The association of *E. coli* and soil particles in overland flow

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Abstract The removal of *E. coli* from overland flow under saturation-excess runoff conditions was investigated in experimental field plots that were 1 m wide and 5 m long. Variation in the attenuation of bacteria and distance transported was quantified under contrasting flow conditions. In addition, the impact of soil tillage upon microbial attenuation was examined by comparing results derived from grassed plots (intact) with those subject to tillage with the soil left bare (cultivated). For intact plots subjected to a flow of 2 L/min, 27% of the *E. coli* in the flow was removed after 5 m with removal following a logarithmic function with respect to distance. For the higher flow rates of 6 L/min and 20 L/min, no attenuation trend was observed over this distance. *E. coli* removal during flow across the cultivated plots was significantly greater compared to the intact plots. This was attributed to a greater infiltration rate in the cultivated plots (due to the tillage) which promoted a greater volume of flow to pass through the soil matrix, providing the opportunity for filtration and adsorption of microbes. Logarithmic trends with respect to distance were observed for all flow rates tested on the cultivated plots (2, 6 and 20 L/min). Total removal after 5 m at a flow rate of 2 L/min was 41% and again removal efficiency decreased as the flow rate increased. Analysis of the transported state of the *E. coli* revealed that the bacteria were being transported predominantly in particles less than 20 μm in diameter and were not attached to large (dense) soil particles. The limited removal (<50%) of bacteria from overland flow under saturation-excess runoff conditions in these experiments appeared, therefore, to be primarily due to a lack of settling or deposition. Instead, most bacteria remained entrained within the overland flow down the length of the plots.

Keywords Attachment; cultivation; grass; riparian; runoff; transport

Introduction

The transportation of microorganisms from agricultural land into waterways is generating concern worldwide (Ferguson *et al.*, 2003; Tyrrel and Quinton, 2003). Vegetative buffer strips (VBS) have been proposed as a measure that can limit the transport of microbes to waterways within overland flow. However, experiments to measure their effectiveness have often produced variable results (Crane *et al.*, 1983; Entry *et al.*, 2000; Fajardo *et al.*, 2001), probably reflecting the variation in soil type, topography and experimental design. In addition, such variability is also likely to reflect variation in the state by which bacteria are transported within overland flow, i.e. individual cells, cells attached to soil particles or large clumps and flocs of cells (Tyrrel and Quinton, 2003). Muirhead *et al.* (2005) demonstrated that *E. coli* cells in runoff direct from cowpats were predominantly eroded as individual cells that are easily transported in water during rainfall events.

Three mechanisms have been proposed as being important in reducing the number of bacteria transported by overland flow. The bacteria may settle on the soil surface, be filtered out of the flow by vegetation or filtered during passage through the soil matrix. Single bacteria are small and neutrally buoyant in water. This limits the potential of
settling or filtration by the vegetation as a removal mechanism (Fiener and Auerswald, 2003), unless they attach to large soil particles or form large flocs during transport. In the absence of attachment and flocculation, the key mechanism that could significantly reduce bacterial numbers in overland flow is filtration by the soil matrix following infiltration of overland flow. Two issues influence the movement of bacteria through the soil matrix. First, when overland flow is generated by saturation-excess conditions infiltration is minimal providing little opportunity for microbes to interact with the soil matrix (Ahuja et al., 1981). Secondly, bacterial transport through some soils tends to be dominated by macro-pore or preferential flow which bypasses the soil matrix (Smith et al., 1985). Lysimeter studies have demonstrated that bacterial removal can be enhanced by repackaging soil so as to remove macropores thereby enhancing flow through the soil matrix (Smith et al., 1985). Here, it is hypothesised that tillage associated with the cultivation of soil will have a similar effect: increasing infiltration, removing macropores and providing greater opportunity for microbial filtration and adsorption. If this hypothesis is correct, cultivation of a buffer strip could be used as a management tool to enhance the attenuation of microbes washed down slope in overland flow whilst also producing a crop that contributes to the economic outputs of the farm.

In this study, the transport of bacteria in overland flow under saturation-excess conditions was investigated as this is the predominant runoff process occurring on agricultural land in New Zealand. Specifically, we examined the effect of flow rate, distance travelled and cultivation on the attenuation of \textit{E. coli} within overland flow. We also investigated the transported state of the \textit{E. coli} in the overland flow.

