

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

Ambroise G. Baker^{1,2*}, Perry Cornelissen³, Shonil A. Bhagwat^{4,5}, Fransciscus W. M. Vera⁶ and Katherine J. Willis^{1,7,8}

¹Oxford Long-Term Ecology Laboratory, Department of Zoology, Biodiversity Institute, University of Oxford, Oxford, OX1 3PS, UK; ²Department of Geography, University College London, London WC1E 6BT, UK; ³State Forestry Service, PO Box 2, 3800 AA Amersfoort, The Netherlands; ⁴Department of Geography, The Open University, Milton Keynes MK7 6AA, UK; ⁵School of Geography and the Environment, University of Oxford, Oxford, OX1 3QY, UK; ⁶University of Groningen, Institute of Evolutionary Life Science, NL-9700 CC Groningen, The Netherlands; ⁷University of Bergen, Department of Biology, PO Box 7803, N-5020 Bergen, Norway; and ⁸Royal Botanic Gardens, Kew, Richmond TW9 3AB, Surrey, UK

Summary

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 relatively passive elements upon the landscape 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 many landscapes in order to increase vegetation heterogeneity and biodiversity through local-scale disturbance regimes. Most of the research examining the relationship between large herbivores and their impact on landscapes has used extant studies. An alternative approach is to estimate the impact of variations in herbivore populations through time using fossil dung fungal spores 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 this method open to varied interpretations.

2. In this study, we developed further the dung fungal spore method and determined the relationship between spore abundance in sediments (number cm⁻² year⁻¹) and herbivore biomass densities (kg ha⁻¹). To establish this relationship, we used the following: (i) the abundance of *Sporormiella* spp., *Sordaria* spp. and *Podospora* spp. spores in modern sediments from ponds and (ii) weekly counts of contemporary wildlife over a period of 5 years from the rewilded site, Oostvaardersplassen, in the Netherlands.

3. Results from this study demonstrate that there is a highly significant relationship between spore abundance and local biomass densities of herbivores that can be used in the calibration of fossil records. Mammal biomass density (comprising Konik horses, Heck cattle and red deer) predicts in a highly significant way the abundance of all dung fungal spores amalgamated together. This relationship is apparent at a very local scale (< 10 m), when the characteristics of the sampled ponds are taken into account (surface area of pond, length of shoreline). In addition, we identify that dung fungal spores are principally transported into ponds by surface run-off from the shores.

4. These results indicate that this method provides a robust quantitative measure of herbivore population size over time.

Key-words: ecosystem engineers, ecosystem function, grazing ecology, herbivory, land management, large mammals, Oostvaardersplassen, palaeoecology, rewilding, *Sporormiella*

Introduction

Large herbivores and other vertebrates that live at high trophic levels play an important role in shaping vegetation cover and community composition across most landscapes (Estes *et al.* 2011; Nuttle *et al.* 2011; Peh & Lewis 2012; Ripple & Beschta 2012; Ritchie *et al.* 2012; Tanentzap & Coomes 2012;

Cromsigt & te Beest 2014). 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, Gerber &

*Correspondence author. E-mail: ambroise.baker@ucl.ac.uk

Wassenaar 2006; Ellis *et al.* 2010) and to over 50% of land, covering semi-natural and wild ecosystems (Ellis *et al.* 2010), where large grazers and browsers are present in abundance. 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 their impact on biodiversity and ecosystem services (Carpenter *et al.* 2009). Increasingly conservation policy is looking to introduce large herbivores as ecosystem engineers where they are absent (Seddon *et al.* 2014; Ceaşu *et al.* 2015; Naundrup & Svenning 2015). This policy is based on the premise that before the late Quaternary extinctions of megafauna (Koch & Barnosky 2006; Stuart 2015), large herbivores were important drivers of ecosystem disturbance, function and biodiversity (Vera 2000; Donlan *et al.* 2005; Corlett 2013; Sandom *et al.* 2014). However, this pre-human baseline scenario is not without its critics, with an alternative view that large herbivores were 'passive' on early landscapes and not ecosystem engineers (Bradshaw, Hannon & Lister 2003; Birks 2005).

One of the main obstacles in this debate has been a limited understanding of past population dynamics of large herbivores (Bradshaw, Hannon & Lister 2003; de Bruyn *et al.* 2011). A number of previous studies have demonstrated the presence of large herbivores on past landscapes and their impact on ecosystems using fossil spores from coprophilous ascomycetes fungi (hereafter dung fungal spores) (Burney, Robinson & Burney 2003; Gill *et al.* 2009; Rule *et al.* 2012; Baker, Bhagwat & Willis 2013; Froyd *et al.* 2014). These 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, microhabitats and types of dung and, when successful, the sticky spores are released explosively onto surrounding vegetation, ready to be ingested (Dix & Webster 1995; Krug, Benny & Keller 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 metres (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 herbivores' digestive tracts to complete their life cycle, the presence of these spores in sediments when recovered during palaeoecological investigations are interpreted as a compelling evidence for the presence of large herbivores (van Geel *et al.* 2003; Davis & Shafer 2006; Baker, Bhagwat & Willis 2013; Johnson *et al.* 2015).

