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Abstract. *Limited data on microbial partitioning between the freely suspended and particulate attached phases during transport along overland flow pathways results in high uncertainty in bacterial fate and transport models. The objectives of this work were to partition E. coli and enterococci between the unattached and attached phases at the edge-of-the-field and to identify the particle sizes to which the attached bacteria preferentially associated. The average E. coli PC for all samples collected was 0.06 which corresponded to 4.8% attached and the average PC for enterococcus was 0.18 which corresponded to 13% attached. E. coli and enterococci PC, PAF, and TC did not change significantly between the rising, peak, and recession limbs of the runoff hydrograph. Additionally, no significant correlations were identified between bacteria fractions or total concentrations and TSS. Fifty eight percent of attached E. coli and enterococci cells were associated with particulates retained by an 8 µm screen, however, the overall low attachment ratios indicate that designing management practices to trap particulates will not sufficiently reduce transport of pathogen indicators to surface waters. Results of this study indicate that best management practices to reduce bacterial loadings from pasturelands with high vegetative cover should focus on retention of pathogen indicators moving through overland flow pathways in the unattached state.*

Keywords. Leave the word "Keywords." then type keywords or key phrases, separated by commas. List both specific and general terms that will aid in searches. The ASABE website has suggested keywords but you are not limited to these.

Introduction

Pathogens are the leading cause of water quality impairments in many parts of the United States. Pathogens originate from many different sources including agricultural operations such as allowing cattle to have direct access to streams; human sources such as leaking septic systems; or wildlife sources such as migratory birds. However, agricultural practices have been cited as the primary contributor to impairments of rivers and streams (USEPA, 2003). The three most common pathogen indicators in the United States include fecal coliforms, *E. coli*, and enterococci (USEPA, 1986). Although fecal coliform have been traditionally used as an indicator to detect the presence of pathogens in surface waters, *E. coli* and enterococci are thought to have a higher degree of association with outbreaks of gastrointestinal illnesses (USEPA, 1986) and are therefore currently the recommended indicator organisms (USEPA, 1998; USEPA, 2002). In an attempt to reduce pollutant loading to the nation's water bodies, Total Maximum Daily Loads (TMDLs) are being developed to assess water quality problems, identify pollution sources, and determine pollutant reductions needed to restore and protect rivers, streams, and lakes. A TMDL is a calculation of the maximum amount of a pollutant that can be introduced to a water body, while still meeting the water quality standards, and an allocation of that amount to the pollutant's sources.

Because of the high costs associated with the development and implementation of TMDLs, it is essential that TMDLs be developed using sound scientific methods that are able to accurately reflect the pollutant loadings from the potential sources within a watershed. Currently, Nonpoint Source (NPS) pollution models are most frequently used to determine the maximum allowable loading rates of bacteria from the identified sources. However, most currently-used NPS models, including SWAT (Soil and Water Assessment Tool) and HSPF (Hydrological Simulation Program – FORTRAN), simulate bacterial transport to surface waters as an unattached or dissolved pollutant (Paul et al., 2004). Cell surface properties such as hydrophobicity of the cell (Fattom and Shilo, 1984; Kinoshita et al., 1993) and the electrostatic nature of the cell envelope (Jamieson et al., 2004) and external factors including availability of attachment sites (Characklis et al., 2005; Fries et al., 2006), ionic strength, and pH of the carrying solution (Jewett et al., 1995; Scholl and Harvey, 1992), and size of the particulate matter (Fontes et al., 1991) have been listed as factors that influence bacterial attachment to soils. Previous studies have determined that fecal bacteria preferentially attached to particulates (Auer and Niehaus, 1993; Henry, 2004; Ling et al., 2002) and statistically indistinguishable release rates between manure particulates and fecal coliforms have been observed through stony soils (Shelton et al., 2003). Very little data is available on bacterial partitioning between the attached and unattached phases during movement along overland transport pathways (Jamieson et al., 2004).

Many researchers and practitioners recognize the shortcomings in the existing methods used to model bacterial fate and transport (Jamieson et al., 2004; Paul et al., 2004). Representing bacteria as a dissolved pollutant might not accurately reflect the transport processes that occur in agricultural watersheds. However, before bacteria transport modeling can be improved, an in-field study of bacteria transport and the related associations with flow, particulates, and water quality indicators is needed. Many models already partition between nutrient phases; thus, identifying correlations between bacterial and nutrient partitioning might improve predictive capabilities of bacterial transport models by modification of existing nutrient overland transport process algorithms. In addition, if attachment is a significant edge-of-field transport factor, design and selection of management practices could be improved to encourage settling of particulates and the attached fecal indicators for reduction of pathogen transport to surface waters.

The goal of this study was to investigate the partitioning of E. coli and enterococci between the unattached and particulate-attached phases during overland flow from pasturelands to improve the predictive capabilities of current NPS models and design of best management practices. Previously published separation techniques were validated and were combined to partition between the unattached and attached phases of E. coli and enterococci at the edge-of-the-field. Correlations between bacterial and nutrient partitioning ratios and loading rates were examined for potential relationships.

