



# Movement of *Cryptosporidium parvum* Oocysts through Soils without Preferential Pathways: Exploratory Test

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Groundwater contamination by oocysts of the waterborne pathogen *Cryptosporidium parvum* is a significant cause of animal and human disease worldwide. Although research has been undertaken in the past to determine how specific physical and chemical properties of soils affect the risk of groundwater contamination by *C. parvum*, there is as yet no clear conclusion concerning the range of mobility of *C. parvum* that one should expect in field soils. In this context, the key objective of this research was to determine the magnitude of *C. parvum* transport in a number of soils, under conditions in which fast and preferential transport has been successfully prevented. *C. parvum* oocysts were applied at the surface of different soils and subjected to artificial rainfall. Apparently for the first time, quantitative PCR was used to detect and enumerate oocysts in the soil columns and in the leachates. The transport of oocysts by infiltrating water, and the considerable retention of oocysts in soil was demonstrated for all soils, although differences in the degree of transport were observed with soils of different types. More oocysts were found in leachates from sandy loam soils than in leachates from loamy sand soils and the retention of oocysts in different soils did not significantly differ. The interaction of various processes of the hydrologic system and biogeochemical mechanisms contributed to the transport of oocysts through the soil matrix. Results suggest that the interplay of clay, organic matter, and Ca<sup>2+</sup> facilitates and mediates the transfer of organic matter from mineral surfaces to oocysts surface, resulting in the enhanced breakthrough of oocysts through matrices of sandy loam soils compared to those of loamy sand soils. Although the number of oocysts that penetrate the soil matrix account for only a small percentage of initial inputs, they still pose a significant threat to human health, especially in groundwater systems with a water table not too distant from the soil surface. The results of the research demonstrate a critical need for the simultaneous study of the interaction of various processes affecting oocysts transport in the subsurface, and for its expansion into complex systems, in order to obtain a coherent picture of the behavior of *C. parvum* oocysts in soils.

**Keywords:** *Cryptosporidium*, microorganisms, groundwater, soil transport, qPCR

## INTRODUCTION

The presence of pathogenic bacteria, viruses, and protozoa in drinking water is a significant cause of animal and human disease in many parts of the world. One of these pathogens, the zoonotic protozoan *Cryptosporidium parvum*, causes cryptosporidiosis, a common gastrointestinal disease associated with severe gastroenteritis and diarrhea (Smith, 1992). Outside of the lower intestines of humans and domestic or wild animals, where *C. parvum* carries out the active part of its cycle, it is present in the environment in the form of 4- to 6- $\mu\text{m}$ -long ovoid-shaped oocysts, with a double wall that is resistant to most oxidation processes (e.g., ozonation and chlorination) typically used for water treatment (Korich et al., 1990). Although the median infective dose of *C. parvum* oocysts for humans is reported at 30 oocysts (Dupont et al., 1995), evidence suggests that as few as ten may be enough to cause infection in humans (Smith, 1992).

During the past three decades, the presence of *C. parvum* in surface waters and groundwaters in the United States and Great Britain (Galbraith et al., 1987; LeChevallier et al., 1991; Rose et al., 1991) has been connected with several major outbreaks of Cryptosporidiosis (Hayes et al., 1989; Mackenzie et al., 1994; Smith and Rose, 1998). The presence of *C. parvum* oocysts in drinking water supplies results from a number of processes. For example, infected hosts, such as cows or deer may defecate in streams and shallow ponds, and their feces may end up on the soil via direct release or land spreading of manure. The land application of municipal or industrial wastewater sludge may also contribute significant numbers of oocysts at the soil surface. Here, runoff may carry oocysts to nearby waterbodies, or rain infiltration may transport oocysts in the subsoil to groundwater. Among the different pathways for transporting oocysts, rain infiltration was generally considered until a few years ago to be of little significance, in line with the common assumption that soils are effective at filtering a wide range of pathogens (Tim et al., 1988). Studies of packed columns with saturated flow by Brush et al. (1999) and Harter et al. (2000) and undisturbed columns with unsaturated flow (Mawdsley et al., 1996a), however, suggested that *C. parvum* oocysts could be transported rapidly downward through the soil. Detailed experimental observations have since confirmed this to be the case, especially through soil macropores and in karstic geological terrain through fractured bedrocks (Boyer and Kuczynska, 2003; Darnault et al., 2003, 2004; Kuczynska et al., 2003; Boyer et al., 2009; Ramirez et al., 2009; Petersen et al., 2012; McLaughlin et al., 2013). In particular, Darnault et al. (2003, 2004) demonstrated that preferential flow in the form of fingered flow and macropores flow in unsaturated soils allowed the fast transport of *C. parvum* oocysts, leading to breakthrough concentrations above infection doses.

