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Demonstration of Methods to Reduce *E. coli* Runoff from Dairy Manure Application Sites

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ABSTRACT

Contamination by bacteria is a leading cause of impairment in U.S. waters, particularly in areas of livestock agriculture. We evaluated the effectiveness of several practices in reducing *Escherichia coli* levels in runoff from fields receiving liquid dairy (*Bos taurus*) manure. Runoff trials were conducted on replicated hay and silage corn (*Zea mays* L.) plots using simulated rainfall. Levels of *E. coli* in runoff were ~10^4 to 10^6 organisms per 100 mL, representing a significant pollution potential. Practices tested were: manure storage, delay between manure application and rainfall, manure incorporation by tillage, and increased hayland vegetation height. Storage of manure for 30 d or more consistently and dramatically lowered *E. coli* counts in our experiments, with longer storage providing greater reductions. Manure *E. coli* declined by >99% after ~90 d of storage. On average, levels of *E. coli* in runoff were 97% lower from plots receiving 30-d-old and >99% lower from plots receiving 90-d-old manure than from plots where fresh manure was applied. Runoff from hayland and cornland plots where manure was applied 3 d before rainfall contained ~50% fewer *E. coli* than did runoff from plots that received manure 1 d before rainfall. Hayland vegetation height alone did not significantly affect *E. coli* levels in runoff, but interactions with rainfall delay and manure age were observed. Manure incorporation alone did not significantly affect *E. coli* levels in cornland plot runoff, but incorporation could reduce bacteria export by reducing field runoff and interaction with rainfall delay was observed. Extended storage that avoids additions of fresh manure, combined with application several days before runoff, incorporation on tilled land, and higher vegetation on hayland at application could substantially reduce microorganism loading from agricultural land.

More than 149 000 river kilometers in the United States are impaired by bacteria levels that routinely exceed water quality standards (USEPA, 2000). Exposure to waterborne pathogens poses a significant and increasing risk to public health (American Society for Microbiology, 1999). Agricultural, urban, and forested land can be sources of microorganisms. However, because of the large quantity of animal waste generated by livestock and applied to the land, runoff of microorganisms from land receiving manure is frequently the cause of impairment and such runoff may pose a threat to human and livestock health and to beneficial use of water.

Livestock agriculture can be a major source of microorganisms to surface and ground waters. Livestock generally shed ~10^6 to 10^7 fecal organisms per gram of waste, or more than ~10^9 to 10^{10} organisms per capita per day (Robbins et al., 1971; Reddy et al., 1981; Moore et al., 1988). In addition to violations of water quality standards, pathogens such as *Salmonella*, *Campylobacter*, *Cryptosporidium*, *Giardia*, and *E. coli* O157:H7 from agricultural operations may directly threaten human health. Data on the occurrence of such pathogens in surface and ground waters are limited; bacterial indicators like *E. coli* are usually measured and reported as evidence of fecal contamination and the potential presence of pathogens. *E. coli* are typically present in fecal material in higher numbers than pathogens and survive at least as long as bacterial pathogens under most environmental conditions, and laboratory analyses are relatively rapid, easy, and inexpensive. The utility of *E. coli* as an indicator organism is evidenced by an association between the presence of *E. coli* and both the occurrence of known gastrointestinal pathogens (Craun et al., 1997; Horman et al., 2004) and the actual incidence of gastroenteritis (Cabelli, 1980; Dufour, 1984).

Runoff from animal waste applied to agricultural land is potentially a major source of microorganisms to surface waters. Levels of 10^4 to 10^6 fecal organisms per 100 mL in runoff from manure application areas are commonly reported (Crane et al., 1983; Baxter-Potter and Gilliland, 1988; Moore et al., 1988). Up to 25% of microorganisms applied in animal waste may be lost in runoff annually (Kunkle, 1970; Robbins et al., 1971; Faust, 1976). The actual quantity of microorganisms transported from a waste application site depends on many factors, including precipitation intensity, runoff volume, time of precipitation relative to application, runoff–infiltration partitioning, vegetation, soil characteristics, slope, application form and method, soil contact time, organism die-off rate, and season (Crane et al., 1983; Moore et al., 1988).

Following excretion from the gastrointestinal tract of animals, fecal microorganisms are subject to a hostile environment and tend to die at rates that depend on environmental conditions. Reductions of bacteria numbers of two to three orders of magnitude have been reported with manure storage for two to six months (Patni et al., 1985; Moore et al., 1988; Trevisan and Dorioz, 1999). Microorganisms in surface-applied wastes may be exposed to high temperatures, desiccation, ultraviolet light, and other stresses, and experience significant die-off after application. Incorporating wastes into the soil by tillage may enhance survival of microorganisms because they are sheltered from lethal stresses, but incorporation also removes microorganisms from interaction with surface runoff. Soils can effectively remove microorganisms from percolating water by adsorption, filtration, and predation (Ellis and McCalla, 1978; Crane and Moore, 1984; Moore et al., 1988; Trevisan et al.,

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2002). The existence of macropores, however, may promote rapid movement of bacteria through the soil; *E. coli* levels of 10^3 to 10^5 organisms per 100 mL have been reported in tile drain flow from grazing land and land receiving animal waste application (Thornley and Bos, 1985; Cook and Baker, 2001; Jamieson et al., 2002).

Practices to specifically control export of microorganisms in agricultural runoff to surface waters have not been as widely developed, tested, or applied as those designed to control sediment and nutrients. However, past studies have identified several promising areas where improved management might reduce runoff losses of microorganisms from agricultural operations, including waste storage, decreasing manure application rates, avoiding application under wet conditions, maintaining buffer strips between application sites and waterways, and fencing livestock away from streams (Moore et al., 1983; Patni et al., 1985; Coyne et al., 1998; Meals, 2001). Due to the sheer numbers of bacteria present in runoff from animal waste application sites, bacteria counts in runoff may continue to contribute to violations of water quality standards even if a 90% reduction can be achieved by implementing an individual management practice. For this reason, a multiple barrier approach consisting of coordinated application of management practices to multiple control points on the farm has been recommended for control of agricultural pathogen losses (Rosen, 2000).

The principal goal of our project was to demonstrate and evaluate several practical methods for controlling pathogens, measured as indicator *E. coli*, from livestock agricultural sources to surface waters as potential components of a multiple barrier approach. Specific objectives included:

- determining the effect of definitive periods of manure storage time (i.e., storage for certain duration without repeated addition of fresh manure) on *E. coli* levels in liquid dairy manure.
- determining the effect of manure age on *E. coli* losses in runoff from hayland and cornland receiving liquid dairy manure;
- determining the effect of manure incorporation on *E. coli* losses in runoff from cornland receiving liquid dairy manure;
- determining the effect of vegetation height on *E. coli* losses in runoff from hayland receiving liquid dairy manure; and
- determining the effect of delay between manure application and rainfall on *E. coli* losses in runoff from hayland and cornland.

The project used a pilot manure storage system, simulated rainfall, and replicated runoff plots in a factorial design to evaluate the effectiveness of these practices, individually and in combination, in reducing *E. coli* losses from manure application sites. Whereas some individual treatments have been tested before, this work focused on evaluating the effectiveness of the treatments and interactions among the treatments simultaneously as potential components of a multiple-barrier approach to reducing bacteria losses. Such evaluation is critical to support the formulation of these treatments into an acceptable best management practice (BMP).

MATERIALS AND METHODS

Study Sites

We conducted a series of experiments in the uplands of the Lake Champlain Basin in northwestern Vermont. The region has a cool, continental climate with cold winters, warm summers, and a short growing season. Annual mean daily air temperature is 5.5°C, with average daily minimum and maximum temperatures of 1 and 12°C. The frost-free period averages 187 d. Annual precipitation averages 880 mm; mean annual snowfall is 2380 mm.

The first manure storage and runoff experiment was conducted on mixed grass hayland in East Montpelier, VT (Fig. 1). Soil on the site is Cabot silt loam (loamy, mixed, active, nonacid, frigid, shallow Typic Humaquepts), a poorly drained soil that formed in dense loamy till. The second experiment was conducted on a silage corn field in Williamstown, VT (Fig. 1). Soil on the site is Buckland stony loam (coarse-loamy, mixed, semiactive, frigid Aquic Dystric Eutrudepts), a moderately drained soil that formed in glacial till and is typically underlain by a fragipan. Both soils are classified as Hydrologic Group C, indicating that they are prone to runoff.

