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Fecal bacterial losses in runoff from conventional and no-till pearl millet fertilized with broiler litter☆

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ABSTRACT

Georgia farmers are increasing preemergence applications of soil residual herbicides to control glyphosate resistant weeds. To improve efficacy these herbicides are often activated by post-application irrigation. Broiler litter is commonly applied to fields before these herbicides. The herbicide wetting-in practice increases surface soil water content and may increase runoff and transport of broiler litter borne fecal bacteria into surface waters during subsequent storm events. Our objective was to determine differences in loads of fecal bacteria, *Escherichia coli* and *Salmonella* spp., in runoff from conventional tillage (CT) and no-till (NT) systems after herbicides were watered into an Ultisol fertilized with broiler litter. On replicated 6 m^2-plots ($n = 3$) simulated rainfall was applied for 70 min with composite runoff samples collected every 5 min and analyzed for *E. coli* and *Salmonella* spp. Although total runoff volume from the CT plots was significantly greater than from NT plots, no significant differences in total load of *E. coli* and *Salmonella* or the percent of total loads of *E. coli* and *Salmonella* recovered in runoff between tillage systems were observed. Total percentage of *Salmonella* recovered in runoff from both tillage systems was, however, four $\log_{10}$ orders of magnitude greater than the percentage of *E. coli* that was recovered. Difference in percentage recovered between the fecal indicator bacterium, *E. coli*, and the pathogen, *Salmonella*, underscores an apparent difference in hydrologic transport characteristics of these two fecal bacteria and casts doubts on the efficacy of *E. coli* as an indicator of risk to public health.

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1. Introduction

Conservation tillage practices such as no-till (NT) have been adapted in many parts of the United States (Endale et al., 2002). These systems accumulate crop residues and organic C at the soil surface and are effective at increasing water infiltration and reducing runoff and soil loss (Langdale et al., 1979; Bruce et al., 1995; Truman et al., 2003, 2005, 2007). With conservation tillage, broiler litter and other animal manures are commonly surface applied and not incorporated into the soil as with conventional tillage (CT). This practice may increase hydrological transport of fecal bacteria during storm events with runoff, contaminate surface water with these pathogens, and put public health at risk (Jenkins et al., 2012).

As a management practice, pre-emergent applications of soil residual herbicides require post-application rainfall or irrigation ("watering-in") to incorporate the active ingredient into the soil matrix (Prostko et al., 2001). Watering-in of soil residual herbicides leads to more effective weed control and their reduced runoff loss during subsequent rain events (Potter et al., 2008, 2011). This practice, however, increases soil antecedent water content (AWC). In turn this may increase runoff, sediment and nutrient loss, and enhance the potential of contaminating surface waters with fecal bacteria when rainfall events closely follow watering-in on fields where manures like broiler litter are used as fertilizer (Moore et al., 1995; Vermang et al., 2009). As part of CT and NT management protocols, broiler litter is applied before watering-in herbicides. In addition to the macronutrients N, P, and K, broiler litter contains fecal bacteria such as the fecal indicator bacterium *Escherichia coli* and pathogenic *Salmonella* spp. (Jeffrey et al., 1998). Their transport in runoff to surface water may adversely impact water quality.

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Our objective was to test the hypothesis that differences will be observed in loads of fecal bacteria in runoff from simulated variable rainfall events between CT and NT systems after herbicides are incorporated by watering-in.

2. Materials and methods

2.1. Study site and rain simulation

The study site was situated in the Atlantic Coastal Plain region near Tifton, GA, USA (N 31°26′, W 83°35′) on a Tifton loamy sand (fine-loamy, kaolinitic, thermic, plinthic, Kandiudult) and with a 3–4% slope. Mean annual precipitation is 1200 mm; climate is humid subtropical. Management of the study site (1998–2009) was previously described (Bosch et al., 2012; Potter et al., 2008; Truman and Nuti, 2010; Truman et al., 2011; Franklin et al., 2012). Half of the area was managed in conventional tillage while the other half was in conservation tillage. CT consisted of fall disking, spring disking and cultivator leveling. Initially strip tillage was practiced that consisted of a 10-cm tilled strip into which a crop was planted. The area in strip-tillage management was transitioned to NT in the Fall of 2009, prior to this study. This involved planting a winter cover crop mixture of rye (*Secale cereal*, 88 l ha^{-1}) and Austrian winter peas (*Lathyrus hirsutus*, 33.6 kg ha^{-1}) on all treatment areas. In the following month of April, 2010 the cover crop was terminated with a glyphosate application. Two weeks later, lime (2.2 Mg ha^{-1}) was applied to both tillage systems; this was followed by rolling the cover crop. Three weeks later, the CT plots were plowed and a field conditioner was run over the plots. In May 2010, pearl millet (*Pennisetum glaucum*, L.R. Br.) was planted. In the NT area the pearl millet was drilled directly into soil covered by the glyphosate killed cover crop. Percent residue at the time of planting in CT and NT treatment areas was 1 and 83%, respectively.

