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Microbial Transport as Affected by Residue Cover and Manure Application Rate

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Abstract. *Manure is applied to cropland areas with varying surface cover to meet single- or multiple-year crop nutrient requirements. The objectives of this field study were to (1) examine microbial transport following land application of manure to sites with and without wheat residue, (2) compare microbial loads following land application to meet the 0, 1, 2, 4, and 8-year P-based requirements for corn, and (3) evaluate the effects of rainfall simulation run on microbial transport. Manure was added and incorporated by disking plots that were 0.75 m wide by 2.0 m long. Three 30 min simulated rainfall events, separated by 24 h intervals, were then applied at an intensity of 70 mm h*$^{-1}$*. Plots containing wheat residue had a total coliform load of 12.6 log CFU ha*$^{-1}$*, which was significantly greater than the 12.4 log CFU ha*$^{-1}$* measured on the plots without wheat residue. The plots with and without wheat residue had transport rates of* E. coli *and enterococci that were not significantly different. The plots on which manure was added at rates varying from 5.4 to 42.8 Mg ha*$^{-1}$* had counts of total coliforms and enterococci that were not significantly different. Rainfall simulation run did not significantly affect measurements of phages, total coliforms, or enterococci. Transport of selected microbes was found to be significantly affected by residue cover, manure application rate, and rainfall simulation run.*

Keywords. *Bacteria, Cattle manure,* E. coli*, Feedlots, Land application, Manure management, Manure runoff, Microbial, Microorganisms, Runoff.*

Sustainable agricultural systems use manure as a valuable source of micro- and macronutrients for crop production. Manure contributes to soil organic matter (Gerzabek et al., 2001; Romanyà et al., 2012; Garcia-Pausas et al., 2016), increases soil microbial biomass, and improves soil quality (Fraser et al., 1988; Doran and Zeiss, 2000; Emeades, 2005). Sustainable agricultural systems must manage manure applications to minimize adverse environmental impacts, including the offsite transfer of bacterial pathogens (Jamieson et al., 2004). Pathogens can be rapidly mobilized and transported from soils by overland flow once manure has been applied to the soil surface (Bradford et al., 2013.) The transport of fecal bacteria in runoff is enhanced if rainfall occurs soon after manure addition (Mishra et al., 2008).

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Incorporation of manure following land application helps to conserve nutrients, reduce odors, and affects microbial concentrations in runoff (Jackson et al., 2003; Gilley et al., 2007). However, tillage practices, such as disking, can increase the potential for erosion and decrease the amount of crop residue on the soil surface. Manure is applied to cropland areas under a variety of tillage and management conditions that result in varying amounts of surface residue cover. The manure application method is known to influence microbial release and subsequent transport from land application areas (Blaustein et al., 2015); however, the specific contributions of residue cover to microbial release from manure are not well understood. Little information is currently available concerning the effects of residue cover on microbial loads in runoff from sites where manure had been applied and then incorporated by tillage.

Manure is often applied each year to meet crop nutrient requirements. However, labor, equipment, and land application costs can be reduced if manure is added to meet multiple-year crop nutrient requirements (Bremer et al., 2007). The amount of manure applied impacts the total number of microorganisms present on the soil surface. The effects of multiple-year manure application on microbial transport have not been well quantified.

The objectives of this field study were to (1) examine microbial transport following land application of manure to sites with and without wheat residue, (2) compare microbial transport following land application of manure to meet the 0, 1, 2, 4, and 8-year P-based requirements for corn, and (3) evaluate the effects of rainfall simulation run on microbial transport.

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MATERIALS AND METHODS

STUDY SITE CHARACTERISTICS

Field tests were conducted in June and July 2010 at the University of Nebraska Roger's Memorial Farm located 18 km east of Lincoln, Nebraska, in Lancaster County. Additional details concerning the experimental study are provided by Thayer et al. (2012). The site had been cropped using a grain sorghum (*Sorghum bicolor* (L.) Moench), soybean (*Glycine max* (L.) Merr.), and winter wheat (*Triticum aestivum* L. cv. Pastiche) rotation, under a no-till management system, and was planted to winter wheat during the 2008-2009 cropping season. Herbicide was applied as needed to control weed growth. The Aksarben (formerly Sharpsburg) silty clay loam at the site (fine, smectitic, mesic Typic Argiudoll) contained 16% sand, 52% silt, 32% clay, 4.6% organic matter, and 2.50% total carbon in the top 8 cm of the soil profile. The soil at the site developed in loess under prairie vegetation and had a mean slope of 6.0%.