Manure Storage Experiments

At each site, we established nine 1.4-m-diameter plastic tanks (depth of ~0.3 m, maximum volume of ~430 L) to document the effects of extended storage on the *E. coli* content of manure and to provide manure of specified age for the field runoff experiments. The intention of the experiments was to achieve storage durations of 30 and 90 d. Tanks representing the "0-d" age manure were filled with fresh manure 3 d before each runoff trial. Three replicate tanks were used for each manure age class.

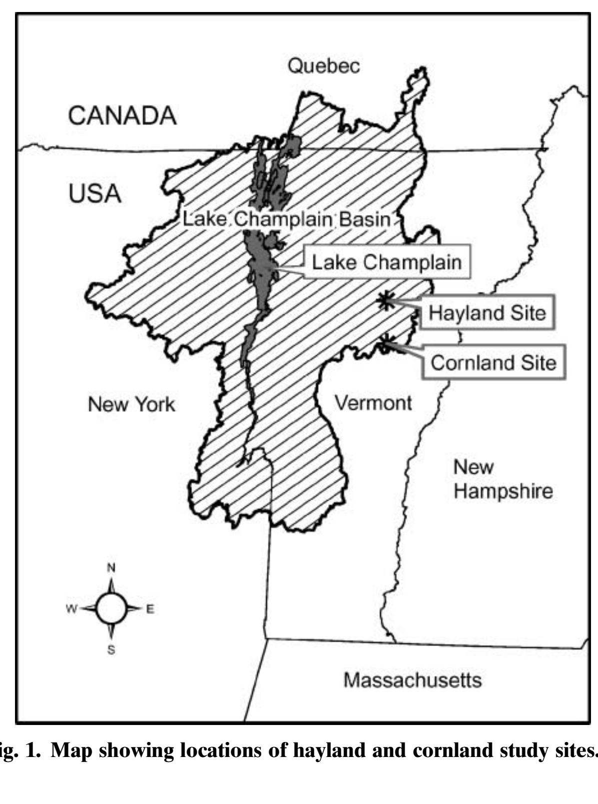

Fig. 1. Map showing locations of hayland and cornland study sites.

With the appropriate lead-time in advance of runoff trials, tanks were filled with fresh liquid dairy manure obtained from a sump below a freestall barn before any storage or treatment. The same source of manure was used in both manure storage experiments. After filling, manure in tanks was allowed to age without further addition under ambient conditions. The actual volume of manure contained in each tank was ~300 L.

The 90-, 30-, and 0-d manure tanks were sampled immediately after filling and again before application in runoff experiments. Contents of each replicate tank were mixed using a canoe paddle, then three subsamples were collected and composited into a sterile polyethylene sample bottle for *E. coli* analysis. Thus, each manure age class was represented by three replicate samples before and after aging.

Runoff Experiments

Two runoff experiments were conducted at separate hayland and cornland sites. For each experiment, 40 1.5- by 3-m plots were created, representing a factorial design of $3 \times 2 \times 2$ treatments, with three replicates per treatment combination, plus three control plots (no manure applied), and one extra plot reserved as a backup.

At each site, plots were arrayed in a grid with the long dimension of each plot parallel to the predominant slope. Plots were isolated from upgradient runoff by a network of ditches and berms that intercepted runoff and conveyed it beyond the plot array. At the bottom of each plot, 15- by 75-cm corrugated metal strips were embedded in the soil in a V-shape to direct runoff into a "dustpan" runoff collector consisting of a 20-cm polyethylene funnel embedded into the soil with a length of 1.3-cm-i.d. polyethylene tubing secured to the funnel outlet. Areas where the metal strips and the funnel entrance met the soil were sealed the day before the trial by brush application of polyurethane to minimize erosion and leakage of runoff water. Each collector drained by gravity to a 19-L polyethylene carboy.

In the hayland experiment, the test plots were laid out in an area of relatively uniform slope of 9.7% in rows oriented to minimize cross-slopes across the plots (Fig. 2). The plots were arranged in inverted V-shaped rows to permit construction of diversion ditches that angled downslope. For the cornland experiment, test plots were laid out in an area of relatively uniform 8.1% slope (Fig. 2). To permit construction of diversion ditches that angled downslope, the plots were arranged in rows diagonally across the field. Plots were oriented approximately perpendicular to the direction of corn rows; row ridges were not large enough to prevent runoff movement downslope to the runoff collectors or to cause appreciable ponding behind ridges.

Treatments applied in both experiments are listed in Table 1. Specific treatments were assigned to plots randomly. Manure of different ages was obtained from the manure storage tanks at each field site. Manure was applied to the plots by hand at a rate equivalent to 42.1 m^3 ha^{-1} (4500 gal ac^{-1}) on hayland and 58.9 m^3 ha^{-1} (6300 gal ac^{-1}) on cornland, rates typical for application to cropland in Vermont. Manure tanks

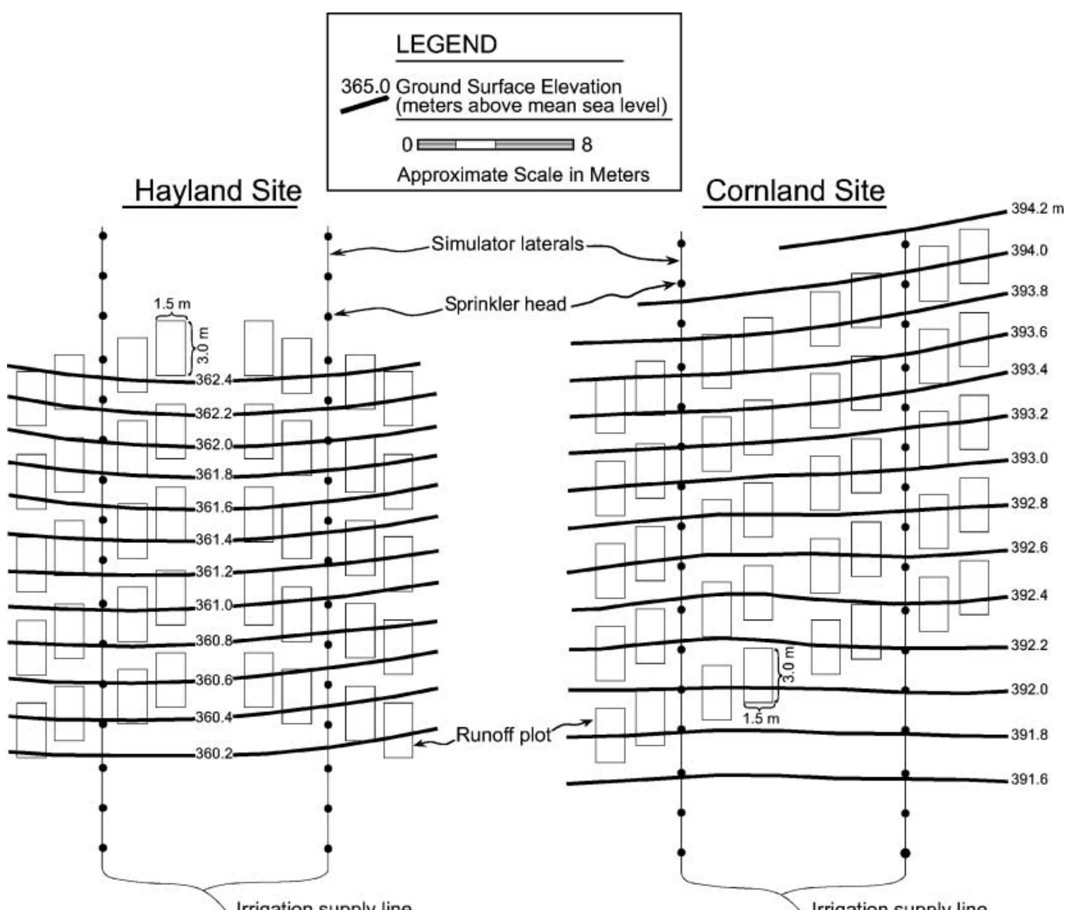

Fig. 2. Schematic of plot layouts for hayland and cornland experiments. Plots were arranged to accommodate berms and drainage ditches that isolated plots from up-gradient runoff and to achieve consistent slope among plots.

