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Basin Program

Demonstration of Methods to Reduce Indicator Bacteria Levels in Agricultural Runoff in Vermont

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and
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Demonstration of Methods to Reduce Indicator Bacteria Levels in Agricultural Runoff in Vermont

FINAL REPORT

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EXECUTIVE SUMMARY

Introduction

Portions of many streams in the Lake Champlain Basin are impaired by bacteria levels that routinely exceed the allowable maximum set by water quality standards (NYS DEC, 2002 and VTDEC, 2000). Livestock agriculture can be a major source of microorganism loading to surface and ground waters, as evidenced by levels of indicator bacteria such as fecal coliform and *Escherichia coli*. Although numerous management practices have been developed and implemented to control sediment and nutrients in agricultural runoff, effective practices to specifically control export of microorganisms to surface waters have not been widely developed, tested, or applied. Because the enormous number of microorganisms present in animal waste so greatly exceeds the number established in water quality standards, the idea of a multiple barrier approach—wherein a series of controls are put in place at several points from the source(s) to the stream—has been proposed to reduce the risk of exposure to pathogens in the surface waters of the Lake Champlain Basin.

Objectives

The principal goals of the project were:

1. Demonstrate and evaluate innovative, practical methods for controlling pathogens, measured as *E. coli*, from livestock agricultural sources to surface waters; and
2. Recommend a multiple barrier approach for reducing pathogens in agricultural runoff in the Lake Champlain Basin.

To achieve these goals, the project undertook several specific objectives:

- Summarize available information on environmental conditions and agricultural practices that influence the export of pathogens from agricultural land.
- Demonstrate and evaluate innovative, practical methods for reducing pathogen loads to surface waters in runoff from hayland and cornland, focusing on a multiple barrier approach.
    - Determine the effect of definitive periods of storage on *E. coli* levels in liquid dairy manure;
    - Determine the effect of manure incorporation on losses of *E. coli* in runoff from cornland receiving an application of liquid dairy manure;
    - Determine the effect of vegetation height on losses of *E. coli* in runoff from hayland receiving an application of liquid dairy manure; and
    - Determine the effect of lag time between manure application and precipitation on losses of *E. coli* in runoff from both hayland and cornland.
- Combine experimental results with conclusions from the scientific literature to recommend a multiple-barrier approach for reducing pathogens in agricultural runoff in the Lake Champlain Basin.

The project used a pilot manure storage system, simulated rainfall, and replicated runoff plots in a factorial design to evaluate the effectiveness of simple, low-cost practices designed to reduce microorganism levels in agricultural runoff from cornland and hayland in the Lake Champlain Basin.

Results

Manure Storage Experiments

- The nutrient content of the liquid manure used in the project was comparable to average values reported from manure analyses conducted by the University of Vermont Agricultural and Environmental Testing Laboratory from 1992 – 1996.
- Mean *E. coli* levels in fresh manure delivered to the experimental sites ranged from 3.1 – 7.5 x 10^5 organisms/g manure wet weight.
- Storage of manure for ~90 days reduced *E. coli* counts by two orders of magnitude, or more than 99%; ~30 day storage reduced *E. coli* counts by 99%.
- Significantly greater reductions in manure *E. coli* occurred over the July – October 90-day storage for the cornland trial than over the April – June 90-day storage for the hayland trial, probably due to higher ambient temperatures during the storage period.

Hayland Runoff Trial (June 24, 2003)

- *E. coli* levels in runoff from manured plots were in the range of values reported in the literature for runoff from agricultural land receiving manure (~10^4 – 10^6 *E. coli*/100 ml) and exceeded those in runoff from unmanured control plots by two to five orders of magnitude.
- Mean runoff *E. coli* decreased significantly with increasing manure age, regardless of other treatments. Compared to a mean of $10^{6.04}$ *E. coli*/100 ml in runoff from application of fresh manure, runoff from 30-day old manure contained an average of $10^{4.51}$ *E. coli*/100 ml, a 97% reduction. Runoff from 90-day old manure contained an average of $10^{3.66}$ *E. coli*/100 ml, a 99.6% reduction compared to runoff from fresh manure.
- Delay to rainfall significantly influenced *E. coli* in runoff from hayland plots. Runoff from plots where manure was applied 1 day before rainfall averaged $10^{4.95}$ *E. coli*/100 ml; runoff from plots where manure was applied 3 days before rainfall averaged $10^{4.65}$ *E. coli*/100 ml. This 49% reduction was smaller than that attributed to manure age.
- Vegetation height alone did not appear to affect *E. coli* in plot runoff. Runoff in plots with high (13-15 cm) grass averaged $10^{4.76}$ *E. coli*/100 ml compared to $10^{4.84}$ *E. coli*/100 ml in runoff from low (5-7 cm) grass plots. The 13% lower *E. coli* in runoff from high grass plots was not statistically significant.
- Mean *E. coli* levels in runoff from plots receiving 90-day manure ($10^{3.480}$ *E. coli*/100 ml) were significantly lower from high grass plots than from low grass plots ($10^{3.927}$ *E. coli*/100 ml), a difference of 71%.

- Mean *E. coli* levels in runoff from high grass plots ($10^{4.382}$ *E. coli*/100 ml) were significantly lower than from low grass plots ($10^{5.029}$ *E. coli*/100 ml)—a 78% difference—only where manure was applied 3 days before rainfall.

Cornland Runoff Trial (October 14, 2003)

- *E. coli* levels in runoff from plots receiving 0-day and 30-day old manure exceeded those in runoff from unmanured control plots by one to three orders of magnitude and were in the range of values reported in the literature for runoff from agricultural land receiving manure (~10^4 – 10^6 /100 ml).
- Mean runoff *E. coli* decreased significantly with increasing manure age, regardless of other treatments. Compared to a mean of $10^{5.70}$ *E. coli*/100 ml in runoff from application of fresh manure, runoff from 30-day old manure contained an average of $10^{4.23}$ *E. coli*/100 ml, a 96.6% reduction.
- Runoff from cornland plots receiving 90-day old manure contained an average of $10^{3.22}$ *E. coli*/100 ml, a 99.6% reduction compared to runoff from fresh manure; *E. coli* levels in runoff from these plots did not differ significantly from *E. coli* levels in runoff from control plots that received no manure.
- Delay to rainfall significantly influenced *E. coli* in runoff from cornland plots. Runoff from plots where manure was applied 1 day before rainfall averaged $10^{4.53}$ *E. coli*/100 ml; runoff from plots where manure was applied 3 days before rainfall averaged $10^{4.23}$ *E. coli*/100 ml. This 50% reduction was smaller than that attributed to manure age.
- Longer delay appeared to have a greater effect on reducing *E. coli* in runoff from fresh manure, compared to runoff from either 30-day or 90-day manure. Where fresh manure was applied to cornland plots, runoff from plots where manure was applied 3 days before rainfall averaged $10^{5.33}$ *E. coli*/100 ml, compared to an average of $10^{6.07}$ *E. coli*/100 ml in runoff from plots where manure was applied 1 day before rainfall. This represents an 81% reduction.
- Manure incorporation alone did not appear to affect *E. coli* in cornland plot runoff. Runoff in plots where manure was not incorporated averaged $10^{4.49}$ *E. coli*/100 ml compared to $10^{4.32}$ *E. coli* /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.
- Incorporation appeared to significantly reduce *E. coli* in runoff when manure was applied 1 day before rainfall, whereas *E. coli* in runoff from 3-day delay plots was not significantly affected by incorporation. Runoff from plots where manure was applied 1 day before rainfall and not incorporated averaged $10^{4.73}$ *E. coli*/100 ml, compared to an average $10^{4.34}$ *E. coli*/100 ml in runoff from similar plots where manure was incorporated, a 60% reduction in bacteria levels.
- Incorporation appeared to delay runoff generation from plots, probably due to differences in surface soil characteristics of incorporated vs. non-incorporated plots. Delayed generation of runoff from tilled plots probably resulted from enhanced infiltration and increased detention storage on the loose, rough soil surface left by tillage.

Conclusions

- Die-off of *E. coli* during experimental storage was dramatic. Manure stored for ~30 days showed a decline of ~99% (2 log) in *E. coli* content; *E. coli* declined by >99% (2 – 3 log) after ~90 days of storage. These declines are consistent with reports from the literature.
- Net bacteria die-off in experimental storage was probably enhanced by the absence of frequent inoculations with fresh manure that would be characteristic of full-scale manure storage systems.
- Manure application to hayland is a potential source of bacterial contamination to surface waters via runoff. For all treatments combined, runoff from hayland plots that received manure averaged 63,222 *E. coli*/100 ml, compared to the average 43 *E. coli*/100 ml from unmanured control plots.
- Manure application to cornland can be a source of bacterial contamination to surface waters via runoff. For all treatments combined, runoff from cornland plots that received fresh manure and manure stored for 30 days averaged 92,145 *E. coli*/100 ml, compared to the average 593 *E. coli*/100 ml from unmanured control plots. Runoff from plots that received manure stored for 90 days, however, contained levels of *E. coli* (1,673/100 ml) that did not differ significantly from those in runoff from unmanured control plots.
- Manure storage is an important factor in reducing the bacteria content of manure to be applied to agricultural land. Storage of manure for 30 days or more dramatically lowered *E. coli* counts in our experiments, with longer storage providing greater reductions. Storage for a specific duration that avoids frequent additions of fresh manure would enhance the bacteria reductions achieved by storage. 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.
- Increased manure storage time resulted in comparably reduced levels of *E. coli* in runoff from the land where the manure was applied. In both the hayland and cornland experiments, manure age was the most significant factor influencing *E. coli* counts in runoff. Use of manure stored for at least 90 days 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 compared to application just one day before runoff. 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 other predicted rainfall events.
- 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. *E. coli* levels 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.

Recommended Multiple-Barrier Approach

Combining the results of our plot studies and the documentation of other bacteria control measures in the literature, we recommend the following set of practices as a multiple barrier approach to reduce indicator bacteria levels in agricultural runoff:

- **Source control**
  - Implement animal treatment/biosecurity practices to reduce the incidence of true pathogens in animal waste;
  - Scrape barnyards regularly, divert runoff flow from upgradient areas away from the barnyard, and control barnyard runoff to direct accumulated manure and runoff into a waste storage facility to eliminate the barnyard as a discrete source; and
  - Store manure for a definitive period of ~90 days without addition of fresh manure;

- **Availability/Runoff control**
  - Prohibit manure application to frozen or snow-covered ground;
  - Prohibit manure application during heavy rainfall, when soil is saturated, or when tile lines are flowing;
  - To the extent feasible, avoid manure application less than 3 days before major storms, storm fronts, or other predicted rainfall events;
  - Apply manure at a maximum rate determined by an approved nutrient management plan based on crop need for nutrients;
  - On cornland, incorporate applied manure by tillage within 24 to 72 hours of application;
  - On hayland, allow vegetation to reach a height of ~14 cm before manure application; and
  - Implement a whole-farm runoff/erosion control plan to promote infiltration and control runoff, reduce movement of bacteria associated with soil particles or manure aggregates, and avoid excessive manure application to sensitive areas or runoff contributing areas. Nutrient management may also help reduce losses of indicator organisms.

- **Delivery control**
  - On pasture land, use fencing or other means to eliminate livestock access to streams and other watercourses;
  - Where possible, use light tillage before or after manure application to disrupt soil macropores; and
  - Although buffers should not be relied upon as a bacteria reduction practice via filtration effects, buffers can be used to ensure that manure applications are set back from watercourses, thereby avoiding accidental direct application of waste to the water.

1. INTRODUCTION

Management plans for the Lake Champlain Basin call for protecting human health by controlling sources of pathogens such as bacteria and viruses to surface waters. Exposure to pathogens in surface waters through contact recreation presents the risk of adverse human health impacts. Portions of many streams in the Basin are impaired by bacteria levels that routinely exceed the allowable maximum set by water quality standards. Water quality standards for indicator bacteria are frequently exceeded in surface waters of the Lake Champlain Basin (NYS DEC, 2002 and VTDEC, 2000); beaches on Lake Champlain are sometimes closed to swimming due to elevated indicator bacteria levels. In three streams draining agricultural watersheds in the Missisquoi River Basin, the Vermont water quality standard for *E. coli* of 77/100 ml was exceeded 50 – 70% of the time from 1995 – 1998; annual median *E. coli* count exceeded the standard for each of the streams in all years (Meals 2001). In the Mad River watershed (Vermont), 54% of warm-weather high flow *E. coli* counts exceeded the Vermont standard (Sargent and Morrissey 2000). Fecal coliform counts at most Missisquoi Bay tributary stations exceeded the Quebec standard of 200/100 ml 25 to >50% of the time from 1999 to 2001 (Simoneau 2003).

Agricultural, urban, and forest 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 agricultural land is frequently the cause of impairment of surface waters in the Basin. Livestock agriculture can be a major source of microorganisms to surface and ground waters; based on sheer numbers and quantities of feces generated, farm animals are likely to be the predominant source of bacteria and other microorganisms in rural/agricultural watersheds characteristic of much of the Lake Champlain Basin. Indicator organisms such as *E. coli* and fecal coliform are commonly found in surface waters draining agricultural land, usually in numbers exceeding water quality criteria (*e.g.*, Crane *et al.* 1983, Baxter-Potter and Gilliland 1988, Meals 1989 and 2001, Niemi and Niemi 1991, Howell *et al.* 1995, Crowther *et al.* 2002). While numbers vary by species, farm animals typically shed ~10^6 - 10^7 fecal coliform organisms per gram of waste, or ~10^9 - 10^{10} organisms per capita per day (Robbins *et al.* 1971, Reddy *et al.* 1981, Moore *et al.* 1988). In addition to benign indicator bacteria such as fecal coliform or *Escherichia coli* that may cause violations of water quality standards, other microorganisms from agricultural operations may directly threaten human health. Pathogenic organisms such as *Salmonella, Campylobacter*, *Listeria*, *Yersinia*, *Mycobacterium*, *Leptospira, Cryptosporidium, Giardia,* and *E. coli* O157:H7, the pathogenic variety of *E. coli,* are sometimes found in animal manures and may be transmitted to the environment to potentially cause severe illness or death to infected persons (Stehman *et al.* 1996). Data on the occurrence of these and other true pathogens in surface and ground waters are rare; bacterial indicators such as *E. coli* are often measured and reported as evidence of fecal contamination and the potential presence of pathogens. There is reasonable evidence of association between presence of indicator bacteria and the occurrence of gastroenteritis (Dufour 1984).

Major sources of microorganisms on dairy farms in the Lake Champlain Basin include:

- **Manure storage**. Collected and stored animal waste represents the primary stock of microorganisms in dairy farm operations.

- **Barnyards and holding facilities.** Concentrated animal holding areas accumulate manure and represent important stocks of fecal microorganisms.
- **Land application.** Runoff from manure application sites is potentially a major source of microorganism loading to surface waters.
- **Grazing.** Animals on pasture deposit microorganisms with their manure; livestock access to streams and runoff from fecal deposits on the land can be important sources of microorganisms.

Although numerous management practices have been developed and implemented to control sediment and nutrients in agricultural runoff, effective practices to specifically control export of microorganisms to surface waters have not been widely developed, tested, or applied. Because the enormous number of microorganisms present in animal waste so greatly exceeds the number established in water quality standards, no single management practice is likely to provide the more than 99.99% reduction necessary to achieve adequate protection of water quality and human health. Consequently, the idea of a multiple barrier approach—wherein a series of controls are put in place at several points from the source(s) to the stream—has been proposed to reduce the risk of exposure to pathogens in the surface waters of the Lake Champlain Basin (Rosen 2000). Given the nascent state of the science, not enough is known at the present time to recommend specific controls with confidence.

2. GOALS AND OBJECTIVES

The project supports the overall goal of protecting humans from waterborne health hazards, as outlined in the long-term management plan for Lake Champlain, *Opportunities for Action: An Evolving Plan for the Future of the Lake Champlain Basin.* The principal goals of the project were:

1. Document the effectiveness of combinations of simple, low-cost practices designed to reduce microorganism levels in agricultural runoff from corn and hay land in the Lake Champlain Basin; and
2. Recommend a multiple barrier approach for reducing pathogens in agricultural runoff in the Lake Champlain Basin.

To achieve these goals, the project undertook several specific objectives:

- Summarize available information on environmental conditions and agricultural practices that influence the export of pathogens from agricultural land.
- Demonstrate and evaluate innovative, practical methods for reducing pathogen loads (measured as indicator *E. coli*) to surface waters in runoff from hayland and cornland, focusing on a multiple barrier approach.
    - Determine the effect of definitive periods of storage on *E. coli* levels in liquid dairy manure;
    - Determine the effect of manure incorporation on losses of *E. coli* in runoff from cornland receiving an application of liquid dairy manure;
    - Determine the effect of vegetation height on losses of *E. coli* in runoff from hayland receiving an application of liquid dairy manure; and
    - Determine the effect of lag time between manure application and precipitation on losses of *E. coli* in runoff from both hayland and cornland.
- Combine experimental results with conclusions from the scientific literature to recommend a multiple-barrier approach for reducing pathogens in agricultural runoff in the Lake Champlain Basin.

The methods evaluated in the experiments were selected based on reports from the literature. Numerous researchers have reported die-off of manure microorganisms associated with storage (e.g., Moore *et al.* 1983, Patni et al. 1985, Walker *et al.* 1990, Trevisan and Dorioz 1999, Jamieson *et al.* 2002). Incorporation of manure on cropland has been proposed as a method to remove manure microorganisms from interaction with surface runoff (Reddy *et al.* 1981, Crane and Moore 1984, Patni et al. 1985). Recent research has suggested that the height of grass at manure application may influence bacteria survival after application, potentially reducing bacteria runoff losses as applied manure adheres to grass stems or enhancing bacteria survival by protection from sunlight and high temperatures (Crane *et al.* 1983, Trevisan *et al.* 2000, Vansteelant 2000). Finally, considerable research has documented the importance of lag time between application and runoff in bacteria losses from manure application sites (Moore *et al.* 1988).

3. METHODS

3.1. Experimental Design

The project used a pilot manure storage system, simulated rainfall, and replicated runoff plots in a factorial design to evaluate the effectiveness of simple, low-cost practices designed to reduce microorganism levels in agricultural runoff from cornland and hayland in the Lake Champlain Basin. The treatments tested in field runoff trials were: (1) duration of manure storage; (2) soil incorporation of manure applied to cornland; (3) vegetation height on hayland receiving manure; and (4) delay between manure application and rainfall. Use of a factorial design allowed assessment of interactions between treatments as part of a multiple barrier approach to control microorganisms in runoff. The overall hypothesis tested was that levels of *E. coli* in runoff from agricultural land can be significantly reduced by a series of simple management practices. This hypothesis was tested by evaluating the effects of treatment using multi-factor Analysis of Variance (ANOVA).

Small-scale experimental manure storage, replicated runoff plots, and simulated rainfall applications are appropriate methods for initial evaluation of innovative techniques before moving to broad application, particularly when underlying physical processes are not believed to be strongly scale-dependent. The key processes affecting bacteria export evaluated in our study—die-off in storage, soil and vegetation interaction, die-off in field between application and rainfall—are not likely to be strongly scale-dependent. Because none of these processes are thought to be a function of runoff time, slope length, or distance of travel, we believe that the small plot size is not a particular disadvantage. Other processes such as erosion or filtration through a vegetated filter strip would be less appropriate to study at this scale. Sharpley and Kleinman (undated) showed that overland flow processes controlling soil P release and transport are independent of simulator type, flow-path length, and plot size, and that small plots were appropriate to determine the factors controlling the relationship between overland flow P and soil P. Our results were consistent with those reported in the literature from field-scale systems.

While appropriate for relative assessments, rainfall simulators and small plots cannot reproduce flow process 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. Microorganism losses as a function of farm-scale management practices cannot be precisely quantified from plot-scale studies because of scale effects on overland flow and runoff. Differences in overland flow, sediment delivery, and P transport as a function of plot size have been documented (Mutchler *et al.* 1988, Truman *et al.* 2001, Sharpley and Kleinman undated).

3.2. Manure Storage Experiments

Two experiments were conducted to document the effects of extended storage on the *E. coli* content of manure and to provide manure of specified ages for the field runoff trials. The hypothesis tested was that *E. coli* levels in manure would decline significantly with increasing age.

At each study site, a pilot manure storage system was established consisting of nine 1.4 m diameter plastic tanks, each with a depth of ~0.28 m and maximum volume of ~430 L (114 gallons) (Appendix A, Photo 1). With the appropriate lead-time in advance of the simulated rainfall trials, tanks were filled with fresh liquid dairy manure and allowed to age under ambient conditions. The actual volume of manure contained in each tank was ~300 L (80 gallons). Three replicate tanks were used for each manure age class.

The source of manure for both the storage experiments was the main dairy barn at Fairmont Farms in East Montpelier, Vermont. In all cases, fresh liquid dairy manure was obtained from a sump below the freestall barn, prior to any on-farm storage or treatment. Some bedding sawdust was unavoidably mixed in with the manure. Manure was transported to the study sites by a contract hauler in a tank truck. The intention of the experiments was to achieve storage durations of 30 and 90 days. Actual storage times (Table 3.1) deviated somewhat due to weather conditions, the schedule of the hauler, and the schedule of the analytical laboratory, which could receive samples for *E. coli* analysis only Mondays and Tuesdays. Throughout this report, the date used as the end of the manure storage period was the date of the second round of plot treatments for each trial, which occurred one day prior to the simulated rainfall runoff event. This date was chosen to represent the end of the manure storage period because the final sampling occurred on this date. Note that the first round of plot treatments occurred two days prior in each case. Tanks representing the “0 day” age manure were filled with fresh manure 3 days prior to the second application date for each trial.

Table 3.1. Delivery dates and actual ages of manure in manure storage experiments

Age Designation	Delivery Date	First Application Date	Actual Age at First Application (days)	Second Application Date (Sampled)	Actual Age at Second Application/ Manure Sampling (days)
Hayland experiment					
90 day	March 31, 2003	June 21, 2003	82	June 23, 2003	**84**
30 day	May 21, 2003	June 21, 2003	31	June 23, 2003	**33**
0 day	June 20, 2003	June 21, 2003	1	June 23, 2003	**3**
Cornland experiment					
90 day	July 22, 2003	Oct. 11, 2003	81	Oct. 13, 2003	**83**
30 day	Sept. 16, 2003	Oct. 11, 2003	25	Oct. 13, 2003	**27**
0 day	Oct. 10, 2003	Oct. 11, 2003	1	Oct. 13, 2003	**3**

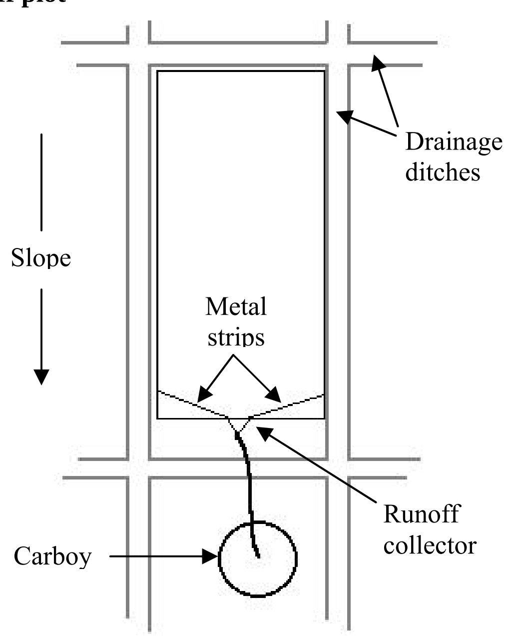
The 90-day and 30-day manure tanks were sampled for *E. coli* and for agronomic nutrient analysis immediately after addition to each tank. Manure in these tanks was sampled again prior to the second round of plot treatments for each trial. The 0-day manure was also sampled at this time. Tank contents were manually mixed using a canoe paddle, then 3 sub-samples were collected from each replicate tank 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. Concurrent with bacteria sampling, a single manure sample for each manure age (a composite of one sub-sample from each replicate tank) was collected for agronomic nutrient analysis. These samples were collected in 1-L polyethylene bottles, frozen within 3 hours of collection, then delivered to the University of Vermont Agricultural and Environmental Testing Laboratory. Field quality control/quality assurance and sample handling and tracking was done in accordance with a Quality Assurance Project Plan (QAPP) approved by U.S. EPA (Braun and Meals 2003).

3.3. Treatment Plots

One runoff trial on hayland and a second trial on cornland were conducted at separate study sites. For each trial, 40 plots were created, representing a factorial design of 3 x 2 x 2 treatments, with three replicates per treatment combination, plus three control plots (no manure applied), and one extra plot reserved as a backup in case of error in application of a plot treatment. Specific treatments were assigned to plots randomly.

At each site, 40 plots 1.5 m by 3 m were arrayed in a grid, with the long dimension of each plot parallel to the predominant slope. The shape of the plot array was adjusted to fit the topography of each site. Plots were isolated from upgradient runoff by a network of ditches (~15 cm deep, created by a *Trenchmaster* gasoline-powered bed-edger) that intercepted runoff and conveyed it beyond the plot array. At the bottom of each plot, ~15-cm by ~75-cm corrugated metal strips were embedded roughly 6 cm into the soil in a V-shape to direct runoff into "dustpan" runoff collectors. Runoff collectors consisted of 20-cm polyethylene funnels 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 located less than 1 m downgradient of the plot. Runoff collection carboys were covered with plastic sheeting to prevent simulated rainfall from entering. A schematic of a plot is shown in Figure 3.1.; photographs are shown in Appendix 8.1—Photos 3 and 13.

Figure 3.1. Schematic of runoff plot



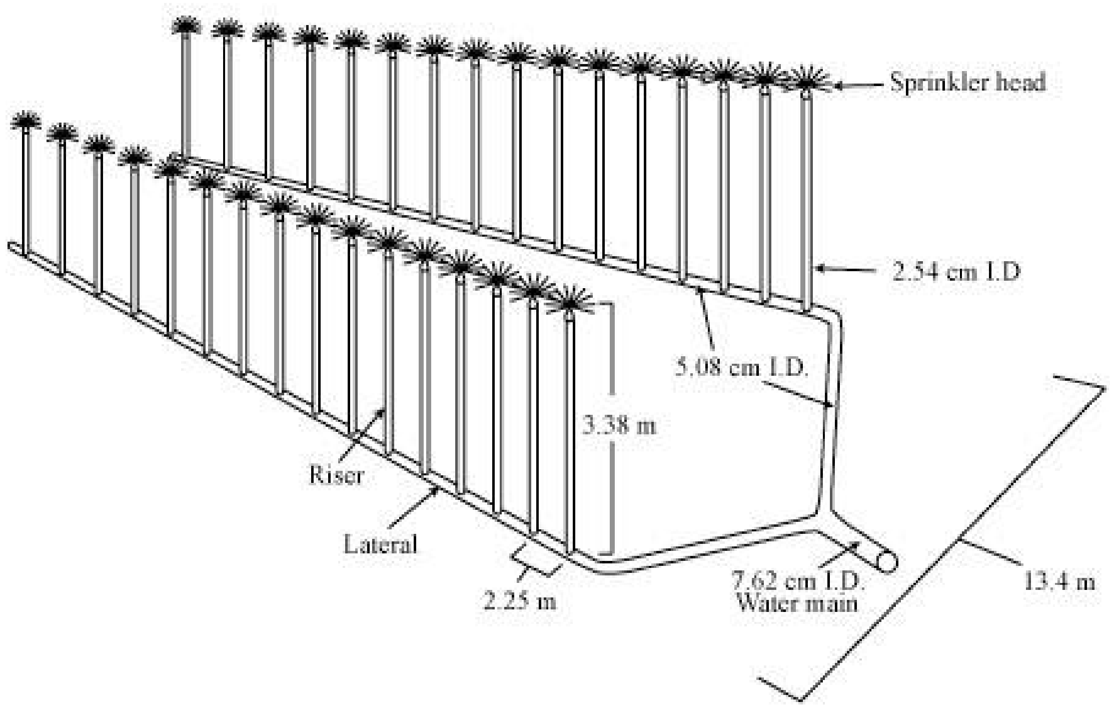
All sample collection apparatus was cleaned before each trial using a 10 percent solution of household chlorine bleach, followed by triple-rinsing with tap water. Prior to each trial, samples of the final rinsate from three randomly-selected carboys were collected for *E. coli* analysis to verify the effectiveness of the sterilization procedures.

For each trial, the first hour or first ~19 L of runoff was collected. No attempt was made to measure or estimate runoff volume; the approximate time of runoff initiation was recorded for each plot. Each carboy was subsampled for *E. coli* by manually agitating for 15 seconds, then pouring an aliquot into a sterile 100-ml polyethylene bottle provided by the Vermont Department of Environmental Conservation (VT DEC) laboratory. Runoff samples for *E. coli* analysis were maintained on ice and transported to the VT DEC laboratory within 3 hours of collection. Field quality control/quality assurance and sample tracking was done in accordance with the Quality Assurance Project Plan (QAPP) approved by U.S. EPA (Braun and Meals 2003).

3.4. Rainfall Simulator

A rainfall simulator was used to generate runoff from the test plots (Figure 3.2; Appendix 8.1, Photo 2). The simulator is a system for continuously and uniformly applying water at an intensity resembling natural rainfall. The rainfall simulator was assembled at each site during the week prior to the runoff event. It consisted of two 5.1-cm (2 in.) i.d. PVC laterals approximately 36 m long, positioned in the test field parallel to the dominant slope. The laterals were equipped with pressure gauges and gate valves and were connected at the downslope end to a 7.6 cm (3 in.) PVC water main. The laterals were spaced 13 m apart, with 20 test plots positioned between them and 10 plots positioned on the outside of each lateral. PVC riser pipes (2.5 cm i.d.) were positioned every 2.25 m along the laterals, with 16 risers per lateral. Each riser was held in place by attachment to a 1.5 m metal T-post driven into the ground. A Nelson S-30 irrigation head fitted with a brass nozzle and an 8° spinner plate was mounted on each riser. The height of the risers with the sprinkler heads in place was 3.4 m (11 ft) above the ground surface. For the cornland trial, a #22 brass nozzle (orifice diameter = 4.4 mm (0.17 in.)) was installed in each irrigation head; for the hayland trial #21 (orifice diameter = 4.2 mm (0.16 in.)) and #23 (orifice diameter = 4.6 mm (0.18 in.)) nozzles were used on alternating irrigation heads. The theoretical water output from these two configurations is essentially equivalent. Each irrigation head contained a 15-psi pressure regulator that provides a constant output rate from the nozzle irrespective of its position along the simulator lateral or the backpressure on the system (assuming the backpressure is above 1.0 – 1.4 kg/cm^2 (15-20 psi)). This serves to maximize the uniformity of water distribution over the test plots. Each irrigation head irrigated a circular area with a radius of approximately 6.7 m (22 ft.). Overlap of these irrigated areas along and between laterals ensured delivery of simulated rainfall to all portions of the plot area.

Figure 3.2. Schematic of rainfall simulator



At both sites, a nearby pond served as the water source for the rainfall simulator. A centrifugal pump positioned next to the pond was used to pump water through the PVC water main to the simulator laterals. A trash screen on the intake line prevented leaves and debris from entering the line and potentially clogging the sprinkler heads. A Y-connection on the water main allowed excess water to be returned to the pond. This diverter line was equipped with a gate valve that could be opened in case of emergency, bypassing the simulator when necessary. The valve on the diverter was opened part way during the first seconds of each simulated rainfall event or simulator test to prevent a pressure surge on the simulator. The valve was then closed after water was observed spraying from the sprinkler heads. Water pressure at the simulator laterals was maintained at ~2 kg/cm^2 (28-30 psi) through each event.

