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Efficacy of Natural Grassland Buffers for Removal of *Cryptosporidium parvum* in Rangeland Runoff

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ABSTRACT

Our goal for this project was to estimate the retention efficiency of natural grassland buffers for *Cryptosporidium parvum.* Three sets of 16 plots (2.0 by 3.0 m) were established at 5, 20, and 35% slopes. Within each set of 16 plots, residual dry vegetation matter treatments of 225, 560, and 900 kg/ha were implemented, along with a noncut control averaging 4,500 kg/ha. Buffer width treatments were implemented by placing cattle fecal material containing known loads of *C. parvum* 0.1, 1.1, or 2.1 m up-slope of the runoff collector. Grassland buffers of 1.1 and 2.1 m generated 3.2- to 8.8-log and 3.6- to 8.8-log retention of *C. parvum,* respectively, across the range of residual dry vegetation matter, land slope, rainfall, and runoff conditions examined during this project. Buffers with an increased percent land slope exhibited improved the retention efficiencies, whereas buffers experiencing larger maximum annual runoff events exhibited reduced retention efficiencies. Water-quality data from the 0.1-m-wide buffer plots (effectively no buffer) demonstrated that the majority of *C. parvum* oocysts (98 to 99.999%) were retained in the fecal matrix for the duration of the storm season, irrespective of the presence of a vegetated buffer. In conclusion, these results support the assertion that grassland buffers are an effective method for reducing animal agricultural inputs of waterborne *C. parvum* into drinking and irrigation water supplies.

Cryptosporidium parvum is a zoonotic protozoal parasite of heightened public health concern because of its ability to be transmitted by a direct waterborne (municipal, recreational) routes of exposure and also through foodborne routes of exposure via to irrigation or the processing of foods with surface water contaminated with infective oocysts *(16).* The probability of excessive waterborne transport of this protozoal pathogen on agricultural watersheds is governed by processes that load a watershed with *C. parvum,* processes that attenuate or inactivate this pathogen load, and the efficiency of the transport process itself. Numerous strategies can be used to minimize the likelihood that a specific animal agricultural operation contaminates surface water with excessive levels of *C. parvum (16).* One such strategy for minimizing the transport potential of *C. parvum* from animal manure to drinking and irrigation water supplies is to place vegetated buffers between animal agricultural operations and vulnerable surface water supplies *(2, 7, 13, 17, 20, 21).*

We and others have determined that relatively short vegetated buffers appear to remove substantial amounts of waterborne zoonotic pathogens such as *C. parvum* from overland flow, interflow, and shallow subsurface flow *(2, 7, 13, 20, 21).* A primary mechanism by which vegetated buffers remove waterborne microbial pathogens is infiltration of overland flow into the soil profile *(2, 7, 13, 20, 21),* followed by such processes as subsurface straining and adsorption of the entrained microbes *(4, 5, 6, 10, 14).* Much of this previous work has been based on simulated buffers, often in the form of soil boxes with either packed or intact soil blocks *(2, 7, 13, 20, 21).* One primary concern with these simulation studies is that the experimental design inadvertently alters the proper functioning of a vegetated buffer, such as artificially elevating the rate of infiltration due to vertical seepage along the wall of the soil box that then leads to elevated estimates of filtration. Our primary goal for this project was to generate improved estimates for the retention efficiency of grassland buffers for *C. parvum* under natural, field-based conditions and to identify management and environmental factors that enhanced or reduced the microbial water-quality benefits generated by these natural grassland buffers on animal agricultural operations.

MATERIALS AND METHODS

Study site. The study site was located at the University of California Sierra Foothill Research and Extension Center (SFREC) 100 km north of Sacramento, Calif. (39°14′22″N, 121°17′46″W). Elevation at the study site is 350 m. Climate is Mediterranean, with cool, moist winters (October through April) and dry, hot summers (May through September). Average annual precipitation is 65 cm, with approximately 90% falling as rainfall October through March *(11).* Located in the foothills of the Sierra Nevada Mountains, the topography at SFREC is hilly, with land slopes ranging from 5 to 45%. The study site soil is a Sobrante-

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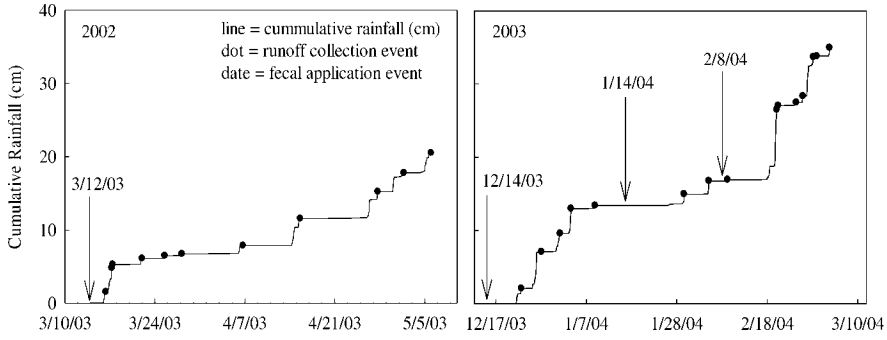

FIGURE 1. *Cumulative rainfall (cm), storm runoff collection timing, and application dates of fecal material with* Cryptosporidium parvum *during the portions of the 2002 to 2003 (12 March 2003 through 2 May 2003) and 2003 to 2004 (14 December 2003 through 2 March 2004) rainfall season included in the study period.*

Timbuctoo gravelly loam complex formed over basic metavolcanic (greenstone) bedrock and classified as Haploxeralfs. 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). Beef cattle, comprising mostly cow-calf operations, is a leading agricultural activity in this region of the Sierra Nevada Range.