Table 1. Treatments applied to test plots.

	Hayland		Cornland	
Treatment	**Condition**	**Code**	**Condition**	**Code**
Manure age	**90 d**	**90**	**90 d**	**90**
	30 d	**30**	**30 d**	**30**
	0 d	**0**	**0 d**	**0**
Delay to rain	**3 d**	**3**	**3 d**	**3**
	1 d	**1**	**1 d**	**1**
Vegetation height	**13–15 cm**	**H**	**NA†**	**NA**
	5–7 cm	**L**	**NA**	**NA**
Incorporation	**NA**	**NA**	**incorporated**	**I**
	NA	**NA**	**non-incorporated**	**N**

† Not applicable.

were completely mixed using a canoe paddle immediately before each batch was removed for application. Each applied batch was a composite from the three replicate tanks representing that age class. Manure was applied as uniformly across each plot as possible, avoiding the area immediately above the runoff collector.

On hayland, vegetation heights were established by hand mowing; grass clippings were bagged and removed from the plots. In both experiments, manure was applied to the 3-d delay plots around mid-day, ~72 h before the scheduled simulated rainfall. Manure was applied to the 1-d delay plots ~24 h before scheduled rainfall. On cornland, manure was incorporated on designated plots by a single pass of a gasoline-powered roto-tiller parallel with the long axis of the plot within 30 min of application.

A rainfall simulator was used to generate runoff from the test plots by continuously and uniformly applying water at an intensity resembling natural rainfall. The simulator consisted of two 5.1-cm-i.d. PVC laterals 36 m long, positioned in the test field parallel to the dominant slope. The laterals were connected at the downslope end to a 7.6-cm-PVC water main. The laterals were spaced 13 m apart, with 20 test plots positioned between them and 10 plots positioned to the outside of each lateral. The PVC riser pipes (2.5-cm i.d.) were positioned every 2.25 m along the laterals, with 16 risers per lateral. An S-30 irrigation head (Nelson Irrigation, Walla Walla, WA) fitted with a brass nozzle and an 8° spinner plate was mounted on each riser 3.4 m above the ground surface. For the cornland trial, a no. 22 brass nozzle (orifice diameter = 4.4 mm) was installed in each irrigation head; for the hayland trial no. 21 (orifice diameter = 4.2 mm) and no. 23 (orifice diameter 4.6 mm) nozzles were used on alternating irrigation heads. The design water output from these two configurations is essentially equivalent. To maximize the uniformity of water distribution over the test plots, each irrigation head contained a 1.0 kg cm^{-2} pressure regulator that provided a constant output rate from the nozzle irrespective of its position along the simulator lateral or the backpressure on the system. Each irrigation head irrigated a circular area with a radius of ~6.7 m. Actual delivery of simulated rainfall was measured by 7.5-cm-diameter catch cups placed on each plot.

At both sites, a nearby pond served as the water source. A centrifugal pump was used to pump water to the rainfall simulator. Water pressure at the simulator laterals was maintained at ~2 kg cm^{-2} through each event. During each experiment, three samples of irrigation water were collected from one of the laterals for *E. coli* analysis.

All sample collection equipment was cleaned before each experiment using a 10% (v/v) solution of household chlorine bleach, followed by triple-rinsing with tap water. Before each experiment, samples of the final rinsate from three randomly selected carboys were collected for *E. coli* analysis. No *E. coli* were detected in rinsate samples, confirming sterilization.

For each experiment, the first hour or first ~19 L of runoff was collected from each plot. The approximate time of runoff initiation was recorded for each plot, but no attempt was made to measure runoff volume. Each carboy was subsampled for *E. coli* by manually agitating for 15 s, then pouring an aliquot into a sterile 100-mL polyethylene bottle. Runoff samples for *E. coli* analysis were maintained on ice and transported to the laboratory within 3 h of collection.

Air and soil temperature, precipitation, relative humidity, barometric pressure, wind direction and velocity, and solar radiation were monitored at each site beginning on the day of the first manure application (3 d before the simulated rainfall event) to characterize weather conditions during the course of plot treatment and runoff. A Vantage Pro meteorologic station (Davis Instruments, Hayward, CA) was erected at each site consisting of a tipping-bucket rain gage, atmospheric thermometer, cup anemometer, wind direction vane, solar pyranometer, relative humidity sensor enclosed in an aspirated shield, and an atmospheric pressure sensor. Soil temperature was measured daily in the center of the plot array at a depth of 10 cm using an Ever-Safe N168 thermometer (Ever Ready Thermometer Company, Dubuque, IA). Additional weather data used to characterize conditions during the manure storage experiments were obtained from a nearby National Weather Service weather station, Montpelier 2 (Coop ID 435273).

Sample Analysis

All *E. coli* analyses were conducted in the Vermont Department of Environmental Conservation Water Quality Laboratory in Waterbury, VT, using the Quanti-Tray method (Method 9223B; American Public Health Association, 1995). Manure samples were pre-processed in the laboratory by suspending a known wet weight of manure in sterile dilution water; results for manure samples were reported as organisms per gram wet weight. Runoff, irrigation source water, and container rinse samples were analyzed by standard Quanti-Tray procedures and results were reported as organisms per 100 mL.

Statistical Analysis

Statistical analysis of *E. coli* data was conducted on $\log_{10}$ transformed data to satisfy the assumptions of normality and equal variances. All statistical tests were performed using JMP software Version 4.0 (SAS Institute, 2000) at an α of 0.1. The effect of treatment on levels of *E. coli* in runoff was evaluated by multi-factor analysis of variance (ANOVA). After an initial pass that included all treatment factors and all possible interactions, nonsignificant ($P > 0.1$) interactions were removed from the model and a final reduced-model ANOVA was conducted. Interpretations of treatment effects were based on the reduced model.

RESULTS

Manure Storage

Results of manure analyses for *E. coli* for each batch of manure at delivery and immediately before application are reported in Table 2. Actual storage periods varied slightly from planned duration. Mean *E. coli* levels in fresh manure delivered to the sites ranged from 3.1 to 7.5×10^5 organisms per gram, levels comparable to those reported by Crane et al. (1983) and Moore et al. (1988). With all data pooled, no significant differences in initial *E. coli* content were noted among different batches of manure (one-way ANOVA). The *E. coli* levels

Table 2. Descriptive statistics for manure *E. coli* analyses.

Manure source	Sample date (2003)	*n*	*E. coli* Median	*E. coli* Mean†	Standard deviation‡	CV§
			organisms per gram wet weight			
			Hayland experiment			
90 d at delivery	31 March	3	326500	308200a	0.093	0.20
90 d at application	23 June	3	1000	1300b	0.174	0.43
30 d at delivery	21 May	3	753500	750300a	0.037	0.09
30 d at application	23 June	3	8600	8100c	0.099	0.22
0 d at application	23 June	3	435000	442200a	0.065	0.15
			Cornland experiment			
90 d at delivery	22 July	3	397000	398500a	0.096	0.22
90 d at application	13 October	3	<100	<110d	0.102	0.25
30 d at delivery	16 September	3	687000	668000a	0.057	0.13
30 d at application	13 October	3	14500	7700c	0.510	0.71
0 d at application	13 October	3	391000	381500a	0.088	0.20

† Anti-log of log mean; means followed by same letter do not differ significantly ($P \leq 0.1$), one-way ANOVA.
‡ Log-transformed data.
§ Coefficient of variation (arithmetic).

were similar among replicate tanks immediately after delivery (coefficient of variation [CV] < 0.22), but variability tended to increase with storage, possibly due to small differences in the storage environment. The *E. coli* levels in 30-d-old manure decreased significantly from initial counts in both experiments (Table 2), and were similar between experiments, ~8×10^3 organisms per gram. The *E. coli* levels in 90-d-old manure were significantly lower than initial or 30-d levels in both experiments, and were significantly lower in the cornland experiment ($<1.1 \times 10^2$ organisms per gram) than in the hayland experiment (1.3×10^3 organisms per gram).

