7 ± 0.03	3.80 ± 0.03
	40–60 cm	4.11 ± 0.0.7	3.75 ± 0.0.04	3.86 ± 0.0.03
Moisture (%)	0-20 cm	78.22 ± 0.38	81.30 ± 0.49	82.89 ± 0.79
	20-40 cm	86.75 ± 0.55	83.31 ± 0.56	86.03 ± 0.36
	40–60 cm	89.27 ± 0.38	86.96 ± 0.53	88.41 ± 0.71
Total N (%)	0-20 cm	1.26 ± 0.02	1.52 ± 0.03	1.36 ± 0.09
	20-40 cm	1.34 ± 0.03	1.18 ± 0.03	1.19 ± 0.02
	40–60 cm	1.41 ± 0.04	1.15 ± 0.04	1.01 ± 0.02
Total C (%)	0-20 cm	50.50 ± 1.06	43.65 ± 0.37	37.87 ± 1.72
	20-40 cm	48.14 ± 0.07	42.61 ± 0.79	36.09 ± 2.46
	40–60 cm	46.74 ± 0.38	43.34 ± 1.74	46.11 ± 0.32
NO_3^- (mg/kg^{-1})	0-20 cm	2.88 ± 0.46	6.45 ± 0.96	3.51 ± 0.24
	20-40 cm	6.89 ± 1.09	3.59 ± 0.42	3.34 ± 0.33
	40–60 cm	4.04 ± 0.36	3.31 ± .0.24	3.18 ± 0.25
NH_4^+ (mg/kg^{-1})	0-20 cm	16.88 ± 1.62	6.86 ± 1.39	4.05 ± 1.29
	20-40 cm	4.24 ± 0.70	2.96 ± 0.51	1.31 ± 0.19
	40–60 cm	3.04 ± 0.44	2.69 ± 0.24	1.19 ± 0.17
Mg (mg/kg^{-1})	0-20 cm	916.4 ± 215.08	316.33 ± 30.09	211.46 ± 14.22
	20-40 cm	457.6 ± 48.77	167.84 ± 9.26	182.93 ± 24.79
	40–60 cm	414.76 ± 32.73	163.69 ± 6.88	167.69 ± 21.00
Ca (mg/kg^{-1})	0-20 cm	1368.7 ± 66.35	1782.00 ± 54.32	1320.9 ± 53.727
	20-40 cm	1842.5 ± 95.47	2194.5 ± 136.34	1754.6 ± 116.72
	40–60 cm	1889.8 ± 67.03	2823.5 ± 113.64	2967.1 ± 117.22
Mn (mg/kg^{-1})	0-20 cm	89.83 ± 4.83	67.89 ± 4.58	37.5 ± 2.97
	20-40 cm	52.67 ± 4.16	47.92 ± 4.50	24.83 ± 1.33
	40–60 cm	52.92 ± 2.43	71.25 ± 8.29	52.08 ± 2.56
Fe (mg/kg^{-1})	0-20 cm	681.51 ± 26.23	1236.93 ± 130.10	777.45 ± 63.75
	20-40 cm	830.03 ± 79.28	1480.23 ± 81.21	824.47 ± 68.569
	40–60 cm	840.16 ± 38.95	2079.39 ± 103.41	1395.4 ± 56.09
Cu (mg/kg^{-1})	0-20 cm	87.75 ± 12.63	59.5 ± 6.58	39.42 ± 4.06
	20-40 cm	14.75 ± 1.03	26.50 ± 3.15	15.00 ± 2.72
	40–60 cm	13.50 ± 1.13	17.50 ± 1.94	15.17 ± 1.05
Zn (mg/kg^{-1})	0-20 cm	271.55 ± 13.70	145.37 ± 19.15	111.89 ± 6.70
	20-40 cm	117.25 ± 2.67	60.66 ± 7.13	44.06 ± 3.33
	40–60 cm	131.64 ± 7.68	63.27 ± 7.20	31.38 ± 4.21
P (mg/kg^{-1})	0-20 cm	245.61 ± 10.96	316.81 ± 43.22	260.19 ± 21.11
	20-40 cm	146.09 ± 19.90	174.68 ± 14.26	101.53 ± 10.57
	40–60 cm	138.3 ± 4.88	193.14 ± 9.50	144.16 ± 4.37
Pb (mg/kg^{-1})	0-20 cm	232.78 ± 14.00	1.874 ± 19.22	118.85 ± 15.73
	20-40 cm	39.79 ± 2.54	21.06 ± 1.79	11.49 ± 1.49
	40–60 cm	25.96 ± 3.29	13.56 ± 2.35	13.27 ± 1.07
K (mg/kg^{-1})	0-20 cm	134.84 ± 7.18	156.25 ± 17.5	140.1 ± 16.87
	20-40 cm	86.43 ± 9.17	56.21 ± 3.31	40.68 ± 4.57
	40–60 cm	96.07 ± 5.84	78.54 ± 7.78	67.48 ± 11.33
Al (mg/kg^{-1})	0-20 cm	463.09 ± 23.35	495.99 ± 56.51	330.26 ± 35.21
	20-40 cm	431.56 ± 30.53	457.01 ± 23.76	249.53 ± 25.26
	40–60 cm	398.56 ± 18.47	561.57 ± 33.22	355.58 ± 31.67

Table 2
PCR primers used for the amplification of microbial populations and functional gene targets. Note: S=C/G, K = G/T, Y=C/T, R = A/G, W = A/T, B = C/G/T, D = A/G/T, N = Any base.

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

potential contamination risk, not only within the lab but also for the standard. The preparation of a gblock fragment is cheap, fast and simple with less risk of contamination. The gblocks were resuspended in 50 µl TE buffer and incubated at 50 °C for 30 min in a water bath to ensure the synthetic oligonucleotides were properly diluted, and the concentration was then measured using a Qubit 4 Fluorometer (Invitrogen, UK). Gene copy number of the standard was calculated using the equation:

$$\text{Standard concentration ng per } \mu\text{l X } 6.023 \text{ X } 10^{23} / \text{Length of amplicon (bp)} 1 \text{ X } 10^{9} \text{x } 660$$

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

2.6. Qpcr

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

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

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

2.7. Statistical analysis

All statistical analysis was carried out using R version 4.0.2 software (R Development Core Team, 2020). Two-way analysis of variance followed by Tukey's honest significant difference (HSD) for multiple comparisons with a $P = 0.05$ grouping baseline was used to test the effects of burn treatment, soil depth and their interaction on the abundance of bacteria, fungi and N-cycling genes, following the Shapiro-Wilk test for normality and Bartlett test for homogeneity of variance. Further, when the interaction was not significant, one-way ANOVA and Tukey's *post-hoc* test for multiple comparisons were used to evaluate differences based on burn treatments within a soil layer, and among the three soil layers within a given burn treatment. Pearson's correlation analysis was used to determine the significant negative or positive correlations between the abundances of bacteria, fungal and N-cycling genes and environmental variables. The correlation coefficients were calculated and plotted using the R package '*corrplot*'. The probability level $P < 0.05$ was considered to be statistically significant. Correlation analysis was performed on the topsoil (0–20 cm) and subsoil (20–60 cm). In this study, any sample below the detection limit was considered as a zero.

3. Results

3.1. Soil physicochemical characteristics across burning and depth gradients

The soil physicochemical data supporting this research are also reported in Allingham et al. (2023; under review). Briefly, burn treatment, soil depth and their interaction had significant effects on soil properties, except for pH, P, K and Al (Table 1). Soil pH was significantly higher in the non-burn control. P was significantly higher in topsoil across burn treatments, being highest in the long rotation plots. K was significantly different in between burn regimes and depth being highest in the non-burn control in the 20–40 cm and 40–60 cm depth profiles. Al was significantly higher in the long rotation burned plots.

3.2. Abundance of bacteria and fungi

The bacterial 16S rRNA copy numbers from all treatments ranged from 5.46×10^7 - 2.17×10^{10} copies g^{-1} dry soil, and were more abundant than the fungal 18S rRNA copy numbers which ranged from 6.19×10^4 - 9.23×10^8 copies g^{-1} dry soil. The abundance of bacterial 16S rRNA gene copy number ranged from 1.95×10^8–2.17×10^{10} copies g^{-1} dry soil in the non-burn control plots, from 5.46×10^7 - 5.22×10^9 copies g^{-1} dry soil in plots under a long rotation regime and 1.27×10^8 - 5.14×10^9 copies g^{-1} dry soil in plots under a short rotation regime. Bacterial abundance was affected by burn treatment, soil depth and their interaction. (Fig. 2A; Table S1). Bacterial abundance was greater in the topsoil (0–20 cm) of the non-burn control and decreased from the topsoil to the 20–40 cm profile.

The abundance of fungal 18S rRNA gene copy number ranged from 1.68×10^5 - 9.23×10^8 copies g^{-1} dry soil in non-burn control plots, 1.22×10^5 - 3.34×10^8 copies g^{-1} dry soil in plots under a long rotation regime and 6.19×10^4 - 4.14×10^8 - copies g^{-1} dry soil in plots under a short rotation regime. Fungal abundance was affected by burn treatment, soil depth and their interaction (Fig. 2B; Table S1). The abundance of fungi was greater in the topsoil of the non-burn control and long rotation regime compared to treatments subject to a short rotation burn.

