Fate of Indicator Microorganisms Under Nutrient Management Plan Conditions

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Nutrient management plans (NMPs) for application of wastewater from concentrated animal feeding operations are designed to meet crop water and nutrient requirements, but implicitly assume that pathogenic microorganisms in the wastewater will be retained and die-off in the root zone. A NMP was implemented on a field plot to test this assumption by monitoring the fate of several fecal indicator microorganisms (*Enterococcus*, fecal coliforms, somatic coliphage, and total *Escherichia coli*). When well-water and wastewater were applied to meet measured evapotranspiration (ET), little advective transport of the indicator microorganisms occurred below the root zone and the remaining microorganisms rapidly died-off (within 1 mo). Additional experiments were conducted in the laboratory to better quantify microorganism transport and survival in the field soil. Batch survival experiments revealed much more rapid die-off rates for the bacterial indicator microorganisms in native than in sterilized soil, suggesting that biotic factors controlled survival. Saturated column experiments with packed field soil, demonstrated much greater transport potential for somatic coliphage than bacterial indicators (*Enterococcus* and total *E. coli*) and that the retention rates for the indicator microorganisms were not log-linear with depth. A worst case transport scenario of ponded infiltration on a large undistributed soil column from the field was also initiated and indicator microorganisms were not detected in the column outflow or in the soil at a depth of 65 cm. All of these observations support the hypothesis that a NMP at this site will protect groundwater supplies from microorganism contamination, especially when applied water and wastewater meet ET.

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Concentrated animal feeding operations (CAFOs) generate large volumes of manure contaminated wastewater, wash water, and storm water runoff which is typically collected and stored on farms in lagoons before land application (USEPA, 2001a). Many pathogenic microorganisms have been found in animal wastes (USDA, 1992; USEPA, 1998; Gerba and Smith, 2005), and water- and food-borne disease outbreaks have frequently been linked to a farm animal source (CDC, 1998; Gerba and Smith, 2005). Macler and Merkle (2000) estimated that pathogenic microorganisms in groundwater cause between 750,000 to 5 million illnesses per year in the United States. Drinking and irrigation water standards to protect human health, however, are largely based on measured concentrations of fecal indicator microorganisms. Bradford et al. (2008) recently presented measured concentrations of several fecal indicator microorganisms in select lagoon water samples from dairy, beef, poultry, and swine CAFOs. These concentrations were found to significantly exceed the recommended U.S. standards for unrestricted irrigation (USEPA, 2004).

The USEPA currently requires farmers to follow approved nutrient management plans (NMPs) when applying CAFO wastewater to agricultural lands. The purpose of a NMP is to meet the water and nutrient needs for plant growth, while minimizing the loss of nutrients to surface and groundwater (USDA, 2000; USEPA, 2003). A NMP is implemented by conducting a mass balance on water and a limiting nutrient for plant growth in the root zone. The NMPs implicitly assume that pathogenic microorganisms in CAFO lagoon water will be retained and inactivated/degraded in the root zone, so that food and water supplies are protected. This assumption has not yet been thoroughly tested.

The human health risks that pathogens pose at NMP application sites are expected to be highly dependent on their survival and transport potential. Pathogen survival in soils is reported to be a complex function of temperature, moisture content, soil solution and solid phase chemistry, soil texture, organic matter, microbial activity, and pathogen type (Yates et al., 1987; Jones, 1999; Guan and Holley, 2003; Wang et al., 2004). Given the right environmental conditions in soils it is also possible that bacterial pathogens can grow in the root zone (Berg et al., 2005). The advective transport potential of pathogens in the root zone is dependent on the water flow regime, which varies with initial

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Abbreviations: CAFO, concentrated animal feeding operation; DAE, day after emergence; ET, evapotranspiration; NMP, nutrient management plan.

soil water status, water application method, application timing and amount, ET, soil hydraulic properties and structure, and surface topography. Pathogen retention processes are known to be a function of many physical (grain size distribution, pathogen size, pore scale hydrodynamics, surface roughness, colloid concentration, and water content), chemical (soil solution and interface chemistry), and microbiological (pathogen type and metabolic activity, motility, biofilms, and microbial community) variables (Elimelech et al., 1998; Ginn et al., 2002; Bradford and Torkzaban, 2008). Furthermore, infiltration and drainage of water in the root zone create transients in water content and soil solution composition that are known to influence these pathogen retention mechanisms (Saiers et al., 2003; Saiers and Lenhart, 2003; Torkzaban et al., 2006).

The objective of this work was to test the hypothesis that fecal indicator microorganisms (*Enterococcus*, total *E. coli*, fecal coliforms, and somatic coliphage) will be retained and die-off in the root zone of a NMP application site. To this end, the fate of indicator microorganisms at a field NMP site was monitored during winter and summer growing seasons. Additional information was obtained in well controlled laboratory experiments to: (i) identify mechanisms that controlled the fate of the indicator microorganisms at the NMP site, and (ii) to develop recommendations for improved NMP performance. Specifically, batch experiments were conducted to quantify the survival characteristics of the indicator microorganisms. Small-scale packed column experiments were conducted to study the influence of the soil matrix on transport and retention behavior. Finally, a large undisturbed column experiment was conducted to assess the influence of soil structure on indicator microorganism transport and survival under a worst case scenario of ponded infiltration and redistribution.

Materials and Methods

Nutrient Management Plan Field Site

The experimental field site is located in San Jacinto, CA (33°50′22′′ N, 117°00′46′′ W) next to a dry river bed and has a shallow perched water table at a depth of –220 cm. The experiments were conducted on a 6 by 6 m plot. The plot was heavily instrumented to a depth of –180 cm with sensors to measure water content (five vertically installed neutron access tubes), soil water pressure head (24 horizontally installed tensiometers, one installed every 30 cm on each plot corner), soil temperature (24 horizontally installed temperature probes, six on each plot corner), solute composition of the soil solution (24 horizontally installed soil solution samplers, one installed every 30 cm on each plot corner), irrigation amount and uniformity (electronic flow meter and rain gauges), and ET (a weighing lysimeter and a weather station equipped with sensors to measure temperature, precipitation, relative humidity, wind speed, and a class A evaporation pan on a balance). Soil cores were periodically collected from the plot boundaries for microbial analysis as described below.

Before initiating the NMP, extensive field and laboratory studies were conducted to characterize the soil hydraulic properties, the water flow behavior, and the transport of a conservative solute tracer on these plots. A detailed description of soil stratigraphy, hydraulic properties, and water flow patterns on the field plots is given by Segal et al. (2008). The most relevant information for this work is that the top 60 cm of the soil surface consists of a uniform sandy loam with a median grain size of close to 50 μm, a bulk density of 1.375 g cm^{-3}, a saturated water content of 0.43 cm^3 cm^{-3}, and a saturated hydraulic conductivity of 2.35 cm h^{-1}. The transport behavior of bromide was assessed and quantified by Segal et al. (2009). The mobile-immobile model (van Genuchten and Wierenga, 1977) accurately characterized the bromide transport in the top 60 cm of the soil profile when the pore water velocity was 0.73 cm h^{-1} using a longitudinal dispersivity of 0.56 cm, a transverse dispersivity of 0.55 cm, an immobile water content of 0.098 cm^3 cm^{-3}, and a mass transfer coefficient between mobile and immobile regions of 0.084 d^{-1}.

For accurate NMP implementation it was necessary to conduct a treatment on the dairy wastewater using an inclined screen, a sedimentation tank, and sand filtration. This treatment significantly decreased the organic load of the wastewater and thus increased the proportion of plant available inorganic N relative to organic N, which was necessary for efficient management of the NMP (Segal et al., 2009). The indicator microbial composition of the dairy wastewater before and after this treatment was measured as part of this work.