Despite the increasing use of dung fungi to study past populations of large herbivores, it remains as yet unclear whether and how the abundance of those spores in sediments can indicate herbivore densities within a landscape (Raper & Bush 2009; Feranec *et al.* 2011; Parker & Williams 2011; Wood & Wilmshurst 2011; Baker, Bhagwat & Willis 2013; Etienne *et al.* 2013). A number of factors can potentially obscure the relationship between spore abundance and herbivore abundance. They include the differential spore production of dung fungal species and their reliance on specific herbivore species, as

well as other taphonomic processes such as average distance travelled by spores and spatial patterns of deposition within waters. These factors are under-researched and evidence can be contradictory. For instance, within water bodies, higher dung fungal spore abundances are reported to be positively related to inflow proximity (Etienne *et al.* 2013), shore proximity (Raper & Bush 2009) and shore distance (Parker & Williams 2011).

The aim of this study was therefore to determine whether the spores can be used as a *quantitative* proxy of herbivore density over time. We addressed this question by calibrating dung fungal spore abundance in relation to contemporary herbivore presence, that is by examining the density of dung spores in modern sediments in relation to known herbivore biomass densities (biomass per surface area, kg ha⁻¹).

The objectives of the study were as follows:

- 1 To determine the way dung fungal spores travel into sediments, that is long-distance transportation by wind, short-distance transportation by wind, transportation with surface runoff or a combination of these transportation mechanisms.
- 2 To understand the relationship between different spore types and different herbivore species.
- 3 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 Lake IJsselmeer in 1968. Rewilding was initiated at this site from 1983 with the introduction of free-ranging Heck cattle (*Bos taurus* Linnaeus) in 1983, Konik horses (*Equus ferus caballus* Linnaeus) in 1984 and red deer (*Cervus elaphus* Linnaeus) in 1992. These herbivores have access to the whole nature reserve but mainly use about 2000 ha of grasslands (e.g. *Lolium perenne* L., *Poa trivialis* L., *Trifolium repens* L.), tall herbs (e.g. *Cirsium* spp., *Urtica dioica* L.), reed (*Phragmites australis* (Cav.) Trin. ex Steud.), *Sambucus nigra* L. scrub and *Salix* spp. shrubs (Fig. 1) of high net primary productivity (Cornelissen & Vulink 2015). The grasslands are visited by large numbers of geese (thousands to tens of thousands of greylag goose *Anser anser* Linnaeus; Barnacle goose *Branta leucopsis* Bechstein; white-fronted goose *Anser albifrons* Scopoli). The site is managed with a policy of minimal intervention, that is the population size of freely roaming large herbivores is not controlled by culling, no supplementary feeding is given during winter and no management intervention is implemented to maintain vegetation.

We sampled modern sediment from 16 ponds (mean surface area: 2573 m², see Table 2) to obtain a good spatial spread across the reserve (see Fig. 1) and a large amplitude of herbivore abundances. These ponds were created between 1985 and 2000 for avian biodiversity. The sediment samples were collected where water was the deepest within each pond and were made up of top sediments representing contemporary deposition. We used a simple tube sampler with sharpened edges. Sedimentary sequences were also collected using a simplified Livingstone corer in order to obtain a sedimentary record of the life span of three ponds since they were first created. To prevent further fungal growth, all samples were stored in sealed plastic bags at 4 °C until processed in laboratory.

