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## Significant *Escherichia coli* Attenuation by Vegetative Buffers on Annual Grasslands

Kenneth W. Tate,\* Edward R. Atwill, James W. Bartolome, and Glenn Nader

### ABSTRACT

A study was conducted to estimate the retention efficiency of vegetative buffers for *Escherichia coli* deposited on grasslands in cattle fecal deposits and subject to natural rainfall-runoff conditions. The study was conducted on annual grasslands in California's northern Sierra Nevada foothills, a region with a distinct wet-dry season Mediterranean climate. We used 48, 2.0- by 3.0-m runoff plots to examine the efficacy of 0.1-, 1.1-, and 2.1-m buffers at three land slopes (5, 20, and 35%) and four dry vegetation matter levels (225, 560, 900, and 4500 kg/ha) across 27 rainfall-runoff events during two rainfall seasons. Buffer width treatments were implemented by placement of cattle fecal material containing known loads of *E. coli* 0.1, 1.1, or 2.1 m upslope of the plot runoff collector. Mean total runoff to total rainfall ratio per plot ranged from 0.014:1 to 0.019:1 and reflected the high infiltration capacity of these soils. Approximately 94.8 to 99.995% of total *E. coli* load applied to each plot appears to be either retained in the fecal pat and/or attenuated within 0.1 m downslope of the fecal pat, irrespective of the presence of a wider vegetated buffer. Relative to a 0.1-m buffer, we found 0.3 to 3.1 log<sub>10</sub> reduction in *E. coli* discharge per additional meter of vegetative buffer across the range of residual dry vegetation matter levels, land slope, and rainfall and runoff conditions experienced during this project. Buffer efficiency was significantly reduced as runoff increased. These results support the assertion that grassland buffers are an effective method for reducing animal agricultural inputs of waterborne *E. coli* into surface waters.

PATHOGENIC bacteria and protozoa, such as *Escherichia coli* O157:H7 and *Cryptosporidium parvum*, are waterborne zoonotic infectious diseases of public health concern found on watersheds with intensive and extensive cattle production systems (Atwill et al., 1999, 2003; Jones, 1999; Renter et al., 2004). Water quality and public health protection agencies commonly utilize fecal coliform and generic *E. coli* as indicators of pathogen contamination in freshwater systems. Waterborne transport of microbial pathogens and indicators, and resulting public health risk, is governed by (i) processes that load a watershed with microbial pollutants, (ii) processes that attenuate or inactivate microbial pollutant load, and (iii) the efficiency of hydrologic transport processes which connect terrestrial pollutants to aquatic components of the watershed. One strategy for minimizing the transport

of microbial pathogens and bacterial indicators from animal agricultural operations to surface water is to create vegetated buffer strips between animal manure sources and vulnerable surface water supplies (Young et al., 1980; Dillaha et al., 1989; Castelle et al., 1994; Younos et al., 1998; Schmitt et al., 1999; Dosskey, 2002). The attenuation efficiency of vegetative buffers varies by pollutant (e.g., sediment, nitrate) and depends on site specific factors such as runoff volume, soil properties, and buffer management (Castelle et al., 1994; Schmitt et al., 1999; Bharati et al., 2002; Bedard-Haughn et al., 2004).

Several studies determined that relatively short vegetated buffers can remove substantial amounts of waterborne bovine genotypes of the protozoa *C. parvum* from overland flow generated under simulated or real rainfall conditions (Mawdsley et al., 1996; Tate et al., 2000, 2004a; Atwill et al., 2002; Davies et al., 2004; Trask et al., 2004). *C. parvum* oocysts (eggs) are spherical with a diameter of 4 to 6 μm. Trask et al. (2004) reported recovery in runoff of 0.6 to 27.2% of *C. parvum* applied to grass covered soil chambers with recovery increasing as simulated rainfall rate increased. Davies et al. (2004) reported similar patterns of *C. parvum* transport and retention for vegetated and bare soil conditions on intact soil blocks during simulated rainfall. Atwill et al. (2002) and Tate et al. (2004a) reported 1.0 to 3.0 log<sub>10</sub> reductions in *C. parvum* transport per meter of grass buffer under soil box and simulated rainfall conditions. Results reported from a series of simulated rainfall, irrigation, and/or soil-vegetation condition experiments are mixed on the efficacy of vegetative buffers to attenuate bacteria in runoff downslope of animal manure application. Enteric bacteria of concern are rod-shaped microorganisms ranging in length from 2 to 5 μm and 0.5 to 1.5 μm in width. Buckhouse and Gifford (1976) reported a negative association between distance (m) downslope of cattle fecal material deposits (fecal pats or fecal pies) and surface runoff fecal coliform concentrations on rangeland in Utah. Larsen et al. (1994) reported 83 and 95% reduction in fecal coliforms in surface runoff from 0.61- and 2.13-m grass-sod buffers below fresh cattle fecal material. Coyne et al. (1995) examined the effectiveness of 9.0-m grass buffer strips to attenuate fecal coliforms entrained in surface runoff following poultry waste application, and reported maximum reductions of 43 to 74% for fecal coliform transport. Coyne et al. (1998) reported trapping efficiencies of 75 and 91% for 4.5- and 9.0-m grass vegetated buffers for fecal coliforms in runoff from poultry waste-amended soil. Chaubey et al. (1994) found that 3.0- and 9.0-m grass vegetated buffers failed to significantly reduce fecal coliform in runoff from plots treated with liquid swine manure. Entry et al. (2000) examined the effect

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**Abbreviations:** RDM, residual dry matter; SFREC, Sierra Foothill Research and Extension Center.

of several combinations of vegetation type on total and fecal coliform concentrations in surface and subsurface water following direct pulse flow application of swine wastewater to 4-m-wide  $\times$  30-m-long riparian filter strips in winter, spring, summer, and fall. This study differed from other studies referenced in that the buffer received direct application of the pollutant of concern. The authors concluded that concentrations in the applied wastewater pulse did not decline as the pulse moved downslope in the filter strip regardless of vegetation type or season. Surface runoff was sufficient to overcome infiltration capacity and discharge from study plots only in winter and spring.

Microbial pollution of surface drinking water sources is a significant management and regulatory issue on California's approximately 7 million ha of annual grasslands. These grasslands are used extensively for cattle grazing during the winter rainfall-growing season, providing approximately 75% of the forage produced on California's rangelands. Approximately two-thirds of the state's drinking water reservoirs are located within annual grasslands (Griffin, 1977; Forest and Rangeland Resources Assessment Program, 1988). The Mediterranean climate of California's annual grasslands creates distinct rainfall and dry seasons. As a result, these grassland watersheds generate runoff and transport land-based contaminants during the period November through April (Tate et al., 1999; Lewis et al., 2000). Cattle densities reach their annual peak on these winter foraging areas during this same time period. The objective of this project was to assess the efficacy of annual grassland buffers to attenuate *E. coli* released from cattle fecal material deposits and entrained in surface runoff under natural rainfall-runoff and hillslope conditions.

## MATERIALS AND METHODS

### Study Site

The study site was located on the University of California Sierra Foothill Research and Extension Center (SFREC) 100 km north of Sacramento, CA (39°14'22" N, 121°17'46" W). Elevation at the study site is 350 m. Climate is Mediterranean with cool rainfall seasons (November through April) and hot, dry summers (May through October). Average annual precipitation is 650 mm, with approximately 90% falling October through March (Lewis et al., 2000). Located in the foothills of the Sierra Nevada mountains, topography at SFREC is hilly with land slopes ranging from 5 to 45%. Study site soil is a Sobrante (fine-loamy, mixed, active, thermic Mollic Haploxeralfs)-Timbuctoo (fine, parasesquic, thermic Typic Rhodoxeralfs) gravelly loam complex formed over basic metavolcanic (greenstone) bedrock and classified as Haploxeralfs (Lytle, 1998). This soil extends to a depth of 1.0 to 1.5 m and overlies relatively massive bedrock. Vegetation on the study site is composed of annual grasses and forbs such as annual ryegrass (*Lolium multiflorum* Lam.), wild oats (*Avena fatua* L.), soft chess (*Bromus mollis* L.), and redstem filaree [*Erodium cicutarium* (L.) L'Her].

