Using Sporormiella to track herbivore biomass within the Hluhluwe-Imfolozi Game Reserve

(Photography by Lindsey Gillson)

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1. Abstract

Historical fossilised spores of *Sporomiella*, a coprophilous fungus that only grows on the dung of herbivores, has been used to infer unknown herbivore abundances or biomass and identify periods of mega-herbivore extinction in the palaeo-record. In Africa, however, mega-herbivores are still extant and there is therefore a unique opportunity to calibrate *Sporomiella* abundance against known herbivore biomass. This study was carried out within the Hluhluwe-Imfolozi Game Reserve, KwaZulu-Natal South Africa (28°00'-28°26'S, 31°43'-32°00'E, Fig 2(a) and (6)). We evaluated the relationship between *Sporomiella* concentration and herbivore abundance, as indicated by total dung abundances. We investigated three aspects of this relationship: [1] the relationship between *Sporomiella* abundance and total herbivore dung abundance, [2] the relationship between *Sporomiella* and individual herbivore species, where we also divided all the herbivores into Mega-herbivores and Meso-herbivores to determine their relationship with *Sporomiella* densities, [3] finally, we tested the differences between the regions of the reserve by comparing the different areas of the park, as each system has its own unique drivers (Hluhluwe (fire driven), Imfolozi (herbivore driven) and the corridor (fire and herbivore driven)), with the concentration of *Sporomiella*.

We found no significant relationships between *Sporomiella* concentration and total herbivore dung abundances, which suggests that the fungus may be selectively growing on certain herbivore species rather than on all herbivore dung and / or the amount of dung for each species is not accurately reflected by dung counts [because amount of dung per species isn't accurately reflected by dung counts?] This isn't reflected in dung counts]. When the sites that had zero *Sporomiella* were excluded from the

analysis, *Sporomiella* concentration was significantly related to elephant and white rhino dung abundance, which could be related to site specific condition. Mega-herbivores and meso-herbivores dung abundance showed no significant relationship with *Sporomiella* concentrations, implying that neither group is the main contributors to *Sporomiella* concentration. There was also no significant difference in *Sporomiella* concentration between the different areas of the park, providing no evidence that spores are differentially distributed throughout the park. *Sporomiella* concentrations showed no significant difference between the different types of vegetation and grasses within the park. This suggests that the spores are not specific to certain vegetation or grass types.

2. Introduction

Savannas are dynamic complex ecosystems involving intricate relationships between fire, herbivores, competition, climate, human management and edaphic conditions which influence system patterns and processes at different spatial and temporal scales (Gillson 2004, 2007). Savanna ecosystems are defined as tropical or near-tropical seasonal systems by a herbaceous layer dominated by C_4 grasses and or sedges and unpredictable densities of shrubs and trees (Frost *et al.* 1986 and Knoop and Walker 1985 as cited by Gillson 2004 and Skarpe 1992). To try and explain the patterns and processes taking place in Savanna ecosystems today it is important to be able to understand the factors that took place in the past.

Palaeoecological studies attempt to do just that, recreating vegetation compositions over thousands of years using fossilised pollen records (Gillson 2004, Gillson 2006 and Gillson & Duffin 2007). This record in conjunction with charcoal records provides a proxy for the historical processes dominate at the time (Gillson 2004, Gillson 2006 and Gillson & Duffin 2007). Fossil pollen records provides information on the species and therefore the type of biomes that were dominate in an area at a certain time while the charcoal records provide an account of fire activity (Gillson 2004, Gillson 2006 and Gillson & Duffin 2007).

Herbivores have long been considered as an important factor affecting Savanna ecosystems (Skarpe 1992 and Ritchie *et al.* 1998). Herbivores can have both direct and indirect effects on plant communities (Huntly 1991 and Ritchie *et al.* 1998). It is therefore thought that mega-herbivores influence the life-history of there food sources making them important factors in shaping Savanna ecosystems (Hemborg & Bond

2006). The palaeo-record also contains a proxy for herbivore abundance, a coprophagous fungal spore, *Sporomiella* (Berrio *et al.* 2004, Burney *et al.* 2003, Koch & Barnosky 2006 and Lejju *et al.* 2005).

Sporomiella is an ascomycete fungus, which is a class of fungi that produce sexual spores endogenously (Davis & Shafer 2006). Coprophilous fungi are fungi that use the excrement of animals as their primary growth substrate (Graf & Chmura 2006). This fungus is only found on the dung of herbivores and is most frequently found on domestic herbivores and mega-herbivore dung (Davis & Shafer 2006 and Graf & Chmura 2006). This genus is prevalent in sub-boreal and temperate regions (Davis & Shafer 2006) but has also been found in Kenya, Uganda and Madagascar (Burney *et al.* 2003, Caretta *et al.* 1998, Lejju *et al.* 2005). Coprophilous fungi are important components of the ecosystem as they aid in the decomposition and mineralization of herbivore faeces, provide a nutritional base for coprophagous and mycophagous arthropods and may even influence the microbial composition and activity in the rumen (Angel & Wicklow 1975).

The spores are dark brown with distinct sigmoid germinal apertures; septa divide the spore into 4 to many cells (Davis & Shafer 2006) (see Figure 1). As their habitat decomposes and shrinks relatively quickly the fungi must be able to efficiently disperse their spores to new fresh substrates for colonization (Graf & Chmura 2006). This fungus has a relatively simple life cycle. Spores are born in sclerotia on the surface of dung and are spread passively (wind, bodies on small invertebrates i.e. arthropods) to nearby vegetation where they are ingested (Davis & Shafer 2006 and Graf & Chmura 2006). Once the faeces of the herbivore have been shed the spores germinate completing their life cycle (Davis & Shafer 2006).

Dispersed spores are generally separated into individual cells (Davis & Shafer 2006). Since the spores are shed close to the ground they are not very efficient at travelling long distances (Davis & Shafer 2006 and Graf & Chmura 2006). This makes them ideal for localised studies as the probability of the spores found in an area being from far away is small (Davis & Shafer 2006 and Graf & Chmura 2006).

Figure 1: Strand of *Sporomiella*, found in Bhavulomu from the Imfolozi side of the Hluhluwe-Imfolozi game reserve (photo by Alicia Thomas).

The fossil spores of this coprophilous fungus have been shown to serve as a reliable proxy for mega-herbivore biomass (Berrio *et al.* 2004, Burney *et al.* 2003, Koch & Barnosky 2006 and Lejju *et al.* 2005). It has been suggested that since the spores of *Sporomiella* are associated with dung they can be used to (1) define which pre-human habitats supported a high biomass of large animals, (2) produce accurate estimates for the

chronological association between mega-faunal biomass decline and other evidence for the extinctions and environmental changes (3) provide a test for the proposed hypotheses for the extinctions, and (4) examine the possible role of live stock extinctions (Berrio *et al.* 2004, Burney *et al.* 2003, Koch & Barnosky 2006 and Lejju *et al.* 2005).

Berrio *et al.* (2004), for example, suggests that the proportion of *Sporomiella* in the fossil record can provide information on human population density and herbivore abundance. The decline in spore abundance in the fossil record was found to closely follow a rise in stratigraphic charcoal levels (Berrio *et al.* 2004, Burney *et al* 2003, Koch & Barnosky 2006, Lejju *et al.* 2005 and Robinson *et al.* 2005). It was therefore inferred that the regional collapse of the mega-herbivore regime was followed by landscape transformation by humans (Robinson *et al.* 2005). Previous studies (mentioned above) have utilised dung fungus in the fossil record to infer unknown herbivore abundances and identify periods of mega-herbivore extinction. In Africa, however, mega-herbivores are still extant and there is therefore a unique opportunity to calibrate *Sporomiella* abundance against known herbivore biomass.

