TREE COVER IN THE EARLY HOLOCENE IN TEMPERATE EUROPE AND IMPLICATIONS FOR THE PRACTICE OF RE-WILDING IN NATURE CONSERVATION

Thesis submitted for the Degree of Doctor of Philosophy,

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Thesis abstract

Tree cover in the early Holocene in temperate Europe and implications for the practice of re-wilding in nature conservation

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Thesis submitted for the degree of Doctor of Philosophy (DPhil), trinity term 2013.

This thesis addresses the methodological challenges of determining the variability of large herbivore populations through time and their impact on European vegetation.

Large herbivores are at the heart of conservation policy however, opinions widely diverge on whether we should aim for fewer herbivores and managed populations or, on the contrary, as advocated by the rewilding movement, more herbivores and self-regulating populations acting as ecosystem engineers. This controversy has roots in a debate regarding the nature of ecosystems before the prevalence of human activities. Baseline ecosystems are either described as continuous forest cover with passive large herbivores, or, in contrast, as mosaics with patchy forest cover driven *inter alia* by bison, aurochs and horses, now rare or extinct in Europe. The main obstacle in moving this debate forward is a poor understanding of large-herbivore densities in the past.

I analysed modern pollen and spore assemblages from known environmental settings to improve palaeoecological interpretation of fossil assemblages dating from the pre-human (baseline) period. The sites investigated are the rewilded grasslands of the Oostvaardersplassen (The Netherlands), the mosaic habitats of The New Forest (UK) and the old-growth closed-canopy forest of Białowieża (Poland).

I demonstrate that the common practice of interpreting pollen percentages fails to estimate past forest cover in situations with natural grazing. As an explanation, I suggest that pollen productivity fluctuates with biotic factors such as herbivory and canopy shading. As a result, new insights into the baseline debate require additional lines of evidence. In this thesis, I develop an existing methodology to reconstruct past herbivore presence using fossil dung fungal spores. I synthesise current knowledge of this method with an emphasis on spore identification and, finally, I demonstrate that dung fungal spore abundance in lake sediments can be translated into large herbivore numbers.

The evidence presented in this thesis contributes to the debate on re-wilding and addresses a fundamental challenge of nature conservation in the human-dominated landscapes of Europe.

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Chapter 1: Thesis Introduction

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Thesis Introduction

Introduction Synthesis

This thesis addresses the methodological challenges of determining the variability of large herbivore populations through time and their impact on European vegetation. Large herbivores are an important part of any ecosystem and increasingly conservation policy is looking to restore large herbivores as ecosystem engineers in landscapes. This policy is based on the premise that before extensive human transformation of the European landscapes, large herbivores were an important driver of disturbance and ecosystem function, and as a result, landscapes were more biodiverse (Vera 2000). However, this pre-human baseline scenario is not without its critics, with an alternative view that large herbivores were 'passive' on the early European landscapes and not ecosystem engineers. This debate has become polarised, with one of the main obstacles in moving it forward being a poor understanding of past densities of large herbivores on the European landscape. The research presented in this thesis aims to examine the different palaeoecological methodologies available to reconstruct past herbivore abundances and their quantitative interpretation (Figure 1). The results of this research are presented in the format of five scientific papers that answer the following research questions:

Paper 1. Can we use proportions of fossil pollen to infer forest cover and herbivore numbers?

Paper 2. To what extent do modern pollen analyses and modelling improve the interpretation of fossil pollen?

Paper 3. Do dung fungal spores make a good proxy for past distribution of large herbivores?

Paper 4. How to make the identification of dung fungal spore comparable between studies?

Paper 5. Can we use dung fungal spore to quantify past abundance of large herbivores?

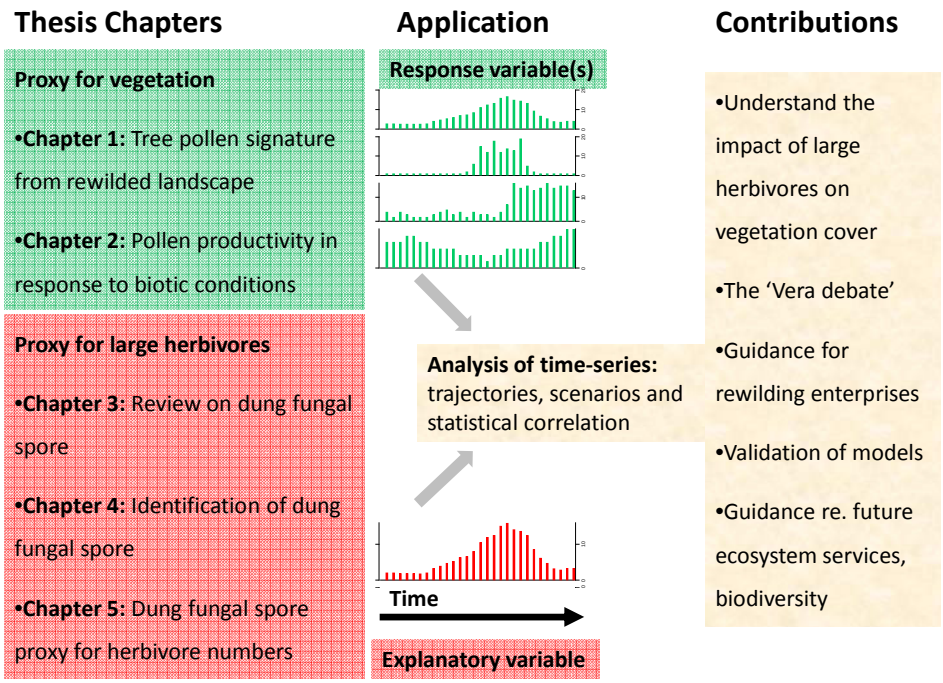


Figure 1: Thesis content and contribution

Aims

This thesis addresses the methodological challenges of determining the variability of large herbivore populations through time and their impact on European vegetation cover. Large herbivores and other large vertebrates are an important part of any ecosystem (Nuttle *et al.* 2011; Estes *et al.* 2011; Ripple & Beschta 2012; Tanentzap & Coomes 2012; Ritchie *et al.* 2012; Peh & Lewis 2012) and understanding their effect on ecosystem functioning is crucial to guide sustainable land use across the globe, at the local, regional or international scale. In this thesis, I focus on temperate Europe (i.e. areas of Europe with Atlantic temperate or continental temperate climate), where there is a pressing need to address the following objectives:

- to gain a better understanding of the impact of different herbivore density and diversity on different environments *and*
- to quantify the numbers and variety of large herbivores that will deliver the benefits expected

In fact, opinions widely diverge on whether we should aim for fewer herbivores and managed populations (conventional perspective) or, on the contrary, more herbivores and self-regulating populations (rewilding). This dilemma is particularly relevant to those areas where agriculture is on the decline, e.g. some parts of Southern Europe and other areas of the world where new management practices remain to be explored (Höchtl *et al.* 2005; Donlan *et al.* 2006; Navarro & Pereira 2012).

The conventional perspective views large herbivores as passive elements of ecosystems that managers can introduce or remove for specific purposes such as:

- The maintenance of landscapes and habitats shaped by traditional agriculture (i.e. pre-WWII) that are no longer economically profitable but are of high heritage and cultural value.
- The protection of forested ecosystems against increasing ungulate populations in the temperate climates of the Northern hemisphere (e.g. Côté *et al.* 2004).
- The protection of rangeland against native and introduced herbivores that compete for resources with livestock (i.e. kangaroo in Australia) (e.g. Gordon 2009).
- The limitation of greenhouse gas emission by reducing the amount of large herbivores (e.g. Goodland 2012).

In contrast, rewilding views large-herbivore and other large-vertebrate populations as ecosystem engineers, whose planned introduction or re-introduction will promote ecological and economical benefits. The objectives most commonly put forward for rewilding programmes are as follows:

- Introduce or re-introduce large vertebrates to restore ecological and evolutionary processes, hence ultimately enhancing biodiversity and ecosystem wealth provision (Donlan *et al.* 2006; Rewilding Europe 2013).

- Explore a novel and inspiring strategy for conservation biology, irrespective of traditional protection status (e.g. protected area, protected habitat, protected species).
- Enlarge the distribution range of endangered large vertebrates (Donlan *et al.* 2006)
- Provide new and viable business opportunities (Donlan *et al.* 2006; Rewilding Europe 2013).
- Mitigate climate change (Zimov 2005).

At the root of this divide of opinions are two questions:

1. How should we manage, if at all, large-herbivore populations in order to achieve our aims?
2. When are large herbivores drivers of and when are they driven by environmental change?

Both questions are highly topical and have been intensively debated in Europe since the publication of Frans Vera's thesis (Vera 2000). The focus of this 'Vera debate' is whether the early Holocene landscapes in Europe are best described as high-forest (continuous forest cover with closed canopy wherever the relief and hydrology permits tree growth) or wood-pasture (patchy forest cover within a mosaic of regeneration stages driven by large herbivores, such as bison, aurochs, horses and others, all native to the area). Early Holocene refers here to the period of time after re-establishment of temperate vegetation cover following the termination of the last glaciation (*c.* 10,000 years ago) but before the advent of agriculture in Europe (from *c*. 6000 years ago) when the first major clearances of land are thought to have occurred.

Why does the Vera-debate and the proposed concept of 'rewilding' polarise opinion?

Rewilding refers to the recent geological and evolutionary time periods (i.e. Pleistocene or Holocene) when populations of large vertebrates were much more diverse than they are now. The Late-Quaternary extinctions of large mammals and slow breeding animals are known to be

due to a combination of climatic variation and human hunting across all continents but Africa (Koch & Barnosky 2006). Pre-extinction is proposed as a baseline for ecological and evolutionary processes and it is argued that by restoring these large herbivores in the landscape, wilderness and associated biodiversity will also be restored. Flagship rewilding projects include Josh Donlan's proposed rewilding in North America (Donlan *et al.* 2005, 2006), the Rewilding Europe initiative inspired by the work of Frans Vera (Rewilding Europe 2013) and finally Sergey Zimov's rewilding to create a Siberian Pleistocene park (Zimov 2005). Rewilding is therefore focused on restoring ecosystem processes rather than the alternative conservation focus of protecting specific areas, habitats or species.

There is a fundamental difference between rewilding projects and more traditional re-introduction programmes as practiced in nature conservation. Re-introduction aims for the introduction of individuals of one species in an area from which historical records attest its presence in the past, thereby aiming to establish self-sustainable populations of that species (see e.g. Armstrong & Seddon 2008). It represents an established tool for action in favour of biodiversity (IUCN 1998, 2012). The justification of such action is generally the recovery of wild populations of one species. This is the preferred type of conservation action in favour of endangered species which have restricted distribution ranges or are found only in captivity (CBD 1992).

In contrast, the ultimate aim of rewilding re-introductions is not only the survival of a species population itself, but also the restoration of ecological processes (e.g. disturbance, seed dispersal) associated with the presence of large herbivores in a landscape (Donlan *et al.* 2006; Caro 2007; Kaiser-Bunbury *et al.* 2010; Griffiths *et al.* 2011). Additional justifications are economic, with the creation of reserves that facilitate both ecotourism value (see e.g. Donlan *et al.* 2006; Navarro & Pereira 2012) and climate mitigation (Zimov 2005; Zimov *et al.* 2012). In such cases, large vertebrates are valued for the function they have in their environment.

Conservationists are responding with passion to rewilding projects with arguments both for and against this approach (see e.g. Caro 2007). Major critiques are concerned with the practicalities of such projects and the difficulties inherent in conducting and assessing a project with unpredicted outcomes (Hughes *et al.* 2011, 2012). There is no doubt that all of these concerns are justified (Donlan *et al.* 2006); nevertheless promoters of rewilding believe they can be overcome and are worth overcoming because rewilding represents a unique opportunity to promote wilderness, biodiversity and wealth provision by ecosystems. Rewilding also faces opposition because it fundamentally questions conservation principles and a long-standing tradition of conservation practices. For instance, the distinction between native and non-native species is at the core of both conservation and restoration ecology. Suggesting the introduction of species considered to be non-native (such as lions in North America) ignores principles accepted by the public, researchers and practitioners as fundamental (Trudgill 2008; Davis *et al.* 2011; Robbins & Moore 2013). In addition, not setting clear spatial objectives regarding the establishment of open versus closed habitats, but instead relying on large herbivores to create a dynamic mosaic clashes with the tradition in nature conservation of defining target habitats to be attained, managed and maintained (see e.g. Fuhlendorf *et al.* 2008).

Was Europe's primary vegetation driven by large herbivores?

Vera (2000) proposed the hypothesis that past land cover of the lowlands in Northern Europe during the early Holocene was a wood-pasture i.e. a dynamic mosaic of grasslands, shrubland and forest. He also proposed that the main driver of such a landscape would have been grazing and browsing by large herbivores, now extinct as a result of the late-Quaternary extinction. In support of this hypothesis the following lines of evidence were put forward:

i. In early Holocene pollen diagrams from North-Western and temperate Europe, the period of high-forest (between *c*. 9500-6000 cal yr BP) is characterised by high

percentages of tree pollen, notably *Corylus avellana* (Hazel) and deciduous *Quercus* spp. (Oaks)

ii. Vera highlighted the fact that native *Quercus* and *Corylus* species in North-Western and temperate Europe do not regenerate in old-growth forest with closed canopy (on the basis of a comprehensive review of today's old-growth forests and today's autecology of tree species).

iii. He therefore concluded that the long persistence of *Quercus* and *Corylus* dominance in fossil pollen assemblages should be interpreted as dynamic landscapes where open habitats were widespread.

Prior to the formulation of this hypothesis and argumentation, primary vegetation had been thought to be closed-canopy forest wherever climate and topography permits (Polis 1999). This traditional thinking viewed large herbivores as a passive presence on the landscape. In temperate Europe a post-glacial succession of vegetation leading to closed-canopy forest for the whole continent, had long been the accepted wisdom (see e.g. Ellenberg 1988) – dating back to the early work of Frederic E Clements and Arthur G Tansley in the first half of the 20th Century (see e.g. Anker 2001).

The concept of succession towards forest is increasingly questioned on the basis of being too simplistic, relying heavily on space-for-time substitution and ignoring disturbance regimes outside large destructive events (Johnson & Miyanishi 2008; Walker *et al.* 2010; Chen & Taylor 2012). Using alternative methods (i.e. Dynamic Global Vegetation Models), for example, Bond *et al.* (2005) demonstrated that fires play a major role in determining global vegetation cover. Bond (2005) added that the interaction between herbivory and fire in determining vegetation cover is one that ought to receive more research attention in the future. In fact, there is an increasing amount of evidence showing that high trophic level vertebrates such as large herbivores play a considerable role in shaping land cover and community composition across

most landscapes (Nuttle *et al.* 2011; Estes *et al.* 2011; Ripple & Beschta 2012; Tanentzap & Coomes 2012; Ritchie *et al.* 2012). In addition, large herbivores appear instrumental in accelerating the decomposition cycle of woody and herbaceous matter and herein transform fire regimes by reducing fuel availability (Gill *et al.* 2009; Rule *et al.* 2012). As a result it appears that large herbivores not only have direct effects on vegetation cover but also indirect effects by modifying fire regimes.

Table 1. Post-2000 studies that contributed evidences towards the 'Vera debate'

Reference	Region	Naturally open habitats	Drivers of open habitats	Line of evidense for past herbivore density	Interaction of drivers	Line of evidence	Methods
Traditional view, e.g. Ellenberg (1988)	Europe	Very limited	Very low or very high water levels	No			
Vera (2000)	Europe	Widespread	Grazing	No			
Svenning (2002)	Europe	Floodplains, dry and poor soils	Hydrological dynamics, grazing, edaphic factors	No	x	Fossil pollen, plant remains and vertebrates	Lit. Review
Bradshaw *et al*. (2003)	Europe	Some	Fire, herbivory, wind throw	No	x	Fossil pollen, vertebrates and invertebrates	Lit. Review
Whitehouse & Smith (2004)	British Isles	Some	Fire, herbivory, wind throw			Fossil invertebrates	Lit. Review
Mitchell (2005)	Europe	Not assessed	Not herbivory	No		Fossil and modern pollen	Meta Analysis
Soepboer & Lotter (2009)	Swiss Plateau	Some, neither high forest nor wood pasture	Not assessed	No		Fossil pollen	Modelling
Whitehouse & Smith (2010)	Great Britain	Some	Mostly not herbivory	Yes		Fossil invertebrates	Meta Analysis
Sommer *et al.* (2011)	Europe	Not assessed	Not herbivory	No		Fossil vertebrates	Meta Analysis
Kunes *et al.* (2011)	Denmark	Some	Nutrients	No		Fossil pollen	Meta Analysis
Hall (2008)	Great Britain	Some	Grazing	No		Fossil vertebrates	Niche modelling
Tolksdorf *et al.* (2013)	N. Germany	Some	Not assessed	No		Fossil aeolien sediments	Sedimentology
Nielsen *et al.* (2013)	N. Germany and Denmark	Some	Edaphic factors	No		Fossil pollen	Meta Analysis, modelling
Fyfe (2007)	Wales	Some	Not assessed	No		Fossil pollen	Meta Analysis

Following the publication of Vera's hypothesis in (2000) new research was initiated to better understand Europe's past land cover before the advent of agriculture. Many studies suggest the presence of more open habitats in temperate Europe throughout the Holocene (Table 1), although this is still the subject of a lively debate (see e.g. Hodder *et al.* 2009; Whitehouse & Smith 2010; Groves *et al.* 2012). One of the key problems in determining the extent of open landscapes in the early Holocene, however, has been the ability to determine the proportions of open versus forested habitats from palaeoecological records. This is difficult to estimate because of methodological limitations, whatever the palaeoecologcial proxy used. Moreover, most drivers of disturbance such as large herbivores cannot be statistically tested against

vegetation cover variation because they are rarely quantified in a satisfactory way. As a result, a body of literature stresses the urgent need for better understanding of the past densities of large herbivores and the use of alternative lines of evidence from fossil pollen (Svenning 2002; Bradshaw *et al.* 2003; Mitchell 2005; Birks 2005), a research target that has not been addressed until today.

What evidence do we need to contribute to those debates?

In order to improve our understanding of the impact of large herbivores on vegetation cover using methods in palaeoecology, we would ideally need more real-world scenarios illustrating the complex interactions between large-herbivore densities, other drivers of change such as climate, and vegetation cover. Such records could, for instance, highlight the potential impact of herbivory and other drivers on ecosystem services, or illustrate the levels of herbivory pressure that are sustainable in a particular situation. However, in the absence of such records, palaeoecology is of particular interest because the methods used allow the study of ecological time-series describing processes at the time scale they operate in, without being dependent on the establishment of long-term permanent plots that require yearly field measurements for decades or more (Willis *et al.* 2007; Silvertown *et al.* 2010; Mitchell 2010).

To study the impact of large herbivores on vegetation cover, it is necessary to gather data for the response variable (vegetation cover) and the explanatory variable (herbivore density) (see Figure 2). Because the organisms studied (such as trees and large vertebrates) have relatively long life spans (>1 year), long-time series such as those collected in palaeoecology are ideal for both variables and potentially for other variables that have influence on vegetation (climate, fire, human activities). Vegetation cover is the classic focus of palaeoecology with the study of fossil pollen sequences from peat and lake sediments. However, as highlighted above, methods to reconstruct past herbivore abundances have traditionally been less well studied (but see Gaillard *et al.* 1992; Mazier *et al.* 2009).

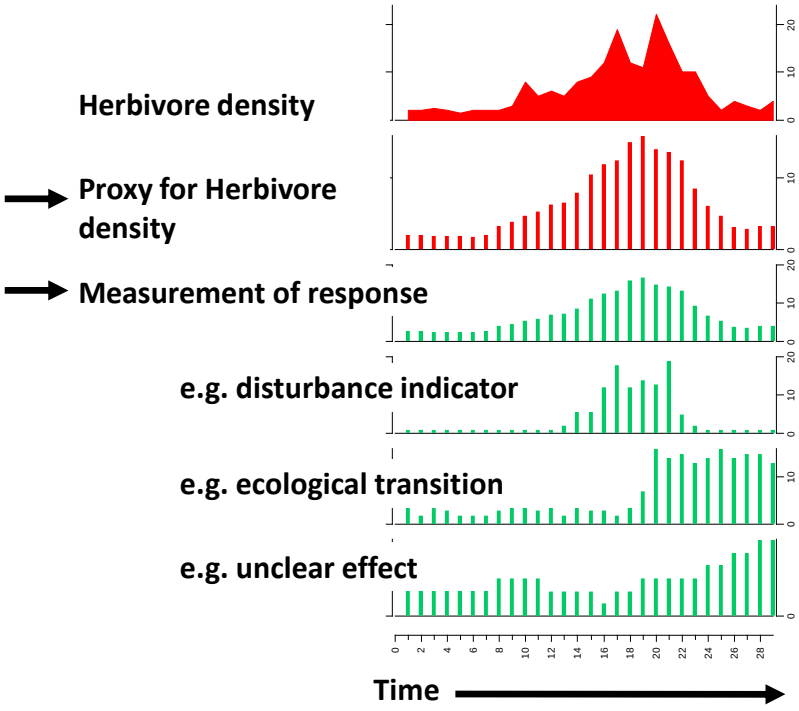


Figure 2: To study the impact of large herbivores on vegetation cover, we need proxies in palaeoecology that are independent, so an explanatory variable can be tested against a response variable.

What does a grazed landscape look like in fossil pollen diagrams?

Expressing fossil pollen in percents is the most common way to interpret pollen assemblages. In particular, the proportion of tree pollen versus herb pollen (often referred to as arboreal pollen and non-arboreal pollen) has been widely used in pollen analysis to determine the openness of a landscape. However, there is evidence demonstrating that in some conditions, tree pollen is not a reliable proxy for tree cover across landscapes (Sugita *et al.* 1999; Favre *et al.* 2008). In addition, there has been very little research on this topic in landscapes where heavy grazing occurs, particularly in temperate Europe. Some indication that grazed landscapes might look very different in terms of their pollen productivity, however, can be ascertained from examining the pollen assemblages from heavily-grazed landscapes in other parts of the world, such as in the Mongolian steppe, for example (Li *et al.* 2006). Here, the pollen signature of this open grassland landscape has a maximum of 10% *Poaceae* pollen despite its lack of trees and

predominance of open-ground herbaceous vegetation types. Such records suggest that the process of grazing itself may influence pollen productivity and henceforth the signature in the pollen record. Thus, without any indication of grazing pressure, it remains unclear as to what extent the abundance of tree pollen and the near absence of *Poaceae* pollen in ancient sediments should be interpreted as continuous forest cover. This question is particularly relevant to the 'Vera debate' because fossil tree pollen is the basis for most arguments supporting either the high-forest or the wood-pasture scenario. I address this knowledge gap (**Paper 1)**, through analyses of modern tree pollen percentages in three herbivore-dominated ecosystems, the old-growth forest of Białowieża (Poland), the forest-heathland-grassland mosaic of the New Forest (United Kingdom) and the grasslands of the Oostvaardersplassen (The Netherlands) (see Table 2 for a description of the sites).

Paper 2 presents the results of a study to address the next knowledge gap and that is the one associated with pollen productivity on these grazed landscapes. The key question I address is as follows: how do biotic factors (e.g. grazing) influence pollen productivity between regions? I utilised the state-of-the-art technique to translate fossil assemblages into past vegetation cover (Sugita 2007a; b; Broström *et al.* 2008; Mazier *et al.* 2012). This technique provides quantitative data for the validation of Dynamic Global Vegetation models (Gaillard *et al.* 2008, 2010) for instance, and is expected to be more appropriate than pollen percentages in testing ecological hypotheses quantitatively. Sugita's methods account for the limitation of percentages (i.e. difference in pollen productivity between taxa, differences in fly ability between pollen types, size of the basin and spatial distribution of vegetation around the sampling point). One crucial parameter in these methods is the relative pollen productivity of the different plant species. So far pollen productivity has been estimated across Europe but we do not know whether the variability of results between studies (Broström *et al.* 2008; Mazier *et al.* 2012) is due to methodological (Bunting & Hjelle 2010; Hjelle & Sugita 2011; Twiddle *et al.* 2012), climatic, or other environmental factors. For this reason I assess in **Paper 2** the biotic factors that can

influence pollen productivity (and in particular *Poaceae* pollen productivity) in the old-growth forest of Białowieża (Poland). This is a fundamental question in pollen analysis because it challenges our ability to interpret fossil pollen assemblages without any preliminary knowledge of the ecosystem they come from.

What is the quantitative relationship between dung fungal spore abundance and large-herbivore density?

Dung fungal spores are increasingly used to track the populations and distributions of past large-herbivores (**Paper 3**, Figure 1) (Figure 3). This method can be used independently from pollen, i.e. independently from the measurement of land cover. Thus, it has great potential for studying the impact of large herbivores on vegetation cover. However, there remain some concerns with regards to the methodology (Raper & Bush 2009; Feranec *et al.* 2011; Wood & Wilmshurst 2011; Parker & Williams 2011). In particular the potential weaknesses that have been highlighted include: complications related to local hydrology, problems with identification, lack of quantitative reconstructions and lack of understanding of the taphonomy (deposition and preservation in different environments). Despite these concerns, in practice it has been possible to demonstrate the worldwide impact of herbivory in ecosystems (Burney *et al.* 2003; Gill *et al.* 2009; Rule *et al.* 2012), supporting previous research in conservation biology and grazing ecology (Owen-Smith 1988; Vera 2000). I review some of the limitations and the potential of dung fungal spores as a proxy for large herbivores in **Paper 3**. I also highlight specific areas of knowledge that need further research to achieve the quantitative reconstruction of herbivore abundance. **Paper 3**, along with **Paper 4**, set solid methodological bases for the implementation of quantitative analysis in **Paper 5**.

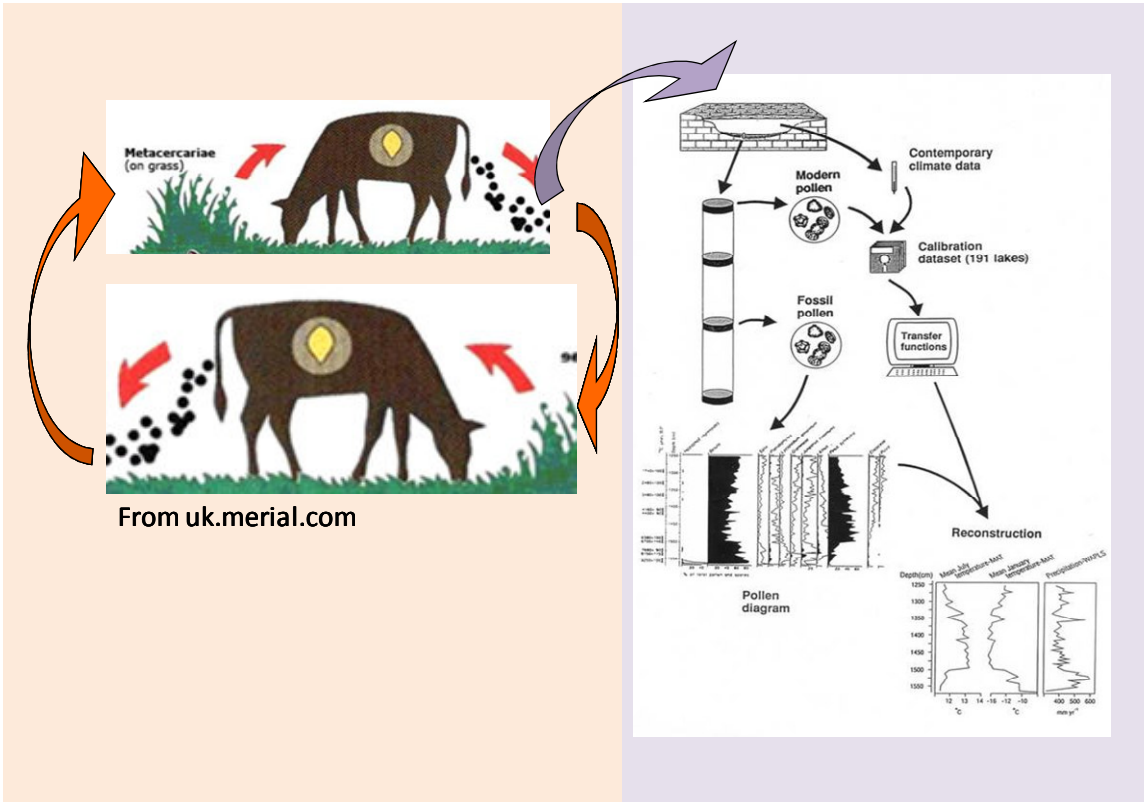


Figure 3: Dung fungi life cycle (orange). Dung fungal spores are unintentionally ingested by large herbivores while feeding on vegetation and germinate after digestion when deposited with dung. Mycelium growth and fructification has species-specific responses to different moisture levels, temperatures, microclimates and microhabitats and when successful, the sticky spores are liberated in an expulsive way to adhere onto surrounding vegetation, ready to be ingested (Dix & Webster 1995; Krug et al. 2004; Bell 2005). Some spores do not accomplish their reproductive function (violet) and end up in lake sediments where scientist sample cores and utilise them for environmental reconstructions. Illustration on right from Birks (2003)

The aspect of dung fungal spore analysis I develop further in **Paper 4** is their identification. The development of robust and consistent characters for the identification of dung fungal spores was a necessary step in order to develop further quantitative analysis (see **Paper 5**). This aspect of my thesis is presented as an identification key in keeping with traditional natural history. This identification tool was designed to be widely disseminated as an online document so that scholars across the world can compare their results. Building on the results of **Paper 3** I assessed the different types of spores likely to be encountered and I provide a taxonomic framework for any future work involving dung fungal spores.

Paper 5 determines whether dung fungal spores can be used as a quantitative proxy of herbivore presence over time. This question is of great importance because one current

knowledge gap is whether it is possible to quantify the number of large herbivores on a landscape from the abundance of dung fungal spores in sediments. I address this question by calibrating dung fungal spore abundance in relation to contemporary herbivore presence, using modern lake surface sediments in relation to known herbivore densities. These modern sediment samples serve as analogues so that the calibrated relationship observed today between animal numbers and dung abundance can be extrapolated to the past.

Study sites and chapter list

A brief description of the three sites studied in my thesis is provided in **Paper 1** and their general characteristics are presented in Table 2. A more thorough description of Białowieża can be found in **Paper 2** and in Falinski (1986) or e.g. Bobiec (2012), one of the New Forest in Tubbs (2001) and of The Oostvaardersplassen in **Paper 5** and Vera (2009).

Table 2: Site characterisitcs. The last column, in italics, present information for the whole of the forest of Białowieża, in which the Białowieża National Park is nested.

	The Oostvaaardersplassen	The New Forest	Białowieża National Park	*Forest of Białowieża*
Highlights	Rewilding experiment and wetland birds	Hotspot for biodiversity and for outdoor activities	Strict reserve at the heart of the last primeval forest in lowland Europe	*Last primeval forest in lowland Europe*
Public access	No	Yes	No	*Yes*
Country	The Netherlands	United Kingdom	Poland	*Poland and Belarus*
Site origin	Reclaimed polder	King's hunting reserve	Nature conservation	*King's hunting reserve*
Date of first protection (year AD)	1986	1079	1921	*1538*
Total area (km square)	56	566	47.5	*1500*
Vegetation cover:				
grassland / heathland / mires	90%	60%	3%	*13%*
forest	10%	40%	97%	*87%*
Large herbivore density (kg/ha)	470 *	200 *	15 *	
Large vertebrates present	cattle, horse, red deer	horse, cattle, pig, fallow deer, roe deer, red deer, sika deer, …	european bison, moose, roe deer, red deer, wild boar, grey wolf, lynx, …	
Data in chapter	1, 4 and 5	1 and 4	1 and 2	
Field season	Summer 2010	Summer 2010	Summer 2011	
Type of sampling	Modern lake sediments from small water bodies	Modern lake sediments from small water bodies	Modern moss mats from forest floor	

* see Paper 1

The list of chapters (**Chapter 1 to 7**) is as follows (please refer to Table 3 for the authors' full names and affiliations):

Chapter 1 - Introduction

Chapter 2 - Paper 1: What is the pollen signature from a naturally grazed landscape?

- To be submitted to Nature (IF: 38.6)
- Baker A, Bhagwat S, Vera F, Zimny M, Latalowa M, Willis K

Chapter 3 - Paper 2: The impact of forest canopy shading, forest age and disturbance regimes on pollen productivity: evidence from the old-growth forest of Białowieża National Park, Poland.

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Chapter 4 - Paper 3: Do dung fungal spores make a good proxy for past distribution of large herbivores?

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- Baker A, Bhagwat S, Willis K

Chapter 5 - Paper 4: Improving the identification of dung fungal spores for estimating past populations of large herbivores

- To be made available on the internet (Biodiversity Institute website)
- Baker A, Bhagwat S, Willis K

Chapter 6 - Paper 5. Quantification of population sizes of large herbivores and their long-term function in ecosystems using dung fungal spores.