### Runoff Plots and Surface Runoff Collection

During September 2001 through February 2002, 48 runoff plots designed to capture surface runoff (overland and litter

flow) during natural rainfall-runoff events were established at the study site (Fig. 1). Each runoff plot was 2 m in width (parallel to slope) and 3 m in length (perpendicular to slope), and was bordered with metal flashing inserted 5 cm into the soil, with 5 cm of flashing extending above the soil surface to isolate the plot from external upslope and/or sideslope surface runoff. A 1-m distance was maintained between boundaries of all adjacent plots (16 plot blocks at same land slope) to minimize cross-plot flow and microbial movement. Aluminum and polyvinylchloride runoff collectors were positioned across the bottom of each plot to capture surface runoff and collect it in a sealed polypropylene container, allowing measurement of discharge volume (L) and enumeration of *E. coli* discharge concentration and load. Collectors were designed, tested, and modified accordingly in the field to eliminate incidental collection of rainfall. Collectors were installed to collect all surface runoff occurring above the litter-mineral soil surface interface. There was a 0.1- to 4.0-cm depth of litter across the surface of all plots depending on residual dry vegetation matter treatment. Surface water discharged from each plot during each rainfall event occurring during trials ( $n = 2$ ) conducted 12 Mar. 2003 to 4 May 2003 (2002-2003 rainfall season) and 14 Dec. 2003 to 2 Mar. 2004 (2003-2004 rainfall season) was collected, volume recorded, and *E. coli* discharge determined for each storm event and rainfall season. Runoff samples were stored at 4°C on collection from the field. An automatic recording tipping bucket rainfall gauge was established at the site to record the occurrence, duration, rate, and amount of each rainfall event realized during the study.

## Treatments

### Land Slope

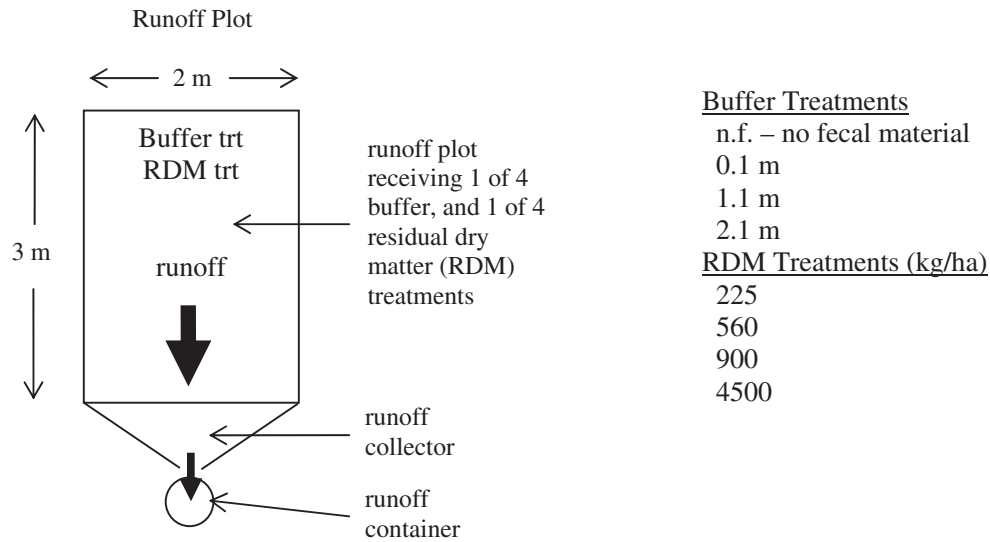
Land slope treatments were implemented by establishing three blocks of runoff plots (16 plots per block) at three locations approximating 5, 20, and 35% slope, respectively (Fig. 1). Actual land slope (%) of each plot was measured following final installation of the plot boundary (metal flashing) and runoff collector. Mean (minimum, maximum, SE mean) plot slope (%) at each block was 6.1 (4.0, 7.5, 0.2), 20.4 (18.0, 24.0, 0.3), and 33.9 (29.0, 42.0, 0.6) for the 5, 20, and 35% land slope blocks, respectively.

### Residual Dry Matter

One of the predominant grazing management recommendations and/or standards on California annual range is the achievement of end of dry season (October) residual dry matter (RDM) levels to promote sustainable forage production and quality levels, soil surface protection from erosion, and other benefits (Bartolome et al., 1980, 2002). Residual dry matter treatments were 225, 560, 900, and 4500 kg/ha. This range mimicked heavy (225 kg/ha) to no grazing (4500 kg/ha) intensity for California annual grasslands. The 225, 560, and 900 kg/ha treatments were implemented annually by hand cutting and removal of vegetation in October. The 4500 kg/ha treatment represented residual dry matter levels without cutting and removal (no grazing). The RDM treatments were implemented annually for 5 yr before this study, as part of an existing experiment examining plant community response to long-term, consistent RDM treatments. Four replicates of each RDM treatment were randomly established and maintained annually in each slope block (Fig. 1).

### Buffer Width

Within each block of plots, three of the four replicate plots (plots with same slope and residual dry matter) were spiked



5% land slope block, randomized buffer and RDM treatment layout (16 runoff plots)

2.1m 900	1.1m 560	0.1m 4500	n.f. 560	0.1m 225	1.1m 4500	2.1m 560	n.f. 900	0.1m 560	2.1m 225	2.1m 4500	0.1m 900	n.f. 225	1.1m 900	1.1m 225	n.f. 4500
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20% land slope block, randomized buffer and RDM treatment layout (16 runoff plots)

n.f. 225	2.1m 225	2.1m 4500	1.1m 900	0.1m 560	n.f. 4500	n.f. 900	1.1m 225	n.f. 560	0.1m 4500	0.1m 900	0.1m 225	2.1m 560	1.1m 560	1.1m 4500	2.1m 900
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35% land slope block, randomized buffer and RDM treatment layout (16 runoff plots)

0.1m 560	0.1m 225	n.f. 4500	n.f. 560	2.1m 900	1.1m 560	1.1m 4500	0.1m 900	2.1m 4500	2.1m 225	1.1m 900	n.f. 225	0.1m 4500	2.1m 560	n.f. 900	1.1m 225
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**Fig. 1.** Runoff plot design and layout of buffer and residual dry matter (RDM) treatments for 5, 20, and 35% land slope treatment blocks.

with cattle fecal material containing generic *E. coli* at either 0.1, 1.1, or 2.1 m upslope from the collector to evaluate the effect of buffer width on *E. coli* pollutant discharge (Fig. 1). The 0.1-m buffer treatment represented almost direct hydrologic linkage between fecal material and the runoff collector (no buffer). A no fecal material application treatment was included on the fourth residual dry matter replicate in each block as a background control, and is considered in this paper as one level of the buffer treatment (0.1 m, 1.1 m, 2.1 m, and no feces control). Thus, each combination of RDM and buffer treatment was present in each slope block. Buffer treatments were allocated in a stratified random manner (random among RDM treatment replicate plots within each block).