Irrigation water consisting of either dairy wastewater or well water was applied to meet the ET and N requirements of Wheat-Rye hybrid (Triticale, Resource Seeds, Inc., Gilroy, CA) and Hybrid Forage Sorghum (Syngenta Global) during winter and summer, respectively. Winter wheat-rye (*Triticum aestivum* L.-*Secale cereale* L.) and summer sorghum [*Sorghum bicolor* (L.) Moench] were irrigated using a pump and nine emitters (R184CT, Raindrip, Fresno, CA; 15 psi nozzles) in 3 m spacing. The application rate was 0.6 to 0.95 cm h^{-1} (depending on the pressure) which is 26 to 40% of the saturated hydraulic conductivity of the soil in the root zone. The Christiansen coefficient of uniformity of water application under low wind conditions was 94%. Water and wastewater application amounts were monitored with a water meter with a resolution of 3.78L ± 1.5% (JSJ075, Carlon meter, Grand Haven, MI).

Detailed information on the NMP design and implementation is provided in Segal et al. (unpublished data, 2009). In brief, measured water balance information in the root zone was used to determine the amount of applied irrigation water during a given time period to meet crop ET. Measured inorganic and organic N balance information in the root zone was used to determine the amount of wastewater that was applied as irrigation water during a given time period to meet the crop nutrient requirements. The dominant sources for plant nitrogen uptake were mineralized soil organic N (52–72%) and applied inorganic N in the dairy wastewater (28–48%). Inorganic N losses due to volatilization were measured (9–32%) and accounted for in the N mass balance. Only minor losses of inorganic N from the wastewater occurred with drainage, and no soil accumulation of inorganic N was observed.

Analysis and Sampling for Indicator Microorganisms

Representative viral (somatic coliphage) and bacterial (total *E. coli*, fecal coliforms, and *Enterococcus*) indicator microorganisms were monitored at the NMP application site and in laboratory experiments discussed below. These microbes are commonly associated with fecal contamination and are typically found in high concentrations in animal wastes (Havelaar, 1986; Bradford et al., 2008). Somatic coliphage, such as ϕ*X174*, are very persistent bacterial viruses that resemble pathogenic viruses in their fate and behavior (Schijven and Hassanizadeh, 2000), but are harmless to humans and can be prepared and quantified easily. Total *E. coli* and *Enterococcus* are frequently used as indicators of bacterial pathogens that are found in the intestinal tract of humans and warm-blooded animals.

The concentration of indigenous somatic coliphage in aqueous samples was determined using the double agar overlay Method 1601 (USEPA, 2001b) with bacterial host *E. coli* CN-13 (ATCC 700609). In brief, 1 mL of log phase host culture and 1-mL sample was added to borosilicate test tubes (Thermo Fisher Scientific, Waltham, MA) containing 4 mL of trypticase soy agar (TSA) (BD Diagnostic Systems, Sparks, MD) supplemented with 1% nadidixic acid (Sigma-Aldrich, St. Louis, MO). The mixture was poured onto sterilized 100 by 15 mm plastic Petri plates (Thermo Fisher Scientific, Waltham, MA) and allowed to solidify for 15 min, after which, they were inverted and incubated for 16 h at 36°C ± 1.0°C. The number of plaque forming units (PFU) in the plates was determined by counting the plaque density under a darkfield colony counter (Leica, Buffalo, NY). All coliphage assays were run in duplicate and diluted as necessary.

Aqueous concentrations of total *E. coli*, fecal coliforms, and *Enterococcus* were determined using the conventional spread plating method (Clesceri et al., 1989). A 100-mL sample was plated on Chromagar ECC (CHROMagar Microbiology, Paris, France) plates for total *E. coli*, on mFC agar (BD Diagnostic Systems, Sparks, MD) plates for fecal coliforms, and on KF agar (EM Science, Gibbstown, NJ) plates for *Enterococcus*. The plates were inverted and incubated at 37°C for total *E. coli* (24 h) and *Enterococcus* (24–48 h), and at 44.5°C for fecal coliform (24 h). The bacterial colony forming units (CFU) were then counted. All bacterial assays were run in duplicate and diluted as necessary.

Dairy wastewater samples were analyzed directly according to the procedures outlined above. Soils samples were collected and weighed under field and oven-dried conditions. For microbial analysis, a 10-g sample of the field soil from each depth increment was placed in a 50- mL sterile polypropylene centrifuge tube (Thermo Fisher Scientific, Waltham, NJ), 20 mL of phosphate buffered solution or deionized water was added, and the solution was gently mixed on a Eberbach shaker (Eberbach corporation, Ann Arbor, MI) for 30 min, and then the soil solution was allowed to settle for 5 min. This solution was subsequently analyzed for microbial concentrations using the outlined procedures, and the concentrations were corrected for the amount of soil and solution in each depth increment. Results from column transport and batch survival experiments discussed below demonstrate that reasonable mass balance and microbe recovery was achieved when using this protocol with soil from the field plot and the considered indicator microorganisms.

For laboratory transport experiments that used spiked concentrations of indicator microorganisms the following protocols were followed. Stock bacteria, *E. coli* (ATCC 11775); *Enterococcus fecalis* (ATCC 19433), and somatic coliphage, ϕ*X174* (ATCC 13706-B1) were grown 24 h before the laboratory experiments. *Escherichia coli* was propagated in trypticase soy broth (TSB) (BD Diagnostic Systems, Sparks, MD) and *Enterococcus fecalis* was grown in LB broth (FisherBiotech, Fair Lawn, NJ). Both bacterial strains were incubated overnight at 37°C ± 1.0°C. The somatic coliphage host, *E. coli* (ATCC 13706) was incubated in TSB for 18 h at 37°C ± 1.0°C. Bacteriophage ϕ*X174* was then added into the broth culture of host bacteria and allowed to propagate overnight. The host, *E. coli*, was removed from the suspension, containing bacteriophage ϕ*X174*, by centrifugation at 17,329 × *g* for 10 min. The other bacteria were harvested from the growth broth, and washed twice in PBS and centrifuged at 17329 × *g* before using them in the transport experiments.

Laboratory Experiments

Batch

Batch experiments were conducted to assess the survival characteristics of the indicator microorganisms. Experiments were conducted by placing 7.5 g of field soil into a 20-mL glass scintillation vial. Two conditions were studied to determine the influence of biotic factors on the survival of the indicator microorganisms. One set of experiments employed native soil and wastewater, and the other used sterilized (autoclaved) soil and wastewater spiked with indicator microorganisms. Lagoon water (0.43 mL) and deionized water were added to the vials to achieve a water saturation of 80%. The porosity of the soil in the vials was estimated from the soil volume and mass to be 0.43. The deionized water and wastewater were then thoroughly mixed with the soil using a metal spatula, and the vial was capped (a pin hole was used for aeration) and wrapped in aluminum foil. The vial were subsequently stored at 25°C and analyzed for indicator microorganism concentrations at desired times. Separate vials were used for each time (0, 6, 10, 24, 48, 80, 144, and 168 h). Hence, a total of eight vials were independently analyzed for native and sterilized batch experiments.

