

Interactions of slope and canopy of herbage of three herbage species on transport of faecal indicator bacteria by rain splash

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Abstract

The movement of faecal pathogens from land to surface and ground-water are of great interest because of the public and livestock health implications. Knowledge of canopy structure and how it might be managed to help mitigate nutrient and pathogen movement in pasture is needed to create management practices that balance livestock production with environmental benefits. An experiment was conducted using a rainfall-simulating device to test whether canopy structure of species common to pastures in Appalachia, USA could be managed to influence dispersion of faecal pathogens. Seven pots (30-cm diameter) of white clover, orchard-grass and perennial ryegrass were lined up on horizontal and sloping surfaces under a rainfall simulator. The centre pot was inoculated at the soil surface with 4×10^{10} faecal coliform bacteria (FC) just before rainfall simulation started. The species were maintained under short, moderate and tall canopy management treatments. White clover exhibited the greatest rates of lateral and vertical dispersion of FC into the canopy, especially in the short canopy management treatment following 30 min of rainfall (about 40 mm). Low concentrations of faecal coliform bacteria also dispersed into the canopies of the grass species but the differences in concentration of FC between the grass species were not different. When the proportion of white clover in a pasture is high, the canopy should be relatively taller to reduce the likelihood of infection associated with faecal coliform-contaminated herbage.

Keywords: rain splash, pathogen dispersal, manure, canopy structure, canopy management, slope

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Introduction

The movement of faecal pathogens from land to surface and ground-water are of concern because of public health implications (ASM, 1999). Non-structural, best-management practices that control the timing, volume and placement of livestock manures are used commonly to limit opportunities for faecal pathogens to enter surface and ground-water channels and reservoirs (NRCS, 1999). Grazing areas in Appalachia, USA tend to be located on slopes, where the sequential downhill movement of faecal coliform bacteria (FC) by repeated rain splash could transport FC directly to water channels or reservoirs or saturated areas. Faecal coliform bacteria are facultatively anaerobic, rod-shaped, gram-negative, non-spore-forming bacteria that ferment lactose and include the faecal-origin genera; *Escherichia*, *Enterobacter*, *Klebsiella* and *Citrobacter*. *Escherichia coli* is usually the predominant genus.

Increased infiltration capacity, water and waste diversions, and vegetated filter strips, are used to control faecal pathogen movement in surface run-off (Lim *et al.*, 1998; Tufford and Marshall, 2002). Faecal pathogens transported by rain splash conceivably could bypass physical barriers. Gregory *et al.* (1959) stated that cells of *E. coli* move readily in rain splash and that dispersal of microorganisms by splash is very likely. Buttlerworth and McCartney (1991) found that rain had the potential to remove and disperse bacteria from leaf surfaces and disperse to nearby foliage. Dunne and Leopold (1978) noted that rain splash on slopes results in net downhill movement of soil particles, with the mathematical formulation for this type of movement being defined by Zaslavsky and Sinai (1981). Although slope was found to have a significant effect on dispersal of FC, Boyer (2008) suggested that plant canopy architecture might affect dispersal by intercepting raindrops and modifying patterns of splash droplet dispersal.

Transport of faecal pathogens by rain splash can be problematical for water quality protection as well as maintenance of the health of livestock at pasture.

The movement of soil particles in rain splash is a well-documented process (Dunne and Leopold, 1978; Ghadiri and Payne, 1979). Faecal pathogens are transported both free-floating and attached to particulate matter suspended in water. Pathogens, adsorbed to particle surfaces, will be transported when the soil particles are transported by rain splash. The transport of plant pathogens (fungal spores and bacteria) by rain splash is well documented (Gregory *et al.*, 1959; Fitt and McCartney, 1986; Walklate, 1989; Walklate *et al.*, 1989; Jenkinson and Parry, 1994; Lovell *et al.*, 1999). Transport of plant pathogens by rain splash might involve simultaneous transport of soil particles depending on whether the particles of plant pathogens are located on soil or plant surfaces (Foster *et al.*, 1985). Like plant pathogens, faecal pathogens are expected to be splashed into the canopy where grazing livestock might ingest sufficient quantities to cause infection and scours, especially in young and stressed animals (Lejeune *et al.*, 2001; Fitzgerald *et al.*, 2003; Looper *et al.*, 2006). Although many faecal pathogens are not problematic to livestock, they can be zoonotic and can affect human health. Infected livestock can then move to other parts of the landscape where pathogen-laden manure might be deposited in or near bodies of water and on storm run-off source areas.

The extensive and meticulous studies of fungal spore dispersal by rain splash conducted by Madden *et al.* (Madden, 1992, 1997; Madden *et al.*, 1996; Madden and Boudreau, 1997; Ntahimpera *et al.*, 1997, 1998, 1999; Saint-Jean *et al.*, 2005) have contributed significantly to understanding of dynamics of rain splash in vegetative canopies and row-crop agriculture. The mechanics of rain splash depend upon raindrop kinetic energy at the point of impact. The kinetic energy is related to factors such as raindrop size, angle of inclination and fall velocity (Stedman, 1979; Park *et al.*, 1983; Walklate, 1989; Walklate *et al.*, 1989; Madden, 1992; Madden *et al.*, 1996; Ntahimpera *et al.*, 1997; Pietravalle *et al.*, 2001; Ma *et al.*, 2008). Surface tension forces are also related to splash dispersal and its relationship with kinetic energy reflected by the dimensionless Weber number has been a subject of recent study (Hoffman *et al.*, 1992). Depth of standing water and surface properties at the impact site are also important (Finney, 1984; Allen, 1988; Huber *et al.*, 1997) for determining characteristics of splash dispersal. Wind velocity and direction also strongly affect patterns of splash dispersal (Erpul *et al.*, 2004). The relationships of spore dispersal to rainfall intensity and storm duration are complicated partially because of wash-off from plant surfaces and soil infiltration (Madden, 1992, 1997; Madden *et al.*, 1996). Increasing surface roughness, plant cover and leaf area index reduces the amount of spore dispersal by rain splash (Madden,

