UNDERSTANDING THE COPROPHILOUS FUNGUS *SPORORMIELLA* AS A PROXY FOR MEGAHERBIVORES

by

Barbara Carol Ulrich

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DEDICATION

For Kaylah, Payton, Braedan, Vicki, Everly, Noah, Burton, and Graeme. I know you will each in your own way make the future a better place. I love you.

In memory of Jerome Regnier, who taught me the value of curiosity – merci mille fois.

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ABSTRACT

In many studies, the presence of the coprophilous fungus *Sporormiella* in Quaternary sediments has been interpreted as evidence of past megaherbivore activity. Despite its use as an ecological proxy, little is known about the taxonomy and life history of *Sporormiella*, and the taphonomic processes that lead to its preservation in lake sediments. This information underlies its utility to interpret past herbivore presence and abundance. Present-day bison (*Bison bison*) dung from Yellowstone National Park was examined to explore the production, dispersal, transportation, deposition, and preservation of *Sporormiella* there. While *Sporormiella* was found in dung samples, sediments from two lakes frequently visited by bison failed to yield *Sporormiella* spores. Laboratory preparation techniques were modified to increase the likelihood of *Sporormiella* spore survival, yet no spores were identified with the new treatment. Although the occurrence of spores in lake-sediment samples may indicate herbivore presence, our study suggests that spore absence does not necessarily indicate an absence of herbivores. We attribute the absence of spores in sediments to local climatic and seasonal factors that may affect production and transport in the watershed, sedimentary processes that may destroy spores after deposition, and harsh laboratory processing techniques that may damage or destroy spores. More research remains to be done to evaluate the importance of these factors before using *Sporormiella* as a reliable proxy of herbivore activity.

UNDERSTANDING THE COPROPHILOUS FUNGUS *SPORORMIELLA* AS A PROXY FOR MEGAHERBIVORES

Introduction

In recent studies, spores of the coprophilous (dung) fungus *Sporormiella* have been used as proxy for past megaherbivore presence (Gill et al., 2009; Johnson et al., 2015; Perrotti, 2018; Robinson et al., 2005; Rule et al., 2012), inasmuch as the life cycle of *Sporormiella* is obligate to dung (Newcombe et al., 2016). *Sporormiella* spores frequently co-occur with pollen in samples retrieved from lake-sediment cores. Dung spores are transported to lakes from dung, soil, and vegetation by surface runoff and air into lakes, and their abundance in sediments has been used to infer past presence and abundance of large-bodied herbivores (e.g. in North America; *Mammut americanum*, *Mammuthus columbi*, and *Megalonyx jeffersonii*) in the watershed. Often these interpretations are part of broader environmental reconstructions that consider past vegetation (based on pollen records) and fire regimes (based on charcoal data) (Gill et al., 2009). Studies in Australia have examined a variety of dung fungi taxa (*Cercophora, Coniochaeta, Sordaria*, and *Podospora* as well as *Sporormiella*) as indicators of the distribution and relative abundance of megaherbivores (Gill et al., 2009; Johnson et al., 2015). *Sporormiella* spores are also found in the feces of birds and other mammals of much smaller size (e.g. *Lagamorpha)*.

Declines in the abundance of *Sporormiella* spores in lake sediments have been used to infer mammal and avian extinctions in many parts of the world. Dung spores in lake sediments and soil cores declined at the time of human arrival in New Zealand, ca. 1280 AD, and are attributed to the extinction of several species of moa by early hunters (Gill et al., 2009; Wood et al., 2011). Rule et al. (2012) note the decline of *Sporormiella* at Lynch's Crater in Australia at

41 ka, coincident with the regional extinction of more than 20 genera of giant marsupials, monotremes, birds, and reptiles.

The disappearance of *Sporormiell*a in lake sediments in North America coincides with the late-Pleistocene extinction of mammoths and mastodons in many locations (Perrotti, 2018). The *Sporormiella* record at Appleman Lake, Indiana (Gill et al., 2009), for example, was used to trace the gradual decline of megafauna over several thousand years. Although the cause of the relatively late Pleistocene extinction of megafauna (defined as 44 kg or more in body weight) in North America has long been debated by paleontologists and archeologists (Faith and Surovell, 2009; Malhi et al., 2016; Martin, 1973; Meltzer, 2015), dung spores have become a widely used tool to infer the temporal and spatial patterns of the extinction event.

Despite its potential as a megaherbivore proxy, there remain significant gaps in our understanding of the taphonomy of the dung fungi spores that are preserved in the sediments of lakes and wetlands. Little is known about the taxonomy and life history of *Sporormiella* or the processes that lead to its transport, deposition, and preservation in lake sediments. Questions also remain about spore dispersal and the effect that local climate conditions, especially moisture availability, have on spore production and preservation (Feranec et al., 2011). This information underlies its utility to interpret past herbivore presence and abundance in paleoecological studies.

Yellowstone National Park presents a unique opportunity for studying *Sporormiella* in association with modern bison (*Bison bison*), the largest and most abundant herbivore in the ecosystem, and the relationship between *Sporormiella* in their dung to presence or absence in the sediments of small lakes (Cannon et al., 2020). By linking *Sporormiella* spores deposited in

lakes and wetlands in watersheds where bison frequent, my interest was to examine fundamental questions about spore taxonomy, taphonomy, and herbivore presence.

The objective of this thesis was to answer these questions:

- Are *Sporormiella* present in modern and fossil samples in northern Yellowstone National Park in the region called the Northern Range?
- What is the history and ecology of modern bison in the Northern Range?
- If present, are *Sporormiella* spores a reliable proxy of past bison presence in the Northern Range?

To address these questions, I examine the presence of *Sporormiella* in modern bison dung and lake sediments in watersheds inhabited by bison, explore the history and ecology of bison in Yellowstone National Park, and determine what relationship exists between *Sporormiella* and bison in the Northern Range.

Site Description

Yellowstone's Northern Range is a 1000 km^2 area within the Greater Yellowstone Ecosystem (GYE) of northwestern Wyoming and southwestern Montana, that includes about 10% of Yellowstone National Park. The Northern Range straddles the Yellowstone and Lamar rivers and their tributaries from east to west, Cache Creek, Soda Butte Creek, Slough Creek, Hellroaring Creek, Blacktail Deer Creek, Lava Creek and the Gardner River. It extends 60 km from Cache Creek on the southeastern edge inside Yellowstone National Park through Yankee Jim Canyon outside of the park to the northwest. (Fig. 1)(Houston, 1982). The Northern Range is

so named because it supports the winter range of the northern Yellowstone elk (*Cervus elaphus*) herd (Metz et al., 2011).

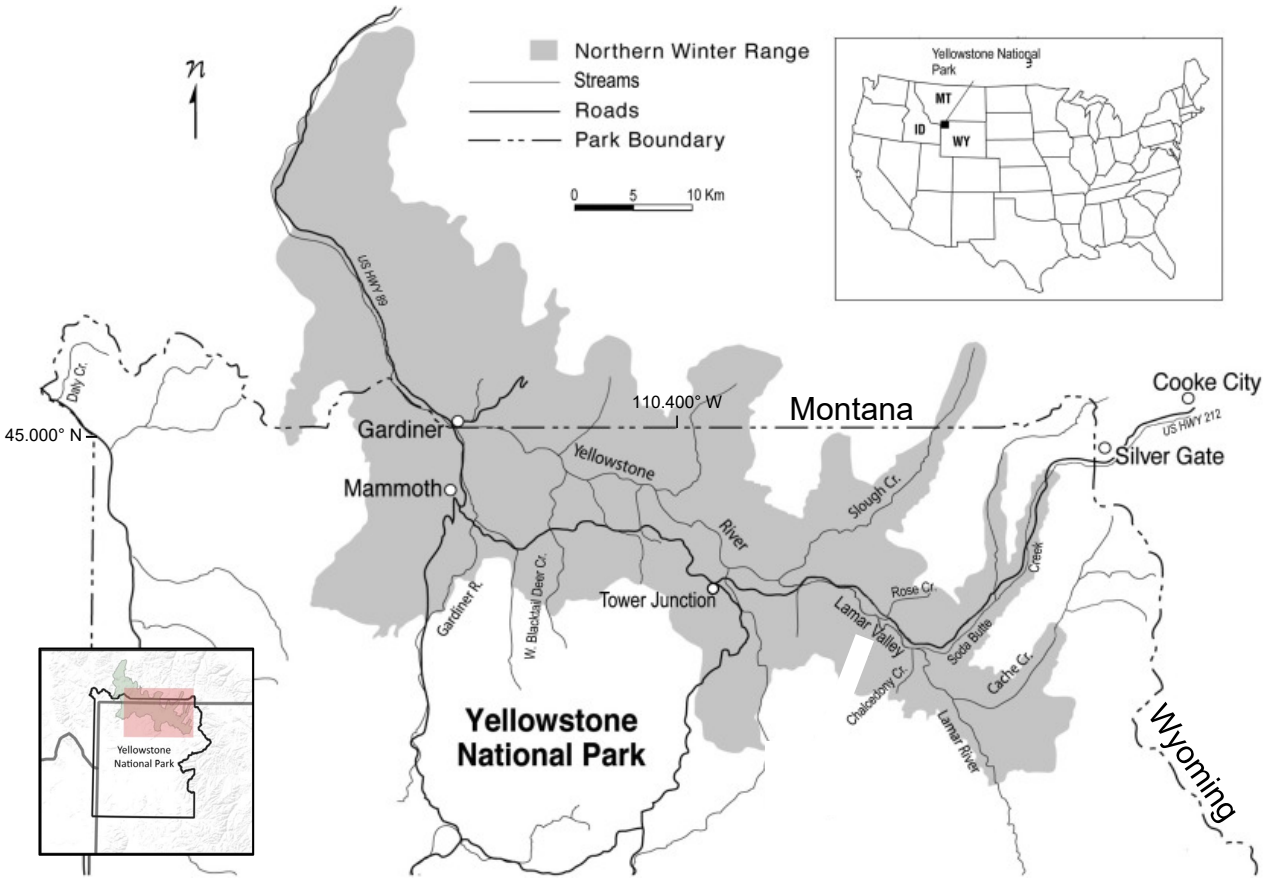


Figure 1. Map showing full extent of the Northern Range (shaded area) within and outside of Yellowstone National Park (NPS, 1997)