Microorganism death/inactivation is typically assumed to follow a simple first-order model (Yates et al., 1987; Schijven and Hassanizadeh, 2000). However, virus inactivation and bacterial growth in soils have been observed to produce more complex survival behavior than the simple first-order model. The following model was proposed by Sim and Chrysikopoulos (1996) to account for observed time dependent inactivation:

$$\frac{dC_T}{dt} = -\lambda_o \exp(-\alpha t) C_T \qquad [1]$$

where C_T [N L^{-3}; where N is the number of microorganisms, and L denotes units of length] is the total microorganism concentration, t [T, denotes units of time] is time, λ_o [T^{-1}] is the initial rate of die-off/inactivation, and α [T^{-1}] is the resistivity

coefficient to die-off/inactivation. The solution to Eq. [1] is given as (Sim and Chrysikopoulos, 1996):

$$\frac{C_T}{C_{Ti}} = \exp\left(\frac{\lambda_o}{\alpha}\left[\exp(-\alpha t) - 1\right]\right) \quad [2]$$

where C_{Ti} [N L^{-3}] is the initial total microorganism concentration. The parameters λ_o and α will be fitted herein to measured batch survival data using a nonlinear least squares optimization routine.

Packed Column

Packed soil column experiments were conducted using top soil from the field site to assess the transport potential of *Enterococcus*, somatic coliphage, and *E. coli* under well controlled conditions. Sterilized field soil and wastewater that was spiked with indicator microorganisms were used for this purpose.

Kontes Chromaflex chromatography columns (Kimble/Kontes, Vineland, NJ) made of borosilicate glass (15 cm long and 4.8 inside diam. were equipped with an adjustable adaptor at the top) were used in the transport studies. The columns were packed dry with oven-dried soil from the surface of the NMP site. Well water from the field was slowly pumped upward through the vertically oriented columns at a steady flow rate for several pore volumes (PV) to saturate the column. A peristaltic pump (Cole-Parmer, Vernon Hills, IL) was used for this purpose. Microorganism transport experiments were subsequently conducted by pumping tracer solution at a steady rate through the column for several PV, after which a three-way valve was used to switch to the well water for several additional PV. Effluent samples were continuously collected in 16 by 150 mm borosilicate test tubes (Thermo Fisher Scientific, Waltham, NJ) using an auto sampler (Isco, Lincoln, NE). Following completion of the transport experiments, the concentration of indicator microorganisms in the effluent and retained in the soil was determined using the previously outlined protocols.

Undisturbed Column

To study the influence of soil structure on the transport and survival of indigenous indicator microorganisms in wastewater a larger undisturbed soil core (core length was 65 cm and the internal diameter was 24 cm) was taken from the field site just outside of the plot. This core was also used in water flow and bromide transport experiments, and details on the collection, handling, instrumentation, hydraulic properties, and solute transport characteristics of this core are provided in the literature (Segal et al., 2009). In brief, the core was encased in an acrylic cylinder that was pushed into the soil with a hydraulic piston and then dug out. The bottom of the core was situated in a temperature controlled room at 25°C on a ceramic plate with a bubbling pressure of 100 kPa and a hanging water column was used to control the water pressure and to collect effluent samples. The soil surface boundary condition was selected to mimic a worst case transport scenario of ponded infiltration in which saturated conditions enable water flow and pathogen transport through macropores and soil structure. In this case, wastewater was instantaneously added to the soil surface to a depth of 7 cm and allowed to infiltrate into the profile. Following application and infiltration of the wastewater, 2 cm diam. and 65 cm long soil cores were collected vertically from the column surface at selected times and analyzed for concentrations of indicator microbes. The sample locations were subsequently plugged with a similar sized PVC tube that was capped.

Results and Discussion

Nutrient Management Plan Field Site

The treatment (inclined screen separator, sedimentation tank, and sand filter) of the dairy wastewater at the field site had a large effect on many microbial properties. Table 1 provides representative information on the concentration of indicator microorganisms before and after this treatment. The treatment produced almost a 2 log decrease in the concentration of the various indicator microorganisms. Concentrations of indicator microorganisms in the treated wastewater, however, still significantly exceeded the recommended U.S. standards for unrestricted irrigation (USEPA, 2004). If pathogenic microorganisms behave similarly to indicators, then care must be taken in using dairy wastewater as a source for irrigation water. To protect surface and groundwater supplies it is therefore essential that pathogenic microorganisms in the dairy wastewater be removed by treatment, or be retained and inactivated/degraded in the root zone of NMP sites.

Water mass balance information for the winter wheat-rye and the summer sorghum growing seasons are presented in Table 2 as a function of day after emergence (DAE). The accurate water application quantities (well water + lagoon water) relative to the actual plant ET restricted changes in water pressure to the upper 60 cm of the soil profile (Segal et al., unpublished data, 2009). Hence, the amounts of drainage below the root zone (Table 2) were very small throughout the winter and summer growing seasons and most of this drainage occurred with well water application (57.6 and 86.9% during winter and summer, respectively). This finding indicates that there exists little potential for advective transport of the indicator microorganisms below the root zone under these NMP management conditions. If the indicator microorganisms survive in the root zone, however, they may still be transported below the root zone when water in excess of ET is applied to this NMP site such as during the fallow period with high precipitation events or during pre-irrigations to leach salts. Hence, pathogen survival in the root zone may pose a risk to food and water resources at NMP sites.

Figure 1a-d present plots of the soil concentration (S, N g^{-1}) of *Enterococcus*, fecal coliform, somatic coliphage, and total *E. coli* as a function of depth at several different DAE during the winter wheat-rye growing season, respectively. Table 2 also includes the amount of well water and dairy wastewater that was applied to the NMP study site. Irrigation water was applied at the end of the intervals provided in Table 2, except for the first interval in which the irrigation water was applied periodically throughout the interval. Low concentrations of total *E. coli*, fecal coliform, and somatic coliphage were initially present in the surface soil of the NMP site. The *Enterococcus* concentrations, however, were initially more abundant. After wastewater addition on DAE 36, the concentrations of the indicator micro-

Table 1. Representative concentrations of indicator microorganisms in raw and treated dairy wastewater, and the percent removal by treatment. Treatment included solid separator, sedimentation tank and sand filter.

Indicator	Raw	Treated	Percent removal
Enterococcus (cfu mL^{-1})	1.56E+06	6.10E+04	96.1
Fecal coliform (cfu mL^{-1})	2.47E+05	6.00E+03	97.6
Somatic coliphage (pfu mL^{-1})	7.50E+03	5.00E+02	93.3
Total *E. coli* (cfu mL^{-1})	9.30E+04	3.00E+03	96.8

Table 2. Actual evapotranspiration (ET), rainfall, well water application, wastewater application, and drainage amounts during the winter (wheat-rye) and summer (sorghum) growing seasons as a function of day after emergence (DAE).

DAE	Actual ET	Rainfall	Well water	Wastewater	Drainage†
	mm				
Winter					
15–29	40.1	7.2	42.9	0.0	10.0
30–36	13.5	0.0	0.0	15.0	1.5
37–50	68.4	4.2	0.0	71.7	7.6
51–58	36.5	4.1	36.3	0.0	4.1
59–65	43.0	13.2	0.0	34.5	4.8
66–72	43.2	0.0	48.0	0.0	4.8
Summer					
5–28	104.0	0.0	135.0	0.0	26.0
29–35	58.5	0.0	62.0	0.0	6.5
36–44	76.2	0.0	0.0	91.51	8.5
45–49	42.4	0.0	48.6	0.0	4.7
50–58	77.4	0.0	86.6	0.0	8.6
59–62	31.7	0.0	35.3	0.0	3.5
63–70	59.1	0.0	66.7	0.0	6.6

† Value estimated from water balance.

organisms in the soil core increased dramatically. The highest concentrations occurred near the soil surface (top 4–6 cm) and then rapidly decreased with soil depth. Over time concentrations of the indicator microorganisms at the soil surface tended to decrease, and by DAE 71 approached the low levels that were found before wastewater application (DAE 36).