Runoff plots and surface runoff collection. During September through February 2002, three blocks of 16 runoff plots (48 total) designed to capture surface runoff (overland and litter flow) during natural rainfall-runoff events were established at the study site. Each runoff plot was 2 m in width (perpendicular to slope) and 3 m in length (parallel to slope) and was bordered with metal flashing inserted 5 cm into the soil to isolate the plot from external up-slope and/or side-slope surface runoff. A 1-m distance was maintained between boundaries of all adjacent plots (16 plots 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 (liter) and enumeration of microbial 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 (i.e., surface runoff from the organic layer of decomposing stems and leaves that rests on top of the mineral soil surface). There was 0.1- to 4.0-cm depth of organic litter across plots depending upon residual dry vegetation matter treatment, described below. All surface water discharged from each plot during the study period (March 2003 to March 2004) was collected, the volumes were recorded, and the microbial discharges were determined. Runoff samples were stored at 4°C upon 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 period.

TABLE 1. *Summary statistics for 2002 to 2003 and 2003 to 2004 rainfall season storm series observed during the study period 12 March 2003 through 2 March 2004*

Rainfall season	Duration (days)[a]	No. of storms	*C. parvum* load (oocysts/plot)[b]	Total rainfall (cm)
2002–2003	53	11	2.0×10^8	20.5
2003–2004	79	16	6.0×10^8	34.9

[a] Days elapsed between placement of first allocation of fecal material on plots and last storm sample collected for each year.

[b] *C. parvum* load applied to each plot in cattle fecal material during each rainfall season.

Treatment of plots. Land slope treatments were implemented by establishing three blocks of runoff plots (16 plots per block) at three locations approximating 5, 20, and 35% slopes, respectively. Actual land slope (percent) of each plot was measured following final installation of the plot boundary (metal flashing) and runoff collector. Mean (minimum, maximum, standard error of the mean) plot slope (percent) 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% blocks, respectively.

Residual dry matter (RDM, e.g., residual dry annual grasses, forbs, and other vegetation) treatments were 225, 560, 900, and 4,500 kg/ha. The 225, 560, and 900 kg/ha treatments were implemented annually by hand cutting and removal of the residual dry vegetation in October until these three levels of RDM were achieved. This range mimics heavy (225 kg/ha, nearly bare) to no grazing (4,500 kg/ha) intensity for California annual grasslands. The 4,500 kg/ha treatment represents RDM levels without cutting and removal (no grazing). Four replicates of each RDM treatment were randomly established and maintained annually in each slope block.

Within each block of plots, three of the four replicate plots (plots with same slope and RDM) were spiked with cattle fecal material containing *C. parvum* at either 0.1, 1.1, or 2.1 m up-slope from the collector to evaluate the effect of buffer width on microbial pollutant discharge. The 0.1-m buffer treatment represents almost direct hydrologic linkage between fecal material and the runoff collector (no buffer). A no-fecal-material application treatment was included as the fourth RDM replicate as a background control and is considered in this article as one level of the buffer treatment (0.1, 1.1, and 2.1 m, and no applied 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 microbial load application. Fresh cattle fecal material was applied to all plots on 12 March 2003 (2002 to 2003 rainfall season spike) and 14 December 2003, 14 January 2004, and 8 February 2004 (2003 to 2004 rainfall season spike) (Fig. 1). Table 1 reports microbial pollutant loads applied to plots each rainfall season. Livestock were excluded from the study site to eliminate incidental fecal deposition within or up-slope of plots. With the exception of the no-fecal-material control plots, each plot received two 1-kg doses of fresh cattle fecal material (approximately 15-cm-diam by 7-m-deep fecal pats) on each application date. Fecal material was placed in the center of the plot 0.1, 1.1, or 2.1 m up-slope of the collector. Multiple fecal material applications were required during the 2003 to 2004 rainfall season to maintain observable concentrations of *C. parvum* across the entire

runoff season. The single application late in the 2002 to 2003 rainfall season (12 March 2003) was sufficient to maintain observable concentrations until the end of that rainfall season (Fig. 1).

For each application event, fresh fecal material was collected from mature cattle and screened to ensure the absence of *C. parvum* and *Cryptosporidium andersoni*. We then added 1×10^8 purified, wild-type *C. parvum* oocysts per kg of fecal material on the dates specified in Figure 1. Naturally infected dairy calves from local commercial dairies were the source of wild-type bovine *C. parvum* oocysts. An acid-fast procedure detected oocysts *(9)*, and samples having more than 50 oocysts per ×400 microscopic field were washed through a series of 40, 100, and 200 mesh sieves. The resulting suspension was decanted off and centrifuged at $1,000 \times g$ for 20 min. Supernatant was discarded and the pellet resuspended 1:1 (vol/vol) in Tween water (0.01% Tween 80 in deionized water, vol/vol). The concentration of purified oocysts was determined as the arithmetic mean of six separate counts with a phase contrast hemacytometer.