3.3. N-cycling functional genes

3.3.1. Nitrification

Archaeal *amoA* gene abundance was below the detection limit of 1×10^4 copies g^{-1} dry soil for 17 % of samples. Detected *AOA amoA* gene copy number abundance ranged from 8.36×10^4 - 9.39×10^7 copies g^{-1} dry soil in unburned control plots, 2.18×10^4–8.71×10^6 copies g^{-1} dry soil in plots under a long rotation regime and 2.88×10^4–9.88×10^7 copies g^{-1} dry soil in plots under a short rotation regime. The abundance of *AOA amoA* was significantly different between burn treatments, being highest in the non-burn control ($P < 0.05$) (Fig. 3A; Table S2). There was a decrease in abundance with depth in the unburned control as an 18 % decrease was observed between 0 and 20 cm and 20–40 cm profiles (Fig. 3A).

Bacterial *amoA* gene abundance was below the detection limit of 5×10^4 copies g^{-1} dry soil for 11 % of samples. Detected *AOB amoA* gene copy number abundance ranged from 1.27×10^5 - 9.34×10^7 copies g^{-1} dry soil in the non-burn control, 1.23×10^5–9.60 x 10^7copies g^{-1} dry soil in plots under a long rotation regime and 1.08×10^5–1.34×10^7 copies g^{-1} dry soil in plots under a short rotation regime. The abundance of *AOB amoA* was significantly different between burn treatments, being highest in the non-burn control ($P < 0.05$) (Fig. 3B; Table S2). *AOB amoA* decreased by 19 % between the 0–20 cm soil profile and the 20–40 cm in the unburned control but increased by 5 % from the 0–20 cm to 20–40 cm in plots under a long rotation burn regime 5 % (Fig. 3B).

3.3.2. Denitrification

The *nirS* gene copy number abundance ranged from 4.64×10^4 - 7.47×10^7 copies g^{-1} dry soil in the non-burn control, 1.06×10^5 - 1.47×10^7 copies g^{-1} dry soil in plots under a long rotation regime and 1.21×10^5–6.39×10^6 copies g^{-1} in plots under a short rotation regime. *NirS* was not significantly affected by burn treatment or soil depth (Fig. 4A; Table S2). There was a 16 % decrease in abundance from the 0–20 cm to 20–40 cm profile in the non-burn control (Fig. 4A). The *nirK* gene copy number ranged from 1.16×10^6–2.30×10^7 copies g^{-1} dry soil in the non-burn control plots, 1.06×10^5–1.25×10^8 copies g^{-1} dry soil in plots under a long rotation regime and 8.06×10^5–7.90×10^8 copies g^{-1} dry soil in plots under a short rotation regime. There was a significant

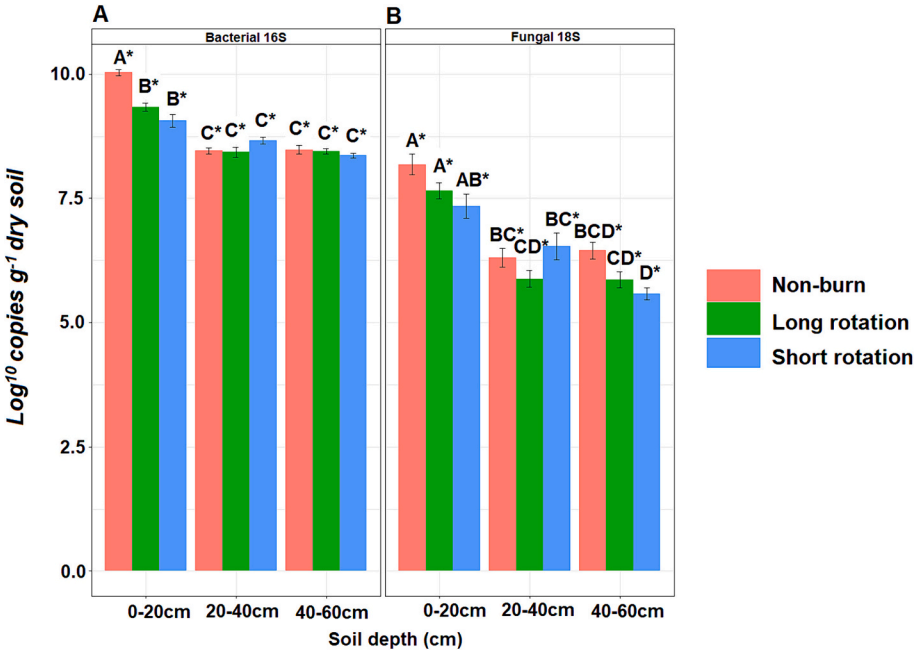

Fig. 2. The abundances of bacterial 16S rRNA gene (A), fungal 18S rRNA gene (B) across three different soil depths under three burn treatments ($n = 12$). The bars indicate the mean values of each treatment, with the error bars representing the standard error. Different letters indicate a significant difference among treatments based on a significant interaction between burn treatment and soil depth (Tukey's HSD, $P < 0.05$).

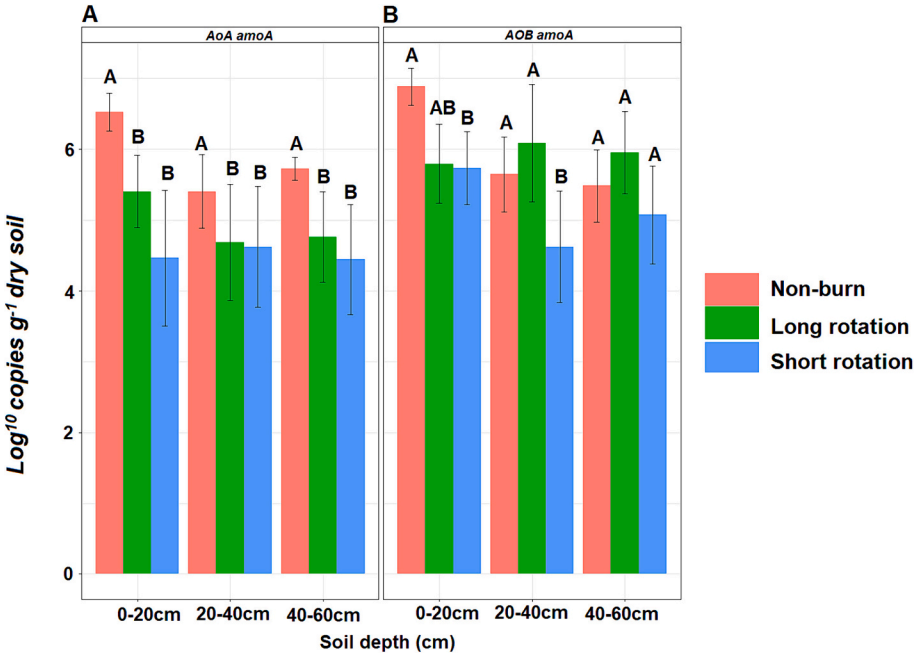

Fig. 3. The abundances of genes for ammonia oxidising archaea (*AOA amoA*) (A) and ammonia oxidising bacteria (*AOB amoA*) (B) across three different soil depths under three burn treatments ($n = 12$). The bars indicate the mean values of each treatment, with the error bars representing the standard error. Different uppercase letters indicate statistically significant differences among the three burn treatments in the same soil layer (Tukey's HSD, $P < 0.05$).

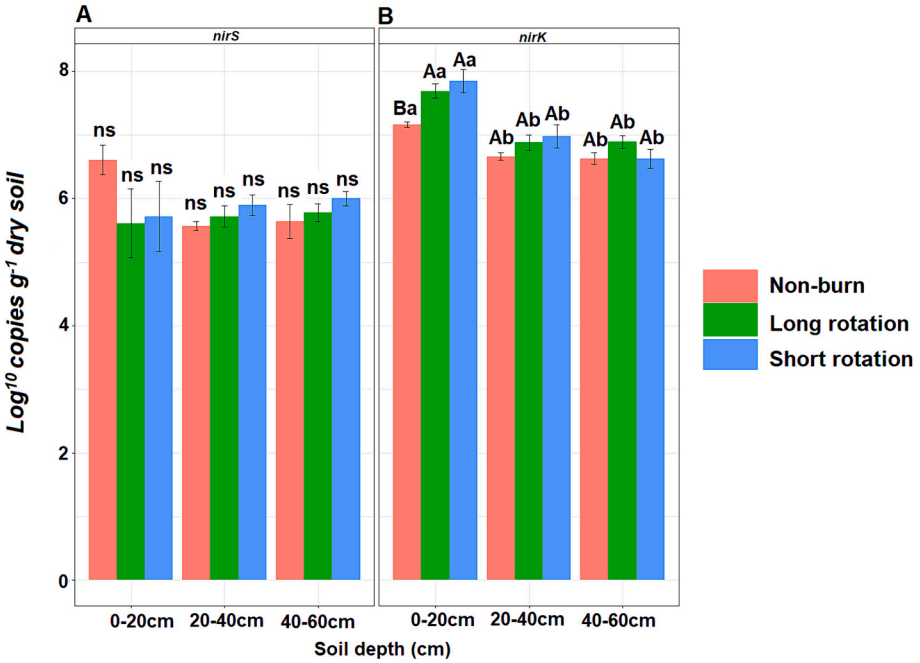

Fig. 4. The abundances of *nirS* (A) and *nirK* (B) across three different soil depths under three burn treatments ($n = 12$). The bars indicate the mean values of each treatment, with the error bars representing the standard error. Different uppercase letters indicate statistically significant differences among the three burn treatments in the same soil layer and different lowercase letters indicate statistically significant differences among the three soil layers across burn treatments (Tukey's HSD, $P < 0.05$) 'ns' = not significant.

difference in the abundance of *nirK* with respect to burn treatment as well as soil depth - being highest in the topsoil of the short rotation regime (Fig. 4B; Table S2). The abundance of *nirK* decreased between the topsoil and intermediate profile in all three burn treatments. There was a 9 % decrease from the 0–20 cm - 40-60 cm in the non-burn control, 11 % decrease in the plots under a long rotation burn and a 16 % decrease plots under a short rotation burn (Fig. 4B).