Similar NMP experiments were conducted during the summer on sorghum. Soil cores were periodically collected from the field site during the summer season and analyzed for indicator microorganism concentrations. Similar to the winter crop, only low levels of *Enterococcus* were found in the surface soil at the beginning and end of the summer season.

A more detailed NMP experiment was initiated the following summer growing season (alfalfa, *Medicago sativa* L.,was the crop) to better understand the transport and fate of the indicator microorganisms. In this case, the sampling frequency (initial, and times = 0, 14, 62, 134, and 206 h after wastewater application) and number of soil cores (composite of three cores) were much higher after a single wastewater application event. In addition, the gravimetric water content with depth was measured at selected times after wastewater application.

Initial soil concentrations were low for the somatic coliphage and total *E. coli* (C_T was 0 CFU cm^{-3}). Conversely, initial soil concentrations for *Enterococcus* (C_T was 5E4 CFU cm^{-3}) and fecal coliform (C_T was 1.3E4 CFU cm^{-3}) were higher. Approximately 10.5 cm of wastewater was applied to the field site at an average rate of 0.95 cm h^{-1}. Changes in the water content occurred over depth and time (Fig. 2a) as a result of infiltration, redistribution, and then evapotranspiration in the root zone (top 60 cm of soil). Figure 2b-e present plots of the normalized soil concentration (S/C_{Ti}; cm^3 g^{-1}) of *Enterococcus*, fecal coliform, somatic coliphage, and total *E. coli* as a function of depth at selected sampling times after wastewater application. Here the soil concentrations were normalized by C_T for a given microorganism determined from the entire soil core taken at time = 0 h, that is, denoted as C_{Ti}. The concentration of indicator microorganisms in the soil was high at time = 0 h, and reflected initial conditions, transport during wastewater infiltration, and retention of the indicator microorganisms in the soil. At later sampling times (≥14 h) infiltration has ceased; redistribution and evapotranspiration of water depletes water from the root zone (Fig. 2a). In general, the concentration of the indicator microorganisms in the soil cores is controlled at earlier sampling times (t = 14 and 62 h) by initial conditions and microbe retention, and at later times (t = 134 and 206) by microbe survival and/or inactivation. Microbe retention tends to be highest at the soil surface and to rapidly decrease with soil depth. Survival of fecal coliforms, somatic coliphage, and total *E. coli* was apparently short lived under these NMP conditions, with soil concentrations approaching low background levels within 134 h after wastewater application. This result is at least partly due to the high summer soil temperature which averaged 22.6°C at a depth of 7 cm during this experiment. Cooler temperatures that were measured during the winter growing season (20.3°C at a depth of 7 cm) are expected to enhance microorganism survival (Reddy et al., 1981; Yates et al., 1987) shown in Fig. 1. Conversely, final soil concentrations of *Enterococcus* were more pronounced than the other indicator microbes. This may occur as a result of differences in survival or due to differences in the initial concentration in these microbes in the soil (C_T was 5E4 and 1.3E4 CFU cm^{-3} for *Enterococcus* and fecal coliform, respectively) and wastewater (C_i in the wastewater was 8.9E04 and 1.7E3 CFU mL^{-1} for *Enterococcus* and fecal coliform, respectively).

A more quantitative determination of the transport and survival of the indicator microorganisms at the NMP site were not attempted due to the relatively low initial concentration levels of these microbes in the irrigation water and our analytical detection limits, potential spatial variability in microbe concentrations in the field, incomplete control of the lower water flow boundary conditions, and transients in water content that were induced by infiltration, redistribution, and ET. Laboratory experiments that are described below were initiated to overcome many of these limitations.

Laboratory Experiments

Figure 3 present results from the batch survival experiments conducted under sterile (Fig. 3a) and native (Fig. 3b) conditions at 80% water saturation for the various indicator microorganisms. A semi-log plot of C_T/C_{Ti} as a function of time is shown in this figure. The considered bacterial indicator microorganisms had much greater die-off under native than sterile conditions. This indicates that biotic factors such as predation and competition in native soil

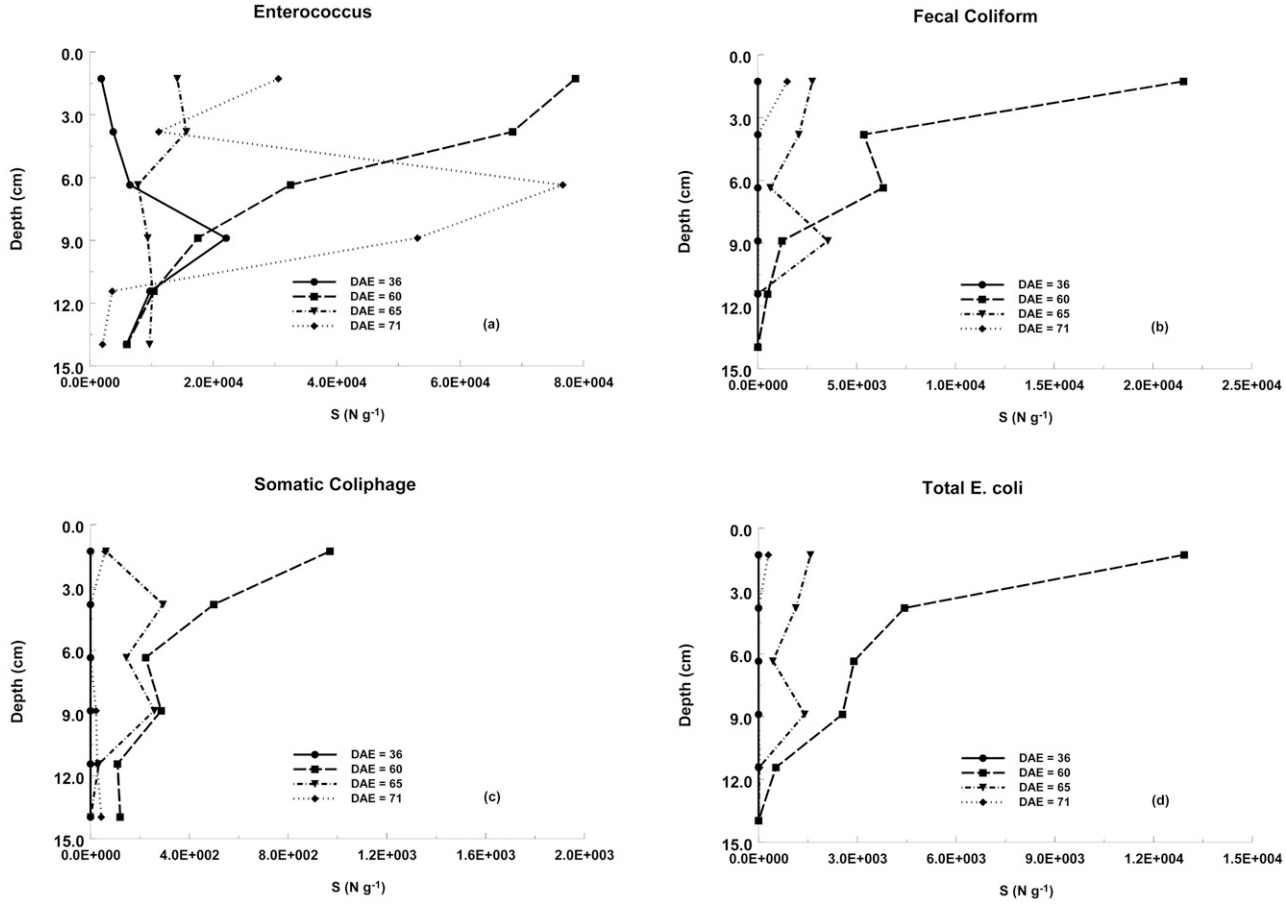

Fig. 1. Plots of the soil concentration (*S*) of *Enterococcus* (Fig. 1a), fecal coliform (Fig. 1b), somatic coliphage (Fig. 1c), and total *E. coli* (Fig. 1d) as a function of depth at several different days after emergance (DAE) during the winter wheat-rye growing season at the field site.