The aim of this study is to determine if the abundance of *Sporomiella* is related to known herbivore abundance, as indicated by total dung abundances (Cromsigt, pers. Comm.) in Hluhluwe-Imfolozi, and whether this relationship can be quantified. We hypotheses that the concentration of *Sporomiella* will be related to the abundance of herbivore dung. In order to test this we investigated three aspects of this possible relationship: [1] the relationship between *Sporomiella* abundance and total herbivore abundance, using herbivore dung abundances as a proxy, [2] testing the relationship between *Sporomiella* and dung abundance from individual herbivore species, which will provide a measure of

Sporomiella's selectivity in terms of their growth medium (*i.e.* which animals' dung they prefer to grow on) and will also allow us to evaluate the relative contribution of mega-herbivore and meso-herbivore dung abundance to the concentration of *Sporomiella* which will show, which group of herbivores contributes the most to the concentrations of *Sporomiella*, and [3] finally, to test differences between the regions of the reserve we compared the different areas of the park (Hluhluwe, Imfolozi and the corridor) with the concentration of *Sporomiella*, by assessing *Sporomiella* abundance in both Hluhluwe (to the North), Imfolozi (to the South) and the corridor in the middle, we can compare spore abundance in a mesic, fire driven habitat, a more arid, herbivore driven habitat and a habitat were these two factors overlap, respectfully (Bond, pers. Comm.), we also looked at the relationship between *Sporomiella* concentrations and, the vegetation type and grass type within the different sites.

The results of this study will be applied in further paleoecological studies. If a quantitative relationship is found between herbivore dung abundance and *Sporomiella* concentrations it can be used in long-term data sets which would shed light on changes in herbivore abundance through time, human population densities and the introduction of domestic herbivores. It might also be useful to determine if different herbivore species have distinctive fungal signatures, a factor being investigated in a separate but complementary study. Several studies have attempted to do just that, looking at the relationships between coprophilous fungi and faecal substrates (Angel & Wicklow 1975 and Parker 1979). Factors such as competition, exploitation, nutritional and environmental conditions have been suggested as possible causes determining the composition of the herbivore vertebrate dung's mycobiota (Caretta *et al.* 1998).

3. Methods

3.1. Study site

This study was carried out within the Hluhluwe-Imfolozi Game Reserve, KwaZulu-Natal South Africa (28°00'-28°26'S, 31°43'-32°00'E, Fig 2(a) and (6)). The reserve was first established in 1895 and is the oldest reserve in Africa. The reserve covers 96000ha which is divided into Hluhluwe in the north, Imfolozi in the south and the corridor in the middle. It is characterized by a hilly topography with an altitudinal range between 60-750m above sea level. The reserve has a moderate coastal climate with a summer rainfall range between 760-1250mm per annum. The mean annual rainfall in Hluhluwe is 990mm while in Imfolozi is around 720mm. The monthly average temperature ranges between 13-33°C. The reserve comprises of bush-veld Savanna with 5 broadly described vegetation types (Walters & Milton 2003). Most of the reserve is located on the Ecca and Beaufort series with Swartland and Sterkspruit soil types (Walters & Milton 2003). The most important difference between the two halves of the park is that they have unique drivers which play a crucial role in determining the dynamics of the park. Hluhluwe (in the North) is a mesic, fire driven ecosystem while Imfolozi is a more arid, herbivore driven ecosystem (Bond pres. Comm.).

Figure 2 (a): Map showing the position of the Hluhluwe-Imfolozi game reserve (www.answer.com)

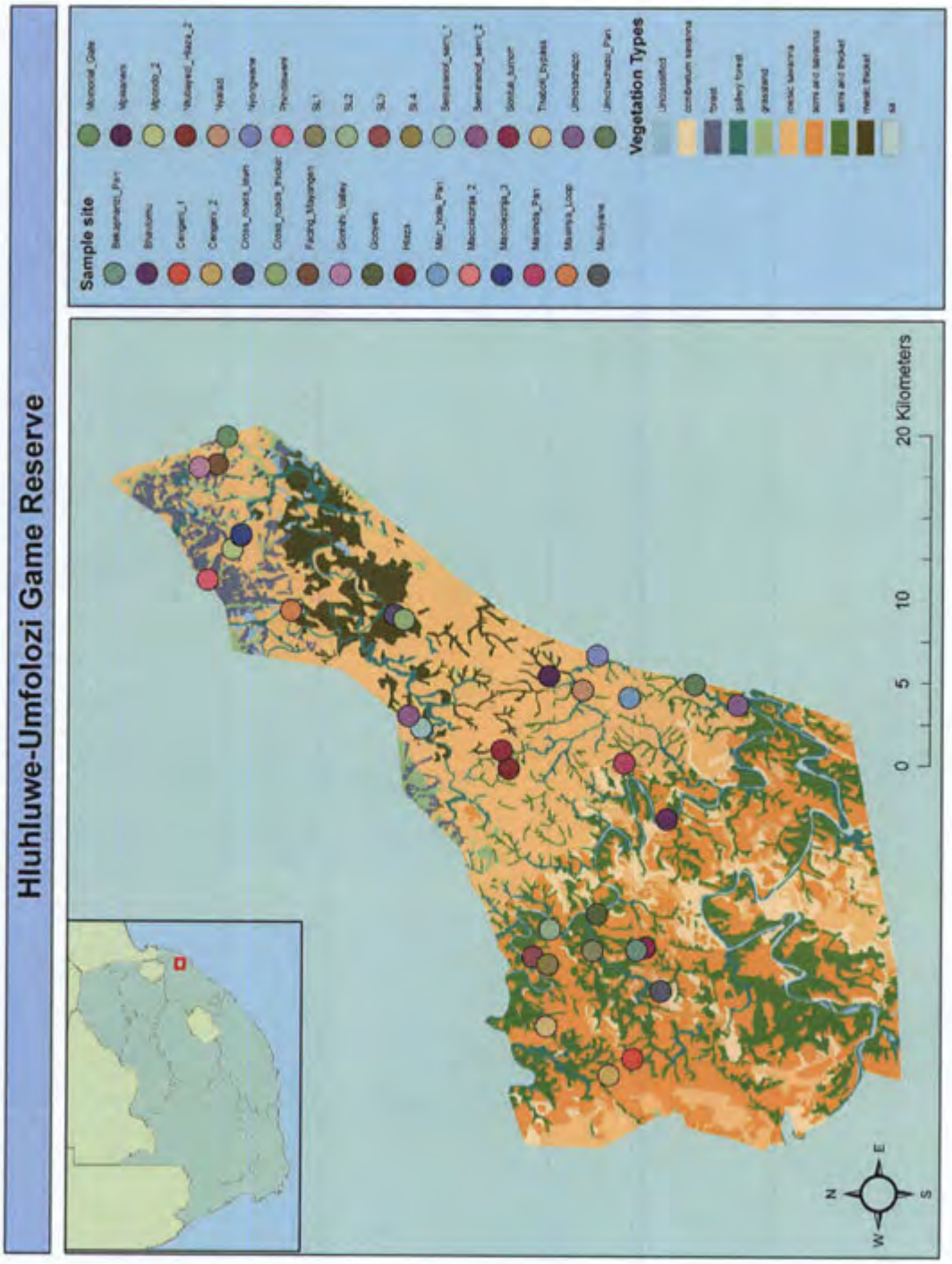


Figure 2(b): Map of the Hluhluwe-Imfolozi game reserve showing the dominate vegetation types and all the data collection points that were counted for *Sporomiella*.

3.2. Sampling

Surface sediment samples were collected from sediment basins throughout Hluhluwe, Imfolozi and the Corridor. We collected a total 39 samples (11 in Hluhluwe, 17 in Imfolozi and 11 in the corridor) for which GPS coordinates were recorded. The samples from each site were taken from pans and other wet areas where sediment accumulates and traps spores, pollen and other palynimorphs. The top 2cm of sediment were removed by hand and placed in labelled sample bags. The samples were then transported back to Cape Town where they were stored at 10°C and analysed for *Sporomiella*. Vegetation at each site was described, by Bond pres. Comm., and divided into forest, gallery forest mesic forest, semi-arid thicket, grass-land, mesic Savanna, Combretum Savanna and semi-arid Savanna. The dominant woody vegetation was noted. Table1 (see appendices) lists the dominant vegetation, woody vegetation and grass types at each of the 39 sites sampled.

3.3. *Sporomiella* analysis

The *Sporomiella* samples were prepared using the standard stepwise pre-treatment techniques used in pollen analysis (Bennet & Willis 2001). One sub-sample was taken from each site and sediment volume (1 cm^3) was determined using volumetric displacement in distilled water. Preceding processing two tablets of known concentration of Lycopodium spores were added to each sample, so that absolute concentrations of *Sporomiella* can be calculated. The samples were sieved through a 140µm sieve to removes macro-particles larger than pollen and spores. The spore extraction included treatments with HCL, NaOH, hot HF, glacial acetic acid and acetolysis. The HCl process removes the carbonates from the samples. The hot NaOH removes humic acids which

are unsaturated organic soil colloids. The HF step removes silica and silicates from the samples. Acetolysis removes the polysaccharides by hydrolising the polymer chain into soluble monosaccharide units (Bennett and Willis 2001). After processing, the sub-sample was then stained using Safranin and mounted in silicone oil. Spore identification was done using 400x magnification and 1000x oil immersion lens, and van Geel (1986) collections.

