11-2009

Escherichia coli Transport from Surface-Applied Manure to Subsurface Drains through Artificial Biopores

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Abstract
Bacteria transport in soils primarily occurs through soil mesopores and macropores (e.g., biopores and cracks). Field research has demonstrated that biopores and subsurface drains can be hydraulically connected. This research was conducted to investigate the importance of surface connected and disconnected (buried) biopores on *Escherichia coli* (*E. coli*) transport when biopores are located near subsurface drains. A soil column (28 by 50 by 95 cm) was packed with loamy sand and sandy loam soils to bulk densities of 1.6 and 1.4 Mg m^{-3}, respectively, and containing an artificial biopore located directly above a subsurface drain. The sandy loam soil was packed using two different methods: moist soil sieved to 4.0 mm and air-dried soil manually crushed and then sieved to 2.8 mm. A 1-cm constant head was induced on the soil surface in three flushes: (i) water, (ii) diluted liquid swine (*Sus scrofa*) manure 48 h later, and (iii) water 48 h after the manure. *Escherichia coli* transport to the drain was observed with either open surface connected or buried biopores. In surface connected biopores, *E. coli* transport was a function of the soil type and the layer thickness between the end of the biopore and drain. Buried biopores contributed flow and *E. coli* in the less sorptive soil (loamy sand) and the sorptive soil (sandy loam) containing a wide (i.e., with mesopores) pore space distribution prevalent due to the moist soil packing technique. Biopores provide a mechanism for rapidly transporting *E. coli* into subsurface drains during flow events.

Disciplines
Agriculture | Bioresource and Agricultural Engineering

Comments
This article is from *JEQ* 38 (2009): 2412–2421, doi:10.2134/jeq2009.0077.

Published November, 2009

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Bacteria transport in soils primarily occurs through soil mesopores and macropores (e.g., biopores and cracks). Field research has demonstrated that biopores and subsurface drains can be hydraulically connected. This research was conducted to investigate the importance of surface connected and disconnected (buried) biopores on *Escherichia coli* (*E. coli*) transport when biopores are located near subsurface drains. A soil column (28 by 50 by 95 cm) was packed with loamy sand and sandy loam soils to bulk densities of 1.6 and 1.4 Mg m^{-3}, respectively, and containing an artificial biopore located directly above a subsurface drain. The sandy loam soil was packed using two different methods: moist soil sieved to 4.0 mm and air-dried soil manually crushed and then sieved to 2.8 mm. A 1-cm constant head was induced on the soil surface in three flushes: (i) water, (ii) diluted liquid swine (*Sus scrofa*) manure 48 h later, and (iii) water 48 h after the manure. *Escherichia coli* transport to the drain was observed with either open surface connected or buried biopores. In surface connected biopores, *E. coli* transport was a function of the soil type and the layer thickness between the end of the biopore and drain. Buried biopores contributed flow and *E. coli* in the less sorptive soil (loamy sand) and the sorptive soil (sandy loam) containing a wide (i.e., with mesopores) pore space distribution prevalent due to the moist soil packing technique. Biopores provide a mechanism for rapidly transporting *E. coli* into subsurface drains during flow events.

Published in J. Environ. Qual. 38:2412–2421 (2009).
doi:10.2134/jeq2009.0077
Published online 11 Sept. 2009.
Received 3 Mar. 2009.
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Animal excretions, slurry, and liquid manure on soil can easily be diluted and transported into the soil by irrigation or rainfall events. Bacteria can be carried by surface runoff, infiltration, and macropore flow to adjacent soils, deeper soils, or drainage systems. Survival of *E. coli* in soils has been reported to range between 60 and 103 d before falling below detectable levels (Stoddard et al., 1998; Sørensen et al., 1999; Wang, 2003).

Pathogenic bacteria can be transported through the soil in the form of suspended cells or they can attach to colloids, organic matter compounds, and mineral particles. Normally, the soil matrix acts as an effective pathogenic control during water infiltration and percolation (Darnault et al., 2004; Pachepsky et al., 2006). The natural soil filtration capacity is a function of bacterium properties, microbial community interaction, sorption processes and porous media characteristics such as texture, organic matter content, temperature, pH, solution ionic strength, and pore space distribution (Fontes et al., 1991; Pachepsky et al., 2006). Normally, these processes are simplified when attempting to analyze fate and transport pathways due to the complexity, specificity, lack of knowledge, and/or insufficient data about specific processes.

Macropores can allow bacteria and pathogens to bypass the soil's natural filter capacity and increase the risk of surface water and groundwater contamination (Reddy et al., 1981; Mawdsley et al., 1996a, 1996b; Guan and Holley, 2003; McGechan and Vinten, 2003; Darnault et al., 2004). Micropores, mesopores, and macropores are defined as pores spaces with equivalent diameters of 5 to 30 μm, 30 to 75 μm, and larger than 75 μm, respectively (SSSA, 2008). With macropores, wetting fronts propagate to significant depths by bypassing matrix pore space. Soil macropores (e.g., pore spaces formed as part of the soil structure) can transport air, water, colloids, organic matter, and microorganisms rapidly from the surface or upper soil (vertically and horizontally) to deeper soil and drainage systems (Lobry de Bruyn and Conacher, 1994; McMahon and Christy, 2000).

Macropores may be subdivided into two major groups based on physical characteristics and origin: natural fractures and cylin-

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Abbreviations: BSD, buried surface disconnected biopore; DG, dry grinding; LS, loamy sand; MPN, most probable number; OSC, open-surface connected biopore; SL, sandy loam; WG, wet grinding.

