Relating dung fungal spore influx rates to animal density in a temperate environment: implications for palaeoecological studies

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Abstract

The management of the remainder of Europe's once extensive forests is hampered by a poor understanding of the character of the vegetation and drivers of change before the onset of clearance for farming. Pollen data indicate a closed-canopy, mixed-deciduous forest, contrasting with the assertion that large herbivores would have maintained a mosaic of open grassland, regenerating scrub and forested groves. Coprophilous fungal spores from sedimentary sequences are increasingly used as a proxy for past herbivore impact on vegetation, but the method faces methodological and taphonomical issues. Using pollen trap data from a long-running experiment in Chillingham Wild Cattle Park, UK, we investigate the first steps in the mechanisms connecting herbivore density to the incorporation of fungal spores in sediments and assess the effects of environmental variables on this relationship.

Herbivore utilization levels correlate with dung fungal spore abundance. Chillingham is densely populated by large herbivores, but dung fungal spore influx is low. Herbivores may thus be present on the landscape but go undetected. The absence of dung fungal spores is therefore less informative than their presence. Dung fungal spores likely enter the sediment record through a different pathway from wind-borne pollen and thus dung fungal abundance is better expressed as influx rates than as % total pollen.

Landscape openness, vegetation type and site wetness do not distort the impact of utilization levels on dung fungal spore representation. However, dung fungal spore influx varies markedly between seasons and years. Spores travel, leading to a background level of spore deposition across the landscape, and at times a depletion of spores, especially under wet weather conditions. Animal behaviour, as well as husbandry practices, can lead to the accumulation of dung, and thus fungal spores, in specific locations on the landscape that do not directly reflect grazing pressure.

Keywords: palaeoecology, coprophilous fungi, vegetation structure, husbandry practices, megafaunal extinction, conservation grazing

1. Introduction

The management of the remainder of Europe's once extensive forests is hampered by a poor understanding of 'virgin forest', or what comprises a 'natural' temperate environment in terms of vegetation abundance and distribution (Birks 2005; Parviainen 2005; Willis and Birks 2006; Kirby and Watkins 2015). During the earlier interglacials of the European Pleistocene and the earlier part of the Holocene, the temperate fauna included a number of important herbivores, with megaherbivores such as elephant and rhinoceros, and a diverse range of other large herbivores, such as elk and aurochs (e.g. Von Koenigswald and Heinrich 1999; Kahlke 1999; Bridgland et al. 2004; Currant and Jacobi 2010). Extant relatives of these herbivores can significantly alter vegetation communities and suppress woodland cover (Owen-Smith 1988; Weisberg and Bugmann 2003; Hester et al. 2006; Gill and Morgan 2010; Ramirez et al. 2018). It can therefore be expected that this large guild of herbivores similarly affected the European Pleistocene and earlier Holocene vegetation. For this reason, the character of the European forest before the onset of clearance, some 6000 years ago, for farming has been highly debated. Early Holocene woodland development may have been a response to the anthropogenic reduction in herbivores (e.g. Sandom et al. 2014). Pollen research and observations of modern forests (e.g. Peterken 1996; Bradshaw and Mitchell 1999; Svenning 2002; Bradshaw et al. 2003; Rackham 2003; Mitchell 2005) have been used as evidence to suggest that temperate Europe was dominated by high, closed-canopy, mixed-deciduous forest. In contrast, other researchers have proposed that as the Holocene landscape

emerged from the last glacial period, large herbivores maintained a mosaic of open grassland, regenerating scrub and forested groves, also known as 'wood-pasture' (Vera 2000; Kirby 2004, 2005; Buckland 2005; Hodder and Bullock 2005a).

Consequently, conservation grazing by large herbivores is increasingly used as a management tool in contemporary landscapes for creating and maintaining mosaics of open woodland to increase biodiversity (Humphrey et al. 1998; Hodder and Bullock 2005b) and reinstate putative natural woodland processes. However, the extent to which wood pasture, instead of closed canopy, is the relevant model for woodland conservation is still unclear. Estimating vegetation openness based on records of pollen (and other microfossils) is problematic due to complexities in pollen dispersal and taphonomic processes (Sugita et al. 1999; Svenning 2002; Smith et al. 2010; Fyfe et al. 2013). A further complicating factor in this debate is the lack of reliable estimates of past large herbivore population sizes. Skeletal records are discontinuous in space and time, and are often not found in the same deposits as plant remains used to reconstruct past vegetation patterns.

Over the past decades, counts of coprophilous fungal spores from sedimentary sequences have increasingly been used in an attempt to improve reconstructions of past herbivore presence and abundance (e.g. Davis 1987; Davis and Shafer 2006; Baker et al. 2013; Perrotti and Van Asperen 2019). Since these spores can be recovered from the same samples that are used for pollen analysis, vegetation records can be directly linked with a potential proxy for large herbivore presence. So far, this proxy has taken on an important role in studies aimed at resolving the timing and impact of the worldwide extinction of megaherbivores at the end of the last ice age (e.g. Gill et al. 2009; Feranec et al. 2011; Rule et al. 2012; Gill 2014; Johnson et al. 2016; Van der Kaars et al. 2017). It is also increasingly used to study aspects of animal husbandry, such as transhumance, overgrazing, the relative importance of small and large

livestock species and dairying (e.g. Feeser and O'Connell 2010; Dietre et al. 2017; Ghosh et al. 2017).

Coprophilous fungi are a diverse group of fungi that grow on animal dung, encompassing genera from most major taxonomic groups (Wicklow 1992; Richardson 2001; Krug et al. 2004). Some of these grow almost exclusively on dung, whilst other species also grow on a variety of other substrates. Many exclusively coprophilous species, especially those belonging to zygomycete or ascomycete genera, actively release their sporangia or individual spores using a variety of explosive mechanisms, propelling them over a short distance (typically a few cm to a few m, with most spores landing within 40 cm of the fruitbody; Ingold and Hadland 1959; Ingold 1961; Trail 2007; Yafetto et al. 2008) onto the surrounding vegetation. The spores can then be ingested by herbivores along with the vegetation, passed through the animal's digestive system and voided with the dung. It is unclear whether the passage through the animal's gut plays any role in the germination of these spores (Janczewski 1871; Massee and Salmon 1902; Krug et al. 2004), but they are rarely found to be active on other substrates (see below; Bell 2005; Doveri 2007; Kruys and Wedin 2009; Guarro et al. 2012; Newcombe et al. 2016). Due to their presence on vegetation, herbivore dung generally harbours a more diverse fungal community than carnivore dung (Lundqvist 1972; Furuya 1990). Many coprophilous fungal spores (primarily from ascomycete genera) are thick-walled, and often the walls contain pigments which protect the spore from exposure to harmful UV radiation (Lundqvist 1972). This also accounts for their long-term survival in soils (Van Asperen et al. 2016) and their consequent preservation in Quaternary sedimentary samples.

Whilst a number of studies have sought to connect modern herbivore density with coprophilous fungal abundance in soil (Blackford and Innes 2006), moss (Cugny et al. 2010;

Dietre et al. 2012) or lake sediments (Raper and Bush 2009; Ekblom and Gillson 2010; Parker and Williams 2012; Etienne et al. 2013), this has exposed a number of problems.

Firstly, there is a methodological issue. Dung fungal spore abundance is often expressed as a percentage of the total pollen sum (TP) or a subgroup of this (Davis and Shafer 2006, Ekblom and Gillson 2010, Feeser and O'Connell 2010, Gill et al. 2009, 2013; Parker and Williams 2012; Johnson et al. 2015). In all likelihood, fungal spores enter the sedimentary record through a different pathway to wind-borne pollen due to differences in dispersal and taphonomy. Furthermore, total pollen influx may vary based on the surrounding cover of wind-blown and insect-pollinated taxa leading to differences between arboreal and non-arboreal site pollen counts (Hicks 2001). These differences may lead to directional shifts in the calculation of fungal spore counts as a percentage of TP. We might expect, for example, lower fungal spore abundance in woodland than in highly grazed pasture. In addition, in wood pasture the 'glade effect' (Feeser and Dörfler 2014), where trees produce more pollen due to increased flowering with broader canopy and access to light, might conceivably reduce the apparent fungal spore quantity as a percentage of TP. Fungal spore counts may therefore not be directly comparable to pollen counts, and the interpretation of fungal spore data as a percentage of the pollen sum may lead to spurious shifts. This is particularly problematic where pollen and/or fungal spore accumulation rates are not constant through time (Hicks 2001; Baker et al. 2013; Etienne et al. 2013; Wood and Wilmshurst 2013; Perrotti and Van Asperen 2019). Since fungal spore influx rates have not been assessed widely, a complete understanding of this is lacking.

