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ESCHERICHIA COLI AND FECAL COLIFORM EXPORT RATES IN TWO AGRICULTURAL WATERSHEDS OF THE U.S. MIDWEST

P. Vidon and **M.A. Campbell:** Department of Earth Sciences and Center of Earth and Environmental Science, SL118, Indiana University–Purdue University, Indianapolis, 723 W. Michigan Street, Indianapolis, Indiana 46202 USA

E. Soyeux: Veolia Environment, Research Department, 36–38 Avenue Kleber, 75116 Paris, France

ABSTRACT. The development of sound regulatory standards for fecal bacterial contamination in streams requires the determination of bacterial export rates at the watershed scale. This study reports *Escherichia coli* (*E. coli*) and fecal coliform bacteria export rate dynamics for two agricultural watersheds in till landscapes of the U.S. midwest. Bacteria concentrations in streams were lowest during the December-February period and were not significantly correlated ($P > 0.05$) to discharge, suggesting that discharge was not a good indicator of bacteria concentration in the study watersheds. Annual *E. coli* and fecal coliform export rates were similar between watersheds and varied between $4.60 \times 10^{+12}$ MPN/km^2/yr and $6.56 \times 10^{+12}$ MPN/km^2/yr for *E. coli* and between $2.56 \times 10^{+14}$ MPN/km^2/yr and $3.33 \times 10^{+14}$ MPN/km^2/yr for fecal coliform (MPN = most probable number). Although discharge was poorly correlated to bacteria concentration, annual *E. coli* and fecal coliform exports were dominated by a few precipitation events during which high flow and high bacteria concentrations occurred simultaneously. In both watersheds, 90% and 50% of annual *E. coli* exports occurred in approximately 16% and 2% of the time, respectively. Similarly, 90% and 50% of annual fecal coliform exports occurred in approximately 18% and 2–2.5% of the time in both watersheds. Considering the importance of some high flow events on annual bacterial export, we propose that management efforts should be focused on best management practices capable of efficiently controlling bacterial transport to streams during storms. Although high bacteria concentrations can occur at baseflow, bacteria loadings at baseflow are small and have limited impact on annual bacterial export rates at the watershed scale.

Keywords: *Escherichia coli*, fecal coliform, export rate, Midwest, watershed

Coliform bacteria are naturally-occurring organisms in the environment and in the feces of all mammals. Although the presence of coliform bacteria in drinking water may not be harmful to humans, it indicates that disease–causing organisms may be present in the water system (Washington Department of Health 2007). The presence of fecal coliform (FC) and *Escherichia coli* (EC) bacteria in freshwater almost always indicates recent fecal contamination, and is an indicator that pathogenic organisms may be present in water. Consequently, FC and EC bacteria are widely considered as water quality indicators and are routinely monitored both in streams and freshwater systems (Nagels et al. 2002; Collins & Rutherford 2004).

Although potential sources of FC and EC bacteria to streams are known (e.g., septic systems and livestock operation), a review of the literature revealed that much uncertainty remains regarding the variables controlling the fate of FC and EC bacteria in the environment after they leave the gastro-intestinal tract of the host organism. For instance, Wickham et al. (2006) indicate that land use and soil characteristics are correlated with fecal bacterial contamination. The authors found that streams in some Maryland watersheds with well-drained and erodible soils, and a high proportion of urban land adjacent to streams, had the highest likelihood of fecal bacterial contamination. Others have shown that flow conditions, sediment transport and precipitation were also related to EC concentration in streams (Mallin et al. 2001; Tyrrel & Quinton 2003; Reeves et al. 2004; Collins & Rutherford 2004). In addition, research indicates that seven-day antecedent precipitation and turbidity influenced the spatial and temporal variations of EC loads in watersheds in Tennessee (mixed land use in karst landscape) and Indiana (agricul-