A standard pollen concentration equation was used to calculate the concentration of *Sporomiella* relative to known concentrations of Lycopodium. The equation

$$S_{conc} = \frac{\text{Lycopodium added}}{\text{Lycopodium counted}} \times \frac{\textit{Sporomiella} \text{ counted}}{\text{Volume}}$$

Where spores added = number of Lycopodium tablets x concentration per tablet
and Volume is the volume of the sub-sample, in this case $1cm^3$

3.4. Herbivore dung abundance

The data for the herbivore dung abundance was provided by Dr Joris Cromsigt (Pers. Comm., Cromsigt *et al*, 2004). He conducted a survey in Hluhluwe-Imfolozi in 2004, counting dung pellets from large herbivore grazer species along transects. These transects were evenly distributed throughout the park encompassing all vegetation types and elevations. The total dung counts and the counts for each species were divided by the length of the corresponding transects (m) to get a measure of the total dung abundance (m^{-1}) and species dung abundance (m^{-1}) respectfully. Dung counts were calculated at a 5X5 Km and a 2.5X2.5

Km resolution. To calculate dung abundance from dung counts we used the equation:

$$\text{Dung abundance} = \frac{\text{Number of pellets counted}}{\text{Length of the transect (m)}}$$

3.5. Statistical Analyses

All data analysis was preformed in Statistica® version 7.0 (StatSoft Inc. 2004). A Kolmogorova-Smirnov test was preformed to test for normality and homogeneity of data. We divided our data analysis into two sections. Some of the sites we sampled had zero *Sporomiella* concentrations which does not necessarily imply that these sites do not contain *Sporomiella*. As only 300 Lycopodium spores were counted due to time constraints we can not be a hundred percent sure that those sites did not contain *Sporomiella*. We therefore looked at the relationship between the *Sporomiella* and dung abundances in two ways. First we analysed the data by excluding the sites with zero *Sporomiella* concentrations and secondly we analysed the data of all the sites with or without *Sporomiella*.

In both cases we investigated three aspects: [1] the relationship between *Sporomiella* abundance and total herbivore abundance was explored by comparing *Sporomiella* concentration (from the above equation) with herbivore abundance, as indicated by total dung abundances (Cromsigt, pers. Comm.). Spore density was plotted against herbivore dung abundance for each sample site, and the relationship between the two variables was then investigated using linear regression and curve fitting. [2] To test the relationship between *Sporomiella* and individual herbivore species we compared the *Sporomiella* concentration (from the above

equation) with individual herbivore abundance, as indicated by the individual species dung abundances (Cromsigt, pers. Comm.). The concentration of the spore was plotted against each individual species to determine the relationship between the two variables. We then divided all the herbivores into Mega-herbivores and Meso-herbivores to determine their relationship with *Sporomiella* densities. [3] Finally, to test differences between the regions of the reserve we compared the different areas of the park (Hluhluwe, Imfolozi and the corridor) with the concentration of *Sporomiella* (from the above equation) and herbivore dung abundances.

The different herbivore species measured including elephant, white rhino, black rhino, buffalo, zebra, wildebeest, kudu, impala, nyala, warthog, duiker, bush pig and giraffe. The herbivores were divided into mega-herbivores (elephant, white rhino, black rhino and buffalo) and meso-herbivores (zebra, wildebeest, kudu, impala, nyala, warthog, duiker, bush pig and giraffe) according to there size. ANOVAs were run to determine if there was a significant difference between the different areas of the park, vegetation type and grass type in terms of *Sporomiella* concentration. All analysis was significant at a 95% significance level. Multiple regressions were preformed to compare *Sporomiella* concentrations with the different herbivore dung abundances to determine which animal is contributing more to the concentration of *Sporomiella*.

4. Results

Of the 39 sediment samples prepared for spore counting, (Fig 1(b)), 6 were not suitable for microscopic analysis due to aggregation of organic matter on the slides. These sites were therefore excluded from the analysis. Mpondo 2 had an anomalously high *Sporomiella* concentration which was probably an artefact of the variation in micro-habitat, in that we most likely picked up dung with the sediment sampled at that site; we therefore excluded this site from the analysis. Umchachazo and Umchachazo Pan were also excluded as no dung counts were available, leaving a total of N= 30 in the analysis at the 5X5 Km resolution. At the 2.5X2.5 Km resolution no dung counts were available for Mpisaneni, Bhavulomu, Umchachazo, Umchachazo Pan and Momorial Gate, leaving a total of N= 27.

Sporomiella concentrations ranged from 0 to 8548.10 with a mean of 1606.66 (±1871.83) and a variance of 7419520.85. The maximum concentration was at the Cross Roads Lawn site with a *Sporomiella* concentration of 8548.10. Hlaza 2, Man Hole Pan and Maqolezinja 2 had the lowest *Sporomiella* concentration of all the sites at 247.88. Several sites had a *Sporomiella* concentration of zero these included: Facing Mayangen, SL3, Sematenof 2, Gqoyeni, Gontshi Valley and Maqolezinja 2. Mpondo 2 which was excluded from the analysis due to its abnormally high density boasted a spore concentration of 13131.99.

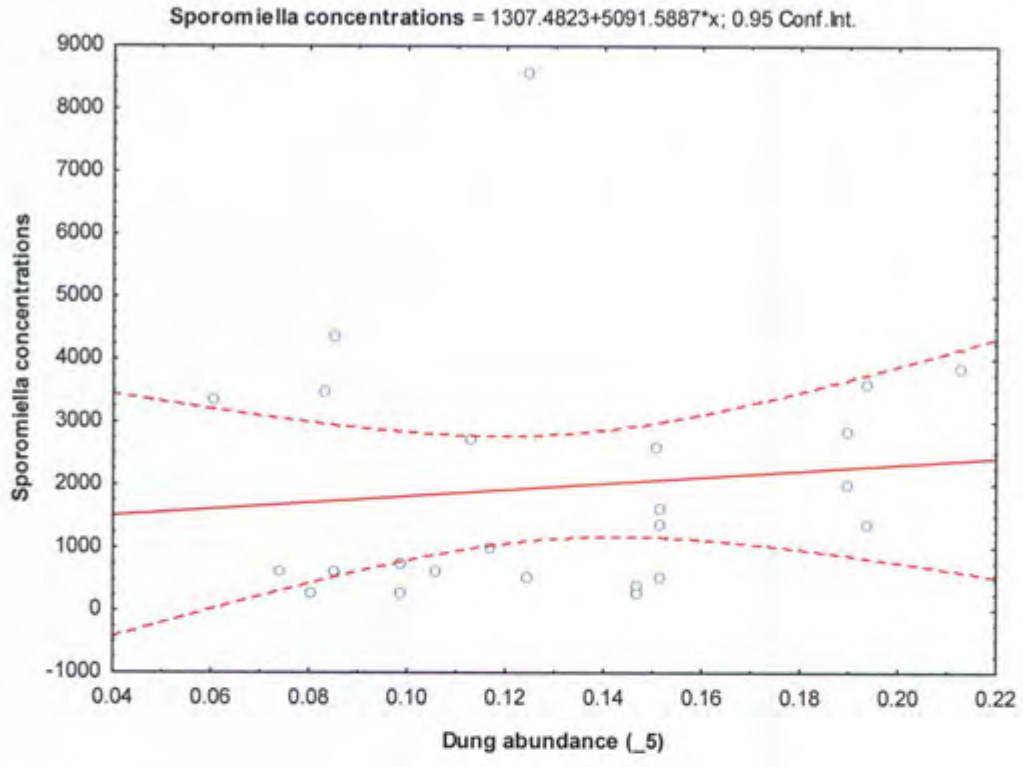
Dung abundance (Cromsigt, pers. Comm.) at a 5X5 Km resolution ranged from 0.060 – 0.213 m^{-1} with a mean of 0.128 (±0.043) and a variance of 0.001. The maximum abundance of dung was found at Thaboti bypass while the lowest was at Phindisweni. In terms of individual species the Black rhino has the highest dung abundance

(1.05) of all the herbivores while the Bushbuck has the lowest (0.001). At the 2.5X2.5 Km resolution dung abundance was the highest at SL1 (0.29) while the lowest abundance was again at Phindisweni (0.027). The dung abundance of individual herbivores shows that the buffalo has the highest dung abundance (0.859) while the bushbuck has the lowest (0.0003).