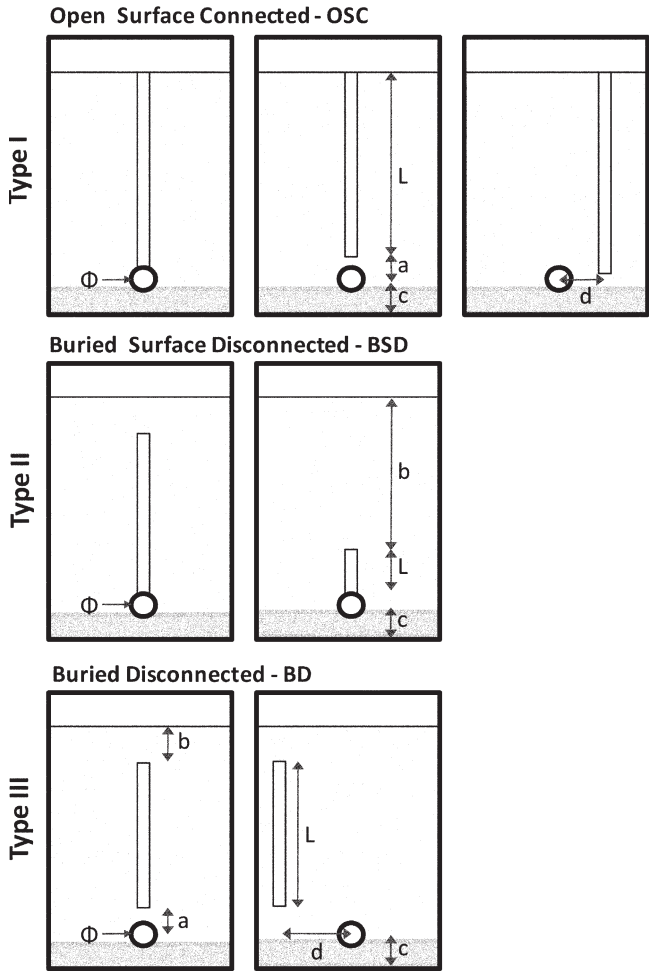

Fig. 1. Potential biopores in relationship to a subsurface drain. OSC: open surface connected; BSD: buried surface disconnected; and BD: buried disconnected; a: soil layer thickness between the center of the drainage pipe to the bottom of the biopores; b: soil layer thickness between the soil surface and the top of the biopores; c: initial saturated soil layer thickness equal to 10 cm for all experiments; d: distance from the center of the drain pipe to the vertical center of the biopores equal to zero for all experiments; L: length of the biopores; Φ: drain pipe diameter equal to 5 cm for all experiments.

drical biopores. Natural fractures originate from soil expansion and contraction or from geological processes. Biopores, on the other hand, are created by tunneling insects, small animals, nematodes, and decaying roots (McMahon and Christy, 2000). Biological (biopores) and mechanical fragmentation (tillage) are common in cultivated lands. Hubert et al. (2007) found that no tillage practices promote biological fragmentation, and biological fragmentation reformed following mechanical fragmentation in soils under annual tillage practices.

Several studies have attempted to investigate the influence of preferential flow pathways on soil pathogen transport (Fontes et al., 1991; Jiang et al., 2007; Garbrecht et al., 2009). For example, Fontes et al. (1991) investigated bacterial transport in homogeneous and heterogeneous sand soil columns (14 cm length). For heterogeneous columns, the preferential path was created by inserting a glass pipe in the center of the column, packing the column with fine sand, filling the glass pipe with coarse sand, and finally removing the pipe. A double peak was observed in the breakthrough curves as a result of flow velocity differences between the preferential flow path and fine sand. They found that the grain size was the most important variable controlling bacterial transport followed by ionic strength and cell size. On the other hand, Jiang et al. (2007), using homogeneous sand columns, concluded that the length of a column (14 cm length) has no effect on the *E. coli* peak concentration. They found that bacteria were mainly retained in the top 10 cm of soil and that grain size had a significant effect on the bacterial transport and retention.

A significant component of pathogen movement to streams commonly identified but not explicitly considered is pathogen movement to subsurface tile drainage systems (Dorner et al., 2006). However, few, if any, studies to date have investigated soil bacteria transport in relation to biopores located in the vicinity of subsurface drains. Figure 1 represents a vertical soil cross-section and conceptual diagram of potential biopore interconnectivity with a subsurface drain. Open-surface connected (OSC), buried surface disconnected (BSD), and buried disconnected biopores are typically found in the vadose zone between the soil surface and the subsurface drain (Akay et al., 2008).

Shipitalo and Gibbs (2000) investigated biopores directly connected to artificial drainage systems by a deep burrowing *Anecic* earthworm species. The interconnectivity was demonstrated in the field using a smoke test, filling the earthworm's channels with resin, and by measuring the biopore flow using infiltrometers. They later excavated the soil to expose the earthworm channel. Akay and Fox (2007) and Akay et al. (2008) investigated the importance of biopores and drainage system interconnectivity in the movement of water using a soil column (28 cm by 50 cm cross-section and 95 cm long) by placing artificial biopores both directly above and shifted away from the drainage pipe without unpacking or disturbing the soil column between experiments. They found that OSC macropores were a highly efficient preferential flow path reducing the breakthrough times to the subsurface drainage outlet as a function of the macropore depth penetration. Simulated BSD macropores diverted as much as 40% of the matrix flow when directly connected to the subsurface drains and after buildup of soil pore-water pressure. Other studies have pointed out the importance of macropore and artificial drainage interconnectivity in the transport of nutrients and pesticides (Villholth et al., 1998; Fox et al., 2004, 2007).

The objective of this research was to investigate the significance of OSC and BSD biopores on *E. coli* transport to subsurface drainage systems. Laboratory experiments of *E. coli* transport through OSC and BSD biopores were performed using the soil column developed by Akay and Fox (2007) with two soils containing different soil organic matter contents and hydraulic conductivities.

Materials and Methods

Transport of *E. coli* in soil was measured using a soil column (28 by 50 by 95 cm) developed by Akay and Fox (2007). Two types of soil were used in the experiments: Dougherty loamy sand (LS; loamy, mixed, active, thermic Arenic Haplustalfs) and Floyd sandy loam (SL; fine-loamy, mixed, superactive,

Table 1. Properties of the loamy sand (LS) and sandy loam (SL) soils used in the soil column experiments.

Soil type	SP†	Bulk density	Specific gravity	Sand	Silt	Clay	SOM‡	θ_s§	θ_r¶	K_s#	n††	α††
		Mg m^{-3}		——————%——————			g kg^{-1}	———cm^3 cm^{-3}———		m s^{-1}		pF^{-1}
LS	–	1.6	2.67	84.5	13.4	2.1	3	0.40	0.01	1.20 x 10^{-5}	3.20	0.40
SL	WG	1.4	2.30	63.6	32.3	4.1	39	0.39	0.21	–	3.59	0.87
SL	DG	1.4	2.30	63.6	32.3	4.1	39	0.39	0.26	1.94 x 10^{-6}	4.73	0.89

† SP, soil preparation technique (WG: wet grinding; DG: dry grinding).