EXPERIMENTAL DESIGN

Thirty plots were established across the slope using a randomized block design (fig. 1). Each of the experimental treatments, which included residue cover (wheat residue or no wheat residue) and manure application rate (0.0, 5.4, 10.7, 21.4, or 42.8 Mg ha^{-1}), were replicated three times. Rainfall simulation tests were performed separately by block over a five-week period from 29 June to 29 July 2010, with tests conducted on six plots each week. Three 30 min rainfall simulation runs separated by approximately 24 h intervals were conducted on each of the plots.

PLOT PREPARATION

Beef cattle manure was collected from feedlot pens located at the U.S. Meat Animal Research Center near Clay Center, Nebraska. Calves born during the spring of 2009 were placed in the pens in October 2009 and fed a corn-based diet. Replicated samples obtained from the feedlot were used to determine the physical and chemical characteristics of the manure. The manure samples were placed in plastic bags and mailed the day of collection by overnight delivery to a commercial laboratory for analyses. The commercial laboratory typically completed the analyses within 24 h after receipt of the samples.

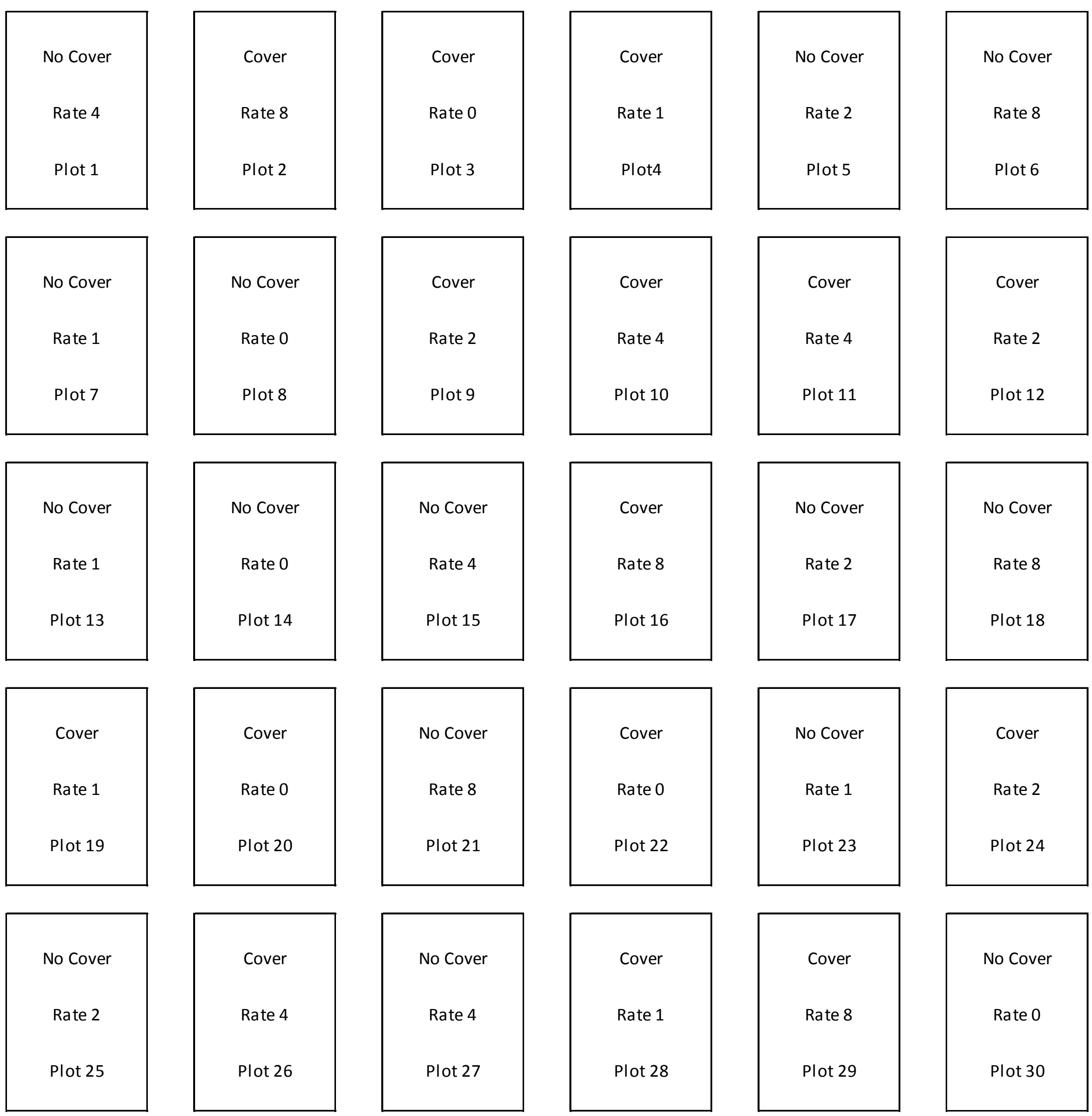

Figure 1. Schematic of plot layout, wheat residue cover, and manure application rate based on 0, 1, 2, 4, or 8-year P requirement for corn.

Wheat residue cover was first removed from 15 of the plots by hand raking, while the other 15 plots remained undisturbed. Manure was then added to the experimental plots on 19 May 2010 at rates of 0.0, 5.4, 10.7, 21.4, or 42.8 Mg ha^{-1} to meet the 0, 1, 2, 4, or 8-year P-based application requirement for corn (25.8 kg P ha^{-1} for an expected yield of 9.4 Mg ha^{-1}) (table 1). A total of 27.7 cm of rainfall occurred between the manure application on 19 May 2010 and the initiation of the rainfall simulation tests on 29 June 2010.

This study was conducted to measure microbial transport in runoff beginning 41 days after manure application and incorporation. The largest number of pathogens in runoff would be expected immediately after land application. Previous studies have been conducted to measure pathogen transport from feedlot surfaces (Gilley et al., 2008, 2009) and from land application sites immediately after manure addition (Durso et al., 2011; Thurston-Enriquez et al., 2005).