Climate

The Northern Range of Yellowstone ranges from 1500 – 2400 m in elevation and is an area of cold harsh climate that varies widely depending on season and elevation. High elevations experience cold temperatures and deep snow cover in winter while low elevations are less harsh and relatively dry year round. Large-scale atmospheric circulation patterns influence annual precipitation in the Northern Range (Whitlock and Bartlein, 1993). The region is characterized by having summer-wet precipitation regime, because, unlike the rest of the Yellowstone region, it receives significant monsoonal moisture from the Gulf of California and Gulf of Mexico in the form of summer convective storms. Winter and spring precipitation, in contrast, comes from Pacific storms (Despain, 1987). Locally, the Gallatin Range to the west of the Northern Range creates an orographic barrier to storms from the west reducing the amount of moisture that falls east of the Gallatin Range (Mock, 1996).

Climate information for the Northern Range is recorded at Tower Falls, Wyoming, which lies less than 25 km from the study sites in this project. The mean annual temperature for the period 1948-2016 at Tower Falls was 2.5ºC. The warmest month is July, with a mean average high temperature of 31.6ºC, and the coldest month is January, with a mean average low temperature of -31.7ºC. Mean annual precipitation for the same time period was 41.85 cm. The wettest month is June and driest months are February and March (Fig. 2).

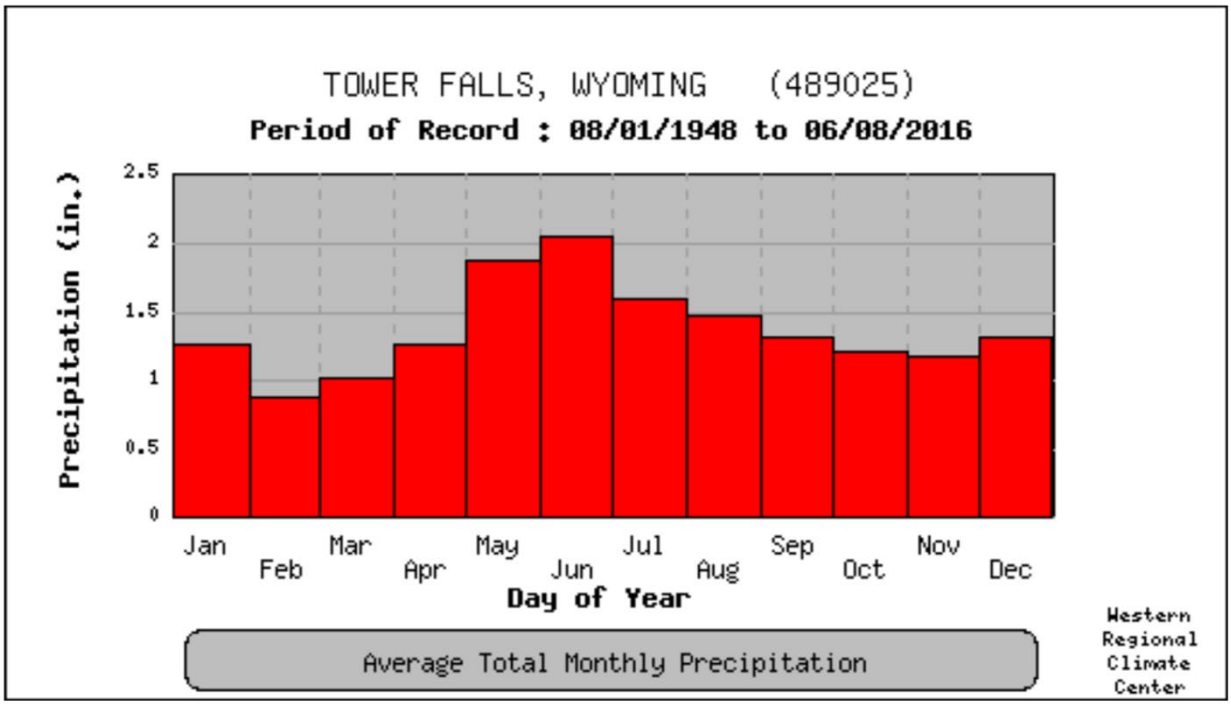


Figure 2. Average total monthly precipitation at Tower Falls, Wyoming from 1948-2016. (WRCC, 2020) https://wrcc.dri.edu/cgi-bin/cliMAIN.pl?wy9025

Vegetation

The vegetation of the Northern Range is controlled by elevation, aspect, substrate, and precipitation (Despain, 1990). Generalized vegetation zones of the Northern Range adapted from USDA Forest Service GTR (Pfister, 1977) are defined by elevation that reflect the length of the growing season and extent of summer drought. At lowest elevations, steppe (<1600 m) is dominated by *Artemisia tridentata* (sagebrush), *Ericameria nauseosa* (rabbit brush) and *Festuca idahoensis* (Idaho fescue), *Pseudoroegneria spicata* (bluebunch wheatgrass), *Koelaria macrantha* (Junegrass), and *Achnatherum richardsonii* (Richardson's needlegrass) in dry areas. *Agropyron caninum* (bearded wheatgrass), *Salix* spp. (willows) and *Carex* spp. (sedges) grow in more moist riparian areas (Singer, 1996). Lower tree line lies between 1600 m to 1900 m

elevation depending on soil moisture. In Northern Yellowstone, a lower parkland (1900 m - 2000 m) is dominated by *Pseudotsuga menziesii* (Douglas-fir) and includes *Pinus flexilis* (limber pine) and *Juniperus scopulorum* (Rocky Mountain juniper) (Iglesias et al., 2018). *Pinus contorta* (lodgepole pine) appears in lower subalpine forest (2000 m – 2400 m elevation) locally controlled by substrate and fire disturbance. The upper subalpine region (2400-2800 m elevation) consists of mixed conifer forest of *Picea englemannii* (Englemann spruce), *Abies lasiocarpa* (subalpine fir), *Pinus contorta* and *Pinus albicaulis* (whitebark pine). Above 2800 m elevation, *Pinus albicaulis* parkland dominates, and above 2900 m elevation is alpine tundra. *Populus tremuloides* (aspen) grows from foothills to subalpine areas wherever soil moisture is adequate. The riparian vegetation of the Northern Range includes *Populus balsamifera* (poplar), *Salix* spp. (willow), *Alnus incana* (alder), *Betula occidentalis* (birch), and *Spiraea douglasii* (spirea) (Engstrom et al., 1991; Romme and Turner, 2015). Wetlands include *Juncus* (rushes), *Scirpus americanus* (bulrush), *Carex* spp., and *Typha latifolia* (cattail).

Modern Dung Sites

Modern dung collection sites are in non-forested areas ranging from steppe to wet meadows. Four locations known to be frequented by bison in summer were selected to maximize the likelihood that bison had ingested fresh vegetation with *Sporormiella* spores attached. From west to east, the sampling locations are Blacktail Plateau (BP) at an elevation of 2011 m, Little America (LA) at 1895 m elevation, Lamar Valley (LV) at 2035 m elevation, and Round Prairie (RP) at 2085 m elevation (Fig. 3)(Table 1).

