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A multiproxy approach to long-term herbivore grazing dynamics in peatlands based on pollen, coprophilous fungi and faecal biomarkers

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Abstract

Herbivory plays a significant role in regulating many contemporary terrestrial plant ecosystems, but remains an imperfectly understood component of past ecosystem dynamics because the diagnostic capability of methods is still being tested and refined. To understand the efficacy of a multiproxy

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approach, we compare the sensitivity of pollen and coprophilous fungal spores (CFS) to changes in grazing intensity over the last 100-150 years in six peat cores from three UK upland areas, and apply faecal lipid biomarkers to two of the cores, using agricultural census data to calculate an independent record of herbivore density. Rising sheep density adversely affected moorland ecology over the last century, which therefore provides a suitable period to test the sensitivity of these proxies. In particular, we assess whether CFS can be used to track variations in large herbivore densities over time, since this has received less attention than their ability to identify high grazing levels. At selected sites, we test whether faecal lipid biomarkers can be used to identify which herbivore species were present. Our results highlight the differential sensitivity of each proxy, demonstrating on peat- and moorlands (i) that peak CFS abundance is a more consistent indicator of ecologically influential (high) herbivore levels than variations in animal density through time; (ii) when recorded with high CFS values, the decline or disappearance of grazing-tolerant pollen taxa is a reliable indicator of high herbivory; and (iii) at low herbivore densities, faecal lipid biomarkers are not an effective indicator of herbivore presence or identity. Quantitative reconstructions of past herbivory and identifying grazer species therefore remain challenging. However, our findings indicate that pollen and CFS provide complementary evidence for high intensity grazing, and emphasise that studies using CFS should aim to define 'high' herbivore levels in terms of the grazing sensitivity of the ecosystem, rather than relative animal abundance.

Keywords: agricultural census; dung fungi; sheep grazing; herbivory; upland ecology

1. Introduction

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Understanding changes in the abundance of large herbivores over time is a key challenge in long-term ecology (Bradshaw et al. 2003), particularly for assessing the role that animals play in disturbance regimes, as drivers of biodiversity change and in ecosystem resilience (Gill, 2014; Bakker et al., 2016; Jeffers et al., 2018; Kuneš et al., 2019). Pollen is the most widely used proxy to reconstruct grazing dynamics, based on the impact of herbivory on vegetation composition and structure. However, without additional sources of evidence, it remains difficult to separate the palynological impacts of large herbivores from those associated with other disturbance mechanisms (Mitchell, 2005; Edwards et al., 2015). The range of tools suitable for understanding animal presence and dynamics in sedimentary sequences now includes coprophilous fungal spores (CFS), faecal lipid biomarkers, ancient DNA and palaeodemographics (Bull et al., 2002; Lorenzen et al., 2011; Baker et al., 2013; Jeffers et al., 2018; Perrotti and van Asperen, 2019; Shillito et al., 2020). Here, we focus on CFS and faecal lipid biomarkers because they are widely applied alongside palynology in palaeoenvironmental and archaeological sciences, yet few studies combine all three (e.g. van Geel et al., 2008, 2011; Guillemot et al., 2017; Anderson et al., 2019). We integrate two indicators (pollen, CFS) at two study areas and combine all three indicators at a third area to evaluate their comparative sensitivity and contribution to our understanding of grazing history.

CFS are increasingly used as an independent proxy for changes in large herbivore biomass (Baker et al., 2013), but the manner in which they are used varies, with some studies proposing a quantitative relationship with large herbivore biomass (Gill et al. 2013; Baker et al., 2016) and others taking a more qualitative approach to interpretation, focused on the relative abundance of CFS (Stivrins et al., 2019). The nature of the relationship between herbivore abundance and CFS values matters because it affects what research questions CFS can most reliably be used to address: (i) qualitative extremes or a quantitative threshold associated with major transitions, such as the functional extinction of megafauna, and periods of intense or prolonged livestock grazing (e.g. Davis, 1987; Gelorini et al. 2012), or (ii) quantitative variations in CFS abundance associated with long-term

fluctuations in animal density, such as variations in ecological impact related to herbivore population size (e.g. Jeffers et al., 2018). It has been suggested that CFS are most reliable for identifying 'high' densities of animals – a relative term that is not always quantified but which is consistent with periods of intense livestock grazing, for example (Davis and Shafer, 2006; Gelorini et al., 2012; Raczka et al, 2016; Davies, 2019; Goethals and Verschuren, 2020). The ability of CFS to track variations in large herbivore densities over time has received less attention.

In addition to herbivore density, CFS production and dispersal are also affected by taphonomic and environmental factors and by animal husbandry practices (Parker and Williams, 2012; Davies, 2019; van Asperen et al., 2020). Comparing CFS to other indicators is therefore valuable for testing uncertainties and improving inferences about herbivory. Faecal lipid biomarkers, in particular, have the potential to extend our understanding of grazing regimes. They are well-preserved in soil, organic-rich and waterlogged environments (Mackenzie et al., 1982; Prost et al., 2017), are established indicators of manuring in buried agricultural soils, and offer insights into changing husbandry practices at archaeological site and landscape levels (Bull et al., 2002; Dubois and Jacob, 2016; Guillemot et al., 2017; Prost et al., 2017; Mackay et al., 2020). Recent advances in faecal lipid biomarker analysis now allow improved differentiation between animal species (Harrault et al., 2019). Identifying which grazers were present is currently impossible using CFS and pollen analysis, so improved species discrimination from biomarkers represents a significant advance for understanding trophic diversity and biotic interactions. Multiproxy pollen/non-pollen palynomorph (NPP)/biomarker studies remain scarce in palaeoecology and archaeology, and have shown either similar (Guillemot et al., 2015) or contrasting trends between faecal lipid biomarkers and CFS (Ortiz et al., 2016). These variable results indicate the need for more comparative analyses to understand whether consistent relationships exist between proxies and grazing regimes.

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Previous studies using faecal lipid biomarkers to study grazing history have focused on lacustrine sediments, rather than peat (D'Anjou et al., 2012; Ortiz et al., 2016; Zocatelli et al., 2017; Argiriadis et al., 2018). Among faecal lipid biomarkers, 5β-stanols and 5β-stanones are most frequently used in environmental studies to track faecal matter inputs as they are produced almost solely in the digestive tracts of animals, and their presence in natural environments with no known faecal input is limited (Bull et al., 2002). While faecal stanols have been identified in peat (Ardiriadis et al. 2020), this proxy has yet to be applied in peatland palaeoecology where it is unclear if faecal lipid biomarkers are present in sufficient quantity to be separated from plant and microbially-derived sterols. Addressing this gap can establish an appropriate multiproxy approach to reconstructing grazing history in peatlands, which constitute a globally significant source of evidence for long-term ecosystem dynamics.

Our case study combines palaeoecological grazing indicators (pollen, CFS), faecal lipid biomarkers, and historical agricultural records of livestock abundance to test the ability of this multiproxy approach to track changing stocking levels and species over the last c.150 years in three UK upland areas. We use historical agricultural census data as an independent, quantitative record of herbivore composition and abundance to test the sensitivity of these proxies. This was a period of significant change in upland vegetation composition across Britain (Stevenson and Thompson, 1993) as well as elsewhere in NW Europe (Berglund et al., 2008). Rising stocking densities are well-documented on national and regional scales (Coppock, 1976; Dallimer et al., 2009) and grazing is implicated in the loss of *Calluna* moorland, broader declines in plant diversity, and biotic homogenisation (Anderson and Yalden, 1981; Milligan et al., 2016). Few palaeoecological studies have used sources other than pollen to understand herbivore impacts during this time period (Hanley et al., 2008; Davies, 2016).

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Using a multiproxy approach in our case study areas in the North Pennines and Peak District (northern England) and in Assynt (northern Scotland), we address two main research questions: (1) Does CFS abundance track known changes in stocking densities over the last c.100-150 years? (2) Can faecal lipid biomarkers from peat sequences be used to identify which species were contributing dung? These questions allow us to consider whether CFS abundance can be used to infer variable herbivore abundance through time, or whether it is more reliable for tracking peak densities. We also assess whether CFS and faecal lipid biomarkers provide identifiable and comparable signals in a relatively simple two-herbivore peatland setting, with seasonal grazing by sheep and a permanent red grouse population (in the Pennines) or red deer population (in Assynt). Our study contributes new data to previously published pollen and (for the Pennines) CFS datasets (Hanley et al., 2008; Davies, 2016, 2019). It represents the first application of faecal lipid biomarker analysis to peat sediment in the UK and also provides the first joint CFS, pollen and faecal lipid biomarker study from peat.