When calculating manure application rates, it was assumed that N and P availability from the beef cattle manure was 40% and 85%, respectively (Eghball et al., 2002). Supplemental urea ($(NH_2)_2CO$) fertilizer N (39-0-0, N-P-K) was added at rates required to meet annual N crop requirements (151 kg N ha^{-1} for an expected yield of 9.4 Mg ha^{-1}). The 4-year P application rate was less than the 1-year N requirement (table 1). Residual soil nutrient content and the concentration of nutrients in the irrigation water were not considered when calculating the manure and supplemental N fertilizer requirements.

The manure was collected from the feedlot and placed in 19 L plastic buckets. After results from the laboratory analyses were available, the amount of manure required to meet P application requirements was calculated. The required amount of manure was then distributed uniformly by hand across the surface using the 19 L buckets. Soil may be transported from its original location as part of the disking operation. Therefore, manure was added to an area slightly larger than the final plot dimensions to provide more uniform application over the experimental area.

A 5 m tandem finishing disk was used to lightly incorporate the applied manure to a depth of approximately 8 cm. The applied manure appeared to be distributed throughout the tillage zone on both the cover and no residue cover treatments. Disking (single pass) occurred up and down the slope in the direction of overland flow. This condition provided a greater runoff and soil loss potential than would have occurred if tillage had been conducted along the contour. If disking had occurred across the slope contour, perpendicular to the direction of overland flow, there would have been a greater opportunity for the transport of soil between plots containing different amounts of manure and/or supplemental N fertilizer. Residue cover at the time of the rainfall simulation tests on the cover and no residue cover treatments was 90% and 5%, respectively.

Table 1. Manure and nutrient application rates.

P Application Interval[a] (year)	Manure Application (Mg ha^{-1})	Total Manure N (kg ha^{-1})	Total Fertilizer N (kg ha^{-1})	Total Manure P (kg ha^{-1})
0	0.0	0	0	0
1	5.4	32	119	26
2	10.7	64	87	52
4	21.4	128	23	103
8	42.8	256	0	206

[a] Manure was applied at rate necessary to meet 0, 1, 2, 4 or 8-year P requirement for corn.

