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Myxomycete diversity on bison dung and cow dung in the Missouri River watershed of the Standing Rock Sioux Reservation, North Dakota

Abstract

The objective of the study reported herein was to document the *myxomycetes* (slime molds) associated with bison dung and cow dung in the temperate prairies of North Dakota. We hypothesized that there is a significant difference between the *myxomycetes* on bison dung and cow dung. The data were analyzed for any significant statistical differences between the two types of dung, using jmp statistical software. We concluded that there was a significant difference between bison dung and cow dung, with statistical p-value <0.0001*; hence, we rejected the null hypothesis. Reciprocal Simpson's diversity indices for bison dung and cow dung were 3.01 and 3.03, respectively. The pH recorded for dung samples ranged between 6.48 and 7.41, with the majority of species associated with weak acidic-weak basic conditions.

Keywords: diversity, coprophilous myxomycetes, bison, cow, watershed, standing rock

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Introduction

The *myxomycetes* have been mistakenly classified as part of the Kingdom Fungi for a long time, but they are now known to be quite unrelated, since one stage in the *myxomycetes* life cycle can move, and these organisms lack chitin in their cell walls. The two groups of *myxomycetes* that have been best described are plasmodial slime molds and cellular slime molds.[1] Slime molds are eukaryotes, and much is not yet known about them, including their association with their various habitats. It is known that the decaying dung of herbivores represents a substrate for *myxomycetes*.[2] Though the vast majority of the species associated with dung are presumed to be secondary inhabitants, a few examples appear to be largely or even completely restricted to this substrate.[3] There have been relatively few studies of some species, such as *coprophilous* (sometimes referred to as fimicolous) myxomycetes, so ecological and distributional data for such species are still limited. *Myxomycetes* diversity varies widely with ecological niches, microhabitats and substrata. Some have narrow ecological niches and are restricted to particular substrata, while others do not. They are important to the environment because they eat decaying vegetation and enhance the nutrient cycle. They also clean the environment by eating bacteria, fungi, and even other slime molds. These characteristics may be beneficial to animals as well as other life forms they associate with. The fact that the decaying dung of herbivores represents a substrate for *myxomycetes* (plasmodial slime molds or myxogastrids) is well established.[2] The vast majority of the species associated with dung are presumed to be secondary inhabitants, and a few examples appear to be largely or even completely restricted to this substrate.[3]

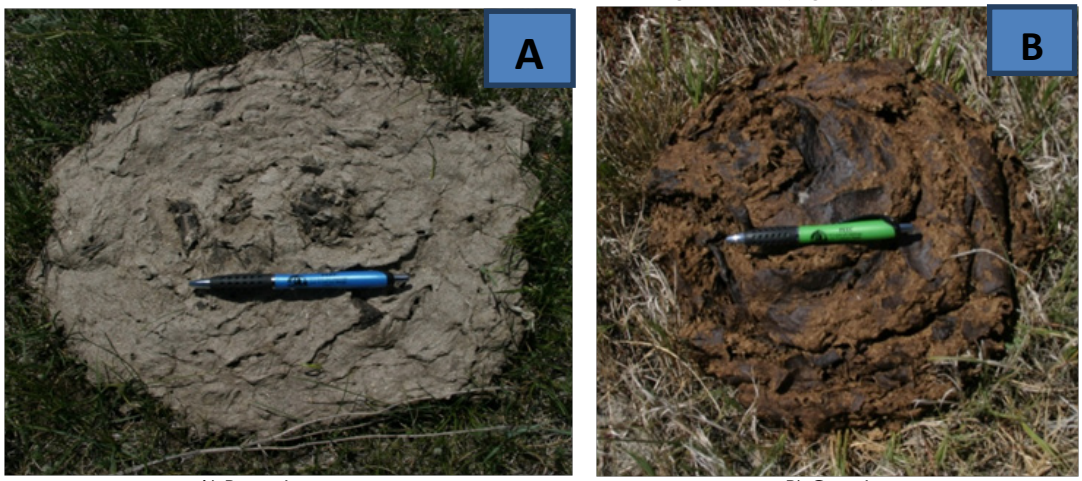
Study areas

The study was conducted on the Missouri River watershed in the Standing Rock Sioux Reservation of North Dakota. The coordinates of the study site were 45^{0}57'35'' N and 100^{0}56'24'' E, and the site was located within the Sitting Bull College bison pasture and an adjacent cow pasture at Selfridge in the Standing Rock Reservation in Sioux County (Figure 1).