Prior to the simulated rainfall events, simulator laterals were flushed and the simulator was tested for leaks under pressure and for proper operation of the irrigation heads. Any leaks detected were repaired and faulty irrigation heads were serviced or replaced. During the event, the simulator was regularly checked for proper operation.

3.5. Meteorological Monitoring

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 applications (3 days before the simulated rainfall event) to characterize ambient conditions during the course of plot treatment and runoff. A *Vantage Pro* meteorologic station (Davis Instruments, Inc., Hayward, CA) was erected at each trial site consisting of a tipping-bucket rain gage, an atmospheric thermometer, a cup anemometer, a wind direction vane, a solar pyranometer, a 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 *Eversafe* thermometer, the calibration of which is traceable to NIST standards. The same thermometer was used to calibrate the Vantage Pro's external temperature sensor. The wind direction vane was calibrated in the field using a *Suunto* compass as a reference.

Additional weather data used to characterize conditions during the manure storage experiments were obtained from a nearby NWS weather station, Montpelier 2 (Coop I.D. 435273).

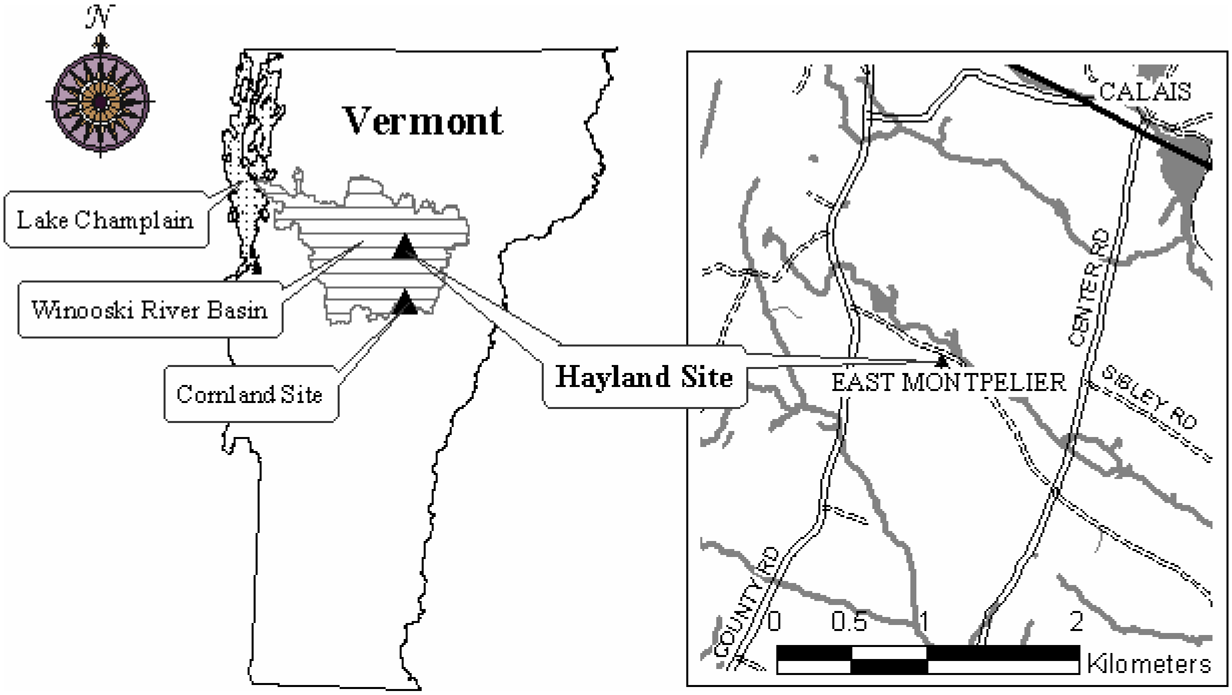
3.6. Hayland Runoff Trial

The hayland runoff trial was scheduled for late June, immediately following the first hay cut of the season. Application of manure to hayland between hay cuts is a typical agronomic practice in the Lake Champlain Basin.

3.6.1. Study site

The test site is a moderately sloping hayfield in East Montpelier, Vermont on Templeton Road, approximately 5 miles north of the city of Montpelier. The site is located directly across the road from Chapells Pond, which was used as the water source for the simulated rainfall irrigation system. The site is mapped in Figure 3.3.

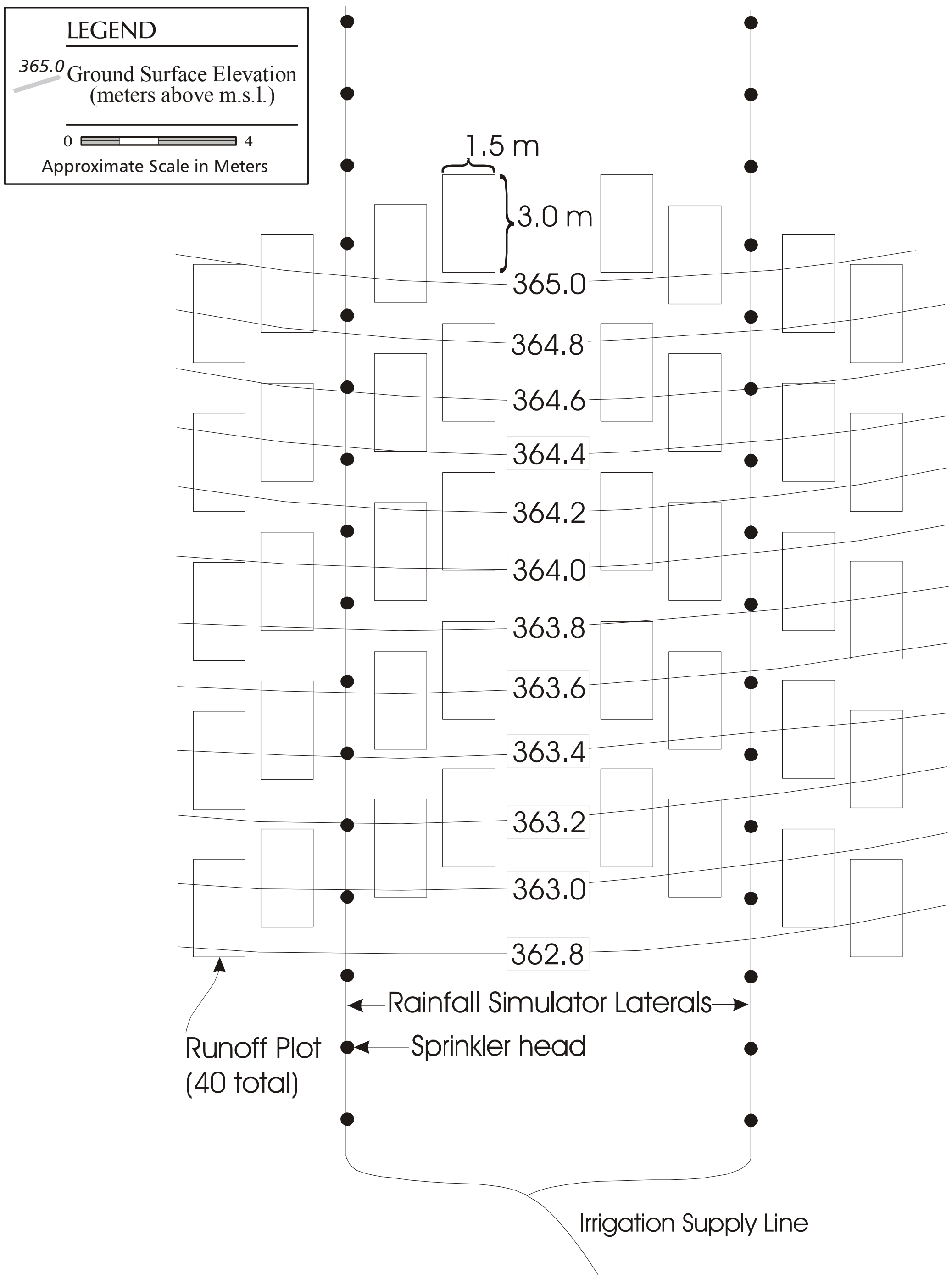
Figure 3.3. Location map of hayland runoff study site



NRCS soil survey maps for Washington County, Vermont (USDA Soil Conservation Service 1992) indicate that the entire site is on Cabot silt loam, 3-8 percent slope (mapping symbol 17B). The Cabot series consists of poorly drained soils that formed in dense loamy till. Cabot soils are shallow to dense till and deep to bedrock. Cabot soils have a perched water table at depths of 0.0 to 2.0 feet below the surface from late fall through late spring. They are classified as hydrologic group C, indicating that they are prone to runoff.

The test plots were laid out in an area of the hayfield that had a relatively uniform slope of 9.7 percent top to bottom. The plots were arrayed in five rows of eight plots per row (Figure 3.4). A transit and stadia rod were used to measure relative elevations across the test field, and the plot array was squared using field tapes. The plot array was oriented in the field to minimize cross-slopes across the plots. To permit construction of diversion ditches that angled downslope, the plots were arranged in inverted V-shaped rows, which conformed to the layout of the drainage ditches.

Figure 3.4. Diagram of hayland runoff study plots



3.6.2. Treatments

Treatments applied to the hayland plots are shown in Table 3.2.

Table 3.2. Treatments applied to hayland plots

Treatment	Condition	Code
Manure Age	90 days	90
	30 days	30
	0 days	0
Vegetation Height	~13 – 15 cm	H
	~5 – 7 cm	L
Delay to Rain	3 days	3
	1 day	1

Each plot was designated with a treatment code that identified its treatment combination (manure age, vegetation height, delay) and its replicate number; for example, plot #20 in the array was designated "30-L-3-2", identifying it as the second replicate of a plot receiving 30-day manure on low vegetation three days before simulated rain. Control plots were designated "C-C-C." Because no errors were made in applying treatments to plots #1 through 39, the backup plot (#40) was used to informally assess "worst-case" conditions; this plot received twice the normal rate of fresh manure on high vegetation immediately before simulated rainfall.

Manure of different ages was generated in the manure storage experiment (Section 3.2). Vegetation height was established by first mowing the entire plot area to ~14 cm with a gasoline-powered push lawnmower, then by resetting the cutting height to 6 cm and mowing the plots receiving the low vegetation treatment. Grass clippings were bagged and removed from the plots. Manure was applied to the 3-day delay plots around mid-day, ~72 hours before the scheduled simulated rainfall. Manure was applied to the 1-day delay plots ~24 hours before scheduled rainfall.

Manure was applied to the surface of plots by hand at a rate equivalent to 4,500 gallons/acre (42.1 m3/ha), a rate typical for application to hayland in Vermont. Manure in the replicate storage tanks was completely mixed using a canoe paddle immediately before use. Each batch of manure to be applied was a composite of subsamples from each of the three replicate tanks representing that age class. Effort was made to apply the manure uniformly across each plot, but to avoid the area immediately above the runoff collector

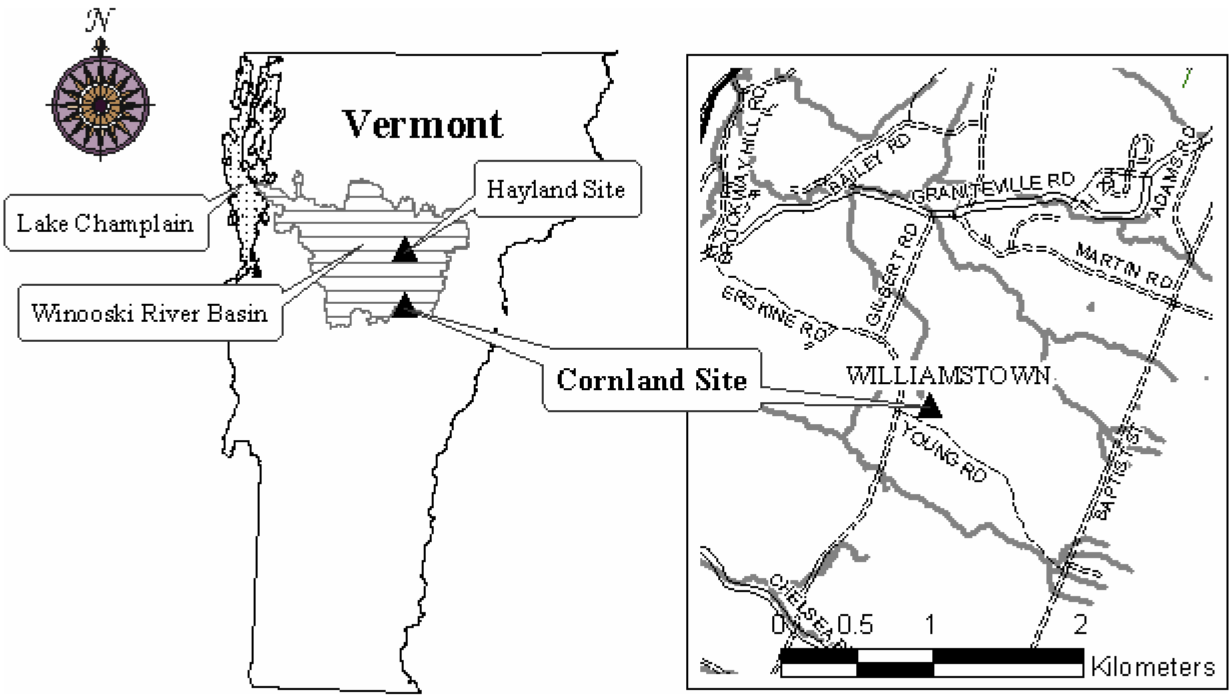
3.7. Cornland Runoff Trial

The cornland runoff trial was scheduled for mid-October, following harvest of corn silage, but before any tillage. Because the corn was harvested for silage, there was minimal residue left on the field, a condition quite typical of cornland in Vermont. Manure application to cornland in the Lake Champlain Basin typically takes place either in spring, prior to planting or in the fall, following harvest. Selection of fall for the cornland trial was driven partially by logistical considerations, including availability of the rainfall simulator and the limits of the overall project schedule. Conducting the trial in the spring would have required manure storage to be initiated in mid-winter, a difficult proposition. More importantly, interference with the farmer's planting schedule would have been a major limitation for conducting the runoff trial after spring manure application. Because fall application of manure to cornland is a common agronomic practice in the Lake Champlain Basin, it was believed that this was an acceptable condition for the runoff trial.

3.7.1. Study site

The test site is a moderately sloping cornfield in Williamstown, Vermont near the intersection of Gilbert Road and Young Road, approximately 2.5 miles east of Williamstown village. The site is mapped in Figure 3.5. A spring fed, recreational pond located ~91 m (300 feet) downslope of the test field was used as the water source for the simulated rainfall irrigation system.

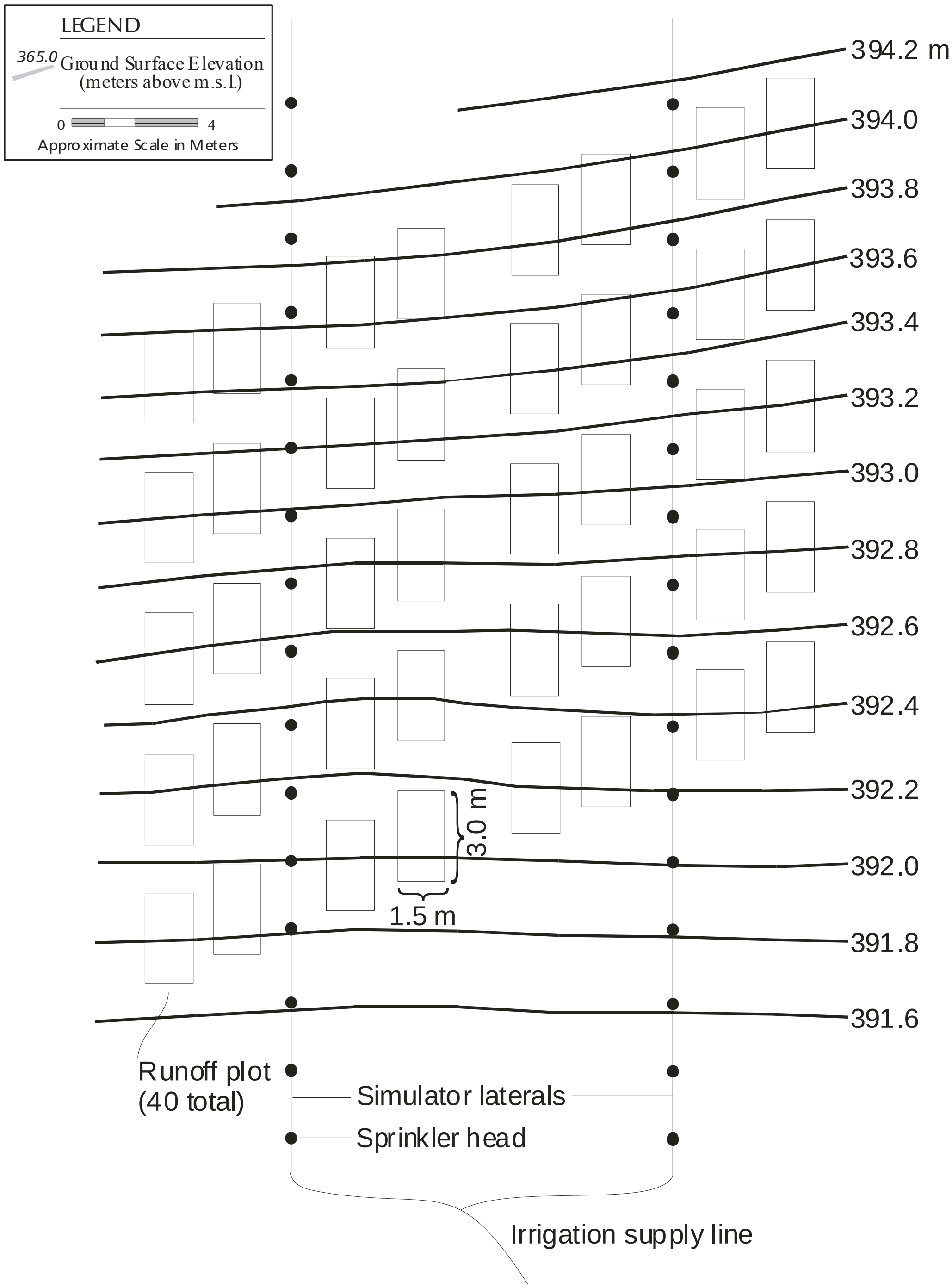
Figure 3.5. Location map of cornland runoff study site



The NRCS soil survey of Orange County, Vermont (Sheehan 1978) indicates that the test field is on Buckland stony loam, 3-8 percent slope (mapping symbol BuB). The Buckland series consists of nearly level to steep, deep (greater than 1.5 m (60 in.)), stony and very stony, well-drained to moderately well drained soils that are underlain by a fragipan at a depth of less than 0.8 m (33 in). Buckland soils formed in glacial till on lower and middle side slopes. Due to the presence of the slowly permeable fragipan, a perched water table typically develops in Buckland soils at a depth of 0.3 – 0.6 m (1.0-2.0 ft) below the surface in the late winter and spring. The Buckland series is classified as hydrologic group C, indicating that these soils are prone to runoff.

The test plots were laid out in an area of the cornfield that had relatively uniform slope ranging from 7.6 to 8.7 percent (average 8.1%) top to bottom. The plots were arrayed in five rows of eight plots per row (Figure 3.6). A transit and a stadia rod were used to measure relative elevations across the test field. The plot array was oriented in the field to minimize cross-slopes across the plots. The plot array was squared using field tapes. To permit construction of diversion ditches that angled downslope, the plots were arranged diagonally across the field to conform to the layout of the drainage ditches. Plots were oriented approximately perpendicular to the direction of corn rows, although row ridges were not large enough to prevent runoff movement downslope to the runoff collectors.

Figure 3.6. Diagram of cornland runoff study plots



3.7.2. Treatments

Treatments applied to the cornland plots are shown in Table 3.3.

Table 3.3. Treatments applied to cornland plots

Treatment	Condition	Code
Manure Age	90 days	90
	30 days	30
	0 days	0
Incorporation	Incorporated	I
	Nonincorporated	N
Delay to Rain	3 days	3
	1 day	1

Each plot was designated with a code that identified its treatment combination (manure age, incorporation, and delay) and its replicate number; for example, plot #3 in the array was designated as "0-N-1-3", identifying it as the third replicate of a plot receiving 0-day manure without incorporation one day before simulated rain. Control plots were designated "C-C-C." As in the hayland trial, the extra plot (#40) was used to informally assess "worst-case" conditions; this plot received twice the normal rate of fresh manure without incorporation immediately before simulated rainfall.

Manure of different ages was generated in the manure storage experiment (Section 3.2). Where designated, manure was incorporated by a single pass of a gasoline-powered rototiller immediately following application. Tillage was done parallel with the long axis of the plot (Appendix 8.1, Photo 11). Manure was applied to the 3-day delay plots around mid-day, 72 hours before the scheduled simulated rainfall. Manure was applied to the 1-day delay plots ~24 hours before scheduled rainfall.

Manure was applied to the plots by hand at a rate equivalent to 6,300 gallons/acre (58.9 m^3/ha), a rate typical for application to silage corn in Vermont (Appendix 8.1, Photo 10). Manure in the replicate storage tanks was 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. Effort was made to apply the manure uniformly across each plot, but to avoid the area immediately above the runoff collector.

3.8. Analytical Methods

All *E. coli* analyses were conducted in the VT DEC Water Quality Laboratory in Waterbury, Vermont using the Quanti-Tray method (APHA 9223B 1995). Manure samples were pre-processed in the laboratory by suspending a known weight of manure (wet weight) in sterile dilution water; results for manure samples were reported as organisms/g. Runoff, irrigation source water, and container rinse samples were analyzed by standard Quanti-Tray procedures and results were reported as organisms/100 ml. All field and laboratory quality assurance/quality control procedures are documented in the project QAPP (Braun and Meals 2003).

Manure samples were analyzed at the University of Vermont Agricultural and Environmental Testing Laboratory in Burlington, Vermont for percent dry matter, total nitrogen, organic nitrogen, ammonium-nitrogen, phosphorus (P_2O_5), potassium (K_2O), calcium, magnesium, and copper by the following methods:

Table 3.4. Methods of manure analysis

Parameter	Method	Reference
Dry matter	Gravimetric at 55 ^oC for 2-3 days	
Total N	Semi-micro Kjeldahl (Cu & Se instead of Hg)	APHA 4500-Norg C.p. 4-94
Organic N	Calculated by difference (TN – NH_4-N)	NA
NH_4-N	Titrimetric (distill w/MgO, KCl into boric acid)	APHA 4500C. p. 4-77
P_2O_5	Microwave digestion in nitric acid, analysis by ICP AES	APHA 3030H p. 3-6
K_2O		
Mg		
Ca		
Cu		

3.9. Data Analysis

Statistical analysis of *E. coli* data was conducted on $\log_{10}$ transformed data to conform with the assumptions of normality and equal variances. All statistical tests were performed using JMP® software ver. 4.0 (SAS Institute 2000). Because of the noise associated with field experiments, all statistical tests used an alpha of 0.10.

4. RESULTS

4.1. Manure Storage Experiments

Results of agronomic analyses of manure used for the runoff experiments are shown in Table 4.1. The nutrient content of the liquid manure used in the project was comparable to average values reported from manure analyses conducted by the University of Vermont (UVM) Agricultural and Environmental Testing Laboratory from 1992 – 1996 (Jokela *et al.* 2002). Dry matter, total nitrogen, and magnesium concentrations in manure delivered for the study tended to be somewhat higher than UVM average values; phosphorus content tended to be lower. Both calcium and potassium levels were similar to UVM averages. Manure composition at application was also comparable to averages reported by UVM, although manure was somewhat lower in P, K, Ca, and Mg than UVM average values. Copper levels in the manure were at background levels (<1 lb/1000 gal), indicating that residual copper was not likely to be a confounding influence on manure bacteria levels. Copper sulfate is sometimes used in dairy cattle foot baths and the spent solution may be discarded in the manure pit; residual copper may have bactericidal effects in manure (Thomas 2003).

Some interesting changes were observed in manure composition with storage, although these changes cannot be confirmed statistically due to the small number of samples. In most cases, dry matter, N, P, K, Ca, and Mg concentrations in manure tended to decline with storage. Some N loss through mineralization and volatilization is to be expected in stored manure. Dry matter loss through organic decomposition would also be expected to occur. Reductions in concentrations of more conservative elements P, K, Ca, and Mg, however, were probably due to net dilution by rainwater. Changes in composition of manure stored for 90 days for the cornland runoff trial were quite large, *e.g.*, dry matter decreased by ~60% from 9.6% to 3.7%, total N declined by ~50%, and P_2O_5 decreased ~33% from 8.3 to 5.5 lb/1000 gallons. The relative contributions of decomposition, volatilization, or dilution to these substantial changes are unknown. Dilution by ~358 mm of rainfall that fell during the storage period may have contributed to significant net dilution of the waste. Changes in composition of manure stored for 30 days for the cornland runoff trial were an exception to this pattern. Dry matter, P, Ca, and Cu content increased with storage and net N loss (<10%) was lower than in other cases (35 – 50%). This “enrichment” may be due to net evaporation loss during the storage period when just 94 mm of rain fell.

Results of manure analyses for *E. coli* are reported in Table 4.2 for each batch of manure at delivery and after experimental storage immediately prior to the second application. Mean *E. coli* levels in fresh manure delivered to the experimental sites ranged from 3.1 – 7.5 x 10^5 /g manure wet weight. This is comparable to the order of magnitude reported elsewhere for fecal coliform bacteria in fresh animal waste (Crane *et al.* 1983, Moore *et al.* 1988). Although *E. coli* levels in manure differed somewhat between batches delivered on different dates, bacteria levels were quite similar among replicate tanks immediately after delivery (C.V. <0.22). There was a tendency for variability among replicate tanks to increase after storage, as indicated by increasing C.V. This may have been due to small differences in the storage environment experienced by different tanks reflecting varying fill levels, different dilution by rainfall due to tank slope, or other factors.

Table 4.1. Agronomic analysis of manure used in runoff experiments

Source	Sample Date	Dry Matter	Total Nitrogen	Ammonium Nitrogen (NH_4-N)	Organic Nitrogen	Phosphorus (as P_2O_5)	Potassium (as K_2O)	Calcium	Magnesium	Copper
		%	lb/1000 gal							
Hayland Trial										
90 d at delivery	3-31-03	----Sample lost, no data-----								
90 d at application	6-23-03	9.4	29.9	8.3	21.5	10.5	19.7	15.3	5.8	0.74
30 d at delivery	5-20-03	8.1	32.9	14.3	18.5	6.4	18.0	11.1	5.3	0.27
30 d at application	6-23-03	6.1	21.5	9.6	11.8	5.4	17.8	9.0	4.8	0.25
0 d at application[2]	6-23-03	9.0	29.4	10.4	19.1	8.4	20.4	10.6	5.6	0.38
Cornland Trial										
90 d at delivery	7-22-03	9.6	30.0	13.0	17.0	8.3	17.4	13.8	4.9	0.55
90 d at application	10-13-03	3.7	16.6	3.3	13.2	5.5	15.5	6.8	3.5	0.41
30 d at delivery	9-16-03	6.1	26.9	10.6	16.3	7.0	18.4	7.6	4.3	0.11
30 d at application	10-13-03	8.5	24.5	8.1	16.4	7.8	15.6	11.3	4.9	0.81
0 d at application[2]	10-13-03	11.2	34.1	13.8	20.4	11.2	19.6	15.1	6.2	0.54
UVM average[1]										
	--	**7.5**	**23**	**11**	**12**	**11**	**20**	**15**	**4**	**--**

[1] From samples of liquid dairy manure analyzed by the UVM Agricultural and Environmental Lab, 1992-1996
[2] Mean of field duplicate samples reported

Table 4.2. Descriptive statistics for manure *E. coli* analyses

Source	Sample Date	n	Median *E. coli* (#/g wet wt.)	Mean[1] *E. coli* (#/g wet wt.)	Std. Dev.[2]	Std. Error Mean[2]	C.V.[3]
Hayland Trial							
90 d at delivery	3-31-03	3	326,500	308,204	0.093	0.054	0.20
90 d at application	6-23-03	3	1,000	1,260	0.174	0.100	0.43
30 d at delivery	5-21-03	3	753,500	750,315	0.037	0.022	0.09
30 d at application	6-23-03	3	8,600	8,098	0.099	0.057	0.22
0 d at application	6-23-03	3	435,000	442,207	0.065	0.037	0.15
Cornland Trial							
90 d at delivery	7-22-03	3	397,000	398,465	0.096	0.055	0.22
90 d at application	10-13-03	3	<100	<114	0.102	0.059	0.25
30 d at delivery	9-16-03	3	687,000	668,040	0.057	0.033	0.13
30 d at application	10-13-03	3	14,500	7,742	0.510	0.294	0.71
0 d at application	10-13-03	3	391,000	381,484	0.088	0.051	0.20

[1] anti-log of log mean [2] log-transformed data [3] coefficient of variation (arithmetic)

Some variation in *E. coli* levels was observed between batches of manure delivered to the experimental sites. Mean manure *E. coli* levels at delivery are compared within each trial based on one-way Analysis of Variance (ANOVA) in Table 4.3 and in Figure 4.1.

Table 4.3. Comparison of initial mean *E. coli* among manure batches, one-way ANOVA

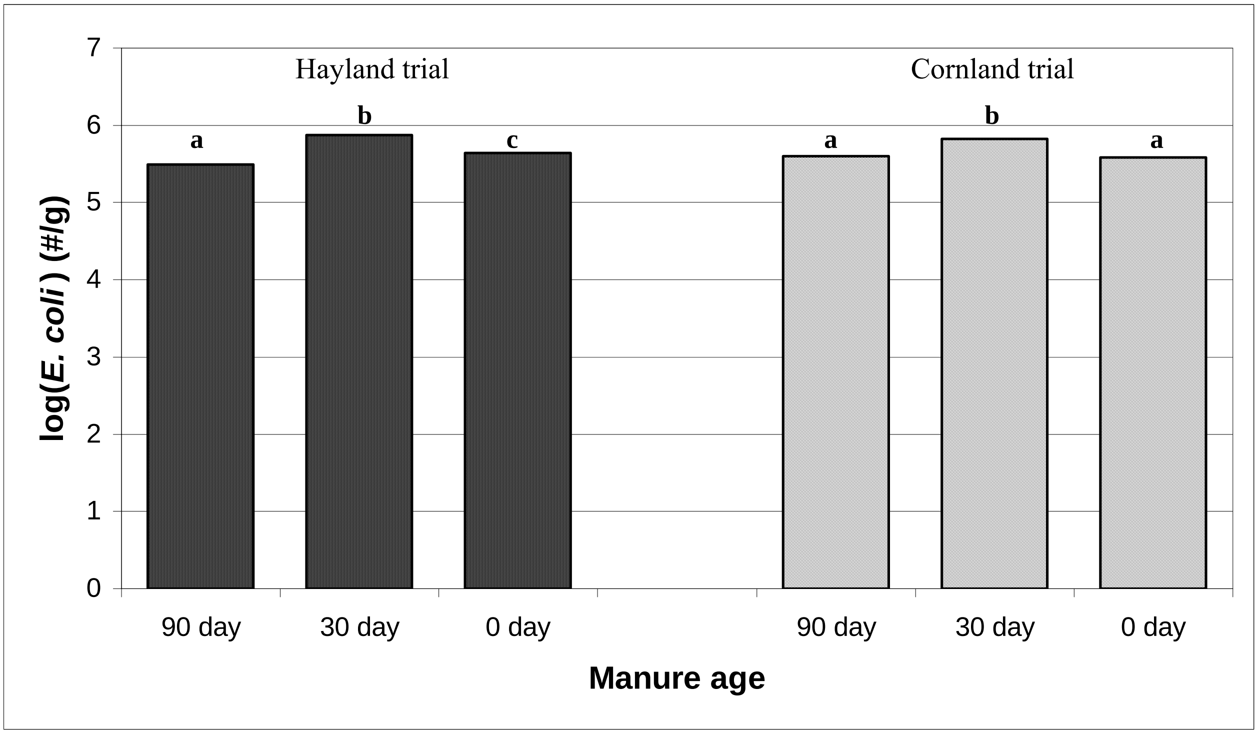
Trial	Manure Age: 90 d	30 d	0 d	F	P
	Manure *E. coli* (#/g wet weight)				
Hayland	308,204 **a**	750,315 **b**	442,207 **c**	23.82	0.001
Cornland	398,465 **a**	668,040 **b**	381,484 **a**	8.150	0.020

Note: Within rows, means followed by the same letter(s) are not significantly different, $P \leq 0.10$

In the hayland trial, initial *E. coli* counts differed significantly among the three batches (Figure 4.1), although all delivered manure contained ~10^5 *E. coli*/g wet weight. Initial *E. coli* counts in manure for the cornland trial were also in the ~10^5 *E. coli*/g wet weight range, with initial bacteria content of the 30-d manure significantly higher than that of either the 90-d or 0-d batches (Figure 4.1).