‡ SOM, soil organic matter content measured from total carbon (TruSpec Carbon and Nitrogen Analyzer, LECO Corp., St. Joseph, MI) using a 1.724 ratio.

§ θ_s, saturated volumetric water content.

¶ θ_r, residual volumetric water content.

K_s, saturated hydraulic conductivity measured by falling head permeameter test.

†† n, α, van Genuchten model parameters, where pF is defined as *–log(h)* and *h* is the pore water pressure in cm.

Table 2. Main experimental variables for the open-surface connected (OSC) and buried surface disconnected (BSD) biopore soil column experiments with loamy sand (LS) and sandy loam (SL) soils. The SL soil was packed using either a wet grinding (WG) or dry grinding (DG) technique.

	Type	Soil	Soil column dimensions			BD‡	Soil preparation	*E. coli* C_o§	*E. coli* recovery	BP¶
			L†	a†	b†					
				cm		Mg m^{-3}		MPN# 100 mL^{-1}		BTT††
1	OSC	LS	65	10	0	1.6		11,517	+	NA‡‡
2	OSC	LS	55	20	0	1.6		15,362	+	NA
3§§	BSD	LS	55	0	17.5	1.6		7140	+	Yes
4§§	BSD	LS	20	0	52.5	1.6		4130	+	Yes
5	OSC	SL	55	20	0	1.4	WG	8355	+	NA
6¶¶	BSD	SL	55	0	17.5	1.4	WG	5771	+	Yes
7¶¶	BSD	SL	20	0	52.5	1.4	DG	15,000	–	No
8¶¶	BSD	SL	55	0	17.5	1.4	DG	16,780	+	Yes

† Dimensions: a = soil layer thickness between the center of the drainage pipe to the bottom of the biopores; b: soil layer thickness between the surface and the top of the biopores; L: length of the biopores; see Fig. 1 for more details.

‡ BD = Bulk density.

§ C_o = Initial *E. coli* concentration in the liquid swine manure.

¶ BP = Biopore.

MPN = most probable number.

†† BTT: Breakthrough time.

‡‡ NA = No measurement directly from the biopores because of experimental setup.

§§ Rhodamine WT, 50 μg L^{-1} added in the final water flush.

¶¶ $CaCl_2$, 0.01 *M* concentration added in the initial water flush; 1 g of Peptone per L of diluted liquid swine manure was added in the manure flush.

mesic aquic Pachic Hapludolls), selected due to the contrasting particle size distribution, soil organic matter content, and saturated hydraulic conductivity (Table 1). Soil organic matter content was estimated from total carbon (TruSpec Carbon and Nitrogen Analyzer, LECO Corp., St. Joseph, MI) using a 1.724 ratio. Saturated hydraulic conductivity was measured using a falling head permeameter (Amoozegar and Wilson, 1999).

Eight experiments were conducted: four with LS and four with SL (Table 2). During the experiments, soil pressure potential was measured at 12 different points at three different depths (20, 50, and 80 cm from the bottom of the column) using pencil-size tensiometers, connected to pressure transducers and a data logger (CR10X, Campbell Scientific, Logan, UT), similar to Akay and Fox (2007).

An artificial biopore built by rolling a metallic mesh around a 6-mm diam. wooden dowel and covered with a plastic mesh was used to simulate OSC biopores with lengths of 55 and 65 cm and BSD biopores with lengths of 20 and 55 cm. All biopores were placed directly above the drain in the center of the soil column (Fig. 1 and 2). A 5-cm diam. perforated tube was placed 6 cm (center of the pipe) from the bottom of the soil column to simulate a zero-pressure head boundary condition, assumed to represent an artificial drain. A 1-cm constant head on top of the column was maintained using a Mariotte infiltrometer. Inflow at the top of the soil column and the outflow from the drain and the biopore were measured every 10 s using weighing scales (Fig. 2).

Each experiment consisted of four stages: (i) packing, (ii) an initial water flush (186–257 mm), (iii) a manure flush (107 mm), and (iv) a final water flush (107 mm). The LS and SL soils were packed at 1.6 and 1.4 Mg m^{-3} bulk density, respectively, for a total length of 85 cm. The LS soil was replaced with new soil after each experiment. For this same LS soil and bulk density, Chu-Agor et al. (2008) reported parameters for the soil moisture (i.e., hydraulic) characteristic curve, derived using the pressure plate extractor method on multiple samples as described by Dane and Hopmans (2002). The SL soil from the Northeast Iowa State University research farm in Nashua was unpacked and reused. The SL was prepared before packing using two processes: (i) moist soil (WG, moisture content 10–20%) forced to pass a 4-mm sieve opening, and (ii) air dried soil, manually crushed by hammering, sieved using a no. 7 sieve, and then moistened to attain a moisture content of <10% before packing. It was hypothesized that the WG pack-

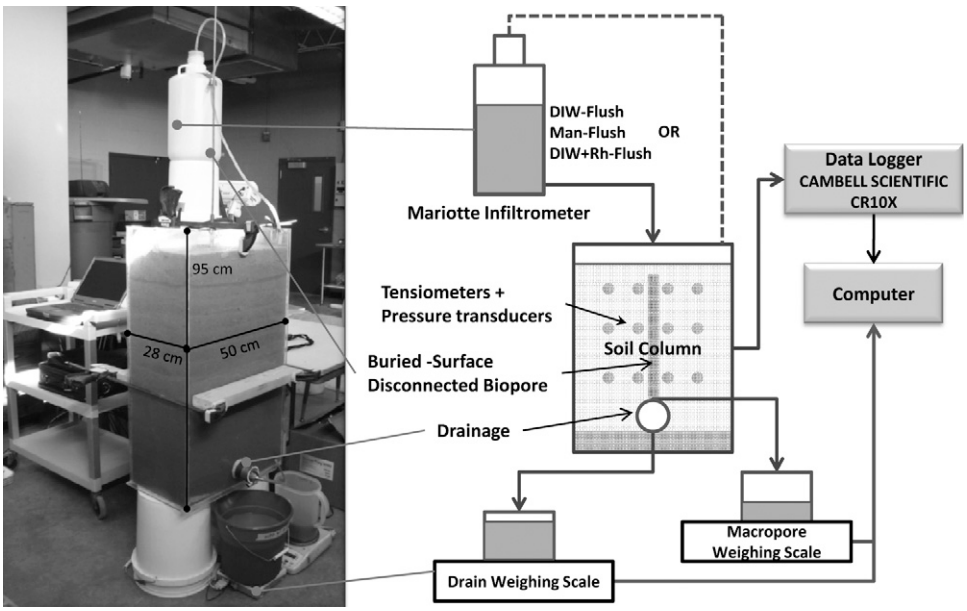

Fig. 2. Descriptive setup of the soil column and instruments used in all experiments.

