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Nitrogen deposition and temperature structure fungal communities associated with alpine moss-sedge heath in the UK

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ABSTRACT

Moss-sedge heath, an important habitat of oceanic alpine regions, is under threat from elevated nitrogen (N) deposition and rising temperatures increasing decomposition rates and fragmentation of the extensive, thick moss mats of *Racomitrium lanuginosum* which characterise this habitat. Here, we examine the potential effects of N deposition and temperature on fungal communities associated with *R. lanuginosum* mats. Fungal community composition in both green moss shoots and underlying soil were significantly influenced by mat temperatures and green moss shoot N contents. Total OTU, Ascomycota, Basidiomycota and saprotrophic fungal richness in shoots were all positively related to temperature, while in soil, the richness of all groups, except Basidiomycota, was positively related to moss tissue N. The observed community changes suggest that increased N loading and increasing temperatures are ameliorating growing conditions for fungi associated with moss-sedge heath. Further study is required to determine if the observed changes in fungal communities contribute to accelerated decomposition of moss mats and degradation of the habitat.

1. Introduction

Deposition of reactive nitrogen (N) is one of the greatest threats to the biodiversity and functioning of terrestrial ecosystems worldwide (Rockström et al., 2009, Ipbes, 2018). Reactive N emitted from agricultural, transport and industrial sources is readily transported over long distances in the atmosphere, and often deposited on ecosystems remote from the places of origin (Galloway et al., 2008; Sutton et al., 2011). Mountain areas can be disproportionally affected, with local enhancement of N deposition compared to surrounding lowlands due to orographic enhancement of rainfall (Crossley et al., 1992; Fowler et al., 1995). Globally, mountain ecosystems are often hotspots of biodiversity where high levels of abiotic heterogeneity promote species coexistence of generally slow-growing plant species adapted to low nutrient availability (Körner, 1995; Grabherr et al., 2003). Under pristine conditions, the productivity in these ecosystems, as in most other temperate and northern ecosystems, is N limited (Lebauer and Treseder, 2008). Enhanced deposition of N in mountain systems can therefore reduce N limitation and can rapidly exceed ecosystem requirements, resulting in cascading changes in community composition and ecosystem function both above and below ground (Galloway et al., 2003; Bobbink et al., 2010; Phoenix et al., 2012; Bowman et al., 2015).

Oceanic alpine regions are notable for the high prevalence of bryophytes within plant communities and for the importance of these bryophytes in the structure and functioning of ecosystems (Rodwell, 1991, 1992; Curtis et al., 2005; Britton et al., 2019). Bryophytes in these systems are highly adapted to oligotrophic conditions, being able to efficiently scavenge nutrients from rainfall (Turetsky, 2003). Deposited nutrients are readily assimilated into moss biomass which, in turn, can form a significant component of the total ecosystem nutrient pool (Turetsky, 2003; Curtis et al., 2005; Britton et al., 2011). Moss necromass is usually highly recalcitrant, with a high C:N ratio and a high prevalence of aromatic compounds (Turetsky, 2003; Lang et al., 2009). This, combined with the prevailing cool, wet conditions, results in slow decomposition and the development of thick mats of moss necromass, which may strongly influence nutrient cycling within the ecosystem (Turetsky, 2003; Lindo and Gonzalez, 2010).

In alpine systems, as in most other terrestrial ecosystems, fungi are the main decomposers of organic matter (Van Der Wal et al., 2013; Christiansen et al., 2018, Voříšková et al., 2019). Significant amounts of

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fungal hyphae are found in moss mats, and they actively decompose moss cell wall materials (Day and Currah, 2011a, 2011b). Fungal activity, as measured by total fungal lengths, is influenced by the bryophyte species, chemical composition, and position within the moss mat (Osono et al., 2012). However, fungi are not restricted to senescing tissue or to moss necromass; fungal biomass is also present in live moss shoots and to a greater degree than is seen in green leaves of vascular plants (Davey et al., 2009). Live moss shoots can support surprisingly diverse endophytic fungal communities, comprising a broad range of functional groups (Kauserud et al., 2008; Pressel et al., 2010; Davey et al., 2013). The fungal communities can also show a high degree of specialisation on particular moss species under similar abiotic conditions (Kauserud et al., 2008; Davey et al., 2013), which has been attributed to variability in species-level chemistry; mainly structural, antimicrobial and bioactive compounds, between individual bryophyte taxa (Lang et al., 2009; Davey et al., 2012, 2014, 2017).

The bryosphere, *sensu* Lindo and Gonzalez (2010), supports complex communities of fungi, and there is evidence that the communities within a single moss species (*Hylocomnium splendens*) can be highly responsive to forest management practices (Davey et al., 2014), including the addition of N fertilisers (Davey et al., 2017). However, as yet, there are no studies that have examined the impacts of elevated atmospheric N deposition on moss endophyte communities across large geographic scales. Here, we determine the fungal communities associated with the moss *Racomitrium lanuginosum* along a N deposition gradient in the mountains of the UK.

Racomitrium heath, an oceanic-alpine community dominated by the moss *R. lanuginosum,* is one of the most extensive near-natural plant communities in alpine areas of the UK. *Racomitrium* heath is highly sensitive to N deposition, with a critical load for N of 5–10 kg N ha^{-1} y^{-1} (Bobbink and Hettelingh, 2011). Elevated N levels have been associated with a significant reduction in moss mat cover and continuity and its replacement by grasses and sedges (Bobbink et al., 2010; Armitage et al., 2014). Previously, we demonstrated that decomposition processes within the moss mat accelerate as moss shoot N content and temperature increase, and that increased decomposition is the likely mechanism by which the cover and thickness of the moss carpet declines (Britton et al., 2018). Changes in decomposition strongly suggest an impact of N deposition on the fungal community associated with the moss mat and the underlying organic soil horizons. However, given the sparsity of studies of N impacts on bryosphere fungal communities, it is difficult to forecast how increases in N deposition and associated changes in the chemical and physical properties of the *R. lanuginosum* moss mat may influence fungal communities. Here, we approach this question by determining the fungal communities associated with *R. lanuginosum* shoots and the underlying soil, in samples collected from *Racomitrium* heath sites across the UK. We examine variability in community composition in relation to the pollution gradient of 6.4–35.4 kg N ha^{-1} y^{-1} and in the context of co-occurring variability in environmental conditions (temperature, rainfall, soil properties). We predicted, based on the commonly observed negative influences of increased N deposition on fungal communities in other ecosystems, that there would be a decline in species richness of fungi associated with *Racomitrium* heath with increasing levels of N deposition.

2. Materials and methods

2.1. Study sites

The fungal communities associated with *Racomitrium* heath [UK National Vegetation Classification U10 (Rodwell, 1992),] were investigated at 15 sites across the UK (Fig. 1). *Racomitrium* heath is typically dominated by *Racomitrium lanuginosum*, which often forms continuous and extensive mats, interspersed by *Carex bigelowii, Festuca ovina/vivipara, Cladonia uncialis, Deschampsia flexuosa,* and *Vaccinium myrtillus* (Rodwell, 1992). However, the past 50 ys has seen marked