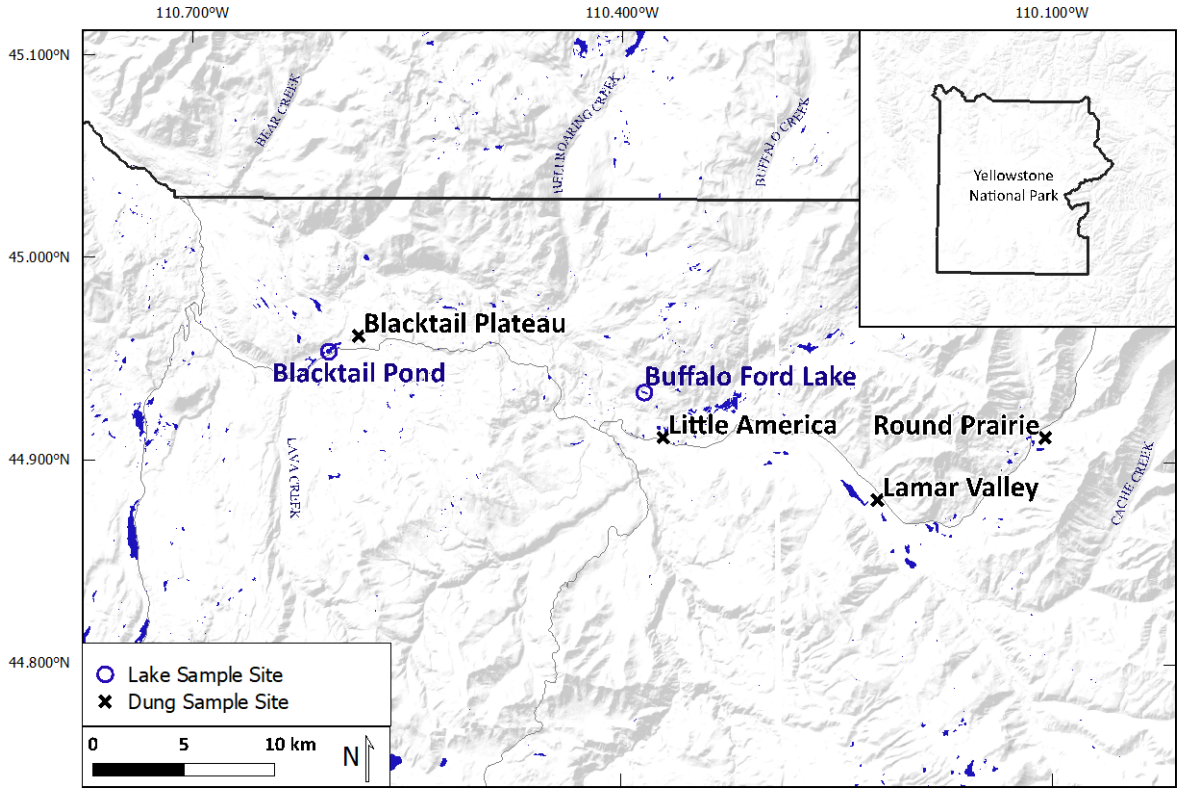


Figure 3. Study area with inset showing the location of the Northern Range within Yellowstone National Park (green) and the study area (pink). Dung sample locations **x** and lakes sampled circled in blue. (ESRI Satellite)

The Blacktail Plateau site lies in steppe dominated by *Artemisia tridentata*, *Festuca idahoensis* and *Ericameria nauseosa*. Bison use the area year-round as part of their annual migration to lower ground in the Gardiner Basin (Geremia et al., 2019). The Little America and Lamar Valley sites lie in an area of *Artemisia tridentata*, *Festuca idahoensis*, *Achnatherum richardsonii* and *Pseudoroegneria spicata* (Kokaly et al., 2003). Bison also use these areas year-round. The Round Prairie collection site lies in the drainage of Soda Butte Creek and is an expansive wet meadow where *Salix* spp., *Carex nebrascensis*, *Lonicera invoucrata* (twinberry),

Potentilla fruticosa (shrubby cinquefoil) and *Rosa* sp. (wild rose) are common with drier locations supporting *Artemisia tridentata* and *Festuca idahoensis* (Ripple et al., 2011). In winter, bull bison use the area, but females and young rarely use this location because of deeper snow depths.

Lake Sites

Buffalo Ford Lake (44.934°N, 110.383°W, 1923 m, elev) and Blacktail Pond (44.953°N, 110.603°W, 2018 m elev) are located in *Artemisia* steppe that grows on nutrient-rich glacial soils of the Northern Range, with adjacent slopes covered by *Pseudotsuga*-dominated parkland and *Pinus contorta* forest. Both areas are frequented by bison throughout the year (Geremia et al., 2019) and have small catchment areas (Table 1). The watersheds provide good grazing conditions for bison and their relatively low elevation offers early spring and winter access to dried grasses, benefitting females during calving as well as young (Gates et al., 2005).

Table 1
Dung and lake sediment sample locations in the Northern Range

Location Name	ID	Elevation	Latitude - Longitude	Dominant Vegetation
Dung Samples				
Blacktail Plateau	BP	2011 m	44.939°N, 110.632°W	*Artemisia tridentata* *Festuca idahoensis*
Little America	LA	1895 m	44.874°N, 110.362°W	*Artemisia tridentata* *Pseudoroegneria spicata* *Achnatherum richardsonii*
Lamar Valley	LV	2035 m	44.883°N, 110.215°W	*Artemisia tridentata* *Pseudoroegneria spicata* *Achnatherum richardsonii*
Round Prairie	RP	2085 m	44.913°N, 110.113°W	*Carex spp.* *Salix bebbiana*
Lake Sediments				
Buffalo Ford Lake	BFL	1923 m	44.934°N, 110.383°W	*Artemisia tridentata*
Blacktail Pond	BTP	2018 m	44.953°N, 110.603°W	*Artemisia tridentata*

Buffalo Ford Lake

Buffalo Ford Lake, also known as Junction Butte Lake, lies north of the Lamar River near its confluence with the Yellowstone River. The lake is at an elevation of 1923 m, is 400 m long and 112 m wide at its widest point with a surface area of 4 hectares (Fig. 4). Watershed geology consists of glacial outwash sediments overlying glacially scoured Precambrian gneiss and schist. The glacial landscape left a series of east-west- trending roches moutonées, and Buffalo Ford Lake lies on the north side of one. The lake is fed by surface runoff and has a small inlet on its north side and a small outlet channel on its west end that drains into the Lamar River. The vegetation consists of *Artemisia* steppe on glacial outwash and parkland of *Pseudotsuga menziesii* on the rocky buttes. There is limited riparian vegetation at the margin.

Previous work of Buffalo Ford Lake includes a study of the sedimentary record to determine historical impacts of ungulate grazing on vegetation, soil stability, and water quality

(Engstrom et al., 1991). The lake was one of several studied in a broad paleoecological study of changes over time in small lakes in the Northern Range.

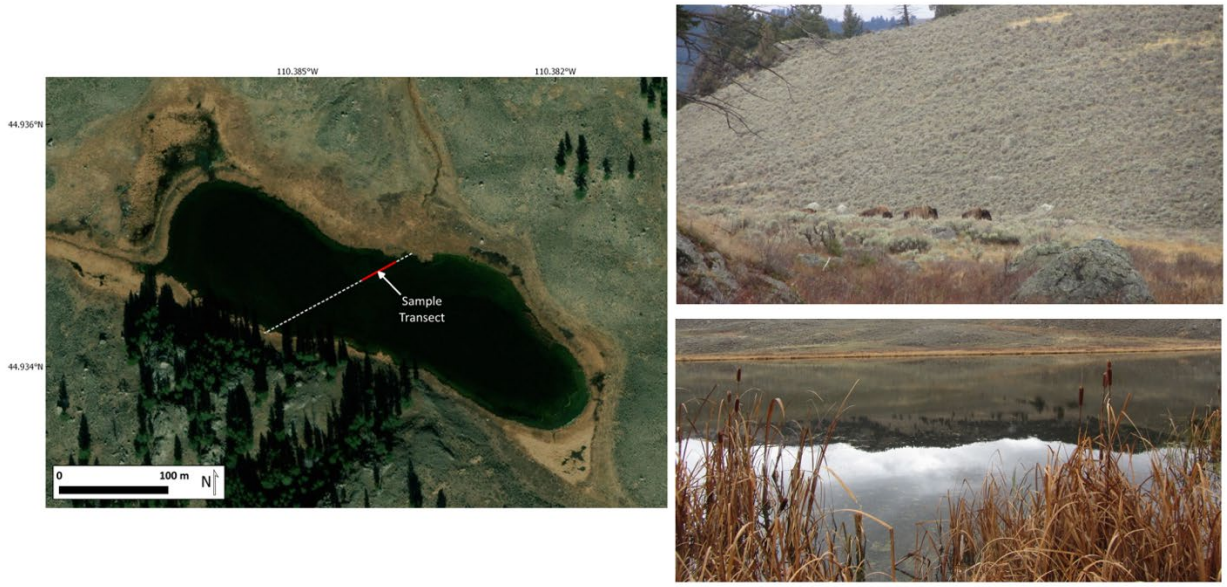


Figure 4. Buffalo Ford Lake with photographs showing the vegetation. The white dotted line depicts the transect across the lake. The portion in red was sampled at 5m intervals. The inlet is visible on the north side with the outlet on the west. (ESRI Satellite)

Blacktail Pond

Blacktail Pond has no inlet or outlet and is fed by surface runoff and groundwater. Watershed geology consists of calcareous glacial outwash, welded ash flows of the Huckleberry Ridge tuff of the Yellowstone Group, and the Junction Butte basalt (USGS, 1972). The pond lies in a remnant ice-marginal channel formed at the end of the Pinedale glaciation (Pierce, 1979).

The pond is at an elevation 2018 m and has 0.25 hectares of surface area (Fig. 5). The site lies in *Artemisia* steppe vegetation and the open water of the lake is surrounded by a marl-rich fen with *Scirpus americanum* and *Typha latifolia*. A thick grass mat floats on the surface of the water along the edges of the pond creating an unstable surface. No bison were observed at the

time of sampling, but a well-scavenged bison carcass was present as well as abundant bison dung.

The sedimentary record at Blacktail Pond has been the focus of considerable paleoecological investigation. A postglacial vegetation history was described by Gennett (1986) and later by Huerta et al. (2009). A multiple proxy examination of the late-glacial to early-Holocene environmental history was also undertaken at Blacktail Pond (Krause and Whitlock, 2013; Lu et al., 2017).

Figure 5. Blacktail Pond with coring location noted in red. The brown areas are vegetation mats floating on the ponds. The light green areas are marl deposition. The photographs show the setting and local vegetation (ESRI Satellite)

METHODS

Dung Samples

Field Methods

Bison dung was abundant at the four study sites, providing ample fresh material to collect. At each site, 12 samples of approximately 150 grams of fresh dung were collected with a trowel or spatula, depending on the condition of the dung, stored in sealed plastic bags, and placed in a cooler. During the first sampling in July, no bison were present on the Blacktail Plateau, likely due to summer heat. Because we wanted to use dung that was deposited within 24 hours of collection, we did not sample this location in July when we were unsure of the freshness of the material. Therefore, only 36 samples were collected in the July sampling event rather than the anticipated 48. In September, a second set of samples was collected from all four locations for a total of 48 samples. The samples were transported to a cool-temperature facility in Gardiner, Montana, where dung gardens were assembled and monitored.