Materials and Methods

Six field plots 3-m (9.8-ft) wide by 18.3-m (60-ft) long were constructed on Groseclose silt loam pasturelands (35% sand, 60% silt, and 5% clay) on an approximate 9-percent slope dominated by a dense stand of Kentucky 31 Tall Fescue. A "V" shaped outlet at the down-slope end of each plot directed runoff into a 0.15-m (6-inch) H-flume equipped with a stilling well and a stage recorder for flow measurement. Surface soil samples (0 - 8 cm depth) were collected with a soil probe from each transport plot. The samples were sieved (2 mm), and stored prior to analysis. Soils were analyzed for Mehlich -1 P (11 mg kg^{-1}), organic matter (2.6%) by a modified Walkley-Black method and pH (6.22) by 1:1 soil to distilled water ratio and solid state pH meter (Donohue and Heckendorn, 1994).

Fresh dairy cattle fecal deposits were collected at the Virginia Tech dairy facility over a 24 hour period. Standard cowpats (Thelin and Gifford, 1983) were formed by mixing the manure in a cement mixer for fifteen minutes. The homogenized manure was placed in molds with a diameter of 20.3 cm (8 in) and a depth of 2.54 cm (1 in) until a weight of 0.9 kg (2.0 lbs) was reached. Manure samples were collected prior to land application and analyzed by the Clemson Agricultural Service Laboratory. Water soluble P, (2.02 g kg^{-1}),was determined by the method proposed by Sharpley and Moyer (2000). The pH, (5.6), was measured potentiometrically in a 1:2 manure/water slurry (Peters et al., 2003). Average moisture content of fresh manure samples was 83.61%. Approximately 106 cowpats were applied to four of the five plots to represent a rotational grazing system or an area where pastured cattle tend to congregate, such as feeding, watering, or shaded areas. One plot received no treatment and was used as a control. Background *E. coli* concentrations averaged 2.0×10^2 (4.2×10^2) cfu 100 mL^{-1} and enterococci concentrations averaged 0.0 cfu 100 mL^{-1}. The background *E. coli* was only detected in two of the ten samples collected during the runoff event and is most likely attributed to wildlife (Doran et al., 1981; Patni et al., 1985).

Due to the unreliability of natural precipitation for short-term field research, a rainfall simulator (Dillaha et al., 1988) generated a uniform rainfall event (2.8 cm/h) to all plots. After the beginning of runoff, discreet grab samples were collected at the outfall of the flumes. Samples were collected at the onset of runoff, at ten minute intervals during the storm event, at the end of the storm event, and four minutes after the precipitation ceased. Three samples were collected during each sampling event, one for bacterial partitioning studies, one for total *E. coli* and enterococci concentration analysis, and one for nutrient analysis. The rainfall event continued until runoff from all plots reached steady state (three hours and 20 minutes) and the longest runoff event lasted 90 minutes (plot 2).

Separation and Dispersion Technique

Collected samples were transported to the laboratory immediately following the end of the rainfall simulation and analyzed for *E. coli*, enterococci, TSS, phosphorous, organic phosphorus, and organic carbon. Partitioning pathogen indicators between attached and unattached phases was achieved by fractional filtration followed by centrifugation. Fractional filtration has been used previously to identify particle sizes to which bacteria are attached (Auer and Niehaus, 1993; Schillinger and Gannon, 1985), and a filter pore size of 8 μm has been identified as a viable method to separate attached and free bacteria (Gordon et al., 2002; Henry, 2004; Mahler et al., 2000; Qualls et al., 1983). The presence of sediments and organic particles in runoff from agricultural lands makes it very likely that the filters could clog and retain free cells, resulting in a higher fraction of cells being classified as attached. To assess retained unattached cells, we rinsed the screens and filters with phosphate buffered water and then centrifuged the re-suspended solution. This technique combines the benefits of fractional filtration, identifying particle sizes to which cells attach, with the more commonly used practice of centrifugation.

Following centrifugation, the solutions associated with each particle size were re-suspended and dispersed prior to enumeration of the total *E. coli* and enterococci concentration. A comparative study was conducted to identify the best method of dispersing wild strains of indicator organisms from sediment and organic matter particles present in runoff from pasturelands. Ten minutes of hand shaking increased *E. coli* concentrations by 60% (*p* value = 0.0018) and additional experiments revealed that the 10-minute hand shaker treatment provided more consistent results for both pathogen indicators and was therefore selected as the treatment for this study.

Nutrient Analysis

Runoff samples were analyzed for nutrient and suspended solids concentrations to examine potential relationships between bacterial and nutrient attachment ratios. Nutrient analysis was performed using a Bran + Luebbe (distributed by SEAL Analytical Ltd, West Sussex, United Kingdom) Traacs 800 Continuous Flow Wet Chemistry Autoanalyzer. The nutrient analysis was performed following procedures in Standard Methods for the Examination of Wastewater (Clesceri et al., 1998). Nutrient analysis included TDP (0.45 μm polyethersulfone filter, Pall Life Sciences, Ann Arbor, MI), TP, DOP, TOP, DOC, and TOC. Total suspended phosphorous was calculated as the difference between TP and TDP and SOP was calculated as the difference between DOP and TOP (Clesceri et al., 1998). Suspended organic carbon was calculated as the difference between TOC and DOC. Total Suspended Solids were analyzed (0.45 μm glass fiber filter, Pall Life Sciences, Ann Arbor, MI) as recommended by Clescersi et al. (1998).