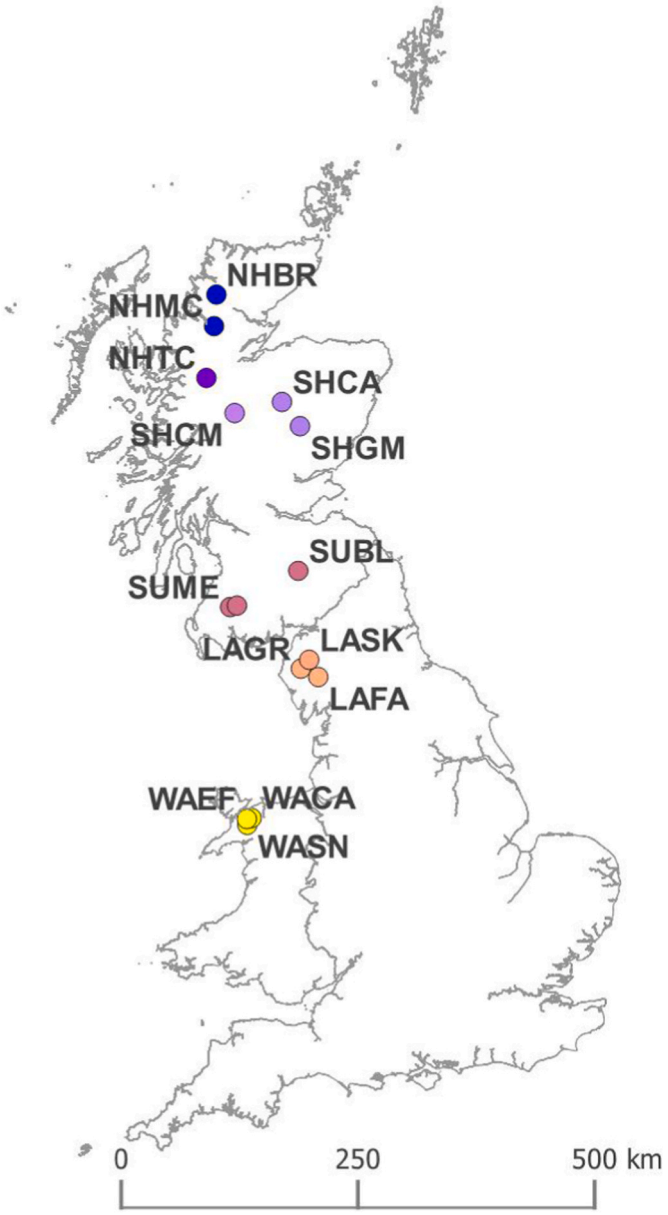

Fig. 1. Distribution of 15 alpine *Racomitrium* heath sites used in the study of fungal community composition in *Racomitrium lanuginosum* moss shoots and underlying soil. See Table 1 for site details.

fragmentation of *Racomitrium* heath in areas receiving higher levels of N deposition and replacement by grass dominated vegetation. See Armitage et al. (2012, 2014) for a discussion of vegetation changes associated with increasing N deposition.

Sites were selected to cover the largest possible gradients of N deposition and climate for this habitat within the UK (Table 1, Figs. S1 and S2) and covered five geographic regions: northern (NH) and southern Scottish Highlands (SH), Southern Uplands of Scotland (SU), Lake District (LA), and north Wales (WA), with three sites in each region. The N deposition range (average annual total deposition for 2010–2012 at 5 km resolution) was 6.4–35.4 kg N ha^{-1} y^{-1}. Fig. S1 shows the relationships between moss mat parameters and environmental variables. It is clear that some variables covary along the transect, in particular the temperature and N contents of the moss mats are strongly positively correlated (Figs. S1 and S2). The mean moss mat temperature range recorded during the study (2011–2013) was over 3 °C (2.55–5.68 °C). Variable covariation means that interpreting variable influence must be made with

Table 1
Location of and environmental conditions at 15 alpine *Racomitrium* heath sites arrange by decreasing latitude in the UK used in an analysis of fungal communities associated with *Racomitrium lanuginosum* moss shoots and underlying soil. N deposition – modelled mean total N deposition for 2010–2012; Rainfall – mean annual rainfall 1971–2000; Moss mat temp – mean temperature measured within the moss mat based on 2-hourly measurements over a 2 y period (2011–2013), Moss % N – mean N content of green moss shoots measured 2007; Soil pH – mean soil pH measured 2011.

Site code	Region	Site name	Latitude	Longitude	Elevation (masl)	N deposition (kg N ha^{-1} y^{-1})	Rainfall (mm)	Moss mat temp (°C)	Moss % N	Soil pH
NHBR	North Highlands	Breabag	58.10	−4.90	751	6.6	3097	4.12	0.45	3.92
NHMC	North Highlands	Meall nan Ceapraichen	57.78	−4.93	955	6.4	2616	3.14	0.40	3.86
NHTC	North Highlands	Tom a' Choinich	57.30	−5.05	945	7.6	2738	3.39	0.42	3.88
SHCA	South Highlands	Cairngorm	57.10	−3.70	1070	11.8	2001	2.55	0.59	3.72
SHCM	South Highlands	Creag Meagaidh	56.98	−4.52	965	9.2	2409	3.12	0.52	3.81
SHGM	South Highlands	Glas Maol	56.87	−3.37	1062	12.7	2145	3.03	0.70	3.74
SUBL	Southern Uplands	Broad Law	55.50	−3.35	835	18.5	2026	4.31	0.74	3.72
SUCO	Southern Uplands	Corserine	55.15	−4.35	810	23.5	2776	4.75	0.76	4.04
SUME	Southern Uplands	Merrick	55.13	−4.47	830	18.8	2670	4.81	0.85	3.82
LASK	Lake District	Skiddaw	54.65	−3.15	820	23.9	2409	4.92	0.89	3.81
LAGR	Lake District	Grasmoor	54.57	−3.27	850	21.4	2464	4.68	0.84	3.80
LAFA	Lake District	Fairfield	54.50	−2.98	857	35.4	2945	4.38	1.07	3.74
WACA	Wales	Carneddau	53.13	−4.00	935	27.9	2849	4.83	0.96	4.00
WAEF	Wales	Elidir Fach	53.13	−4.08	783	20.6	2657	5.68	0.84	3.99
WASN	Wales	Snowdon	53.07	−4.07	1020	21.3	4645	4.35	0.92	4.10

caution (see results and discussion).

At each site, a representative homogeneous 1 ha area of heath was selected for study. Between late July and early September 2011, samples of *R. lanuginosum* moss shoots and associated organic soil were collected from eight plots distributed across each 1ha area, for determination of fungal community composition. In this study, the depth of the moss mat varied significantly across the sites from ca. 5 cm in the less polluted sites to 2.5–3 cm in sites with higher N deposition. Samples comprised a small bundle of live moss shoots (all of the green portion plus a variable amount of brown material to the depth of 5 cm) from a single point and approximately 25 g fresh weight (ca. 100 ml) of the underlying organic soil to a maximum depth of 5 cm. The organic soil layer was situated beneath the necromass of the moss and comprised decomposed organic material in which recognisable moss shoots were no longer present. Samples were kept on ice during transport and frozen at minus 20 °C on return to the lab. Measurements of soil pH, temperature within the moss mat, decomposition rate of moss shoots, and moss N content were also made for each site as described in Armitage et al. (2012) and Britton et al. (2018). Briefly, soil pH was measured in 2011 for 10 samples of organic horizon soil collected from across the 1 ha sampling area at each site. Mean moss mat temperature was monitored over a 2 y period (2011–2013) using 10 i-Button temperature loggers per site, inserted into the moss mat (2–5 cm depth) which recorded temperature every 2 h. Moss shoot decomposition rate was measured over the same 2 y period (Britton et al., 2018) using litter bags inserted into the moss mat (10 bags per site, filled with litter sourced on site). Moss N contents was determined in the green portions of moss shoots collected at 10 points in each 1 ha sampling area in 2007. Previous studies (Britton et al., 2018) and data from this study (Fig. S1) have shown that the N contents of the green and brown portions of the moss shoots are very similar, suggesting that there is little mobilisation of N from senescing shoots.

2.2. Preparation of soil and moss shoot fungal communities

Moss shoot samples were prepared for sequencing by pooling the terminal green portions of 3–4 shoots from each sample. Green shoots were chosen as these were the substrate used in previous studies which demonstrated enhanced decomposition at the study sites (Britton et al., 2018). DNA was extracted from 20 mg each of lyophilised and ball-milled green moss shoots and soil as described below. Soil samples from the three sites in north Wales were compromised during storage and were not used, resulting in a dataset with 15 sites for the moss shoots but 12 sites for the soil.

DNA was extracted using the E.Z.N.A. Plant DS Mini Extraction Kit (Omega bio-tek, Norcross, USA). fITS7 and ITS4 primers from Ihrmark et al. (2012) were used to amplify the ITS2 region of fungi. Both primers were tagged with Illumina overhang adapters (Illumina tech-support Document # 1000000002694 v06, https://emea.illumina.com/). The cycling was as follows: initial denaturation at 94 °C for 2 min followed by 30 cycles of 94 °C for 30s, 54 °C for 30s, 72 °C for 30s and a final elongation step at 72 °C for 5 min. PCRs were conducted twice per sample and PCR products were pooled and purified using the AMPure XP PCR purification kit (Beckman Coulter, High Wycombe, UK) according to the manufacturer's protocol. Nextera XT indices and Illumina sequencing adaptors were attached using Nextera XT Index Kit v2, Set A, B & C according to manufacturer's instructions (Illumina, Essex, UK). Illumina MiSeq sequencing of moss and soil samples was conducted in three runs using MiSeq Reagents Kit v3 (2 × 300 cycles) (Illumina, Essex, UK) at the James Hutton Institute in Dundee, UK.