Laboratory Procedures

Approximately 50 grams from each sample was placed in a hard-plastic box that measured 15 cm square. An effort was made to use dung from both the surface and interior of the sample. Each box contained a composite of four individual samples: 1-4, 5-8, and 9-12, and thus each location had three dung gardens (Fig. 6). Moistened organic filter paper was placed in the bottom of each box to prevent desiccation. The four samples were physically separated in the corners of the box. Samples were lightly sprayed with de-ionized water twice a week during the incubation period to keep them moist. Some samples required more water than others to keep

from drying. Dung gardens were kept at a minimum of 10ºC in natural light and ambient temperature (Mungai et al., 2012). The boxes were tightly covered by lids to avoid contamination from airborne spores.

Figure 6. A dung garden after sporulation. Visible are the four samples in each box. The tubes contain composites of those samples ready to be rehydrated and processed.

Dung gardens were allowed to sporulate for four weeks because ascospores require two to four weeks to reach maturity (Bell, 1983). Samples were visually inspected regularly to see what, if anything, was present. At four weeks, despite the fact that pseudoperithecia were not visible, the dung gardens were transported to the Paleoecology Laboratory at Montana State University in Bozeman where they were prepared for analysis.

On August 11, 2017, a processing trial was conducted. Three samples of 15 g each were taken from the Round Prairie, Lamar Valley, and Little America dung gardens. A fourth sample was included from a trial dung garden that contained dried dung that had been collected prior to the main sampling event. Samples were placed in tubes and filled with deionized water to the

20 ml mark. Ten drops of a generic surfactant were added to each tube, and the tube was capped and vortexed for 1 minute. After this minimal treatment, they were given for 5 days to disaggregate. Upon examination, it was clear that additional treatment would be necessary to prepare the samples for microscopic examination.

Using information from the processing trial, a composite sample of approximately 20 g of dung was taken from each of the 21 dung-gardens. Each sample was placed in a 50 ml plastic tube, filled with de-ionized water, and shaken to make a slurry. The tubes were covered for three days to thoroughly rehydrate the material. Each tube was then shaken to disaggregate as much material as possible, the tubes were then centrifuged and the suspension was decanted. After one washing, the residual material was suspended in ethanol (95%), centrifuged, and decanted twice more. At this stage of preparation, samples were examined under the microscope by placing a drop of processed material onto a slide. With this amount of gentle processing, spores were identifiable at a magnification of 400X on a Nikon Eclipse 50i compound microscope.

Lake Sediments

Field Methods

Lake-surface sediments were collected from Buffalo Ford Lake with a Klein gravity corer to retrieve an undisturbed mud-water interface. The upper 2 cm of sediment were sampled at 5-m intervals along a transect from the inlet on the north shore towards the deeper part of the lake (Fig 4). This sampling strategy was undertaken to determine if *Sporormiella* settled preferentially in shallow water after being washed into the lake. Water depth was recorded at each of the 8 coring locations and ranged from 30 cm depth near shore to 460 cm depth.

Samples were stored in plastic bags, refrigerated, and transported back to the Montana State University Paleoecology Laboratory for analysis.

In May 2016, a 47-cm-long core was recovered from Blacktail Pond with a piston-fitted plastic tube in 3.90 m of water adjacent to the western fen margin. An abrupt drop off from the floating fen margin accounts for deep water relatively near the edge of the lake (Fig. 5). The core was extruded into plastic bags in the field at 0.5 cm intervals, starting with the sediment-water interface. Samples were transported to the Montana State University Paleoecology Laboratory and refrigerated. All field samples were split. Half of each sample remains in cold storage.

Laboratory Procedures

Two methods were used to prepare the Buffalo Ford Lake (BFL) samples, A-H. Method 1 followed the standard procedures for pollen analysis described by Bennett and Willis (2001) (Table 2). Method 2 was modified to eliminate hydrofluoric acid (HF) and acetolysis treatments, which have been reported in other studies to damage spore material (Van Asperen et al., 2016). Method 2 involved adding 25 ml of sediment to small test tubes, which was then washed and centrifuged three times, once with de-ionized water and twice with 95% ethanol. After initial processing, sample BFL-A, the sample collected nearest to shoreline at 30 cm depth, was examined under a microscope to determine if it was sufficiently clean of organic debris to identify spores. Although clumps of organic material were still present, the material was clean enough to determine if *Sporormiella* or other spores were present. Some spore fragments were present, but no *Sporormiella* was observed. No further treatment was performed on this sample. Samples that still contained a lot of debris were treated with 5% potassium hydroxide (KOH) to remove humic acids and further disaggregate the material. Samples were placed in a hot water

bath for 2-3 minutes and residues were washed through a screen residue (180 µm mesh size) with distilled water. Samples were then treated with 10% hydrochloric acid (HCl) to remove carbonates and water washes. Samples were examined at this point without further chemical treatment.

Table 2
Description of the steps in the two preparation methods

Method 1 (Bennett & Willis, 2001) Processed 1/9 and 10/2018	Method 2
Lycopodium tracer tablets added	Centrifuged 3X; once in H_2O, twice in 95% Ethanol
Heated with 5% KOH followed by 6 rinses with distilled water	*Lycopodium* tracer tablets added
Sieved out the >180 µm fraction	Heated with 5% KOH followed by 3 rinses with distilled water
Washed with 10% HCl followed by 3 rinses with distilled water	Washed residue through > 180 µm mesh screen
Treatment with 48% HF followed by 5 rinses with distilled water	Treatment with 10% HCl followed by 5 rinses with distilled water
Acetolysis – washed with acetic acid, heated with concentrated sulfuric acid and acetic anhydride for 1 minute	
Washed with 95% Ethanol Mounted with silicone oil	Washed with 95% Ethanol Mounted with silicone oil

RESULTS

Dung Samples

Every dung sample collected in July contained *Sporormiella*. Spores were healthy, well-preserved, and identifiable by their intact four-segmented chambers and diagnostic sigmoid germination slit. Most samples collected in September had *Sporormiella* present; however, despite using the same preparation method, spores from these samples were scant and in poor condition compared to the July group (Table 3).

Table 3
Results of dung garden samples collected in July and September 2017

Sample ID [1]	*Sporormiella* Present/absent +/-	Collection Date	ID Date	Comments
JUL_717				Garden assembly: 7/11/17 Processing: 9/25/17
LA 1-4	+	7/11/17	10/1/17	2 double segments, 2 singles, no intact segments
LA 5-8	+	7/11/17	9/25/17	3 intact segments
LA 9-12	+	7/11/17	10/1/17	4 intact segments
LV 1-4	+	7/10/17	9/28/17	Cluster of segments
LV 5-8	+	7/10/17	9/28/17	Complete segments
LV 9-12	+	7/10/17	9/28/17	4 segments clustered together
RP 1-4	+	7/10/17	9/28/17	Intact segments
RP 5-8	+	7/10/17	9/28/17	Intact segments, individual spores, 2 with visible germ slits
RP 9-12	+	7/10/17	9/28/17	Intact segments, segments of 2
SEP_917				Garden assembly: 9/9/17 Processing: 9/25/17
BP 1-4	+	9/9/17	9/25/17	Several intact segments
BP 5-8	+	9/9/17	10/18/17	Several intact segments
BP 9-12	+	9/9/17	10/12/17	Single spores in poor condition
LA 1-4	+	9/9/17	10/1/17	1 single spore

LA 5-8	+	9/9/17	10/1/17	1 intact segments, several single spores
LA 9-12	+	9/9/17	10/12/17	Single spores in poor condition
LV 1-4	+	9/9/17	10/1/17	Single spores
LV 5-8	+	9/9/17	10/1/17	1 intact segments, 1 double segment, 1 triple segment
LV 9-12	+	9/9/17	10/1/17	1 intact segments, 1 double segment
RP 1-4	+	9/9/17	10/1/17	1 complete segments with additional single spores
RP 5-8	+	9/9/17	10/1/17	1 complete segments
RP 9-12	+	9/9/17	9/25/17	2 individual spores

[1] BP = Blacktail Plateau, LA = Little America, LV = Lamar Valley, RP = Round Prairie

Lake Sediments

Material from Buffalo Ford Lake was processed with both Method 1 and Method 2 and mounted on slides in silicone oil. Each slide was examined for spores using a grid system of the entire slide. With Method 1, none of the samples contained *Sporormiella*. Spores of other fungi were observed, but most were broken or damaged (Table 4). Samples processed with Method 2 also did not have *Sporormiella* spores, although the *Lycopodium* tracer and *Pinus* pollen were present. Sample BFL-A was examined under a microscope after initial processing (washing and centrifuging) to determine if it was adequately cleaned to identify spores. A significant amount of organic material remained following the initial preparation, making identification difficult, and no *Sporormiella* were observed. As with samples prepared with Method 1, several unidentifiable fragments of spore material were noted. Neither method resulted in the presence of *Sporormiella* in any sample (Table 5).

The average sedimentation rate at Buffalo Ford Lake was estimated to be 0.031 g/cm, based on previous ^{210}Pb dating of a previously collected core (Engstrom et al. 1991). This information suggests that the upper 2 cm of sediment in our cores accumulated since ca. 1987.