Secondly, the mechanism connecting herbivore density (in particular differential use of areas within a landscape) and the incorporation of fungal spores in sediments is poorly understood. Whilst studies of modern pollen dispersal have calibrated our interpretation of palynological records (e.g. Janssen 1967; Wright et al. 1967; Overpeck et al. 1985; Prentice 1985;

Wilmshurst and McGlone 2005; Broström et al. 2008), similar studies have so far not been carried out for dung fungal spores. Although the depositional environments studied so far approximate the types of samples used in palaeoecological studies, it is paramount to first understand how fungal spores become incorporated in these records before drawing conclusions about herbivore density from counts in palaeoecological samples (Feranec et al. 2011).

Although it seems that dung fungal spore active dispersal distances are short (Ingold and Hadland 1959; Ingold 1961; Trail 2007; Yafetto et al. 2008), it is unclear whether, and if so, how far, spores can move around the landscape through other taphonomic processes such as wind and water transport (Raper and Bush 2009; Johnson et al. 2015; Raczka et al. 2016).

Thirdly, at present, we have a limited understanding of the role of confounding environmental factors such as seasonality, vegetation cover and wetness that could bias the deposition, preservation and recovery of spores. Whilst we have limited ability to test the mechanisms by which fungal spores become part of the palaeoecological record, unravelling the complexities these confounding factors present is a good place to start.

To our knowledge, only a single study (Gill et al. 2013) used Tauber traps to study the relationships between herbivore abundance and dung fungal spore influx rates. This investigation used bison enclosures with known grazing densities to show a clear relationship between bison grazing and presence of a single fungal genus, *Sporomiella*. However, Gill et al. (2013) did not study environmental variables and these are potentially important. For example, Wood and Wilmshurst (2012) show that soil moisture has an impact on dung fungal spore presence. Testing the relationship between a range of dung fungal spores and herbivore utilization levels, in differing soil moisture conditions is, therefore, an important step. Incorporating a wider range of dung fungal taxa in the analysis better indicates herbivore presence and abundance, especially since *Sporormiella* is not always common on the dung of

extant herbivores (Johnson et al. 2015; Baker et al. 2016; Van Asperen 2017). This study aims to resolve some of these uncertainties by investigating the impact of vegetation cover, wetness and grazing density on the abundance of a range of dung fungal genera in a long-running experiment with cattle and deer in northeast England.

2. Study site and methods

2.1 Study site and field methods

To investigate influx rates of coprophilous fungal spores across a range of habitats in a mosaic of wood pasture with an overall known herbivore density but varying levels of use of different parts of the landscape, pollen (modified Tauber) traps (Tauber 1974; Hicks and Hyvärinen 1986) with a volume of 5 l were placed at 11 locations in Chillingham Wild Cattle Park, Northumberland, UK (table 1, figure 1). A feral herd of Chillingham cattle has occupied this area since at least 1646, and no human handling or veterinary intervention takes place, apart from occasional culling of aged or diseased animals (Hall 2007, 2013). At present, the herd consists of about 100 animals ranging freely over an area of 123.4 ha, equivalent to a cattle biomass of about 186 kg/ha (Bunce and Hall 2013). In winter (January to mid-April; Hall 1988) limited supplementary, locally harvested hay and compound feed is provided only if necessary, and in previous years (1980-2004), limestone has been applied to the grazing areas to prevent dietary magnesium deficiency (Hall et al. 2005). The park is also frequented by fallow and roe deer, badgers, foxes and a variety of smaller mammals. The park therefore represents an opportunity to gather data in as near-natural grazing conditions as is possible in this part of Western Europe to provide a reasonable analogue for palaeoecological studies.

Although the overall herbivore density of the park is known (0.81 cattle per ha, plus occasional deer presence), not all parts of the park are used equally intensively, and different parts of the park are used in different ways. Utilization patterns are notoriously difficult to quantify at the local scale. In a study from June 1977 to January 1981, when the cattle herd consisted of about 50 animals and the park was also still grazed by about 300 sheep, the spatial behaviour of the cattle was closely monitored (Hall 1988). The overall utilization patterns have remained the same (C. Leyland / D. Oard, pers. comm.). In summer, the cattle preferentially graze in the lower-lying grassland areas and the ash/alder woodland (Hall and Bunce 1984; Hall 1988). Since the lower-lying grassland areas tend to be quite wet, in winter the cattle move to the less nutritious but dryer upland grasslands. The shaded woodlands are used less intensively, with the exception of the coniferous woodland on the south-western boundary of the park, which is sheltered from the prevailing winds. Although there is little undergrowth and therefore not much food for the cattle, this area has some of the largest accumulations of dung in the park, since it is heavily used in adverse weather conditions.

Traps were placed within a variety of settings to encompass the mosaic of utilization intensity, habitats types and soils moisture levels. Utilization intensity was based on Hall (1988) and information from the park wardens (C. Leyland / D. Oard, pers. comm.). Each trap was assigned a value of low, medium or high utilization, and since utilization levels vary between seasons, besides a year-round value, each trap was also assigned a winter and summer utilization value.

The sampling locations represent a range of vegetation types. The vegetation classification (table 1) follows Hall and Bunce (1984), but we have split their 'dense shade' (S) habitat into two categories, coniferous and deciduous woodland (including their ash/alder (A) in the deciduous woodland category), and merged their two categories of good grassland (G) and second-rate grassland (M) into a single category with upland grassland (U). The stands of

woodland in the park are small, but pollen assemblages from these traps are dominated by local deposition (E. van Asperen, unpublished data). Soil moisture at the pollen trap locations was measured at a depth of 5 cm every 6 months (in April and October) using a MoonCity soil moisture sensor, which measures soil moisture on a scale of 1-10 (1-3 dry, 4-6 medium, 6-10 wet; table 1).

Two traps were situated in existing exclosures (approximately 15m in diameter) within the park, to which the cattle do not have access (traps CT1 and CT2). One trap was placed just outside the park (trap CT5) approximately 7m from the fence line; this area could not be accessed by the cattle but other free-roaming species did have access. The remaining traps were placed to cover areas that are intensively used by the cattle as well as areas that are used less intensively. Apart from traps CT1, CT2 and CT5, the traps were surrounded by three wooden poles with barbed wire to protect the traps from trampling (figure 2a). The poles were placed as close to the trap as possible to make sure the trap was within the dispersal distance of fungi growing on nearby dung (Ingold 1961; Trail 2007; Yafetto et al. 2008).

The traps were buried into the ground so that the collar and opening were about 5 cm above ground level (figure 2b; Hicks et al. 1996). 10 ml of glycerol and 10 ml of anti-algal barley straw extract (Blagdon) were added to the trap. A coarse mesh was placed over the opening to prevent small animals from falling into the trap (Hicks et al. 1999). The first traps were placed in October 2014. Traps were collected and replaced twice a year (in April and October) to assess seasonal variation in spore influx. Here we present data for the first two years of sampling (October 2014 – September 2016). Analysis of samples for later years is ongoing.

Weather data for the sampling period were obtained from the Met Office Integrated Data Archive (MIDAS) Surface Weather Stations network, provided by the British Atmospheric Data Centre (BADC; http://badc.nerc.ac.uk, last accessed 16 May 2017), from the weather

station at Chillingham Barns (Lat: 55.530N, Lon: 1.917W, alt: 70 m asl). Mean monthly temperature and monthly total precipitation were calculated from hourly values (figures 3a and 3b).

2.2. Laboratory methods

Upon collection from the field, traps were kept in cold storage and processed as soon as possible. Trap volume was measured, and based on a visual inspection of the amount of sediment present in the trap, 2-6 *Lycopodium* tablets dissolved in 10 ml 10% HCl were added to the trap contents to enable quantification of influx rates (Hicks et al. 1999). Items larger than 125 µm were removed by sieving. The remaining trap contents were centrifuged to concentrate the samples. To maximize fungal spore recovery (Van Asperen et al. 2016), treatment with highly corrosive or acidic chemicals was avoided. The samples were first heated in a 10% KOH solution. Heavy particles were removed by swirling, whilst particles <6 µm were removed with a fine mesh sieve. The samples were then treated with 10% HCl, stained with Safranine and mounted in silicon oil using tertiary butyl alcohol. Pollen and fungal spores were counted at 400x magnification until 350 *Lycopodium* spores had been counted (Etienne and Jouffroy-Bapicot 2014).