Figure 4.1. Mean initial manure *E. coli* counts for hayland and cornland trials

Note: Within each trial, bars labeled with different letter(s) differ significantly, P< 0.10.



The effect of experimental manure storage on *E. coli* is summarized in Table 4.4 and Figure 4.2. In the hayland trial, ~90 day storage reduced *E. coli* counts by two orders of magnitude, or 99.6%; ~30 day storage also reduced *E. coli* counts by 98.9%. Both differences were significant at $P \leq 0.10$ by Student's t Test. Similar results were observed during storage for the cornland trial, with reductions of 98.8% and >99.9% for ~30 and ~90 day storage, respectively.

Table 4.4. Results of manure storage experiments

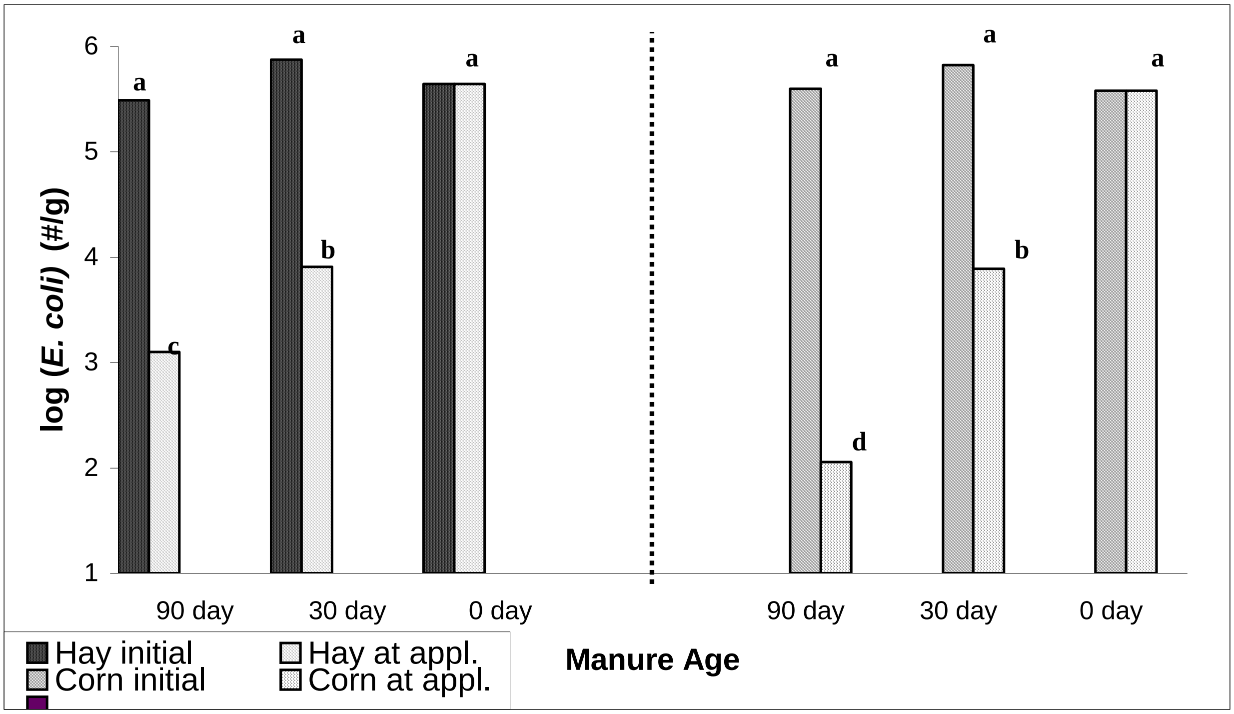
Storage time (days)		Mean[2] *E. coli* Start	Mean[2] *E. coli* End	t-Test		% Reduction
Nominal	Actual[1]	#/g		t	P	
Hayland Trial						
30	33	750,315	8,098	-32.31	<0.001	98.9
90	84	308,204	1,260	-20.97	<0.001	99.6
Cornland Trial						
30	27	668,040	7,742	-6.50	0.003	98.8
90	83	398,465	114	-43.86	<0.001	99.9

[1]Actual storage time from filling of storage tanks to the second manure applications to test plots

[2] anti-log of log mean

Figure 4.2. Mean manure *E. coli* counts at delivery and at second application for hayland and cornland trials

Note: Identical bars are shown for 0-day manure for comparative purposes. Bars labeled with different letter(s) differ significantly, one-way ANOVA, P ≤ 0.10, all data pooled



As shown in Figure 4.2, when all data are pooled, initial manure *E. coli* levels did not differ significantly among the delivered batches of manure. Bacteria levels in 30-day manure, which decreased significantly from initial counts in both trials (Table 4.4), were similar between the hayland and cornland trials, ~8 x 10^3 *E. coli*/g. Bacteria levels in 90-day manure in both trials were significantly lower than initial or 30-day levels. *E. coli* levels in 90-day manure in the cornland trial (<1.1 x 10^2 *E. coli*/g) were significantly lower than those in 90-day manure in the hayland trial (1.3 x 10^3 *E. coli*/g). Significantly greater reductions in manure *E. coli* occurred in 90-day storage for the cornland trial than for the hayland trial. This difference may be due to differences in environmental conditions during storage. Selected weather data from the Montpelier 2 weather station (Coop I.D. 435273) are shown in Table 4.5.

Table 4.5. Selected weather data over manure storage period

Manure Treatment	Period	Mean Daily Air Temp. (°C)	Mean Maximum Daily Air Temp. (°C)	Cooling Degree Days[1]	Total Precip. (mm)	Mean Precip./day (mm/d)	Days With Precip. (# / %)
Hayland—90 d	3/31 – 6/23	11.3	18.0	29	246	7.9	31 / 36%
Hayland—30 d	5/21 – 6/23	16.9	23.4	27	102	7.8	13 / 38%
Cornland—90 d	7/22 – 10/13	18.1	24.9	292	358	12.8	28 / 33%
Cornland—30 d	9/16 – 10/13	13.4	20.5	9	94	9.4	10 / 36%

[1] Cooling degree day = daily number of degrees Fahrenheit by which mean temperature exceeds 65 °F.
Source: National Climatic Data Center, NOAA, http://www.ncdc.noaa.gov/oa/ncdc.html

As shown in Table 4.5, air temperatures were higher during the July – October 90-day manure storage period for the cornland trial (mean daily air temperature 18.1 °C; 292 cooling degree days) than during the April – June 90 days of storage for the hayland trial (mean daily air temperature 11.3 °C, 29 cooling degree days). Substantially more rain was recorded during the 90-day storage for the cornland trial (358 mm) than for the comparable storage period for the hayland trial (246 mm). Both warmer temperatures and greater dilution by rainfall may have contributed to the lower *E. coli* content of the 90-day manure for the cornland trial. Weather conditions over the 30-day storage period were more similar for the hayland and cornland trials, although slightly higher air temperatures and cooling degree-days were reported for the May/June 30-day storage period for the hayland trial than during the September/October 30-day storage period for the cornland trial. Rainfall amounts were comparable for the two 30-day storage periods.

4.2. Hayland Runoff Trial

The hayland runoff event was conducted on June 24, 2003. Construction of the rainfall simulator and preparation of the runoff plots took place over the preceding week, as described in Section 3 (Methods).

4.2.1. Weather

Weather data collected on site over the trial period from June 21 (the day of first manure application) through June 24 (the day of runoff) are summarized for selected hours in Table 4.6 and all hourly data for selected variables over the same period are plotted in Figures 4.3 and 4.4. Complete hourly weather data for the hayland runoff trial are given in Appendix 8.2.

Table 4.6. Summary of on-site weather data for hayland runoff trial

Date	Time	Air Temp. (^oC)	Relative Humidity (%)	Barometric Pressure (kPa)	Wind Speed (km/hr)	Wind Direction	Precip. (mm)	Solar Radiation (watts/m^2)
June 21, 2003	00:30	13.9	87	100.98	0.0	---	0	0
	06:30	11.7	93	101.06	0.0	---	0	44
	12:30	26.1	48	100.73	4.0	variable	0	788
	18:30	25.5	58	100.40	1.2	NW/W	0	126
June 22, 2003	00:30	17.1	91	100.49	0.8	S	0.25	0
	06:30	16.1	95	100.38	0.0	---	0	18
	12:30	21.7	79	100.32	2.4	variable	0	577
	18:30	24.6	61	100.08	1.6	NNE/N	0	158
June 23, 2003	00:30	16.6	93	100.21	0.4	SW	0	0
	06:30	14.9	96	100.21	0.0	---	0	80
	12:30	28.4	49	100.25	5.6	variable	0	782
	18:30	29.6	48	100.32	8.4	N/NW	0	238
June 24, 2003	00:30	18.8	91	100.67	0.0	---	0	0
	06:30	16.9	95	100.80	0.0	---	0	70
	12:30	31.8	44	100.83	3.6	variable	0	837
	18:30	32.2	35	100.73	4.4	NW	0	288

Figure 4.3. Plot of hourly on-site air temperature and relative humidity during hayland runoff trial

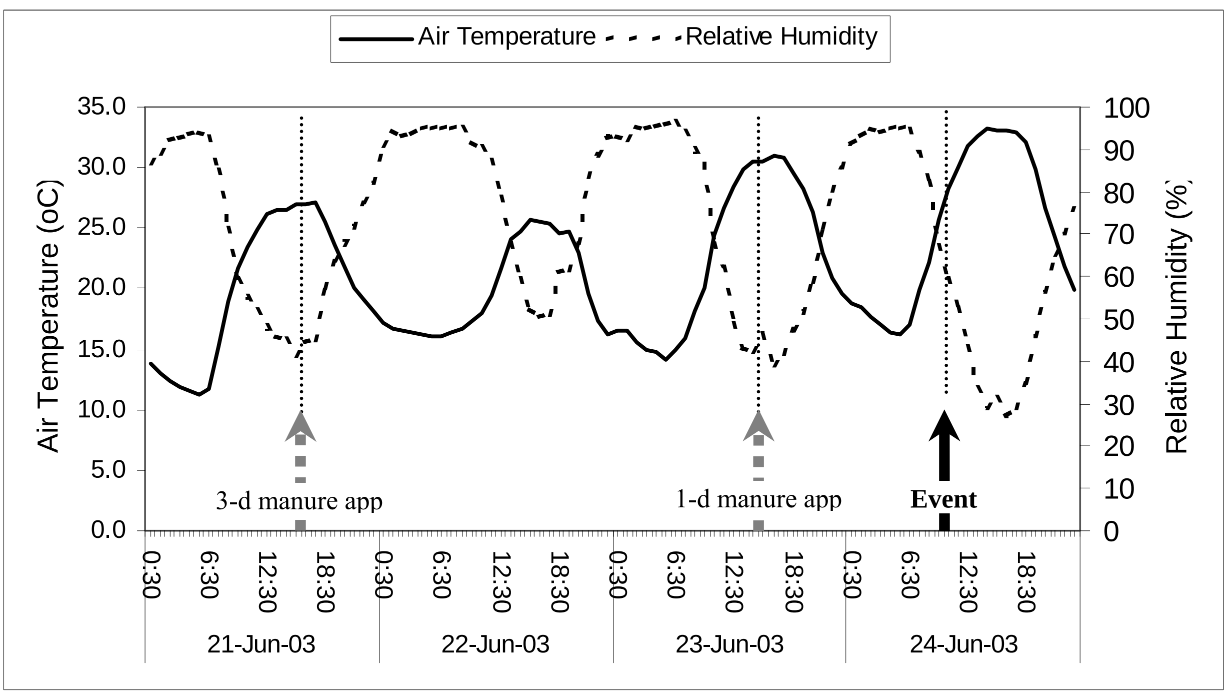
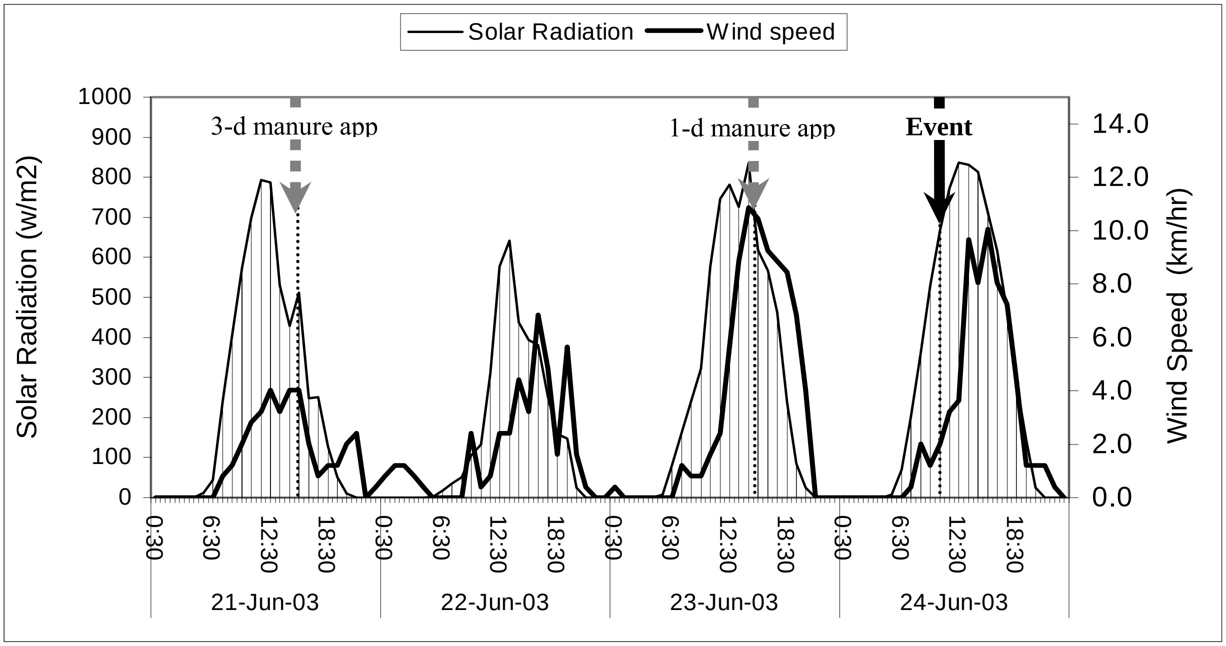


Figure 4.4. Plot of hourly on-site solar radiation and wind speed during hayland runoff trial



Mean air temperature over the trial period was 21.8 °C. Minimum air temperature of 11.2 °C occurred at 05:00 on June 21; maximum air temperature during the trial period was 33.4 °C during the afternoon of June 24. Relative humidity peaked at ~95% during night, but generally dropped to ~50% at midday through the period. Barometric pressure was steady through the trial period, averaging 100.53 kPa. Winds were light and variable. There was almost no precipitation during the trial period (a total of 1 mm on June 22); some of this trace amount recorded in early morning hours was probably the result of condensation. Skies were mainly clear over the trial period, with maximum solar radiation of ~640 – 970 watts/m^2 recorded at midday.

Soil conditions were fairly dry; little or no rainfall was recorded on the site from the beginning of weather monitoring (June 19); in the 30 days preceding the runoff event, a total of 97 mm of rain was recorded at the Montpelier weather station; only 0.1 mm of rainfall was recorded in the 8 days immediately prior to the event. Soil temperatures ranged from 20 – 28 °C during the trial period (Table 4.7).

Table 4.7. Soil temperatures on the runoff area during the hayland runoff trial

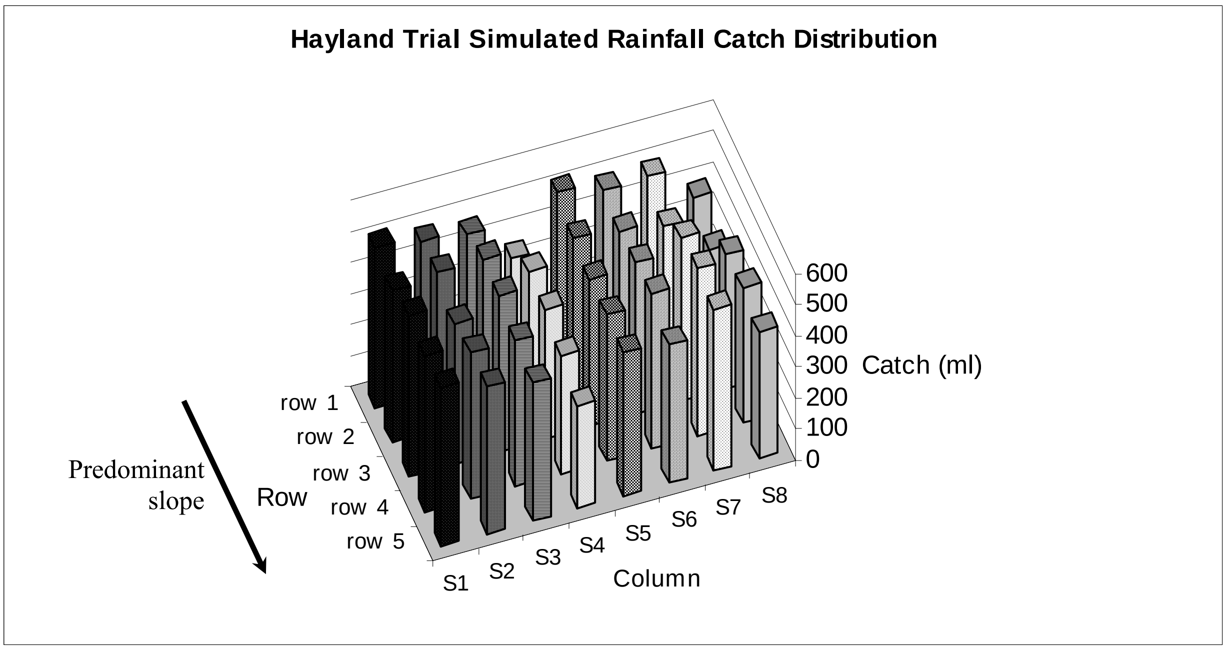
Date	Time	Soil Temperature (°C)
June 21, 2003	14:41	24.5
June 22, 2003	12:05	20.0
June 23, 2003	16:15	24.0
June 24, 2003	10:39	21.1
June 24, 2003	15:21	27.8

4.2.2. Rainfall simulation

Simulated rainfall application began at 11:05 on June 24 and continued until 15:11. Measured rainfall application averaged 102 mm among the test plots, for an average intensity of ~ 25 mm/hour. An average of 460 L (122 gallons) of water was applied to each test plot. Due to the long duration of this intense simulated rainfall event, it exceeded a 100-year storm in Central Vermont (McKay and Wilks 1995). A one-hour duration storm of equivalent intensity has a 5-year return period, and the majority of plots generated runoff within about 75 minutes.

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; the standard deviation was 52 ml, yielding a coefficient of variation of 11%. Catch of simulated rainfall is shown graphically in Figure 4.5.

Figure 4.5. Bar chart of total catch of simulated rainfall by test plots during hayland runoff event



The difference between treatment mean rainfall catches and the mean catch across all plots ranged from 0.1% to 10.7%. This satisfied the criterion for acceptable uniformity of delivered rainfall as stated in the QAPP, *i.e.*, the treatment mean rainfall catches were all within ±1 standard deviation (11%) of the event mean rainfall catch (Braun and Meals 2003). Rainfall catch was therefore not used as a variable in evaluating runoff *E. coli* data for the effects of treatment in the hayland trial.

As described in Section 3 (Methods), simulated rainfall was sampled three times during the hayland runoff event for *E. coli* bacteria. Results are shown in Table 4.8.

Table 4.8. *E. coli* content of simulated rainfall—hayland runoff trial

Sample time	*E. coli* (#/100 ml)
11:10	2
12:05	<3
15:03	21

As shown in Table 4.8, the *E. coli* content of irrigation water was minimal during the hayland runoff event. Simulated rainfall added a negligible quantity of *E. coli* bacteria to the experimental plots.

4.2.3. Hayland runoff

The first plot runoff was recorded at 11:35, 30 minutes after the beginning of simulated rainfall. The first plot to generate runoff was #40, the "extra" plot that had received manure immediately before the beginning of simulated rain at twice the rate of other plots. Other plots began to generate runoff shortly thereafter. Simulated rainfall and runoff collection continued through 15:11, at which time runoff had been generated from all but plots #3 and #26. Based on approximate times that runoff began from each plot, there was no discernible pattern in the order in which plots/treatments began to generate runoff. Treatment did not appear to affect the onset of runoff. The two plots that never generated runoff both received 90-day manure, but represented different vegetation heights and different delays to rainfall. Differences among plots in characteristics such as micro-topography, soil condition, vegetation density, or wind exposure were probably the major determinants of runoff timing.

E. coli data from plot runoff are summarized in Table 4.9; raw data are reported in Appendix 8.3. No *E. coli* were detected in rinsate from carboys collected prior to the runoff event, confirming that runoff collection containers were effectively sterilized.

Table 4.9. Summary of *E. coli* data from hayland runoff

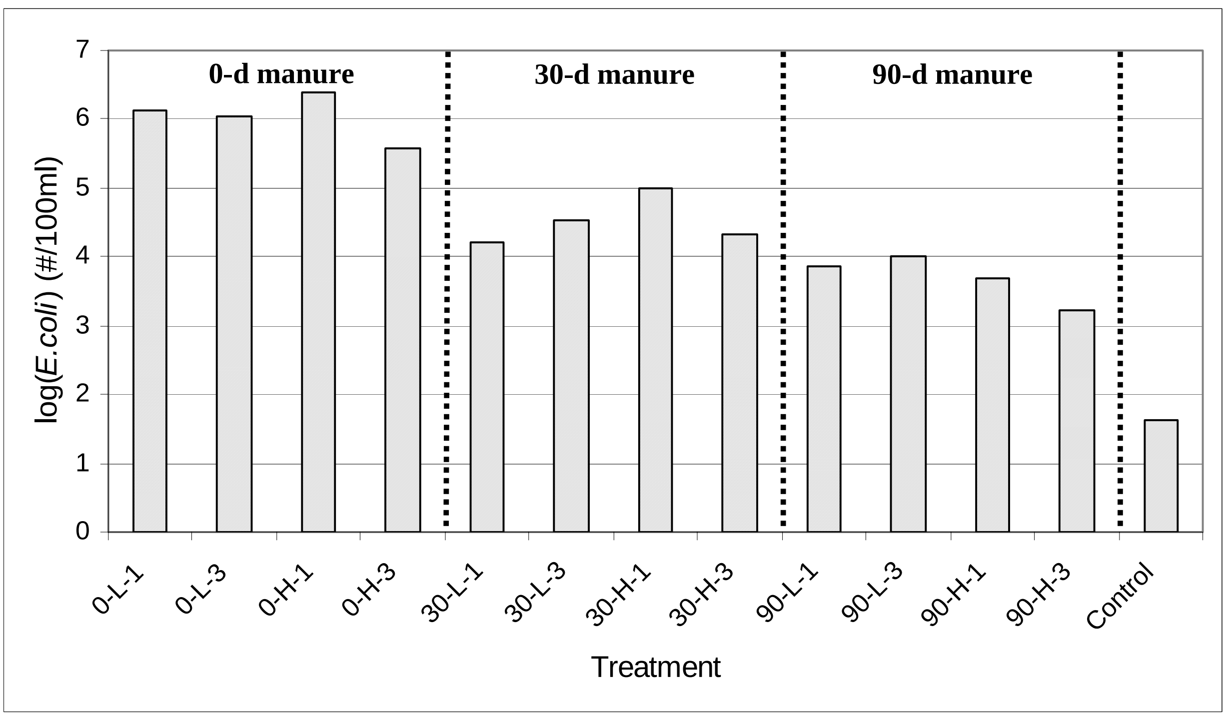
Manure Age (d)	Vegetation Height	Delay to Rain (d)	n	Median *E. coli*	Mean[1] *E. coli*	Std. Dev.[2]	Std. Error Mean[2]	C.V.[3]
				#/100 ml				
0	Low	1	3	>2,420,000	>1,365,757	0.430	0.248	0.65
		3	3	>1,200,000	>1,102,129	0.361	0.209	0.73
	High	1	3	>2,420,000	>2,420,000	0.000	0.000	0.00
		3	3	314,500	374,030	0.543	0.313	1.13
30	Low	1	3	16,000	16,676	0.305	0.174	0.67
		3	3	38,900	32,950	0.370	0.214	0.70
	High	1	3	77,100	95,814	0.282	0.163	0.69
		3	3	27,400	21,705	0.325	0.188	0.60
90	Low	1	3	11,500	7,259	0.514	0297	0.77
		3	2	12,700	9,944	0.447	0.316	0.88
	High	1	2	4,805	4,805	0.001	<0.001	<0.01
		3	3	1,080	1,727	0.502	0.290	1.16
Control			2	98	43	0.896	0.634	1.29

[1] anti-log of log mean [2] log-transformed data [3] coefficient of variation (arithmetic)

Contamination of runoff from one control plot with flow from a diversion ditch carrying runoff from up-slope plots caused rejection of data from one of the control plots. *E. coli* levels in runoff from the valid control plots (which received no manure) were very low, indicating that background contributions of *E. coli*, *e.g.*, from soil or wildlife, were probably negligible in comparison to contributions from applied manure. Two treatments (90-L-3 and 90-H –1) had data from only two replicate plots due to the lack of runoff from one plot in each treatment. Most runoff samples from plots that received 0-day manure exceeded the maximum range for the *E. coli* analysis; means reported in Table 4.9 are reported as "greater than" in those cases. However, in subsequent statistical analysis, this condition is dropped and the values are used as real numbers. Coefficients of variation across replicates were reasonably low, less than 1.00 for most treatments. This indicates fairly similar performance among replicate plots.

Mean *E. coli* levels in plot runoff from the hayland trial are plotted in Figure 4.6. Note that the vertical axis is on a log scale.

Figure 4.6. Mean *E. coli* levels in plot runoff—hayland trial



E. coli levels in runoff were in the range of values reported in the literature for runoff from agricultural land receiving manure (~10^4 – 10^6 /100 ml). Bacteria levels in runoff from manured plots exceeded those in runoff from unmanured control plots by two to five orders of magnitude. Clearly, runoff from hayland receiving manure has a large bacteriological pollution potential.

Figure 4.6 shows that *E. coli* levels in plot runoff tended to decrease with increasing manure age. Runoff *E. coli* levels declined by at least an order of magnitude in treatments with successively older manure.

This is not surprising, given that significant decreases in manure *E. coli* content with manure age were documented in the manure storage experiments (see Section 4.1); the initial bacteria content of the manure determines the stock of bacteria available to runoff.

The effect of treatment on levels of *E. coli* in runoff was evaluated by multi-factor Analysis of Variance (ANOVA) using the following approach. An initial pass used the full model, including all treatment factors (manure age, "Age;" vegetation height, "Veg Ht;" and delay to rain, "Delay") and all possible interactions (Age*Veg Ht, Age*Delay, Veg Ht*Delay, and Age*Veg Ht*Delay), without regard to the significance of factors or interactions. After the initial pass, if the three-factor interaction was non-significant (P > 0.10), it was removed and ANOVA was repeated using all of the main factors and two-way interactions. After the second pass, non-significant factors and interactions were removed and a final "reduced model" ANOVA was conducted. Interpretations of treatment effects are based on the reduced model. Least-square means differences among treatments were assessed using Tukey's HSD and Student's t tests.

The full model ANOVA documented that significant differences existed among treatments, but showed that the three-way interaction Age*Veg Ht*Delay was non-significant (P = 0.861). The second ANOVA run with the three-way interaction removed showed that both the Veg Ht factor and the Age*Delay interaction were non-significant (P=0.460 and P=0.571, respectively). The final reduced model included all three main factors, Age, Veg Ht, and Delay, and the Age*Veg Ht and Veg Ht*Delay interactions. Because the interactions with Veg Ht were significant, Veg Ht had to be included in the analysis, even though it was non-significant as a main factor. Results of the reduced model ANOVA are given in Table 4.10.

Table 4.10. ANOVA table for hayland runoff trial, final reduced model

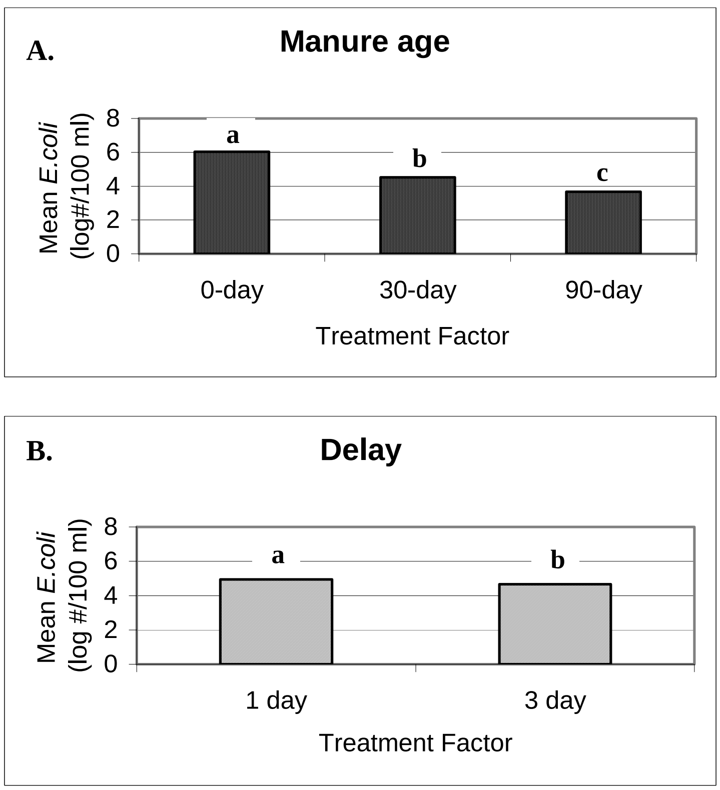
Analysis of Variance					
Source	**DF**	**SS**	**MS**	**F Ratio**	**P**
Model	7	34.8385	4.9769	37.135	<0.001
Error	26	3.4846	0.1340		
Total	33	38.3231			
Effects Tests					
Source	**DF**	**SS**		**F Ratio**	**P**
Age	2	31.1188		116.096	<0.001
Veg Ht	1	0.0673		0.502	0.485
Delay	1	0.602		4.494	0.044
Age*Veg Ht	2	0.7427		2.771	0.081
Veg Ht*Delay	1	1.2076		9.011	0.006

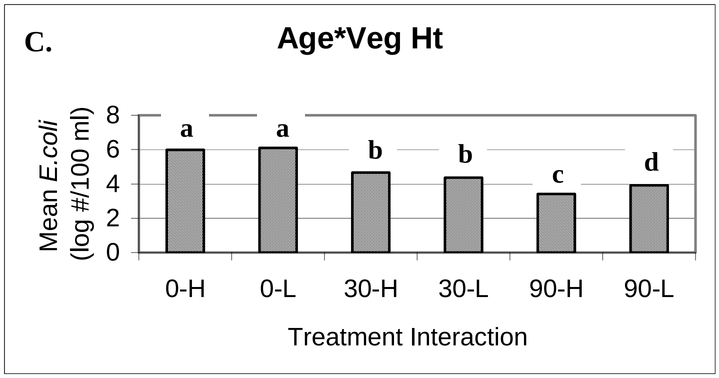
Mean *E. coli* in runoff grouped by main factors and interactions are shown in Figure 4.7. As shown in Figure 4.7.A., mean runoff *E. coli* decreased significantly with increasing manure age, regardless of other treatments. Compared to a mean of $10^{6.04}$ *E. coli*/100 ml in runoff from application of fresh manure, runoff from 30-day old manure contained an average of $10^{4.51}$ *E. coli*/100 ml, a 97% reduction. Runoff from 90-day old manure contained an average of $10^{3.66}$ *E. coli*/100 ml, a 99.6% reduction compared to runoff from fresh manure.