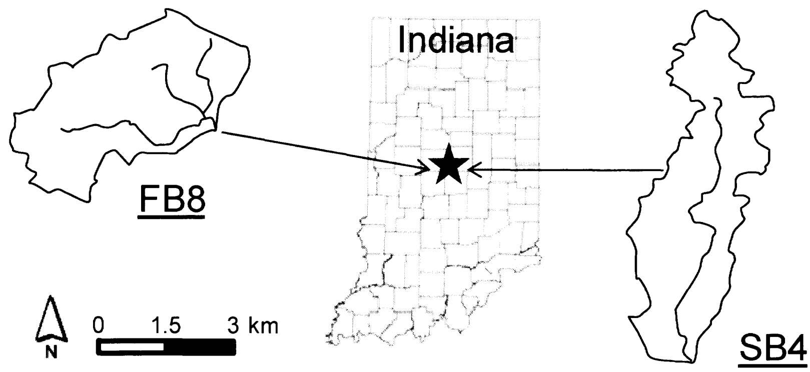


Figure 1.—Locations of FB8 and SB4 watersheds in Indiana.

tural land use in till landscape) (Gentry et al. 2006; Vidon et al. 2008a).

Much uncertainty therefore remains surrounding the processes controlling FC and EC dynamics in streams. This strongly limits our ability to predict hot spots of bacterial contamination as well as total maximum daily loads for regulatory purposes. Although organisms react to concentration, load determination is essential to determine bacterial loading of downstream reservoirs and to develop better predictive models of bacteria export at the watershed scale. Indeed, one critical step toward the development of sound regulatory standards for bacterial contamination in freshwater streams is the assessment of bacterial load at the watershed scale for a variety of land uses and geomophological settings. This includes the determination of bacterial export rates (MPN/km^2/yr) for inclusion in export and risk assessment models. With the exception of Line et al. (2008), who reported FC export rates between $1.81 \times 10^{+12}$ MPN/km^2/yr and $1.9 \times 10^{+13}$ MPN/km^2/yr for residential and low density industrial watersheds in North Carolina, very few studies report actual bacterial export rates at the watershed scale. Gentry et al. (2006) and Vidon et al. (2008a) report EC daily loads but do not report annual export rates. A limited number of studies reporting annual bacterial loads is likely due to the high variability of bacterial concentration in streams and subsequent difficulty to determine accurately stream bacterial load. Nevertheless, although errors in bacterial load estimates are certainly high, determining annual export rates is of critical importance to proper watershed management.

This study reports monthly and annual export rates of EC and FC bacteria for two agricultural watersheds in till landscapes of the U.S. Midwest, near Indianapolis, Indiana. The objectives of this study were: 1) to determine EC and FC export rates for two watersheds with similar land uses, and 2) to determine whether discharge could be used as a good indicator of EC and FC export at the watershed scale. The implications of our results for watershed management are briefly discussed.

METHODS

Site description.—The two experimental subwatersheds used in this study (FB8 and SB4) are located in Eagle Creek watershed (39°55′15″ N, 86°21′01″ W) in the nearly flat Tipton Till Plain near Indianapolis, Indiana (Fig. 1). Indiana has a temperate continental and humid climate. The average annual temperature for central Indiana is 11.7 °C with an average January temperature for Eagle Creek Watershed of −3.0 °C and an average July temperature of 23.7 °C. The long-term average annual precipitation (1971–2000) in the watershed is 105 cm (NOAA 2005). Precipitation is relatively evenly distributed throughout the year, which typically precludes the need for irrigation in summer time. Average stream discharge nevertheless varies with seasons owing to higher evapotranspiration in summer months. Highest stream discharge is observed in March while the lowest discharge typically occurs in September (Clark 1980). Artificial drainage of agricultural soils is common in these two watersheds where soils are generally poorly to somewhat poorly drained (Campling et al. 2002) and belong for the most part to the

Table 1.—Land use and site characteristics for FB8 and SB4 watersheds.