Based on experimental evidence, it seems well established that *C. parvum* oocysts can move in soils through preferential pathways in relatively large amounts. It is also possible however that in many situations, typical rainfall events are of insufficient intensity for macropores to conduct much water or for fingers to develop to transport *C. parvum*. Such migration then occurs

through the soil matrix, where oocysts are subjected closely to a number of physico-chemical processes, many of them similar to those that have been documented extensively for solutes. Some of the key mechanisms involved have been studied in detail. Changes in the solution chemistry induce the modification of van der Waals interactions, steric repulsions, and cation bridging that impact the processes controlling the attachment of *C. parvum* oocysts to collector in porous media (LeChevallier et al., 1991; Byrd and Walz, 2005, 2007; Liu et al., 2009; Janjaroen et al., 2010; Park et al., 2012; Balthazard-Accou et al., 2014). Steric interactions have been shown to hinder the impact of ionic strength on the oocysts' Debye length (Liu et al., 2009; Janjaroen et al., 2010). The presence of  $\text{Ca}^{2+}$  also enhances the deposition of *C. parvum* on silica surface compared to  $\text{Na}^{2+}$  solution (Janjaroen et al., 2010). Studies of the influence of ionic strength, natural organic matter (NOM), and surface charge on *C. parvum* oocyst transport demonstrate that electrostatic effects dominate hydrophobic effect (Hsu et al., 2001; Dai and Hozalski, 2002; Bradford and Bettahar, 2005; Cortis et al., 2006; Kim et al., 2010; Metge et al., 2010). Straining and release of *C. parvum* oocysts in porous media have been demonstrated to be a key mechanism controlling the transport and retention of *C. parvum* oocysts (Harter et al., 2000; Logan et al., 2001).

Whereas several of the processes that can, theoretically, affect the transport of *C. parvum* oocysts in soils have been studied separately, a coherent picture has yet to emerge of how these various processes interact in any given soil, and of the extent to which they determine the transport of oocysts. A complete analysis of these questions would clearly require very extensive experiments, with different types of soils and a full factorial design in each case. Unfortunately, such a program is extremely onerous, in part because each measurement of oocyst concentration in water and especially in soil samples costs a very significant amount of money. Therefore, preliminary experiments are recommended to elucidate the range of behaviors expected among soils with contrasting properties prior to any intensive and expensive research effort. If the transport of oocysts among selected soils with varied physico-chemical conditions does not vary appreciably, and in no case is cause for serious concern, there may be little justification for conducting the type of detailed factorial experiment needed to assess which parameters or combinations of parameters are most influential, and under what conditions.

In this context, the primary objective of the research detailed here involves examining the difference in the transport and retention of *C. parvum* oocysts in soils among a set of soil columns with contrasting properties, subjected to simulated rainfall under laboratory conditions. Comparisons are made in terms of traditional breakthrough curves (BTCs) of *C. parvum* oocysts, but also relative to distribution profiles of *C. parvum* oocysts within the soil columns. Based on the outcome of these comparisons, a secondary objective is to determine the need for further research to improve our ability to predict *C. parvum* oocysts in field soils.