RAINFALL SIMULATION PROCEDURES

The rainfall simulation procedures adopted by the National Phosphorus Research Project were employed in this study (Sharpley and Kleinman, 2003). A portable rainfall simulator based on the design by Humphry et al. (2002) was used to apply rainfall to 0.75 m wide × 2 m long paired plots. The distance between paired plots was set at approximately 5 m to accommodate the tandem disk used for tillage. The simulator was used to apply rainfall for 30 min at an intensity of 70 mm h^{-1}. Two additional rainfall simulation tests were conducted for the same duration and intensity at approximately 24 h intervals. Two rain gauges were placed along the outer edge of each plot, and one rain gauge was located between the plots. Some drying on the soil surface occurred between rainfall simulation runs.

Water was first added to the plots before the initial rainfall simulation run using a hose on which a garden watering wand was attached. The addition of water continued until runoff began to provide more uniform antecedent soil water conditions among plots. The amount of water introduced to each plot was not the same because the initial soil water conditions varied during the five-week study period depending on rainfall events and weather conditions. The addition of inflow was stopped once saturated soil water conditions were established and runoff began. Soil samples were not collected at the time of the rainfall simulation events to identify soil water capacity. The amount of runoff that occurred before the initial rainfall simulation run was minimal, and it was not collected. A large rubber mat with numerous holes was placed across the soil surface before the addition of water to allow more uniform distribution and to protect the soil surface during wetting. The rubber matt was only used for soil wetting.

Plot borders channeled runoff into a sheet metal lip that emptied into a collection trough located across the bottom of each plot. The trough diverted runoff into plastic buckets. A sump pump was then used to transfer runoff into larger plastic storage containers. The storage containers were weighed at the completion of each run to determine the total runoff mass. Because of the relatively large amount of runoff that was collected, measurement of runoff mass was more accurate than determining runoff volume. The relatively small variations in runoff mass caused by differences in water temperature were not considered in the analyses.

Accumulated runoff was agitated immediately before a sample was collected for microbial analyses. The runoff samples were obtained within a few minutes following completion of the rainfall simulation tests. The samples were placed in a cooler containing ice packs before they were transported to the laboratory.

SAMPLE ANALYSES

Somatic phages were assayed using the single-layer agar method (USEPA, 2001), resulting in counts of plaque-forming units (PFU) that are displayed as individual clear lysis zones on a lawn of bacterial hosts. PFU counts represent the growth of a single virus, or a clump of virions that result in a single plaque.

Enumeration of total coliforms, *E. coli*, and enterococci was performed using the EPA-approved Quanti-Tray system (IDEXX Laboratories, Westbrook, Maine). For these, 10 g of sample was combined with 90 mL of phosphate-buffered saline and manually mixed prior to inoculation. All samples were incubated for 24 h. *E. coli* and total coliform assays were incubated at 37°C, and enterococcus trays were maintained at 42°C (APHA, 2014).

Centrifuged and filtered runoff samples of a known volume were analyzed for dissolved P (DP) (Murphy and Riley, 1962) and NO_3-N and NH_4-N (measured with a flow injection analyzer using spectrophotometry; AutoAnalyzer 3, SEAL Analytical Ltd., Southampton, U.K.). Samples that were not centrifuged were analyzed for total phosphorus (TP) (Johnson and Ulrich, 1959), total nitrogen (TN) (Tate, 1994), pH, and electrical conductivity (EC) (Klute, 1986). Particulate phosphorus (PP) was obtained by subtracting DP measurements from TP values.

Statistical Analyses

Analysis of variance (ANOVA, SAS Mixed Procedure; SAS, 2003) was performed to determine the effects of the three experimental treatments on microbial transport: (1) wheat residue (yes or no), (2) manure application rate (0.0, 5.4, 10.7, 21.4, or 42.8 Mg ha^{-1}), and (3) rainfall simulation run (first, second, or third). The experimental treatments were replicated three times, so the number of observations for each of the variables was 90. If a significant difference was identified, the least significant difference (LSD) test was used to identify differences among experimental treatments. A probability level of <0.10 was considered significant. Pearson correlation coefficients (SAS COOR Procedure) were used to determine the relationships between microbial transport and selected water quality characteristics. A correlation coefficient was considered significant at the 95% level.

