

Research Article

Cover Crop and Fertility Effects on *Escherichia coli* Abundance in a Composted Poultry Litter-Amended Silt Loam Soil

Sait Sarr,¹ Mark Coyne ,² Maheteme Gebremedhin ,³ Avinash Topè,³ and Shreya Patel³

¹School of Urban and Public Affairs, University of Louisville, Louisville, KY 40208, USA

²Department of Plant and Soil Sciences, University of Kentucky, Lexington, KY 40546, USA

³College of Agriculture, Communities, and the Environment, Kentucky State University, Frankfort, KY 40601, USA

Correspondence should be addressed to Maheteme Gebremedhin; maheteme.gebremedhin@kysu.edu

Received 18 October 2019; Accepted 23 January 2020; Published 20 February 2020

Academic Editor: Amaresh K. Nayak

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Soil conservation practices such as cover crops can improve crop production, soil quality, and water quality. Cover crops can also influence soil microbial growth and activity. Cover cropped and manured soils can potentially store and transmit fecal bacteria (e.g., *E. coli*) to surface water if runoff and subsurface seepage occur. While many studies have shown the soil health benefits of cover crops, fewer studies have evaluated the extent to which cover crops influence the abundance of potential waste-borne pathogens. A two-year study (2015–2017) was conducted on a limited-resource farm in Logan County, Kentucky, USA, to quantify the abundance of the fecal indicator *Escherichia coli* (*E. coli*) bacteria (as a proxy for fecal coliforms) in treatments with and without cover crops or composted poultry litter. The cover crop consisted of a cereal rye (*Secale cereale* L.), Austrian winter pea (*Pisum sativum* L.), and crimson clover (*Trifolium incarnatum* L.) mix. Summer crops consisted of a no-till maize (*Zea mays* L.)—soybean (*Glycine max* L.) rotation. Soil samples were taken before and after each summer crop season and assessed to detect and enumerate *E. coli*. At the end of the study period, no significant treatment differences in the *E. coli* abundance in soil were detected (ca. 10^4 CFU·g⁻¹) ($p > 0.05$). However, season/time was a significant factor ($p < 0.05$). We conclude that the background *E. coli* already present in soil was sufficiently high, inhibiting the detection of the influence of added composted litter. These indigenous *E. coli* were unaffected by cover crop and nutrient management but did fluctuate on a seasonal basis.

1. Introduction

Conservation management practices can improve crop production and soil quality [1, 2]. There are numerous benefits derived from using cover crops, including protecting the soil from erosion [3, 4], enhancing soil mineralization [2, 5], suppressing weeds and soilborne diseases [6], more efficient nutrient cycling [7], and improving soil quality and yield [8, 9]. From a microbial perspective, cover crops improve soil aeration, increase water holding capacity, provide available carbon (C), and enhance soil microbial growth and activity [10]. However, soil can be a reservoir for transmitting fecal pathogens if use of manure is one of the conservation practices [11], and soil-borne pathogens can contaminate farm produce, particularly vegetables, at the crop production level.

Efficient integration and application processes along with cover cropping in the long-term and sustainable use of animal manure can control pathogens [12], but there have been few studies investigating how cover crops influence the persistence of fecal bacteria in soil. Schutter et al. [13] observed that season was the key determinant of microbial community structure, rather than winter or summer cover crop use; *E. coli* numbers declined as temperatures fell. Jiang et al. [14] also found rapid decline in *E. coli* numbers in manured soils at lower temperatures. Other factors influencing the presence and survival of fecal bacteria include soil type, pH, manure application rate, nutrient availability, and competition [15]. Reed-Jones et al. [11] showed that time, bacterial species, temperature, precipitation, and tillage influenced the relationship between cover crops and bacterial numbers in soils. Cover crop was a factor in *E. coli*

persistence in one year, but elevated populations did not persist year-to-year.

Untreated manure can carry harmful bacteria (e.g., pathogens) to soil, which can survive for weeks and transfer to the edible crop parts with time [16]. Land-application of animal manure also enables bacterial pathogens to reach groundwater and adversely affect ecosystem and human health [17–20]. Though it is normally a benign commensal organism and used as a proxy for enteric pathogens, several *E. coli* strains are pathogenic and can cause severe hemorrhagic colitis and hemolytic uremia in humans [15, 21–23].