	FB8 watershed	SB4 watershed
Area (km^2)	13.29	13.67
Stream length (km)	9.41	7.88
Drainage density	0.71	0.58
Mean slope (%)	0.47	0.39
Agricultural land use (%)	82.2	87.0
Urban land use (%)	4.3	3.4
Forested land use (%)	5.9	3.2
Herbaceous land use (%)	8.4	6.2

Crosby-Treaty-Miami association (Hall 1999). Land use is similar in both watersheds (Table 1) and is dominated by agriculture (mainly corn-soybean rotation) (82–87%). A limited number of household using septic systems are located in the watershed (exact number is unknown). Those septic systems could act as a possible point source of EC and FC in the watersheds. No confined animal feeding operations are located in the watersheds.

Hydrological and water quality measurements.—Watershed boundaries and channel stream lengths were established using ArcGIS surface hydrology tools and 30 m U.S. Geological Survey digital elevation model (DEM) data. The 2004 Natural Resources Conservation Service 1 m imagery was used to determine land use in each of the watersheds studied. Stream discharge (Q) at the outlet of watersheds FB8 and SB4 was estimated based on daily discharge measurements made at the nearby USGS Zionsville stream gauging station (Station #3353200) following the general equation:

$$Q_{station} = [A_{station}/A_{zionville}]Q_{zionsville} \quad (1)$$

where $Q_{station}$ is the discharge for each stream monitoring station (m^3/s), $Q_{zionsville}$ is the discharge measured at the USGS Zionsville stream gauging station (m^3/s), $A_{zionville}$ is the area upstream from Zionsville monitoring station (km^2) and $A_{station}$ the area upstream from each station (km^2) (USGS 2005). This equation was used because discharge typically scales linearly or nearly linearly with contributing area (Dunne & Leopold 1978; Pazzaglia et al.1998). Instantaneous discharge was also measured in the field in 2005 to check for the accuracy of estimated discharge using a Doppler velocity meter (SONTEK Flow Tracker). In this study, high flow is defined as the 75th percentile for discharge (Q_{75}), i.e., the discharge exceeded 25% of the time based on long-term discharge measurements obtained at the USGS stream gauging station.

Water samples were collected on a bi-weekly to monthly basis between storms, with additional sampling during storms between April 2005 and March 2006. Over a 12-month period, a total of 23 samples was collected in each watershed, with 10 of them collected during high flow conditions. Over the course of the study period, 12 precipitation events generated high flow conditions ($Q > Q_{75}$) and one water sample at high flow was collected for 10 out of 12 events (Fig. 2). Field blanks and triplicate analysis of selected samples were performed for quality control and samples were kept on ice after sampling until return to the laboratory. FC and EC concentrations (most probable number (MPN) of colony forming unit per 100 ml) were measured within a few hours of collection for a total of 46 samples. FC concentration was determined using membrane filtration technique (standard method SM9221D), and EC concentration was measured using the *E. coli* Test using EC-MUG Medium and read using a fluorometer (long-wavelength UV) (standard methods SM9221-F) (Eaton et al. 2005).

Bacterial export rates were determined by summing storm and non-storm bacterial export rates (Line et al. 2008). Specifically, non-storm export rates were calculated by multiplying EC or FC concentrations for each non-storm period by the total discharge volume for each period. Storm export rates were determined by multiplying the bacterial concentration in the grab sample for each storm studied by the total discharge volume for each storm. Storm samples were generally collected near peak flow during each of the storms studied. The two storms for which bacterial concentration were not available (storms 2 and 12) were small storms where streamflow barely exceed the 75th discharge percentile. For these two storms, bacterial concentrations were interpolated based on concentration before and after the storms. It is important to note that considering that daily bacteria loads between baseflow and high flow vary by approximately two orders of magnitude, and that EC concentrations significantly increase during storms (Vidon et al. 2008a), there is likely a large error in estimated bacterial loads in this study. Although we believe that export rates are of the correct order of magnitude, the potential impact of

