Effect of manure application on soil microbial activity

J.R. de Freitas, J.J. Schoenau, S.M. Boyetchko & S.A. Blechinger
Department of Soil Science - University of Saskatchewan; Agriculture and Agri-Food Canada - Saskatoon[1].

Abstract

Soils from field trials treated with hog and cattle manure in spring of 1997 were sampled in the fall after harvest and soil microbial activity assessed. CO_2 evolution measurements indicated that 6 months after addition, hog and cattle manure had little influence on measured microbial activity. In contrast, manure additions greatly stimulated soil microbial activity immediately after application when manure was freshly applied to the soils in the laboratory. For example, the highest level of microbial activity was observed at 3 days after treating the soil in the lab with 400 kg N-ha-l of cattle or hog manure. These initial results indicate that time of sampling is critical when assessing the effect of manure on soil microorganisms, with measurements required immediately after application as well as at longer time intervals. The fresh application of manure stimulated microbial activity by lo-fold compared to that measured in samples taken 6 months after application. However, microbial activity decreased with time and amounts of C evolved leveled out at about 20 $\mu g\ C \cdot g^{-1}$ soil after 38 days, similar to activity observed in the samples after 6 months. Preliminary results on the effects of manure application on the incidence of soil entero-pathogens (fecal coliforms) and wheat root rot incidence are discussed.

Introduction

The use of animal manures as a soil amendment is an important option for crop rotations and nutrient recycling. This system can contribute to better long term soil quality and fewer chemical inputs. Unfortunately, each year bacterial and fungal diseases cause damage to Saskatchewan crops, thus it is important to determine whether manure additions to soil are related to increase or decrease in diseases in the production system. In addition to controlling the release and availability of many important plant nutrients, the soil microbial community is the main source of soil - borne plant pathogens, thus it directly affects the sustainability and health of cropping systems. The successful application of manure to soil systems in the future will require an understanding of the microbial characteristics of the soil. Hence, it is imperative to assess the general microbial community and in particular the incidence of plant diseases and fecal coliforms *i.e.*, indicator organisms for the presence of human entero-pathogens, if any, as affected by manure applications in the soil. The objective of this study was to assess the impact of manure application on soil microbial community dynamics. Microbial populations expected to impact on human (entero-pathogens) and plant (fungi) diseases were assessed.

Materials and Methods

In 1997, two field sites were established in the Black soil zone near Humboldt, Saskatchewan. The sites are located on a highly productive clay loam soil Blaine Lake association) near Dixon and a marginal sandy loam (Meota association) near Burr, respectively. A replicated randomized complete block design was set up at each site. Treatments were imposed in the fall and spring including: (i) Control - no application, (ii) Hog manure, (iii) Cattle manure; and, (iv) Urea. Hog manure treatments were injected or surface applied (wide x narrow row injection spacing) in spring of 1997 and again in late fall after harvest. Cattle manure treatments were broadcast and incorporated or broadcast without incorporation in the spring of 1997 and again in late fall. Manure applications (kg of available N-ha-l) included hog (75, 150, 300) and cattle (120, 240, 480) at the Dixon site; and, hog (200,400, 800) and cattle (225,450, 900) at the Burr site. Urea was applied at 0, 50, 100 or 200 kg of N-ha-1 at each site for comparison purposes. Both Dixon and Burr field sites were cropped to canola and spring wheat in 1997 and 1998, respectively. Soil

samples were taken from each plot by taking soil cores (5 cm diameter x 30 cm depth) in the fall of 1997, after harvest and in spring of 1998 and fall 98. Samples were brought to the laboratory and stored at 4°C until use. At each sampling time, a total of 44 soil samples were obtained from each site i.e., 16 samples (4 rates x 4 replicates) and 12 samples (3 rates x 4 replicates) for hog/cattle and urea, respectively. Parameters assessed included: microbial activity, microbial populations; and, degree of disease infection (human and phytopathogenic microorganisms).

- Microbial activity: Assessed by the CO_2 evolution method using gas chromatography - GC.