Our previous research suggests that combining cover crops and poultry litter has additive effects on soil nitrogen (N) mineralization [2] and crop biomass [24]. The objective of this study was to quantify abundance of fecal indicator bacteria (*E. coli*—a proxy for fecal coliforms and more serious pathogens) in different poultry litter compost-amended and chemical fertilizer treatments, with and without cover crops. The study was conducted over a 2-year period. Based on previous research, our hypothesis was that cover crops would have no effect on the abundance and survival of fecal indicator bacteria.

2. Materials and Methods

2.1. Research Site and Experiment Design. Starting in Fall 2015, a 0.32 ha study site was established at a farm in Logan County, Kentucky (36.88°N; 86.60°W, altitude 183 m). The site has a temperate humid continental climate with mean annual temperature and precipitation ranging between 7 and 21°C and 991 to 1524 mm, respectively (Figure 1). The area is frost-free much of the year (154–219 frost-free days) with an average growing season from May to September, and an average temperature of 22°C during the growing season (Figure 1). According to USDA soil taxonomy, the soil is classified as Crider silt loam (fine-silty, mixed, active, mesic Typic Paleudalfs), well-drained with 2–6% slopes. During the study period, maize (*Zea mays* L.) and soybean (*Glycine max* L.) were grown in a rotation with a cover crop mix of cereal rye (*Secale cereale*), Austrian winter pea (*Pisum sativum*), and crimson clover (*Trifolium incarnatum*).

The field experiment was a randomized complete block design with four replications (blocks). Six treatments were randomly applied to plots within blocks: (1) an unamended control; (2) cover crops (CC); (3) poultry litter compost (PL); (4) poultry litter compost and cover crop (PL + CC); (5) synthetic fertilizer (NPK); and (6) fertilizer and cover crop (NPK + CC). Poultry litter compost was applied on an N-rate basis, and chemical fertilizers consisting of synthetic compounds were applied at established rates recommended by the University of Kentucky's Cooperative Extension Service [2, 26]. Poultry litter compost was applied at a rate of 1.78 Mg·ha⁻¹ in Spring 2016 when the summer crop was soybean and 26.3 Mg·ha⁻¹ in Spring 2017 when the summer crop was maize.

2.2. Soil Collection and Laboratory Methods. Twenty-four soil core samples (consisting of four composite soil cores each) were collected from surface soil (0–15 cm depth) prior

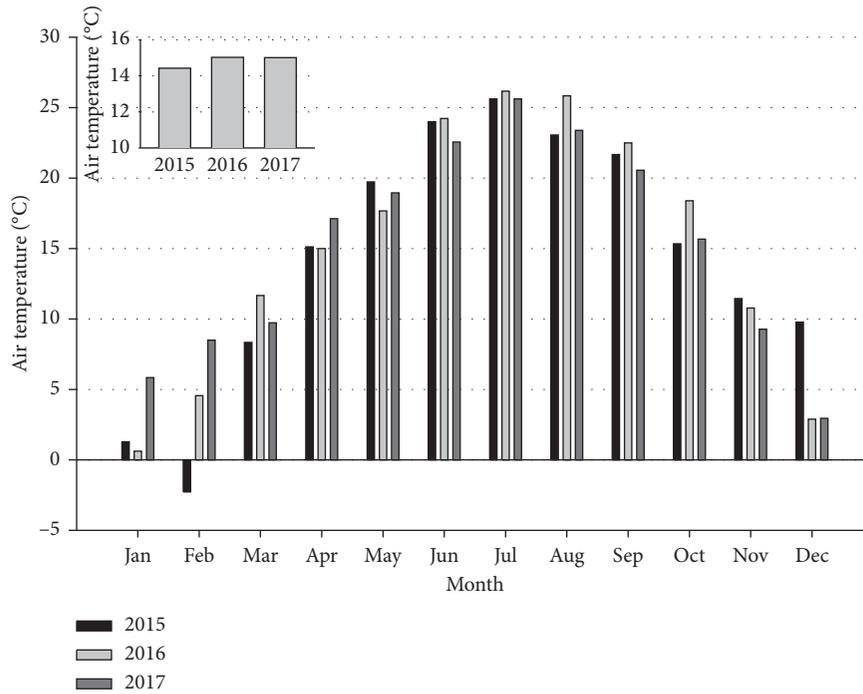
to treatment applications in Spring and at harvest in Fall each year (Table 1). Soil cores were obtained using a soil core sampler (inner diam = 7 cm), and samples were taken from between rows within each treatment plot. Plant residues and nonsoil materials were removed, and large soil clods were broken by hand.