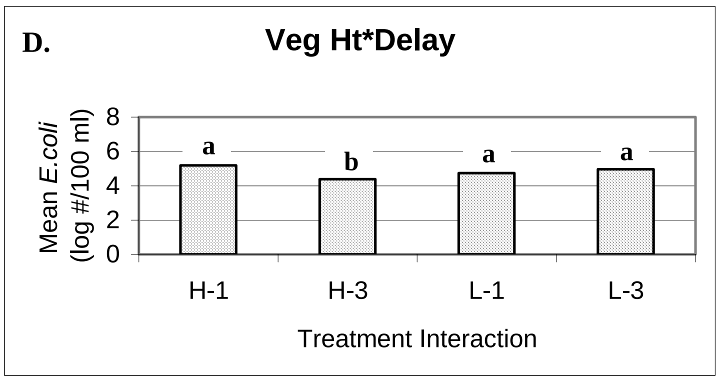
Delay to rainfall also significantly influenced *E. coli* in runoff from hayland plots (Fig. 4.7.B.). Runoff from plots where manure was applied 1 day before rainfall averaged $10^{4.95}$ *E. coli*/100 ml; runoff from plots where manure was applied 3 days before rainfall averaged $10^{4.65}$ *E. coli*/100 ml. This 49% reduction was smaller than that attributed to manure age, but was statistically significant.

Vegetation height (not plotted in Fig. 4.7) did not appear to affect *E. coli* in plot runoff. Runoff in plots with high grass averaged $10^{4.76}$ *E. coli*/100 ml compared to $10^{4.84}$ *E. coli*/100 ml in runoff from low grass plots. The 13% lower *E. coli* in runoff from high grass plots was not statistically significant.

Figure 4.7. Plots of mean *E. coli* levels in hayland runoff grouped by main factor and interactions
Note: In each plot, bars labeled with different letter(s) differ significantly, P≤0.10.







Interactions between main factors are more difficult to interpret. A significant interaction indicates that the relationship between variables is not the same for different classes. As shown in Figure 4.7.C., vegetation height appears to influence *E. coli* in runoff differently for different manure age classes. Whereas no significant differences were observed between runoff from high vs. low grass plots for plots receiving either 0-day old and 30-day old manure, mean *E. coli* levels in runoff from plots receiving 90-day manure were significantly lower from high grass plots than from low grass plots, $10^{3.480}$ *E. coli*/100 ml and $10^{3.927}$ *E. coli*/100 ml, respectively, a difference of 71%. Similarly, the interaction between delay and vegetation height (Figure 4.7.D.) indicates that mean *E. coli* levels in runoff from high grass plots ($10^{4.382}$ *E. coli*/100 ml) were significantly lower than from low grass plots ($10^{5.029}$ *E. coli*/100 ml)—a 78% difference—only where manure was applied 3 days before rainfall.

4.3. Cornland Runoff Trial

The cornland runoff trial was conducted on October 14, 2003. Construction of the rainfall simulator and preparation of the runoff test plots took place over the preceding week, as described in Section 3 (Methods).

4.3.1. Weather

Weather data collected on site over the trial period from October 11 (the day of first manure application) through October 14 (the day of runoff) are summarized for selected hours in Table 4.11 and complete hourly data for selected variables over the same period are plotted in Figures 4.8 and 4.9. Complete hourly weather data for the cornland runoff trial are given in Appendix 8.2.

Table 4.11. Summary of on-site weather data for cornland runoff trial

Date	Time	Air Temp. (°C)	Relative Humidity (%)	Barometric Pressure (kPa)	Wind Speed (km/hr)	Wind Direction	Precip. (mm)	Solar Radiation (watts/m²)
Oct. 11, 2003	00:30	13.8	86	101.56	5.2	SW	0	0
	06:30	9.4	97	101.67	1.2	S	0	0
	12:30	21.2	59	101.54	4.4	WSW/W	0	558
	18:30	18.0	70	101.26	8.0	SSE	0	12
Oct. 12, 2003	00:30	12.4	88	101.10	2.0	SSW	0	0
	06:30	8.3	97	100.83	2.4	SSE/SE	0.25	0
	12:30	18.8	61	100.41	12.1	WSW/SW	0	574
	18:30	14.2	86	100.13	3.6	SW/S	0	8
Oct. 13, 2003	00:30	11.3	96	100.11	9.7	N	0	0
	06:30	8.0	88	100.37	4.0	NNE	0	0
	12:30	13.3	58	100.41	22.1	N	0.25	569
	18:30	12.5	58	100.43	4.8	NNW	0	10
Oct. 14, 2003	00:30	7.0	80	100.69	5.2	SSE	0	0
	06:30	3.4	94	100.80	1.2	SSE	0	0
	12:30	15.1	51	100.42	18.1	S/SSW	0	556
	18:30	12.6	62	99.89	17.3	S/SSE	0	6

Figure 4.8. Plots of hourly on-site air temperature and relative humidity during cornland runoff trial

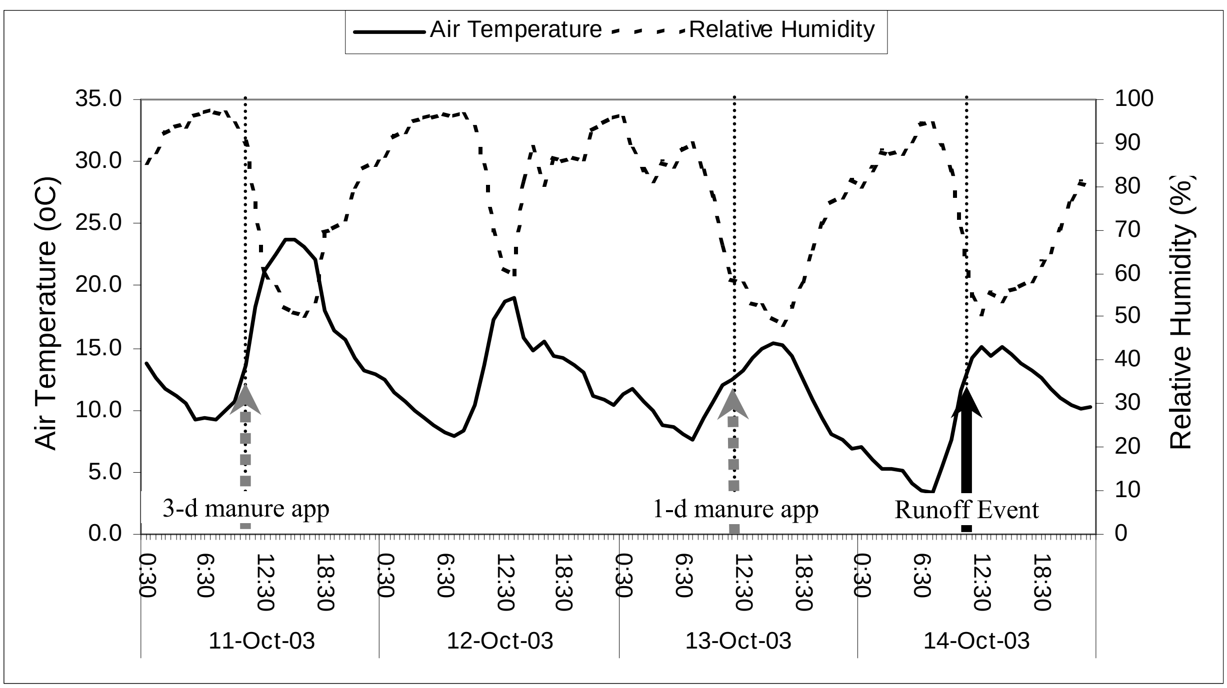
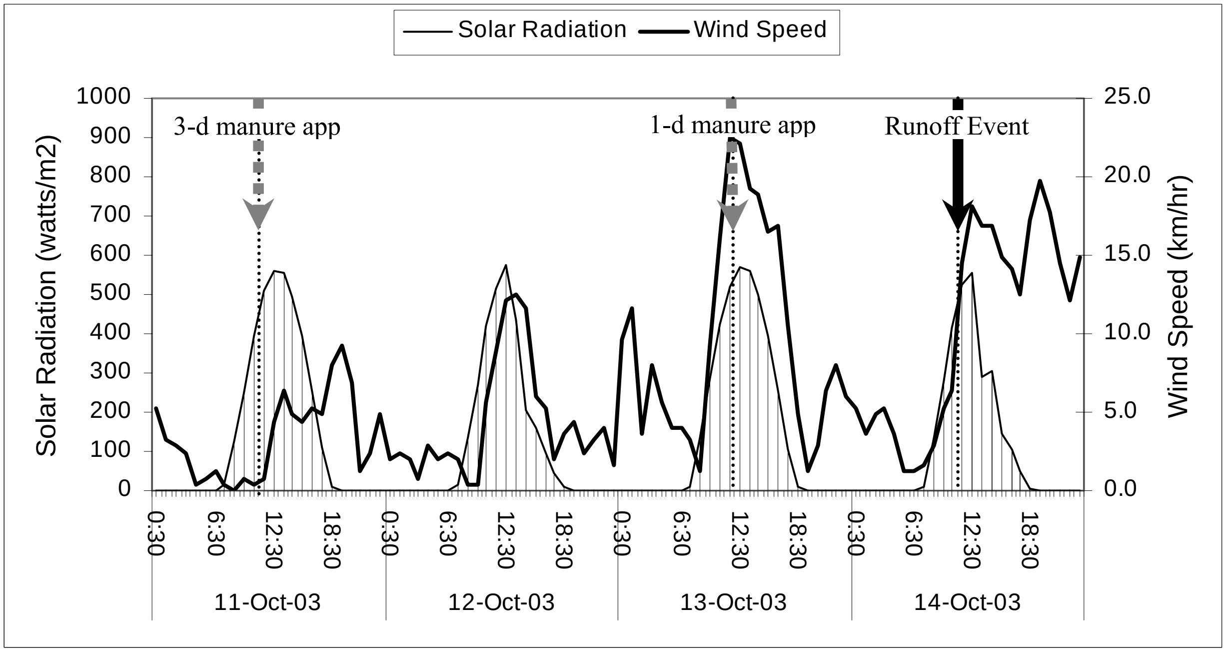


Figure 4.9. Plots of hourly on-site solar radiation and wind speed during cornland runoff trial



Mean air temperature over the trial period was 12.1 °C. Minimum air temperature of 3.2°C occurred at 07:30 on October 14; maximum air temperature during the trial period was 24.4 °C during the afternoon of October 11, just after the 3-day manure application. Air temperatures were substantially lower during the cornland runoff trial than during the June hayland trial, when mean and maximum air temperatures were 21.8 and 33.2 °C, respectively. Relative humidity averaged ~78% over the trial period, peaking at ~90% or above during night, but generally dropping to ~50-60% in mid-afternoon through the period. Barometric pressure averaged 100.67 kPa and generally declined through the trial period. Humidity and pressure were similar to conditions observed during the hayland runoff trial. Winds were moderate and variable and became stronger during the last two days of the trial, including the day of the rainfall event. With maximum speeds up to ~20 km/hr, winds were noticeably higher during the cornland runoff trial compared to the ~10 km/hr winds during the hayland runoff trial. There were only trace amounts of precipitation during the trial period (a total of 0.75 mm); some of this amount was probably the result of condensation. Skies were mainly clear over the trial period, with maximum solar radiation of ~560 – 590 watts/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; little or no rainfall was recorded on the site from the beginning of weather monitoring (October 9); in the 30 days preceding the runoff event, a total of only 94 mm of rain was recorded at the Montpelier weather station; no rain fell during the 8 days preceding the event. Soil temperatures ranged from 7.0 – 14.5 °C during the trial period (Table 4.12).

Table 4.12. Soil temperatures on the runoff area during the cornland runoff trial

Date	Time	Soil Temperature (^oC)
Oct. 11, 2003	15:00	14.5
Oct. 13, 2003	14:55	12.0
Oct. 14, 2003	07:45	7.0
Oct. 14, 2003	12:15	9.8
Oct. 14, 2003	15:33	11.0

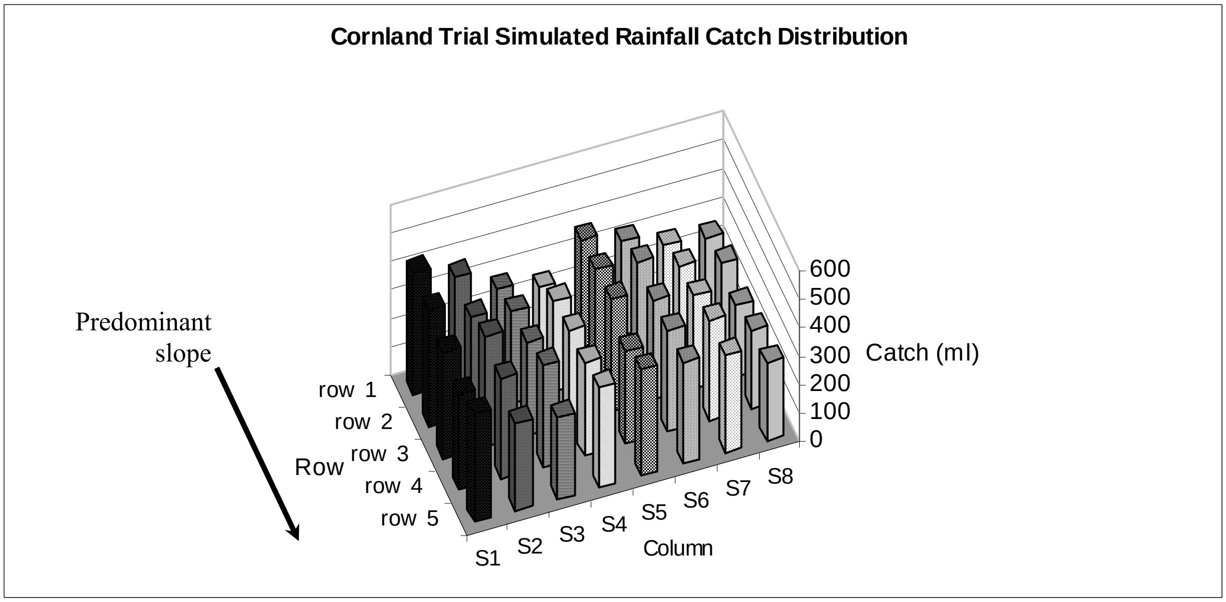
Soil temperatures during the cornland trial period were substantially lower than the 20 – 28 ^oC observed on the hayland site in June.

4.3.2. Rainfall simulation

Simulated rainfall application began at 09:18 on October 14 and continued until 12:02. Measured rainfall application averaged 73 mm among the test plots, for an average intensity of ~ 27 mm/hour. An average of 330 L (87 gallons) of water was applied to each test plot. Note that the plots received 28% less simulated rain in the cornland trial than during the hayland trial. The rate of application was essentially the same for the two trials; the difference in volume applied was due to the shorter irrigation period for the cornland trial. Due to the long duration of this intense simulated rainfall event, it exceeded a 100-year storm in Central Vermont (McKay and Wilks 1995). Note, however, that all plots began to generate runoff within one hour, and that a one-hour duration storm of equivalent intensity has a 5-year return period.

Distribution of simulated rain across the test field was less uniform than hoped for due to winds during rainfall application. Mean volume captured in catch cups located at the center of each plot was 334 ml; the standard deviation was 43 ml, yielding a coefficient of variation of 13%. Catch of simulated rainfall is shown graphically in Figure 4.10. The difference between treatment mean rainfall catches and the mean catch across all plots ranged from 1.3% to 17.2%. Because rainfall received by one treatment (0-I-1) differed from the test field mean by more than one standard deviation, rainfall catch was considered as an independent variable in the subsequent analysis, as called for in the QAPP (Braun and Meals 2003).

Figure 4.10. Bar chart of total catch of simulated rainfall by test plots during cornland runoff event



As in the hayland runoff trial, simulated rainfall was sampled three times during the cornland runoff event for *E. coli* bacteria. Results are shown in Table 4.13. Simulated rainfall added a negligible quantity of *E. coli* bacteria to the experimental plots.

Table 4.13. *E. coli* content of simulated rainfall—cornland runoff trial

Sample Time	*E. coli* (#/100 ml)
09:21	1
10:10	1
12:05	<1

4.3.3. Cornland runoff

Simulated rainfall began at 09:18 and the first plot runoff was recorded 9 minutes later at 09:27. Runoff occurred on all plots within ~60 minutes of the onset of rainfall. Simulated rainfall and runoff collection continued through 12:02, at which time a minimum of several liters of runoff had been collected from each plot.

Observation of approximate times that runoff began from each plot suggested that treatment affected the onset of runoff. The first 10 plots to generate runoff were all non-incorporated (untilled); of the first 20 plots to generate runoff, 17 were non-incorporated. This result was probably due to differences in surface soil texture of incorporated vs. non-incorporated plots. The soil surface of the cornfield was generally smooth and relatively compacted; the presence of liquid manure on the surface probably decreased infiltration capacity further. Incorporation by tillage loosened the soil, mixed the manure into the soil, and left the surface rough. The delayed generation of runoff from tilled plots was probably the result of both enhanced infiltration and increased detention storage on the loose, rough soil surface. As in the hayland trial, differences among plots in characteristics such as micro-topography, soil condition, vegetation density, or wind exposure probably also affected runoff timing.

E. coli data from cornland trial plot runoff are summarized in Table 4.14; raw data are reported in Appendix 8.3. No *E. coli* were detected in rinsate from carboys collected prior to the simulated rainfall event, confirming that runoff collection containers were effectively sterilized.

Table 4.14. Summary of *E. coli* data from cornland runoff trial

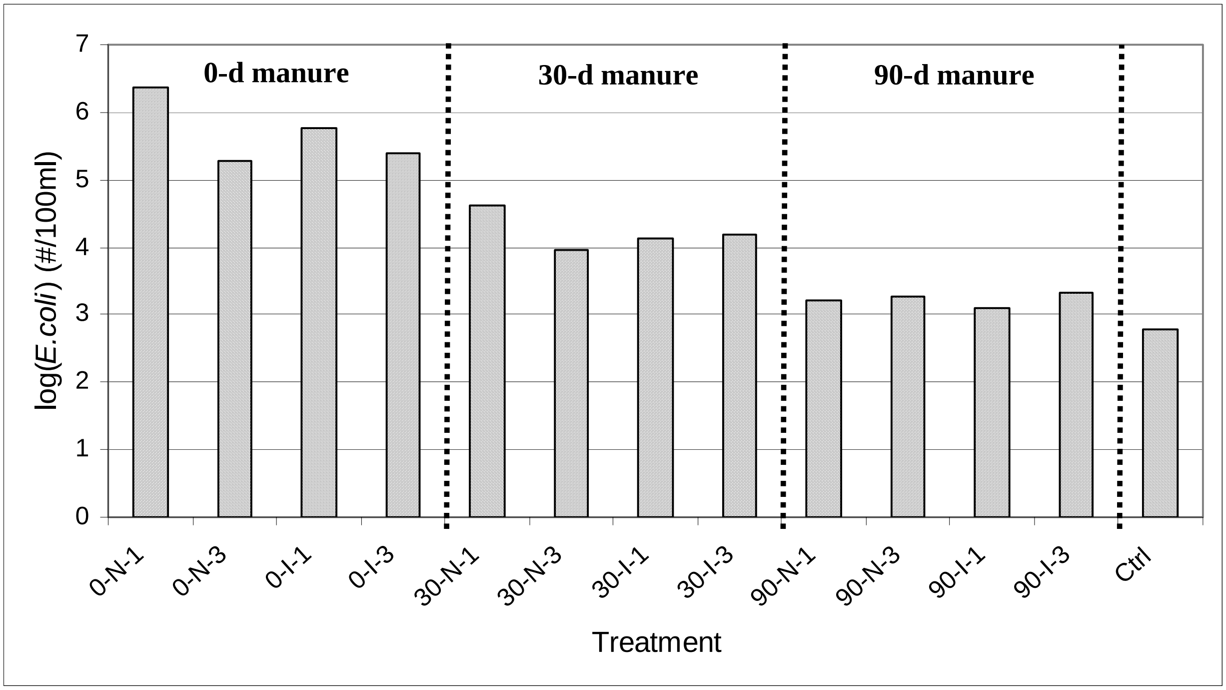
Manure Age (d)	Incorporation	Delay to Rain (d)	n	Median *E. coli* #/100 ml	Mean[1] *E. coli* #/100 ml	Std. Dev.[2]	Std. Error Mean[2]	C.V.[3]
0	Non-Incorp.	1	3	2,010,000	2,342,437	0.242	0.140	0.59
		3	3	175,000	190,326	0.174	0.100	0.41
	Incorp.	1	3	657,000	582,060	0.097	0.056	0.21
		3	3	529,000	239,622	0.776	0.448	0.87
30	Non-Incorp.	1	3	52,900	41,971	0.232	0.134	0.44
		3	3	7,400	8,993	0.290	0.167	0.70
	Incorp.	1	3	12,100	13,934	0.246	0.142	0.59
		3	3	16,000	15,892	0.038	0.022	0.09
90	Non-Incorp.	1	3	1,000	1,600	0.354	0.204	0.88
		3	3	1,000	1,847	0.461	0.266	1.10
	Incorp.	1	3	1,000	1,260	0.174	0.100	0.43
		3	3	1,500	2,103	0.416	0.240	0.99
Control			3	860	593	0.415	0.240	0.68

[1] anti-log of log mean [2] log-transformed data [3] coefficient of variation (arithmetic)

Improved ditching to isolate plots and better prediction of *E. coli* levels in runoff samples resulted in complete data for the cornland trial. *E. coli* levels in runoff from the control plots were low, but were higher than the levels observed in control plot runoff in the hayland trial (mean of 593/100 ml vs. 43/100 ml for the hayland trial). Coefficients of variation across replicates were again reasonably low, less than 1.00 for all but one of the treatments. This indicates fairly similar performance among replicate plots.

Mean *E. coli* levels in plot runoff from the cornland trial are plotted in Figure 4.11. Note that the vertical axis is on a log scale.

Figure 4.11. Mean *E. coli* levels in plot runoff—cornland trial



For plots receiving 0-d and 30-d old manure, *E. coli* levels in runoff were in the range of values reported in the literature for runoff from agricultural land receiving manure (~10^4 – 10^6 /100 ml). Runoff from plots receiving 90-d old manure contained ~10^3 *E. coli*/100ml. Bacteria levels in runoff from plots receiving 0-d and 30-d old manure exceeded those in runoff from unmanured control plots by one to three orders of magnitude. Clearly, runoff from cornland receiving manure has a large bacteriological pollution potential.

It is obvious from Figure 4.11 that *E. coli* levels in plot runoff tended to decrease with increasing manure age, just as they did in the hayland trial. Runoff *E. coli* levels declined by at least an order of magnitude in successively older manure treatments. Runoff from plots receiving 90-d old manure contained *E. coli* levels similar to those observed from the control plots. This is not surprising, given the significant decreases in manure *E. coli* content with manure age documented in the manure storage experiments (see Section 4.1), especially for 90 days of storage.

The effect of treatment on *E. coli* runoff was evaluated by multi-factor Analysis of Variance (ANOVA) using the same approach described in Section 4.2.3. For the cornland data, rainfall catch was also included in the full model, as discussed in Section 4.3.2. The initial pass with the full model included all treatment factors and all possible interactions.

- Manure age, "Age"
- Incorporation "Incorp"
- Delay to rain, "Delay"
- Rainfall catch, "Catch"
- Age*Incorp
- Age*Delay
- Age*Catch
- Incorp*Delay
- Incorp*Catch
- Delay*Catch
- Age*Incorp*Delay
- Age*Delay*Catch
- Age*Incorp*Catch
- Incorp*Delay*Catch
- Age*Incorp*Delay*Catch

After the initial pass, if the four-factor interaction was non-significant (P > 0.10), it was removed and ANOVA was repeated with main factors and two- and three-way interactions. After the second pass, non-significant factors and interactions were removed and a final "reduced model" ANOVA was conducted. Interpretations of treatment effects are based on the reduced model.

The full model ANOVA documented that significant differences existed among treatments, but showed that the four-way interaction Age*Incorp*Delay*Catch was non-significant (P = 0.432). The second ANOVA run with the four-way interaction removed showed that the Catch factor was non-significant (P=0.928), as were all interaction terms involving Catch. The third ANOVA run with Catch removed showed that main factors Age and Delay were both statistically significant, while Incorp was nonsignificant (P=0.269); the Age*Incorp and Age*Incorp*Delay interactions were also nonsignificant (P=0.722 and P=0.535, respectively). Because the Incorp*Delay interaction was statistically significant, Incorp remained in the final model. Results of the final reduced model ANOVA are given in Table 4.15.

Table 4.15. ANOVA table for cornland runoff trial, final reduced model

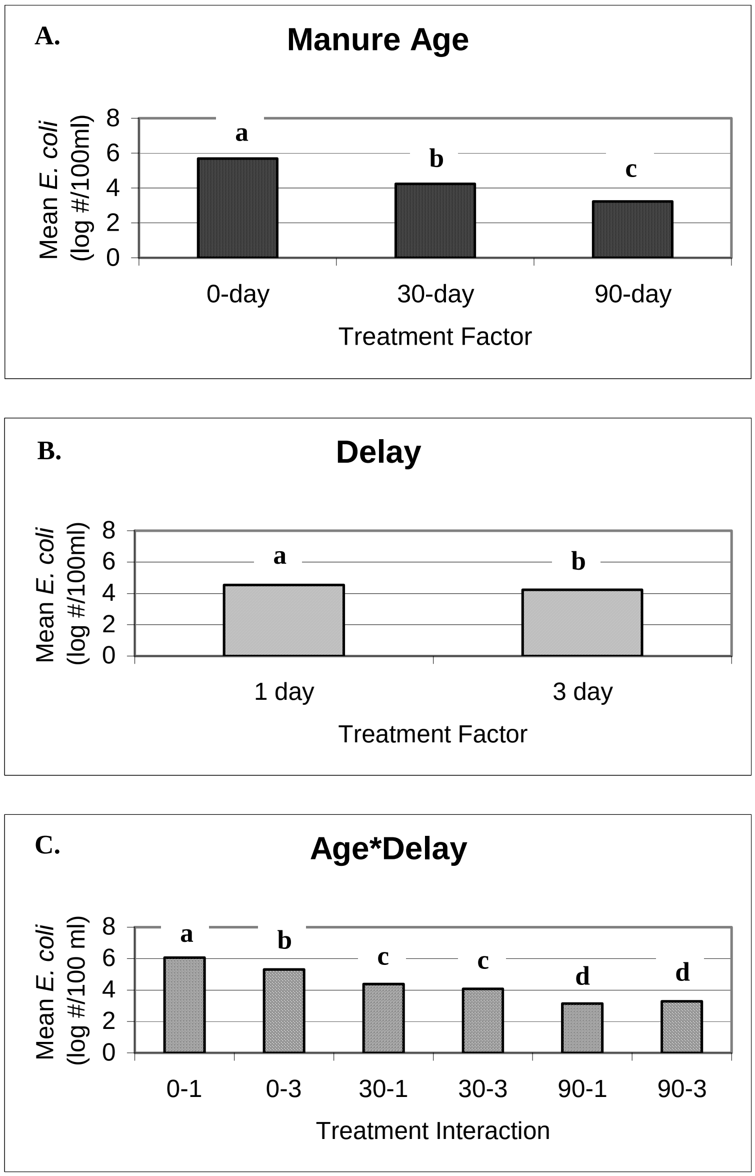
Analysis of Variance					
Source	**DF**	**SS**	**MS**	**F Ratio**	**P**
Model	7	39.9381	5.7054	51.273	<0.001
Error	28	3.1157	0.1113		
Total	35	43.0538			
Effects Tests					
Source	**DF**	**SS**		**F Ratio**	**P**
Age	2	37.1768		167.049	<0.001
Incorp	1	0.1536		1.380	0.250
Delay	1	0.8126		7.303	0.012
Age*Delay	2	1.1621		5.222	0.012
Incorp*Delay	1	0.6330		5.688	0.024

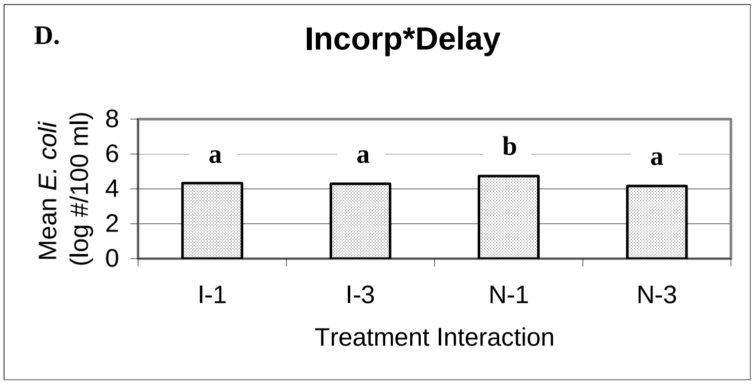
Mean *E. coli* in cornland runoff grouped by main factors and interactions are shown in Figure 4.12. As shown in Figure 4.12.A., mean runoff *E. coli* decreased significantly with increasing manure age, regardless of other treatments. Compared to a mean of $10^{5.70}$ *E. coli*/100 ml in runoff from application of fresh manure, runoff from 30-day old manure contained an average of $10^{4.23}$ *E. coli*/100 ml, a 96.6%

reduction. Runoff from 90-day old manure contained an average of $10^{3.22}$ *E. coli*/100 ml, a 99.6% reduction compared to runoff from fresh manure. In fact, *E. coli* levels in runoff from plots receiving 90-day old manure did not differ significantly from *E. coli* levels in runoff from control plots that received no manure (by one-way ANOVA on manure age).

Figure 4.12. Plots of mean *E. coli* levels in cornland runoff grouped by main factor and interactions

Note: In each plot, bars labeled with different letter(s) differ significantly, P≤0.10





Delay to rainfall also significantly influenced *E. coli* in runoff from cornland plots (Fig. 4.12.B). Runoff from plots where manure was applied 1 day before rainfall averaged $10^{4.53}$ *E. coli*/100 ml; runoff from plots where manure was applied 3 days before rainfall averaged $10^{4.23}$ *E. coli*/100 ml. This 50% reduction was smaller than that attributed to manure age, but was statistically significant.