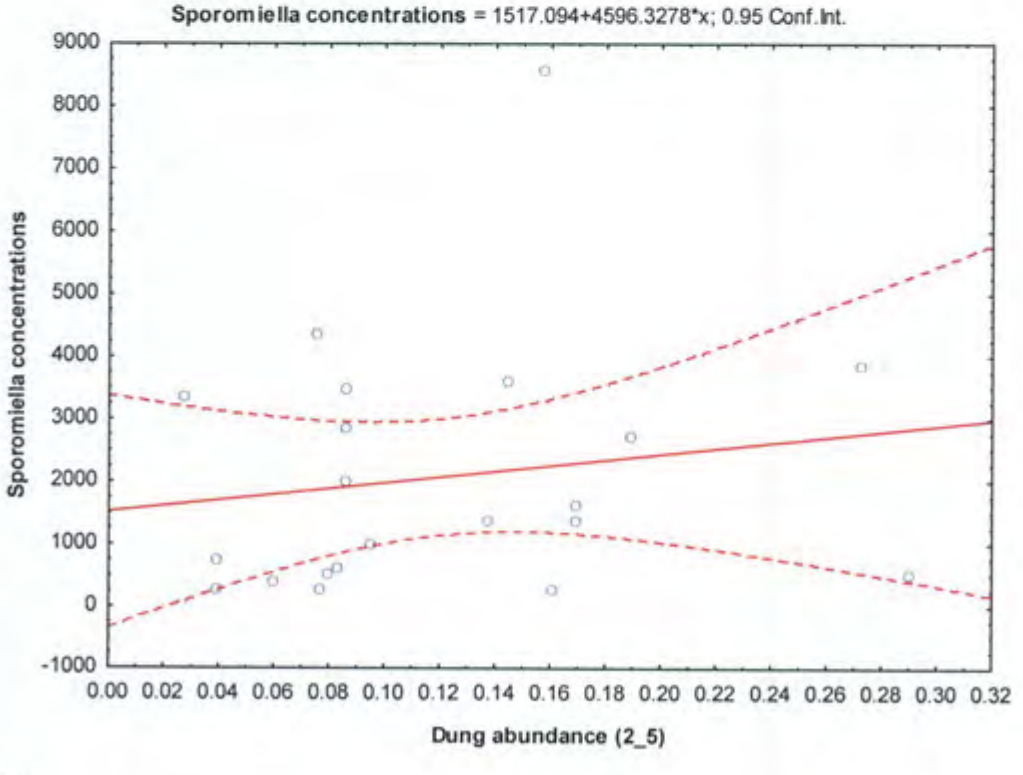
Our data were normally distributed with p-values > 0.20 for all variables measured.

4.1. Analysis excluding the sites where *Sporomiella* were absent

Firstly we analysed the relationship between *Sporomiella* concentration and herbivore dung abundance, a proxy for herbivore abundance (Fig 3 (a) and (b)). Linear regressions and curve fitting (logarithmic, polynomial, power and exponential) were used to determine if there were any significant relationship. No significant liner relationship was found between *Sporomiella* concentration and total dung abundance at both the 5X5 Km ($p = 0.5904$) and 2.5X2.5 Km resolution ($p = 0.4808$). None of the curves fitted to *Sporomiella* concentration plotted against herbivore dung abundance showed any significant relationship ($p > 0.05$).


Sporomiella concentrations = 1307.4823+5091.5887*x; 0.95 Conf.Int.
Sporomiella concentrations
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
0.04
0.06
0.08
0.10
0.12
0.14
0.16
0.18
0.20
0.22
Dung abundance (_5)

(a)


Sporomiella concentrations = 1517.094+4596.3278*x; 0.95 Conf.Int.
Sporomiella concentrations
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
0.00 0.02 0.04 0.06 0.08 0.10 0.12 0.14 0.16 0.18 0.20 0.22 0.24 0.26 0.28 0.30 0.32
Dung abundance (2_5)

(b)

Figure 3: *Sporomiella* concentrations compared to the abundance of dung within the Hluhluwe- Imfolozi game reserve at 5X5 Km (a) and a 2.5X2.5 Km (b) resolution excluding the site with a *Sporomiella* concentration of zero.

Secondly we looked at the relationship between the concentration of *Sporomiella* and the dung abundances of the individual herbivore species. *Sporomiella* concentration compared to the different animal dung abundance at both the 5X5 Km and the 2.5X2.5 Km resolution only showed a significant relationship between the elephant (Fig 4, r^2=0.2015, p =0.0412) and the white rhino (Fig 5, r^2 = 0.1622, p =0.0702) at the 2.5X2.5 Km scale. There was no significant difference between the dung abundance of mega-herbivores and meso-herbivores when compared to *Sporomiella* concentrations at both resolutions (p>0.05). Comparing the correlation of these two herbivore species found that they are significantly different from each other (Fig 6) as the two regression lines do not fall in the same confidence limits. The *Sporomiella* concentrations compared to the elephant dung abundances yielded a linear equation: y =1285.4786+1.2476^{5}x while the white rhino dung abundances generated the linear equation: y = 779.309 + 4.3667^{5}x.

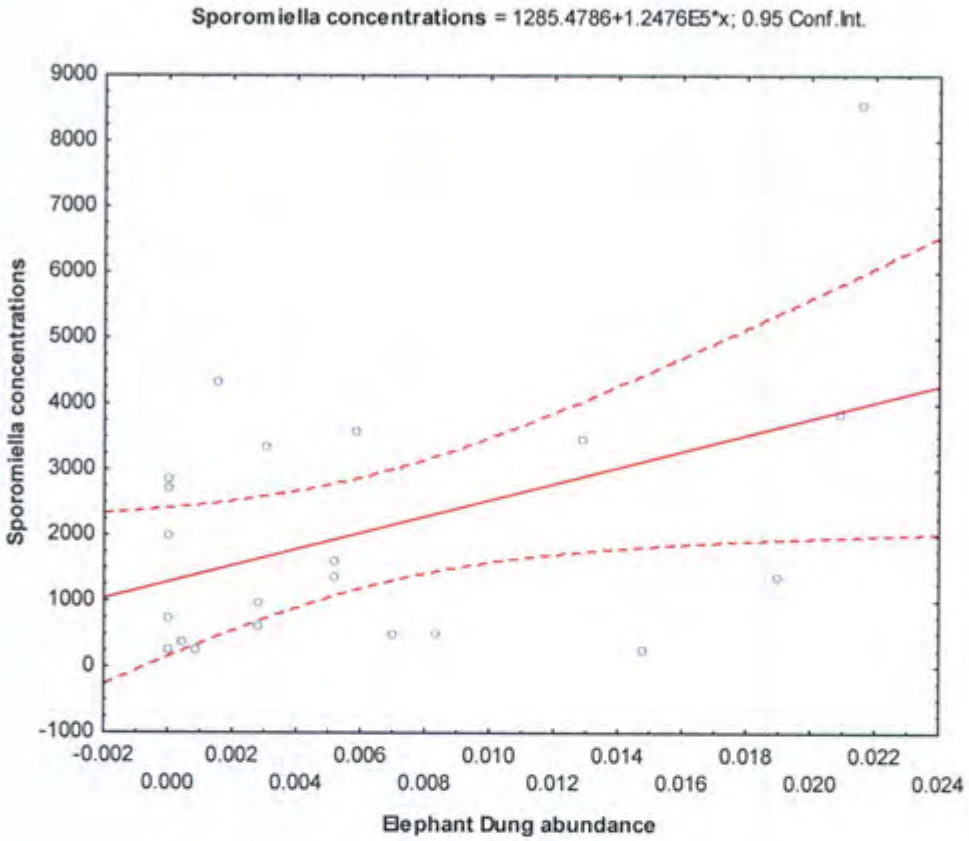


Figure 4: *Sporomiella* concentrations compared to elephant dung abundance excluding the site with a *Sporomiella* concentration of zero.