To assess reproducibility and potential contamination, samples of known composition and duplicate soil samples were extracted and sequenced in each sequencing run. Samples of known composition contained fungal fruitbody DNA from commercially available *Agaricus bisporus, Boletus edulis, Pleurotus ostreatus* and *Lentinula edodes*. Analysis of the processed data showed that replicate soil samples processed independently had Bray-Curtis similarity indices of 69 ± 2.9% (mean ± sd), whereas across all samples, the Bray-Curtis index was 13.5 ± 3.5%. As an indication of low contamination between samples, sequences of commercial mushrooms were retrieved from the samples of known composition but were not recovered in any other samples.

2.3. Bioinformatic analysis

Sequence and metadata data are deposited with NCBI (BioProject ID:

PRJNA804870). Quality of sequences was initially assessed by evaluating the phred quality scores using FastQC (Andrews, 2010). Low quality sequences and chimeric sequences were removed from sequence libraries using the big data routine (without fragment size restriction) implemented in DADA2 v1.6 (Callahan et al., 2016) within the R statistical environment (R Development Core Team, 2015) using the standard settings with some modifications. In brief, the filterAndTrim command was used with settings maxN = 0, maxEE = c(2,2), truncQ = 2, rm.phix = TRUE, sequencing runs were joined with mergeSequenceTables and chimeric sequences were removed from the joined dataset with removeBimeraDenovo(seqtab, method = "consensus"). DADA2 assigned sequence variant (ASV) outputs were initially screened for non-fungal sequence motives using ITSx (Bengtsson-Palme et al., 2013) and imported into the mothur v. 1.3.95 pipeline for further analysis (Schloss et al., 2009). To reduce taxon inflation in the ASVs resulting from identification of intraspecific ITS variability, de-novo hierarchical agglomerative clustering of DADA2 ASVs was performed with the Vsearch agc module with 2.5% dissimilarity threshold. The resulting OTUs were classified using the naïve Bayesian classifier incorporated into mothur (Wang et al., 2007) against the UNITE database ver. 7.2; 2017-10-10 (https://unite.ut.ee/index.php) at the default threshold of 80% bootstrap confidence. Remaining non-fungal lineages were removed and OTU representative sequences were selected by majority representation. OTUs represented by 2 or fewer reads were removed from the data set and sequence data were rarefied by random subsampling to the lowest sequence count in the sample set using the sub.sample routine in Mothur with the persample option. Finally, the OTU table was parsed into the online FUNGuild database (Nguyen et al., 2016) to assign OTUs to ecologically meaningful functional groups, using taxonomic keywords and functional metadata. The FUNGuild assignments were then checked by an expert (A. Taylor) and refinements based on knowledge of the fungal groups' ecology were made where possible.

2.4. Statistical analysis

Data analysis was performed in R v 4.0.0 (R Development Core Team, 2015) and Canoco v 5.11 (Ter Braak and Smilauer, 2012) for multivariate analyses. To visualise fungal community composition in the combined moss and soil dataset (n = 216), sequence count data were first transformed into a sample-level presence-absence matrix before multivariate analysis using Detrended Correspondence Analysis (DCA). The fungal community data were then split into separate presence-absence matrices for moss (n = 120) and soil (n = 96) for subsequent analyses. Environmental data (rainfall, moss mat temperature, green moss N content, soil pH) were available at the site level only (n = 15 for moss and n = 12 for soil), so for analysis of fungal community-environment relationships, the fungal community data were summarised as site-level frequency data (number of occupied samples per site). Fungal OTU frequency matrices were analysed by Redundancy Analysis (RDA) with interactive forward selection of significant explanatory variables. For moss shoot communities, rainfall, moss mat temperature, and green moss N content were tested as potential explanatory variables, and for soil communities soil pH was additionally included. As already stated green moss N content was used as a proxy for N deposition in these analyses, as it is known to be strongly correlated with N deposition and could provide a more accurate reflection of local N deposition rates than modelled N deposition data which are produced at 5 km resolution (Armitage et al., 2012; Pitcairn et al., 2006). Significance of the first axis and of all axes together was tested using a multivariate permutation test with 999 permutations. Variation partitioning was then used to examine the amount of unique and shared variation associated with each significant environmental variable.

Significance of differences in the OTU richness of taxonomic and functional groups between sites, nested within regions to account for spatial structure, was tested using Generalized Linear Models (function glm) with a Poisson distribution and log link function. Relationships between the mean taxonomic/functional group richness at each site and potential environmental drivers (rainfall, moss mat temperature and moss N content for both communities, soil pH for soil communities) were explored using multiple linear regression modelling. The interpretation of the importance some variables needs to be made with caution as rainfall and soil pH were correlated as were moss mat temperature and the N content of moss shoots (Figs. S1 and S2). Models were first run with all potential environmental drivers included. Models were then refined by sequentially removing non-significant variables to achieve the most parsimonious model. Finally, relationships were assessed between site mean taxonomic/functional group richness in moss shoots and moss decomposition rate, and between site mean richness of taxonomic groups in moss vs soil, using simple linear regression.

3. Results

The Illumina runs yielded 5 212 694 reads of sufficient quality to be included in further analyses. These were clustered into 1862 OTUs: 688 OTUs in the 120 moss shoot samples and 1620 OTUs in the 96 soil samples, 446 OTUs were common to both moss and soil samples. Species accumulation curves (Fig. S3) showed that the recovered 1862 OTUs were only a limited proportion of the potential fungal richness in the alpine moss-sedge heath habitat. The subsampled dataset, which was rarefied to the lowest read number recovered from a sample (2233 reads), contained 1809 OTUs in total. Of these 687 OTUs occurred in moss (Chao 2 estimated total community richness = 1062), 1555 OTUs occurred in soil (Chao 2 estimated total community richness = 2619), and 433 OTUs were common to both moss and soil. These subsampled data were used for all subsequent analyses.

Analysis of the resolution at which OTUs could be assigned taxonomically showed that 25% (452), could be assigned no further than kingdom. However, most fungal sequences (75%) were assigned to at least phylum level, with a total of 12 phyla identified (Table S1). Ascomycota were highly dominant (916 OTUs; 50.6%), followed by Basidiomycota (330 OTUs; 18.2%). The remaining 10 phyla each made up 2% or less of the total community.

A total of 605 OTUs were assigned functional groups by FUNGuild but a significant proportion of these were very broad and of very limited use. OTUs with only high-level taxonomic status (e.g. Agaricales, Tremellales) were assigned to multiple groups. These broad designations were excluded from any analyses of functional groups to provide more precise functional data on the OTUs. A combination of FUNGuild, and expert knowledge (A. Taylor), enabled 546 OTUs (30%) to be allocated to functional groups which reflected more precisely the ecology of the OTUs.

3.1. Total community composition

A DCA of the combined moss and soil community data (Fig. 2) showed firstly, a clear separation between moss and soil on the first axis and, secondly, similar gradients of sites arranged approximately south to north along the second axis for both moss and soil, coinciding with the combined gradients in temperature and N deposition. However, there was no clear correspondence between site mean OTU richness in shoots and in the underlying soil, either for all OTUs together or for any taxonomic or functional group (all $P > 0.1$, data not shown). All subsequent analyses were carried out on the moss and soil communities separately.