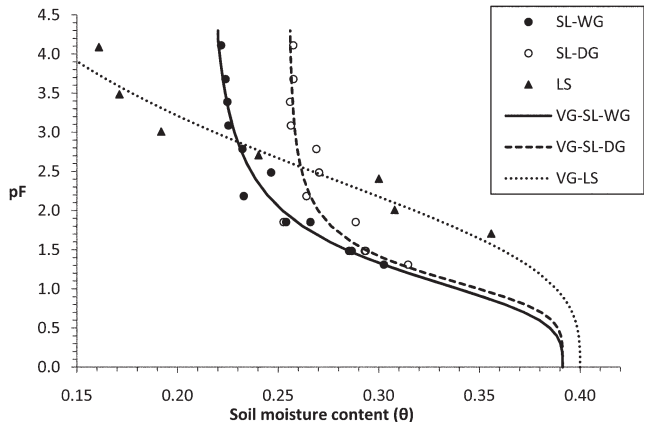

Fig. 3. Hydraulic (i.e., soil moisture) characteristic curve for the loamy sand (LS) and sandy loam (SL) soil and its two preparation techniques (WG, wet soil; DG, dry soil) and fit to the van Genuchten (1980) model (VG). Data represent the means for six cores extracted from two SL columns. Data for the LS are reported by Chu-Agor et al. (2008) for the same bulk density as used in these experiments.

ing resulted in a greater distribution of soil pore spaces compared to the DG packing due to the differences in soil structure.

During the unpacking of two experiments (i.e., SL-WG and SL-DG, BSD, 55-cm), six soil core samples from each soil preparation were taken at different depths to define the hydraulic characteristic curve. Data from the pressure cell using the 12 SL core samples (WG and DG soil preparation techniques) were grouped and averaged according to the different pressures applied. The final data were plotted and fit with the van Genuchten (1980) model. The fit model was used to compare the LS vs. SL soils and DG vs. WG packing techniques in terms of their soil moisture characteristic curves and to explain activation of the artificial biopore.

After packing and instrument setup, the initial water flush followed immediately. The manure flush and the final flush followed 48 and 96 h later, respectively. During the initial flush, only matrix flow was allowed by clogging the biopore with a wooden dowel. The initial flush in all experiments provided a hydrostatic, initial moisture content profile, allowed initial consolidation of the soil, established the saturated zone, and verified that there was no initial biopore flow. Water samples were taken in the drain after the initial flush to verify that *E. coli* was not present. Before the manure flush, the wooden dowel was pulled out to the desired biopore length. Diluted liquid swine manure was applied at the top of the soil column with different concentrations (Table 2). The liquid swine manure used in all experiments was collected in a 5-d composite sample from the Swine Research and Educational Center at Oklahoma State University and stored at 4°C.

Before each experiment, a manure dilution using distilled water was prepared to assure a proper *E. coli* concentration to minimize dilution during sample analysis and to mimic the effect of rainfall or irrigation in the field. During the manure flush in the SL experiments, 1 g of peptone (Special Peptone L0072, Oxoid, Lenexa, KS) per L of diluted liquid manure was used to reduce bacteria die-off in the Mariotte bottle due to the long infiltration time. The peptone concentration used (1 g per L) is typically recommended to provide or equilibrate a media at a steady bacteria concentration (e.g., growth equivalent to die-off). *Escherichia coli* samples were collected periodically from the Mariotte bottle and water at the top of the soil column and demonstrated that *E. coli* concentrations remained approximately stable during the experiments. Preliminary experiments indicated that the *E. coli* from diluted liquid swine manure required 8 to 10 h (e.g., lag time) to begin using nutrients from sources other than the manure media.

Distilled as opposed to tap water was used in the initial flush to avoid chlorine residuals in the soil solution that might affect the bacteria population and transport. However, the use of distilled water most likely decreased the bulk soil solution ionic strength as water infiltrated due to the ionic interchange between the soil

minerals and the displaced wetting front. This decrease in solution ionic strength may have promoted soil dispersion and progressive clogging of pore spaces as a result of the soil mineral double layer expanding. During preliminary experiments, soil dispersion and clogging was observed when the SL soil was used. To minimize soil dispersion in the SL soil, $CaCl_2$ (0.01 mol L^{-1} concentration) was added during the initial flush. The authors considered that the addition of $CaCl_2$ at this concentration allowed equilibrating the soil solution ionic strength to avoid soil dispersion and clogging and only marginally affected the fate and transport results.

Distilled water was used for the final flush of experiments with LS soil while tap water was used for SL soil. The use of tap water for SL was to further minimize soil dispersion and clogging of the soil column. In the LS soil, dispersion was not a problem. Rhodamine WT (50 μg L^{-1} concentration) was used in some experiments during the final flush to investigate flow conditions and verify no additional preferential flow along the soil column walls. Rhodamine WT is considered a conservative tracer and therefore minimally adsorbed to soil particles during the soil column experiments. Rhodamine WT was not expected to be used by the bacteria in significant quantities to affect fate and transport in the time frame of the experiments. The final flush in all cases acted as a replicated experiment of the hydrologic response of the system.

Water samples and flow rates from the drain and the macropore were collected after each flushing for *E. coli* and total Coliform quantification. *Escherichia coli* was used as an indicator organism of fecal contamination and total Coliform for environmental bacterial activity. The semi-automated Quanti-Tray Method (IDEXX, Westbrook, ME), which provides counts from 0.0 to 2419.6 per 100 mL, was used to quantify the *E. coli* and total Coliform concentration by the most probable number (MPN) technique. Initial *E. coli* and total Coliform concentration were estimated as the average concentration between the beginning and end of the manure flush taken from the Mariotte bottle. Discharge and *E. coli* breakthrough time were defined as the point in time in which the constant cumulative discharge gradient changed and *E. coli* was continuously detected, respectively. After each experiment, the soil column was unpacked, disinfected and packed again with soil.