Based on the known number of *Lycopodium* spores added, the spore influx rate per cm^2 per 6 months was calculated for the coprophilous genera *Podospora*, *Sordaria* and *Sporormiella*, as well as an overall coprophilous spore influx rate (Baker et al. 2013; Perrotti and Van Asperen 2019), using the formula: ((n *Lycopodium* added / n *Lycopodium* counted) * n fungal spores counted) / 19.6 (the surface area of the trap opening in cm^2) (Hicks et al. 1999). A small number of other coprophilous genera (*Arnium*, *Delitschia*, *Trichodelitschia*; Bell 2005: 46, 51; Doveri 2007: 872; Guarro et al. 2012: 59, 159) were encountered very rarely, and where

present, these were included in the total coprophilous spore influx rate. The presence of other spore types of genera that contain a mixture of coprophilous and non-coprophilous species (*Apiosordaria*, *Cercophora*, *Coniochaeta*; Krug et al. 2004; Bell 2005: 39; Doveri 2007: 810; Guarro et al. 2012: 47–51, 132–142) was noted, but these genera were not included in the total coprophilous spore influx rate.

2.3. Fungal spore taxonomy, morphology and ecology

The genera *Podospora, Sordaria* and *Sporormiella* are generally regarded as being among the strongest indicators of dung in palaeoecological studies (e.g. Baker et al. 2013; Perrotti & Van Asperen 2019). These genera have highly recognisable pigmented spores which survive well in soil and pollen preparations (modified method of Faegri and Iversen 1989; Moore et al. 1991; see Van Asperen et al. 2016; figure 4). Species in the genus *Podospora* are mostly coprophilous (Bell 2005: 14; Doveri 2007: 905; Guarro et al. 202: 340; Schlütz & Shumilovskikh 2017). *Podospora* species are not commonly isolated from other substrates, whereas some species of the closely similar genus *Cercophora* are (Bell 2005: 40; Doveri 2007: 847; Guarro et al. 2012: 111). The pigmented cells of the spores of the latter genus tend to be relatively small (<25 × 15 μm) compared with the mostly larger pigmented cells of *Podospora* spores (Bell 2005; Doveri 2007). In addition, *Cercophora* spores tend to remain hyaline until after maturation and discharge (Lundqvist 1972) and such thin-walled, unpigmented spores are less likely to survive in soil and pollen preparations (Van Asperen et al. 2016). *Sordaria* is almost exclusively coprophilous (Bell 2005: 36; Doveri 2007: 826), although some species are frequently isolated from soil (Guarro et al. 2012), and there are some indications the genus can also reproduce on certain plants (Newcombe et al. 2016). However, such occurrences are sufficiently rare that it can be assumed that most *Sordaria*

spores isolated from soil result from growth on dung. The spores of *Sporormiella* are indistinguishable from those of the genus *Preussia* (Kruys and Wedin 2009), leading some authors to include *Sporormiella* in *Preussia* as a later synonym (e.g. Kruys and Wedin 2009; Guarro et al. 2012). Since non-pollen palynomorph (NPP) analysts have so far used the generic name *Sporormiella* for 4- to many-celled spores with germ slits, this name is used here. Most species of *Sporormiella* and *Preussia* grow on dung, but plant debris, wood or soil also serve as substrates (Doveri 2007: 613; Kruys and Wedin 2009; Guarro et al. 2012), so the use of these spores as obligate indicators of past herbivore abundance must be approached with caution.

2.4. Statistical methods

To test the validity of displaying and analysing abundance of fungal spores as a percentage of total pollen, correlations between pollen and dung fungal spore counts and influx rates were tested using Pearson's product-moment correlation. Each data set was examined by sampling season and year, and for all samples together. Given the likely fluctuations in total pollen, dung fungal spores are also correlated here with Poaceae pollen values. Poaceae is used as a reference taxon in relative pollen abundance quantification as it has a reasonably stable linear relationship with plant abundance (Broström et al. 2004). Finally, the correlation between dung fungal spore influx rates and counts expressed as %TP is tested to examine whether an increase in dung fungal spores as %TP reflects an increase in influx of these spores.

To test whether environmental factors impact on dung fungal spore influx rates, correlations between dung fungal spore influx and vegetation cover, vegetation type, soil moisture and utilization level (see table 1) were each tested separately with Spearman's rank correlation coefficient. Differences in dung fungal spore influx in pollen traps between different habitats

and utilization levels were tested with the Mann-Whitney U test for environmental variables with two categories and the Kruskal-Wallis test for environmental variables with three categories. Correlations and tests for difference were first carried out by individual sampling season, then for both winter seasons together and both summer seasons together, for each sampling year (October-September) and for all samples together. All statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS) version 24. Results for the tests were considered significant if $p \leq 0.05$.

3. Results

3.1. Correlation between fungal spore and pollen influx

When all samples are taken together, there is no correlation between total pollen and total dung fungal spore influx (Supplementary Information). However, this correlation is significant for the summer 2015 traps (p=0.034), the winter 2015-2016 traps (p=0.010), and all winter samples together (p=0.037), due primarily to a correlation between pollen and *Podospora* influx rates (p=0.056 for summer 2015, p=0.021 for winter 2015-2016, and p=0.026 for all winter samples). Poaceae influx is related to total dung fungal spore (p=0.006), *Podospora* (p=0.002) and *Sordaria* (p=0.044) influx for all winter samples together, while the correlation with *Sporormiella* influx is also close to significance (p=0.076).

Dung fungal spore influx rates are strongly positively correlated with dung fungal spore counts expressed as %TP (table 2) for all samples taken together (p<0.001; Supplementary Information) and for the year from October 2014 to September 2015 (p<0.001). In contrast, for the year from October 2015 to September 2016, the correlation is not significant.

3.2 Fungal spore influx variation between seasons and sites

Apart from the 2014-2015 winter traps, most traps contained very low numbers of dung fungal spores, ranging from 0 to 85 spores per cm^2 in the summer 2015, winter 2015-2016 and summer 2016 traps, and between 33 and 423 spores per cm^2 for the winter 2014-2015 traps (table 3). In the winter of 2015-2016, 5 out of 11 traps were lost; three washed out of position due to high groundwater levels in combination with sheet flow due to high rainfall, and two were dug out by a badger. The limited data from the smaller number of surviving traps is more difficult to interpret. With only two traps lost in summer 2015 and one in summer 2016 (both washed out during wet weather) the data are more secure for these sampling periods. In all four sampling seasons, traps in the areas that are not accessible to the cattle (exclosures CT1 and CT2, location outside the park CT5) have similar influx rates of dung fungal spores to traps in areas of low grazing intensity.

Influx rates are quantified using counts of *Lycopodium* marker grains, and this process masks the fact that although for each sample, at least 350 (and up to 1000) *Lycopodium* grains were counted, alongside at least 100 (and up to 2380) pollen grains, in absolute numbers for most traps fewer than 10 dung fungal spores were counted, except for the five richest traps (all from winter 2014-2015) for which 29 to 54 dung fungal spores were counted. This also means that in none of the summer 2015, winter 2015-2016 and summer 2016 traps, dung fungal spore counts expressed as a percentage of TP is above the 2% value that is often quoted as representing 'background deposition' and functions as the cut-off for meaningful interpretation (table 2; Davis 1987; Gill et al. 2013; Baker et al. 2016; Raczka et al. 2016). In contrast, in nearly all winter 2014-2015 traps dung fungal spore frequencies make up over 2% of TP.

Podospora and *Sordaria* were the most commonly encountered types of dung fungal spores (figure 4). *Podospora* reaches maximum influx rates between 150 and 250 spores per cm^2 per 6 months in a few samples from winter 2014-15, whilst *Sordaria* reaches 185 spores per cm^2 per 6 months in some samples from winter 2014-15. *Sporormiella* is more rarely encountered, with a maximum influx rate of 76 spores per cm^2 per 6 months.

3.3 Vegetation cover

Vegetation cover (open vs. closed, p=0.009) and vegetation type (grassland, deciduous woodland and coniferous woodland, p=0.005) are significantly positively correlated with *Sordaria* influx for all samples analysed together (figure 5a; Supplementary Information). *Sordaria* influx rates are significantly different between the three vegetation types (p=0.024). This implies that this genus is more common in samples from woodland, especially coniferous woodland.