1992; Madden and Boudreau, 1997; Ntahimpera *et al.*, 1998). Canopy characteristics also significantly affect rain-splash dispersal by intercepting incoming raindrops as well as outgoing splash droplets. Lovell *et al.* (1997) found that penetration of raindrops to the soil surface was much less in winter wheat crops with thicker canopies resulting from N fertilization than less dense, low-N fertility canopies. In a study of splash dispersal of fungal spores in wheat, Paul *et al.* (2004) found that spores were routinely splashed to upper parts of the canopy and that rain splash played an important role in the spread of *Fusarium* head blight.

In this study, it was hypothesized that canopy architecture of herbage and terrain configuration influences the geometry of rain splash and dispersion of faecal pathogens. Knowledge about factors affecting farm-level transmission of pathogens is needed to design management systems that protect livestock and human health, and the environment (Looper *et al.*, 2006). The results of this study should contribute to that base of knowledge.

Materials and methods

Treatments

Canopies with different architecture based on leaf characteristics and practical management considerations in terms of mean sward height were created. The herbage species used were orchardgrass (*Dactylis glomerata* L.), perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.). Orchardgrass has coarse tillers with vertical to slightly decumbent, wide leaves. Perennial ryegrass was selected for its fine, densely-packed tillers with vertical, narrow leaves. White clover has horizontal leaves and leaflets, with leaflet size depending on canopy management interacting with genetic factors. The forages were grown as pure swards in a rooting medium mixture of soil, sand and potting mix (proportionately 0.44, 0.42 and 0.14 on a weight basis respectively) in 30-cm diameter pots (volume 10.1 L). Once established, plants were supplied with fertilizer in a soluble form to sustain vigorous growth and stimulate formation of a sod. Clipping was initiated to maintain sward height as soon as practical, based on growth and development of the plants. Table 1 shows the sward heights maintained for each of the species for the short (S), moderate (M) and tall (T) canopy management treatments. Leaf area index was not measured but all of the pots maintained complete canopy closure.

Rainfall simulation

The rainfall simulator was built in house and was modelled after the Tlaloc 3000 portable rainfall

Table 1 Canopy height (cm) treatments for each of the species.

Canopy height treatment	Species		
	Orchardgrass	Perennial ryegrass	White clover
Tall (T)	>25	>20	>15
Moderate (M)	15–25	10–20	5–15
Short (S)	<15	<10	<5

simulator, based on the design of Miller (1987). A rotating nozzle placed 3 m above the surface of the ground provided a relatively uniform rainfall within a 2 m × 3 m area. A pressure regulator was used to establish a water flow rate of 8.1 L min⁻¹. Rainfall was applied at a constant intensity of 1.35 mm min⁻¹ over a 30-min period. The mean total storm depth for all simulations was 40.5 (s.d. of mean, 6.54) mm. The distribution of raindrop size was determined using the flour pellet technique described by Laws and Parsons (1943). Median raindrop diameter was 2.68 mm (mean was 2.90 mm) with a range of 2.35 mm to 6.39 mm. The velocity of the median raindrops was 6.7 m s⁻¹ according to the equations presented in Park *et al.* (1983). Six small plastic rain gauges (orifice diameter of 43 mm) were located at ground level in the simulator along the two long edges of the pots (three per side) in order to assess uniformity of rainfall distribution on the pots. The coefficient of variation (standard deviation divided by mean × 100) for total rainfall in the six rain gauges was 0.63 per cent across all simulations. The coefficients of variation for individual simulations ranged from 0.14 to 1.42 per cent.

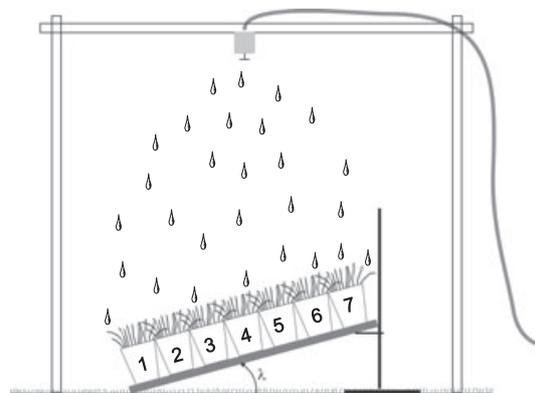
During each rainfall simulation, rain was collected in a sterile vessel placed out of reach of the rain splash so the application water could be checked for the presence of FC. Fifty of the 55 simulations tested negative for FC in the application water. The eight simulations that tested positive for FC had very low concentrations and were not considered to constitute a problem except possibly for two simulations when 73 colony forming units (cfu) 100 mL⁻¹ and 22 cfu 100 mL⁻¹ were detected in the application water. Those positive tests were simulations on short clover on a 26.8 per cent slope and short orchardgrass on a level surface respectively. It is suspected that birds contaminated the water stored in the source tanks by landing on the tanks, which are stored outside, and defaecating near the filler portal. The tanks were flushed and cleansed around the filler portal following the contaminated runs and FC were not detected in the application water of the next simulation. Faecal coliform bacteria were not detected on the vegetation of pots 1 and 7 of the simulations using FC positive application water. Therefore, the

contaminated water was not considered a major problem and the simulation run results were retained in the statistical analyses.