Results and Discussion

Soil Property Characterization

A uniform soil pore space distribution was observed after packing the LS soil. Packing the SL soil to the designated bulk density generated an approximately equivalent average pore size between the DG and WG processes. However, the distribution of the pore spaces around this average (i.e., more homogeneous soil pore sizes in the DG and more widely varying in the WG) was hypothesized to be different and qualitatively observed during the experiments. During the SL soil preparation using the WG process, formation of soil aggregates were observed after forcing the soil to pass the sieve mesh. Soil macropore formation within the soil aggregates was observed to be distributed irregularly along the soil column. On the other hand, the DG process resulted in a uniform pore size distribution with no soil aggregate development and no observable large pore spaces.

After the manure flush, some small cracks formed at the surface during the soil column depletion period. These cracks were also observed during the pressure cell experiments in most of the DG samples. Data from the pressure cell and van Genuchten (1980) model confirmed the previous observation (Table 1 and Fig. 3). Parameters of the model indicated a difference in the macropore activation pressure for the two SL soil preparations primarily in the air-entry pressure value. Estimates from the model based on six replicated samples implied an air-entry pressure difference in the range of 0.3 to 2.3 cm higher in the DG as well as the development of higher water suction values as moisture content decreased (Fig. 3).

Water and Manure Suspension Flow

For a specific soil type and packing, discharge breakthrough time was inversely proportional to the biopore length in the OSC and BSD experiments. The only exception was for the LS, OSC, 55-cm experiment during the final flush, probably a result of additional preferential flow between the soil and the soil column walls. In general, the breakthrough time in the biopore occurred later than in the drain with the time difference decreasing as a function of the biopore length (Table 3). During the manure flush, the discharge breakthrough time for the SL, OSC, 55-cm experiment (i.e., WG soil preparation) was detected earlier than expected, especially in comparison with the LS OSC experiments. This early discharge breakthrough time was probably the result of the soil macropore formation around the soil aggregates.

Reduction in the cumulative matrix flow, measured 24 h after flush initialization, was observed between the manure and final flush (Table 4). This phenomenon was a function of the biopore length and soil type, with a stronger effect in the SL soil. It was hypothesized that soil dispersion was the result of distilled water utilization that might change the soil solution ionic strength and promote soil dispersion followed by clogging. Therefore, $CaCl_2$ (0.01mol L^{-1}) was used during the initial water flush to equilibrate the soil solution ionic strength. However, results indicate that the reduction in flow discharge was a complex combination of soil swelling and dispersion, soil minerals-organic matter aggregation, and bacteria straining. In the SL soil, the larger proportion of small pore spaces and the higher soil organic matter and clay content was hypothesized to promote sorption of colloids, straining of fine particles in suspension, and straining of bacteria, all of which favored the clogging process.

In the biopore flow, a reduction in the cumulative discharge after 24 h for the SL soil and an increasing discharge for the LS soil were observed (Table 4). In the SL soil, this was hypothesized to be the result of pore space clogging as previously discussed. In the LS soil, the biopore discharge increase can be explained by expansion of the biopore effective radius due to internal erosion along the biopore wall (observed qualitatively based on turbidity in the outflow water) and/or the reduction in the soil matrix suction around the biopore after the manure flush (Table 3). In general, during the unpacking of the soil column, it was observed that the soil in contact with the biopore wall was saturated in most of its length 48 h after final flush initialization. The occurrence of this condition after the manure flush in com-

Table 3. Drainage (Drain) and biopore (BP) breakthrough time (minutes) after manure flush and final water flush for open-surface connected (OSC) and buried surface disconnected (BSD) biopores in loamy sand (LS) and sandy loam (SL) soils. The SL soil was packed using either a wet grinding (WG) or dry grinding (DG) technique.

Experiment			Manure-Flush				Final-Flush			
			Discharge		*E. Coli*		Discharge		*E. Coli*	
	Type-length	Soil type	Drain	BP	Drain	BP	Drain	BP	Drain	BP
	cm									
1	OSC-65	LS	3.0	NA†	17.5	NA	3.3	NA	4.5	NA
2	OSC-55	LS	7.7	NA	18	NA	2.7	NA	3.0	NA
3	BSD-55	LS	24.5	25.9	37.0‡	26.1	24.7	25.1	25.5	25.4
4	BSD-20	LS	34.7	47.4	70.0§	50	33.8	52.1	106¶	53.8
5	OSC-55	SL-WG	1.2	NA	60	NA	≈ 4.0	NA	4.0	NA
6	BSD-55	SL-WG	11.5	≈ 132	170.0#	132	234.5	No Flow	No BTT††	No Flow
7	BSD-20	SL-DG	≈ 30	No Flow	No BTT	No Flow	NA	No Flow	No BTT	No Flow
8	BSD-55	SL-DG	6.0	60.2	No BTT	No BTT	13.0	20.2	No BTT	No BTT

† NA = no measurement directly from the biopore because of experimental setup.

‡ *Escherichia coli* was initially detected at 25.4 min followed by no continuous detection until 37 min.

§ *Escherichia coli* was initially detected at 57 min followed by no continuous detection until 70 min.

¶ *Escherichia coli* was initially detected at 9 min followed by no continuous detection until 106 min.

Escherichia coli was not detected continuously; data in the table correspond to the first detection.

†† BTT = Breakthrough time.

Table 4. Cumulative matrix (Drain) and biopore (BP) discharge in the open-surface connected (OSC) and buried surface disconnected (BSD) biopore experiments with loamy sand (LS) and sandy loam (SL) soils. The SL soil was packed using either a wet grinding (WG) or dry grinding (DG) technique. Cumulative discharge measured 24 h after flush initialization.