Calculations and Statistical Analysis

The attached cells were assumed to be the difference between the unattached and total *E. coli* and enterococci concentrations. The partitioning coefficient was calculated using Equation 1 and the particulate associated fraction was calculated using Equation 2. The attached portion associated with each screen size was divided by the TSS associated with respective screen size to obtain the colony forming units per gram of particulates and determine the particle sizes to which *E. coli* and enterococci preferentially attach.

$$Partitioning\,Coefficient = \frac{attached}{planktonic} \quad (1)$$

$$Partitculate\ Attached\ Fraction = \frac{attached}{attached + planktonic} \quad (2)$$

Statistical analysis of data was performed using the Statistical Analysis System (SAS Institute, 2004). The nonparametric Kruskal-Wallis rank test was used to test for significant differences between partitioning ratios, particulate associated fractions, and total concentrations during the rising, peak, and receding limbs of the runoff hydrograph. Pearson correlation coefficients between bacteria partitioning and concentrations and runoff, TSS, and nutrients were determined using PROC CORR and a *p*-test was performed to test for statistical significance (SAS Institute, 2004). Data were normalized prior to analysis and statistical significance was determined when $p \leq 0.05$.

Results and Discussion

The average *E. coli*, enterococci, and nutrient flow-weighted concentrations and loads are presented in Table 1. Flow-weighted concentrations were calculated by multiplying the sample concentrations by the subsequent runoff volume and then dividing by the total runoff volume from each plot. Bacterial and nutrient loads were calculated by multiplying the sample concentrations by the subsequent runoff volume and converting the plot area to a per hectare basis. Plot 3 was excluded from bacterial load calculations because of missing data points. High standard deviations in load calculations are to be expected because of the differences in total runoff volumes from each plot (ranging from 0.12 to 0.50 m^3). Nutrient analysis included total and organic forms of phosphorus to account for inorganic residual from fall fertilizer application and the organic forms present in fresh cowpats.

Table 1. Average *E. coli*, enterococci, total suspended solids, and nutrient flow-weighted concentrations and loads

	Flow-weighted concentration	units		Mean Load (Standard Deviation)	units
E. coli	6.96×10^5	cfu 100 mL^{-1}	*E. coli* attached	4.39×10^5 (3.77×10^5)	cfu ha^{-1}
enterococcus	3.63×10^5	cfu 100 mL^{-1}	*E. coli* unattached	1.12×10^6 (1.43×10^7)	cfu ha^{-1}
TDP	2.67	mg L^{-1}	enterococci attached	1.83×10^5 (2.08×10^5)	cfu ha^{-1}
TSP	1.41	mg L^{-1}	enterococci unattached	7.28×10^6 (1.21×10^7)	cfu ha^{-1}
TP	4.08	mg L^{-1}	TDP	0.11 (0.09)	kg ha^{-1}
DOP	1.36	mg L^{-1}	TSP	0.049 (0.030)	kg ha^{-1}
SOP	0.75	mg L^{-1}	DOP	0.056 (0.046)	kg ha^{-1}
TOP	2.11	mg L^{-1}	SOP	0.025 (0.018)	kg ha^{-1}
DOC	14.65	mg L^{-1}	DOC	2.57 (2.13)	kg ha^{-1}
SOC	1.02	mg L^{-1}	SOC	0.19 (0.12)	kg ha^{-1}
TOC	15.67	mg L^{-1}	TSS	761 (242)	kg ha^{-1}
TSS	152.22	mg L^{-1}			

Bacterial partitioning related to flow regime

As indicated previously, following the onset of runoff from the plots, samples were collected at ten minute increments from the outfall of the flume. Bacterial partitioning might be impacted by flow velocities (Guber et al., 2005a; Krometis et al., 2007) so bacterial and nutrient partitioning was separated into rising, peak, and receding limbs of the overland flow hydrograph (Figure 1). The number of samples collected from each plot varied due to different beginning of runoff times but ranged from seven to eleven samples. Nineteen samples were included in the rising limb analysis, ten samples in the peak limb analysis, and 6 samples in the falling limb analysis. Partitioning coefficients and PAF are presented as box and whisker plots illustrating 10^{th}, 25^{th}, 75^{th}, and 90^{th} percentiles. The *E. coli* PC and PAF medians are both slightly lower during the rising and falling limbs of the hydrograph following the same pattern as TSS concentrations

(Figure 2). Enterococci, however followed the opposite trend; the mean PC and PAF increased during the rising and falling limbs of the runoff hydrograph. The total bacterial concentrations decreased as the runoff hydrograph progressed (Figure 2), similar to what you would expect from a first flush effect (Novotny, 2003).