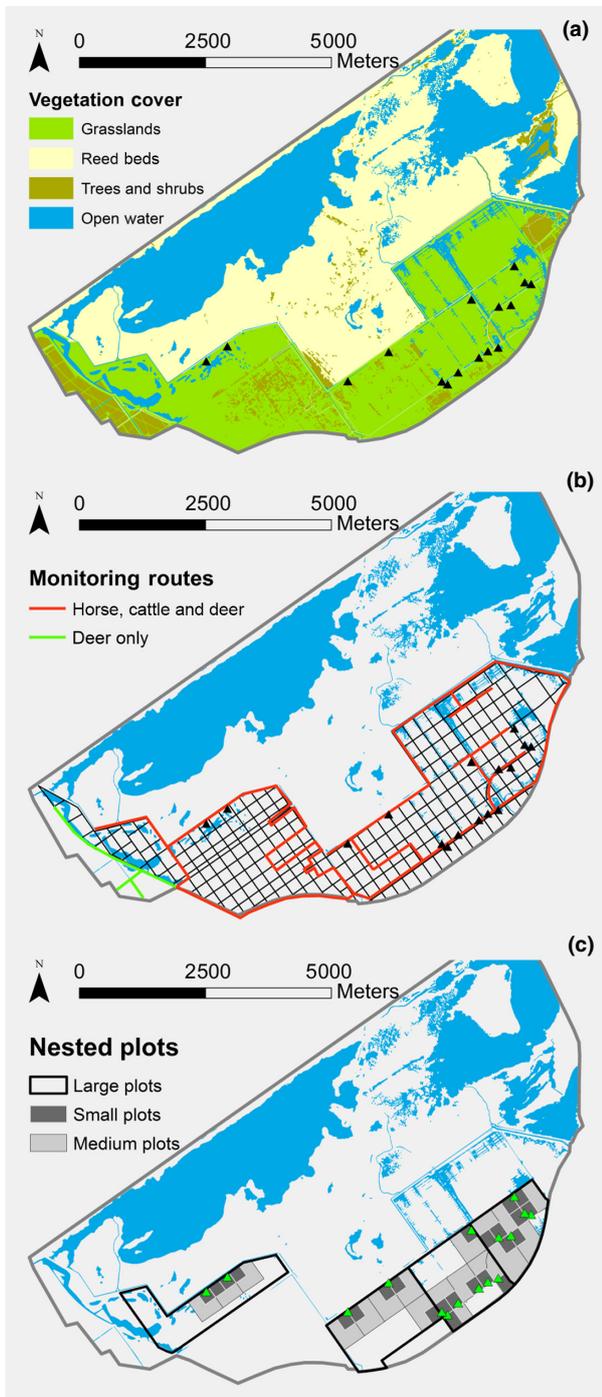


Fig. 1. Maps of the Oostvaardersplassen, sample locations indicated with triangles. (a) Vegetation map highlighting the grasslands where the herbivores spend most of their time. (b) Monitoring method, the number of herbivores is recorded weekly for each of the small plots following established routes, in red. (c) The nested plots utilised in this study (aggregation of the small plots shown in figure b).

RESPONSE VARIABLE: SPORE ABUNDANCE IN SEDIMENTS

The spores were extracted from one 1 cm^3 subsamples per sediment sample collected from the 16 ponds. We followed a standard extraction method in pollen analysis to isolate spores, estimate their concentration

using *Lycopodium* spore tablets (batch 938934, Lund University) and carry out identifications at a $400\times$ magnification (Willis & Bennett 2001). Spore identification and spore association with obligate dung fungi were based on the literature reviewed in Baker, Bhagwat & Willis (2013). The abundance of spores in sediments was calculated as accumulation rates (spore $\text{cm}^{-2}\text{ year}^{-1}$) using the spore concentration (spore cm^{-3}) and the sedimentation rate (cm year^{-1}) (Maher 1981; Bennett 1994; Willis & Bennett 2001). Using our three cores, we estimated sedimentation rates on the basis of the age of the ponds and the depth of sediment deposited since creation as detailed in Appendix S1 (Supporting information). We averaged these sedimentation rates and applied the average to all samples from our 16 ponds. Applying a constant sedimentation rate to all our samples means that the analysis undertaken returns exactly the same results whether we use spore concentration or spore accumulation rates. We opted for analysing and presenting realistic spore accumulation rates throughout this article in order to facilitate comparison with similar studies in the future.

EXPLANATORY VARIABLES: HERBIVORE BIOMASS DENSITIES AND PHYSICAL VARIABLES

The distribution of herbivores (cattle, horses, deer and geese) was monitored by the reserve wardens on a weekly basis at the Oostvaardersplassen (see Fig. 1b). The data analysed in this article 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 Fig. 1c). Small plots were the basic unit for monitoring large herbivores. For ponds overlaying two small plots, data from the relevant small plots were aggregated. Medium plots included the relevant small plot(s) plus adjacent small plots freely accessible by large herbivores. Large plots represented uninterrupted grasslands delimited by ditches or abrupt vegetation changes known to be of relevance for large herbivore movement. Other animals including foxes and large birds represent a negligible herbivore biomass in comparison with those monitored and there are 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 used herbivore biomass as a proxy for the dung production. We transformed herbivore numbers into herbivore biomass density (i.e. biomass per surface area, kg ha^{-1}) using an average biomass of individuals per species. The biomass of herbivore species was compiled from Dunning (2007) for geese, and from the long-term monitoring of large herbivore biomass by the RWS Water Service and the State Forestry Office, The Netherlands, for cattle, horses and deer (see Appendix S2).

Physical variables of each pond (i.e. pond surface area, pond 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 $0.5 \times 0.5\text{ m}$ resolution georeferenced aerial photographs taken in 2010 (Ministry of Infrastructure and the Environment and the Ministry of Economic Affairs, the Netherlands).

DATA ANALYSIS

In order to determine the predominant mechanism of transport of dung fungal spores into the ponds, 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 three explanatory variables. The factors included in each of the transportation mechanisms are detailed in Table 1. They all account for herbivore biomass density around the

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, ‘pond i’ is any of the 16 ponds sampled

	Transport agent	Location of dung	Factors included
Run-off	Surface run-off, very short flight, erosion	Pond shoreline and slopes	$H_{A,i}$: Herbivore biomass density within distance A of pond 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 biomass density within distance A of pond 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 biomass density within distance A of pond 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)

ponds and for the surface area of the pond, in keeping with the well-studied transportation of pollen grains from vegetation into water bodies (Sugita 1993; Giesecke & Fontana 2008). They differ as follows: ‘Surface run-off’ accounts for the pond perimeters, or shoreline lengths, to distinguish spores produced on the shore of the sampled ponds. ‘Wind transportation from local shores’ accounts for the total length of shorelines around the pond, to distinguish spores produced from habitats with permanent moisture supply, an important factor for dung fungal growth (Dix & Webster 1995; Krug, Benny & Keller 2004). ‘Wind transportation from local grasslands’ accounts for the surface area of grassland around the ponds to distinguish spores produced from the overall density of dung around the ponds. An additional transportation mechanism, background spore rain (i.e. constant deposition of spores across the reserve) was accounted for as the intercept of our models. We used generalised linear models (GLM) in R (R Core Team 2015) to examine this relationship. Because overdispersion 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 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).