							Drain		BP			
	Type	Soil type	Flush	Flush	Drain	BP	Percent of flush	Percent change	Percent of flush	Percent change	Percent of drain	% Change
				mL			%					
1	OSC-65	LS	Manure	15,000	14,601	NA†	97.3	−8.6	NA	NA	NA	NA
			Final flush	15,000	13,311	NA	88.7		NA		NA	
2	OSC-55	LS	Manure	15,000	14,962	NA	99.7	−9.7	NA	NA	NA	NA
			Final flush	15,000	13,500	NA	90.0		NA		NA	
3	BSD-55	LS	Manure	15,000	10,688	3387	71.3	−11.7	22.6	1.8	31.7	9.3
			Final flush	15,000	8930	3659	59.5		24.4		41.0	
4	BSD-20	LS	Manure	15,000	12,567	934	83.8	−13.1	6.2	2.2	7.4	4.5
			Final flush	15,000	10,600	1261	70.7		8.4		11.9	
5	OSC-55	SL-WG	Manure	15,000	11,623	NA	77.5	DL‡	NA	NA	NA	NA
			Final flush	15,000	DL	NA	DL		NA		NA	
6	BSD-55	SL-WG	Manure	15,000	6435	1235	42.9	−36.0	8.2	−8.2	19.2	−19.2
			Final flush	15,000	1028	0	6.9		0.0		0.0	
7	BSD-20	SL-DG	Manure	15,000	10,000	0	66.7	−58.2	0.0	0.0	0.0	0.0
			Final flush	15,000	1264	0	8.4		0.0		0.0	
8	BSD-55	SL-DG	Manure	15,000	10,506	564	70.0	−25.5	3.8	−3.0	5.4	−3.7
			Final flush	15,000	6676	110	44.5		0.7		1.6	

† NA = no measurement directly from the biopore because of experimental setup.

‡ DL = No data available because data were lost due to datalogger failure.

bination with a lower air-entry pressure (higher pF value) in the LS favored macropore discharge during the final flush.

In all BSD experiments, discharge from the biopore and into the macropore weighing scale occurred suddenly. This condition was studied by Akay and Fox (2007) and occurred at the moment in which the matrix suction decreased along the biopore as the water pressure increased near the drain. Comparison between the SL-DG and SL-WG BSD, 55-cm experiments indicated the importance of pore size in the biopore activation due to changes in the soil structure. The SL-DG contained uniform pore spaces and maintained higher pore water suction than SL-WG around the soil in contact with the biopore wall after depletion. This condition reduced the capacity of water to move into the biopore while at the same time moved water from the walls of the biopore into the soil matrix as the wetting front progressed downward. These effects are also applicable when comparing total biopore discharge in the LS and SL soils (Table 3).

Escherichia coli Transport: Soil Type and Packing

Water samples taken during the initial flush indicated no initial *E. coli* concentration capable of desorbing into the matrix flow. However, total Coliform was always detected in the drain at concentrations greater than 500 MPN/100 mL. During the manure flush, with the exception of the SL-DG experiments where no activation of the biopore occurred, *E. coli* and discharge breakthrough time indicated that *E. coli* moved

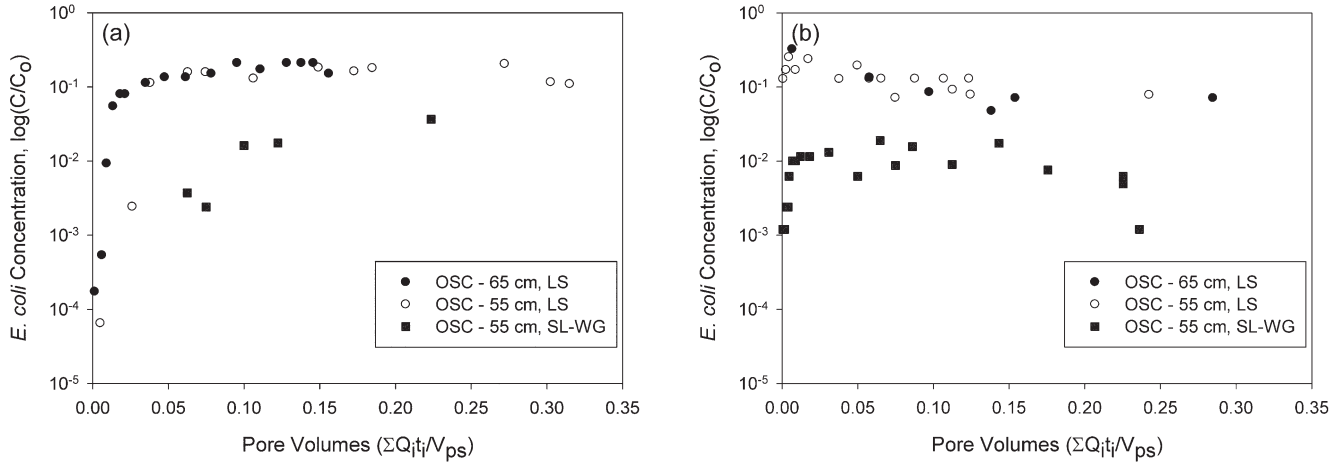

Fig. 4. *Escherichia coli* concentrations from the drain (C) relative to the inflow *E. coli* concentration (C_o) for open-surface connected (OSC) biopore experiments (i.e., 55 and 65 cm biopores lengths) as a function of the number of pore volumes (i.e., product of flow rate, Q, and time, t, divided by the volume of pore space, V_{ps}, of the soil column) of inflow water (a) during manure flush and (b) during the final water application. Experiments correspond to numbers 1, 2, and 5 in Tables 2 to 5.

slower than water in the soil matrix, as represented by the drain discharge in Table 3. On the other hand, in the biopore, *E. coli* was always detected at approximately the same time as the discharge (Table 3). The difference between the discharge and *E. coli* breakthrough time in the biopore and matrix was hypothesized to be the result of *E. coli* resistance to be transported through pore spaces by using a combination of motile capabilities and adhesion (Hill et al., 2007). In the soil solution, the sorptive properties of the soil, primarily determined by fine particles (i.e., clay and silt) and organic matter content, established the *E. coli* concentration available for transport. Then, *E. coli* in planktonic forms in the soil solution, attached to colloids in suspension, or weakly attached could be transported as water moved through soil pore spaces.

The presence of a larger proportion of small pore spaces (e.g., micropores and small mesopores) in the soil promoted straining (physical and biological) and induced large hydraulic energy losses. Energy losses were translated as a reduction in the pore space velocity distribution and allowed *E. coli* to resist transport (adhesion and/or auto-propulsion) in the direction of flow. In cases where the soil contained a larger proportion of soil macropores, flow and shear stress forces can more effectively mobilize available bacteria in solution, bacteria that are weakly attached, or colloids and aggregates previously colonized by bacteria. In fact, Smith et al. (1985) made similar observations in reporting that the transport of bacteria through sieved or mixed soil columns was negligible when compared to more structured soils.