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Chapter 7 - Conclusions. Synthesis of findings and future work.

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Chapter 2: Paper1

What is the fossil pollen signature from a landscape with natural grazing?

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What is the signature of landscapes with natural grazing in fossil pollen diagrams?

Abstract

There is widespread debate around the openness of the vegetation cover in North-western Europe, before the advent of agriculture *c*. 6000 years ago (Birks 2005)**. Fossil pollen records from this period of time, which demonstrate high percentages of tree pollen, are traditionally interpreted as continuous forest cover. However, others have put forward an alternative interpretation suggesting that these records instead indicate more open landscapes as a result of early Holocene grazing by large herbivores** (Vera 2000; Svenning 2002)**. In these early Holocene fossil pollen records, this assumption of high tree pollen indicating a closed-canopy forest is based on modern-day pollen analogues from wooded and agricultural landscapes. In contrast little is known about pollen signatures from natural landscapes grazed by wild herbivores and without human intervention since these are rare in Europe due to a long history of land management. Here we present pollen assemblages from the Oostvaarderplassen, a protected area rewilded since 1983 with Heck cattle, Konik horses and red deer. We show that the pollen signature of this open landscape with natural grazing can give a pollen signature that is similar to a landscape with abundant forest cover. We compare these results with two other European flagship ecosystems, the New Forest and the forest of Białowieża, and conclude that the percentage of tree pollen, a measure commonly used to interpret the percentage of trees on past landscapes, is a poor indication of forest cover and herbivore numbers. As a consequence, alternative lines of evidence for past vegetation cover and past herbivore numbers are necessary to interpret openness of the early Holocene landscapes in Europe.**

Introduction

Two contrasting interpretations have been put forward for pollen assemblages pre-dating the advent of agriculture (*c*. 6000 years BP) (Birks 2005). The high-forest scenario describes a continuous forest cover in areas where the relief and hydrology permit tree growth. In contrast, the wood-pasture scenario describes forest cover as patchy within a mosaic of regeneration stages driven by large herbivores such as bison, aurochs and horses, all native to Europe but extinct in the wild since the middle ages. The debate between the proponents of these scenarios also echoes two contrasting baselines for conservation in Europe, one that promotes conservation of open and biodiverse cultural landscapes by managing cultural activities (e.g. burning, traditional agricultural land management) to maintain open vegetation, and another that promotes rewilding with large herbivores in order to introduce or re-introduce ecological disturbance. Usually the large herbivores used in rewilding projects are analogues to their now-extinct counterparts and there is an emphasis on restoring their function of ecosystem engineers rather than the traditional restoration practice targeted on species and habitat types.

Support of the early postglacial high-forest scenario is principally based on fossil pollen from the pre-agricultural period. The percentage of tree pollen is usually high and it is argued that beyond a certain threshold (e.g. 60% (Mitchell 2005; Birks 2005) or 85-90% (Favre *et al.* 2008)) we can safely infer closed-canopy forest. In contrast, the main argument in favour of wood-pasture scenario is the abundance of pollen of *Quercus robur*, *Q. petraea* and *Corylus avellana*. In pollen assemblages dominated by tree pollen the presence of these species indicates conditions favourable for their abundant regeneration in temperate European landscape. Long-term ecological evidence shows unequivocally that *Quercus robur*, *Q. petaea* and *Corylus avellana* require open habitats to regenerate (Vera 2000). In addition, large-herbivore activity enhances the regeneration of these species (Vera 2000; Bobiec *et al.* 2011). Therefore, continuous abundance of their pollen in sedimentary archives over long period of time (>1000 years) could be indicative of a mosaic of open and forested habitat rather than continuous

dense forest cover. This study aimed to test whether modern pollen assemblages from present-day landscapes with natural grazing (wood-pasture scenario) display a signature that is distinct from that of forested landscapes (high-forest scenario). It is hypothesised that the higher the tree cover, the higher is the tree pollen percentage. Following this logic, rewilded grasslands with natural grazing should have very distinct pollen signature with very low tree pollen percentages. On the other hand, if a gradient of tree cover does not correspond with a gradient of tree pollen percentages, then the evidence in support of high-forest scenario is questionable.

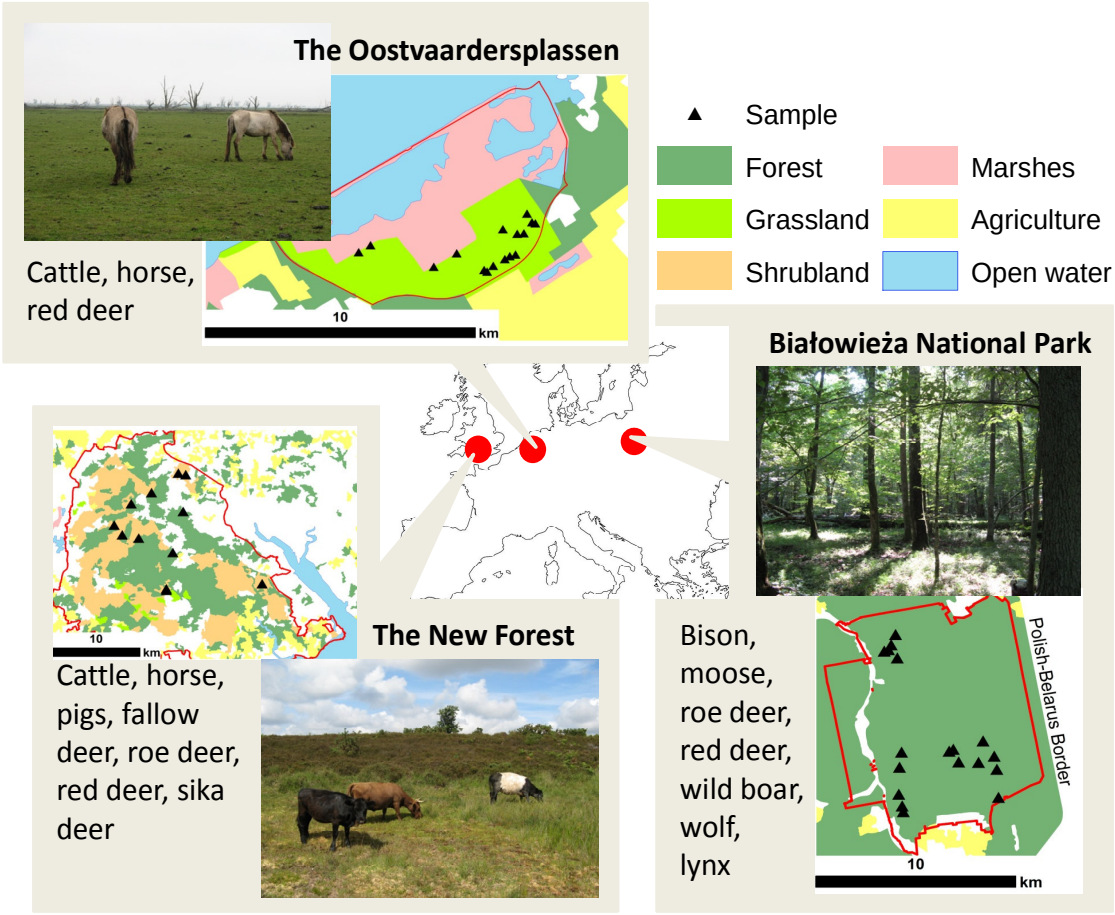


Figure 1: Map for the three areas studied and their large-vertebrate diversity

Methods

In order to assess the modern-day pollen signature from grazed landscapes we reconstructed present-day pollen assemblages from 16 surface sediment samples collected from water bodies in the Oostvaardersplassen (the Netherlands), 12 surface sediment samples from water bodies

in the New Forest (United Kingdom) and 18 modern moss samples in the forest of Białowieża (Poland) (see figure 1). We collected these 46 samples following standard protocol (Broström *et al.* 2008) and extracted pollen using standard laboratory procedures (Willis & Bennett 2001). We identified and counted all pollen grains to calculate the proportion of tree pollen present in each samples. Tree cover was established using Corine land cover (European Environment Agency 2007) around each sampling point within a radius distance of 1 km and 10km. In Białowieża, we extrapolated the forest cover from aerial photographs on the Belarus side because the Corine scheme does not cover this country. Herbivore densities for each study area were compiled from field data, published literature and government reports (SI). We tested the relationship between tree pollen percentage and tree cover using mixed effect models with a link function for binomial distribution (Bates *et al.* 2011). We used the study areas as random effect explanatory variables, tree cover as fixed explanatory variable and the proportion of tree pollen in the samples as response variable.

Study sites

The Oostvaardersplassen is the first rewilding experiment in Europe, where herbivores numbers are not regulated. The biomass density of large herbivore is currently estimated at 474 kg ha^{-1} (SI) and because of the grazing pressure, there is limited tree regeneration within the 56 km^2 of grassland and reed beds. The New Forest, spread over 566 km^2, is an herbivore-dominated ecosystem consisting of a mosaic of grassland, shrubland and forest. Herbivore numbers are regulated by ancient rights of grazing dating back to the protection of the area as a royal hunting reserve from AD1079 (currently about 200 kg ha^{-1} large herbivore biomass density, SI). The Białowieża National Park (Poland) is nested at the heart of the forest of Białowieża, the largest fragment of temperate forest in lowland Europe spanning 1500 km^2. Herbivore numbers are regulated through hunting and culling to maintain continuous closed-canopy forest and currently there is a biomass density of large herbivore of about 15 kg ha^{-1} large (SI).

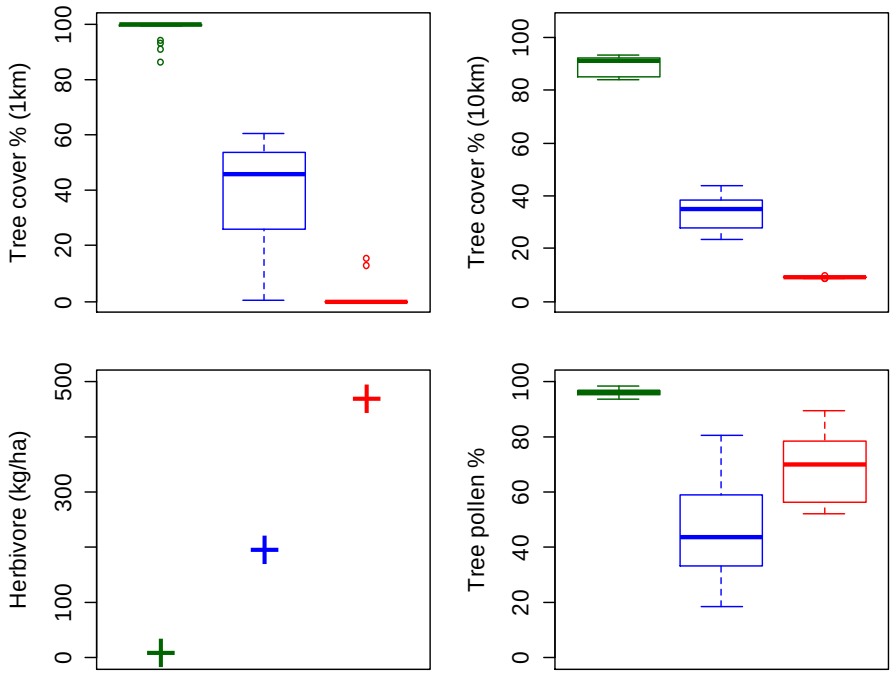


Figure 2: Present-day tree cover, overall herbivore densities and tree pollen percentages from surface samples for Białowieża (green, n= 18), the New Forest (blue, n= 12) and the Oostvaardersplassen (red, n = 16). See methods for descriptions of measurement of present-day tree cover and herbivore density.

Results

Tree cover within 1 km of our sampling sites is high in Białowieża (near 100 %), intermediate in the New Forest (between 28 and 55 %) and low in the Oostvaardersplassen (near 0 %). The situation is similar within 10 km, yet in a less pronounced way (respectively 90 %, 35 %, and 8 %). Herbivore numbers show the reverse trend with low density in Białowieża (15 kg ha^{-1}), intermediate density in the New Forest (200 kg ha^{-1}) and high density in the Oostvaarderplassen (470 kg ha^{-1}) (Figure 2).

Results from our analyses (Figure 2) indicate that present-day tree pollen percentages obtained from lake surface sediments from the rewilded grassland of Oostvaardersplassen are intermediate between those of the New Forest and Białowieża (Figure 2). However, the tree cover at Oostvaardersplassen is the lowest among our three sites, within the radii of 1 km and 10 km both. There does not therefore appear to be a positive linear relationship between tree cover and percentage of tree pollen, even when taking into account the random effect induced

by comparing three different spatial areas (1km: p=0.112 ; 10km: p=0.070). Our results therefore demonstrate that tree pollen percentage does not always indicate tree cover or herbivore numbers in ecosystems with natural grazing. As a consequence, landscapes that have experienced natural grazing in the past may remain undetected in fossil pollen assemblages used to support the high-forest scenario.

Discussion

Our results show that rewilded landscape with natural grazing has a pollen signature that is remarkably close to that normally interpreted as closed forest cover (i.e. between 60-80 % tree pollen). The most dramatic case is the Oostvaardesplassen where, despite this being an almost open landscape containing more than 4000 large herbivores, tree pollen percentages are above 60 % in nearly all samples measured (n= 16). There is also no linear relationship apparent between grazing density, tree cover and percentage of tree pollen between the three sites, suggesting that indicators other than percentage of tree pollen should be looked at when estimating past tree cover. This result suggests that in order to resolve the debate about past openness of the vegetation cover in North-western Europe, lines of evidence other than tree pollen proportion are urgently needed (Svenning 2002; Bradshaw *et al.* 2003; Baker *et al.* 2013).

There may be several mechanisms for over-representation of tree pollen in all three sites but particularly in the Oostvaardersplassen. These mechanisms are as follows (i) Vera (2000) has hypothesised that pollen productivity is strongly suppressed in heavily-grazed situations for the simple reason that flower heads are systematically eaten by herbivores. This was shown to be true in one case study (Groenman-van Waateringe 1993) but further work on this subject will be necessary to improve our understanding of the direct effect of grazing on flowering of grasses; (ii) trees and shrubs that escape grazing and browsing in open habitats receive more light than in shaded situations such as in forested environments. Increased light availability can enhance the general fitness of trees and therefore increase their pollen productivity. Again, this

phenomenon has received little attention by palaeoecologists and how it might influence fossil pollen analyses remains to be quantified; (iii) the spatial distribution of forest and open vegetation around the sampled basins and the size of vegetation patches in relation to basin size are two other factors that also need accounting for in order to translate the tree pollen percentages to forest cover (Sugita *et al.* 1999; Birks 2005). Although great progress has been made in reconstructing spatial distribution of vegetation from past pollen assemblages (Sugita 2007a; b), modelling techniques of this type are not yet applied systematically to palaeoecological studies. These measures will enable future research to test the high-forest and wood-pasture scenarios against quantitative land cover reconstruction across temperate Europe. In the Oostvaardersplassen, pollen tree over-representation is likely to be due to a combination of intense herbivory and enhanced light availability for isolated trees.

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Author contribution

KW, AB, SB and FV designed the study; AB, MZ and ML collected and analysed the samples; AB analysed the data; AB, KW and SB wrote the paper.

Supplementary Information

Herbivore biomass densities in the Oostvaardersplassen, the New Forest and the forest of Białowieża

In order to estimate the biomass density of large herbivore present in the three protected areas, we first compiled herbivore numbers (Oostvaardersplassen and New Forest) as recorded by counting surveys from the relevant state agencies (State Forestry Service, the Netherlands, and the Forestry Commission, UK, respectively) and herbivore density (forest of Białowieża) as published in [1]. As a second step we compiled data on the body mass of large herbivores. The body mass of cattle, horse and red deer in the Oostvaardersplassen was compiled from two sources: first, regular measurements in the Oostvaardersplassen by the State Forestry Service, the Netherlands, for the period 2008-2012 and second, measurement of all Konik horses and Highland cattle from the wetland nature reserve Zoutkamperplaat, The Netherland, by the RWS Water Service for the period 1991-1994. (Heck cattle have similar body weight to Highland cattle). Because the exact male/female ratio and the age structure of the Oostvaardersplassen populations are unknown, we made the assumption that all genders and age classes were equally represented. The body mass of herbivores in the New Forest and the forest of Białowieża was compiled from [2] and various online resources. Supplementary Table 1 summarises the data compiled and the biomass densities of large herbivores in the three protected areas.

Supplementary Table 1: Biomass density of large herbivores

	Total density (kg/ha)	Numbers (N)	Density (N/ha)	Body mass (kg)	Area (ha)
The Oostvaardersplassen	**474.3**				
Cattle		320		465	1708
Horse		1090		375	1708
Red deer		2500		119	2012
The New Forest	**201.2**				
Fallow deer		1227		60	18375
Roe deer		410		25	18375
Red deer		129		170	18375
Sika deer		98		50	18375
Muntjac deer		35		14	18375
Ponies		4160		500	18375
Cattle		2316		600	18375
Pigs		266		300	18375
Sheep		80		90	18375
Donkeys		99		300	18375
Bialowieza	**15.3**				
Red deer (mostly)			0.09	170	

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Chapter 3: Paper2

The impact of forest canopy shading, forest age and disturbance regimes on pollen productivity: evidence from the old-growth forest of Białowieża National Park, Poland.

To be submitted to the journal **The Holocene,** after modification and agreement with all co-authors:

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The impact of forest canopy shading, forest age and disturbance regimes on pollen productivity: evidence from the old-growth forest of Białowieża National Park, Poland.

Abstract

Estimates of pollen productivity are important in determining changes in land cover and biomass through time. Fossil pollen records contained in lake sedimentary sequences can provide an important tool to estimate the changes in land cover and biomass in response to environmental change. Determining changes from fossil pollen records, however, also requires an understanding of the relative pollen productivity of different plants and their contribution to the pollen rain. In the past decade, a number of important studies have been carried out to determine relative pollen productivity of different species. The vast majority of these studies, however, have been located in the cultural landscapes of lowland temperate Europe where vegetation cover is preponderantly manipulated by human activities such as farming, forestry, industrialisation and urbanisation. The aim of this paper is to ascertain whether pollen productivity from the closed canopy old-growth forest in the Białowieża National Park, Poland where human impact has been minimal for nearly a century, is different from that calculated in cultural landscapes. The key research question we address is: do anthropogenic disturbances alter the amount of pollen released from a plant? We implemented maximum likelihood estimation of relative pollen productivity using data from 18 modern pollen surface samples and distance-weighted plant abundance of seven trees and *Poaceae* located around the surface sample sites. Our results demonstrate that the ratio of pollen productivity between high producers (*Pinus sylvestris* and *Quercus robur*) and low producers (*Poaceae* and *Corylus avellana*) is on an average six times greater in Białowieża than across European cultural

landscapes, i.e. the pollen of forest *Poaceae* and *Corylus avellana* is six times more underrepresented in old-growth forest than hitherto estimated from cultural landscapes. We surmise from this finding that pollen productivity varies depending on the prevailing biotic conditions and this study is a first step towards quantifying this variability. In particular, disturbance regimes and cultural impact over time on the landscape needs to be taken into account when using pollen productivity estimates to determine past land cover and biomass.

Key words

Pollen productivity, old-growth forest, Białowieża National Park, disturbance regime, vegetation cover, *Poaceae*

Introduction

An understanding of future changes in vegetation biomass and land cover in response to external drivers is important to inform land-use planning and policies regarding sustainable land use, ecosystem service provision and food security. The interaction between climate change, nitrogen deposition, habitat fragmentation and a variety of other factors that drive global change makes it challenging to predict future changes (Carpenter *et al.* 2009). Current predictions and future scenarios regarding environmental change rely predominantly on results from modelling (Coreau *et al.* 2009). Some of the most commonly used modelling methods include species envelope models that extrapolate today's species distributions to obtain insight into future distributions under new climatic conditions. Dynamic global vegetation models also predict potential vegetation cover by simulating physiological growth of plant functional groups under the constraint of climatic and soil conditions. However, the outcome of these and other models can vary greatly depending on the algorithm implemented and the parameters used (Fisher *et al.* 2010; Hartig *et al.* 2012). Given the limitations of these algorithms and parameters (Heikkinen *et al.* 2006; Thuiller *et al.* 2008; Kissling *et al.* 2012) it is desirable to test the validity of model outcomes before they are used to inform decision making in the real world. One major

difficulty in testing methods that predict future environmental conditions is that the model outcomes cannot be compared to empirical data because future is unknown. Validation using past trajectories is regularly put forward and palaeoecological data have the potential to provide independent quantitative data for this purpose (Seppä & Hicks 2006; Gaillard *et al.* 2010).

Advances in quantitative reconstruction of vegetation cover using fossil pollen over the last decade have made it possible to estimate land cover throughout the Holocene for the whole of Europe (Gaillard *et al.* 2008, 2010; Hellman *et al.* 2008; Mazier *et al.* 2012). This achievement is due to the conceptual and technical progress in landscape reconstruction algorithms that translate pollen assemblages from small and large lakes into the estimation of percent vegetation cover or biomass, for example, Sugita's Landscape Reconstruction Algorithm (Sugita 2007a; b). Sugita's methodology used in these models solves a major difficulty in the analysis of fossil pollen, i.e. the confounding influence of spatial distribution of vegetation onto pollen proportion in sediments (Davis 2000). One crucial parameter within Sugita's algorithms is the estimation of relative pollen productivity that is specific to different plant species (Hellman *et al.* 2008; Broström *et al.* 2008). Pollen productivity is the number of pollen grains produced by surface cover or biomass of one species (Hicks & Hyvärinen 1999; Sjögren *et al.* 2008; Seppä *et al.* 2009) and relative pollen productivity is the ratio between the pollen productivity of two species (Sugita *et al.* 1999).

To date, relative pollen productivity has been established for all the major tree species throughout cultural landscapes of temperate Europe (Sugita *et al.* 1999; Nielsen 2004; Bunting *et al.* 2005; Soepboer *et al.* 2007; Mazier *et al.* 2008; Poska *et al.* 2011; Twiddle *et al.* 2012; Abraham & Kozáková 2012; Theuerkauf *et al.* 2012; Matthias *et al.* 2012). The cultural landscapes utilised in these studies are made up of mosaics of vegetation types where agriculture and urbanisation directly modify between 40 and 90% of the landscape (Table 1).

There are then two further studies that estimate pollen productivity for various herbs but no trees (Hjelle 1998; Broström *et al.* 2004). Also, there are studies from boreal and subalpine environment where climate is significantly colder and where lower pollen productivity is predictable (Räsänen *et al.* 2007; von Stedingk *et al.* 2008) (Table 1). However, what is still unknown is whether results from cultural landscapes can be utilised to interpret fossil pollen assemblages from landscapes that have undergone different disturbance regimes, for instance old-growth forests or ancient landscapes with vegetation cover relatively untouched by agriculture.

Table 1. Vegetation cover and climatic characteristics of pollen productivity studies using the same methodology as in this paper. Climatic data from Hijmans et al. (2005): AMT = annual mean temperature, TS = temperature seasonality (SD of all months), TAP = total annual precipitation, PS = precipitation seasonality (SD of all months).

Reference	Country	Region	% Forest	% Grassland and arable land	% Others	Forest	Sample media	AMT (° C)	TS (° C)	TAP (mm)	PS (mm)
This Paper	Poland	Bialowieza National Park (Miscicki, 2012)	97	3		Temperate	moss	7.0	8.1	579	31
		Forest of Bialowieza (Mikusinska et al., 2013)	87	10	3	Temperate					
Nielsen (2004)	Denmark	Denmark	10	65	25	Temperate	lake	7.7	6.0	611	22
Theuerkauf *et al.* (2012)	Germany	States of Mecklenburg-Vorpommern and Brandenburg	22	59	19	Temperate	lake	8.1	6.7	579	22
Soepboer *et al.* (2007)	Switzerland	Swiss Plateau	25	50	25	Temperate	lake	8.7	6.4	1103	25
Matthias *et al.* (2012)	Germany	State of Brandenburg	37	45	18	Temperate	lake	9.0	6.8	567	22
Sugita *et al.* (1999)	Sweden	i. Southern Skane	20-40	60-80		Temperate	moss	7.3	6.7	494	24
		ii. Nothern Skane and Smaland	50-60	40-50		Temperate					
Poska *et al.* (2011)	Estonia	South Estonia	51	31	18	Temperate/Boreal	lake	5.0	8.7	605	33
Mazier *et al.* (2008)	Switzerland	Parc Jurassien Vaudois	60	40		Montane	moss	4.5	6.1	1622	18
Abraham & Kozáková (2012)	Czech Rep.	Central Bohemia	Forest fragments within agricultural landscape			Temperate	moss	7.9	6.9	533	40
Bunting *et al.* (2005)	England	Norfolk	Forest fragments within agricultural landscape			Temperate	moss	10.3	4.8	618	15
Twiddle *et al.* (2012)	Scotland	Cairngorm National Park	Forest fragments within agricultural landscape			Temperate/Boreal	moss	7.2	4.3	785	16
von Stedingk *et al.* (2008)	Sweden	Skalstugan, Scandes Mountains	<30		>70	Boreal	moss	1.6	7.0	962	21
Räsänen *et al.* (2007)	Finland	Northern Finland	Large pine forests, with treeless mires and mountain tops			Boreal	moss	-0.8	8.8	438	42

This study is situated in the Białowieża National Park, an area of extensive old-growth forest (not exploited by forestry and other human activities) nested at the heart of the largest fragment of temperate forest in lowland Europe. This is therefore a landscape that has had minimum human impact over time and can serve as an important contrast to the cultural landscapes that have been used to previously calculate estimates of pollen productivity.

The overarching aim of this study therefore was to estimate pollen productivity from trees and *Poaceae* from the Białowieża National Park and compare it with pollen productivity from cultural landscapes (where human activity is a preponderant source of disturbance). Our key objectives of this study were as follows:

i. To establish relative pollen productivity estimates for the main trees and *Poaceae* in the Forest of Białowieża.
ii. To compare pollen productivity estimates from Białowieża to those obtained in cultural landscapes across Europe.
iii. To discuss any differences in the light of the disturbance regimes prevailing in old-growth forests and cultural landscapes.

Study Area

The Forest of Białowieża (hereafter Białowieża) is a flagship ecosystem in European nature conservation (Peterken 1996; Bobiec 2002). It covers about 1500 km^2 straddling the border between Poland and Belarus. This expanse of lowland temperate closed canopy forest is unique for the complete assemblage of native trees, large herbivores and carnivores, for its size and for its antiquity (Faliński 1986). The main natural disturbance factors that have been shaping the forest composition and structure are: large herbivore selective browsing (Kuijper *et al.* 2010b; Smit *et al.* 2012), pathogen outbreaks such as the European spruce bark beetle (*Ips typographus*) (Bobiec *et al.* 2011; Miścicki 2012), fire regimes (Niklasson *et al.* 2010) and wind throws (Faliński 1978). The historical variation of these disturbance regimes explains much of the forest diversity today (Pigott 1975; Mitchell & Cole 1998; Bobiec 2012), along with underground water levels. Large herbivores and carnivores in this forest include bison (*Bison bonasus*), elk (*Alces alces*), red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), wild boar (*Sus scofa*), wolves (*Canis lupus*), lynx (*Lynx lynx*) and others. The bison population is highly managed with supplementary feeding and regular yearly culling that target around 10% of the

population. Hunting is also an important factor in determining large herbivores population size in Białowieża. For instance, it significantly influences the red deer population in terms of behaviour and number of individuals (Jedrezejewski *et al.* 2006). The main trees found in the forest are: *Picea abies*, *Quercus robur*, *Carpinus betulus*, *Alnus glutinosa*, *Tilia cordata*, *Pinus sylvestris*, *Fraxinus excelsior*, *Acer platanoides*, *Betula pendula* and *B. pubescens* (hereafter *Betula*), *Populus tremula*, *Ulmus glabra* and *U. minor* (Miścicki 2012).

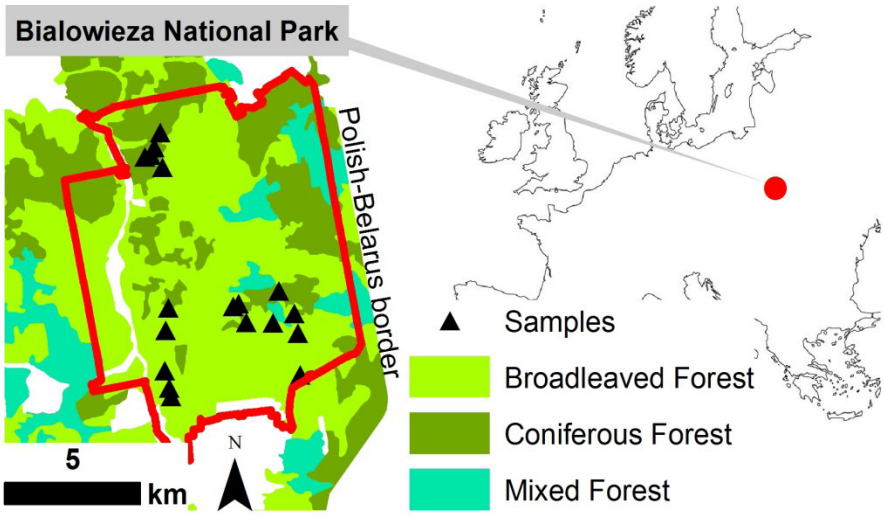


Figure 1. Map of the Białowieża National Park and sample location. The vegetation cover categories follow the CORINE land use (Belarus not covered).

The Forest of Białowieża has been under protection from agricultural development from the Middle Ages when it became a game reserve for Lithuanian and Polish rulers (Samojlik 2005). As a result, it has remained remarkably untouched by human activities in contrast with most of the rest of Europe (Faliński 1986). No destructive activity such as large-scale agriculture or industry has even been prominent, although small-scale activities such as subsistence farming, potash baking, tar, charcoal and pitch making, honey harvesting and hay making are all reported from historical archives (summarised in Bobiec, 2012). The area has experienced exceptional stability with regards to land cover for the last 200 years (Mikusinska *et al.* 2013). Today it has international protection as a UNESCO Biosphere Reserve and World Heritage site, a Ramsar site, and a Natura 2000 site as well as having protected status at the national and local level. The

most restrictive management applies to 105 km^2 of strict protection within the Polish Białowieża National Park (Figure 1) and to 157 km^2 at the heart of the Belarus *Belovezhskaya Pushcha* National Park, where no human intervention is allowed and access is limited to research or restrictive tourism. Outside this area of strict protection both hunting and forestry are current practice, thereby directly influencing the large herbivore and tree populations.

Methods

Pollen

All the field work was carried out in the strict reserve of the Polish Białowieża National Park. Forest floor mosses were collected for the modern pollen they contain, including both green and brown parts (Räsänen *et al.* 2004). We sampled 3-4 moss sub-samples within a 1 m^2 quadrat using a ring of 21.2 cm^2. The sub-samples were mixed in a plastic bag and kept in dark conditions at 4^o C. In the laboratory, the moss polsters were rinsed in distilled water, *Lycopodium* tablets were added and dissolved in HCl 10%, they were then boiled in 10% KOH for a short time (5 minutes), followed by Erdman's acetolysis treatment, staining and mounting in glycerine (Willis & Bennett 2001). The extracted pollen was identified with pollen atlases (eg Beug 2004) and the reference collection of the Laboratory of Palaeoecology and Archaeobotany, University of Gdansk.

Vegetation

The vegetation survey followed standard practice for pollen productivity estimation (Bunting 2003; Bunting & Hjelle 2010) and was conducted around each 1 m^2 quadrat sampled for pollen. The ground flora was recorded using four transects of four 1 m^2 quadrats to 10 m away from the sampling point (distances from 0.5, 1.5, 3.5 and 7.5 m). Damages from large herbivores were recorded for each species. The canopy cover was also recorded using four transects along a distance of 100 m. For each transect the canopy composition and percent cover was recorded

within four 5m-radius relevés (distance 15, 30, 50 and 90 m), while canopy directly above the sampling quadrat was recorded in a 10-m radius relevé. All plant identifications followed the Polish Flora (Rutkowski 1998) or other European Floras when necessary, and Eggenberg and Möhl (2008) for vegetative characters. The rest of the vegetation data was drawn from the Białowieża National Park cartography of forest types (Michalczuk 2001). For each forest type we compiled from the relevant phytosociological literature a list of tree and grass species and their average percent cover within the community. In a GIS environment (ArcGIS 10.1), we compiled the survey and forest map data into a single vegetation map and split it into adjoining concentric rings at regular distances from the sampling point (0.5, 1.5, 3, 6, 10, 23.7, 41.2, 72.8, 100 meters and then every 50 m from 100 m to 1000 m). Thus, we obtained the surface cover of all tree and herbs species (in m^2 or %) within each concentric ring.