In order to understand the relationship between the different spore types and different herbivore species, we analysed separately herbivore densities (kg ha⁻¹) of geese, Konik horses, Heck cattle, red deer and the three mammalian large herbivores together (five sets of explanatory variables) against each of the three main dung fungal spore types (*Sporormiella*, *Sordaria* and *Podospora*, see Baker, Bhagwat & Willis 2013) and their sum (therefore four 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).

Results

A total of 21 modern sediment samples were collected from 16 ponds of similar morphology (mean surface area: 2573 m²) 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

Table 2. List of ponds, their characteristics and samples analysed. Note that five ponds were sampled twice and therefore have two sample years, two sample codes and two sample depths

Pond	Surface (m ²)	Shore length (m)	Total shore length within medium plot (m)		Year(s) sampled	Sample code(s)	Sample depth(s) (cm)	
			North	East				
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	2009, 2010	Oost-203, Oost-204	10, 5
OO17	1353	247	6209	52-43784175	5-39378056	2010	Oost-205	7

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 biomass 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 showed little variation within the Oostvaardersplassen (see Appendix S1).

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

The background spore deposition is highly significantly different from zero and positive, implying a spatially constant atmospheric input of spores across the Oostvaardersplassen. The maximum likelihood estimation of the background spore deposition was 318.7 (dung fungal 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. 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.

Total mammal biomass density of large herbivores related better to total spore abundance than any of the herbivore biomass densities taken individually. While biomass densities of Heck cattle or Konik horses both showed a good fit with the models, biomass densities of red deer were never significantly related to any of the spore types using the methodology adopted. Total biomass densities of geese only showed significant relationship with *Sordaria* abundance.

Sordaria and *Sporormiella* taken separately show very similar patterns. They are best explained by total biomass density of large herbivores. However, the significance levels are lower for *Sporormiella*. On the contrary, *Podospora* is better explained by biomass densities of Konik horse 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.

Discussion

In the introduction, we highlighted the factors that can potentially obscure the relationship between herbivore biomass densities and dung fungal spores in sediments. These factors relate to spore production by different fungal species and whether they rely on specific herbivores, as well as to the taphonomic processes that the spores experience between their release in the air and their deposition into sediments. Our results demonstrate a highly significant relationship between spore

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 three plot scales for three transportation mechanisms)

Response variables	Biomass density (kg ha ⁻¹) for:	AIC	Significance levels				Optimum plot scales		
			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

(-) indicates a negative relationship and small, medium and large, the nested plots (see text and Fig. 1c); x: mechanism excluded by model simplification; in bold, best model for each fungal spore type, as determined by AIC.

Significance codes for *P*-values: ***<0.001; **<0.01; *<0.05; ns otherwise.

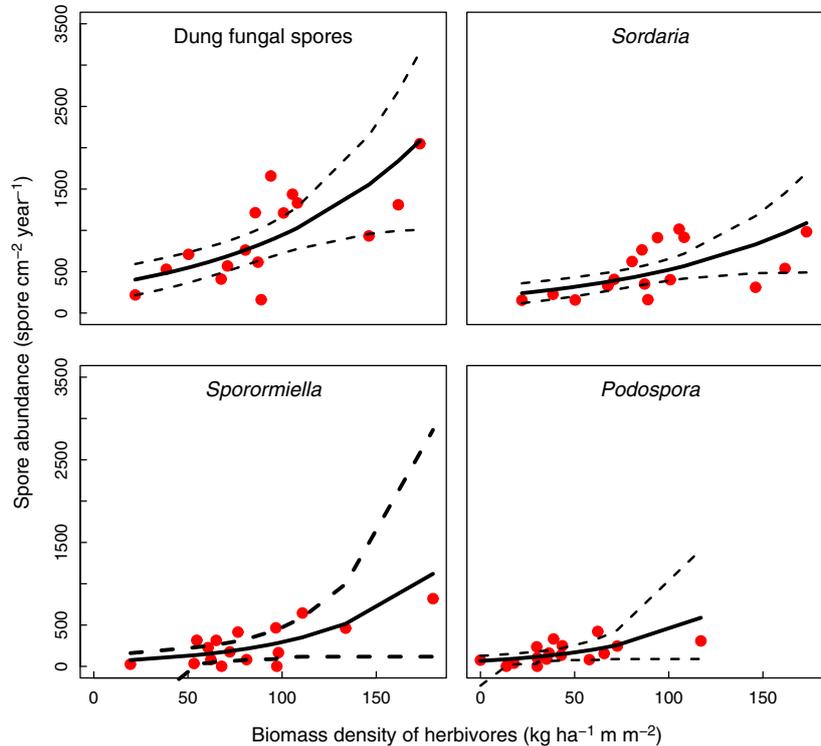


Fig. 2. Optimum relationships between spore abundances and herbivore biomass densities. On the x -axis is the run-off transportation mechanism, that is the product of local herbivore biomass density (only Konik horses for *Podospora*), pond shore length and pond surface area. Dots in red are the observed data, in black the best model prediction as in Table 3 (dashed, 0.95 confidence interval).

abundance and local biomass densities of large herbivores when these biological and taphonomic factors are taken into account.

