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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 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 ii) weekly counts of contemporary wildlife over a period of five 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 (<10m), 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 function, ecosystem engineers, 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 (Nuttle *et al.* 2011; Estes *et al.* 2011; Ripple & Beschta 2012; Tanentzap & Coomes 2012; Ritchie *et al.* 2012; Peh & Lewis 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 & 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; Ceausu *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 (Donlan *et al.* 2005; Vera 2000; Sandom *et al*. 2014; Corlett, 2013). 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 Bryun *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 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 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; Wood & Wilmshurst 2011; Parker & Williams 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, i.e. 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:

(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 understand the relationship between different spore types and different herbivore species.

(iii) 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. Re-wilding 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 (Figure 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, i.e. 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 m^2, see Table 2) to obtain a good spatial spread across the reserve (see Figure 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 lifespan of three ponds since they were first created. To prevent further fungal growth, all samples were stored in sealed plastic bags at 4^{0}C until 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 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 400x 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 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). 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 1. 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 paper 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 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). 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 2).

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 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

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 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 generalized linear models (GLM) in R (R Core Team, 2012) to examine this relationship. 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 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 (5 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 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).

Results

A total of 21 modern sediment samples was collected from 16 ponds of similar morphology (mean surface area: 2573 m^2) 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 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 1).

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 Figure 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 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, i.e. 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 limits our ability to infer which specific large herbivore species they are associated with. However, our data on dung fugal 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 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 goose 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 less than 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 2) in comparison to the surrounding land (mostly arable land, built-up areas and open water, where large herbivores are overall in low density) suggest 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 meters) 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 worldwide: 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.

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Data Accessibility

The data used are archived as DRYAD entry: doi:10.5061/dryad.5v8b7

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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)

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 square)	Shore length (m)	Total shore length within medium plot (m)	North	East	Year(s) sampled	Sample code(s)	Sample depth(s) (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	2009, 2010	Oost-203, Oost-204	10, 5
OO17	1353	247	6209	52.43784175	5.39378056	2010	Oost-205	7

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.

			Significance levels				Optimum plot scales		
Response variables	Biomass density ($kg\ ha^{-1}$) 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

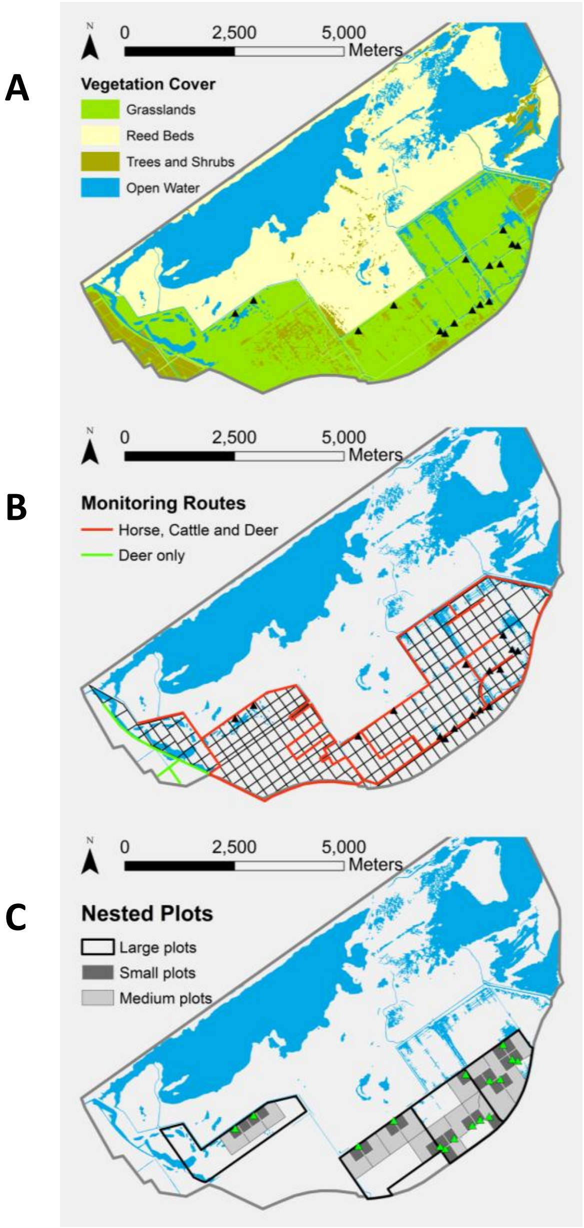


Figure 1: Maps of the Oostvaardersplassen, sample locations indicated with triangles. A. Vegetation map highlighting the grasslands where the herbivores spend most of their time. B. Monitoring method, the number of herbivores is recorded weekly for each of the small plots following established routes,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