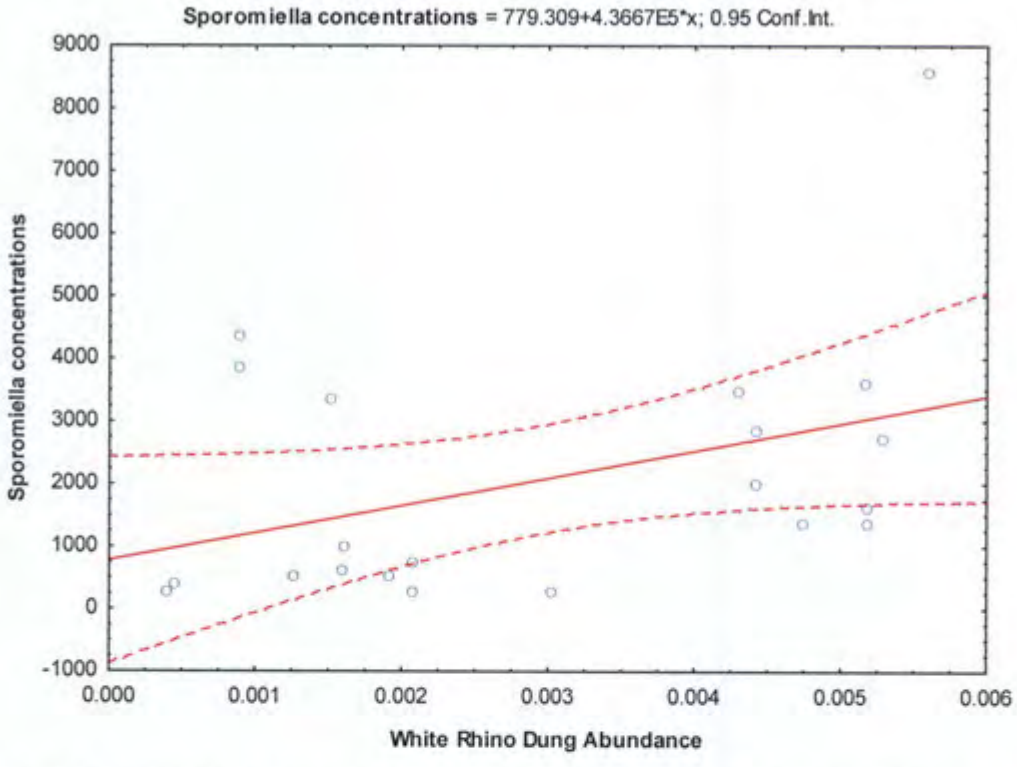


Figure 5: *Sporomiella* concentrations compared to white rhino dung abundance excluding the site with a *Sporomiella* concentration of zero.

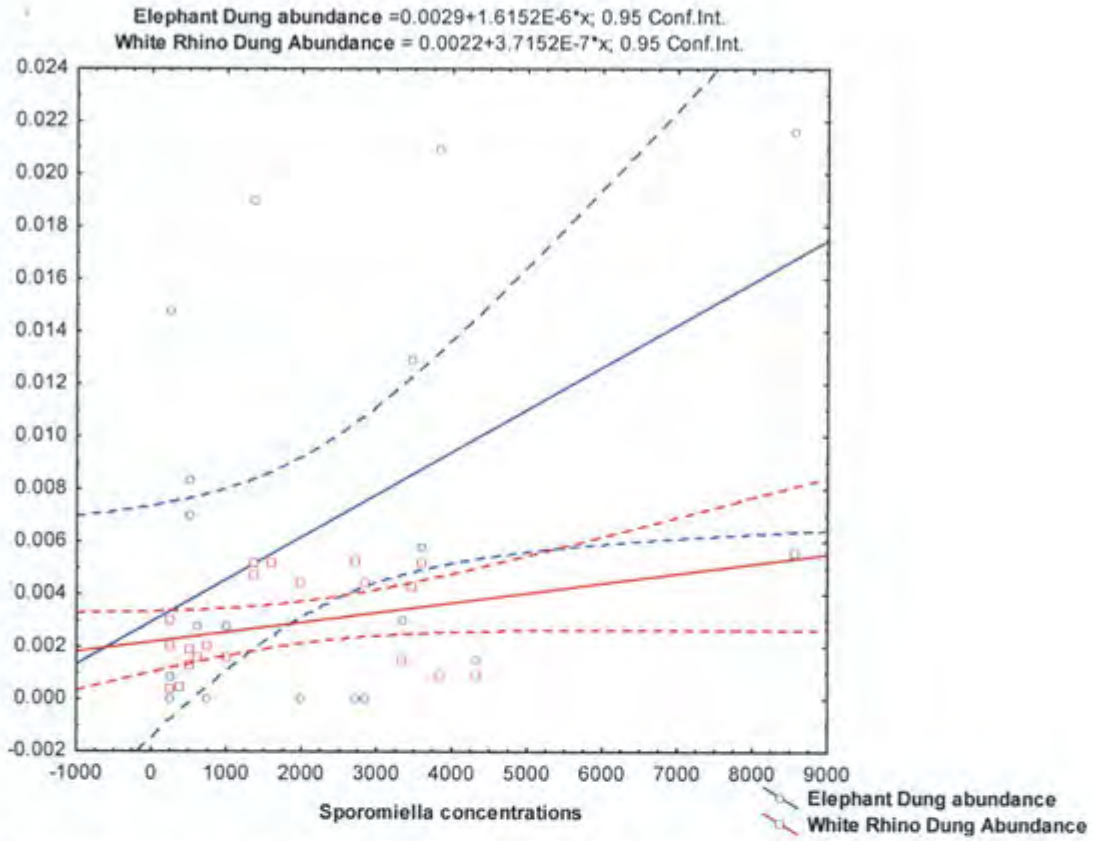


Figure 6: Comparing *Sporomiella* concentrations between elephant and white rhino dung abundance, excluding the site with a *Sporomiella* concentration of zero.

Thirdly we examined the relationship between the concentration of *Sporomiella* and the abundance of dung with the three different areas of the park: Hluhluwe, Imfolozi and the corridor. At the 5x5 km resolution no significant difference was found between *Sporomiella* concentration and the three areas of the park ($df = 2$, $F = 0.02$, $p = 0.980135$). Comparing the herbivore dung abundances between the different areas of the park at this resolution shows a significant differences ($df = 2$, $F = 20.60682$, $p = 0.000011$). The corridor and Hluhluwe were significantly different from Imfolozi ($p < 0.05$) but there was no significant difference between the corridor and Hluhluwe ($p > 0.05$). The 2.5X2.5 km resolution

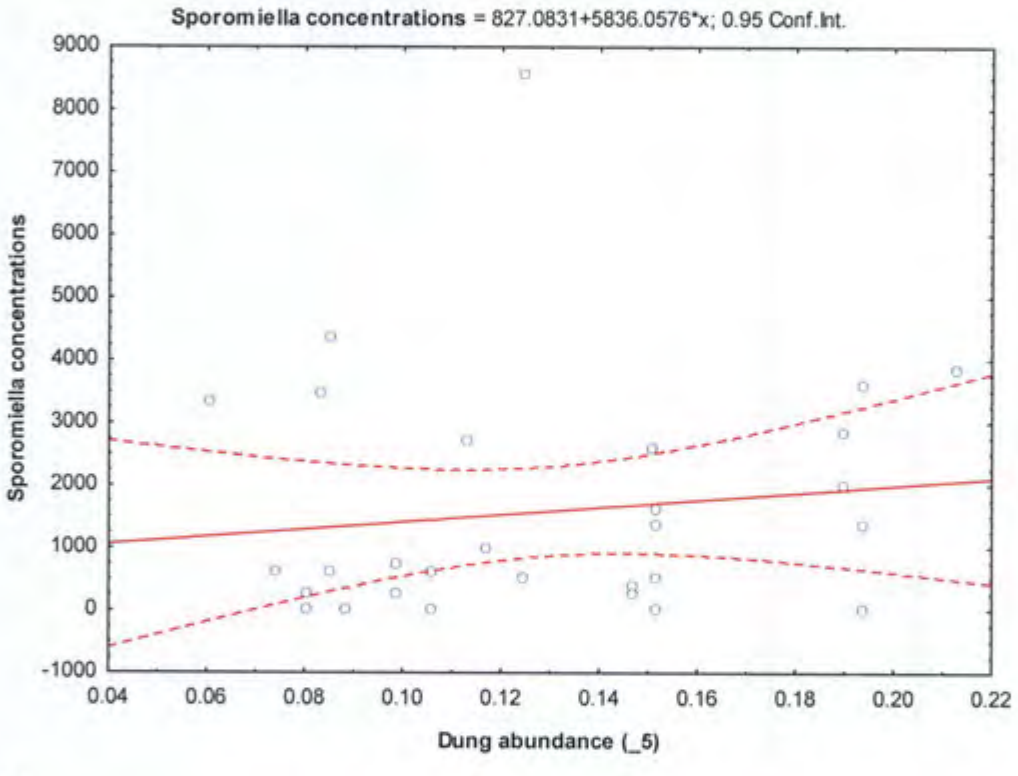
shows a similar pattern to the 5X5 Km in that *Sporomiella* concentrations show no significant differences with area of the park (df =2, F =0.03158, p =0.96896). There is also a significant difference between the herbivore dung abundance and the areas of the park (df =2, F =3.588635, p =0.048795) but none of the area are significantly different form each other (p >0.05).