3.3.3. Nitrogen fixation and N decomposition

The *nifH* gene copy number abundance ranged from 1.08×10^5–9.64×10^8 copies g^{-1} dry soil in non-burn control plots, 8.05×10^4 - 1.44×10^8 copies g^{-1} dry soil in plots under a long rotation regime and 7.42×10^4 - 6.75×10^7 copies g^{-1} dry soil in plots under a short rotation regime (Fig. 5A; Table S2). There was a significant difference in abundance between different burn treatments and with soil depth ($P < 0.05$).

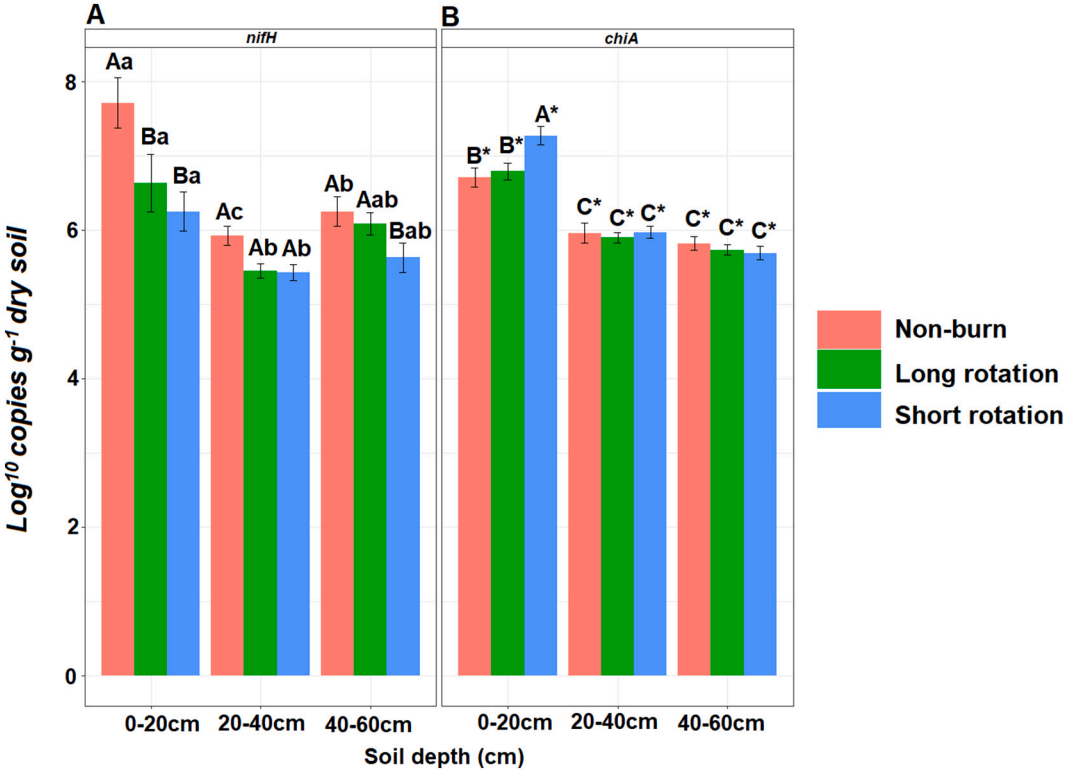

Fig. 5. The abundances of *nifH* (A) and *chiA* (B) across three different soil depths under three burn treatments ($n = 12$). The bars indicate the mean values of each treatment, with the error bars representing the standard error. Different uppercase letters indicate statistically significant differences among the three burn treatments in the same soil layer, different lowercase letters indicate statistically significant differences among the three soil layers across burn treatments and different letters with an asterisk indicate a significant difference among treatments based on a significant interaction between burn treatment and soil depth (Tukey's HSD, $P < 0.05$).

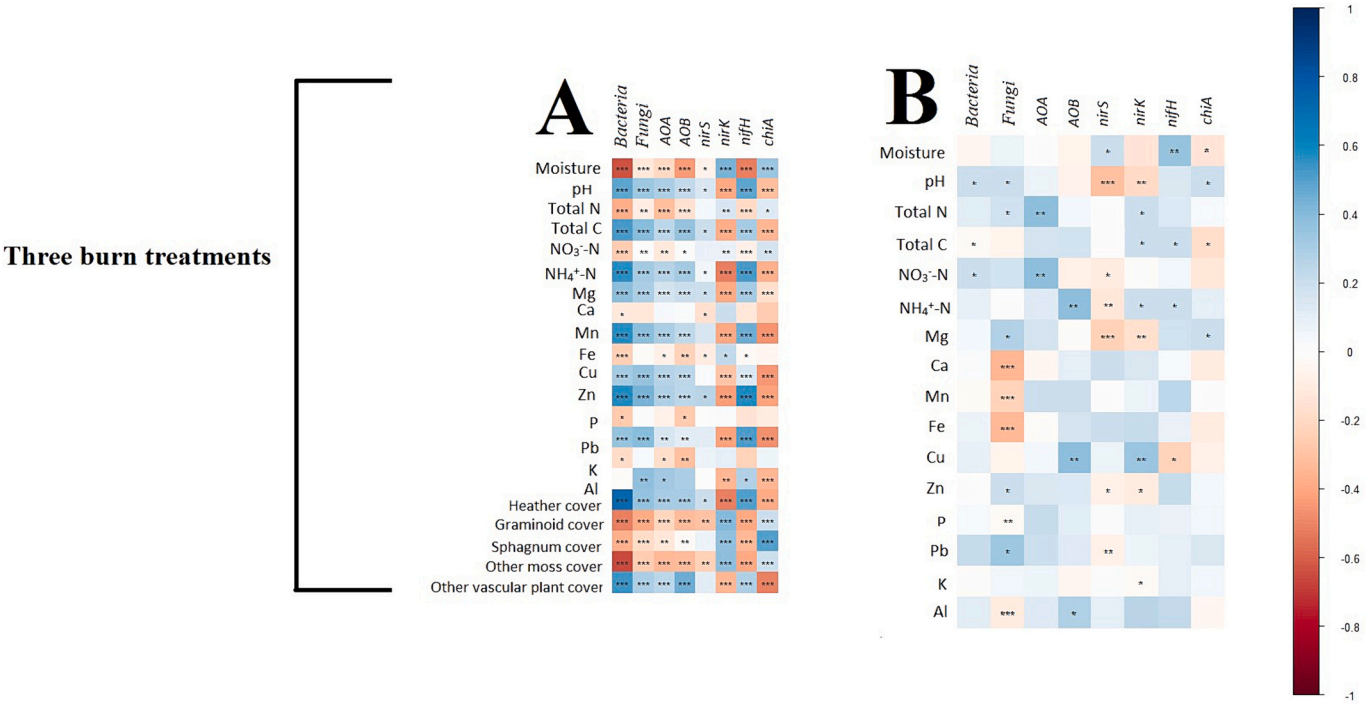

Fig. 6. Correlogram representing Pearson's correlation coefficient between environmental parameters and abundances of bacterial populations, fungal populations and N-cycling genes. (A) Topsoil (0-20 cm), (B) Subsoil (20-60 cm). The correlation relationships ranging from negative to positive are indicated by the intensity of colour changing from red to blue. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

The abundance of *nifH* was significantly higher in the topsoil compared to the subsoil as a decrease of 24 % in abundance was observed in the non-burn control, 18 % in the plots under a long rotation burn and 14 % in plots under a short rotation burn between the 0–20 cm and 20–40 cm soil profiles respectively (Fig. 5A). The *nifH* copy number was significantly higher in the non-burn control in surface soils (Fig. 5A). *ChiA* copy numbers ranged from 3.25×10^5–2.60×10^7 copies –g^{-1} dry soil in the non-burn control, 1.70×10^5–1.52×10^7 copies g^{-1} dry soil in plots under a long rotation regime and 3.25×10^5–8.54×10^7 copies g^{-1} dry soil in plots under a short rotation regime. *ChiA* abundance was affected by burn treatment, soil depth and their interaction (Fig. 5B; Table S2). *ChiA* abundance was greater in the topsoil of plots under a short rotation burn regime and decreased with depth as a 12 % decrease in abundance was observed in the non-burn control, 14 % in plots under long rotation burns and 18 % in plots under short rotation burns between the 0–20 cm and 20–40 cm soil profiles respectively (Fig. 5B).