## MATERIALS AND METHODS

### *Cryptosporidium parvum* Oocysts

Original stock suspensions of purified and viable *C. parvum* oocysts (Iowa isolate–Cat# P102C@1 × 10<sup>9</sup>) were obtained from Waterborne Inc. (New Orleans, LA). The concentration of the stock oocyst suspension (10<sup>9</sup> oocysts in 50 mL) was determined using a Neubauer hemocytometer at a magnification of 200×. The stock of *C. parvum* oocysts was suspended in 50 mL de-ionized (DI) water with penicillin, streptomycin, gentamicin and amphotericin B. The *C. parvum* oocysts were stored in the dark at 4°C for 18 months. Prior to each experiment, the stock oocyst suspension was mixed in a vortexer at 3,000 rpm for 15 min to disperse the *C. parvum* oocysts that had settled to the bottom of the 50 mL centrifuge tube. This mixing process was designed to optimize the uniformity and consistency of the inoculum used to prepare the experimental oocysts concentration for use in the soil column experiments.

### Soils

The four different soils used in this study were collected in bulk form from fallow and cultivated pasture fields in the U.S. states of Illinois and Utah. The difference in the textural characteristics of these soils resulted in their classification as loamy sand and sandy loam based on their particle size analyses. One of the loamy sand soils collected in Kankakee County, IL is classified as a coarse-loamy, mixed, superactive, mesic Typic Endoaquoll belonging to the Gilford series which is a very deep, poorly drained or very poorly drained soil, and that formed in loamy over sandy sediments on outwash plains, near-shore zones (relict), and flood-plain steps. The Gilford soil series has a negligible potential for surface runoff, with a saturated hydraulic conductivity that is high in the upper part and very rapid in the lower part. Its soil permeability is moderately rapid in the upper part and rapid in the lower part. The other loamy sand soil, from Kankakee County, belongs to the Sparta series. The Sparta series is classified as a coarse-loamy, mixed, superactive, mesic Typic Endoaquoll, which is a very deep, and excessively drained soil. Formed in sandy outwash that has been reworked by wind these soils have a saturated hydraulic conductivity ranging from 10.00 to 100.00 micrometers per second. The Greenon sandy loam soils collected in Cache County (Utah) are classified as fine-silty, mixed, superactive, mesic Oxyaquic Calcixerolls; they are very deep, somewhat poorly drained, or moderately well-drained soils that formed from lacustrine deposits. Found on low lake terraces, the Greenon soil series is either somewhat poorly drained or moderately well-drained with a low-to-medium surface runoff, a slow-to-moderate permeability (moderately low to high saturated hydraulic conductivity), and a generally high organic matter content, from 3 to 9%. Collected in Cache County, UT, the Lewiston sandy loam soil is classified as coarse-loamy, mixed, superactive, mesic Aquic Calcixeroll that is a very deep, somewhat poorly drained soil formed in lacustrine sediments. Also found on lake terraces, the soil is somewhat poorly drained, has slow or very slow runoff and moderate permeability.

The soil organic matter content ranges generally from 1 to 3%.

The physicochemical characteristics of these soils, presented in **Table 1**, were determined by the Utah State University Analytical Laboratories (USUAL). Soil texture and particle size analyses were performed using the hydrometer method (Gee and Bauder, 1986). Total carbon analyses were performed using a Primac<sup>SLC</sup> Analyzer (Skalar Inc., Buford, GA, USA). Saturated soil paste extracts were prepared for the electrical conductivity and pH measurements (Rhoades, 1996). The cation exchange capacities of the soils were measured using the sodium acetate/ammonium acetate replacement method. Soil elements and ions were determined by ion chromatography using a Dionex ICS-3000 Ion Chromatography (IC) system. The soils were air-dried at 37°C, and sieved (2 mm), which were then stored in lidded buckets at room temperature until used.

**TABLE 1** | Physicochemical properties of loamy sand and sandy loam soils from the four series of soils examined–Sparta, Lewiston, Gilford, and Greenon.