Enumeration of *C. parvum* concentration in runoff. Quantitative immunofluorescent microscopy, adjusted for percent recovery, was used to enumerate *C. parvum* oocysts in runoff water samples *(2, 20)*. Depending on storm intensity and runoff volumes per plot, up to 1 liter of a water sample was partitioned into four 250-ml conical tubes to which 62.5 μl of 10% Tween 80 and 10% sodium dodecyl sulfate were added. The suspension was mixed on a wrist action shaker (model 75, Burrel Scientific, Pittsburg, Pa.) at setting 7 for 5 min, followed by centrifugation at $1,100 \times g$ for 15 min, with the supernatant then removed. For low turbidity samples, the pellets were resuspended in sterile distilled water and pooled into either 1.5- or 10-ml centrifuge tubes, and the resultant suspension was centrifuged for 10 min at either 11,600 or $1,000 \times g$, respectively. The supernatant was removed until a 1:1 (supernatant:pellet) volume remained. If the combined total volume was ≤50 μl (method A), the entire suspension was transferred to a commercially prepared well slide for immunofluorescence microscopy using a fluorescein isothiocyanate–labeled anti-*Cryptosporidium* antibody (Meridian Diagnostics, Cincinnati, Ohio). If the final volume was >50 μl (method B), then 15 to 50 μl was transferred to a commercially prepared well slide and immunofluorescent microscopy performed as above. For higher turbidity samples (method C), the pellet was resuspended in distilled water with 0.2% Tween 20, 10 ml overlaid onto 20 ml of sucrose solution (specific gravity 1.18), and centrifuged for 10 min at $1,000 \times g$. Oocyst suspension was collected into a 15-ml tube and centrifuged for 10 min at $1,000 \times g$, and the supernatant was removed. The residual pellet was resuspended and transferred to a commercially prepared well slide and immunofluorescent microscopy was performed as above. Positive and negative controls were included with each run.

Adjustment of *C. parvum* concentration for percent recovery. To determine percent recovery for immunofluorescent microscopy, we collected storm runoff from negative control plots that tested negative for *C. parvum* and added purified bovine *C. parvum* oocysts (purification method described above) to a final concentration of either 100, 500, 1000, 5000, or 10,000 oocysts per liter. The immunofluorescent microscopy procedure was then performed as described above for a total of 116 spiked samples.

Statistical analyses. Percent recovery for the immunofluorescent microscopy procedure was estimated by fitting a negative binomial regression model to the observed number of oocysts, with the number of spiked or expected oocysts functioning as the offset variable *(2, 8, 20)*.

Linear mixed effects regression was used to determine the effect of land slope (percent), residual dry vegetative matter (kilogram per hectare), total rainfall season runoff (liter) per plot, maximum individual storm event runoff volume (liter) per plot per rainfall season, rainfall season (2002 to 2003, 2003 to 2004), and vegetative buffer treatment (0.1 m, 1.1 m, 2.1 m, no fecal application control) on *C. parvum* discharge from the plot and estimated vegetated buffer filtration *(2)*. 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 by using restricted maximum likelihood, and the *P* value for each coefficient was estimated by using the conditional *t* test *(15)*. The potential for correlated data induced by repeated measures on the same experimental unit (plot) was assessed by setting the plot identification number as a group term and conducting a likelihood ratio test on the significance of this term *(15)*, which would be dropped if found to be not significant, resulting in the use of a generalized least squares regression model instead.

The dependent variable for *C. parvum* was oocysts discharged per plot per rainfall season. Raw discharge data were transformed as log(1 + oocysts) to help normalize and account for heteroscadiscity in the residuals. Sample size was 96 (48 plots × 2 rainfall seasons). Total rainfall season discharge of *C. parvum* per plot was calculated as the sum of *C. parvum* discharges for each storm event of the rainfall season for each plot. Storm event *C. parvum* discharge per plot was calculated as the product of concentration (number of oocysts per liter) and storm event runoff (liter) for each plot. All calculations and statistical analyses were conducted on data adjusted for percent recovery of *C. parvum*.

RESULTS

Adjustments to the observed *C. parvum* concentrations. The percent recovery for methods A, B, and C for enumerating *C. parvum* was 11.5% (95% CI, 5.9 to 22.6), 23.9% (95% CI, 20.3 to 28.2), and 13.9% (95% CI, 8.9 to 21.8), respectively. As such, observed concentrations of waterborne *C. parvum* in plot runoff were inflated 4.2- to 8.7-fold for statistical analyses, depending on the method used on each water sample. The ability of analytical methods A, B, and C to detect *C. parvum* (sensitivity curves) is shown in Figure 2. Equations for calculating these sensitivity functions have been presented previously in Atwill et al. *(1)*.

Weather conditions, storm events, and runoff. Figure 1 illustrates surface runoff collection dates and cumulative rainfall for the 2002 to 2003 and 2003 to 2004 rainfall seasons following first fecal application date until the last runoff-generating storm of the season. Eleven rainfall-runoff events were realized and collected following fecal application on 12 March 2003 for the 2002 to 2003 rainfall season, and 16 events were captured over the course of the 2003 to 2004 rainfall season following the 14 December 2003 fecal application (Table 1).