3.4. Relationships between microbial abundance, functional gene abundance and environmental parameters

The correlation between environmental parameters and the abundances of bacterial 16S rRNA gene, fungal 18S rRNA gene and N-cycling functional genes were compared using Pearson's correlation coefficients. Soil pH, total C, NH_4^+, and heather cover were positively correlated with bacterial 16 s rRNA gene abundance in the topsoil (Fig. 6A) and pH and NO_3^- were positively correlated in the subsoil (Fig. 6B). Likewise, pH, total C and NH_4^+ were correlated with fungal 18 s rRNA gene abundance in the topsoil (Fig. 6A) while pH, total N, Mg, Zn and Pb were significantly correlated in the subsoil (Fig. 6B). *AOA*, *AOB* and *nifH* gene abundances were significantly correlated with pH, total C, NH_4^+, Mg, Mn, Cu, Zn, Pb and heather cover in the topsoil (Fig. 6A). Furthermore, *nirK* was positively correlated with moisture, total N, NO_3^-, graminoid cover, *Sphagnum* cover and other moss cover (Fig. 6A).

In the subsoil there was a positive correlation between *AOA* abundance and total N and NO_3^- while *AOB* was positively correlated with NH_4^+, Al and Cu. There was a positive correlation between *nirS* abundance and moisture while *nirK* was positively correlated with total N, total C and NH_4^+ and negatively correlated with Mg (Fig. 6B). *NifH* was positively correlated with moisture, total C and NH_4^+, while *chiA* was positively correlated with pH and Mg (Fig. 6B).

4. Discussion

Key steps in N-cycling can be significantly affected by human activity such as management regimes. N cycling genes play a crucial role in the cycling of soil nutrients and plant productivity (Xie et al., 2014). Conversely, environmental conditions including nutrient availability and vegetation community determine the composition and abundance of soil microbial groups and N cycling genes. This study focused on the effects of different burning regimes on the abundance of bacteria and fungi as well as N-cycling genes at different soil depths. The results show that the abundance of bacteria, fungi and N cycling genes varied significantly with burning regime as well as soil depth.

4.1. Differences in the abundance of bacteria and fungi across different burn treatments and depths

The interaction between burn treatment and soil depth was found to have a significant impact on bacterial and fungal abundance, with bacterial abundance being greater in the topsoil of the non-burn control and the abundance of fungi being greater in the topsoil of the non-burn control and long rotation regime (Fig. 2; Table S1) supporting our first hypothesis that the abundance of bacteria and fungi will decrease with the frequency of burn regime and be higher in the non-burn control plots. Both bacteria and fungi were more abundant in the topsoil than in the subsoil (Fig. 2). A decrease in microbial abundance with depth has been observed in previous studies in a Quaternary terrace (Fierer et al., 2003), arable soils (Kramer et al., 2013) and rice paddy soils (Wang et al., 2017). The topsoil is likely to have more favourable environmental conditions such as nutrient resources that support the growth of bacteria and fungi. Although the abundance of bacteria and fungi decreased with soil depth, the abundance of fungi decreased more than bacteria. This is in line with previous research on community structure, which found that soil depth impacted fungal communities' more than bacterial communities in rice paddy soils (Liu et al., 2016; Wang et al., 2017) and is likely to be due to the anaerobic soil at the lower depths in peatlands. Fungi are recognised as essential decomposers of complex carbon polymers in these habitats, and play an essential role in nutrient cycling (Myers et al., 2012). Despite the fungi isolated and identified from peatlands being mostly aerobic (Andersen et al., 2013), fungi isolated from anaerobic lower peat layers exhibit a tolerance to these conditions (Thormann and Rice, 2007). By modifying plant communities, eliminating plant biomass, burning soil organic residues like carbon and nitrogen, shifting fuel loads, and affecting fungal populations, fire regimes cause adjustments in ecosystem carbon and nutrient cycles (Pellegrini et al., 2015). Previous research in Canada (Day et al., 2019), China (Yang et al., 2020a, 2020b), the boreal forests of United States (Holden et al., 2016) and Australian shrublands (Muñoz-Rojas et al., 2016) have found a reduction of fungal abundance and diversity one-year following wildfires. Research on how long it takes for fungal abundance to recover following fire effects has shown a range of conclusions. For example, Muñoz-Rojas et al. (2016) report that fungal abundance reaches pre-burn levels in 5 years and Holden et al. (2013) report 12 to 24 years, respectively, while Yang et al. (2020a, 2020b) show that reduced fungal abundance persists 126 years after a wildfire. Clearly, further study is needed to determine the long-term effects of prescribed burning on fungal abundance.

4.2. Changes in functional gene abundance across burn regimes

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

The non-burn control plots have not been burned since 1954 and provide a context to ecosystem recovery through the succession of vegetation. The marker genes *AOA- amoA* and *AOB- amoA* have an essential role in the transformation of energy and are often used to study nitrification in ecosystems (Norton et al., 2002). The results indicate that *AOA* and *AOB* were sensitive to burn treatment as both were more abundant in the topsoil of the non-burn control (Fig. 3; Table S2) where NH_4^+ was significantly correlated with *AOA* and *AOB* overall (Fig. 6A). This is in agreement with our second hypothesis that N-cycling genes will vary across burn treatment and be higher in the non-burn control plots. *AOA-amoA* genes have been discovered in a variety of environments, including ocean sediments (Francis et al., 2007) and soils (Di et al., 2010; Leininger et al., 2006). *AOB-amoA* genes have been found to be more abundant than *AOA-amoA* in some soils (Di et al., 2010). This is contrary to the results from Zhang et al. (2018) who found that *AOA* and *AOB* increased after fire in a suburban forest ecosystem of subtropical Australia, Long et al. (2014) who found an increase in *AOB* after burning in wet sclerophyll forest and Yang et al. (2020a, 2020b) who found fire did not significantly impact *AOA* abundance in a Californian grassland. It has been noted that the concentration of microbial enzymes involved in organic N decomposition can increase or decrease after fires (Docherty et al., 2012).

In this study *AOB* was found to be more abundant than *AOA* despite previous studies showing *AOA-amoA* to be more dominant in wetland type environments (Baolan et al., 2012; Chen et al., 2008). One possible explanation for this is that *AOB* is more likely to prefer higher NH_4^+ than *AOA* (Dong et al., 2020; Liu et al., 2011). Therefore, higher NH_4^+ content could increase the competitiveness of *AOB* within this environment. Plant species richness, root biomass, and total C have been found to be strongly related to the *AOA* and *AOB* abundance (Boyle-Yarwood et al., 2008; Rasche et al., 2011; Rooney et al., 2010; Szukics et al., 2010; Szukics et al., 2012; Zeglin et al., 2011). These soil parameters can be altered rapidly after vegetation burning affecting the abundance of *AOA* and *AOB*. *AOA* and *AOB* are slow growing which could explain their relatively low abundance compared with other functional genes in this study (Prosser and Nicol, 2008).

Denitrification is critical to the nitrogen cycle and involves four reaction steps that convert nitrate to nitrous oxide or dinitrogen gas (Hayatsu et al., 2008). The results indicate that the dentrification gene *nirK* varied across burn treatments, being greater in the topsoil of the short rotation and long rotation plots (Fig. 4B). This result is in agreement with Zhang et al. (2018) who found the abundance of *nirK* increased 3 months after prescribed burning and Li et al. (2023) who found that *nirK* increased in abundance 6 years and 11 years after fire. Indicating the fire disturbance can have a promotion effect on soil denitrification (Liu et al., 2017). There was no significant difference between the abundance of *nirS* and burn treatment or soil depth (Fig. 4A; Table S2), indicating that microorganisms harbouring the '*nir*' gene select for different habitats when soil is affected by anthropogenic activity (Levy-Booth et al., 2014). Previous studies indicate that the niche of *nir*-habouring bacteria (*nirS* and *nirK*) also change their respective behaviour in relation to land-use (Bu et al., 2020; Li et al., 2018). However, in this study *nirK* was more abundant overall. This is in agreement with Li et al. (2018) where *nirK* was more abundant in every stage of restoration indicating that *nirK* is a good indicator for denitrification. *NirS* has been identified to be more resilient during land-use changes (Chen et al., 2010) but in this study *nirK* was more resilient to prescribed burning. The differences in abundance may be due to differences in the soil environment. For example, soil moisture has been identified as a major driver for *nirK* abundance as moisture primarily regulates denitrification (Klemedtsson et al., 1988). Therefore, the change in soil moisture in burned rotations can explain the relatively high abundance of *nirK*.

The activity of the *nifH* gene is related to the ability of nitrogen-fixing archaea and bacteria to fix N_2 (Bürgmann et al., 2003). In this study, *nifH* abundance correlated with total C overall (Fig. 6A). Previous research has found a positive correlation between the *nifH* gene and carbon (Kennedy and Egger, 2010; Levy-Booth and Winder, 2010; Morales et al., 2010) because diazotrophs are dependent on carbon for provisioning the large amount of adenosine triphosphate (ATP) consumed by N fixation (Chen et al., 2010). In line with our second hypothesis, the *nifH* gene was most abundant in the non-burn control (Fig. 5A) which also had the highest amount of carbon present compared to the burn treatments (Table 1). There was a positive relationship between the *nifH* gene and heather cover (%) (Fig. 6A) suggesting that N_2 fixation was aligned with decomposing plant material and previous studies have shown that N fixation is achieved with a symbiotic relationship with plant roots (Hayden et al., 2010). Because bulk soils were sampled in this study, the *nifH* genes detected were expected to be primarily from N-fixing bacteria that are free-living. There was a negative correlation between *nifH* gene abundance and total N overall (Fig. 6A) and particularly in soils subject to short rotation burns (Fig. S1 C & F), suggesting that short rotation burning could inhibit dinitrogenase reductase and lead to a reduction in the biological capacity for nitrogen fixation.