		Soil Series			
		Sparta	Lewiston	Gilford	Greenon
Soil Texture	Sand	82.00	79.70	84.40	66.90
	Silt	8.40	7.70	7.70	13.60
	Clay	9.50	12.50	7.90	19.50
	USDA texture	LS	SL	LS	SL
pH		6.90	7.50	5.20	7.40
Soil Carbon	TC (%)	1.93	1.28	2.91	2.00
Organic Material (%)	Walkey-Black	2.80	0.60	3.30	2.80
	Loss on Ignition	3.40	0.80	4.20	3.80
Total Nitrogen (%)		0.15	0.13	0.19	0.08
EC (dS·m <sup>-1</sup> )		0.36	1.01	0.68	0.75
CEC (mmol <sub>c</sub> ·kg <sup>-1</sup> )		86	115	84	175
Elements and Ions	Sodium (mg·kg <sup>-1</sup> )	3.27	11.29	1.87	13.55
	Potassium (mg·kg <sup>-1</sup> )	13.80	22.00	26.70	10.80
	Magnesium (mg·kg <sup>-1</sup> )	4.84	14.91	5.71	12.00
	Calcium (mg·kg <sup>-1</sup> )	9.36	37.10	14.64	42.09
	Chloride (mg·L <sup>-1</sup> )	10.40	53.20	44.60	49.90
	Sulfur (mg·kg <sup>-1</sup> )	1.79	7.50	3.73	8.40
	Nitrate-N (mg·L <sup>-1</sup> )	12.60	0.40	44.10	0.90
	Boron (mg·kg <sup>-1</sup> )	0.02	0.04	0.02	0.02
	Carbonate + Bicarbonate (mmol <sub>c</sub> ·L <sup>-1</sup> )	1.80	8.41	0.50	6.04

## Artificial Rainfall Solutions and *C. parvum* Oocysts Inoculum Preparation

All solutions were prepared with deionized water ( $18 \text{ M}\Omega\cdot\text{cm}^{-1}$  resistivity; Milli-Q, Millipore Corp, Bedford, MA). For all flow-through column experiments, the artificial rainfall solutions contained 1 mM KCl as a background electrolyte. Potassium bromide (KBr) was used as a conservative tracer and was measured using an Orion™ Bromide Electrode (ThermoFisher Scientific, Waltham, Massachusetts, USA). The preparation of the *C. parvum* oocysts inoculum consisted of adding about  $1.5 \times 10^6$  *C. parvum* oocysts (quantified and enumerated using the qPCR method) from the stock oocysts suspension to 53 mL of artificial rainfall/background solution (1 mM KCl) that was spiked with 10 mM KBr tracer. Fifty of the 55 mL that constituted the inoculum were used in the flow-through column experiments and 5 mL were kept for further quantification of the initial input concentration ( $C_0$ ) of *C. parvum* oocysts applied to each column. The inoculum applied to the surface of each column contained a measured concentration of *C. parvum* oocysts of about  $2.85 \times 10^4 \text{ mL}^{-1}$ , corresponding to a total of  $1.56 \times 10^6 \pm 575,000$  oocysts.