Figure 1 A representative view of the study site (a temperate prairie near Standing Rock, Sioux County, North Dakota).

Materials and methods

A series of samples of bison dung and cow dung samples were collected from Sitting Bull College bison pasture and an adjacent cow pasture at Selfridge in Standing Rock Reservation, Sioux County. All samples were placed in paper bags, the bags numbered, marked with the type of dung they contained and returned to the laboratory, where they were allowed to air-dry for several days. Later, the entire set of samples was sent to the Eumycetozoan laboratory at the University of Arkansas for processing. At the University of Arkansas, the samples were used to prepare moist chamber cultures. These were prepared in the manner described by Stephenson & Stempen[2] and consisted of plastic disposable Petri dishes (100mm diameter) lined with filter paper. Two Petri dishes were prepared from each sample giving a total of twenty (20) Petri dishes (10each for bison and cow dung samples) from 5sample plots. A sample plot was represented by grazing fields within the larger pasture. The grazing fields were demarcated by fences. Enough sample material was placed in each dish to cover most of the bottom, and then the material was moistened with distilled water. After a period of approximately 24hours, the pH of each culture was determined with a portable pH meter and then excess water in the Petri dish was poured off. Moist chamber cultures were placed out of direct sunlight and maintained at room temperature. Water was added to these cultures when necessary to maintain moist conditions, and the cultures were checked at least twice each week for evidence (either plasmodia or fruiting bodies) of *myxomycetes* over a period of three months. Data were collected through observation, and recorded species diversity and abundance on each sample. A hand magnifying lens and microscopes were used for this investigation. Whenever fruiting bodies were observed, they were recorded, removed from the moist chamber culture, air-dried and placed in small pasteboard boxes for long-term storage. A specimen was defined as a record of the occurrence of one or more fruiting bodies of a particular species of *myxomycetes* in a single culture. Some specimens consisted of only a single fruiting body, whereas others consisted of numerous fruiting bodies in a single culture (Figure 2).



A) Bison dung B) Cow dung

Figure 2 Representative examples of the two types of dung.

Results and discussion

A total of 6species were recorded from the thirty dung substrate cultures. Bison dung substrate had a lower mean pH (Figure 3), but higher frequency mean (Figure 4). The species found in bison dung had higher frequency compared to those found in cow dung. *Arcyria cinerea, Didymium difforme* and *Kelleromyxa fimicola* do well at a lower pH compared to *Perichaena depressa, Perichaena liceoides* and *Physarum* sp. (Figure 5). The term pH refers to the negative logarithm of hydrogen ion concentration (-log [H^+]). There was statistical significant difference between *myxomycetes* species found in bison and cow dung substrates with the Wald p-value of less than 0.001 ([Wald p-Value <.0001*] Table 1). The Walden test computes the score test for coefficient of generalized linear model between two means. It is also referred to as chi-square. The analysis of the data revealed that the standard deviation of the sample distribution mean (standard error) remained very low at 0.18, while the percent variance explained by the model value (R-square) was high at 0.86 (86%) (Table 2). This implies that the regression line perfectly fits the real data point. The variation in dependent variable (*myxomycetes* species) is well explained by independent variable (the subjects). The subjects here refer to bison and cow dung substrates. Root mean square error (RMSE) is a measure of how much error exists between two data sets. It remains low (0.135), indicating a better fit (Table 2). Of the 29 *coprophilous myxomycetes* recorded, about 55%, 35% and 10% were recorded in the first, second and third months respectively (Table 3). *Arcyria cinerea* species was the laggard, coming out only in the third month, while *Didymium difforme* and *Perichaena liceoides* species were found in each of the three months period. No single species was recorded from cow dung in the third month.