Table 4
Buffalo Ford Lake results using method 1

Sample ID	Distance from shore (m)	Depth (cm)	Sample condition
BFL16-A	0	28	Clumps of organic material still present, clear enough to determine if *Sporormiella* are present. Other types of spores may be present. No *Sporormiella* observed.
BFL16-B	5	55	Organics well-disaggregated, material very fragmented, *Lycopodium* tracer and *Pinus* pollen intact. No *Sporormiella* observed.
BFL16-C	10	151	Organics well-disaggregated, material fragmented, spike and pine pollen recognizable. No *Sporormiella* observed.
BFL16-D	15	307	Organics well-disaggregated, material fragmented, spike and pine pollen recognizable. No *Sporormiella* observed.
BFL16-E	20	430	Organics well-disaggregated, material fragmented, spike and pine pollen recognizable. No *Sporormiella* observed.
BFL16-F	25	452	Organics well-disaggregated, material fragmented, spike and pine pollen recognizable. No *Sporormiella* observed.
BFL17-G	30	461	Organics well-disaggregated, material fragmented, spike and pine pollen recognizable. No *Sporormiella* observed.
BFL17-H	35	475	Organics well-disaggregated, material fragmented, spike and pine pollen recognizable. No *Sporormiella* observed.

Table 5
Buffalo Ford Lake results using method 2

Sample ID	Distance from shore (m)	Water depth (cm)	Sample condition
BFL16-A	0	28	Sample adequately clean, *Lycopodium* tracer noted, no *Sporormiella* 3-segmented spore, damaged
BFL16-B	5	55	Dark material, clumped, Fungus present but no *Sporormiella*
BFL16-C	10	151	Clear, transparent No *Sporormiella*
BFL16-D	15	307	Clear, transparent, *Lycopodium* tracer visible and intact, unidentifiable broken pieces
BFL16-E	20	430	Well-washed, abundant clumped detritus, *Lycopodium* visible, *Pinus* pollen visible
BFL16-F	25	452	Organic material, clumped detritus
BFL17-G	30	461	Organic material, deformed spores, damaged *Pinus* pollen, broken pieces of pollen
BFL16-H	35	475	Hash, intact *Pinus* pollen

The 47-cm-long core from Blacktail Pond was composed of coarse detritus gyttja from 0-6 cm depth and had a fining downward sequence of medium detritus gyttja from 6-45.5 cm depth. A 1.5-cm-thick marl unit was present from 45.5-47 cm depth. Six samples of 2 cc volume were taken at 3-cm intervals from 3.0 to 18.0 cm depth and processed according to Method 1 (Table 6). None of the samples from the core contained *Sporormiella* and no damaged spores or spore fragments were observed.

Huerta et al. (2009) developed chronology for a short core taken from Blacktail Pond, and ^{210}Pb dates from that core suggest that sediments at 21 cm depth had an age of ~1941 CE.

The deepest samples processed at Blacktail Pond used in this study are from 18.0-18.5 cm depth, which we estimate would represent 1969 CE according to their age model.

Table 6
Blacktail Pond samples from the core (BTP 16-01) obtained April 2016 and processed July 2016

Sample ID	Depth (cm)	Sediment volume (cm^3)	Comments	Presence/Absence of *Sporormiella*
BTP16-01	3.0-3.5	2	organics	No *Sporormiella*, no damaged spores or spore fragments.
BTP16-01	6.0-6.5	2	organics, shells, woody debris	No *Sporormiella*, no damaged spores or spore fragments.
BTP16-01	9.0-9.5	2	organics, woody debris	No *Sporormiella*, no damaged spores or spore fragments.
BTP16-01	12.0-12.5	2	organics	No *Sporormiella*, no damaged spores or spore fragments.
BTP16-01	15.0-15.5	2	organics	No *Sporormiella*, no damaged spores or spore fragments.
BTP16-01	18.0-18.5	2	organics	No *Sporormiella*, no damaged spores or spore fragments.

DISCUSSION

To interpret our results of the analysis of modern dung and lake sediments in northern Yellowstone National Park, it is important to understand the life cycle of *Sporormiella* and how dung fungal spores may be transported to the lakes and ultimately buried in the sediments. It is also necessary to understand the ecology and history of bison in the Northern Range of Yellowstone. Based on these considerations, I discuss aspects of *Sporormiella* life cycle and the history of bison in northern Yellowstone National Park. I also discuss what was learned about growing *Sporormiella* in the laboratory and present a conceptual model to discuss where spores might have been lost or destroyed in the steps between successful spore production on dung and retrieval of sediments by standard sampling methods.

Life Cycle of *Sporormiella*

Dung fungi have the potential to be a megaherbivore proxy for several reasons: (1) They are produced continuously during the life of an herbivore; (2) The fungus sporulates exclusively in the dung of herbivores and to complete its life cycle, the spore must pass through an herbivore's digestive tract where, unlike non-coprophilous spores, it is protected against the strong enzymes present in the digestive track. Once voided, viable spores are situated in a medium favorable for their germination, fruiting and discharge onto vegetation where they go back into the ecosystem to be consumed again and repeat the cycle (Baker et al., 2013); (3) The fungus is found in many different ecosystems worldwide, making it universal among herbivores (Johnson et al., 2015); (4) The fungus is associated with herbivores of all sizes, but the volume produced by large herbivores is proportionally more (Baker et al., 2013; Feranec et al., 2011;

Gill et al., 2013); (5) The presence of dung fungal spores in lake sediments in other studies appears to be closely associated with the presence of the megafauna allegedly responsible for its deposition (Baker et al., 2013; Feranec et al., 2011; Gill et al., 2013); and (6) Other proxies (pollen and charcoal) preserved in lake sediments can be helpful in interpreting the vegetation history at the time of deposition (Feranec et al., 2011; Johnson et al., 2015).

Sporormiella is a genus of fungi, part of the largest Phylum of dung fungi called Ascomycota, in the Family Sporomiaceae (Bell, 1983). They are in the class Dothideomycetes, order Pleosporales, which is characterized by having a pseudothecium that serves as a container for spore development. The asci that develop inside the pseudothecium are bitunicate, meaning that the ascus is double walled (Bell, 1983). Inside each ascus are eight dark-colored spores, each composed of 4-14 cells, depending on the species. Sometimes, the cells remain clustered together, but depending on the condition of the sample, the cells may have broken apart and appear individually. Note that in species with 4-celled spores, the two distal cells are tapered, while the two medial cells are more rectangular. A helpful diagnostic tool is the sigmoid germination slit visible on individual spores (Mungai et al., 2012).

The reproductive sequence typical for dung fungi occurs after passing through the gastrointestinal tract of an herbivore (Fig. 7). Meiosis occurs on the dung, resulting in the creation of ascospores (Newcombe et al., 2016). The incubation period for *Sporormiella* is estimated to be fairly long compared to other dung fungi. Richardson (2002) estimates that mean time for first appearance of fruiting bodies is 8.8 days. Bell (1983) suggests that Ascomycetes fungi that develop fruiting bodies require a longer time to mature. Typically within 24 hours, Zygomycetes produce sporangia for the first few days, followed by a second phase of succession

(the order in which the fungi begin to grow in the dung once the incubation period begins) when the Ascomycetes develop fruiting bodies and, because they are more complex, take longer to mature, 2-4 weeks. Once mature, spores are discharged through the air but close to the ground and are unlikely to disperse more than two meters away from the dung on which they grew (Baker et al., 2013). Aided by a sticky gelatinous coating, they then attach to vegetation where they may be re-ingested by another herbivore. Spores that do not successfully attach to vegetation may be deposited within the two-meter dispersal distance of the dung. Spores that remain in or on the dung can enter the ecosystem by surface water runoff (Johnson et al., 2015).