FUNGAL AND HERBIVORE SPECIES

Total spore abundance, *Sordaria* abundance and *Sporormiella* abundances were each best explained by total biomass density of mammals (kg ha^{-1}). This demonstrates that dung fungal spores as identified in this study do not indicate any herbivore species in particular but instead indicate Heck cattle, Konik horse and red deer collectively. This finding differs from some other studies of dung fungal diversity (e.g. Richardson 1972, 2001); however, these earlier studies identify dung fungi to the species level using whole living organisms, that is with a greater taxonomic precision than is currently possible using spore morphology alone (Baker, Bhagwat & Willis 2013). Therefore, it would appear that the current limitations in the identification of dung fungal spores from sediments limit our ability to infer which specific large herbivore species they are associated with. However, our data on dung fungal spore abundance suggest that there is a direct link between spore production and the total biomass density of large herbivores. *Podospora* abundance was associated with Konik horse biomass density, but the validity of the modelling method for this spore type should be interpreted with some caution. This limitation is probably due to the low number of individual *Podospora* spores recovered and does not concern *Sporormiella* and *Sordaria* types that were significantly more abundant.

Similarly, although *Sordaria* spore explained goose abundance significantly, this herbivore is less likely than mammals to be the main source of fungal spore according to the AIC selection method used. 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 geese and other birds of similar size (Jano, Jefferies & Rockwell 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 in New Zealand can be tracked using the spores of *Sporormiella* spp. (Wood *et al.* 2011). Therefore, more studies such as ours but in different environments and featuring other species of herbivore (e.g. Froyd *et al.* 2014) will be required to fully assess how our results can be applied to other situations.

Using our model selection methodology, it was not possible to statistically determine whether it is preferable to aggregate all dung fungal spores into one indicator or to keep them separate. This was because there is no widely accepted method to calculate absolute goodness-of-fit, or R^2 , for GLM. Nevertheless, the confidence intervals plotted in Fig. 2 highlight that aggregated dung fungal spores would have a higher predictive power for palaeoecological reconstructions than spore types taken individually. In fact, significant increases in aggregated spore abundance appear to be a systematic reflection of an increase in herbivore biomass densities. Subtle changes, and

spore types taken individually, may be more difficult to interpret. In addition, studies of dung fungal diversity (e.g. Richardson 1972, 2001) highlight the preference of certain species for certain types of dung. Moreover, the large body of evidence reviewed by Dix & Webster (1995) and Krug, Benny & Keller (2004) shows that dung fungi have species-specific responses to different environmental conditions. The consequent assumption is that the greater the diversity of spore types, the more likely it is to capture all large herbivore activities. Dix & Webster (1995) also highlight the importance of competition between species as a driver for the composition of the dung fungal community. 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 herbivore biomass.

TAPHONOMIC FACTORS

Our analysis demonstrates that surface run-off from the shoreline and surrounding slopes (as opposed to longer distances by wind) explains dung fungal spore abundances highly significantly. This is the first-time evidence is gained regarding the source area of dung fungal spores from water bodies (Feranec *et al.* 2011; Baker, Bhagwat & Willis 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 <10 m away from pond shore). 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 abundance quantified with dung fungal spore actually represents herbivore abundance in a broader landscape. In our samples, there is in addition a significant influx of spores that is not related to the local distribution of herbivores within the reserve and that we identify as background spore deposition. The extremely high herbivore biomass density prevailing in the reserve (474.3 kg ha⁻¹, see Appendix S2) in comparison with the surrounding land (mostly arable land, built-up areas and open water, where large herbivores are overall in low density) suggests that much of this background spore influx originates within the reserve. This indicates that the influx of wind-dispersed spores in our case represents a signal from the overall abundance of large herbivores within the reserve that is not specific to the exact location of sampling. This contrasts with Gill *et al.*'s (2013) study in North America which demonstrated the importance of short-distance wind dispersal (<100 m) to explain the significant relationship between bison local distribution and abundance of dung fungal spores. Their study was conducted in terrestrial habitats away from water, so further research will be necessary to assess fully the relative importance of run-off and wind transportation in different deposition environments.

We found that biomass density of large herbivores explains dung fungal spore abundance in a highly significant way when accounted in conjunction with the morphological characteristics of the sampled pond (i.e. pond surface area and pond shoreline length). Therefore, changes in spore abundance through time can be used to indicate large herbivore population size variation. However, this is with the caveat that the water body has stayed approximately the same size during the same interval in time. If drastic hydrological changes are suspected, there are several means to assess water level in palaeoecology, notably using macrofossils of aquatic plants (Hannon & Gaillard 1997; Dieffenbacher-Krall & Halteman 2000).