#### Fecal Material and *E. coli* Load Application

There were two trials conducted during the course of this experiment. The 2002–2003 rainfall season trial occurred from the period 12 Mar. 2003 to 4 May 2003, and the 2003–2004 rainfall season trial occurred from the period 14 Dec. 2003 to 2 Mar. 2004 (Fig. 2). During the 2002–2003 rainfall season trial fresh cattle fecal material was applied to all plots on 12 Mar. 2003 (2002–2003 rainfall season spike). During the 2003–2004 rainfall season trial fresh cattle fecal material was applied to all plots on 14 Dec. 2003, 14 Jan. 2004, and 8 Feb. 2004 (2003–2004 rainfall season spike) (Fig. 2). Generic *E. coli* is commonly

present in high concentrations in fresh cattle fecal material. For each application event, a sufficient volume of fresh cattle fecal material was hand collected directly from the rectums of cattle into several large containers and thoroughly mixed, and 1-kg loads were randomly allocated for application to each plot. Mean *E. coli* concentration in feces used for each application was determined for every other 1.0-kg spike ( $n = 36$ ) by dispersing randomly collected aliquots of 1.0 g of feces in 39 mL of phosphate buffered solution (PBS) using a rotational mixer for 5 min. The feces–PBS solution was then serially diluted ( $10^1$ ,  $10^2$ ,  $10^3$ ). The *E. coli* concentration in diluted feces–PBS solution was determined by direct membrane filtration and culturing onto CHROMagar EC (Chromagar Microbiology, Paris, France) at 44.5°C for 24 h (American Public Health Association, 1989).

Table 1 reports total *E. coli* load (cfu) applied to plots for each rainfall season. Livestock were excluded from the study site to eliminate incidental fecal deposition within or upslope of plots. With the exception of the no fecal material application plots, each plot received two 1-kg doses (approximately 15-cm-diameter by 7-cm-deep fecal pats) of fresh cattle fecal material on each application date. Fecal material was placed in the center of the plot 0.1, 1.1, or 2.1 m upslope of the collector. Multiple fecal material applications were required during the 2003–2004 rainfall season to maintain observable concentrations of *E. coli* across the entire runoff season. The single

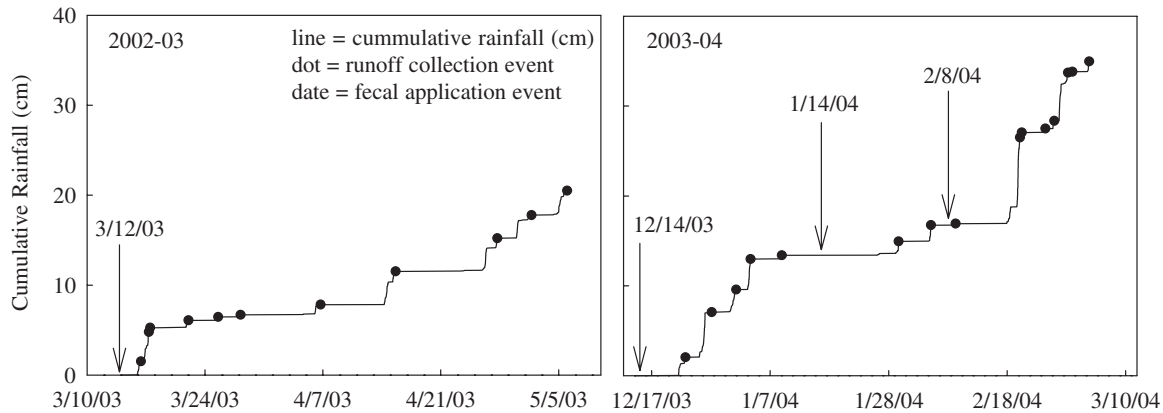


Fig. 2. Cumulative rainfall (cm), storm runoff collection timing, and fecal material application dates during the portions of the 2002 rainfall season (12 Mar. 2003 to 4 May 2003) and 2003 rainfall season (14 Dec. 2003 to 2 Mar. 2004) included in the study period.

application late in the 2002–2003 rainfall season (12 Mar. 2003) was sufficient to maintain observable concentrations until the end of that rainfall season (Fig. 2).

### Enumeration of *E. coli* Concentration in Runoff

The *E. coli* concentration (cfu/100 mL) in surface runoff water was determined by direct membrane filtration and culturing the membrane onto CHROMagar EC at 44.5 C for 24 h (American Public Health Association, 1989). We observed that runoff water turbidity (ntu) was closely linked to transport of suspended and dissolved solids (and thus microbial pollutants) from feces on these plots. Thus, we estimated sample dilution requirements before filtration based on sample turbidity. Aliquots of several volumes ranging from 1 to 100 mL were directly filtered for samples with turbidity of <100 ntu. Turbid samples (>100 ntu) were serially diluted ( $10^3$ ,  $10^4$ ,  $10^5$ ,  $10^6$ ) and all dilutions filtered and cultured.

### Adjustment of *E. Coli* Concentration for Variable Hold Times

Variation in time elapsed from surface runoff sample collection and sample processing (hold time) was inherent in this project due to differences in time of collection and time of processing caused by storm to storm variation in storm duration and storm end time. Standard methods require either (i) sample processing within 6 to 24 h post collection or (ii) development of a quantitative method to adjust sample concentrations for inconsistency in sample hold time (American Public Health Association, 1989). We developed a quantitative relationship between hold time (6, 24, 48, 72 h) and *E. coli* concentration (cfu/100 mL) to adjust all concentrations to a 24-h hold time from the mid-point of each storm event. Sample hold times in this study ranged from 4 to 72 h.

To develop this relationship, surface runoff water was collected from six plots (two from each block) with a no fecal material application treatment and thoroughly mixed into a single container. Background *E. coli* concentration was determined via direct membrane filtration and culturing as described for runoff water analysis. Following culturing, several *E. coli* colonies were randomly selected from the membranes, suspended, and dispersed in 40 mL of PBS via rotational mixing for 15 min. The concentration of this 40-mL *E. coli*–PBS solution was then determined via serial dilution and direct membrane filtration. Following enumeration of the *E. coli*–PBS solution concentration, we used serial dilution of aliquots from this solution to generate aliquots with concentrations of approximately  $10^2$ ,  $10^4$ ,  $10^6$ , and  $10^7$  cfu/100 mL. These concentrations represent the range observed during the course of the study. Five replicates of each concentration were developed for each of four hold times (6, 24, 48, and 72 h) for a total of 80 aliquots. Within a concentration group, each aliquot was randomly assigned to a hold time group. Individual aliquots were then held at 4°C and processed (appropriately diluted, filtered, and cultured at 44.5°C) at 6, 24, 48, or 72 h post generation according to their hold time assignment.

### Statistical Analyses

#### Analysis for *E. coli* Hold Time Adjustment

A linear mixed effects analysis (Pinheiro and Bates, 2000) was used to generate the time-dependent decay coefficient(s) for *E. coli* in our source water. The  $\log_{10}$  concentration of *E. coli* was used as the outcome variable, dose and time (i.e., duration in hours between water collection in the field and *E. coli* enumeration in the laboratory) were set as fixed effects or covariates, and replicates within a group were set as repeated measures or a group random effect to control for lack

Table 1. Total duration, storm number, rainfall, and *E. coli* load-discharge statistics for the 2002–2003 (12 Mar. 2003 to 4 May 2003) and 2003–2004 (14 Dec. 2003 to 2 Mar. 2004) rainfall seasons.

Rainfall season	Duration <sup>†</sup>	Storms	Total rainfall	<i>E. coli</i>	
				Load <sup>‡</sup>	Discharge <sup>§</sup>
	d		mm	cfu/plot	
2002–2003	53	11	205	$1.27 \times 10^{10}$	$2.27 \times 10^7$
2003–2004	79	16	349	$2.88 \times 10^{10}$	$4.36 \times 10^6$

<sup>†</sup> Days elapsed between placement of fecal material on plots and last storm sample collected for each year.