3.4 Site wetness

Summer wetness impacts on *Sordaria* representation negatively (figure 5b; Supplementary Information, p=0.022), but although there is a significant difference (p=0.016) between dry, medium and wet sites, this is mainly because the medium wet sites are characterised by lower *Sordaria* influx, whereas both dry and wet sites have higher *Sordaria* influx. Overall, site wetness does not seem to have a major impact on dung fungal spore presence in the pollen traps.

3.5 Utilization level

Surprisingly, year-round utilization level (low, medium and high) does not correlate significantly with dung fungal spore influx rates (Supplementary Information; tables 3 and 4; figures 5c and 6). However, winter utilization level does correlate significantly positively with total dung fungal spore ($p=0.029$) and *Sordaria* ($p=0.021$) influx, and it is also marginally significantly correlated with *Podospora* influx ($p=0.087$). This is also borne out by the Mann-Whitney U test, which indicates a significant difference in total dung fungal spore ($p=0.032$) and *Sordaria* ($p=0.024$) influx rates between sites with low and high winter utilization levels, and once again a marginally significant difference in *Podospora* influx ($p=0.087$). *Sordaria* influx rates are also different between sites with low and high year-round utilization levels ($p=0.033$).

4. Discussion

4.1 Fungal spore production, deposition and preservation

The dung fungal spore counts from the Chillingham pollen traps highlight some significant issues. Apart from a few exceptions, fungal spore numbers from most traps are very low (table 2), meaning that our interpretations are based on very limited increases and decreases of spore counts. There are also marked differences in fungal spore influx from season to season and year to year. The evidence from Chillingham shows that the dominant fungal spore types can vary between landscapes, and reveals a complex relationship between fungal spore percentages and pollen percentages.

Firstly, the low numbers of fungal spores found are not unusual; the fungal spore counts are in the same range as reported elsewhere. Many papers (37 out of 47 according to Baker et al. 2013) use the relative measure of %TP because inaccurate dating of sediment precludes the calculation of influx rates. In these studies, dung fungal spore percentages above 4% are exceptional, which, with a generally used total pollen sum of 250, translates into a spore count of 10 or less. Davis and Schafer (2006) highlight percentages of *Sporomiella* spores up to 29% in areas where animals have been corralled but below 4% in extensively grazed meadows. They also cite studies with an absence of *Sporomiella* in grazed meadows and attribute this to climatic factors, vegetation cover and soil conditions. These previous studies seem in reasonable agreement with the data from Chillingham, regardless of the fact that they report findings from different sample types. Trap CT4 has comparatively high values because it is situated in a dung-rich sheltering spot which might equate to the corralling sites mentioned by Davis and Shafer (2006). The fungal spore presence in other samples and seasons is very limited, rarely reaching the 2% threshold used by other studies to infer herbivore presence (Feranec et al. 2011; Gill et al. 2013) and in agreement with Davis and Schafer (2006) that dung fungal spores may be a poor detector of low utilization levels in meadow habitats.

In contrast to the 2014-2015 samples, the samples from the second winter season (2015-2016) were poor in dung fungal spores. Unfortunately the evidence from this sampling period is sparse due to the loss of five traps to the very wet conditions. However, the explanation for the low number of spores may lie precisely in the wet weather (figures 3 and 6). Dung fungal growth is suppressed when the substrate is too wet (Kuthubutheen and Webster 1986; see also Wood and Wilmshurst 2012 for a discussion of the impact of soil hydrology on dung fungal spore representation). Furthermore, pollen influx rates were also very low for this period (average 839 pollen grains per cm^2 per 6 months compared to 5,538 for winter 2014-

2015, and 15,857 and 23,286 for summer 2015 and 2016, respectively). This may indicate that the very high level of rainfall in November 2015 – January 2016 either prevented dispersal from taking place as usual, or may have increased runoff and transport of pollen and spores downhill, although at present it is unknown whether spores are easily transported by water flowing downhill through vegetation.

Secondly, in the Chillingham samples, *Podospora* is by far the most commonly encountered dung fungal spore in the pollen trap samples (~60% of all dung fungal spores; table 3). Although *Sporormiella* is the dung fungal genus most commonly used in palaeoecological studies, this genus was rare in the Chillingham pollen traps. Since the dung fungal spores in the pollen traps are hypothesised to originate from the dung of herbivores in the area, it is instructive to compare the range of dung fungal types encountered in the pollen traps and the diversity of dung fungi growing on the dung itself to check the representativeness and reliability of the dung fungal spore record in the pollen traps. The most abundant genera on cattle dung from Chillingham incubated in the laboratory are *Pilobolus* and *Cheilymenia* spp. (Van Asperen 2017). *Podospora* spp. is common on cattle dung and abundant on deer dung from Chillingham, whilst *Sordaria* and *Sporormiella* are only encountered occasionally on cattle dung.

The low numbers of dung fungal spores recovered from the pollen traps can thus be explained by the fact that the most common dung fungi on the cattle dung, *Pilobolus* and *Cheilymenia*, produce spores that are very small (*Pilobolus*) and therefore likely lost in pollen preparations, or thin-walled and hyaline (both genera) and therefore do not preserve well and are strongly adversely affected by chemical preparation methods (Van Asperen et al. 2016). The relative abundance of *Podospora* spores in the dung fungal spore assemblage may reflect the higher abundance of the genus on deer dung as well as common presence on cattle dung

from Chillingham, whilst the relative lack of *Sporormiella* spores corresponds to a sparse occurrence of the genus on the dung itself (Van Asperen 2017).

Although most coprophilous fungi can grow on a wide range of dung types (Richardson 1972, 2001), some genera are more abundant on certain dung types (Van Asperen 2017). This is especially important where it concerns ruminants such as cattle and deer, whose dung is dominated by fungal species with hyaline, thin-walled spores that do not survive well in palaeoecological samples (Lundqvist 1972; Richardson 1972; Bell 2005; Van Asperen 2017). In this case, the deer, although they are less abundant, contribute more spores to the dung fungal signal than the more abundant herbivore (in this case the cattle), because the deer dung also produced significant numbers of fungal fruitbodies with thick-walled spores (Van Asperen 2017), whilst such species were rare on the cattle dung.

Thirdly, the lack of correlation between total pollen influx and the total dung fungal spore influx shows that dung fungal spores enter the sediment record through a different pathway than wind-borne pollen, which likely reflect a much larger, potentially distant source area than the more local dung fungal signal. The fact that dung fungal spore influx correlates well with dung fungal counts expressed as %TP shows that in this case, this potential discrepancy in source area size has a limited effect. However, this relationship breaks down for the year from October 2015 to September 2016, due to pollen influx being extremely low during the winter of 2015-2016, probably due to the wet weather, leading to higher dung fungi expressed as %TP, even though dung fungal influx rates were low too. %TP should therefore be used with caution as a measure for dung fungal abundance, and where possible, influx rates provide a more accurate measure (Etienne et al. 2013; Wood and Wilmshurst 2013; Johnson et al. 2015; Perrotti and Van Asperen 2019).

Overall, the low influx of dung fungal spores in the traps show that a landscape can be densely populated by large herbivores that do not always leave a dung fungal spore signal

that is strong enough to distinguish it from background deposition rates. The absence of dung fungal spores in palaeoecological samples may therefore be less informative than their presence (Raper and Bush 2009; Jones et al. 2017; Perrotti and Van Asperen 2019). Although in some cases *Sporormiella* spore abundance alone may indicate herbivore presence and/or abundance, the low levels of *Sporormiella* spore recovery, from both the Chillingham pollen traps and the dung, underline the importance of analysing all dung fungal spore taxa encountered in a palaeoecological sample, rather than limiting the analysis to a single spore type (Johnson et al. 2015; Baker et al. 2016; Perrotti and Van Asperen 2019).

4.2 Temporal and spatial variability in fungal spore recovery

Spore recovery over time and across the landscape also shows some interesting patterns. Firstly, traps in areas that are not accessible to the cattle have similar influx rates of dung fungal spores to traps in areas of low utilization level. For the trap located outside the park (CT5) this is not surprising, since this site is accessible to deer and other animals. The fact that the traps placed in exclosures (CT1 and CT2) also contained dung fungal spores could indicate that the spores travel further across the landscape than previously thought (e.g. Graf and Chmura 2006). At present it is unclear how far fungal spores can travel. Active dispersal distances are not likely to exceed 1m (Ingold and Hadland 1959; Ingold 1961; Trail 2007; Yafetto et al. 2008), but more research into passive dispersal, e.g. by water or wind transport, is needed. The two traps in exclosures are both located at relatively high elevation. This makes it unlikely, especially for CT1, that water transport plays a major role. For CT2, water transport could have some role, since this trap is located in a waterlogged part of the park. At times of high rainfall, sheet flow or even flooding could transport spores into this area. Given that some of the traps contained much more water than would be expected from rainfall

levels (though never more than the 5 l volume of the traps; table 5), this seems a likely mechanism.