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 certainty 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 world-wide: for instance, agricultural abandonment in marginal areas, the growing need for food production and the adoption of novel conservation strategies such as rewilding. 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 palaeoecology, we provide here the foundations for the quantitative reconstruction of long time series of herbivore densities using fossil dung spores contained in sedimentary sequences.

Using modern surface sediments, we found that there is a significant relationship between biomass density of large herbivores 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.

Acknowledgements

We thank Hans Breeveld for access to the Oostvaardersplassen nature reserve. The herbivore data were provided by the State Forestry Service of the Netherlands. This work was carried out as part of a UK Natural Environment Research Council studentship to AGB. We thank John Birks, David Etienne and an anonymous reviewer for their insightful comments and recommendations.

Data accessibility

The data used are archived as Dryad entry: <http://dx.doi.org/10.5061/dryad.5v8b7> (Baker *et al.* 2016).

References

- Asner, G.P., Elmore, A.J., Olander, L.P., Martin, R.E. & Harris, A.T. (2004) Grazing systems, ecosystem responses, and global change. *Annual Review of Environment and Resources*, **29**, 261–299.
- Baker, A.G., Bhagwat, S.A. & Willis, K.J. (2013) Do dung fungal spores make a good proxy for past distribution of large herbivores? *Quaternary Science Reviews*, **62**, 21–31.