<sup>‡</sup> *E. coli* load applied to each plot in cattle fecal material during each rainfall season.

<sup>§</sup> Calculated as the mean *E. coli* discharge of all 48 plots for each season.

of independence of *E. coli* concentration within replicate samples. A group was comprised of five replicate samples for each dosage and time. In addition, given marked heteroscedasticity of the error term across different strata of the *E. coli* dosage, different variance terms were fitted for each dosage (Pinheiro and Bates, 2000). Level of significance for the covariate terms was set at a *P* value of <0.05, based on a conditional *t* test.

To adjust the *E. coli* concentration in each water sample tested *x* hours ( $t = x$ ) after initial time of collection ( $t = 0$ ) to a single 24-h standard ( $t = 24$ ), we first assumed the following basic model:

$$\log_{10}(EC_{t=x}) = \log_{10}(EC_{t=0}) + \beta(t = x) \quad [1]$$

where  $\log_{10}(EC_{t=x})$  was the observed  $\log_{10}$  concentration of *E. coli* determined *x* hours ( $t = x$ ) after initial time of collection,  $\log_{10}(EC_{t=0})$  was the modeled  $\log_{10}$  concentration of *E. coli* at the initial time of collection ( $t = 0$ ), and  $\beta(t = x)$  was the fitted decay coefficient(s) generated by the linear mixed effects model described above. The term  $\beta(t = x)$  was allowed to be a univariate or polynomial term depending on whether the raw data signified a first or second-order time-dependent decay process for the *E. coli* concentration in our source water. The decay process was for water samples held at approximately 4°C. Once  $\beta(t = x)$  was obtained, Eq. [2] was used to adjust each sample to a single 24-h standard ( $t = 24$ ):

$$EC_{t=24} = (EC_{t=x})10^{\beta(24-x)} \quad [2]$$

where  $EC_{t=24}$  was the fitted or expected concentration of *E. coli* at a 24-h standard,  $EC_{t=x}$  was the observed concentration of *E. coli* determined *x* hours ( $t = x$ ) after initial time of collection, and  $10^{\beta(24-x)}$  was the expected decay coefficient adjustment factor raised to the power of 10 which allowed us to model concentrations of *E. coli* directly instead of  $\log_{10}$  values.

### Analysis of Treatment Effect

Linear mixed effects analysis was used to determine the effect of land slope, residual dry vegetative matter, runoff parameters, and buffer width on *E. coli* discharge and thus buffer attenuation efficacy ( $\log_{10}$  reduction) per rainfall season ( $n = 2$ ). This is an analysis approach we have employed successfully in similar experiments to evaluate vegetated buffer efficacy for attenuating *C. parvum* (Atwill et al., 2002) and nitrogen (Bedard-Haughn et al., 2004). A forward stepping approach was used to develop a final model, with *P* < 0.10 set as the criterion for inclusion of a variable in the final model. Final model coefficients were estimated using restricted maximum likelihood, and *P* value for each coefficient was estimated using the conditional *t* test (Pinheiro and Bates, 2000). Plot identity was set as a group effect to account for potentially correlated data induced by repeated measures on the same experimental unit (plot).

The dependent variable was total *E. coli* colony forming units (cfu) discharged per plot per rainfall season. The raw discharge data were transformed [ $\log_{10}(1 + \text{cfu})$ ] to help normalize and account for heteroscedasticity in residuals. Sample size was 96 (48 plots  $\times$  2 rainfall seasons). Total rainfall season *E. coli* discharge per plot was calculated as the sum of discharges for each storm event of the rainfall season for each plot. Storm event *E. coli* discharge per plot was calculated as the product of concentration (cfu/100 mL) and storm event runoff (L) for each plot. All calculations and analysis were conducted on data adjusted for hold time. Independent variables introduced in the forward stepping analysis were land slope (%), residual dry matter (kg/ha), buffer treatment (0.1 m, 1.1 m, 2.1 m, no fecal material application), rainfall

season (2002–2003, 2003–2004), total runoff (L) per plot per rainfall season, and maximum event runoff (L) per plot per rainfall season. The quadratic form of residual dry matter ( $\text{RDM}^2$ ), land slope ( $\text{land slope}^2$ ), total runoff ( $\text{total runoff}^2$ ), and maximum runoff event ( $\text{maximum runoff event}^2$ ) were also tested for inclusion into the final model. All two-way interactions of each independent variable were tested for inclusion in the final model.

## RESULTS

### Adjustments to the Observed *E. coli* Concentrations

Figure 3 illustrates the relationship between  $\log_{10}$  *E. coli* concentration and hold time (sample age) for hold times ranging from 6 to 72 h. The  $\log_{10}$  concentration of *E. coli* followed a first order decay process, such that the decay coefficient  $\beta$  from the expected decay coefficient adjustment factor raised to the power of 10 [i.e.,  $10^{\beta(24-x)}$  in Eq. [2]] was  $-0.00356$  (95% CI,  $-0.0054$ ,  $-0.0017$ ), with units of time in hours. The *P* value was >0.05 for an interaction term between time and *E. coli* concentration, indicating that a single decay coefficient could be used for adjusting *E. coli* concentrations at  $t = x$  to a 24-h standard ( $t = 24$ ) for all samples collected in this study, regardless of concentration. The value for this decay coefficient ( $-0.00356$ ) signifies that for each additional hour of holding time at 4°C, the concentration of *E. coli* in our source water declined by about 0.8%.

### Weather Conditions, Storm Events, and Runoff

Figure 2 reports surface runoff collection dates and cumulative rainfall for the 2002–2003 and 2003–2004 rainfall seasons from first fecal application date until the last runoff generating storm of the season. Eleven rainfall-

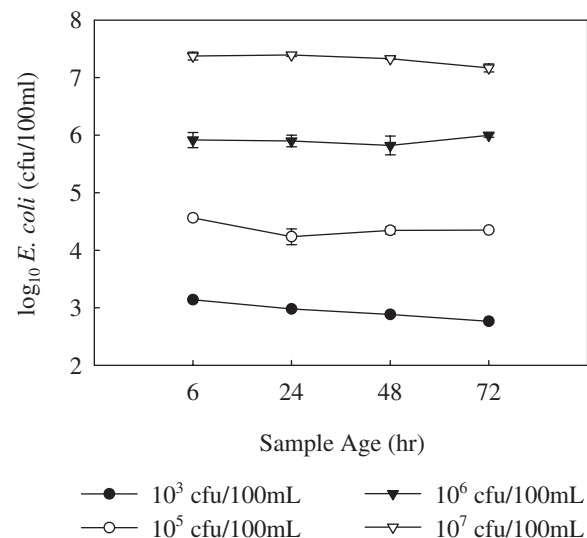


Fig. 3. Change in mean concentration of *E. coli* (cfu/100 mL) in surface water runoff samples held for 6, 24, 48, and 72 h from collection (time = 0 h) before processing. Data points represent the mean concentration of five replicates with an initial concentration of  $10^3$ ,  $10^4$ ,  $10^6$ , and  $10^7$  cfu/100 mL, respectively. Error bars report one standard error of the mean.

**Table 2. Storm event duration, rainfall amount, rainfall rate statistics for the storms occurring during the 2002–2003 (12 Mar. 2003 to 4 May 2003) and the 2003–2004 (14 Dec. 2003 to 2 Mar. 2004) rainfall seasons.**

Rainfall season	Statistic†	Rainfall		
		Total‡	Duration§	Rate¶
		mm	h	mm/h
2002–2003	minimum	2.29	0.28	0.33
	mean	18.24	14.42	2.71
	maximum	36.58	39.48	16.14
2003–2004	minimum	1.52	0.22	0.32
	mean	21.48	8.90	4.31
	maximum	76.96	21.93	12.24

† Minimum, mean, and maximum observed across 11 and 16 storm events during the 2002–2003 and 2003–2004 rainfall seasons, respectively.