Data Handling and Modelling

To estimate the pollen productivity of main trees in Bialowieza, we undertook a whole modelling approach similar to that of Prentice and Sugita (Parsons & Prentice 1981; Prentice & Parsons 1983; Sugita 1993; Sugita *et al.* 1999). This approach estimates relative pollen productivity by maximum likelihood using pollen counts as a response variable and vegetation data as explanatory variable. We implemented three types of distance-weighting transformation on the vegetation data (Prentice & Parsons 1983; Prentice 1985; Sugita 1993; Sugita *et al.* 1999) by down-weighting plant species surface cover in the concentric rings, proportionally with distances from the sampling points. This method simulates pollen dispersal such that pollen accumulation is influenced by the distance of a sampling point from the pollen source. The three methods are inverse distance (1/d), inverse square distance ($1/d^2$) and pollen-type specific (pt-s) distance-weighting (Prentice 1985). Pollen-type specific distance-weighting is based on the travelling of small particles in turbulent air above the canopy and has been successfully applied in similar situations (Broström *et al.* 2008).

Three maximum likelihood algorithms were used to obtain relative pollen productivity estimates. These were implemented using vegetation data, distance-weighted in three ways as presented above, resulting in nine combinations of algorithm-distance-weighting. The algorithms are named erv-1, erv-2 and erv-3 (Prentice & Parsons 1983; Sugita 1993) and make different assumptions regarding the background pollen contribution across sites (summarised in Broström *et al.* 2008). There is no empirical or theoretical reason to favour one algorithm over another, so all three are usually applied (Broström *et al.* 2008). The relevant source area of pollen (Sugita 1994) was established by implementing each of the nine combinations of algorithm-distance-weighting at the same regular distances used for distance-weighting. We used the log-likelihood indicator to select the distance and combination of algorithm-distance-weighting that fitted best our pollen and vegetation data. The relevant source area of this best combination of parameters is reached when the log likelihood indicator stops improving with distance (ie it plateaus off when plotted against distance) (see Figure 3).

The final selection of plant species was obtained by repeating the analysis above until all pollen productivity estimate values returned by the modelling were plausible (between 0.01 and 100) and had reasonable standard errors (<10% of the pollen productivity estimate). The distance-weighting and maximum likelihood calculations were implemented with the 'ERV.Analysis.v1.3.0' programme (S. Sugita, unpublished).

To compare the pollen productivity estimates obtained in Białowieża with those of other studies, we compiled from the published literature all relevant estimates obtained with similar methods (Sugita *et al.* 1999; Nielsen 2004; Bunting *et al.* 2005; Soepboer *et al.* 2007; Mazier *et al.* 2008; Poska *et al.* 2011; Twiddle *et al.* 2012; Abraham & Kozáková 2012; Theuerkauf *et al.* 2012; Matthias *et al.* 2012). We also added to this list the most recent consensus values compiled by Mazier et al. (2012).

Results

A total of 18 samples were collected in August 2011 from various locations and with a variety of sample characteristics (Table 2; Figure 1). All samples had abundant pollen. Damage from herbivory was virtually absent in herbaceous plants (including *Poaceae*) but was relatively common in juvenile woody plants in the shrub layer of the forest's undergrowth.

Table 2. Sample locations and characteristics. Moss growth form follows Vanderpoorten and Goffinet (2009).

Site code	North	East	Forest community	No of sub-samples	Moss growth form	Pollen sum
site 1	52.72464	23.83144	fresh broadleaf forest	3	rough mat	673
site 2	52.73317	23.83069	fresh broadleaf forest	3	rough mat	586
site 3	52.72742	23.83142	fresh broadleaf forest	3	rough mat & weft	928
site 4	52.74947	23.87708	fresh mixed broadleaf forest	3	large cushion & weft	990
site 5	52.75086	23.90003	fresh mixed conifer forest	3	tall turf, weft & large cushion	901
site 6	52.74308	23.87919	moist broadleaf forest	3	weft & rough mat	1006
site 7	52.74892	23.87383	fresh mixed broadleaf forest	3	large cushion & weft	934
site 8	52.74567	23.83531	moist mixed broadleaf forest	4	worled-branche turf	1011
site 9	52.75253	23.83903	fresh mixed broadleaf forest	3	tall turf	1024
site 10	52.80144	23.84486	fresh conifer forest	3	weft	1179
site 11	52.79686	23.85008	moist broadleaf forest	3	weft	1256
site 12	52.74278	23.90583	fresh broadleaf forest	4	rough mat	1173
site 13	52.74119	23.89347	fresh broadleaf forest	4	weft	1035
site 14	52.73644	23.90583	moist broadleaf forest	3	weft	1082
site 15	52.72322	23.90278	fresh broadleaf forest	3	tall turf	931
site 16	52.80347	23.84758	fresh conifer forest	3	weft	1213
site 17	52.80786	23.85231	bog conifer forest	4	whorled-branch turf	1169
site 18	52.80111	23.84156	fresh conifer forest	3	weft	1125

The species analysed were *Alnus glutinosa, Betula* spp. (including *Betula pendula* and *Betula pubescens*), *Carpinus betulus, Corylus avellana, Pinus sylvestris, Poaceae* species, *Quercus robur* and *Tilia cordata* (Figure 2). Other pollen-types were not suitable for modelling with the methodology used. Notably *Picea abies*, an important component in Białowieża's forest, did not have a positive relationship between pollen percent and distance-weighted percent cover, so the models returned unrealistic results with very large standard errors. *Cyperaceae* species did not show a good enough spread of data either, also producing very large standard errors and unrealistic results.

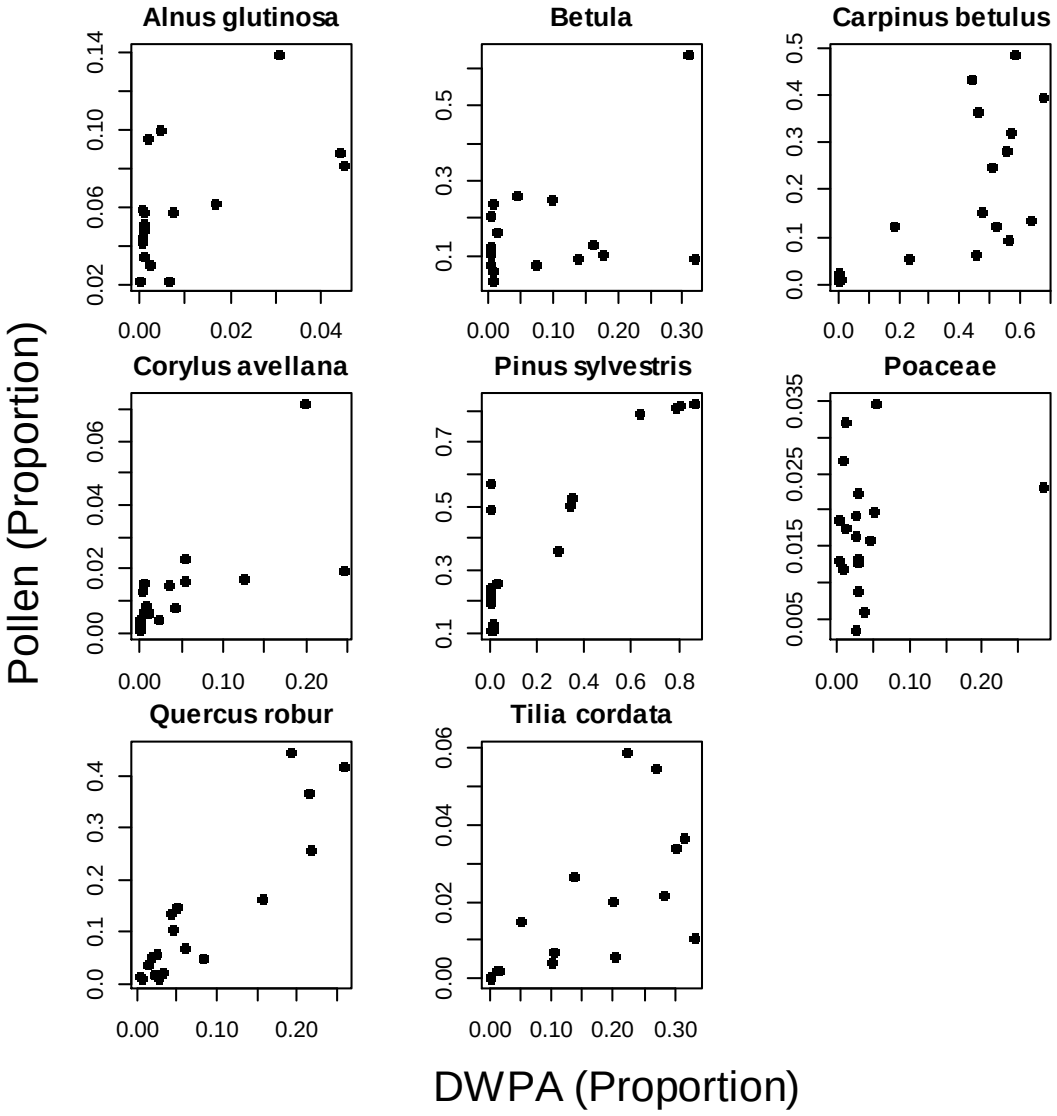


Figure 2 A. Pollen proportions plotted against the distance weighted abundance of taxa. The distance weighting method presented here is inverse distance (1/d).

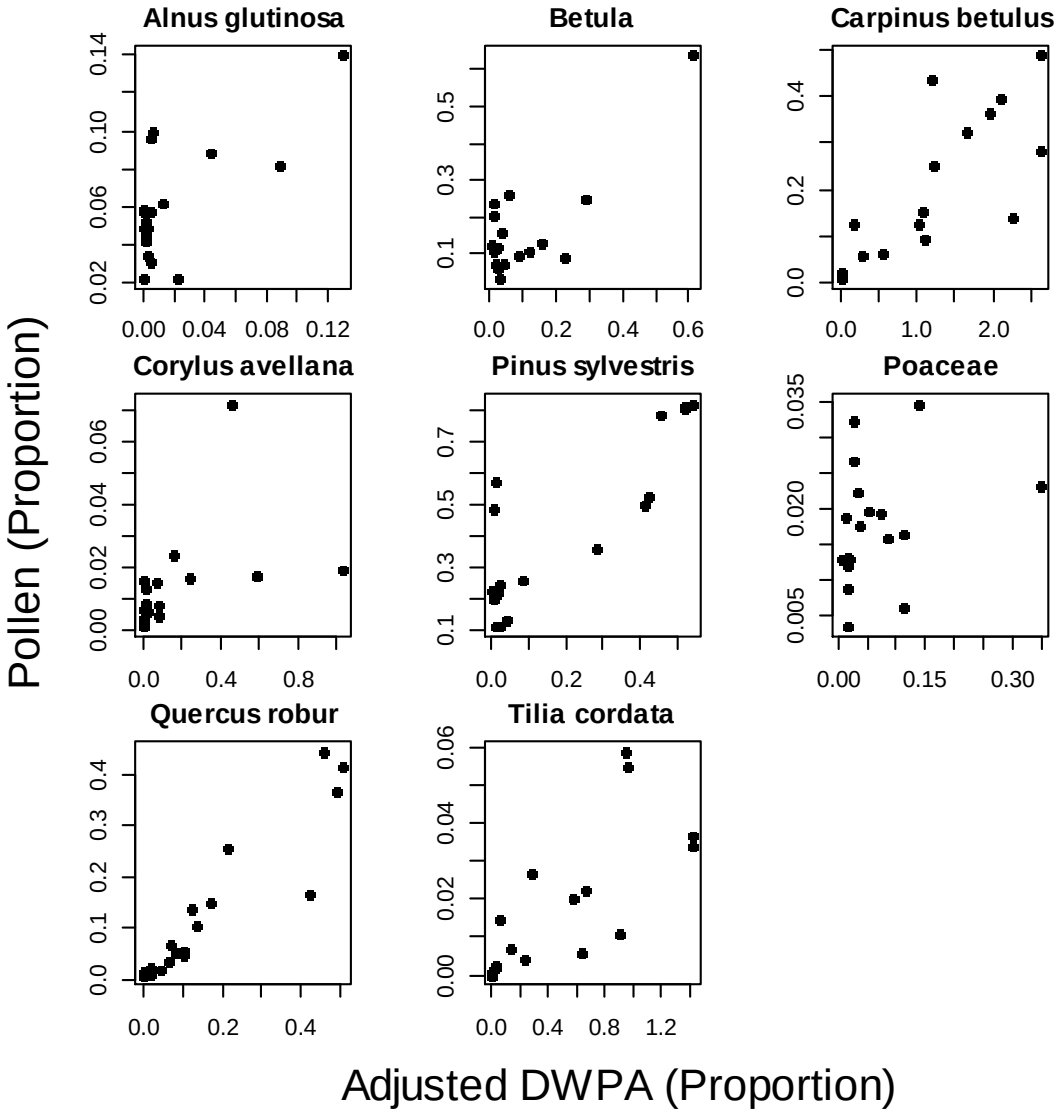


Figure 2 B. Pollen proportions plotted against distance weighted abundance as in Fig 2 A but this time adjusted according to the pollen productivity estimates and the background pollen rain as estimated by the algorithm erv-1. The distance weighting method used is inverse distance (1/d), the wind speed was estimated at 3 m/s, and the model used is erv-1.

Amongst distance-weighting methods, 1/d and pt-s returned nearly equally good results with all three algorithms (Figure 3 A-C), while $1/d^2$ systematically returned worse log-likelihoods. In such situations it is appropriate to recommend 1/d, the simplest distance-weighting method. Although pt-s distance-weighting is theoretically more accurate because different pollen-types have different flight abilities, the model currently in use (Prentice 1985; Sugita *et al.* 1999) only reflect pollen dispersal above the forest canopy (Jackson & Lyford 1999; Theuerkauf *et al.* 2012).

In our case, pollen samples were collected under the canopy and therefore this type of distance-weighting may not be directly applicable.

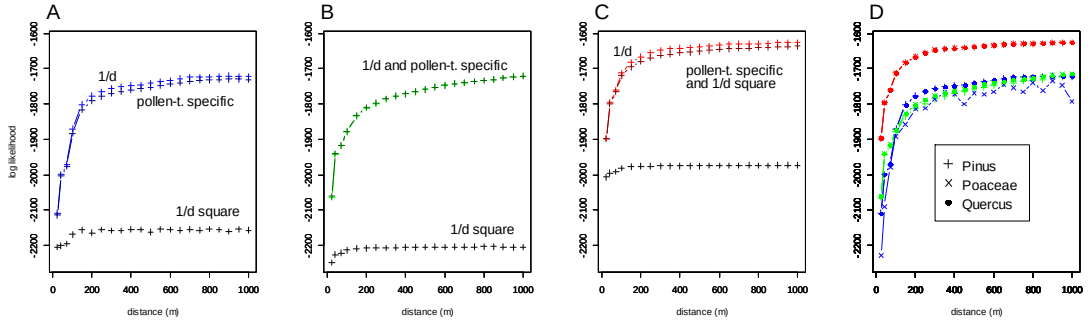


Figure 3. Log Likelihood values for the selection of the distance weighting method (A-C) and of the selection of reference species (D). Shades of blue are for algorithm erv-1, shades of green, for erv-2, shades of red, for erv-3. 1/d: inverse distance.

When using *Poaceae* as a reference taxa (Broström *et al.* 2008), the erv-1 algorithm did not perform as consistently as erv-2 and erv-3, as shown by the variability of the log-likelihood when plotted against the distance gradient (Figure 3 D). Because this behaviour is not observed when using *Pinus sylvestris* or *Quercus robur* as reference species, it was concluded that the data collected for *Poaceae* was less suitable to be used as reference taxa (Figure 3). As a result, we suggest that using *Pinus sylvestris* (Theuerkauf *et al.* 2012; Matthias *et al.* 2012) or *Quercus* (Bunting *et al.* 2005) is more appropriate in our case but we also provide pollen productivity values relative to *Poaceae*, for comparison with similar published analyses (Table 3).

Table 3. Relative pollen productivity estimates in the Forest of Białowieża

	relative to Poaceae				relative to Pinus sylvestris				relative to Quercus robur			
	erv-1	SE	erv-3	SE	erv-1	SE	erv-3	SE	erv-1	SE	erv-3	SE
Poaceae	1.00	na	1.00	na	0.051	0.0048	0.039	0.0246	0.066	0.0066	0.049	0.0406
Alnus glutinosa	33.28	0.8581	15.95	0.6622	0.488	0.0143	0.680	0.0280	0.697	0.0230	0.873	0.0404
Betula	30.08	0.4084	13.94	0.2293	0.505	0.0072	0.604	0.0095	0.726	0.0104	0.758	0.0199
Carpinus betulus	10.43	0.1461	4.48	0.0301	0.162	0.0015	0.195	0.0020	0.234	0.0025	0.244	0.0066
Corylus avallana	2.78	0.1418	1.35	0.0512	0.045	0.0018	0.057	0.0021	0.066	0.0026	0.072	0.0032
Pinus sylvestris	51.38	0.7011	23.12	0.2388	1.000	na	1.000	na	1.457	0.0217	1.256	0.0323
Quercus robur	44.76	0.5937	18.47	0.1032	0.694	0.0077	0.808	0.0071	1.000	na	1.000	na
Tilia cordata	2.18	0.0596	0.98	0.0263	0.034	0.0008	0.043	0.0010	0.049	0.0011	0.053	0.0020

The relevant source area is within a 400m radius distance from sampling points with erv-1 and erv-3. Using these algorithms, estimated pollen productivity values for all species stabilise before 400m along a distance gradient (data not shown). In contrast the pollen productivity

estimates obtained with the algorithm erv-2 do not show any stable pattern along the distance gradient while log-likelihood values do not reach a horizontal asymptote as expected with this method (Sugita 1994). We decided not to retain results of model erv-2 on this basis, although the pollen productivity estimates were in the same range of values than those obtained with models erv-1 and erv-3.

The pollen productivity estimates relative to *Pinus sylvestris*, *Poaceae* and *Quercus robur* are presented in Table 3. This table only presents the pollen productivity estimates obtained with the best setting identified for the Białowieża data, ie algorithms erv-1 and erv-3, 1/d distance-weighting and wind speed of 3 m s-1. Results indicate that *Alnus glutinosa*, *Betula*, *Quercus robur* and *Pinus sylvestris* are high pollen producers, *Carpinus betulus* is an intermediate pollen producer and *Poaceae*, *Corylus avellana* and *Tilia cordata* are relatively very low producers. There is between 23- and 50-fold difference of pollen productivity between the highest producers (*Pinus sylvestris, Quercus robur*) and the lowest producers (*Poaceae* and *Tilia cordata*).

Table 4. Ratio between high pollen producers and low pollen producers in this study and across Europe.

		Highest producers		Lowest producers		Range of ratio between high and low producers
	Location	*Pinus*	*Quercus*	*Corylus*	*Poaceae*	
This study - erv1	Poland	51.38	44.76	2.78	1	51 - 16
This study - erv3	Poland	23.12	18.47	1.35	1	23 - 14
Mazier *et al.* (2012)	Europe-wide	6.38	5.83	1.99	1	6 - 3
Räsänen *et al.* (2007)	Finland	8.4	-	-	1	8
Sugita *et al.* (1999)	S Sweden	5.66	7.53	1.4	1	8 - 4
Bunting *et al.* (2005)	England	-	5.83	1.51	1	6 - 4
Poska *et al.* (2011)	Estonia	5.07	7.39	-	1	7 - 5
Soepboer *et al.* (2007)	Switzerland	1.35	2.56	2.58	1	3 - 0.5
Abraham & Kozáková (2012)	Czech Rep.	6.17	-	-	1	6

Comparing our results to values from similar studies across Europe indicates that the difference between low and high producers is much higher in Białowieża than in any other place in the rest of Europe (Table 4). The ratio of pollen productivity between the highest producers (*Pinus*

sylvestris and *Quercus robur*) and the lowest producers (*Poaceae*, *Corylus avellana*) is on average six times greater in Białowieża than across European cultural landscapes. This means that either closed-canopy forest *Poaceae* and *Corylus avellana* are six times more underrepresented than hitherto estimated from cultural landscapes, or *Pinus sylvestris* and *Quercus robur* are six times more over-represented, or any mixture between these two extreme explanation. However, because it is based on ratios, the modelling method used here does not allow us to ascertain whether high producers in Białowieża are higher producers in absolute term, or whether low producers produce an absolute lower quantity of pollen. Figure 4 also highlights that the four lower pollen producers (*Poaceae*, *Tilia cordata*, *Corylus avellana* and *Carpinus betulus*) are lower producers in Białowieża than in the rest of Europe in relation to *Pinus sylvestris*. When expressed in relation to *Poaceae*, however, *Carpinus betulus* and *Tilia cordata* are higher producers than in the rest of Europe. This suggests that *Carpinus betulus* and *Tilia cordata* have a similar pollen productivity in Bialowieza than in cultural landscapes, unlike the reference species used (*Poaceae*, *Quercus robur* and *Pinus sylvestris*). Also the relative pollen productivity of *Corylus avellana* to *Poaceae* has a similar relationship in Białowieża to the rest of Europe, suggesting their pollen productivity is robust across space.

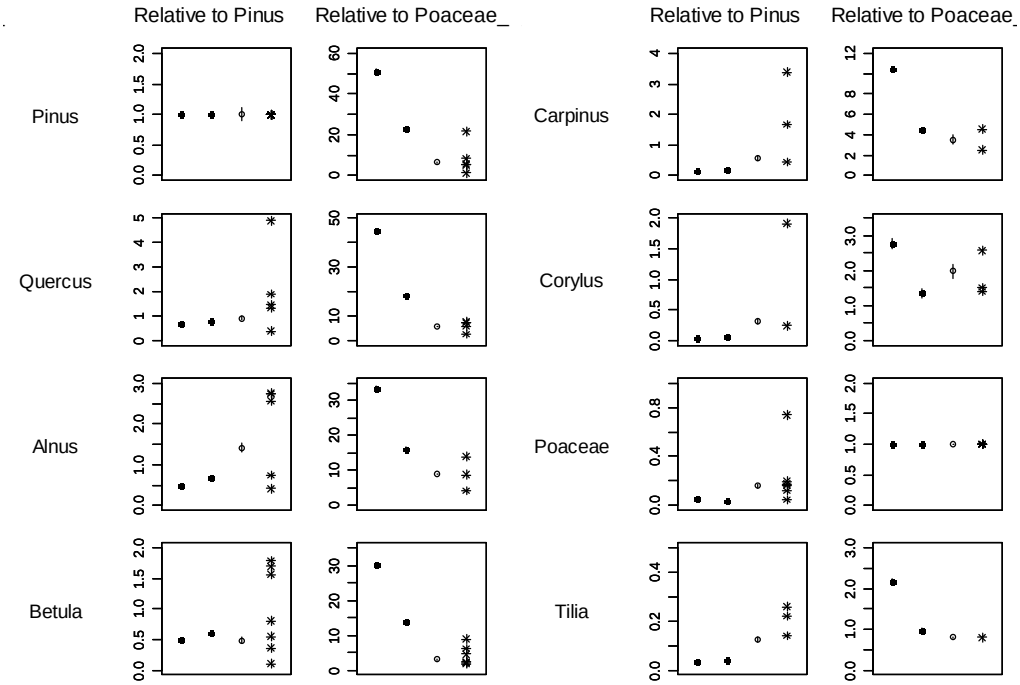


Figure 4. Comparison of the pollen productivity estimates obtained in this study with those from the rest of Europe. The two first back dots in each plot are this study's erv-1 and erv-3 results, the empty dot the recommended productivity from Mazier et al. (2012), the stars are the productivity estimates in all other studies from temperate Europe (see Table 1 for references). Note that sometimes stars can overlap and that y axes have different scales. See text for the species names.

Discussion

We demonstrate that for some taxa (*Pinus sylvestris, Quercus robur, Poaceae*, and *Corylus avellana*) pollen productivity estimates measured in Białowieża old-growth forest show a distinct signature in comparison with those calculated from cultural landscapes across the rest of Europe. For this study, we defined cultural landscapes as those showing a mosaic of habitats shaped by human activities like farming, industrialisation and urbanisation. In particular, the ratio of pollen productivity between high producers (*Pinus sylvestris* and *Quercus robur*) and low producers (*Poaceae*, *Corylus avellana*) is on an average six times greater in Białowieża than across European cultural landscapes. There are several reasons why this might be the case including different methodologies between studies, extrinsic abiotic factors such as climate influencing pollen productivity rates and intrinsic biotic factors such as the composition of the

vegetation and the disturbance regimes that the forest has been undergoing. Each of these will be discussed in turn in the next sections.

Media sampled and vegetation survey

First, we needed to address whether or not the differences in results obtained were due to different sample media (moss or lake sediments), as previous studies have suggested that in samples from lake sediments, littoral vegetation can be over-represented (Broström *et al.* 2008). To remove this bias we used moss samples away from open water. Also, as seen on Table 1, sampling mosses is the most common way to acquire modern pollen assemblages, so our results are comparable to most other studies. In addition, new data indicate that pollen assemblages collected from different media do not show significant differences (Lisitsyna *et al.* 2012); this finding is in contrast with previous results from the Southern Hemisphere (Wilmshurst & McGlone 2005).

Another consideration of type of media is that pollen assemblages in mosses may be biased by occasional extreme meteorological events, which induce variation in absolute pollen productivity of some plant species (Sjögren *et al.* 2006). We addressed this by sampling mosses forming mats as thick as possible, including the lower brown parts, and representing as many years as possible. Moreover, we sampled similar types of forest floor mosses (pleurocarps or *Sphagnum*) throughout the study area so as to avoid large discrepancies in rain accumulation time between samples (see Table 2).

Methods used to survey the plant species abundances around moss samples have also been shown to influence the pollen productivity estimates of various herbs and *Ericaceae* (Bunting & Hjelle 2010). However, the relevant source area of pollen of the habitats studied in that piece of research is very low (around 1-2 m) in comparison with ours (400 m) giving far more weight to the field survey data in the estimation of pollen productivity than in our case. In addition our

study principally deals with tree pollen productivity and we followed the same methodology as other similar studies.

The influence of extrinsic abiotic factors such as climate

Plant species experience limited fitness towards the extreme edge of their distribution range because of sub-optimal climatic conditions and as a result produce pollen less abundantly. Of the tree species dealt with here, four are at the centre of their distribution range (*Betula*, *Pinus sylvestris*, *Tilia cordata*, *Corylus avellana*), while the three others (*Alnus glutinosa*, *Quercus robur*, *Carpinus betulus*) are in the eastern part of their distribution but not towards the edge (Jalas & Suominen 1973, 1976). Therefore, it is unlikely that the pollen productivity estimates in Białowieża differ because the tree species studied are not experiencing optimal climatic conditions for growth and flowering in the area.

Also, some pollen types may comprise different species in different climatic regions of Europe. For instance, the *Quercus* pollen recorded from our samples comes from the Białowieża native *Q. robur*. However, in other studies (Soepboer *et al.* 2007; Theuerkauf *et al.* 2012) both *Quercus robur* and *Q. petraea* abundance were included for the estimation of *Quercus* spp. pollen productivity . There is a similar situation with *Alnus incana*, a common tree in central Europe that produces pollen morphologically similar to that of *A. glutinosa* but that is not present in Białowieża. However, it is sometimes included in pollen productivity estimation of *Alnus* spp. (Poska *et al.* 2011). We are not aware of any direct evidence specifically demonstrating that these species (*Q. robur*-*Q. petraea* and *A. glutinosa*-*A. incana*) have the same pollen productivity but it is likely to be the case because they show extremely similar growth form, vigour, phenology and are genetically very closely related (respectively, Muir *et al.* 2001; Chen & Li 2004). Consequently there is no reason to believe that the species assemblage present in the climatic condition of Białowieża is sufficient to explain the unique characteristics of our results.

Age of trees

Matthias *et al.* (2012) suggest that in cultural landscapes where forests are exploited, calculated pollen productivity is likely to be lower than what is expected from a closed canopy old-growth forest. They argue that forest stands reach sexual maturity only after 10 to 50 years depending on the tree species (and hardly produce any pollen until then). As a result, young tree plantations would not produce pollen, yet this fraction of forest cover tends to be included in datasets for the estimation of pollen productivity. The result would be an over-estimation of the tree species' surface cover, resulting in an under-estimation of pollen productivity per meter square. However, the old-growth forest of the Białowieża National Park is also a dynamic ecosystem where regeneration of young trees is integral part of the vegetation cover (Faliński 1986; Sokolowski 1993). In particular, there is an increasing body of evidence showing that Białowieża's tree recruitment is increasing (Miścicki 2012) and being constantly shaped by disturbance factors like browsing (Kuijper *et al.* 2010a; b) and fires (Niklasson *et al.* 2010). It is difficult to predict how juvenile trees (whether clustered together in cultural landscapes or found in admixture with older trees in large close canopy old-growth forest) impact our calculations but it is unlikely that in our research area, the proportion of young trees is significantly lower.

Canopy structure and light limitation

In Białowieża, *Corylus avellana* is restricted to very shaded forest undergrowth and very rarely reaches the canopy (Sokolowski 1993; AGB's personal observation); this is in direct contrast to its presence on other cultural landscapes. Therefore light limitation is likely to reduce drastically the reproductive fitness of *Corylus avellana*, which is generally associated with open or semi-open habitats such as hedgerows in cultural landscapes (eg Ellenberg 1988). We suggest that this might well account for the much lower pollen productivity than hitherto assumed and the

values calculated in this study should be used in the interpretation of fossil pollen from past forested environments.

Poaceae flowering is also possibly reduced by the low light levels occurring in forest understorey in comparison to agricultural situation. It has been highlighted that *Poaceae* comprises numerous species and as a result, pollen productivity from different areas may be different depending on which species is present (Broström *et al.* 2008). In fact, many of the *Poaceae* species found in Białowieża during our survey are specialists of forest environments (*Poa nemoralis*, *Festuca altissima*, *Deschampsia cespitosa* subsp. *parviflora*, *Dactylis aschersoniana*, *Brachypodium sylvaticum* and *Melica nutans*) that are expected to be lower pollen producers than plants species growing in full light conditions outside forest. In addition other more ubiquitous *Poaceae* recorded during our survey (*Phragmites australis*, *Molinia caerulea* sl, *Festuca ovina*, *Deschampsia flexuosa*, *Danthonia decumbens, Calamagrostis arundinacea*) are expected to show reduced fitness as a result of the shading from the canopy.

Grazing, Browsing and nutrient cycling by large herbivores

Large grazers directly impact on herbaceous plant flowering, either by physically suppressing flowering in a continuous way (Groenman-van Waateringe 1993; Vera 2000) or by enhancing the production of inflorescence and lengthening the flowering season, a phenomenon known as over-compensation (see eg Massad 2013). However, in Białowieża, grazing is unlikely to explain the low *Poaceae* pollen productivity for three reasons. First, we did not find any signs of grazing during our survey and *Poaceae* cover is low in the quadrats near our sampling points (mean= 3.1 %, n=306), i.e. it never consists in a grazing lawn likely to sustain regular grazing. Second, ungulates in Białowieża tend to graze and browse preferentially in forest gaps (Kuijper *et al.* 2009) not covered in our study. Third, bison is the only large herbivore truly adapted to grazing within the Białowieża faunal assemblage (Kerley *et al.* 2012; Cromsigt *et al.* 2012) but receives winter supplementary feeding and thus does not depend for survival on forest floor *Poaceae*.

The other large herbivores are primarily browsers. Browsing has a pervasive effect on the forest composition in Bialowieza, where herbivores literally select the canopy trees of tomorrow (Kuijper *et al.* 2010a). However they can only reach young seedling and sapling and in this respect, browsing herbivores do not impact pollen productivity in a direct way in the short term.

An alternative potential impact is enhanced nitrogen availability promoted by large herbivores (carcases, dung and urine). More nutrients can induce enhanced general fitness and flowering, hence higher pollen productivity. The density of large herbivores in Białowieża is particularly high and the assemblage of native European large vertebrate is one of the most complete on the continent (eg Jędrzejewska *et al.* 1997). Long-term manipulation field experiments in the UK shows that flowering can increase up to 3 times in woody plants but decreases in herbaceous species with nitrogen addition (Phoenix *et al.* 2012). Moreover, North American *Quercus* spp. have been shown to enhance their flowering in response to nitrogen addition (Callahan *et al.* 2007). Large herbivore activity alone does not add nitrogen to the system but redistributes it in a more heterogeneous way (Augustine & Frank 2001; Hobbs 2006; Bump *et al.* 2009; Murray *et al.* 2013) and modifies the cycling of nutrients (reviewed in Pastor *et al.* 2006). As a consequence it is difficult to assess the impact of nutrient manipulation by large vertebrates on our results, because the exact distribution of the animals within the forest and at our 18 sampling sites is not sufficiently known. However, this is a factor that needs further consideration. Moreover, we do not currently hold any explanation as to why the impact of nutrient would be different for on one hand *Pinus sylvestris*, *Quercus robur, Alnus glutinosa* and *Betula* (higher producers than expected) and for, on the other hand, *Tilia cordata*, *Carpinus betulus* and *Corylus avellana* (lower producers than expected).