Plots under a short burn rotation regime harboured a higher abundance of the *chiA* gene (Fig. 5B). There was a positive correlation with moisture, nitrogen, NO_3, graminoid cover, *Sphagnum* cover and other non-*Sphagnum* moss cover, but a negative correlation with NH_4^+ (Fig. 6A). It is not surprising that *chiA* correlated with nitrogen content as decomposition of chitin contributes to both the labile nitrogen pools in the soil. These results show that these microorganisms select for different habitats when soil is affected by anthropogenic activity. However, it is also important to acknowledge that chitin degradation is commonly associated with fungi as well as bacteria (Talbot and Treseder, 2010), hence care must be taken when assessing the true biological potential of these genes.

4.3. Changes in functional gene abundance across soil profiles

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

In contrast with our fourth hypothesis, *nirS* was not significantly affected by soil depth while *nirK* was significantly higher in the topsoil suggesting *nirK*-harbouring denitrifiers thrived in the topsoil. This result is consistent with a previous study where *nirK* were more abundant in the topsoil of paddy soils (Wang et al., 2017) and desert soils (Bu et al., 2020) However, this is in contrast to a previous studies (Castellano-Hinojosa et al., 2018; Song et al., 2019) which found the gene abundance of *nirK* to increase with soil depth. This variation could be attributed to changes in soil environmental conditions (Levy-Booth et al., 2014) and nir-harbouring denitifyers (*nirK* and *nirS*) occupying different niches within soil profiles (Levy-Booth et al., 2014). This is supported by Tang et al. (2016) and Bu et al. (2020) who showed that *nirS* and *nirK* can differ across soil profiles as a result of differing environmental factors. Previous research has shown that soil properties (soil pH, C and N contents) has a major influence on denitrifier communities (Bru et al., 2011; Clark et al., 2012). For example there was a positive correlation between *nirK* and total N (Fig. 6A). Earlier studies have found *nirK* abundance to be positively correlated with total N (Clark et al., 2012; Wang et al., 2017; Jiang et al., 2021). In this study soil physicochemical properties showed significant changes along the soil depth gradient or among the three treatments, which might represent an important effect on the distribution of *nirS* and *nirK*-containing denitrifiers.

The inputs of N depend on biological N fixation which is processed by nitrogen-fixing bacteria (Zehr, 2011). In this study the *nifH* gene abundance in peat significantly declined from the topsoil to subsoil across all treatments. This supports our third hypothesis that the abundance of *nifH* will be higher in the topsoil and agrees with previous research in other ecosystems that the fixation of N_2 occurs mainly at the topsoil such as in desert soil (Bu et al., 2020), agricultural soil (Li et al., 2018), grassland soil (Song et al., 2019) and paddy soil (Wang et al., 2017). A higher abundance of heterotrophic decomposers with higher nitrogen requirements could explain the higher abundance in surface soils.

Organic nitrogen obtained from the detritus of soil microorganisms is a critical substrate for microbes carrying the *chiA* gene. Here, *chiA*

showed a decrease with soil depth in all treatments. The results here are in contrast to Li et al. (2018) where *chiA* increased with depth in forest soils. The cause of different responses may be due to specific environmental conditions which are very different between a forest with deep rooting trees and typical wetter peatlands exhibiting shrub-dominated vegetation. This study provides new data on the distribution of microbes and functional genes in peatlands under different burning regimes, and the microbial response is expected to be unique in many respects compared to different environmental situations.

4.4. Implications for N cycling processes in peatlands under disturbance regimes

The observed associations between microbial gene abundances and environmental parameters have significant implications for the biogeochemical processes of N cycling in peatland ecosystems (Limpens et al., 2006). For instance, the results suggest that disturbances that degrade peatland moisture conditions and reduce the concentration of total C and NH_4^+ might affect the microbial process of N fixation. This was evident in the positive correlation between *nifH* gene abundance and moisture, total C, and NH_4^+. Similarly, the correlations between *AOA* and *AOB* gene abundances with edaphic factors like pH, NH_4^+, and metal concentrations suggest that changes in soil chemistry associated with the addition of ash and oxidized forms of nutrients in burned plots can influence the rate of nitrification, and subsequently impact the availability of nitrate in the peatland ecosystem. This can be attributed to the sensitivity of nitrifying microorganisms to changes in soil acidity and nutrient availability (Prosser and Nicol, 2012). Consistent with previous studies, our results also suggest that the process of denitrification in peatlands is a function of availability of N, soil moisture and vegetation cover (Castaldi and Aragosa, 2002; Fang et al., 2020), which was evident in their correlation with *nirK* and *nirS* gene abundances (Philippot et al., 2011).

These associations collectively indicate that in the event of peatland disturbance, microbial functional groups mediating different biogeochemical processes respond differently to modifications in environmental conditions. Depending on the prevailing conditions, the balance between nitrogen fixation, nitrification, and denitrification can be altered, affecting the overall availability of nitrogen compounds and the potential for nitrogen loss from the ecosystem. Additionally, the correlations between environmental parameters and microbial gene abundances also underscore the complex interplay of biotic and abiotic factors that regulate microbial community structure and their critical role of mediating essential biogeochemical processes in peatland ecosystems. The insights gained from these associations can inform peatland management strategies and the impacts of different disturbance regimes on nitrogen cycling processes.

5. Conclusions

Previous research has shown that soil properties and vegetation cover play a vital role in determining the distribution of nitrogen cycling genes in the environment, providing insight into the genetic potential for nitrogen cycling under different situations (Bu et al., 2020; Castellano-Hinojosa et al., 2018; Song et al., 2019). In this work, we extended prior knowledge from other ecosystems by assessing the abundance of nitrogen cycling related genes in a UK upland peat bog under differential management with respect to burning regimes. The long-term cessation of burning increased the abundance of bacteria, fungi, and improved microbial N turnover potential as evidenced by an increase in the abundance of N-cycling genes. The abundance of *nirK*, *nifH* and *chiA* showed a reduction with depth from the topsoil to the subsoil, indicating that these genes have different niche requirements which may be a consequence of biochemical constraints of the processes which they facilitate. Contrasting correlation results among soil properties in the topsoil and subsoil indicate a complex structured environment with respect to depth which is modified by burning. Further exploration of these relationships would be valuable to help achieve a better understanding of peatland soil processes in order to support sustainable land management approaches. For instance, present results indicate that the avoidance of burning in peatlands would enhance nitrogen cycling and thus support ecosystem services which rely upon the provisioning of nitrogen (e.g. plant growth). Furthermore, this research has shown that there are functional implications in the deeper soil for land management actions which take place on the surface.

CRediT authorship contribution statement

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

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2024.105426.

References

Andersen, R., Wells, C., Macrae, M., Price, J., 2013. Nutrient mineralisation and microbial functional diversity in a restored bog approach natural conditions 10 years post restoration. Soil Biol. Biochem. 64, 37–47. https://doi.org/10.1016/j.soilbio.2013.04.004.

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.

Baolan, H., Shuai, L., Lidong, S., Ping, Z., Xiangyang, X., Liping, L., 2012. Effect of different ammonia concentrations on community succession of ammonia-oxidizing microorganisms in a simulated paddy soil column. PloS one 7 (8), e44122 pmid: 22952893. https://doi.org/10.1371/journal.pone.0044122.

Berthrong, S.T., Buckley, D.H., Drinkwater, L.E., 2013. Agricultural management and labile carbon additions affect soil microbial community structure and interact with carbon and nitrogen cycling. Microbial. Ecol. 66 (1), 158–170. https://doi.org/10.1007/s00248-013-0225-0.

Blodau, C., Zajac, K., 2015. Carbon and nitrogen dynamics in mesocosms of five different European peatlands. In: EGU General Assembly Conference Abstracts, Vol. 17. April.

Borneman, J., Hartin, R.J., 2000. (PCR) primers that amplify fungal rRNA genes from environmental samples. Appl. Environ. Microbiol. 66 (10), 4356–4360. https://doi.org/10.1128/AEM.66.10.4356-4360.2000.

Boyle-Yarwood, S.A., Bottomley, P.J., Myrold, D.D., 2008. Community composition of ammonia-oxidizing bacteria and archaea in soils under stands of red alder and Douglas fir in Oregon. Environ. Microbiol. 10 (11), 2956–2965. https://doi.org/10.1111/j.1462-2920.2008.01600.x.

Bru, D., Ramette, A., Saby, N.P.A., Dequiedt, S., Ranjard, L., Jolivet, C., Arrouays, D., Philippot, L., 2011. Determinants of the distribution of nitrogen-cycling microbial communities at the landscape scale. ISME J. 5 (3), 532–542. https://doi.org/10.1038/ismej.2010.130.

Bu, L., Peng, Z., Tian, J., Song, F., Wei, G., Wang, H., 2020. Distinct abundance patterns of nitrogen functional microbes in desert soil profiles regulate soil nitrogen storage potential along a desertification development gradient. Catena 194, 104716. https://doi.org/10.1016/j.catena.2020.104716.