‡ Total storm event rainfall amount.

§ Duration of rainfall during individual storm events in hours.

¶ Mean storm event rainfall calculated as rainfall amount (mm) divided by rainfall duration (h).

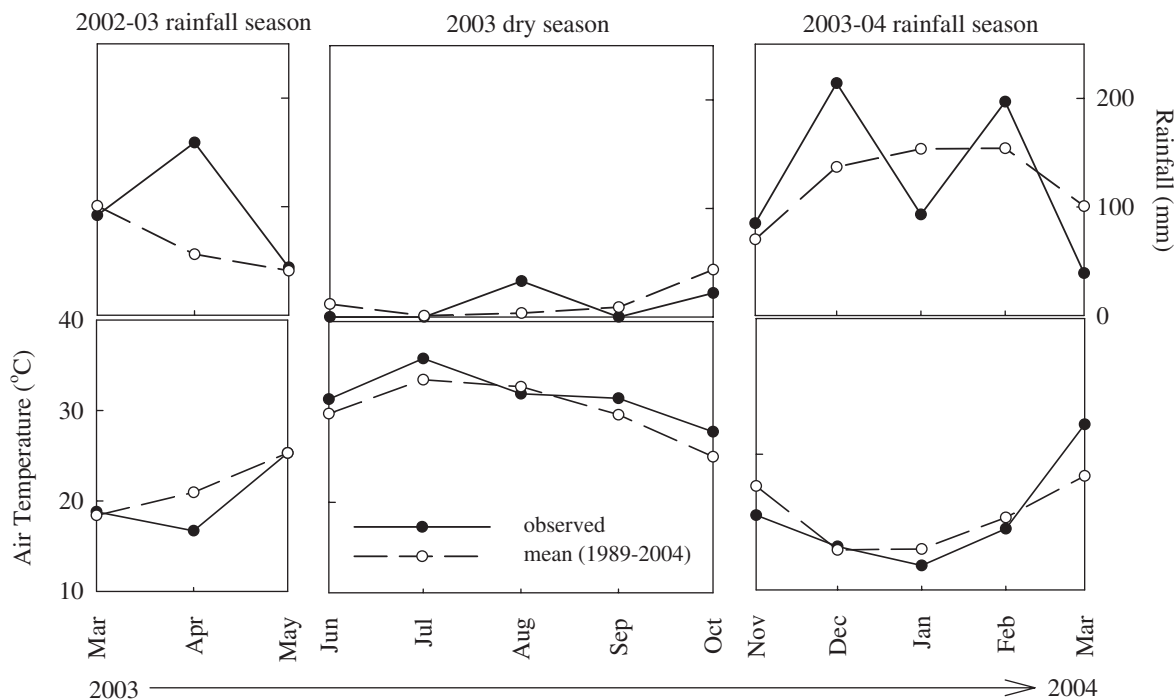
runoff events were realized and collected following fecal application on 12 Mar. 2003 for the 2002–2003 rainfall season, and 16 events were captured over the course of the 2003–2004 rainfall season following the 14 Dec. 2003 fecal application (Table 1). Table 2 reports summary statistics for storm event rainfall totals, durations, and rates realized during the study period. Figure 4 displays monthly rainfall and maximum daily air temperature realized during the 2002–2003 rainfall season, the 2003–2004 rainfall season, and the intervening 2003 dry season which occurred during this study. Mean monthly rainfall and maximum daily air temperature from the long-term record (1989–2004) at SFREC are also included for reference. Rainfall during April 2003, December 2003, and February 2004 was above average, while rainfall

during January 2004 was below average. Air temperature realized during the study period was representative of mean conditions.

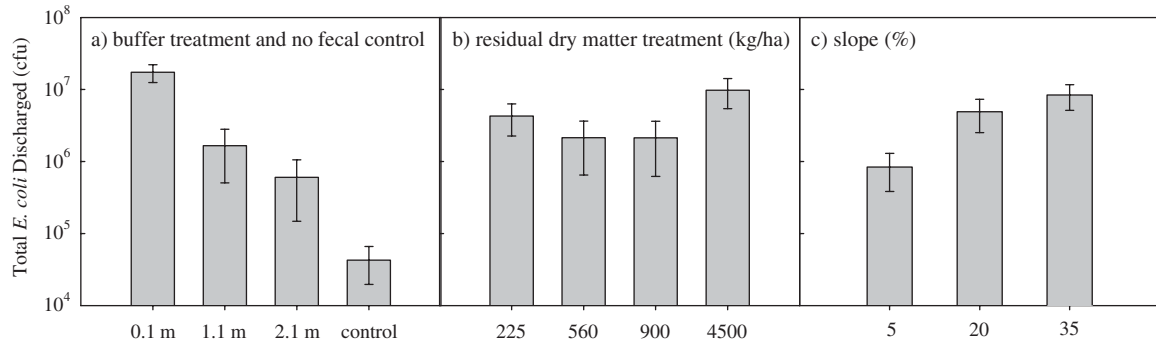
During the 2002–2003 rainfall season total surface runoff ranged from 0.4 to 50.0 L/plot, with a mean of 16.7 L/plot. Maximum single storm event runoff ranged from 0.3 to 14.1 L/plot, with a mean of 5.2 L/plot. During the 2003–2004 rainfall season total surface runoff ranged from 9.7 to 116.2 L/plot, with a mean of 40.4 L/plot; maximum single storm event runoff ranged from 2.6 to 17.0 L/plot, with a mean of 13.2 L/plot. On average, the maximum single storm event runoff volume accounted for 37.0 and 39.3% of total plot runoff during the 2002–2003 and 2003–2004 rainfall seasons, respectively. On average, each plot received a cumulative rainfall volume of 1230 (200 × 300 × 20.5 cm) and 2094 L (200 × 300 × 34.9 cm) during the 2002–2003 and 2003–2004 rainfall season, respectively. Thus, the mean total runoff to total rainfall ratio per plot for each season was 0.014:1 and 0.019:1, respectively. These relatively low runoff-to-rainfall ratios reflect the high infiltration capacity of these soils, the predominance of shallow subsurface flow paths and variable source areas in stream flow generation on these watersheds, and the relatively low intensity (mm/h) of frontal storm events typical of this region of California (Lewis et al., 2000).

### *E. coli* Discharge Patterns

Table 1 reports overall mean *E. coli* discharge per plot per rainfall season, calculated as the mean discharge of all 48 plots for each rainfall season. During the 2002–2003 rainfall season the percent of total *E. coli* load



**Fig. 4. Total rainfall (mm) and mean maximum daily temperature (°C) observed for each month of the 2002–2003 rainfall season, the 2003 dry season, and the 2003–2004 rainfall season with long term monthly means for the period of record (1989 through 2004) at the U.C. Sierra Foothill Research and Extension Center official weather station (California Irrigation Management Information System, Station #84).**



**Fig. 5.** Mean total *E. coli* (cfu) discharged from 48, 2.0-m-wide by 3.0-m-long annual grassland runoff plots across the 2002–2003 and 2003–2004 rainfall seasons following application of a total of  $41.5 \times 10^9$  *E. coli* (cfu) in cattle fecal material. (a) Mean discharge for buffer treatments of 0.1-m buffer, 1.1-m buffer, 2.1-m buffer, and a no fecal application negative control; calculated by combining rainfall season, land slope, and residual dry matter treatments. (b) Mean discharge for residual dry vegetation matter in October treatments of 225, 560, 900, and 4500 kg/ha; calculated by combining rainfall season, land slope, and buffer treatments. (c) Mean discharge for land slope treatments of 5, 20, and 35%; calculated by combining rainfall season, buffer, and residual dry matter treatments. Error bars report one standard error of the mean.

applied per plot that discharged as surface runoff ranged from 0.00 to 5.20%, with an arithmetic mean of 0.22%. During the 2003–2004 rainfall season the percent of total *E. coli* load applied per plot that discharged as surface runoff ranged from 0.00 to 0.23%, with an arithmetic mean of 0.02%. These numbers indicate that a significant portion, conservatively >90%, of the microbial pollutant load applied to each plot was either retained within the cattle fecal material, filtered by vegetative litter and soil surface organic matter, or entered the soil profile via infiltration.