Escherichia coli Transport: Open-Surface Connected versus Buried Surface Disconnected Biopores

In the OSC experiments, *E. coli* transport to the drain was mainly a function of the soil layer thickness between the end of the biopore and the drain and the *E. coli* concentration along the biopore wall (Fig. 1 and Table 2). The latter was a function of the energy head in the biopore. The OSC biopores allowed rapid *E. coli* transport from the surface to deeper soil layers at the end of the biopore, followed by slower wetting front movement through the remaining soil profile (i.e., 10 or 20 cm depending of the biopore length) before reaching the drainage pipe. These conditions were verified by the soil tensiometer data. This 10 to 20 cm soil buffer layer in the LS was not important in the *E. coli* breakthrough time. However, changing the soil texture as indicated in the SL experiment (WG, OSC, 55-cm) resulted in an extended *E. coli* breakthrough time. Soil type and organic matter content impacted the straining (physical and biological) and sorption mechanisms as well as allowed *E. coli* to resist being transported when soil pore water shear forces were low (Table 3 and Fig. 4).

Transport of *E. coli* in BSD biopores was subjected to two different processes: the soil-biopore interaction as the wetting front moved downward and the soil water suction relaxation along the biopore wall that allowed water movement into the biopore. In the first case, small water fluxes from the soil solution moved into the biopore as the wetting front moved downward, with a possibility of *E. coli* transport if soil pore space allowed bacteria movement. As water moving into the biopore wall entered in contact with soil containing higher pore water suction, water moved back into the soil matrix. *E. coli* stayed in the soil solution, attached to the soil surrounding the biopore, or moved back into the biopore when the wetting front from matrix flow reached the surrounding soil. In the second case, *E. coli* was transported from the soil solution or detached by stress forces when biopore fluxes created shear forces that exceeded *E. coli* attachment forces. These conditions can explain the large *E. coli* recovery concentration at the beginning of the biopore discharge in Fig. 5.

During the final flush for most experiments, *E. coli* and discharge breakthrough time in the drain and biopore occurred at approximately the same time (Table 3). This demonstrated that *E. coli* was previously established in the soil solution or weakly attached when water fluxes started moving into the biopore. The manure flush provided the initial *E. coli* concentration at different depths through soil matrix and/or biopore domains, followed by an *E. coli* regrowth period. During the final flush, *E. coli*