Rain splash experiments on a level surface

In the first set of experiments seven pots were lined up edge-to-edge on a level surface (Figure 1). Each species and sward height was replicated three times for a total of 27 rainfall simulations. A control pot was also placed outside near the rainfall simulation but out of reach of the rainfall simulator. The control received the same treatment as the other pots but received no rain during the simulation. The centre pot was designated the source pot (inoculated with faecal bacteria) and the other six pots were target pots (initially free of faecal bacteria). Immediately before simulation of rainfall began, a solution containing 4 × 10¹⁰ cfu of FC was sprayed onto the soil surface of the source pot using a small hand-spray bottle.

After simulation of rainfall ended, the pots were moved to the laboratory where the herbage in each pot was clipped from ground level at 5-cm height increments (subsequently called horizons). The top 1 cm of soil was sampled from three randomly selected locations in each pot. The herbage clippings from each pot and horizon were divided into two parts and weighed before placing one part in a 250 mL plastic bottle and the other part in a pre-weighed paper bag. The bagged clippings were oven-dried (105°C) so that dry matter (DM) content could be determined. Sterile phosphate buffer dilution water (200 mL) was added to the bottles to cover the clippings. The bottles were shaken on a reciprocating shaker for 30 min. Aliquots of the eluent were plated on mFc nutrient agar (Membrane Faecal Coliform Agar; Oxoid, Basingstoke, UK; prepared according to standard methods) to determine FC

**Figure 1** Diagram of experimental setup. Slope inclination is represented by λ .

concentration, which was expressed as cfu g⁻¹ DM herbage mm⁻¹ rain. The three soil samples from each pot were combined in a 150-mL bottle with 100 mL of sterile phosphate buffer dilution water. Aliquots of the eluent were plated on mFc nutrient agar to determine FC concentration. The remaining soil solution was passed through filter paper, oven-dried (105°C) and weighed so that soil FC concentrations could be expressed as cfu g⁻¹ soil mm⁻¹ rain.

Rain splash experiments on sloping surfaces

The experimental procedures for the sloping surface studies were identical to the level surface experiment except as noted here. The pots were placed edge-to-edge on a board raised on one end (see Figure 1) to create slopes of 8.7, 17.6, 26.8, 36.4, 46.6, 70 and 100 per cent. The slope experiments were run on white clover and perennial ryegrass short and moderate canopy management combinations (Table 1). The rain-fall simulations were run once for each species, canopy management treatment and slope combination for a total of 28 simulations.

Statistical analyses

Statistical analyses were performed with the SAS Version 9.1 (SAS Institute, Inc., Cary, NC, USA). Statistical tests of significance were accepted at the $P < 0.05$ level of significance unless stated otherwise.

A method for modelling skewed data with many zeros (Fletcher *et al.*, 2005) was used to model the expected abundance of FC within the canopies. The level surface data were modelled first and then slope was added in the second model. Briefly, logistic regression was first used to model the probability that bacteria occurred at specific locations, $\Pr(Z = 1)$, where Z was set as a binary variable equal to 0 when FC were not present or 1 when FC were present. Multiple regression was then used on the set of FC log abundance data (zeros removed from the data set) to model expected abundance $E(Y|Z = 1)$. Combining the models gives the expected value of Y as:

$$E(Y) = \Pr(Z = 1)E(Y|Z = 1). \quad (1)$$

Detailed mathematical and statistical descriptions of the modelling technique are available in Fletcher *et al.* (2005).

Logistic regression predicted the logit for $\Pr(Z = 1)$ given the classification variables for canopy height management (S, M and T), as described in Table 1, and for the horizons within the canopy (horizon centres at 2.5, 7.5, 12.5, 17.5, 22.5 and 27.5 cm) and splash distance from the centre target pot (0, 30, 60 and 90 cm; 1 pot distance equals 30 cm). Slope angle was added as an

effects variable in the sloping surface experiment. If the logit is represented by $f(X)$ with X being the set of effects variables then the $\Pr(Z = 1)$ equals

$$\frac{1}{1 + e^{-f(X)}}. \quad (2)$$

A forward selection method available in the SAS LOGISTIC procedure was used to select the effects variables for $f(X)$. The effects variables tested were canopy management, horizon, splash distance and slope angle (in the sloping surface experiment) as previously described. In the SAS forward selection method of the LOGISTIC procedure, statistically significant effects were added one at a time, with no removal of previously added effects, until no more added effects were statistically significant. In order to gain the most information about canopy management and slope effects on FC splash, each species was analysed separately. Species could have been added as an effects variable but some important information about splash dynamics might have been lost through pooling of data.

The same effects variables were tested in a general linear regression (SAS GLM procedure) to predict the log concentrations of FC given that FC were present. Because the FC data were highly skewed to the right, concentrations were log (base 10) transformed prior to analysis.

Results

Rain-splash experiments on a level surface

Most of the splashed FC occurred within the lowest horizon of the canopies of the target pots for all species. Figure 2 illustrates the distributions of bacteria within the canopies among pots. The highest FC concentrations splashed onto the herbage were observed in the lowest horizon (0–5 cm) above the soil surface. Concentrations at that horizon ranged from 18 cfu g⁻¹ DM mm⁻¹ for perennial ryegrass with the short canopy management treatment to 14 096 cfu g⁻¹ DM mm⁻¹ for orchardgrass with the moderate canopy management treatment.