Manure incorporation (not plotted in Fig. 4.12) did not appear to affect *E. coli* in cornland plot runoff. Runoff in plots where manure was not incorporated averaged $10^{4.49}$ *E. coli*/100 ml compared to $10^{4.32}$ *E. coli* /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 Figure 4.12.C., longer delay appeared to have a greater effect on reducing *E. coli* in runoff from fresh manure, compared to runoff from either 30-day or 90-day manure. Where fresh manure was applied, runoff from plots where manure was applied 3 days before rainfall averaged $10^{5.33}$ *E. coli*/100 ml, compared to an average of $10^{6.07}$ *E. coli*/100 ml in runoff from plots where manure was applied 1 day before rainfall. This represents 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 day before rainfall (Figure 4.12.D.). Whereas *E. coli* in runoff from 3-day delay plots was not significantly affected by incorporation, *E. coli* levels in runoff from 1-day delay plots were significantly reduced by incorporation of manure into the soil. Runoff from plots where manure was applied 1 day before rainfall and not incorporated averaged $10^{4.73}$ *E. coli*/100 ml, compared to an average $10^{4.34}$ *E. coli*/100 ml in runoff from similar plots where manure was incorporated. This represents a 60% reduction in bacteria levels in runoff due to incorporation.

5. DISCUSSION

5.1. Manure Storage Experiments

The dairy manure used in the storage experiments was reasonably representative of liquid dairy manure from Vermont farms. Manure dry matter, Total N, P_2O_5, and K_2O were all within the average range reported by the UVM Agricultural and Environmental Testing Laboratory from 1992 – 1996 (UVM Extension 2004). All batches of manure delivered to the storage tanks at both test sites contained ~10^5 *E. coli*/g wet weight, consistent with the bacterial content reported for liquid dairy manure in the literature (Crane *et al.* 1983, Moore *et al.* 1988).

Die-off of *E. coli* during experimental storage was dramatic. Manure stored for ~30 days showed a decline of ~99% (2 log) in *E. coli* content; *E. coli* declined by >99% (2 – 3 log) after ~90 days of storage. These declines are consistent with reports from the literature (*e.g.*, Patni *et al.* 1985, Trevisan and Dorioz 1999, Conboy and Goss 2001).

Although no intermediate samples were taken during the manure storage period, it is possible to estimate the rate of *E. coli* decline from samples taken at the beginning and end of the storage period. It is generally accepted that decay in bacterial populations follows simple first-order kinetics (Crane and Moore 1986) according to this equation:

$$N_t/N_o = 10^{-kt}$$

where:
- N_t = number of bacteria at time t
- N_o = number of bacteria at time 0
- t = time in days
- k = first order or die-off rate constant

Based on this assumption, die-off rate constants calculated for our storage experiments are shown in Table 5.1. Also shown in Table 5.1 are values of T_{90}, another common gauge of bacterial decay, defined as the number of days required to reduce initial *E. coli* levels by 90%. Measured values of T_{90} are reported in the literature; values for our experiments in Table 5.1. are estimated from a first-order decay at the given rate constant.

Table 5.1. First-order *E. coli* die-off rate constants (k) from manure storage experiments

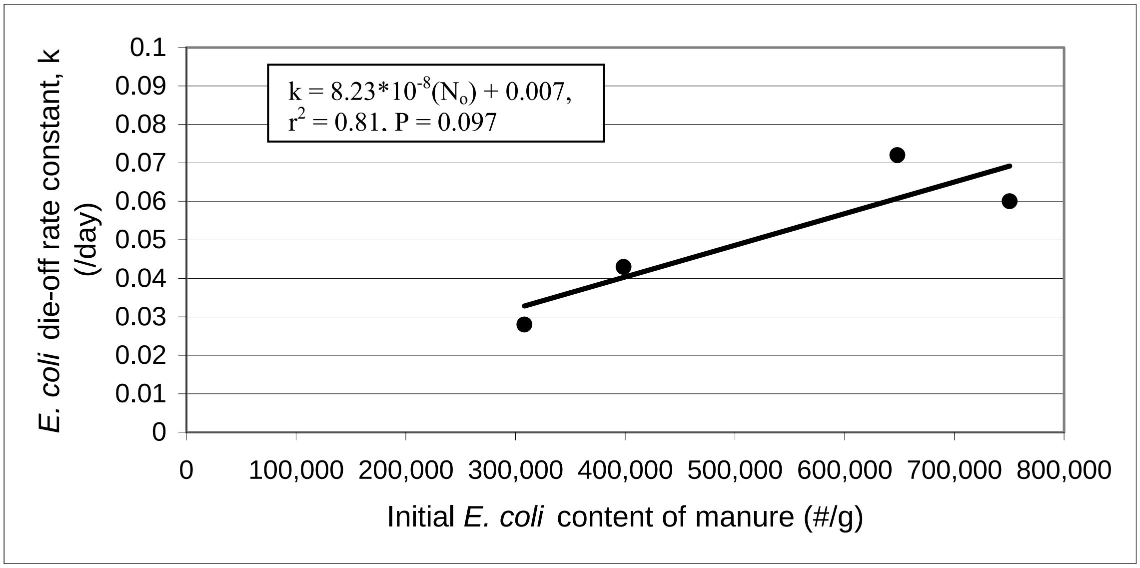
Storage Time (days)	*E. coli*—Start (#/g)	*E. coli*—End (#/g)	k (day $^{-1}$)	T_{90}[1] (days)
Hayland Trial				
33	750,315	8,098	0.060	17
84	308,204	1,260	0.028	36
Cornland Trial				
27	668,040	7,742	0.072	14
83	398,465	114	0.043	23

[1] T_{90} = time required to reduce initial *E. coli* levels by 90%, estimated from first-order decay at given k value

The *E. coli* die-off rates calculated in our experimental storage are lower than the range of 0.1 – 0.3 day^{-1} for dairy manure most often reported in the literature (Rankin and Taylor 1969, Burrows and Rankin 1970, Kovacs and Tamasi 1979), although comparable values of 0.032 day^{-1} have also been reported (Conboy and Goss 2001). Most reported die-off rates, however, are developed from laboratory experiments that may not be directly comparable to our outdoor, meso-scale experiments. Estimated values of T_{90} (Table 5.1) were generally comparable to values reported in the literature from full-scale storage systems. For example, Jones (1980) reported a T_{90} of 14 to 28 days in unaerated storage. Kearney *et al.* (1993b) determined a T_{90} of ~77 days 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 days in solid manure and from 2 to 35 days in slurry.

It is interesting to note that in both storage experiments, die-off rates for manure stored for ~30 days were considerably higher than for the manure stored for ~90 days. The reason for this is not certain, but may be related to the initial *E. coli* content of the manure. In the hayland storage experiment, for example, the initial *E. coli* level in the 30-day manure was more than double the initial level in the 90-day manure. Moreover, when values of k are plotted against initial *E. coli* concentration for all stored manure (Figure 5.1), a positive linear relationship between initial *E. coli* level and die-off rate is apparent: die-off is more rapid when bacteria levels are higher. This kind of relationship is not widely reported in the literature, but it has been noted that competition for limited nutrients is one factor in bacterial die-off (Crane and Moore 1986). Such competition would probably be higher in larger bacterial populations, possibly leading to more rapid initial mortality. It is also possible that bacteria die-off did not follow a simple first-order decay, but experienced higher initial die-off rates that dominated the shorter storage periods.

Figure 5.1. ***E. coli*** **die-off rate in manure storage experiments vs. initial** ***E. coli*** **content**



It is also important to note that *E. coli* die-off was more rapid in the ~90-day storage for the cornland trial than for the similar period in advance of the hayland trial, as evidenced by the higher k value, lower T_{90}, and lower final *E. coli* count (Table 5.1). As noted in Section 4.1, air temperatures were higher during the July – October 90-day manure storage period for the corn trial (mean daily air temperature 18.1 ^{o}C; 292 cooling degree days) than during the April – June 90 days of storage for the hayland trial (mean daily air temperature 11.3 ^{o}C, 29 cooling degree days). Temperature has been widely noted as one of the most important factors in bacterial survival, with higher temperatures generally enhancing die-off (Crane and Moore 1986, Moore *et al.* 1988). It is likely that the higher temperatures contributed to the greater die-off during the July – October storage.

Although conditions in our storage experiments may not have replicated those in actual full-scale manure storage structures, these results clearly support the notion that manure storage can be an important factor in reducing the bacteria content of manure to be applied to agricultural land. The die-off rates observed in our experiments were probably higher than those in typical full-scale storage systems. Temperature extremes and variability in 300 L above ground plastic tanks, for example, were likely greater than those to be expected in a below-ground earthen manure lagoon, possibly contributing to greater die-off in our experiments than would occur in the real world. The lack of frequent inoculation with fresh manure additions in our definitive storage period may have enhanced net *E. coli* loss. Such definitive storage would probably enhance bacteria die-off in full-scale manure storage. On the other hand, a surface crust developed quickly on the manure in each of our storage tanks; conditions were almost certainly anaerobic below the crust, as manure stored in a full-scale structure would be. Although conditions of our storage experiments may not have replicated actual full-scale conditions, our results are not inconsistent with data reported from actual storage facilities and tend to confirm the potential for definitive storage as one element of a multi-barrier approach to reducing microorganism losses from agricultural land. Bacteria die-off rates in full-scale manure storage systems deserve further investigation.

5.2. Hayland Runoff

Microorganism counts in runoff from agricultural land have been widely reported to exceed water quality standards (Baxter-Potter and Gilliland 1988). Our results confirm that manure application to hayland is a potential source of bacterial contamination to surface waters via runoff. For all treatments combined, runoff from hayland plots that received manure averaged 63,222 *E. coli*/100 ml, compared to the average 43 *E. coli*/100 ml from unmanured control plots. *E. coli* counts in hayland plot runoff in our study were comparable to levels reported in the literature. Counts of 10^4 – 10^6 fecal coliform/100 ml in runoff from manure application areas are commonly reported in the literature (Crane *et al.* 1983, Moore *et al.* 1988, Irvine and Pettibone 1996). Surface slurry application to perennial grassland in Finland, for example, led to runoff losses of *E. coli* of 4.4 – 4.8 x 10^3/100 ml (Heinonen-Tanski and Uusi-Kamppa 2000).

The treatment that had the greatest influence on *E. coli* in runoff from hayland in our experiments was manure age. This is not surprising, as *E. coli* levels declined significantly with increasing storage time in our manure storage experiments (Section 4.2). It would be expected that runoff losses of microorganisms should tend to be proportional to the quantity of microorganisms available for transport (Van Donsel *et al.* 1967, McCaskey *et al.* 1971, Robbins *et al.* 1971). Runoff from plots treated with 30-day old manure averaged 97% fewer *E. coli* organisms than did runoff from plots on which fresh manure was applied; runoff from plots treated with 90-day old manure had >99% fewer *E. coli* than runoff from fresh manure treated plots. Note that the manure age effect is probably even greater than indicated by these data, as *E. coli* levels in runoff from six of twelve plots receiving fresh manure exceeded the range of the analysis and were almost certainly considerably higher than the censored values used in our analysis.

Delay between manure application and rainfall/runoff was also a significant influence on *E. coli* in hayland runoff in our experiments. Runoff plots where manure was applied 3 days before rainfall averaged $10^{4.65}$ *E. coli* /100 ml, a 49% reduction from the average $10^{4.95}$ *E. coli* /100 ml in runoff from plots where manure was applied 1 day before rainfall. The significance of time elapsed between manure application and rainfall has been widely documented elsewhere. 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, 58-90% of fecal coliform were lost in runoff. If residence time increased from 1 to 3 days, only 10-22% of fecal coliform were lost.

Increasing residence time of manure on the land surface before rainfall/runoff increases the opportunity for bacteria die-off. Following land application, microorganisms in manure are subjected to a number of potentially lethal conditions, most notably solar radiation, temperature extremes, and desiccation (Crane and Moore 1986, Moore *et al.* 1988). During the 3-day delay between manure application and simulated rainfall in our hayland experiment, peak daily solar radiation was ~640 – 970 watts/m^2, midday air temperatures exceeded 25 ^{o}C, and essentially no natural precipitation fell. Such conditions would have been inimical to bacteria survival on the soil surface. Adsorption/fixation of bacteria to surface soils and vegetation and competition and predation within the microorganism population are also believed to contribute to reductions in bacteria runoff following manure application (Moore *et al.* 1988).

As a result of such factors, die-off of *E. coli* bacteria after land application has been observed to follow a first-order decay, with reported values of k ranging from 0.303 – 0.697/day, with greatest die-off in warm weather (Klein and Casida 1967, Van Donsel *et al.* 1967, Taylor and Burrows 1971). At a hypothetical k of 0.5/day, *E. coli* in surface-applied manure containing 500,000 *E. coli*/g would be expected to decline to 15,800 *E. coli*/g (a 97% reduction) in three days.

Vegetation height alone was not found to exert a significant effect on levels of *E. coli* in runoff from hayland plots. This treatment was selected for our experiment because literature reports have related the condition of hayland vegetation to bacteria runoff. Two contrasting mechanisms of effects on *E. coli* levels have been proposed. First, when manure is applied to hayland, some of the material is intercepted and captured on vegetation before it reaches soil, affecting bacteria availability for loss and overall survival rates. In greenhouse studies, Brown *et al.* (1980) observed that fecal coliform from sludge application to grasses adhered to leaves and were difficult to wash off. When slurry was applied to grassland in the French Alps, Vansteelant (2000) found *E. coli* in ~equal numbers on plant stems and the soil surface just after spreading, suggesting that half of the organisms were intercepted on the vegetation. A gradual decrease in *E. coli* on grass leaves was observed over 8 weeks. Trevisan *et al.* (2000) 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 proposed that bacteria die-off can be enhanced when manure is applied to thin or short vegetation. Crane *et al.* (1983) observed a vegetation height effect, noting that cutting pastures reduced bacterial survival times by enhancing drying and exposure to solar radiation. In haylands of the French Alps, Trevisan *et al.* (2000) reported that fecal coliform disappeared from the plant stems at different rates, depending on the thickness of the vegetation. When the biomass of the plant canopy was low, die-off was more rapid, probably 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 most intensively managed hay meadows (those with best vegetation stands) maintain higher bacteria numbers.

Despite these potential modes of action, 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 (~6 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. We did observe interception of liquid manure on vegetation during the manure application process, but the difference in interception between low and high grass may not have been large enough to make a difference in *E. coli* levels in plot runoff. Vegetation on the entire hay field before the experiment was somewhat thin and the 8 cm height difference between the two treatments may not have been enough to provide a sheltering effect.

There was, however, a significant vegetation height effect observed for plots that received 90-day manure (Veg Ht*Age interaction). Mean *E. coli* levels in runoff from plots receiving 90-day manure were significantly lower from high grass plots ($10^{3.480}$ *E. coli* /100 ml) than from low grass plots ($10^{3.927}$ *E. coli* /100 ml), a difference of 71%. Manure stored for 90 days contained far fewer *E. coli* (~1260/g) than did the manure from the 30 or 0 day storage treatments. The effect of interception and resulting *E. coli* die-off on high vegetation may have been large enough to be detectable at the bacteria densities of the 90-day old manure, but too small to be detectable in higher bacteria content manure. Furthermore, our ability to detect significant differences among plots receiving 0-day 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 days before simulated rainfall, runoff from high vegetation plots contained significantly fewer *E. coli* 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. Mean *E. coli* levels in runoff from high grass plots ($10^{4.382}$ *E. coli* /100 ml) were significantly lower than from low grass plots ($10^{5.029}$ *E. coli* /100 ml) only where manure was applied 3 days before rainfall, a 78% difference. In this case, the three days of 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 one day.

Thus, for manure application to hayland, storage of manure for definite time periods (weeks to months) and the timing of manure application to avoid spreading within several days of runoff appear to significantly reduce *E. coli* losses in runoff. Application of manure to relatively high vegetation, while not consistently effective, appears to offer significant reductions in bacteria losses under some conditions.

5.3. Cornland Runoff

Our results confirm that manure application to cornland can be a source of bacterial contamination to surface waters via runoff. For all treatments combined, runoff from cornland plots that received manure averaged 24,218 *E. coli*/100 ml, compared to the average 593 *E. coli*/100 ml from unmanured control plots. Runoff from plots that received manure stored for 90 days, however, contained levels of *E. coli* (1,673/100 ml) that did not differ significantly from those in runoff from unmanured control plots. Runoff from cornland plots that received fresh manure and manure stored for 30 days averaged 92,145 *E. coli*/100 ml. These *E. coli* counts were comparable to the levels 10^3 – 10^6 fecal coliform/100 ml in runoff from manure application areas reported in the literature (Crane *et al.* 1983, Moore *et al.* 1988), although few measurements specifically from silage cornfields have been reported.

As in the hayland trial, the treatment that had the greatest influence on *E. coli* in runoff from cornland plots was manure age. Again, this is likely a direct result of the observed declines in *E. coli* levels with increasing storage time in our manure storage experiments (Section 4.2) and the resulting reduction in the number of microorganisms available for transport in runoff. Runoff from plots treated with 30-day old manure averaged 97% fewer *E. coli* than did runoff from plots that received fresh manure; runoff from plots receiving 90-day old manure had >99% fewer *E. coli* than runoff from plots that received fresh manure. Runoff from plots receiving 90-day old manure contained *E. coli* levels similar to those in runoff from unmanured plots.

It should be noted that runoff from unmanured cornland plots contained higher levels of *E. coli* (average 593/100 ml) than did runoff from unmanured hayland plots (average 43/100 ml). This may be due to higher levels of *E. coli* in the soil of the cornfield than the hayfield or to more recent manure application. Long-term manure application has been reported to promote bacteria survival in soils compared to soils that have not been manured (Dazzo *et al.* 1973). High levels of organic matter and nutrients in soils support the survival, and in some cases the regrowth, of indicator organisms in the soil (Jamieson *et al.* 2002). The study area for the cornland trial had been in continuous corn for the past eight years and received annual manure applications in the spring prior to planting. The cornland study area had received manure in Spring 2003, ~six months before the runoff event. In contrast, the hayland site was seeded in 2000 and received an annual application of liquid manure between 2000 and 2003. Prior to the hayland runoff event, the most recent manure application had been in July 2003, ~11 months earlier.

In addition, higher *E. coli* levels in runoff from unmanured cornland plots compared to unmanured hayland plots may have been due to the higher runoff potential from the nearly bare soil of the cornland plots, compared to the vegetated hayland plots.

Consistent with results from the hayland trial, delay to rainfall significantly influenced *E. coli* in runoff from cornland plots. Runoff from plots where manure was applied 1 day before rainfall averaged $10^{4.53}$ *E. coli* /100 ml; runoff from plots where manure was applied 3 days before rainfall averaged $10^{4.23}$ *E. coli* /100 ml. A delay of 3 days between manure application and simulated rainfall yielded a 50% reduction in runoff *E. coli* compared to manure application the day before rainfall. Just as on hayland, it is likely that the longer residence time of the manure on the land provided greater opportunities for bacterial die-off due to solar radiation, high temperatures, dessication, and competition and predation within the microorganism population. Even though environmental conditions were less harsh than during the hayland trial (midday solar radiation peaks were ~560-590 watts/m^2, about 25% lower than for the June hayland trial; mean daily air temperatures of 12 ^oC were about 10 ^oC lower than for the hayland trial), the lack of cover on the nearly bare surface of the cornfield probably resulted in greater exposure of bacteria to inimical conditions.

Longer delay between manure application and runoff had a greater effect in reducing *E. coli* numbers in runoff from fresh manure treated plots than from plots treated with stored manure (Age*Delay interaction). Where fresh manure was applied, runoff from plots where manure was applied 3 days before rainfall averaged 82% fewer *E. coli* ($10^{5.33}$ *E. coli* /100 ml) than an average $10^{6.07}$ *E. coli* /100 ml in runoff from plots where fresh manure was applied 1 day before rainfall. This effect may have been due to higher initial die-off rates in the higher *E. coli* populations in the fresh manure.

Incorporation of manure following application to plots did not exert a significant direct effect on levels of *E. coli* in runoff from cornland plots. This treatment was selected for our experiment because of longstanding recommendations for manure incorporation to reduce runoff losses of nutrients and other constituents. Some literature reports have documented substantial bacteria die-off in the soil due to hostile environmental conditions and predation; other reports have suggested that mixing bacteria into the soil may promote immobilization of organisms through adsorption onto soil particles. Reddy *et al.* (1981), for example, reported first-order die-off rate constants in the range of 0.15 - 6.39 day^{-1} for *E. coli* in the soil-water-plant system. Sjogren (1994) reported somewhat lower die-off rates of 0.03 – 0.06 day^{-1} for *E. coli* in laboratory soil microcosms. In contrast, some authors have suggested that long-term bacterial survival may actually be enhanced when manure is incorporated because organisms are protected from desiccation, sunlight, and high temperatures (Dazzo *et al.* 1973, Walker *et al.* 1990, Jamieson *et al.* 2002).

In addition, incorporation is thought to reduce the availability of microorganisms for loss in runoff because most of the microorganisms are below the soil surface, away from the zone of interaction with surface runoff.

Despite these potential effects, we observed no significant difference in *E. coli* levels in cornland plot runoff due to incorporation alone. This result may be somewhat misleading, however, if our results are to be applied to larger-scale settings. Incorporation did have a major observed effect on the generation of runoff from plots. Although this effect was not quantified because we did not measure plot runoff quantity, incorporation appeared to cause a substantial delay in runoff generation from our plots. Recall that in the cornland trial, the first 10 plots to generate runoff were all non-incorporated (untilled); of the first 20 plots to generate runoff, 17 were non-incorporated. This result was probably due to differences in surface soil characteristics of incorporated vs. non-incorporated plots, with delayed generation of runoff from tilled plots resulting from both enhanced infiltration and increased detention storage on the loose, rough soil surface. In a full-scale field application, this would potentially 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 storm events. 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* in runoff was significantly affected by incorporation when the manure was applied 1 day before simulated rainfall (Incorp*Delay interaction), but not when applied 3 days before. Runoff from plots where manure was applied and incorporated the day before simulated rainfall averaged 60% fewer E. coli ($10^{4.34}$ *E. coli* /100) compared to an average $10^{4.73}$ *E. coli* /100 ml in 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 bacterial immobilization through soil interactions was greater than the opportunity for bacteria die-off on the surface over just one day before runoff. Alternatively, this result could suggest that a 3 day 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 soil surface environment. This conclusion is supported by the fact that *E. coli* in runoff from non-incorporated plots treated 3 days prior to rainfall ($10^{4.17}$ *E. coli* /100 ml) was 73 percent lower than *E. coli* in runoff from non-incorporated plots where manure was applied 1 day before rainfall ($10^{4.73}$ *E. coli* /100 ml), a statistically significant difference (P= 0.024). The difference in *E. coli* in runoff due to delay was nonsignificant for incorporated plots.

These results on cornland lend further support to the principle of providing long-term manure storage as a means to reduce *E. coli* losses in runoff from agricultural land. As on hayland, manure application to cornland several days in advance of runoff appears to also significantly reduce bacteria losses in runoff, especially for fresh manure with high initial bacteria content. Although we did not document a significant effect of manure incorporation alone as a factor in *E. coli* levels in runoff, the observed effect of incorporation on runoff generation from plots and the apparent ability of incorporation to reduce runoff *E. coli* levels in manure applied the day before runoff indicate that incorporation of manure into the soil can still be recommended as a practice that will assist in reducing the loss of microorganisms in runoff from cornland.

5.4. Comparison of Hayland and Cornland Runoff

Although the study was not designed to quantitatively compare microorganism losses from hayland and cornland, some preliminary inferences can be drawn from plot runoff data. Several important differences between trials must be recognized, however, as these differences limit direct comparison of E. coli data in runoff from hayland and cornland plots. First, cornland plots received 40% more manure (and generally more E. coli) than did hayland plots. The different rates are representative of normal agronomic practice on Vermont cropland. Second, although the E. coli content of the manure applied in the two trials was generally comparable between manure ages, the bacteria content of the 90-day manure differed dramatically between the two trials. Third, hayland plots required considerably more simulated rainfall to generate runoff than did cornland plots. These and other dissimilarities may have contributed to the observed differences between the hayland and cornland E. coli runoff data.

Mean E. coli counts in plot runoff are shown in Table 5.2 for hayland and cornland plots under different sets of treatments. Note that there is some ambiguity in direct comparisons because there is not a one-to-one correspondence in plot treatments between the hayland and cornland trials. However, the treatments shown in Table 5.2 probably encompass the broad range of conditions and management of hayland and cornland in the Lake Champlain Basin.

Table 5.2. Comparison of mean *E. coli* counts in plot runoff from hayland and cornland trials

Plot Treatment	Mean[1] *E. coli* in runoff (#/100 ml)	
	Hayland	**Cornland**
All plots	63,222	24,218
90-day manure plots	4,627	1,673
30-day manure plots	32,695	17,003
0-day manure plots	1,080,389	499,356
3-day rain delay plots	45,065	17,136
1-day rain delay plots	88,693	34,228

[1] anti-log of log mean

As shown in Table 5.2, runoff from hayland plots tended to be substantially higher in E. coli than runoff from cornland plots. Comparing runoff from all plots regardless of treatment, hayland runoff contained nearly three times more E. coli on average than did cornland runoff; the actual difference is probably even greater because E. coli data for several of the hayland plots are reported as "greater than" (>) values. Bacteria levels in runoff from hayland plots were higher than in runoff from cornland plots for all three ages of manure tested. Runoff from hayland plots receiving fresh manure averaged about twice the E. coli as runoff from cornland plots that received fresh manure. It should be noted that some of these differences were probably influenced by differences in the E. coli content of the manure applied in the trials. For example, the 90-day manure applied to cornland plots contained fewer E. coli organisms than did 90-day manure applied to hayland plots, <114 E. coli/g vs. 1,260 E. coli/g, respectively. The fresh manure applied to cornland plots was also lower in E. coli than the fresh manure applied in the hayland trial.

Bacteria levels in runoff from hayland plots were higher than in cornland plot runoff for both 3-day and 1-day delay before simulated rainfall, with all other treatments combined. In both cases, E. coli levels in runoff from hayland plots were ~2.5 times those in runoff from cornland plots.

Manure applied to hayland cannot be incorporated into the soil, whereas incorporation is possible on cornland; under some circumstances, incorporation did influence E. coli levels in plot runoff. Furthermore, in the cornland runoff experiment, we observed a clear effect of soil incorporation on runoff generation. To control for this fundamental difference between hayland and cornland, we compared mean runoff E. coli between hayland plots and non-incorporated cornland plots under different conditions of manure age and delay to rainfall (Table 5.3). Plots that received 90-day manure were not compared because of the lower bacteria content of the 90-day manure applied in the cornland trial.

Table 5.3. Comparison of mean *E. coli* counts in runoff from hayland plots and cornland plots where manure was applied without incorporation

Plot Treatment	Mean[1] *E. coli* in runoff (#/100 ml)	
	Hayland	Cornland
0-d manure/1-d rain delay	1,817,294	2,342,448
0-d manure/3-d rain delay	642,052	190,327
30-d manure/1-d rain delay	39,972	41,971
30-d manure/3-d rain delay	26,742	8,993
0-d manure/1+3-d rain delay	1,080,389	667,698
30-d manure/1+3-d rain delay	32,695	19,428
0+30-d manure/1+3-d rain delay	187,945	113,894

[1] anti-log of log mean

Again, in most cases, mean E. coli levels in hayland plot runoff were substantially higher than those in cornland plot runoff. The only case where E. coli levels in cornland plot runoff were markedly higher than those of hayland plot runoff was for fresh manure applied the day before rainfall; this result is ambiguous because of the number of “>” values for runoff from hayland plots receiving fresh manure.

Finally, to make more realistic comparisons between runoff from hayland and cornland, Table 5.4 compares treatments that are analogous to current common practice in Vermont and treatments that might be considered to be Best Management Practices.

Table 5.4. Comparison of mean *E. coli* counts in plot runoff from hayland and cornland trials: Current practice vs. BMP

Plot Treatment	Mean[1] *E. coli* in runoff (#/100 ml)	
	Hayland	Cornland
~Current practice[2]	16,676	41,971
~BMP[3]	1,727	2,103

[1] anti-log of log mean [2] hayland: 30-L-1; cornland: 30-N-1

[3] hayland: 90-H-3; cornland: 90-I-3

In this analysis, current standard practice for manure application to hayland is represented by the application of 30-day old manure on low vegetation one day before rainfall (30-L-1). Common practice for manure application to cornland is represented by 30-day old manure without incorporation one day before rainfall. While actual practices no doubt vary widely, these conditions are probably the most representative among the conditions of our experiments. Note that 30-day manure for both trials contained approximately equivalent levels of E. coli.

Under conditions representing current practice, E. coli levels in cornland runoff were ~2.5 times higher than levels in hayland runoff, suggesting that at least for a single runoff event, runoff from cornland may be a more potent source of microorganisms than runoff from hayland. Under “BMP” conditions, the difference in E. coli levels in runoff from hayland and cornland was very small, suggesting that application of improved management techniques to both hayland and cornland might be effective not only in reducing microorganism runoff from both types of land but also in reducing the difference in pollution potential between the two land types.

These inferences should be viewed with a great deal of caution, as important confounding influences result from fundamental differences between the two trials. The most important of such influences is the difference in simulated rainfall and runoff generation between the two events. Even though simulated rainfall was applied at the same rate in both events, the duration – and therefore the total quantity of water applied – was greater for the hayland event than for the cornland event. Runoff generation was quite rapid on the cornland plots – the first runoff was generated after less than 10 minutes of simulated rainfall and all plots began to generate runoff within ~60 minutes. Runoff was generated more slowly on hayland plots; initial runoff was observed after 30 minutes of simulated rainfall, two plots never generated measurable runoff, and ~180 minutes of simulated rainfall were required to generate runoff from all of the remaining plots. In general, then, for a storm of short duration, less runoff would be expected from a hay field than from a cornfield, possibly offsetting the tendency for greater E. coli levels in hayland runoff that we observed. Thus, it is difficult to extrapolate differences between cornland and hayland to natural rainfall events at the field scale to evaluate overall pollution potential.

Furthermore, patterns of manure application to hayland and cornland must be considered. In Vermont, cornland generally receives a single annual manure application in the spring, although a second application in the fall after harvest is not uncommon. In contrast, hayland in Vermont receives more frequent manure applications – typically two or three annually (one after each hay cut), and potentially an additional early season application. Thus, even though the probability of runoff from hayland may be lower than from cornland, more frequent manure applications to hayland would tend to increase the risk of microorganism losses over the course of a year.

Thus, it is quite difficult to compare the overall pollution potential of hayland vs. cornland from our limited plot studies. Such an assessment would best be done through field-scale monitoring over a year or more.

6. CONCLUSIONS

The state of the art in agricultural management practices aimed at reducing microorganism losses is not as advanced as for sediment or nutrient losses. Few Best Management Practices (BMPs) have been developed, tested, or applied that are aimed specifically at reduction of microorganisms.

Reduction of microorganism populations and losses through improved farm management presents an additional challenge due to the sheer magnitude of microorganisms available for loss at waste application sites, compared to acceptable levels in receiving waters. Reduction rates for *E. coli* bacteria in runoff that would be impressive for phosphorus or sediment would generally be inadequate to comply with water quality criteria for bacteria. Even after a 99% reduction in *E. coli* levels—from 10^6 organisms/100 ml to 10^4 organisms/100 ml for example—runoff would still represent a significant threat to receiving water quality, where the recreational water quality standard is two orders of magnitude lower still. For this reason, a multiple barrier approach wherein different measures are applied at different points on the farm, offers the best chance for reducing microorganism export from agricultural land to an acceptable level.

This section combines evidence from the scientific literature with results from our field experiments to derive a recommended multiple-barrier approach for application in the Lake Champlain Basin.