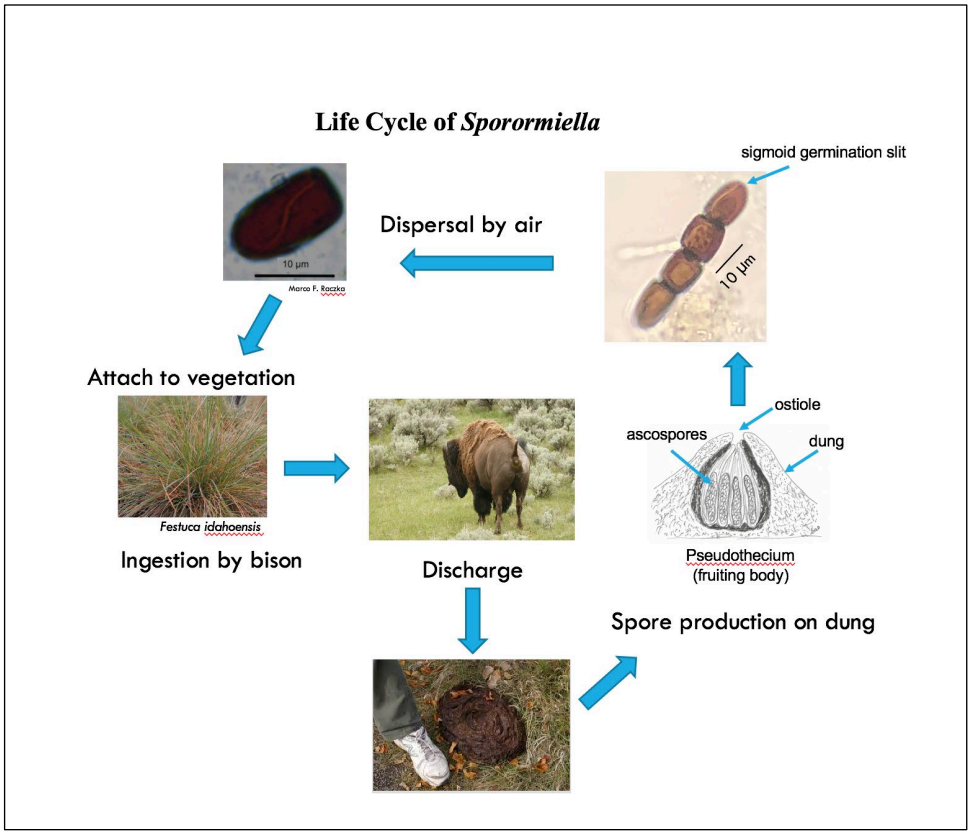
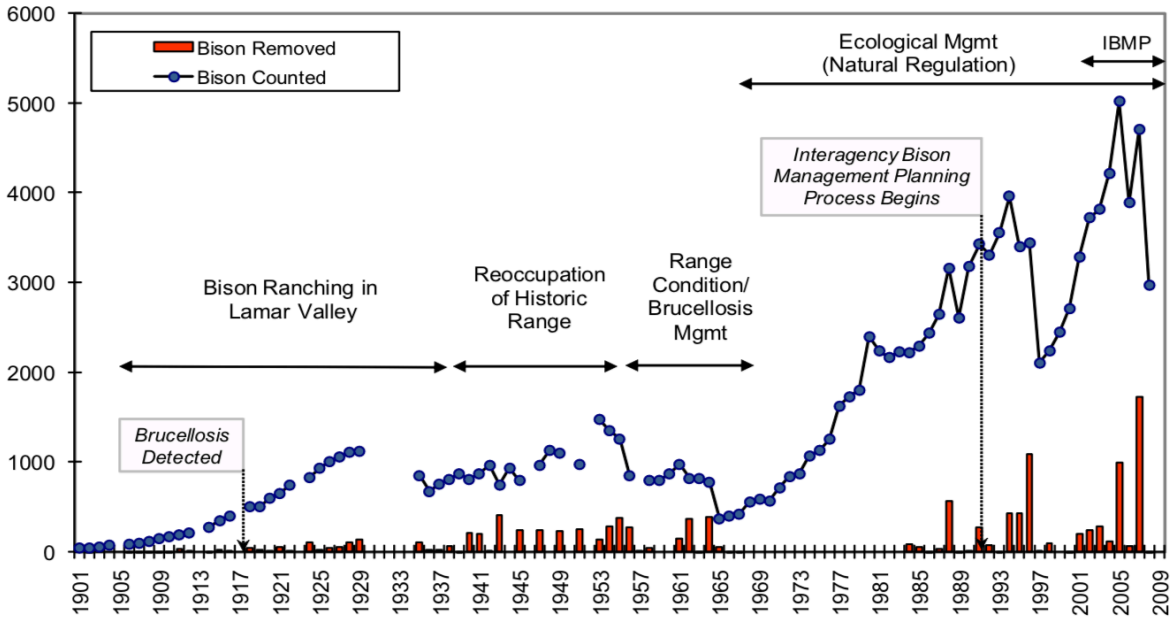


Figure 7. Life cycle of *Sporormiella* from discharge into the ecosystem in dung, to sporulation on dung, airborne dispersal, attachment to vegetation, and re-ingestion to repeat the cycle.

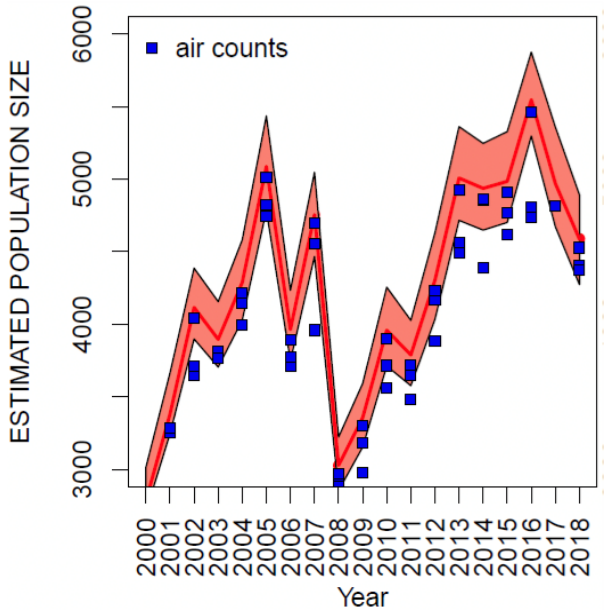
Ecology and History of Bison in Northern Yellowstone National Park

Bison have inhabited the Yellowstone region since before Euro-American exploration (Meagher, 1973). By 1901, extermination of bison through commercial and sport hunting reduced the population of wild bison in Yellowstone National Park to less than two dozen. In 1902, in an attempt to preserve the last remaining wild bison in North America, the US Army began a captive breeding program in Yellowstone. By 1952, the population reached 1,000 individuals through intense management and supplemental feeding. Since that time, bison have been left to forage on their own and population size is managed through periodic culling operations (Fig. 8a)(White et al., 2011).

In 2000, the Interagency Bison Management Team (IBMT) was formed to enable state and federal collaboration in bison management across the GYE, largely in response to concerns about the transmission of brucellosis (*Brucella abortus*) from bison to domestic cattle. The IBMT is composed of Animal and Plant Health Inspection Service, Confederated Salish and Kootenai Tribes, Inter Tribal Buffalo Council, Montana Department of Livestock, Montana Department of Fish, Wildlife and Parks, National Park Service (NPS), Nez Perce Tribe, and the US Forest Service. One goal of the Interagency Bison Management Plan IBMP, which was implemented by the IBMT, is to maintain a wild free-ranging population of bison in the Park at a minimum of 3,500 individuals. From 2000 to 2018, the Yellowstone population estimated from air counts was between 3,000 and 4,500 animals (Fig. 8b). As reported in the IBMP 2019 Annual Report, the population was estimated at 5,500 animals, with distribution in most parts of the Park.



(a)



(b)

Figure 8. Bison population in Yellowstone National Park. (a) Bison numbers in the park from 1900 to 2010 showing counts and removals (Nishi, 2010). (b) Estimated bison population from 2000 to 2018 based on counts from aircraft (IBMP, 2019).

Bison occupy a relatively small portion of the Yellowstone and are the largest ungulate species in the Park. Because they concentrate in large numbers in valleys and wetlands, they are good herbivore to use in a study of dung spores. Yellowstone National Park supports two geographically distinct herds - the northern herd and the central herd, but individuals move freely within and between the two herds. The bison observed and sampled for this study are members of the northern herd in Yellowstone's Northern Range.

Bison are matriarchal herd animals forming groups of 200 to 1,000 led by an older female, or matriarch. Bison sexually segregate for most of the year (Berini, 2017). Males travel with the herd only during the rut in August and can be found in bachelor groups during other seasons of the year. They concentrate in less than 4% of the park when they occupy their summer breeding range in July and August (Fig. 9) (Geremia, 2015).

National Park Service data indicate that the northern herd increased from about 600 individuals in 2000 to 3,628 in 2015. The central herd declined from 3500 individuals to about 1200 over the same period. The reason for this shift is not known. Spring migration for bison in the Northern Range follows a "Green Wave" or the changing elevational timing of the green-up of grasses from spring into summer. The timing and route of bison migration are synchronized with plant phenology to optimize grazing potential (Geremia et al., 2019). Bison begin their annual migration in early March from the lower elevations of the Northern Range and reach the headwaters of the Lamar River by late August (Fig 9). This migration pattern is contained within the boundaries of the Northern Range. In winter, when snow covers the upper reaches of the Northern Range, bison migrate to lower elevations inside and beyond Park boundaries to the north and west where there is less snowpack.

Grasses and sedges comprise 90% of bison diet (Meagher, 1973). Cool-season grasses such as *Pseudoroegneria spicata* and *Carex* spp. are highest in nutrients in early spring and decline as the season progresses. Bison have peak forage intake from June through September, spending nine to eleven hours daily foraging (Geremia, 2015).