and wastewater had a significant influence on the survival of the considered bacterial indicator microorganisms. Other researchers have reported similar observations and this literature have been reviewed by Van Veen et al. (1997). Conversely, the somatic coliphage exhibited similar survival/inactivation under both sterile and native conditions, suggesting that abiotic factors controlled die-off/inactivation for this coliphage. The native condition is expected to be most representative of field conditions at the NMP site. Table 3 therefore provides a summary of the fitted die-off/inactivation rates (Eq. [2]) and the standard error coefficient on these parameters for the various indicator microorganisms under native conditions at 80% water saturation. The *P* values for statistical significance of the correlation coefficients were <0.00002. It is interesting to note that fecal coliforms had a lower die-off rate than *Enterococcus* under native conditions (Fig. 3b and Table 3). This finding implies that the observed differences in final soil concentrations of these microbes shown in Fig. 2 was likely due to differences in the initial soil and wastewater concentrations of these organisms.

Packed soil column experiments were conducted using sterilized soil (sandy loam) from the root zone of the NMP field site to better understand the transport of the indicator microorganisms. The tracer solution consisted of sterilized (autoclaved) wastewater spiked with a known concentration of specific microorganisms, and sterilized well water was employed as the resident and eluant solutions. Figure 4a presents breakthrough curves for *Enterococcus*, total *E. coli*, and ϕ*X174* (a somatic coliphage) in the packed soil column. Relevant experimental conditions are provided in the figure caption. The coliphage ϕ*X174* exhibited much greater transport potential (87.1%) than the bacteria *Enterococcus* (0.6%) or *E. coli* (0.3%). This is likely due to differences in the size and surface chemistry of these microorganisms. In particular, ϕ*X174* is much smaller (20 nm) than *Enterococcus* or *E. coli* (0.5–2 microns). Straining is therefore expected to play a more dominant role in retention of the bacterial cells than the coliphage based on size considerations (Bradford et al., 2003). It is interesting to note that the breakthrough curve for both *Enterococcus* and *E. coli* exhibited a similar shape and magnitude in the relative concentrations, with initially low effluent concentrations that slowly increased with time. This observation suggests the potential for low but persistent amounts of bacteria transport that were likely due to slow release of cells from the solid phase due to detachment, hydrodynamic shearing, or diffusion from low velocity regions (Bradford and Torkzaban, 2008).

After recovery of the breakthrough curve, the soil in the column was excavated to recover the remaining cells. Figure 4b presents the microorganism retention profiles for *Enterococcus*, total *E. coli*, and ϕ*X174* in the packed soil column. Consistent with

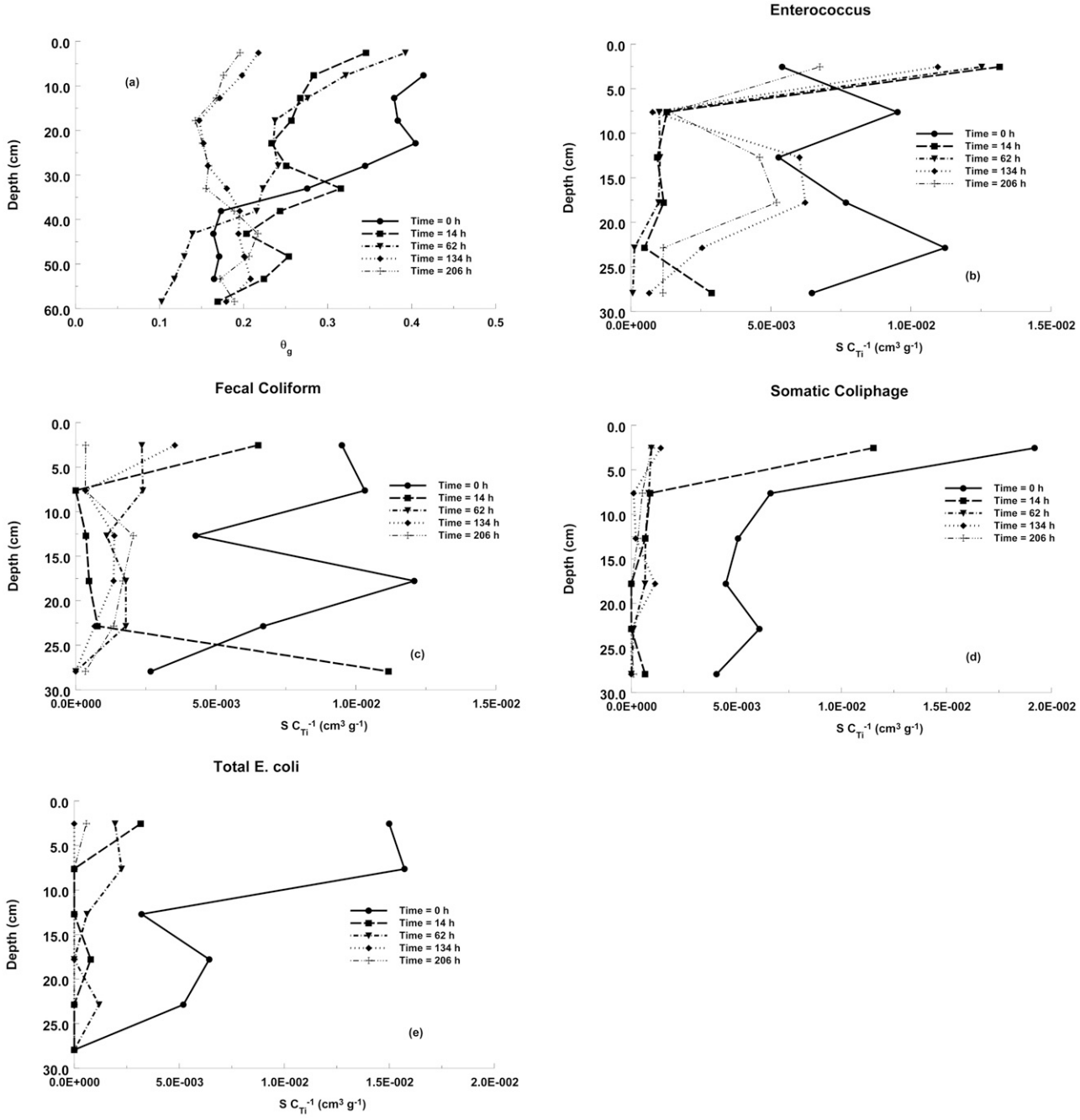

Fig. 2. Plots of the gravimetric water content (θ_g) (Fig. 2e), and the normalized soil concentration (S/C_{Ti}) of *Enterococcus* (Fig. 2b), fecal coliform (Fig. 2c), somatic coliphage (Fig. 2d), and total *E. coli* (Fig. 2e) as a function of depth at selected sampling times (initial, and times = 0, 14, 62, 134, and 206 h after wastewater application) during the summer alfalfa growing season at the field site. Microbial concentrations were determined from a composite of three soil cores at the indicated depth and time, and normalized by the total concentration of a given microorganism measured in the entire core at time = 0 (C_{Ti}).