During the 2002 to 2003 rainfall season, total surface runoff ranged from 0.0 to 38.0 liters per plot, with a mean of 13.67 liters per plot (standard deviation [SD], 10.91). Maximum single storm event runoff ranged from 0.0 to 14.15 liters per plot, with a mean of 3.87 liters per plot

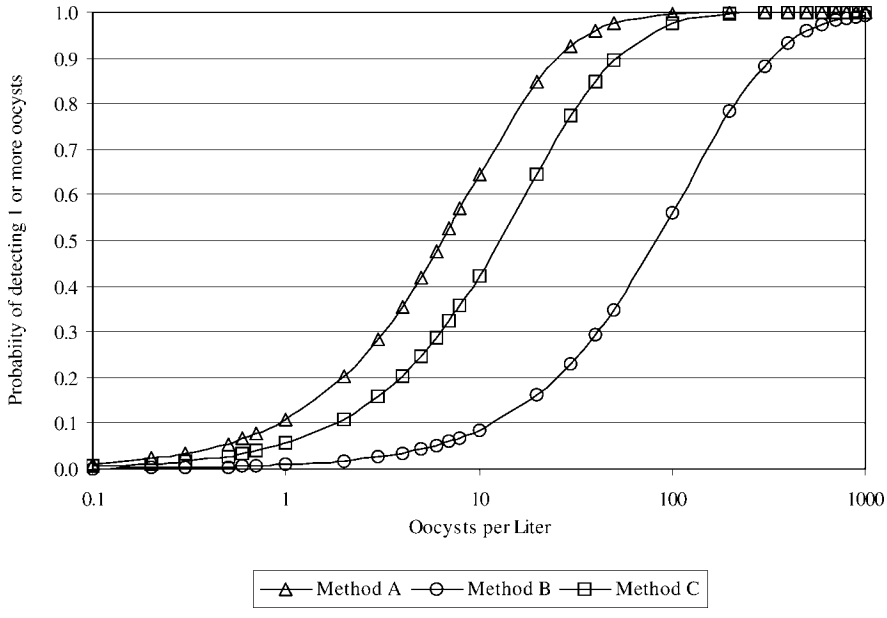

FIGURE 2. *Sensitivity of the three immunofluorescent microscopy assays (methods A, B, C) used to enumerate* Cryptosporidium parvum *in runoff water samples.*

(SD, 3.54). During the 2003 to 2004 rainfall season, total surface runoff ranged from 9.66 to 116.17 liters per plot, with a mean of 40.38 liters per plot (SD, 26.78); maximum single storm event runoff ranged from 2.55 to 17.02 liters per plot, with a mean of 13.15 liters per plot (SD, 5.29). Each plot received an annual cumulative rainfall volume of 1,230 (200 by 300 by 20.5 cm) and 2,094 liters (200 by 300 by 34.9 cm) during the 2002 to 2003 and 2003 to 2004 rainfall seasons, 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 (millimeters per hour) of frontal storm events typical of this region of California *(11).*

***C. parvum* discharge patterns.** During the 2002 to 2003 rainfall season, the percent applied *C. parvum* discharged per plot ranged from 0.00 to 2.03%, with an arithmetic mean of 0.12%. During the 2003 to 2004 rainfall season, the percent of applied *C. parvum* discharged per plot ranged from 0.00 to 0.19%, with an arithmetic mean of 0.02%. These numbers indicate that during the course of the study, on average, ≥99.88% of the initial *C. parvum* 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 within 0.1- to 2.1-m distance.

Figure 3 illustrates mean total *C. parvum* oocysts discharged per plot averaged across both rainfall seasons for buffer widths and land slope treatment levels. The overall treatment means reported in Figure 3 indicate, in general, a reduction in total *C. parvum* discharge as buffer width