## Column Experimental and Rainfall Simulator Set-Up

The columns used in these experiments were composed of plastic rings with dimensions of 20 cm in length and 9.5 cm in internal diameter. A ring 5 cm in height was placed at the bottom of the column, followed by the placement of 2-cm rings to achieve a total column height of 30 cm, of which 20 cm were used for the experiments. Rings were compressed together with top and bottom column holders, which were held with four rods positioned parallel to the length of the column on the outside perimeter of the rings, and bolts to prevent sliding. The rods were bolted through a screen section of a funnel that also had an additional mesh to prevent soil particles from moving through. The column was then clamped to an individual stand. A separate clamp allowed connection of the bottom part of the funnel below the column and an additional rod built in the stand allowed connection of a nozzle above the column. The soil was packed into each column. To achieve a uniform and homogeneous soil packing and a soil bulk density of  $1.5 \text{ g}\cdot\text{cm}^{-3}$  of soil, the total amount of soil (2,125 g) used to form the soil column was poured in three equal amounts and compacted with a rod. Four experimental columns were run simultaneously. One pump equipped with four cartridges dispensed the artificial rainfall solutions to the four nozzles, and a single air tube with brass couplings was used to pump air into these four nozzles. Rainfall was applied to the surface of each column using an individual nozzle (XA nozzle system  $\frac{1}{4}$ , 303 from BETE Fog Nozzle Inc., Greenfield, MA). The nozzle was connected to the water on one side through a peristaltic pump (Cole-Parmer, Vernon Hills, Illinois, USA) and to the air on the other side. The flow rate and the air pressure at the nozzle were adjusted at the pump and through a Parker Watts miniature precision regulator gauge (1/4 in; 60 psi; Parker Hannifin Corp., Cleveland, OH) to ensure that the nozzle spray covered the entire soil

surface. The height of the nozzle above the soil surface was 10 cm.

## Experimental Procedures/Rainfall Treatments and Leachate Collection

Each soil column experiment was performed in duplicate. Once the experimental columns were loaded with soils and placed in their stands, rainfall simulation was initiated with the artificial rainfall. Subsequent to achieving steady state and equilibrium conditions and a determination of constant outflow, *C. parvum* oocysts were released at the soil surface by pouring the 50 mL inoculum into the column. No rainfall was applied during the duration of the inoculum release at the soil surface until the inoculum had infiltrated. Rainfall was then resumed. In soil column experiments characterized by an occurrence of water ponding at the soil surface, the rainfall was discontinued until the ponded water had infiltrated and was then resumed. The soil flow velocities ranged from 0.09 to  $0.16 \text{ cm}\cdot\text{h}^{-1}$  with all of the column outflows collected in 50 mL centrifuge tubes. After about 6 pore volumes (PV) had passed through the columns, the rainfall was stopped, the column set-up was dismantled, and the columns were sliced with a thin metal sheet. A total of 10 column layers were sliced: 1 cm for the top layer, 2 cm for each of the following seven rings, with the remaining 5 cm ring divided in two, to obtain two layers of approximately 2.5 cm each.

## Soil Water Content

Each soil layer was sampled to determine the water content and the concentration of *C. parvum* oocysts in the soil profile. To establish the water content in the soil layers, three wet soil samples of 5 g each were collected randomly in each soil layer. These wet soil samples were then placed in aluminum foil cup holders, weighed, and oven dried at  $105^\circ\text{C}$  for 24 h. The weight of the dried samples was then recorded and the water content was established gravimetrically.

## Isolation of *C. parvum* Oocysts from Soil

All of the soil remaining in each layer after sampling for water content was collected for purposes of measuring the concentration of *C. parvum* oocysts in the soil. 25 g of wet soil was placed in each of between 9 and 11 50 mL plastic centrifuge tubes, depending on the layer. The method developed by Koken et al. (2013) was used to isolate *C. parvum* oocysts from the soil. The *C. parvum* oocysts attached to soil particles were released by the addition of 20 mL of Tween 80 at two critical micelle concentrations (CMC) to each of the 50 mL centrifuge tubes, which were then fixed in a rotational shaker, perpendicular to the axis of rotation, for 24 h. Two tubes from each soil layer were selected to undergo the *C. parvum* oocyst isolation procedure, while the remaining tubes were stored. In each of the selected tubes, the soil was underlaid by 10 mL of cold sucrose solution with a density of  $1.18 \text{ g}\cdot\text{mL}^{-1}$ . These tubes were then centrifuged at  $2,500 \times g$  for 15 min to construct a sucrose concentration gradient and retain the *C. parvum* oocysts. The resulting supernatant was then transferred into a new 50 mL conical-bottomed centrifuge tube and subjected to centrifugation at  $2,500 \times g$  for 15 min. Thereafter, the supernatant was removed,