the breakthrough curve information the total amounts of microbes recovered in the soil were 7.8, 83, and 109% for ϕ*X174*, *E. coli*, and *Enterococcus*, respectively. Reasonable mass balance was achieved in this packed column experiment (83–109%). Batch results shown in Fig. 3a indicate that little death or growth of the indicator microorganisms was likely to occur during the column experiments (<6.25 h). Hence, the mass balance information supports our methodology for determining microorganism concentrations in the field. It should also be mentioned that the collected retention profiles for the microorganisms were not log–linear with depth; that is, the rate of retention was not first-order. The observed nonmonotonic shape of the retention profiles for the bacteria cells likely reflected the release and continued slow migration of cells through the soil. Other researchers have reported nonmonotonic profiles for bacteria in porous media and this shape has been reported to be sensitive to the soil grain size, the system hydrodynamics, and the sampling time (Tong et al., 2005; Bradford et al., 2006b). In contrast, the retention profile

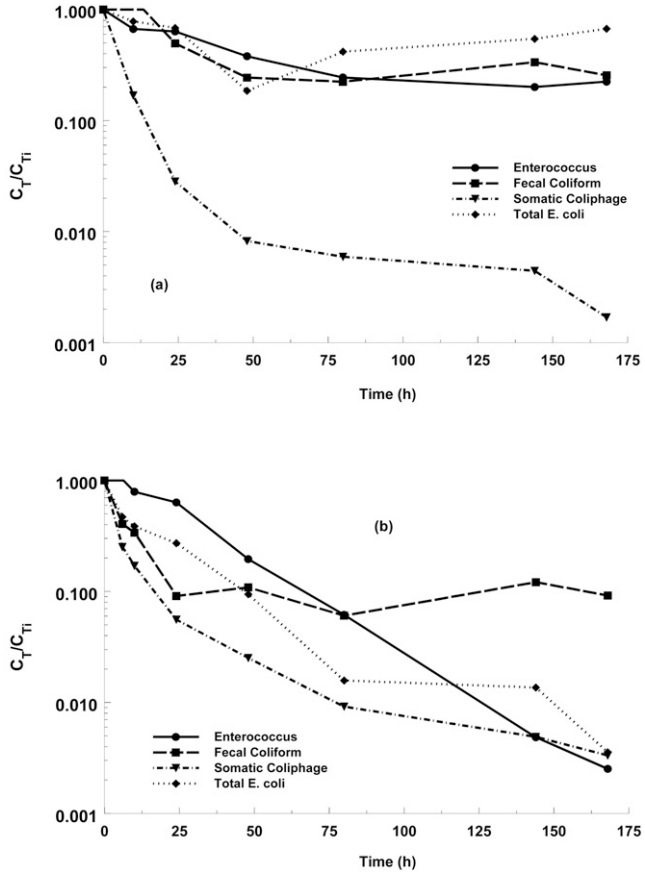

Fig. 3. A semi-log plot of the relative total concentration (C_T/C_{Ti}) as a function of time in batch survival experiments under sterile (Fig. 3a) and native (Fig. 3b) conditions at 80% water saturation for the various indicator microorganisms.

Table 3. Fitted parameters (λ_o and α) from the time dependent die-off/inactivation model (Eq. [2]) for the various indicator microorganisms under native conditions at 80% water saturation. The goodness of model fit is quantified by the coefficient of linear regression (R^2) on natural log transformed data, and standard error coefficient of the fitted model parameters is provided in brackets.

Indicator	λ_o	α	R^2
	h^{-1}		
Enterococcus	3.52E-02 (1.31E-03)	3.54E-11 (4.32E-04)	0.992
Fecal coliform	1.90E-01 (4.03E-02)	7.81E-02 (1.86E-02)	0.933
Somatic coliphage	1.61E-01 (2.05E-02)	2.95E-02 (4.65E-03)	0.973
Total *E. coli*	7.29E-02 (1.22E-02)	1.25E-02 (3.55E-03)	0.963

for $\phi X174$ shows enhanced retention near the column inlet that decreased with distance. In the literature this type of depth dependent retention has been attributed to straining (Bradford et al., 2006a) as well as chemical heterogeneity of the microorganisms (Li et al., 2004; Tufenkji and Elimelech, 2005).

Collectively, Fig. 4a and 4b demonstrate the complexity of microorganism transport and retention behavior in soil from the NMP site. In this work we do not attempt to quantify and simulate the exact retention mechanisms, as this would require additional experiments and model development that are beyond the scope of this manuscript. In addition, application of laboratory retention information collected under saturated conditions to predict transport of the microorganisms at the NMP site is

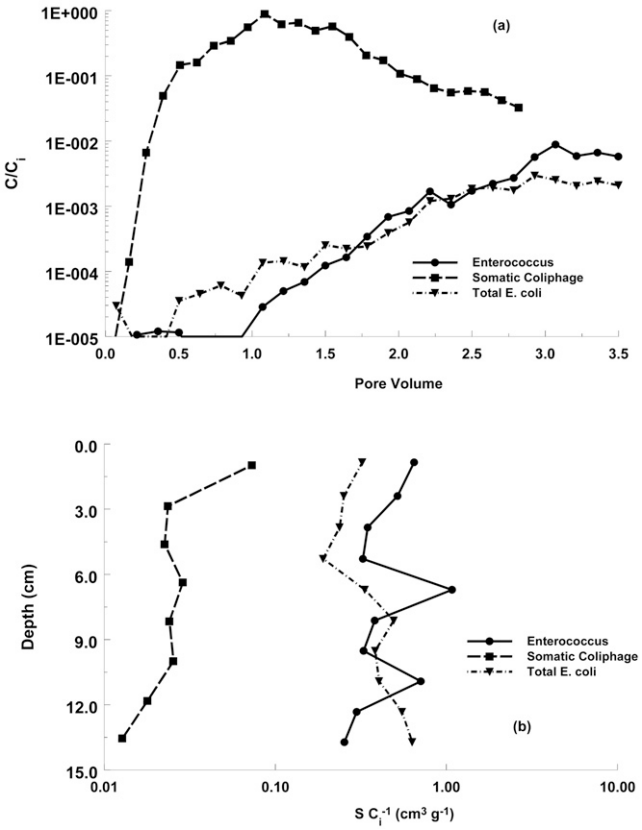

Fig. 4. Breakthrough curves (Fig. 4a) and retention profiles (Fig. 4b) for *Enterococcus*, total *E. coli*, and $\phi X174$ (a somatic coliphage) in a column experiment packed with sterilized field soil. In Fig. 4a log relative concentrations (C/C_i; where C is the aqueous concentration and C_i is the initial concentration in the influent suspension) are plotted as a function of pore volumes. In Fig. 4b log relative soil concentrations, (S/C_i), is plotted as a function of distance away from the inlet. Relevant experimental conditions include the following parameters: porosity = 0.50, column length = 14.4 cm, tracer pulse duration = 112.5 min, and the Darcy water velocity = 3.36 cm h^{-1}.

complicated by the sensitivity of microorganism retention to water content, transients, and fluid velocity. The interested reader is referred to the following literature that discusses current challenges in modeling microorganism retention in porous media (Bradford and Toride, 2007; Bradford and Torkzaban, 2008).