In a densely occupied area such as Chillingham Park, movement of spores around the landscape appears to result in a homogeneous, though low, background level of spore deposition, with influx rates generally <100 spores per cm^2 per 6 months and a %TP <2%. Such low levels of spore deposition cannot be reliably distinguished from background deposition from other sources than large herbivores. Smaller herbivores, such as lagomorphs and rodents, as well as herbivorous birds, could contribute to the background signal, but unless their densities are high, which is not the case at Chillingham, their dung is unlikely to be a significant source of dung fungal spores (Baker et al. 2016). An alternative explanation could be that these spores are present because they use a wider range of substrates than exclusively dung, but previous research shows that this is unlikely (see summary in Perrotti and Van Asperen 2019; Bell 2005; Doveri 2007; Kruys and Wedin 2009; Guarro et al. 2012; Newcombe et al. 2016).

Secondly, although correlations between utilization levels and dung fungal spore influx are relatively weak, a number of locations that are used relatively intensively are characterised by larger numbers of spores. This concerns mainly locations where the cattle find shelter from adverse weather conditions (CT4 and to a lesser extent CT10), areas with heavy summer grazing (CT3 and CT9), as well as an area that is used intensively by a small number of individuals (CT11). The fact that two of these sites are in coniferous (CT4) and deciduous woodland (CT10) likely partially explain the correlation between *Sordaria* influx and vegetation type. Both sites are also relatively dry, contributing to the negative correlation between *Sordaria* and wetness.

Dung does not necessarily concentrate in areas where animals are grazing, and can accumulate in locations where the animals are resting, chewing the cud, watering or

sheltering. Furthermore, some herbivores defecate in latrines, leading to an accumulation of dung in one location (e.g. domestic horses; Ödberg and Francis-Smith 1976; and rhinoceroses; Groves 1972; Groves and Leslie 2011; Basumatary et al. 2017). Some species do not use latrines but avoid grazing near dung (e.g. cattle and sheep; Forbes and Hodgson 1985; Lütge et al. 1995), whilst other species do defecate in grazing areas (e.g. wild horses; Lamoot et al. 2004). It is therefore important to consider animal behaviour when interpreting dung fungal records. In archaeological contexts, husbandry techniques (e.g. penning of animals at night time or during some parts of the year, use of watering locations; see Davis and Shafer 2006; Kammerling et al. 2017) can also lead to spatial patterning of utilization intensity.

The correlations between winter utilization level and dung fungal spore influx also partially explain the correlations between dung fungal spore influx and Poaceae pollen influx in the winter samples, since the cattle heavily graze the grassland sites that are characterised by high Poaceae representation. Low intensity grazing has been shown to increase Poaceae pollen production (Groenman-van Waateringe 1993; Innes and Blackford 2003; Fyfe et al. 2008; Davies 2016), whilst high intensity grazing suppresses it. The correlation between winter utilization and Poaceae pollen may thus be due to the lower utilization levels of especially the upland areas in summer, allowing the grasses to flower. Winter grazing tends to have little effect on Poaceae pollen production (Groenman-van Waateringe 1993: 160). Thus, although grazing utilization is high in winter, a larger pollen load in the area is reflected in the pollen traps. Since Poaceae on average account for 24.1% of the total pollen sum, this also impacts on the somewhat weaker correlation between dung fungal spore influx and total pollen influx.

Thirdly, the 2014-2015 winter samples were rich in dung fungal spores, whereas the summer samples contained few spores. Although the evidence is limited and needs further

corroboration, dung fungal diversity in temperate latitudes is known to be higher in winter than in summer (Wicklow 1992; Richardson 2001; Krug et al. 2004; Van Asperen 2017). This is due to an interaction between the effects of temperature and moisture availability on dung fungal growth on the one hand, and the higher activity levels of other dung-inhabiting species, such as dung beetles and fly larvae, in summer on the other hand (Perrotti and Van Asperen 2019, and references therein). Such differential growth patterns become important where animals migrate seasonally or transhumance is practiced. The implications are that the presence of large numbers of herbivores may go more or less undetected if they are only present on the landscape during the season in which dung fungal growth is suppressed.

4.3 Implications for palaeoecology

The results from this study raise some important considerations for assessing herbivore presence in palaeoecological landscapes. It is clear that a wider range of fungal types are informative when assessing past herbivore presence, rather than relying on one indicator taxon such as *Sporomiella* (Perrotti and Van Asperen 2019). However, even with a range of fungal spores the data presented here suggest that some levels of grazing in wetter meadows might not be detectable or may require painstaking searches for sparse fungal spores in quantities insufficient to draw robust conclusions from. Equally, although high levels of fungal spores within woodlands might indicate the presence of sheltering animals, these may not indicate grazing at that particular location. Dung fungal spores found beside stratigraphic deposits from places such as watering holes might reflect a small number of herbivores in the wider environment, condensed into an area at particular times, and therefore a widespread impact of herbivores or a mosaic of open landscape areas proposed by Vera (2000) may not be inferred directly. To be utilised in the openness debate dung fungal spores may have to be

found in density, or at least consistently, at several sites throughout the landscape before large numbers of herbivores can be inferred. This indicates an important role for the continued study of networks of local-scale palaeoecological sites (*sensu* Jacobson and Bradshaw 1981). It is often a goal of palaeoecological studies to sample at fine temporal resolution, attempting annual sampling in suitable varved deposits. The temporal shifts in fungal spore deposition observed across the two years of this study so far sampled show that fine temporal scales might be more difficult to interpret and could lead to spurious results, as year to year variations may be of a greater amplitude than site variations. Averages over a few years of deposition might be more valuable, however, this requires further testing with expanded long-term datasets.

Conclusion

Our study shows that there is some correlation between herbivore utilization levels of different parts of the landscape and dung fungal spore abundance in pollen traps. However, herbivores may be present on the landscape at high utilization levels and yet go potentially undetected. The presence of dung fungal spores is therefore more significant than their absence, and drops in dung fungal spore levels should be interpreted with caution.

Results from pollen traps may not be directly transferable to the interpretations of palaeoecological samples. Pollen traps represent a very short sampling period, whereas palaeoecological samples typically represent longer time periods, which may lead to certain pollen types, and potentially also NPP types, to be under- or overrepresented (Pardoe et al. 2010).

Studies of dung fungal spores are often based on extremely low spore counts. Any conclusions drawn from such small datasets should be treated with caution. It may be

advisable to count a minimum of e.g. 100 spores (Blackford and Innes 2006; Dietre et al. 2012), but this must balance what is practically achievable against what added information can be gained through this extra time investment, and may not be practically achievable for all sample types. Furthermore, some dung types can be dominated by dung fungi that leave no trace in the palaeoecological record, with the types that do preserve remaining below detection levels. For these reasons, as large a range of spore types possible should be used. Furthermore, because dung fungal spores follow different taphonomic pathways from pollen, interpretations of dung fungal spore counts expressed as %TP must be accompanied by an assessment of confounding factors related to fluctuations in pollen influx. Where possible, influx rates rather than %TP should be used to provide a more accurate measure of dung fungal spore abundance.

Landscape openness, vegetation type and site wetness do not distort the impact of utilization levels on dung fungal spore representation. However, spores travel, leading to a background level of spore deposition across the landscape, and at times a depletion of spores, especially under wet weather conditions. So far, very little is known about the processes that move spores around the landscape, and more research into passive dispersal, e.g. by water or wind transport, is needed. Furthermore, there seems to be a seasonal signal related to both higher fungal activity in winter and higher levels of competition with other dung-inhabiting organisms in summer, which can be an important factor when seasonal migration or transhumance takes place.

Finally, animal behaviour, as well as husbandry practices, can lead to the accumulation of dung in specific locations on the landscape that do not necessarily reflect grazing in that location. Such complexities need to be considered when interpreting dung fungal spore signals, but can also add richness to our understanding of landscape utilization.