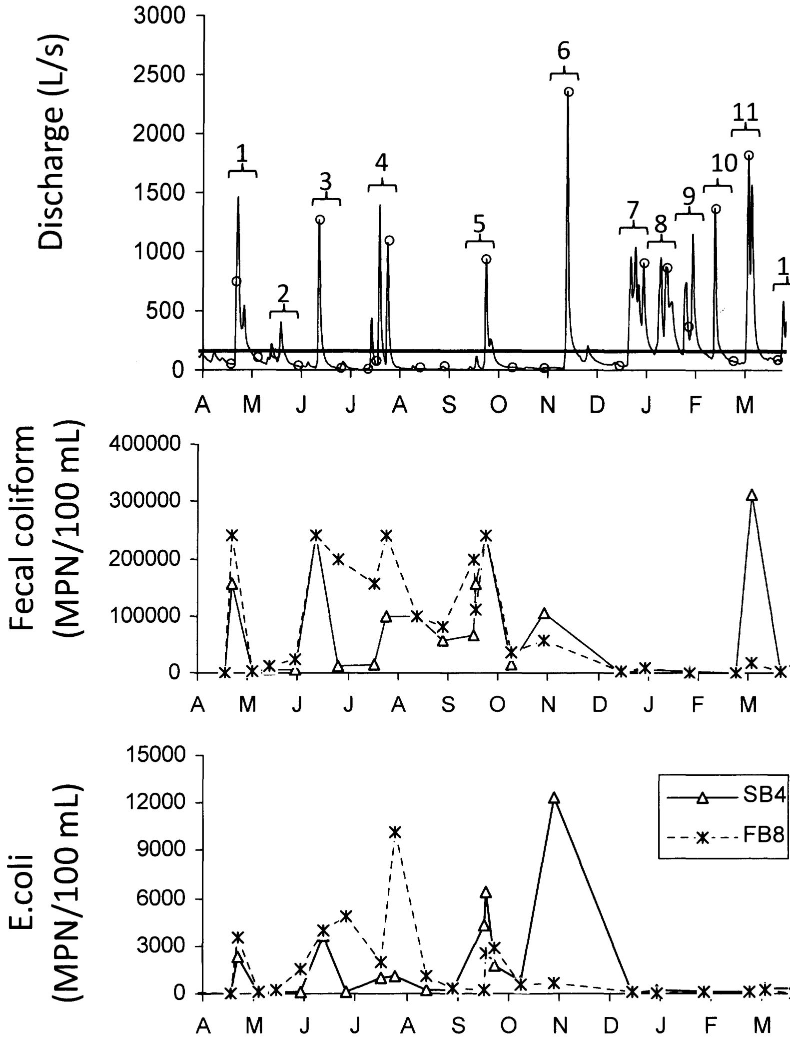


Figure 2.—Discharge (Q), fecal coliform and *E. coli* concentrations between April 2005 and March 2006 in the streams at the outlets of watersheds SB4 and FB8. Upper panel: horizontal solid line indicates the 75^{th} percentile for discharge (Q_{75}). Numbers indicate storm events that generated high flow in the watersheds studied ($Q > Q_{75}$). Clear circles indicate when grab samples for bacterial analysis were collected in relation to discharge. Hydrographs for SB4 and FB8 overlap owing to similar discharge patterns in both watersheds.

inherent calculation errors for load estimates should be taken into account when interpreting results. Simple *t*-tests are used to determine significant difference between samples.

RESULTS AND DISCUSSION

EC and FC concentrations in streams.—FC and EC bacteria concentrations are shown for SB4 and FB8 watersheds in Fig. 2. A grab sample for bacterial concentration measurements was collected for each of the storm events shown in Fig. 2 (upper panel) except for storms 2 and 12. However, compared to the other 10 events for which bacterial concentration were monitored, these events were small. Not having FC or EC concentration data for these two events is therefore unlikely to significantly affect bacterial loads estimated on an annual basis. It is possible, however, that the absence of samples for storm 2 (May 2007) affected monthly estimates of EC and FC loads for the month of May. Median FC concentrations were 14850 MPN/100 ml and 30366 MPN/100 ml in SB4 and FB8, respectively.

