

Introduction

>Nutrient accumulation from manure application

•Poultry litter is high in P and some micronutrients in relationship to the quantities

required by plants (Sistani et al., 2004). •Application of higher litter rates resulted in accumulations of P, K, Cu, and Zn in the top 5 cm of soil (Adeli et al., 2008).

•Broiler litter applied to meet N requirements can give 8x as much P as needed (Franzluebbers et al., 2002)

•In a 5 year bermudagrass study using poultry litter, Zn concentrations were found to be 2.1 times higher in soils with poultry litter application in comparison to inorganic fertilization (Franzluebbers et al., 2004).

>Microbial Survival after manure application

• Most of the previous research on pathogen/microbial survival has been in vegetable crop production.

• In a study by Natvig et al.(2002), it was found that *Salmonella enterica* and *Escherichia coli* survival was influenced most by temperature, moisture and length of time in the environment.

•Escherichia coli and Salmonella enterica were reduced by 99% after 1 hour in 55-65 °C laboratory conditions (Wilkinson et al., 2011). As would be anticipated, moisture (65% vs. 30%) made these pathogens survive for a longer time in the laboratory, up to 21 days. •High soil moisture coupled with low temperatures was found in most studies to favor enteric pathogens persistence in soil (Entry et al., 2000)

•Other factors that seem to also correlate with soil moisture and survival are season, presence of plant root systems, and decaying materials all increase soil microbial populations (Dowe et al. 1997).

• Poultry litter compost that was windrowed, led to increased E. coli densities when

incubated in a lab for up to 21 days (Wilkinson et al, 2011).

Objective

> To determine if select microbes survival is influenced by fertilizer source in tall fescue soils and on tall fescue grass blades.

Materials and Methods

➤General Study Information

Sample Site: WKU Agricultural Research and Education Complex, Bowling Green, KY

Fertilizer application: May 17, 2011

Fescue harvest and soil nutrient analysis dates: June 21, Aug. 9, and Oct. 6, 2011.

Microbial sampling: May 17, 18, 19, 21, 24, June 2, 8, 22, 29, July 13, Aug. 9 and Oct. 6, 2011.

Soil samples: Fifteen random soil cores were taken from each plot

before fertilizer and at each harvest.

Soil type: Crider silt loam (Typic Paleudalf)

≻Soil slope: 0-2%

>Statistical Design: split plot

➤ main plot variable=tillage

> subplot variable= fertilizer source

Table 1. Fertility treatments (total amount on a dry weight) basis) Voon

rear	DNI Mo ha ⁻¹ PI	PL	ı kg ha ⁻¹	C
			ng nu	
2011	5.5	16.3	N 225	0
			$P_2O_5 45 K_2O 180$	0
			K₂O 180	0

➢ Microbial Sampling Protocol

>25 g of manure or soil placed in 100 ml Buffered Peptone Water (BPW), shaken 10 mins at 200 rpm.

>500 μ l of BPW mix place in 4.5 ml of 0.1 XPBS for plating.

≻5 ml of BPW mix added to Bolton and UVM Broth for selective enrichment. >1 ml of BPW mix saved for molecular analysis and duplication.

After removal of the above aliquots, the remaining BPW was incubated at 37°C for 24 hours for broth inoculations.

▶ 5 organisms were targeted for detection: *Enterococci*, *E. coli*,

Campylobacter, *Listeria*, and *Salmonella* on their respective media and by molecular analysis.

Relationship of Soil Nutrient Content from Poultry Litter and Dairy Manure on Microbial Survival in Fescue Soils.

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> **Results and Discussion Comparison of Soil Measurements by Fertilizer Source and Tillage** (Tables 2 & 3)

-After a 2 factor repeated measure ANOVA showed no differences in fertilizer x time, tillage x time, fertilizer x tillage, and fertilizer x tillage x time interactions, data were averaged across the three harvests for statistical analysis (p<=0.05).

–pH were similar by tillage and fertility treatment (p<=0.05).

–P and K were equal by tillage treatment. P and K were equal in all fertility treatments except C which was lower than DM (p<0.05). - No differences between tillage or fertility treatments were detected in regards to Cu accumulations (p <= 0.05).

-Zn concentrations were higher in no-till compared to tilled fields. DM and PL had the highest Zn levels. PL, I, and C were all similar in Zn levels (p <= 0.05).