In short, dung fungal spores are a promising proxy, but more research is needed into the taphonomic pathways by which these spores become incorporated into palaeoecological records. Pollen traps only document the first step in this process. As a next step, pollen trap influx rates must be compared with moss samples, which are more similar to palaeoecological samples as they represent averaging out of the pollen and NPP assemblage over a longer period of time (Pardoe et al. 2010). The extent to which spores are preserved in soil, as well as experimental studies of spore movement across the landscape (see also Raper and Bush 2009; Johnson et al. 2015; Raczka et al. 2016), should further elucidate the taphonomy these spores. Analyses of moss and soil samples, as well as coring of a small forest hollow, are ongoing at Chillingham. Furthermore, it is crucial to repeat such studies in different landscapes and climatic conditions to ascertain whether these processes vary between different environmental conditions. Finally, it is advisable to use the dung fungal spore method in conjunction with other proxies for dung or grazing animals, such as dung spherulites, faecal biomarkers, or environmental DNA of dung fungi (Lydolph et al. 2005; Shahack-Gross 2011; Lancelotti and Madella 2012; Prost et al. 2017). Such methods will be particularly useful in archaeological excavations, where the contexts of the samples provide further corroboration of the results, or in continuous records, where fluctuations in the different proxies can be compared.

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Table 1. Locations of pollen traps in Chillingham Wild Cattle Park

Trap no.	Latitude/ Longitude	Elevation (m OSL)	Vegetation cover[1]	Wetness[2]	Utilization level
CT1	55.5308N/ 1.8891W	130	Closed – deciduous woodland (*Acer, Betula, Fraxinus, Quercus*)[S]	Wet [6.3]	Low – exclosure
CT2	55.5255N/ 1.8889W	111	Closed – deciduous woodland (*Alnus, Fraxinus*)[A]	Wet [7.3]	Low – exclosure
CT3	55.5253N/ 1.8892W	109	Closed – deciduous woodland (*Alnus, Fraxinus*)[A]	Wet [6.0]	Medium (high in summer, low in winter)
CT4	55.5225N/ 1.8920W	110	Closed – coniferous woodland [S]	Dry [3.4]	High – shelter area in adverse weather
CT5	55.5223N/ 1.8899W	114	Closed – coniferous woodland [S]	Dry [3.4]	Low – outside park boundaries
CT6	55.5245N/ 1.8942W	95	Open – grassland [M]	Medium [5.5]	Medium (high in summer, low in winter)
CT7	55.5240N/ 1.8943W	98	Open – grassland [M]	Medium [5.6]	Medium (high in summer, low in winter)
CT8	55.5249N/ 1.8942W	95	Open – grassland [G]	Wet [6.6]	Medium (high in summer, low in winter)
CT9	55.5261N/ 1.8931W	105	Open – grassland [G]	Medium [5.3]	Medium (high in summer, low in winter)
CT10	55.5265N/ 1.8925W	106	Closed – deciduous woodland (*Acer, Fagus, Quercus*) [S]	Dry [2.9]	Low (medium in summer, low in winter)
CT11	55.5300N/ 1.8794W	156	Open – upland grassland [U]	Medium [4.6]	Medium (low in summer, high in winter)

[1] in []: vegetation classification according to Hall & Bunce 1984: A = ash/alder; G = good grassland; M = second-rate grassland; S = dense shade; U = upland grassland

[2] in []:soil moisture sensor average over the sampling period; 1-3 dry, 4-6 medium, 6-10 wet

Table 2. Dung fungal spores counts expressed as %TP

	Winter 2014-2015		Summer 2015		Winter 2015-2016		Summer 2016	
	Count	%TP	Count	%TP	Count	%TP	Count	%TP
CT1	5	4.2	1	0.1	3	1.7	0	0.0
CT2	54	4.4	4	0.3	5	1.5	3	0.3
CT3	39	17.3						
CT4	34	1.5	5	0.3			6	0.6
CT5	8	0.5	0	0.0			2	0.1
CT6	5	4.3	0	0.0	0	0.0	0	0.0
CT7	3	1.5	1	0.1			3	0.2
CT8	7	4.9			0	0.0	0	0.0
CT9	29	8.1	2	0.2			0	0.0
CT10	4	1.6	5	0.3	5	1.9	1	0.0
CT11	33	6.1	5	0.5	4	2.1	1	0.2

Table 3. Pollen trap influx rates per cm^2 per 6 months of dung fungal spores, total pollen and Poaceae pollen

Winter 2014-2015							
Trap no.	Total dung fungi	*Podospora*	*Sordaria*	*Sporormiella*	Other dung fungi	Total pollen	Poaceae pollen
CT1	54.18	43.34	10.84	0.00	0.00	1278.67	238.40
CT2	292.58	146.29	70.43	70.43	5.42	8089.19	861.47
CT3	422.61	184.21	184.21	54.18	0.00	16113.36	368.43
CT4	368.43	227.56	108.36	32.51	0.00	2676.53	3142.48
CT5	86.69	43.34	21.67	21.67	0.00	2438.13	1126.96
CT6	54.18	43.34	0.00	0.00	10.84	15896.63	335.92
CT7	32.51	21.67	0.00	10.84	0.00	1246.16	281.74
CT8	75.85	65.02	10.84	0.00	0.00	2232.25	227.56
CT9	314.25	184.21	43.34	75.85	10.84	1549.57	823.55
CT10	43.34	21.67	21.67	0.00	0.00	3727.64	260.07
CT11	357.59	249.23	54.18	43.34	10.84	5667.31	996.93
Summer 2015							
Trap no.	Total dung fungi	*Podospora*	*Sordaria*	*Sporormiella*	Other dung fungi	Total pollen	Poaceae pollen
CT1	10.84	0.00	10.84	0.00	0.00	9882.57	1625.42
CT2	43.34	32.51	10.84	0.00	0.00	13826.93	563.48
CT4	58.53	35.12	23.41	0.00	0.00	23192.99	7128.78
CT5	0.00	0.00	0.00	0.00	0.00	23235.85	3640.95
CT6	0.00	0.00	0.00	0.00	0.00	18833.23	4562.02
CT7	10.84	0.00	0.00	10.84	0.00	13025.05	8051.26
CT9	21.67	10.84	0.00	10.84	0.00	17170.09	5201.35
CT10	70.50	42.30	28.20	0.00	0.00	10868.66	5005.17
CT11	79.34	47.61	31.74	0.00	0.00	12678.30	9330.88
Winter 2015-2016							
Trap no.	Total dung fungi	*Podospora*	*Sordaria*	*Sporormiella*	Other dung fungi	Total pollen	Poaceae pollen
CT1	11.38	3.79	7.59	0.00	0.00	656.13	117.57
CT2	18.96	7.59	7.59	3.79	0.00	1251.58	261.69
CT6	0.00	0.00	0.00	0.00	0.00	587.86	53.10
CT8	0.00	0.00	0.00	0.00	0.00	341.34	45.51
CT10	27.09	21.67	5.42	0.00	0.00	1462.88	189.63
CT11	15.17	0.00	7.59	7.59	0.00	735.77	75.85
Summer 2016							
Trap no.	Total dung fungi	*Podospora*	*Sordaria*	*Sporormiella*	Other dung fungi	Total pollen	Poaceae pollen
CT1	0.00	0.00	0.00	0.00	0.00	10801.87	3499.87
CT2	66.49	66.49	0.00	0.00	0.00	21832.17	4809.73
CT4	84.54	42.27	42.27	0.00	0.00	33850.73	4818.91
CT5	29.89	0.00	29.89	0.00	0.00	14273.55	6515.73
CT6	0.00	0.00	0.00	0.00	0.00	13711.87	7612.42

CT7	59.18	59.18	0.00	0.00	0.00	15690.74	10632.60
CT8	0.00	0.00	0.00	0.00	0.00	41090.36	7716.55
CT9	0.00	0.00	0.00	0.00	0.00	19761.14	7782.03
CT10	19.73	19.73	0.00	0.00	0.00	30095.60	1814.84
CT11	34.61	34.61	0.00	0.00	0.00	31756.73	11455.23

Table 4. Average dung fungal spore influx rate per cm^2 per 6 months with standard deviation per sampling season