Bürgmann, H., Widmer, F., Sigler, W.V., Zeyer, J., 2003. mRNA extraction and reverse transcription-PCR protocol for detection of nifH gene expression by *Azotobacter vinelandii* in soil. Appl. Environ. Microbiol. 69 (4), 1928–1935. https://doi.org/10.1128/AEM.69.4.1928-1935.2003.

Castaldi, S., Aragosa, D., 2002. Factors influencing nitrification and denitrification variability in a natural and fire-disturbed Mediterranean shrubland. Biol. Fertil. Soils 36, 418–425. https://doi.org/10.1007/s00374-002-0549-2.

Castellano-Hinojosa, A., González-López, J., Bedmar, E.J., 2018. Distinct effect of nitrogen fertilisation and soil depth on nitrous oxide emissions and nitrifiers and denitrifiers abundance. Biol. Fertil. Soils 54 (7), 829–840. https://doi.org/10.1007/s00374-018-1310-9.

Che, R., Qin, J., Tahmasbian, I., Wang, F., Zhou, S., Xu, Z., Cui, X., 2018. Litter amendment rather than phosphorus can dramatically change inorganic nitrogen pools in a degraded grassland soil by affecting nitrogen-cycling microbes. Soil Biol. Biochem. 120, 145–152. https://doi.org/10.1016/j.soilbio.2018.02.006.

Chen, X.P., Zhu, Y.G., Xia, Y., Shen, J.P., He, J.Z., 2008. Ammonia-oxidizing archaea: important players in paddy rhizosphere soil? Environ. Microbiol. 10 (8), 1978–1987. https://doi.org/10.1111/j.1462-2920.2008.01613.x.

Chen, Z., Luo, X., Hu, R., Wu, M., Wu, J., Wei, W., 2010. Impact of long-term fertilization on the composition of denitrifier communities based on nitrite reductase analyses in a paddy soil. Microbial. Ecol 60 (4), 850–861. https://doi.org/10.1007/s00248-010-9700-z.

Ciccolini, V., Ercoli, L., Davison, J., Vasar, M., Öpik, M., Pellegrino, E., 2016. Land-use intensity and host plant simultaneously shape the composition of arbuscular mycorrhizal fungal communities in a Mediterranean drained peatland. FEMS Microbiol. Ecol. 92 (12) https://doi.org/10.1093/femsec/fiw186 p.fiw186.

Clark, I.M., Buchkina, N., Jhurreea, D., Goulding, K.W., Hirsch, P.R., 2012. Impacts of nitrogen application rates on the activity and diversity of denitrifying bacteria in the Broadbalk wheat experiment. Philos. Trans. R. Soc., B 367 (1593), 1235–1244. https://doi.org/10.1098/rstb.2011.0314.

Clay, G.D., Worrall, F., Clark, E., Fraser, E.D., 2009. Hydrological responses to managed burning and grazing in an upland blanket bog. J. Hydrol. 376 (3–4), 486–495. https://doi.org/10.1016/j.jhydrol.2009.07.055.

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).

Day, N.J., Dunfield, K.E., Johnstone, J.F., Mack, M.C., Turetsky, M.R., Walker, X.J., White, A.L., Baltzer, J.L., 2019. Wildfire severity reduces richness and alters composition of soil fungal communities in boreal forests of western Canada. Glob. Chang. Biol. 25 (7), 2310–2324. https://doi.org/10.1111/gcb.14641.

Di, H.J., Cameron, K.C., Shen, J.P., Winefield, C.S., O'Callaghan, M., Bowatte, S., He, J. Z., 2010. Ammonia-oxidizing bacteria and archaea grow under contrasting soil nitrogen conditions. FEMS Microbiol. Ecol. 72 (3), 386–394. https://doi.org/10.1111/j.1574-6941.2010.00861.x.

Docherty, K.M., Balser, T.C., Bohannan, B.J., Gutknecht, J.L., 2012. Soil microbial responses to fire and interacting global change factors in a California annual grassland. Biogeochemistry 109, 63–83. https://doi.org/10.1007/s10533-011-9654-3.

Dong, J., Che, R., Jia, S., Wang, F., Zhang, B., Cui, X., Wang, S., Wang, S., 2020. Responses of ammonia-oxidizing archaea and bacteria to nitrogen and phosphorus amendments in an alpine steppe. Eur. J. Soil Sci. 71 (5), 940–954. https://doi.org/10.1111/ejss.12911.

Douglas, D.J., Buchanan, G.M., Thompson, P., Amar, A., Fielding, D.A., Redpath, S.M., Wilson, J.D., 2015. Vegetation burning for game management in the UK uplands is increasing and overlaps spatially with soil carbon and protected areas. Biol. Conserv. 191, 243–250. https://doi.org/10.1016/j.biocon.2015.06.014.

Espenberg, M., Truu, M., Mander, Ü., Kasak, K., Nõlvak, H., Ligi, T., Oopkaup, K., Maddison, M., Truu, J., 2018. Differences in microbial community structure and nitrogen cycling in natural and drained tropical peatland soils. Sci. Rep. 8 (1), 4742. https://doi.org/10.1038/s41598-018-23032-y.

Evans, C.D., Bonn, A., Holden, J., Reed, M.S., Evans, M.G., Worrall, F., Couwenberg, J., Parnell, M., 2014a. 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.

Evans, C.D., Chadwick, T., Norris, D., Rowe, E.C., Heaton, T.H., Brown, P., Battarbee, R. W., 2014b. Persistent surface water acidification in an organic soil-dominated upland region subject to high atmospheric deposition: the North York Moors, UK. Ecol. Indic 37, 304–316. https://doi.org/10.1016/j.ecolind.2012.02.018.

Fang, X., Zheng, R., Guo, X., Fu, Q., Zhang, K., 2020. Responses of denitrification rate and denitrifying bacterial communities carrying nirS and nirK genes to grazing in peatland. J. Soil. Plant. Nutr 20, 1249–1260. https://doi.org/10.1007/s42729-020-00209-x.

Fierer, N., Schimel, J.P., Holden, P.A., 2003. Variations in microbial community composition through two soil depth profiles. Soil Biol. Biochem. 35 (1), 167–176. https://doi.org/10.1016/S0038-0717(02)00251-1.

Fierer, N., Jackson, J.A., Vilgalys, R., Jackson, R.B., 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Appl. Environ. Microbiol. 71 (7), 4117–4120. https://doi.org/10.1128/AEM.71.7.4117-4120.2005.

Fisk, M.C., Ruether, K.F., Yavitt, J.B., 2003. Microbial activity and functional composition among northern peatland ecosystems. Soil Biol. Biochem. 35 (4), 591–602. https://doi.org/10.1016/S0038-0717(03)00053-1.

Francis, C.A., Roberts, K.J., Beman, J.M., Santoro, A.E., Oakley, B.B., 2005. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. PNAS 102 (41), 14683–14688. https://doi.org/10.1073/pnas.0506625102.

Francis, C.A., Beman, J.M., Kuypers, M.M., 2007. New processes and players in the nitrogen cycle: the microbial ecology of anaerobic and archaeal ammonia oxidation. ISME J. 1 (1), 19–27. https://doi.org/10.1038/ismej.2007.8.

Hallin, S., Lindgren, P.E., 1999. PCR detection of genes encoding nitrite reductase in denitrifying bacteria. Appl. Environ. Microbiol. 65 (4), 1652–1657. https://doi.org/10.1128/AEM.65.4.1652-1657.1999.

Harper, A.R., Doerr, S.H., Santin, C., Froyd, C.A., Sinnadurai, P., 2018. Prescribed fire and its impacts on ecosystem services in the UK. Sci. Total Environ. 624, 691–703. https://doi.org/10.1016/j.scitotenv.2017.12.161.

Harte, J., Saleska, S.R., Levy, C., 2015. Convergent ecosystem responses to 23-year ambient and manipulated warming link advancing snowmelt and shrub encroachment to transient and long-term climate–soil carbon feedback. Glob. Chang. Biol. 21 (6), 2349–2356. https://doi.org/10.1111/gcb.12831.

Hayatsu, M., Tago, K., Saito, M., 2008. Various players in the nitrogen cycle: diversity and functions of the microorganisms involved in nitrification and denitrification. Soil Sci. Plant Nutr. 54 (1), 33–45. https://doi.org/10.1111/j.1747-0765.2007.00195.x.

Hayden, H.L., Drake, J., Imhof, M., Oxley, A.P., Norng, S., Mele, P.M., 2010. The abundance of nitrogen cycle genes amoA and nifH depends on land-uses and soil types in south-eastern Australia. Soil Biol. Biochem. 42 (10), 1774–1783. https://doi.org/10.1016/j.soilbio.2010.06.015.

Holden, S.R., Gutierrez, A., Treseder, K.K., 2013. Changes in soil fungal communities, extracellular enzyme activities, and litter decomposition across a fire chronosequence in Alaskan boreal forests. Ecosystems 16, 34–46. https://doi.org/10.1007/s10021-012-9594-3.

Holden, J., Wearing, C., Palmer, S., Jackson, B., Johnston, K., Brown, L.E., 2014. Fire decreases near-surface hydraulic conductivity and macropore flow in blanket peat. Hydrol. Process. 28 (5), 2868–2876. https://doi.org/10.1002/hyp.9875.

Holden, J., Palmer, S.M., Johnston, K., Wearing, C., Irvine, B., Brown, L.E., 2015. Impact of prescribed burning on blanket peat hydrology. Water Resour. Res. 51 (8), 6472–6484. https://doi.org/10.1002/2014WR016782.