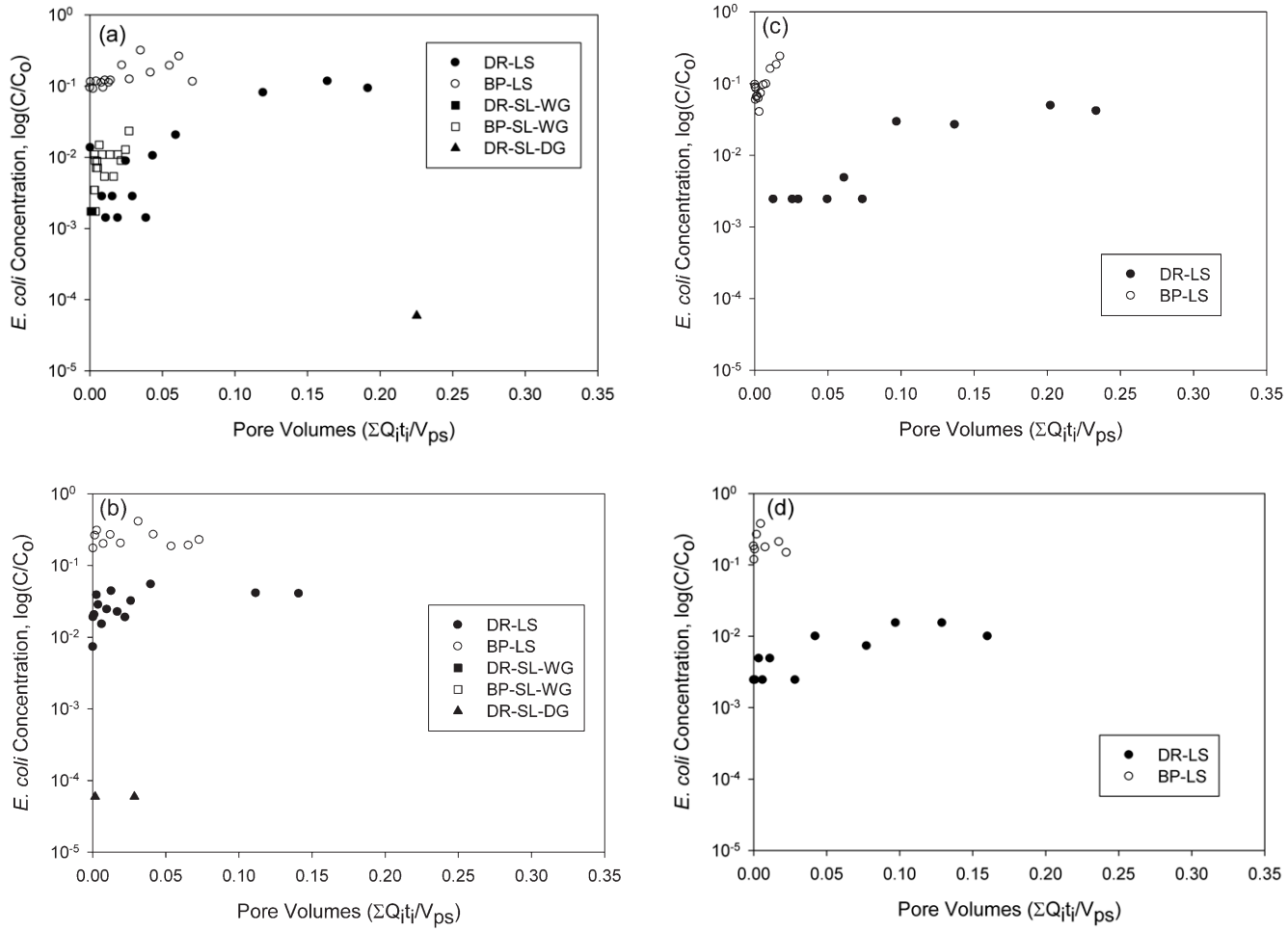

Fig. 5. *Escherichia coli* concentrations from the drain (C) relative to the inflow *E. coli* concentration (C_o) for buried surface disconnected (BSD) biopore experiments (i.e., 55 and 20 cm macropore lengths) as a function of the number of pore volumes (i.e., product of flow rate, Q, and time, t, divided by the volume of pore space, V_{ps}, of the soil column) of inflow water. Figures (a) and (b) are during the manure flush and final water application for the 55-cm BSD biopores, respectively (i.e., Exp. 3, 6, and 8 in Tables 2–5). Figures (c) and (d) are during the manure flush and final water application for 20-cm BSD biopores, respectively (i.e., Exp. 4 in Tables 2–5 with Exp. 7 having no observed *E. coli* from the matrix or biopore). DR = drain flow, BP = biopore flow, LS = loamy sand, SL = sandy loam, WG = wet soil preparation and DG = dried soil preparation.

were flushed out from the soil closest to the drain and/or biopore wall, providing the initial *E. coli* concentration observed in the breakthrough curve. The *E. coli* concentrated then peaked and decreased over time in most cases (Fig. 4b, 5c-d). These results have implications in terms of the impact of pathogenic bacteria transport to subsurface drainage from storm events beyond those immediately following manure applications. The OSC and BSD biopores may provide a mechanism for transporting bacteria to more favorable survival and growth conditions and therefore may contribute *E. coli* in subsequent drain flow events.

For all LS experiments, *E. coli* was always detected in the drain flow for the OSC and BSD biopores with the highest recovery concentrations during the final flush (Table 5). Note than in the case of OSC biopore experiments, *E. coli* recovery and discharge cannot be measured at the biopore due to the experimental setup. The LS soil possessed larger pore spaces and less sorptive properties than the SL soil. Therefore, *E. coli* moved easily through the soil macropores and eventually through mesopores during the manure and final flush. During the final flush, *E. coli* regrowth and lower straining conditions explained the highest concentration recovery in Table 5. On the other hand, in the SL soil, the highest *E. coli* recovery occurred during the manure flush indicating the importance of transport restriction due to smaller pore spaces that can easily promote straining. This hypothesis was also associated with the observed flow reduction between the manure and final flush described previously.

Summary and Conclusions

Results from this study indicated the efficiency of *E. coli* transport to drainage systems under the presence of interconnected open-surface or buried biopores. Soil macropores and large mesopores play an important role in allowing the movement of pathogens to deeper soils after irrigation or rainfall events. Soils with small soil pore spaces (e.g., micropore and small mesopores) and sorptive properties can filter *E. coli* in most of the cases due to the development of physical and biological straining as well as adsorption. Additionally, low velocities in the soil pore spaces may allow *E. coli* to resist being transported by its auto-propulsion and adhesion capabilities.

Table 5. Macropore length, *Escherichia coli* initial concentration (C_o), and maximum *E. coli* recovery from the matrix and macropore flow for the open-surface connected (OSC) and buried surface disconnected (BSD) biopore experiments with loamy sand (LS) and sandy loam (SL) soils. The SL soil was packed using either a wet grinding (WG) or dry grinding (DG) technique.

					Matrix flow		Macropore flow	
	Type	Soil type	Macropore	*E. coli* C_o	Man-Flush	Final-Flush	Man-Flush	Final-Flush
			cm	MPN 100 mL^{-1}				
1	OSC	LS	65	11,500	0.21Co†	0.32Co	NA‡	NA
2	OSC	LS	55	15,400	0.20Co	0.25Co	NA	NA
3	BSD	LS	55	7,140	0.12Co	0.05Co	0.32Co	0.41Co
4	BSD	LS	20	4,130	0.05Co	0.01Co	0.24Co	0.37Co
5	OSC	SL-WG	55	8,355	0.04Co	0.02Co	NA	NA
6	BSD	SL-WG	55	5,771	0.002Co	0	0.023Co	No Flow
7	BSD	SL-DG	20	15,000	0	0	No Flow	No Flow
8	BSD	SL-DG	55	16,780	$6\times10^{-5}C_o$	$6\times10^{-5}C_o$	0	0

† *Escherichia coli* exceeded the upper limit of the test procedure (i.e., 2419.6 MPN 100 mL^{-1}) for two samples.

‡ NA = not measured because of experimental setup (no measurement directly from the biopores).

On the other hand, soils with large pore spaces such as large mesopores and soil macropores favored *E. coli* transport. The development of shear forces under these conditions may promote transport and detachment of bacteria or colloids colonized by bacteria during wetting front displacement.

Biopores that are directly connected to subsurface drainage systems can provide a direct conduit for *E. coli* transport from the soil surface into tile drainage that may impact drain flow *E. coli* concentrations in immediate and subsequent storm or irrigation events following manure applications. In these experiments, it was clear that biopore activation occurred later than discharge in the drain. In the biopore, *E. coli* and flow were simultaneously detected. In the open-surface connected biopore, *E. coli* transport to the drain was mainly a function of the soil type and/or soil layer thickness between the end of the macropore and the drain. In the LS soil, the thickness of the layer was not important in regard to the *E. coli* peak concentration in the drain; however, in the SL soil, *E. coli* transport to the drain was clearly limited by the soil properties. This study indicated that sorption of *E. coli* to soil determined residual *E. coli* concentrations in the soil solution or attached to soil particles and colloids in solution after manure application. For these reasons, adsorption and adhesion mechanisms of pathogenic bacteria should be further investigated. Buried surface disconnected biopores can be an effective *E. coli* pathway through soils when they are in contact with other soil macropores or large mesopores. However, under the presence of small and homogeneous soil pore spaces, the soil filter capacity will limit the transport of *E. coli* to the biopore and then to the interconnected drainage system.

Acknowledgments

The authors acknowledge the financial assistance of a 2007-2010 United States Department of Agriculture (USDA), Cooperative State Research, Extension, and Education Service (CSREES) National Research Initiative Grant, Award no. 2007-35102-18242. The authors acknowledge Amanda K. Fox, Stillwater, OK, for reviewing an earlier version of this manuscript.

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