Figure 5 reports mean *E. coli* discharge per plot per season for buffer, RDM, and land slope treatments. Buffer treatment means reported in Fig. 5 were calculated as the mean of all 48 plots by combining rainfall season, residual dry matter treatment, and land slope treatment. Residual dry matter treatment means reported in Fig. 5 were calculated as the mean of all 48 plots by combining rainfall season, buffer treatment, and land slope treatment. Land slope treatment means reported in Fig. 5 indicate an apparent reduction in total *E. coli* discharge as buffer width increases from 0.1 to 1.1 to 2.1 m. *E. coli* were discharged from plots with no cattle fecal application, indicating that background levels were not zero. *E. coli* discharge also appears to increase as land slope increases from 5 to 35%, but these mean raw-data values are weighted heavily toward the data generated by the 0.1-m plots. *E. coli* discharge decreases as RDM level increases from 225 to 900 kg/ha, but *E. coli* discharge then appeared to increase as RDM level rises to 4500 kg/ha (no vegetation removal control).

### Analysis of Effects of Buffer Width and Associated Covariates on *E. coli* Discharge

Table 3 reports the final linear mixed effects analysis identifying significant relationships between *E. coli* discharge,  $\log_{10}$  reductions, and the various plot treatments. Figure 6 plots observed *E. coli* discharge [ $\log_{10}(\text{cfu} + 1)$

transformed] against values predicted by the linear mixed effects model reported in Table 3, allowing evaluation of model fit. Figures 7 and 8 graphically display *E. coli* discharges and  $\log_{10}$  reductions as a function of buffer width, total plot runoff, land slope, and RDM. Due to the  $\log_{10}$  transformation, coefficients reported in Table 3 can be directly interpreted as  $\log_{10}$  changes in annual *E. coli* discharge associated with each factor.

Water quality data from the 0.1-m buffer plots indicates that 94.8 to 99.995% of total *E. coli* load applied

**Table 3.** Linear mixed effects model for the effect of buffer width and associated covariates on discharge of *E. coli* from 2.0-m-wide by 3.0-m-long annual grassland runoff plots across 11 and 16 storm events during the 2002–2003 and 2003–2004 rainfall seasons, respectively.

Factor	Coefficient†	95% CI	P value‡
<b>Rainfall season</b>			
2002–2003§	0.00	–	–
2003–2004	–1.06	(–1.76, –0.37)	0.003
<b>Buffer treatment</b>			
0.1 m§	0.00	–	–
1.1 m	–2.79	(–3.97, –1.60)	<0.001
2.1 m	–3.60	(–4.86, –2.34)	<0.001
Control	–4.55	(–5.81, –3.50)	<0.001
<b>RDM (kg/ha)</b>	–0.002	(–0.0034, –0.0004)	0.017
<b>RDM<sup>2</sup> (kg/ha)</b>	$3.8 \times 10^{-7}$	( $6.9 \times 10^{-8}$ , $6.9 \times 10^{-7}$ )	0.017
<b>Land slope (%)</b>	0.03	(0.008, 0.059)	0.01
<b>Total runoff (L)</b>	–0.004	(–0.032, 0.024)	0.76
<b>Maximum runoff event (L)</b>	0.12	(0.046, 0.2)	0.002
<b>Buffer × total runoff (RO)</b>			
0.1 m × RO§	0.00	–	–
1.1 m × RO	0.037	(0.003, 0.073)	0.04
2.1 m × RO	0.031	(–0.003, 0.065)	0.07
Control × RO	0.034	(0.004, 0.064)	0.02
<b>Intercept</b>	6.32	(5.02, 7.62)	<0.001

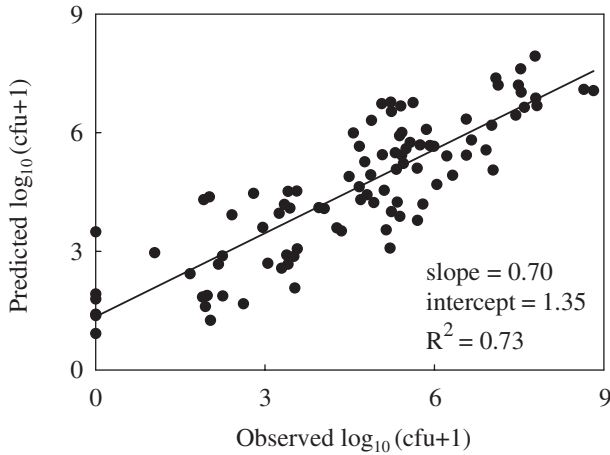
† Total cfu of *E. coli* discharged per plot across all storms occurring during each rainfall season of the study period (2002–2003 and 2003–2004) was set as the dependent variable; year, buffer, residual dry matter, land slope, and runoff were set as fixed and independent effects; plot identity set as a group effect to account for repeated measures. Coefficients are for transformed total *E. coli* discharge [ $\log_{10}(\text{cfu} + 1)$ ].

‡ Significance (coefficient not equal to 0) was determined at a P value of  $\leq 0.10$ , using a conditional t test.

§ Referent condition to which other levels of the categorical factor were compared.

|| Negative control plots to which no cattle fecal material with *E. coli* was applied.