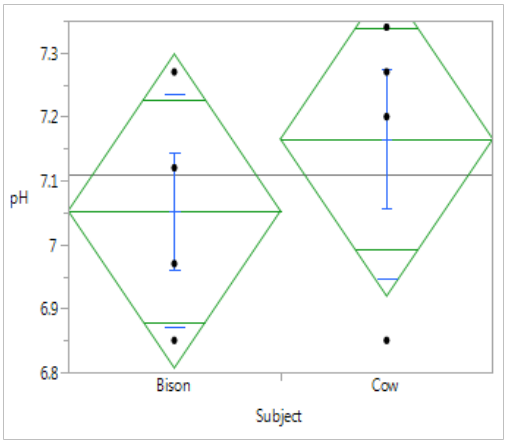


Figure 3 Fit pH by Subject

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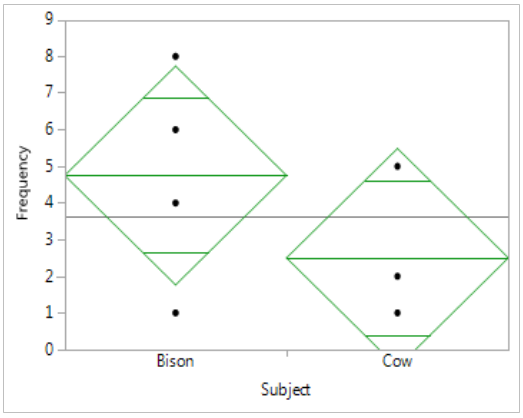

Figure 4 Fit Frequency by Subject

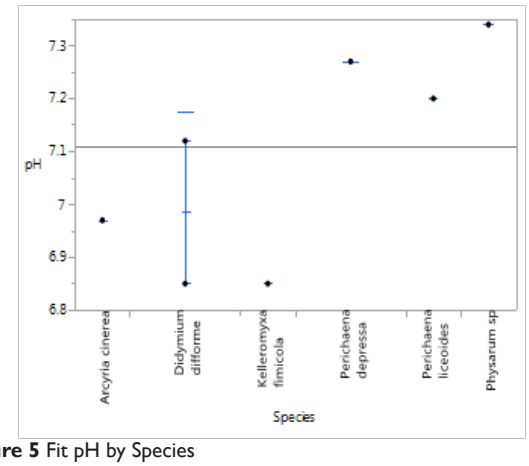

Figure 5 Fit pH by Species

Table 1 REML Variance Component Estimates

Random Effect	Var Ratio	Var Component	Std Error	95% Lower	95% Upper	Wald p-Value	Pct of Total
Subject[Species]		0.018225	0.018225	0.0049405	0.719849	<.0001*	100.000
Total		0.018225	0.018225	0.0049405	0.719849		100.000

Species frequency on bison dung were 8, 6, 4 and 1 for *Kelleromyxa fimicola*, *Perichaena liceoides*, *Didymium difforme*, and *Arcyria cinerea* respectively. On cow dung, the values were 5, 2, 2, and 1 for *Perichaena liceoides*, *Perichaena depressa*, *Didymium difforme* and *Physarum* species respectively (Table 4 & Figure 4). The result shows that the genus *Didymium* is the most frequent, while *Physarum* is the least frequent (Figure 6). Species abundance (the number of individuals per species) was higher on the bison dung, the values being nineteen (19), and only ten (10) on cow dung substrate. *Coprophilous myxomycetes* biological diversity (biodiversity) refers to the variety of *myxomycetes* species on the dung substrates. Biodiversity index estimation takes into consideration species richness and species evenness. We defined species evenness as how evenly the *myxomycetes* species were represented in the dung substrates (Table 4). Both bison dung and cow dung samples had four (4) species each, thus the same species richness (the number of different species present in a substrate) value. *Arcyria cinerea*, *Didymium difforme*, *Kelleromyxa fimicola*, and *Perichaena liceoides*, were on bison dung substrate while *Didymium difforme*, *Perichaena depressa*, *Perichaena liceoides*, and *Physarum* species were on cow dung substrate (Table 2 & Table 4).

Table 2 Summary of Fit

Item	Value	Explanation
RSquare	0.863528	R-Square: Percent variance explained by the model value
RSquare Adj	0.522348	Adjust the statistics based on the number of the independent variables in the model
Root Mean Square Error (RMSE)	0.135	The distance of a data point from the fitted line, measured along a vertical line.
Mean of Response	7.10875	The mean of the y population associated with xd