Similarly, median EC concentrations during the study period were 200 and 280 MPN/100 mL in SB4 and FB8. Data therefore indicate that bacterial concentrations tended to be slightly higher in FB8 than SB4. Although some natural variability in bacterial concentration was expected between watersheds SB4 and FB8, it is unclear why bacterial concentrations were significantly ($P > 0.05$) higher in FB8 than in SB4. Considering the small surface area of the watersheds studied (13–14 km^2), it is possible that a single source of bacterial contamination in FB8 could have created these differences. For instance, a failing septic system in one of the farm households located in FB8 (4.31% urban) could have lead to this variability.

Data in Fig. 2 also indicate higher FC concentrations during the March-September period than during the rest of the year. Similarly, EC concentrations in both watersheds were higher between April and November than during the rest of the year. With the exception of SB4 watershed where FC concentration was significantly correlated to discharge (correlation coefficient = 0.79, $P < 0.01$), correlation analysis showed no significant correlation (correlation coefficient < 0.3, $P > 0.05$) between discharge and FC in FB8 watershed or EC in either of the watersheds studied. The lack of significant relationship between discharge and bacterial concentration in the watersheds studied is especially clear during the December to March period during which five high flow events occurred while FC and EC concentrations remained low most of the time. The only exception is for FC in SB4 watershed for storm 11, where FC reached its highest value for the study period. These results are consistent with those reported by Gentry et al. (2006) in a mixed land use watershed in Tennessee where the authors indicated a poor correlation (correlation coefficient = 0.06) between EC concentration and discharge. They are, however, in contrast with those reported by Kay et al. (2008) for a series of watersheds in the UK where fecal bacteria concentrations were typically more than one order of magnitude higher at high flow than during baseflow conditions.

Higher EC concentrations in the April-November period were consistent with the results reported by Vidon et al. (2008b) indicating that there was a higher probability of high EC concentration in the spring/summer/fall than in the winter for the watersheds studied. Kay et al. (2008) also found higher fecal bacteria concentrations in summer than winter, especially during high flow conditions. It is possible that the higher concentration of bacteria in the spring and summer occurred because FC and EC colonies were more likely to thrive in the stream at higher temperatures during the summer when flow is low, than during winter. Nevertheless, there was no evidence in the data allowing us to validate this hypothesis.

Overall, discharge appears to be a poor indicator of EC and FC concentration in streams in this region of the country. Seasons, on the other hand, may control to some extent bacterial concentration in the streams studied. More studies need to be conducted to further determine the parameters controlling temporal variability in bacterial concentration in the watersheds studied (e.g., temperature, antecedent moisture conditions).

FC and EC export rates.—Monthly and annual FC and EC export rates between April 2005 and March 2006 are shown in Fig. 3 for watersheds SB4 and FB8. Annual EC and FC export rates were similar (same order of magnitude) between watersheds SB4 and FB8. Specifically, annual EC export rates were $6.56 \times 10^{+12}$ MPN/km^2/yr and $4.60 \times 10^{+12}$ MPN/km^2/yr in SB4 and FB8 watersheds, respectively. FC export rates were $3.33 \times 10^{+14}$ MPN/km^2/yr and $2.56 \times 10^{+14}$ MPN/km^2/yr in SB4 and FB8, respectively. Line et al. (2008) reported FC export rates varying between $1.81 \times 10^{+12}$ MPN/km^2/yr and $1.9 \times 10^{+13}$ MPN/km^2/yr for residential and low density industrial watersheds in North Carolina. Annual FC export rates reported in this study are therefore 1 to 2 orders of magnitude higher than in the Line et al. (2008) study. Most houses located in our watersheds were on septic systems and were built before regulations on septic system design and installation were implemented in the state of Indiana. It is therefore highly likely that a small number of failing septic systems in both SB4 and FB8 contributed to the higher bacterial contamination in our watersheds than in the North Carolina study. Other factors such as soil and animal activity may also have affected our results. Vidon et al. (2008a) also reported that EC concentrations in the watersheds studied were higher than in a mixed land use watershed in karstic landscape of Tennessee. Finally, Tedesco et al. (2005) reported acute contamina-