6.1. Approaches to Bacteria Reduction Described in the Literature

Prior to initiating the field experiments, we evaluated information from published scientific literature on environmental, seasonal, and agricultural conditions and practices that influence the survival, transport, and export of microorganisms from agricultural land representative of conditions on dairy farms in the LCB. The purpose of this review was two-fold. First, it provided support and context for the specific treatment conditions tested in the field studies. Second, the review provides documentation on pathogen reduction measures evaluated elsewhere that may become part of a multiple barrier approach for controlling pathogens in runoff from agricultural land in the Lake Champlain Basin. This literature review has been published separately (Meals and Braun 2004) and is summarized in Appendix 8.4 of this report.

This section presents measures identified in the literature that may be useful in the Lake Champlain Basin to reduce bacteria populations within agricultural systems and/or export from agricultural land, and also notes measures found to be relatively ineffective. The reader is encouraged to refer to the full literature review for a complete discussion of these measures. The measures identified below are specifically focused on issues of microorganism reduction. The effects of such measures on other pollutants should be considered when proposing any of these measures in a specific case. Applying manure in the fall, for example, may help reduce bacteria survival, but may promote nitrogen loss and thus conflict with good nutrient management practice. Such trade-offs must be evaluated in any farm management plan.

Effective measures documented in the literature include:

- Animal treatment/biosecurity practices such as good veterinary practices and careful control of animal purchase, food and manure handling, and sanitation can reduce the incidence of true pathogens in animal waste (HACCP Alliance 2003);
- Barnyard runoff management practices that include paving, regular scraping, and diversion of clean water from upgradient can virtually eliminate the barnyard as a discrete source of bacteria (Moore *et al.* 1983, Cassell and Meals 2002);
- Long-term storage of livestock wastes prior to land application reduces bacteria counts in manure and in runoff; although continual inoculation from fresh manure added approximately daily from the barn, barnyard, and other animal holding areas may reduce the effectiveness of storage as a means of microorganism control (Moore *et al.* 1983, Walker *et al.* 1990, Trevisan and Dorioz 1999, Jamieson *et al.* 2002);
- Avoidance of winter manure application throughout the Lake Champlain Basin should be considered as a BMP for reducing the runoff of indicator organisms. Manure application on frozen or snow-covered ground can significantly increase microorganism losses in runoff from agricultural land compared to applications in other seasons (*e.g.*, Thompson *et al.* 1979, Reddy *et al.* 1981, Clausen 1990 and 1991, Fayer and Nerad 1996, Melvin and Lorimor 1996);
- Several researchers have recommended that manure not be applied to soils that are wet or when tile drains are flowing due to the high risk of bacteria transmission in leachate (Joy *et al.* 1998, Abu-Ashour *et al.* 1998). Microorganisms can leach downward rapidly through soil macropores and this leachate can be delivered quickly to surface waters if intercepted by artificial drainage; and
- Exclusion of animals from streams, either through fencing or simply by providing alternative drinking water sources, can significantly reduce indicator organism counts in streams draining grazing areas by preventing the direct deposition of manure into surface waters (Larsen *et al.* 1994, Sheffield *et al.* 1997, Meals 2000).

Measures not shown to be reliably effective include:

- Manure composting has been suggested as a means of reducing levels of microorganisms in manure, but results reported in the literature are inconsistent (Mote *et al.* 1988, Kudva *et al.* 1998, Larney *et al.* 2003). Unless carefully managed to achieve temperatures sufficiently high to kill microorganisms, composting should not be considered a reliable approach to reduce microorganism levels in animal waste;
- While excessive rates of manure application should clearly be avoided, there seems to be little definitive evidence in the literature to recommend control of waste application rate as a specific pathogen-reduction BMP beyond what would normally be specified in a good nutrient management plan (Jamieson *et al.* 2002). Similarly, the choice of solid or liquid manure or application method does not seem to offer a particular advantage or disadvantage for control of microorganism losses (Moore *et al.*1988); and

- Reported performance of buffers or filter strips in bacteria removal has been contradictory (*e.g.*, Young *et al.* 1980, Dickey and Vanderholm 1981, Srivastava *et al.* 1996, Lim *et al.* 1998, Entry *et al.* 2000a and 2000b). Although modest bacteria reductions can be achieved with buffer strips, the consensus of the recent literature suggests that grass filters or buffers alone will be insufficient to reduce bacterial concentrations in runoff from manured areas to meet water quality goals (Walker *et al.* 1990, Coyne and Blevins 1995, Entry *et al.* 2000a and 2000b).

There are several additional practices that appear to have significant potential as components of a multiple barrier system in the Lake Champlain Basin, but that need further investigation. These include:

- Manure or animal treatment to reduce microorganism content, *e.g.*, alkali/carbonate (Diez-Gonzalez *et al.* 2000), lime (NLA 2001, Hogan *et al.*1999), chlorate (Callaway *et al.* 2002), or careful composting (Kudva *et al.* 1998, Lung *et al.* 2001, Larney et al. 2003);
- Favoring fall manure application over spring application to enhance bacteria die-off (Stoddard *et al.* 1998, Warnemuende and Kanwar 2002);
- Use of light tillage prior to manure application to break up soil macropores (Abu-Ashour *et al.* 1998) and
- Specialized buffers, such as a vegetated filter strips for agricultural point sources or application of the synthetic polymer coagulant polyacrylamide (PAM) (Entry and Sojka 2000) to buffer strips, to reduce microorganisms in runoff to critical resources.

6.2. Approaches to Bacteria Reduction Identified in Field Trials

The results of our field experiments support the practice of several bacteria control measures suggested in the literature. While our results were consistent with effects reported in the literature, it should be emphasized that results from experimental plots should be applied to the design of recommended farm management practices with some caution. On the one hand, because the key processes affecting bacteria export evaluated in our plot studies—die-off in storage, soil and vegetation interaction, die-off in the field between application and rainfall—are not likely to be strongly scale-dependent, our results should be applicable to the field scale. However, we recognize that microorganism losses as a function of farm-scale management practices cannot be precisely quantified from plot-scale studies because of scale effects on overland flow and runoff. 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 are more useful as indicators of relative comparisons, rather than absolute values.

In this context, management recommendations based on our field experiments are:

- Manure storage is an important factor in reducing the bacteria content of manure to be applied to agricultural land. Reductions of >99% in the *E. coli* content of manure were documented in pilot storage experiments. Storage of manure for 30 days or more consistently and dramatically lowered *E. coli* counts in our experiments, with longer storage providing greater reductions. These results are consistent with data reported from actual storage facilities. We believe that storage for a specific duration that avoids frequent additions of fresh manure would enhance the bacteria reductions achieved by storage. 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.

- Increased manure storage time reduced the *E. coli* content of manure at application and resulted in comparably reduced levels of *E. coli* in runoff from the land where the manure was applied. In both the hayland and cornland experiments, manure age was the most significant factor influencing *E. coli* counts in runoff. Runoff from land receiving stored manure contained significantly fewer *E. coli* organisms as the manure age increased. 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 days 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. This result is widely confirmed in the literature. We can therefore recommend reducing or restricting manure applications within ~3 days of significant rainfall as a measure to reduce microorgansm losses from land receiving manure. 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 predicted rain events. There is some precedent elsewhere in the U.S. for conditioning manure application on weather (Woiwode, 2001).
- On hayland, vegetation height alone did not have a significant effect on *E. coli* losses in runoff, but interaction effects with manure age and delay between manure application and runoff suggest that maintaining higher vegetation (~14 cm in our experiments) on hayland at manure application may be beneficial in some circumstances. We can therefore propose that hayland vegetation reach a height of ~14 cm before manure application. 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 of manure into the soil alone was not a significant factor in *E. coli* levels in runoff. However, because incorporation appeared to delay the generation of runoff from plots and because *E. coli* levels in runoff were significantly reduced when manure applied the day before runoff was incorporated, we can recommend prompt incorporation of applied manure as a practice that will assist in reducing the loss of microorganisms in runoff from cornland under many circumstances.

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 BMP to be applied at the farm or watershed level. We would therefore foresee additional evaluation at the farm scale before positive results from this study were codified as a BMP (see Section 7).

6.3. Recommended Multiple Barrier Approach

Combining the results of our plot studies and the documentation of other bacteria control measures in the literature, we recommend the following set of practices as a multiple barrier approach to reduce indicator bacteria levels in agricultural runoff:

- **Source control**
    - Implement animal treatment/biosecurity practices to reduce the incidence of true pathogens in animal waste;
    - Scrape barnyards regularly, divert runoff flow from upgradient areas away from the barnyard, and control barnyard runoff to direct accumulated manure and runoff into a waste storage facility to eliminate the barnyard as a discrete source; and
    - Store manure for a definitive period of ~90 days without addition of fresh manure.

- **Availability/Runoff control**
    - Prohibit manure application to frozen or snow-covered ground;
    - Prohibit manure application during heavy rainfall, when soil is saturated, or when tile lines are flowing;
    - To the extent feasible, avoid manure application less than 3 days before major storms, storm fronts, or other predicted rainfall events;
    - Apply manure at a maximum rate determined by an approved nutrient management plan based on crop need for nutrients;
    - On cornland, incorporate applied manure by tillage within 24 to 72 hours of application;
    - On hayland, allow vegetation to reach a height of ~14 cm before manure application; and
    - Implement a whole-farm runoff/erosion control plan to promote infiltration and control runoff, reduce movement of bacteria associated with soil particles or manure aggregates, and avoid excessive manure application to sensitive areas or runoff contributing areas (Gilley *et al.* 2002). Nutrient management may also help reduce losses of indicator organisms.

- **Delivery control**
    - On pasture land, use fencing or other means to eliminate livestock access to streams and other watercourses;
    - Where possible, use light tillage before or after manure application to disrupt macropores; and
    - Although buffers should not be relied upon as a bacteria reduction practice via filtration effects, buffers can be used to ensure that manure applications are set back from watercourses, thereby avoiding accidental direct application of waste to the water.

There are several additional practices that appear to have significant potential as components of a multiple barrier system in the Lake Champlain Basin, but that need further investigation. These include:

- Manure or animal treatment to reduce microorganism content, *e.g.*, lime, chlorate, or composting;
- Favoring fall manure application over spring application to enhance bacteria die-off; and
- Specialized buffers, such as a vegetated filter strips for agricultural point sources or application of PAM to buffer strips, to reduce microorganisms in runoff to critical resources.

7. RECOMMENDATIONS FOR FUTURE WORK

Our pilot-scale experiments demonstrated the potential for several specific practices to reduce runoff losses of indicator organisms from land receiving manure. There remain, however, many questions that must be answered before these practices can be fully incorporated into the farm management toolbox. For example, this study tested runoff from a single manure application in a single rainfall event. It is known that additional phosphorus can be washed out of a single manure application by successive rainfall events (Kleinman and Sharpley 2003); how does *E. coli* behave in such circumstances? Would rates of *E. coli* loss from successive storms differ between hayland runoff where manure remains on the surface and cornland where manure is incorporated? What is the comparative bacterial pollution potential between cornland and hayland in the Lake Champlain Basin under current or future conditions? What are the prevalence of true pathogens in animal waste and the water resources of the Lake Champlain Basin?

Such questions cannot be adequately answered with kiddie pools, simulated rainfall, 4.5 m^2 plots, and analysis of *E. coli* alone. The next step in confirming and refining methods to control microorganism losses must be done at a field or farm scale, and over at least a full seasonal cycle. Specifically, we recommend the following steps for future work:

1. Document bacterial dynamics in real-world manure storage facilities over at least one complete annual cycle. This effort should focus on both current practice and the kind of definitive storage suggested by this study. Such a study could also include evaluation of the incidence of common pathogenic microorganisms, such as *E. coli* O157:H7, *Salmonella, Campylobacter*, *Cryptosporidium,* and *Giardia*, in manure in the Lake Champlain Basin.

2. Evaluate proposed practices on field-scale watersheds (*e.g.*, 1 – 10 ha) with natural weather for at least two full seasonal cycles. In addition to scale issues, such a study could address the dynamics of successive storms and successive manure applications, as well as differences in seasons of application. Such a study would be best accomplished by a paired-watershed design, wherein a pre-treatment calibration period would yield useful data on microorganism losses from successive storms and manure applications, as well as comparisons between hayland and cornland losses, while data from a post-treatment period would quantify the effectiveness of proposed practices at the field scale. Automated flow monitoring and sampling of runoff from paired field-scale watersheds would be needed to allow computation of input/output mass balances of water and bacteria.

3. Evaluate some promising innovative treatments/practices, including:
   - Animal treatment with chlorate to reduce excreted *E. coli* and pathogens
   - Manure treatment to reduce *E. coli* content, including composting and chemical addition
   - Specialized buffer practices, such as PAM treatment

4. Evaluate the impact of different waste types and treatments on *E. coli* losses, including

- Liquid vs. solid manure
- Digested manure
- Solids removal
- Composted manure

8. APPENDICES

8.1. Photographs of Runoff Trials

Photo 1: Pilot manure storage for hayland trial

Photo 2: Rainfall simulator on hayland site

Photo 3: Sampling set-up on hayland plot

Photo 4: Manure applied to low-vegetation hayland plot

Photo 5: Manure applied to high-vegetation hayland plot

Photo 6: Hayland runoff trial, June 24, 2003

Photo 7: Runoff from hayland plot

Photo 8: Cornland runoff trial site, Williamstown, VT

Photo 9: Creating diversion ditches to isolate plots

Photo 10: Spreading manure on cornland plot

Photo 11: Incorporating manure on cornland plot

Photo 12: Surface of non-incorporated plot (left) and incorporated plot (right)

Photo 13: Sampling set-up on cornland plot

Photo 14: Cornland runoff trial, October 14, 2003

Photo 15: Simulated rainfall and runoff from cornland plot

8.2. On-Site Weather Data for Runoff Trials

Table 8.1. Mean hourly weather data during hayland trial (precipitation data are hourly totals)

Date	Time	Air Temp. (°C)	Relative Humidity (%)	Barometric Pressure (kPa)	Wind Speed (km/hr)	Wind Direction	Total Precip. (mm)
21-Jun-03	00:00 - 01:00	13.9	87	100.98	0.0	---	0
21-Jun-03	01:00 - 02:00	13.0	89	100.99	0.0	---	0
21-Jun-03	02:00 - 03:00	12.4	92	100.99	0.0	---	0
21-Jun-03	03:00 - 04:00	11.9	93	101.01	0.0	---	0
21-Jun-03	04:00 - 05:00	11.6	94	101.01	0.0	---	0
21-Jun-03	05:00 - 06:00	11.2	94	101.04	0.0	---	0
21-Jun-03	06:00 - 07:00	11.7	93	101.06	0.0	---	0
21-Jun-03	07:00 - 08:00	15.3	85	100.98	0.8	NNW	0
21-Jun-03	08:00 - 09:00	18.9	72	100.93	1.2	E	0
21-Jun-03	09:00 - 10:00	21.6	60	100.92	2.0	ESE/SW	0
21-Jun-03	10:00 - 11:00	23.4	55	100.85	2.8	ENE	0
21-Jun-03	11:00 - 12:00	24.8	52	100.80	3.2	ENE	0
21-Jun-03	12:00 - 13:00	26.1	48	100.73	4.0	variable	0
21-Jun-03	13:00 - 14:00	26.5	46	100.67	3.2	variable	0
21-Jun-03	14:00 - 15:00	26.5	45	100.59	4.0	NNE	0
21-Jun-03	15:00 - 16:00	27.0	42	100.53	4.0	NE/N	0
21-Jun-03	16:00 - 17:00	26.9	45	100.47	2.0	N	0
21-Jun-03	17:00 - 18:00	27.1	45	100.41	0.8	WNW	0
21-Jun-03	18:00 - 19:00	25.5	58	100.40	1.2	NW/W	0
21-Jun-03	19:00 - 20:00	23.6	64	100.42	1.2	S/SW	0
21-Jun-03	20:00 - 21:00	21.8	68	100.45	2.0	SE	0
21-Jun-03	21:00 - 22:00	20.1	72	100.50	2.4	SE	0
21-Jun-03	22:00 - 23:00	19.0	78	100.51	0.0	---	0
21-Jun-03	23:00 - 24:00	18.1	82	100.47	0.4	S	0
22-Jun-03	00:00 - 01:00	17.1	91	100.49	0.8	S	0.254
22-Jun-03	01:00 - 02:00	16.7	95	100.46	1.2	variable	0.254
22-Jun-03	02:00 - 03:00	16.5	93	100.35	1.2	NW/WNW	0
22-Jun-03	03:00 - 04:00	16.4	94	100.30	0.8	SW/SSE	0
22-Jun-03	04:00 - 05:00	16.2	95	100.29	0.4	SSE	0
22-Jun-03	05:00 - 06:00	16.0	95	100.35	0.0	---	0
22-Jun-03	06:00 - 07:00	16.1	95	100.38	0.0	---	0
22-Jun-03	07:00 - 08:00	16.4	95	100.38	0.0	---	0
22-Jun-03	08:00 - 09:00	16.7	95	100.44	0.0	---	0.254
22-Jun-03	09:00 - 10:00	17.3	92	100.39	2.4	WNW/NW	0
22-Jun-03	10:00 - 11:00	18.1	90	100.40	0.4	W	0
22-Jun-03	11:00 - 12:00	19.4	88	100.36	0.8	variable	0
22-Jun-03	12:00 - 13:00	21.7	79	100.32	2.4	variable	0
22-Jun-03	13:00 - 14:00	24.0	68	100.28	2.4	variable	0
22-Jun-03	14:00 - 15:00	24.7	60	100.25	4.4	NE/N	0.254
22-Jun-03	15:00 - 16:00	25.7	52	100.18	3.2	N/NNE	0
22-Jun-03	16:00 - 17:00	25.5	50	100.16	6.8	NNW/N	0
22-Jun-03	17:00 - 18:00	25.4	51	100.11	4.8	NW/N	0
22-Jun-03	18:00 - 19:00	24.6	61	100.08	1.6	NNE/N	0
22-Jun-03	19:00 - 20:00	24.7	61	100.08	5.6	N/NW	0
22-Jun-03	20:00 - 21:00	23.0	68	100.10	1.6	N	0
22-Jun-03	21:00 - 22:00	19.5	84	100.20	0.4	WNW	0

Date	Time	Air Temp. (^oC)	Relative Humidity (%)	Barometric Pressure (kPa)	Wind Speed (km/hr)	Wind Direction	Total Precip. (mm)
22-Jun-03	22:00 - 23:00	17.4	89	100.24	0.0	---	0
22-Jun-03	23:00 - 24:00	16.3	93	100.23	0.0	---	0
23-Jun-03	00:00 - 01:00	16.6	93	100.21	0.4	SW	0
23-Jun-03	01:00 - 02:00	16.5	93	100.19	0.0	---	0
23-Jun-03	02:00 - 03:00	15.6	96	100.17	0.0	---	0
23-Jun-03	03:00 - 04:00	15.0	95	100.14	0.0	---	0
23-Jun-03	04:00 - 05:00	14.7	95	100.14	0.0	---	0
23-Jun-03	05:00 - 06:00	14.1	96	100.18	0.0	---	0
23-Jun-03	06:00 - 07:00	14.9	96	100.21	0.0	---	0
23-Jun-03	07:00 - 08:00	16.0	94	100.26	1.2	S	0
23-Jun-03	08:00 - 09:00	18.2	90	100.29	0.8	SE/ESE	0
23-Jun-03	09:00 - 10:00	20.0	87	100.35	0.8	ESE/ENE	0
23-Jun-03	10:00 - 11:00	24.5	68	100.32	1.6	variable	0
23-Jun-03	11:00 - 12:00	26.7	62	100.29	2.4	variable	0
23-Jun-03	12:00 - 13:00	28.4	49	100.25	5.6	variable	0
23-Jun-03	13:00 - 14:00	29.8	43	100.24	8.8	N/NW	0
23-Jun-03	14:00 - 15:00	30.5	43	100.24	10.9	NW/N	0
23-Jun-03	15:00 - 16:00	30.5	46	100.25	10.5	NW/N	0
23-Jun-03	16:00 - 17:00	30.9	40	100.26	9.3	N/NNE	0
23-Jun-03	17:00 - 18:00	30.9	42	100.27	8.8	N/NW	0
23-Jun-03	18:00 - 19:00	29.6	48	100.32	8.4	NW/NNW	0
23-Jun-03	19:00 - 20:00	28.2	51	100.41	6.8	NW	0
23-Jun-03	20:00 - 21:00	26.3	59	100.47	4.0	NW	0
23-Jun-03	21:00 - 22:00	22.9	72	100.52	0.0	---	0
23-Jun-03	22:00 - 23:00	20.9	81	100.60	0.0	---	0
23-Jun-03	23:00 - 24:00	19.5	86	100.64	0.0	---	0
24-Jun-03	00:00 - 01:00	18.8	91	100.67	0.0	---	0
24-Jun-03	01:00 - 02:00	18.5	93	100.67	0.0	---	0
24-Jun-03	02:00 - 03:00	17.7	95	100.68	0.0	---	0
24-Jun-03	03:00 - 04:00	17.1	94	100.68	0.0	---	0
24-Jun-03	04:00 - 05:00	16.4	95	100.70	0.0	---	0
24-Jun-03	05:00 - 06:00	16.2	95	100.77	0.0	---	0
24-Jun-03	06:00 - 07:00	16.9	95	100.80	0.0	---	0
24-Jun-03	07:00 - 08:00	19.9	89	100.80	0.4	NW	0
24-Jun-03	08:00 - 09:00	22.1	83	100.82	2.0	NNE/ENE	0
24-Jun-03	09:00 - 10:00	25.7	67	100.83	1.2	ENE	0
24-Jun-03	10:00 - 11:00	28.3	60	100.84	2.0	ENE	0
24-Jun-03	11:00 - 12:00	30.0	53	100.86	3.2	variable	0
24-Jun-03	12:00 - 13:00	31.8	44	100.83	3.6	variable	0
24-Jun-03	13:00 - 14:00	32.7	34	100.82	9.7	WNW/NW	0
24-Jun-03	14:00 - 15:00	33.2	29	100.79	8.0	W/NW	0
24-Jun-03	15:00 - 16:00	33.0	32	100.78	10.1	NW	0
24-Jun-03	16:00 - 17:00	33.0	27	100.78	8.0	NW	0
24-Jun-03	17:00 - 18:00	32.9	29	100.75	7.2	NW	0
24-Jun-03	18:00 - 19:00	32.2	35	100.73	4.4	NW	0
24-Jun-03	19:00 - 20:00	29.9	46	100.76	1.2	WNW/W	0
24-Jun-03	20:00 - 21:00	26.6	56	100.79	1.2	WSW	0
24-Jun-03	21:00 - 22:00	24.2	65	100.83	1.2	NW	0
24-Jun-03	22:00 - 23:00	21.8	70	100.89	0.4	W	0
24-Jun-03	23:00 - 24:00	19.9	76	100.91	0.0	---	0

Shaded area indicates period of hayland runoff event

Table 8.2. Mean hourly weather data during cornland trial (precipitation data are hourly totals)

Date	Time	Air Temp. (^oC)	Relative Humidity (%)	Barometric Pressure (kPa)	Wind Speed (km/hr)	Wind Direction	Total Precip. (mm)
11-Oct-03	00:00 - 01:00	13.8	86	101.56	5.2	SW	0.00
11-Oct-03	01:00 - 02:00	12.7	88	101.58	3.2	SSE	0.00
11-Oct-03	02:00 - 03:00	11.7	92	101.59	2.8	SSE	0.00
11-Oct-03	03:00 - 04:00	11.2	94	101.57	2.4	SSE	0.00
11-Oct-03	04:00 - 05:00	10.6	94	101.59	0.4	SSE	0.00
11-Oct-03	05:00 - 06:00	9.2	96	101.63	0.8	S	0.00
11-Oct-03	06:00 - 07:00	9.4	97	101.67	1.2	S	0.00
11-Oct-03	07:00 - 08:00	9.2	97	101.71	0.4	SSE	0.00
11-Oct-03	08:00 - 09:00	10.0	97	101.73	0.0	---	0.00
11-Oct-03	09:00 - 10:00	10.8	94	101.74	0.8	SSE	0.00
11-Oct-03	10:00 - 11:00	13.6	90	101.69	0.4	ESE	0.00
11-Oct-03	11:00 - 12:00	18.3	76	101.61	0.8	N	0.00
11-Oct-03	12:00 - 13:00	21.2	59	101.54	4.4	WSW/W	0.00
11-Oct-03	13:00 - 14:00	22.6	57	101.43	6.4	W/SW	0.00
11-Oct-03	14:00 - 15:00	23.8	53	101.33	4.8	WSW/W	0.00
11-Oct-03	15:00 - 16:00	23.7	51	101.25	4.4	WNW/WSW	0.00
11-Oct-03	16:00 - 17:00	23.2	51	101.20	5.2	WSW/SW	0.00
11-Oct-03	17:00 - 18:00	22.1	54	101.22	4.8	SW/S	0.00
11-Oct-03	18:00 - 19:00	18.0	70	101.26	8.0	SSE	0.00
11-Oct-03	19:00 - 20:00	16.5	71	101.27	9.3	SSE	0.00
11-Oct-03	20:00 - 21:00	15.7	73	101.25	6.8	SSE	0.00
11-Oct-03	21:00 - 22:00	14.2	80	101.25	1.2	SSE	0.00
11-Oct-03	22:00 - 23:00	13.2	84	101.20	2.4	SW/SE	0.00
11-Oct-03	23:00 - 24:00	12.9	85	101.15	4.8	SSE	0.00
12-Oct-03	00:00 - 01:00	12.4	88	101.10	2.0	SSW	0.00
12-Oct-03	01:00 - 02:00	11.4	91	101.05	2.4	S	0.00
12-Oct-03	02:00 - 03:00	10.7	93	100.97	2.0	SSE	0.00
12-Oct-03	03:00 - 04:00	10.0	95	100.90	0.8	SSE	0.00
12-Oct-03	04:00 - 05:00	9.4	96	100.85	2.8	SSE	0.00
12-Oct-03	05:00 - 06:00	8.7	96	100.85	2.0	SSE	0.00
12-Oct-03	06:00 - 07:00	8.3	97	100.83	2.4	SSE/SE	0.25
12-Oct-03	07:00 - 08:00	7.9	96	100.85	2.0	SSE/SSW	0.00
12-Oct-03	08:00 - 09:00	8.3	96	100.83	0.4	S	0.00
12-Oct-03	09:00 - 10:00	10.3	94	100.75	0.4	NW	0.00
12-Oct-03	10:00 - 11:00	13.7	85	100.64	5.6	WSW	0.00
12-Oct-03	11:00 - 12:00	17.2	70	100.53	8.8	WSW/SW	0.00
12-Oct-03	12:00 - 13:00	18.8	61	100.41	12.1	WSW/SW	0.00
12-Oct-03	13:00 - 14:00	19.0	60	100.32	12.5	SW/S	0.00
12-Oct-03	14:00 - 15:00	15.8	82	100.27	11.7	SW/WSW	0.00
12-Oct-03	15:00 - 16:00	14.7	89	100.23	6.0	WSW/SSW	0.00
12-Oct-03	16:00 - 17:00	15.5	81	100.16	5.2	SW/WSW	0.25
12-Oct-03	17:00 - 18:00	14.4	87	100.16	2.0	SW	0.00
12-Oct-03	18:00 - 19:00	14.2	86	100.13	3.6	SW/S	0.00
12-Oct-03	19:00 - 20:00	13.6	87	100.12	4.4	SSW/SSE	0.00
12-Oct-03	20:00 - 21:00	13.0	86	100.08	2.4	SE	0.00
12-Oct-03	21:00 - 22:00	11.1	93	100.06	3.2	SE	0.00
12-Oct-03	22:00 - 23:00	10.8	95	100.04	4.0	SE	0.00
12-Oct-03	23:00 - 24:00	10.4	96	100.05	1.6	SE	0.00
13-Oct-03	00:00 - 01:00	11.3	96	100.11	9.7	N	0.00

Date	Time	Air Temp. (^oC)	Relative Humidity (%)	Barometric Pressure (kPa)	Wind Speed (km/hr)	Wind Direction	Total Precip. (mm)
13-Oct-03	01:00 - 02:00	11.8	89	100.17	11.7	N	0.00
13-Oct-03	02:00 - 03:00	10.6	84	100.21	3.6	NNE/N	0.00
13-Oct-03	03:00 - 04:00	9.9	82	100.20	8.0	NW/N	0.00
13-Oct-03	04:00 - 05:00	8.8	85	100.27	5.6	N/NNW	0.00
13-Oct-03	05:00 - 06:00	8.7	85	100.32	4.0	N	0.00
13-Oct-03	06:00 - 07:00	8.0	88	100.37	4.0	NNE	0.00
13-Oct-03	07:00 - 08:00	7.7	90	100.40	3.2	ENE/NE	0.00
13-Oct-03	08:00 - 09:00	9.4	84	100.44	1.2	NE/NNE	0.00
13-Oct-03	09:00 - 10:00	10.8	78	100.45	9.3	N/NNW	0.00
13-Oct-03	10:00 - 11:00	12.0	66	100.47	16.1	NNW/N	0.00
13-Oct-03	11:00 - 12:00	12.5	59	100.43	22.5	N/NNW	0.00
13-Oct-03	12:00 - 13:00	13.3	58	100.41	22.1	N	0.25
13-Oct-03	13:00 - 14:00	14.2	53	100.34	19.3	N/NNW	0.00
13-Oct-03	14:00 - 15:00	14.9	52	100.30	18.9	N/NNW	0.00
13-Oct-03	15:00 - 16:00	15.4	50	100.29	16.5	N	0.00
13-Oct-03	16:00 - 17:00	15.3	48	100.29	16.9	N	0.00
13-Oct-03	17:00 - 18:00	14.4	53	100.33	10.5	N/NNW	0.00
13-Oct-03	18:00 - 19:00	12.5	58	100.43	4.8	NNW	0.00
13-Oct-03	19:00 - 20:00	10.8	66	100.53	1.2	N	0.00
13-Oct-03	20:00 - 21:00	9.3	72	100.59	2.8	E/ENE	0.00
13-Oct-03	21:00 - 22:00	8.1	76	100.64	6.4	E/SSE	0.00
13-Oct-03	22:00 - 23:00	7.6	77	100.65	8.0	SSE	0.00
13-Oct-03	23:00 - 24:00	6.9	82	100.67	6.0	SSE	0.00
14-Oct-03	00:00 - 01:00	7.0	80	100.69	5.2	SSE	0.00
14-Oct-03	01:00 - 02:00	6.1	84	100.69	3.6	SSE/S	0.00
14-Oct-03	02:00 - 03:00	5.3	88	100.72	4.8	SSE	0.00
14-Oct-03	03:00 - 04:00	5.3	87	100.72	5.2	SSW/SSE	0.00
14-Oct-03	04:00 - 05:00	5.2	88	100.71	3.6	SSE	0.00
14-Oct-03	05:00 - 06:00	4.1	91	100.75	1.2	SSE	0.00
14-Oct-03	06:00 - 07:00	3.4	94	100.80	1.2	SSE	0.00
14-Oct-03	07:00 - 08:00	3.4	95	100.81	1.6	SSE	0.00
14-Oct-03	08:00 - 09:00	5.5	90	100.78	2.8	SSE/SSW	0.00
14-Oct-03	09:00 - 10:00	7.6	84	100.73	5.2	SW/WSW	0.00
14-Oct-03	10:00 - 11:00	11.5	70	100.64	6.4	SW/S	0.00
14-Oct-03	11:00 - 12:00	14.3	55	100.51	14.5	SSW/S	0.00
14-Oct-03	12:00 - 13:00	15.1	51	100.42	18.1	S/SSW	0.00
14-Oct-03	13:00 - 14:00	14.4	56	100.37	16.9	S	0.00
14-Oct-03	14:00 - 15:00	15.2	54	100.20	16.9	S/SSE	0.00
14-Oct-03	15:00 - 16:00	14.4	56	100.10	14.9	S	0.00
14-Oct-03	16:00 - 17:00	13.8	57	100.05	14.1	S	0.00
14-Oct-03	17:00 - 18:00	13.3	59	99.98	12.5	S	0.00
14-Oct-03	18:00 - 19:00	12.6	62	99.89	17.3	S/SSE	0.00
14-Oct-03	19:00 - 20:00	11.7	65	99.78	19.7	S	0.00
14-Oct-03	20:00 - 21:00	11.0	70	99.71	17.7	S	0.00
14-Oct-03	21:00 - 22:00	10.4	77	99.61	14.5	S	0.00
14-Oct-03	22:00 - 23:00	10.1	81	99.46	12.1	SSE/S	0.00
14-Oct-03	23:00 - 24:00	10.2	80	99.32	14.9	SSE/S	0.00