- Baker, A.G., Cornelissen, P., Bhagwat, S.A., Vera, F. & Willis, K.J. (2016) Data from: Quantification of population sizes of large herbivores and their long-term functional role in ecosystems using dung fungal spores. *Methods in Ecology and Evolution*, <http://dx.doi.org/10.5061/dryad.5v8b7>.
- Bell, A. (2005) *An Illustrated Guide to the Coprophilous Ascomycetes of Australia*. APS Press, St-Paul.
- Bennett, K.D. (1994) Confidence intervals for age estimates and deposition times in late-Quaternary sediment sequences. *The Holocene*, **4**, 337–348.
- Birks, H.J.B. (2005) Mind the gap: how open were European primeval forests? *Trends in Ecology and Evolution*, **20**, 154–156.
- Bradshaw, R.H.W., Hannon, G.E. & Lister, A.M. (2003) A long-term perspective on ungulate–vegetation interactions. *Forest Ecology and Management*, **181**, 267–280.
- de Bruyn, M., Hoelzel, A.R., Carvalho, G.R. & Hofreiter, M. (2011) Faunal histories from Holocene ancient DNA. *Trends in Ecology & Evolution*, **26**, 405–413.
- Burney, D.A., Robinson, G.S. & Burney, L.P. (2003) *Sporormiella* and the late Holocene extinctions in Madagascar. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 10800–10805.
- Carpenter, S.R., Mooney, H.A., Agard, J., Capistrano, D., Defries, R.S., Díaz, S. et al. (2009) Science for managing ecosystem services: beyond the Millennium Ecosystem Assessment. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 1305–1312.
- Ceausu, S., Hofmann, M., Navarro, L.M., Carver, S., Verburg, P.H. & Pereira, H.M. (2015) Mapping opportunities and challenges for rewilding in Europe. *Conservation Biology*, **29**, 1017–1027.
- Corlett, R.T. (2013) The shifted baseline: prehistoric defaunation in the tropics and its consequences for biodiversity conservation. *Biological Conservation*, **163**, 13–21.
- Cornelissen, P. & Vulink, J.T. (2015) Density-dependent diet selection and body condition of cattle and horses in heterogeneous landscapes. *Applied Animal Behaviour Science*, **163**, 28–38.
- Crawley, M.J. (2007) *The R Book*. Wiley, Chichester, UK.
- Cromsigt, J.P.G.M. & te Beest, M. (2014) Restoration of a megaherbivore: landscape-level impacts of white rhinoceros in Kruger National Park, South Africa. *Journal of Ecology*, **102**, 566–575.
- Davis, O.K. & Shafer, D.S. (2006) *Sporormiella* fungal spores, a palynological means of detecting herbivore density. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **237**, 40–50.
- Dieffenbacher-Krall, A. & Halteman, W. (2000) The relationship of modern plant remains to water depth in alkaline lakes in New England, USA. *Journal of Paleolimnology*, **24**, 213–229.
- Dix, N.J. & Webster, J. (1995) *Fungal Ecology*. Chapman & Hall, London, UK.
- Donlan, J.C., Berger, J., Bock, C.E., Bock, J.H., Burney, D.A., Estes, J.A. et al. (2005) Re-wilding North America. *Nature*, **436**, 913–914.
- Doveri, F. (2007) *Fungi Fimicoli Italici: A Guide to the Recognition of Basidiomycetes and Ascomycetes Living on Faecal Material*. Associazione Micologica Bresadola, Trento.
- Dunning, J.B. (2007) *CRC Handbook of Avian Body Masses*, 2nd edn. CRC Press, London.
- Ellis, E.C., Klein Goldewijk, K., Siebert, S., Lightman, D. & Ramankutty, N. (2010) Anthropogenic transformation of the biomes, 1700 to 2000. *Global Ecology and Biogeography*, **19**, 589–606.
- Estes, J.A., Terborgh, J., Brashares, J.S., Power, M.E., Berger, J., Bond, W.J., Carpenter, S.R. & Essington, T.E. (2011) Trophic downgrading of planet Earth. *Science*, **333**, 301–306.
- Etienne, D., Wilhelm, B., Sabatier, P., Reys, J.-L. & Arnaud, F. (2013) Influence of sample location and livestock numbers on *Sporormiella* concentrations and accumulation rates in surface sediments of Lake Allos, French Alps. *Journal of Paleolimnology*, **49**, 117–127.
- Feranec, R.S., Miller, N.G., Lothrop, J.C. & Graham, R.W. (2011) The *Sporormiella* proxy and end-Pleistocene megafaunal extinction: a perspective. *Quaternary International*, **245**, 333–338.
- Froyd, C.A., Coffey, E.E.D., van der Kraak, W.O., van Leeuwen, J.F.N., Tye, A. & Willis, K.J. (2014) The ecological consequences of megafaunal loss: giant tortoises and wetland biodiversity. *Ecology Letters*, **17**, 144–154.
- van Geel, B., Buurman, J., Brinkkemper, O., Schelvis, J., Aptroot, A., van Reenen, G.B.A. & Hakbijl, T. (2003) Environmental reconstruction of a Roman Period settlement site in Uitgeest (The Netherlands), with special reference to coprophilous fungi. *Journal of Archaeological Science*, **30**, 873–883.
- Giesecke, T. & Fontana, S.L. (2008) Revisiting pollen accumulation rates from Swedish lake sediments. *The Holocene*, **18**, 293–305.
- Gill, J.L., Williams, J.W., Jackson, S.T., Lininger, K.B. & Robinson, G.S. (2009) Pleistocene megafaunal collapse, novel plant communities, and enhanced fire regimes in North America. *Science*, **326**, 1100–1103.
- Gill, J.L., McLauchlan, K.K., Skibbe, A.M., Goring, S., Zirbel, C.R. & Williams, J.W. (2013) Linking abundances of the dung fungus *Sporormiella* to the density of bison: implications for assessing grazing by megaherbivores in palaeorecords. *Journal of Ecology*, **101**, 1125–1136.
- Gonias, M., Neonakis, I., Darivianaki, E., Gonias, I., Bouros, D. & Kontou-Fili, K. (2005) Airborne Ascomycota on the island of Crete: seasonal patterns based on an 8-year volumetric survey. *Aerobiologia*, **21**, 69–74.
- Hannon, G.E. & Gaillard, M.-J. (1997) The plant-macrofossil record of past lake-level changes. *Journal of Paleolimnology*, **18**, 15–28.
- Hernández Trejo, F., Muñoz Rodríguez, A.F., Tormo Molina, R. & Silva Palacios, I. (2011) Airborne ascospores in Mérida (SW Spain) and the effect of rain and other meteorological parameters on their concentration. *Aerobiologia*, **28**, 13–26.
- Hoef, J.Ver & Boveng, P. (2007) Quasi-Poisson vs. negative binomial regression: how should we model overdispersed count data? *Ecology*, **88**, 2766–2772.
- Jackson, S.T. & Lyford, M.E. (1999) Pollen dispersal models in Quaternary plant ecology: assumptions, parameters, and prescriptions. *The Botanical Review*, **65**, 39–75.
- Jano, A., Jefferies, R. & Rockwell, R. (1998) The detection of vegetational change by multitemporal analysis of LANDSAT data: the effects of goose foraging. *Journal of Ecology*, **86**, 93–99.
- Jefferies, R. & Rockwell, R. (2002) Foraging geese, vegetation loss and soil degradation in an Arctic salt marsh. *Applied Vegetation Science*, **5**, 7–16.
- Johnson, C.N., Rule, S., Haberle, S.G., Turney, C.S.M., Kershaw, A.P. & Brook, B.W. (2015) Using dung fungi to interpret decline and extinction of megaherbivores: problems and solutions. *Quaternary Science Reviews*, **110**, 107–113.
- Koch, P.L. & Barnosky, A.D. (2006) Late quaternary extinctions: state of the debate. *Annual Review of Ecology, Evolution, and Systematics*, **37**, 215–250.
- Krug, J.C., Benny, G.L. & Keller, H.W. (2004) Coprophilous fungi. *Biodiversity of Fungi* (eds G.M. Mueller, G.F. Bills & M.S. Foster), pp. 468–499. Elsevier, Amsterdam.
- Maher, L.J. (1981) Statistics for microfossil concentration measurements employing samples spiked with marker grains. *Review of Palaeobotany and Palynology*, **32**, 153–191.
- Naundrup, P.J. & Svenning, J.-C. (2015) A geographic assessment of the global scope for rewilding with wild-living horses (*Equus ferus*). *PLoS One*, **10**, e0132359.
- Nuttall, T., Yerger, E.H., Stoleson, S.H. & Ristau, T.E. (2011) Legacy of top-down herbivore pressure ricochets back up multiple trophic levels in forest canopies over 30 years. *Ecosphere*, **2**, art4.
- O'Hara, R.B. & Kotze, D.J. (2010) Do not log-transform count data. *Methods in Ecology and Evolution*, **1**, 118–122.
- Parker, N.E. & Williams, J.W. (2011) Influences of climate, cattle density, and lake morphology on *Sporormiella* abundances in modern lake sediments in the US Great Plains. *The Holocene*, **22**, 475–483.
- Peh, K.S.-H. & Lewis, S.L. (2012) Conservation implications of recent advances in biodiversity–functioning research. *Biological Conservation*, **151**, 26–31.
- Putzfarken, D., Dengler, J., Lehmann, S. & Härdtle, W. (2008) Site use of grazing cattle and sheep in a large-scale pasture landscape: a GPS/GIS assessment. *Applied Animal Behaviour Science*, **111**, 54–67.
- R Core Team (2015) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna. URL <https://www.R-project.org/>.
- Raper, D. & Bush, M. (2009) A test of *Sporormiella* representation as a predictor of megaherbivore presence and abundance. *Quaternary Research*, **71**, 490–496.
- Richardson, M.J. (1972) Coprophilous ascomycetes on different dung types. *Transactions of the British Mycological Society*, **58**, 37–48.
- Richardson, M.J. (2001) Diversity and occurrence of coprophilous fungi. *Mycological Research*, **105**, 387–402.
- Ripple, W.J. & Beschta, R.L. (2012) Trophic cascades in Yellowstone: the first 15 years after wolf reintroduction. *Biological Conservation*, **145**, 205–213.
- Ritchie, E.G., Elmhagen, B., Glen, A.S., Letnic, M., Ludwig, G. & McDonald, R.A. (2012) Ecosystem restoration with teeth: what role for predators? *Trends in Ecology & Evolution*, **27**, 265–271.
- Rule, S., Brook, B.W., Haberle, S.G., Turney, C.S.M., Kershaw, A.P. & Johnson, C.N. (2012) The aftermath of megafaunal extinction: ecosystem transformation in Pleistocene Australia. *Science*, **335**, 1483–1486.
- Sandom, C.J., Ejrnaes, R., Hansen, M.D.D. & Svenning, J.-C. (2014) High herbivore density associated with vegetation diversity in interglacial ecosystems. *Proceedings of the National Academy of Sciences of the United States of America*, **111**, 4162–4167.
- Seddon, P.J., Griffiths, C.J., Soorae, P.S. & Armstrong, D.P. (2014) Reversing defaunation: restoring species in a changing world. *Science*, **345**, 406–412.