Few FC splashed away from the centre pot (FC source) in perennial ryegrass. Some FC splashed to the first pot in orchardgrass, especially on the tall canopy management treatment. Faecal coliform bacteria splash away from the centre pot was greatest in white clover with FC splashed to the first (closest) pot in all three canopy management treatments. Faecal coliform bacteria splashed to the second and third pots in the short canopy management treatment of white clover. Concentrations of FC in the pot surface soils (Table 2) at the end of the simulations closely followed the trends observed on the herbage and are not further discussed in this paper.

Distance (DIST) was highly significant in estimating the probability of occurrence of FC for all three species.

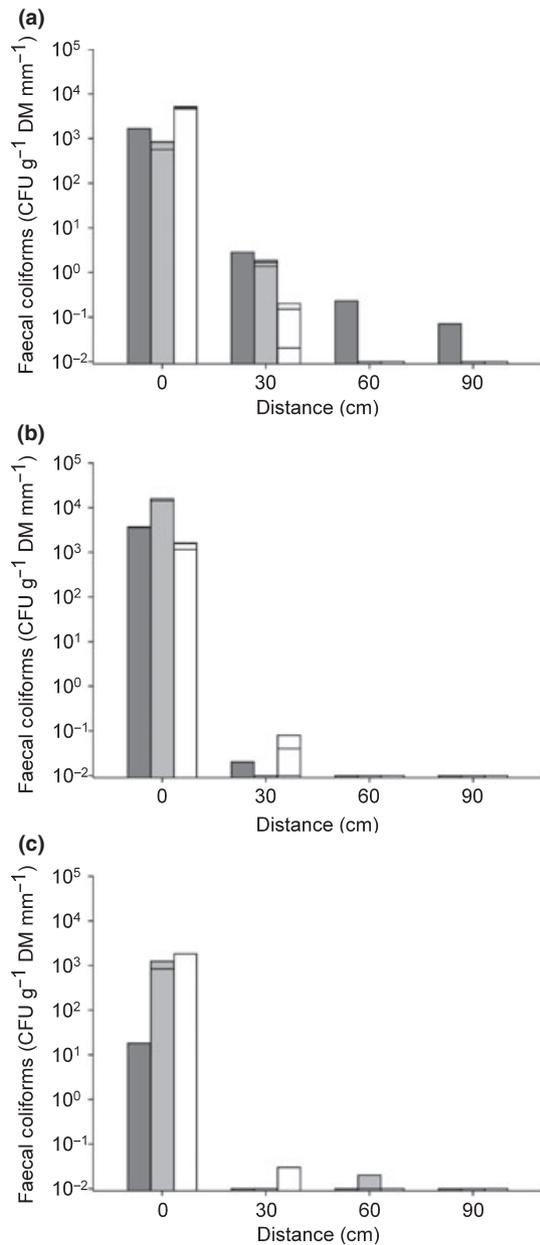


Figure 2 Geometric mean faecal coliform concentrations (cfu g^{-1} DM herbage mm^{-1} of rain) within the canopies for each of the canopy management treatments [short (dark bar), moderate (grey bar), high (white bar)] on a level surface at each distance from the centre pot for (a) white clover, (b) orchardgrass and (c) perennial ryegrass. All horizons are accumulated in each bar with the lowest section of each bar representing the geometric mean concentration in the 0–5 cm horizon.

With white clover, canopy treatment (FM) was the only other significant variable for estimating the logit for the probability of occurrence of FC. The final $f(X)$ results for

Table 2 Geometric mean faecal coliform bacteria concentrations (cfu g^{-1} soil) in the pot soils of the level surface experiment with three species (white clover, orchardgrass and perennial ryegrass) and three canopy height treatments [short (S), medium (M) and tall (T); see Table 1 for full description] following rainfall. The distance refers to distance from contaminated centre pot in experimental design.

Species and treatment	Distance (cm)			
	0	30	60	90
White clover				
S	54.98	0.48	0.04	0
M	10.38	0.51	0	0
T	56.26	0.03	0	0
Orchardgrass				
S	80.00	0.14	0	0
M	48.85	0.17	0	0
T	26.59	0	0	0
Perennial ryegrass				
S	24.09	0	0	0
M	47.38	0	0	0
T	29.31	0.35	0	0

white clover (standard errors of the parameter estimates are shown in parentheses) were:

$$f(X) = 5.2788(1.0510) - 0.1198(0.0215)\text{DIST} + \beta_{\text{FM}}, \quad (3)$$

where β_{FM} was 4.3858 (0.9482), -1.1574 (0.5005), and -3.2284 (0.6523) for canopy management treatments S, M and T, respectively. No variables other than DIST were significant for estimating the logit for the probability of occurrence of FC in the two grass species. The final model results were

$$f(X) = 2.4432(0.6013) - 0.1473(0.0227)\text{DIST} \quad (4)$$

for orchardgrass and

$$f(X) = 1.2980(0.4594) - 0.1152(0.0198)\text{DIST} \quad (5)$$

for perennial ryegrass.

Given that FC were present, the regression model results for estimating the log concentrations of FC all used DIST as a predictor variable. Height increment within the canopy (CH) was an important predictor for both grass species, and FM was important in the orchardgrass model. The regression model for estimating log concentration of FC in white clover was:

$$\text{Log}(\text{FC}) = 2.1951(0.2159) - 0.0526(0.0066)\text{DIST}, \\ R^2 = 0.56. \quad (6)$$

The regression model for perennial ryegrass was:

$$\text{Log}(\text{FC}) = -0.0766(0.0091)\text{DIST} + \beta_{\text{CH}}, R^2 = 0.81, \quad (7)$$

where β_{CH} was 2.6659 (0.2525), 2.5892 (0.3272), 0.8625 (0.3142), 0.0198 (0.4443) and -0.1732 (0.7696) for horizons 0–5 cm, 5–10 cm, 10–15 cm, 15–20 cm, and 20–25 cm, respectively. β_{CH} for the 15–20 cm and 20–25 cm horizons was not significantly different from zero.