Broiler litter (4.98 Mg ha^{-1}, containing *E. coli* and *Salmonella* loads of 12.8 and 6.9 $\log_{10}$ cells ha^{-1}) obtained from a local poultry production facility was broadcast over CT and NT areas before planting. Previous studies (Jenkins et al., 2006, 2008a) quantified soil background concentrations of *E. coli* and *Salmonella* to be 3–4 $\log_{10}$ cells kg^{-1} surface (0–5 cm depth) soil and not detectable, respectively. Litter applied to the CT plots was incorporated (10–15 cm depth) with a field cultivator. The NT plots were not disturbed. Three replicate rainfall simulation plots (2 m × 3 m) were then randomly assigned to CT and NT treatments at time of planting pearl millet (six 2 m × 3 m plots total). Following a protocol for activating the herbicide, Callisto®, fertilized plots were pre-wetted with 12.7 mm (25 mm h^{-1} for 30 min) of well (irrigation) water using the rainfall simulator (Truman et al., 2011).

Rainfall simulations were initiated 24 h after the herbicide was watered-in. Simulated rainfall was applied with an oscillating nozzle that used 80,150 Veejet nozzles (Potter et al., 2008; Truman et al., 2007; Truman and Nuti, 2010) for 70 min at a variable rate of intensity as Truman et al. (2011) described. Mean total simulated rainfall applied to CT and NT plots was 54.7 and 52.8 mm, respectively. The water used for simulations had a pH of 7.0, an electrical conductivity of 0.002 S cm^{-1}, and temperature of 22.4 °C.

Runoff was collected at the down-slope end of each 6-m^2 plot every five min in separate sterile, stainless steel containers. Total runoff volumes and sediment loads were quantified (Truman et al., 2011). Twenty-five-mL aliquots were taken from each 5-min interval sample for microbiological analysis and composited for time intervals 0–15, 20–25, 30–35, 40–45, 50–55, and 60–70 min. Ten-mL aliquots were either diluted by additional 10-fold dilutions or used directly to inoculate 90 mL of Colilert substrate as described below. For *Salmonella* determinations the remaining volume of each composited sample was concentrated by centrifugation at 10,000 × *g* for 30 min at 5 °C. Pellets from each composited sample were themselves composited and a final volume of 5-mL in sterile phosphate buffered saline (PBS) (Clesceri et al., 1998) was used to inoculate a five-tube, three-by-three 10-fold dilution scheme described below. Thus, determination of the load of *Salmonella* was for the total volume of runoff.

Table 1
Mean total applied rainfall, and mean total runoff by tillage practice, and mean percentage of *E. coli* and *Salmonella* recovered in runoff from their respective loads in the litter applied to the conventionally tilled (CT) and no-till (NT) treatment plots.

Treatment	Total applied rainfall (L)	Total runoff (L)	*E. coli* (%)	*Salmonella* (%)
CT	328.2[a]	217.0[a]	2.07×10^{-3}[a]	25.5[a]
NT	316.8[a]	64.9[b]	2.05×10^{-3}[a]	16.7[a]

Different letters by means per treatment indicate differences at $P < 0.05$.

2.2. Microbiological analysis

Concentrations of *E. coli* in runoff and broiler litter samples were determined with a commercial Colilert kit (IDEXX, Atlanta, GA) (Jenkins et al., 2008a). This kit represents a defined substrate technology (Edberg et al., 1990), and is a semi-automated most probable number (MPN) methodology. A modification of the MPN method for determining concentrations of *Salmonella* spp. in environmental samples (Jenkins et al., 2008b, 2012) was made: Rappaport-Vassiliadis (RV) broth instead of tetrathionate broth was used. From RV tubes showing growth, 1-mL aliquots were removed and washed by centrifugation in PBS, resuspended in PBS and assayed directly with a TaqMan PCR assay (Jenkins et al., 2012). Most probable number (MPN) determinations for *Salmonella* were calculated by the number of positive tubes with all determinations having improbability ratios >0.0001 (Briones and Reichardt, 1999; Garthright and Blodgett, 2003; Blodgett, 2005; FDA, 2013).

2.3. Data analysis

The load of *E. coli* and *Salmonella* spp. in runoff was the product of their concentrations and runoff volume for each respective composited time interval per plot and expressed per hectares. Total loads of *E. coli* and *Salmonella* per tillage treatment were the sum of the loads per sampling time intervals and expressed per hectares. Percent load of *E. coli* and *Salmonella* was determined by: % load = (load of *E. coli* or *Salmonella* recovered in runoff)/(load of *E. coli* or *Salmonella* applied with litter) × 100. Bacterial concentrations were transformed to natural log before performing analysis of variance and regression analyses. Analysis of variance with significant differences ($P \leq 0.05$) between treatments was undertaken with Proc Mixed (SAS, 2004; Littell et al., 2006). Relations between runoff volumes and sediment mass in runoff and *E. coli* loads were analyzed with Proc Reg (SAS, 2004).

3. Results and discussion

3.1. Runoff

Significant differences in runoff volumes were evident between the two tillage practices (Fig. 1). Mean total volume from NT plots was >150 L less than that of CT plots (Table 1). As expected, the greatest runoff rates were observed during periods of greatest rainfall intensity (rise, peak, and fall of the hydrograph) which occurred between the 15 and 40 min sampling intervals. In a previous rainfall simulation study, Jenkins et al. (2008a) reported a similar observation for a variable intensity rainfall simulation on a Southern Piedmont Cecil soil. In a prior study at the site used in the