Why the Similarities between Bioactive Phosphorus and Enterobacteria Released to Runoff?

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ABSTRACT

Objective: Compare the release of manure phosphorus (P), *Escherichia coli*, and *Enterococci* to runoff to evaluate the hypothesis that the bacteria were associated with organic P-containing particulates during the release.

Methods and Results: We applied dairy manure on grass-covered soil and made periodic sampling of simulated runoff. Concentrations and mass loads of bioactive P and bacteria in runoff over time were log-normally distributed. High correlations were found between water turbidity, bacteria and phosphohydrolase-labile P (PHP), a fraction associated with particulate manure, or the more-inclusive total bioactive P fraction. Delayed bacteria and P concentrations and mass loads indicated live leaf and bacteria interactions that impeded their releases to runoff. Resultant deviations in linearity between WEP and bacteria distributions showed that manure-borne bacteria were released while associated with organic P-containing particulates.

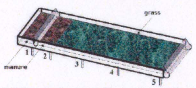
Conclusions and Significance: Analogies between turbidity, organic P, and bacteria distributions showed that determination of bioactive P forms complemented bacteria measurements to confirm enteric bacteria release. Given the micrometer size range of suspended particles, grass strips may not effectively filter and eliminate lateral transport of colloid-associated bacteria and organic P.

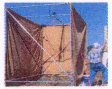
INTRODUCTION

Vegetative filter strips contribute to the improvement of water quality of nearby waterways and reservoirs by reducing water velocity, enhancing infiltration, deposition of sediments, and sorption of chemicals or microorganisms. Limited information is available on the concurrent release and transport of manure phosphorus (P) and enteric bacteria. No consensus exists on whether those mechanisms have similar quantitative effects on their transport. The correlation in TP and fecal coliforms runoff concentrations may be related to the transport of P in bacterial cells. This implies that different forms of P may differ in their correlations with pathogenic indicators. Although bacteria may move as free-living organisms (Muirhead et al., 2005), bacteria were observed to adhere and colonize C-rich aggregates in aquatic ecosystems (Knoll et al., 2001; Kiørboe et al., 2002). As manure is composed of partly digested feed materials which are primarily organic C in nature, we compare releases of enteric bacteria and bioactive P forms to evaluate the hypothesis of co-transport of these manure-borne contaminants.

MATERIALS AND METHODS

A. **Rainfall Simulations:** Runoff boxes (200 cm x 41 cm x 10 cm) were made from commercial lumber. A 5-cm layer of tall fescue (*Festuca arundinacea* Schreb.) sod was placed in each runoff box. Three replicated soil-grass boxes were placed on an 4% incline inside a simulator frame area for each grass treatment in separate simulations (Guber et al., 2007). Constant rainfall intensity of 32.4 ± 0.6 mm h^{-1} was maintained for 90 min.





B. **Runoff Phosphorus Fractionation:** Bioactive P fractions in manure and runoff samples were determined according to the procedure developed by Dao (2003; Dao et al., 2006). In summary, manure water-extractable P in runoff was measured in duplicate aliquots following centrifugation at 10000 x g for 15 min prior to phosphate-P analysis. Another set of aliquots was agitated with a 0.05 *M* EDTA (1 to 10, w/v) for 1h. After centrifugation, ligand-exchangeable inorganic phosphates (EEPi) were determined by comparing the WEP and EDTA-P results of phosphate-P analyses. Total bioactive P was determined following the EEPi measurement; an aliquot (0.05 unit ml^{-1}) of *Aspergillus ficuum* phosphohydrolases that were prepared in our laboratory (Hoang and Dao, 2007; Dao et al., 2007) was added. The mixture was equilibrated for 24 h, then aliquots of the mixture were centrifuged prior to P analysis.

C. ***Enterococci* and *E. coli* Enumeration:** *Enterococci* in water and manure samples were enumerated by plating a 50-μl exponential spiral onto culture plates containing membrane *Enterococcus* agar. Red-colored CFU that developed after 48 h incubation at 37 oC were counted. Similarly, numbers of *E. coli* CFU in manure and runoff water aliquots were determined by plating 50 μl exponential spirals on MacConkey agar. Red-colored CFU developed after overnight incubation at 44 oC were counted.

D. **Statistical Analysis:** Distributions of concentrations and mass loads of bacteria, WEP, EEPi, and EDTA-PHP forms in runoff were fitted to the log-normal distribution function. Linear regression equations were fitted to paired chemical and bacteria concentrations for each simulation.