Results and Discussion

Microbial Transport as Affected by Wheat Residue Cover

A significant residue cover by manure application rate interaction was indicated by ANOVA (table 2). The phage count of 8.92 log PFU ha^{-1} measured on the treatment containing wheat residue on which manure was applied at a rate of 42.8 Mg ha^{-1} (8-year P-based application requirement for corn) was significantly greater than any of the other experimental treatments (fig. 2). No significant differences in phage transport were found among plots with and without residue cover for the other manure application rates. The load of total coliforms in runoff of 12.6 log CFU ha^{-1} measured on the plots containing wheat residue was significantly greater than the 12.4 log CFU ha^{-1} measured on the plots without residue (table 2). The group of microorganisms represented by total coliforms includes several plant-associated organisms, and it is possible that the increased numbers of total coliforms on the residue plots were due to the increased plant biomass on these plots. No significant differences were found in counts of *E. coli* and enterococci between the plots with and without wheat residue.

The load of manure-borne microorganisms in runoff following application of beef cattle manure at a rate required to meet the annual N requirements for corn was measured by Thurston-Enriquez et al. (2005). The loads of *E. coli* and enterococci from the 0.75 m × 2 m plots on which cattle manure was applied but not incorporated were 12.7 and 12.1 log CFU ha^{-1}, respectively. These values represent mean measurements from three consecutive 30 min simulated rainfall events, producing 35 mm of rainfall and separated by 24 h intervals. In the present study, the mean transport rates for *E. coli* and enterococci on the plots where beef cattle manure was applied at rates varying from 5.4 to 42.8 Mg ha^{-1} were 9.93 and 11.0 log CFU ha^{-1}, respectively (table 2). The larger microbial transport values reported by Thurston-Enriquez et al. (2005) were for plots on which beef cattle

Table 2. Microbial transport as affected by residue cover, manure application rate, and rainfall simulation run.[a]

Variable	Value	Phages (log PFU ha^{-1})	Total Coliforms (log CFU ha^{-1})	*E. coli* (log CFU ha^{-1})	Enterococci (log CFU ha^{-1})
Residue cover	Wheat residue	8.45 a	12.6 a	9.83	11.0
	No wheat residue	8.35 b	12.4 b	9.57	10.9
	Standard error	0.051	0.197	0.137	0.200
Manure application rate[b]	0 Mg ha^{-1}	8.39 b	12.2 b	8.77 b	10.5
	5.4 Mg ha^{-1}	8.38 b	12.6 ab	9.67 a	11.1
	10.7 Mg ha^{-1}	8.26 b	12.5 ab	9.66 a	10.9
	21.4 Mg ha^{-1}	8.32 b	12.7 a	10.1 a	10.9
	42.8 Mg ha^{-1}	8.66 a	12.7 a	10.3 a	11.2
	Standard error	0.067	0.218	0.217	0.247
Rainfall simulation run[c]	First rainfall event	8.38	12.6	9.83 a	11.0
	Second rainfall event	8.41	12.6	9.76 ab	10.9
	Third rainfall event	8.42	12.5	9.50 b	10.9
	Standard error	0.055	0.194	0.128	0.193
ANOVA (Pr > F)	Residue cover	0.07	0.09	0.20	0.42
	Manure rate	0.01	0.09	0.01	0.13
	Rainfall simulation run	0.69	0.11	0.07	0.58
	Residue cover × Manure rate	0.02	0.40	0.92	0.81
	Residue cover × Run	0.79	0.53	0.46	0.83
	Manure rate × Run	0.50	0.27	0.56	0.83
	Manure rate × Run × Residue cover	0.63	0.66	0.70	0.95

[a] Values followed by different letters are significantly different at the 0.10 probability level based on LSD tests.
[b] Beef cattle manure was applied at rates required to meet the 0, 1, 2, 4, or 8-year annual P requirement for corn.
[c] Rainfall was applied for 30 min at an intensity of 70 mm h^{-1} during three rainfall events separated by 24 h intervals.