The nonparametric Kruskal-Wallis rank test was used to identify statistically significant differences between PC, PAF, and TC during the rising, peak, and recession phases of the runoff hydrograph. Neither *E. coli* or enterococci PC, PAF, or TC were significantly different between the rising, peak, and recession limbs of the runoff hydrograph. Only organic carbon exhibited statistically significant differences among the partitioning coefficients ($p \leq 0.0300$) and particulate associated fractions ($p \leq 0.0300$). Concentrations of TP, TOP, and TOC all significantly differed between phases of the runoff hydrograph ($p \leq 0.0003$, $p \leq 0.0187$, and $p \leq 0.0045$, respectively). A Wilcoxon pairwise comparison found the concentration of TP to differ among all three phases while TOP concentrations only differed between the peak and rising limbs of the hydrograph. Total organic carbon differed between the peak and the rising limb and the peak and recession limb while the PC and PAF differed between the peak and recession and rising and recession limbs.

The average *E. coli* PC for all samples collected was 0.06 which corresponded to a PAF of 4.8% and the average PC for enterococcus was 0.18 with corresponding PAF of 13%. Partitioning coefficients and PAF were calculated using Equations 1 and 2. The average PAF was higher for enterococci (13%) than *E. coli* (4.8%). Characklis et al. (2005) found *E. coli*, fecal coliforms, and enterococci all displayed relatively similar partitioning behavior from background samples, but during storm events the attached fractions of fecal coliforms and enterococci increased at all three sites while the attached fraction of *E. coli* decreased at two of the three sites. While it is difficult to attribute a single factor to the increased attachment exhibited by enterococci, enterococci cells have a tendency to occur in pairs or short chains during the exponential or log phase of the growth curve (Holt et al., 1993). Enterococci were likely in the active growth stage since the rainfall simulation occurred within 24 hours after deposition and several decay studies have reported that bacteria increase in fresh fecal deposits for up to two weeks before die-off begins (Conner and Kotrola, 1995; Crane et al., 1980; Muirhead et al., 2005; Wang, et al., 1996; Wang, et al., 2004).

Muirhead et al. (2005) studied the transport state of *E. coli* cells by placing cowpats and fecal-material–soil mixtures on a metal tray 250 mm long and 200 mm wide, and runoff was created by placing a rainfall simulator nozzle 250 cm above the soil. On average only 8% of *E. coli* cells attached to sediment particles and most cells were not bioflocculated (attached to one another and form aggregates), but instead transported in runoff as single cells. Low attachment of *E. coli* to particulates in runoff from lands receiving manure applications might be explained by the presence of organic matter and carbon. Equilibrium batch experiments examining the effect of dairy manure on *E. coli* attachment to soils found increasing manure content resulted in decreased attachment (Guber et al., 2005b). Other studies found that removal of dissolved organic carbon from a bacterial suspension increased attachment (Scholl and Harvey, 1992), and bacteria adsorption on quartz and iron-quartz particles was decreased by the addition of organic matter (Johnson and Logan, 1996). Guber et al. (2005a) hypothesized that the decreased bacterial attachment to particulates in the presence of manures could be caused by a variety of factors including competition between bacteria and dissolved organic matter for