2. Materials and methods

2.1 Study sites

This study included six peat cores from three upland areas, two in northern England, and one in northwest Scotland (Table 1, Fig. 1). Two study areas lie within the Pennines, the main area of deep blanket peat in England (JNCC, 2011), where we sampled two sites in the Peak District, on the south-eastern range limits for extensive, deep blanket peat in Britain, and a further two from a long-term ecological experiment in the North Pennines (Bonn et al., 2009). The sites have contrasting ecologies, which may influence their palatability to grazers and sensitivity to grazing (Table 1) (Thompson et al., 1995). All sites in northern England are grazed by sheep. Heather cover on Emlin Dike has undergone rotational patch burning for red grouse management since AD 1950 (Estate manager, 2010, personal communication). Hard Hill in the North Pennines is located on Moor House National Nature Reserve

(NNR), which has been managed since 1954 as an ecological experiment to study the long-term impacts of sheep grazing and burning on upland ecology and carbon sequestration (Garnett et al., 2000; Ward et al., 2007; Lee et al., 2013; Milligan et al., 2016). Moor House is subject to low-intensity sheep grazing during the summer, with fences protecting experimental exclosure plots from stock ingress. Palaeoecological and ecological data suggest that grazing has, along with fire, strongly affected *Calluna* cover at the English sites over the last two centuries, at least (Rawes, 1983; Chambers et al., 2017; Davies, 2016, 2019). The final two sampling sites are located in Assynt, in the far north-west of Scotland. Although red deer are the currently the main herbivore and a source of ecological concern (Clifford and Mackenzie, 2017), historically and into the late twentieth century, extensive sheep grazing was an important land-use (Hanley et al., 2008).

A single peat core was extracted from each site using a 10 cm diameter golf-hole corer modified to sample the top 50 cm of sediment. These were closest together at the site in the North Pennines, where a core was taken from one exclosed (HHE) plot and one grazed (HHG) plot on Hard Hill to assess whether the difference in experimental treatment is reflected in proxy records. All sites have a predominantly local pollen source area owing to contributions from surface vegetation on the peat. Vegetation growing within c.2–50 m is likely to dominate the pollen signal, particularly for herbaceous and heath taxa, with smaller contributions from the surrounding 400–1000 m (Bunting, 2003; Brostrom et al., 2005). Dispersal distances for CFS are estimated to range from <10 m to around 80-100 m (Gill et al., 2013; Davies, 2019). The sites thus record community variability on a spatial scale comparable with ecological monitoring. Fresh sheep (*Ovis aries*) and red grouse (*Lagopus lagopus scotica*) dung were collected to provide reference material for comparison with the biomarker lipid extraction from peat samples. Pellets from individual red grouse and sheep were collected with latex/nitrile gloves at Hard Hill in July 2019 and black grouse (*Tetrao tetrix*) dung was collected in Suollagavallda, northwest Sweden (67°48'03.6"N 16°44'38.0"E) in July 2016 for wider comparison. The dung samples were placed in plastic bags before being freeze-dried in the lab.

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165

166 *2.2 Chronology*

167 Chronologies were constructed slightly differently for each area, since this paper combines
168 data from three projects to provide new analyses. More details are provided in the earlier publications
169 cited below. In the Peak District and Assynt, a composite chronology was constructed for each site
170 using the probability-weighted average of calibrated AMS ^{14}C dates to provide basal age estimates,
171 with ^{210}Pb and spheroidal carbonaceous particle (SCP) ages to constrain the chronology from AD
172 c.1850 to the present (Davies, 2011; 2016). SCPs were extracted and quantified independently of
173 pollen and NPPs. In the North Pennines, approximate chronologies were derived from SCP
174 concentrations on pollen slides, which were used to identify three key dating horizons attributable to
175 broad-scale changes in fossil fuel use over the last c.250 years: the earliest appearance of SCPs (AD
176 1850 ± 25), rapid rise (AD 1955 ± 15), and peak concentration (AD 1974 ± 4) (Rose and Appleby, 2005;
177 Davies, 2019). At each site, a linear rate of peat accumulation is assumed between dated samples. All
178 dates are quoted in calibrated/calendar years AD and estimated ages are rounded to the nearest 5
179 years. Given the relatively small errors associated with ^{210}Pb dates and key SCP horizons, these
180 chronologies are considered appropriate for comparison with historically-derived stocking records.

181

182 *2.3 Pollen and spore analysis*

183 Peat cores were subsampled in c.0.5 cm thick slices, and analysed for pollen and selected
184 NPPs. The subsamples, each with a volume of 0.5-1.0 cm^3, were processed using standard pollen
185 analytical techniques, including acetolysis and the addition of *Lycopodium clavatum* spores to allow
186 pollen concentrations to be calculated, but without hydrofluoric acid (Stockmarr, 1971; Moore et al.,
187 1991). Pollen identification was based on standard pollen keys (Moore et al., 1991), following the

nomenclature of Bennett (1994). A minimum of 300 (for Assynt and Peak District) or 500 (for North Pennines) total land pollen grains was counted for each sample (TLP, excluding aquatic taxa, plant and fungal spores). Samples span the full depth of the 50 cm deep cores from Assynt and the Peak District, but were taken from the upper 20 cm of the North Pennines cores to focus on the experimental period. Three pollen taxa were selected as grazing disturbance indicators based on known relationships with grazing disturbance: *Plantago lanceolata*, *Rumex* and *Urtica* (Sagar and Harper, 1964; Behre, 1981; Bunting, 2003). These taxa are not characteristic of peat and moorland communities so their presence in the pollen record is commonly used as an indicator of grazing disturbance. The summed value of these pollen disturbance indicators (PDI) is presented. The coprophilous fungal spore types *Sporormiella* HdV-113, *Sordaria*-type HdV-55A and *Podospora*-type HdV-368 were quantified on pollen slides. All have a strong, possibly obligate, preference for dung, show a strong association with the presence of large herbivores, and preserve well in peat and lake sediments (Baker et al., 2013; van Asperen et al., 2016; Perrotti and van Asperen, 2019). Fungal spore nomenclature follows Miola (2012), but lab identifiers (e.g. HdV) are omitted in subsequent text for brevity. Summary pollen diagrams (%TLP) are provided to indicate the dominant pollen types around each site over the study period. Pollen diagrams were constructed using TILIA and TILIA*GRAPH, with local pollen assemblage zones defined at each site using constrained sum of squares analysis (Grimm, 1987) to identify periods of similar pollen composition.

2.4 Faecal lipid biomarker analysis

Five horizons were selected for faecal lipid biomarker analysis in each peat core from Hard Hill. Sampling depths were based on pollen and CFS data which show similar trends in each core (Davies, 2019). These include pre- and post-experimental samples, peaks or troughs in both PDI and CFS data, and peaks in CFS but not pollen. One centimetre thick subsamples of peat, of c.5 g (wet

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weight) were freeze-dried and powdered prior to lipid extraction. Grouse and sheep pellets from Hard Hill and the grouse pellet from Suollagavallda were processed in the same manner to extend the existing reference database of animal faecal lipid signatures.

Our analysis focused on Δ^5-sterols, stanols and stanones. Bile acids potentially provide complementary faecal lipid biomarkers as they are exclusively produced by vertebrates (Danielsson and Sjövall, 1985; Setchell et al., 1988), but their concentrations in soils can be very low (e.g. Guillemot et al., 2015; 2017). We therefore focus only on 'neutral' steroids. The extraction and analysis of steroid biomarkers from peat and faecal samples was adapted from Harrault et al. (2019). Freeze-dried samples were milled and sieved at 500 µm, then c.200 mg of peat, grouse or sheep faeces were extracted three times by sonication (room temperature, 15 min) with 10 mL of dichloromethane/methanol (DCM/MeOH, 3:1, v/v). After each extraction, samples were centrifuged (3500 rpm, 15 min, 10˚C) and the supernatant transferred to a vial and concentrated under N_2. This operation was repeated twice and the three supernatants were pooled and N_2-concentrated. These total lipid extracts were then separated on a silica-filled glass column into apolar and polar fractions. The former was eluted with 3 mL of *n*-heptane and 3 mL of a *n*-heptane/DCM mixture (3:1, v/v), and the latter fraction was eluted with 4 mL of a DCM/MeOH mixture (3:1, v/v). Polar fractions were then dried under N_2 and redissolved in DCM, and aliquots were transferred to GC vials, dried, and derivatized with a mixture of N,O-bis(trimethylsilyl)trifluoroacetamide/trimethylchlorosilane (99:1, v/v) for 45 mins at 70˚C after addition of 50 ng of 5α-cholestane as an injection standard. Derivatized polar fractions were analysed by an Agilent GC6890N-MS5973N gas chromatograph-mass spectrometer (GC-MS). 1 µL of fractions was injected in splitless mode at 280°C. Helium was used a carrier gas (1 mL/min) through a Restek Rxi5Sil MS capillary column (30 m x 0.25 mm x 0.5 µm). The oven programme started at 200°C with a first ramp at 20°C/min to 275°C, a second ramp at 0.8°C/min to 300°C, and a final ramp at 10°C/min to 310°C, held for 20 min. The transfer line temperature was set at 310°C, the ion source at 220 °C and the electron ionization was carried out at 70 eV. Detection

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and semi-quantification of steroids were performed in selective ion monitoring mode (detailed information in Table S1) with internal calibration curves of standards with relevant fragments. In addition to the eleven compounds described in Harrault et al. (2019), a twelfth compound since identified as helpful for species-level biomarker fingerprinting was also targeted: 5β-epilichestanol (24-methyl-5β-cholesta-8(9),22E-dien-3α-ol). Dwelling time was 20 ms. A signal over noise ratio (SN) of 3 was used as a limit of detection and the limit of quantification was SN = 9.