Conclusion

To date, pollen productivity has only been estimated in temperate Europe from cultural landscapes where human activities such as agriculture, industrialisation and urbanisation are a

preponderant source of disturbance. We found that pollen productivity in the closed canopy old-growth Forest of Białowieża (where disturbance by human activities is not preponderant) was different from that measured in cultural landscapes. In fact the difference of productivity between high pollen producers (i.e. *Alnus glutinosa*, *Betula*, *Querus robur* and *Pinus sylvestris*) and low pollen producers (i.e. *Poaceae*, *Corylus avellana*) is distinctively more pronounced in old-growth forest than hitherto estimated. We proposed that this is related to the forest structure and composition, where *Poaceae* and *Corylus avellana* grow in a relatively shaded situation and do not receive sufficient light for abundant pollen production. Besides light, another factor that may influence the production of pollen is the nutrient availability as a result of modification of nitrogen cycling by large herbivores. The main consequence of our findings is that pollen productivity can fluctuate depending on the prevailing biotic conditions and disturbance regimes. We present a first attempt to quantify this variability but further work will be needed in order to integrate this variability into vegetation cover reconstructions using fossil pollen.

Acknowledgements

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Chapter 4: Paper3

Do dung fungal spores make a good proxy for past distribution of large herbivores?

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Do dung fungal spores make a good proxy for past distribution of large herbivores?

Abstract

The importance of herbivory as a long-term driver of ecosystem change is a topic that has been hotly debated over the past few years. An understanding of the interaction between herbivores and ecosystems is particularly important for conservation policies aimed at re-wilding. Dung fungal spores have been highlighted as an important potential proxy to reconstruct large herbivore densities across past landscapes. However, this proxy appears to have been used and interpreted in a variety of ways in addition to highly variable taxonomic identification of dung fungal spores. Here we review studies that have utilised fungal spore assemblages to assess past herbivore presence and test the validity of this method. We aim to determine whether there is a set of identifiable dung fungal spores that can unequivocally track variation of large herbivore activity through time and across regions. Our meta-analysis identifies: (1) spore types that are commonly found to be indicative of large herbivores and their geographical ranges, (2) linkages between these spores and their biological origin, and (3) the most appropriate quantitative method to express their abundance for comparisons through time and across sites.

Keywords

Dung fungal spore; herbivory; *Sporormiella*; non-pollen palynomorphs; palaeoecology; reference collection

Introduction

Large herbivores are increasingly accepted as playing an important role as ecosystem engineers across most of the Earth's biomes (Chase *et al.* 2000; Hopcraft *et al.* 2009; Griffiths *et al.* 2011; Estes *et al.* 2011). The disturbance regimes they create are often significant enough to modify vegetation structure and composition. Landscapes altered by domesticated and wild herbivores

range from cultural landscapes such as the New Forest in the UK (Putman 1986; Tubbs 1986, 2001) through to wilderness area like the Serengeti in Tanzania (Sinclair & Norton-Griffiths 1979; Sinclair & Arcese 1995). In these herbivore-dominated ecosystems, grazing can represent one of the main drivers of disturbance along with fire regimes, human activity and climatic variability (Jeffers *et al.* 2012).

The past occurrence of large vertebrate herbivores and their impact on the Quaternary landscape has been traditionally studied on the basis of sub-fossilised bones recovered from places such as archaeological sites, caves, lacustrine and fluvial sediments. However these remains provide little opportunity to estimate distribution range, population size, or dates of exact colonisation/extinction events.

Obligate coprophilous fungi (hereafter referred to as dung fungi) provide a promising additional proxy to assess the past presence of large herbivore populations. These fungi strictly rely on terrestrial warm-blooded vertebrates such as elephants, deer, rabbits and larger birds to complete their life cycle. In fact their spores are ingested by herbivores while feeding on plants, survive digestion and then germinate in the dung where they are deposited (for dung fungi ecology and biology, see Bell 1983, 2005; Dix & Webster 1995). Fruiting bodies are produced after few to many days and, from those, spores are discharged to a distance of up to two meters (e.g. Ingold 1971; Yafetto *et al.* 2008). Hence the underlying assumption is that the larger the herbivore biomass in an ecosystem, the larger is the fungal biomass, and the greater the quantity of spores produced. Following this line of reasoning, the abundance of these spores in Quaternary sediments is interpreted to infer the presence and abundance of large herbivores in the past (Davis 1987; van Geel *et al.* 2003; Davis & Shafer 2006). In fact, the utilisation of non-pollen palynomorphs such as dung fungal spores is a valuable complement to pollen analysis because fungal, algal, insect and other biological remains often provide proxies for environmental variables for which pollen alone is not informative (van Geel 2001).

Dung spore presence, absence or abundance in sedimentary sequences have been used to determine dramatic wild herbivore population crashes during the late Quaternary in Madagascar (Burney *et al.* 2003), North America (Robinson *et al.* 2005; Davis & Shafer 2006; Gill *et al.* 2009), New Zealand (Wood *et al.* 2011) and Australia (Rule *et al.* 2012). Fluctuations in domesticated herbivore populations have also been noted using dung spores in sedimentary sequences from the historic period in North America (Davis & Shafer 2006) and Europe (see Mazier *et al.* 2009).

In spite of an increasing number of studies (Figure 1), there remain some concerns with regards to the methodology (Raper & Bush 2009; Feranec *et al.* 2011; Wood & Wilmshurst 2011). In particular the potential weaknesses that have been highlighted include: complications related to local hydrology, problems with identification, lack of quantitative reconstructions and lack of understanding of the taphonomy (deposition and preservation in different environments). Despite these weaknesses, in practice it has been possible to demonstrate the worldwide impact of herbivory in ecosystems (Burney *et al.* 2003; Gill *et al.* 2009; Rule *et al.* 2012), supporting previous research in conservation biology, grazing ecology and palaeobiology (e.g. Owen-Smith, 1988; Vera, 2000; Johnson, 2009).

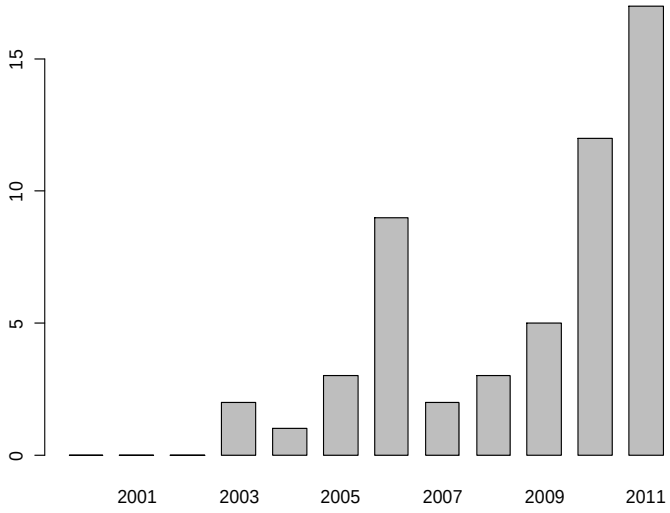


Figure 1: Number of published articles using coprophilous organism as a proxy for large herbivore. Publications selected from Reuters' ISI Web of Knowledge using the key words "coprophilous" or "Sporormiella".

Building on previous research, here we review studies that have utilised dung fungal spore assemblages to assess past herbivore presence or those that have tested the validity of this method. Our aim was to determine whether there is a set of identifiable dung fungal spores that can unequivocally track variation of large herbivore activity through time and across regions. Specifically, we review (1) spore types that have been found, in practice, to be indicative of large herbivores and their geographical ranges, (2) linkages between these spores and their biological origin, and (3) the most appropriate quantitative method to express their abundance for comparisons through time and between sites. We then discuss the future potential of this method for reconstruction of large herbivore past populations.

Which spores provide unequivocal evidence of the presence of large herbivores?

What fungal spore types are commonly recorded, on what basis and where

Spore types that are found to be indicative of large herbivores are listed in Table 1 and their geographical ranges are mapped in Figure 2.

Table 1: List of types reviewed, their codes following the nomenclature initiated by Bas van Geel, see van Geel (2001), the original publication and what the authors thought to be the best photographic reference published for identification purposes.

Type	Code	Original Publication	Best Published Pictures for Identification
Sporormiella-type	HdV - 113	Davis, 1975; Davis et al., 1977	van Geel et al., 2003
Sordaria-type	HdV - 55A	van Geel, 1976, 1978	Cugny et al., 2010; van Geel et al., 2003
Podospora-type	HdV - 368	van Geel et al., 1981	van Geel et al., 2003
Bombardioidea-type	HdV - 575	Bos et al., 2005	Refer to original publication
Pteridiosperma sp.-type	TM-021	van Geel and Aptroot, 2006	Refer to original publication
Rhytidospora cf. *tetraspora*-t.	HdV - 171	van Geel et al., 1983	van Geel and Aptroot, 2006
Apiosordaria-type	HdV - 169	van Geel et al., 1983	van Geel and Aptroot, 2006; Feeser and O'Connell, 2009
Chaetomium-type	HdV - 7A	van Geel, 1976, 1978	van Geel et al., 2003
Trichodelitschia-type	HdV - 546	Unpublished report	Cugny et al., 2010
Cercophora-type	HdV - 112	van Geel et al., 1981	Cugny et al., 2010; van Geel et al., 2003
Coniochaeta-type	HdV - 172	van Geel et al., 1983	Cugny et al., 2010
Anthostomella fuegiana-type	HdV - 4	van Geel 1976, 1978	van Geel and Aptroot, 2006
Schizothecium conicum-type	BRN7	Feeser and O'Connell, 2009	Refer to original publication
HdV - 324	HdV - 324	van Geel et al., 1981	Refer to original publication
HdV - 3A	HdV - 3A	van Geel 1976, 1978	Refer to original publication
HdV - 494	HdV - 494	van Smeerdijk, 1989	Refer to original publication
HdV - 8A	HdV - 8A	van Geel 1976, 1978	Refer to original publication
Microthyrium-type	HdV - 8B	van Geel 1976, 1978	van Geel and Aptroot, 2006
Arnium-type *	HdV - 261, HdV - 262	van Geel et al., 2003	Cugny et al., 2010, as HdV - 55B and TM-H); van Geel et al., 2003
BRN8	BRN8	Feeser and O'Connell, 2009	Refer to original publication
Coniochaeta xylariispora-t.	HdV - 6	van Geel, 1976, 1978	Refer to original publication
Coniochaeta ligniaria-type	HdV - 172	van Geel et al., 1983	Refer to original publication
Gelasinospora-type	HdV - 1 & 2	van Geel 1976, 1978	van Geel and Aptroot, 2006
Delitschia-type	TM-006	Jarzen and Elisk, 1986	Cugny et al., 2010
Coniochaeta-type A	TM-016	Cugny et al., 2010	Refer to original publication
Coniochaeta-type B	TM-211	Cugny et al., 2010	Refer to original publication
HdV - 10	HdV - 10	van Geel, 1976, 1978	Refer to original publication
HdV - 19	HdV - 19	van Geel, 1976, 1978	Refer to original publication
Sordaria (2-pored)-type *	HdV - 55B	van Geel, 1976, 1978	Cugny et al., 2010
HdV - 123	HdV - 123	Pals et al., 1980	Refer to original publication
HdV - 8E	HdV - 8E	van Geel, 1976, 1978	Refer to original publication
Pleospora-type	HdV - 3B	van Geel, 1976, 1978	van Geel and Aptroot, 2006

* in this study HdV - 261, HdV - 262 and HdV - 55B are treated as one broad type

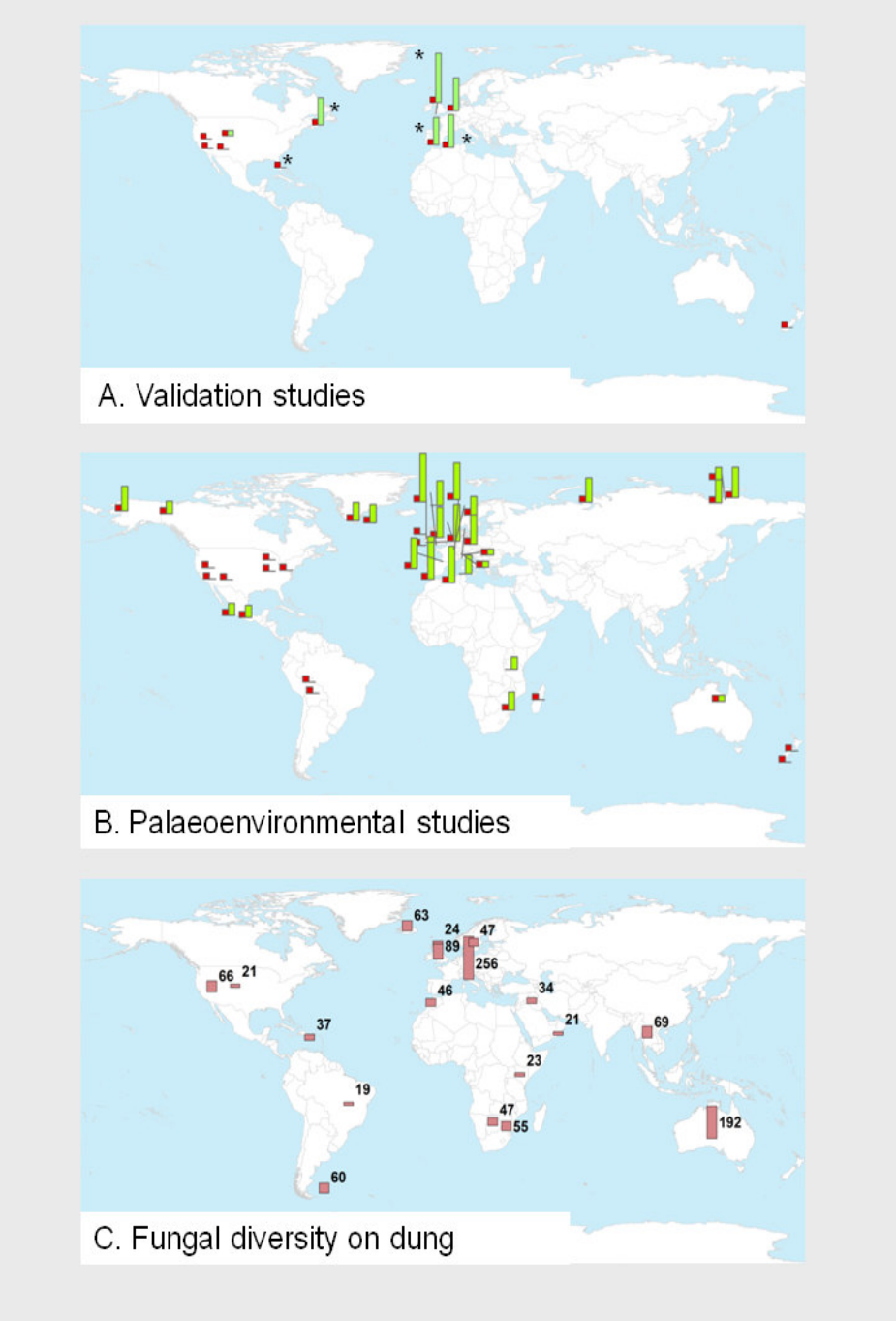


Figure 2: Distribution maps of dung fungal spore. A. Validation studies using modern (*) and ancient sediments, dark red bars show the presence of Sporormiella-type, light green bars the number of other dung fungal spore types, if any. B. Palaeoenvironmental studies using dung fungal spores, same legend. C. Biodiversity of dung fungi (in number of species), all studies include the genus Sporormiella. See Appendix for the comprehensive reference list.

A total of 33 fungal spore types identified in the published palynological literature are related to large herbivore activity (Table 1). This list includes types that have been identified as confirmed bioindicators (e.g. *Sporormiella*-type and *Sordaria*-type) and potential indicators in need of further research (e.g. *Arnium*-type and *Trichodelitschia*-type).

Many of those types have been the subject of validation studies (e.g. Davis & Shafer 2006; Raper & Bush 2009; Cugny *et al.* 2010). These studies seek empirical evidence from modern and ancient sediments that show whether a spore type is consistently found in greater abundances in situations where herbivory is also known to be higher. Such evidence is necessary in order to demonstrate the applicability of dung fungal spores to palaeoenvironmental reconstructions. However, they do not provide any information regarding the process explaining the relationship between a spore type and herbivory. In addition, they rely on an accurate measurement of herbivore densities and on quantitative estimation of the distance fungal spores can travel.

Spore types supported by empirical evidence are listed in Table 2. Six of these fungal types were found several times to indicate herbivore activity. Of these, *Sporormiella*- and *Apiosordaria*-type show consistently positive evidence. For the remaining four types, *Sordaria*- and *Podospora*-, *Chaetomium*- and *Cercophora*-type, the positive association with herbivory has been questioned on at least one occasion each (van Geel *et al.* 2003; Blackford & Innes 2006; van Geel & Aptroot 2006; Cugny *et al.* 2010; Ejarque *et al.* 2011; Parker & Williams 2011).

Table 2: Evidence of bioindication from the literature (+: positive, -: negative, ~: equivocal). The references in the last two columns appear in the same order as in the two other columns. The references are, 1: Davis (1987), 2: van Geel et al. (2003), 3: Blackford and Innes (2006), 4: Davis and Shafer (2006), 5: Graf and Chmura (2006), 6: van Geel and Aptroot (2006), 7: Raper and Bush (2009), 8: Cugny et al. (2010), 9: Cordova et al. (2011), 10: Wood and Wilmshurst (2011), 11: Ejarque et al. (2011), 12: Parker and Williams (2011).

Type	Evidence from		References for	
	Modern Sediments	Ancient Sediments	Modern Sediments	Ancient Sediments
Sporormiella-type	+, +, +, +, +, +, +	+, +, +, +, +	3, 5, 7, 8, 9, 11, 12	1, 2, 4, 6, 10
Sordaria-type	~, +, +, +	+, +,	3, 5, 8, 11	2, 6
Podospora-type	+, +, ~, ~	+, +,	5, 8, 11, 12	2, 6
Chaetomium-type	+, +, ~	~, ~,	5,8, 11	2, 6
Cercophora-type	+, +, -,	+, +,	3, 5, 8	2, 6
Apiosordaria-type	+, +,	+, +,	5,8	2, 6
Delitschia-type	+		8	
Coniochaeta-type	+		11	
Anthostomella fuegiana-type	+		3	
HdV - 324	+		3	
HdV - 3A	+		3	
HdV - 494	+		3	
HdV - 8A	+		3	
Microthyrium-type	+		3	
Trichuris ovis-type	+		11	
Arnium-type	~	+	11	6
HdV - 10	~		3	
HdV - 19	~		3	
Soradaria (2-pored)-type	~		3	
Trichodelitschia-type	~		8	
Coniochaeta xylariispora-t.		~		6

Other spore types that are promising, but whose occurrence in sediments has been studied even less well are: *Coniochaeta-*, *Arnium-*, *Delitschia-*, *Trichodelitschia-*, *Microthyrium-*, *Anthostomella fuegiana*-type and a series of unidentified types (see Table 2). These fungal spores were not linked to herbivory in more than one study and therefore need further research. Other spore types were not reported to have been associated with high herbivore densities. Noteworthy, however, are the identifiable eggs of various parasitic worms regularly observed in archaeological deposits (see references in Chaves *et al.* 2006) and in particular those of *Trichuris ovis*, recorded by Ejarque *et al.* (2011) within a sedimentary archive. Although this type of microfossil deserves to be the subject of further research, it is beyond the scope of this paper.

Validation studies that aim to establish association between fungal spores and herbivores are mapped on Figure 2A. They are mostly restricted to North America and Europe, and with a further study in New Zealand. This strongly contrasts with the worldwide distribution of palaeoenvironmental reconstructions using these same spore types (Figure 2B). In fact, the map of Figure 2B shows that fungal spores have been identified in sedimentary archives and interpreted as indicative of herbivory from most biomes and continents on Earth, from the tropics (e.g. Mumbi *et al.* 2008) to the arctic (e.g. Gauthier *et al.* 2010). Despite this variety of locations, North America and Europe show a much greater density of studies than the rest of the World. In particular, not a single published dung fungal spore record was found from Southern Asia.

In addition, the number of spore types assumed to indicate large herbivore activity varies greatly between palaeoecological studies and regions of the world. Figure 2B shows that records from the United States, South America, Madagascar and Australasia only report *Sporormiella*-type, depicted by dark red bars. By contrast, in Europe, Mexico and Africa 3-9 types are used per study (depicted by the length of the light green bars), generally including *Sporormiella*-type, except in three instances (out of 37), where it was not observed (Blackford & Innes 2006;

Mumbi *et al.* 2008; Ponel *et al.* 2011). But do these patterns reflect the actual biological distribution of the dung fungi concerned?

A series of taxonomic studies have analysed the diversity of dung fungi by collecting herbivore dung from the field and subsequently incubating it in growth chambers (Figure 2C). This is the standard method to allow the investigator to identify, using a taxonomic key, the diversity of fungi developing on such substrates (Lundqvist 1972; Krug *et al.* 2004). The higher number of species reported from Australia (Bell 2005) and Italy (Doveri 2007) are related to the intensive work carried out in these two countries. With the exception of Wicklow & Angel's (1983) study from the United States, all studies mapped on Figure 2C have recorded the genus *Sporormiella* Ellis & Everh. It is common knowledge that at the genus level, dung fungi are cosmopolitan (Krug *et al.* 2004; Kirk *et al.* 2008), and may follow a decreasing diversity with latitude (Richardson 2001, 2006; Krug *et al.* 2004). In view of this, the geographical patterns seen in Figure 2B (palaeoenvironmental studies) are likely to be a consequence of different research practices.

In summary, there are two possible approaches to track past herbivore activities using fungal spores. In both cases, empirical validation studies using modern and ancient sediments are heavily biased towards North America and Europe. Future research needs to focus on both a better understanding of *Sporormiella*-type and the other types, as well as a much wider geographical distribution.

Taxonomic origin and ecology of the types recorded

The linkages between assumed dung spores and their biological origins are presented in Table 3. Whilst it is generally assumed that all these spores identified from sediments are indeed dung fungi, the evidence for this linking is often lacking in publications. In order to confirm the empirical evidence compiled (Table 1 and 2), here we establish the link between the spore types observed in sediments and actual fungus taxa, described in the mycological literature. We

consulted a wide body of mycological literature to establish associations between our compiled list of dung spore types and existing fungal taxa. Within this literature, we specifically focused on Ascomycetes that feature in the illustration plates, keys and descriptions of various identification handbooks dealing with both dung fungi and fungi growing on other substrates. Texts which focussed on dung fungi include Lundqvist (1972), Bell (1983, 2005), Richardson & Watling (1997) and Doveri (2007); and books on other fungi include Dennis (1978), Ellis & Ellis (1997, 1998) and Watanabe (2010).

This mycological literature highlighted a variety of coprophilous and non-coprophilous taxa which are potentially associated with the types identified in palynological literature (Table 3). This literature review confirms the link with obligate coprophilous ecology for the *Sporormiella*-, *Sordaria*-, *Podospora*-, *Delitschia*-, *Trichodelitschia*- and *Bombardioidea*-type. Of these six types, *Trichodelitschia*-type has very distinct morphology but empirical evidence restricted to the Pyrenees Mountain range in Southern Europe. In addition, along with type HdV – 19, for instance, *Trichodelitschia*-type is part of a large group of 1-septate spore that can potentially be produced by several different taxa, e.g. within the *Pleosporales* (Zhang *et al.* 2011), the *Sorardiales* (Huhndorf *et al.* 2004) and also in diverse lichenized fungi (e.g. Smith *et al.* 2009), many of which are as yet unidentified (see Table 3). Similarly, empirical evidence still remains to be established for *Bombardioidea*- and *Delitschia*-type. This lack of evidence is probably due to the small number of studies and the lack of systematic identification on pollen slides, as these types are only occasionally reported from palynological literature (Jarzen & Elsik 1986; Bos *et al.* 2005; Cugny *et al.* 2010). In the case of *Apiosordaria*-type the situation is exactly opposite. Although highlighted empirically, the taxonomic origin of this type remains unclear. For this reason, the processes by which it happens to co-occur with high animal densities in four published studies (see Table 2) cannot be established. This co-occurrence could be due for instance to coincidence as well as circumstances not directly related to herbivory.

Table 3: Taxonomic origins of spore types, their substrate preferences and some key references consulted in addition to the general identification handbooks cited in the text. Both Preussia and Podospora are s.l.. Bracketed genera indicate a small minority of their species. + : Strictly or largely coprophilous, - : Partly Coprophilous or not coprophilous, ~ : unclear, p.p.: (pro parte) for part, … : and possibly other taxa.

Type	Biological Origin and Ecology			
	Dung Fungi	Other Fungi	Conclusion	Key References
Bombardioidea-type	*Bombardioidea*, *Fimetariella*?		+	Krug and Scott, 1994; Krug, 1995
Sporormiella-type	*Preussia*	(*Preussia*)	+	Kruys and Wedin, 2009;
Trichodelitschia-type	*Trichodelitschia*, *Zygospermella*?	*Melanconiella spodiaea*?	+	
Delitschia-type	*Delitschia*	(*Delitschia*)	+	Hyde and Steinke, 1996
Podospora-type	*Podospora* p.p., *Zygopleurage zygospora*?	(*Podospora*)	+	
Sordaria-type	*Sordaria*, *Podospora* p.p.	(*Sordaria*), (*Podospora*), *Anthostomella lugubris*?	+	
Chaetomium-type	*Chaetomium* p.p., ...	*Chaetomium* p.p., ...	-	
Coniochaeta-type	*Coniochata* p.p., *Ascotricha*?, ...	*Coniochata* p.p., *Hyploxylon*?, *Eurotium*?, *Ascotricha*?, ...	-	García et al., 2006
Sporormia-type	*Sporormia* p.p.	*Sporormia* p.p.	-	Kruys and Wedin, 2009
Gelasinospora-type		*Gelasinospora*	-	
Anthostomella fuegiana-type	*Anthostomella* p.p., ...	*Anthostomella* p.p., ...	~	Lu, 2000
Arnium-type	*Arnium* p.p.	*Arnium* p.p., *Melanospora brevirostris*?, ...	~	
Cercophora-type	*Cercophora* p.p.	*Cercophora* p.p., *Farlowiella carmichaeliana*?, *Persiciospora masonii*?, *Immersiella caudata*?, ...	~	Hanlin, 1999
Apiosordaria-type	*Zopfiella?*, *Apiosordaria* p.p.?, *Triangularia*?, ...	*Zopfiella*?, *Bombardia bombarda*?,	~	Guarro and Cano, 1988; Krug et al., 1983; Stchigel et al., 2000; García et al., 2003
BRN8	*Hypocopra*?, *Podosordaria*?, *Poronia*?,		~	
BRN6	see *Podospora*-type			
Coniochaeta xylariispora-type	sub-type within *Coniochaeta*-type			
Coniochaeta ligniaria-type	sub-type within *Coniochaeta*-type			
Coniochaeta-type A	sub-type within *Coniochaeta*-type			
Coniochaeta-type B	sub-type within *Coniochaeta*-type			
Soradaria (2-pored)-type	see *Arnium*-type			
Sordaria (shouldered)-type	sub-type within *Podospora*-type or *Sordaria*-type			
Schizothecium conicum-type	sub-type within *Podospora*-type or *Sordaria*-type			
Microthyrium-type	Not Assessed - Limited Identification Material			
Pleospora-type	Not Assessed - probably not coprophilous			
Pteridiosperma sp.-type	Not Assessed - Limited Identification Material			
Rhytidospora cf. *tetraspora*-t.	Not Assessed - Limited Identification Material			
BRN2	Not Assessed - Origin Unknown			
HdV - 10	Not Assessed - Origin Unknown			
HdV - 123 & QM10	Not Assessed - Origin Unknown			
HdV - 19	Not Assessed - Origin Unknown			
HdV - 324	Not Assessed - Origin Unknown			
HdV - 3A	Not Assessed - Origin Unknown			
HdV - 494	Not Assessed - Origin Unknown			
HdV - 65	Not Assessed - Origin Unknown			
HdV - 8A	Not Assessed - Origin Unknown			
HdV - 8E	Not Assessed - Origin Unknown			

Most types would benefit from further research in order to establish their specific origins. This is particularly true for the many types that could not be assessed during the course of this review

because of a lack of identification characters provided in the literature, or, in many cases, simply because no initial hypothesis regarding their origin was formulated. In fact many types still do not have any taxon name (see Miola, 2012). Consequently, *Sporormiella*-, *Sordaria*- and *Podospora*-types represent the most reliable set of types on the basis of our current knowledge on the matter; however, it can be expected that, with further research, additional types will be included.

Two confusing issues deserve to be mentioned here, both due to a lack of consensus regarding the taxonomy of the genera *Sporormiella* and *Podospora* Ces. The genus *Sporormiella* has traditionally been understood in a strict sense, i.e. to the exclusion of the genus *Preussia* Fuckel (Ahmed & Cain 1972; Lundqvist 1972). However recent molecular work by Kruys & Wedin (2009) has shown that *Sporormiella* and *Preussia* may be merged in the future into *Preussia* s.l. (*sensu lato*, in a broader sense). This work also demonstrates that all the members of this larger genus show germination slit on their spore cells. As a consequence it is likely that the *Sporormiella*-type is produced by any member of *Preussia* s.l. In addition, small *Sporormiella*-type-like spores without germination slits (personal observation, AGB) are likely to be related to the genus *Sporormia* De Not., which comprises a significant proportion of coprophilous species (van Geel *et al.* 2003; Kruys & Wedin 2009; Zhang *et al.* 2011). The second issue concerns the current debate regarding the phylogeny of the genera *Podospora* and *Schizothecium* Corda. They are sometimes merged together into *Podospora* s.l. (Kirk *et al.* 2008) but this question remains unresolved yet and far beyond the scope of this study. It is certain though that *Podospora*-type is produced by one to several members of *Podospora* s.l., and that some members of this enlarged genus are probably also the origin of spores usually referred to as the *Sordaria*-type (see e.g. Doveri, 2007). Similarly to the *Sordaria*-type, the *Schizothecium conicum*-type reported by Feeser & O'Connell (2009) corresponds to spores of a (or some) member(s) of *Sordaria* Ces. & De Not. or *Podospora* s.l. The high level of uncertainty for the specific origin of these two types is not an impediment to bioindication, because the genera involved are, with

rare exceptions, dung-dwellers. As will be discussed in section 4.1, this may actually be an opportunity to refine spore identification to smaller groups of taxa sharing narrower ecological preferences related to dung quality, for instance. Also, these refined identifications may be necessary in order to exclude anecdotic non-coprophilous taxa producing spores of similar morphology. As an example, the spores of *Anthostomella lugubris* (Roberge ex Desm.) Sacc. may well be included in the *Sordaria*-type if encountered on pollen slides but this fungus grows on the dead leaves of sand-loving grass *Ammophila* Host, not on dung (Ellis & Ellis 1997).

The *Apiosordaria*-type (which is also referred to as *Tripterospora*-type, or sometimes *Apiosordaria verruculosa*-type) is not likely to be produced by *Apiosordaria verruculosa* (C.N. Jensen) Arx & W. Gams, which has warty spores, nor by *Apiosordaria tuberculata* J.C. Krug, Udagawa & Jeng (used to be *Tripterospora verruculosa* Cailleux) as the spores of the genus *Apiosordaria* Arx & W. Gams are systematically composed of an ornamented dark cell and a small hyaline cell (Krug *et al.* 1983; Stchigel *et al.* 2000; García *et al.* 2003). The origin of this type remains thus uncertain, although it may be worth investigating the related genera *Triangularia* Boedijn and *Zopfiella* G. Winter. These two genera are reported to have spores similar in morphology to *Apiosordaria* but for their smooth surface (Guarro & Cano 1988; García *et al.* 2003).

In the case of *Cercophora*- and *Arnium*-type, refining the identifications into sub-types is likely to pinpoint groups of species more informative than they currently are. With today's definition, these morphological types can also originate from taxa not specifically related to herbivory. For *Cercophora*-type, those taxa mentioned in the Table 3 are only examples and in fact, there are in addition several non-coprophilous *Cercophora* Fuckel (see e.g. Ellis & Ellis 1997). For *Arnium*-type, size ranges of *Arnium* Nitschke ex G. Winter spores may allow the differentiation of subgroups of species strongly related to dung and thus discard the others. In this connection, it is to be noted that the coprophilous *Arnium arizonense* (Griffiths) N. Lundq. & J.C. Krug has only

1 pore (Bell 1983; Richardson & Watling 1997) unlike other members of this genus and would in this respect be included into the *Sordaria*-type.

Finally, in three instances, we have highlighted the potentially unfounded link between spore types and dung fungi. In fact *Chaetomium*- and *Coniochatea*-types comprise many non-coprophilous taxa (Ellis & Ellis, 1997, 1998; Garcia *et al.* 2006) and *Anthostomella fuegina*-type cannot be related to *Anthostomella fuegiana* Speg. with any certainity because of the large number of species producing similar spores (Ellis & Ellis 1997; Lu 2000). This makes these three types unsuitable for the reconstruction of past herbivore activity.