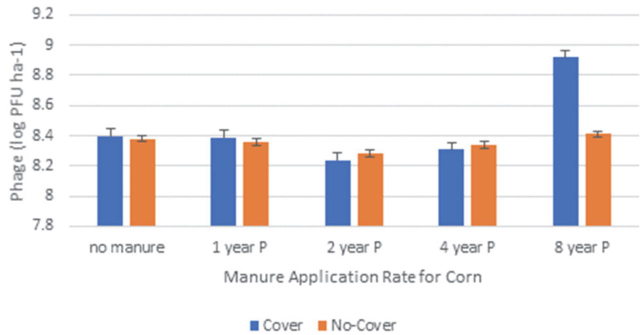

Figure 2. Phage transport as affected by manure application rate for sites with and without residue cover. Vertical bars are standard errors.

manure was broadcast but not incorporated, and rainfall simulation tests were performed immediately following manure application. The time between manure application and rainfall events impacts the amount of *E. coli* detected in runoff (Stocker et al., 2018). Each of the plots examined in this study was disked following manure application, which reduced the amount of manure remaining on the soil surface. In this study, a total of 27.7 cm of rainfall occurred between the time manure was applied and the initiation of the rainfall simulation tests 41 days later.

Microbial Transport as Affected by Manure Application Rate

No significant differences in mean counts of total coliforms, which ranged from 12.5 to 12.7 log CFU ha^{-1}, and enterococci, which ranged from 10.9 to 11.2 log CFU ha^{-1}, were found among the plots on which manure was applied at rates varying from 5.4 to 42.8 Mg ha^{-1} (table 2). The *E. coli* load of 8.77 log CFU ha^{-1} measured on the plots without manure was significantly less than the values of 9.67 to 10.3 log CFU ha^{-1} measured on the plots where manure was added.

The transport on *E. coli* in runoff increased in a linear fashion with manure application rate (fig. 3). The derived regression equation relating *E. coli* load (y) in CFU ha^{-1} to manure application rate in Mg ha^{-1} is:

$$y = 5.0\ E^{8}(x) + 1.0\ E^{9} \quad (R^2 = 0.97) \qquad (1)$$

The transport of total coliforms in runoff also increased in a linear fashion with manure application rate (not shown). The derived regression equation relating total coliform load (y) in CFU ha^{-1} to manure application rate in Mg ha^{-1} is:

$$y = 7.0\ E^{10}(x) + 3.0\ E^{12} \quad (R^2 = 0.61) \qquad (2)$$

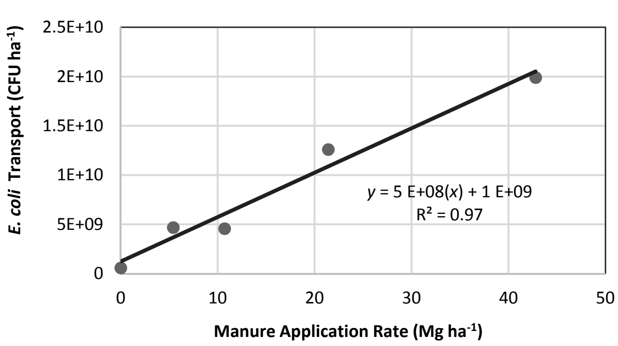

Figure 3. *E. coli* transport as affected by manure application rate.

The effects of animal diet, manure application rate, and tillage on the transport of selected microorganisms from plots on which beef cattle manure was applied but not incorporated were measured by Durso et al. (2011). Transport rates of phages, total coliforms, and *E. coli* immediately following manure application increased from 10.4 to 11.2 log PFU ha^{-1}, from 12.7 to 13.2 log CFU ha^{-1}, and from 12.3 to 13.0 log CFU ha^{-1}, respectively, as the manure application rate increased from a 1-year to a 4-year P application requirement for corn. The smaller microbial transport loads obtained in the present study are attributed to the incorporation of manure following application and the occurrence of 27.7 cm of precipitation during the 41 days preceding the rainfall simulation tests.