3.2. Moss shoot fungal community composition and richness

Seven phyla of fungi were present in the moss shoots (Table S1), but the community was dominated by the Ascomycota (67% of OTUs). Functional group assignments were made for 218 of the 687 OTUs (31%). Thirteen functional groups of fungi were present (Fig. 3A), but

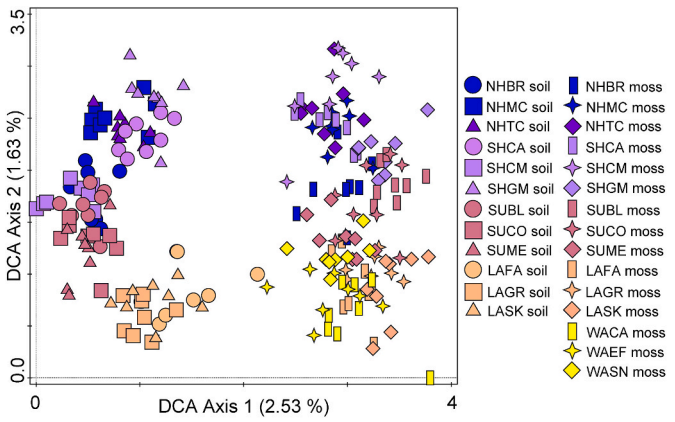

Fig. 2. Detrended Correspondence Analysis of fungal community composition in moss shoots and organic soil at 15 *Racomitrium* heath sites across the UK. Sites are coloured according to latitude, from north (blue) to south (yellow), approximately coinciding with gradients of low to higher nitrogen deposition and temperature. For site details see Table 1 and Fig. 1.

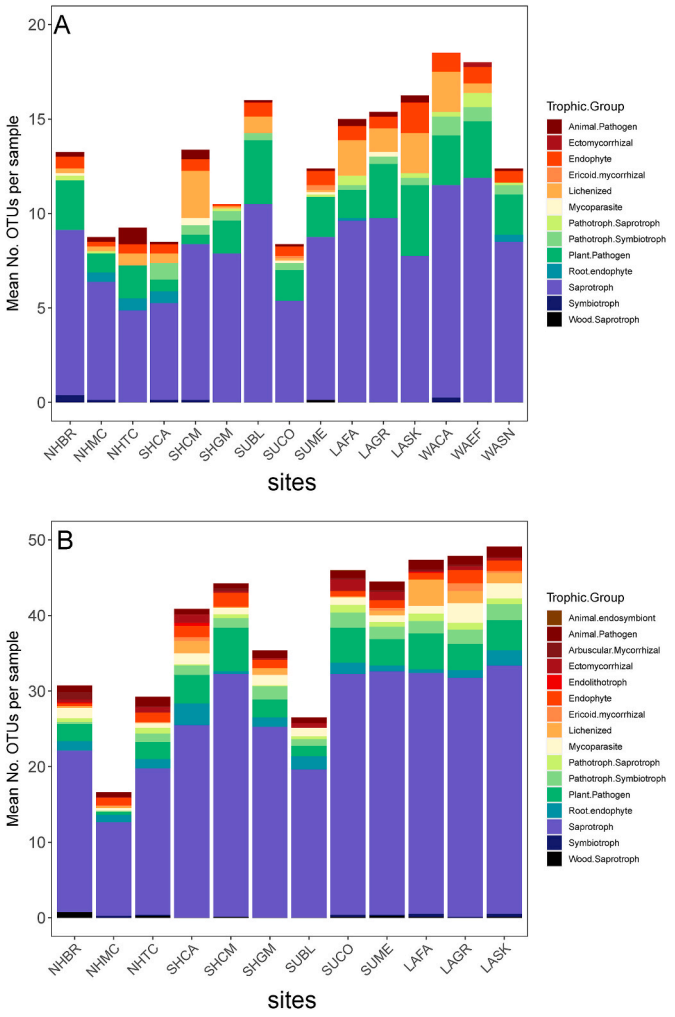

Fig. 3. Mean richness of fungal functional groups in a) moss shoots and b) organic soil at 15 (moss) or 12 (soil) *Racomitrium* heath sites across the UK, n = 8 samples per site. Sites are arranged by latitude from north (left) to south (right). For site details see Table 1 and Fig. 1.

the fungal communities at all 15 sites were dominated by saprotrophic species, with an average of 8.3 OTUs per sample across all sites, 63% of all OTUs assigned to a functional group. Plant pathogens, lichenised fungi, endophytes and pathotroph-symbiotroph fungi were also present at most sites, with an average of 0.4–2.1 OTUs per sample, across all sites. Overall, the mean number of OTUs per moss sample was 39.7 ± 1.2 (range = 12–80). The OTU richness per sample differed significantly between sites (Fig. 4) for total OTU richness ($X^2 = 121.04$, $P < 0.001$), Ascomycota OTUs ($X^2 = 115.47$, $P < 0.001$), Basidiomycota OTUs ($X^2 = 20.26$, $P = 0.027$) and saprotrophic fungi ($X^2 = 37.26$, $P < 0.001$). All other taxonomic and functional groups had too few OTUs to be analysed.

3.3. Relationships between moss shoot fungal community and environmental factors

Mean richness of all OTUs combined, and richness of Basidiomycota and Ascomycota were all significantly and positively related to moss mat temperature (Table 2, Fig. 4). The relationships between moss shoot fungal community composition and rainfall, moss mat temperature and moss N content (Table 1) were investigated at the site level (n = 15) using OTU frequency data. Redundancy Analysis with forward selection of significant variables showed that moss mat temperature and moss N content were significant predictors of moss shoot fungal community composition (Fig. 5A). Combined, these two variables explained 16.1% of the variation in community composition. Variation partitioning showed that moss mat temperature explained 5.1% of the variation and moss N content explained 3.8%, with a further 7.2% of shared variation. Nearly half of the fungi showing an association with moss mat temperature and moss shoot N% could only be classified as far as the fungal kingdom. All the classified fungi belong to the Ascomycota and were mainly leaf and litter saprotrophs. The exception to this was *Mycosphaerella tassiana*, which is a plant pathogen with a wide range of hosts.

Relationships with environmental drivers were also sought for the subset of OTUs allocated to the saprotrophic functional group (Fig. 5B, Table 2). Forward selection of significant environmental variables showed that for this functional group, moss mat temperature was the main influence on community composition, explaining 8.80% of total variation. All of the saprotrophic fungi in the shoots with distributions showing an association with moss mat temperature were from the Ascomycota, with most being generalist decay fungi, although some could also be endophytes or associated with animal dung. The overall richness of saprotrophic OTUs was also significantly and positively related to moss mat temperature (Table 2, Fig. 4).

3.4. Soil fungal community composition and richness

Overall, the mean number of OTUs per soil sample was 103.4 ± 4.1 (range = 19–199). The fungal communities recovered from the 96 soil samples were therefore 2.5 times as species rich as those associated with the shoots. Of the 1555 OTUs present in the soil, 75% could be assigned at least to phylum level, and 12 phyla were present (Table S1). The soil fungal community was dominated by Ascomycota (50% of OTUs) but to a lower degree than seen in the moss shoots (Table S1). Functions were assigned to 485 (31%) of the OTUs present in the soil with sixteen functional groups of fungi identified (Fig. 3B). Animal endosymbiont, arbuscular mycorrhizal fungi and endolithotroph groups were additionally present in the soil compared with the moss shoot fungal community. The soil fungal community was again heavily dominated by saprotrophic fungi with a total of 69% of OTUs in this category (mean of 26.3 OTUs per sample across all sites). The next most numerous functional groups: plant pathogens, pathotroph-symbiotrophs, root endophytes, mycoparasites and endophytic fungi, all had 1-3 OTUs per sample across all sites.