The soil samples, placed on ice, were transported to Kentucky State University for further analysis. Microbial analyses started within 24 h of field data collection. The soil samples were analyzed to detect and enumerate *E. coli*. Approximately, 5 g of soil was weighed and added to 10 ml of sterile saline solution. One millilitre of this mixture was used to perform a serial dilution up to 1000 folds. One millilitre of the mixture from the 1000-fold dilution was used to inoculate triplicate plates of 3 M™ Petrifilms to test for the presence of *E. coli*. The Petrifilms were incubated at 37°C for 48 hours, after which positive colonies on each Petrifilm were enumerated. *E. coli* in the poultry litter compost was also tested by a similar procedure prior to application. All results are expressed as colony forming units per gram of soil or poultry litter compost on a dry weight basis. The values of the fecal bacteria (*E. coli*) count were log transformed for statistical purposes. For reference purposes, baseline samples were taken in Fall 2015 before the start of the study.

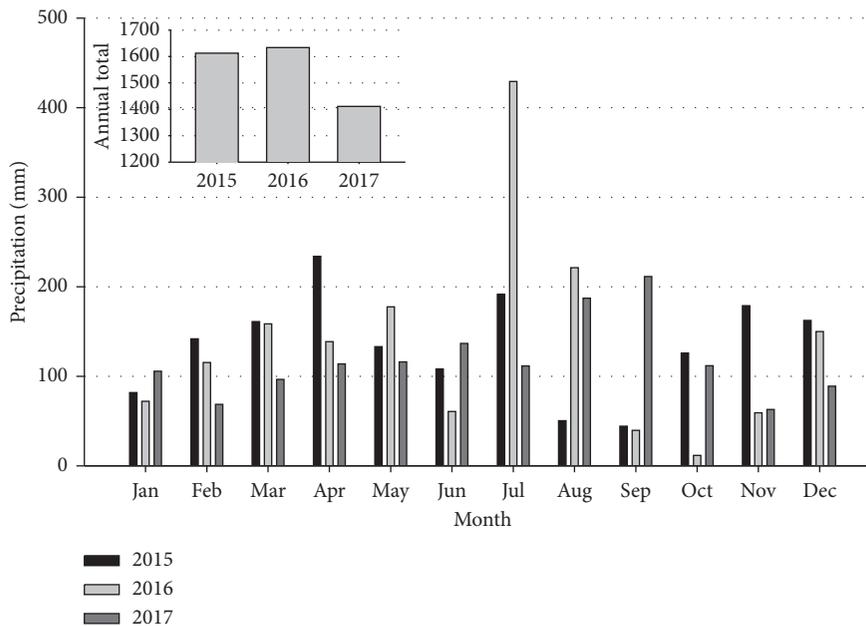
2.3. Statistical Methods. Treatment effects on fecal bacterial (*E. coli*) numbers with time were assessed by comparing treatments, including the cover crop treated plots and the noncover crop treated plots. We also compared treatment means between sampling periods in a given year. A one-way analysis of variance (ANOVA) to compare means was used based on Tukey's test (a single-step, multiple comparison statistical test used to find means that are significantly different from each other). A significant *F*-test was declared at $p < 0.05$. Analysis was performed using SPSS (IBM SPSS Statistics version 22).

3. Results and Discussion

The site had a baseline mean *E. coli* count of 6.3×10^4 CFU·g⁻¹ soil in Fall 2015 prior to planting cover crops. This was attributed to the site having been fertilized with poultry litter for the past five years (personal communication with the owner). A newly poultry litter-amended silt loam soil in Kentucky studied by Coopridge and Coyne [27] had soil fecal coliform numbers between 2×10^3 and 6.4×10^4 CFU·g⁻¹, while a similar unamended soil had fecal coliform numbers of $1\text{--}2 \times 10^2$ CFU·g⁻¹ [28]. The litter from the poultry source throughout the study was composted rather than fresh. Consequently, the *E. coli* count in the poultry litter compost itself prior to application was only 66 CFU·g⁻¹ in 2016 and 6.3×10^3 CFU·g⁻¹ in 2017. These numbers are quite low relative to the reported values of *E. coli* in fresh poultry litter (ca. 10^7 ·g⁻¹) [29]. It is reasonable to expect low *E. coli* count in composted and dried material. From the perspective of land application a decline in *E. coli* count is expected, thus making it a safer option to fresh litter. Considering treatments without poultry litter compost



(a)



(b)

FIGURE 1: Average monthly air temperature (yearly average inset) and precipitation (yearly average inset) at the Logan farm for 2015, 2016, and 2017. Precipitation records are from the Logan County Mesonet Weather Data (38.12°N; 84.88°W) [25].

amendment each year had statistically the same *E. coli* counts as poultry litter compost-treated soils, we can conclude that poultry litter compost amendment in and of itself did not change the *E. coli* concentration either year. In 2016, the litter compost application rates would have changed soil *E. coli* concentrations <0.01% and in 2017 <0.5%. Consequently, our study principally evaluated how adding the poultry litter composts itself and cover crops influenced the resident soil *E. coli* concentration.