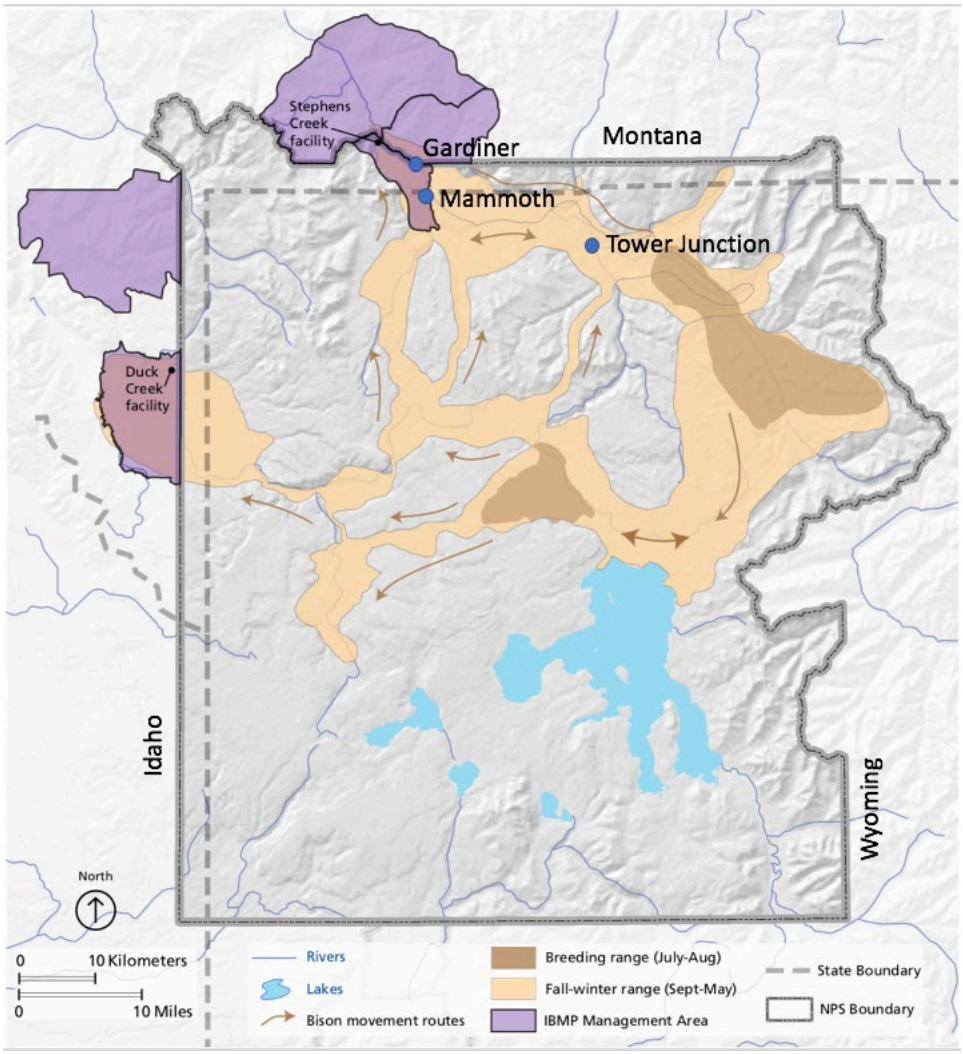


Figure 9. Map showing seasonal distribution of Yellowstone bison. In July and August, the northern herd occupies the eastern portion of bison range with breeding grounds (in dark brown) in the Lamar River valley above Tower Junction. Arrows indicate traditional migration pathways for both the northern herd and the central herd. The tan color indicates the area occupied for winter range (September – May). For the northern herd, winter range extends out of Yellowstone National Park beyond the North Entrance (Atlas of Yellowstone, 2012).

Sporormiella as a Reliable Proxy of Bison Presence in the Northern Range

Our study indicates that *Sporormiella* spores were present in the dung of bison in northern Yellowstone National Park, but not in lake sediments from watersheds where bison are known to frequent. These results call into question the use of *Sporormiella* as an indicator of large ungulate presence in the Northern Range. A conceptual model of *Sporormiella* taphonomy helps identify where in the life cycle and subsequent transportation and deposition spores may have been lost (Figure 10). Several interpretations might explain the absence of *Sporormiella* in lake sediments in our study: (1) steppe and near-shore environments in Yellowstone National Park may not have provided suitable conditions for sporulation; (2) the short dispersal distance of spores (<2 meters) was not sufficient to transport spores from dung to lakes; (3) the semiarid conditions of the Northern Range did not provide enough surface runoff to carry spores into lakes; (4) the thick grass mat and other riparian vegetation may have prevented spores from reaching the lakes; (5) subaqueous conditions may not have been favorable for spore preservation and deposition; and (6) spore processing techniques may have been too harsh and damaged or destroyed dung spores.

Conceptual Model

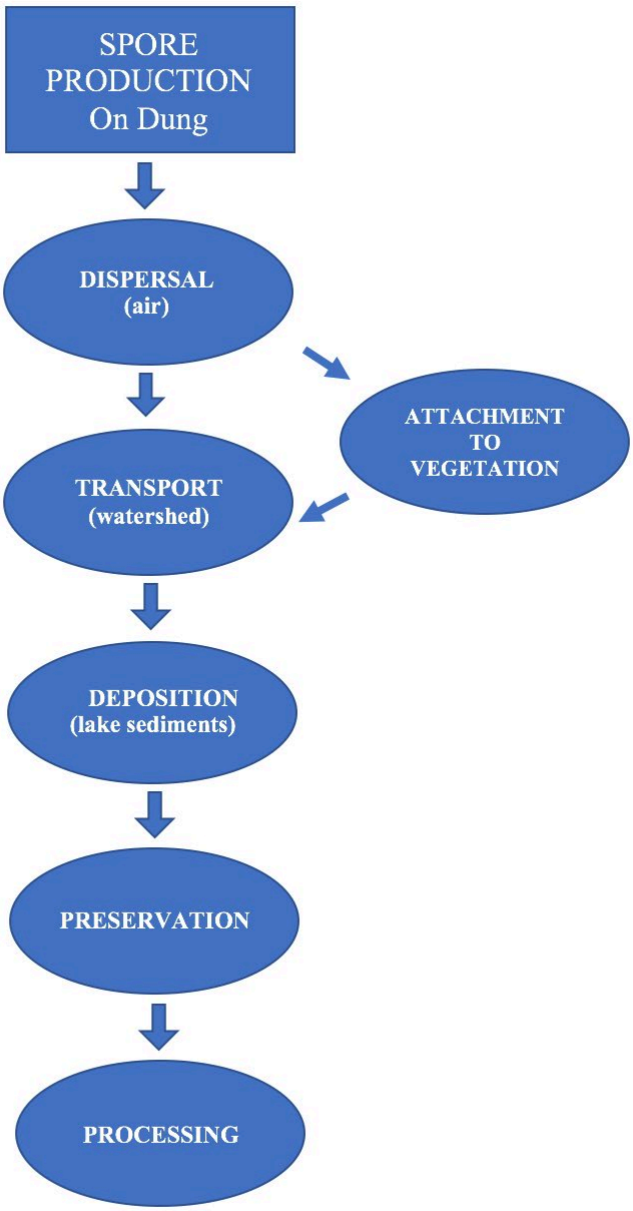


Figure 10. Conceptual model of how *Sporormiella* gets in lake-sediment samples. The taphonomy of *Sporormiella* spores in the northern Yellowstone ecosystem begins with production on dung and dispersal into the environment. To maintain its life cycle, spores must attach to vegetation; spores that do not attach may be transported in the watershed, deposited in lake sediments and preserved. Once sampled, laboratory extraction techniques must not destroy spores.

Production

Results of our dung garden study suggest that *Sporormiella* sporulate well on fresh dung if it is kept moist at moderate temperatures (between 10°C and 25°C). Spores grown from the July sampling were well-preserved whereas many from the September sampling were fragmented (Table 3). Unfavorably dry conditions between the July and September sampling events may have affected the preservation and abundance of spores on grasses ingested by bison prior to sampling. In our first attempt at dung gardens, several samples dried out, which probably prevented sporulation. We later corrected this by consistently moistening dung gardens with deionized water. Variations in seasonal climate may similarly impact spore production, given our observations in the laboratory that successful sporulation depended on moisture levels that were neither too dry nor too wet. We suggest that dry conditions in summer and fall may have desiccated dung and spores and prevented sporulation. If the dung forms a hard crust as a result of drying or freezing, spores cannot escape. Snowmelt and abundant spring and summer rain might have over-saturated dung and impeded aerial dispersal at our sites.

Other studies of modern and fossil *Sporormiella* have also noted the importance of the terrestrial conditions for spore production. In a study of cattle density on the US Great Plains, Parker and Williams (2011) noted that *Sporormiella* spore abundance decreased along a west-to-east transect from South Dakota to Wisconsin in which precipitation increased. They suggested that in the wetter eastern part of their transect, spores were not able to disperse as far as in drier areas to the west and the wet conditions decreased the chance of spores directly reaching lake sediments. In New Zealand, Wood et al. (2011) found that fossil *Sporormiella* spores were present in relatively dry forest soils in association with extinct avifauna and introduced livestock

and their presence accurately reflected known avian extinctions and the timing of European settlement. However, in saturated soils from nearby wetlands, *Sporormiella* abundance varied in pre-extinction horizons in association with fluctuations in local hydrological conditions, as inferred from pollen data (Wood and Wilmshurst, 2012). They offer two reasons for their observations: 1) wet areas may have been avoided by herbivores either because of unpalatable vegetation types or the possibility of becoming mired, and 2) water availability plays a major role in sporulation such that too much water can cause the dung substrate to disintegrate prior to sporulation or conditions that are too dry would allow the dung to desiccate prior to sporulation and create a "false-extinction."

The optimal level of moisture to sporulate varies among fungal spore species; for example, Nyberg and Persson (2002) studied the effect of habitat on dung fungus diversity by comparing the growth of coprophilous fungus on moose dung in spruce and pine forests and on an open mire. Dung in moist spruce forest had low diversity whereas, dung in the dry pine forest and mire had three times the spore diversity found in spruce forest. In total, there were 26 species of fungi on moose dung, 20 in the pine forest, 11 in the spruce forest, and 21 in the mire. These findings suggest an inverse relationship between the water content of the dung and species richness. *Sporormiella* was present in all three habitats but was the most common species in the spruce forest, where moisture content was highest. Their study suggests that optimal growth conditions vary among dung fungi, and that *Sporormiella* production was better under the relatively more moist conditions of the spruce forest.

Our findings and other studies thus suggest that the environment where the dung is deposited greatly influences fungal growth. The most important factor may be obtaining a critical

moisture level necessary for successful sporulation. More research is needed on the autecology of dung fungal species, and this topic requires further investigation before we can use dung fungi as a reliable indicator of herbivores.