Table 3 Species of Coprophilous Myxomycetes

Plots	pH	Sample type	Month 1. *myxomycetes*	Month 2. *myxomycetes*	Month 3. *myxomycetes*
1B	6.82	Bison	*Didymium difforme* (33184)	*Kelleromyxa fimicola* (33185)	N
1B	7.41	Bison	*Didymium difforme* (33148)	*Kelleromyxa fimicola* (nc)	N
2B	6.48	Bison	*Kelleromyxa fimicola* (nc)	*Didymium difforme* (nc)	*Perichaena liceoides* (nc)
2B	6.91	Bison	*Perichaena liceoides* (33231)	*Kelleromyxa fimicola* (nc)	*Arcyria cinerea* (nc)
3B	6.65	Bison	*Kelleromyxa fimicola* (33227)	*Perichaena liceoides* (nc)	
3B	6.71	Bison	*Kelleromyxa fimicola* (nc)	*Perichaena liceoides* (nc)	*Didymium difforme* (nc)
4B	7.19	Bison	*Kelleromyxa fimicola* (nc)	N	N
4B	7.03	Bison	*Perichaena liceoides* (33252)	N	N
5B	6.99	Bison	N	N	N

Table 3 Continued...

Plots	pH	Sample type	Month 1. *myxomycetes*	Month 2. *myxomycetes*	Month 3. *myxomycetes*
5B	7.2	Bison	*Kelleromyxa fimicola* (nc)	*Perichaena liceoides* (nc)	N
1C	7.37	Cow	*Didymium difforme* (33247)	N	N
1C	6.85	Cow	*Didymium difforme* (33246)	N	N
2C	7.25	Cow	N	N	N
2C	7.06	Cow	*Perichaena liceoides* (33230)	*Perichaena liceoides* (nc)	N
3C	7.25	Cow	N	N	N
3C	6.84	Cow	N	N	N
4C	7.2	Cow	*Perichaena depressa* (33229)	N	N
4C	7.34	Cow	*Perichaena depressa* (33183)	*Perichaena liceoides* (nc)	N
5C	7.2	Cow	*Perichaena liceoides* (nc)	N	N
5C	7.34	Cow	*Perichaena liceoides* (nc)	*Physarum sp*. (33254)	N

Key: B = Bison; C = Cow; N = No Myxomycete growth; nc = not collected

Table 4 Coprophilous myxomycetes frequency, monthly establishment, abundance, diversity indexes and pH ranges.

#	Species	Frequency												pH	Condition
		Bison dung						Cow dung							
		M1	M2	M3	Su	Pi	Pi²	M1	M2	M3	Su	Pi	Pi²		
1	*Arcyria cinerea*	0	0	1	1	.053	.003	0	0	0	0	000	000	6.91	AD
2	*Didymium difforme*	2	1	1	4	.211	.045	2	0	0	2	.200	.040	6.85-7.37	AB
3	*Kelleromyxa fimicola*	5	3	0	8	.421	.177	0	0	0	0	000	000	6.48-7.41	AB
4	*Perichaena depressa*	0	0	0	0	000	000	2	0	0	2	.200	.040	7.2-7.30	BS
5	*Perichaena liceoides*	2	3	1	6	.316	.100	3	2	0	5	.500	.250	6.48-7.34	AB
6	*Physarum sp*	0	0	0	0	000	000	00	00	1	1	.100		7.34	BS
	Grand Total	9	7	3	19		D = .325	7	2	1	10		D = .330		
	Reciprocal Diversity Index (1/D) =						3.01	1/D =					3.03		

Key: AB=Acid-Base Condition AD=Acid Condition BS=Basic Condition

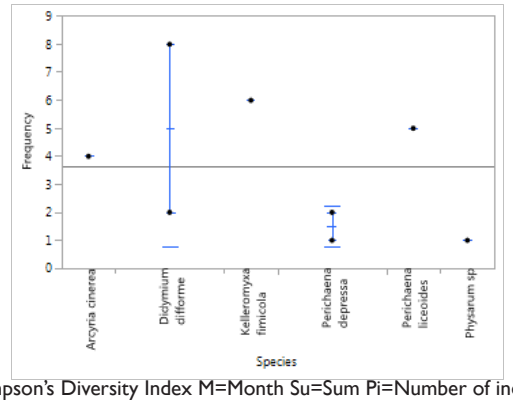

Figure 6 Fit Frequency by Species.

D=Simpson's Diversity Index M=Month Su=Sum Pi=Number of individuals of a species / total number of samples

Conclusion

The study reveals that there is a significant difference in species composition between bison dung and cow dung substrates. Some *myxomycetes* species take more than two months to establish on dung substrates. *Coprophilous myxomycetes* are found in both weak acidic and weak basic substrate conditions. Future study will concentrate on the species' characterization using molecular tools.[4–20]

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Conflict of interest

The authors declare no conflict of interest.

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