In Spring 2016, neither poultry litter compost nor cover crop treatment were significant factors ($p > 0.05$; Figure 1) for *E. coli* concentration. *E. coli* counts among all treatments sharply decreased from the Fall 2015 assessment. In contrast, *E. coli* counts significantly increased from Spring 2016 (before poultry litter compost application) to Fall 2016 ($p < 0.05$; Figure 1). The greatest increase in *E. coli* count (+3.1 log CFU·g⁻¹ soil) occurred with the NPK + CC treatment, and the least increase (+2.2 log CFU·g⁻¹ soil) occurred

TABLE 1: Soil sampling, and cover crop planting and termination dates.

Year	Season	Cover crop/main crop	Planting date	Harvest/termination date
2015-2016	Fall	Mix of cereal rye, Austrian winter pea, and crimson clover	15 October 2015	5 May 2016
	Summer	Soybean	25 May 2016	26 October 2016
2016-2017	Fall	Mix of cereal rye, Austrian winter pea, and crimson clover	27 October 2016	5 May 2017
	Summer	Field maize	7 June 2017	25 October 2017
2017-2018	Fall	Mix of cereal rye, Austrian winter pea, and crimson clover	26 October 2017	30 May 2018

with the PL + CC treatment. Neither cover crop nor poultry litter compost treatment was significant. Other studies [11, 18, 28] found a strong correlation between *E. coli* persistence in Spring and Fall sampling periods, with weather-related factors such as temperature and precipitation causing significant declines during the season. So, the decline from Fall 2015 to Spring 2016 was predictable. The increase in *E. coli* counts during the season was not predicted. In a no-till practice, cover crops can conserve soil moisture and serve as insulators that buffer soil temperature fluctuations [30, 31]. Spring 2016 was colder than in 2017, while the months from February to August were wetter (Figure 1), and this may account for the resilience of the resident soil *E. coli* present from prior years (Figure 2).

In 2017, *E. coli* counts usually declined among all treatments from Spring 2017 to Fall 2017 (Figure 3). The greatest decline in the *E. coli* ($-1.3 \log \text{CFU} \cdot \text{g}^{-1} \text{soil}$) counts occurred with the PL + CC treatment, but differences among treatments were not statistically significant ($p > 0.05$). The decline was not nearly as considerable as one would expect from freshly added fecal bacteria as demonstrated by Reed-Jones et al. [11] and Stoddard et al. [18] in which non-detectable baseline *E. coli* values were reached several months after application.

The *E. coli* count in Spring 2017 was much higher than in Spring 2016 regardless of treatment. January and February 2016 were substantially colder than the corresponding months in 2017, which could account for the difference in persistence. Despite the higher litter compost application rate and *E. coli* counts, the trend was for *E. coli* counts to slightly decline with time in 2017 in contrast to 2016 when counts increased; 2016 had slightly more precipitation than 2017 and substantially more in the key summer months of July and August, which may account for the different trends.

The absence of a treatment effect and the similarity of *E. coli* counts in soil without any added poultry litter compost