The 5α-sitostanol/(5α-sitostanol+sitosterol) ratio was used as proxy for post-depositional phytosterol degradation (Naafs et al., 2019). As supplementary assessments of 5β-stanol post-depositional formation and 5β-stanol post-depositional epimerisation, we also generate the 24-ethylcoprostanol/(24-ethylcoprostanol+sitosterol) and 24-ethylcoprostanol/24-ethylepicoprostanol ratios, respectively.

2.5 Agricultural census data on livestock abundance

Historical stocking densities were calculated from animal abundance data presented in June Agricultural Census (JAC) records. These are an annual survey of farm holdings which has been undertaken since the 1860s. They include land area and livestock numbers, from which we calculated sheep stocking densities (abundance per square km). To preserve confidentiality at the individual farm level, archived census data are available at local administrative levels (parish or ward), so they reflect a landscape-level average for each study site. The parish data are held in the National Archives (England) and National Records of Scotland. The JAC data were originally collected for separate projects and livestock densities were calculated slightly differently for each area.

The Peak District dataset incorporates 32 parishes (totalling c.556 km^2, of which c.366 km^2 is classed as agricultural land). Our JAC data for the Peak District cover the area for which vegetation

maps are available since 1913, rather than the full extent of this region, and comprise approximately 62% of the area encompassed by the Peak District National Park (Dallimer et al., 2009). Records were extracted every 10 years from 1900-1988 and stocking densities were calculated per square kilometre of agricultural land to reflect changes in the proportion of land used for agriculture over time (Dallimer et al., 2009; Zayed and Loft, 2019). Both the aggregated average stocking density for the 32 parishes and the individual parish density for each study site are presented to account for geographical variability and possible inaccuracies at smaller scales (Clark et al., 1983). Emlin Dike is located in Bradfield Parish (c.142 km^2, 68 km^2 agricultural land), while Withens Moor is located in Tinwistle parish (c.48 km^2, 31.5 km^2 agricultural land).

Parish-level JAC data were not obtained for Hard Hill (North Pennines) because the initial project compared pollen and CFS dispersal with local grazing patterns (Davies, 2019). However, wider-scale county level data are available online from the UK government Department for Environment, Food and Rural Affairs (https://www.gov.uk/government/statistical-data-sets/structure-of-the-agricultural-industry-in-england-and-the-uk-at-june) and data relating to Moor House were extracted every 10 years from 1905-2016. In this case, the density of sheep is calculated per square kilometre of total agricultural land since the categories used for pastoral ground vary between surveys. Administrative boundaries also changed over the survey period: the study site has been included in JAC surveys for Westmorland (1905-1965), Cumbria (1975-1985), and East Cumbria (1995-2016). This generated significant changes in total land area between surveys (ranging from 1850-6722 km^2). These boundary transition dates do not coincide with major changes in sheep density, suggesting that the administrative shifts do not have a major effect on trends in sheep densities. The Westmorland/Cumbria county-level sheep densities are compared with site-level estimates for the area within the long-term ecological experiment at Moor House (38 km^2).

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283 For Assynt, stocking densities were calculated for the whole parish (estimated at c.447 km^2
284 from the Land Cover Map 2000), rather than the area of land suitable for agriculture, to account for
285 lack of clarity over how the area of agricultural land changed through time. To calculate sheep
286 densities, livestock data were collated from 1866 and for each decade until 2000 (Hanley et al., 2008).
287 The study site at Veyatie lies on the boundary of Assynt and Loch Broom parishes, but only the data
288 for Assynt parish are presented because both study sites formed part of a single land holding (estate)
289 until 2005.

290 For all study areas, historical livestock densities are only presented for sheep since they were
291 the most abundant animal type, particularly on the hills and moors where the peat cores were
292 obtained. Currently, red deer are the main wild herbivore in Assynt, roe deer numbers are considered
293 low in the English sites, and a small red grouse population is resident at Hard Hill, although information
294 on their historical densities is limited. We assume that the parish and county level stocking data reflect
295 landscape scale patterns that are representative of herbivore densities around each site as well as
296 contributing an airborne background CFS signal, particularly when landscape-level animal densities
297 are high (Baker et al., 2016; van Asperen et al., 2020). However, we recognise that the geographical
298 scale of the stocking data is an imperfect match to the predominantly local production and dispersal
299 of pollen, spore and biomarkers, and that stocking density is likely to have varied within each parish
300 and county.

301

302 *2.6 Analytical approach*

303 Differences in how CFS were recorded and quantified limits comparability between previous
304 studies. We follow the recommendation of van Asperen et al. (2021) by presenting and evaluating
305 individual and summed CFS values in relative (percentage) and absolute (concentration, influx)
306 formats. To examine how quantification choices can influence the CFS signal and potential correlations

with herbivore abundance, we also examine the diagnostic value of a single (e.g. *Sporormiella*) versus a composite (*Sporomiella-Sordaria-Podospora*) CFS indicator for herbivory. We examine the relative abundance of each spore type over space and time, and apply Spearman's rank correlation coefficient to test for correlations between CFS types and between CFS and total pollen influx to better understand taphonomic influences.

To address research question one on the ability of grazing proxies to track stocking densities calculated from the JAC, we compare trends in the abundance of PDI and CFS with the JAC data. Based on existing literature, we hypothesise that the CFS signal will most consistently correlate with peak stocking densities, whereas grazing indicator pollen taxa will be sensitive to variations in sheep numbers and peak animal densities (Hanley et al., 2008; Raczka et al., 2016). The relatively short timeseries in our study prevent the use of methods like changepoint analysis to assess potential causal relations (cf. Gill et al., 2012; Wood et al., 2016). Instead, we apply Spearman's rank correlation coefficient to test for an association between indicators, using the results from the correlation above to select the most appropriate CFS quantification method. It is not possible to run a simple correlation between JAC and the palaeo-indicators because sample ages of the historical and sedimentary sources are not identical.

To address the second research question on the value of faecal lipid biomarkers as an additional grazing proxy and indicator of herbivore species in peat ecosystems, we compare trends in pollen, CFS and faecal lipid biomarker abundance with local estimated sheep density and regional stocking levels at Hard Hill. We also examine whether it is possible to distinguish faecal lipid signatures of different animals based on the abundance of twelve 5β-stanol compounds. We hypothesise that faecal lipid biomarker abundance for sheep will be higher in the grazed experimental plot than the fenced exclosure plot, whereas faecal lipid biomarkers associated with red grouse will be present in similar quantities in both plots, since the fences do not exclude small animals. Bird faeces is recognised

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as a poorer fungal growth substrate than mammal dung (Richardson, 2001). However, since duration and density of herbivore activity both affect the CFS signal (Kamerling et al., 2017; Goethals and Verschuren, 2020), we explore whether year-round, low density habitation by red grouse compared with seasonal low density sheep-grazing generates an identifiable faecal signal that allows the use of CFS as a proxy for bird faunas (Wood et al., 201; Baker et al., 2016). We also test whether biomarkers can be correlated with animal density (Dubois and Jacob, 2016; Zocatelli et al., 2017).

3. Results

3.1 Comparison of pollen and fungal indicator signals with livestock census data

Summary pollen and CFS diagrams for the last 140-400 years show the dominance of open pollen assemblages, particularly *Calluna vulgaris*, which was the main heath species at all sites (Fig. 2). Emlin Dike provides the shortest record, with the base of the 50 cm deep core dated to AD c.1870. Withens Moor provides the oldest record, with 50 cm dated to AD 830 (Davies 2016); the last 180 years are shown here. Only the upper 20 cm of the Hard Hill cores were analysed since SCP abundance indicated that this spans the last c.150 years. *P. lanceolata* is the most abundant PDI (Fig. 2). CFS values are often higher than PDI frequencies (Fig. 3). PDI and CFS values show similar (e.g. zones WM1, HHE2a, VEY2b) and contrasting peaks and trends (e.g. zones ED3, HHG1c, AGM1b).