Transport Within the Watershed

Sporormiella spores are transported primarily by surface runoff and short-distance aerial transport from dung (Van Geel et al., 2003). Spores are also transported in the gut of their herbivore host (Feranec et al., 2011). Spores are released from dung close to the ground and covered with a gelatinous material that helps them attach to nearby vegetation where they may be ingested by browsers and start the cycle again (Ahmed, 1972; Davis and Shafer, 2006). Unlike arboreal pollen, dung spores cannot travel more than about two meters from the dung where it is produced (Davis and Shafer, 2006; Raper and Bush, 2009). Spores that fail to attach to vegetation may be dispersed to a nearby waterbody by airborne transport or they may fall to the ground where they can be carried by surface runoff. The short dispersal distance alone could explain the absence of spores in lake sediments in our study because it requires defecation close to the shore. At Blacktail Pond, bison avoid the floating fen margin, especially in spring, when they risk becoming mired.

At Cuddie Springs in the Sahul of New South Wales, Dodson and Field (2018) studied *Sporormiella* in lacustrine deposits containing well-preserved megaherbivore fossils in two units separated by a fragipan layer. The pollen/spore record from the lower unit (SU9), which was deposited from 580 to 379 ka, indicates moist, wooded conditions, whereas the upper unit (SU6), deposited from ca. 40 to 30 ka, is interpreted as a shallow, low-energy lacustrine environment suggesting a time of water scarcity. Despite the presence of megaherbivore fossils in the lower

deposit, *Sporormiella* spores were present only at low values and in some samples not at all. *Sporormiella* spores were much more abundant in the upper unit (SU6). The authors suggest that focused megaherbivore use around watering holes was greater during the dry period, which would have concentrated dung locally, affording spores a better opportunity to enter the water. During deposition of the lower unit, dung was locally less abundant, likely because animals were more dispersed due to widespread availability of water.

Studying a captive bison herd in a tallgrass prairie system in Kansas, Gill et al. (2013, 2014) link known bison biomass to *Sporormiella* spore abundance. The study used 28 Tauber traps to collect airborne pollen and spores inside and outside a 1,000 ha bison enclosure that contained 386 bison. The bison were fitted with GPS collars to track their grazing intensity with respect to the traps that were set 500 m apart to collect pollen and spores from a very local source area. The results showed *Sporormiella* abundances were significantly higher within the bison enclosure with the strongest relationship between spore abundance in Tauber traps and areas grazed by bison when traps were within 25 m to bison dung. Their definition of short distance dispersal is greater than the 2 m suggested by Baker et al. (2013) however, their conclusion that *Sporormiella* spores are a good local-scale indicator of megaherbivore presence in grassland systems is consistent with other studies.

Once spores reach a lake, they have the potential to become incorporated in the sediments. Local climate conditions can have a significant impact on the surface transport of spores to the lake. In a semiarid ecosystem with little runoff, like the Yellowstone's Northern Range, transport of dung fungal spores to a lake by run-off or snowmelt seems unlikely except for spores deposited very close to the lake margin. Local hydrology, thus, has the potential to

influence where and how far spores are carried. Riparian vegetation could also trap spores, inhibiting their travel from dung to lake margin. In the case of Blacktail Pond, the presence of a floating fen margin may have contributed to the absence of *Sporormiella* in lake sediments there. The fen margin, which developed 11,000 years ago (Huerta et al., 2009), may have hindered *Sporormiella* transported and potentially trapped spores before entering the lake. Riparian vegetation is limited at Buffalo Ford Lake so this does not explain their absence there.

Deposition in Lake Sediments

Our study used two approaches for sediment collection from lakes. At Buffalo Ford Lake, we took surface sediment samples from the shoreline to the center of the lake and at Blacktail Pond, we sampled the small southwest inlet in 4 m of water approximately 20 m away from the edge of the grass mat. We hoped this approach would allow us to observe differences in spore distribution within water bodies; however, the absence of *Sporormiella* spores in the sediments of both sites limited this line of inquiry.

Several factors can affect the deposition of *Sporormiella* in lake sediments. In a study of *Sporormiella* in lakes in central Florida, Raper and Bush (2009) found that lakes in watersheds with cattle had more *Sporormiella* than lakes in ungrazed locations. In grazed settings, *Sporormiella* abundance in lake sediments decreased with increasing water depth. For example, *Sporormiella* was found in 77% of samples in shallow water and in only 37% of samples from the lake center. In lakes where there was no grazing in the watershed, *Sporormiella* was absent in deep water and present in low concentrations close to shore. In these ungrazed sites, the authors attributed the shallow-water *Sporormiella* to animals other than cattle (small mammals and birds).

In a study of *Sporormiella* abundance in natural and dammed lakes in the Great Plains, Parker and Williams (2012) found *Sporormiella* concentrations were highest in lake-margin samples, whereas samples far from shore were barren. The findings were consistent with those of the Florida study, namely that *Sporormiella* was most abundant in shallow-water samples. We were surprised by the absence of *Sporormiella* in lake sediments close to shore at Buffalo Ford Lake and Blacktail Pond.

Preservation in Lake Sediments

Although we did not find *Sporormiella* spores preserved in lake sediments, we did find fragments of unidentified spores in the sediments from Buffalo Ford Lake. Spore fragments were observed in samples processed by both Method 1 and Method 2 (Table 2). Spores that successfully reached the lake and were deposited along with other environmental proxy, such as pollen and charcoal, may have been degraded and damaged in the water column as a result of shifts in currents and sediment re-deposition.

At Appleman Lake, Indiana, a change in lithology from fine-grained to coarse-grained sediment occurred at the same time as the decline in *Sporormiella* (Gill et al., 2009). Feranec et al. (2011) suggest that the decline of *Sporormiella* at Appleman Lake may have been caused by a change in depositional environment and burial conditions that were unfavorable to spore preservation. Coarse-grained sediments apparently did not preserve spores as well as finer-grained sediments, and thus variations in *Sporormiella* abundance may have reflected changes in preservation potential. Other factors may also cause spore degradation, for example, changes in lake water chemistry, subaqueous bioturbation, ingestion by zooplankton or benthic organisms,

or subaerial exposure during times of lower lake level. These possibilities all bear further investigation.

Processing

We modified standard pollen processing techniques to be as gentle as possible in extracting spores. Fresh spores from the dung gardens were treated only with deionized water and 95% ethanol. These spores exhibited no fragmentation or corrosion with this treatment.

When core sediments from Blacktail Pond were processed with standard pollen preparation methods (Bennett and Willis, 2001), no spores were found. The surface sediments from Buffalo Ford Lake were also processed with this protocol and yielded fragments of dung fungal spores but nothing identifiable to *Sporormiella* (Table 4). When the technique was modified to eliminate hydrofluoric acid and acetolysis treatments, we found dung fungal spore fragments, but none were *Sporormiella* (Table 5).

Recovery of dung fungal spores from sediments in our study may have been affected by the chemical processing. In general, we posit that fewer chemical procedures would be better when analyzing sediment for *Sporormiella*. Van Asperen et al. (2016) also concluded that pollen preparation protocols impacted dung fungal spore recovery. Their study found that acetolysis, in particular, damaged *Sporormiella* and the best results were achieved by first sieving the samples (>125µm and <6µm mesh size) and then following with treatments with 10% KOH, 10% HCl, heavy liquid flotation, and hot 10% NaOH.

More evaluation of different chemical treatments to extract spores from fresh dung and from lake sediments is needed to determine which techniques yield optimal spore preservation and abundance. It would be helpful to develop a spore processing technique that could be

universally applied and evaluated independent of other paleoecological proxies, as is done for macroscopic charcoal analysis. Use of a standardized processing protocol would enable better comparison of results from different settings and regions and provide greater confidence in interpreting negative results. Although many studies have observed *Sporormiella* spores in fossil pollen samples that were prepared with standard pollen processing procedures, including the use of hydrofluoric acid and acetolysis, we question the extent to which dung fungal spores may have been destroyed by these harsh treatments.

CONCLUSIONS

Sporormiella was identified in modern bison dung in northern Yellowstone National Park; however, using standard collection methods, we were not able to find *Sporormiella* spores in the sediments of lakes from watersheds that are known to have supported large numbers of bison since the 1920s. We modified laboratory preparation techniques to improve *Sporormiella* spore detection, yet still none were identified. We attribute the absence of spores in lake sediments in Yellowstone's Northern Range to (1) unsuitable climate and seasonal moisture variations that may have interfered with spore production and dispersal; (2) the semiarid climate that may not have provided adequate rainfall for surface runoff into lakes; (3) wetland conditions that bison may have avoided to avoid miring; (4) riparian vegetation (grass mat) that may have impeded spore transport to lake margins; (5) subaqueous processes that may have degraded dung fungal spores prior to or soon after burial; and (6) the processing treatments that may have destroyed spores in the laboratory.

Our study has identified areas of research that warrant further study to help interpret the presence or absence of *Sporormiella* in lake sediments. For example, the effect of Yellowstone's climate and moisture conditions on spore viability, production, and distribution in the ecosystem should be examined more rigorously as a key to accurately interpret spore presence or absence. A better understanding of how spores are transported to lakes, whether by water or air, and buried in lake sediments is also needed. A study of spore viability in dung collected during all four seasons might shed light on the effect of climate extremes, including freezing and desiccation, on *Sporormiella* spore production, transport, and preservation. Finally, a standard sampling protocol specifically for dung fungal spores is needed to enable results from different

sites, vegetation types, and climate settings to be evaluated and compared. A control sample using *Sporormiella* spores to test the preparation method should be included to ensure the results are not being biased. Further study in the Yellowstone's Northern Range may yield useful information to clarify some of these issues.

In summary, the presence of *Sporormiella* spores may be a useful proxy for identifying the presence of megaherbivores; however, our study suggests that the absence of *Sporormiella* does not indicate an absence of bison. Much research remains before drawing any conclusions about herbivore presence or absence based on dung spores.

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