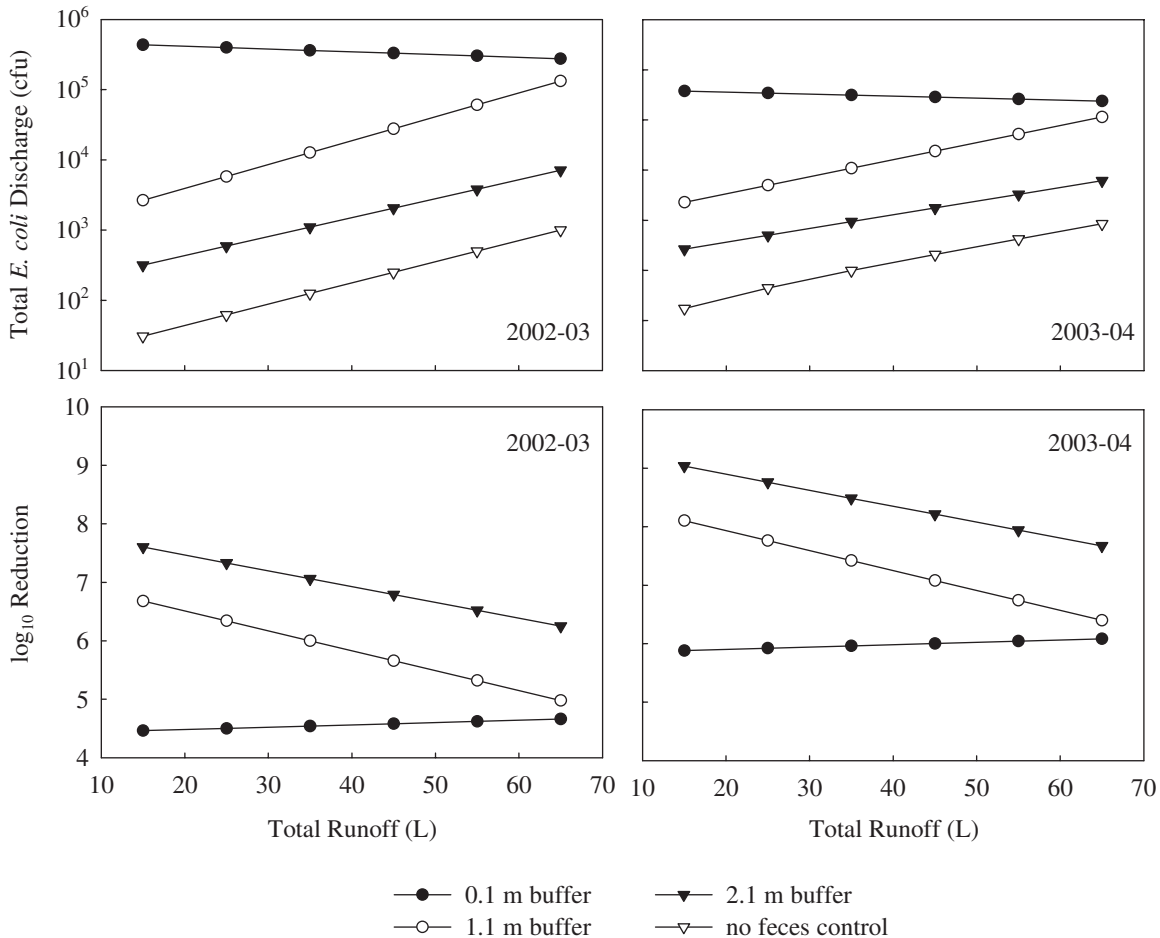


**Fig. 6.** Observed total *E. coli* [ $\log_{10}(\text{cfu} + 1)$ ] discharged from annual grassland runoff plots versus values predicted by linear mixed effects model which contained year, buffer width, residual dry vegetative matter, land slope, and runoff as independent factors.

to each plot appears to be either retained in the fecal pat and/or in the narrow 10 cm of soil for the duration of the storm season, irrespective of the presence of a wider

vegetated buffer. The significant interaction between buffer width and total runoff per plot per rainfall season indicates that the effect of buffer width on *E. coli* discharge was in part dependent on total runoff (Table 3). *E. coli* attenuation for 1.1- to 2.1-m buffer widths compared to 0.1-m buffer width ranged from an additional 2.22 to 0.31  $\log_{10}$  reduction, respectively, as total plot runoff increased from 15 to 65 L (Fig. 6). *E. coli* discharge from no feces control plots ranged from 4.15 to 2.44  $\log_{10}$  lower than 0.1-m buffer plots as total plot runoff increased from 15 to 65 L. Figure 7 illustrates that as total runoff increases, the  $\log_{10}$  reductions in waterborne *E. coli* for wider buffers are substantially reduced and begin to approach the microbial water quality from plots having no effective buffer (0.1 m). *E. coli* discharge was also significantly associated with maximum single storm event runoff volume such that each 1-L increase in maximum runoff event runoff resulted in a 0.12  $\log_{10}$  (approximately 32%) increase in *E. coli* discharge (Table 3).

*E. coli* discharge was significantly related to RDM levels. The relationship is quadratic in nature as evident by the significance of the quadratic residual dry matter term ( $\text{RDM}^2$ ) in the model. Figure 8 illustrates that



**Fig. 7.** Relationship of total *E. coli* discharge (cfu) and  $\log_{10}$  reduction in discharge due to buffer width and total runoff for storm series observed during portions of the 2002–2003 and 2003–2004 rainfall seasons contained in the study period. Slope and residual dry matter level set to 20% and 900 kg/ha, respectively.

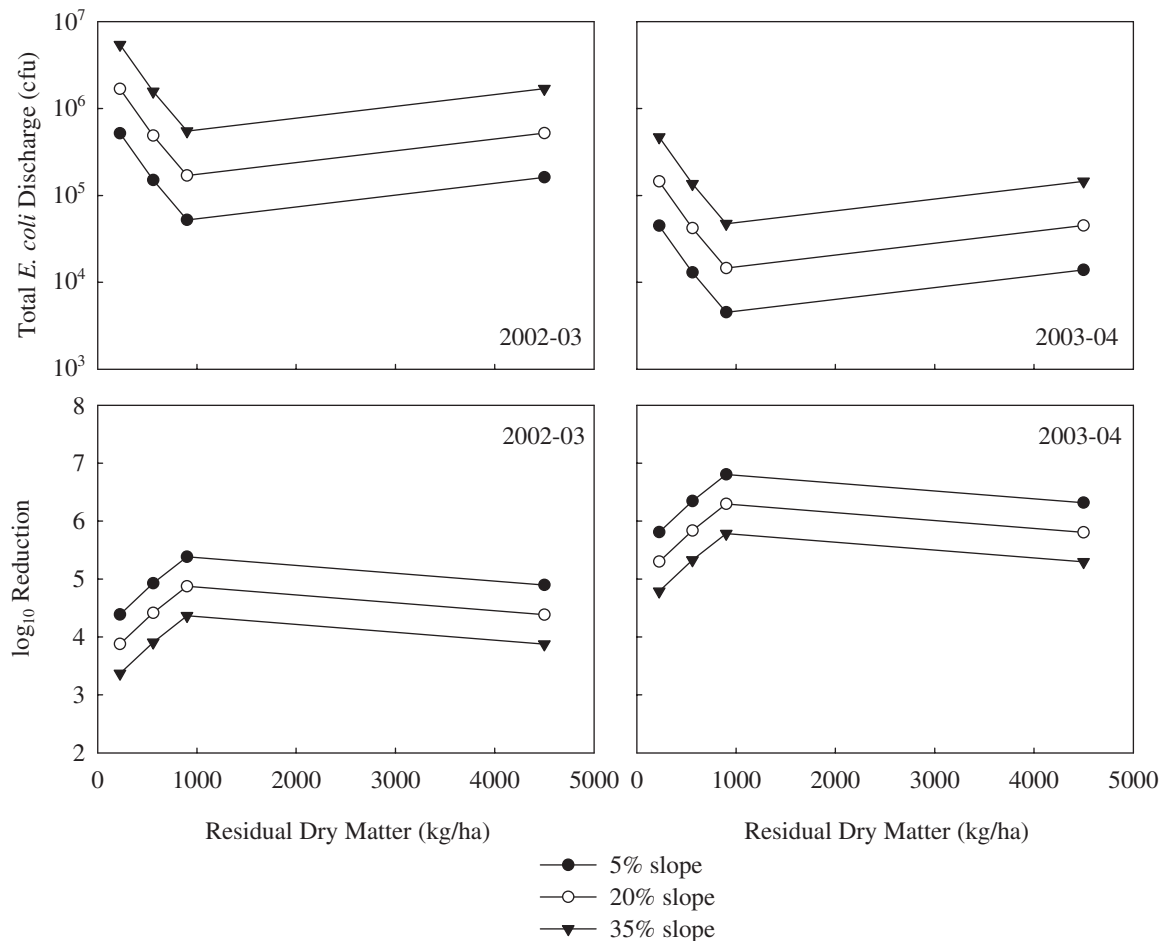


Fig. 8. Relationship of total *E. coli* discharge (cfu) and log<sub>10</sub> reduction in discharge due to residual dry vegetation matter and land slope for storm series observed during portions of the 2002–2003 and 2003–2004 rainfall season contained in the study period.