RESULTS AND DISCUSSION

Bacteria concentrations peaked early during the simulation then declined in the runoff over time, as described by the log-normal density distribution (Fig. 1A). The dead grass cover delivered peak concentration or load sooner than live grass cover. Total mass loadings to runoff averaged 9.8 x 10^6 and 20 x 10^6 *E coli* CFU and 0.4 x 10^6 and 2.3 x 10^6 *Enterococci* CFU for the live and dead grass treatments. A greater precision was attained with the bioactive P measurements (Fig. 1B-C). Manure WEP concentration fluxes and mass loadings displayed distribution patterns similar to those of bacteria. Similarly, the state of the grass cover affected the timing of peak concentration and mass load of any of the bioactive P forms delivered to runoff.

Linear relationships between concentrations of P forms and bacteria were found in the majority of cases (Fig. 2A-C). Manure WEP was not correlated to either *E. coli* and *Enterococci* bacteria concentrations, i.e., r^2 of 0.64 and 0.72, respectively. Manure WEP concentrations in runoff had the lowest correlation with water turbidity from the live grass treatment (Table 1); the curvilinear relationship would suggest a very rapid increase in the delivery of manure WEP at the early stage of the simulation, as compared to the delivery of organic particulate manure components and bacteria to runoff.

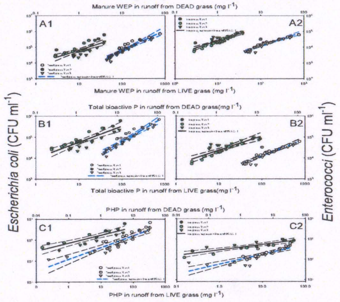


Fig. 2 Binary relationships between concentrations of manure water-extractable P (A), total bioactive P (B), phosphohydrolase-labile P (C), and *E. coli* and *Enterococci* bacteria in runoff from grass-applied dairy manure over 90 min of simulated rain applied at 3.2 cm h^{-1}.

The enzyme-hydrolysable organic P fraction (PHP) was directly correlated to both *E. coli* and *Enterococci* releases (Fig. 2C). Mean particle-size distributions derived from water turbidity for each grass treatment showed that there was a continuum of particle sizes down to the sub-μm range in the runoff (Fig. 3).

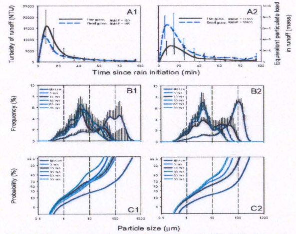


Fig. 3. Water turbidity and changes in particle size (means and +SD) distributions and associated probability of occurrence of suspended material in runoff and a dairy manure suspension spread on live (B1-C1) and dead (B2-C2) tall fescue sod over 90 min of simulated rain applied at 3.2 cm h^{-1}.

Table 1. Relationships between runoff water turbidity (NTU) and manure water-extractable P or phosphohydrolase-hydrolysable P in runoff from grass-applied dairy manure over 90 min of simulated rain applied at 3.2 cm h^{-1}.

	Manure Water-extractable P			Phosphohydrolase-hydrolysable P		
Run	Slope	RMSE[†]	r^2	Slope	RMSE	r^2
Live grass	463.6**	2637	0.783	376.2	825	0.979
Dead grass	197.0**	1000	0.918	321.4	836	0.943
All	263.8	2780	0.642	356.4	1082	0.946

[†] RMSE = Root mean square error
** significantly different at the 0.05 level of probability

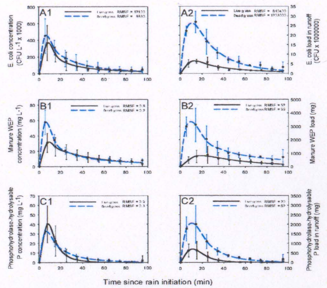


Fig. 1. Distributions of concentrations and mass loads of *Escherichia coli* (A), manure water-extractable P (B) and phosphohydrolase -hydrolysable P (C) in runoff from grass-applied dairy manure over 90 min of simulated rain applied at 3.2 cm h^{-1}.

CONCLUSIONS

High correlations were found between water turbidity, bacteria and phosphohydrolase-labile P (PHP), a fraction associated with particulate manure. The differential effect of the state of the grass cover on manure WEP and bacterial distribution in runoff provided additional support to the particulate-associated release of *E. coli* and *Enterococci* to runoff. The retardation of particulate loading to runoff, arising from interactions of live grass surfaces with manure particulates and bacterial cells, resulted in large deviations from linearity of the WEP-bacteria concentration relationships. This is in contrast to the improved correlation between these two variables when water and particulate movement were unimpeded in the dead grass cover. Therefore, the similarities between the distributions of both manure-borne pollutants and manure particulates suggested that, mechanistically, *E. coli*, and to a lesser extent, *Enterococci*, were released along with organic P-containing colloids or larger manure particulates during the dispersal of the manure layer upon raindrops' impact. These manure components appeared to be predominantly released to the water-phase in the 0.1 to 10 micrometer range and are rather difficult to be filtered out by grass filter strips.

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