**Materials and methods**

Eight plots (1 m wide, 5 m long) were constructed by inserting sheet metal 0.2 m (depth of the A horizon) into the ground in intact soil previously used as a sheep pasture. Four of the plots were left intact with their existing pasture cover of grass mown to 5 cm height. The other four plots were cultivated by first turning with a spade followed by cultivation with a rotary hoe; these plots were not re-sown. The soil was a Warepa silt loam classified as a Mottled Fragic Pallic soil with a very low saturated hydraulic conductivity \([K_{sat}] < 1 \text{ mm/h}\) due to severely impeded drainage in the B horizon. Dairy cow faeces, collected from the concrete base of a farm dairy, were mixed and the number of \textit{E. coli} in a sub-sample estimated using the Colilert/Quanti-Tray method (Muirhead et al., 2004). Tap water was added to the faeces to derive liquid effluent with a target \textit{E. coli} concentration of \(5 \times 10^5\) MPN/100 mL. The effluent was evenly distributed onto the top of the plots \textit{via} a manifold that delivered flow rates of either 2, 6 or 20 L/min. In the intact and cultivated soils, collectors were positioned at distances of 1, 2, 3 and 4 m down-slope of the inlet flow in the centre of each plot. For the intact treatment, surface flows were collected. For the cultivated treatment, a combined surface and sub-surface flow sample was obtained from a sampler installed at 15 cm depth. Five metres from the top of each plot, channels collected the overland flow and directed it to V-notch weirs where the flow rate was measured and samples were collected for analysis.

For each experiment, the effluent was added to the plot at flow rate of 2 L/min until the outflow at the lower end of the plot was constant. Constant outflow was deemed to be two identical flow rate measurements taken 5 min apart; this typically took 30–60 min. Samples were collected from the outlet, then the surface (or sub-surface) collectors followed by the outlet again. The flow rate onto the plot was then increased to 6 L/min and once the outlet flow rate was constant (which took \(<\) 20 min) samples were again collected as described above. The flow was then increased to 20 L/min and the sampling regime repeated once outflow was again constant (which took \(<\) 5 min). Samples of the
inlet water were also collected during the experiments. The procedure for increasing inflow during the experiment was adopted to reflect the increase in overland flow often observed as a natural rain event progresses. *E. coli* concentrations were determined in all samples using the Colilert/Quanti-Tray method (Muirhead *et al.*, 2004). Preliminary experiments at all three flow rates were carried out using tap water only in order to determine background counts. The first attenuation experiment using effluent was run at a flow rate of 2 L/min only and in the following three attenuation experiments the plots were exposed to all three flowrates.

The in- and out-flow samples from some of the runoff experiments were further analysed to determine the association between the bacteria and soil particles. The number of *E. coli* attached to dense soil particles was estimated using a buoyant density separation technique described (Muirhead *et al.*, 2005). The number of *E. coli* cells attached to large particles was estimated by determining cell concentrations in a column of water before and after settling for 20 min during which time all soil particles >3 μm diameter would have settled a distance of 1 cm (Tanner and Jackson, 1947). Size analysis of the particles was conducted by gravity filtering a sub-sample through a 20 μm filter paper (Grade 41, Whatman International Ltd, Maidstone, England).

For statistical analysis the *E. coli* concentrations were log-transformed before analysis with GENSTAT version 8. ANOVA was conducted for each distance down the plot to test for treatment and flow rate effects using the inlet concentration as a covariate. The percentage removal of *E. coli* for each sample was calculated with respect to the inlet concentration.

**Results**

**Preliminary experiments**

The preliminary runoff experiment, using tap water only, generated concentrations of *E. coli* in the runoff ranging up to $2.5 \times 10^4$ MPN/100 mL (Figure 1). In both the intact and cultivated plots, concentrations decreased as the flow rate increased due to a dilution

![Figure 1](https://iwaponline.com/wst/article-pdf/54/3/153/431635/153.pdf)
effect (Figure 1). At flow rates of 2, 6 or 20 L/min, the mass discharge rates for the intact plots at the outlet (5 m) were $8.7 \times 10^3$, $1.9 \times 10^4$ and $2.3 \times 10^4$ MPN/s, respectively. For the cultivated plots, the mass discharge rates at the outlet were $5.1 \times 10^3$, $8.5 \times 10^3$ and $2.1 \times 10^4$ MPN/s at the flow rates of 2, 6 or 20 L/min, respectively. On the intact plots, overland flow E. coli concentrations increased with distance down slope indicating that the soil provided a source of E. coli that became entrained within the overland flow (Figure 1). In the cultivated plots, the increase in overland flow E. coli concentrations was not as steep as observed for the intact plots (Figure 1). There were statistically significant differences in concentrations of E. coli between the flow rates ($P < 0.01$) but no difference between the cultivated and intact plots.