CFS were present in the majority of samples (Table 2). The records are dominated by *Sporormiella* and *Sordaria*, with relatively scarce and discontinuous records for *Podospora* (Table 2, Fig. 2). *Sporormiella* and *Sordaria* frequencies are highly or well-correlated, except at Hard Hill (Table 3). There are significant correlations between percentage, concentration and influx values for the CFS sums (Table 2) and only percentage values are therefore presented (Figs. 2-3). With the exception of

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Withens Moor, the values for individual CFS taxa and the CFS sum do not correlate closely with TLP influx.

In the Peak District and North Pennines (Westmorland/Cumbria), the JAC records show rising sheep densities from the middle of the twentieth century and a steeper increase around the AD 1970s (Fig. 3). In Assynt two maxima are recorded, at AD 1870-1880 and AD 1950-1970. Maximum livestock densities show strong regional differences, ranging from 470-500 sheep/km^2 in the Peak District and Westmorland/Cumbria, to 220-320 sheep/km^2 at parish level for the Peak District study sites, and a maximum of just 60 sheep/km^2 in Assynt, the northernmost study area. On finer spatial scales, stocking trends are rather variable, with Bradfield Parish (including the relatively dry moorland around Emlin Dike) following the regional trend, whereas Tintwistle Parish (including extensive blanket peat on Withens Moor) shows a more modest and shorter-lived rise (Fig. 3b). Local grazing levels at Hard Hill are also significantly lower than county-level densities and have declined since the start of the ecological experiment in 1954 rather than following the county-level rise (Fig. 3a).

The CFS and PDI records show both sustained trends and single sample peaks or declines (Fig. 2). Based on the finding that percentages are representative of CFS trends (Table 3) and similar tests of PDI percentages against concentration and influx (all significant at $p<0.05$ or $p<0.1$, not shown), Spearman correlation shows that the relationships between CFS and PDI vary from positive (strongest in Assynt), to slightly negative (strongest for the Hard Hill exclosure site), but all are non-significant (Table 4). This indicates that dung and vegetation indicators provide differing information regarding herbivores.

To compare patterns between the grazing proxies and sheep density records, we focus on periods when stocking densities underwent marked, often multi-decadal shifts, and on trends that are maintained for three or more samples in each proxy record before considering shorter-lived CFS and pollen peaks associated with JAC maxima (Fig. 3). Twentieth-century increases in sheep densities

correspond with rising CFS values at Veyatie (AD 1930-1940) and Withens Moor (AD 1960-2000). At Emlin Dike, a sustained rise in CFS abundance (AD 1980-2000) begins during the steep increase in sheep numbers (AD 1960-2000). At Hard Hill, the county-level rise in sheep density from the 1950s to 1990s corresponds with an on-site reduction in sheep numbers, during which CFS abundance falls (AD 1950-1980) and then rises in both cores. In Assynt, single sample peaks and declines in CFS at Allt na Glaic Moire and Veyatie coincide with the AD 1880 peak and subsequent fall in sheep numbers.

Many PDI trends go in the opposite direction to JAC values. These include reductions in PDI abundance at Withens Moor (AD 1915-70), both Hard Hill plots (AD 1955-2000) and Veyatie (AD 1870-1900) as regional sheep densities rise, and a recovery in PDI frequencies in the grazed Hard Hill plot (AD 1995-2010) and at Allt na Glaic Moire (AD 1965-1995) as JAC values fall. PDI values at Emlin Dike show no clear trends and sustained falls in PDI at Allt na Glaic Moire (AD 1875-1940) and Veyatie (AD 1915-1980) occur through both rising and falling sheep densities.

If only peaks are compared, CFS maxima correspond with JAC maxima at Allt na Glaic Moire (AD 1880, 1915), Veyatie (AD 1880, 1940), the Hard Hill grazed plot (AD 1995-2000), Withens Moor (AD 2000) and Emlin Dike (AD 2000). PDI abundances display minima with peak stocking densities at Allt na Glaic Moire (AD 1965) and Withens Moor (AD 1975).

3.2 Faecal lipid biomarkers as an additional proxy for herbivory

The steroid content of grouse faeces was analysed for the first time as part of this project. Red grouse faeces contains low amounts of steroids and is dominated by sitosterol, with low amounts of other sterols and 5α-stanols and 5α-stanones; no 5β-stanols and 5β-stanones were detected (Table S2). The black grouse sample has higher concentrations of steroids overall, dominated by sitosterol and containing significant amounts of 24-ethylcoprostanol, with the other steroids detected in lower

amounts (Fig. 4). In contrast, sheep faeces are richer in steroids, and are dominated by 24-ethylcoprostanol, followed by sitosterol, 5α-sitostanol, 24-ethylepicoprostanol and coprostanol, and lower contributions of other steroids.

The steroids in the Hard Hill samples are largely dominated by sitosterol, and contain lower amounts of 5α-sitostanol and very low amounts of other stanols and stanones, with no particular differences between the exclosure and grazed plot (Table S2). In both profiles, the concentration of 5β-stanols is low and consists mainly of 24-ethylcoprostanol and 24-ethylepicoprostanol, except for one sample in the exclosure, which displays significant contributions of coprostanol, epicoprostanol and 5β-campestanol (Fig. 5). None of the peat sample 5β-stanol distributions match the reference samples of grouse or sheep faeces. In both peat sequences, the 5α-sitostanol/(5α-sitostanol+sitosterol) ratio tends to decrease with depth, while the 24-ethylcoprostanol/(24-ethylcoprostanol+sitosterol) and the 24-ethylcoprostanol/24-ethylepicoprostanol ratios do not display particular trends (Fig. 5). The stanol/sterol ratios do not show clear differences between the exclosure and the grazed plot. These findings indicate that it is not appropriate to apply a Spearman test to the relationship between faecal lipid biomarkers and CFS or PDI data.

4. Interpretation and discussion

4.1 Selection and quantification of grazing proxies

Significant correlations between *Sporormiella* and *Sordaria*, the two most abundant CFS types, show that composite CFS abundance can be used to represent trends (Table 3). This correlation indicates that using multiple CFS taxa or the aggregate abundance of CFS may be preferable to relying on one fungal type alone: similar signals in multiple spore types can strengthen the evidence, while uncorrelated signals may provide complementary evidence for ecosystem or fungal processes (van

Asperen et al., 2021). Reasons for the abundance of particular coprophilous spores cannot yet be explained, such as the scarcity of *Podospora* in this study compared with its abundance in van Asperen et al. (2020), but providing this basic information on the CFS assemblage remains important for developing our understanding of the method. Strong correlations between percentage, concentration and influx values for the CFS sum suggest that the choice of quantification method does not significantly affect CFS trends in this study, despite marked changes in dominant vegetation at Emlin Dike and Withens Moor (Wood and Wilmshurst, 2013; Davies, 2016). With the exception of Withens Moor, the frequency of individual spore types and the CFS sums do not correlate closely with TLP influx, confirming that, in peat, fungal spores enter the record via a different pathway to pollen (van Asperen et al., 2020) and/or that the digestive, depositional and microenvironmental attributes that control the abundance of CFS are very different from the floristic dispersal mechanisms that determine pollen abundance (Bunting et al., 2013; Perrotti and van Asperen, 2019).

4.2 The use of CFS to understand changing herbivore density

At the scale of regions and counties, the JAC show strong trends over the last c.100 years (Fig. 3), marked by rising sheep densities from the middle of the twentieth century (post-WWII) and a steeper increase around the 1970s linked to UK entry into the European Economic Community (Condliffe, 2009). On finer spatial scales, the stocking trends are more variable, reflecting variations in ecology and land management decisions (Dallimer et al., 2009). Lower stocking densities in Assynt reflect lower productivity in more northerly regions and reduced levels between 1880 and 1950 may reflect vulnerability to competition from more productive areas in the UK and abroad (Hanley et al., 2008). These marked national and regional scale trends provide a suitable context for analysing the sensitivity and replicability of our CFS and pollen signals to grazing dynamics.

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The palaeoecological results present a mixed but complementary picture regarding the potential of CFS and PDI to track changing herbivore density through time. The three main findings are: (1) that trends in JAC and CFS are similar for more periods and sites than for JAC and PDI, (2) where the timing of trends does coincide, PDI values tend to show an inverse relationship with stocking levels, and (3) the most consistent correlation between stocking data and palaeoecological indicators occurs at peak herbivory levels, when CFS maxima occur in all study regions. We evaluate the CFS findings before discussing the pollen results.