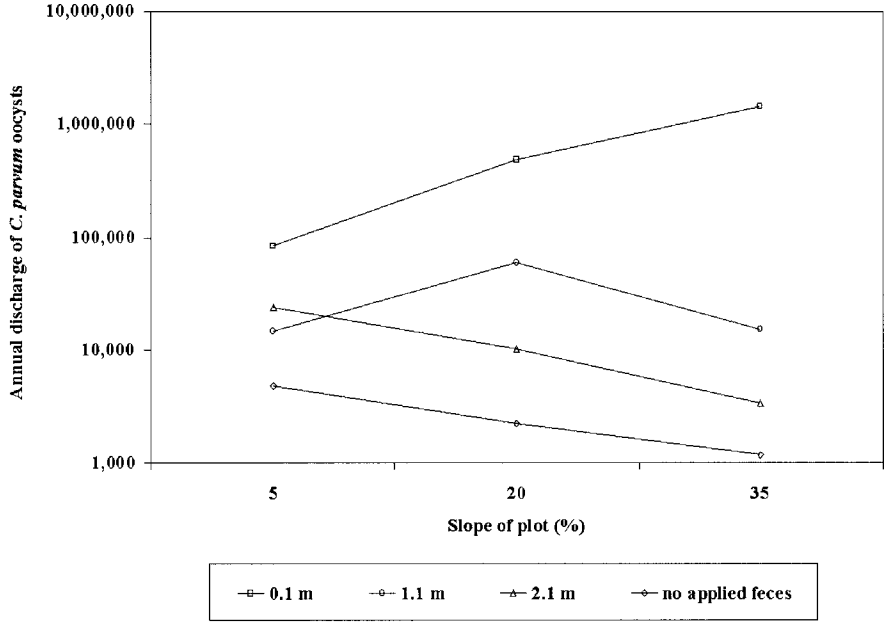

FIGURE 3. *Average annual total* Cryptosporidium parvum *oocysts discharged from 2.0-m-wide by 3.0-m-long annual grassland runoff plots during the portions of the 2002 to 2003 (12 March 2003 through 2 May 2003) and 2003 to 2004 (14 December 2003 through 2 March 2004) rainfall season, stratified by width of grass buffer (0.1, 1.1, 2.1 m, negative control of no feces). Approximately* 2×10^8 *and* 6×10^8 *oocysts in cattle fecal material were applied during the 2002 to 2003 and 2003 to 2004 rainfall season, respectively.*

TABLE 2. *Generalized least squares model for the effect of buffer width and associated covariates on discharge of* Cryptosporidium parvum *from 2.0-m-wide by 3.0-m-long annual grassland runoff plots across 11 and 16 storm events during 2002 to 2003 and 2003 to 2004 rainfall season, respectively*

Factor	Coefficient[a]	95% CI[b]	*P* value[c]
Buffer width (m)			
0.1[d]	0.0	—	—
1.1	−0.78	(−2.26, 0.69)	0.29
2.1	−1.46	(−2.98, 0.06)	0.06
Negative control[e]	−4.25	(−5.74, −2.76)	<0.001
Land slope (%)	0.061	(0.02, 0.10)	0.002
Maximum runoff event (liter)	0.0015	(−0.07, 0.07)	0.97
Buffer width (m) × land slope (%) interaction			
0.1 × slope[d]	0.0	—	—
1.1 × slope	−0.089	(−0.14, −0.03)	0.002
2.1 × slope	−0.077	(−0.13, −0.02)	0.004
Negative control × slope	−0.026	(−0.08, 0.03)	0.35
Buffer width (m) × maximum runoff event (liter) interaction			
0.1 × maximum runoff event[d]	0.0	—	—
1.1 × maximum runoff event	0.085	(−0.01, 0.18)	0.086
2.1 × maximum runoff event	0.12	(0.01, 0.22)	0.033
Negative control × maximum runoff event	0.19	(0.09, 0.29)	<0.001
Intercept	3.93	(2.92, 4.94)	<0.001

[a] Total *C. parvum* discharged per plot across all storms during each rainfall season of the study period (2002 to 2003 and 2003 to 2004) was set as the dependent variable; buffer, land slope, and runoff were set as fixed independent effects. Plot identity set as a random group effect to account for repeated measures was not significant ($P > 0.10$). Coefficients are for transformed total *C. parvum* discharge (log[oocysts + 1]transformed).

[b] CI, confidence interval.

[c] Significance (coefficient $\neq 0$) was determined by $P \leq 0.10$ a conditional *t* test.

[d] Referent condition with which other levels of the categorical factor are compared.

[e] Negative control plots to which no bovine fecal material or *C. parvum* was applied.

increased from 0.1 to 1.1 to 2.1 m. Low levels of presumptive *Cryptosporidium* spp. oocysts were discharged from plots containing no feces and no applied *C. parvum* (negative controls), indicating that background levels for these microbial constituents were not zero on these annual grassland sites. Moreover, none of the buffer treatments were capable of achieving these low background levels of *Cryptosporidium* (Fig. 3). We did not genotype nor speciate the presumptive *Cryptosporidium* isolated from these negative control plots; hence, we cannot classify these oocysts as *C. parvum* and they may, in fact, constitute *Cryptosporidium* oocysts from wildlife common to the area *(3)*. For 0.1-m buffers (i.e., fecal pat 10 cm from the collector, effectively no buffer), oocyst discharge increased as land slope increased from 5 to 35%, but in general the opposite effect occurred for 1.1 m, 2.1 m, and our negative control plots (increasing slope reduced oocyst discharge, thereby improving buffer retention efficiency). There was no readily apparent relationship between *C. parvum* discharge and RDM levels (data not shown).

Effect of buffer width and associated covariates on *C. parvum* discharge and log retention. Table 2 reports the final generalized least squares regression model identifying significant relationships between *C. parvum* removal and the various plot treatments. A linear mixed effects regression model was not used because of the lack of significance of the group term (i.e., repeated measures on each plot). Because of the log transformation, coefficients reported in Table 2 can be directly interpreted as log changes in annual *C. parvum* discharge or log buffer retention associated with each factor. Width of the grassland buffer was strongly associated with the total number of oocysts discharging off the buffer and, therefore, the buffer's predicted log retention efficiency for this waterborne pathogen, but the magnitude of this beneficial effect of reducing oocyst discharge by increasing buffer width was conditional on the buffer's percent land slope and the maximum annual runoff event (Table 2 and Fig. 4). For example, buffers of 1.1- or 2.1-m width generated substantially higher log retention values compared with buffers of 0.1 m (i.e., no buffer per se), with 2.1-m buffers providing the highest retention of all, but these differences became less pronounced as the maximum annual runoff event increased from 1 to 14 liters (Fig. 4). In fact, the added benefit of having 2.1- versus 1.1-m-wide buffers for reducing oocyst discharge becomes negligible for buffers experiencing a maximum annual runoff event ≥10 liters, especially for higher sloped buffers. Buffers 1.1 or 2.1 m wide also exhibited higher log retention values when located at higher compared with lower percent slopes, but the opposite effect occurred for 0.1-m buffers (i.e., increasing slope lead to substantially larger amounts of oocysts discharging off the plot across the rain-