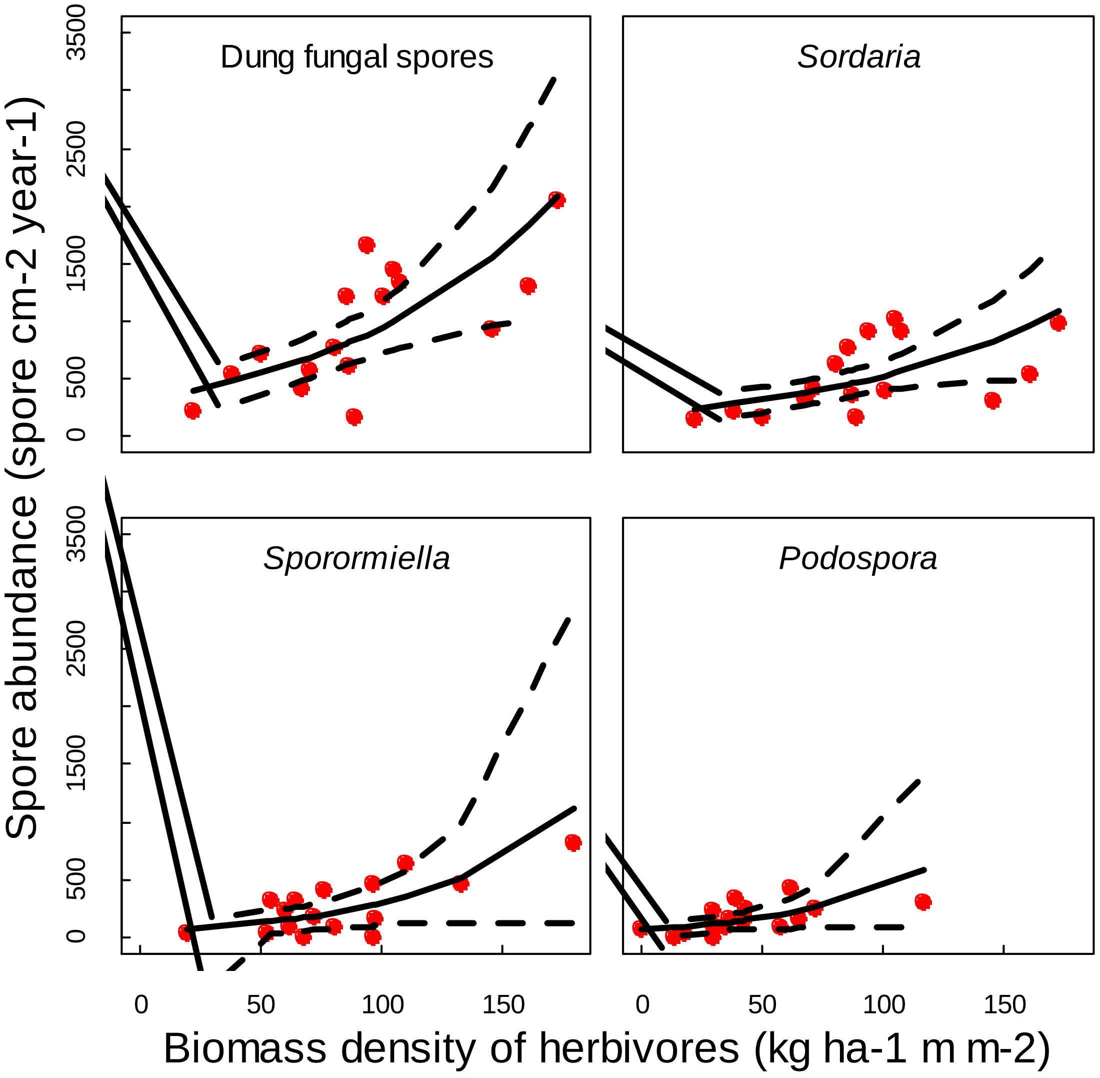


Figure 2: Optimum relationships between spore abundances and herbivore biomass densities. On the x axis is the run-off transportation mechanism, i.e. the product of local herbivore biomass density (only Konik horses for *Podospora*), pond shore length and pond surface area. Dots in red are the observed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

Appendix 1

Estimation of sedimentation rates in the ponds of the Oosvaardersplassen

Introduction

The aim of this appendix is to calculate the sedimentation rate (cm year^{-1}) in the ponds 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 ponds 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 ponds of the Oostvaardersplassen.