Changes in *E. coli* levels with manure storage are summarized in Table 3. In the hayland experiment, storage for about 30 d reduced *E. coli* counts by nearly two orders of magnitude, or 98.9%; ~90 d of storage reduced *E. coli* counts by 99.6%. Similar results were observed during storage for the cornland experiment, with reductions of 98.8% for ~30 d and >99.9% for ~90 d of storage. All differences were significant at $P \leq 0.1$ by Student's *t* test.

Hayland Experiment

The hayland runoff experiment was conducted on 24 June 2003. During the trial period, 21 June (day of first manure application) through 24 June (day of runoff), mean air temperature was 21.8°C. Winds were light and variable. Relative humidity peaked at ~95% during night, and generally dropped to ~50% at midday. Only 1 mm of precipitation was recorded onsite; some of this trace amount was probably condensation. Skies were mainly clear, with maximum solar radiation of ~640 to 970 W m^{-2} recorded at midday. The soil surface appeared to be fairly dry at the time of manure application; in the 30 d preceding the runoff event, 97 mm of rain were recorded at the Montpelier weather station and only 0.1 mm was recorded in the final 8 d immediately before the event. Soil temperatures on the plot area at the 10-cm depth ranged from 20 to 28°C during the trial period.

Simulated rainfall was applied on 24 June from 1105 to 1511 h. Measured rainfall application averaged 102 mm among the plots, yielding an average intensity of ~25 mm h^{-1}. The majority of plots generated runoff within about 75 min of the beginning of rainfall. A 1-h storm of equivalent intensity has a 5-yr return period in the region (McKay and Wilks, 1995). The distribution of simulated rain across the plot area was relatively uniform. The mean volume captured in catch cups located at the center of each plot was 466 mL, with a standard deviation of 52 mL and a CV of 11%. The *E. coli* content of irrigation water was <3 to 21 *E. coli* per 100 mL; therefore, simulated rainfall added a negligible quantity of *E. coli* bacteria to the plots.

Table 3. Changes in manure *E. coli* in storage and estimated first-order *E. coli* die-off rate constants (k) and time required to reduce initial *E. coli* levels by 90% (T_{90}).

Storage time Nominal	Storage time Actual†	Mean *E. coli*‡ Start	Mean *E. coli*‡ End	*t* Test *t*	*t* Test *P*	Reduction	k	T_{90}§
d	d	organisms per gram wet weight				%	d^{-1}	d
				Hayland experiment				
30	33	750300	8100	−32.31	<0.001	98.9	0.060	17
90	84	308200	1300	−20.97	<0.001	99.6	0.028	36
				Cornland experiment				
30	27	668000	7700	−6.50	0.003	98.8	0.072	14
90	83	398500	100	−43.86	<0.001	99.9	0.043	23

† Actual storage time from filling of storage tanks to manure applications to test plots.
‡ Anti-log of log mean.
§ Estimated from first-order decay at given k value.

Table 4. Summary of *E. coli* data from hayland runoff plots.

Manure age	Vegetation height	Delay to rain	*n*	*E. coli* Median	Mean† *E. coli*	Standard deviation‡	CV§
d		d		organisms per 100 mL			
0	low	1	3	>2420000	>1 366000a	0.430	0.65
		3	3	>1200000	>1 102000a	0.361	0.73
	high	1	3	>2420000	>2 420000a	0.000	0.00
		3	3	314500	374000a	0.543	1.13
30	low	1	3	16000	16700bcd	0.305	0.67
		3	3	38900	33000bc	0.370	0.70
	high	1	3	77100	95800b	0.282	0.69
		3	3	27400	21700bc	0.325	0.60
90	low	1	3	11500	7300cd	0.514	0.77
		3	2	12700	9900bcd	0.447	0.88
	high	1	2	4800	4800cd	0.001	<0.01
		3	3	1100	1730d	0.502	1.16
	control		2	100	40e	0.896	1.29

† Anti-log of log mean; means followed by same letter(s) do not differ significantly ($P \leq 0.1$), one-way ANOVA.
‡ Log-transformed data.
§ Coefficient of variation (arithmetic).

The first plot runoff was recorded 30 min after the beginning of simulated rainfall. Simulated rainfall and runoff collection continued through 1511 h, at which time runoff had been generated from all but two plots. There was no discernible pattern in the order in which plots/treatments began to generate runoff. Variation in micro-topography, soil condition, vegetation density, or wind exposure were probably the major determinants of runoff timing.

The *E. coli* data from hayland plot runoff are summarized in Table 4. The result from one control plot was rejected because contamination by runoff from up-slope treatment plots was observed. The *E. coli* levels in runoff from the remaining control plots were very low, indicating that background levels of *E. coli* (e.g., from previous manure applications, soil, or wildlife) were negligible in comparison to contributions from applied manure. Two treatments (90-d-old manure applied to low vegetation 3 d before runoff [90-L-3] and 90-d-old manure applied to high vegetation 1 d before runoff [90-H-1]) had data from only two replicate plots due to the lack of runoff on the third plot. Most runoff samples from plots that received 0-d manure exceeded the maximum range for the *E. coli* analysis; means presented in Table 4 are reported as "greater than" in these cases. However, in subsequent statistical analysis, this condition is dropped and the values are used as real numbers.

The *E. coli* levels in runoff were in the range of ~10^4 to 10^6 organisms per 100 mL reported in the literature for runoff from agricultural land receiving manure (Crane et al., 1983; Baxter-Potter and Gilliland, 1988; Moore et al., 1988). Bacteria levels in runoff from manured plots exceeded those in runoff from control plots by two to five orders of magnitude. The *E. coli* levels in plot runoff declined by at least an order of magnitude in plots treated with successively older manure.

The effect of treatment on levels of *E. coli* in runoff from hayland plots was first evaluated using a multifactor ANOVA model that included all treatment factors (manure age, vegetation height, and delay to rain) and all possible interactions. The full-model ANOVA indicated significant differences among treatments ($P <$ 0.001), but showed that the three-way interaction age × vegetation height × delay was nonsignificant (P = 0.861). A second ANOVA run with the three-way interaction removed showed that both the vegetation height factor and the age × delay to rain interaction were nonsignificant (P = 0.460 and P = 0.571, respectively). The final reduced model included all three main factors plus the age × vegetation height and vegetation height × delay to rain interactions. Results of the reduced model ANOVA are given in Table 5. Mean *E. coli* in runoff grouped by main factors and interactions are shown in Fig. 3. As shown in Fig. 3A, mean runoff *E. coli* decreased significantly with increasing manure age, irrespective of other treatments. Compared to a mean of $10^{6.04}$ *E. coli* per 100 mL in runoff from application of fresh manure, runoff from 30-d-old manure contained an average of $10^{4.51}$ *E. coli* per 100 mL, a 97% reduction. Runoff from 90-d-old manure contained an average of $10^{3.66}$ *E. coli* per 100 mL, a 99.7% reduction compared to runoff from fresh manure.

Delay to rainfall also significantly influenced *E. coli* in runoff from hayland plots (Fig. 3B). Runoff from plots where manure was applied 1 d before rainfall averaged $10^{4.95}$ *E. coli* per 100 mL; runoff from plots where manure was applied 3 d before rainfall averaged $10^{4.65}$ *E. coli* per 100 mL. This 49% reduction was smaller than

Table 5. Analysis of variance table for hayland runoff experiment, final reduced model.