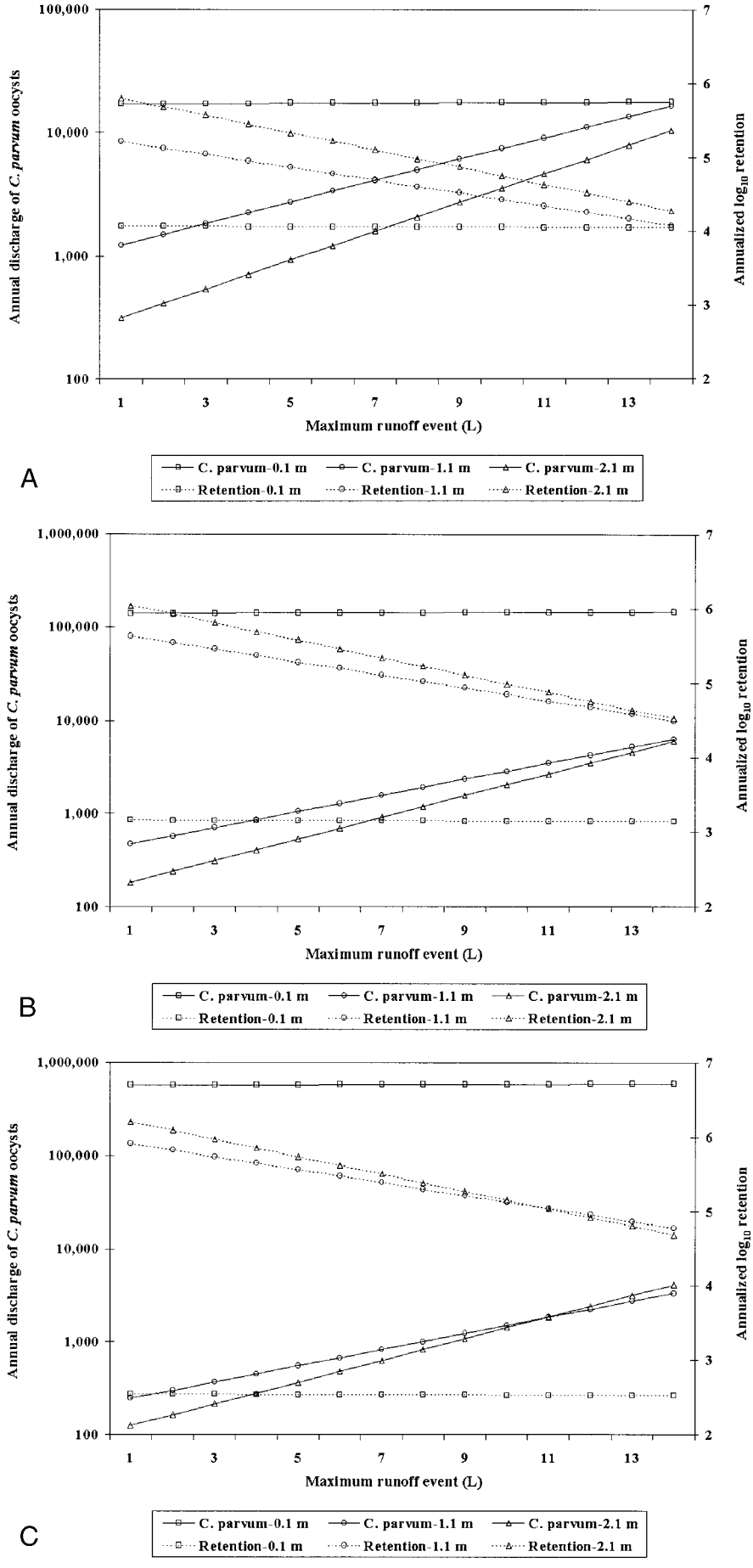

FIGURE 4. *Predicted retention efficiency for* Cryptosporidium parvum *by annual grassland buffers on a 5, 20, and 30% land slope, set as a function of buffer width (0.1, 1.1, 2.1 m) and the maximum annual runoff event (liter). The initial load was 2 × 10^8 and 6 × 10^8 oocysts during the 2002 to 2003 and 2003 to 2004 rainfall season, respectively. Plots A, B, and C represent slopes 5, 20, and 30%, respectively.*

fall season, reducing the log retention value from 4.0 to 2.5 for this 10-cm wide or effectively a nonbuffered plot). This divergence of buffer retention efficiency for 0.1-m compared with 1.1- and 2.1-m-wide buffers as a function of land slope was reflected in the raw data (Fig. 3).

Given that buffer width, land slope, and maximum annual runoff event were in the model, residual dry vegetation matter, rain year, or total annual runoff per plot were not significant predictors of *C. parvum* buffer retention ($P > 0.10$).