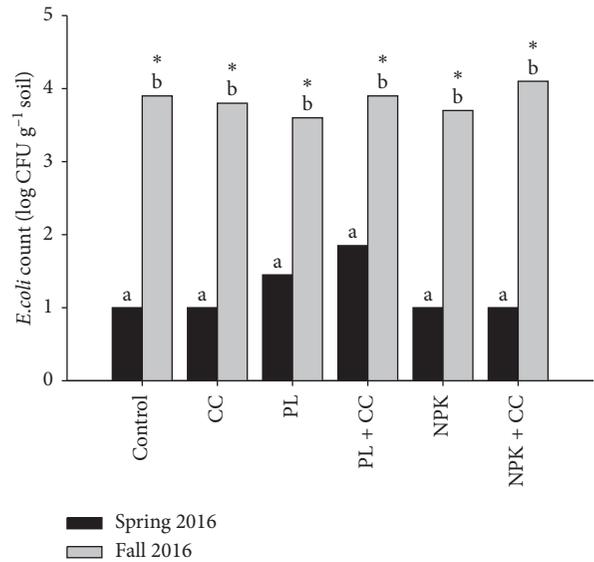


FIGURE 2: Treatment effects of poultry litter compost (PL) and chemical fertilizers (NPK) with or without cover crops (CC) and an unamended control on the numbers of *E. coli* ($\log \text{CFU} \cdot \text{g}^{-1} \text{soil}$) in Spring and Fall of 2016. Different letters above each bar indicate significant differences between treatments within a sample period at $p < 0.05$. Within a treatment, asterisks indicate significant difference between sampling periods (Spring and Fall in a given year) at ($p < 0.05$). Only sample season was a significant effect.

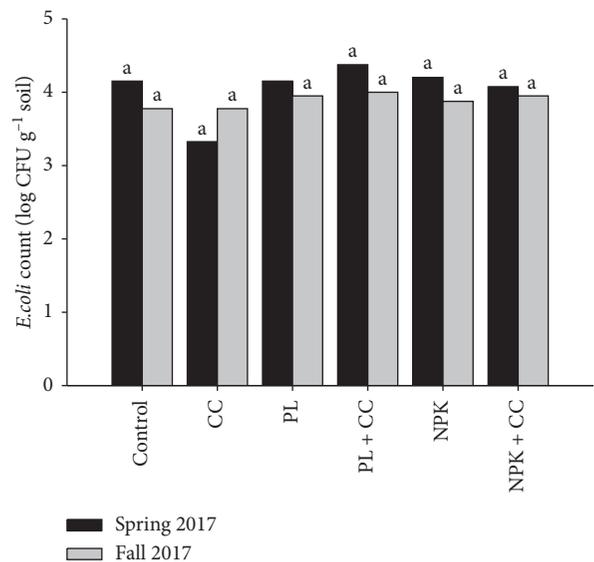


FIGURE 3: Treatment effects of poultry litter compost (PL) and chemical fertilizers (NPK) with or without cover crops (CC) and unamended control on the numbers of *E. coli* ($\log \text{CFU} \cdot \text{g}^{-1} \text{soil}$) in Spring and Fall of 2017. Different letters above each bar indicate significant differences between treatments within a sample period at $p < 0.05$. There were no significant time or treatment effects.

strongly suggest seasonal changes in the indigenous soil *E. coli* rather than an influence of the *E. coli* in poultry litter compost. Cover crop presence had no effect. The reduction in soil *E. coli* from 2015 to 2017 may suggest a trend to lower

background *E. coli* from season to season as poultry litter compost is added at agronomically relevant rates instead of fresh litter. Litter effects on the soil microbial community persisted for as long as 4 years in a study with poultry litter amended cotton in Arizona [32] and, overall, increased diversity of the resident population even as the culturable fecal coliforms in the soil became undetectable. The benefits of poultry litter compost addition on the soil microbial population, therefore, appear to outweigh the potential detriment of adding fecal organisms.

4. Conclusion

Cover crops had no influence on *E. coli* dynamics. There was no significance to the combined effects of cover crops and poultry litter compost compared to the use of either or alone on the number of fecal bacteria (e.g., *E. coli*) in either year (2016 and 2017). The effect of the crop treatment could have been limited by the interaction of the different cover crop species (soybean vs. maize), precipitation, temperature, and time. The fluctuation in the number of *E. coli* was statistically significant between sampling periods in a given year, but the time between sampling periods to detect *E. coli* was also long. Many interactions that play a role in the population dynamics of *E. coli* could have been missed between the Spring and Fall sampling periods. Prior work has shown that temperature and moisture (rainfall) are key drivers in the population dynamics of *E. coli* and that also appears to be demonstrated in this study. Cover crops neither increased nor decreased the soil *E. coli* counts.

Data Availability

Data used for this study can be obtained upon request made to Maheteme Gebremedhin (Maheteme.gebremedhin@kysu.edu).

Disclosure

The content is solely the responsibility of the authors and does not necessarily represent the official views of Kentucky NRCS.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments

This study was fully supported by Kentucky's NRCS Conservation Innovation Program (Grant no. 68-5C16-15-5251). Sait Sarr acknowledges the graduate assistantship from the College of Agriculture, Communities, and the Environment at Kentucky State University. The authors extend their gratitude to the following individuals for their assistance during the design, field sampling, data collection, and laboratory analysis: Jason Simmons, Tierra Freeman-Taylor, Edwin Chavous, Ronald Bunton, and Leroy Ballew.

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