Shaded area indicates period of cornland runoff event

8.3. Analytical Data

Table 8.3. Raw *E. coli* data for hayland trial samples

Date Collected	Field Sample #	DEC I.D.	Sample Type	Plot #	Treatment	E. coli (#/100 ml)	Comment
Manure Storage							
31-Mar-03	1	69563	manure	---	90_start_1	243,000	
31-Mar-03	2	69564	manure	---	90_start_2	378,000	
31-Mar-03	3	69565	manure	---	90_start_2d[1]	275,000	
31-Mar-03	4	69566	manure	---	90_start_3	369,000	
21-May-03	6	70737	manure	---	30_start_1	816,000	
21-May-03	7	70738	manure	---	30_start_2	457,000	
21-May-03	8	70739	manure	---	30_start_2d	1,050,000	
21-May-03	9	70740	manure	---	30_start_3	687,000	
23-Jun-03	11	71635	manure	---	90_end_1	1,000	
23-Jun-03	12	71636	manure	---	90_end_2	2,000	
23-Jun-03	13	71637	manure	---	90_end_3	<1,000	
23-Jun-03	14	71632	manure	---	30_end_1	9,800	
23-Jun-03	15	71633	manure	---	30_end_2	8,600	
23-Jun-03	16	71634	manure	---	30_end_3	6,300	
23-Jun-03	17	71628	manure	---	0_end_1	517,000	
23-Jun-03	18	71629	manure	---	0_end_2	435,000	
23-Jun-03	19	71630	manure	---	0_end_3	308,000	
23-Jun-03	20	71631	manure	---	0_end_3d	461,000	
Plot Runoff							
24-Jun-03	25	71653	runoff	1	H-0-H-3-3	1,410,000	
24-Jun-03	26	71654	runoff	2	H-30-H-1-3	77,100	
24-Jun-03	27	-9[2]	runoff	3	H-90-L-3-2	N.S.	No runoff collected
24-Jun-03	28	71655	runoff	4	H-30-L-1-1	34,100	
24-Jun-03	29	71656	runoff	5	H-90-H-1-3	4,810	
24-Jun-03	30	71657	runoff	6	H-C-C-C-2	185	
24-Jun-03	31	71658	runoff	7	H-0-H-1-2	>2,420,000	
24-Jun-03	32	71659	runoff	8	H-30-H-1-1	199,000	
24-Jun-03	33	71660	runoff	9	H-90-L-3-3	20,600	
24-Jun-03	34	71661	runoff	10	H-0-H-1-1	>2,420,000	
24-Jun-03	35	71662	runoff	11	H-30-L-3-1	70,200	
24-Jun-03	36	71663	runoff	12	H-0-H-1-3	>2,420,000	
24-Jun-03	37	71664	runoff	13	H-0-L-3-2	1,200,000	
24-Jun-03	38	71665	runoff	14	H-30-H-3-1	39,700	
24-Jun-03	39	71666	runoff	15	H-90-H-3-1	6,440	
24-Jun-03	40	71667	runoff	16	H-30-L-3-3	12,200	
24-Jun-03	67	71693	runoff	16d		14,000	
24-Jun-03	41	71668	runoff	17	H-C-C-C-1	>24,200	Contaminated by flow from diversion ditch
24-Jun-03	42	71669	runoff	18	H-30-H-3-2	8,600	
24-Jun-03	68	71694	runoff	18d		10,200	
24-Jun-03	43	71670	runoff	19	H-0-H-3-1	397,000	
24-Jun-03	66	71692	runoff	19d		242,000	
24-Jun-03	44	71671	runoff	20	H-30-L-3-2	38,900	

Date Collected	Field Sample #	DEC I.D.	Sample Type	Plot #	Treatment	E. coli (#/100 ml)	Comment
24-Jun-03	45	71672	runoff	21	H-30-H-3-3	27,400	
24-Jun-03	46	71673	runoff	22	H-90-L-3-1	4,800	
24-Jun-03	47	74674	runoff	23	H-0-L-3-3	>2,420,000	
24-Jun-03	48	71675	runoff	24	H-90-H-1-1	4,800	
24-Jun-03	49	71676	runoff	25	H-30-L-1-2	16,000	
24-Jun-03	50	-9	runoff	26	H-90-H-1-2	N.S.	No runoff collected
24-Jun-03	51	71677	runoff	27	H-0-L-1-2	>2,420,000	
24-Jun-03	52	71678	runoff	28	H-30-H-1-2	57,300	
24-Jun-03	53	71679	runoff	29	H-90-L-1-1	1,890	
24-Jun-03	54	71680	runoff	30	H-0-H-3-2	118,000	
24-Jun-03	55	71681	runoff	31	H-30-L-1-3	8,500	
24-Jun-03	56	71682	runoff	32	H-90-L-1-2	18,500	
24-Jun-03	65	71691	runoff	32d		16,700	
24-Jun-03	57	71683	runoff	33	H-90-L-1-3	11,500	
24-Jun-03	58	71684	runoff	34	H-90-H-3-2	1,080	
24-Jun-03	59	71685	runoff	35	H-0-L-1-1	435,000	
24-Jun-03	60	71686	runoff	36	H-0-L-1-3	>2,420,000	
24-Jun-03	61	71687	runoff	37	H-0-L-3-1	461,000	
24-Jun-03	62	71688	runoff	38	H-C-C-C-3	10	
24-Jun-03	63	71689	runoff	39	H-90-H-3-3	740	
24-Jun-03	64	71690	runoff	40	H-0-H-0-2X	>2,420,000	
Container Rinse							
24-Jun-03	73	71699	rinse	7	---	<1	
24-Jun-03	74	71700	rinse	19	---	<1	
24-Jun-03	75	71701	rinse	31	---	<1	
24-Jun-03	76	71702	rinse	31d		<1	
Irrigation Water							
24-Jun-03	69	71695	irrigation	1	---	2	
24-Jun-03	70	71696	irrigation	2	---	<1	
24-Jun-03	71	71697	irrigation	2d		5	
24-Jun-03	72	71698	irrigation	3	---	21	

[1] "d" denotes field duplicate sample
[2] "N.S." denotes no sample collected

Table 8.4. Raw *E. coli* data for cornland trial samples

Date Collected	Field Sample #	DEC I.D.	Sample Type	Plot #	Treatment	E. coli (#/100 ml)	Comment
Manure Storage							
22-Jul-03	77	74923	manure	---	90_start_1	479,000	
22-Jul-03	78	74924	manure	---	90_start_1d[1]	517,000	
22-Jul-03	79	74925	manure	---	90_start_2	320,000	
22-Jul-03	80	74926	manure	---	90_start_3	397,000	
16-Sep-03	82	75799	manure	---	30_start_1	579,000	
16-Sep-03	83	75800	manure	---	30_start_2	687,000	
16-Sep-03	84	75801	manure	---	30_start_3	866,000	
16-Sep-03	85	75802	manure	---	30_start_3d	633,000	
13-Oct-03	87	76493	manure	---	90_end_1	<100	
13-Oct-03	88	76494	manure	---	90_end_2	150	
13-Oct-03	89	76495	manure	---	90_end_3	<100	
13-Oct-03	90	76496	manure	---	30_end_1	16,000	
13-Oct-03	91	76497	manure	---	30_end_2	2,000	
13-Oct-03	92	76498	manure	---	30_end_3	14,500	
13-Oct-03	93	76499	manure	---	0_end_1	461,000	
13-Oct-03	94	76500	manure	---	0_end_2	308,000	
13-Oct-03	95	76501	manure	---	0_end_3	326,000	
13-Oct-03	96	76502	manure	---	0_end_3d	456,000	
Plot Runoff							
14-Oct-03	105	76513	runoff	1	C-30-N-1-1	52,900	
14-Oct-03	106	76514	runoff	2	C-30-I-3-1	17,300	
14-Oct-03	107	76155	runoff	3	C-0-N-1-3	1,470,000	
14-Oct-03	108	76516	runoff	4	C-0-I-1-2	474,000	
14-Oct-03	146	76554	runoff	4d		426,000	
14-Oct-03	109	76517	runoff	5	C-0-I-3-3	529,000	
14-Oct-03	110	76518	runoff	6	C-0-I-1-3	667,000	
14-Oct-03	111	76519	runoff	7	C-30-N-3-1	18,900	
14-Oct-03	112	76520	runoff	8	C-30-N-1-3	61,300	
14-Oct-03	113	76521	runoff	9	C-90-N-1-1	<1,000	
14-Oct-03	114	76522	runoff	10	C-30-I-3-2	16,000	
14-Oct-03	115	76523	runoff	11	C-0-N-3-1	175,000	
14-Oct-03	116	76524	runoff	12	C-0-N-1-2	4,350,000	
14-Oct-03	117	76525	runoff	13	C-30-N-1-2	22,800	
14-Oct-03	118	76526	runoff	14	C-90-I-1-3	2,000	
14-Oct-03	119	76527	runoff	15	C-90-I-1-1	<1,000	
14-Oct-03	120	76528	runoff	16	C-0-I-3-2	839,000	
14-Oct-03	121	76529	runoff	17	C-0-N-3-3	243,000	
14-Oct-03	145	76553	runoff	17d		345,000	
14-Oct-03	122	76530	runoff	18	C-0-N-1-1	2,010,000	
14-Oct-03	123	76531	runoff	19	C-90-N-3-2	6,300	
14-Oct-03	124	76532	runoff	20	C-30-I-3-3	14,500	
14-Oct-03	125	76533	runoff	21	C-30-N-3-2	7,400	
14-Oct-03	126	76534	runoff	22	C-90-N-3-3	<1,000	
14-Oct-03	127	76535	runoff	23	C-90-I-3-1	<1,000	
14-Oct-03	147	76555	runoff	23d		<1,000	
14-Oct-03	128	76536	runoff	24	C-0-I-1-1	657,000	
14-Oct-03	129	76537	runoff	25	C-90-N-1-3	1,000	

14-Oct-03	130	76538	runoff	26	C-90-I-3-3	1,500	
14-Oct-03	148	76556	runoff	26d		1,500	
14-Oct-03	131	76539	runoff	27	C-30-N-3-3	5,200	
14-Oct-03	132	76540	runoff	28	C-90-N-1-2	4,100	
14-Oct-03	133	76541	runoff	29	C-C-C-C-2	1,210	
14-Oct-03	134	76542	runoff	30	C-C-C-C-3	860	
14-Oct-03	135	76543	runoff	31	C-30-I-1-2	8,600	
14-Oct-03	136	76544	runoff	32	C-30-I-1-3	12,100	
14-Oct-03	137	76545	runoff	33	C-C-C-C-1	200	
14-Oct-03	138	76546	runoff	34	C-0-I-3-1	31,000	
14-Oct-03	139	76547	runoff	35	C-90-I-1-2	<1,000	
14-Oct-03	140	76548	runoff	36	C-30-I-1-1	26,000	
14-Oct-03	141	76549	runoff	37	C-0-N-3-2	134,000	
14-Oct-03	142	76550	runoff	38	C-90-I-3-2	6,200	
14-Oct-03	143	76551	runoff	39	C-90-N-3-1	<1,000	
14-Oct-03	144	76552	runoff	40	C-0-N-0-2X	12,400,000	
Container Rinse							
14-Oct-03	101	76503	rinse	4	---	<1	
14-Oct-03	104	76506	rinse	4d		<1	
14-Oct-03	102	76504	rinse	11	---	<1	
14-Oct-03	103	76505	rinse	29	---	<1	
Irrigation Water							
14-Oct-03	149	76557	irrigation	1	---	1	
14-Oct-03	150	76558	irrigation	2	---	1	
14-Oct-03	151	76559	irrigation	2d		1	
14-Oct-03	152	76560	irrigation	3	---	<1	

[1] "d" denotes field duplicate sample

8.4. Summary of Literature Review

Sources of Microorganisms on Agricultural Land

- Livestock agriculture can be a major source of microorganisms to surface and ground waters in rural agricultural watersheds. While numbers vary by species, farm animals shed ~10^6 - 10^7 fecal coliform organisms per gram of waste, or ~10^9 - 10^{10} organisms per capita per day (Robbins *et al.* 1971, Reddy *et al.* 1981, Moore *et al.* 1988).
- Based on these numbers, dairy cows (~216,700 dairy animal units) in the Vermont and Quebec portions of the Lake Champlain Basin shed ~2.2 x 10^{14} – 2.2 x 10^{15} fecal coliform organisms/day. In the Missisquoi Bay subbasin alone, an estimated 99,285 dairy animal units could produce some 10^{14} to 10^{15} fecal coliform organisms each day.

In addition to indicator organisms like fecal coliform and *E. coli* which are the basis of water quality standards in the Lake Champlain Basin, pathogenic microorganisms such as *Salmonella, Campylobacter*, *Cryptosporidium, Giardia,* and *E. coli* O157:H7 from agricultural operations may directly threaten human health (Ongerth and Stibbs 1989, Stehman *et al.* 1996, Pell 1997). A significant proportion of dairy herds across the U.S. are reported to be positive for *E. coli* O157:H7 (Wang *et al.* 1996, Hancock *et al.* 1997, Pell 1997). No systematic data on pathogens in animal waste in the Lake Champlain Basin are available.

Indicator organisms originate in animal waste; the collection, storage, management, and distribution of that waste within a farm create several distinct reservoirs of microorganisms that behave differently.

Manure storage

- Collected and stored animal waste represents the primary stock of microorganisms in dairy farm operations. Unless microorganism populations have been reduced by die-off in long-term storage, bacteria counts in manure in storage are similar to those for excreted waste, ranging from 10^5 to 10^8 organisms/g of waste (Moore *et al.* 1983, Culley and Phillips 1982, Conboy and Goss 2001). Microorganism levels in stored waste vary by waste form, addition of bedding materials, or dilution by other wastewater, but in general the differences in microorganism levels due to such variation are not large (Moore *et al.* 1983). In storage, bacteria levels in waste may be affected by dilution, die-off, or other factors.

Barnyards and holding facilities

- Barnyards, feedlots, and other concentrated animal holding areas accumulate manure and consequently represent important stocks of fecal microorganisms. Studies have shown that runoff from barnyards laden with stacked animal wastes may have the highest pollution potential of any agricultural activity (Moore *et al.* 1983). Runoff from concentrated animal holding areas may contain 10^5 – 10^8 fecal coliform organisms/100 ml (Clausen 1989).
- The stock of microorganisms in a holding facility depends on stocking rate, frequency of use, facility type (*e.g.*, paved vs. unpaved), and management (*e.g.*, frequency of scraping) (Cassell and Meals

2002). It is believed that poorly managed facilities (*i.e.*, those cleaned infrequently) store relatively large numbers of organisms and represent significant potential sources of bacteria during runoff events.

Land application

- Most agricultural animal waste produced in the Lake Champlain Basin is ultimately applied to the land to provide nutrients for crop production, organic matter for maintenance of soil quality, or as a disposal method. Such application may deliver $10^9 – 10^{12}$ *E. coli*/ac ($10^8 – 10^{11}$ *E. coli*/ha) to cornland and hayland in the basin annually. Depending on subsequent events such as weather, precipitation, runoff, and land management, microorganisms in the land waste may be available for transport and delivery to surface or ground waters.

Grazing

- Animals on pasture deposit microorganisms with their manure; such deposition may be a loading to the land over a significant portion of the year. In the Lake Champlain Basin, the pasture season typically extends from mid-May through late October or 47% of the year.
- The actual extent of manure deposition on pastureland depends on livestock density or stocking rate, which varies with the type of agricultural enterprise.
- Most studies report that fecal bacteria levels in runoff from grazed pastures exceed levels in runoff from ungrazed areas (e.g., Coltharp and Darling 1975, Sewell and Alphin 1976, Tiedemann *et al.* 1988, Edwards *et al.* 2000). Such relationships are not always directly associated with the presence of animals on pasture because bacteria may survive for extended periods in soils or fecal deposits after the animals are removed (Thelin and Gifford 1983).
- Livestock access to streams can be a source of direct deposit of microorganisms to surface waters. Extremely high *E. coli* and fecal coliform counts ($10^4 – 10^5$ /100 ml) have been observed in streams draining agricultural watersheds in northern Vermont where free access to streams is common (Meals 2000).

Human sources

- Wastewater from municipal treatment systems is typically disinfected before discharge to surface water. In runoff from rural/agricultural watersheds in the Lake Champlain Basin, the dominant source of indicator bacteria of human origin is likely to be on-site wastewater systems, *i.e.*, septic systems.
- While the contents of septic systems prior to soil infiltration are potent sources of *E. coli* and other microorganisms, leachate from a properly functioning septic system is not likely to represent a major contributor of indicator bacteria to surface waters. Except in cases of major system failure involving surfacing of untreated effluent, bacterial contamination from septic systems is likely to be confined to soils and shallow groundwater near the source (Hagedorn *et al.* 1978, Crane and Moore 1984).
- Significant levels of bacterial contamination in coastal areas has been associated with high densities of septic systems and where contamination from systems installed close to artificial drainage or agricultural tile lines was delivered rapidly to creeks and tidal waters (Duda and Cromartie 1982).

- Elevated bacteria levels have been reported in streams draining heavily used recreation/camping areas (Varness *et al.* 1978).

Wildlife

- Because all warm-blooded animals excrete indicator bacteria in their feces, wildlife contributes to the pool of microorganisms available in agricultural watersheds. Monitoring has consistently shown that wildlife can contribute to a small, but significant level of background bacterial contamination, even in “pristine” watersheds (Kunkle 1970a, Moore *et al.* 1988, Niemi and Niemi 1991, Entry *et al.* 2000a).
- Waterfowl represent a particularly potent source of indicator microorganisms to surface waters. Significant contamination of reservoirs and coastal areas has been attributed to the presence of ducks, geese, and gulls (Benton *et al.*, 1983, Valiela *et al.* 1991, Levesque *et al.* 1993).
- Wildlife is also an important source of other microorganisms such as *Giardia* and *Cryptosporidium* (Roach *et al.* 1993).

Losses of Microorganisms from Agricultural Operations

Indicator organisms and other microorganisms may be lost from agricultural operations through runoff, leaching, and direct deposition in surface waters.

Animal holding facilities

- Large quantities of manure are deposited on feedlot and barnyard surfaces that are devoid of vegetation, subject to severe hoof action, and highly compacted or paved. If runoff occurs, such major reservoirs of microorganisms can be direct sources of microbe loading to surface waters.
- Reported levels of fecal coliform in runoff from feedlots and barnyards range from ~10^5 to 10^8 organisms/100 ml (Dickey and Vanderholm 1981, Crane *et al.* 1983, Baxter-Potter and Gilliland 1988).
- Data from Vermont and elsewhere suggest that paved barnyards may export more microorganisms than do unpaved facilities, probably because of efficiency of runoff transport from paved surfaces versus depression storage on irregular unpaved surfaces (Clausen 1989).

Land runoff

Indicator bacteria counts in streams draining agricultural watersheds frequently exceed water quality criteria (Harms *et al.* 1975, Baxter-Potter and Gilliland 1988). Microorganisms may reach surface waters by a variety of means from a variety of sources.

- Most research has shown that overland flow is the dominant means of bacteria transport from land to receiving water (e.g., McDonald *et al.* 1982, Faust 1982, Irvine and Pettibone 1996). Bacteria in runoff from cropland are usually related to waste application to the land (Crane *et al.* 1983, Moore *et al.* 1988).
- Under ideal conditions (*i.e.*, immediate incorporation of stored manure on flat, well-drained soils during warm, dry weather), the potential for bacterial pollution of runoff from manured cropland can be low (Patni *et al.* 1985). However, organism counts of $10^4 – 10^6$ fecal coliform/100 ml in runoff

from manure application areas are much more commonly reported (e.g., Crane *et al.* 1983, Moore *et al.* 1988). Studies have estimated that up to 25% of microorganisms from applied animal waste may be lost in runoff annually (Robbins *et al.* 1971, Kunkle 1970b, Faust, 1976).

- The actual quantity of microorganisms transported from a waste application site depends on interaction of many factors, including: precipitation intensity, runoff volume, time of precipitation relative to application, runoff/infiltration partitioning, vegetation, soils, slope, application form and method, soil contact time, organism die-off rate, and season.
- Although numerous studies have shown that bacterial concentrations in runoff from manured land generally exceed water quality criteria, there have been relatively few studies isolating the effects of specific individual management practices on bacteria levels in runoff.
- Except in circumstances of gross over application, manure application rate alone does not appear to be a major driver of microorganism levels in land runoff (Crane *et al.* 1980, Cook and Baker 2001).
- Residence time of manure on the land surface after waste application to pasture is believed to be a key factor for bacteria loss in runoff. Runoff occurring on the day of manure application may carry substantially more microorganisms than runoff after manure residence time is increased from to 3 days (Moore *et al.* 1988).
- Consistent differences between methods of manure application – liquid, semi-solid, solid – with respect to bacteria levels in runoff have not been reported (Moore *et al.* 1988). Lowest total losses of bacteria tend to be observed after applying liquid manure, while the greatest losses have occurred using solid spreading techniques, but the differences are not generally large.
- Reports on the relationship between bacteria runoff and hydrology are conflicting. Precipitation is an obvious driver of bacterial transport in runoff and associations between high runoff flows and bacteria numbers have been reported. However, relationships between streamflow and bacteria levels are often confounded by resuspension of organisms from streambeds, strong seasonality in bacteria levels, and direct deposition of waste by livestock in streams during low-flow periods (Bohn and Buckhouse 1985, Wyer *et al.* 1996, Baudart *et al.* 2000, Meals 2001).
- Bacteria loss from grazing land has been studied extensively. Levels of indicator organisms in runoff from grazed land (10^4 – 10^6 /100 ml) can exceed levels in runoff from other types of agricultural land (Faust 1982, Crane *et al.* 1983, Moore *et al.* 1988, Edwards *et al.* 2000). Most bacteria reaching the stream are thought to come from pasture areas near the stream channel (Kunkle 1970b).
- Most published work indicates that the presence of livestock on pasture increases bacteria levels in runoff (Gary *et al.* 1983, Jawson *et al.* 1982, Crane *et al.* 1983, Boyer and Perry 1987, Tiedemann *et al.* 1988, Howell *et al.* 1995, Edwards *et al.* 1997b). However, results on the magnitude and duration of the increase have been contradictory, probably due to the wide variation in stocking rates and management of grazing lands.
- An additional confounding influence on relating bacteria losses in runoff to grazing activity is the ability of bacteria to persist in soils or fecal deposits on grazing land after livestock are removed (Thelin and Gifford 1983, Kress and Gifford 1984). Enhanced bacterial survival in "cow pies" has been well documented. Fecal deposits as old as 100 days old may remain a potential source of fecal coliform.

- Most data on the influence of grazing management on bacteria levels in runoff have been reported from the western U.S. and are of limited relevance to the Lake Champlain Basin. No data have been reported on the influence of management intensive grazing in the Northeast on bacteria losses in surface runoff.

Soil profile

- Microorganisms deposited on the soil surface may enter the soil, either carried in by infiltrating water or by deliberate mixing (*e.g.*, plowing). Within the soil, organisms may die, be trapped, or may move into groundwater. Although many microorganisms are usually filtered out through soils, movement of microorganisms through soil can be rapid and microorganisms can migrate significant distances in the field under some circumstances.
- Microbial movement through porous media is governed by physical processes (convection or advection and hydrodynamic dispersion) and by biological processes (organism mobility and chemotaxis) (Abu-Ashour *et al.* 1994). Geophysical processes such as filtration, adsorption, and sedimentation tend to attenuate microbial transport through soils. Laboratory studies have reported that average velocity of microorganisms moving through soil can exceed the velocity of ambient groundwater flow and the velocity of a chemical tracer due to chemotaxis, *i.e.*, preferential movement along a chemical concentration gradient (Abu-Ashour *et al.* 1994).
- Physical filtration in the soil profile is believed to be the primary process limiting bacteria mobility in the soil (Crane and Moore 1984, Jamieson *et al.* 2002). Soil texture and porosity are reported to be the main factors determining soil's ability to filter microorganisms; coarse-grained soils are less efficient than fine soils in bacterial removal. Numerous studies report that nearly all *E. coli* may be filtered out of infiltrating ground water in the first centimeter of soil; most of the remaining organisms are typically retained in the next 4 cm of soil (e.g., Ellis and McCalla 1978, Moore *et al.* 1988, Huysman and Verstraete 1993, Zyman and Sorber 1988). Retention of bacteria at the soil surface, however, increases the likelihood of losses during surface runoff.
- Microorganisms are not always effectively filtered or captured rapidly in soils. Lysimeter studies have shown that manure application can significantly increase fecal bacteria in leachate through 90 cm of soil compared to unmanured treatment (Stoddard *et al.* 1998, Gagliardi and Karns 2000).
- The existence of macropores, relatively large channels in soil resulting from worm-holes, voids left by decayed plant roots, etc., can bypass soil filtration. Preferential flow through macropores, cracks, and fractures is thought to be the main reason for rapid movement of microorganisms through soils and the dominant transport pathway for bacteria through soils (Smith *et al.* 1985, Hunter *et al.* 1992, Abu-Ashour *et al.* 1998, Vansteelant 2000, Jamieson *et al.* 2002).
- Although surface runoff represents the greatest contamination risk for surface waters, under some circumstances such as very heavy waste applications and extensive macropore flow, microorganisms may reach artificial drainage lines. If leachate from manure-amended fields reaches subsurface tile drains, discharge from tile drainage can be an important source of indicator organisms (Evans and Owens 1972, Dean and Foran 1992, Geohring et al. 1998, Jamieson *et al.* 2002).

Ground water

- Once in the soil, microorganisms may move with water movement (Valiela *et al.* 1991), although relatively little work has been reported on movement or survival in the subsurface environment.
- Fecal bacteria have been detected in groundwater beneath pastures and irrigated land, but movement is thought to be slow (Entry and Farmer 2001). Longer bacteria survival times in ground water may offset the slower movement, suggesting that bacteria can threaten ground water over longer time periods and larger areas than previously assumed (Conboy and Goss 2001).
- Bacteria movement in ground water in karst regions can be rapid and significant, but this is of limited relevance to the Lake Champlain Basin.

Watershed export

- Local monitoring data have documented very high indicator organism levels in streams draining agricultural regions of the Lake Champlain Basin, suggesting that losses from agricultural land can be significant (VT RCWP Coord. Comm. 1986, Meals 1998, Simoneau 2003).
- Bacteria counts in monitored streams in the Basin have demonstrated pronounced seasonal cycles with a range of more than 5 orders of magnitude. Lowest counts were common during winter months and highest counts dominated during the summer. *E. coli* counts in three streams in northern Vermont exceeded the Vermont water quality criterion for recreation (77 organisms/100 ml) 50 to 60 percent of the time (Meals 1998).
- The seasonal cycle of bacteria in streamflow is at least partially driven by temperature, which relates to rates of bacteria survival outside their host, but also frequently coincides with the cropping and grazing season - the period from ~May through October when manure is applied to agricultural land and livestock are out on pasture in the watersheds (Hunter and McDonald 1991, Edwards *et al.* 1997a, Meals 1998).
- Not all microorganisms discharged from a watershed necessarily result from recent overland or subsurface flow from land sources. Bacteria have been shown to accumulate in aquatic sediments and biofilms (Stephenson and Rychert 1982, Burton *et al.* 1987, Sherer *et al.* 1988, Struck 1988, Sherer *et al.* 1992). Stocks of bacteria surviving in the stream could sustain high counts of indicator organisms in the absence of new organisms in land runoff. Increases in bacteria counts in streams draining agricultural watersheds have been attributed solely to scour or resuspension by high flows or livestock trampling (McDonald *et al.* 1982). Studies in Vermont have suggested that bacteria stocks in streambeds were able to persist for several months and were replenished from land runoff during the growing season (Meals 2001).

Processes for Microbial Reduction

Natural die-off

- Outside of their natural habitat (the gastrointestinal tract of warm-blooded animals), indicator organisms are subject to a hostile environment where temperature, moisture, nutrient, and other conditions are inimical to their survival. Therefore, following excretion, bacteria tend to die at rates that depend on environmental conditions. Temperature, moisture, pH, nutrient supply, and solar radiation are believed to have the greatest effect on bacterial survival (Crane and Moore 1986, Moore *et al.* 1988):

  - Lower temperatures increase survival time; elevated temperatures (especially with dry conditions) increase die-off rates.
  - Temperature extremes – either hot or cold – seem to be most disruptive to bacterial survival.
  - In soils, moisture appears to be a major factor when it is significantly lowered.
  - Extremes in pH are detrimental to microorganism survival; generally a neutral pH favors bacterial survival.
  - Nutrient supply is critical – it has been proposed that a major cause of bacteria die-off is the inability of organisms to lower their metabolic requirements in situations of low nutrient availability.
  - Solar radiation (UV) is effective in reducing bacteria numbers on the land surface or on vegetation sprayed with wastes.

- It is widely accepted that decay in bacterial population follows simple first-order kinetics (Crane and Moore 1986):

$$N_t/N_o = 10^{-kt}$$

where: N_t = number of bacteria at time t
N_o = number of bacteria at time 0
t = time in days
k = first order or die-off rate constant

- Reported values for k range from 0.172 – 0.697/d for *E. coli* and 0.045 – 0.470/d for fecal coliform (Crane *et al.* 1980).

Die-off in storage

- In general, manure storage results in a significant reduction of bacteria numbers, compared to those in fresh waste; reduction of fecal coliform levels by 2 – 3 orders of magnitude are typical with storage for 2 – 6 months (e.g., Patni *et al.* 1985, Crane and Moore 1986, Moore *et al.* 1988, Conboy and Goss 2001).
- Decline of microorganism numbers in stored manure is temperature-dependent; organisms in stored slurry decline more rapidly at higher temperatures (Jones 1980, Kearney *et al.* 1993a and 1993b, Stehman *et al.* 1996, Himathongkham *et al.* 1999).
- Oxygen status also influences bacteria survival in storage. Aeration reportedly enhances die-off, with many microorganisms surviving up to 15 days in aerated liquid manure, compared to 39 days in nonaerated liquid manure (Jones 1980, Strauch 1987, Curtis *et al.* 1992).
- Manure digestion can enhance microorganism die-off in storage. Anaerobic digestion at mesophilic temperatures (~35 ^oC) reportedly decreased *E. coli* numbers by 90% in less than one day during batch digestion, in contrast to bacteria survival in manure slurry of up to 77 days (Stehman *et al.* 1996).

- Reports of bacteria die-off in composting manure have been conflicting. If properly managed, composting may offer significant initial reductions of bacteria numbers due to high temperatures (Kudva *et al.* 1998, Larney et al. 2003), but regrowth of bacterial populations after temperatures decline has been reported (Mote *et al.* 1988). Because bacteria have been reported to increase to numbers approaching those in original dairy waste solids, some authors suggested that composting offers little benefit toward net reduction of coliform bacteria in dairy waste.