Our study indicates that CFS consistently reflect high or peak herbivore levels. Late twentieth century CFS maxima are recorded at all sites except Veyatie, where a peak in CFS frequencies at AD c.1935 may reflect the increase in sheep densities from AD 1930-1950. In addition, there is good correspondence between late nineteenth century AD maxima in sheep density and CFS at both Assynt sites (Fig. 3). CFS abundance also tracks rising herbivory levels, with similar trends in spore and stocking curves detected for some periods at all sites, but this evidence is less consistent than peak correlations. For instance, rising CFS abundance through the twentieth century AD at Emlin Dike, Withens Moor and Veyatie corresponds with sustained regional increases in sheep density, while CFS values from Allt na Glaic Moire and Veyatie correspond with both the regional peak and decline in sheep stocks during the late nineteenth century AD. In contrast, CFS frequencies at Hard Hill do not consistently correspond with either locally declining or regionally rising sheep densities since AD c.1950. Overall, this strengthens evidence derived from lakes that CFS are reliable indicators of high or peak herbivore levels (Gelorini et al., 2012; Raczka et al., 2016) and should be used cautiously to infer animal population dynamics. In the present study, this applies across drier and wetter moor- and peatland communities and different grazing levels, which provides increased confidence in the sensitivity of this indicator.

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469 It is not unexpected to find that CFS abundance does not always follow regional-level stocking
470 trends, since local environmental factors, husbandry decisions and animal feeding preferences all
471 influence the distribution of herbivores, and CFS production and dispersal (Parker and Williams, 2012;
472 van Asperen et al., 2020, 2021). In peat sequences, which are dominated by fungal spore production
473 and dispersal on scales of c.10-100 m, these factors generate spatial variability in the CFS record
474 (Kamerling et al., 2017; van Asperen et al., 2020). Farm-level stocking decisions may, for instance,
475 explain why the mid-twentieth century peak in sheep at parish level is evident at Veyatie, but not at
476 Allt na Glaic Moire. Multiple cores are therefore needed to understand how variations in herbivore
477 population size over time influence ecological mosaics in heterogeneous upland terrain (Ejarque et
478 al., 2011; Ghosh et al., 2017). Spatially detailed historical grazing records are seldom available to test
479 interactions on finer spatial scales (Davies and Watson, 2007). In this study, even the smallest scale
480 stocking record, from Hard Hill, relates to a more extensive area than the estimated fungal spore and
481 pollen dispersal distances (Davies, 2019). This emphasises the importance of using multiple proxies to
482 produce robust reconstructions of herbivore abundance and impact on peatlands, particularly when
483 only a single coring site is available. At a landscape scale, it is important to emphasise that there are
484 good regional matches in Assynt around the 1870-1890 JAC peak and in the Pennines around the AD
485 2000 maximum in sheep densities (Fig. 3), indicating that there can be consistent correlations at peak
486 stocking densities, beyond what might be anticipated for locally-dispersed indicators. As a result,
487 regional-scale trends can be recorded, despite locally heterogeneous grazing patterns.

488 In contrast to the positive relationship between CFS and JAC, the abundance of PDI repeatedly
489 shows an inverse relationship with sheep densities. This applies to both trends and peak levels. At
490 their peak, sheep herbivory exceeded the capacity for *Calluna* regeneration in the Peak District and
491 Cumbria (Anderson and Yalden, 1981; Hulme et al., 2002; Pakeman et al., 2003). In combination with
492 the competition and regeneration pressures brought about by burning and atmospheric pollution,
493 high intensity grazing is likely to have reduced flowering, even in taxa that are generally considered

494 tolerant of grazing and trampling (Sagar and Harper, 1964; Davies, 2016). This can explain patterns in
495 the Peak District. In contrast, the establishment of the ecological experiment at Hard Hill in the AD
496 1950s decoupled stocking from the regional trend and decades of sheep exclusion have generated an
497 increase in *Calluna* cover on exclosed blanket bog (Milligan et al., 2016). Declines in *Plantago*, in
498 particular, around the mid-twentieth century may therefore reflect the local reduction in stocking
499 levels (Davies, 2019) (Fig. 2). Although JAC stocking densities in Assynt remained below levels that are
500 currently considered to be ecologically damaging, some heath communities may have been sensitive
501 to prolonged grazing under northerly growing conditions, leading to a decline in PDI at Allt na Glaic
502 Moire from AD 1875-1965, whereas CFS peaks coincide with higher JAC values around AD 1875 and
503 1915 (Holden et al., 2007; Hanley et al., 2008; Davies, 2011). PDI and CFS signals thus provide
504 complementary sources of evidence for high intensity grazing, where 'high' is defined in terms of the
505 sensitivity of the ecosystem to grazing pressure.

506

507 *4.3 The use of faecal lipid biomarkers as a proxy for grazing on peatland*

508 In contrast to CFS and pollen, faecal lipid biomarkers were not an effective proxy for herbivore
509 presence or stocking levels. The low concentrations and low diversity of 5β-stanols and 5β-stanones
510 in the peat samples from Hard Hill, the absence of clear differences between the grazed and ungrazed
511 plots, and limited correspondence with local stocking data or CFS profiles suggest that these
512 biomarkers cannot be reliably used to detect low density herbivore populations on blanket peat. While
513 this conclusion is based on a relatively small number of samples from one study area, the results from
514 both peat cores were comparable and the low concentrations and diversity of 5β-stanols and 5β-
515 stanones are comparable to the only previous peatland faecal lipid biomarker study, as indicated
516 below. There may be several reasons for this.

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First, since peats are made up of plant materials, their lipid assemblage is dominated by plant-derived sitosterol and its main metabolite, 5α-sitostanol, which may have diluted the signal of faecally-derived 5β-metabolites (5β-stanols and 5β-stanones). However, our analyses were conducted in selected ion monitoring (SIM) mode to overcome this limitation, by targeting specific and significant diagnostic fragments. Moreover, our extraction procedure was similar to that applied by Argiriadis et al. (2020) to blanket peat and similar concentrations of 5α-stanols and Δ^5-sterols were recorded in both studies, suggesting that low abundance may be characteristic of sites with low herbivore densities. While Argiriadis et al. suggest that concentrations of 5β-stanols (coprostanol and epicoprostanol) below 0.5 μ/g may be attributable to human activities, our results indicate that such low values may be below robust interpretation limits.

The second possible explanation for the low abundance of 5β-stanols, post-depositional degradation, seems unlikely, since there were no visible decreasing trends with depth/age of the 24-ethylcoprostanol/(24-ethylcoprostanol + sitosterol) and the 24-ethylcoprostanol/24-ethylepicoprostanol ratios (Fig. 5). On the contrary, the post-depositional formation of 5α-sitostanol from sitosterol is highlighted by the increase with depth/age of the 5α-sitostanol/(5α-sitostanol+sitosterol) ratio (Fig. 5). These results suggest that selective degradation of phytosterol occurs in peat sequences, but it seems limited for 'faecal' 5β-stanols.

The third and most likely explanation for the low abundance of 5β-metabolites is the low density of sheep grazing the site, and the hyper-local mechanisms by which lipids enter peat sequences. Sheep densities on the blanket peat at Hard Hill may be as low as 10-30 sheep/km^2 (Rawes and Welch, 1969). At this density, considering that a sheep can defecate c.369 g of dry faeces per day (Welch, 1982), and that stanol concentrations are c.2000 μg per gram of dry faeces (Table S3), and assuming that dung deposition has been homogeneous in the area since the beginning of the experiment, then the maximum concentration of 5β-stanol which could be encountered in the peat

sample would be c.9-154 µg/g (detailed calculation in Table S3). Even at these low input concentrations, at least some 5β-stanols should be detectable in the peat samples, but this was not the case, as the samples contained few 5β-stanols except for 24-ethylcoprostanol and 24-ethylepicoprostanol. In contrast, the modern reference sample of sheep faeces from Hard Hill contained all twelve 5β-stanols (Fig. 4). Stanol concentrations are more diverse in the exclosure plot and higher in the lower sections of both Hard Hill cores (Fig. 5). This corresponds with regionally stable stocking levels prior to the local reduction in sheep numbers after the experiment began. While this signal could therefore be attributed to localised faecal inputs, the 5β-stanol signature is not comparable to those of grouse or sheep dung pellets. This reinforces the conclusion that the input of sheep faecal material was too low to be detected by the current methodology. The very local deposition and integration of faecal lipid biomarkers on peat occurs on a finer spatial scale than the dispersal and deposition of pollen and fungal spores through wind and water, and this is likely to contribute to differential sensitivity of these proxies.

Finally, it should be noted that the grouse faecal reference samples did not present a clear lipid fingerprint, which limits our ability to differentiate between animal species on the basis of their faecal lipid biomarker signature using current reference materials. The presence of 5β-stanols in the grouse faeces from Sweden, and their absence from the grouse faeces from Hard Hill, suggests that faeces composition can vary with locality, due to its influence over diet and gut microbiota (Leeming et al., 1996). This highlights the importance of expanding faecal lipid biomarker databases in environmental and geoarchaeological studies to ensure that locally representative material is available and to provide a better understanding of intra-species variability (Prost et al., 2017; Harrault et al., 2019).

