



### 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 <sup>-1</sup> PI	PL	ı kg ha <sup>-1</sup>	C
			ng nu	
2011	5.5	16.3	N 225	0
			$P_2O_5  45 K_2O  180$	0
			<b>K<sub>2</sub>O</b> 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  $\mu$ 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  $1.98 \times 10^{10}$  (+/-  $5.6 \times 10^{9}$ ) cells g<sup>-1</sup> of poultry litter

and  $1.10X10^7$  (+/- 7.4x10<sup>6</sup>) cells g<sup>-1</sup> in dairy manure. -Enterococci cell counts were  $1.22 \times 10^9$  (+/-  $3.33 \times 10^8$ ) cells g<sup>-1</sup> of poultry litter and  $1.53 \times 10^6$  (+/-  $5.72 \times 10^5$ ) cells g<sup>-1</sup> 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)	<b>Dairy Manure</b>	<b>Poultry litter</b>				
Measure		(DM)	(PL)				
pH	6.6 (±0.20) <sup>a</sup>	6.7 (±0.15) <sup>a</sup>	6.3 (±0.12) <sup>a</sup>				
		mg kg <sup>-1</sup>					
Р	41.91 (±11.51) <sup>b</sup>	69.55 (±44.41) <sup>a</sup>	55.62 (±9.87) <sup>ab</sup>				
K	88.32	203.58	165.64				
	(±17.53) <sup>b</sup>	(±24.94) <sup>a</sup>	(±15.78) <sup>ab</sup>				
Cu	4.18 (±0.63) <sup>a</sup>	8.73 (±2.26) <sup>a</sup>	4.57 (±0.55) <sup>a</sup>				
Zn	4.27 (±0.76) <sup>b</sup>	6.72 (±4.05) <sup>a</sup>	5.44 (±1.06) <sup>ab</sup>				
p<=0.05							

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

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# Inorganic **Fertilizer** (I)