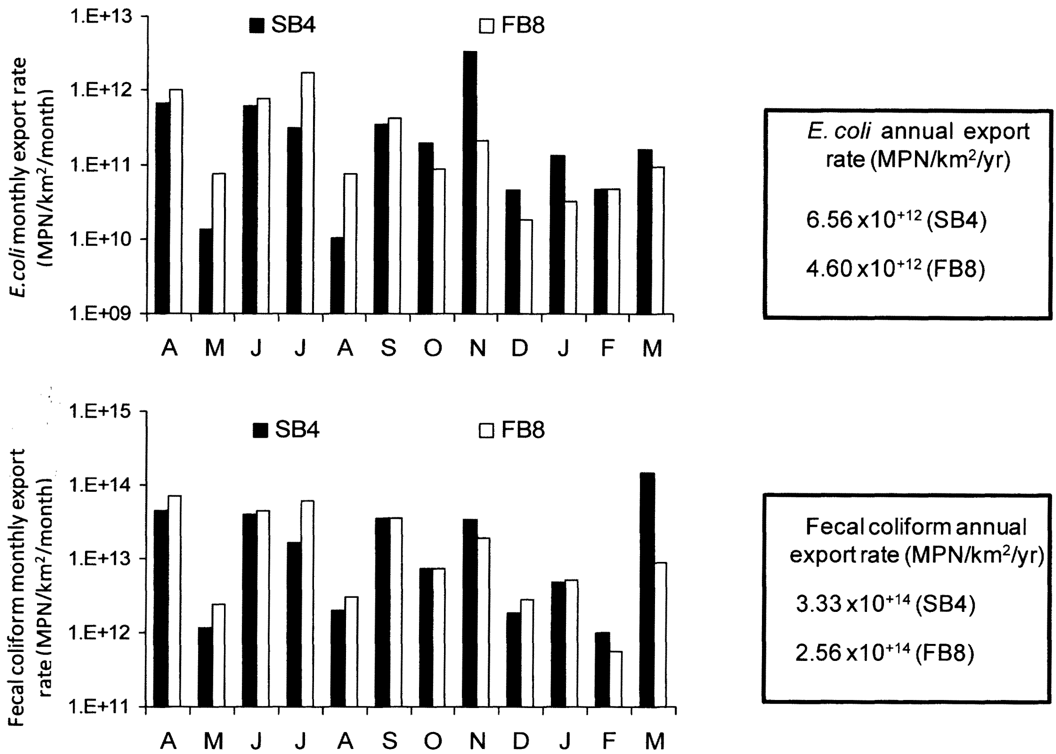
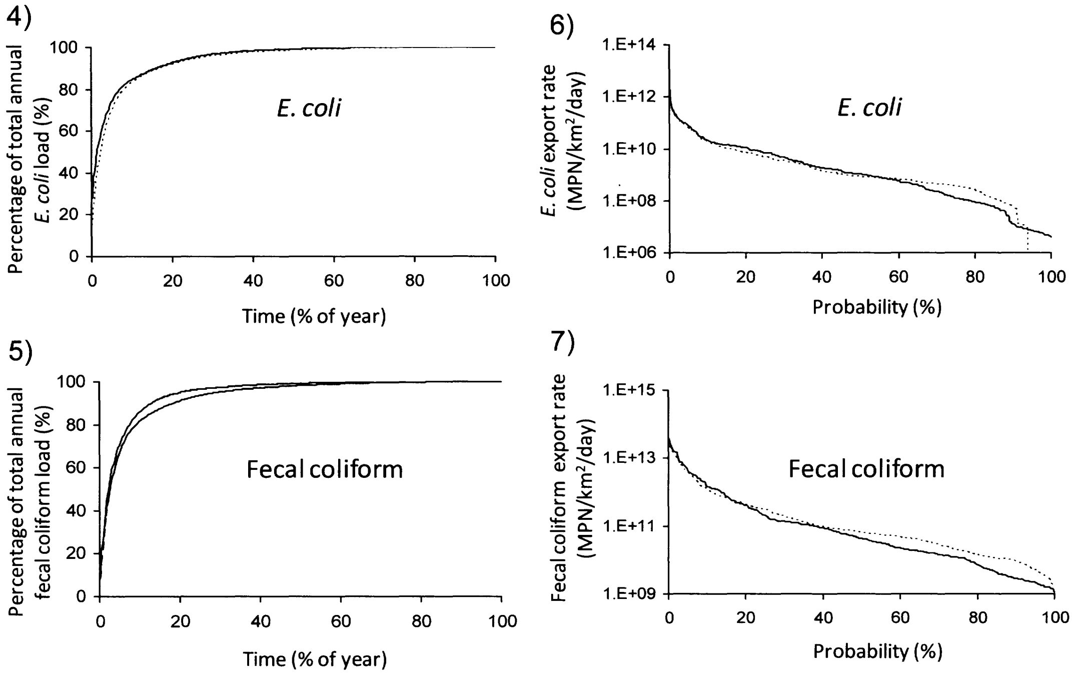