A. Lab study: Soil freshly treated with manure. This study provided important information as to determine the length of time for the soil microbial activity to peak after fresh additions of manure. Untreated surface soil (O-30 cm) was collected from the Dixon site, air dried, sieved (2 mm) and 200 g placed into 1.0 L Mason jars. Treatments (n=7) included: (i) Untreated control, (i/j Swine manure, (iii) Cattle manure; and, (iv) Urea, each applied at 100 and 400 $\mu g \cdot g^{-1}$ soil (240 and 960 kg of $N \cdot ha^{-1}$, respectively). Stock solutions were prepared based on the hog manure and/or urea-N content *i.e.*, 0.2% and 46%, respectively. Aliquots were used to bring the soil to 60% moisture holding capacity - MHC (ca. field capacity @ 27%), whereas the cattle manure (1.41% N) treatment was accomplished by incorporation into the soil, followed by addition of water to 60% MHC. Mason jars were hermetically sealed and replicates (n=4) arranged in a completely randomized design. All jars were incubated at 24°C and samples analyzed for CO2 evolution (described above) at 3,7, 14,21,28, 38, 46 and 54 days.

B. Soils sampled from field plots (both sites) receiving manure in 1997 - 1998. Soils subjected to manure and/or urea applied at field rates above were collected. Fresh (60% MHC) soil samples (100 g) were incubated at 24°C, and microbial activity (mg C $day \cdot g^{-1}$) was assessed as described above in samples (n=4) periodically up to 58 days.

- Microbial populations: Isolation of microbial community from the manure plots at Dixon and Burr sites. Microbial populations have been assessed in soils and hog, cattle and poultry manure using the agar plate count method (Bagley & Seidler, 1978; Foster & Rovira, 1975). Assessment of human pathogenic bacteria *(Enterobucteriaceae, Klebsiellae* and fecal coliforms) has been performed in pure manure and soil samples from the two field locations using selective culture media. Fresh manure or soil samples were suspended in H20 and then serially diluted (l/10). Aliquots of appropriate dilutions were spread plated onto the following culture media: (i) MacConkey's, (ii) MacConkey's plus carbenicillin to separate presumptive *Enterobacteriaceae* and *non-Enterobacteriaceae;* and, (iii) Trypticase soy agar for total heterotroph bacteria counts (Bagley & Seidler, 1978; Foster & Rovira, 1975). The Levine Eosin Broth Agar (EMB) medium was used to assess *E. coli* population in fresh manure. Inoculated plates (n=4) were incubated at 28°C and 37°C for *Enterobacteriaceae* and heterotrophs, respectively and bacterial colony-forming units (cfu) determined at 72 to 120 h of incubation. Fecal coliforms were assessed by the Most Probable Number (MPN) method. Fecal coliforms were used as indicator organisms for the possible presence of human enteropathogens (Franson *et al.*, 1989). A random number table was consulted and 50 colonies from a plate were isolated. Heterotroph populations including pathogenic bacteria were streaked on original isolation medium, incubated for 48 h at the appropriate temperature, checked for purity and stored at -80°C.

- Degree of plant disease infection (foliar and root rot) caused bv phytopathogenic microorganisms.

A plant disease-survey was performed at the Dixon field site during the Summer 98 to assess disease incidence and severity. Infested plants and roots were brought to the laboratory and characterized for the presence of pathogenic fungi. Disease incidence and severity were evaluated on a O-l 1 scale, while cereal root diseases were assessed by sampling 40 roots, washing and evaluating sub-crown internodes for root symptoms.