		Total dung fungi		*Podospora*		*Sordaria*		*Sporormiella*		Other dung fungi	
		mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
Totals	W14-15	191.11	157.13	111.81	87.44	47.78	56.14	28.08	29.16	3.45	5.01
	S15	32.78	30.82	18.71	20.35	11.67	13.04	2.41	4.78	0.00	
	W15-16	12.10	10.72	5.51	8.48	4.70	3.73	1.90	3.17	0.00	
	S16	29.44	31.41	22.23	26.58	7.22	15.49	0.00		0.00	
Low grazing	W14-15	152.91	147.73	83.68	68.06	40.33	58.42	25.89	32.03	3.01	4.78
	S15	33.38	35.75	20.03	23.93	13.35	13.28	0.00		0.00	
	W15-16	11.49	11.87	6.61	8.99	4.12	3.86	0.76	1.70	0.00	
	S16	32.75	27.22	25.28	31.96	7.47	14.94	0.00		0.00	
Medium-high grazing	W14-15	363.01	7.66	238.40	15.32	81.27	38.31	37.93	7.66	5.42	7.67
	S15	32.31	30.67	17.65	19.89	10.32	14.23	4.33	5.94	0.00	
	W15-16	15.17		0.00		7.59		7.59		0.00	
	S16	27.24	36.29	20.20	25.42	7.05	17.26	0.00		0.00	

Table 5. Trap content volume and expected volume from rainfall (based on local precipitation levels) in ml

	Winter 2014-2015	Summer 2015	Winter 2015-2016	Summer 2016
CT1	400	260	720	270
CT2	475	275	1830	1100
CT3	425			
CT4	350	250		440
CT5	200	350		430
CT6	5000	350	940	300
CT7	2500	260		320
CT8	5000		850	120
CT9	5000	160		5000
CT10	400	300	750	300
CT11	275	130	835	100
Rainfall (mm)	258.2	264.4	554.8	305.6
Expected volume	506	518	1087	599

Figure 1. Locations of pollen traps in Chillingham Wild Cattle Park

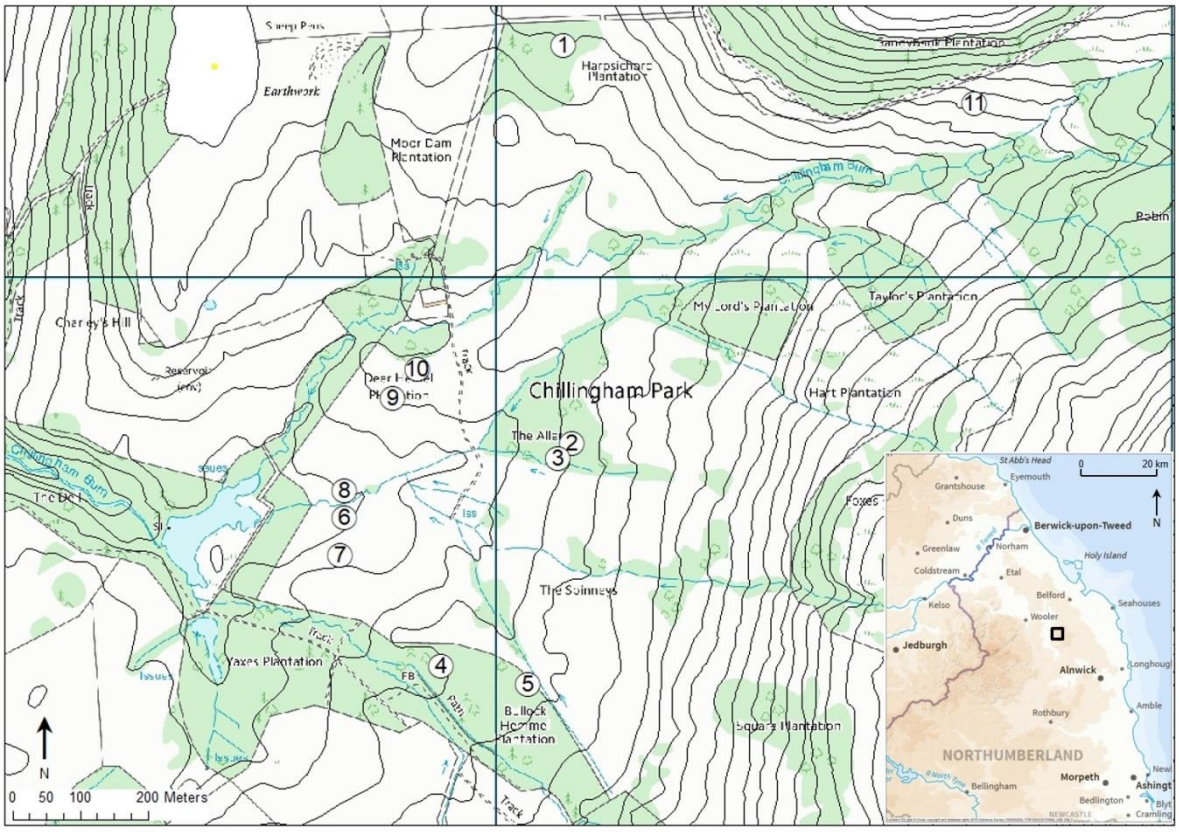

Figure 2. Pollen trap in field position; a. trap CT3 with surrounding posts and barbed wire; b. trap CT1 sunk into the ground

12 Figure 3. Weather data for the sampling period (October 2014 – September 2016); a. mean
13 monthly temperature; b. monthly total precipitation

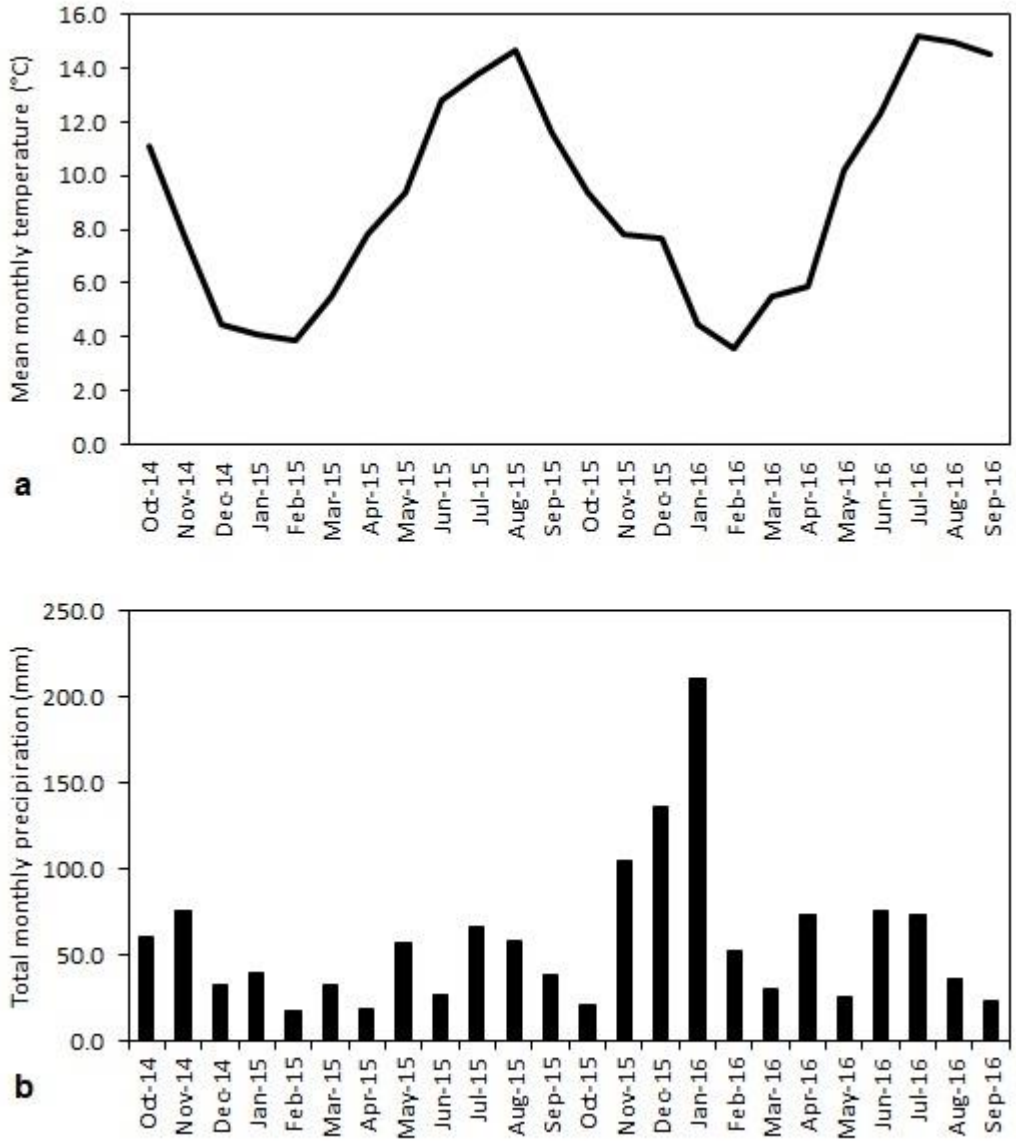

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Figure 4. Common fungal spore types encountered in the Chillingham pollen traps; a. *Podospora*; b. *Sordaria*; c. *Sporormiella*; d. *Arnium*; e. *Delitschia*; f. *Trichodelitschia*; g. *Apiosordaria*; h. *Cercophora*; i. *Coniochaeta*; scale bar: 10 µm