In summary, *Podospora*-, *Sordaria*- and *Sporormiella*-type are the most reliable indicators of large herbivore activity, with our current state of knowledge. These three types are the only ones showing strong empirical evidence of presence in areas with high animal densities and whose morphology can be clearly related to obligate coprophilous fungi.

Do dung fungal spores provide quantitative evidence for herbivore abundance?

The next question to address is whether abundance of spore types in the sedimentary record can be used as an indicator of herbivore abundance. The first difficulty is to interpret the absence of dung fungal spores in sedimentary archives. The current knowledge of fungal spore dispersal and deposition onto sediments is in urgent need of further empirical studies in order to address this question. Owing to the reduced height at which these spores are released into turbulent air, it can be postulated that most of the spores do not travel more than a few metres (Jackson & Lyford 1999). This potentially limits the accumulation of fungal spores beyond the immediate proximity to large herbivores. A number of studies have shown that once spores reach a water body, remixing within the waters (Davis & Brubaker 1973) appears weak (Raper & Bush 2009; Parker & Williams 2011; Etienne *et al.* 2012). However, these authors put forward contradictory findings regarding the location within a water body where spore accumulation is the highest (in decreasing order, these are: near the shore, near the lake centre, near stream

inlets). In the absence of further insights into spore deposition, it can be expected that the smaller the basin, whether pond or peat, the more likely it is to indicate the local abundance of herbivores. On the contrary, the interpretation of both abundance and absence of those fungal spores in larger sedimentary basins should be more cautious. In addition, variation between sites is likely to arise with respect to the behaviour of large herbivores and drinking water availability from the sampling basin, but again no published empirical data provides us with guidance regarding this aspect.

Secondly, palaeoecologists need to decide whether different dung fungal spores can be aggregated into a single proxy or whether they should be regarded as separate lines of evidence. Studies of dung fungus diversity (e.g. Richardson, 1972, 2001) highlight the preference of certain species for certain types of dung. The consequent assumption is that the greater the spore type diversity, the more likely it is to capture all large herbivore activities. In addition, the large body of evidence reviewed by Dix and Webster (1995) and Krug *et al.* (2004) shows that dung fungi have species-specific responses to different moisture levels, temperatures, microclimates and microhabitats. As a consequence, the presence of large herbivores is more likely to be reliably tracked using a range of types as broad as possible, representing as many obligate coprophilous taxa as possible, rather than relying on one taxon.

Dix and Webster (1995) also highlight the importance of competition between species as a driver for the composition of the dung fungal community, particularly in the form of competition for resources and allelopathy. This suggests that dung fungal biomass, and thus ultimately spore production, is strongly limited by factors such as space, nutrient and moisture availability. The ecology of dung fungi therefore suggests that the sum of individual dung spores, irrespective of the type encountered, provides the most appropriate measurement of fungal biomass. However, what is as yet unclear is how to translate the number of spores in sediments into past herbivore densities.

Thirdly, dung fungal spore counts have been expressed relative to the total number of pollen grains in 37 out of the 47 publications reviewed (see Appendix). What constitutes the pollen sum is, however, considerably variable from one study to another, largely due to different research traditions, type of sedimentary archives, regions, and environments. The ratio of dung spores to the pollen sum is generally called a "percentage" on pollen diagrams and will be referred to thereafter as relative quantification. Less commonly, spore abundance is expressed as concentration, either relative to a volume of sediments (Davis 1987; Raper & Bush 2009; Cordova *et al.* 2011) or rarely to grams of sediments (Ponel *et al.* 2011). Also, accumulation rates (or influx) have been used (Davis 1987; Hausmann *et al.* 2002; Jeffers *et al.* 2011, 2012), and in some instances the simple presence of spores is considered informative (van Geel *et al.* 2007, 2011), but which of these four methods is most appropriate?

Relative quantification has several drawbacks that have been well studied in palynology. It assumes pollen accumulation to be sufficiently constant through time, so variation of spore influx can be detected between samples. For this reason, the interpretation of such data is difficult when passing through major climatic thresholds for instance, because the overall pollen productivity of a system can be directly affected (Davis 1969; Hicks & Hyvärinen 1999). Independently from animal density, post-glacial alleviation of net primary production limiting factors like cold and drought, for example, would have dramatic repercussions on relative curves of dung fungal spores. In fact, when vegetation growth increases, so does pollen production (e.g. Davis, 2000), hence a relatively lower quantity of spores. Assuming a negligible effect of herbivory on vegetation, relative quantification may also be sound; however, as stated in the introduction, there is an increasing demand for assessing the impact of herbivory itself. In this case, it appears unwise to express spores relative to vegetation productivity which, in certain cases, could introduce large noise in the data.

Relative quantification unfortunately impedes comparison between sites, especially if biomes are crossed, or sites of different nature are considered. In fact, pollen productivity varies from

one ecosystem to another (Faegri *et al.* 1989 p.123 and references within; Gosling *et al.* 2009), and the characteristics of the deposition basin influences the abundance of pollen (reviewed by Giesecke & Fontana 2008). Despite this, Davis (1987) and Davis & Shafer (2006) found relative data informative for comparing sites within south-west North America, though, maximum ratio values at those sites were systematically lower than on the rest of that continent (Robinson *et al.* 2005; Graf & Chmura 2006; Gill *et al.* 2009, 2012; Parker & Williams 2011) (detail shown in Appendix). Because of the nature of relative quantification it is not possible to assess whether those differences correspond to an artefact of the methodology or to actual changes in fungal spore abundance. Looking worldwide at all studies available at the time of writing, Figure 3 illustrates the seeming lack of significant regional trends regarding the relative abundance of fungal spores. It is to be noted that, for most continents, the number of studies is extremely low for any statistical inferences, and even null in Southern Asia.

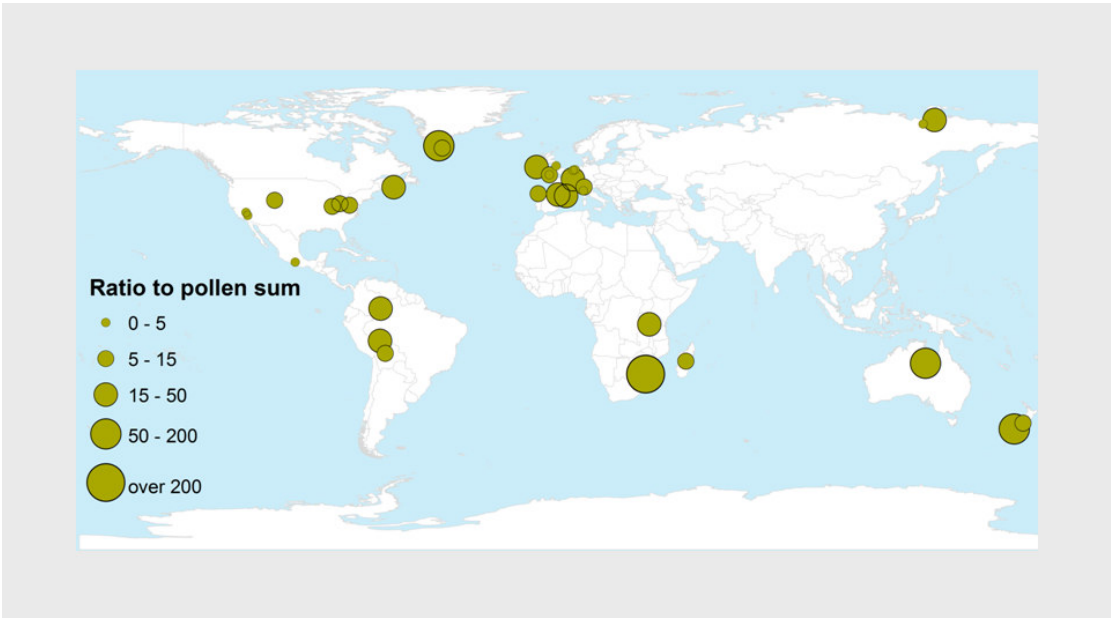


Figure 3: Maximum percentages of dung fungal spores from studies across the World. * indicates 5 sites within those values and **, 20 sites within those values. See Appendix for the comprehensive reference list.

As an alternative to relative quantification, there is concentration, which expresses the number of spores per volume of sediments. Concentration measures heavily depend on sedimentary rates, so sites of different nature, or levels from different environments within the same site

would not be comparable. For this reason it has not been widely used (Davis 1987; Ponel *et al.* 2011; Cordova *et al.* 2011) but it proved appropriate for the calibration study of Raper & Bush (2009).

Finally, spore accumulation rates are totally independent of the surrounding vegetation and on the sedimentary environment but rely on the quality of dating and of the age model developed (Telford *et al.* 2004). In cases where the quantitative impact of herbivory is assessed against other disturbance factors, this is the favoured data form (Jeffers *et al.* 2011, 2012). In addition, in his attempt to compare sites, Davis (1987) provides the estimated spore accumulation rates for three of his sites. Although not used systematically because of technical difficulties associated with it (Bennett 1994), accumulation rates represent the best means not only to calculate an independent proxy for herbivory, but also to compare sites with each others. Such a truly independent line of evidence could be in addition compared with alternative palynological means to quantify herbivory pressure using indicator pollen types (Gaillard *et al.* 1992; Mazier *et al.* 2009).

In summary, the interpretation of dung fungal spore abundance or absence should be limited to very small sedimentary basins such as forest hollows, small lakes and small peat bogs, until further research about spore dispersal and deposition is available. On the basis of the biology and the ecology of dung fungi, a spore sum, including all reliable indicator types, is the most appropriate manner to track past herbivore densities. All methods to express this measurement of spore abundance have drawbacks, however, and only accumulation rates are truly independent from vegetation and sedimentary changes. Obtaining a line of evidence independent from environmental changes is particularly relevant when cause-and-effect relationships between herbivore and their environment are studied.

Potential for Future Research

Areas of potential developments

In addition to new empirical research, there are several ways to improve the best set of indicator types identified in this paper in order to reconstruct herbivore densities. Most obviously, the basic taxonomic origin remains to be established for many distinct spore types. In keeping with our focus on herbivory, it may be more time-efficient in those instances to track on pollen slides the morphologies that are known to be related to dung, instead of focussing on all the existing unidentified spore types. Moreover, even for widely recognised types there may be the possibility to refine identification, which may lead to important palaeoecological progress. Refining identification to smaller group of species has firstly the potential to exclude non-coprophilous taxa producing similar spores. Secondly, it could allow us to link morphologies to herbivore-specific dung fungi.

In the case of *Sporormiella*-type, two characters illustrated in Bell (2005) can help to refine identification. Not only the distal cells may be tapered or cylindrical, but also the germ slit itself may have different orientation: parallel, oblique, diagonal, transverse, kinked or right-angled. Moreover, it is worth noting that the ratio of distal and proximal cells in the counts can as well be a good indicator of the origin of the spores. In fact the genus *Preussia* s.l. has typically 4- to 8-celled spores while *Sporormia* has typically 16 or 32 (Richardson & Watling 1997; Ellis & Ellis 1998; Kruys & Wedin 2009). For instance a series of *Sporormiella*-type spores showing a (distal cell : proximal cell) ratio not different from one, would suggest 4-celled spores species. This would link the fossil spores to a narrower set of 13 species of *Preussia* s.l. out of the 21 most common species recorded in the UK (Richardson & Watling 1997). In all those three cases (shape of distal cells, germ slit orientation and distal: proximal cell ratio) it becomes apparent that new in-depth morphometric studies are desirable. A major drawback is that this work can be extremely time-consuming; thus, resorting to alternative means for identification and counting should be considered. Automated image recognition as described by Holt *et al*. (2011) in this respect may be a great opportunity to improve identification of *Sporormiella*-type and indeed for many other spore types.

In his studies of dung fungi (Richardson 1972, 2001) analysed dung samples mostly from the United Kingdom and found statistical evidences for groups of fungal species specific to three categories of herbivore's dung. Either like *Podospora decipiens* (G. Winter ex Fuckel) Niessl they preferred ruminants (cattle, deer, sheep) or like *Sporormiella intermedia* (Auersw.) S.I. Ahmed & Cain ex Kobayasi they preferred lagomorphs (hare, rabbit) or like *S. minima* (Auersw.) S.I. Ahmed & Cain they preferred large bird's dung (grouse) or they were relatively non host-specific. This conclusion is supported by data presented by Doveri (2011), on the basis of his Italian samples and from a worldwide publication review, but without any statistical analysis. This last study suggests that generally speaking horse preference is most related to the ruminants' preference recorded by Richardson, and also suggests the existence of a group of species principally linked to deer (e.g. *Arnium cervinum* N. Lundq.) but not other ruminants. These three articles clearly show that fungi have species-specific dung type preferences but, at a higher taxonomic rank, no fungus genus appears to show a trend for a certain type of dung.

As a consequence, as is the case for pollen analysis, only refined palynological types are likely to provide additional information to the palaeoecologist, regarding the abundance of different herbivore species. With improvements in identification like those exemplified above for *Sporormiella*-type, this does not appear far out of reach though. In fact, for instance, the authors of this review have observed spores belonging to the *Sporormiella*-type but according to their mean sizes and distal cell shapes, they were, on average, likely to belong to *Sporormiella intermedia* and *S. australis* (Speg.) S.I. Ahmed & Cain (Richardson & Watling 1997), both being common species strongly related to lagomorphs (Richardson 2001; Doveri 2011). The analysis of assemblages of refined palynological types would most certainly enable researchers to infer trends of herbivore community composition through time within sedimentary archives.

To achieve this result it will be necessary to compare more adequately modern spores of known origin to those observed on pollen slides. In the more advanced field of pollen grain identification, more than 80 years of collegial experience has led to the conclusion that access

to a comprehensive modern reference collection is *essential* in order to carry out accurate identifications and counts (Willis & Bennett 2001).

Current Methodological Limitations

In view of these potential developments, there is a need to refine identification. However, matching spore morphologies to illustration and description found in the mycological literature, the methodology currently used, presents many limitations and pitfalls; the main ones are illustrated below.

First of all, with the advent of molecular classification, mycologists are increasingly recognising that within the *Sordariales* Chadef. ex D. Hawksw. & O.E. Erikss. for example, ascospore morphology is not a good phylogeny predictor (Cai *et al.* 2005; Miller & Huhndorf 2005). The consequences are that palynological types are likely to encompass biologically unnatural group of species, as is illustrated in detail in the section "Taxonomic origin and ecology of the types recorded". It is thus unlikely that genus-based type names like *Podospora*-type and *Sordaria*-type will include all members of a single genus. Moreover, the picture matching approach requires comprehensive floras like that of Doveri (2007) for Italy, that of Bell (2005) for Australia or that of Bell (1983) for New Zealand; however, such works are not available in such detail for other parts of the world.

Second, it is important to put dung fungal spores into their ecological context. In fact, there tend to be a minority of non-coprophilous species within most coprophilous families. For instance, the unrelated *Sporormiaceae* Munk, *Phaeotrichaceae* Cain and *Delitschiaceae* M.E. Barr within the *Dothideomycetes* O.E. Erikss. & Winka all predominantly show coprophilous ecology but as well show members preferring other substrates (Kruys *et al.* 2006; Schoch *et al.* 2009). These authors highlight that adaptation to a new substrate is a common evolutionary step within this botanical class. Following this example, four out of about 50 members of the genus *Delitschia* Auersw. grow on submerged wood while the rest of the family is restricted to

dung (Hyde & Steinke 1996; Doveri 2007). As a result, spores of the *Delitschia*-type can occasionally originate from alternative substrates. It can be expected that most coprophilous clades show these types of discrepancies, whether due to ecological reversal or to the persistence of ancestral characteristics.

Third, apart from those biological considerations, it must be emphasised that although of taxonomic and identification value, spore morphology is not always the centre of research in the mycological literature. As a result, there is no systematic description of spores for all taxa in sufficient amount of morphological detail that would be required for species identification on the basis of spores alone. Similarly, the variability of spore morphologies within taxa is relatively poorly understood. As an illustration, the two most recent monographs on dung fungi provide spore dimensions on the basis of 10 (Bell 2005) or 20 spores (Doveri 2007). Although representing invaluable biological information, this may not be statistically sufficient in palynology to establish with high level of certainty the identity of individual spores. In this regard, it should be noted that macrofossil of fungus asci (spore-containing body in the Ascomycetes) as recovered by van Geel *et al*. (2011), can add valuable information to microfossils because mycological identification keys often rely on ascus morphology.

Fourth, the comparison of spore measurements made from freshly fruiting fungi, with those of spore ending up on pollen slides, is not straightforward. In the case of pollen grains and fern spores for instance, it is a well established fact that using different mounting agent (glycerol-jelly or silicone oil), or different laboratory chemical treatments will induce significant changes in sizes and shapes (Reitsma, 1969; Large & Braggins, 1990; Meltsov *et al*., 2008; and references cited therein). For this reason, there is a need for measurements on spores of known taxonomic origin that have experienced the same laboratory protocol than the spores recovered from sedimentary archives.

Alternative method

As already hinted above, there are alternative methods available that together can advance research towards more accurate identification and towards establishing the taxonomic identity of dung fungal spores. This would not only enhance the accuracy of reconstructed herbivore densities but can also provide insights into the species composition of herbivore populations.

A modern reference collection can be built using herbarium specimens, when suitable, and new field collections, for instance from the same region a piece of palaeoecological research is being conducted. As an indication, the very well studied coprophilous fungal flora of Italy comprises 338 species, 252 of which belong to the Ascomycetes from which most types dealt with are though to originate. A collection could start with the most commonly encountered species in the research region. For instance Richardson (2001) found out that 32 species out of the 282 identified in his study represented 64% of all his observation on 425 dung samples mostly from the UK. Hence a reference collection can rapidly become a powerful identification tool even with a relatively small number of species.

In addition, there are probably many diagnostic characters for which the human eye is difficult to train. Size measurements using eye graticule are certainly not as accurate as computerised measurements on microphotographs using calibration software, and various degrees of curvature are only observable with a great deal of experience. If much effort is invested in automated image recognition as described by Holt *et al*. (2011), this method may quickly become as effective at recognising spores types, if not more so, than a palynologist. A modern reference collection can serve as a training set along with pollen collection and as a result dung fungal spores could become an integral part of this new computerised identification journey.

Conclusion

The dung fungal spore *Sporormiella*-type, *Sordaria*-type and *Podospora*-type are currently the three most reliable indicators of large herbivore activity. They are the only types that have been recurrently shown to be related to herbivory and that can be linked to obligate coprophilous

fungus taxa. Further research is needed, however, to improve identifications for other less common types to ensure that they become robust and reliable indicators.

Once spores are correctly identified, in order to reconstruct herbivore densities, the use of accumulation rates (number of spores/cm^2/year) appears to be more appropriate for comparison of changes within a sequence and between sequences from different sites. This is in contrast to the currently preferred method that expresses fungal spores as a percentage in relation to the pollen sum; this relative method does not provide a proxy for herbivory that is independent from vegetation changes.

Many dung fungi show narrow ecological requirements, resulting in them being found significantly more often on the dung of a specific herbivore. It may be possible in the future to link certain spore morphologies to herbivores groups such as lagomorphs (rabbits, etc.), cervids (deer, etc.), other ruminants and horses, or birds. From those groups, indices can be built in palaeoecology and trends through time can be inferred. In order for this to become possible, however, the development of a robust set of indicator types is needed, and further research towards refined identification is necessary.

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Appendix

Appendix: List of publication for figures 2 and 3, and the maximum values recorded from pollen diagrams at the site(s) examined. * 5 sites within those values, ** 20 sites within those values.

		Figure						Ascomycetes			Concentration			
	Continent	2A	2B	2C	3	number of spore types recorded	presence of Sporormiella (type or taxa)	number of species	number of genera	Ratio to pollen sum	relative to mass	relative to volume	Accumulation Rates	Presence
Abdullah (1982)	Asia			1			1	34	15					
Almeida-Lenero et al. (2005)	North America		1		1	3	1			0 to 5				
Andreev et al. (2012)	Asia		1		1	5	1			5 to 15				
Aptroot and van Geel (2006)	Asia		1			6	1							X
Bell (2005)	Australasia			1			1	192	?					
Berrio et al. (2005)	North America		1		1	3	1			0 to 5				
Blackford and Innes (2006)	Europe	1			1	10	1			5 to 15				
Blackford et al. (2006)	Europe		1		1	4				0 to 5				
Bos et al. (2006)	Europe		1		1	7	1			0 to 5				
Burney et al. (2003)	Africa		1		1	1	1			5 to 15				
Caretta (1998)	Africa			1			1	23	?					
Cordova et al. (2011)	North America	1	1		1	1	1					1800		
Cugny et al. (2010)	Europe	1	1		1	6	1			15 to 50				
Davis (1987)	North America	1	1		1	1	1			0 to 5 *				
Davis and Shafer (2006)	North America	1	1		1	1	1			0 to 5 **				
Davis et al. (2002)	North America		1		1	1	1			5 to 15				
Doveri (2011) & Doveri (2007)	Europe			1			1	256	47					
Ebersohn and Eicker (1992)	Africa			1			1	55	20					
Ejarque et al. (2011)	Europe	1	1		1	7	1			15 to 50				
Ekblom and Gillson (2010)	Africa		1		1	4	1			> 200				
Elshafie (2005)	Asia			1			1	21	12					
Etienne et al. (2011)	Europe		1		1	4	1			15 to 50				
Feeser and O'Connell (2009)	Europe		1		1	9	1			15 to 50				
Feeser and O'Connell (2010)	Europe				1	9	1			15 to 50				
Finsinger (2001)	Europe		1		1	2	1			5 to 15				
Gauthier et al. (2010)	Greenland		1		1	4	1			5 to 15				
Gill et al. (2009)	North America		1		1	1	1			5 to 15				
Gill et al. (2012)	North America		1		1	1	1			5 to 15				
Graf and Chmura (2006)	North America	1			1	6	1			15 to 50				
Hausmann et al. (2002)	Europe		1		1	2	1						300	
Jeffers et al. (2011)	Europe		1		1	1	1						1000	
Jeffers et al. (2012)	Europe		1		1	1	1						1500	
Kruys and Ericson (2008)	Europe			1			1	47	16					
Masunga et al. (2006)	Africa			1			1	47	29					
Mazier et al. (2009)	Europe				1	6	1			50 to 200				
Menozzi et al. (2010)	Europe		1		1	6	1			0 to 5				
Mighall et al. (2010)	Europe		1		1	8	1			5 to 15				
Montoya et al. (2010)	South America				1	2	1			15 to 50				
Mumbi et al. (2008)	Africa		1		1	2				15 to 50				
Mungai et al. (2011)	Asia			1			1	69	39					
Parker and Williams (2011)	North America				1	2	1			5 to 15				
Ponel et al. (2011)	Europe		1		1	3					2000			
Raper and Bush (2009)	North America	1			1	1	1					600		
Richardson (2001a)	Europe			1			1	89	19					
Richardson (2001b)	South America			1			1	19	11					
Richardson (2003)	North America			1			1	66	17					
Richardson (2004a)	Africa			1			1	46	18					
Richardson (2004b)	Europe			1			1	63	22					
Richardson (2006)	Europe			1			1	24	17					
Richardson (2008)	Carabeen			1			1	37	12					
Robinson et al. (2005)	North America		1		1	1	1			5 to 15				
Rule et al. (2012)	Australasia		1		1	2	1			50 to 200				
Ryan and Blackford (2010)	Europe		1		1	6	1			0 to 5				
Schofield and Edwards (2011)	Greenland		1		1	4	1			50 to 200				
van Geel et al. (2003)	Europe	1	1		1	7	1			0 to 5				
van Geel et al. (2007)	North America		1		1	3	1							X
van Geel et al. (2011a)	Asia		1			5	1							X
van Geel et al. (2011b)	North America		1			5	1							X
Watling and Richardson (2010)	South America			1			1	60	23					
Wetterich et al. (2008)	Asia		1		1	3	1			15 to 50				
Wicklow and Angel (1983)	North America			1			1	21	10					
Williams et al. (2011a)	South America		1		1	1	1			15 to 50				
Williams et al. (2011b)	South America		1		1	1	1			5 to 15				
Wood and Wilmshurst (2011)	Australasia	1	1		1	1	1			50 to 200				
Wood et al. (2011)	Australasia		1		1	1	1			5 to 15				

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Chapter 5: Paper4

Improving the identification of dung fungal spore for estimating past population of large herbivore

To be made available on the internet for download

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Improving the identification of dung fungal spore for estimating past population of large herbivore

Abstract

Dung fungal spores are increasingly used to identify densities of large herbivores over time. However which spores are recorded varies between studies. We provide here an identification key to improve comparability between pieces of research. In addition, using this key, we identified 353 individual spores and we ascertained that there may be more variation in spore length and width than reported in the literature. This compilation of identification material is going to greatly facilitate comparison between studies.

Key words: identification key, dung fungal spore, ascomycetes, coprophilous fungus, large herbivores, palaeoecology

Introduction

Dung fungal spores are increasingly used to identify past large herbivore densities. They offer new research opportunities because there is no other means to study past densities of large herbivores. However, when reviewing how dung fungal spore have been used so far, we encountered many studies that only report the presence of *Sporormiella* spp. spore in sediment samples, while others report *Sporormiella* spp. and at least two other spore types assumed to come from dung fungi (Baker *et al.* 2013 fig. 2). This is not in agreement with studies of modern fungal diversity, which report all the genera to be cosmopolitan (world-wide distributed). One possible origin of this discrepancy is the lack of a comprehensive up-to-date guide to the identification of dung fungal spores and the other spore types of similar morphology.

Our aim was to clarify the process of dung fungal spore identification from sedimentary archives (lake sediments or peats) in order to improve the accuracy and comparability between studies assessing past herbivore densities. We had three objectives:

1. To compile identification characters into a key so dung fungal spores from North-Western Europe can be identified in a consistent way;
2. Identify spores from sedimentary archives using this key, and record the dimensions of individual spores; and
3. Compare the spore dimensions we found to those reported in the literature.

Methods

Objective 1

We wrote a key using information from European and international Floras (Bell 1983, 2005; Richardson & Watling 1997; Doveri 2007) and the references reviewed in Baker *et al*. (2013 Table 1 and 3). We included spores from both dung fungi and fungi showing other ecologies, to distinguish between spores indicative of large herbivores and those that are not.

Objective 2

We identified spores from 28 surface samples from small water bodies and nine samples from the top of nine peat sequences. The samples were prepared following standard fossil pollen laboratory methods (Willis & Bennett 2001). The characteristics of all samples used are detailed in Table 1. Every time we encountered a fungal spore we went through the key written as per objective 1 and recorded the spore's length and width (both in µm) at 400x magnification on a Nikon Elipse E400 microscope using an eyepiece graticule calibrated to a stage micrometer. For *Coniachaeta* spp. only the longest diameter's length was recorded.

Table 1 list of samples used

Sample name	Location	North	East	sample type	depth sampled	Year sampled
OO2	The Oostvaardersplassen, NL	52.431	5.394	lake sediments	7	2010
OO3	The Oostvaardersplassen, NL	52.438	5.397	lake sediments	9	2010
OO4	The Oostvaardersplassen, NL	52.442	5.401	lake sediments	20	2009
OO5	The Oostvaardersplassen, NL	52.445	5.398	lake sediments	20	2009
OO6	The Oostvaardersplassen, NL	52.439	5.386	lake sediments	1.5	2010
OO7	The Oostvaardersplassen, NL	52.429	5.388	lake sediments	8	2010
OO8	The Oostvaardersplassen, NL	52.424	5.350	lake sediments	20	2009
OO9	The Oostvaardersplassen, NL	52.430	5.362	lake sediments	20	2009
OO10	The Oostvaardersplassen, NL	52.431	5.315	lake sediments	20	2009
OO11	The Oostvaardersplassen, NL	52.428	5.308	lake sediments	20	2009
OO12	The Oostvaardersplassen, NL	52.430	5.391	lake sediments	10	2010
OO13	The Oostvaardersplassen, NL	52.424	5.379	lake sediments	10	2010
OO14	The Oostvaardersplassen, NL	52.426	5.382	lake sediments	8	2010
OO15	The Oostvaardersplassen, NL	52.424	5.377	lake sediments	7	2010
OO16	The Oostvaardersplassen, NL	52.442	5.403	lake sediments	5	2010
OO17	The Oostvaardersplassen, NL	52.438	5.394	lake sediments	7	2010
Long Cross	The New Forest, UK	50.936	-1.650	lake sediments	8	2010
Plaitford Common	The New Forest, UK	50.961	-1.612	lake sediments	8	2010
Fritham Plain	The New Forest, UK	50.921	-1.679	lake sediments	8	2010
Broomy Plain	The New Forest, UK	50.895	-1.701	lake sediments	8	2010
No Name	The New Forest, UK	50.886	-1.685	lake sediments	8	2010
James's Hill	The New Forest, UK	50.875	-1.600	lake sediments	8	2010
Fletchers Green	The New Forest, UK	50.836	-1.600	lake sediments	8	2010
Fletchers Green S	The New Forest, UK	50.834	-1.600	lake sediments	8	2010
Dibden Bottom	The New Forest, UK	50.857	-1.449	lake sediments	8	2010
Cadnam	The New Forest, UK	50.921	-1.594	lake sediments	8	2010
Mogshade	The New Forest, UK	50.885	-1.658	lake sediments	8	2010
Cranes Moor	The New Forest, UK	50.817	-1.730	peat	10	2003
Barrow Moor	The New Forest, UK	50.921	-1.710	peat	10	2003
Church Moor	The New Forest, UK	50.861	-1.648	peat	12	2003
Stephill Bottom	The New Forest, UK	50.843	-1.503	peat	26	2003
The Noads Bog	The New Forest, UK	50.853	-1.435	peat	22	2003
Alderhill Bottom	The New Forest, UK	50.922	-1.714	peat	22	2003
Hive Gran Bottom	The New Forest, UK	50.934	-1.720	peat	20	2003
Rake brakes Bottom	The New Forest, UK	50.912	-1.684	peat	22	2003
Gritnam Bog	The New Forest, UK	50.859	-1.596	peat	8	2003

Objective 3

We compared our measurements to those published for fossil spores by van Geel *et al*. (2003) (except for *Schizothecium*-type, by Freeser & O'Connell (2009)) and those published for living material by Richardson & Walter (1997).

Results

Objective 1

The key is presented in Figure 1 and a glossary to the key in Figure 2.

In **bold** are the types that have been identified by Baker *et al.* (2013) as originating from dung fungal spores. Other types are either not coprophilous or in need of further research but are included in order to allow a better discrimination of the types that rely on large herbivores to complete their life cycle.

This key may not enable identification of every single spore, bearing in mind that:

- Spore shape and size is variable within species, genus and types.
- Not all spore types have been described, hence spore types not reported so far in the literature are likely to be encountered
- If this key does not lead to the identification of the target spore(s), it is unlikely to be a coprophilous fungi

First step: Is it worth going through the Key?

0. Body between 8 and 50 µm in its maximum length, dark brown in colour, external surface of the wall perfectly smooth, rarely with any ornamentation, wall generally simple (but may be covered by an additional wrinkly, or loose layer) ..... Yes it is, go to couplet 1

0. Body orange, pink, light orangy-pinky brown, to deep reddish brown but never chocolate brown, wall composed of more than one layer (this applies to saffarin-stained samples) ..... No, it is not, pollen grain

0. Any other palynological microfossil ..... No, it is not, Exit

- ‡ = "Not Observed by the authors, refer to the original publication(s)"
- Dimensions are between square brackets [].