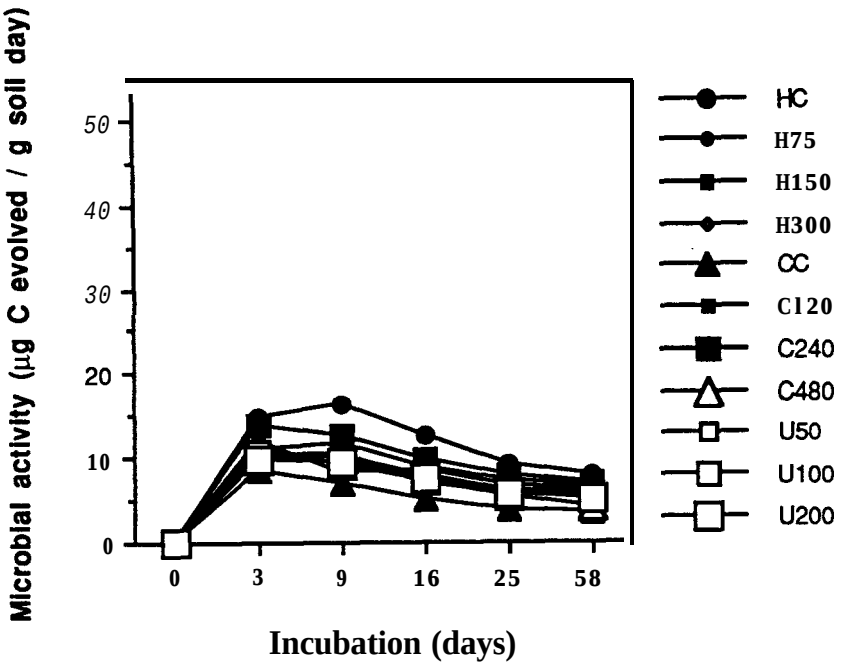
Results

Study I. Soils sampled from field plots (both sites) receiving manure in 1997

- *Did manure applications affect soil microbial activity at the two sites?*

Application of urea, hog or cattle manure had no effect on microbial activity *i.e.*, CO2 evolution rates on the clay loam soil at the Dixon site 6 months after application (Fig. la). Conversely, on the sandy loam soil at Burr site, the hog or cattle manure produced higher CO_2 evolution rates compared to the control or urea treated soils (Fig. lb).

A



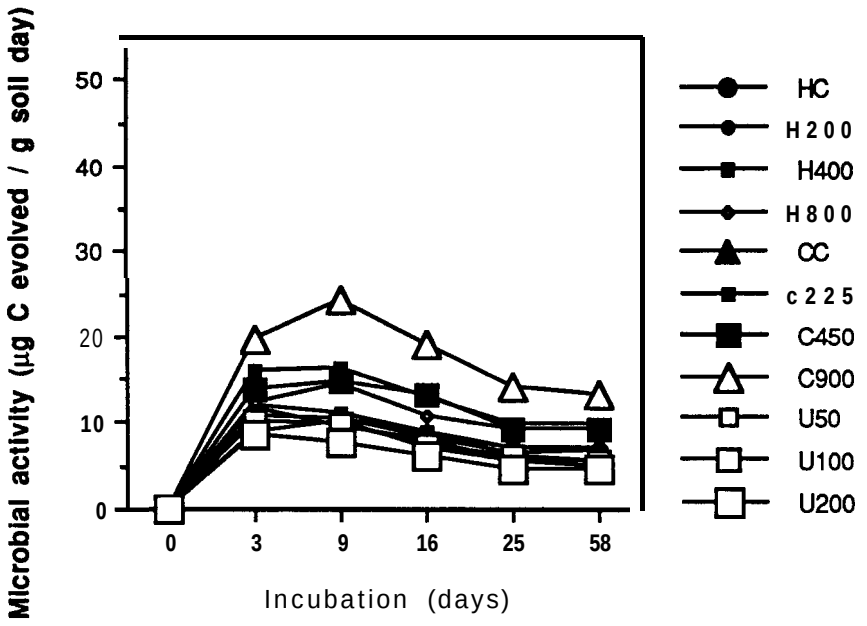
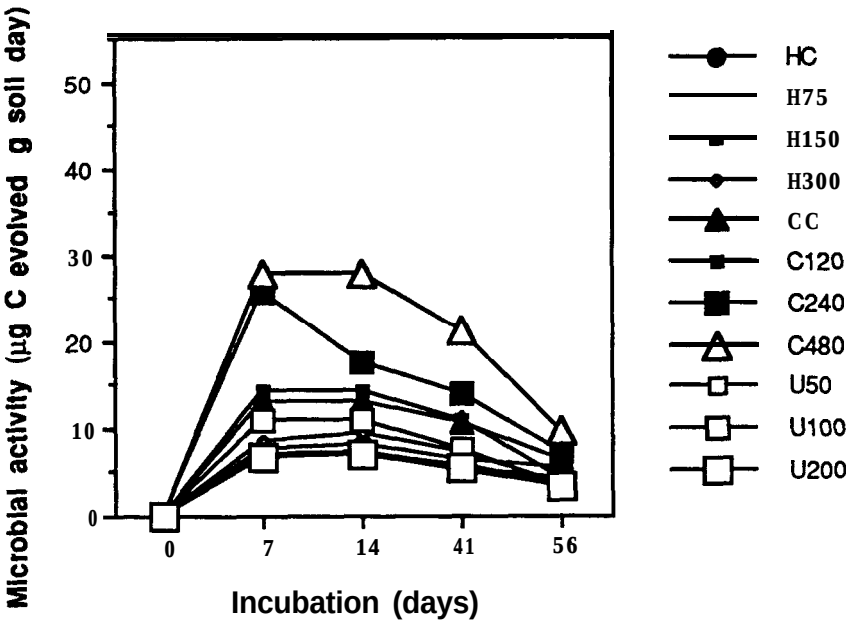