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

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46.03 (±11.99)<sup>ab</sup> 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											
<b>D0</b>	]	D1 D2	2 D4	<b>D7</b>	D15	D21	D35	D42	D57	<b>D84</b>	D148
				C	Cell no. g	g <sup>-1</sup> soil ·					
Fertility Treatment											
<b>6</b> <sup>a</sup>	<b>37</b> <sup>a</sup>	156 <sup>a</sup>	<b>637</b> <sup>a</sup>	<b>481</b> <sup>a</sup>	1843 <sup>a</sup>	<b>975</b> <sup>a</sup>	162 <sup>a</sup>	6212 <sup>a</sup>	<b>68</b> <sup>a</sup>	1556 <sup>a</sup>	137 <sup>a</sup>
17	69	351	1685	821	4288	1853	360	7875	133	1896	109
62 a	125 a	<b>46875</b> <sup>a</sup>	62 <sup>a</sup>	14881 <sup>a</sup>	837 <sup>a</sup>	<b>931</b> <sup>a</sup>	<b>793</b> <sup>a</sup>	<b>3643</b> ª	75 <sup>a</sup>	<b>7706</b> <sup>a</sup>	<b>531</b> <sup>a</sup>
62	220	130566	138	2446	914	2554	1829	2802	125	11763	669
25 a	140125 <sup>b</sup>	542812 <sup>b</sup>	5362500 <sup>b</sup>	<b>1894275</b> ª	239375 <sup>b</sup>	<b>40687</b> <sup>b</sup>	1156 <sup>a</sup>	17481 <sup>a</sup>	<b>1912</b> <sup>b</sup>	<b>14706</b> <sup>a</sup>	<b>718</b> <sup>a</sup>
6	73606	520956	4314655	3154278	169798	24065	1742	37615	2110	17951	1102
Tillage Treatment											
<b>41</b> <sup>a</sup>	<b>45729</b> <sup>a</sup>	<b>293458</b> <sup>a</sup>	<b>1971295</b> <sup>a</sup>	<b>1770</b> <sup>a</sup>	<b>59379</b> <sup>a</sup>	12533 <sup>a</sup>	<b>708</b> <sup>a</sup>	3587a	<b>362</b> <sup>a</sup>	<b>9516</b> <sup>a</sup>	<b>650</b> <sup>a</sup>
11	88075	480804	3945197	3727	92159	25552	1509	3524	621	15325	973
<b>8</b> a	<b>47795</b> <sup>a</sup>	<b>99770</b> <sup>a</sup>	<b>1604170</b> <sup>a</sup>	1271320 <sup>a</sup>	<b>101991</b> <sup>a</sup>	15862 <sup>a</sup>	<b>700</b> ª	14637 <sup>a</sup>	<b>1008</b> <sup>a</sup>	<b>6462</b> <sup>a</sup>	275 <sup>a</sup>
19	72091	251916	3185794	2679267	190651	21940	1493	30734	1970	10882	412
	D0 y Tr 6 <sup>a</sup> 17 62 <sup>a</sup> 62 25 <sup>a</sup> 6 25 <sup>a</sup> 6 17 41 <sup>a</sup> 11 8 <sup>a</sup>	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 <sup>-1</sup> 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 <sup>-1</sup> soil         Treatment         G <sup>a</sup> 37 <sup>a</sup> 156 <sup>a</sup> 637 <sup>a</sup> 481 <sup>a</sup> 1843 <sup>a</sup> 975 <sup>a</sup> 162 <sup>a</sup> 17       69       351       1685       821       4288       1853       360         6 <sup>a</sup> 125 <sup>a</sup> 46875 <sup>a</sup> 62 <sup>a</sup> 14881 <sup>a</sup> 837 <sup>a</sup> 931 <sup>a</sup> 793 <sup>a</sup> 62       220       130566       138       2446       914       2554       1829         25 a 140125 <sup>b</sup> 542812 <sup>b</sup> 536250 <sup>b</sup> 1894275 <sup>a</sup> 239375 <sup>b</sup> 40687 <sup>b</sup> 1156 <sup>a</sup> 6       73606       520956       4314655       3154278       169798       24065       1742         Treatment         41 <sup>a</sup> 45729 <sup>a</sup> 293458 <sup>a</sup> 1971295 <sup>a</sup> 1770 <sup>a</sup> 59379 <sup>a</sup> 12533 <sup>a</sup> 708 <sup>a</sup> 11       88075       480804       3945197       3727       92159       25552       1509         a       99770 <sup>a</sup>	Do         D1         D2         D4         D7         D15         D21         D35         D42           O         D1         D2         D4         D7         D15         D21         D35         D42           Cell no. g <sup>-1</sup> soil           Cell no. g <sup>-1</sup> soil           Televite            Televite </th <th>Days after application         D0       D1       D2       D4       D7       D15       D21       D35       D42       D57         Cell no. g<sup>-1</sup> soil         Cell no. g<sup>-1</sup> soil         Treatment         6<sup>a</sup>       37<sup>a</sup>       156<sup>a</sup>       637<sup>a</sup>       481<sup>a</sup>       1843<sup>a</sup>       975<sup>a</sup>       162<sup>a</sup>       6212<sup>a</sup>       68<sup>a</sup>         17       69       351       1685       821       4288       1853       360       7875       133         62<sup>a</sup>       125<sup>a</sup>       46875<sup>a</sup>       62<sup>a</sup>       14881<sup>a</sup>       837<sup>a</sup>       931<sup>a</sup>       793<sup>a</sup>       3643<sup>a</sup>       75<sup>a</sup>         62       220       130566       138       2446       914       2554       1829       2802       125         54       140125<sup>b</sup>       542812<sup>b</sup>       5362500<sup>b</sup>       1894275<sup>a</sup>       239375<sup>b</sup>       40687<sup>b</sup>       1156<sup>a</sup>       17481<sup>a</sup>       1912<sup>b</sup>         6       73606       520956       4314655       3154278       169798       24065       1742       37615       2110         Treatment         41<sup>a</sup>       45729<sup>a</sup>       293458<sup>a</sup>       1971295<sup>a</sup>       170<sup>a</sup></th> <th>D0         D1         D2         D4         D7         D15         D21         D35         D42         D57         D84           or         Cell no.         g<sup>-1</sup> soil         Cell no.         ge<sup>-1</sup> soil         Cell no.         geometry         D1         D2         D84         D7         D15         D21         D35         D42         D57         D84           or         Cell no.         g<sup>-1</sup> soil         Cell no.         g<sup>-1</sup> 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 <sup>-1</sup> soil         Cell no. g <sup>-1</sup> soil         Treatment         6 <sup>a</sup> 37 <sup>a</sup> 156 <sup>a</sup> 637 <sup>a</sup> 481 <sup>a</sup> 1843 <sup>a</sup> 975 <sup>a</sup> 162 <sup>a</sup> 6212 <sup>a</sup> 68 <sup>a</sup> 17       69       351       1685       821       4288       1853       360       7875       133         62 <sup>a</sup> 125 <sup>a</sup> 46875 <sup>a</sup> 62 <sup>a</sup> 14881 <sup>a</sup> 837 <sup>a</sup> 931 <sup>a</sup> 793 <sup>a</sup> 3643 <sup>a</sup> 75 <sup>a</sup> 62       220       130566       138       2446       914       2554       1829       2802       125         54       140125 <sup>b</sup> 542812 <sup>b</sup> 5362500 <sup>b</sup> 1894275 <sup>a</sup> 239375 <sup>b</sup> 40687 <sup>b</sup> 1156 <sup>a</sup> 17481 <sup>a</sup> 1912 <sup>b</sup> 6       73606       520956       4314655       3154278       169798       24065       1742       37615       2110         Treatment         41 <sup>a</sup> 45729 <sup>a</sup> 293458 <sup>a</sup> 1971295 <sup>a</sup> 170 <sup>a</sup>	D0         D1         D2         D4         D7         D15         D21         D35         D42         D57         D84           or         Cell no.         g <sup>-1</sup> soil         Cell no.         ge <sup>-1</sup> soil         Cell no.         geometry         D1         D2         D84         D7         D15         D21         D35         D42         D57         D84           or         Cell no.         g <sup>-1</sup> soil         Cell no.         g <sup>-1</sup> 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