Master key to groups A B and C:

1. Microfossil not septate, may have slit(s) or pore(s) but always formed of one single body. 2

1. Microfossil septate, formed of a couple to several bodies or cells ..... **Key A**

2. Absence of both slit and pore ..... Exit

2. Presence of either slit(s) or pore(s) 3

3. Presence of a slit ..... **Key B**

3. Presence of one to several pores ..... **Key C**

Key A: septate microfossils

1. Radially septate	‡ Types HdV - 8 (A-G)
1. Either transversally sepate, or septate in an irregular way but not radially	2
2. 1-septate, i.e. formed of two cells	3
2. 2- to more-septate	7
3. Brown wall of the main body covered with an extra wrinkly layer	Valsaria-type
3. Brown wall of the main body not covered.	4
4. Cells of different dimensions, or absence of both pore(s) and slit(s)	Check couplet 9, otherwise Exit
4. Cells of same dimension, presence of either pore(s) or slit(s)	5
5. Presence of a pore centred in the polar region of each cell, with thick rim around half the diameter of the spore's diameter	**Trichodelitschia-type**
5. Presence of a slit on each cell (running from one polar area to another)	6
6. Both polar areas rounded and of similar shape	**Delitschia-type**
6. Polar areas either both truncated or one truncated, one rounded (2 cells)	**Sporormiella-type**
7. Multi septate	‡ HdV - 3A, or Exit
7. 2 to 7-septate	8
8. 2 to 3-septate, presence of a slit on each cell	**Sporormiella-type** (3 or 4 cells)
8. 2 to 7-septate, absence of any slit or pore	‡ HdV - 10, ‡ HdV – 494, or Exit

Key B: One-celled spores with slit(s)

1. Slit curved, either S-shaped, or following the arcuate edge of the spores, spore not or hardly elongated	2
1. Slit straight and flat, spore clearly longer than large and wide, i.e. elongated	3
2. Spore lentil-shaped (of the shape of a culinary lentil) with a slit on arcuate edge, body slightly ellipsoidal from polar view	Coniochaeta-types
2. Spore +/- cylindrical, rounded at one end or truncated at both, slit running from one polar area to the other	**Sporormiella-type**
3. Both polar areas rounded, [45 x 21 µm (Feeser & O'Connell 2009)]	‡ BRN8

3. One polar area sharply acute, the other truncated, [18-25 x 5-7 µm (van Geel & Aptroot 2006)] ‡ Anthostomella fuegiana-type

Key C: One-celled spore with pore(s)

1. Pores more than 2 **2**
1. Pores 1 or 2 **3**

2. Pores (pits) larger, covering regularly the whole surface of the spore Gelasinospora-type
2. Pores smaller, main one polar, the others irregularly scattered over the surface, polar area without pore slightly truncated, large spore **Bombardioidea-type**

3. Pore 2 (of similar diameter, otherwise: Exit) 4
3. Pore 1 7

4. Spore wall covered with a loose outer layer ‡ Rhytidospora-type, ‡ Pteridospora-type
4. Spore wall formed of only one layer 5

5. Spore clearly heteropolar, large [37 x 18 µm (Feeser & O'Connell 2009)] one polar area roundly tapering, 2 pores according to (Feeser & O'Connell 2009) but only 1 pore observed. type BRN2
5. Spores homopolar 6

6. Small [6–12 x 4–9 µm (van Geel *et al.* 2003)], constricted at both ends thus having a lemon shape Chaetomium-type
6. Medium to large, not constricted at both ends Arnium-type or Soradria (2-pored)-type

7. Spore either perfectly spherical or clearly inequilateral, i.e. having one side straight, or bean shaped Exit
7. Spore equilateral and elongated 8

8. Pore perfectly centred in the polar area (apical) 9
8. Pore slightly to clearly off-centre (sub-apical), and as a result at least partly visible from equatorial view 12

9. Spore outline homopolar, to nearly homopolar 10
9. Spore outline clearly heteropolar 11

10. Pore clearly extruding, polar area obtuse **Schizothecium conicum-type**

10. Pore not extruding or only slightly, polar areas obtuse to acute, ... **Sordaria-type**
(possible presence of a slight truncation)

11. Spore about as long as wide, one end truncated, the other slight ..... Apiosordaria-type
constricted, occasional presence of a transparent appendage at the truncated end
11. Spore longer than wide, large [37 x 18 µm ..... Type BRN2
(Feeser & O'Connell 2009)], one polar area acutely tapering into a
narrow point, the other polar area roundly tapering, 2 pores according
to (Feeser & O'Connell 2009) but only 1 pore observed

12. Widest section often nearer the polar area without pore, internal ..... Type HdV - 65
wall of irregular thickness, very variable in shape
12. Widest section usually nearer the middle, outer and inner 13
surface perfectly smooth

13. Polar area with pore often constricted, the other, truncated ..... Cercophora-type
[13–27 x 6–14 µm (van Geel *et al.* 2003)]
13. Neither constricted nor truncated, slight truncation located ..... **Podospora-type**
in the polar area without pore [39–48 x 16–23 µm (van Geel
et al. 2003)]

Figure 1. Key to fossil spores of dung fungi

Septate: formed of more than one cell, or sometimes of one cell that shows a membranous internal separation (a septa), or simply a body not recognisable as cellular in origin but patterns suggesting some sort of partitioning. A 1-septed body is made up of 2 cells.
Slit: An elongated rupture of the spore wall.
Pore: A round opening of the spore wall.
Extruding pore: raised pore area sticking out from the outline curve of the spore.
Polar area: apices or ends, in spores that are elongated, for other shapes, top and bottom. Here the polar areas are generally those two areas of the spores that show a radial symmetry.
Polar view: viewed from above the polar area, so that one of the polar areas is fully visible and the other polar area is fully concealed behind it.
Equatorial view: viewed from the side, so that one side of the equatorial area is fully visible and half of both polar area too.
Homopolar: Whose polar areas are identical, symmetric (note that the pore and extruding pores are not taken into account for this character).
Heteropolar: Whose polar areas are dissimilar, asymmetric (note that the pore and extruding pores are not taken into account for this character).
Inequilateral: with uneven equatorial area, with no reflection symmetry from the equatorial view.

Constricted / constriction: whose outline become abruptly narrower.
Truncated / truncation: whose polar area is abruptly flattened. This generally has an ontogenetic/developmental origin, whereby the growth of the forming cell is physically limited by the presence of another cell (see e.g. Hanlin (1999) for a description and pictures of the process in *Cercophora palmicola*).

Figure 2. Glossary to the key to fossil spores of dung fungi (Figure 1)

Objective 2 and 3

We identified and measured 353 individual spores. The average length and width for each spore types is presented in Table 2, along with published dimensions for comparison.

Table 2: Measurements of fungal spores as identified in this paper an in the published literature

	Fossil spores (van Geel *et al*. 2003)				Living spores (Richardson & Watling 1997)				Fossil spores, this paper					
	Length		Width		Length		Width		Length			Width		
	min	*max*	*min*	*max*	*min*	*max*	*min*	*max*	*mean*	*sd*	*N*	*mean*	*sd*	*N*
Sordaria-type	16	31	10	17	8	35	4	22	22.1	5.4	132	13.1	3.1	126
Sporormiella-type	14	21	11	17	4.8	26	4	20	13.2	2.6	39	9.2	2.4	39
HdV - 65									34.0	3.7	37	18.6	2.5	37
Podospora-type	39	48	16	23	18	68	11	32	37.0	2.8	33	19.6	2.1	32
HdV - 123									12.6	1.8	22	6.9	1.1	22
Coniochaeta-type					6	23	5	19	18.2	2.6	19			
Cercophora-type	13	27	6	14					19.4	3.4	13	10.6	2.6	13
Arnium-type	38	86	18	36					30.3	9.6	11	15.9	4.6	11
Delitschia-type					8	65	3	33	36.1	8.1	11	16.0	3.2	11
Schizothecium conicum-type		25		16					20.0	2.4	7	12.9	1.9	7
Apiosordaria-type	19	24	1	17					18.3	2.5	6	13.3	1.5	6
Chaetomium-type	6	12	4	9					12.8	1.4	5	9.3	1.1	5
Trichodelitschia-type					18	34	6	12	37.5	2.5	3	12.5	0.0	3

Discussion

Our aim was to clarify the identification of fossil dung fungal spores in order to improve the accuracy and comparability of studies. The identification key presented deals with all known fossil dung fungal spores as well as all spores that could be mistaken for dung fungal spores. In addition we present data on the dimensions of the most common spore types. This compilation of information is going to facilitate comparison between studies.

The identification key and spore dimensions presented here are based on literature and material mainly from north-western Europe. However, most genera of dung fungal spores being cosmopolitan it can be expected that the diagnostic characters can be applied across the globe.

Regional variation is likely and for instance some studies from Africa reported spore types not seen any where else (e.g. Ekblom & Gillson 2010).

The number of spores used for the determination of dimension in this study is relatively small to capture the variability of dung fungal spores. However for some types this is much higher than routinely practiced for the description of species in mycology: 10 measurements in Doveri (2007) and 20 measurements in Bell (2005).

The differences in dimensions highlighted in Table 2 demonstrate that more work will be required to discriminate between the explanations as follows: spore size changes in the process of fossilisation, spore size changes depending on laboratory protocol, and fossil spores originate from a very small sample within the array of species described in Floras.

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Chapter 6: Paper5

Quantification of population sizes of large herbivores and their long-term function in ecosystems using dung fungal spores

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Quantification of population sizes of large herbivores and their long-term function in ecosystems using dung fungal spores

Running title

Large herbivore population size over time

Abstract

1. The relationship between large herbivore numbers and landscape cover over time is poorly understood. There are two schools of thought: one views large herbivores as passive and the other as ecosystem engineers driving vegetation succession. The latter relationship has been used as an argument to support reintroductions of large herbivores onto landscapes in order to restore biodiversity.
2. Most of the research to examine this relationship between large herbivores and landscape change has used extant studies. An alternative approach is to estimate variations in herbivore populations through time and their impact on landscape change using fossil dung fungal spore and pollen in sedimentary sequences. However, to date there has been little quantification of fossil dung fungal spore records and their relationship to herbivore numbers, leaving the dung spore method open to varied interpretations. In this study we aim to develop this method and to calibrate the abundance of dung fungal spores (number cm^{-2} $year^{-1}$) in modern sediments from water bodies in relation to herbivore densities (kg ha^{-1}) using extant records from the rewilded site, the Oostvaardersplassen, in the Netherlands.
3. Results from this study demonstrate that there is a highly significant relationship between spore abundance and local herbivore densities. In addition we identify which transportation mechanisms, herbivore species and physical variables best explain the

abundance of dung fungal spores in sediments. Mammal density (comprising Konik horses, Heck cattle and red deer) best predicted the abundance of total dung fungal spores.

4. A highly significant relationship is apparent between dung fungal spore abundance and mammal density at a very local scale (<10m), when the characteristics of the sampled water bodies are taken into account (surface area of water body, length of shoreline).
5. We found that dung fungal spores from *Sporormiella* spp., *Sordaria* spp. and *Podospora* spp. were principally transported into water bodies by surface run-off from the shores.
6. These results indicate that this method provides a robust quantitative measure of herbivore population size over time; therefore, this method may prove instrumental in providing guidance to policy and management.

Key Words

Dung fungal spores, ecosystem function, ecosystem engineers, grazing ecology, herbivory, land management, large mammals, Oostvaardersplassen, paleoecology, rewilding

Introduction

High trophic level vertebrates such as large herbivores play an important role in shaping vegetation cover and community composition across most landscapes (Nuttle *et al.* 2011; Estes *et al.* 2011; Ripple & Beschta 2012; Tanentzap & Coomes 2012; Ritchie *et al.* 2012; Peh & Lewis 2012). However, what is still widely debated is when and where top-down processes (by which large herbivore populations modify ecosystems) override bottom-up processes (by which climate and soil productivity determine ecosystem structures and composition, including herbivore population size). These processes and their interaction with population dynamics are highly relevant to over 25% of land on earth that is intentionally managed as grazing systems for food production (Asner *et al.* 2004; Steinfeld *et al.* 2006; Ellis *et al.* 2010) and to over 50% of land, covering semi-natural and wild ecosystems (Ellis *et al.* 2010), where there is strong to

moderate presence of large grazers and browsers. Therefore, quantifying the effects of wild and domesticated large herbivore pressure on ecosystems is extremely important if we are to gain understanding of future land cover changes and the impact on biodiversity and ecosystem services (Carpenter *et al.* 2009).

There have been a number of previous studies where the presence of large herbivores on past landscapes and their impact on ecosystems has been studied using fossil spores from coprophilous ascomycetes fungi (hereafter dung fungal spores) (Burney *et al.* 2003; Gill *et al.* 2009; Rule *et al.* 2012; reviewed in Baker *et al.* 2013). Dung fungal spores are unintentionally ingested by large herbivores while feeding on vegetation and germinate after digestion when deposited with dung. Mycelium growth and fructifications have species-specific responses to different moisture levels, temperatures, microclimates and microhabitats and, when successful, the sticky spores are released explosively onto surrounding vegetation, ready to be ingested (Dix & Webster 1995; Krug *et al.* 2004; Bell 2005). Without wind, dung fungal spores are typically ejected up to 30 cm away from the fruiting body (e.g. Yafetto *et al.* 2008). With wind, the majority of spores are deposited within meters (Jackson & Lyford 1999); however, occasionally spores can travel further away (e.g. Gonianakis *et al.* 2005, Hernández Trejo *et al.* 2011). Because dung fungi are strictly reliant on large herbivore's digestive tracts to complete their life cycle, the presence of these spores in lake sediments when recovered during palaeoecological investigations is interpreted as a compelling evidence for the presence of large herbivores (van Geel *et al.* 2003; Davis & Shafer 2006; Baker *et al.* 2013). Despite an increasing use of dung fungi to study past populations of large herbivores, the mechanisms explaining how dung fungal spores are transported into lake sediments have not been addressed and it remains as yet unclear whether and how the abundance of those spores in lake sediments can indicate herbivore *densities* within a landscape (Raper & Bush 2009; Feranec *et al.* 2011; Wood & Wilmshurst 2011; Parker & Williams 2011; Baker *et al.* 2013).

The aim of this research is to determine whether the spores can be used as quantitative proxy of herbivore density over time. We address this question by calibrating dung fungal spore abundance in relation to contemporary herbivore presence, using modern lake surface sediments in relation to known herbivore densities (biomass per surface area). These modern sediment samples serve as analogues to those used in paleoecology, so the calibrated relationship observed today can be extrapolated to the past.

Our objectives are:

(i) To determine the way dung fungal spores travel into sediments i.e. long distance transportation by wind, short distance transportation by wind, transportation with surface run-off, or a combination of these transportation mechanisms.

(ii) To determine whether different spore types indicate different herbivore species.

(iii) To determine what factors most influence the abundance of spores in the sediment i.e. basin size, water body shoreline length, herbivore densities and others factors influencing the transportation mechanisms; and

(iv) Finally, to ascertain whether changes in fossil spore abundances through time in a single sequence can be used to infer changes in herbivore density on the surrounding landscape.

Methods

Study site and sample collection

The Oostvaardersplassen nature reserve, The Netherlands, was established on polder land reclaimed from the sea in 1968. Re-wilding was initiated at this site from 1983 with the introduction of free-ranging Heck cattle (*Bos primigenius*) in 1983, Konik horses (*Equus ferus caballus*) in 1984 and red deer (*Cervus elaphus*) in 1992. These herbivores have access to *c*. 2000 ha of grasslands (e.g. *Lolium perenne, Poa trivialis, Trifolium repens*), tall herbs (e.g. *Cirsium* spp, *Urtica dioica*), reed (*Phragmites australis*), *Sambucus nigra* scrub and *Salix* spp. shrubs (Figure

1). The reserve is visited by large numbers of geese (thousands to tens of thousands of greylag goose, *Anser anser*; Barnacle goose, *Branta leucopsis*; white-fronted goose, *Anser albifrons*). The site is managed with a policy of minimal intervention, i.e. the population size of freely roaming large herbivore is not controlled by culling and no management intervention is implemented to maintain vegetation.

We sampled modern sediment from 16 small water bodies created between 1985 and 2000 to enhance avian biodiversity. Surface sediment samples from the deeper part of the water bodies were collected using a simple tube sampler with sharpened edges. Sedimentary sequences were also collected using simplified Livingstone corer in order to obtain a sedimentary record of the lifespan of three water bodies since they were first created (see Appendix 1). All samples were stored in sealed plastic bags at 4 deg C until being processed in laboratory.

Response variable: spore abundance in sediments

The spores were extracted from one 1 cm^3 sub-samples per sediment sample collected from the 16 water bodies. We followed a standard extraction method in pollen analysis to isolate spores and mount them for microscopic examination at a 400x magnification (Willis & Bennett 2001). Spore identification was based on the literature reviewed in Baker *et al.* (2013). The abundance of spores in sediments was calculated as accumulation rates (spore cm^{-2} $year^{-1}$) using the spore concentration (spore cm^{-3}) and the sedimentation rate (cm $year^{-1}$) (Maher 1981; Bennett 1994; Willis & Bennett 2001). One average sedimentation rate was estimated for all the water bodies sampled using the three cores (Appendix 1).

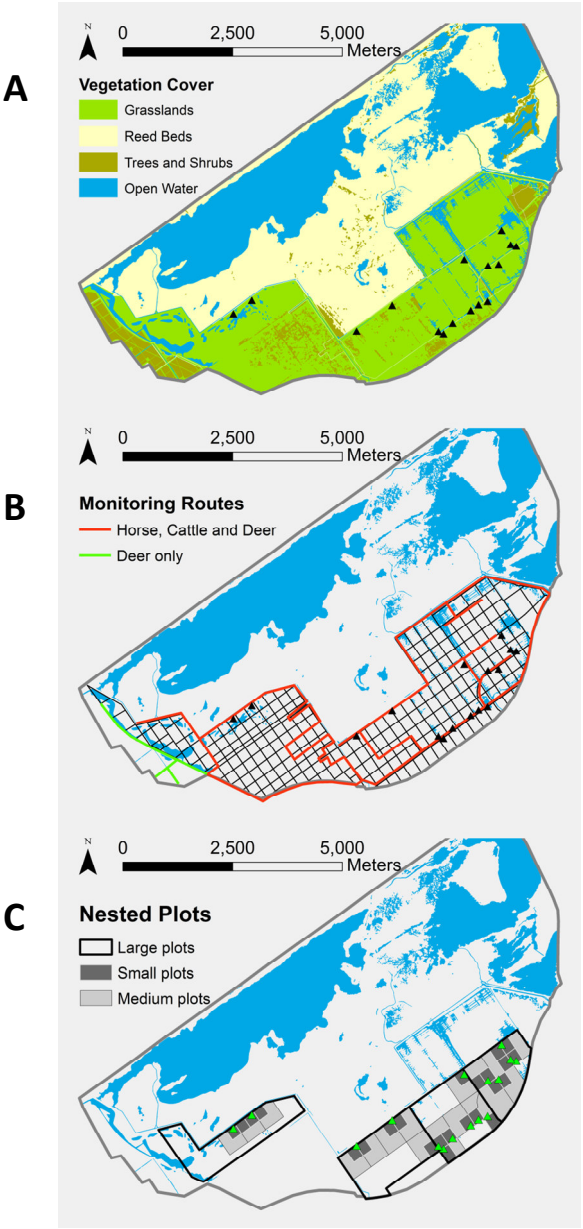


Figure 1: Maps of the Oostvaardersplassen, sample locations indicated with triangles. A. Vegetation map highlighting the grasslands where the herbivores spend most of their time. B. Monitoring method, the number of herbivore is recorded weekly for each of the small plots following established routes. C. The nested plots utilised in this study (aggregation of the small plots shown in B).

Explanatory variables: herbivore densities and physical variables

The distribution of herbivores (cattle, horses, deer and geese) is currently monitored by the reserve wardens on a weekly basis at the Oostvaardersplassen (see Figure 1 B). The data analysed in this paper span the period 2005-2009, for which we established the average number

of individuals for every species in each small, medium and large nested plot (see Figure 1 C). Other animals like foxes and large birds represent a negligible herbivore biomass in comparison with those monitored and there is only incomplete associated data regarding their numbers. To account for the difference of dung production in goose species, red deer, Konik horse and Heck cattle, we transformed herbivore numbers into herbivore biomass density (i.e. biomass per surface area, kg ha^{-1}) using the average biomass of individuals per species. The biomass of herbivore species was compiled from Dunning (2007) for goose, and from the long-term monitoring of large mammal biomass by the RWS Water Service and the State Forestry Office, The Netherlands (see Appendix 2).

Physical variables of each water body (i.e. water body surface area, water body shore length) and surrounding habitat (i.e. total length of shores within small, medium and large nested plots, total surface area of grassland within small, medium and large nested plots) were calculated on the basis of 0.5 x 0.5 m resolution georeferenced aerial photographs taken in 2010 (Ministry of Infrastructure and the Environment and the Ministry of Economic Affairs, The Netherlands).

Data analysis step 1: (i) to determine the way dung fungal spore travel into sediments and (ii) whether different spore types indicate different herbivore species

We used spore abundance as the response variable and compared it to three spore transportation mechanisms (surface run-off, wind-transportation from local shores and wind-transportation from local grasslands) as explanatory variables (Table 1) in generalized linear models (GLM) in R (R Core Team, 2012). A fourth transportation mechanism (background spore rain) is constant across the reserve and is represented by the intercept in GLM outputs. Because over-dispersion of our count data was highlighted by our initial analyses using a link function for Poisson distribution, we used negative binomial regression throughout the analysis. This was chosen over the quasi-Poisson alternative, because more weight on sampling points with higher

spore counts was not deemed appropriate in our case (Hoef & Boveng 2007; O'Hara & Kotze 2010). Models were first fitted using all three explanatory variables without interaction and stepwise-simplified using the function stepAIC of the MASS package (Venables & Ripley 2002) in order to recover the minimal adequate model, aka optimum model (Crawley 2007).

Table 1: Transportation mechanism used for the analysis. 'distance A' can be either small, medium or large nested plots for which we had herbivore densities, 'water body i' is any of the 16 water bodies sampled.

	Transport agent	Location of dung	Variables included
Run-off	Surface run-off, erosion	Water body shoreline and slopes	$H_{A,i}$: Herbivore density within distance A of water body i (kg ha^{-1}) P_i: Perimeter of pond i (m) S_i^{-1}: (Surface area of pond i)$^{-1}$ (m^{-2})
Wind (shore)	Turbulent air	Shorelines and slopes from nearby water bodies	$H_{A,i}$: Herbivore density within distance A of water body i (kg ha^{-1}) $SL_{A,i}$: Shore length of other water bodies within distance A of pond i (m) S_i^{-1}: (Surface area of pond i)$^{-1}$ (m^{-2})
Wind (land)	Turbulent air	Nearby grasslands	$H_{A,i}$: Herbivore density within distance A of water body i (kg ha^{-1}) $L_{A,i}$: Surface area of grassland within distance A of pond i (m^2) S_i^{-1}: (Surface area of pond i)$^{-1}$ (m^{-2})
Background	Turbulent air	Unspecified	(Model intercept)

We analysed separately herbivore densities (kg ha^{-1}) of goose, Konik horses, Heck cattle, red deer and the three mammals together (5 sets of explanatory variables) against each of the three main dung fungal spore types (*Sporormiella*, *Sordaria* and *Podospora*) and their sum (therefore 4 response variables), resulting in 20 distinct optimum models. Each of these 20 models had been initially selected, on the basis of AICs, out of the 27 optimum models representing all combinations of scales the transportation mechanisms were available (three transportation mechanisms measured each at three nested plot sizes).

Data analysis step 2: (iii) to determine what factors most influence the abundance of spores in the sediment

On the basis of the transportation mechanisms identified in the first step of the analysis as significantly related to spore abundance, we took a second step to identify what exact factor (within the transportation mechanisms, see Table 1) influences spore abundance most. This was

implemented by applying GLMs with the same settings as above (link function for negative binomial distribution and automatic stepwise simplification) using the individual factors included in the transportation mechanism (Table 1) as explanatory variables and allowing the assessment of interactions.

Table 2: List of ponds, their characteristics and samples analysed

Pond code	Surface (m square)	Shore length (m)	Total shore length within medium plot (m)	North	East	Year sampled	Sample code(s)	Sample depth (cm)
OO2	7081	414	1657	52.43050800	5.39363240	2009, 2010	Oost-2, Oost-213	20, 7
OO3	3092	487	4583	52.43812010	5.39735413	2009, 2010	Oost-3, Oost-222	20, 9
OO4	4403	525	6776	52.44220897	5.40135547	2009	Oost-4	20
OO5	4419	696	6776	52.44513021	5.39834122	2009	Oost-5	20
OO6	2687	322	2274	52.43912714	5.38580996	2009, 2010	Oost-6, Oost-3/1	20, 1.5
OO7	1521	204	4250	52.42868373	5.38800097	2009, 2010	Oost-7, Oost-217	20, 8
OO8	203	56	172	52.42444476	5.34973321	2009	Oost-8	20
OO9	1063	171	658	52.42969659	5.36165301	2009	Oost-9	20
OO10	5705	323	3209	52.43070174	5.31460139	2009	Oost-10	20
OO11	2011	169	5412	52.42800156	5.30843074	2009	Oost-11	20
OO12	1998	250	4250	52.42982508	5.39061816	2010	Oost-214	10
OO13	443	76	75	52.42389313	5.37887143	2010	Oost-216	10
OO14	2368	274	4338	52.42603232	5.38194373	2010	Oost-201	8
OO15	1206	178	2428	52.42438731	5.37715128	2010	Oost-202	7
OO16	1621	254	6776	52.44189417	5.40323788	2010	Oost-203, Oost-204	10, 5
OO17	1353	247	6209	52.43784175	5.39378056	2010	Oost-205	7

Results

A total of 21 modern sediment samples was collected from 16 ponds of similar morphology (on average about 2600 m^2 in surface) across the reserve (Table 2). Throughout those samples, 17 fungal spore types were identified but only those from *Sporormiella* spp.*, Sordaria* spp. and *Podospora* spp. had regular occurrence and made up c. 70% of all 370 fungal spore identified. Dung fungal spore abundance varied between 161 and 2049 (spore cm^{-2} $year^{-1}$) (mean=945 sd=537 N=16) and herbivore densities between 308 and 1863 (kg ha^{-1}) (mean=728 sd=448 N=16) around the ponds (small plot scale). The cores had an overall average sedimentation rate of 1.14 (cm $year^{-1}$) and a variation within the Oostvaardersplassen of same order of magnitude than

those observed from well dated sedimentary sequences (Bennett *et al.* 1992; Bennett 1994) (see Appendix 1).

(i) How does method of transportation affect dung fungal spore abundances?

Our results demonstrate that there is a quantitative relationship between total dung fungal spore abundance and large mammal density measured in biomass per surface area (Table 3). In particular, shore run-off explained, in a highly significant way, total spore abundance. Plots of the significant relationships between the spores types and transportation by surface run-offs are shown in Figure 2. Local wind dispersal, whether from the grasslands or other nearby water-body shorelines, is not positively contributing to spore influx into the sediments.

Table 3: Identification of transportation mechanisms. Each line summarises the optimum model with the lowest AIC out of a series of 27 optimum models (all combinations of 3 plot scales for 3 transportation mechanisms). Significance codes for p-values: '***' <0.001; '**' <0.01; '*' <0.05; ns otherwise. (-) indicates a negative relationship, and small, medium and large for the nested plots (see text and Figure 1 C); x: mechanism excluded by model simplification.

Response variables			Significance levels				Optimum plots scales		
	Herbivore density for:	AIC	run-offs (shore)	wind (shores)	wind (land)	Intercept	run-off (shore)	wind (shores)	wind (land)
Dung Fungal Spore Total	**mammal**	**240.9**	*******	**(-) ns**		*******	**small**	**medium**	**x**
Dung Fungal Spore Total	Heck cattle	242.8	***	(-) *		***	small	medium	x
Dung Fungal Spore Total	Konik horse	241.7	***	(-) **		***	small	large	x
Dung Fungal Spore Total	red deer	246.4		(-) *	ns	***	x	medium	medium
Dung Fungal Spore Total	goose	247.3		(-) ns	*	***	x	large	large
***Sordaria*-type**	**mammal**	**224.6**	******			*******	**small**	**x**	**x**
Sordaria-type	Heck cattle	226.4	**	(-) *		***	small	medium	**x**
Sordaria-type	Konik horse	226.5	**	(-) ns		***	small	large	x
Sordaria-type	red deer	228.9				***	x	x	x
Sordaria-type	goose	227.2	**	(-) **	*	***	medium	medium	large
***Sporormiella*-type**	**mammal**	**210.5**	*****			*******	**medium**	**x**	**x**
Sporormiella-type	Heck cattle	211.7			ns	***	x	x	large
Sporormiella-type	Konik horse	212.1			ns	***	x	x	large
Sporormiella-type	red deer	211.8	ns			***	large	x	x
Sporormiella-type	goose	212.7				***	x	x	x
Podospora-type	mammal	199.0	ns			***	small	x	x
Podospora-type	Heck cattle	199.3				***	x	x	x
***Podospora*-type**	**Konik horse**	**197.5**	******	**(-) ns**		*******	**small**	**large**	**x**
Podospora-type	red deer	197.9		(-) *		***	x	medium	x
Podospora-type	goose	199.3				***	x	x	x

The background spore deposition is highly significantly different from zero and positive, meaning a spatially constant atmospheric input of spores across the Oostvaardersplassen. The maximum likelihood estimation of the background spore deposition was 318.7 (spore cm^{-2} $year^{-}$

[1]) (95% CI between 428.5 and 237.1) and was in the vicinity of this value for all models presented in Tables 3 and 4. The absence of spatial autocorrelation for the spore abundance in our sediment samples (Moran's I test, observed =0.05787573, expected = -0.06666667, sd = 0.1011752, p = 0.2183387), supports the very local origin of spore abundance in sediments.

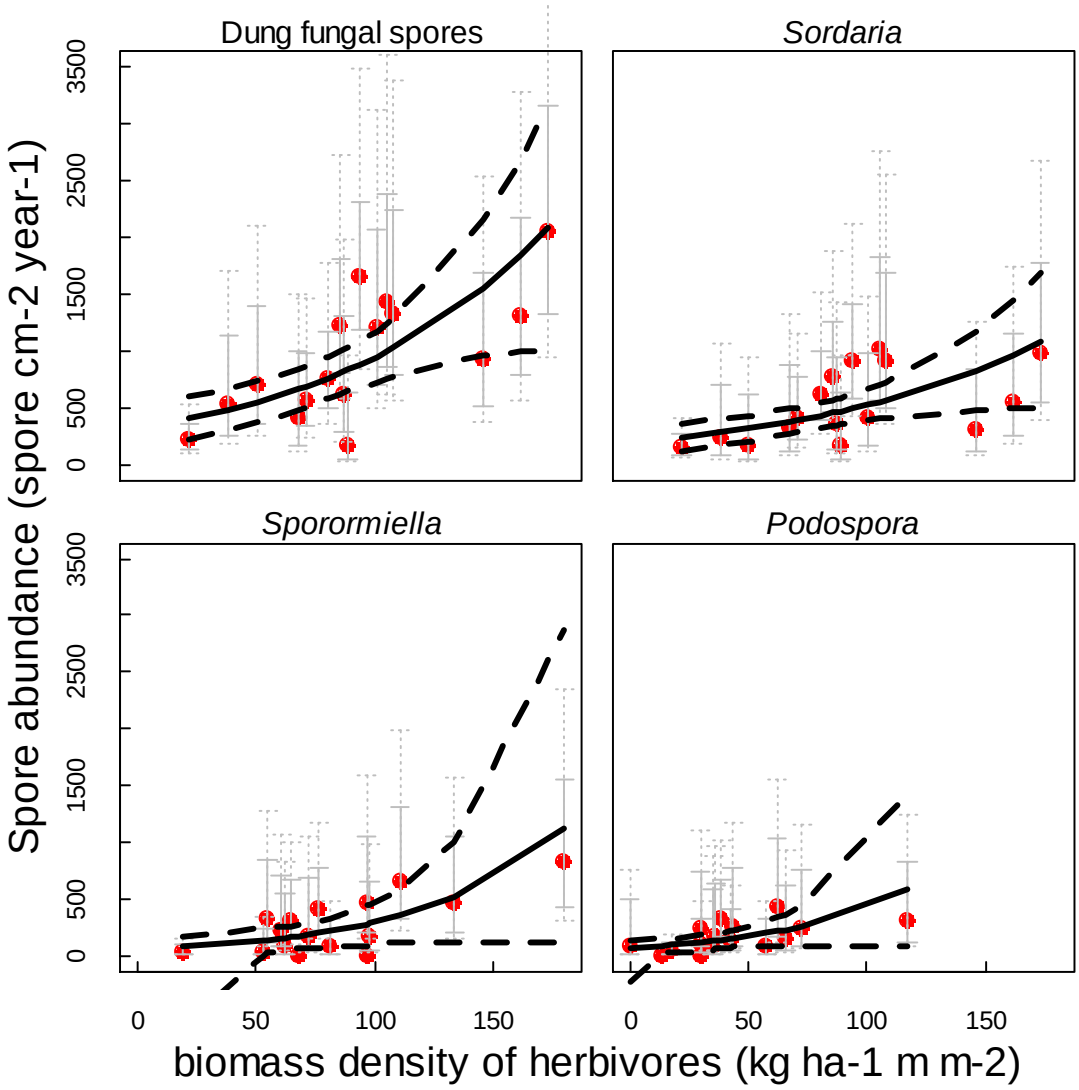


Figure 2: Optimum relationships between spore abundances and the biomass density of herbivores. On the x axis is the interaction between local mammalian biomass density (only Konik horses for Podospora), water body shore length and water body surface area (i.e. surface run-off transportation). Dots in red are the observed data, in black the best model prediction as in Table 3 (dashed, 0.95 confidence interval) and in grey the 0.95 confidence interval relative to spore abundance (dashed, incurred by the sedimentation rate).

(ii) Do different spore types indicate different herbivore species?

Mammal density (irrespective of species) compared better to total spore abundance than any of the herbivore densities taken individually. While Heck cattle and Konik horses both show a good fit with the models, red deer was never significantly related to any of the spore types, and geese only showed significant relationship with *Sordaria* abundance.