In this study, microbial transport on the control plots where manure was not applied was substantial (table 2). In previous field studies, the transport of microbes in runoff has been reported to be similar for grazed and nongrazed watersheds. Doran et al. (1981) measured the bacteriological quality of runoff from a pasture grazed by beef cattle and from a nongrazed control pasture in south central Nebraska from 1976 to 1978. For rainfall events, mean counts of total coliforms, fecal coliforms, and fecal streptococci from the grazed pasture were 5.75, 5.06, and 5.53 log CFU per 100 mL, respectively, compared to 5.65, 4.12, and 6.01 log CFU per 100 mL from the nongrazed pasture. The relatively large bacteriological counts on the nongrazed pasture were attributed to wildlife activity, including field mice and rabbits.

Microbial transport in runoff from grazed and nongrazed watersheds in the Pacific Northwest was monitored by Jawson et al. (1982). Mean annual weighted-average counts of total coliforms, fecal coliforms, and fecal streptococci from the grazed pasture during the 1978 water year were 5.89, 3.18, and 3.34 log CFU per 100 mL, respectively, compared to 5.04, 1.81, and 3.40 log CFU per 100 mL from the nongrazed watershed. The presence of wildlife on the field site used in the present investigation may have contributed to the relatively large microbial transport values obtained on the plots where manure was not applied.

Microbial Transport as Affected by Rainfall Simulation Run

The rainfall simulation protocols used in this study called for three 30 min rainfall simulation events separated by 24 h intervals. Rainfall simulation run did not significantly affect measurements of phages, total coliforms, or enterococci (table 2). However, the *E. coli* count of 9.83 log CFU ha^{-1} measured for the first rainfall simulation run was significantly greater than the count of 9.50 log CFU ha^{-1} measured for the third rainfall simulation run. No significant differences in *E. coli* transport values were found between the second and third rainfall simulation runs.

Thurston-Enriquez et al. (2005) measured the load of manure-borne microorganisms in runoff immediately following surface application of beef cattle manure. Transport rates of *E. coli* and enterococci increased from 10.8 to 13.0 log CFU ha^{-1} and from 11.5 to 12.2 log CFU ha^{-1}, respectively, between the first and second rainfall simulation runs, suggesting bacterial growth. Each of the plots examined in the present study was disked following manure application, resulting in reduced quantities of manure on the soil surface. Microbial growth did not appear to be substantially enhanced following the addition of rainfall on the soil surface, which contained reduced quantities of manure following incorporation.

Correlation between Microbial Transport and Runoff Characteristics

Oun et al. (2014) recommended assessing the associations between bacterial communities and nutrient and chemical concentrations to determine if specific microbial community structures could be associated with individual chemical inputs or land uses. Determination of microbial populations in runoff is labor-intensive, time-consuming, and expensive. If more easily measured surrogates for microbial transport could be identified, information on the presence of pathogens in surface waters could be more readily available. Therefore, the microbial transport values (log PFU ha^{-1} or log CFU ha^{-1}) obtained in this study were correlated to selected nutrient loads (kg ha^{-1}) and measurements of electrical conductivity (dS m^{-1}) and pH (table 3).

The transport of phages was positively correlated to runoff loads of DP, PP, and TP (table 3). Total coliform transport was positively correlated to DP, TP, and TN and negatively correlated to pH. The transport of *E. coli* was positively correlated to DP, TP, NH_4-N, TN, NO_3-N, and EC and negatively correlated to pH. Enterococci transport was positively correlated to DP and TP and negatively correlated pH.

Each of the three bacterial constituents was positively correlated to DP and TP and negatively correlated to pH. Phosphorus concentrations were previously found to influence the survival of *E. coli* in drinking water (Juhna et al., 2007). Therefore, there may be a positive correlation between microbial populations and phosphorus concentrations.

The pH of the manure used in this study was 8.2. Thayer et al. (2012) reported that the pH of runoff in their study varied from 7.93 on plots with no manure application to 7.91 on treatment plots where 42.8 Mg ha^{-1} of manure was added. The populations of total coliforms, *E. coli*, and enterococci on land application areas appeared to be very sensitive to small changes in soil pH.