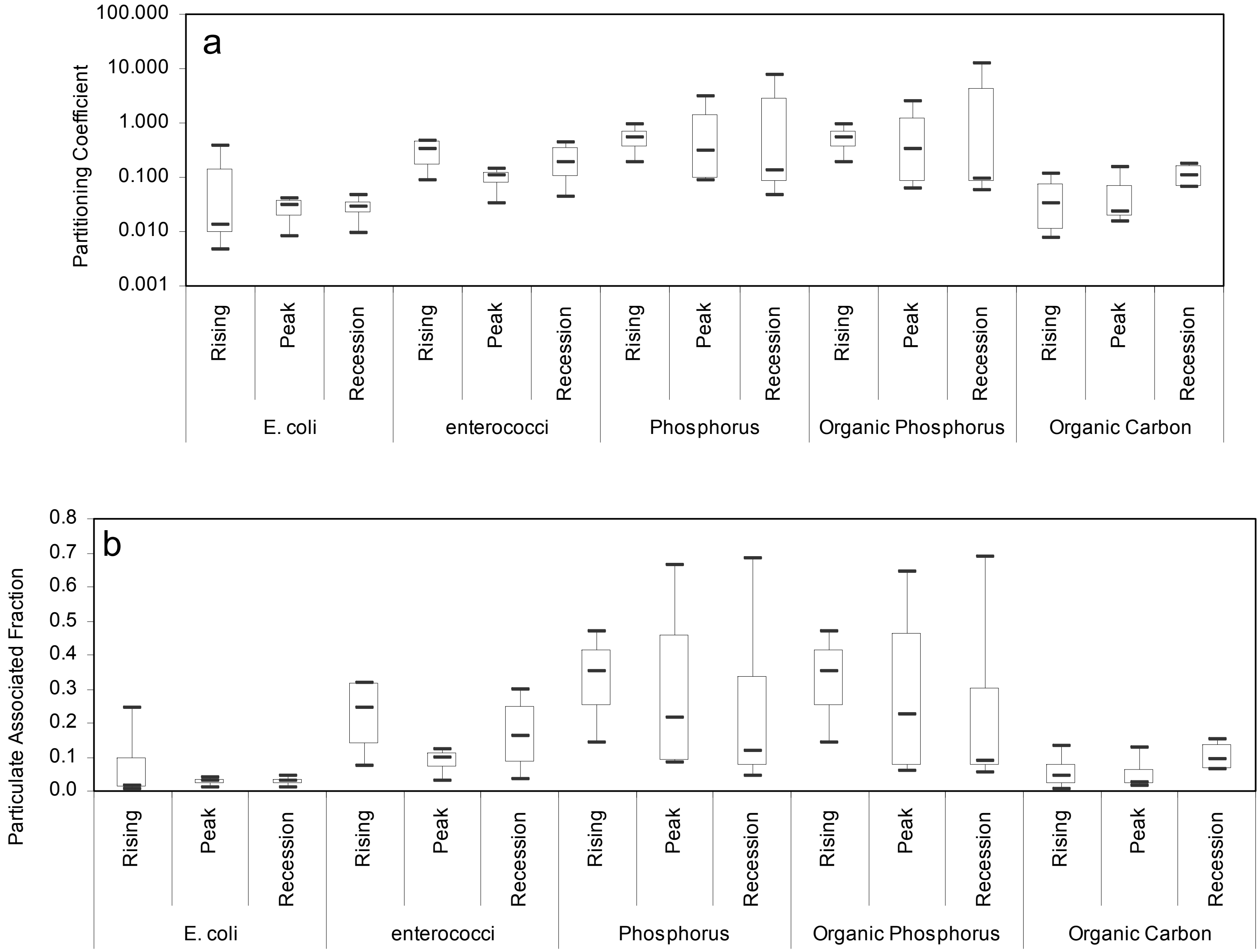

Figure 1. Partitioning coefficients (a) and particulate associated fractions (b) of *E. coli*, enterococci, phosphorus, organic phosphorus, and organic carbon in runoff from pasturelands during the rising, peak, and recession limbs of a runoff hydrograph.