Analysis of variance					
Source	df	Sum of squares	Mean of squares	*F* ratio	*P*
Model	7	34.8385	4.9769	37.135	<0.001
Error	26	3.4846	0.1340		
Total	33	38.32311			
Effects tests					
Source	df	Sum of squares		*F* ratio	*P*
Manure age	2	31.1188		116.096	<0.001
Vegetation height	1	0.0673		0.502	0.485
Delay to rain	1	0.602		4.494	0.044
Manure age × vegetation height	2	0.7427		2.771	0.081
Vegetation height × delay to rain	1	1.2076		9.011	0.006

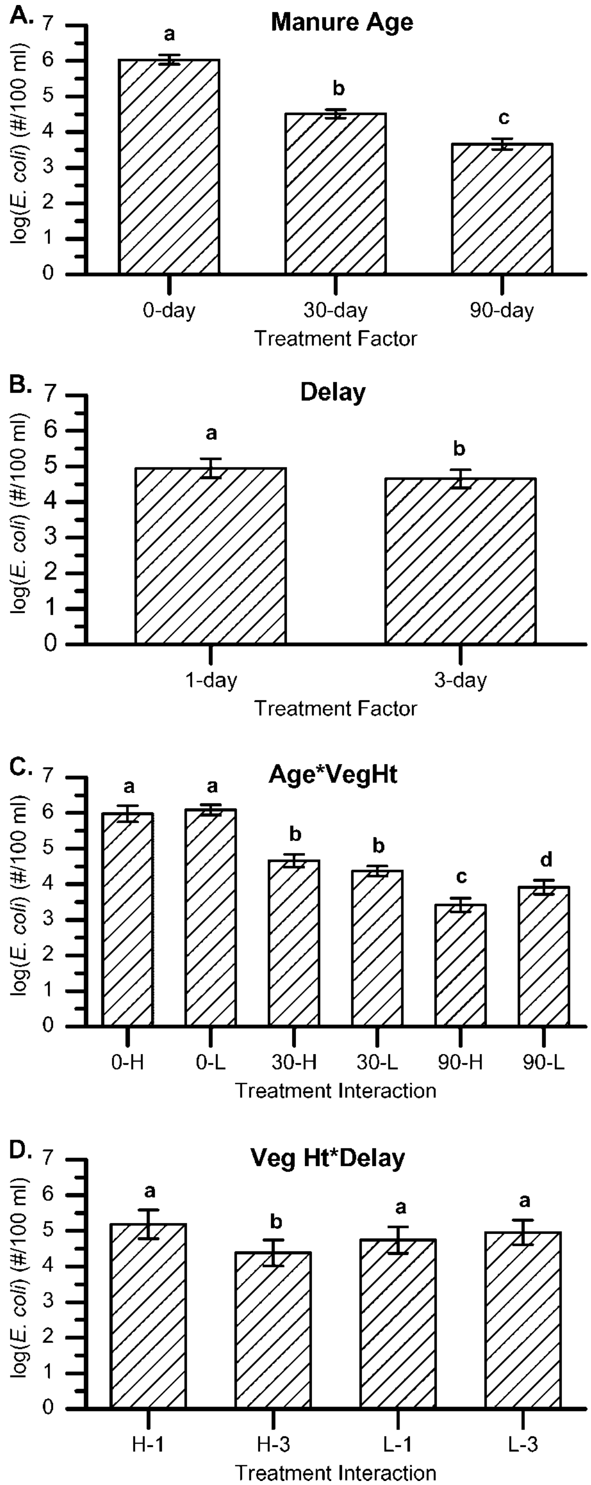

Fig. 3. Levels of *E. coli* in hayland plot runoff by treatment factors and interactions. Error bars represent ±1 standard deviation; bars labeled with different letter(s) differ significantly ($P \leq 0.1$). In (C), treatment codes 0, 30, and 90 indicate age of applied manure in days; H (high) and L (low) indicate vegetation height. In (D), treatment codes H and L indicate high and low vegetation; codes 1 and 3 indicate delay between manure application and simulated rainfall in days.

that attributed to manure age, but was statistically significant ($P = 0.044$).

Vegetation height (not plotted in Fig. 3) did not appear to affect *E. coli* in plot runoff. Runoff from plots with high grass averaged $10^{4.76}$ *E. coli* per 100 mL compared to $10^{4.84}$ *E. coli* per 100 mL in runoff from low grass plots. The 13% lower *E. coli* in runoff from high grass plots was not statistically significant.

Two interactions between main factors were significant. As shown in Fig. 3C, vegetation height appears to influence *E. coli* in runoff differently for different manure age classes. Whereas no significant differences were observed between *E. coli* levels in runoff from high vs. low grass plots receiving 0- or 30-d manure, mean *E. coli* levels in runoff from plots receiving 90-d manure were significantly lower from high grass plots ($10^{3.48}$ *E. coli* per 100 mL) than from low grass plots ($10^{3.93}$ *E. coli* per 100 mL), a difference of 71%. Similarly, the interaction between delay and vegetation height (Fig. 3D) was significant only for the 3-d delay treatments, where mean *E. coli* levels in runoff from high grass plots ($10^{4.38}$ *E. coli* per 100 mL) were 78% lower than from low grass plots ($10^{5.03}$ *E. coli* per 100 mL).

Cornland Experiment

The cornland runoff trial was conducted on 14 Oct. 2003. During the trial period, 11 October (day of first manure application) through 14 October (day of runoff), mean air temperature was 12.1°C, substantially lower than during the June hayland trial. Relative humidity was similar to conditions observed during the hayland runoff trial, averaging ~78%. Winds were moderate and variable and became stronger during the last 2 d of the trial, including the day of the rainfall event. With maximum speeds up to ~20 km h^{-1}, winds were noticeably higher during the cornland runoff trial compared to the ~10 km h^{-1} winds during the hayland runoff trial. There were only trace amounts (0.75 mm) of precipitation recorded onsite during the trial period; some of this amount was probably condensation. Skies were mainly clear, with maximum solar radiation of ~560 to 590 W m^{-2} recorded at midday. Despite clear skies, maximum solar input during the October cornland trial was about 25% lower than that recorded in June in the hayland trial. Soil conditions were fairly dry; in the 30 d preceding the runoff event, 94 mm of rain were recorded at the Montpelier weather station and no rain was recorded during the final 8 d preceding the event. The soil surface appeared smooth and slightly compacted at the time of manure application; the soil surface on incorporated plots was loose and rough following roto-tilling. Soil temperatures on the plot area at 10 cm ranged from 7.0 to 14.5°C during the trial period, substantially lower than the 20 to 28°C observed on the hayland site in June.

Simulated rainfall was applied on 14 October from 0918 to 1202 h. Measured rainfall application averaged 73 mm among the plots, yielding an average intensity of ~27 mm h^{-1}. The rate of application was essentially the same for the cornland and hayland trials, but the plots

Table 6. Summary of *E. coli* data from cornland runoff plots.

Manure age	Incorporation	Delay to rain	*n*	*E. coli* Median	*E. coli* Mean†	Standard deviation‡	CV§
d		d		— organisms per 100 mL —			
0	non-incorporated	1	3	2 010 000	2 342 000a	0.242	0.59
		3	3	175 000	190 000bc	0.174	0.41
	incorporated	1	3	657 000	582 000ab	0.097	0.21
		3	3	529 000	240 000abc	0.776	0.87
30	non-incorporated	1	3	52 900	42 000cd	0.232	0.44
		3	3	7 400	9 000def	0.290	0.70
	incorporated	1	3	12 100	13 900de	0.246	0.59
		3	3	16 000	15 900de	0.038	0.09
90	non-incorporated	1	3	1 000	1 600efg	0.354	0.88
		3	3	1 000	1 800efg	0.461	1.10
	incorporated	1	3	1 000	1 300fg	0.174	0.43
		3	3	1 500	2 100efg	0.416	0.99
	control		3	9 000	600g	0.415	0.68

† Anti-log of log mean; means followed by same letter(s) do not differ significantly ($P \leq 0.1$), one-way ANOVA.
‡ Log-transformed data.
§ Coefficient of variation (arithmetic).

received 28% less simulated rain during the cornland trial due to the shorter irrigation period. All plots began to generate runoff within 1 h; a 1-h duration storm of equivalent intensity has a 5-yr return period in the region (McKay and Wilks, 1995).

Distribution of simulated rain across the test field was somewhat variable due to wind. The mean volume captured in plot catch cups was 334 mL, with a standard deviation of 43 mL and a CV of 13%. Because of variability in rainfall catch among sets of plot replicates, catch was included initially as an independent variable in analysis of the effects of treatment. The *E. coli* content of irrigation water was <1 to 1 *E. coli* per 100 mL, confirming that simulated rainfall added a negligible quantity of *E. coli* bacteria to the plots.

The first plot runoff was recorded 9 min after the beginning of simulated rainfall. Simulated rainfall and runoff collection continued through 1202 h, when a minimum of several liters of runoff had been collected from each plot. Runoff was generated first on the non-incorporated (untilled) plots. The first 10 plots to generate runoff were all non-incorporated; of the first 20 plots to generate runoff, 17 were non-incorporated.