5. Conclusions

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In a study of centennial grazing dynamics on peat using pollen and CFS with the addition of faecal lipid biomarkers in one of three study areas, we find that (i) peak CFS abundance provides a more consistent indicator of 'high' (meaning ecologically influential) herbivore levels across a range of peat and moorland ecosystems than of variations in animal abundance through time; (ii) inverse relationships between PDI and stocking densities indicate that the decline or loss of grazing-tolerant taxa provides a reliable indicator of high levels of herbivory when accompanied by high CFS abundance; and (iii) at low herbivore densities, faecal lipid biomarkers are not an effective indicator of herbivore presence, abundance or identity in peatlands.

These findings reinforce and extend evidence derived from lakes that CFS are reliable indicators of high or peak herbivore levels. CFS abundance tracks rising and falling herbivory levels in some cases, but this relationship is not consistent enough to allow quantitative reconstructions of herbivore population size through time. Given this uncertainty and differential sensitivity to grazing amongst plant species, 'high' or 'peak' herbivore levels should be defined in terms of ecological sensitivity and impacts, rather than (relative) animal abundance. Our results emphasise the differential sensitivity of each indicator, reflecting the differing mechanisms and spatial scales of dispersal and deposition. These differences in sensitivity can generate complementary insights in multiproxy studies. For instance, peak CFS and low PDI suggest herbivory levels above ecological carrying capacity, rather than low grazing levels – the potential inference if pollen alone is used. The presence of CFS and PDI with low faecal lipid biomarker concentrations and poor representation of the major lipid compounds found in reference dung samples suggests low herbivory levels, below the functional ecological threshold for the ecosystem. In this situation, variations in CFS or PDI should be interpreted with caution. As this represents the first peatland study to compare faecal lipid biomarkers with CFS and PDI and derives from one area, further testing is required, but our findings stress the importance of using multiple lines of evidence when working with diffuse faecal sources, like those associated with wide-ranging animals, rather than concentrations of animals within settlements or

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corrals. More validation work is required on sites with a moderate to high grazing intensity to establish whether faecal lipid biomarkers can provide evidence for animal presence/absence and identify grazer species on peatlands and buried soil sequences, or whether the method should only be applied to lake sediments which may concentrate the signal from the catchment. The potential of faecal lipids to differentiate which species of grazers were present presents an exciting opportunity for understanding trophic diversity and interactions, but our reference samples suggest that faeces lipid composition can vary with environment for functionally similar animals, such as grouse. While this result derives from a small sample, it is an important consideration in studies where past vegetation assemblages differ from modern reference conditions or non-analogue communities are inferred.

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Data availability

Raw pollen and fungal spore data from the Peak District and Assynt have been archived in http://dx.doi.org/10.5255/UKDA-SN-6791-1. Raw pollen and fungal spore data from the North Pennines are being prepared for archiving in Neotoma.

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830 Tables

831 Table 1. Location and current ecology of each sampling site, including dominant vegetation cover

832 and main herbivore species present, with estimated animal density where recent data are available.

Site and location	Current ecology and sampling site
Peak District: Emlin Dike (ED) 53° 25' 55" N 01° 39' 27" W, 390 m OD	*Calluna*-dominated dwarf shrub heath and blanket bog. Red grouse present year-round, summer sheep grazing. Sediment core from flushed fen in natural drainage channel (placename element 'dike' refers to the stream).
Peak district: Withens Moor (WM) 53° 31' 09" N 01° 50' 59" W, 440 m OD	*Molinia* and *Juncus*-dominated grassmoor overlying deep blanket peat. Summer sheep grazing. Sediment core from sloping deep blanket peat.
North Pennines: Hard Hill experimental plots (HHE, HHG) on Moor House NNR 54° 41' 30" N 02° 23' 57" W, 590 m OD	Intact *Calluna-Eriophorum* blanket peat. Red grouse present year-round in low numbers with high interannual variability (c.25-140 grouse/km^2, low density summer sheep grazing (c.10-30 sheep/km^2, roe deer numbers considered very low (Davies, 2019). Peat cores from 30 x 30 m unfenced experimental plot (Hard Hill grazed, HHG) and exclosure plot (Hard Hill exclosure, HHE), around 80 m apart, both established 1954.
Assynt: Allt na Glaic Moire (AGM) 58° 08' 33" N 04° 57' 10" W, 204 m OD	Mosaic of base-rich grassland, acidic wet heath and blanket peat. Red deer resident (see above), summer sheep grazing. Peat core from c.5 m diameter valley-side flushed basin.
Assynt: Veyatie (VEY) 58° 03' 41" N 05° 03' 04" W, 120 m OD	Undulating blanket peat with fragmentary *Betula* woodland along south shore of loch. Red deer resident (c.7-9

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	deer/km^2 across Assynt; Albon et al., 2017). Peat core from c.10-15 m diameter basin in blanket peat.

833

834 Table 2. Summary of fungal spore counts showing the dominance of *Sporormiella* and *Sordaria*, and
835 the range of variability in abundance within and between sites. Influx data were not calculated at
836 Hard Hill House owing to the less robust chronology. CFS percentage, concentration and influx
837 values are averages with minimum and maximum values in brackets. Sites: ED = Emlin Dike, WM =
838 Withens Moor, HHE = Hard Hill exclosure (ungrazed), HHG = Hard Hill grazed, AGM = Allt na Glaic
839 Moire, VEY = Veyatie.

	ED	WH	HHE	HHG	AGM	VEY
Estimated age range (AD)	1866-2008	830-2008	1850-2019	1850-2019	1570-2008	1580-2008
Total no. spores counted	1298	341	260	181	1129	294
Sporormiella total count	1250	69	173	109	573	90
Sordaria total count	45	265	87	70	538	201
Podospora total count	3	7	0	2	18	3
Samples with CFS	17 of 20 (85%)	19 of 24 (79%)	13 of 13 (100%)	14 of 14 (100%)	24 of 26 (92%)	18 of 23 (82%
Samples with *Sporormiella* (% total no.)	15 (75%)	18 (75%)	12 (92%)	13 (93%)	21 (81%)	15 (65%)
Samples with *Sordaria* (% total no.)	17 (85%)	17 (71%)	12 (92%)	13 (93%)	22 (85%)	17 (74%)
Samples with *Podospora* (% of total no.)	3 (15%)	4 (17 %)	0	2 (14%)	11 (42%)	3 (13%)

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CFS percentages	21.1 (0, 373.5)	4.1 (0, 14.2)	4.3 (0.6, 13.3)	2.4 (0.2, 5.7)	13.0 (9.3, 40)	4.4 (0, 16.8)
CFS concentration (spores/cm3)	4594.4 (0, 79157)	2947.6 (0, 17798)	993.2 (162, 2226)	1236.5 (78, 2914)	3454.4 (0, 12078)	958.9 (0, 5630)
CFS influx (spores/cm2/yr)	3051.2 (0, 52772)	294.4 (0, 2083)	n/a	n/a	438.5 (0, 1441)	92.5 (0, 462)

Table 3. Spearman rank correlation between fungal spore types and between fungal spores and total pollen influx. Significant results ($p<0.05$) are shown in bold font. ρ = Spearman's coefficient. Conc = concentration. n/a = data not available. *Podospora* was not tested separately analysis due to infrequent occurrence. Data relate to the full age range of each sequence (see Table 2). Sites: AGM = Allt na Glaic Moire, VEY = Veyatie, ED = Emlin Dike, WM = Withens Moor, HHE = Hard Hill exclosure (ungrazed), HHG = Hard Hill grazed.

Site	Statistic	*Sporormiella*-*Sordaria* (%)	*Sporormiella*-*Sordaria* (conc)	*Sporormiella*-*Sordaria* (influx)	CFS %-conc	CFS%-influx	*Sporormiella*-TLP (influx)	*Sordaria*-TLP (influx)	CFS-TLP (influx)
ED	ρ	0.287	0.505	0.627	0.855	0.863	0.206	0.435	0.346
	p-value	0.220	**0.023**	**0.003**	**0.000**	**0.000**	0.383	0.055	0.135
WM	ρ	0.682	0.750	0.826	0.934	0.944	0.676	0.694	0.722
	p-value	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
HHE	ρ	0.080	-0.055	n/a	0.905	n/a	n/a	n/a	n/a
	p-value	0.796	0.863	n/a	**0.000**	n/a	n/a	n/a	n/a
HHG	ρ	0.142	0.297	n/a	0.763	n/a	n/a	n/a	n/a
	p-value	0.628	0.302	n/a	**0.001**	n/a	n/a	n/a	n/a
AGM	ρ	0.688	0.645	0.609	0.887	0.933	-0.084	0.307	0.143

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	p-value	**0.000**	**0.000**	**0.001**	**0.000**	**0.000**	0.684	0.127	0.486
VEY	ρ	0.828	0.809	0.821	0.969	0.962	-0.182	-0.183	-0.181
	p-value	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.405	0.404	0.409

847

848 Table 4. Spearman rank correlation between summed CFS and PDI data, expressed as percentages of

849 TLP. Symbols and abbreviations are identical to those used in Table 3.

Site	Rho	p-value
ED	-0.05555557	0.816
WM	-0.1015763	0.7534
HHE	-0.2248278	0.4602
HHG	0.1431718	0.6253
AGM	0.3750857	0.1379
VEY	0.40645	0.1681

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852 Figure captions

853 Figure 1. Locations of study sites, showing parish and county boundaries that define the geographical
854 extent of the agricultural census data. (a) North Pennines, (b) Peak District, and (c) Assynt.