Sordaria and *Sporormiella* taken separately show very similar patterns. They are best explained by mammalian densities; however the significance levels are lower for *Sporormiella*. On the contrary, *Podospora* is better explained by Konik horse densities alone. In this case though, the results should be interpreted cautiously because two samples stood out as outliers on model checking plots, suggesting potential heteroscedasticity and potential non-normal errors.

(iii) What factors most influence the abundance of spores in sediments?

Breaking down run-off transportation into its elements (i.e. herbivore density at the small, medium and large nested plot scale, water body surface area and water body shoreline length, see Table 1), only the three-way interaction between local mammal density, water body shoreline length and water body surface area explained highly significantly the total spore abundance, to the exclusion of all two-way interactions and the three factors on their own (Table 4). This result clearly shows that spore abundance is a function of a combination of the local density of large herbivores and the morphology of the sampled water body. Looking at the spore types individually provided a similar message for *Sordaria* and *Sporormiella*. However, this was not significant for *Podospora*.

Table 4: Optimum models identifying which run-off factors and interactions explain best dung fungal spore abundance. Significance codes for p-values: '***' <0.001; '**' <0.01; '*' <0.05

	Estimate	Std. Error	z value	Pr(>|z|)	
Total dung fungal spores					
Intercept	5.7644	0.2960	19.4760	< 2e-16	***
Mammal Density : Pond Perimeter : (1/Pond Surface Area)	0.0108	0.0029	3.6970	0.0002	***
Minimum adequate model: residual deviance: 16.598 on 14 df; AIC: 241.32; 2 x logl: -235.322					
(Maximal Model: residual deviance: 16.508 on 8 df; AIC: 248.65; 2 x logl: -230.646)					
Sordaria					
(Intercept)	6.6650	0.5755	11.5810	< 2e-16	***
Mammal Density	-0.0026	0.0008	-3.3750	0.0007	***
Pond Perimeter	-0.0057	0.0017	-3.4260	0.0006	***
Mammal Density : Pond Perimeter	0.0000	0.0000	3.7470	0.0002	***
Mammal Density : Pond Perimeter : (1/ Pond Surface Area)	0.0121	0.0036	3.3970	0.0007	***
Minimum adequate model : residual deviance : 16.343 on 11 df; AIC : 221.19; 2 x logl : -209.188					
(Maximal Model : residual deviance : 16.250 on 8 df; AIC : 220.9; 2 x logl : -202.904)					
Sporormiella					
(Intercept)	8.9481	2.3344	3.8330	0.0001	***
Mammal Density	-0.0065	0.0030	-2.1320	0.0330	*
(1/ Pond Surface Area) : Pond Perimeter	-44.4672	21.3301	-2.0850	0.0371	*
Mammal Density : (1/ Pond Surface Area) : Pond Perimeter	0.0792	0.0312	2.5360	0.0112	*
Minimum adequate model : residual deviance : 18.625 on 12 df; AIC : 212.92; 2 x logl : -202.922					
(Maximal Model : residual deviance : 18.123 on 8 df; AIC : 217.98; 2 x logl : -199.984)					
Podospora					
(Intercept)	4.4539	0.3809	11.6930	<2e-16	***
Konik pony Density	0.0016	0.0009	1.8530	0.0639	.
Minimum adequate model : residual deviance : 18.225 on 14 df; AIC : 198.31; 2 x logl : -192.311					
(Maximal Model : residual deviance : 18.122 on 8 df; AIC : 207.59; 2 x logl : -189.588)					

Discussion

Our aim was to calibrate the abundance of dung fungal spores to contemporary herbivore densities (kg ha^{-1}) using modern surface sediments. Results from this study demonstrate that that there is a highly significant relationship between spore abundance and local herbivore densities. In addition we identify which transportation mechanisms, herbivore species and physical variables best explain the abundance of dung fungal spores in sediments. Therefore, our results provide significant advances in understanding the use of dung fungal spore to determine the population size of herbivores in the past. It is expected that this new understanding will enable the quantitative comparison of long time-series of herbivore

population size to response or explanatory variables, for instance, vegetation cover dynamic or hunting policies.

(i) How does method of transportation affect dung fungal spore abundances?

Our analysis demonstrates that surface run-off from the shoreline and surrounding slopes (as opposed to longer distances by wind) highly significantly explains dung fungal spore abundances. This is the first time evidence is gained regarding the source area of dung fungal spores from water bodies (Feranec *et al.* 2011; Baker *et al.* 2013). The main implication of this finding is that a time-series of spore abundance tracks herbivore abundance in the close proximity of the sampled water body (in our case less than 10 m away from water). At the same time, drinking water from water features such as those sampled in this study directly determine the daily movements of wild and domesticated herbivores (e.g. Putfarken *et al.* 2008; Shannon *et al.* 2009). Thus, water features are a strategic location to sample and it can be postulated that the local herbivore density quantified with dung fungal spore actually represents herbivore abundance in a broader landscape.

Evidence for local wind-dispersal is very limited. In contrast the wind-driven background spore rain proved systematically different from zero in a highly significant way, comprising a non-negligible influx of spores. The extremely high herbivore density prevailing in the reserve (474.3 kg ha^{-1}, see Appendix 2) in comparison to the surrounding land (mostly arable land, built-up areas and open water, where large herbivores are scarce or absent) suggest that much of this background spore influx originated within the reserve. This indicates that the influx of wind-dispersed spores produces a signal from the overall abundance of large herbivores within the reserve that is not specific to the exact location of sampling.

(ii) Do different spore types indicate different herbivore species?

The total biomass per hectare of mammals proved the best way to measure herbivore density in order to explain significantly total, *Sordaria* and *Sporormiella* spore abundances but not *Podospora*'s. For *Podospora*, there was a relationship trend with Konik horse biomass; however, this was not significant (Table 4). Our results demonstrate that the spores of *Sordaria* and *Sporormiella* as identified in this study do not indicate an herbivore species in particular. This partly differs from the results presented by studies of dung fungus diversity (Richardson 1972, 2001) however these studies are carried out at a finer taxonomic level than spore identification allows. It can be hoped that spore identification will be developed further (Baker *et al.* 2013). In practice, the total dung fungal spore abundance, independently from the various types, currently represent the most effective method to study the size of past herbivores populations as a whole, whatever the mammal species present. Although goose abundance explained *Sordaria* spore abundance significantly, this herbivore is less likely than mammals to be the main source of fungal spore. This result is congruent with bird dung being reported to be a substrate less suitable for fungal growth (Richardson 2001; Doveri 2007). As a result, the important grazing pressure from goose and other birds of similar size (Jano *et al.* 1998; Jefferies & Rockwell 2002) may not be well captured by the abundance of dung fungal spores. Application of our method away from arctic wetlands or other areas favoured by geese would therefore convey total grazing pressure with greater accuracy. However there is clear evidence that the past presence of larger flightless herbivorous birds such as those native to New Zealand can be tracked using the spores of *Sporormiella* spp. (Wood *et al.* 2011).

(iii) What biotic and abiotic factors most influence the abundance of spores in sediments?

We found that mammalian density in conjunction with the morphological characteristics of the sampled water body (i.e. water body surface area and water body shoreline length) explains in a

highly significant way dung fungal spore abundance. Therefore, changes in spore abundance variation through time can be used to indicate large herbivore population size variation whenever we can make the assumption that the water body has stayed approximately the same size. If drastic hydrological changes are suspected, there are several means to assess water level in paleoecology, notably using macrofossils of aquatic plants (Hannon & Gaillard 1997; Dieffenbacher-Krall & Halteman 2000). Using literature review and empirical examples, Giesecke and Fontana (2008) similarly conclude that fossil pollen accumulation rates in lakes can depend on physical variables such as basin size.

The highly significant results using the water body surface area suggest that once transported from the shore to the water body, spores are subject to mixing in the waters and they sediment evenly across the whole surface area flooded. However, studies from North America and the European Alps focusing solely on *Sporormiella* spores suggested otherwise. Raper and Bush (2009), for instance, observed the existence of a negative distance-from-shore gradient for spore abundance. On the other hand, Parker & Williams (2011) found little correlation between *Sporormiella* spore abundances in sediments from the centre and the margin of their lakes. Finally, Etienne *et al.* (2013) found a significant correlation between spore abundance and the proximity of small stream inlets within a small alpine lake. In our case, the small size of the sampled water bodies (mean= 2573.4 m^2, sd= 1926.5, N=16, see Table 2) and the absence of water inlets probably facilitated even mixing within waters.

Conclusion

There is much debate regarding the long-term impact of large herbivores on their environment. As a consequence there is great difficulty in predicting with certitude the impact that wild and domestic large herbivores might have, particularly in conjunction with the unpredictable effects of global change. Several factors can influence fluctuations in large herbivore population dynamics worldwide: for instance, agricultural abandonment in marginal areas, the growing

need for food production and the adoption of novel management strategies such as re-wilding. Our aim was to develop a method for the measurement of long time-series of large herbivore population sizes in relation to environmental factors affecting or impacted by those populations because this is a critical step towards improving our understanding of herbivore-dominated ecosystems. Based on an existing method in paleoecology, we provide here the foundations for the quantitative reconstruction of long time-series of herbivore densities using fossil dung spores contained in lake sedimentary sequences.

Using modern surface sediments, we found that there is a significant relationship between mammalian densities and dung fungal spore abundance in sediments. To extrapolate this relationship into the past we ascertained that when the morphology of the water body sampled remains the same, accurate quantitative reconstructions are possible.

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Appendix 1

Estimation of sedimentation rates in the water bodies of the Oosvaardersplassen

Introduction

The aim of this appendix is to calculate the sedimentation rate (cm $year^{-1}$) in the water bodies of the Oostvaardersplassen. These sedimentation rates are used to calculate spore accumulation rates (spore cm^{-2} $year^{-1}$) from spore concentrations (spore cm^{-3}) (Bennett 1994). All water bodies sampled were dug the same year, in the same bedrock material and, today, they all have similar shape, water depth and vegetation in and around water. As a consequence we expected to have similar sedimentation rates across the water bodies of the Oostvaardersplassen.

Methods

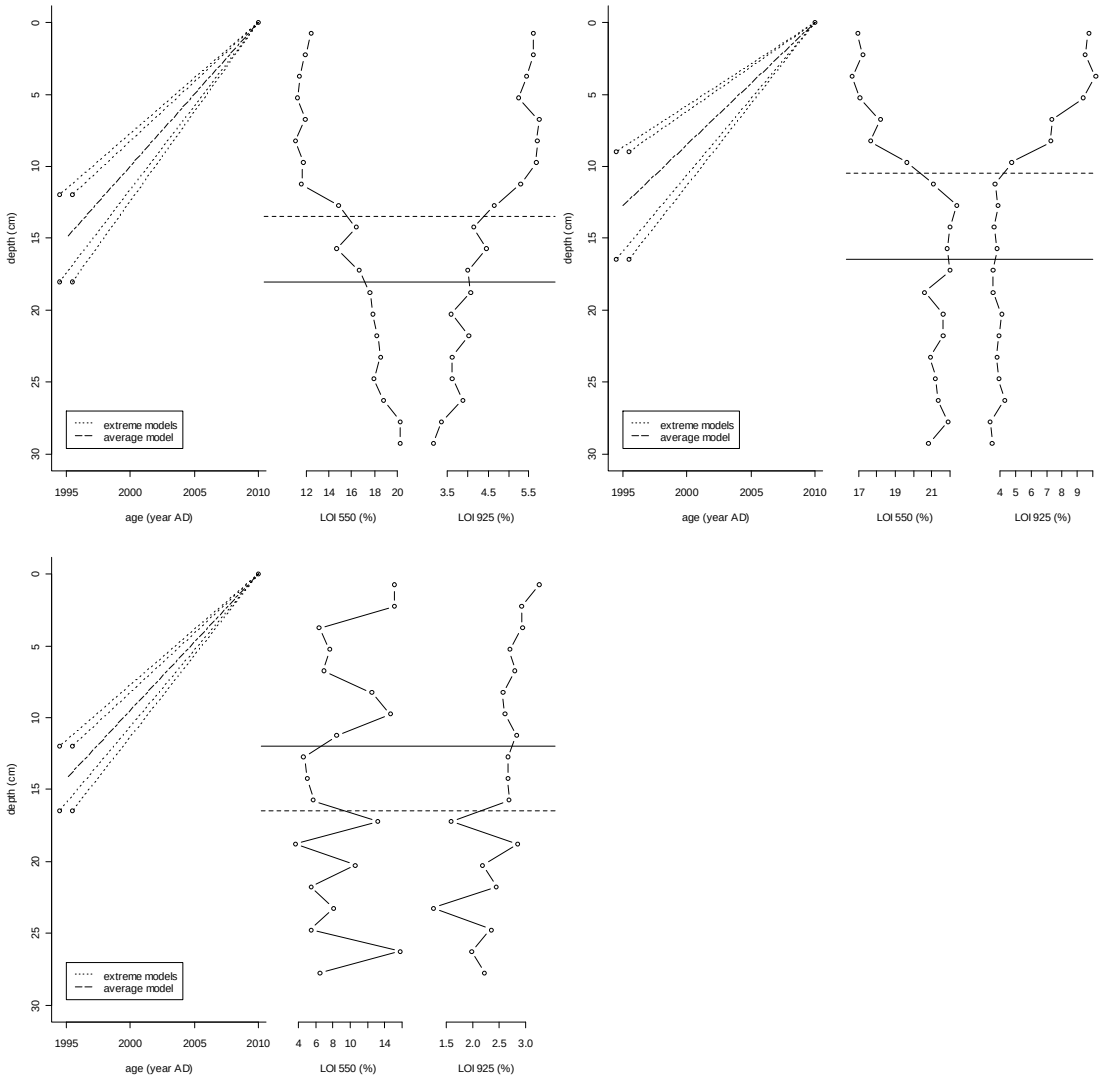
Three short cores were extracted from three different water bodies in July 2010 (OO6, OO14, OO15, see Table 2), using a simplified Livingstone corer. This corer was made with a transparent coring tube, with sharpened edges. Once the core was extracted from the deepest part of the pond, it was slowly extruded in the field from top to bottom, in slices of 1.5 cm. Special care was taken not to disturb the very runny top sediments. The three water bodies that we cored were initially dug in 1995 (month unknown), resulting in between 14.5 and 15.5 years of water body existence represented in the cores.

We determined the transition between pre-reclamation sediments and water body sediments using the coincidence of sedimentary changes. For the sedimentary changes we used Loss-on-Ignition (LOI) at 550 and 925 deg C (Heiri *et al.* 2001), colorimetry (Munsell's charts), and visual assessment of the texture and consistency of the sediments.

Using this data, we calculated four extreme age models per core, corresponding to the minimum and maximum age of the pond (14.5 and 15.5 year) and the maximum and minimum depth of the sedimentary transition zone (between pre-reclamation and post-pond inception). The best estimate of the sedimentation rate was calculated as the average between the minimum and the maximum sedimentation rates.

Results

Appendix 1 Figure 1 shows the sedimentary data and the minimum, maximum and average age-depth models for the three cores.



Appendix 1 Figure 1. Sedimetnary characteristics of the cores and age models. The horizontal plain lines represent discrete texture and consistency transition, the horizontal dashed lines, gradual colour transition.

Appendix 1 Table 1 summarises the sedimentation rates (cm year^{-1}) in the Oostvaardersplassen

Appendix 1Table 1. Calculated sedimentation rates.

	Core 1	Core 2	Core 3	Whole site
Minimum	-0.88	-0.88	-0.81	-0.81
	-0.94	-0.94	-0.86	
	-1.61	-1.21	-1.21	
Maximum	-1.72	-1.29	-1.29	-1.72
Average	-1.29	-1.08	-1.04	-1.14

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Appendix 2

Body mass of large herbivores in the Oostvaardersplassen

The body mass of cattle, horse and deer (Appendix 2 Table 1) was compiled from two sources.

- Regular measurements in the reserve by the State Forestry Service for the period 2008-2012 (Unpublished data).
- Mesurement of all Konik horses and Highland cattle from the wetland nature reserve Zoutkamperplaat, The Netherland, by the RWS Water Service for the period 1991-1994 (Unpublished data). Heck cattle has similar body weigh than Highland cattle.

Appendix 2 Table 1: Body weight of cattle, horse and deer in the Oostvaarderplassen

	Body Weight in Kg					**Average Population**
Heck cattle	1 year	2 year	3 year	4 year	5 years and older	
female	250	350	400	450	500	
male	300	450	550	650	750	**465**
Konik horses	1 year	2 year	3 year	4 years and older		
female	250	350	400	450		
male	275	375	425	475		**375**
Red deer	1 year	2 year	3 year	4 years and older		
female	50	80	100	125		**119**
male	60	110	175	250		

Because the exact male/female ratio and the age structure of the Oostvaardersplassen populations are unknown, we made the assumption that the all genders and age class were equally represented.

Chapter 7: Thesis Conclusion

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Thesis Conclusion

The questions addressed

The research undertaken for my thesis aimed to address the main methodological challenges of determining the variability of large herbivore populations through time and their impact on European vegetation. Large vertebrates are an important part of any ecosystem and, increasingly, conservation policy is looking to restore large herbivores as ecosystem engineers (Zimov 2005; Donlan *et al.* 2006; Kaiser-Bunbury *et al.* 2010; Griffiths *et al.* 2011; Ritchie *et al.* 2012). This policy is based on the premise that before extensive human transformation of the European landscapes, large herbivores were an important driver of disturbance and ecosystem function, and as a result, landscapes were more biodiverse (Vera 2000). With large herbivores shaping the vegetation, it is assumed that primary vegetation in Europe was a mosaic of open habitats and closed-canopy forest (the wood-pasture scenario). However, this pre-human baseline scenario is not without its critics, with an alternative view that in the early European landscape large herbivores were passive and did not modify ecosystems significantly. In the absence of a disturbance regime driven by large herbivores, primary vegetation is assumed to have developed into a dense closed-canopy forest across temperate Europe (the high-forest scenario). The introduction to this thesis identified the main obstacles in palaeoecology to moving this debate forward. These are:

- Understanding the limitations of fossil pollen to identify past land cover. This field of research has been intensively developed in recent years (Sugita 2007a; b; Hellman *et al.* 2008; Mazier *et al.* 2012).
- A poor understanding of past densities of large herbivores in the European landscape. This impediment to our knowledge has not been addressed until today (Svenning 2002; Bradshaw *et al.* 2003; Mitchell 2005; Birks 2005).

- A poor understanding of what a landscape with natural grazing looks like in the pollen record (Mitchell 2005; Birks 2005).

For this reason my thesis addressed five research questions as follows:

Paper 1. Can we use proportions of fossil pollen to infer forest cover and herbivore numbers?

Paper 2. To what extent do pollen analyses such as those following Sugita's approach (2007a; b) improve the interpretation of fossil pollen?

Paper 3. Do dung fungal spores make a good proxy for past distribution of large herbivores?

Paper 4. How can we make the identification of dung fungal spores comparable across studies from different regions?

Paper 5. Can we use dung fungal spores to quantify past abundance of large herbivores?

Below I discuss the main findings from this work and how my results form a framework to understand the impact of large herbivore on vegetation cover through time. The implications for future research are then presented.

Results: greater accuracy for reconstruction of forest cover and herbivores numbers

I first demonstrated that tree pollen percentages from a landscape with natural grazing - in this case, the Oostvaardersplassen, a rewilded nature reserve - does not always provide the expected pollen signature for low tree cover and high herbivore density (**Paper 1**). This contributes to the debate between proponents of the wood-pasture scenario and proponent of the high-forest scenario because my results appear to suggest that naturally grazed landscapes may remain undetected in the fossil pollen record. This shows that pollen percentages alone are not a method that can provide conclusive evidence to determine the influence of large herbivores on vegetation cover. In addition my results strongly suggest that some biological

interaction (here herbivory) can modify relative pollen productivity to such an extent that interpretation of vegetation from the pollen signature alone becomes difficult on a grazed landscape.

In **Paper 2** I developed this understanding of pollen productivity further to find out that *Poaceae* and *Corylus avellana* appear to produce around six time less pollen in closed-canopy forest (old-growth forest of Białowieża) than in open cultural landscapes across Europe (as reported in the literature from seven other studies using these species and the same methodology). Although the literature explicitly acknowledges the potential influence of biotic factors on pollen productivity (e.g. Gaillard *et al.* 2008), the quantification of their impact had never been attempted before. Therefore my results lay an important foundation to intergrate pollen productivity variability when reconstructing vegetation cover from fossil pollen. How in practice biotic interaction could be integrated within a modelling scheme such as the Landscape Reconstruction Algorithm (Sugita 2007a; b) remains to be ascertained.

In **Paper 3**, I highlight the great potential of analysing dung fungal spore as a proxy for large herbivore activity through undertaking a large review of the published work to date that uses this palaeoecological proxy. Results from this exercise indicate that only three spore types (from the genus *Sporormiella*, *Podospora* and *Sordaria*) are widespread enough and have received enough research attention to be used with confidence to determine past herbivore presence on a landscape. However, much remains to be investigated, in particular the use of other spore types, their worldwide distribution in sediments and how their abundance in sedimentary archives can be translated into herbivore presence and densities. On the basis of the literature reviewed, I developed an identification tool based on diagnostic characters and compiled them into a user-friendly key (**Paper 4**). This identification key can be used by scholars around the world in combination with the published microphotographs such as those pointed out in Figure 1 in **Paper 3** to achieve consistent identification across studies. Applying this key to 353

individual spores, I ascertained that size variability may be greater than reported in the literature so far.

Results from **Papers 3 and 4** guided data collection for **Paper 5**. In this chapter I demonstrated that the abundance of dung fungal spore in water body sediments, when expressed as an accumulation rate (number of spore / cm^2 / year), shows a strong correlation with herbivore density and two other variables related to the characteristics of the water body sampled (size of the water body and length of shoreline). The results presented in **Paper 5** also show that dung fungal spores are mainly transported into water with surface run-off, as opposed to wind-transportation. Altogether, my results on dung fungal spores indicate that variation in population size of large herbivore can be reliably reconstructed from small basins such as forest hollows.

Limitations to my experimental work

With **Papers 1, 2 and 5** I undertook the calibration of modern pollen and dung fungal spore in order to improve interpretation of fossil pollen and spore assemblages in sedimentary archives. There is no evidence demonstrating that today's array of ecosystems available for research is sufficient to illustrate past ecosystems (Jackson & Williams 2004). Therefore our ability to interpret the impact of grazing from past pollen and spore assemblages - whether in a qualitative or in a quantitative way - is limited by the type of grazing systems existing today, in particular in the human-dominated landscapes of Europe. This limitation is of course not specific to my thesis and I attempted to reduce it as much as possible by targeting ecosystems with contrasting grazing regimes that are thought to have been more widespread in the past.

While work of **Paper 1** is a necessary step in order to establish the pollen signature of landscapes showing natural grazing, it is not sufficient to provide definitive evidence explaining the mechanism(s) leading to the results presented (Jackson 2012). However, the empirical evidence presented strongly suggests that the process of grazing and browsing by large

herbivores influences pollen productivity in herbaceous and shrubby plants, and in particular in *Poaceae.* This limitation, however, opens up opportunities for future research described below.

Because I collected the modern pollen samples for **Paper 2** under the forest canopy, it may not be appropriate to apply pollen-type specific distance-weighting. In fact the models of pollen deposition currently utilised are based on fine particles dispersion above the forest canopy (Prentice 1985) (but have also been utilised for open landscapes and mosaics landscapes (Broström *et al.* 2008)). The use of inverse distance distance-weighting instead assumes all pollen types have the same flight ability. This limitation was possibly responsible for the poor performance of models including *Picea abies*, a tree species producing pollen grain with much higher fall speed (0.056 m/s) than the other plants studied here (mean = 0.0319 m/s; SD=0.0078 m/s) (e.g. Mazier *et al.* 2012). However, the assumption of same-flight-ability is reasonable within the final set of pollen-types selected in my analysis and has been proved to be acceptable in other similar studies (e.g. Mazier *et al.* 2008).

Paper 5, as a methodological paper, deals with all the experimental limitation of the work on dung fungal spore.

Suggested future research: pollen

Pollen percentages are difficult to interpret quantitatively (**Paper 1**) and therefore more sophisticated approaches such as those developed by Sugita (2007a; b) should be the focus of future research aiming at reconstructing past forest cover. I would like to focus on elucidating the exact mechanism(s) through which grazing and other processes specific to the Oostvaardersplassen modify pollen productivity and assemblages. One approach will be to establish absolute pollen productivity of *Poaceae* and other herbs in the Oostvaardersplassen and in other research areas. It will be necessary to sample from a wider range of ecosystems with a wider range of grazing regimes and other potential factors such as soil fertility. One particular research question that can be addressed with this experimental design is in which

situation grazing decreases pollen productivity (Groenman-van Waateringe 1993; Vera 2000) or on the contrary in which it induces further flowering through over-compensation (Strauss & Agrawal 1999). The balance between pollen productivity suppression and over-compensation is likely to depend on herbivore variety, herbivore numbers, seasonality in herbivory, *Poaceae* species and soil characteristics (e.g. humidity status, fertility and pH). The main difficulty in implementing such research design is to find research areas where the local herbivory pressure is measured in an accurate way, as it is the case at the Oostvaardersplassen (see Paper 5). Failing this, resorting to average pollen accumulation rates and overall herbivore densities within the study area may be practicable, although the number of samples to analyse will be high (hundreds). In such cases, focussing on one or two pollen types and possibly training a machine to carry out the counting will facilitate the task (Holt *et al.* 2011).

There is also an urgent need to develop the conceptual framework and the numerical methodology to integrate pollen productivity variability within a scheme such as Sugita's Landscape Reconstruction Algorithm (Sugita 2007a; b). First, pollen productivity estimates (whether absolute or relative) should be measured from a wider array of ecosystems to ascertain the variability of pollen productivity in response to the diverse types and levels of biotic (e.g. herbivory, canopy shading, presence of pathogen) and abiotic (e.g. climate, soil fertility, and fire) factors. I will focus as a priority on ecosystems showing disturbance regimes such as wild fires, herbivory, river systems with natural flood plains and pathogen outbreaks. Second, I would like to assess the impact of variability in pollen productivity when applied to quantitative reconstruction such as Nielsen *et al*. (2012) and Soepboer *et al*. (2010). Mazier *et al*. (2012) found out that pollen productivity variation (as compiled from published studies) does not induce significant differences in reconstructions but my results show considerably larger variation in pollen productivity. Therefore this need to be assessed again, given the new evidence presented in **Paper 2**.

Suggested future research: dung fungal spore

I would like to develop the work presented in **Paper 3, 4 and 5** by studying the relationship between large herbivores and dung fungal spores in more diverse ecosystems. In particular one objective will be to study areas where the availability of drinking water is less than in the Oostvaardersplassen (**Paper 5**). Water availability across a landscape is likely to influence greatly the visitation frequency by large herbivores to water points, where palaeoecological sampling takes place. As a result, the abundance of dung fungal spores in the sediments of that water point also depends on water availability in the wider landscape (Figure 1). Therefore I would like to demonstrate how dung fungal spores can be used to estimate large herbivore numbers in this wider landscape. There is much scope for improving our understanding of herbivore population sizes in the past, for instance in relation to migration and transhumance. A second objective will be to ascertain whether the positive relationship highlighted in **Paper 5** also applies to systems with other range of herbivore densities.

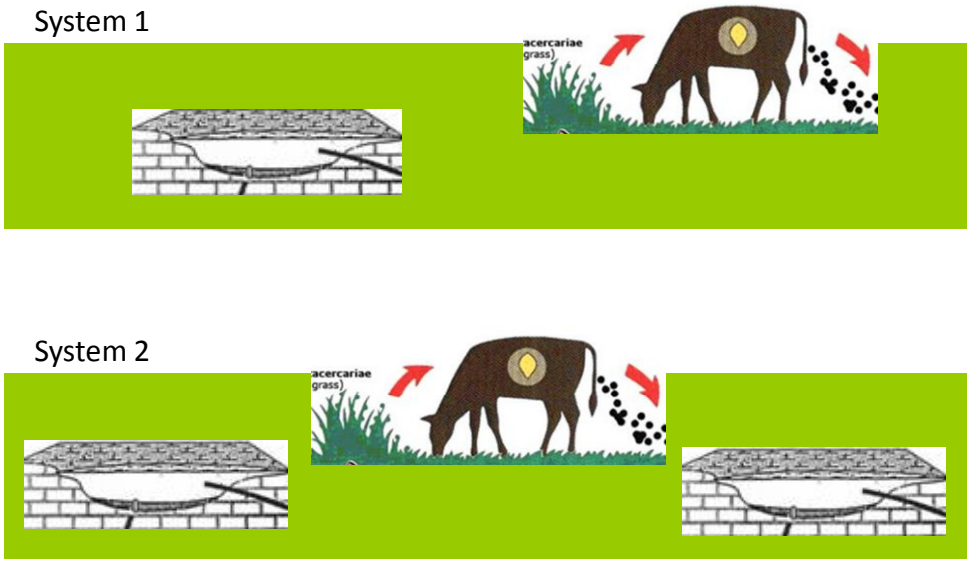


Figure 1: Water availability within the reach of herbivores can influence dung fungal spore abundance in sediments. Here in System 1, the pond will be visited two times more than in System 2. As a result it is likely to have two times more dung along its shore and ultimately a greater abundance of spores in the sediments.

Towards reconstructing ecosystem functioning

My thesis is a contribution to improving studies of large-herbivore population variability through time and their impact on European vegetation cover. Quantitative long-time series collected using fossil pollen and fossil dung fungal spores is the first step towards reconstructing past ecosystem functioning. Lines of evidence for other abiotic (climate, soil fertility, fire, etc.) and biotic factors (canopy shading, pathogen outbreaks, colonisation of organisms new to an area, etc.) are also needed to gain a deeper understanding of interaction between the many components of ecosystems. Improving our understanding of those interactions is needed to better anticipate future environmental changes and promote biodiversity (Estes *et al.* 2011; Blois *et al.* 2013). For instance, the work presented here is directly relevant to the production of evidence to establish appropriate policies with regards to land abandonment and rewilding initiatives in Europe. Although the calibration of proxies in this thesis is geographically restricted to temperate Europe, with modification, this work is also relevant to many other parts of the world where large herbivores are an important feature of the terrestrial system.

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Appendix 1

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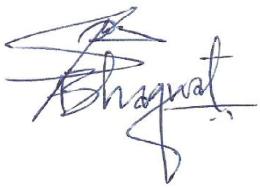
October 11 2013

To whom it may concern

We declare that each of the seven chapters presented in this thesis are substantially (>50%) the work of Mr Ambroise Baker, and that our role has been limited to supervisory input and comments on drafts. The table attached summarises the contribution of each co-author for Chapter 2 to Chapter 6.



Professor Kathy J. Willis
Tasso Leventis Chair in Biodiversity
Director of Biodiversity Institute



Dr Shonil Bhagwat
Senior Research Associate
Biodiversity Institute

				Contributions			
		% of work carried out by Ambroise		All sites	New Forest	Oostvaarders-plassen	Bialowieza
Chapter 2. Paper 1. What is the signature of landscapes with natural grazing in fossil pollen diagrams?							
	Study Design	50		AB, KW, SB			
	Field work	83			AB	AB	AB, MZ
	Laboratory work	66			AB	AB	MZ
	Data analysis	100		AB			
	writing	75		AB, KW, SB, FV			
	TOTAL	**75**					
Chapter 3. Paper 2. The impact of forest canopy shading, forest age and disturbance regimes on pollen productivity: evidence from the old-growth forest of Bialowieza National Park, Poland.							
	Study Design	75					AB, MZ
	Field work	50					AB, MZ
	Laboratory work	0					MZ
	Data analysis	100					AB
	writing	75					AB, KW, SB
	TOTAL	**60**					
Chapter 4. Paper 3. Do dung fungal spores make a good proxy for past distribution of large herbivores?							
	Study Design	75		AB, KW, SB			
	Literature review	100		AB			
	writing	75		AB, KW, SB			
	TOTAL	**83**					
Chapter 5. Paper 4. Improving the identification of dung fungal spore for estimating past population of large herbivore							
	Study Design	100		AB			
	Literature review	100		AB			
	writing	100		AB			
	TOTAL	**100**					
Chapter 6. Paper 5. Quantification of population sizes of large herbivores and their long-term function in ecosystems using dung fungal spores.							
	Study Design	100				AB	
	Field work	100				AB	
	Laboratory work	100				AB	
	Data analysis	80				AB, PC	
	writing	75				AB, PC, KW, SB, FV	
	TOTAL	**91**					
Authors							
AB	Ambroise Baker						
KW	Kathy Willis						
SB	Shonil Bhagwat						
PC	Perry Cornelissen						
MZ	Marcelina Zimny and Malgorzata Latalowa						
FV	Frans Vera						

Appendix 2

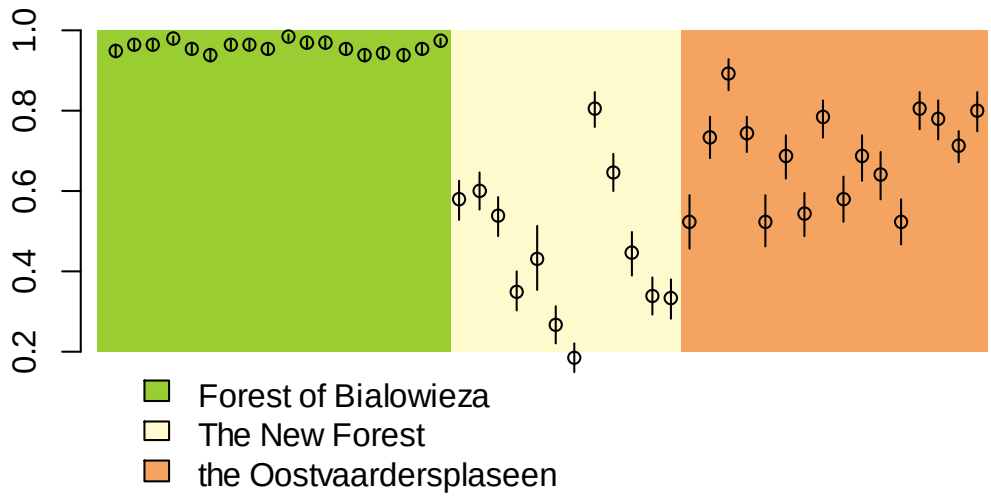
Raw data of the pollen and spore counts for chapter 2, 3, 5 and 6.