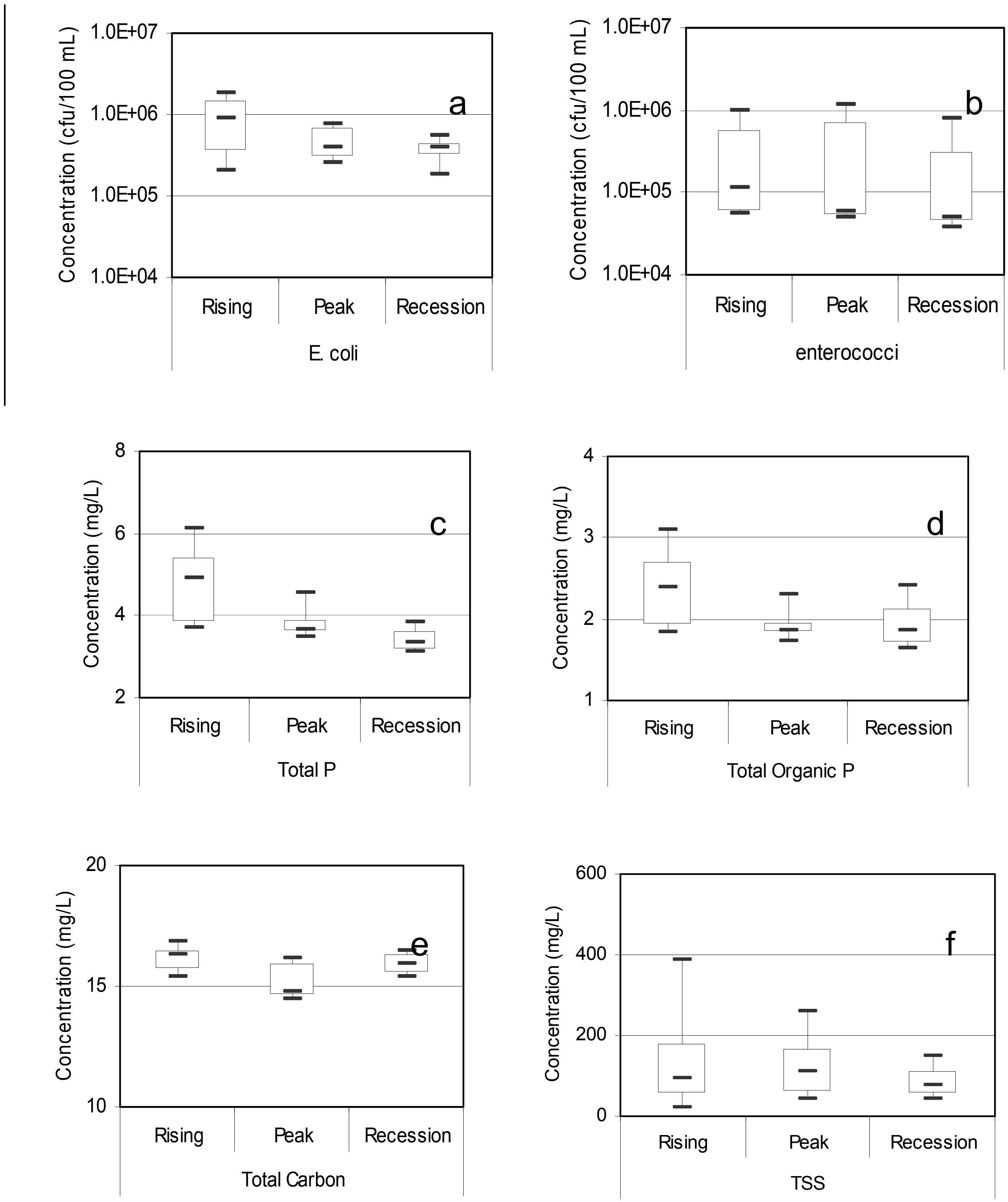

Figure 2. Bacterial and nutrient concentrations in runoff from pasturelands during the rising, peak, and recession limbs of a runoff hydrograph for (a) *E. coli*, (b) enterococci, (c) total phosphorus, (d) total organic phosphorus, (e) total carbon, and (f) total suspended solids.

attachment sites on soil, modification of soil mineral surfaces by soluble manure constituents, or modification of bacterial surfaces by dissolved organic matter.

These rates of *E. coli* and enterococci attachment are lower than the majority of studies which have focused on in-stream background and storm event partitioning. Jeng et al. (2005) found *E. coli* attachment to range from 21.8% to 30.4% in stormwater samples while Characklis et al. (2005) found an average attachment ranging from 20% to 35% in grab samples collected during storm events. Differences between this study and previous findings are likely due to the increased presence of manure particulates in runoff from a fresh fecal source; however, several additional factors could have contributed to these discrepancies including the laboratory methods used to partition between unattached and attached phases. Muirhead et al. (2005) separated between phases by injecting a Nycodenz solution below the suspension and then centrifuging the samples, Jeng et al. (2005) used a static settling technique, allowing particulates to settle for five hours before assessing the suspended portion while Characklis et al. (2005) separated between the two phases by centrifugation.

Different time periods between introduction of the fecal sources into the environment and sample collection could also partially explain differences in bacterial partitioning rates. Cells exposed to an oligotrophic (nutrient limited) environment are more likely to attach to particulates in an effort to obtain nutrients and increase survival (Morita, 1997). The short time (24 hours) between manure application and runoff in our study could help to explain the low bacterial attachment rates. Bacteria present in runoff from lands treated with a fresh manure source are unlikely to be stressed since nutrients and moisture are in abundant supply. Sources from which the fecal indicators originated might also explain differences between the results of our study and previous studies. The source of *E. coli* from stormwater samples (Characklis et al., 2005; Jeng et al., 2005; Krometis et al., 2007) is unknown and there is some indication that strains of *E. coli* introduced into a system from different sources (eg. waterfowl, cattle, domestic pets) may exhibit different attachment properties (Lago, 2005). Nevertheless, many different environmental strains of dairy cow *E. coli* were also applied to the plots in this study and attachment rates also differ among strains from the same source (Muirhead et al., 2005). Therefore, even though the source species in the stormwater studies are unknown, it is difficult to attribute lower attachment ratios to differences in environmental strains without knowledge of the strains present in each study.

Bacterial attachment and TSS concentrations

Raindrops detached fecal material from cowpats but the thick vegetation aided to reduce particulate transport. The TSS flow-weighted concentration of 152 mg L^{-1} (Table 1) is similar to TSS concentrations in runoff from previous pastureland studies and slightly higher than pastureland plots receiving liquid dairy and poultry litter applications (Soupir et al., 2006). A correlation analysis found no significant linear relationships between bacterial concentrations, PC, or PAF and TSS concentrations. High levels of organic carbon present in runoff samples might explain this dissimilarity; however, Characklis et al. (2005) identified potential linkages between in-stream fecal coliform partitioning and particle number concentrations (R^2 = 0.51), and Fries et al. (2006) found that concentrations of *E. coli* and enterococci increased along with particulates in suspension following storm events. After being transported into the stream and prolonged exposure to the external environment, bacteria attachment might increase to ensure survival (Morita, 1997). The exposed cell surface of the attached cell is decreased and the attached portion of the cell does not participate in substrate uptake. In addition to surface attachment, bacteria also bioflocculate, usually when substrates are depleted and bacteria are stressed for nutrients (Morita, 1997). Therefore, once in- stream, the availability of attachment sites could influence attachment. If in fact attachment is limited by availability of attachment sites, bacterial attachment from poorly managed pasturelands with lower vegetative cover and erosive soils might be higher than the PAF presented in this study.