855 Figure 2. Summary diagrams for each site over the last 140-400 years, showing changes in the
856 dominant pollen types and the frequencies of pollen disturbance indicators (PDI) and coprophilous
857 fungal spores (CFS) over time (%TLP). Peak District: (a) Emlin Dike, (b) Withens Moor; North Pennines:
858 (c) Hard Hill exclosure plot, (d) Hard Hill grazed plot; Assynt: (e) Allt na Glaic Moire and (f) Veyatie.
859 Clear curves show x10 exaggeration for clarity.

860 Figure 3. Comparison of corprophilous fungal spores (CFS) and pollen disturbance indicators (PDI)
861 with sheep stocking density over the last c.200 years for (a) Peak District: Emlin Dike (ED) and
862 Withens Moor (WM), (b) North Pennines: Hard Hill exclosed (HHE) and grazed (HHG) plots, and (c)
863 Assynt: Allt na Glaic Moire (AGM) and Veyatie (VEY). CFS sum shown in solid line and triangles, PDI
864 sum shown in dotted line and open circles. Sheep densities for the Peak District are shown as the 32
865 parish average (solid line and filled squares) and at individual parish-level for Bradfield (location of
866 ED, open squares) and Tintwhistle (location of WM, crossed squares). Sheep densities for Hard Hill
867 are shown at county level (solid line and filled squares), and NNR level (dotted line and open
868 squares). Vertical blue bars show peak sheep densities from JAC data and dotted green lines show
869 start of experiment at Hard Hill in 1954. Note differences in y-axis scales (truncated axis for single
870 high CFS value at ED, high CFS abundance at AGM, lower CFS and PDI abundances at HHG, and lower
871 sheep density in Assynt).

872 Figure 4. Distribution of 5β-stanols in faecal reference samples. Black bars represent pellets from a
873 black grouse sampled in Suollagavallda, Sweden, and grey bars represent sheep faeces from Hard Hill.
874 Red grouse sample from Hard Hill is not shown as steroid content was very low. Compound details
875 can be found in Table S1.

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876 Figure 5. Variations in (a) 5β-stanol concentration and distribution and steroid ratio variations of
877 peat sequences from Hard Hill exclosure (left) and grazed plot (right), relative to (b) CFS and PDI
878 frequencies, and (c) regional and local sheep densities. 5β-stanol distributions show, from black to
879 very light grey, respectively: coprostanol, epicoprostanol, 5β-campestanol, 24-ethylcoprostanol and
880 24-ethylepicoprostanol. CFS sum shown in solid line and triangles, PDI sum shown in dotted line and
881 open circles. Sheep densities shown at county (solid line and filled squares) and NNR levels (dotted
882 line and open squares). Vertical blue bars show peak sheep densities from JAC data and dotted
883 green lines show start of experiment at Hard Hill in 1954. Note differences in y-axis scales between
884 sites.

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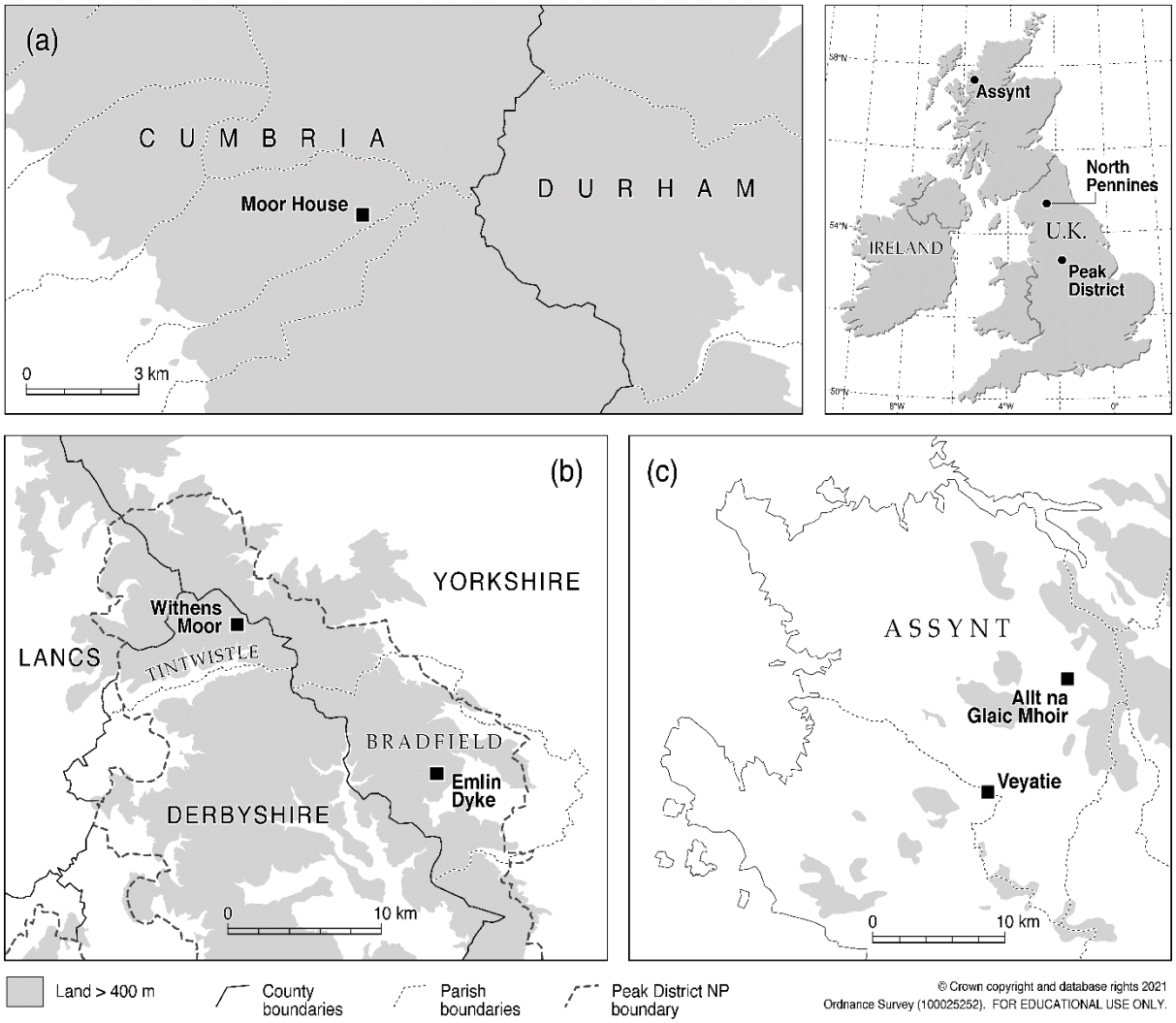