Die-off following land application

- Microorganisms in land-applied waste are subject to mortality from high temperatures, desiccation, UV light, and other stresses (Moore *et al.* 1983 and 1988). Die-off of indicator bacteria after land application follows a first-order decay, with reported values of k ranging from 0.195 – 0.667/d, with greatest die-off in warm weather, longest survival in cold weather (Crane and Moore 1986, Moore *et al.*1983 and 1988).
- Surface-applied wastes may be exposed to greater stresses than wastes mixed with the soil and therefore demonstrate greater die-off after application (Reddy *et al.* 1981). However, the opposite has also been reported; little decline in bacterial numbers when manure was surface-applied to bare soil, possibly due to limited interaction with soil (Crane *et al.* 1980).
- When manure is applied to hayland, some may be intercepted and captured on vegetation before it reaches soil, affecting overall bacteria survival rates (Vansteelant 2000). It has been reported that when the biomass of the plant canopy is low, die-off of fecal coliform applied in manure is rapid due to effects of UV light and drying, whereas bacteria counts were maintained better when the canopy biomass was higher, suggesting a protective effect of the vegetation (Trevisan *et al.* 2000). Cutting pastures appears to reduce bacterial survival times by enhancing drying and exposure to solar radiation (Crane *et al.* 1983).
- Enhanced bacterial survival in intact animal fecal deposits (*i.e.*, "cow pies") has been documented, although microorganism release from old fecal deposits is usually small compared to release from fresh deposits (Thelin and Gifford 1983, Kress and Gifford 1984).

Soil interactions

Incorporation of waste into soils is a common practice thought to reduce bacteria available for runoff loss. Bacteria reduction or removal with movement into the soil profile is accomplished by adsorption, filtration, and die-off (Crane and Moore 1984). Nearly complete removal of bacteria within the top 5 cm of soil is commonly reported.

- There is considerable evidence that *E. coli* and fecal coliform tend to die off in a matter of days or weeks in soils; some reports suggest that two to three months is sufficient in most cases to reduce pathogens to negligible numbers once they have been applied to soil (e.g., Dazzo *et al.* 1973, Chandler *et al.*1981, Faust 1982, Zhai *et al.* 1995). However, there is also evidence of extended survival of indicator organisms in soils; survival for as long as 5 years has been documented (Rudolfs *et al.* 1950; Gerba and Bitton 1984, Vansteelant 2000). Regrowth of indicator organisms in soil systems has also been reported (Van Donsel *et al.* 1967, Howell *et al.* 1996, Gagliardi and Karns 2000).

- The principal controls on indicator bacteria survival in soil appear to be temperature and moisture, with several other factors exerting an influence (Gerba *et al.* 1975, Jamieson *et al.* 2002). Limited moisture availability in the soil reduces the survival rates of enteric bacteria in manure amended soils (Moore *et al.* 1983). An inverse relationship between temperature and bacterial mortality has been reported, with higher temperatures decreasing survival times (Van Donsel *et al.* 1967). Reduced bacteria survival in acid soils and increased survival and possible regrowth when organic matter is high have been reported. High soil nutrient levels such as those resulting from long-term manure application may improve microorganism survival (Dazzo *et al.* 1973).
- Soils of fine texture and high organic matter have been reported to support microbial populations three times larger than coarse textured soils (Tate 1978, Gerba and Bitton 1984, Jamieson *et al.* 2002). This pattern is thought to be a function of availability of micronutrients, provision of new microhabitats, or protection from predation, as well as soil moisture retention.
- Although soils can be effective filters for microorganisms, the existence of macropores promotes rapid infiltration of bacteria (Moore *et al.* 1988, Abu-Ashour *et al.* 1998). *E. coli* levels of 10^3 – 10^5/100 ml have been reported in tile drain flow from grazing land and land receiving animal waste application (Dean and Foran 1992, Geohring et al. 1998).

Competition/Predation

- Significant declines in bacteria numbers have been attributed to competition and predation from native soil microorganisms such as streptomycetes, myxobacter, and *Bdellovibrio*, and larger soil organisms such as protozoa and nematodes (Abu-Ashour *et al.* 1994).
- Laboratory studies have shown that *E. coli* survival is dramatically reduced in nonsterile soil solutions compared to sterile soil solutions (Tate 1978, McCoy and Hagedorn 1979).
- Predation by protozoa can be a significant factor in bacteria reduction in soils. Significant relationships between fecal coliform mortality and protozoan activity have been observed in soils, suggesting a predator-prey relation whose characteristics depend on soil conditions, temperature, and bacterial concentration (England *et al.* 1993, Trevisan *et al.* 2000). Some researchers suggest that reported effects of temperature and moisture on bacteria die-off may be due in part to conditions more or less favorable for protozoans (Trevisan *et al.* 2000).

Management Practices for Microbial Reduction

The state of the art in agricultural management practices aimed at reducing microorganism losses is not as advanced as for sediment or nutrient losses. Few Best Management Practices (BMPs) exist today that are aimed specifically at reduction of microorganisms.

Reduction of microorganism populations and losses through improved management presents an additional challenge due to the sheer magnitude of microorganisms available for loss, compared to acceptable levels in receiving waters. Reduction rates in *E. coli* bacteria that would be welcomed for phosphorus or sediment would generally be inadequate to comply with water quality criteria for bacteria. Even after a

99% reduction in *E. coli*, from 10^6 organisms/100 ml to 10^4 organisms/100 ml for example, runoff would still represent a significant threat to receiving water quality.

Animal management and biosecurity

- The presence of pathogenic organisms on farms can be a serious concern; these concerns are usually addressed by programs of biosecurity. Biosecurity consists of measures taken on the farm to control the introduction, spread, and dissemination of pathogens and disease among livestock and humans, both on and off the farm (HACCP Alliance 2003).
- Good biosecurity practices may reduce the incidence of actual pathogen presence in manure, thereby reducing the risk of transmission in runoff from agricultural land. However, biosecurity programs do not and cannot normally address generic indicator organisms like fecal coliform and *E. coli* that are a normal part of the animals' digestive system.
- There is considerable work currently underway to reduce the incidence of *E. coli* O157:H7 in cattle as a food safety measure (e.g., Behrends *et al.* 2002, Moxley 2002). Most of these approaches are specific to *E. coli* O157:H7, do not affect ordinary *E. coli*, and thereby would not be generally effective in reducing indicator organism populations in animal waste.
- Application of chlorate ($NaClO_3$) to reduce *E. coli* O157:H7 in cattle prior to slaughter may offer some promise (Callaway *et al.* 2002). Chlorate is bactericidal only against nitrate reductase-positive bacteria (*e.g.*, *E. coli*). Cattle can be treated without harm to the other gastro-intestinal organisms necessary for fermentation and digestion. When supplied in drinking water, sodium chlorate reduced the population of *E. coli* O157:H7 and also reduced total coliforms and generic *E. coli* by several orders of magnitude through the gastro-intestinal tract.
- It should be noted that unless any treatment simultaneously reduced **all other** pathogens in animal feces, the reduction in indicator bacteria alone would be counter-productive to efforts to protect water quality and human health.

Animal holding facilities

- Although there are no specific data on bacteria reductions reported in the literature, a barnyard runoff management BMP could include control and delivery of runoff into the manure structure. The microorganisms then become subject to other waste management and land runoff control practices (including die-off in waste storage) and the barnyard is essentially eliminated as a separate source of bacteria.
- Unpaved barnyards may present a slightly lower bacteria pollution potential compared to paved barnyards (Crane *et al.* 1983, Baxter-Potter and Gilliland 1988, Clausen 1989). If barnyards are paved, the frequent cleaning will reduce pollution potential (Cassell and Meals 2002). If it is not possible to capture barnyard runoff and combine it with stored manure, runoff should be treated by other means (Moore *et al.* 1983).

Animal waste storage

- Considerable research has documented the tendency for microorganisms to die in waste storage (Moore *et al.* 1983, Walker *et al.* 1990, Trevisan and Dorioz 1999, Jamieson *et al.* 2002). Most researchers have concluded that long-term storage of livestock wastes prior to land application has the greatest impact on reducing bacterial transport from agricultural land to water. Long-term storage is therefore an effective practice for reducing fecal coliform counts in runoff.

- In actual operation, manure storage in the Lake Champlain Basin may be substantially less effective than controlled studies suggest. In practice, stored manure does not age uniformly; fresh manure is typically added ~daily from the barn, barnyard, and other animal holding areas. This continued inoculation of microorganisms may reduce the effectiveness of storage as a means of microorganism control. Conversely, definitive storage, where no fresh manure is added for an extended period, may yield better microorganism control than reported in the literature.

Animal waste treatment

- As farms in the Lake Champlain Basin increase in size and the quantity of manure to be collected, stored, and managed increases, treatment of waste to reduce microbial content may become practical. Literature reports on manure treatment suggest that this may be a promising avenue for microbial reduction.
- Composting has been suggested as a means of reducing levels of microorganisms in manure. Results reported in the literature have, however, been mixed (Mote *et al.* 1988, Kudva *et al.* 1998, Larney et al. 2003). Composting may offer significant initial reductions of bacteria numbers due to high temperatures and/or drying, but regrowth of bacterial populations after temperatures decline has been observed (Mote *et al.* 1988).
- Treatment of manure with lime (calcium hydroxide, calcium oxide) has been proposed as a means to reduce pathogens in animal waste, just as it is used in materials to reduce pathogens and odors in biosolids (sewage sludge) (NLA 2001, Hogan *et al.*1999). There is, however, scant information in the scientific literature directly concerning animal waste treatment.
- The use of sodium carbonate and alkali to eliminate *E. coli* from dairy cattle manure has shown some promise in laboratory experiments (Diez-Gonzalez *et al.* 2000). Virtually complete elimination of *E. coli* in manure, including *E. coli* O157:H7 as well as other pathogens such as *Salmonella* and *Klebsiella*, has been reported in response to treatment. Although no full-scale tests have been reported, researchers have proposed that stabilization of dairy manure with sodium carbonate and sodium hydroxide to virtually eliminate *E. coli* could be done for a cost as low as $10 per cow per year.

Land application

Application rate

- Once microorganisms are applied to the land with animal waste, management options exist that may be used to reduce indicator bacteria losses. Some of the factors influencing the quantity of microorganisms transported from a waste application site can be varied by management.
- Management effects on controlling runoff or leaching losses of microorganisms are probably more important and feasible than efforts to influence organism die-off rate.

- Within the range of typical agronomic rates, there is little strong documentation in the literature of a relationship between manure application rate and bacteria counts in surface runoff (Jamieson *et al.* 2002). Normal manure application rates do not appear to influence bacterial survival in soils, although long-term manure application may improve bacteria survival over soils that have not been manured (Dazzo *et al.* 1973).
- Some reports suggest that high liquid manure application rates pose a significant threat of bacterial contamination through leaching, especially to tile lines (Cook and Baker 2001).
- While excessive rates of manure application should clearly be avoided, there seems to be little definitive evidence in the literature to recommend control of waste application rate as a specific pathogen-reduction BMP.

Application method

- Conventional wisdom suggests that incorporation or injection of manure into soils may reduce the availability of microorganisms and other manure components for transport in surface runoff (Patni *et al.* 1985). Incorporation of manure would also subject microorganisms to interactions with the soil and to predation, all of which would tend to reduce bacteria populations. However, soil incorporation may protect bacteria from damaging UV light and desiccation and promote extended survival; organisms could be lost through soil erosion. Subsurface injection may reduce manure contact with surface soils and tend to increase bacteria transport to tile drains or ground water.
- Surface applied manure may be subject to the effects of sunlight, desiccation, and high temperatures, all of which may increase die-off rate. However, manure remaining at the land surface is more available for runoff losses (Reddy *et al.* 1981). In addition, microorganisms in the soil may move down through macropores or laterally through drainage systems.
- Tillage before or after manure application may be useful in disrupting macropores and reducing downward transport of bacteria (Abu-Ashour *et al.* 1998).
- The choice of solid or liquid manure does not seem to offer a particular advantage. Application of solid manure may enhance bacteria survival in larger aggregates (Vansteelant 2000), but larger aggregates may be more resistant to erosion losses. Initial bacteria die-off may be greater in manure applied as a liquid, but infiltration into the soil may enhance long-term survival of microorganisms.
- There seems to be no clear preference for manure application method or form for minimizing microorganism losses; each method may have its advantages and disadvantages (Moore *et al.* 1988, Walker *et al.* 1990, Jamieson *et al.* 2002). On the whole, there is no solid basis for recommending a particular waste application method as a BMP to minimize losses of indicator organisms under all circumstances.

Application timing

- Runoff from manure application on frozen or snow-covered ground can significantly increase microorganism losses in runoff from agricultural land compared to applications in other seasons (e.g., Thompson et al. 1979, Reddy et al. 1981, Clausen 1990 and 1991, Melvin and Lorimor

1996). In addition to the potential for high levels of runoff during snowmelt, microorganism survival may be enhanced by winter application (Carrington and Ransome 1994, Fayer and Nerad 1996). Because incorporation or injection of manure is impossible in winter applications, filtration and adsorption through soil contact is prevented. Winter manure application has been generally prohibited in Vermont and Quebec. Avoidance of winter manure application throughout the Lake Champlain Basin should be considered as a BMP for reducing the runoff of indicator organisms.

- In some cases, season of manure application may affect leaching of microorganisms. Greater mortality of fecal coliform bacteria in soils has been observed after fall manure application, compared to spring application (Stoddard et al. 1998, Warnemuende and Kanwar 2002). Enhanced die-off to freezing and freeze/thaw cycles in conjunction with high soil moisture may lead to lower bacteria levels in leachate from fall manure-applied soil columns than in spring manure-applied columns. Thus, favoring fall application of manure over spring may tend to reduce indicator organism levels in soils. It should be noted that disadvantages of fall application such as excessive N loss, must be considered in making any such recommendation.
- On a shorter temporal scale, the timing of manure applications relative to precipitation and runoff events must be considered (Dunigan and Dick 1980, Moore et al. 1988). Clearly, the longer microorganisms remain on the land surface and/or in the soil, the greater the die-off from numerous inimical factors is likely to be. Conversely, the sooner runoff occurs, the higher the runoff of indicator organisms will be.
- Studies of E. coli movement from manured fields to tile drains have shown that timing of manure application was a critical factor in E. coli loss in soil leachate (Joy et al. 1998, Abu-Ashour et al. 1998, Jamieson et al. 2002). The highest bacteria counts in leachate occurred when field drains were already flowing when the manure was applied (Joy et al. 1998). Under these conditions, E. coli rapidly penetrated the soil profile and reached drain tiles. Several researchers have recommended that manure not be applied when tile lines are flowing (Jamieson et al. 2002).
- To reduce the potential for indicator bacteria losses in runoff from agricultural land, manure should not be applied when the soil is very wet, during heavy rainfall, when tile drains are flowing, or when a major storm event is impending.

Other cropland issues

Some research suggests that thick protective vegetation stands promote longer bacteria survival after manure application to hayland (Crane *et al.* 1983, Trevisan *et al.* 2000). Reducing vegetation height on hayland when manure is spread may enhance bacteria die-off through exposure to UV light, higher temperatures, and desiccation. Because of the large potential of bacteria movement through soil macropores, manure application should be carefully controlled or limited on soils susceptible to shrinking or cracking (Jamieson *et al.* 2002).

Grazing management

- Stocking rate – the number of animals per unit area – has an influence on indicator organism loss from grazing land, most likely because animal numbers determine the stock of organisms available for runoff. Very low stocking rates may lead to little or no increase in microorganism loss (Buckhouse and Gifford 1976, Crane *et al.* 1983); losses generally increase as stocking rate increases (Saxton and Elliot 1980). Although differences reported are not dramatic, lower pasture stocking rates would tend to reduce runoff losses of indicator organisms from grazing land.

- Little has been reported on the influence of grazing management on bacteria losses from pastureland. Duration of grazing is reported to influence bacteria levels in runoff; one study noted significantly higher fecal coliform levels in runoff after 12 weeks of simulated grazing compared to 4 weeks of grazing (Edwards, *et al.* 2000). The same study reported that bacteria levels in pasture runoff did not depend on whether conventional or rotational grazing took place.
- Free access to streams and riparian areas is commonplace in pastures in the Lake Champlain Basin. In warm weather, livestock may spend much of their time in and around streams for cooling and for drinking water. Numerous studies, including one in the Vermont portion of the Basin (Meals 2000), have documented significant reductions in bacteria levels in streams by excluding livestock from the stream and riparian area, either by fencing or by other means (e.g., Larsen *et al.* 1994, Sheffield *et al.* 1997).
- To reduce bacteria contributions from grazing land, keeping pasture stocking rate low may yield modest reductions in runoff of indicator organisms. Reducing the duration of grazing may also give modest benefits. There is no evidence in the literature to support a recommendation for rotational grazing as a BMP for this purpose. Exclusion of animals from streams, either through fencing or simply by providing alternative drinking water sources, can significantly reduce indicator organism counts in streams draining grazing areas.

Buffers

Application of vegetated buffers or grass filter strips in the agricultural sector can be considered in two general areas: treatment of runoff from animal holding areas and treatment of runoff from farm fields.

- Bacteria reductions of 30 – 70% have been reported in vegetated filter strips treating runoff from barnyards and feedlots (Young *et al.* 1980, Dickey and Vanderholm 1981, Schellinger and Clausen 1992). However , not all studies reported significant reductions.
- Results of vegetated filter strip treatment of cropland runoff have been contradictory. Some studies have reported up to 90% reduction in fecal coliform counts in runoff after passage through a filter strip (Coyne and Blevins 1995, Coyne *et al.* 1995 and 1998, Lim *et al.* 1998). However, in some cases bacteria counts increased again during the end of a runoff event (Srivastava *et al.* 1996). Filter strips can apparently become reservoirs for sediment-bound fecal coliform trapped from surface runoff subject to detachment by rainfall impact and flowing water. After breaking down of larger aggregates, bacteria may become more mobile in grass filters (Coyne and Blevins 1995).
- Even where significant reductions have been reported, output from filter strips and buffers can still contain levels of indicator organisms that far exceed water quality standards. Construction of grass filters of sufficient length to adequately remove bacteria from runoff would be impractical in agricultural settings The consensus of the recent literature suggests that grass filters or buffers alone will be insufficient to reduce bacterial concentrations in runoff from manured areas to meet

water quality goals (Walker *et al.* 1990, Coyne and Blevins 1995, Entry *et al.* 2000a and 2000b). It should be noted, however, the performance of filter strips and vegetated buffers for sediment and nutrient removal has been well-documented and their use may be justified on those grounds alone.

Other BMPs

- Several researchers have suggested that reworking the top 2 cm layer of soil may reduce transmission of *E. coli* to drainage water by blocking or disrupting macropore flow (Abu-Ashour *et al.* 1998). Where manure is applied to row cropland, light tillage before spring manure application may enhance trapping of microorganisms in surface soils and reduce movement of bacteria into tile drains or ground water. This could be beneficial where soils are fall plowed and macropores could develop over winter.
- Management practices such as avoidance of soil compaction and maintenance of adequate soil moisture may foster high levels of soil protozoa and reduce *E. coli* levels in soils due to predation (Trevisan *et al.* 2000).
- Laboratory studies have documented that application of the flocculent polyacrylamide (PAM) can reduce losses of fecal coliform bacteria in soil leachate and in runoff by 90 to 99.9% (Entry and Sojka 2000). Although such treatments have not been field-tested and are not likely to be practical for widespread application across the agricultural landscape, it is possible that their use in critical areas in specific applications such as a vegetative filter strip enhancement might be feasible in the future.
- Systems of BMPs or whole-farm plans that include runoff and erosion control and improved animal waste management may contribute indirectly to reduction of microorganism losses (Gilley et al.1992; Meals 1989, 1992, 1996; Stuntebeck and Bannerman 1998). Practices that promote infiltration and control runoff such as contouring, strip cropping, conservation tillage, terraces, and buffer strips could potentially reduce bacteria transport by reducing surface runoff and may promote bacterial die-off through soil contact. Erosion control practices would reduce movement of bacteria associated with soil particles or manure aggregates. By managing manure applications and avoiding application in sensitive areas or runoff contributing areas, nutrient management may also help reduce losses of indicator organisms (Gagliardi and Karns 2000). Secondary containment systems, sedimentation basins, or ponds may be used to intercept bacteria-laden runoff.

8.5. QA/QC Report

Summary of QA/QC Program

Sample collection, transport, and analysis, as well as all other aspects of project data collection, were conducted in accordance with the Quality Assurance Project Plan (QAPP) dated March 14, 2003, and approved by US EPA on March 18, 2003. Laboratory quality control/quality assurance procedures and instrument calibration and maintenance for the *E. coli* analysis were performed by the VT DEC laboratory under its EPA-approved QA plan dated July, 2002. Instrument and equipment testing, inspection, and maintenance for manure analysis were conducted under the normal QA programs in force at the UVM Agricultural and Environmental Testing Laboratory.

Field quality control activities for sample collection on this project included the following:

- Manure samples: 1 field duplicate per manure sampling event for *E. coli* analysis.
- Irrigation source water: One of the three samples of water collected from the rainfall simulator during each simulated rainfall event was field duplicated for *E. coli* analysis.
- Runoff collector rinsate: One of the three samples of collection container rinsate collected prior to each simulated rainfall event was field duplicated for *E. coli* analysis.
- Plot runoff: Field duplicates of 10% of runoff samples from randomly-selected plots, a minimum of 4 duplicated samples per rainfall simulation event.

The following sections present laboratory quality control data for *E. coli* reported by the VT DEC lab, results of field quality control sampling, and results of data review, verification, and validation.

Laboratory QC Data

The VT DEC laboratory reported data from laboratory duplicate *E. coli* analyses of manure and water samples. The relative percent differences (RPDs) between lab-duplicated samples are as follows:

SEI Sample #	Lab ID #	Result[1] (*E. coli*/100 ml)	RPD
4	69566	369,000	48%
9	70740	687,000	0%
35	71662	70,200	20%
45	71672	27,400	11%
56	71682	18,500	43%
85	75802	633,000	17%
88	76494	150	67%
110	76518	667,000	5%
120	76528	839,000	16%
130	76538	1,500	67%
140	76548	26,000	9%
144	76552	12,400,000	80%
148	76556	1,500	67%

[1] Mean of duplicate analyses reported by laboratory

Field QC Data

One of the five manure samples collected from each storage experiment for agronomic analysis at the UVM Agricultural and Environmental Testing Laboratory was duplicated in the field, a duplication rate of 20%. The relative percent differences (RPD) between field-duplicated manure samples are as follows:

Source	Sample Date	Dry Matter	Total N	Ammonium N (NH_4-N)	Organic N	P (as P_2O_5)	Potassium (as K_2O)	Ca	Mg	Cu
		%	lb/1000 gal							
Hayland 0-day	6-23-03	8.5	30.2	9.8	20.4	8.4	20.1	10.3	5.5	0.38
		9.6	28.7	10.9	17.8	8.5	20.6	10.9	5.6	0.39
RPD		11%	5%	10%	13%	1%	2%	6%	2%	2%
Cornland 0-day	10-13-03	11.1	35.3	14.3	21.0	11.5	20.0	15.5	6.3	0.55
		11.3	32.9	13.3	19.7	10.8	19.2	14.7	6.1	0.54
RPD		2%	7%	7%	6%	6%	4%	5%	3%	2%

Each time manure samples were collected for *E. coli* analysis, one sample was duplicated in the field for *E. coli* analysis at the VT DEC laboratory. Of the 15 manure samples collected for *E. coli* analysis during each storage experiment, three were duplicated, for a duplication rate of 20%. The relative percent differences between duplicate manure samples are as follows:

Source	SEI Sample #	Lab ID #	Result (*E. coli*/100 ml)	RPD
Hayland 90-day start	2	69564	378000	27%
	3	69565	275000	
Hayland 30-day start	7	70738	457000	56%
	8	70739	1050000	
Hayland 0-day	19	71630	308,000	33%
	20	71631	461,000	
Cornland 90-day start	77	74923	479,000	7%
	78	74924	517,000	
Cornland 30-day start	84	75801	866,000	27%
	85	75802	633,000	
Cornland 0-day	95	76501	326,000	28%
	96	76502	456,000	

Results of all field duplicate water samples collected during the hayland and cornland runoff events are given below:

Source	SEI Sample #	Lab ID #	Result (*E. coli*/100 ml)	RPD
Hayland Runoff Event				
Container rinse	75	71700	<1	0%
	76	71701	<1	
Irrigation water	70	71696	<1	80%
	71	71697	5	
Plot runoff	40	71667	12,200	13%
	67	71693	14,000	
Plot runoff	42	71669	8,600	16%
	68	71694	10,200	
Plot runoff	43	71670	397,000	39%
	66	71692	242,000	
Plot runoff	56	71682	18,500	10%
	65	71691	16,700	
Cornland Runoff Event				
Container rinse	101	76503	<1	0%
	104	76506	<1	
Irrigation water	150	76558	1	0%
	151	76559	1	
Plot runoff	108	76516	474,000	10%
	146	76554	426,000	
Plot runoff	121	76529	243,000	30%
	145	76553	345,000	
Plot runoff	127	76535	<1,000	0%
	147	76555	<1,000	
Plot runoff	130	76538	1,500	0%
	148	76556	1,500	

Data Review, Verification, and Validation

All data were collected with documentation that permitted each analytical result to be traced from collection through analysis and reporting. According to the QAPP, the data quality objective for traceability with respect to all primary data analyses for all samples was 100 percent and this was achieved.

The QAPP discussed several potential field or data conditions that could cause data to be questioned or rejected. These conditions included:

- Excessive natural rainfall during simulation;
- Non-uniform simulated rainfall application;
- Excessive *E. coli* in irrigation water; and
- Violations of statistical assumptions for parametric statistical analysis.

None of these conditions seriously affected data collection or analysis. No natural rainfall occurred during either simulated rainfall event. Essentially no *E. coli* were detected in irrigation water in either the hayland or cornland event. Simulated rainfall met criteria for uniformity in the hayland event; rainfall application in the cornland event was slightly non-uniform, but subsequent statistical analysis showed that this was not a significant factor in runoff losses of bacteria. Raw data were not normally distributed, but normality was achieved using a standard logarithmic transformation. Equality of variance among treatments was confirmed using several tests, including Levene's and Brown-Forsythe.

Field-collected and laboratory analytical data were reviewed by project personnel and were accepted for the study unless there was a noted occurrence of adverse field conditions or instrumentation malfunction, or a laboratory note indicating that the required analysis was not performed in accordance with one or more of the criteria associated with the particular analysis. Only one data value was rejected for such reasons. In the hayland study, one control plot was observed to be contaminated by significant runoff from other plots running from a diversion ditch. This contamination was reflected in a very high *E. coli* count (>24,200 / 100 ml) in runoff collected from the control plot. Due to the field contamination, this value was rejected and excluded from subsequent data analysis.

All *E. coli* data from the VT DEC laboratory were accepted even when the RPD exceeded 50% (4 samples); it was assumed that data reported from the DEC laboratory met the QA objectives of the laboratory and were usable for our study. The RPD for two field duplicates exceeded 50%; one sample of manure and one of irrigation water. In both cases, the individual values of the duplicates were not dramatically different from the range of other comparable samples and the values were not rejected. For both laboratory and field duplicates, the mean of the duplicate pair was used in subsequent data analysis.

As noted in the QAPP, internal assessments and response actions with regard to *E. coli* analysis within the VT DEC laboratory occurred under the terms of the lab's approved QA plan. Examination of these assessments included in *Internal Remark and Justification Codes* reported with the data indicated that analytical results did not generally exceed internal control limits. No samples, for example, were flagged with the "W" code (warm on arrival), indicating that cooling in the field and during transport was adequate. No samples were flagged with the "J" code (samples cannot be processed within 8 hours), indicating that all analyses were conducted in the laboratory within the specified time interval. In the hayland trial, six samples were noted as exceeding the 8 hour holding time by ~30 minutes. The affected samples were those from container rinse (collected before the rainfall event) and from irrigation water taken early in the progress of the event. All of these samples were kept on ice as soon as collected and none contained significant numbers of *E. coli*. Data from these samples were accepted for use in subsequent analysis. None of the data from plot runoff were similarly affected.

The most significant problem with data usability occurred when *E. coli* exceeded the maximum range for enumeration in some samples, resulting in data reported as "greater than" the maximum value. Despite our best efforts to predict the levels of *E. coli* expected in plot runoff, seven samples of hayland plot runoff exceeded the maximum range. No samples from the cornland runoff plots exceeded the maximum range. Similarly, some samples contained fewer *E. coli* than anticipated and results were reported as "less than" a minimum value. This occurred for one manure sample from the hayland manure storage experiment and for five water samples in the hayland runoff trial. In the case of the water samples, all of the samples were from container rinse or irrigation water. Because these samples were not expected to contain many bacteria, the "less than" results are probably equivalent to "below detection limit," rather than the result of an incorrect dilution. In the cornland runoff trial, 14 samples were reported with "less than" the minimum number of *E. coli*. As in the hayland trial, many of these samples were from container rinse and irrigation water and probably represent "below detection limit." Some values, however, were from 90-day old manure and from runoff from plots receiving 90-day old manure. These cases were the result of dilutions to account for erroneously high predicted bacteria levels. Recall that the die-off of *E. coli* in summer storage was unexpectedly high and that runoff from plots receiving this manure had very low levels of *E. coli*, not significantly different from the control plots.

To allow evaluation of all treatments, there was no alternative but to include the data reported as *greater than/less than* as real values. As noted in the discussion, the net effect of this inclusion was probably some loss of statistical inference, wherein a statistical test might have achieved statistical significance if the actual higher (or lower) values had been known. However, this effect was likely limited to the hayland treatments using fresh manure and should not affect the overall conclusions of the project.

Data completeness is a measure of the percentage of planned samples collected or the percentage of usable data points obtained that meet criteria for accuracy, precision, and representativeness. As stated in the QAPP, the minimum completeness objective for the E. *coli* counts in manure and runoff water was 95 percent. Considering all 152 planned samples, the only samples not collected were those from two hayland plots that failed to generate runoff. Thus, 99% of all planned samples were collected. Because one manure sample collected for agronomic analysis was lost in the laboratory and one hayland plot runoff sample was contaminated, 148 of 152 planned samples generated usable data, a completeness percentage of 97%. The project objective for data completeness was achieved.

8.6. List of Project Work Products

This final report is the main product of this project. Because this project involved both original research and a significant education and outreach component, there were many additional documents and presentations provided, as follows:

- Comprehensive literature review on the behavior, survival, transport, and export of microorganisms from agricultural land. Available as a separate volume from LCBP
- Quality Assurance Project Plan, incorporating detailed experimental protocol
- Seven quarterly progress reports
- Two presentations to LCBP Technical Advisory Committee—interim and final project results
- An article for LCBP's *Casin' the Basin* newsletter and for inclusion on the LCBP web site
- Coverage of field trials in Champlain 2000 television program and the Times Argus newspaper
- Project summary developed for distribution to the public
- Presentation to the Natural Resources Conservation Service State Office, January 12, 2004
- Presentation of the recommended multiple barrier system of practices to farmers and agricultural service professionals through the State Department of Agriculture's *Agriview* newsletter
- Submission of a paper for publication in a peer-reviewed scientific journal
- Two oral presentations at professional conferences:
    - EPA's 12th National Nonpoint Source Monitoring Workshop (September 2004) in Ocean City, Maryland (Don Meals)
    - American Water Resources Association annual conference (November 3, 2004) in Orlando Florida (Dave Braun)

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