The *E. coli* data from cornland plot runoff are summarized in Table 6. Improved ditching to isolate plots and better prediction of *E. coli* levels in runoff samples resulted in complete data for the cornland trial. The *E. coli* levels in runoff from the control plots were low, but higher than the levels observed in control plot runoff in the hayland trial (mean of 593 *E. coli* per 100 mL vs. 43 *E. coli* per 100 mL in the hayland trial).

The *E. coli* levels in cornland plot runoff decreased with increasing manure age, as they did in the hayland experiment. For plots receiving 0- and 30-d old manure, *E. coli* levels in runoff were in the range of ~10^4 to 10^6 organisms per 100 mL reported in the literature for runoff from agricultural land receiving manure (Crane et al., 1983; Baxter-Potter and Gilliland, 1988; Moore et al., 1988), exceeding levels in runoff from control plots by one to three orders of magnitude. Runoff from plots receiving 90-d-old manure contained ~10^3 *E. coli* per 100 mL, which did not differ significantly from levels observed from the control plots ($P < 0.001$, one-way ANOVA by manure age). The decrease in runoff *E. coli* levels with manure age reflects the magnitude of the decrease in manure *E. coli* content with storage time for the cornland experiment.

The effect of treatment on levels of *E. coli* in runoff from cornland plots was first evaluated using a full multifactor ANOVA model that included all treatment factors (manure age, incorporation, delay to rain, and rainfall catch) and all possible interactions. The full model ANOVA documented significant differences among treatments ($P < 0.001$), but showed that the four-way interaction age × incorporation × delay to rain × rainfall catch was nonsignificant ($P = 0.432$). A second ANOVA run with the four-way interaction removed showed that the rainfall catch factor was nonsignificant ($P = 0.928$), as were all interaction terms involving rainfall catch. A third ANOVA run with rainfall catch removed showed that main factors manure age and delay to rain were both statistically significant, whereas incorporation was nonsignificant ($P = 0.269$); the age × incorporation and age × incorporation × delay to rain interactions were also nonsignificant ($P = 0.722$ and $P = 0.535$, respectively). Results of the final reduced model ANOVA are given in Table 7. Mean *E. coli* in cornland runoff grouped by main factors and interactions are shown in Fig. 4. As shown in Fig. 4A, mean runoff *E. coli* decreased significantly with

Table 7. Analysis of variance table for cornland runoff trial, final reduced model.

Analysis of variance					
Source	df	Sum of squares	Mean of squares	*F* ratio	*P*
Model	7	39.9381	5.7054	51.273	<0.001
Error	28	3.1157	0.1113		
Total	35	43.0538			
Effects tests					
Source	df	Sum of squares		*F* ratio	*P*
Manure age	2	37.1768		167.049	<0.001
Incorporation	1	0.1536		1.380	0.250
Delay to rain	1	0.8126		7.303	0.012
Manure age × delay to rain	2	1.1621		5.222	0.012
Incorporation × delay to rain	1	0.6330		5.688	0.024

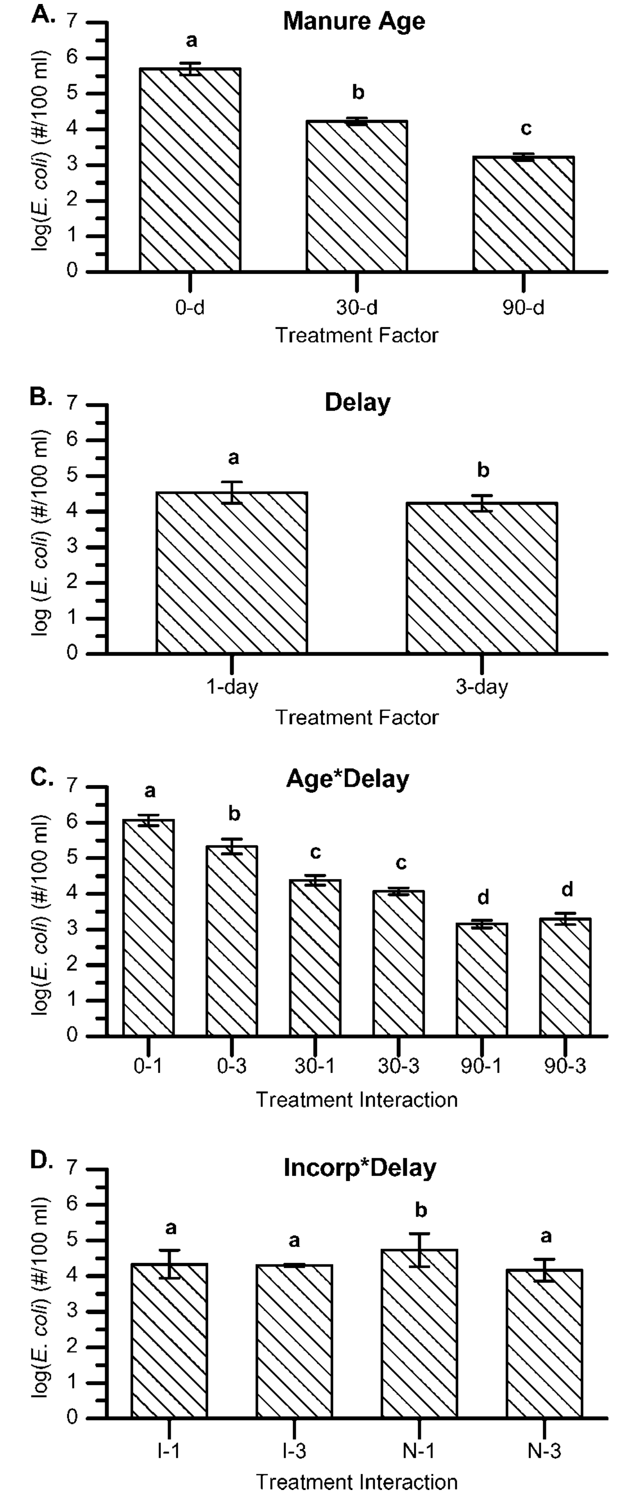

Fig. 4. Levels of *E. coli* in cornland plot runoff by treatment factors and interactions. Error bars represent ±1 standard deviation; bars labeled with different letter(s) differ significantly ($P \leq 0.1$). In (C), treatment codes 0, 30, and 90 indicate age of applied manure in days; codes 1 and 3 indicate delay between manure application and simulated rainfall in days. In (D), treatment codes I and N indicate manure incorporated or not incorporated; codes 1 and 3 indicate delay between manure application and simulated rainfall in days.

increasing manure age, irrespective of other treatments. Compared to a mean of $10^{5.70}$ *E. coli* per 100 mL in runoff from application of fresh manure, runoff from 30-d manure contained an average of $10^{4.23}$ *E. coli* per 100 mL, a 96.6% reduction. Runoff from 90-d manure contained an average of $10^{3.22}$ *E. coli* per 100 mL, a 99.6% reduction compared to runoff from fresh manure.

Delay to rainfall also significantly influenced *E. coli* in runoff from cornland plots (Fig. 4B). Runoff from plots where manure was applied 1 d before rainfall averaged $10^{4.53}$ *E. coli* per 100 mL; runoff from plots where manure was applied 3 d before rainfall averaged $10^{4.23}$ *E. coli* per 100 mL. This 50% reduction was smaller than that attributed to manure age, but was statistically significant ($P = 0.012$).

Manure incorporation alone (not plotted in Fig. 4) did not appear to affect *E. coli* in cornland plot runoff. Runoff from plots where manure was not incorporated averaged $10^{4.49}$ *E. coli* per 100 mL compared to $10^{4.32}$ *E. coli* per 100 mL in runoff from plots where manure was incorporated. The 26% lower *E. coli* in runoff from plots where manure was incorporated was not statistically significant.

As shown in Fig. 4C, longer delay appeared to have a greater effect on reducing *E. coli* in runoff from fresh manure, compared to runoff from either 30- or 90-d-old manure. Where fresh manure was applied, runoff from plots where manure was applied 3 d before rainfall averaged $10^{5..33}$ *E. coli* per 100 mL, compared to an average of $10^{6.07}$ *E. coli* per 100 mL in runoff from plots where manure was applied 1 d before rainfall, an 81% reduction.