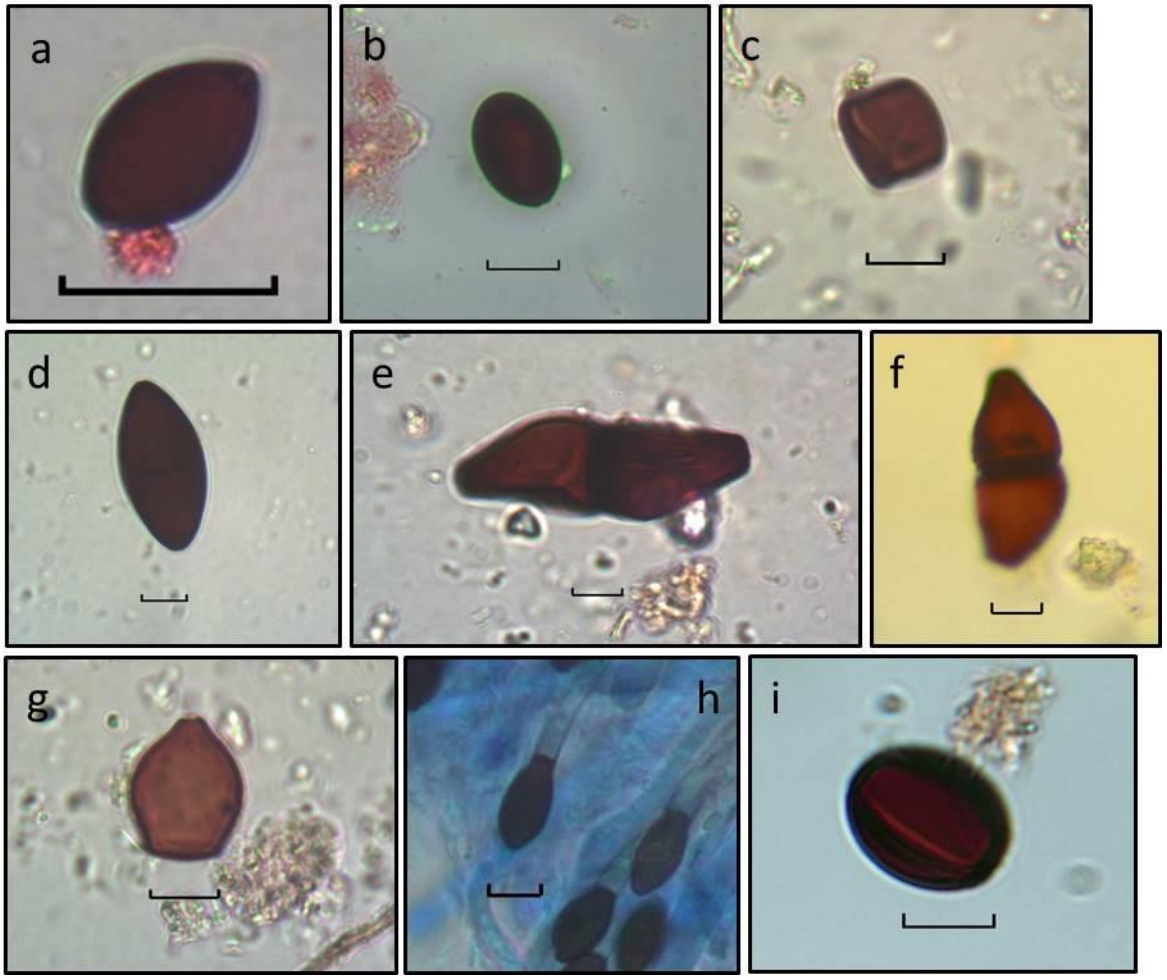

Figure 5. Boxplots of dung fungal spore influx rates per cm^2 per 6 months for each sampling season for the environmental variables measured; a. vegetation type; b. site wetness; c. utilization levels

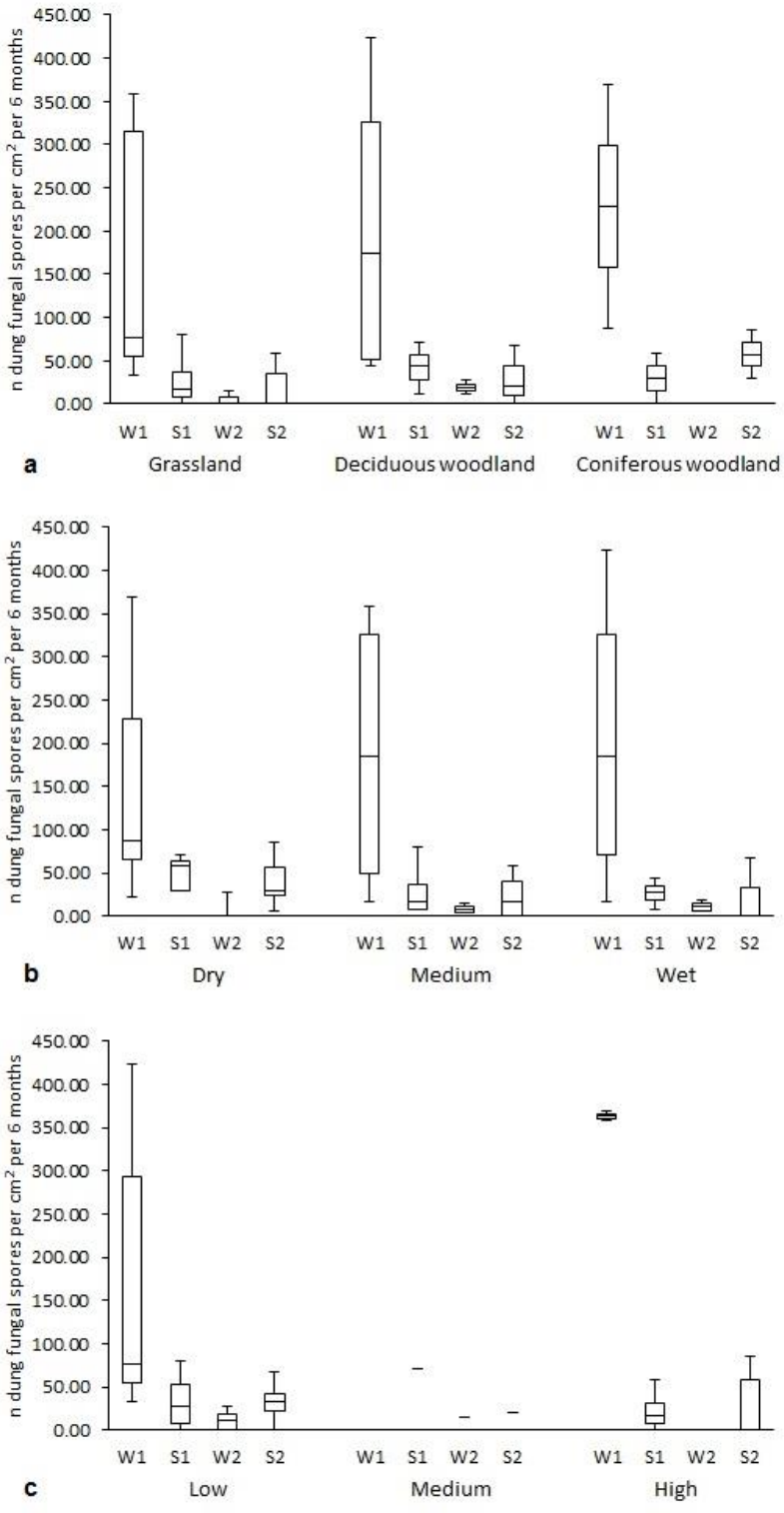

Figure 6. Average dung fungal spore influx rates per cm^2 per 6 months for all traps, for traps with low year-round utilization levels and for traps with medium-high year-round utilization levels for the four sampling seasons