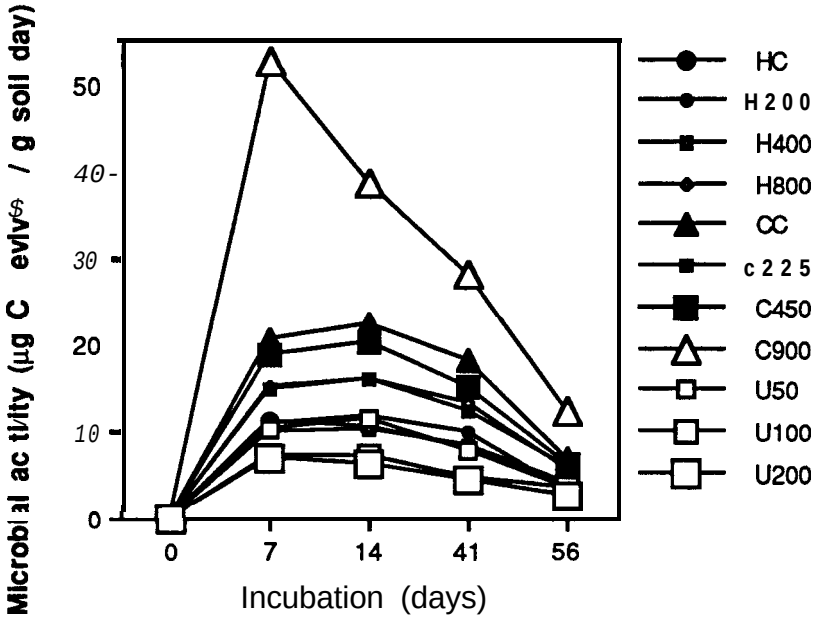
Figure la - b. Soil microbial activity ($\mu g\ C \cdot g^{-1}$ soil) determined 6 months after field application of 50, 100 or 200 kg $N \cdot ha^{-1}$ urea (U), cattle (C) and/or hog'(S) manure. Data are average of 4 replicates incubated for 3, 9, 16, 25, and 58 days in soil from the Dixon (a) and Burr (b) sites.

. Did manure applications affect soil microbial activity one year after application?

Yes. Application of cattle manure at rates higher than 240 kg $N \cdot ha^{-1}$ stimulated microbial activity in both sites. The highest activity was observed at 7 days incubation (Figs. 2a-b). Application of urea produced the lowest increment.

A


Microbial activity (µg C evolved g soil day)
50
40
30
20
10
0
0
7
14
41
56
Incubation (days)
HC
H75
H150
H300
CC
C120
C240
C480
U50
U100
U200



Figures 2a-b. Soil microbial activity ($\mu g\ C \cdot g^{-1}$ soil) determined 12 months (Spring 98) after field application of 50, 100 or 200 kg $N \cdot ha^{-1}$ urea (U), cattle (C) and hog (S) manure. Data are average of 4 replicates incubated for 7, 14, 41, and 56 days in soil from the Dixon (A) and Burr (B) sites.

Study II. Soil freshly treated with manure

- *Did fresh manure applications affect short term soil microbial activity?*

Yes, with the highest level observed at 3 days after treating the soil with 400 kg $N \cdot ha^{-1}$ of cattle or hog manure (Fig. 3). However, the hog manure peaks reduced more rapidly than those correspondent to the cattle manure, thus it may be an indication that the hog manure is more readily used as a substrate for energy, nutrient and biomass production by the soil microbial population.

Urea fertilizer had relatively little impact on microbial activity (Fig. 3).