Chapter 2: Pollen data used for Figure 1

1. Confidence interval on pollen proportions
2. Raw data, the Oostvaardersplassen
3. Raw data, the New Forest
4. Raw data, Białowieża

1. Proportion of tree pollen for each individual sample, with 95% confidence interval (Maher 1972).

Maher, L.J., 1972. Nomograms for computing 0.95 confidence limits of pollen data. Rev. Palaeobot. Palynol. 13, 85–93.



2. Raw pollen counts: Oostvaardersplassen

Oostvaardersplassen																
	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11	Site 12	Site 13	Site 14	Site 15	Site 16	Site 17
Arboreal Pollen																
Alnus glutinosa	18	61	85	97	43	56	56	78	56	41	52	43	80	68	119	60
Corylus avellana-ty	17	52	79	72	22	52	62	63	46	49	34	23	52	57	118	63
Quercus	14	38	27	46	18	23	22	36	26	32	28	25	42	23	63	41
Betula	22	16	11	16	11	26	21	24	13	16	23	19	16	31	34	19
Pinaceae	11.5	20.5	15.5	29	19	14.5	11	18.5	29	31.5	12.5	28	10.5	26	25.5	15.5
Fagus sylvatica	6	10	7	5	2	3	1	5	3	0	3	6	11	3	13	9
Salix	7	4	2	3	4	1	5	4	3	2	2	3	2	1	6	4
Ulmus	0	2	2	2	1	2	3	1	0	6	7	2	3	2	7	1
Fraxinus excelsior	12	1		3	1	5	2	1	0	1	4	0	6	1	2	2
Sambucus nigra	6	0	0	0	0	0	0	0	0	0	1	2	1	0	1	0
And also rarer grains: Carpinus betulus, Tilia.																
Non-Arboreal Pollen																
Poaceae	68	58	20	73	74	62	112	46	97	61	61	109	46	49	130	43
Chenopodiaceae	4	8	6	9	8	3	5	9	13	9	8	5	8	2	11	6
Urtica	18	0	0	1	6	5	16	1	4	5	9	11	0	6	2	0
Plantago major/me	3	2	0	1	8	8	17	3	4	0	3	5	0	1	4	3
Lactuceae	4	3	0	5	3	5	2	0	1	4	6	5	0	1	3	1
Plantago lanceolata	4	2	1	4	2	0	1	1	7	3	3	1	0	0	3	0
Rumex	1	1	0	1	5	0		2	2	0	1	0	0	1	4	0
Veronica	2	0	0	0	4	0	2	2	0	0	2	2	1	0	0	0
And also rarer grains such as: Brassicaceae, Mentha-type, Stachys-type and Scutellaria-type, Polygonum, Fabaceae, etc.																
Excluded Aquatics																
Cyperaceae	95	11	3	8	58	16	8	13	16	44	0	34	6	3	42	17
Potamogeton natar	8	2	1	1	6	22	1	1	4	2	36	2	0	1	5	1
Trilete spore	1	6	4	13	1	0	0	4	4	5	0	1	5	1	7	4
Typha latifolia	0	1	3	2	0	10	1	3	4	1	7	0	0	4	3	0
Myriophyllum spica	22	0	0	2	2	0	0	1	0	0	5	2	1	0	0	0
Zig-zag cf Equisetur	3	1	0	3	1	0	2	1	1	0	0	4	0	5	2	0
And also rarer grains: Sparganium (abundant in one sample), Callitriche.																
Excluded because not part of the 1 km and 10 km vegeation: Calluna, Vaccinium-type and Sphagnum or equivocal ecology: monolete Pteropsida																

3. Raw pollen counts: New Forest

The New Forest												
	Site1	Site2	Site3	Site4	Site5	Site6	Site7	Site8	Site9	Site10	Site11	Site12
Arboreal Pollen												
Pinus sylvestris	11.5	30.5	5	37.5	1	36.5	28	36	164	68	60	6
Corylus avellana-ty	132	7	83	9	27	19	11	8	29	15	16	32
Quercus	23	57	23	34	11	21	13	74	23	18	24	22
Betula	13	48	45	17	6	8	14	29	33	26	12	27
Fagus sylvatica	2	75	1	15	0	4	3	99	13	5	2	9
Alnus glutinosa	41	6	48	10	12	1	11	7	10	8	3	6
Salix	1	16	1	0	2	2	0	5	3	2	1	2
Fraxinus excelsior	2	1	2	6	1	3	1	2	5	0	3	8
Ilex aquifolium	1	0	0	2	0	0	1	6	0	0	3	3
Taxodiaceae?	0	0	0	1	1	1	0	0	2	0	0	5
Also the rarer grain: Frangula alnus and Sambucus nigra												
Non-Arboreal Pollen												
Poaceae	86	54	108	139	29	146	203	27	49	52	109	91
Calluna vulgaris	38	8	36	52	34	46	52	13	29	103	75	94
Cyperaceae	21	87	0	5	13	15	3	7	67	11	8	4
Lactuceae	8	0	6	10	0	27	37	3	3	0	10	13
Plantago lanceolata	4	2	3	7	0	0	6	0	1	1	3	17
Vaccinium-type	2	0	3	1	4	8	3	0	2	2	13	6
Rosaceae	1	4	2	3	0	6	8	3	1	0	10	5
Plantago coronopus	2	2	4	8	0	0	15	0	0	1	3	0
Echinate Asteraceae	2	0	5	3	0	5	6	0	0	0	2	6
Rumex	0	0	0	4	0	3	3	4	1	4	5	3
Fabaceae	0	2	1	0	0	0	18	1	1	1	0	0
Ranunculus acris-ty	4	0	2	3	0	0	0	0	0	2	3	2
Lotus	0	1	2	2	1	0	4	4	0	1	0	0
Veronica	0	0	0	1	0	3	6	0	0	0	0	0
Rubiaceae	0	0	1	0	0	6	0	0	0	0	0	1
Ranunculaceae	0	0	3	0	0	0	0	1	0	0	3	0
Also the rarer grains: Rhinanthus-type, periporate Caryophylaceae, Trifolium-type, Urtica, Platago major/media												
Excluded Aquatics												
Sphagnum	35	27	44	0	8	1	2	0	8	0	1	0
Callitriche	1	11	0	3	0	0	0	51	32	0	3	0
Potamogeton natan	5	3	0	0	1	2	0	0	0	0	0	1
Also the rarer grains: Sparganium emersum and Lemnaceae												
Also excluded Pteridium aquilinum (9 grains in total) because this can be from wooded or open habitats												

4. Raw pollen counts: Białowieża

Bialowieza: pollen counted by Marcelina Zimny																		
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18
Arboreal pollen																		
Pinus sylvestris	385	287	183	257	453	249	123	363	544	964	141	129	220	240	209	1002	926	911
Carpinus betulus	93	166	86	395	51	441	301	126	63	14	155	294	507	397	142	15	27	7
Betula	22	45	192	160	234	63	233	131	247	111	802	125	120	129	117	93	106	120
Picea abies	341	418	123	153	218	173	108	107	65	36	36	47	216	140	138	33	37	47
Quercus	100	22	387	105	44	166	128	259	71	26	22	523	55	61	341	12	18	10
Alnus	15	25	29	48	90	35	90	90	53	58	103	52	61	151	21	70	73	65
Tilia	37	20	25	6	2	11	19	15	7	0	3	26	38	64	4	0	1	1
Corylus avellana	3	10	14	13	6	6	22	7	16	2	10	10	17	21	67	5	3	1
Fraxinus excelsior	3	1	0	0	1	8	0	0	2	0	1	0	0	1	2	0	2	0
Ulmus	5	2	0	1	0	0	1	0	1	0	2	0	1	0	3	0	1	1
Acer	7	2	0	0	0	1	2	0	0	0	0	0	5	0	0	0	0	0
Salix	0	0	0	0	0	1	0	0	1	1	2	1	1	0	0	1	1	0
And also rarer grains such as: Fagus, Populus, Sambucus, Viburnum, Abies, Julgans, Frangula alnus.																		
Non-Arboreal pollen																		
Sum	53	39	37	23	51	74	38	41	53	19	39	39	58	80	62	82	59	32
Poaceae	18	11	12	6	21	35	18	20	23	4	20	14	17	19	30	16	15	10
Cyperaceae	3	2	0	1	1	4	1	0	0	0	2	0	0	0	3	1	0	1
Counts for other types not shared by my colleagues at Gdansk.																		

Chapter 3: Raw data used for the analysis (same data as for chapter 2)

Samples counted by Marcelina Zimny, marcelina.zimny@univ.gda.pl								
	***Betula* spp.**	***Pinus sylvestris***	***Quercus robur***	***Carpinus betulus***	***Tilia cordata***	***Corylus avellana***	***Alnus glutinosa***	***Poaceae* species**
Site 1	22	385	100	93	37	3	15	18
Site 2	45	287	22	166	20	10	25	11
Site 3	192	183	387	86	25	14	29	12
Site 4	160	257	105	395	6	13	48	6
Site 5	234	453	44	51	2	6	90	21
Site 6	63	249	166	441	11	6	35	35
Site 7	233	123	128	301	19	22	90	18
Site 8	131	363	259	126	15	7	90	20
Site 9	247	544	71	63	7	16	53	23
Site 10	111	964	26	14	0	2	58	4
Site 11	802	141	22	155	3	10	103	20
Site 12	125	129	523	294	26	10	52	14
Site 13	120	220	55	507	38	17	61	17
Site 14	129	240	61	397	64	21	151	19
Site 15	117	209	341	142	4	67	21	30
Site 16	93	1002	12	15	0	5	70	16
Site 17	106	926	18	27	1	3	73	15
Site 18	120	911	10	7	1	1	65	10

Chapter 5: Raw data relative Table 2

(All measurements are in µm and OVD stands for the Oostvaardersplassen)

type	Length	Width	Truncation length	Proximal/ Distal cell (1)	Diameter (2)	Area	Site (as in Table 1, Chapter 5)
Cf. HdV - 123	12.5	6.25				New Forest	Rake Brakes Bottom
Cf. HdV - 123	10	5				New Forest	Rake Brakes Bottom
Sporormiella	8.75	5		D		New Forest	Rake Brakes Bottom
Delitschia	47.5	17.5				New Forest	Rake Brakes Bottom
Schizothecium conicum	23.75	15				New Forest	Rake Brakes Bottom
Schizothecium conicum	18.75	10				New Forest	Hive Gran Bottom
Sporormiella	10	6.25		P		New Forest	Hive Gran Bottom
Podospora	40	17.5				New Forest	Hive Gran Bottom
Sordaria	0	10				New Forest	Alderhill Bottom
Sordaria	22.5	12.5				New Forest	Alderhill Bottom
Cercophora	17.5	12.5	0.25			New Forest	Alderhill Bottom
Coniochaeta	0	0			22.5	New Forest	Alderhill Bottom
Un-ID	15	10				New Forest	Alderhill Bottom
Sordaria	50	25				New Forest	Alderhill Bottom
Podospora	37.5	20				New Forest	Alderhill Bottom
Sordaria	25	12.5				New Forest	Alderhill Bottom
Sordaria	22.5	16.25				New Forest	Alderhill Bottom
Podospora	35	17.5				New Forest	Alderhill Bottom
Sordaria	25	15				New Forest	Alderhill Bottom
Sporormiella	11.25	6.25		D		New Forest	Alderhill Bottom
Sordaria	47.5	22.5				New Forest	Alderhill Bottom
Cercophora	20	10	5			New Forest	Alderhill Bottom
Sordaria	47.5	22.5				New Forest	Alderhill Bottom
Arnium	15	10				New Forest	Alderhill Bottom
Sordaria	22.5	12.5				New Forest	The Noads Bog
Delitschia	32.5	17.5				New	The Noads

						Forest	Bog
Schizothecium conicum	20	12.5				New Forest	The Noads Bog
Coniochaeta	0	0			20	New Forest	The Noads Bog
Sporormiella	12.5	7.5		P		New Forest	The Noads Bog
Podospora	35	16.25				New Forest	The Noads Bog
Sordaria	21.25	12.5				New Forest	The Noads Bog
Sordaria	25	13.75				New Forest	The Noads Bog
Sordaria	25	17.5				New Forest	The Noads Bog
BRN2	33.75	17.5				New Forest	The Noads Bog
Schizothecium conicum	22.5	15				New Forest	The Noads Bog
Delitschia	37.5	15				New Forest	The Noads Bog
Delitschia	40	20				New Forest	The Noads Bog
Sordaria	27.5	16.25				New Forest	The Noads Bog
Delitschia	42.5	20				New Forest	The Noads Bog
Sordaria	25	12.5				New Forest	The Noads Bog
Delitschia	0	0				New Forest	Stephill Bottom
Tripterospora	18.75	15	10			New Forest	Stephill Bottom
Delitschia	40	17.5				New Forest	Stephill Bottom
Coniochaeta	0	0			15	New Forest	Stephill Bottom
Sordaria	27.5	17.5				New Forest	Stephill Bottom
Coniochaeta	0	0			15	New Forest	Stephill Bottom
Delitschia	40	17.5				New Forest	Stephill Bottom
Sordaria	17.5	0				New Forest	Church Moor
Sordaria	20	10				New Forest	Church Moor
Sordaria	22.5	15				New Forest	Church Moor
Sordaria	22.5	12.5				New Forest	Church Moor
Sordaria	25	12.5				New Forest	Church Moor
Sordaria	22.5	15				New Forest	Church Moor
Sporormiella	13.75	10		P		New Forest	Church Moor
Sordaria	22.5	12.5				New	Church Moor

						Forest	
Cercophora	17.5	10	5			New Forest	Church Moor
Sporormiella	15	7.5		D		New Forest	Church Moor
Sordaria	22.5	12.5				New Forest	Church Moor
Sordaria	22.5	12.5				New Forest	Church Moor
Sordaria	21.25	10				New Forest	Church Moor
Sordaria	27.5	15				New Forest	Church Moor
Podospora	37.5	20				New Forest	Church Moor
Sordaria	17.5	10				New Forest	Church Moor
Sordaria	20	0				New Forest	Church Moor
Sordaria	20	12.5				New Forest	Church Moor
Sordaria	20	10				New Forest	Church Moor
Sordaria	17.5	8.75				New Forest	Church Moor
Sordaria	20	12.5				New Forest	Church Moor
Sordaria	20	12.5				New Forest	Church Moor
Sordaria	18.75	10				New Forest	Church Moor
Sordaria	20	0				New Forest	Church Moor
Sordaria	21.25	12.5				New Forest	Church Moor
Sporormiella	15	11.25				New Forest	Church Moor
Sordaria	20	12.5				New Forest	Church Moor
Sordaria	16.25	11.25				New Forest	Church Moor
Sordaria	20	11.25				New Forest	Church Moor
Sordaria	22.5	12.5				New Forest	Church Moor
Sordaria	21.25	10				New Forest	Church Moor
Sordaria	25	15				New Forest	Church Moor
Cf. Sordaria (shouldered)	20	10				New Forest	Church Moor
BRN2	35	15				New Forest	Church Moor
Cf. Sordaria (shouldered)	20	12.5				New Forest	Church Moor
Sporormiella	12.5	10		P		New Forest	Church Moor
Sordaria	20	12.5				New	Barrow Moor

						Forest	
Sordaria	22.5	15				New Forest	Barrow Moor
Sordaria	22.5	15				New Forest	Barrow Moor
Sordaria	22.5	15				New Forest	Barrow Moor
Sordaria	17.5	12.5				New Forest	Barrow Moor
Sordaria	22.5	15				New Forest	Barrow Moor
Sordaria	21.25	11.25				New Forest	Barrow Moor
Sordaria	23.75	15				New Forest	Barrow Moor
Sordaria	22.5	13.75				New Forest	Barrow Moor
Sordaria	20	12.5				New Forest	Barrow Moor
Sordaria	22.5	15				New Forest	Barrow Moor
Sordaria	18.75	11.25				New Forest	Barrow Moor
Sordaria	30	17.5				New Forest	Gritnam Bog
Podospora	35	17.5				New Forest	Gritnam Bog
Cf. HdV - 65	30	20				New Forest	Gritnam Bog
Cf. HdV - 65	37.5	17.5				New Forest	Gritnam Bog
Cf. HdV - 65	30	20				New Forest	Gritnam Bog
Cf. HdV - 65	35	20				New Forest	Gritnam Bog
Cf. HdV - 65	40	21.25				New Forest	Gritnam Bog
Cf. HdV - 65	40	22.5				New Forest	Gritnam Bog
Cf. HdV - 65	40	22.5				New Forest	Gritnam Bog
Cf. HdV - 65	33.75	17.5				New Forest	Gritnam Bog
Cf. HdV - 65	35	20				New Forest	Gritnam Bog
Cf. HdV - 65	30	20				New Forest	Gritnam Bog
Sordaria	22.5	10				New Forest	Gritnam Bog
Cf. HdV - 65	40	22.5				New Forest	Gritnam Bog
Podospora	37.5	20				New Forest	Gritnam Bog
Cf. HdV - 65	32.5	17.5				New Forest	Gritnam Bog
Sordaria	30	15				New Forest	Gritnam Bog
Cf. HdV - 65	42.5	20				New Forest	Gritnam Bog

Cf. HdV - 65	35	17.5				New Forest	Gritnam Bog
Sordaria	25	10				New Forest	Gritnam Bog
Sordaria	32.5	20				New Forest	Gritnam Bog
Cf. HdV - 65	32.5	17.5				New Forest	Gritnam Bog
Cf. HdV - 65	32.5	20				New Forest	Gritnam Bog
Cf. HdV - 65	32.5	20				New Forest	Gritnam Bog
Cf. HdV - 65	27.5	12.5				New Forest	Gritnam Bog
Cf. HdV - 65	32.5	17.5				New Forest	Gritnam Bog
Cf. HdV - 65	32.5	15				New Forest	Gritnam Bog
Cf. HdV - 65	37.5	15				New Forest	Gritnam Bog
Schizothecium conicum	17.5	11.25				New Forest	Gritnam Bog
Cf. HdV - 65	32.5	20				New Forest	Gritnam Bog
Cf. HdV - 65	30	15				New Forest	Gritnam Bog
Cf. HdV - 65	35	16.25				New Forest	Gritnam Bog
Cf. HdV - 65	25	12.5				New Forest	Gritnam Bog
Cf. HdV - 65	32.5	17.5				New Forest	Gritnam Bog
Cf. HdV - 123	15	7.5				New Forest	Gritnam Bog
Cf. HdV - 65	35	20				New Forest	Gritnam Bog
Cf. HdV - 65	32.5	16.25				New Forest	Gritnam Bog
Sporormiella	11.25	8.75		P		New Forest	Gritnam Bog
Cf. HdV - 65	37.5	20				New Forest	Gritnam Bog
Cf. HdV - 65	32.5	17.5				New Forest	Gritnam Bog
Cf. HdV - 65	37.5	18.75				New Forest	Gritnam Bog
Cf. HdV - 65	35	22.5				New Forest	Gritnam Bog
Podospora	35	18.75				New Forest	Gritnam Bog
Cf. HdV - 65	32.5	20				New Forest	Gritnam Bog
Cf. HdV - 65	35	20				New Forest	Gritnam Bog
Cf. HdV - 65	32.5	20				New Forest	Gritnam Bog
Cf. HdV - 65	35	20				New Forest	Gritnam Bog

Podospora	37.5	20				New Forest	Gritnam Bog
Cercophora	27.5	15	5			New Forest	Gritnam Bog
Sordaria	22.5	12.5				New Forest	Gritnam Bog
Cercophora	12.5	7.5	2.5			New Forest	Gritnam Bog
Podospora	37.5	20				New Forest	Gritnam Bog
Sporormiella	10	6.25		D		New Forest	Gritnam Bog
Sordaria	17.5	10				New Forest	Gritnam Bog
Sordaria	26.25	15				New Forest	Gritnam Bog
Cercophora	20	10	5			New Forest	Gritnam Bog
Podospora	40	21.25				New Forest	Gritnam Bog
Podospora	35	20				New Forest	Gritnam Bog
Cercophora	20	16.25	5			New Forest	Gritnam Bog
Sporormiella	10	6.25		D		New Forest	Gritnam Bog
Sporormiella	15	10		P		New Forest	Gritnam Bog
Cercophora	17.5	7.5	5			New Forest	Gritnam Bog
Coniochaeta	0	0			21.25	New Forest	Gritnam Bog
Coniochaeta	0	0			20	New Forest	Gritnam Bog
Sordaria	20	12.5				New Forest	Gritnam Bog
Podospora	40	20				New Forest	Gritnam Bog
Sordaria	20	10				New Forest	Gritnam Bog
Sporormiella	11.25	7.5				New Forest	Gritnam Bog
Sordaria	17.5	10				New Forest	Gritnam Bog
Sordaria	20	11.25				New Forest	Church Moor
Tripterospora	17.5	12.5				New Forest	Church Moor
Sordaria	22.5	10				New Forest	Church Moor
Tripterospora	18.75	13.75				New Forest	Church Moor
Tripterospora	17.5	11.25				New Forest	Church Moor
Sordaria	20	13.75				New Forest	Church Moor
Sordaria	20	12.5				New Forest	Church Moor
Sordaria	17.5	15				New	Church Moor

						Forest	
Sordaria	20	13.75				New Forest	Church Moor
Podospora	40	22.5				New Forest	Church Moor
Arnium	42.5	22.5				New Forest	Church Moor
Cercophora	20	10	5			New Forest	Church Moor
Sordaria	17.5	12.5				New Forest	Church Moor
Coniochaeta	0	0			22.5	New Forest	Church Moor
Podospora	35	20				New Forest	Church Moor
Podospora	37.5	17.5				New Forest	Church Moor
Podospora	35	22.5				New Forest	Church Moor
Sordaria	22.5	12.5				OVD	OO1
Coniochaeta	0	0			20	OVD	OO1
Coniochaeta	0	0			17.5	OVD	OO1
Coniochaeta	0	0			15	OVD	OO1
Sporormiella	12.5	6.25		D		OVD	OO15
Cf. HdV - 123	10	6.25				OVD	OO15
Sordaria	25	0				OVD	OO15
Podospora	40	20				OVD	OO15
Cf. HdV - 123	12.5	7.5				OVD	OO15
Sordaria	20	13.75				OVD	OO15
Cf. HdV - 123	12.5	7.5				OVD	OO15
Podospora	35	20				OVD	OO15
Cf. HdV - 123	13.75	7.5				OVD	OO15
Sordaria	20	12.5				OVD	OO15
Cf. Sordaria (shouldered)	17.5	10				OVD	OO15
Sporormiella	15	10		P		OVD	OO16
Sordaria	20	12.5				OVD	OO16
Sordaria	27.5	15				OVD	OO13
Sporormiella	15	12.5		P		OVD	OO13
Delitschia	27.5	16.25				OVD	OO13
Sporormiella	17.5	10				OVD	OO13
Sordaria	20	12.5				OVD	OO13
Podospora	35	22.5				OVD	OO13
Chaetomium	12.5	10				OVD	OO3
Sordaria	20	11.25				OVD	OO4
Sordaria	25	17.5				OVD	OO5
Arnium	40	20				OVD	OO6

Sporormiella	15	10		D		OVD	OO9
Arnium	27.5	20				OVD	OO10
Sordaria	17.5	10				OVD	OO11
Sporormiella	15	10		D		OVD	OO12
Delitschia	17.5	10				OVD	OO3
Sordaria	20	12.5				OVD	OO3
Delitschia	37.5	12.5				OVD	OO3
Sordaria	12.5	7.5				OVD	OO4
Sporormiella	15	10		P		OVD	OO6
Sordaria	15	7.5				OVD	OO7
Sordaria	17.5	10				OVD	OO7
Sordaria	20	12.5				OVD	OO8
Cf. HdV - 123	15	7.5				OVD	OO9
Sordaria	30	15				OVD	OO10
Sporormiella	7.5	5		P		OVD	OO11
Sporormiella	12.5	10		P		OVD	OO12
Sporormiella	15	12.5		P		OVD	OO13
Podospora	30	15				OVD	OO5
Sporormiella	12.5	10				OVD	OO5
Sordaria	17.5	10				OVD	OO5
Cf. Sordaria (shouldered)	17.5	12.5				OVD	OO10
Cercophora	20	10	5			OVD	OO10
Sordaria	17.5	10				OVD	OO10
Podospora	30	20				OVD	OO10
Sordaria	20	10				OVD	OO3
Sordaria	25	15				OVD	OO15
Coniochaeta	0	0			20	OVD	OO15
Podospora	40	20				OVD	OO16
Sordaria	17.5	10				OVD	OO16
Sordaria	17.5	10				OVD	OO16
Arnium	20	10				OVD	OO16
Arnium	27.5	12.5				OVD	OO14
Sordaria	20	15				OVD	OO14
Coniochaeta	0	0			17.5	OVD	OO14
Sporormiella	12.5	10				OVD	OO14
Delitschia	35	12.5				OVD	OO17
Cercophora	22.5	10				OVD	OO17
Trichodelitschia	37.5	12.5				OVD	OO17
Cf. HdV - 123	12.5	7.5				OVD	OO17
Sordaria	20	10				OVD	OO17
Cf. HdV - 123	12.5	7.5				OVD	OO2
Sporormiella	17.5	12.5				OVD	OO12
Trichodelitschia	40	12.5				OVD	OO12

Cf. HdV - 123	11.25	7.5				OVD	OO12
Sordaria	27.5	15				OVD	OO12
Cf. Sordaria (shouldered)	15	12.5				OVD	OO12
Sporormiella	15	12.5		P		OVD	OO13
Sordaria	27.5	17.5				OVD	OO13
Cf. HdV - 123	11.25	6.25				OVD	OO13
Cf. HdV - 123	12.5	7.5				OVD	OO13
Sporormiella	15	13.75		P		OVD	OO13
Sporormiella	15	12.5		P		OVD	OO13
Cf. HdV - 123	15	7.5				OVD	OO13
Sordaria	25	0				OVD	OO7
Sordaria	25	15				OVD	OO7
Cf. HdV - 123	12.5	5				OVD	OO7
Sordaria	20	12.5				OVD	OO7
Podospora	35	17.5				OVD	OO7
Sordaria	15	10				OVD	OO7
Sordaria	20	11.25				OVD	OO7
Arnium	30	15				OVD	OO7
Sordaria	17.5	10				OVD	OO7
Chaetomium	15	8.75				OVD	OO7
Sordaria	27.5	17.5				OVD	OO7
Podospora	37.5	20				OVD	OO7
Coniochaeta	0	0			17.5	OVD	OO7
Cf. HdV - 123	15	7.5				OVD	OO7
Coniochaeta	0	0			20	OVD	OO3
Podospora	40	25				OVD	OO3
Sordaria	20	10				OVD	OO3
Cf. HdV - 123	12.5	6.25				OVD	OO3
Cf. Sordaria (shouldered)	25	15				OVD	OO3
Sordaria	17.5	11.25				OVD	OO3
Tripterospora	15	12.5				OVD	OO3
Sordaria	17.5	11.25				OVD	OO3
Gelasinospora	22.5	15				OVD	OO3
Sordaria	27.5	17.5				OVD	OO3
Sordaria	17.5	10				OVD	OO3
Sporormiella	15	10		D		OVD	OO3
Sordaria	25	15				OVD	OO3
Sordaria	20	10				OVD	OO3
Sordaria	25	16.25				OVD	OO3

Sordaria	22.5	12.5				OVD	OO3
Cercophora	17.5	8.75				OVD	OO12
Sordaria	25	13.75				OVD	OO12
Sporormiella	15	12.5		P		OVD	OO12
Sordaria	20	12.5				OVD	OO6
Sporormiella	15	10				OVD	OO6
Cf. HdV - 65	30	17.5				OVD	OO17
Sordaria	20	15				OVD	OO6
Schizothecium conicum	20	13.75				OVD	OO6
Tripterospora	22.5	15	10			OVD	OO6
Arnium	35	17.5				OVD	OO6
Sordaria	27.5	20				OVD	OO6
Podospora	37.5	18.75				OVD	OO6
Sordaria	17.5	12.5				OVD	OO6
Podospora	35	17.5				OVD	OO6
Arnium	42.5	20				OVD	OO6
Cf. HdV - 123	10	6.25				OVD	OO6
Arnium	18.75	10				OVD	OO6
Sporormiella	15	10		D		OVD	OO6
Sordaria	17.5	10				OVD	OO6
Sordaria	20	10				OVD	OO6
Sporormiella	12.5	10		D		OVD	OO6
Podospora	40	20				OVD	OO6
Coniochaeta	0	0			17.5	OVD	OO6
Sordaria	17.5	0				OVD	OO6
Sordaria	20	12.5				OVD	OO6
Sporormiella	12.5	7.5		D		OVD	OO6
Sordaria	20	10				OVD	OO6
Sordaria	0	0				OVD	OO8
Coniochaeta	0	0			17.5	OVD	OO8
Sordaria	20	12.5				OVD	OO8
Sporormiella	15	10				OVD	OO8
Sordaria	17.5	12.5				OVD	OO8
Podospora	40	20				OVD	OO8
Sordaria	17.5	11.25				OVD	OO14
Sordaria	30	22.5				OVD	OO14
Coniochaeta	0	0			1.25	OVD	OO14
Cf. HdV - 123	12.5	7.5				OVD	OO14
Coniochaeta	0	0			15	OVD	OO14
Trichodelitschia	35	12.5				OVD	OO14
Coniochaeta	0	0			15	OVD	OO15

Cercophora	20	10	5			OVD	OO15
Chaetomium	12.5	10				OVD	OO15
Sporormiella	7.5	5				OVD	OO15
Cf. HdV - 123	10	5				OVD	OO15
Chaetomium	11.25	7.5				OVD	OO15
Podospora	37.5	17.5				OVD	OO15
Cf. HdV - 123	16.25	10				OVD	OO15
Chaetomium	12.5	10				OVD	OO15
Cf. HdV - 123	12.5	6.25				OVD	OO15
Podospora	35	0				OVD	OO15
Arnium	35	17.5				OVD	OO15
Sordaria	25	17.5				OVD	OO6
Sporormiella	15	10		D		OVD	OO6
Sordaria	17.5	10				OVD	OO6
Sporormiella	7.5	5		P		OVD	OO6
Sordaria	27.5	15				OVD	OO6
Sordaria	17.5	10				OVD	OO6
Sporormiaceae without slit	10	6.25				OVD	OO6
Podospora	42.5	22.5				OVD	OO6
Schizothecium conicum	17.5	12.5				OVD	OO6
Gelasinospora	27.5	17.5				OVD	OO6

(1) Sporormiella type only

(2) Coniochaeta type only

Chapter 6: Raw data of spore counts relative to modelling work as presented in Table 3

			Counts				
	Number of lycopodium tablets added	Number of Lycopodium spore per tablet	Podospora	Sordaria	Sporormiella	Lycopodium	Percent Lycopodium spore counted
OO2	4	18583	3	12	2	3136	4.218909756
OO3	4	18583	3	18	1	1112	1.495990959
OO4	2	18583	3	2	4	537	1.444868966
OO5	2	18583	1	4	2	482	1.296884249
OO6	4	18583	8	22	10	1022	1.374912554
OO7	4	18583	3	17	7	992	1.334553086
OO8	2	18583	3	12	10	517	1.391056342
OO9	2	18583	2	5	8	525	1.412581392
OO10	2	18583	5	12	0	501	1.348006242
OO11	2	18583	2	4	6	545	1.466394016
OO12	2	18583	1	3	3	509	1.369531292
OO13	2	18583	4	7	6	500	1.345315611
OO14	2	18583	0	4	1	516	1.388365711
OO15	2	18583	3	11	2	509	1.369531292
OO16	4	18583	2	10	2	992	1.334553086
OO17	2	18583	0	2	0	525	1.412581392