The regression model for orchardgrass was:

$$\text{Log(FC)} = -0.0637(0.0076)\text{DIST} + \beta_{CH} + \beta_{FM},$$

$$R^2 = 0.78, \quad (8)$$

where β_{CH} was 3.1007 (0.3304), 2.0307 (0.2947), 1.3609 (0.3682), 1.0026 (0.3682), 0.9931 (0.4097) and 0.8097 (0.4685) for horizons 0–5 cm, 5–10 cm, 10–15 cm, 15–20 cm, 20–25 cm and 25–30 cm, respectively, and β_{FM} was 0.1551 (0.3821), 0.9579 (0.3212) and 0 for canopy management treatments S, M and T, respectively. β_{CH} was not significantly different from zero for the 25–30 cm interval and β_{FM} was not significantly different than zero for the S and T canopy management treatments. Combining the two models as in Equation 1, produced the expected log concentrations of FC for the species, horizons, canopy management treatments and distance. Graphical results for the 0–5, 5–10 and 10–15 cm horizons are shown in Figure 3.

Rain-splash experiments on sloping surfaces

Redistribution of FC in the down-slope direction was most apparent in the M canopy management treatment for white clover and the S canopy management treatment for perennial ryegrass (Figure 4). The redistribution of FC down-slope was especially evident at the steeper slopes. Although there was also a redistribution of FC in the S canopy management treatment for white clover and the M canopy management treatment for perennial ryegrass, the visual evidence was not very strong.

Up-slope and down-slope probabilities of occurrence and concentrations of FC were modelled separately because of the differential FC redistributions in the two slope directions. The model for estimating the logit for the probability of occurrence of FC in the down-slope direction in white clover used distance (DIST), slope (SL) and canopy management (FM) as independent variables and classifications. The resulting model for white clover and down-slope (standard errors of the parameter estimates are shown in parentheses) was:

$$f(X) = 3.7040(0.6803) - 0.0824(0.0126)\text{DIST}$$

$$+ 0.0307(0.0091)\text{SL} + \beta_{FM}, \quad (9)$$

where β_{FM} was 0.8502 (0.3165) and -0.8502 (0.3165) for S and M canopy managements, respectively. The model for estimating the logit for the probability of occurrence of FC in the up-slope direction did not use

slope as a predictor variable. The resulting model (white clover, up-slope) was

$$f(X) = 4.6025(0.8439) - 0.1000(0.0170)\text{DIST} + \beta_{FM}, \quad (10)$$

where β_{FM} was 1.8979 (0.4448) and -1.8979 (0.4448) for S and M canopy managements, respectively.

The down-slope model for estimating the logit for the probability of occurrence of FC in perennial ryegrass used distance (DIST), slope (SL) and canopy horizon (CH). The only significant parameters for the up-slope model were distance and slope. The down-slope model was:

$$f(X) = 1.1259(0.3995) - 0.0481(0.0080)\text{DIST}$$

$$+ 0.0343(0.0081)\text{SL} + \beta_{CH}, \quad (11)$$

where β_{CH} was 0.9012 (0.2969), -0.0196 (0.3249), and -0.8816 (0.3409) for 0–5, 5–10, and 10–15 cm horizons, respectively. β_{CH} for the 5–10 cm horizon was not significantly different from zero. The up-slope model for perennial ryegrass was

$$f(X) = 1.2741(0.3943) - 0.0602(0.0091)\text{DIST}$$

$$+ 0.0182(0.0073)\text{SL} \quad (12)$$

Given that FC were present, the following regression equations for estimating the log concentrations of FC were derived:

$$\text{(white clover, down-slope)Log(FC)}$$

$$= -0.0543(0.0042)\text{DIST} + 0.0165(0.0032)\text{SL}$$

$$+ \beta_{CH}, R^2 = 0.68, \quad (13)$$

where β_{CH} was 2.4127 (0.1967), 1.7105 (0.2481) and 1.4684 (0.2735) for horizons 0–5 cm, 5–10 cm, and 10–15 cm, respectively;

$$\text{(white clover, up-slope)Log(FC)}$$

$$= -0.0699(0.0049)\text{DIST} + 0.0112(0.0034)\text{SL}$$

$$+ \beta_{CH}, R^2 = 0.78 \quad (14)$$

where β_{CH} was 2.7147 (0.1976), 2.1762 (0.2486) and 1.8455 (0.2789) for the same horizons;

$$\text{(perennial ryegrass, down-slope)Log(FC)}$$

$$= 2.2455(0.1484) - 0.0430(0.0035)\text{DIST},$$

$$R^2 = 0.65 \quad (15)$$

and

$$\text{(perennial ryegrass, up-slope)Log(FC)}$$

$$= 2.4310(0.1635) - 0.0549(0.0051)\text{DIST},$$

$$R^2 = 0.66. \quad (16)$$

Combining the two models as in Equation 1, produced the expected log concentrations of FC for the species, canopy managements, distance and slopes for