Initial Indicator Organisms in Manures (Data not shown) -Total cell counts were 1.98×10^{10} (+/- 5.6×10^{9}) cells g⁻¹ of poultry litter

and $1.10X10^7$ (+/- 7.4x10⁶) cells g⁻¹ in dairy manure. -Enterococci cell counts were 1.22×10^9 (+/- 3.33×10^8) cells g⁻¹ of poultry litter and 1.53×10^6 (+/- 5.72×10^5) cells g⁻¹ dairy manure.

Microbial Survival in Soils (Table 4)

-Statistical analysis was a two factor repeated measures ANOVA design.

-Fertilizer source (C vs. DM vs. PL) was significant over time with the *Enterococci* population (p<=0.05)(Figure 1).

-DM and C treatments did not change *Enterococci* populations over time. Populations in these treatments were similar to day 0 levels. -Enterococci populations spiked day 1 to 15 with the PL treatment with day 4 being highest. (Figure 1)

-Over time, both tillage treatments showed similar changes in regards to *Enterococci* populations (p<0.05). Both had peak *Enterococci* populations by day 4 and diminished from there (Figure 2).

 Table 2. Selected soil properties based upon fertility treatment.

		Fertility Treatment					
Soil	Control (C)	Dairy Manure	Poultry litter				
Measure		(DM)	(PL)				
pH	6.6 (±0.20) ^a	6.7 (±0.15) ^a	6.3 (±0.12) ^a				
		mg kg ⁻¹					
Р	41.91 (±11.51) ^b	69.55 (±44.41) ^a	55.62 (±9.87) ^{ab}				
K	88.32	203.58	165.64				
	(±17.53) ^b	(±24.94) ^a	(±15.78) ^{ab}				
Cu	4.18 (±0.63) ^a	8.73 (±2.26) ^a	4.57 (±0.55) ^a				
Zn	4.27 (±0.76) ^b	6.72 (±4.05) ^a	5.44 (±1.06) ^{ab}				
p<=0.05							

Soil	Tilla	Tillage Treatment					
Measurement	Till	No till					
рН	6.4 (±0.18) ^a	6.5 (±0.29) ^a					
		mg kg ⁻¹					
Ρ	58.00 (±25.50) ^a	48.60 (±25.31) ^a					
Κ	162.35 (±47.00) ^a	122.84 (±43.89) ^a					
Cu	6.48 (±0.86) ^a	4.18 (±0.92) ^a					
Zn	5.69 (±2.81) ^b	4.55 (±1.53) ^a					

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Inorganic **Fertilizer** (I)

 $6.3 (\pm 0.24)^{a}$

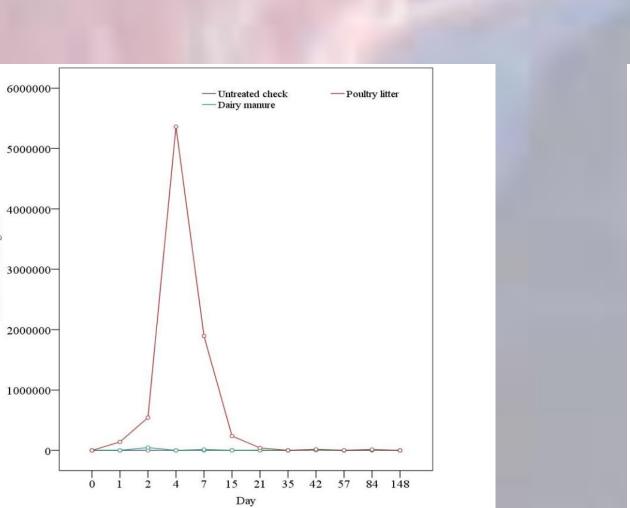
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46.03 (±11.99)^{ab} 1126.83 $(\pm 20.62)^{ab}$ $3.84 (\pm 0.34)^{a}$ $4.06 (\pm 0.45)^{b}$