Figure 3.—Monthly and annual export rates for *E. coli* and fecal coliform bacteria in FB8 and SB4 watersheds between April 2005 and March 2006.

tion of fresh water by EC bacteria in most areas of the larger Eagle Creek watershed where the sites are located. Further research is needed to determine why bacterial contamination is more acute in these agricultural watersheds of the U.S. Midwest than in North Carolina coastal watersheds or karstic watersheds in Tennessee. We hypothesize that a small number of failing septic systems or unreported manure application as fertilizer in the spring may explain why bacterial contaminations were so high in the watersheds studied.

Consistent with higher bacteria concentration in spring/summer/fall than in winter (December–January–February), monthly export rates tend to be higher in the spring/summer/fall period than in winter. In FB8, lowest EC export rates occurred in December, January and February in spite of 25% of precipitation events occurring during these three months (events 7–10) (Fig. 2). In SB4, EC monthly loads were also low in December, January and February compared to spring (April, June, July) or Fall (November). FC export rates in both watersheds presented a similar seasonal pattern as did EC export rates in SB4. Specifically, FC export rates were lowest in December, January and February, and highest in March, April, June, July, September and November. Low loads in May were likely due the fact that no samples were collected for storm 2 (May 2007). Low loads in August were likely related to extremely low flow and the absence of high flow events in August (Fig. 2). Although loads were low in May and August, overall, loads tended to be higher in spring/summer than in winter. Our results are consistent with those reported by Line et al. (2008) and Kay et al. (2008) who indicated lower bacterial export rate in winter than during the rest of the year.

Although instantaneous EC and FC concentrations may be poorly correlated to instantaneous discharge, further analysis of the data revealed that annual EC and FC export rates were driven by a few events during which both high flow and high EC or FC concentrations occurred simultaneously. Figure 4 shows the cumulative EC and FC loads as a function of time (Figs. 4, 5), as well as the distribution of



Figures 4–7.—Cumulative percentage of total annual *E. coli* (4) and fecal coliform (5) loads as a function of time between April 2005 and March 2006. Probability of exceedence for *E. coli* (6) and fecal coliform (7) daily loads between April 2005 and March 2006. Data for watershed SB4 are indicated by a solid line, and data for watershed FB8 are indicated by a broken line. MPN = the most probable number of colony-forming units.