Bacterial attachment and nutrient partitioning

NPS pollution models assess sources of microbial loadings in watersheds and identify reductions necessary from these sources to meet water quality standards. These models already have mechanisms in place to partition between dissolved and suspended forms of nutrients, so relationships developed between bacterial partitioning and nutrient partitioning could be easily incorporated in these models. Partitioning coefficients and PAF were calculated for phosphorus, organic phosphorus, and organic carbon to compare trends between the pathogen indicator and nutrient fractions. A correlation analysis was conducted to investigate the potential linear relationships between bacterial and nutrient partitioning. Data were normalized and statistically significant ($p \leq 0.05$) correlation coefficients are presented in Table 2. The correlation analysis provides an initial indication as to which nutrient variables could predict bacterial partitioning by water quality models. This analysis indicates that *E. coli* PC is most closely related to TP PC (r = 0.50800); correlations also exist between TP PAF (r = 0.48819), TOP PC (r = 0.49983), and TOP PAF (r = 0.49100). Correlations between enterococcus partitioning and phosphorous and organic carbon were not as strong, but TOP PC (r = 0.37557) and TOC (r = 0.42220 and r = 0.41358) might both aid in prediction of enterococcus partitioning at the edge-of-the-field. Nutrients and nutrient fractions not significantly correlated with bacteria PC or PAF included TP, TP PAF, DOC, TOC PC, and TOC PAF. Total concentrations of *E. coli* and enterococci also were not significantly correlated with nutrient TC, PC or PAF.

Table 2. Pearson correlation coefficients identify statistically significant relationships between nutrient parameters and bacterial partitioning.

	TDP TC	TP PC	TP PAF	DOP	TOP	TOP PC	TOP PAF	TOC
E. coli PC	-0.40074 †	0.50800	0.49235	-0.40593	NS	0.49983	0.49541	0.37521
E. coli PAF	-0.39995	0.50414	0.48819	-0.40493	NS	0.49622	0.49100	0.36824
enterococci PC	NS	0.38811	0.39793	NS	0.43041	0.37557	0.37895	0.42220
enterococci PAF	NS	0.39312	0.40209	NS	0.41113	0.37808	0.37996	0.41358

†All values presented significant at the $p \leq 0.05$ level, data normalized prior to analysis

Bacterial and nutrient loading rates

Loading curves describing the bacterial partitioning over the duration of a storm event from a single plot are presented in Figure 3. Plot 2 was selected because runoff first began from this plot, resulting in the highest number of samples. The unattached fraction consistently exceeded the attached fraction by as much as two orders of magnitude. The overwhelming loading of indicators in the unattached state suggests that management practices should focus on removal of unattached cells to improve water quality in agricultural areas dominated by rotationally grazed pasturelands.

Because loading curves appeared to follow similar trends as nutrients, a second correlation analysis was conducted to investigate the potential linear relationships between bacterial and nutrient loading rates. Again, data were normalized and statistically significant ($p \leq 0.05$) correlation coefficients are presented in Table 3. The correlation analysis provides an initial indication as to which nutrient variables could predict bacterial partitioning by water quality