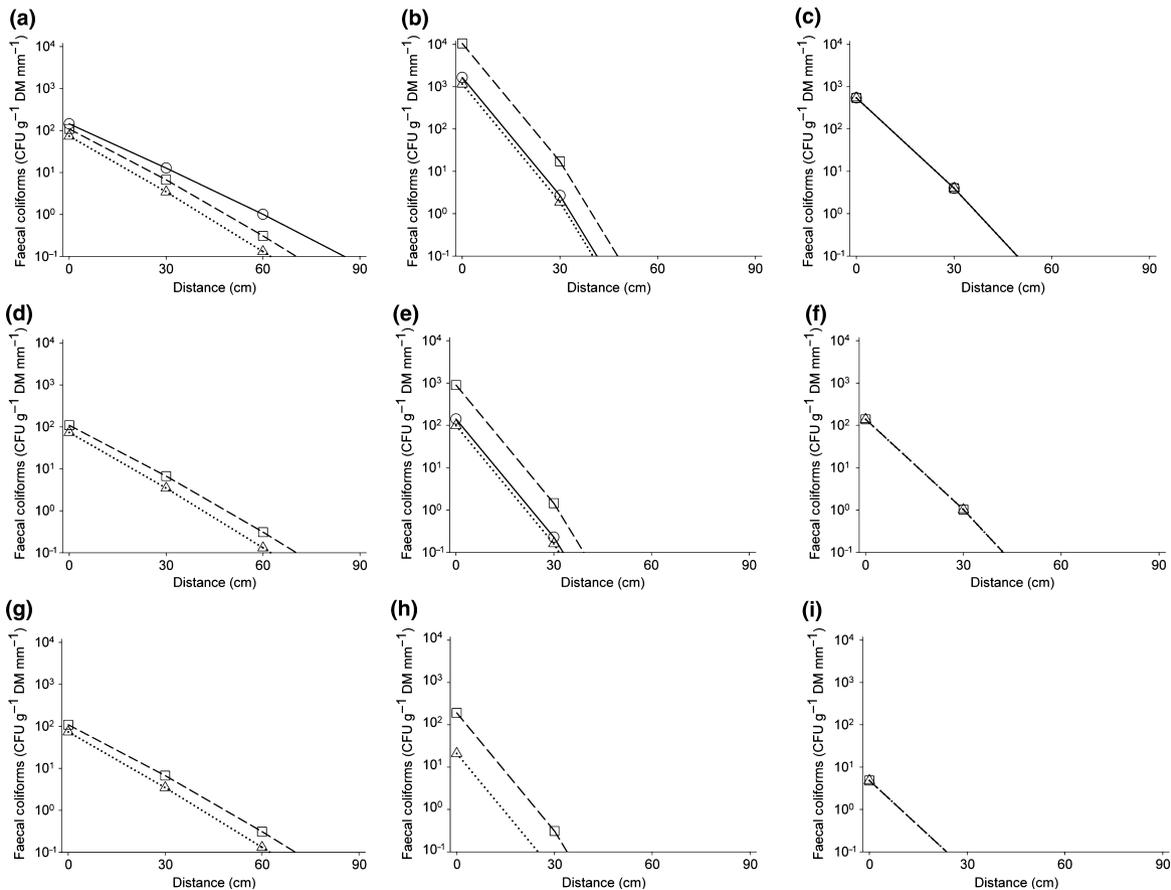


Figure 3 Modelled expected values of faecal coliform concentrations in the canopy management treatments [short (solid line), moderate (dashed line), high (dotted line)] on a level surface at each distance from the centre pot. Results are presented for canopy horizons of 0–5 cm [graphs (a), (b) and (c)], 5–10 cm [graphs (d), (e) and (f)] and 10–15 cm [graphs (g), (h) and (i)] for white clover [graphs (a), (d) and (g)], orchardgrass [graphs (b), (e) and (h)] and perennial ryegrass [graphs (c), (f) and (i)].

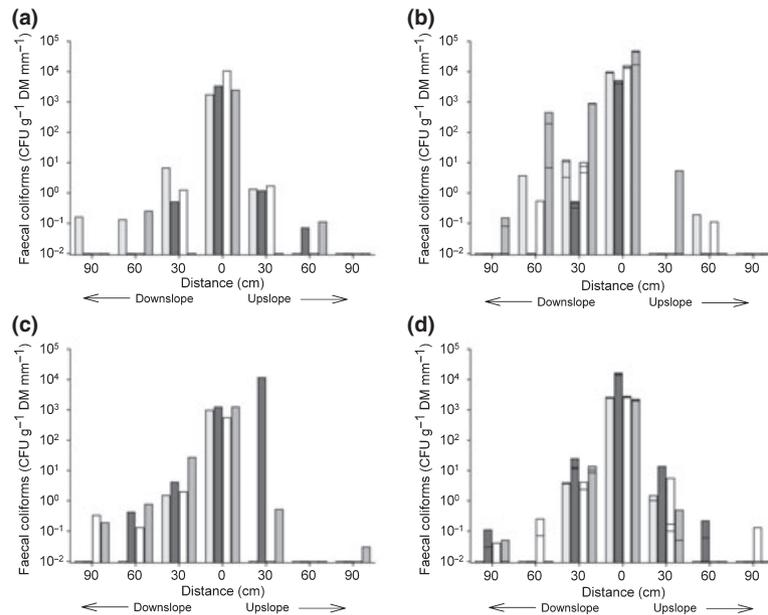
the 0–5 cm horizon (Figure 5). The combined models for S and M canopy management treatments of perennial ryegrass were identical because canopy management was not a significant variable influencing the probability of occurrence or the concentration, given occurrence, models.