EC and FC daily loads and their respective probability of occurrence for the study period (Figs. 6, 7). In both SB4 and FB8 watersheds, 90% of EC exports at the watershed scale occurred in approximately 16% of the time and 50% of EC exports occurred in only in 2% of the time (Fig. 4). This suggests that annual EC export rates at the watershed scale are dominated by a few high flow periods during which both flow and EC concentration happen to be high. Indeed, even though the total annual export rates in SB4 and FB8 watersheds were between $6.56 \times 10^{+12}$ MPN/km^2/yr and $4.60 \times 10^{+12}$ MPN/km^2/yr, daily EC export rates above 10^{+12} MPN/km^2/day occurred only 0.2% of the time in SB4 and never occurred in FB8. EC export rates above 10^{+11} MPN/km^2/day occurred only approximately 3.5% of the time in either SB4 or FB8 watersheds and EC export rates above 10^{+10} MPN/km^2/day occurred only 21% of the time in either watershed (Fig. 6). Daily EC export rates above 10^{+11} MPN/km^2/day all occurred during storms 1, 3, 5, and 8 in SB4 (Fig. 2). In FB8 watershed, daily EC export rates above 10^{+11} MPN/km^2/day all occurred during storms 1, 3, 4, and 5 (data not shown). During these events, export rates were 2 to 3 orders of magnitude higher than during the rest of the year. These storms were not necessarily those which generated the highest discharge (Fig. 2) but corresponded to storms for which a combination of relatively high flow and high EC concentration in the streams were observed simultaneously. This is consistent with the poor correlation previously reported between EC concentration and discharge in the watersheds studied for a 12 month period.

Patterns of FC export rates were very similar to those of EC bacteria (Figs. 5, 7). Specifically, 90% of FC exports at the watershed scale occurred in 13% of the time in SB4 watershed and in 18% of the time in FB8 watershed (Fig. 5). Similarly, 50% of FC exports occurred in 2.2% and 2.5% of the time in SB4 and FB8 watersheds, respectively. Similar to EC, high FC daily loads mainly occurred during a limited number of precipitation events during the year. In SB4 watershed, FC daily loads above 10^{+12} MPN/km^2/day occurred for only storms 1, 3, 5, 8, and 11. In FB8 watersheds, FC daily loads above 10^{+12} MPN/km^2/day only occurred for storms 1, 3, 4, and 5 (data not shown).

Overall, this pattern of dominance of a few storms combining high flow and high bacteria concentration in annual contaminant loads is consistent with what has been observed by others for contaminants like pesticides that are exported via overland flow in a very similar way to EC or FC. For instance, Shipitalo & Owens (2006) indicate that herbicide transport for seven small watersheds (0.45–0.79 ha) in Ohio was dominated by hot moments of pesticide transport in precipitation driven overland flow during precipitation events. Out of a total of 1800 storm events monitored, 60–99% of herbicide loss was due to the five largest transport events during the 9-year study period.

WATERSHED MANAGEMENT IMPLICATIONS

Overall, data indicated that annual bacterial export rates were dominated by discharge events during which high discharge and high EC and FC concentrations occurred simultaneously, in spite of the lack of consistent positive correlation between bacteria concentration and flow over a 12 month period. We propose that large concentration and loads of EC and FC bacteria during selected storm events may have long-lasting effects on downstream water quality even after return to baseflow. Indeed, there is evidence in the literature that EC bacteria and other coliform bacteria can be transported downstream and persist in the environment for over a year in some instances (Palmateer et al. 1989; Lang & Smith 2007). More specifically, Koirala et al. (2008) reported short-term and long-term persistence for coliform bacteria in streams ranging from four days to approximately one year, respectively. Minimizing bacterial export rates during storms is therefore critical in order to improve water quality at the watershed scale. In spite of the limitations associated with load calculations owing to the variability of bacterial concentrations in streams, we believe that the trends observed in this study can be used to develop better management strategies at the watershed scale. We propose that management efforts to minimize bacterial contamination of freshwater systems should be focused on best management practices capable of efficiently controlling bacterial transport to streams during storms as minimizing bacterial concentration in streams at baseflow would have only a limited impact on downstream bacterial loading as both high flow and high bacteria concentration are necessary to significantly impact loading.

Conducting similar studies in a variety of watersheds with various land uses and contrasting geomorphological characteristics and climates would help further generalize the results of this study.

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