Although incorporation of manure was not statistically significant as a main factor, incorporation appeared to significantly reduce *E. coli* in runoff when manure was applied 1 d before rainfall (Fig. 4D). Runoff from plots where manure was applied 1 d before rainfall and not incorporated averaged $10^{4.73}$ *E. coli* per 100 mL, compared to an average of $10^{4.34}$ *E. coli* per 100 mL in runoff from similar plots where manure was incorporated into the soil, a 60% reduction due to incorporation. There was no statistically significant difference between *E. coli* levels in runoff from incorporated and non-incorporated 3-d delay plots.

DISCUSSION

While many of our results were consistent with effects reported in the literature, results from experimental plots should be applied to the design of farm management practices with considerable caution. To the extent that some key processes affecting bacteria export—die-off in storage, interaction with soil and vegetation, time between application and rainfall—are not likely to be strongly scale-dependent, our results may be directly applicable to the field scale. However, microorganism losses as a function of farm-scale management practices cannot be precisely quantified from plot-scale studies because of scale effects on runoff accumulation and overland flow. Rainfall simulators and small plots cannot reproduce flow processes occurring over a landscape and therefore results of our study will not be directly transferable to predicting absolute values of microorganism export from fields or farms in response to innovative management.

Our results confirm that manure application to agricultural land can be a potent source of *E. coli* contamination to surface waters via runoff. Runoff from hayland plots that received manure averaged 63 200 *E. coli* per 100 mL, compared to the average 43 *E. coli* per 100 mL from unmanured control plots. Runoff from cornland plots that received 0- or 30-d manure averaged 92 100 *E. coli* per 100 mL, compared to the average 593 *E. coli* per 100 mL from unmanured control plots. The *E. coli* counts we observed in plot runoff were comparable to the levels 10^4 to 10^6 per 100 mL in runoff from manure application areas reported in the literature (Crane et al., 1983; Baxter-Potter and Gilliland, 1988; Moore et al., 1988; Irvine and Pettibone, 1996; Heinonen-Tanski and Uusi-Kamppa, 2001).

Our experiments were conducted in different seasons, which may have influenced some of our results. Air temperatures and solar radiation, for example, differed substantially between the two experiments. Our results should be reasonably applicable to the agricultural calendar because the timing of our experiments coincided with typical agricultural schedules in Vermont. Manure is typically applied to hayland in late June, after the first hay cut, whereas manure is typically applied to cornland after harvest, in part to distribute manure before the onset of winter.

Effects of Manure Storage

Die-off of *E. coli* during experimental storage was dramatic. From an initial *E. coli* content of ~10^5 organisms per gram at delivery, *E. coli* content of manure stored for ~30 d declined by ~99% (2 log); *E. coli* declined by >99% (2–3 log) after ~90 d of storage. Both the initial bacteria content and the observed declines were consistent with reports from the literature (e.g., Crane et al., 1983; Moore et al., 1988; Patni et al., 1985; Trevisan and Dorioz, 1999; Conboy and Goss, 2001). The two to three orders-of-magnitude declines in *E. coli* counts with storage tend to confirm the potential for manure storage to be an important factor in reducing the bacteria content of manure to be applied to agricultural land.

It is generally accepted that die-off in bacteria populations follows simple first-order kinetics (Crane and Moore, 1986). Estimates of first-order die-off rate constants based on data from the beginning and end of our storage periods are shown in Table 3, along with values of T_{90} (time required to reduce initial *E. coli* levels by 90%) derived from the given rate constants. Although our storage experiments may not have replicated conditions in full-scale manure storage structures, our results are consistent with data reported from actual storage facilities. For example, Jones (1980) reported a T_{90} of 14 to 28 d for *Salmonella* sp. in unaerated storage. Kearney et al. (1993) determined a T_{90} of ~77 d for *E. coli* in an anaerobic manure digester. Himathongkham et al. (1999) observed exponential decay of *E. coli* O157:H7 and *Salmonella typhimurium* in both solid manure and manure slurry, with values of T_{90} from 6 to 21 d in solid manure and from 2 to 35 d in slurry.

The *E. coli* levels declined more rapidly in the ~90-d storage tanks established for the cornland trial than in those used for the hayland trial. This difference may have been due to higher air temperatures during the July–October storage period preceding the cornland experiment (mean daily air temperature 18.1°C, 292 cooling degree days; cooling degree day = daily number of degrees Fahrenheit by which mean temperature exceeds 65°F/18.3°C) compared to the March–June storage period for the hayland trial (mean daily air temperature 11.3°C, 29 cooling degree days). Temperature has been widely noted as one of the most important factors affecting bacterial survival, with higher temperatures generally enhancing die-off (Crane and Moore, 1986; Moore et al., 1988). Weather conditions over the 30-d storage periods before the hayland and cornland trials were more comparable. The estimated k values in Table 3 also demonstrate that the estimated die-off rate in both experiments was more rapid over the ~30-d storage period than over the ~90-d period. This may be an artifact of our crude estimates of k values based only on initial and final bacteria numbers. If real, however, this result may indicate that *E. coli* die-off was not in fact first-order over the longer period. We speculate that the higher die-off rates over ~30 d (k = 0.06–0.07) may have been due to an initial rapid die-off of the most sensitive portion of the *E. coli* population on first exposure to inimical conditions, while more hardy portions of the population may have survived longer. If this pattern is valid in full-scale storage, it suggests that the largest proportional benefit in reducing *E. coli* occurs within the first ~30 d of storage, making storage a more practical and cost-effective practice for producers than would be the case if ~90-d storage was required.

Manure age had the greatest influence on *E. coli* in runoff from hayland and cornland in our experiments. This is not surprising, as runoff losses of *E. coli* should tend to be proportional to the quantity of microorganisms available for transport (VanDonsel et al., 1967; McCaskey et al., 1971; Robbins et al., 1971). Runoff from hayland and cornland plots treated with 30-d-old manure averaged 97% fewer *E. coli* than did runoff from plots that received fresh manure. Runoff from hayland and cornland plots treated with 90-d-old manure contained 99% (hayland trial) and >99% (cornland trial) fewer *E. coli* than runoff from fresh manure treated plots. Runoff from cornland plots receiving 90-d manure contained *E. coli* levels similar to those in runoff from unmanured plots.

Effects of Delay between Application and Runoff

Delay between manure application and rainfall and runoff was a significant influence on *E. coli* levels in runoff in our experiments. Runoff from both hayland and cornland plots where manure was applied 3 d before rainfall contained ~50% fewer *E. coli* than did runoff from plots that received manure 1 d before rainfall.

The significance of time elapsed between manure application and rainfall has been widely documented. Increasing residence time of manure on the land surface before rainfall and runoff increases the opportunity for bacteria die-off and immobilization of bacteria through adsorption–fixation to surface soils and vegetation and exposure to UV radiation and dessication. Moore et al. (1988) reported that the residence time of manure on the land surface after application of liquid swine waste to pasture was the controlling factor for bacteria loss in runoff. If runoff occurred on the day of application, Moore et al. found that 58 to 90% of fecal coliform were lost in runoff, but if residence time increased to 3 d, only 10 to 22% of fecal coliform were lost. During the 3-d delay between manure application and simulated rainfall in our hayland experiment, peak daily solar radiation was ~640 to 970 W m^{-2}, midday air temperatures exceeded 25°C, and essentially no natural precipitation fell. Such conditions would have been inimical to bacteria survival on the land surface. Even though environmental conditions during the cornland trial in October were less harsh than those during the hayland trial in June (peak daily solar radiation ~560 to 590 W m^{-2}, about 25% lower; mean daily air temperature of 12°C, about 10°C lower than in the June trial), the lack of cover on the nearly bare surface of the corn field probably resulted in greater exposure of bacteria to lethal conditions. Competition and predation within the microorganism population are also believed to contribute to *E. coli* die-off (Moore et al., 1988). As a result of such factors, die-off of *E. coli* after land application has been observed to follow a first-order decay, with reported values of k ranging from 0.303 to 0.697 d^{-1}, with greatest die-off in warm weather (Klein and Casida, 1967; Van Donsel et al., 1967; Taylor and Burrows, 1971).

Whereas a 3-d delay between manure application and runoff reduced *E. coli* in runoff from all ages of manure, the longer delay had a greater effect in reducing *E. coli* numbers in runoff from fresh manure treated cornland plots than from cornland plots treated with stored manure. This effect may have been due to higher initial die-off rates in the fresh manure on application.