- Shannon, G., Matthews, W.S., Page, B.R., Parker, G.E. & Smith, R.J. (2009) The affects of artificial water availability on large herbivore ranging patterns in savanna habitats: a new approach based on modelling elephant path distributions. *Diversity and Distributions*, **15**, 776–783.
- Steinfeld, H., Gerber, P. & Wassenaar, T. (2006) *Livestock's Long Shadow*. FAO, Rome.
- Stuart, A.J. (2015) Late Quaternary megafaunal extinctions on the continents: a short review. *Geological Journal*, **50**, 338–363.
- Sugita, S. (1993) A model of pollen source area for an entire lake surface. *Quaternary Research*, **39**, 239–244.
- Tanentzap, A.J. & Coomes, D.A. (2012) Carbon storage in terrestrial ecosystems: do browsing and grazing herbivores matter? *Biological Reviews of the Cambridge Philosophical Society*, **87**, 72–94.
- Venables, W.N. & Ripley, B.D. (2002) *Modern Applied Statistics with S*, 4th edn. Springer, New York.
- Vera, F.W.M. (2000) *Grazing Ecology and Forest History*. CABI, Wallingford.
- Willis, K.J. & Bennett, K.D. (2001) Pollen. *Tracking Environmental Change Using Lake Sediments Volume 3 Terrestrial, Algal and Siliceous Indicators* (eds J.P. Smol, H.J.B. Birks & W.M. Last), pp. 5–32. Kluwer Academic Publishers, Dordrecht.
- Wood, J.R. & Wilmshurst, J.M. (2011) Wetland soil moisture complicates the use of *Sporormiella* to trace past herbivore populations. *Journal of Quaternary Science*, **27**, 254–259.
- Wood, J.R., Wilmshurst, J.M., Worthy, T.H. & Cooper, A. (2011) *Sporormiella* as a proxy for non-mammalian herbivores in island ecosystems. *Quaternary Science Reviews*, **30**, 915–920.
- Yafetto, L., Carroll, L., Cui, Y., Davis, D.J., Fischer, M.W.F., Henterly, A.C. *et al.* (2008) The fastest flights in nature: high-speed spore discharge mechanisms among fungi (A.S. Gladfelter, Ed.). *PLoS One*, **3**, e3237.

Received 10 January 2016; accepted 8 April 2016

Handling Editor: David Murrell

Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Appendix S1. Estimation of sedimentation rates in the ponds of the Oostvaardersplassen.

Appendix S2. Body mass of large herbivores in the Oostvaardersplassen.