Methods

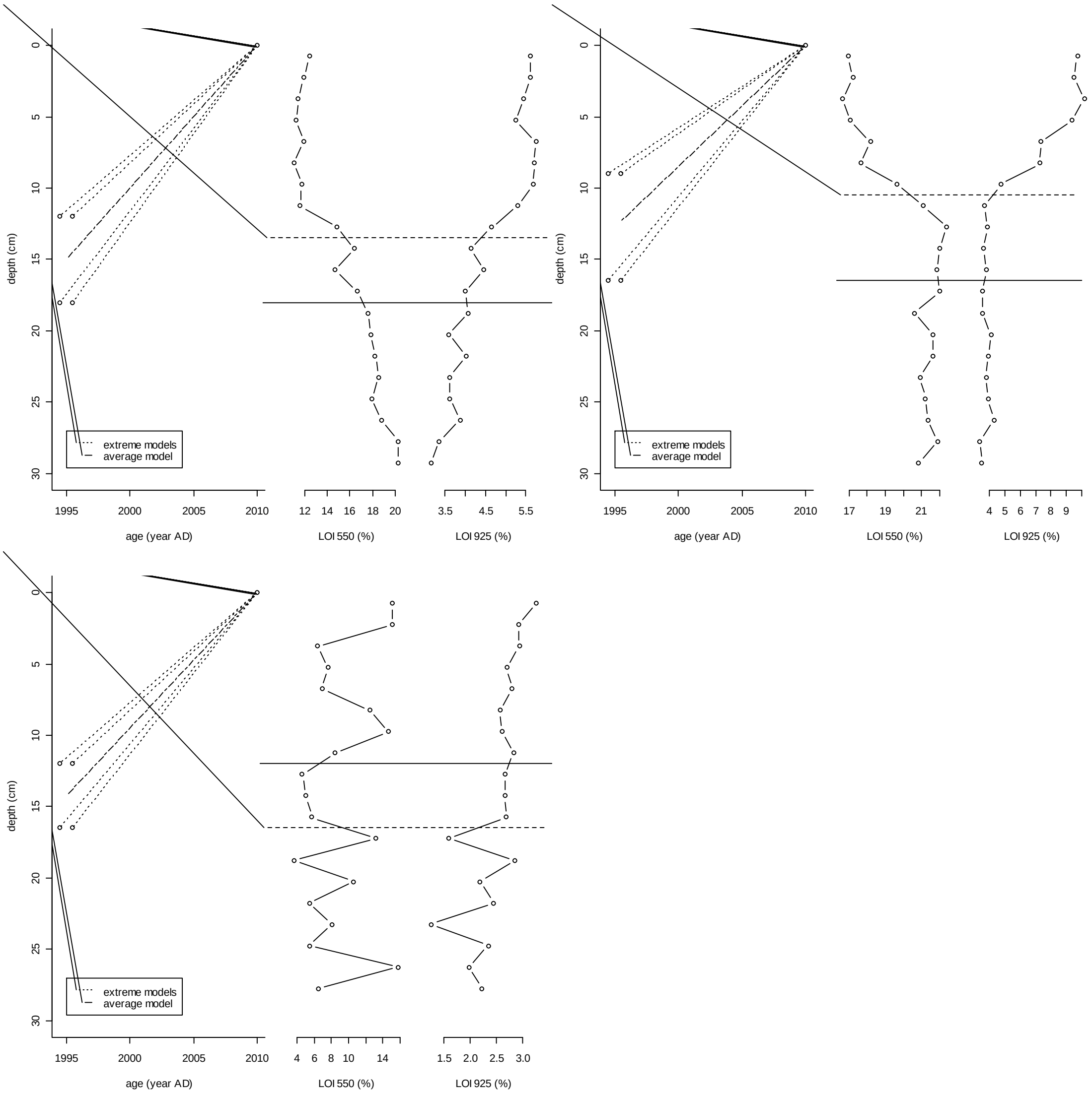
Three short cores were extracted from three different ponds in July 2010 (OO6, OO14, OO15, see Table 2 main text), using a simplified Livingstone corer. This corer was made with a transparent coring tube, with sharpened edges. Once the core was extracted from the deepest part of the pond, it was slowly 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 ponds that we cored were initially dug in 1995 (month unknown), resulting in between 14.5 and 15.5 years of ponds existence represented in the cores.

We determined the transition between pre-reclamation sediments and pond 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 these 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. Sedimentary 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).
- 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 (Unpublished data). Heck cattle has similar body weight 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.