Effects of Vegetation Height

Vegetation height alone was not found to significantly affect levels of *E. coli* in runoff from our hayland plots. Two contrasting influences on *E. coli* levels have been proposed in relating the condition of hayland vegetation to bacteria runoff. Interception of some manure on vegetation is thought to affect bacteria availability for loss as well as overall survival rates. In greenhouse studies, Brown et al. (1980) observed that fecal coliform bacteria from sludge application to grasses adhered to leaves and were difficult to wash off. J.Y. Vansteelant at Institut National de la Recherche Agronimique (INRA; personal communication, 2000) found that about half of the *E. coli* applied to grassland in the French Alps was intercepted on the vegetation. Trevisan et al. (2002) reported that fecal coliform from manure slurry applied to grassland declined in number rapidly on the vegetation, particularly during dry periods. Thus, bacteria in manure intercepted by vegetation could be more resistant to being washed off by rain or could die more quickly than on soil. Under this scenario, fields with greater standing vegetation would tend to reduce the quantity of microorganisms lost in runoff.

Alternatively, it has been suggested that bacteria die-off can be enhanced when manure is applied to thin or short vegetation. Crane et al. (1983) observed that cutting pastures reduced bacteria survival times by enhancing manure drying and exposure to solar radiation. In haylands of the French Alps, Trevisan et al. (2002) reported that when plant canopy biomass was low, fecal coliform die-off was rapid due to the effect of increased UV light penetration and/or drying; fecal coliform counts remained higher when the canopy biomass was greater, suggesting a protective effect of the vegetation. The authors concluded that after slurry application, the hay meadows with the best vegetation stands maintain higher bacteria numbers.

We observed no significant difference in *E. coli* levels in hayland plot runoff due to vegetation height alone. It is possible that the difference in vegetation heights between the low treatment (~7 cm) and the high treatment (~14 cm) was not large enough for either a protective effect or an interception effect to be detectable, or that such effects offset each other. Vegetation on the entire hay field before the experiment was somewhat thin and even the 7-cm height difference between the two treatments may not have been enough to provide a sheltering effect.

However, mean *E. coli* levels in runoff from plots receiving 90-d manure were 68% lower from high than low grass plots. Manure stored for 90 d contained far fewer *E. coli* (~1260 organisms per gram) than did the manure from the 30- or 0-d storage treatments (Table 3). The effect of *E. coli* interception die-off on high vegetation may have been large enough to be detected at the lower initial bacteria densities of the 90-d-old manure, but too small to be detectable in higher bacteria content manure. Furthermore, our ability to detect significant differences among plots receiving 0-d manure was compromised by the fact that many of the *E. coli* counts exceeded the analytical range.

We also observed a significant interaction between vegetation height and delay. When manure was applied 3 d before simulated rainfall, runoff from high vegetation plots contained significantly fewer *E. coli* (78% less) than did runoff from low vegetation plots. No significant difference was observed in runoff from plots of different vegetation height where manure was applied the day before the rainfall event. The 3-d delay before rainfall may have been sufficient for the effects of enhanced die-off on high vegetation to be observed, while these effects were not apparent over just 1 d.

Effects of Incorporation

Although manure incorporation following application to cropland has long been recommended to reduce runoff losses of nutrients, potential influences of incorporation on bacteria losses are contradictory. Some studies have documented substantial bacteria die-off when waste is mixed into the soil due to predation or immobilization of organisms through adsorption onto soil particles. Reddy et al. (1981) reported die-off rate constants in the range of 0.15 to 6.39 d^{-1} for *E. coli* in the soil–water–plant system. Sjogren (1994) reported lower die-off rates of 0.03 to 0.06 d^{-1} for *E. coli* in laboratory soil microcosms. Additionally, incorporation is thought to reduce the availability of microorganisms for loss in runoff because most of the microorganisms are moved away from interaction with surface runoff. In contrast, some research has suggested that long-term bacterial survival may be enhanced when manure is incorporated into the soil because organisms are protected from desiccation, sunlight, and high temperatures (Dazzo et al., 1973; Walker et al., 1990; Jamieson et al., 2002).

Despite these potential effects, we observed no significant difference in *E. coli* levels in cornland plot runoff due to incorporation alone. Our tillage with a roto-tiller was less aggressive than moldboard plowing, which inverts the plow layer; not all manure *E. coli* may have been removed from interaction with surface runoff. A conclusion that incorporation will have no effect on bacteria losses would be misleading, however, if our results are applied to larger-scale settings. We observed that incorporation caused a substantial delay in runoff generation from our plots. This result was probably due to enhanced infiltration and increased detention storage on the loose, rough soil surface of the tilled plots. In a full-scale field application, these characteristics could be a major determinant of *E. coli* transport because runoff volume would tend to be greatly reduced or even eliminated from a field where manure had been recently incorporated, especially during small storms. Thus, export of bacteria could be reduced by manure incorporation simply as the result of reduction of field runoff.

We also observed that *E. coli* levels in runoff from cornland plots were significantly affected by incorporation when manure was applied 1 d before simulated rainfall but not when applied 3 d before. Runoff from plots where manure was applied and incorporated the day before simulated rainfall averaged 60% fewer *E. coli* compared to runoff from similar plots where manure was not incorporated. This result can be interpreted in different ways. It is possible that the effect was observed because bacteria immobilization through burial and soil interactions was greater than the opportunity for bacteria die-off on the surface over just a 1-d period. Alternatively, this result could suggest that a 3-d delay resulted in greater *E. coli* die-off without manure incorporation than with incorporation because incorporation protected the bacteria from the lethal effects of the surface environment. This conclusion is supported by the fact that *E. coli* levels were 73% lower, a statistically significant difference ($P = 0.024$), in runoff from non-incorporated plots treated 3 d before rainfall than from non-incorporated plots treated 1 d before rainfall, whereas delay did not significantly effect *E. coli* levels in runoff from incorporated plots.

CONCLUSIONS

The results of our experiments support several recommendations for improved manure management to reduce microorganism export from manure application sites. Manure storage is an important factor in reducing the bacteria content of manure to be applied to agricultural land. Reductions of more than 99% in the *E. coli* content of manure were documented in pilot storage experiments. Storage of manure for 30 d or more consistently and dramatically lowered *E. coli* counts in our experiments, with longer storage providing greater reductions. We believe that storage for a specific duration that avoids repeated additions of fresh manure would reduce bacteria levels relative to conventional storage practices. Such definitive storage could take the form of multiple-pit or compartmentalized storage structures, or multiple, sequential stacking areas for farm operations that lack a storage facility. Management of such systems would require that the oldest manure be applied first.

In both the hayland and cornland experiments, manure age was the most significant factor influencing *E. coli* counts in runoff. In the cornland trial, runoff from plots that received the oldest manure did not contain significantly more *E. coli* than runoff from plots that received no manure. Use of manure stored for at least 90 d before application to hayland or cornland should yield substantial reductions in the loss of microorganisms from agricultural land.

Manure application several days in advance of runoff significantly reduced *E. coli* losses in runoff from both hayland and cornland by ~50%. Although it is clearly not possible to completely control this variable because of uncertainty in short-term weather forecasting, it should still be possible to avoid manure application in advance of major frontal storm systems or long-term weather patterns.

On hayland, maintaining higher vegetation (~14 cm) at manure application may be beneficial in some circumstances. For applications following hay cuts, this translates to raising the mowing height or, alternatively, to waiting about a week between a cut and manure application.

On cornland, incorporation appeared to delay the generation of runoff from plots, an effect that would tend to reduce runoff volume and total bacteria export from fields over a series of real-world storm events. Levels of *E. coli* in runoff were significantly reduced when manure applied the day before runoff was incorporated. Thus, prompt incorporation of applied manure should assist in reducing the loss of microorganisms in runoff from cornland under a variety of circumstances.

The treatments tested in this study could be important components of a multiple barrier approach for control of agricultural pathogen losses. In the process of establishing new management practices, we believe that plot studies are a necessary first step, but are not sufficient alone to define a management practice to be applied at the farm or watershed level. We would therefore urge additional evaluation at the farm scale before positive results from this study were codified as a best management practice.

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