Comparison of Microbial Transport Rates with Previously Reported Values

Durso et al. (2011) examined the effects of animal diet (corn or distiller's grain), manure application rate (1, 2, or 4-year corn P requirement), and tillage (till or no-till) on the transport of microorganisms immediately following manure application. A grain sorghum-soybean-winter wheat crop rotation under no-till management was used at the study site, and the soil surface contained sorghum residue from the previous year. Mean transport rates of phages, total coliforms, *E. coli*, and enterococci for the plots on which manure from cattle fed a corn diet was applied just prior to the rainfall simulation tests were 10.2 log PFU ha^{-1}, 13.0 log CFU ha^{-1}, 12.7 log CFU ha^{-1}, and 11.9 log CFU ha^{-1}, respectively. In the present study, manure from beef cattle fed a corn diet was also applied to 0.75 wide × 2.0 m long plots at rates required to meet the 0, 1, 2, 4, or 8-year P requirement for corn. Mean transport rates of 8.45 log PFU ha^{-1}, 12.6 log CFU ha^{-1}, 9.83 log CFU ha^{-1}, and 11.0 log CFU ha^{-1} were measured in the present study for phages, total coliforms, *E. coli*, and enterococci, respectively, on the plots with wheat residue. The smaller microbial counts obtained in the present study may have been influenced by the 41-day delay between manure application and the initiation of the rainfall simulation tests, the variations in soil characteristics, tillage conditions, weather patterns, the presence of competing microbes, and other factors.

Conclusions

Manure is applied to cropland areas managed using a variety of cropping and tillage conditions that result in varying amounts of surface residue. In this study, the load of total coliforms in runoff of 12.6 log CFU ha^{-1} measured on the plots with 90% wheat residue cover was significantly greater than the 12.4 log CFU ha^{-1} measured on the plots with 5% residue cover. No significant differences were found in the quantities of *E. coli* and enterococci transported in runoff between the plots with and without residue.

Manure can be applied to meet single- or multiple-year crop nutrient requirements. No significant differences in mean counts of total coliforms, which ranged from 12.5 to 12.7 log CFU ha^{-1}, and *E. coli*, which ranged from 9.66 to 10.3 log CFU ha^{-1}, were found among the plots on which manure was applied at rates varying from 5.4 to 42.8 Mg ha^{-1} (1 to 8-year P-based requirements for corn). The *E. coli* load of 8.77 log CFU ha^{-1} measured on the plots without manure was significantly less than the values measured on the plots where selected amounts of manure were applied.

Rainfall simulation run did not significantly affect measurements of phages, total coliforms, or enterococci. However, counts of *E. coli* were significantly affected by rainfall simulation run, with values varying from 9.50 to 9.83 log CFU ha^{-1}. Measurements of *E. coli* were significantly greater

Table 3. Correlation coefficients of microbial transport with water quality characteristics.[a]

Microbial Constituent	DP	PP	TP	NH_4-N	TN	NO_3-N	EC	pH
Phages	**0.47**	**0.46**	**0.55**	-0.04	0.12	0.17	0.05	-0.01
	(0.01)	(0.01)	(0.01)	(0.74)	(0.24)	(0.12)	(0.66)	(0.94)
Total coliforms	**0.28**	0.08	**0.27**	0.09	**0.30**	0.21	-0.01	**-0.28**
	(0.01)	(0.44)	(0.01)	(0.41)	(0.01)	(0.06)	(0.95)	(0.01)
E. coli	**0.43**	0.06	**0.39**	**0.27**	**0.41**	**0.52**	**0.30**	**-0.24**
	(0.01)	(0.60)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.02)
Enterococci	**0.27**	0.07	**0.25**	0.06	0.12	0.15	0.06	**-0.25**
	(0.01)	(0.51)	(0.02)	(0.60)	(0.26)	(0.16)	(0.61)	(0.02)

[a] Correlation coefficients are significant (shown in **bold**) at the 95% level if |correlation| > 0.21 for n = 90. Values in parentheses represent Pr > |r|. DP = dissolved phosphorus, PP = particulate phosphorus, TP = total phosphorus, TN = total nitrogen, and EC = electrical conductivity.

for the first rainfall simulation run than for the third run.

Each of the three bacterial constituents was positively correlated to DP and TP and negatively correlated to pH. The effects of residue cover, manure application rate, and rainfall simulation run on microbial transport varied significantly among microbes.

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