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.

IUCN, 2020. International Union for Conservation of Nature UK Peatland Programme: Burning & Peatlands Position Paper. Version 2, 31st March, 2020. IUCN, Edinburgh, UK. Accessed at: https://www.iucn-uk-peatlandprogramme.org/sites/default/files/2020-03/IUCN%20UK%20PP%20Burning%20and%20Peatlands%20Position%20Paper%.

Jiang, L., Song, Y., Sun, L., Ma, X., Wang, X., Song, C., Hou, A., Wang, L., 2021. Distribution of carbon and nitrogen cycle microbes along permafrost peatland profile in Northeast China. Environ. Prog. Sustain. Energy 40 (5), e13707. https://doi.org/10.1002/ep.13707.

Kennedy, N., Egger, K.N., 2010. Impact of wildfire intensity and logging on fungal and nitrogen-cycling bacterial communities in British Columbia forest soils. For. Ecol. Manage. 260 (5), 787–794. https://doi.org/10.1016/j.foreco.2010.05.037.

Kim, J.G., Jung, M.Y., Park, S.J., Rijpstra, W.I.C., Sinninghe Damsté, J.S., Madsen, E.L., Min, D., Kim, J.S., Kim, G.J., Rhee, S.K., 2012. Cultivation of a highly enriched ammonia-oxidizing archaeon of thaumarchaeotal group I. 1b from an agricultural soil. Environ. Microbiol. 14 (6), 1528–1543. https://doi.org/10.1111/j.1462-2920.2012.02740.x.

Klemedtsson, L., Svensson, B.H., Rosswall, T., 1988. Relationships between soil moisture content and nitrous oxide production during nitrification and denitrification. Biol. Fertil. Soils 6 (2), 106–111. https://doi.org/10.1007/BF00257658.

Kramer, S., Marhan, S., Haslwimmer, H., Ruess, L., Kandeler, E., 2013. Temporal variation in surface and subsoil abundance and function of the soil microbial community in an arable soil. Soil Bio. Biochem 61, 76–85. https://doi.org/10.1016/j.soilbio.2013.02.006.

Lee, H., Alday, J.G., Rose, R.J., O'Reilly, J., Marrs, R.H., 2013a. 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.

Lee, H., Alday, J.G., Rosenburgh, A., Harris, M., McAllister, H., & Marrs, R.H. (2013b). Change in propagule banks during prescribed burning: a tale of two contrasting moorlands. Biol. Conserv. 165(0), 187–197. https://doi.org/10.1016/j.biocon.2013.05.023.

Leininger, S., Urich, T., Schloter, M., Schwark, L., Qi, J., Nicol, G.W., Prosser, J.I., Schuster, S.C., Schleper, C., 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442 (7104), 806–809. https://doi.org/10.1038/nature04983.

Levy-Booth, D.J., Winder, R.S., 2010. Quantification of nitrogen reductase and nitrite reductase genes in soil of thinned and clear-cut Douglas-fir stands by using real-time PCR. Appl. Environ. Microbiol. 76 (21), 7116–7125. https://doi.org/10.1128/AEM.02188-09.

Levy-Booth, D.J., Prescott, C.E., Grayston, S.J., 2014. Microbial functional genes involved in nitrogen fixation, nitrification and denitrification in forest ecosystems. Soil Biol. Biochem. 75, 11–25. https://doi.org/10.1016/j.soilbio.2014.03.021.

Li, H., Chen, J., Ding, Z., Shu, Y., Wei, J., Zhao, P., Zhou, M., Wang, Y., Zhang, Y., 2023. Effects of Fire Disturbance on Soil Nitrogen Fractions and Functional Genes of