Attenuation experiments

The E. coli concentrations in the effluent used to generate overland flow ranged from $1.6 \times 10^5$ to $1.1 \times 10^6$ MPN/100 mL with an overall average of $3.9 \times 10^5$ MPN/100 mL which was slightly less than the target concentration of $5 \times 10^5$/100 mL. As expected for this soil type, in all experiments the runoff flow rates at steady state equalled the inflow rates, indicating saturation-excess runoff conditions. In the cultivated plots, any water that infiltrated the cultivated soils travelled horizontally through the cultivated soil matrix, resurfaced and re-emerged as overland flow 5 m down slope where the flow reached intact soils again.

The mean percentage of E. coli removed from overland flow is illustrated as a function of flow rate and distance down slope from the inlet for both intact and cultivated treatments (Figure 2). Statistical analysis of the removal of E. coli from the overland flow showed that there was a significant difference between the intact and cultivated soil treatments at all distances down slope from the inlet ($P < 0.05$). Analysis of the effect of flow rate on the removal of E. coli from the overland flow found no significant difference between the flow rates at 1 m distance, but significant differences at 2, 3 and 4 m

Figure 2  Attenuation of E. coli by the intact and cultivated soil treatments. The mean percentage of E. coli removed from the runoff is plotted against the distance down slope that the effluent has flowed from the inlet. Solid symbols represent intact plots; hollow symbols represent cultivated plots for flow rates of 2 (●), 6 (■) or 20 (▲) L/min. The logarithmic trend lines are represented as a solid line for the intact treatment (2 L/min only). Dashed lines show logarithmic trend lines for the cultivated treatment at all flow rates.
(P < 0.05) and a highly significant result was observed at 5 m (P < 0.001). Within each treatment (intact or cultivated soils) there were no significant differences in the attenuation of E. coli between individual plots or between the repeated runoff experiments.

For the intact plots, runoff flowed over the surface of each plot at all flow rates and samples of overland flow were able to be collected from all experiments. The percentage removal of E. coli from overland flow at the flow rate of 2 L/min was 14% after 1 m and increased to 27% after 5 m (Figure 2). The logarithmic trend line through the 2 L/min data points for the intact plots (R² = 0.56) indicated that the removal was not linear with respect to the distance the overland flow travelled over the plots (Figure 2). The percentage removal of E. coli from overland flow at the 6 and 20 L/min flow rates was positive at 1 and 5 m but negative at 2, 3 and 4 m, indicating that the overall trend was less than the variability of the individual samples analysed. Therefore, no trend lines were placed through these data sets (Figure 2).

On the cultivated plots, at the 2 L/min flow rate, all runoff flowed through the soil matrix during all experiments. In contrast, at 20 L/min, runoff was observed to travel over the soil surface in all experiments. At the 6 L/min flow rate for the preliminary and first attenuation experiment, runoff travelled through the soil matrix. However, by the last two attenuation experiments the cultivated soil had compacted (due to experimental runoff and natural rainfall) and some overland flow was observed. The percentage of E. coli removed at the 2 L/min flow rate was 24% at 1 m down slope and increased every metre thereafter to a maximum of 41% removed at 5 m. This removal pattern appeared to follow a logarithmic trend (R² = 0.98) with respect to distance (Figure 2). The percentage of E. coli removed by the cultivated plots at 6 and 20 L/min was less than at 2 L/min and was more variable with R² values for the logarithmic trends of 0.17 and 0.71, respectively (Figure 2).

Analysis of selected samples of the runoff by buoyant density separation technique revealed that approximately 9% of the E. coli cells were attached to dense particles. There was no significant difference (P > 0.05) between the E. coli concentrations in settled and un-settled samples, indicating that the E. coli cells were not attaching to large dense soil particles. The number of E. coli in the filtered samples was significantly less (P < 0.001) than that observed in the unfiltered samples (data not shown), with approximately 80% of the E. coli cells passing through a 20 μm filter.