Since the corridor and Hluhluwe were not significantly different from each other at the 5X5 Km resolution we combined them to test there significance with Imfolozi. The *Sporomiella* concentrations remained insignificant when compared to the areas of the park (df =1, F =0.041627, p =0.84020) and the herbivore dung abundance was significantly different compared to the two areas (Imfolozi and Hluhluwe + corridor). A significant difference of p = 0.000163 was found between Imfolozi and Hluhluwe + corridor. There was no significant relationship between *Sporomiella* concentration and vegetation type (df=19, F=0.35598, p=0.8975) or grass type (df=15, F=0.0144, p=0.9606) within the different areas of the park.

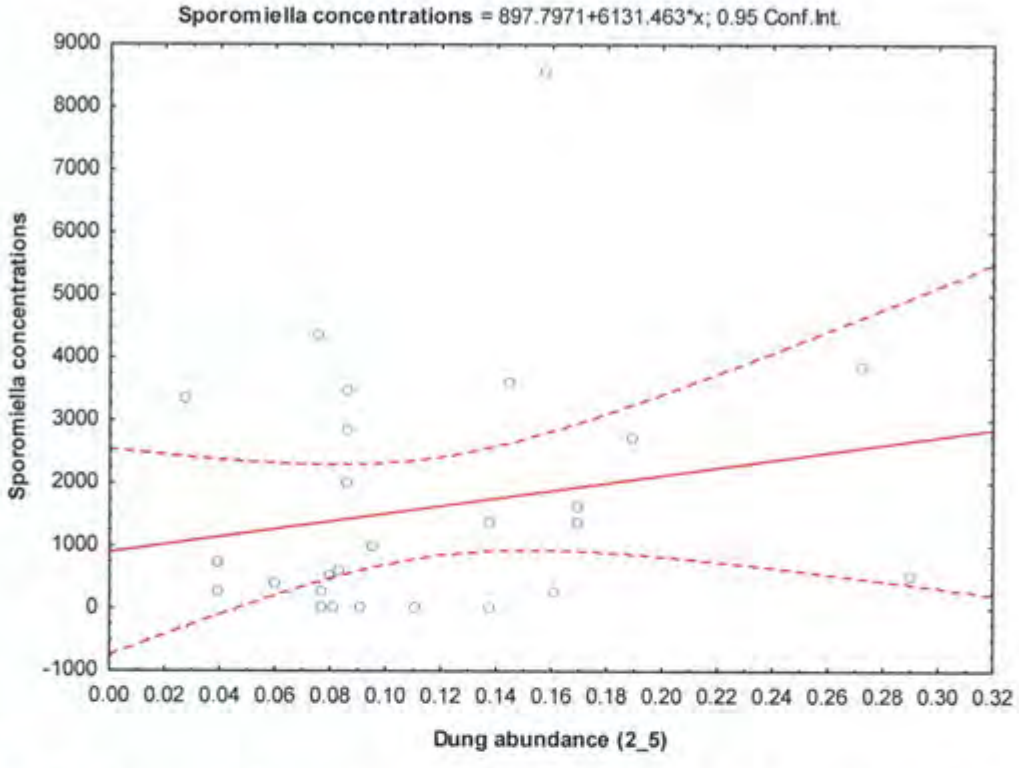
Comparing *Sporomiella* concentration with all the herbivore dung abundances for the different animals using multiple regression shows that at the 5X5 Km resolution there is no significant difference (r^2 =0.3547, F= (14, 9)= 0.35348, p<0.96033). At the 2.5X2.5 Km resolution there is only a significant difference with elephant dung abundance (p=0.020087) all the other herbivores were insignificant (r^2 =0.8390, F (13, 7) =2.807, p <0.08806).

4.2. Analyses with all the sites included

Following on the same format as above, we first analysed the relationship between *Sporomiella* concentrations and total dung abundance at both resolutions (5X5 Km and 2.5X2.5Km). No significant relationship was found at both resolutions ($p>0.05$).



(a)



(b)

Figure 7: *Sporomiella* concentrations compared to the abundance of dung within the Hluhluwe- Imfolozi game reserve at 5X5 Km (a) and a 2.5X2.5 Km (b) resolution for all sites including sites with zero *Sporomiella*.

Secondly we tested the relationship between *Sporomiella* concentrations and individual herbivore species dung abundances. No significant difference was found for any of the animals at both the 5X5 Km and the 2.5X2.5 Km resolution ($p>0.05$). Grouping the herbivore species into mega-herbivores and meso-herbivores showed no significant difference with *Sporomiella* concentrations ($p>0.05$). Lastly we looked at the relationship between *Sporomiella* concentrations and the three different areas of the park. At the 5X5Km resolution *Sporomiella* concentrations were not significantly different between the different areas of the park ($p>0.05$). There was however a significant difference ($p<0.0001$) in dung abundance. Imfolozi was significantly different from Hluhluwe ($p =$

0.000130) and the corridor (p = 0.000133). Hluhluwe and the corridor were not significantly different from each other (p>0.05). There was also no significant difference at the 2.5X2.5 Km resolution between *Sporomiella* and the different areas of the park (p>0.05). There was a significant difference between dung abundance and the different areas of the park (p = 0.0359). The dung abundances between the areas of the park were however not significantly different from each other (p>0.05). Comparing the concentration of the park with the different vegetation types (df = 25, F = 0.3714, p = 0.8901) and grass types (df = 17, F = 0.0208, p = 0.8868) showed no significant relationship. The multiple regressions between *Sporomiella* and the individual herbivore dung abundances showed no significant relationship at either the 5X5 Km resolution (r^2=0.3792, F (14, 15) = 0.6545, p<0.7828) or the 2.5X2.5KM resolution (r2= 0.5001, F (13, 13) =1.0006, P<0.49961).

5. Discussion

The results from this study are by no means clear-cut with different patterns emerging depending on the resolution of the dung abundance and whether the sites without any *Sporomiella* are analysed. The thinking behind analysing the data by first excluding the sites without *Sporomiella* is simply that the absence of *Sporomiella* from these sites does not indicate they are not present in the area. We therefore decided to analysis the data both ways to see the effect. It is interesting to note that at the 5X5 Km resolution none of our sites contained any waterbuck dung while at the 2.5X2.5 Km resolution there were no waterbuck or bushbuck dung.

5.1. Excluding the sites with zero *Sporomiella*

The results show that the total abundance of herbivore dung within the Hluhluwe-Imfolozi game reserve does not have a significant linear relationship or any other curve fitting relationship with the concentration of *Sporomiella*. This implies that the fungus may be selectively growing on certain herbivore species rather than on all herbivore dung, or that dung counts do not reflect the differences in dung amounts in the landscape. To test [the former] this we compared *Sporomiella* concentration to the different animal dung abundances at both the 5X5 Km and the 2.5X2.5 Km resolution. The only significant relationship was found with elephant (Fig 3) and the white rhino (Fig 4) at the 2.5X2.5 Km scale, which implies that the *Sporomiella* are selectively growing on certain herbivore dung.