Discussion

Redistribution of FC from the soil surface to the surrounding canopy was differentially modified by canopy architecture associated with the respective plant species, canopy management, slope and distance. The presence of vegetation reduced the amount of lateral transport of FC relative to that observed occurring on bare soil by Boyer (2008). Boyer (2008) found that the concentration of bacteria splashed to 30 cm was proportionately about 0.20 of the concentration splashed in the near-vicinity of

the FC source on horizontal surfaces. In this study the concentration of FC splashed to the first pot was proportionately 0.0017, <0.0001 and <0.0001 of the FC splashed to vegetation within the FC source pot for the short canopy management treatment of white clover, orchardgrass and perennial ryegrass respectively. Similar differences were also observed for bare-soil slopes where Boyer (2008) found that the concentration of bacteria splashed down-slope to 30 and 60 cm was proportionately about 0.24 and 0.09 on a 17.6 per cent slope, respectively, of the concentration splashed in the near-vicinity of the FC source. On a 86.9 per cent slope, the proportions were 0.44 and 0.21, respectively. The proportions of FC splashed onto the vegetation of the first and second down-slope pots relative to the FC splashed onto the vegetation of the FC source pot were less than 0.025 (Figure 6) for white clover and perennial ryegrass on all slopes in this experiment.

Figure 4 Distributions of faecal coliform bacteria in the down-slope and up-slope directions for 26.8, 46.6, 10.0, and 100 per cent slopes (left to right in each bar group, respectively) for white clover on (a) short and (b) moderate canopy management treatments and perennial ryegrass on (c) short and (d) moderate canopy management treatments. All canopy horizons are accumulated in each bar with the lowest section of each bar representing the geometric mean concentration in the 0–5 cm horizon.



Low concentrations of bacteria were splashed into the canopy of white clover down-slope of the source pot except when slopes were less than 46.6 per cent and splash was observed to 30 cm with the short canopy treatment (Figure 6). There was a sharp rise in relative concentrations of FC splashed to 30 and 60 cm with the moderate canopy treatment of white clover at the 100 per cent slope (Figure 6). Relative concentrations of FC splashed to 60 cm in the short and moderate canopy management treatments of perennial ryegrass were low at all slopes (Figure 6). The splash transport of FC to 30 cm increased steadily with slope greater than 36.4 per cent with a noticeable increase at the 100 per cent slope in perennial ryegrass (Figure 6). The large increase in relative concentration of FC splashed in the short canopy management treatment of perennial ryegrass at the 8.7 per cent slope indicates that lateral short-distance splash transport of FC is important at low slopes.

Results of the statistical modelling showed that species differed with respect to the log concentrations of FC splashed, supporting the hypothesis that canopy architecture influences precipitation splash geometry and dispersion of faecal pathogens. Log concentrations of FC splashed in white clover were greater than for the grass species studied.

The broad leaves of white clover probably offered a near horizontal surface on which splashed FC could accumulate and subsequently be transported to other areas of the canopy by sequential splash transport from leaf to leaf. Other studies have found this to be an important mode of transport for plant pathogens (fungal spores and bacteria) in a number of plant species

(Gregory *et al.*, 1959; Fitt and McCartney, 1986; Walklate, 1989; Walklate *et al.*, 1989; Jenkinson and Parry, 1994; Lovell *et al.*, 1999). More FC splashed in short than in moderate or tall canopy management treatments on level surfaces. The taller white clover canopies were probably intercepting more raindrops and reducing raindrop kinetic energy thereby reducing the number of splashed FC. There is experimental evidence that large diameter drops can be created on leaves and the larger drops, even with a short fall distance, can exert enough impact energy to dislodge and disperse soil particles and microorganisms. Butler and Huband (1985) found that about 0.40 of small rainfall events can be intercepted by wheat canopies. Plant canopies have finite storage potential for interception and interception decreases as a proportion of storm amount as storm size increases (Hoffman *et al.*, 1992). Inertial forces of raindrop splash are supplied by kinetic energy. Another factor controlling splash is the surface tension force. Saint-Jean *et al.* (2006) studied the dimensionless Weber number which compares the inertial forces to tension force and found a relationship with splash dispersal of fungal spores.

Slope geometry apparently modified canopy management effects on splash of FC. As slope increases, there is the chance that the top of the canopy of down-slope plants can be lower in elevation than the base of up-slope plants. Greater proportions of FC splashed into the upper parts of the canopies on slopes (Figure 4) in comparison to canopies on level surfaces (Figure 2). It is interesting that upper portions of the perennial ryegrass canopy of up-slope plants were also accumulating FC.