DISCUSSION

These results demonstrate the efficacy of vegetated buffers to retain *C. parvum* oocysts deposited in bovine fecal matrices (fecal pats) on annual grasslands under natural rainfall and rangeland conditions, thereby minimizing potential contamination of nearby drinking and irrigation water supplies with infective oocysts. Based on these results and within the environmental conditions of the study, grassland buffers of 1.1- to 2.1-m width with residual dry vegetation matter between 225 and 4,500 kg/ha, land slopes of 5 to 35%, and the rainfall and runoff conditions experienced during this project generated between 3.2- to 8.8-log retention of *C. parvum.* Moreover, water-quality data from the 0.1-m-wide buffer plots demonstrated that the majority of *C. parvum* oocysts (98 to 99.999%) appear to be either retained in the fecal matrix 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. This range of values for oocyst retention in bovine fecal pats is somewhat higher than the 90.4 to 99.1% retention that Bradford and Schijven *(4)* observed for fresh fecal pats under simulated rainfall of varying salinity. In an attempt to simulate natural defecation patterns of cattle *(18),* we applied fecal pats only intermittently to our natural grassland buffers during this project (Fig. 1), allowing for such processes as drying of fecal pats and excystation of oocysts leading to rapid sporozoite inactivation within the fecal matrix that collectively reduced the elutable amount of oocysts in a fecal matrix *(12).* It is important to note that these estimates of oocyst retention are based on formed bovine feces. Given the propensity of *C. parvum* infection to induce watery feces in young stock, it is feasible that if diarrheic instead of formed feces were used different estimates of oocyst retention would have been obtained for these grassland buffers.

The results of this project agree with recent studies conducted under simulated rainfall and soil-vegetation conditions *(2, 7, 20, 21).* For example, Davies et al. *(7)* found that 1-m bare compared with 1-m vegetated (55 to 80% cover) buffers generated a 2.5- compared with a 6.8-log retention of *C. parvum* oocysts. Their experiment used intact loam soil blocks set at 5° or 10° slope and simulated rainfall of 25 or 55 mm/h. Atwill et al. *(2)* reported a 2- to 3-log reduction in surface and shallow subsurface waterborne *C. parvum* oocysts with 1-m vegetated buffers. This experiment used sealed wooden boxes containing repacked soil from the same study site as where this current project was conducted (SFREC site, loam soil), with ≥85% grass cover, 5 to 20% land slope, and rainfall rates of 15 or 40 mm/h. Similar to the present study, they found that the log reduction of *C. parvum* increased as slope of the buffer was increased, the specific amount dependent on the soil type and bulk density. This result is counterintuitive, but we speculated previously that as slope of a vegetated buffer is increased, the vertical thickness of Hortonian sheet flow is decreased due to an increase in sheet flow velocity across the face of the buffer, potentially increasing the ability of a buffer to entrap or filter entrained oocysts *(2).* In this case, our low slope plots were located at lower topographic positions (slope toe at the head of a swale), whereas 20 and 35% higher slope plots were located at up-slope positions. Mean annual total runoff decreased significantly with slope ($P < 0.05$), suggesting that different hydrologic processes were occurring at low compared with higher slope plots. We suspect that because of their topographic position, low slope plots were receiving lateral subsurface return flow from up-slope positions compared with higher slope plots. This effectively created more saturated soil conditions for low compared with higher slope plots, enhancing surface runoff and thus surface hydrologic transport capacity of low compared to steeper slope plots. A more controlled study is needed to better resolve this finding. Lastly, in a soil box experiment using soil and vegetation typical of southern Sierra Nevada annual grasslands, Tate et al. *(20)* observed mean log retention of *C. parvum* of 1.2 to 1.4 for 1-m vegetated buffers set at 5 to 20% land slope. Rainfall application rate (millimeter per hour) 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 reduction for every additional millimeter per hour of rainfall applied to the soil box (each additional millimeter per hour of rainfall generated an additional 0.5 liters of runoff per hour under field-saturated, steady-state conditions). In the present study, as annual maximum runoff event per plot was increased by an additional liter, the log retention of *C. parvum* for 1.1- or 2.1-m-wide buffers was reduced by 1.5 to 2.5%.

Runoff volume and associated hydrologic transport capacity are important factors that determine buffer efficiency and microbial discharge on these Sierra Nevada foothill grasslands. Because of 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 microbial pollutant transport can occur during single storm events *(11, 19).* Maximum storm event runoff per rainfall season accounted for almost 40% of total runoff per plot per season and was a significant factor determining when buffers began to fail for *C. parvum* retention. This was especially true for the lower sloped buffers (5%), with *C. parvum* concentrations in rangeland runoff from 1.1- and 2.1-m-wide buffers approaching nonbuffered sites (0.1 m) as their annual maximum runoff event reached 10 to 15 liters (Fig. 4). Fortunately, these extreme events for our annual grassland sites in the central Sierra Nevada are rare,

suggesting that under normal precipitation patterns these grassland buffers of 1.1- to 2.1-m typically retain ≥99.9% of the *C. parvum* load.

The results from this experiment suggest that strategically placed vegetated buffers can function as one of several beneficial management practices that an animal agricultural operation can use to minimize the risk of waterborne *C. parvum* contamination of drinking and irrigation water supplies.