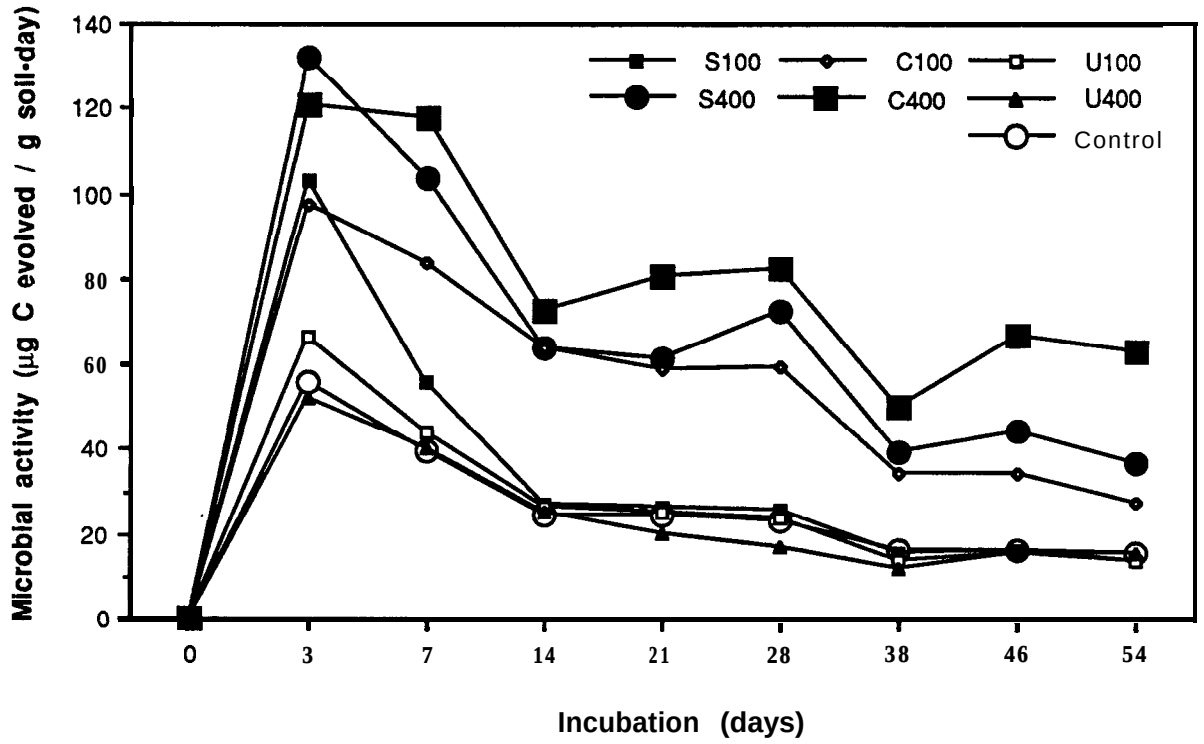


Figure 3. Effect of urea, cattle and/or hog manure application (50, 100 or 200 kg $N \cdot ha^{-1}$) on short-term soil microbial activity (pg Cg-1 soil). Data are average of 4 replicates assessed at 3,7, 14,21,28,38,46 and 54 days after application of manure.

Microbial populations

. ***Were*** E. coli or *presumptive* Klebsiellae ***isolated from fresh hog manure?***

No. Escherichia coli bacteria were not detected in hog or cattle manure. However, ***Klebsiellae*** were present in cattle manure. In contrast, fresh poultry manure sustained a high incidence ***i.e., ca. 50%*** of these two pathogenic bacteria (Table 1). Of the three manure assessed, hog manure also had the lowest incidence of total ***Enterobacteriaceae*** (Table 1).

Table 1. Assessment of microbial populations present in hog, cattle and poultry fresh manure using selective culture media.

Microbial populations	Hog	Cattle	Poultry
	.....	(cfu·g^{-1} soil*)	.....
Escherichia *coli*	ND§	*ND*	1.5×10^{6}
Presumptive Klebsiellae	ND	2.1x105	1.6×10^{5}
Total Enterobacteriaceae	4.5x105	6.4×10^{7}	2.7×10^{8}
Total heterotrophs	3.5×10^{8}	1.1×10^{10}	1.5×10^{11}

*Fresh weight basis; §Not detected.

. Did hog or cattle manure applications at the Dixon site affect the culturable human pathogenic bacteria (Enterobacteriaceae) ***population the soil?***

Yes. At the Dixon site, total heterotrophs, ***Enterobacteriaceae*** and presumptive ***Klebsiellae*** populations increased slightly with increasing rates of hog manure. Conversely, when cattle manure was applied Enterobacteriaceae and presumptive ***Klebsiellae*** populations increased a ten fold as compared to the untreated control (Table 2).

Table 2. Culturable bacteria populations (cfu·g^{-1} soil) present in soil collected at the Dixon site during the Fall 97.