The OTU richness per sample differed between sites for all fungi ($X^2 = 230.1$, $P < 0.001$), Ascomycota ($X^2 = 116.6$, $P < 0.001$) and Basidiomycota ($X^2 = 82.54$, $P < 0.001$, Fig. 6). The richness of OTUs

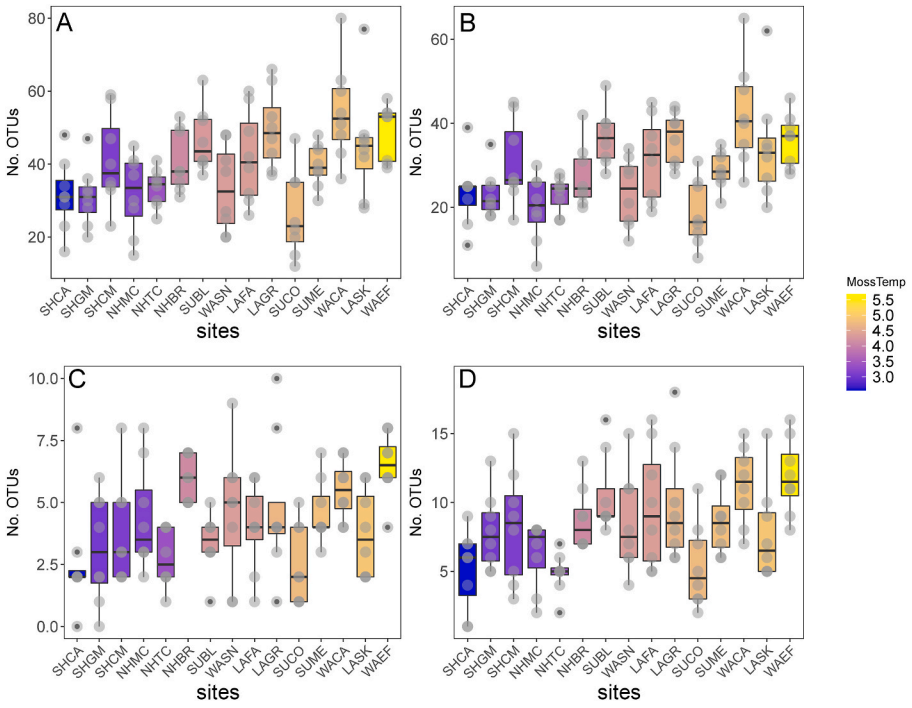

Fig. 4. OTU richness per sample for A) all OTUs, B) Ascomycota, C) Basidiomycota, D) saprotrophs in moss shoots collected from 15 *Racomitrium* heath sites across the UK, n = 8 samples per site. Box plots show median, 25th and 75th centiles, with whiskers indicating 10th and 90th centiles. Sites are arranged and coloured according to annual mean temperature (°C) in the moss mat.

Table 2
Significant environmental variables in multiple linear regressions of site mean fungal taxonomic and functional group richness in moss shoots (n = 15) and underlying soil (n = 12) at alpine *Racomitrium* heath sites in the UK. The variables in the best fit models are shown with their t-values. Empty cells reflect non-significant variables, 'x' indicates variables not tested. Temp – mean moss mat temperature, moss % N – mean green moss N content, Rainfall – mean annual rainfall, pH – mean soil pH.

Dependent variable	Temp	Moss % N	Rainfall	pH	R^2 adj	*P*
Moss shoot communities						
Total OTU richness	2.599			x	0.291	0.022
Ascomycota richness	2.480			x	0.269	0.028
Basidiomycota richness	2.485			x	0.270	0.027
Saprotroph richness	3.015			x	0.366	0.010
Soil communities						
Total OTU richness		2.544			0.332	0.029
Ascomycota richness		3.660			0.530	0.004
Basidiomycota richness						ns
Mortierellomycota richness						ns
Saprotroph richness		3.230			0.462	0.009

assigned to saprotrophic fungi also differed between sites ($X^2 = 61.52$, $P < 0.001$, Fig. 6).

3.5. Relationships between soil fungal community and environmental factors

Relationships between soil fungal community composition and rainfall, moss mat temperature, moss N content and soil pH were tested at the site level (n = 12) using OTU frequency data. Redundancy Analysis with forward selection of significant variables showed that, as for the moss shoots, both moss mat temperature and moss N content were significant predictors of fungal community composition in soil (Fig. 7A). Combined, these two variables explained 17.4% of the variation in community composition. Variation partitioning showed that moss mat temperature explained 7.3% of the variation and moss N content explained 6.4%, with a further 3.7% of shared variation. The soil fungi showing an association with moss mat temperature and moss shoot N% represented a wider taxonomic selection than those in the shoots, with representatives from the Mortierellomycota, Ascomycota, and Kickxellomycota being present. Taxa which could be classified sufficiently to determine functional group were all saprotrophs.

Moss N content was also a significant predictor of total OTU richness in the soil (Table 2, Fig. 6). This was primarily due to a strong positive relationship between moss N content and Ascomycota OTU richness; Basidiomycota and Mortierellomycota richness were not related to moss N content.

Relationships with environmental variables were also examined for the soil saprotrophic community. The overall richness of saprotrophic OTUs was significantly and positively related to moss N content (Table 2, Fig. 6). Redundancy analysis and interactive forward selection of significant environmental variables showed that the saprotrophic community composition was related only to moss mat temperature (Fig. 7B), which explained 11.35% of total variation. The saprotrophic community in the soil had the most fungi showing an association with moss mat temperature. While most could be considered generalist decay fungi, the *Filobasidiella* sp deserves special mention as the group contains *Filobasidiella neoformans* (*Cryptococcus neoformans*) which can cause cryptococcosis in humans. The *Filobasidiella* sp. was widespread being found in 56 soil samples but only one moss shoot sample.

4. Discussion

In this study we examined fungal communities associated with *Racomitrium* heath, an ecologically important and iconic alpine habitat which has declined significantly in both quality and extent in the UK (Armitage et al., 2012; Hayhow et al., 2015; Britton et al., 2018). This decline has been largely attributed to increased deposition of N, leading to enhanced decomposition and fragmentation of the moss mat, followed by invasion of vascular plant species (Armitage et al., 2012, 2014;

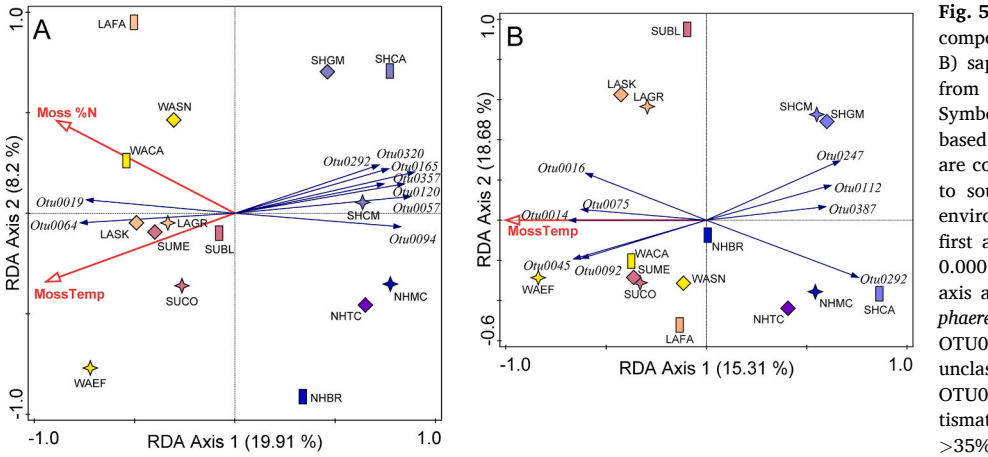

Fig. 5. Redundancy Analysis of site-level community composition for A) total shoot fungal community and B) saprotroph community in moss shoots collected from 15 Racomitrium heath sites across the UK. Symbols show community composition at each site based on OTU frequency in 8 samples per site. Sites are coloured according to latitude from north (blue) to south (yellow). Red arrows indicate significant environmental variables (see text). Significance of first axis P = 0.0002, significance of all axes P = 0.0002. In A) nine OTUs with a fit of >50% to the first axis are shown as blue arrows: OTU0019 – *Mycosphaerella tassiana*; OTU0057, OTU0064, OTU0094, OTU0120– Fungi unclass.; OTU0165 – Ascomycota unclass.; OTU0292 – Hyaloscyphaceae unclass.; OTU0320 – Helotiales unclass.; OTU0357 – Rhytismataceae unclass. In B) nine OTUs with a fit of >35% to the first axis are shown as blue arrows: OTU0014 – *Lachnum* sp.; OTU0016 – *Catenulifera* sp.; OTU0045 – Chaetosphaeriacae unclass.; OTU0075 – *Coniochaeta* unclass.; OTU0092, OTU0387 – *Cladophialophora humicola*; OTU0112 – *Hyaloscypha* sp.; OTU0247 – *Cheilymenia* sp.; OTU0292 – Hyaloscyphaceae unclass.