Discussion
The results from the preliminary experiments (using tap water only) showed that there were high background concentrations of E. coli in overland flow on these plots. This indicated that soils could act as both a source and a sink for microorganisms and that the overall performance of a buffer strip would typically be a balance between the two processes. During the attenuation experiments, overland flow E. coli concentrations decreased with distance down slope and increased with increasing flow rate indicating that the strips primarily acted as a sink for E. coli. However, it was likely that some background bacteria simultaneously provided a source and were entrained within the passing overland flow (Collins et al., 2004).

The removal of E. coli in overland flow from the intact plots at a flow rate of 2 L/min at 5 m distance was relatively poor (<27%) which under saturation-excess conditions was not unexpected (Collins et al., 2004). Coyne et al. (1998) observed that faecal coliform concentrations in the overland flow did not decrease as the water flowed through a 9 m buffer strip and that mass removal was achieved by infiltration. In our data, we showed a small decrease in concentration with distance but the important observation was that more than half of the attenuation occurred in the first metre of the experimental
strip. Under saturation-excess conditions, overland flow has very little interaction with the soil matrix (Ahuja et al., 1981) and, therefore, the key bacterial removal mechanisms are potentially the settling of microbes onto the soil surface and filtration by vegetation (Fiener and Auerswald, 2003). Our analysis indicated that E. coli travelling 5 m through the experimental strips were not attached to large soil (dense) particles but were instead transported as small particles, predominantly less than 20 μm, with neutral buoyancy. Therefore, bacteria were not easily removed from overland flow by the mechanisms operating under saturation-excess conditions. It is very likely that any large particles or bacteria attached to large dense soil particles were rapidly removed from the overland flow during the first metre as observed for sediment removal in buffer strips (Helmers et al., 2005). Once these large particles have been removed from the overland flow the ability of the buffer strip to further trap microorganisms would be decreased.

As flow rate increased on the intact plots, E. coli attenuation appeared to stop with any overall trend being less than the variability of the data. There were two possible explanations for this observation: (1) as the flow rate increased so the travel time of effluent through the plot decreased providing less opportunity for bacteria to settle out or be filtered; (2) at the increased flow rates bacteria that had previously been trapped at the lower flow rate were entrained into the flow resulting in no net deposition within the buffer strip.

The cultivation treatment was implemented to increase the infiltration capacity of the soil and to disrupt any macropores thus promoting matrix flow through the soil. Our hypothesis that enhanced matrix flow through the soil would increase the attenuation of the E. coli in the experimental strips was shown to be correct. The attenuation pattern with respect to distance in the cultivated strips was the same as the intact strips with the majority of the removal occurring in the first metre. Water flow through soils is thought to occur mainly through pores that are greater than 30 μm diameter (McLaren and Cameron, 1996). Our results showing that the majority of the E. coli were in particles smaller than 20 μm suggested that the bacteria were also travelling through these large soil pores.

Most buffer strip studies have concluded that infiltration is the dominant removal mechanism for attenuating microbes in overland flow (e.g. Coyne et al., 1998). Our results showing limited attenuation under saturation-excess runoff conditions thus appeared to be realistic for what is effectively a worst-case (limited or no infiltration) scenario. However, to fully quantify long-term buffer performance requires experiments to be conducted under variable antecedent conditions for a full range of hydrological events.

Conclusions

- Vegetative buffer strips can act as both a source and a sink for microorganisms and, therefore, the overall performance is a balance between these two processes.
- Attenuation of E. coli by buffer strips appears to follow a logarithmic function with distance travelled.
- E. coli attenuation is decreased as the flow rate through the buffer strips increases.
- Tillage associated with cultivation appears to enhance bacterial attenuation by promoting flow through the soil matrix providing opportunity for filtration and adsorption. Cultivated buffer strips thus have the potential to both (a) enhance the attenuation of microbes and other diffuse pollutants and (b) contribute to the economic outputs of a farm.
- Under saturation-excess runoff conditions the removal of bacteria in these experimental strips was <50% primarily because the bacteria were transported as small particles of neutral buoyancy that predominantly remained entrained within the overland flow.
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