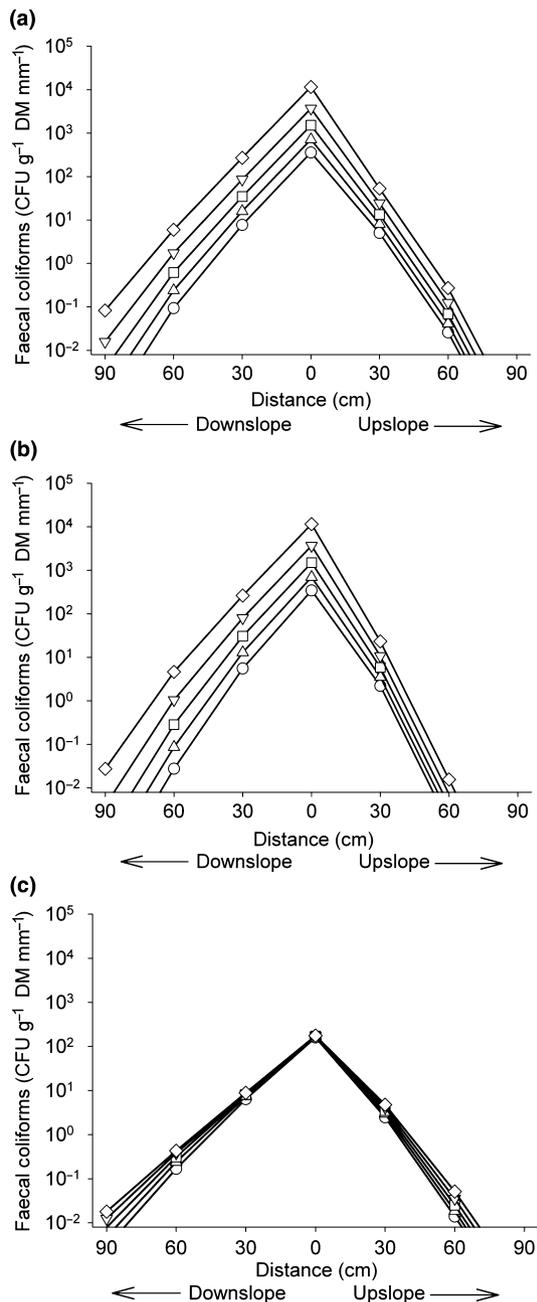


Figure 5 Modelled expected values of faecal coliform concentrations in the 0–5 cm horizon at each distance from the centre pot in the canopies on sloping surfaces of 8.7 (○), 26.8 (△), 46.6 (□), 70.0 (▽) and 100 (◇) per cent for white clover under (a) short and (b) moderate canopy management treatments and perennial ryegrass under (c) short canopy management treatment.

That might have been a result of the fine blades of grass bending over the down-slope pots under the weight of accumulated intercepted rainfall.

The combined models for deriving expected values of FC concentrations in the canopies are presented for comparisons within this study. The models are not expected to apply outside the boundaries of this study. However, the results of the models can be used to guide further studies of the effects of canopy – slope interactions coupled with rainfall characteristics. For example, it is of interest to determine how expected values of FC concentrations in the canopies change when raindrop size and rainfall intensity change.

The FC concentrations were standardized to number of bacteria g^{-1} DM herbage mm^{-1} of rain to account for spatial and temporal differences in DM production of herbage and small differences in total rainfall applied across the experiments. Many of the FC concentrations presented in this study appear small. However, if one considers that an average growing steer (about 300 kg) consumes proportionately about 0.03 of its live weight as herbage (about 9 kg DM) daily, then the total FC that could be ingested can be high.

For example, if a 40-mm rainfall event is considered, which was the average for this study, then 1 cfu g^{-1} DM mm^{-1} translates to 3.6×10^5 FC ingested d^{-1} and 100 cfu g^{-1} DM mm^{-1} translates to 3.6×10^7 FC ingested d^{-1} and so on. Some of the observed FC concentrations on herbage exceeded $20\,000 \text{ g}^{-1}$ DM mm^{-1} . It is unknown what quantity of faecal pathogens will cause ill health in livestock, but it is known that young and stressed livestock are susceptible to infection by ingesting pathogens with herbage resulting in the animals shedding an increased numbers of pathogens in faeces (Lejeune *et al.*, 2001; Fitzgerald *et al.*, 2003; Looper *et al.*, 2006).

This series of experiments demonstrated that rainfall splash transport of faecal bacteria into canopies is a possible source of contamination of adjacent herbage and infection of livestock consuming such herbage. Although lateral dispersal was modified by the presence of vegetation, there was still dispersion into the canopy caused by rain splash. Only a small soil surface area ($<0.07 \text{ m}^2$) contaminated with FC was used in this experiment. A pasture will have many source areas depending on movement of grazing livestock and where dung pats are deposited. Closely paired source areas could result in much higher concentrations of FC in the canopy than what was observed in this study. The greatest rates of lateral and vertical dispersion of FC into the canopy occurred with white clover, especially with the short canopy management treatment. From this it seems that a somewhat greater canopy height would be appropriate to minimize splash dispersion of faecal contaminants in canopies with an abundance of broad-leaved species. The results suggest that young or stressed grazing livestock should be kept off contaminated pastures during rainy periods. The results add to

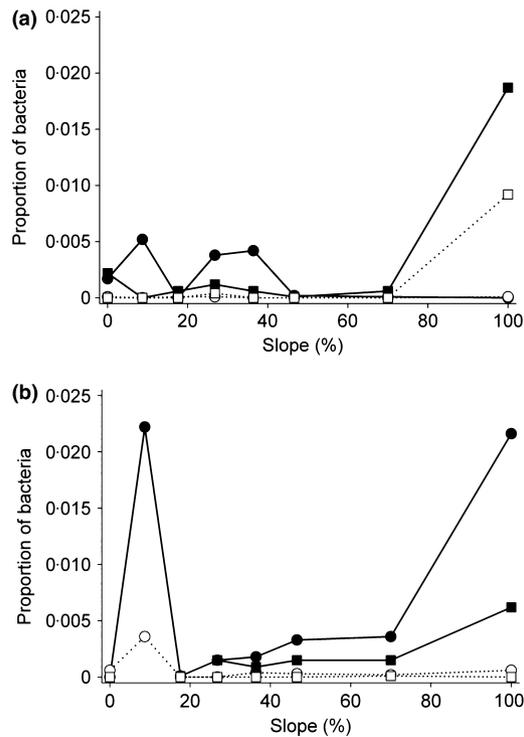


Figure 6 Proportion of faecal coliform bacteria on the vegetation at 30 cm (first pot, filled symbols) and 60 cm (second pot, open symbols) down-slope relative to the number of faecal coliform bacteria on vegetation in the source pot for (a) white clover and (b) perennial ryegrass for short (○) and moderate (□) canopy management treatments.

knowledge about farm-level transmission of faecal microorganisms and contribute to the ability to design and implement management practices that protect human and livestock health as well as water resources in the environment.

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