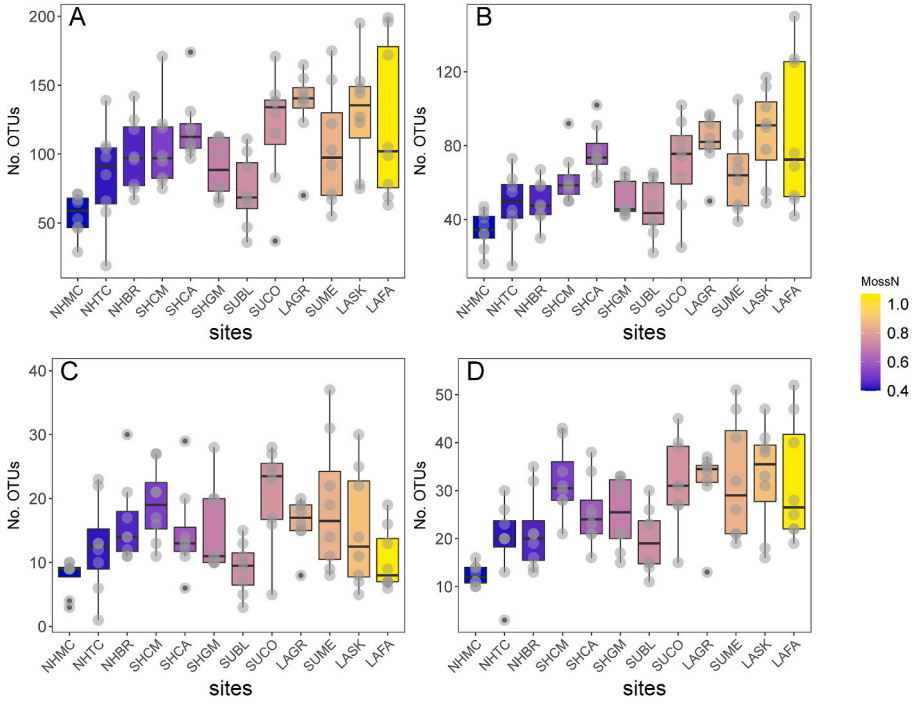

Fig. 6. OTU richness per sample for A) all OTUs, B) Ascomycota, C) Basidiomycota, D) saprotrophs in organic soil collected from 12 *Racomitrium* heath sites across the UK, n = 8 samples per site. Box plots show median, 25th and 75th centiles, with whiskers indicating 10th and 90th centiles. Sites are arranged and coloured according to site mean N content (%) in green moss tissue.

Britton et al., 2018). Our detailed characterisation of fungal communities associated with both the moss mat and the underlying soil has found that this deterioration in habitat quality is accompanied by major changes in the fungal communities. However, contrary to our expectation of increasing N deposition being associated with a decline in fungal species richness, there were significant increases in richness, particularly of saprotrophic species. Both N deposition and temperature were strong predictors of the structure and composition of the fungal community, with species richness positively related to moss tissue N contents and temperature.

4.1. Community composition and species richness

Both the moss shoots and the underlying soil supported taxonomically and functionally diverse fungal communities. Although nearly 70% of the 688 OTUs found in the moss shoots were also found in the underlying soil, the green shoots of *R. lanuginosum* clearly provide a distinct habitat supporting additional unique fungal taxa. The failure of the species accumulation curves for the shoot and soil communities to reach an asymptote suggests that a considerable proportion of the fungal richness of this habitat was not captured and that more intensive sampling would be required to comprehensively capture the fungal communities associated with this moss-dominated habitat.

Davey et al. (2012, 2013, 2014, 2017) examined the fungi associated with several species of woodland mosses and recovered communities which were generally more species rich than that found associated with *R. lanuginosum* in the present study. In all of the studies by Davey et al., just as in the present study, species accumulation curves failed to reach

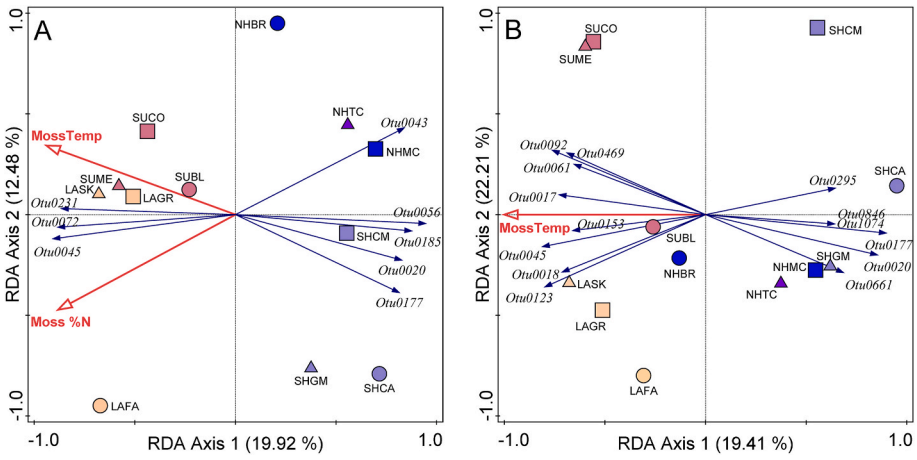

Fig. 7. Redundancy analysis of site-level community composition for A) total soil fungal community and B) saprotroph community in organic soil collected from 12 *Racomitrium* heath sites across the UK. Symbols show community composition at each site based on OTU frequency in 8 samples per site. Sites are coloured according to latitude from north (blue) to south (yellow). Red arrows indicate significant environmental variables (see text). Significance of first axis P = 0.0028, significance of all axes P = 0.0002. In A) Eight OTUs with a fit of >65% to the first axis are shown as blue arrows: OTU0020 – *Mortierella macrocystopsis*; OTU0043 – Fungi unclass.; OTU0045 Chaetosphaeriaceae unclass.; OTU0056 – Ascomycota unclass.; OTU0072 – Pleosporales unclass.; OTU0177 - *Mortierella* sp.; OTU0185 – *Leptodontidium trabellum*; OTU0231 – GS19 sp. In B) 14 OTUs with a fit of >45% to the first axis are shown as blue arrows: OTU0017 – *Filobasidiella* sp; OTU0018 – Sordaria unclass.; OTU0020 – *Mortierella macrocystopsis*; OTU0045 – Chaetosphaeriaceae unclass.; OTU0061 – *Entoloma conferendum*; OTU0092 – *Cladophialophora humicola*; OTU0123 – Sporomiaceae unclass.; OTU0153, OTU0177– *Mortierella* sp.; OTU0295 – *Ascocoryne* sp.; OTU0469 - Sporomiaceae unclass.; OTU0661 – *Clavaria* sp.; OTU0846 – *Galerina* sp.; OTU1074 – Mucor sp.

the asymptote, so overall richness was likely to be much higher than that recovered. They found that species richness was strongly influenced by moss host species and by the moss substrate analysed (green *versus* brown shoots). For *Hylocomnium splendens* they recovered 2337 OTUs (Davey et al., 2014), considerably more than the 688 OTUs we found in *R. lanuginosum*. The larger number of OTUs in *H. splendens* could be at least partly explained by the greater sampling intensity in that study (10 shoots *versus* 4). The higher richness found by Davey et al. (2014) is even more impressive as they surface sterilised the moss shoots before extraction, meaning that most taxa recovered would have been endophytes from within the moss shoots. The communities in the present study will include both endophytes and phyllosphere taxa, which may be one reason why the number of OTUs representing lichens was high. Gomes et al. (2018) found that endophyte and phyllosphere communities associated with the leaves of olive trees reacted differently to environmental factors. It is therefore possible that examining the two communities separately could provide further insights into fungal responses to changing conditions in *Racomitrium* heath.

The broad taxonomic distribution of fungal taxa in *Racomitrium* shoot communities was similar to that found previously in other moss species (Davey et al., 2012, 2013, 2014, 2017), with the communities dominated by the Ascomycota (56–62% of OTUs in Davey et al., 66.5% in this study). However, the proportion of Basidiomycota in this study (14.4%) was less than half that recovered in the four studies of Davey et al. (30–40%), and the proportion of unassigned OTUs in the present study (ca. 25%) was much higher than in Davey et al. (5–11%). The studies by Davey et al. are primarily from forest habitats and the fungal communities include many symbiotic Basidiomycota and taxa associated with woody substrates. In general, the taxa and fungal communities in woodlands have been much more comprehensively studied and representation of taxa from these habitats in sequence databases is likely to be much higher than for the much less-studied alpine habitats.