Treatment	Nitrogen (kg·ha^{-1})	Total heterotrophs (cfu·g^{-1} soil) (×10^{8})	*Enterobacteriaceae* (cfu·g^{-1} soil) (×10^{2})	Incidence (cpm)*	Presumptive ***Klebsiellae*** (cfu·g^{-1} soil) (×10^{2})	Incidence (cpm)
Control			41		24	4.9
	0	5		2.0		1.0
Hog	75	66	120	0.9	26	0.5
Hog	300	35	31		110	3.1
					34	
Control	0	120	390	2.8	27	0.3
Cattle	120	49	140	67.6		0.6
Cattle	240	49	3300		150	3.0
Cattle	480	140	5800	40.1	310	2.1
Urea	50	170	250	1.5	400	2.3
						1.4
Urea	200	150	880	7.8	330	3.5

* Incidence (cpm): Counts per million of total heterotroph population.

• ***Did hog or cattle manure applications at the Burr site affect the cultruable human pathogenic bacteria*** (Enterobacteriaceae) ***population the soil?***

Yes. Similarly to the Dixon site, ***Enterobacteriaceae*** and presumptive ***Klebsiellae*** populations increased slightly with manure applications, but total counts were not higher than those obtained for the urea treatments (Table 3). Cattle manure showed an increase in the ***Enterobacteriaceae*** and presumptive ***Klebsiellae*** populations as compared to the untreated control (Table 3).

Table 3. Culturable bacteria populations (cfu·g^{-1} soil) present in soil collected at the Burr site during the Fall 97.

Treatment	Nitrogen (kg-ha-l)	Total heterotrophs (cfu·g^{-1} soil) (×10^9)	*Enterobacteriaceae* (cfu·g^{-1} soil) (×10^4)	Incidence (cpm)*	Presumptive *Klebsiellae* (cfu·g^{-1} soil) (×10^4)	Incidence (cpm)
Control	0	2	2	10.3	11	56.7
Hog	200	17	24	14.1	320	188.9
Hog	400	5	0.1	0.1	6	11.2
Hog	800	5	5i	109.1	11	24.5
Control		24	32	13.4	19	
Cattle	2%	15	1 22	694.2	1500	881.1
Cattle	450	18	220	14.4	26	16.9
Cattle	900			117.8	72	39.4
Urea	50	2 9	25	113.6	34	153.2
Urea	100		10	11.7	2	1.9
Urea	200	7	2	3.1	8	11.6

* Incidence (cpm): **Counts** per million of total heterotroph population.

Incidence of soil entero-pathogens (fecal coiiforms)

• ***Did hog or cattle manure applications increase the fecal coliform (entero-pathogens) population in the soil?***

Yes. Fecal coliforms were isolated and confirmed in soils sampled within a few after receiving hog or cattle manure. However, there was no indication that increased manure rates also increased fecal coliform populations. Counts increased slightly with manure applications, but total counts were not higher than those obtained for the urea treatments (Table 4). No fecal coliforms were found in soil receiving urea (Table 4).

Table 4. Presumptive and confirmatory tests of fecal coliforms in soil and manure from the Dixon site sampled in the Fall 1998.

Treatment	Nitrogen ($kg \cdot ha^{-1}$)	Most Probable Number	
		Presumptive	Confirmatory
		..... [counts ($\times 10^3$) per 100 ml]	
Control	0	1.0	ND*
Hog	75	5.5	1.1
Hog	150	2.6	1.0
Hog	300	2.6	1.2
Control	0	1.5	ND
Cattle	120	8.0	0.2
Cattle	240	8.0	0.4
Cattle	480		1.0
Urea	50	0.2	ND
Urea	100	ND	ND
Urea	200	0.3	ND
Hog manure		11.0	2.7

*Not detected.

- ***Did hog or cattle manure applications increase foliar plant disease in the first year?***

No. Application of hog or cattle manure did not cause visual foliar disease in wheat. In fact, our results indicate that manure applications improved crop health. For example, leaf spot, septoria and tan spot incidence were consistently lower in plants grown in soil which received manure or urea applications as compared to those grown in the untreated control (Table 5).

Table 5. Foliar disease assessment in wheat during the summer 98. Plants were grown in soil previously treated with hog manure at Dixon Site. Data are average of 4 replications.