Wastewater application under ponded infiltration conditions represents a worst case scenario for microorganism transport, because larger more conductive pore spaces and soil structure are water filled under saturated conditions. An additional transport experiment was therefore conducted on an undisturbed column from the NMP site (core length was 65 cm and the internal diameter was 24 cm), with wastewater instantaneously added to the soil surface to a depth of 7 cm. Figure 5 presents plots of the normalized soil concentration of the indicator microorganisms as a function of depth at a sampling time of 24 h. In this case, the infiltrate front has already passed through the column and water is slowing redistributing (data not shown). High concentrations of the indicator microorganisms occur near the soil surface and then tend to rapidly decrease with depth. This mainly reflects microbe retention behavior. Figure 5 also indicates the presence of isolated

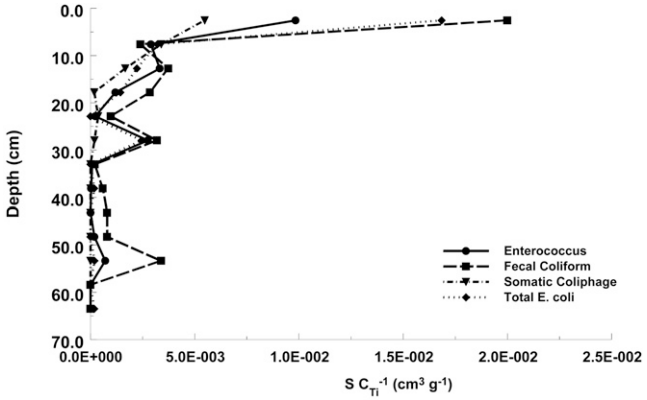

Fig. 5. Plots of the normalized soil concentration (S/C_{Ti}) of the indicator microorganisms as a function of depth at a sampling time of 24 h after ponded infiltration of wastewater ceased on the undisturbed soil core from the site. Here the soil concentration is normalized by the total concentration of a given microorganism measured in the entire core at time = 0 (C_{Ti}).

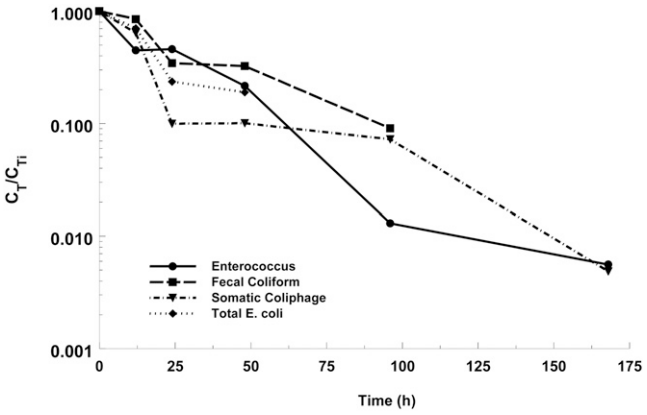

Fig. 6. A semi-log plot of the relative total concentration (C_T/C_{Ti}) of the various indicator microorganisms as a function of time in the ponded infiltration undisturbed soil column experiment. Here the total soil concentration is normalized by the total soil concentration of a given microorganism measured in the entire core at time = 0 (C_{Ti}).

low concentrations of bacterial indicators at several deeper depths (27.94 and 53.34 cm). The concentrations at these locations likely reflect the influence of soil structure or low levels of bacteria release and migration as previously identified in Fig. 4.

It should be mentioned that analysis of the column effluent and soil at the column outlet from the undisturbed core did not reveal any indicator microorganisms for all core sampling times (24, 48, 96, and 176 h). Soil cores taken at later sampling times primarily reflected the influence of indicator microorganism survival and may be analyzed in a similar fashion to the batch survival experiments by plotting the log of C_T/C_{Ti} for the entire core as a function of time in Fig. 6. Survival information obtained from batch experiments under native conditions and 80% water saturation were generally consistent with results shown in Fig. 6. After 176 h the batch and undisturbed column values of C_T/C_{Ti} were 0.003 and 0.006 for *Enterococcus*, and 0.003 and 0.005 for somatic coliphage, respectively. The total concentrations of fecal coliform and total *E. coli* in the undisturbed column could only be detected up to 96 and 48 h, respectively, but were similar in magnitude to native batch experiments at these times (compare with Fig. 3b).

Summary and Conclusions

Farmers in the United States are currently required to follow approved NMPs when applying CAFO lagoon water to agricultural lands. The NMPs are designed to meet the water and nutrient needs of crops and thereby protect surface and groundwater supplies from contamination. However, NMPs implicitly assume that pathogenic microorganisms in the lagoon water will be retained and die-off in the root zone. To test this assumption in regards to groundwater protection, a NMP was implemented in which dairy wastewater application rates were limited by crop N requirements and the rate of evapotranspiration on a small-scale field plot (6 by 6 m). The transport and fate of indicator microorganisms were monitored during winter and summer growing seasons.

To efficiently implement the NMP the dairy wastewater was treated (screen, sedimentation tank, and sand filter) to minimize the organic load in the wastewater. This treatment also produced close to a 2-log reduction in concentrations for *Enterococcus*, fecal coliform, total *E. coli*, and somatic coliphage. When well-water and treated wastewater were applied to the field site to meet ET and plant nutrient requirements, little advective transport of the indicator microorganisms occurred below the root zone (60 cm). The remaining concentrations of these indicator microorganisms in the root zone died-off during the winter and summer growing seasons. These observations support the hypothesis that a well-designed and implemented NMP at this site will protect groundwater supplies from microorganism contamination.

Additional experiments were conducted in the laboratory to better quantify microorganism transport and survival in the field soil. Batch survival experiments revealed much more rapid die-off rates for the bacterial indicator microorganisms in native than in sterilized soil, suggesting that the biotic factors played a dominant role in survival behavior. Saturated column experiments with packed field soil, demonstrated much greater transport potential for somatic coliphage than bacterial indicators (*Enterococcus* and total *E. coli*). Retention rates for the indicator microorganisms were not log-linear with depth (not first-order with respect to concentration), demonstrating the complexity of the microorganism transport behavior. A worst case transport scenario of ponded infiltration on a large undistributed soil column from the field was also investigated. Concentrations of the indicator microorganisms were not detected in the column outflow and in the soil at a depth of 65 cm. The remaining concentrations of the indicator microorganisms in the column rapidly decreased toward low concentrations within 1 wk at a temperature of 25°C. Both of these observations were consistent with field NMP results and conclusions.

Results from field and laboratory experiments demonstrate that the fate of microorganisms at NMP sites will depend on both transport and survival characteristics. Although transport and survival of microorganisms in NMP soils were shown to be compli-

cated and are likely to be site specific, a few recommendations for NMP implementation can be developed. The transport potential of microorganisms can be significantly reduced by minimizing water leaching below the root zone and surface water runoff. This can be achieved by: (i) precise estimation of ET rate; (ii) uniform application of wastewater; and (iii) selecting water application timing and quantities based on considerations of soil permeability and ET. Special caution is warranted in coarse textured and structured soils and during water flow transients where enhanced microorganism transport potential has been reported in the literature (Natsch et al., 1996; Bradford et al., 2003; Saiers et al., 2003). Survival characteristics of microorganisms are also likely to be site specific, but may be quantified through relatively simple batch type experiments that mimic natural conditions. Timing of water application should allow for adequate die-off of microorganisms before leaching the root zone by irrigation or natural precipitation. Finally, the potential for groundwater contamination will increase with shorter travel times and distances. The water table depth is therefore another important consideration for environmentally protective NMPs. Implementation of these NMP recommendations will undoubtedly be technically challenging, and may not be economically feasible in some instances. Nevertheless, these recommendations provide guidance to minimize the potential risks of pathogen contamination of water resources at NMP sites.

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References

Berg, G., L. Eberl, and A. Hartmann. 2005. The rhizosphere as a reservoir for opportunistic pathogenic bacteria. Environ. Microbiol. 7:1673–1685.

Bradford, S.A., E. Segal, W. Zheng, Q. Wang, and S.R. Hutchins. 2008. Reuse of concentrated animal feeding operation wastewater on agricultural lands. J. Environ. Qual. 37:S97–S115.