This result also suggests that mega-herbivores are the main contributes to *Sporomiella* concentrations within the park. We tested this idea by

grouping the mega-herbivores and the meso-herbivores together and then comparing them to *Sporomiella* concentrations. We found no significant difference between the mega-herbivores and the meso-herbivores at both dung abundance resolutions ($p>0.05$) suggesting that the main drivers for *Sporomiella* abundance are elephants and white rhino. This result leads further support to the idea that *Sporomiella* are selective in the dung they inhabit. The regressions of these two species were then compared to determine if there was any significant difference between them. The regressions were significantly different (Fig 5) from each other confirming that both these species are affecting the *Sporomiella* concentrations within the park. The equations developed from these two regressions can be used in the future to estimate elephant and white rhino abundance using *Sporomiella* concentrations.

The park is divided into three different regions with Hluhluwe in the north, Imfolozi in the south and the corridor connecting the two. The north of the park is a mesic, fire driven habitat while the south is a more arid, herbivore driven habitat (Bond, pers. Comm.). As Imfolozi is a herbivore driven ecosystem one would expect that the concentration of *Sporomiella* would be higher than in Hluhluwe. Our results do not support this hypothesis as we found no significant difference in *Sporomiella* concentration between the different areas of the park. We did however find a significant difference between the dung abundances. Imfolozi is significantly different from Hluhluwe and the corridor but Hluhluwe is not significantly different from the corridor. This suggests that the corridor and Hluhluwe have similar herbivore abundances and that Imfolozi is different. Since Hluhluwe and the corridor were so similar we combined them and tested to see if there was a significant difference in *Sporomiella* concentration between Imfolozi and Hluhluwe + the corridor. Once again there was no significant difference in *Sporomiella*

concentrations between the different areas of the park. This result implies that the *Sporomiella* concentrations are similar through out the park suggesting that elephant and white rhinos are equally distributed. Elephants are known for there large territories and travel all over the park while white rhinos are more territorial with larger densities within Imfolozi (Bond, pers. Comm.). We found no significant relationship between *Sporomiella* concentration compared to vegetation type and grass type within the park, which suggests that *Sporomiella* are not specific to areas with certain vegetation types and grass types. A multiple regression between *Sporomiella* concentration and herbivore dung abundance showed that elephant ($p=0.020$) was the only herbivore affecting the concentration of *Sporomiella* when compared to all other herbivores at the 2.5X2.5 Km resolution. This implies that although white rhino has a significant regression the elephant is contributing the most to the concentration of *Sporomiella* throughout the park, which is what you would predict if elephants have a large home range.

5.2. Including the sites with *Sporomiella*

Including the sites without *Sporomiella* showed similar results to not including them in that *Sporomiella* concentrations had no significant relationship with herbivore dung abundance. The most important difference between the two sets of analysis was that the elephant and white rhino no longer had a significant relationship with *Sporomiella* concentrations; in fact none of the herbivores had a significant relationship with *Sporomiella*. Dividing the herbivores in to mega-herbivores and meso-hebivores showed no significant difference with *Sporomiella* abundance. There was no significant difference in the area of the park at both resolutions in terms of *Sporomiella* concentrations which implies that the concentration of *Sporomiella* is uniform

throughout the park which is consistent with the results from the first analyses. *Sporomiella* concentrations did not have a significant relationship with vegetation type or grass type supporting the idea that *Sporomiella* are not restricted to areas with specific types of vegetation and grass. Multiple regression between *Sporomiella* and the individual herbivores dung abundance showed no significant differences ($p>0.05$).

One major limiting factor in this study is that we were unable to compare *Sporomiella* concentrations to herbivore biomass or abundance as the data for this is unavailable for the Hluhluwe-Imfolozi game reserve. Comparing the dung of different herbivore species as a proxy for herbivore abundance is not a very accurate measure of herbivore numbers or Biomass within an area. The weight of the dung between the different species is also very variable as the size of the herbivores range between a bush pig and an elephant, which could also lead to inconsistency in the data as bigger herbivores, produce more dung. We therefore suggest that herbivore numbers rather than dung abundances be used in further studies.

6. Conclusions

In this study we have shown that there was a significant relationship between *Sporomiella* abundance the dung abundance from white rhinos, but only if sites with zero *Sporomiella* are excluded form the analysis. Neither mega-herbivore nor meso-herbovore dung abundance had a significant relationship with *Sporomiella* concentration. There was no significant relationship between *Sporomiella* concentration and dung abundance when the sites with zero *Sporomiella* were included in the analysis. There was also no significant difference between the different areas of the park in terms of *Sporomiella* concentrations. The data suggests that *Sporomiella* is dominant on elephant and white rhino dung over the whole park. There was no significant relationship between vegetation or grass type and *Sporomiella* concentrations suggesting that the spores are not specific to areas with certain vegetation and grass types. Cross Roads Lawn had the highest *Sporomiella* concentration (8548.10) of all the sites measured; this area is known for its high herbivore density and is heavily grazed (Bond, pers. Comm.); we therefore anticipated a high *Sporomiella* concentration for this area. It is surprising though that this area does not have the highest dung abundance. Further suggesting that it's not the quantity of the dung that's important it's the quality *i.e.* they only grow on certain species of dung. Though *Sporomiella* have great potential in indicating herbivore presence in the palaeo record, further calibration work will be needed before the relationship between *Sporomiella* concentration and herbivore abundance can be quantified.

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8. References:

Angel. Sr. K. and Wicklow. D. T. 1975. Relationships between coprophilous fungi and fecal substrates in a Colorado grassland. *Mycologia* **67(1)**: 63-74.

Bennett K.D. and Willis K.J. 2001. Pollen. In: Tracking Environmental Change using Lake Sediments. Kluwer, Dordrecht, pp. 355-361.

Berrio. J. C., Hooghiemstra. H., van Geel. B. and Ludlow-Wiechers. B. 2006. Environmental history of the dry forest biome of Guerrero, Mexico, and human impact during the last c. 2700 years. *The Holocene* **16(1)**: 63-80.

Burney. D. A., Robinson. G. S. and Burney. L. P. 2003. *Sporormiella* and the late Holocene extinctions in Madagascar. *PNAS* **100(19)**: 10800-10805.

Caretta. G., Piontelli. E., Savino. E. and Bulgheroni. A. 1998. Some corprophilous fungi from Kenya. *Mycopathologia* **142(3)**: 125-134.

Cromsigt. J. P. G. M., Prins. H. H. T. and Olff. H. 2004. Diversity of habitat use by large grazers: an interaction between body mass and digestive strategy. Landscape use differences among large grazers. Chapter 3. Manuscript.

Davis. O. K. and Shafer. D. S. 2006. *Sporormiella* fungal spores, a palynological means of detecting herbivore density. *Palaeogeography, Palaeoclimatology, Palaeoecology* **237**:40-50.

Graf. M. T. and Chmura. G. L. 2006. Development of modern analogues for natural, mowed and grazed grasslands using pollen assemblages and coprophilous fungi. *Review of Paleobotony and Palynology* **141**:139-149

Hemborg. A. M. and Bond. W. J. 2006. Do browsing elephants damage female tress more? *African Journal of Ecology* **45**: 41-48.

Huntly. N. 1991. Herbivores and the dynamics of communities and ecosystems. *Annual Review of Ecology and Systematics.* **22**: 477-503.

Koch. P. L. and Barnosky. A. D. 2006. Late Quaternary Extinctions: State of the debate. *Annu. Rev. Ecol. Evol. Syst.* **37**: 215-250.

Lejju. B. J., Taylor. D. and Robertshaw. P. 2005. Late-Holocene environmental variability at Munsa archaeological site, Uganda: a multicore, multiproxy approach. *The Holocene* **15(7)**: 1044-1061.

Parker. A. D. 1979. Associations between coprophilous ascomycetes and fecal substrates in Illinois. *Mycologia* **71(6)**: 1206-1214.

Ritchie. M. E., Tilman. D. and Knops. M. H. 1998. Herbivore effects on plant and nitrogen dynamics in Oak Savanna. *Ecology* **79(1)**: 165-177.
Robinson. G. S., Burney. L. P. and Burney. D. A. 2005. Landscape paleoecology and megafaunal extinctions in southeastern New York State. *Ecological Monographs* **75(3)**: 295-315.

Skarpe. C. 1992. Dynamics of savanna ecosystems. *Journal of vegetation Sciences* **3**: 293-300.