Treatment	Rate (kg $N \cdot ha^{-1}$)	Growth stage	Leaf spot rating*	Septoria	Tan spot
Control	0	milk & soft dough	10	2.2	1
Hog	75	milk & soft dough	9	2	1
Hog	150	milk & soft dough	7	1	1,2
Hog	300	milk & soft dough	6.2	1	1
Control	0	soft dough	10	2	3
Cattle	120	soft dough	10	2	1.5
Cattle	240	soft dough	9.5	2	1.2
Cattle	480	soft dough	8.5	2	1
Urea	50	milk & soft dough	9	2.2	1
Urea	100	milk & soft dough	7	1.5	1.2
Urea	200	milk & soft dough	5.7	1	0.7

*Percentage of leaf area with lesions in the upper, middle and lower canopies.

- ***Did hog or cattle manure applications increase plant root fungal disease in*** the *first* ***year?***

Yes. application of hog and cattle manure increased root rot incidence. Results were compared to the hog and cattle controls which had 57.5% and 62% root rot incidence, respectively (Table 6).

Table 6. Common root rot on sub-crown internodes of wheat cultivated in soil previously treated with hog manure at the Dixon site. Data are average of 4 replications. Sampling was performed after the harvested in the Fall 98.

Treatment	Rate (kg N·ha^{-1})	Disease rating (%)	Disease incidence (%)	Confiition by plating
Control	0	15.8	57.5	14B* 6F 4U
Hog	75	32.0	85.0	15B 9F 12U
Hog	150	21.3	70.0	20B 7F 1C 6U
Hog	300	41.3	95.0	29B 5F 1C 7U
Urea	50	20.8	77.5	27B 14F 6U
Urea	100	24.0	80.0	20B 12F 9U
Urea	200	23.5	75.0	28B 12F 1C 1U
	0			
Control	120	23.0	62.5	12B 17F 12U
Cattle		24.8	67.5	20B 9F 13U
Cattle	240	32.8	75.0	23B 15F 18U
Cattle	480	25.8	70.0	18B 9F 5C 7U

*Number of plates infected with both ***Bipolaris*** (B) and/or ***Fusarium*** (F); ***C:*** clean; U: disease other than ***Bipolaris*** or ***Fusarium.***

Summary and Conclusions

- Application of urea, hog or cattle manure had no effect on respiration (CO_2 evolution) on the clay loam soil at the Dixon site 6 months after application. Conversely, on the sandy loam soil at Burr site, where higher rates were applied, hog or cattle manure produced higher activity compared to the control or urea treated soils.

- Fresh application of manure increased microbial activity with the highest level observed at 3 days after treating the soil with 400 kg N·ha^{-1} of cattle or hog manure. However, the hog manure peaks reduced more rapidly than those correspondent to the cattle manure, thus it may be an indication that the hog manure is more readily used as a substrate for energy, nutrients and biomass production by the soil microbial population.

- ***Escherichia coli*** bacteria were not detected in hog or cattle manure. However, ***Klebsiellae*** were present in cattle manure. In contrast, fresh poultry manure sustained a high incidence ***i.e., ca.*** 50% of these two pathogenic bacteria. Of the three manure assessed, hog manure also had the lowest incidence of total ***Enterobacteriaceae.***

- At the Dixon site, total heterotrophs, ***Enterobacteriaceae*** and presumptive ***Klebsiellae*** populations increased slightly with increasing rates of manure. Conversely, when cattle manure was applied ***Enterobacteriaceae*** and presumptive ***Klebsiellae*** populations increased a ten fold as compared to the untreated control.

Enterobacteriaceae and presumptive ***Klebsiellae*** populations increased slightly with manure applications, but total counts were not higher than those obtained for the urea treatments. Cattle manure exerted a larger increase in the ***Enterobacteriaceae*** and presumptive ***Klebsiellae*** populations as compared to the untreated control. Fecal coliforms were isolated and confirmed in soils which received hog or cattle manure. However, there was no indication that increased manure rates also increased fecal coliform populations.

Application of hog or cattle manure did not cause visual foliar disease in wheat. In fact, our results indicate that manure applications improved crop health. For example, leaf spot, septoria and tan spot incidences were consistently lower in plants grown in soil which received manure or urea applications as compared to those grown in the untreated control. Application of hog and cattle manure did increase root rot incidence.

Acknowledgments

This work was supported by a grant from the Saskatchewan Agriculture Development Fund SADF.

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