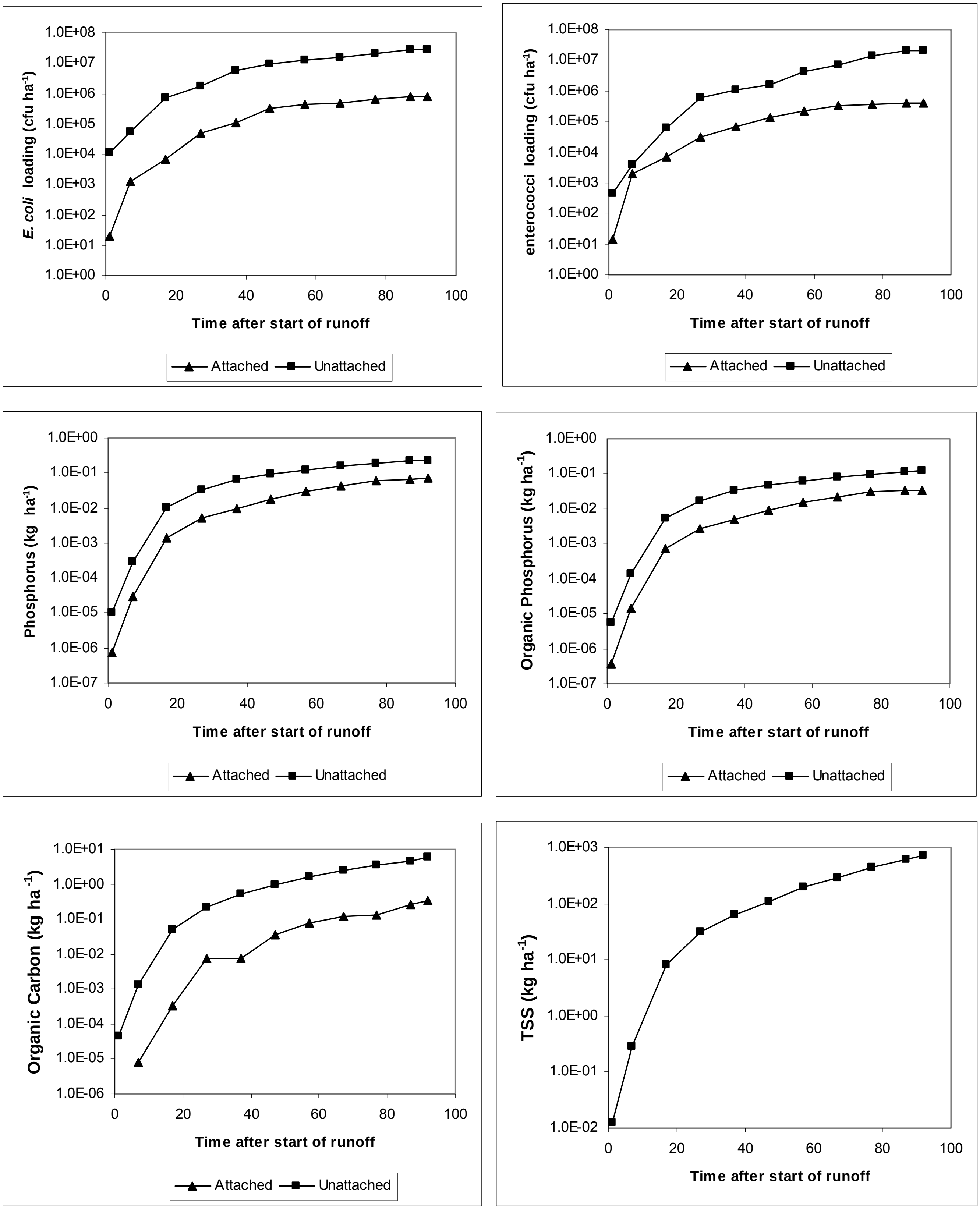

Figure 3. *E. coli* and enterococci loading rates from a single plot (plot 2) treated with cowpats during an overland flow event.

models. Significant correlations were not found when relating ratios of partitioned bacteria and nutrient phases. A more promising approach could be to model separately the attached and unattached phases *of E. coli* and enterococci. Attached *E. coli* loads were significantly correlated with SOP (r = 0.84682). Unattached *E. coli* (r = 0.93166) and both attached and unattached enterococci loading curves (r = 0.92257 and 0.87567, respectively) were most closely related to DOC.

Table 3. Pearson correlation coefficients identify statistically significant relationships between bacteria and nutrient partitioning loads

	TSP	TDP	P ratio	SOP	DOP	Organic P ratio	TSC	DOC	Organic C ratio	TSS
E. coli attached	0.83974[†]	0.74154	NS	0.84682	0.74581	NS	0.69583	0.83832	NS	0.74991
E. coli unattached	0.82664	0.88770	0.46104	0.82786	0.88980	0.39711	0.82757	0.93166	NS	0.83984
E. coli ratio	0.60040	0.35792	NS	0.60993	0.36235	NS	NS	0.45832	NS	0.41334
enterococci attached	0.87319	0.89986	0.38204	0.87499	0.90161	NS	0.76396	0.92257	NS	0.85686
enterococci unattached	0.77906	0.84105	0.46726	0.77963	0.84248	0.39569	0.74988	0.87567	NS	0.77922
enterococci ratio	NS	NS	NS	NS	NS	NS	-0.37S046	NS	NS	NS

[†]All values presented significant at the $p \leq 0.05$ level, data normalized prior to analysis

Summary and Conclusions

A field study was conducted to evaluate the partitioning of *E. coli* and enterococci between the unattached and attached phases in runoff from pasturelands and to identify the particle sizes to which the fecal indicators preferentially attach. Field plots were constructed on well-managed pastureland with high vegetative cover to determine partitioning ratios of *E. coli* and enterococci in runoff samples collected at the edge-of-the-field.

Results indicate that the majority of bacterial indicator organisms are transported from a fresh manure source in the unattached state. Average PC for *E. coli* was 0.06 which corresponds to 4.8% attachment and 0.18 for enterococci corresponding to 13% attachment. Low attachment rates might be best explained by the low TSS concentrations (relative to poorly managed and other agricultural landuses) and competition for attachment sites between fecal indicators and organic carbon. Linear correlations existed between *E. coli* loading rates and SOP while unattached *E. coli* and both attached and unattached enterococci loading rates were most closely related to DOC. Comparison of unattached and attached indicator loading rates found that the unattached fraction exceeded the attached fraction by at least two orders of magnitude.

Partitioning ratios developed from this study can be incorporated into NPS models that allow for partitioning between the attached and unattached phases. Relationships between bacterial loading rates and phosphorous and organic carbon loading rates should prove useful in improving predictions of bacterial transport from grazed pasturelands. The majority of cells were transported in the unattached state from pasturelands with high vegetative cover receiving fresh fecal deposits and therefore, development of best management practices for well-managed pastureland scenarios should focus on reduction of unattached pathogen indicators. Future study is recommended to determine partitioning of indicators from different landuses with higher transport of suspended solids and from aged fecal sources.

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