The broad taxonomic and functional diversity of fungi associated with the green moss shoots mirrors that found in other studies of fungal endophytes across a wide range of plant taxa and ecosystems (e.g. Rim et al., 2021; Wenndt et al., 2021). A common feature of these communities is the high proportion of saprotrophic taxa, many of which are also found in the decaying litter of the plant host (Guerreiro et al., 2018), which is also the case in the present study.

The communities of fungi in mountain ecosystems are very poorly known and the present study represents the first broad scale study of fungal communities in an alpine habitat in the UK (but see Hesling [2013] for ectomycorrhizal fungi associated with alpine ectomycorrhizal host plants). Several of the species detected were previously considered very rare in the UK. A good example is *Squamanita contortipes* (99% match with UNITE species hypothesis *S. contortipes* SH179 6373.08FU; sequence data provided Table S2) previously known only from a single record of a sporocarp in a meadow at Rothiemurchus, Scotland in 1957 (http://data.rbge.org.uk/herb/E00159492). In the present study, this species was recorded at eight of our 15 sites, seven in Scotland. At Cairngorm (elevation 1070 m) in the southern Highlands, *S. contortipes* sequences were detected in five of the eight samples taken. These data strongly suggest that this fungus is much more widespread and common – as mycelia – than considered from sporocarp records. *Squamanita contortipes*, like other members of the genus, produce their sporocarps by colonising and taking over the sporocarps of other agarics. In the case of *S. contortipes* the 'hosts' are species of the saprotrophic genus *Galerina*. Seven OTUs found in the present study could be assigned to *Galerina*, but there was no discernible pattern to the distribution of *S. contortipes* in relation to any of the *Galerina* sp., with only two of the 13 occurrences of the *Squamanita* coinciding with *Galerina* OTUs.

Another very rare fungus found, but from a different functional group, is the ectomycorrhizal fungus, *Bankera violescens* (99% match with UNITE submission UDB019672; DOI: SH2557096.08FU; sequence data provided Table S2), known from only three UK sites, all in NE Scotland. This species was recorded in the present study from one site (NH-BR: Breabag) in the extreme NW of Scotland. Ectomycorrhizal hosts which could occur as part of the vegetation in *Racomitrium* heath, include *Salix herbacea, Arctostaphylos uva-ursi* and *A. alpinus*. The preferred hosts for *B. violescens* are *Picea* spp., so it is most likely that if it exists as an active mycelium then it would be associated with an *Arctostaphylos* spp. as these are known to associated with a wide range of ECM fungi, including those considered specifics on other hosts (see Hesling and Taylor, 2013; Hesling, 2013).

Further studies of soil fungal communities in alpine habitats will undoubtedly discover many other examples of fungal taxa considered rare in other habitats and will provide more comprehensive and realistic data on fungal distribution.

4.2. Fungal communities and environmental variables

The interpretation of relationships between fungal communities

associated with the moss mats and individual environmental variables is somewhat complicated due to covariation along the transect (Figs. S1 and S2). As already stated, caution must therefore be exercised in the interpretation of the importance of individual variables in determining the composition and richness of the fungal communities. Additionally, relationships between environmental variables and rates of moss decomposition of moss are not necessarily simple nor direct. Moss mat temperatures and mat thickness are negatively correlated (Fig. S1), potentially suggesting increased decomposition with higher temperatures. But thinning of the mat will also allow greater and more rapid fluctuations in temperature and moisture contents of the mat and the underlying soil (Soudzilovskaia et al., 2013), which will strongly impact on decomposition rates.

However, it is clear that fungal communities associated with the moss shoots and the underlying soil in *Racomitrium* heath are strongly structured, with communities within regions generally more similar than between regions, and both moss and soil communities changing in a comparable way from north to south (Fig. 2). The north-south compositional changes in communities were also associated with increases in taxon richness, particularly in saprotrophic Ascomycota. Of the environmental variables included in the study, only moss mat temperature and shoot N content (a proxy for N deposition) were found to be important for community compositional changes, with their combined influence explaining 16.1% and 17.4% of the community composition in shoots and soils, respectively. While part of this explained variation can be attributed to the combined effects of N and temperature (shared variation in shoots = 3.7%, and soils = 7.2%), each variable was also significant in explaining part of community composition. Although the importance of shoot N content and temperature are discussed separately below, the combined influences of the variables on the fungal communities are fully acknowledged.

4.2.1. Nitrogen

The sites included in the present study encompass a substantial N deposition gradient (6.4–35.4 kg N ha^{-1} yr^{-1}) and moss N content (as a proxy for N deposition) was found to be a major factor structuring fungal communities. The strongest effects were found in the soil, with both fungal community composition and richness significantly related to N, while only the composition of the shoot communities was affected.

Changes in N availability have been shown on numerous occasions to impact on soil fungal community composition and richness along both natural (Sterkenburg et al., 2015) and anthropogenically induced gradients, across a broad range of ecosystems and scales (Zak et al., 2011, Van Der Linde et al., 2018). Although a recent meta-analysis suggested an overall negative impact of increasing N availability on fungal biomass (Zhang et al., 2018), community responses can be highly variable, and even within defined functional groups, responses can be taxon specific. Ectomycorrhizal fungi appear particularly sensitive to increases in soil N, with many taxa rapidly decreasing in abundance while others proliferate (Van Der Linde et al., 2018; Lilleskov et al., 2019). Similar response variations have been observed in free-living soil saprotrophic fungal communities. Sterkenburg et al. (2015) found that saprotrophic Ascomycota declined in forest ecosystems as nutrient availability increased along a natural fertility gradient, whereas Morrison et al. (2018) found an increase in saprotroph richness, mainly basidiomycete yeasts, in hardwood forests under simulated N deposition.

The composition of fungal communities associated with *R. lanuginosum* shoots was strongly affected by increased N deposition and this supports previous studies which have shown endophyte and phyllosphere communities to be sensitive to a wide range of factors, including host identity (Hoffman and Arnold, 2008; Vincent et al., 2016), leaf chemistry (Larkin et al., 2012; Oono et al., 2020), leaf structure (González-Teuber et al., 2021), season (Jumpponen and Jones, 2010), and temperature (Gomes et al., 2018; Oita et al., 2021). There are few studies however, which have examined impacts of N availability in single plant species. Jumpponen and Jones (2010) suggested that leaf N content related to nutrient availability in urban environments was important in determining endophyte community composition in burr oak leaves. Davey et al. (2017) examined the impact of forest fertilizer additions on the endophytes associated with the mosses *Dicranum scoparium, Hylocomium splendens* and *Pleurozium schreberi* and reported complex reactions of fungal communities to the N additions. Fertilizer additions, which had been applied for 7 years at the time of sampling, were the equivalent of 47 kg N ha^{-1} yr^{-1} and had strong effects on community composition with ca. 26% of OTUs found only in the control or the N plots. The strong impacts of fertilization on community composition mirrors the high turnover of species found in this study with *R. lanuginosum* in both the significant compositional differences among regions and the progressive differences along the whole N gradient.

Davey et al. (2017) also found that responses to N varied between fungal functional groups, with ectomycorrhizal fungi showing a general decline with increasing N additions, while plant pathogens increased. Saprotrophic taxon response ranged from increased frequency to no change, to a reduction in frequency. Davey et al. (2017) found that overall richness did not differ between control and fertilized plots, but they did find an increase in OTU richness in the green shoots of the mosses with increasing N additions.

4.2.2. Temperature

The sites in the present study encompass a gradient of over 3 °C (2.55–5.68 °C) in moss mat temperature, and temperature emerged as a strong driver of fungal community composition in both moss shoots and soil and of richness in the moss shoots. Soudzilovskaia et al. (2013) demonstrated significantly greater amplitudes of temperature fluctuations in mats of *R. lanuginosum* compared to the underlying soil but also showed that annual mean temperatures were similar within the mats and the soil. The lack of a temperature effect on soil community richness may reflect buffering against the potentially wider fluctuation in temperature experienced by the shoots.