Nitrogen Cycling in Soil of Larix gmelinii Forests. Ecol. Environ. 32 (9), 1563. https://doi.org/10.16258/j.cnki.1674-5906.2023.09.003.
Li, D., Zhang, X., Green, S.M., Dungait, J.A., Wen, X., Tang, Y., Guo, Z., Yang, Y., Sun, X., Quine, T.A., 2018. Nitrogen functional gene activity in soil profiles under progressive vegetative recovery after abandonment of agriculture at the Puding Karst Critical Zone Observatory, SW China. Soil.Biol. Biochem. 125, 93–102. https://doi.org/10.1016/j.soilbio.2018.07.004.
Limpens, J., Heijmans, M.M., Berendse, F., 2006. The nitrogen cycle in boreal peatlands. In: Boreal Peatland Ecosystems. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 195–230.
Liu, X., Chen, C.R., Hughes, J.M., Wang, W.J., Lewis, T., 2017. Temporal changes rather than long-term repeated burning predominately control the shift in the abundance of soil denitrifying community in an Australian sclerophyll forest. Microb.Ecol 73, 177–187. https://doi.org/10.1007/s00248-016-0894-6.
Liu, Z., Huang, S., Sun, G., Xu, Z., Xu, M., 2011. Diversity and abundance of ammonia-oxidizing archaea in the Dongjiang River, China. Microbiol. Res. 166 (5), 337–345. https://doi.org/10.1016/j.micres.2010.08.002.
Liu, Y., Wang, P., Pan, G., Crowley, D., Li, L., Zheng, J., Zhang, X., Zheng, J., 2016. Functional and structural responses of bacterial and fungal communities from paddy fields following long-term rice cultivation. J. Soil. Sediment. 16 (5), 1460–1471. https://doi.org/10.1007/s11368-015-1343-8.
Long, X.E., Chen, C., Xu, Z., He, J.Z., 2014. Shifts in the abundance and community structure of soil ammonia oxidizers in a wet sclerophyll forest under long-term prescribed burning. Sci. Total Environ. 470, 578–586.
Martens-Habbena, W., Berube, P.M., Urakawa, H., José, R., Stahl, D.A., 2009. Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria. Nature 461 (7266), 976–979. https://doi.org/10.1038/nature08465.
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.
Morales, S.E., Cosart, T., Holben, W.E., 2010. Bacterial gene abundances as indicators of greenhouse gas emission in soils. ISME J. 4 (6), 799–808. https://doi.org/10.1038/ismej.2010.8.
Muñoz-Rojas, M., Erickson, T.E., Martini, D., Dixon, K.W., Merritt, D.J., 2016. Soil physicochemical and microbiological indicators of short, medium and long term post-fire recovery in semi-arid ecosystems. Ecol. Indic. 63, 14–22. https://doi.org/10.1016/j.ecolind.2015.11.038.
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.
Nelson, M.B., Berlemont, R., Martiny, A.C., Martiny, J.B., 2015. Nitrogen cycling potential of a grassland litter microbial community. Appl. Environ. Microbiol. 81 (20), 7012–7022. https://doi.org/10.1128/AEM.02222-15.
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.
Norton, J.M., Alzerreca, J.J., Suwa, Y., Klotz, M.G., 2002. Diversity of ammonia monooxygenase operon in autotrophic ammonia-oxidizing bacteria. Arch. Microbiol. 177 (2), 139–149. https://doi.org/10.1007/s00203-001-0369-z.
Nurulita, Y., Adetutu, E.M., Gunawan, H., Zul, D., Ball, A.S., 2016. Restoration of tropical peat soils: the application of soil microbiology for monitoring the success of the restoration process. Agric. Ecosyst. Environ. 216, 293–303. https://doi.org/10.1016/j.agee.2015.09.031.
Pajares, S., Bohannan, B.J., 2016. Ecology of nitrogen fixing, nitrifying, and denitrifying microorganisms in tropical forest soils. Front. Microbiol. 7, 1045. https://doi.org/10.3389/fmic/2016.01045.
Pellegrini, A.F., Hedin, L.O., Staver, A.C., Govender, N., 2015. Fire alters ecosystem carbon and nutrients but not plant nutrient stoichiometry or composition in tropical savanna. Ecology 96 (5), 1275–1285. https://doi.org/10.1890/14-1158.1.
Philippot, L., Andert, J., Jones, C.M., Bru, D., Hallin, S., 2011. Importance of denitrifiers lacking the genes encoding the nitrous oxide reductase for N2O emissions from soil. Glob. Chang. Biol. 17 (3), 1497–1504. https://doi.org/10.1111/j.1365-2486.2010.02334.x.
Prosser, J.I., Nicol, G.W., 2008. Relative contributions of archaea and bacteria to aerobic ammonia oxidation in the environment. Environ. Microbiol. 10 (11), 2931–2941. https://doi.org/10.1111/j.1462-2920.2008.01775.x.
Prosser, J.I., Nicol, G.W., 2012. Archaeal and bacterial ammonia-oxidisers in soil: the quest for niche specialisation and differentiation. Trends Microbiol. 20 (11), 523–531. https://doi.org/10.1016/j.tim.2012.08.001.
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/.
Rasche, F., Knapp, D., Kaiser, C., Koranda, M., Kitzler, B., Zechmeister-Boltenstern, S., Richter, A., Sessitsch, A., 2011. Seasonality and resource availability control bacterial and archaeal communities in soils of a temperate beech forest. ISME J. 5 (3), 389–402. https://doi.org/10.1038/ismej.2010.138.
Rennie, S., Andrews, C., Atkinson, S., Beaumont, D., Benham, S., Bowmaker, V., Dick, J., Dodd, B., McKenna, C., Pallett, D., Rose, R., 2020. The UK environmental change network datasets–integrated and co-located data for long-term environmental research (1993–2015). Earth Syst. Sci. Data 12 (1), 87–107. https://doi.org/10.5194/essd-12-87-2020.
Rodwell, J.S., 1998. British Plant Communities, vol. 2. Cambridge University Press.
Rooney, D.C., Kennedy, N.M., Gleeson, D.B., Clipson, N.J., 2010. Responses of ammonia-oxidising bacterial communities to nitrogen, lime, and plant species in upland grassland soil. Appl. Environ. Soil Sci. 2010 https://doi.org/10.1155/2010/319721.
Rösch, C., Mergel, A., Bothe, H., 2002. Biodiversity of denitrifying and dinitrogen-fixing bacteria in an acid forest soil. Appl. Environ. Microbiol. 68 (8), 3818–3829. https://doi.org/10.1128/AEM.68.8.3818-3829.2002.
Rotthauwe, J.H., Witzel, K.P., Liesack, W., 1997. The ammonia monooxygenase structural gene amoA as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. Appl. Environ. Microbiol. 63 (12), 4704–4712. https://doi.org/10.1128/aem.63.12.4704-4712.1997.
Rowell, D.L., 2014. Soil Science: Methods & Applications. Routledge.
Shukla, S., Shukla, K., Mishra, A., Jindal, T., Sharma, S., Upadhyay, D., Singh, V., 2021. Ecological perspectives on soil microbial community involved in nitrogen cycling. In: Soil Nitrogen Ecology. Springer, Cham, pp. 51–91.
Simmons, I.G., 2003. The Moorlands of England and Wales: An Environmental History 8000 BC to AD 2000. Edinburgh University Press.
Song, Z., Wang, J., Liu, G., Zhang, C., 2019. Changes in nitrogen functional genes in soil profiles of grassland under long-term grazing prohibition in a semiarid area. Sci. Total Environ. 673, 92–101. https://doi.org/10.1016/j.scitotenv.2019.04.026.
Stone, M.M., Kan, J., Plante, A.F., 2015. Parent material and vegetation influence bacterial community structure and nitrogen functional genes along deep tropical soil profiles at the Luquillo critical zone observatory. Soil Biol. Biochem. 80, 273–282. https://doi.org/10.1016/j.soilbio.2014.10.019.
Szukics, U., Abell, G.C., Hödl, V., Mitter, B., Sessitsch, A., Hackl, E., Zechmeister-Boltenstern, S., 2010. Nitrifiers and denitrifiers respond rapidly to changed moisture and increasing temperature in a pristine forest soil. FEMS Microbiol. Ecol. 72 (3), 395–406. https://doi.org/10.1111/j.1574-6941.2010.00853.x.
Szukics, U., Hackl, E., Zechmeister-Boltenstern, S., Sessitsch, A., 2012. Rapid and dissimilar response of ammonia oxidizing archaea and bacteria to nitrogen and water amendment in two temperate forest soils. Microbiol. Res. 167 (2), 103–109. https://doi.org/10.1016/j.micres.2011.04.002.
Talbot, J.M., Treseder, K.K., 2010. Controls over mycorrhizal uptake of organic nitrogen. Pedobiologia 53 (3), 169–179. https://doi.org/10.1016/j.pedobi.2009.12.001.
Tang, Y., Yu, G., Zhang, X., Wang, Q., Ge, J., Liu, S., 2018. Changes in nitrogen-cycling microbial communities with depth in temperate and subtropical forest soils. Appl. Soil Ecol. 124, 218–228. https://doi.org/10.1016/j.apsoil.2017.10.029.
Tang, Y., Zhang, X., Li, D., Wang, H., Chen, F., Fu, X., Fang, X., Sun, X., Yu, G., 2016. Impacts of nitrogen and phosphorus additions on the abundance and community structure of ammonia oxidizers and denitrifying bacteria in Chinese fir plantations. Soil Biol. Biochem. 103, 284–293.
Thacker, J.I., Yallop, A.R., Clutterbuck, B., 2000. Burning in the English Uplands—A Review, Reconciliation and Comparison of Results of Natural england's Burn Monitoring: 2005–2014. Improvement Programme for England's Natura.
Thompson, K.A., Bent, E., James, K., Carlyle, C.N., Quideau, S., Bork, E.W., 2020. Access mats partially mitigate direct traffic impacts on soil microbial communities in temperate grasslands. Appl. Soil Ecol. 145, 103353. https://doi.org/10.1016/j.apsoil.2019.09.003.
Thormann, M.N., Rice, A.V., 2007. Fungi from peatlands. Fungal Divers. 24 (2415), 299.
Throbäck, I.N., Enwall, K., Jarvis, Å., Hallin, S., 2004. Reassessing PCR primers targeting nirS, nirK and nosZ genes for community surveys of denitrifying bacteria with DGGE. FEMS Microbiol. Ecol. 49 (3), 401–417. https://doi.org/10.1016/j.femsec.2004.04.011.
Tian, X.F., Hu, H.W., Ding, Q., Song, M.H., Xu, X.L., Zheng, Y., Guo, L.D., 2014. Influence of nitrogen fertilization on soil ammonia oxidizer and denitrifier abundance, microbial biomass, and enzyme activities in an alpine meadow. Biol. Fertile. Soils 50 (4), 703–713. https://doi.org/10.1007/s00374-013-0889-0.
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, H.T., Su, J.Q., Zheng, T.L., Yang, X.R., 2014. Impacts of vegetation, tidal process, and depth on the activities, abundances, and community compositions of denitrifiers in mangrove sediment. Appl. Microbiol. Biotechnol. 98 (22), 9375–9387. https://doi.org/10.1007/s00253-014-6017-8.
Wang, H., Li, X., Li, X., Li, X., Wang, J. & Zhang, H., (2017). Changes of microbial population and N-cycling function genes with depth in three Chinese paddy soils. PloS One, 12 (12), p.e0189506. https://doi.org/10.1371/journal.pone.0189506.
Williamson, N., Brian, P., Wellington, E.M.H., 2000. Molecular detection of bacterial and streptomycete chitinases in the environment. Antonie Van Leeuwenhoek 78 (3), 315–321. https://doi.org/10.1023/A:1010225909148.
Xie, Z., Le Roux, X., Wang, C., Gu, Z., An, M., Nan, H., Chen, B., Li, F., Liu, Y., Du, G., Feng, H., 2014. Identifying response groups of soil nitrifiers and denitrifiers to grazing and associated soil environmental drivers in Tibetan alpine meadows. Soil Biol. Biochem. 77, 89–99. https://doi.org/10.1016/j.soilbio.2014.06.024.
Xu, J., Morris, P.J., Liu, J., Holden, J., 2018. PEATMAP: refining estimates of global peatland distribution based on a meta-analysis. Catena 160, 134–140. https://doi.org/10.1016/j.catena.2017.09.010.
Yallop, A., Thacker, J., Clutterbuck, B., 2006. Mapping extent of burn management in the north Pennines: review of extent year 2001-2003. English Nature Research Reports 698.
Yallop, A.R., Clutterbuck, B., Thacker, J., 2010. Increases in humic dissolved organic carbon export from upland peat catchments: the role of temperature, declining Sulphur deposition and changes in land management. Clim. Res. 45, 43–56. https://doi.org/10.3354/cr00884.
Yang, T., Tedersoo, L., Lin, X., Fitzpatrick, M.C., Jia, Y., Liu, X., Yingying, N., Yu, S., Pengpeng, L., Jianguo, Z., Chu, H., 2020a. Distinct fungal successional trajectories

following wildfire between soil horizons in a cold-temperate forest. New Phytol. 227 (2), 572–587. https://doi.org/10.1111/nph.16531.

Yang, S., Zheng, Q., Yang, Y., Yuan, M., Ma, X., Chiariello, N.R., Docherty, K.M., Field, C. B., Gutknecht, J.L.M., Hungate, B.A., Niboyet, A., Roux, X.L., Zhou, J., 2020b. Fire affects the taxonomic and functional composition of soil microbial communities, with cascading effects on grassland ecosystem functioning. Glob. Change Biol 26 (2), 431–442. https://doi.org/10.1111/gcb.14852.

Zeglin, L.H., Taylor, A.E., Myrold, D.D., Bottomley, P.J., 2011. Bacterial and archaeal amoA gene distribution covaries with soil nitrification properties across a range of land uses. Environ. Microbiol. Rep. 3 (6), 717–726. https://doi.org/10.1111/j.1758-2229.2011.00290.x.

Zehr, J.P., 2011. Nitrogen fixation by marine cyanobacteria. Trends Microbiol. 19 (4), 162–173. https://doi.org/10.1016/j.tim.2010.12.004.

Zhang, M., Wang, W., Wang, D., Heenan, M., Xu, Z., 2018. Short-term responses of soil nitrogen mineralization, nitrification and denitrification to prescribed burning in a suburban forest ecosystem of subtropical Australia. Sci. Total Environ. 642, 879–886. https://doi.org/10.1016/j.scitotenv.2018.06.144.

Zhang, G., Huang, J., Jia, M., Liu, F., Yang, Y., Wang, Z., Han, G., 2019. Ammonia-oxidizing bacteria and archaea: response to simulated climate warming and nitrogen supplementation. Soil Sci. Soc. Am. 83 (6), 1683–1695. https://doi.org/10.2136/sssaj2019.05.0134.