Bradford, S.A., J. Simunek, M. Bettahar, M.Th. van Genuchten, and S.R. Yates. 2003. Modeling colloid attachment, straining, and exclusion in saturated porous media. Environ. Sci. Technol. 37:2242–2250.

Bradford, S.A., J. Simunek, M. Bettahar, M.Th. van Genuchten, and S.R. Yates. 2006a. Significance of straining in colloid deposition: Evidence and implications. Water Resour. Res. 42, W12S15, doi:10.1029/2005WR004791.

Bradford, S.A., J. Simunek, and S.L. Walker. 2006b. Transport and straining of E. coli O157:H7 in saturated porous media. Water Resour. Res. 42, W12S12, doi:10.1029/2005WR004805.

Bradford, S.A., and N. Toride. 2007. A stochastic model for colloid transport and deposition. J. Environ. Qual. 36:1346–1356.

Bradford, S.A., and S. Torkzaban. 2008. Colloid transport and retention in unsaturated porous media: A review of interface, collector, and pore scale processes and models. Vadose Zone J. 7:667–681.

CDC. 1998. Surveys of waterborne disease outbreaks compiled by the Center for Disease Control and Prevention from 1986 to 1998, published in Morbidity and Mortality Weekly Reports. Centers for Disease Control and Prevention, Atlanta, GA.

Clesceri, L.S., A.E. Greenberg, and R.R. Trussell. 1989. Standard methods for the examination of water and wastewater. 17th ed. American Public Health Assoc., Washington, DC.

Elimelech, M., J. Gregory, X. Jia, and R.A. Williams. 1998. Particle deposition and aggregation measurement, modeling and simulation. Butterworth-Heinemann, Woburn, MA.

Gerba, C.P., and J.E. Smith, Jr. 2005. Sources of pathogenic microorganisms and their fate during land application of wastes. J. Environ. Qual. 34:42–48.

Ginn, T.R., B.D. Wood, K.E. Nelson, T.D. Schiebe, E.M. Murphy, and T.P. Clement. 2002. Processes in microbial transport in the natural subsurface. Adv. Water Res. 25:1017–1042.

Guan, T.Y., and R.A. Holley. 2003. Pathogen survival in swine manure environments and transmission of human enteric illness—A review. J. Environ. Qual. 32:383–392.

Havelaar, A.H. 1986. F-specific bacteriophages as model viruses in water treatment processes. Ph.D. thesis. Univ. of Utrecht, Utrecht, the Netherlands.

Jones, D.I. 1999. Potential health risks associated with the persistence of *Escherichia coli* 0157 in agricultural environments. Soil Use Manage. 15:76–83.

Li, X., T.D. Scheibe, and W.P. Johnson. 2004. Apparent decreases in colloid deposition rate coefficient with distance of transport under unfavorable deposition conditions: A general phenomenon. Environ. Sci. Technol. 38:5616–5625.

Macler, B.A., and J.C. Merkle. 2000. Current knowledge on groundwater microbial pathogens and their control. Hydrogeol. J. 8:29–40.

Natsch, A., C. Keel, J. Troxler, M. Zala, N. Von Albertini, and G. De´fago. 1996. Importance of preferential flow and soil management in vertical transport of a biocontrol strain of *Pseudomonas fluorescens* in structured field soil. Appl. Environ. Microbiol. 62:33–40.

Reddy, K.R., R. Khaleel, and M.R. Overcash. 1981. Behavior and transport of microbial pathogens and indicator organisms in soils treated with organic wastes. J. Environ. Qual. 10:255–266.

Saiers, J.E., G.M. Hornberger, D.B. Gower, and J.S. Herman. 2003. The role of moving air-water interfaces in colloid mobilization within the vadose zone. Geophys. Res. Lett. 30:2083, doi:10.1029/2003GL1018418.

Saiers, J.E., and J.J. Lenhart. 2003. Colloid mobilization and transport within unsaturated porous media under transient-flow conditions. Water Resour. Res. 39(1):1019, doi:10.1029/2002WR001370.

Schijven, J.K., and S.M. Hassanizadeh. 2000. Removal of viruses by soil passage: Overview of modeling, processes, and parameters. Crit. Rev. Environ. Sci. Technol. 30:49–127.

Segal, E., S.A. Bradford, P. Shouse, N. Lazarovitch, and D. Corwin. 2008. Integration of hard and soft data to characterize field-scale hydraulic properties for flow and transport studies. Vadose Zone J. 7:878–889.

Segal, E., P. Shouse, and S.A. Bradford. 2009. Deterministic analysis and upscaling of bromide transport in a heterogeneous vadose zone. Vadose Zone J. (in press).

Sim, Y., and C.V. Chrysikopoulos. 1996. One-dimensional virus transport in porous media with time-dependent inactivation rate coefficients. Water Resour. Res. 32:2607–2611.

Tong, M., X. Li, C.N. Brow, and W.P. Johnson. 2005. Detachment-influenced transport of an adhension-deficient bacterial strain within water-reactive porous media. Environ. Sci. Technol. 39:2500–2508.

Torkzaban, S., S.M. Hassanizadeh, J.F. Schijven, and H.H.J.L. van den Berg. 2006. Role of air-water interfaces on retention of viruses under unsaturated conditions. Water Resour. Res. 42, W12S14, doi:10.1029/2006WR004904.

Tufenkji, N., and M. Elimelech. 2005. Breakdown of colloid filtration theory: Role of the secondary energy minimum and surface charge heterogeneities. Langmuir 21:841–852.

USDA. 1992. National engineering handbook: Agricultural waste management field handbook. Part 651 (210-AWMFH, 4/92). Ch. 3, p 1–29. USDA, Washington, DC.

USDA. 2000. National nutrient management (Code 590). Natural Resource Conserv. Serv., Conservation Practice Standard. Available at ftp:j//ftp-fc.sc.egov.usda.gov/NHQ/practice-standards/standards/590.pdf (verified 11 Apr. 2009).

USEPA. 1998. Environmental impacts of animal feeding operations. U.S. Environ. Protection Agency, Office of Water, Standards and Applied Sci. Div., Washington, DC.

USEPA. 2001a. Development document for the proposed revisions to the national pollutant discharge elimination system permit regulation and effluent limitation guidelines and standards for concentrated animal feeding operations (CAFOs). EPA-821-R-01–003. U.S. Environ. Protection Agency, Washington, DC.

USEPA. 2001b. Method 1601: Male-specific (F+) and somatic coliphage in water by two-step enrichment procedure. EPA-821-R-01–030. U.S. Environ. Protection Agency , Office of Water, Engineering and Analysis Div., Washington, DC.

USEPA. 2003. National pollutant discharge elimination system permit regulation and effluent limitation guidelines and standards for concentrated animal feeding operations (CAFOs). Fed. Regist. 69(29):7176–7274.

USEPA. 2004. Guidelines for water reuse. EPA 625/R-04/108. U.S. Environ. Protection Agency, Cincinnati, OH.

van Genuchten, M.Th., and P.J. Wierenga. 1977. Mass transfer studies in sorbing porous media: I. Analytical Solutions. Soil Sci. Soc. Am. J. 40:473–480.

Van Veen, J.A., L.S. van Overbeek, and J.D. van Elsas. 1997. Fate and activity of microorganisms introduced into soil. Microbiol. Mol. Biol. Rev. 61:121–135.

Wang, H., G. Magesan, and N.S. Bolan. 2004. An overview of the environmental effects of land application of farm effluents. N. Z. J. Agric. Res. 47:389–403.

Yates, M.V., S.R. Yates, J. Wagner, and C.P. Gerba. 1987. Modeling virus survival and transport in the subsurface. J. Contam. Hydrol. 1:329–345.