Temperature has been shown previously to be an important factor in determining fungal distribution and abundance at global (Vetrovsky et al., 2019), regional (Van Der Linde et al., 2018) and local scales (Bahram et al., 2012). Altitudinal gradient studies of soil fungal communities have shown that temperature can be a strong driver of total fungal community composition (Shen et al., 2020), and of plant fungal mutualists (Jarvis et al., 2015). The latter study found that soil temperature was a strong predictor of mutualistic ectomycorrhizal community composition along 300m altitudinal gradients in the Cairngorm mountains of the UK. This gradient represented a potential change of ca. 2–3 °C, similar to that in the present study.

Manipulation of soil temperature in warming experiments has also shown that fungal biomass (Clemmensen et al., 2006), community composition (Morgado et al., 2015) and function (Christiansen et al., 2018) can be influenced by increasing soil temperatures. However, concomitant changes in plant community composition and biomass can make it difficult to partition variation in community responses solely due to changing temperatures (Clemmensen et al., 2006, Deslippe et al., 2012). In a recent metanalysis of the impacts of warming on soil organisms, Salazar et al. (2020) found evidence for a general increase in fungal abundance with increasing temperatures in cold ecosystems. However, responses are unlikely to be universal as there is also evidence that the growth of some cold-adapted fungi is inhibited by increasing temperatures within expected realistic ranges (Misiak et al., 2021).

The gradient of temperature in the present study represents an average difference of only 3 °C yet appeared to have a strong impact on the fungal communities associated with *Racomitrium* heath. These data support the suggestions from earlier, more localised elevational studies (Jarvis et al., 2015), that fungal communities are highly responsive to climate change.

4.3. Decomposition

In areas without elevated N deposition, the N content of *R. lanuginosum* shoot tissue is extremely low (Britton et al., 2018, Table 1), which is typical of many bryophytes that produce recalcitrant litter – low in N and high in polyphenols and structural carbohydrates (Turetsky, 2003; Lindo and Gonzalez, 2010). This litter decomposes extremely slowly relative to other plants and bryophytes that are more N rich (Turetsky, 2003; Lang et al., 2009; Lindo and Gonzalez, 2010). However, we have previously shown that changes in the C:N ratio of *R. lanuginosum*, linked to N deposition, is the major driver of decomposition rate of the moss (Britton et al., 2018). Rates are relatively stable down to a C:N ratio of 100, but then there is a four-fold increase in decomposition rate as the ratio approaches 40. Bragazza et al. (2006) observed a similar increase in *Sphagnum* decomposition with increasing N deposition, and this may be a common mechanism for the depletion of bryophyte carpets under enhanced N deposition (Curtis et al., 2005; Gundale et al., 2011).

In the present study, it was the saprotrophic fungi, primarily those in the Ascomycota, in the soil communities which showed the greatest response to increasing N levels, with species richness increasing along the N gradient. It is tempting to consider a link between increasing species richness and increased decomposition rates as there were strong positive relationships between OTU richness and decomposition rates in both shoots and soil (Fig. S4). However, there are a number of confounding factors which make this inference problematic, including changes in the physical structure of the moss mat which could enhance decomposition.

Saprotrophic Ascomycota are often considered generalist decomposers (Martinez et al., 2005), and their significant increase may suggest that N deposition leads to a general increase in decomposable substrate via a decrease in moss tissue C:N (Britton et al., 2018), thereby providing more opportunities for generalist taxa with similar functional attributes to coexist. By contrast, specialised wood decay fungi were very uncommon in the study with only six species found in a total of 15 out of 216 samples (soil and shoots combined). The scarcity of woody substrate requiring specialised degradative enzymatic systems is likely to be the main reason for this rarity. This is a situation not unlike agricultural systems where non-woody plant tissues are the major substrate and Ascomycota predominate as the saprotrophic community (Ma et al., 2013).

Lang et al. (2009) determined the decomposition rates of litter from a range of vascular plants, bryophytes and lichens under field conditions in subarctic Sweden. They confirmed that as a group, bryophytes had the slowest rates of decomposition, and also showed that *R. lanuginosum* had the lowest rate of all the non-Sphagna mosses tested. Under low N deposition conditions, mats of *R. lanuginosum* can be 20 cm deep in the UK, including a thick layer of moss necromass, and they are the major biomass and above ground pool of C and N in *Racomitrium* heath (Britton et al., 2019). Increased N deposition alters the balance between moss shoot growth and decomposition and has resulted in thinning and fragmentation of the moss mat at sites with high N deposition (Armitage et al., 2012; Britton et al., 2018).

In N limited environments, decomposition can be constrained by the availability of N for the production of extracellular catabolic enzymes (Schimel and Weintraub, 2003), and both the activity of fungal extracellular hydrolytic enzymes involved in litter decomposition, and fungal abundance have been shown to be stimulated at higher litter nutrient contents (Schneider et al., 2012). Increasing N availability can also lead to increased decomposition by priming the degradation of recalcitrant carbon substrates and this is often associated with increased microbial biomass (Kuzyakov et al., 2000). Without detailed experimental analysis examining incorporation of labelled substrates, such as those described by Chen et al. (2014), it is not possible to say which mechanism – enhanced enzymatic production or substrate priming – underlies the enhanced decomposition rates of the moss mats in *Racomitrium* heath. However, irrespective of the mechanism(s) involved, it is very clear that increasing N deposition has altered community composition in the shoots and has enabled the co-existence of greater diversities of soil fungi. Since soil communities were characterised from uniform amounts of soil, the increasing richness must indicate an increase in taxon richness per unit soil.

In addition to the mechanisms outlined above, increased decomposition could also result from an increase in the functional diversity of the fungal community enabling the breakdown of more complex recalcitrant substrates. However, this mechanism does not seem to be supported by the present study as most saprotrophs appeared to be general decomposers. The caveat here is that there is no significant functional diversity hidden within the taxa to which functional group status could not be attributed. Our results are slightly at odds with those of Morrison et al. (2018) who also demonstrated an increase in saprotrophic fungi with simulated N deposition but found that the favoured taxa were yeasts with limited potential of decomposition. Morrison et al. (2018) suggested that this could lead to the accumulation of organic matter – the opposite of the effects recorded here.

4.4. Temperature and decomposition

The interpretation of the role of increasing species richness in increasing decomposition is complicated by the positive relationship between decomposition rate of moss necromass and moss mat temperature across the sites of the present study. Relationships between decomposition of organic material and temperature can be both direct via increasing rates of chemical reactions with increasing temperature (Wei et al., 2021) or more complex and indirect. The latter may include, in N limited systems, increased availability of N from increased rates of mineralisation with soil warming (Lukewille and Wright, 1997; Rustad et al., 2001). More complex influences include temperature effects on the resistance of organic matter to decomposition via alterations of the physical structure of organic matter and changes to its physico-chemical protection (Conant et al., 2011). Without experimentation to specifically test the physical impacts of temperature on decomposition of moss necromass along the gradient, it is not possible to partition variation between purely physical phenomena and biological acceleration. But it is likely that both are important in the observed degradation of *Racomitrium* mats.

5. Summary

In summary, the fungal communities associated with *Racomitrium* heath are diverse and species rich, with species composition strongly structured by the combined effects of N deposition and temperature. Elevated N levels appeared to release the fungal communities in soil from N limitation and species richness increased significantly along the gradient of N deposition. Temperature strongly affected community composition in both shoots and soil, though richness was not affected in the latter. Detailed proteomic (Schneider et al., 2012) or metagenomic studies (Purahong et al., 2016) would provide valuable insights into the mechanisms and the fungal community dynamics underlying these changes. In addition, the strong link between fungal community composition and temperature suggests that climate change will cause further alteration in the communities as conditions in our alpine zone continue to ameliorate. It is therefore likely that both N deposition and climate change will contribute to the continued loss of this iconic and important alpine habitat.

The discovery that green shoots of mosses contain numerous fungal endophytes is relatively new, but recent work (Davey et al., 2012; Davey et al., 2013; Davey et al., 2014; Davey et al., 2017) and the present study highlight the potential taxonomic and functional diversity of these communities and some of the environmental variables important in structuring these communities. Further studies, including a broad range of host species in less well-known habitats such as alpine systems, are required to explore the generality of these findings.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funeco.2022.101191.

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