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LITERATURE CITED

1. Atwill, E. R., B. Hoar, M. das Gracas Cabral Pereira, K. W. Tate, F. Rulofson, and G. Nader. 2003. Improved quantitative estimates of low environmental loading and sporadic periparturient shedding of *Cryptosporidium parvum* in adult beef cattle. *Appl. Environ. Microbiol.* 69:4604–4610.
2. Atwill, E. R., L. Hou, B. M. Karle, T. Harter, K. W. Tate, and R. A. Dahlgren. 2002. Transport of *Cryptosporidium parvum* oocysts through vegetated buffer strips and estimated filtration efficiency. *Appl. Environ. Microbiol.* 68:5517–5527.
3. Atwill, E. R., R. Phillips, M. Das Graças C. Pereira, X. Li, and B. McCowan. 2004. Seasonal shedding of multiple *Cryptosporidium* genotypes in California ground squirrels (*Spermophilus beecheyi*). *Appl. Environ. Microbiol.* 70:6748–6752.
4. Bradford, S. A., and J. Schijven. 2002. Release of *Cryptosporidium* and *Giardia* from dairy calf manure: impact of solution salinity. *Environ. Sci. Technol.* 36:3916–3923.
5. Bradford, S. A., J. Simunek, M. Bettahar, M. T. VanGenuchten, and S. R. Yates. 2003. Modeling colloid attachment, straining, and exclusion in saturated porous media. *Environ. Sci. Technol.* 37:2242–2250.
6. Bradford, S. A., S. R. Yates, M. Bettahar, and J. Simunek. 2002. Physical factors affecting the transport and fate of colloids in saturated porous media. *Water Resour. Res.* 38:1029/2002WR001340.
7. Davies, C. M., C. M. Ferguson, C. Kaucner, M. Krogh, N. Altavilla, D. A. Deere, and N. J. Ashbolt. 2004. Dispersion and transport of *Cryptosporidium* oocysts from fecal pats under simulated rainfall events. *Appl. Environ. Microbiol.* 70:1151–1159.
8. Hardin, J., and J. Hilbe. 2001. The negative binomial family, p. 141–158. *In* J. Hardin and J. Hilbe (ed.), Generalized linear models and extensions. Stata Press, College Station, Tex.
9. Harp, J. A., P. Jardon, E. R. Atwill, M. Zylstra, S. Checel, J. P. Goff, and C. Desimone. 1996. Field testing of prophylactic measures against *Cryptosporidium parvum* infection in calves in a California dairy herd. *Am. J. Vet. Res.* 57:1586–1588.
10. Harter, T., S. Wagner, and E. R. Atwill. 2000. Colloid transport and filtration of *Cryptosporidium parvum* in sandy porous media (soils and groundwater). *Environ. Sci. Technol.* 34:62–70.
11. Lewis, D. J., M. J. Singer, R. A. Dahlgren, and K. W. Tate. 2000. Hydrology in a California oak woodland watershed: a 17-year study. *J. Hydrol.* 230:106–117.
12. Li, X., E. R. Atwill, L. A. Dunbar, T. Jones, J. Hook, K. W. Tate. 2005. Seasonal temperature fluctuations induces rapid inactivation of *Cryptosporidium parvum*. *Environ. Sci. Technol.* 39:4484–4489.
13. Mawdsley, J. L., A. E. Brooks, R. J. Merry, and B. F. Pain. 1996. Use of a novel tilting table apparatus to demonstrate the horizontal and vertical movement of the protozoan *Cryptosporidium parvum* in soil. *Biol. Fertil. Soils* 23:215–220.
14. McDowell-Boyer, L. M., J. R. Hunt, and N. Sitar. 1986. Particle transport through porous media. *Water Resour. Res.* 22:1901–1921.
15. Pinheiro, J. C., and D. M. Bates. 2000. Theory and computational methods for LME models, p. 57–96. *In* J. C. Pinheiro and D. M. Bates (ed.), Mixed Effects Model in S and S-Plus. Springer, New York.
16. Rosen, B. H., R. Croft, E. R. Atwill, S. Wade, and S. Stehman. 2000. Waterborne pathogens in agricultural watersheds. Tech. note 2. Watershed Science Institute, University of Vermont, Natural Resources Conservation Service, U.S. Department of Agriculture, Burlington, Vt.
17. Tate, K. W., E. R. Atwill, M. R. George, N. K. McDougald, and R. E. Larsen. 2000. *Cryptosporidium parvum* transport from cattle fecal deposits on California rangeland. *J. Range Manage.* 53:295–299.
18. Tate, K. W., E. R. Atwill, N. K. McDougald, and M. R. George. 2003. Spatial and temporal patterns of cattle feces deposition on annual rangeland watersheds. *J. Range Manage.* 56:432–438.
19. Tate, K. W., R. A. Dahlgren, M. J. Singer, B. Allen-Diaz, and E. R. Atwill. 1999. On California rangeland watersheds: timing, frequency of sampling affect accuracy of water quality monitoring. *Calif. Agric.* 53:44–48.
20. Tate, K. W., M. Das Gracas C. Pereira, and E. R. Atwill. 2004. Efficacy of vegetated buffer strips for retaining *Cryptosporidium parvum*. *J. Environ. Qual.* 33:2243–2251.
21. Trask, J. R., P. K. Kalita, M. S. Kuhlenschmidt, R. D. Smith, and T. L. Funk. 2004. Overland and near-surface transport of *Cryptosporidium parvum* from vegetated and nonvegetated surfaces. *J. Environ. Qual.* 33:984–993.