Table 4. Average Enterococci populations over 148 day period from application.											
Days after application											
D0]	D1 D2	2 D4	D7	D15	D21	D35	D42	D57	D84	D148
				C	Cell no. g	g ⁻¹ soil ·					
Fertility Treatment											
6 ^a	37 ^a	156 ^a	637 ^a	481 ^a	1843 ^a	975 ^a	162 ^a	6212 ^a	68 ^a	1556 ^a	137 ^a
17	69	351	1685	821	4288	1853	360	7875	133	1896	109
62 a	125 a	46875 ^a	62 ^a	14881 ^a	837 ^a	931 ^a	793 ^a	3643 ª	75 ^a	7706 ^a	531 ^a
62	220	130566	138	2446	914	2554	1829	2802	125	11763	669
25 a	140125 ^b	542812 ^b	5362500 ^b	1894275 ª	239375 ^b	40687 ^b	1156 ^a	17481 ^a	1912 ^b	14706 ^a	718 ^a
6	73606	520956	4314655	3154278	169798	24065	1742	37615	2110	17951	1102
Tillage Treatment											
41 ^a	45729 ^a	293458 ^a	1971295 ^a	1770 ^a	59379 ^a	12533 ^a	708 ^a	3587a	362 ^a	9516 ^a	650 ^a
11	88075	480804	3945197	3727	92159	25552	1509	3524	621	15325	973
8 a	47795 ^a	99770 ^a	1604170 ^a	1271320 ^a	101991 ^a	15862 ^a	700 ª	14637 ^a	1008 ^a	6462 ^a	275 ^a
19	72091	251916	3185794	2679267	190651	21940	1493	30734	1970	10882	412
	D0 y Tr 6 ^a 17 62 ^a 62 25 ^a 6 25 ^a 6 17 41 ^a 11 8 ^a	D0 I J00 I J17 G9 G2 I J17 G9 J10 I J11 J J11 J	D0 D1 D2 D0 D1 D2 D1 D2 D2 D1 D2 D2 D1 D2 D2 D1 D2 D2 D2 D3 D2 D3 D3 D2 D3 D3 D2 D3 D3 D3 D3 D3 D3	D0 D1 D2 D4 6a 37 a 156a 637a 17 69 351 1685 62 a 125 a 46875a 62a 62 a 125 a 5362500b 138 62 a 73606 520956 4314655 71 a 45729a 293458a 1971295a 11 a 88075 480804 3945197 8a 47795a 99770a 1604170a	D0 D1 D2 D4 D7 O1 D2 D4 D7 D7 G1 D3 D4 D7 D7 G1 J1 D5 D4 D7 G1 J1 D5 637a 481a J7 G9 J51 16850 821 G2 J20 J30566 J38 2446 G2 J201 J30566 J382500 1894275a G1 J30606 520956 J314655 J154278 G1 45729a 293458a 1971295a 1770a J1 88075 480804 3945197 3727 Ra 47795a 99770a 1604170a 1271320a	D0 D1 D2 D4 D7 D15 D0 D1 D2 D4 D7 D15 Cell no. g Cell no. g Cell no. g Cell no. g y Trestment Cell no. g Cell no. g Cell no. g y Trestment J56° 637° 481° 1843° j 7 69 351 1685 821 4288 j 62° 125° 46875° 62° 14881° 837° j 69 351 1685 821 4288 j 69 351 26° 3237° j 62° 120° 337° 138 2446 914 j 62° 130566 138 2446 914 j 63006 520956 4314655 3154278 169798 j 64872° 520956 4314655 3154278 59379° j 11 88075 480804 3945197 3727 92159 j 604170° j 271320° 101991°	Days after application Days after application D0 D1 D2 D4 D7 D15 D21 Cell no. g ⁻¹ soil y Trestment 6 a 37 a 156a 637a 481a 1843a 975a 6 a 37 a 156a 637a 481a 1843a 975a 6 a 37 a 46875a 62a 14881a 837a 931a 62a 125 a 46875a 62a 14881a 837a 931a 62a 140125 542812b 5362500b 1894275a 239375b 40687b 62 3154278 149125 3154278 239375b 24065 Trestment 41a 85729a 293458a 1971295a 1770a 59379a 12533a 170a 59379a 25552 88 075 480804 3945197 3727 92159 25552 88 075 25252 88 075 480804 3945197 3727 92159 25552 88 075 99770a 1604170a 1271320a 101991a 15862a	Days after application D0 D1 D2 D4 D7 D15 D21 D35 Cell no. g ⁻¹ soil Treatment G ^a 37 ^a 156 ^a 637 ^a 481 ^a 1843 ^a 975 ^a 162 ^a 17 69 351 1685 821 4288 1853 360 6 ^a 125 ^a 46875 ^a 62 ^a 14881 ^a 837 ^a 931 ^a 793 ^a 62 220 130566 138 2446 914 2554 1829 25 a 140125 ^b 542812 ^b 536250 ^b 1894275 ^a 239375 ^b 40687 ^b 1156 ^a 6 73606 520956 4314655 3154278 169798 24065 1742 Treatment 41 ^a 45729 ^a 293458 ^a 1971295 ^a 1770 ^a 59379 ^a 12533 ^a 708 ^a 11 88075 480804 3945197 3727 92159 25552 1509 a 99770 ^a	Do D1 D2 D4 D7 D15 D21 D35 D42 O D1 D2 D4 D7 D15 D21 D35 D42 Cell no. g ⁻¹ soil Cell no. g ⁻¹ soil Televite Televite </th <th>Days after application D0 D1 D2 D4 D7 D15 D21 D35 D42 D57 Cell no. g⁻¹ soil Cell no. g⁻¹ soil Treatment 6^a 37^a 156^a 637^a 481^a 1843^a 975^a 162^a 6212^a 68^a 17 69 351 1685 821 4288 1853 360 7875 133 62^a 125^a 46875^a 62^a 14881^a 837^a 931^a 793^a 3643^a 75^a 62 220 130566 138 2446 914 2554 1829 2802 125 54 140125^b 542812^b 5362500^b 1894275^a 239375^b 40687^b 1156^a 17481^a 1912^b 6 73606 520956 4314655 3154278 169798 24065 1742 37615 2110 Treatment 41^a 45729^a 293458^a 1971295^a 170^a</th> <th>D0 D1 D2 D4 D7 D15 D21 D35 D42 D57 D84 or Cell no. g⁻¹ soil Cell no. ge⁻¹ soil Cell no. geometry D1 D2 D84 D7 D15 D21 D35 D42 D57 D84 or Cell no. g⁻¹ soil Cell no. g⁻¹ soil Cell no. geometry D1 D2 D84 D56 D21 D35 D42 D57 D84 or String Geometry String G16 D35 D42 D57 D84 or String G16 String M31 D84 D56 D1 D56 D1 D56 D1 D56 D1 D1 D1 D1 D2 D35 D46 D35 D42 D57 D84 O2 D35 D45 String D35 D45 D35 D35 D46 <thd35< th=""> <thd36< th=""> <thd36< th=""></thd36<></thd36<></thd35<></th>	Days after application D0 D1 D2 D4 D7 D15 D21 D35 D42 D57 Cell no. g ⁻¹ soil Cell no. g ⁻¹ soil Treatment 6 ^a 37 ^a 156 ^a 637 ^a 481 ^a 1843 ^a 975 ^a 162 ^a 6212 ^a 68 ^a 17 69 351 1685 821 4288 1853 360 7875 133 62 ^a 125 ^a 46875 ^a 62 ^a 14881 ^a 837 ^a 931 ^a 793 ^a 3643 ^a 75 ^a 62 220 130566 138 2446 914 2554 1829 2802 125 54 140125 ^b 542812 ^b 5362500 ^b 1894275 ^a 239375 ^b 40687 ^b 1156 ^a 17481 ^a 1912 ^b 6 73606 520956 4314655 3154278 169798 24065 1742 37615 2110 Treatment 41 ^a 45729 ^a 293458 ^a 1971295 ^a 170 ^a	D0 D1 D2 D4 D7 D15 D21 D35 D42 D57 D84 or Cell no. g ⁻¹ soil Cell no. ge ⁻¹ soil Cell no. geometry D1 D2 D84 D7 D15 D21 D35 D42 D57 D84 or Cell no. g ⁻¹ soil Cell no. g ⁻¹ soil Cell no. geometry D1 D2 D84 D56 D21 D35 D42 D57 D84 or String Geometry String G16 D35 D42 D57 D84 or String G16 String M31 D84 D56 D1 D56 D1 D56 D1 D56 D1 D1 D1 D1 D2 D35 D46 D35 D42 D57 D84 O2 D35 D45 String D35 D45 D35 D35 D46 <thd35< th=""> <thd36< th=""> <thd36< th=""></thd36<></thd36<></thd35<>

p<=0.05

Figure 1. Average Enterococci population change over time as influenced by fertilizer source



Summary

≻P and K concentrations were similar in all fertility treatments except C. >Zn concentrations were higher in no-till compared to tilled fields. DM and PL had the highest Zn levels.

>DM and C treatments did not change *Enterococci* populations over time with similar populations to day 0.

Enterococci populations spiked day 1 through 15 with the PL treatment with day 4 being highest.

References

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Figure 2. Average Enterococci population change over time influenced by tillage