Van Geel. B. 1986: Application of fungal and algal remains and other microorganisms in palynological analysis. In Berglund, B.E., editor, Handbook of Holocene palaeoecology and palaeohydrology. Chichester: John Wiley, 497-505.

Walters. M and Milton. S. 2003. The production, storage and viability of seeds of *Acacia karroo* and *A. nilotica* in a grassy savanna in KwaZulu-Natal South Africa. *African Journal of Ecology* **41**: 211-217.

9. Appendix

Table 1: The lists of dominant vegetation type, area of the park, woody vegetation and grass types at each of the 39 sites sampled.

Site	Vegetation type	Area of Park	Grass type	Woody vegetation
Masinda Pan	Mesic Savanna	Corridor	-	*A. karoo* *A. nilotica* *Dicrostacus* *Maytenus* *seuegaleusis* *Marula* *termite mound* *thicket with Euclea*
Masinya Loop	Mesic Thicket	Hluhluwe	tall bunched grass	*Dicrostacus* *A. karoo* *Ziziphous* *Maytenus* *seuegaleusis* *Sideroxiglei* *Marules* *A. robusta* *E.divonorum* *E. schimperii*
Mpisaneni	Mesic Savanna	Corridor	tall bunched grasses	*Sporobulus* *A. nilotica* *A. karoo* *Sderocarya* *Dicrostacus* *Maytenus* *heteriophylla* *Euchea shimperii* *Cassini* *Maytenus* *seuegaleusis*
Facing Magangeui	Mesic thicket	Hluhluwe	Short grass	*A.robusta* *M. sevagelensii* *B. xeri* *Ziziphous* *Dicrostacus* *E. schimperii*
Bhavlomu	Semi arid Savanna	Imfolozi	short grass	*Ziziphous* *Schotia* *Maytenus* *heteriophylla* *A. graudiconuta* *Spirostachys* *(tauhoti) (salt tolerent)*
Gontshi Valley	Mesic Thicket	Hluhluwe	Tall bunched	*A .karro*

			grass	*A. cathra* *Maytenus* *Dicrostacus*
Nyalazi	Mesic Savanna	Corridor	tall bunched grass	*Dicrostacus* *A. karoo* *A. burkeii* *Ziziphous* *Cambuetum molii* *Maytenus seuegaleusis* *Rhius perthei*
Ntobayezi (Hlaza 2)	Savanna	Corridor	Short grass	*A. karoo (saplings)*
Sontuli turnoff	Semi arid thicket	Imfolozi	short grass	*Dicrostacus* *Tauboti/ spiro* *E. divonorum* *E. crispa* *Schotia brachypetala*
Phindisweni	Forest	Hluhluwe	short grass	*Celtis africamer* *Rhus chirdenses* *Angularphytum notalensii* *Albusia Zuluensis (rare spp)* *Bridelea* *A. ataxicantha (forest type)* *few sedges* *Sporobulus*
Nyongwane	Flood plain grass land	Corridor	Flood plain grass land	*Digiteria* *Elysorii* *A. rhobusta* *Albizzia versicula* *A. karoo* *Dicrostacus* *Ficus sycamosus (asclepiaeh)*
Cross roads lawn	Mesic Savanna	Corridor	short grass	*A. nilotica* *Dicrostacus* *Sporobulus* *Digiteria longifloria* *Liliaceae* *Bothriocloa* *Tragus* *E. divonorum* *Maytenus seuegaleusis* *A. burbesii (indicates sandy soils)*

Cross roads thicket	Mesic Thicket	Corridor	-	*E. divonorum* *Spirostachys (tauhoti) (salt tolerent)* *Maytenus heteriophylla* *Schotia brachypetala (cordia rhudis)- on termite mond* *E. schimperii (rare)*
SL1	Semi arid thicket	Imfolozi	-	*Euclea spp* *schotia capitala*
Man hole Pan	Mesic Savanna	Corridor	Short grass	*A. karoo* *Dicrostacus* *Maytenus seuegaleusis* *A. nilotica* *E. divonorum* *E. schrimperii* *Tarchisteus* *Cassini* *Schotia brachypetala (cordia rhudis)* *Lippia*
SL2	Semi arid Thicket	Imfolozi	-	*Tamboti* *A. nigrescens* *A. graudiconuta* *E. crispa* *E. divonorum* *M. heteriophylla* *Aizaeua tetracautho (thicket clump)* *Ziziphous schotia capitala*
SL3	Semi arid Thicket	Imfolozi	-	*A. nigrescens* *Dicrostacus* *A. tortilis* *M. heteriophylla* *Euclea* *Capparis*
SL4	Semi arid Thicket	Imfolozi	-	*A. graudiconuta* *A. tortilis* *Dicrostacus* *Ziziphous* *Capparis* *schotia capitala* *Plectroniella amata* *Maytenus heteriophylla*

Hlaza	Mesic Savanna	Corridor	tall bunched grasses	*A. karoo (saplings)* *Faclea* *Maytenus*
Cengeni 1	Semi arid semi arid Thicket	Imfolozi	short grass	*A. nigresenus (wood land)* *A. graudiconuta* *A. tortilis* *tamboti* *Maytenus* *heteriophylla* *sideroxylon ineuve*
Cengeni 2	Semi arid Thicket	Imfolozi	short grass	*A. graudiconuta* *A. tortilis* *Maytenus* *heteriophylla*
Sematenof (semi 1)	Mesic Savanna	Corridor	short grass	*Dicrostacus* *A. karro* *Mevula* *A. tortilis* *M. seuegaleusis* *E. divonorum*
Sematenof (Semi 2)	Mesic Savanna	Corridor	short grass	*Dicrostacus* *A. karro* *Mevula* *A. tortilis* *M. seuegaleusis* *E. divonorum*
Thaboti bypass	Semi arid Thicket	Imfolozi	-	*A. nigrescens* *Dicrostacus* *Maytenus* *heteriophylla*
Mautiyane	Semi arid Savanna	Imfolozi	short grass	*A. nilotica* *Dicrostacus* *E. divonorum*
Momorial Gate	Mesic Savanna	Hluhluwe	tall bunched grasses	*Rhus* *Mytinus*
Bekaphanzi Pan	Semi arid Savanna	Imfolozi	short grass	*tauboti/ spiro* *A. tortilis* *Gardenia* *E. divonorum* *E.undulata* *schotia capitala* *Dicrostacus* *Ziziphous* *Boscia*
Gqoyeni		Imfolozi	Tall bunched grass	*M. heteriophylla* *Ziziphous* *A. graudiconuta* *S. capitala* *S. brachypetala*

Maqolezinja 2	Mesic Savanna	Hluhluwe	Tall bunched grass	*Dicrostacus* *Ziziphous* *Mytinus* *A. rhobusta* *Uliphia*
Maqolezinja 3	Mesic Savanna	Hluhluwe	tall bunched grasses	*Dicrostacus* *Ziziphous* *Mytinus* *A. rhobusta* *Uliphia*
Mpondo 1 (excluded)	Mesic	Hluhluwe	Tall bunched grass	*Dicrostacus* *A. karro* *Ziziphous* *Rhus* *Rhoicissus* *Dombaya*
Mpondo 2 (excluded)	Mesic Savanna	Hluhluwe	Tall bunched grass	*Sporobolus* *Hyperthelia*
Mpondo 3 (excluded)	Mesic Savanna	Hluhluwe	Tall bunched grass	*Sporobolus* *Hyperthelia*
Maqolezinja 1 (excluded)	Mesic Savanna	Hluhluwe	Tall bunched grass	*Dicrostacus* *Maytenus*
Bhengu Pan (excluded)	Semi arid Savanna	Imfolozi	Short grass	*Dicrostacus* *E. divonorum*
Sontuli Turnoff 2 (excluded)	Semi arid thicket	Imfolozi	Short grass	*Dicrostacus* *Maytenus* *Euclea* *A. tortilis* *A. luderizzi* *B. albitrunca*
Inyathi (excluded)	Semi arid thicket	Imfolozi	Short grass	*A. tortilis* *Boscia* *A.granicormuta*
Umchachazo (excluded)	Reed bed	Imfolozi	-	*Phragmites* *A.nigresceins* *Taumbothi* *A. tortilis*
Umchachazo Pan (excluded)	Reed bed	Imfolozi	-	*Taumbothi* *M. heterophylla* *E.schimperii* *Eulea* *Acauthaveae* *A. karoo*