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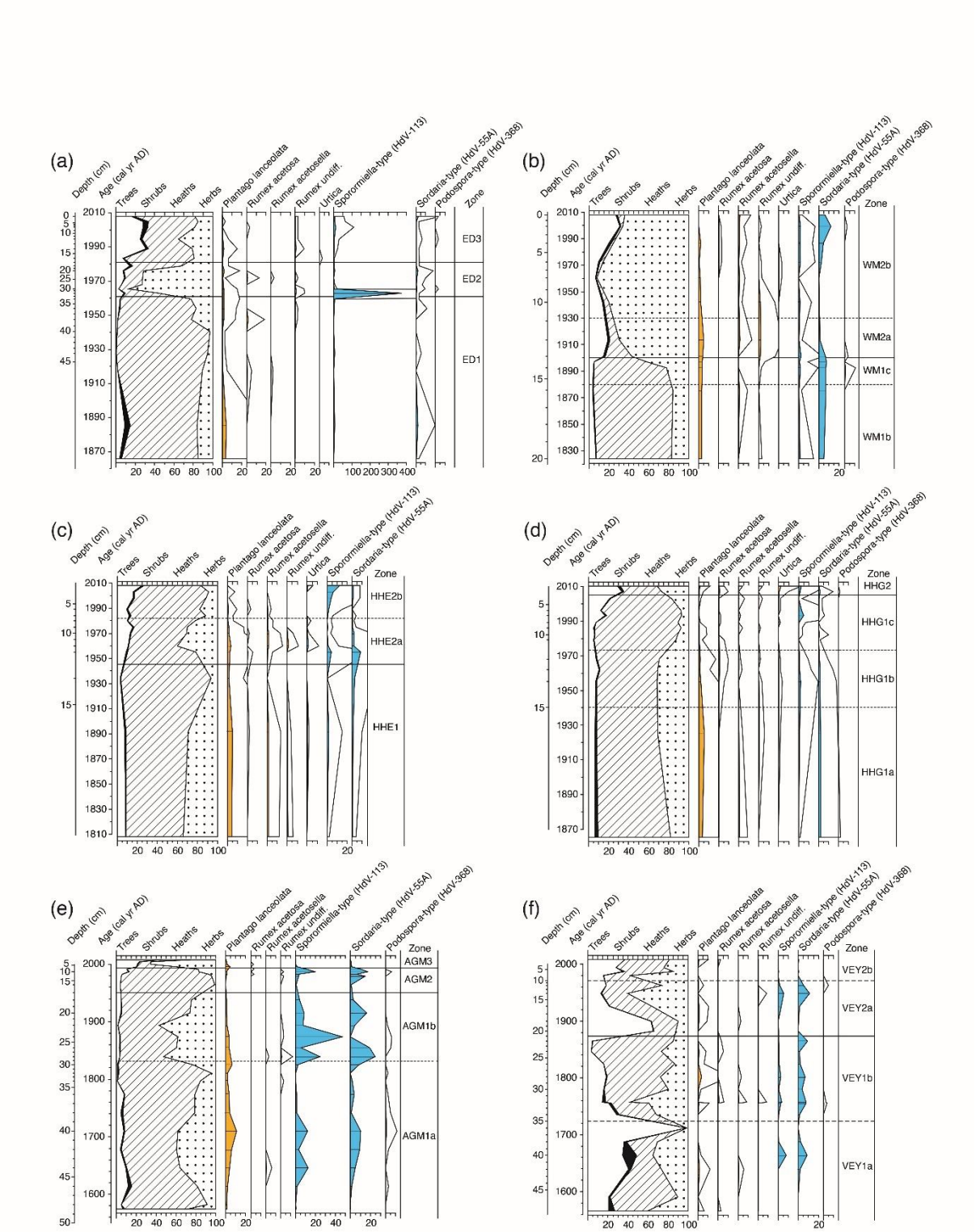

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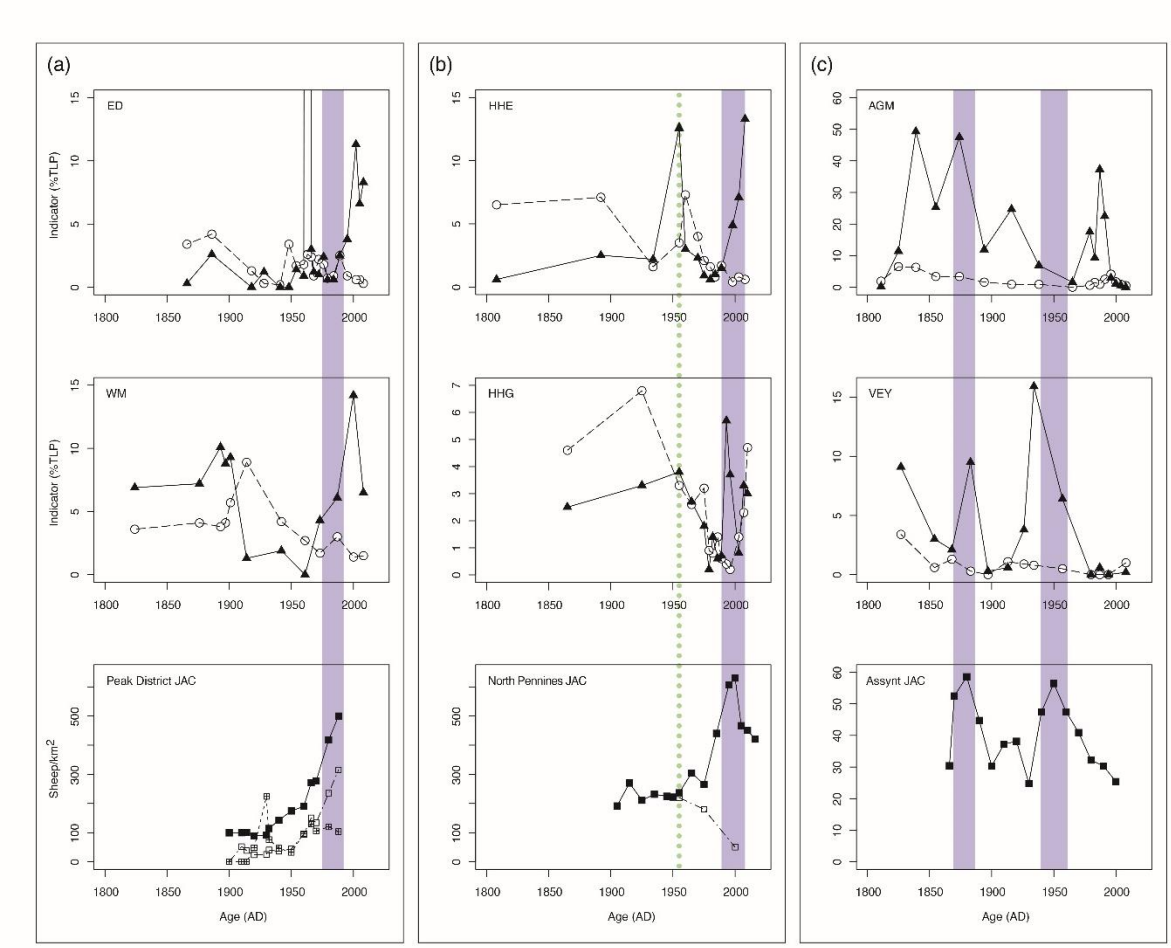

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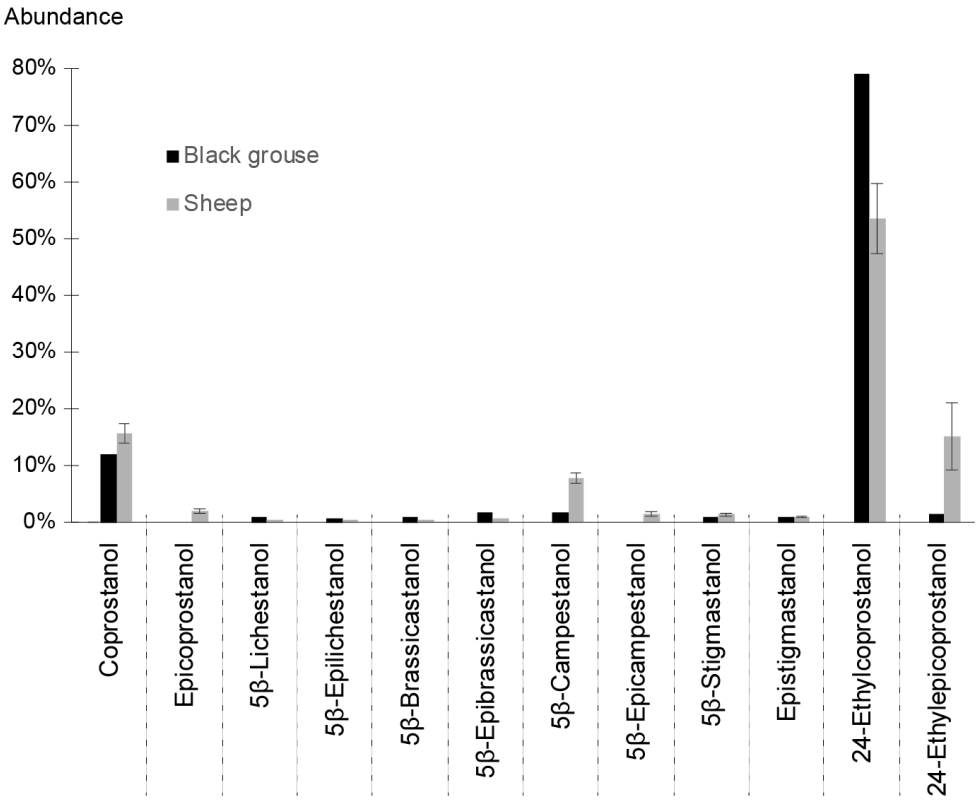

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