*E. coli* discharge decreased as RDM increased from 225 to 900 kg/ha, but that *E. coli* discharge increased as RDM then increased to 4500 kg/ha. *E. coli* discharge increased 0.03 log<sub>10</sub> (approximately 7%) with each percent increase in land slope. *E. coli* discharge was –1.06 log<sub>10</sub> less (91% reduction) during the 2003–2004 rainfall season compared to the 2002–2003 rainfall season (Table 3).

## DISCUSSION

These results demonstrate the significant capacity of vegetative buffers to attenuate waterborne *E. coli* deposited in cattle fecal material (fecal pats) on annual grasslands under natural rainfall and hillslope conditions. Relative to the 0.1-m buffer, it is reasonable to expect 0.3 to 3.1 log<sub>10</sub> reduction in *E. coli* discharge per additional meter of vegetative buffer across the range of residual dry vegetation matter, land slope, and rainfall and runoff conditions experienced during this project. Moreover, water quality data from the 0.1-m-wide buffer plots demonstrate that the majority of *E. coli* (94.8 to 99.995%) appears to be retained in the fecal material for the duration of the rainfall season, irrespective of the presence of a vegetated buffer. Hence, technical risk

analyses regarding minimum buffer widths for expected environmental loading rates of these microorganisms from livestock operations should be adjusted accordingly (i.e., >90% of *E. coli* deposited on the terrestrial component of the landscape in cattle fecal pats will not be entrained in overland flow, presenting minimal waterborne disease risk to downstream communities).

Under natural hillslope and rainfall-runoff conditions we found grassland buffers to be relatively more efficient at attenuating *E. coli* than reported in much of the existing literature on bacterial indicators (Larsen et al., 1994; Chaubey et al., 1994; Coyne et al., 1995, 1998; Entry et al., 2000). This is not unreasonable given that many of the existing studies purposely simulated worst case rainfall-runoff and microbial transport scenarios. We found that buffer efficacy for *E. coli* attenuation declined as total runoff volume increased (i.e., approaching potential worst case microbial transport conditions where buffers can fail). Many of the published studies examined liquid and solid animal waste product application at rates significantly higher than typically found on the extensively grazed annual grasslands represented in our study. Variable reporting of load versus concentration discharge reductions also complicates direct comparison of study results. The results of this project agree with recent

studies of the transport and attenuation of the pathogenic protozoa *C. parvum* conducted under simulated rainfall and soil-vegetation conditions (Atwill et al., 2002; Davies et al., 2004; Tate et al., 2004a; Trask et al., 2004). Atwill et al. (2002) reported a 2 to 3 log<sub>10</sub> mean reduction in surface and shallow subsurface waterborne *C. parvum* oocysts using a 1-m-long soil box with 85 to 99% grass cover and under conditions of 5 to 20% land slope, repacked soil from the same study site where this project was conducted (i.e., SFREC site, loam soil), and rainfall rates of 15 or 40 mm/h. In a soil box experiment using soil and vegetation typical of southern Sierra Nevada annual grasslands, Tate et al. (2004a) observed mean log<sub>10</sub> reductions of *C. parvum* per meter of vegetated buffer of 1.44, 1.19, and 1.18 for buffers set at 5, 12, and 20% land slope, respectively. Rainfall application rate (mm/h) in this soil box study was strongly associated with oocyst discharge from these vegetated buffers, resulting in a decrease of 2 to 4% in the log<sub>10</sub> reduction per meter buffer for every additional mm/h of rainfall applied to the soil box.

Runoff volume and associated hydrologic transport capacity is an important factor determining vegetative buffer efficiency and microbial discharge on these annual grasslands. Due to the inherently high infiltration capacity of these soils and the low intensity frontal system storm events typical of the region, the ratio of total runoff to total rainfall is exceedingly low. Surface runoff is generated on these plots when sufficient rainfall has occurred to create saturated antecedent soil conditions. Once these conditions are achieved, significant runoff and nonpoint-source pollutant transport can occur during single storm events (Tate et al., 1999; Lewis et al., 2000). Maximum storm event runoff per rainfall season accounted for almost 40% of total plot runoff per season, and was a significant factor determining *E. coli* discharge and conditions when buffers can be expected to fail. Total plot runoff per rainfall season was positively associated with *E. coli* discharge. The significant interaction between buffer width and total runoff volume in determining *E. coli* discharge illustrates that buffer efficiency (log<sub>10</sub> reduction) is diminished as hydrologic transport capacity increases (Fig. 7). Surface hydrologic transport capacity is a limiting factor in the discharge of microbial pollutants from these annual grassland sites, with episodic large transport events likely accounting for the majority of microbial discharge across a rainfall season. This limited surface hydrologic transport potential is also likely a major mechanism for the relatively high efficiency of these annual grasslands to buffer microbial pollutants in surface runoff during the majority of storm events.

Lower overall *E. coli* discharge in 2003–2004 compared to 2002–2003 is potentially due to differences in timing of fecal material application relative to the subsequent occurrence of storm events (Fig. 2). Kress and Gifford (1984) report significant reductions in *E. coli* release from cattle fecal pats as pat age increased from 2 to 100 d. Preliminary examination of storm event *E. coli* discharge data from this project (data not shown) indicates that *E. coli* discharge decreased as fecal mate-

rial age increased. Differences in event rainfall intensity and duration also exist between years (Table 2).

Residual dry vegetation matter was a significant factor in determining *E. coli* discharge (Fig. 8). Recommended RDM levels for the study site would range from 500 to 900 kg/ha depending on slope. *E. coli* transport decreased as RDM increased from 225 to 900 kg/ha, but increased as RDM increased to 4500 kg/ha. The reduction in *E. coli* transport as RDM increased from 250 to 900 kg/ha could be due to reductions in soil surface infiltration capacity for low RDM level treatments applied over the five years previous to this study. In annual grasslands in the southern Sierra Nevada foothills, Tate et al. (2004b) found that soil surface bulk density, a measure of soil compaction and inversely correlated to infiltration capacity, was significantly increased by 0.08, 0.18, and 0.21 g/cm<sup>3</sup> at sites with long-term (15 yr) RDM levels of >1100, 670 to 900, and <450 kg/ha compared to sites not grazed by cattle for >26 yr. It is further possible that the mechanism behind the increase in *E. coli* as RDM levels increase from 900 to 4500 kg/ha is ecological rather than hydrological. At the 4500 kg/ha RDM level there is significant accumulation of vegetation and litter that moderates fluctuations in temperature and moisture, thereby providing a relatively moist, cool, nutrient rich environment in which *E. coli* could survive and multiply. Increased survival and replication of bacteria in high RDM plots could offset increased infiltration capacity and lead to an increase in *E. coli* that discharges off the site.

The results of this and several experiments indicate the potential microbial pollution risk reduction benefits of physically establishing vegetated buffers around drinking water storage reservoirs and their primary tributaries (Mawdsley et al., 1996; Atwill et al., 2002; Davies et al., 2004; Trask et al., 2004; Tate et al., 2004a). Our results indicate that light to moderate cutting and removal of vegetation in buffers may reduce *E. coli* discharge relative to high RDM conditions. Concerns exist about buildup of excessive wildfire fuel loads, and subsequent public safety risk and liability, on grasslands along California's expansive urban-grassland interface (Fried et al., 2004). Public health concerns exist about the excessive buildup of natural organic carbon levels near surface drinking water sources due to potential subsequent formation of disinfection by-products at drinking water treatment facilities (Bull et al., 2001; Jassby and Cloern, 2000; Krasner et al., 1989). The potential for buffers to serve as sources rather than sinks for some nutrients is dependent on vegetation harvest and removal (Bedard-Haughn et al., 2005; Mendez et al., 1999; Jackson et al., 1988). Collectively, these issues indicate that prudent management of vegetation within any fixed buffer system is warranted to optimally achieve multiple public health and safety benefits near critical drinking water surface supplies.

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