leaving 4 mL in the tube. A first wash was performed by vortexing the tube, re-suspending the pellet by adding 35 mL of deionized water, followed by centrifugation at  $2,500 \times g$  for 15 min. A second wash was conducted following the procedure described in the first wash. For the third wash, supernatants were removed and the remaining 4 mL of the two tubes from each layer were vortexed and transferred into one 15 mL centrifuge tube. Deionized water was added to reduce the final volume to 12 mL. These 15 mL tubes were centrifuged at  $2,500 \times g$  for 15 min. In each case, the supernatant was removed to leave 3 mL of pellet in the 15 mL tube, and the pellet was re-suspended by vortex. All centrifugations involving 50 and 15 mL tubes were performed using a refrigerated centrifuge (Model 5810R, Eppendorf AG, Hamburg, Germany). A 300  $\mu$ L aliquot was taken from the 3 mL, and placed in a microcentrifuge tube to which 900  $\mu$ L of TE buffer was added. After vortexing briefly, the microcentrifuge tube was centrifuged at  $14,000 \times g$  in a microcentrifuge (Model 5424, Eppendorf AG) for 5 min. The supernatant was then removed, leaving a 0.5 mL sample for DNA extraction and PCR analyses.

## Concentration of *C. parvum* Oocysts in Effluents

Soil effluent samples were collected in 50 mL centrifuge tubes and the method developed by Koken et al. (2013) was again used to concentrate *C. parvum* oocysts from the soil. Selected tubes were centrifuged at  $2,500 \times g$  for 15 min to concentrate the *C. parvum* oocysts in the pellet. The supernatants were discarded and the remaining 5 mL containing the pellets were transferred into 15 mL centrifuge tubes. Deionized water was added to each 15 mL tube to bring the final volume to 8 mL. The tubes were then spun at  $2,500 \times g$  for 15 min. The supernatant was removed and the remaining 1.7 mL were transferred to microcentrifuge tubes. These tubes were centrifuged at  $14,000 \times g$  for 5 min. The supernatants were removed, leaving 0.5 mL, to which 900  $\mu$ L of TE buffer was added. After being vortexed, the microcentrifuge tubes were centrifuged at  $14,000 \times g$  for 5 min, and the supernatant was removed to leave 0.25 mL of pellet to which 0.25 mL of TE buffer was added, yielding a 0.5 mL of sample for DNA extraction and PCR analyses. All centrifugation processes involving 50 mL and 15 mL tubes were performed using an Eppendorf 5810 R refrigerated centrifuge. All centrifugations involving microcentrifuge tubes were performed in an Eppendorf 5424 microfuge.

## DNA Extraction and qPCR Analyses of *C. parvum* Oocysts

The molecular biological methods used for the detection and enumeration of *C. parvum* oocysts, which include DNA extraction and qPCR analyses, using the procedures described in detail by Koken et al. (2013). Briefly, 7-point standard curves were prepared with DNA from 10,000; 1,000; 100; 10; 1; 0.1; and 0.01 oocysts (per 5  $\mu$ L volume), which were obtained by pooling two samples from the *C. parvum* oocyst stock suspension. Each standard curve was used during the analysis of six microtiter plates containing samples obtained from soil and leachates. Six plates were run, with each plate containing its own set of

standards for the standard curve. After DNA isolation, each sample was resuspended in 30  $\mu$ L of DNA elution buffer (Cat# D3004-4; Zymo Research, Irvine, CA). A 5  $\mu$ L sample DNA was used for each qPCR reaction, and each sample was tested in triplicate. Samples were analyzed in a 20  $\mu$ L reaction volume with 50 cycles per run using a QuantStudio 12K Flex Real Time PCR system (Life Technologies, Carlsbad, CA, USA). Taqman<sup>®</sup> chemistry was used, and the primers and probe indicated in Koken et al. (2013) were synthesized as PrimeTime<sup>®</sup> qPCR assays by Integrated DNA Technologies (Coralville, Iowa, USA) on the basis of GenBank accession number AF190627 by Jothikumar et al. (2008) as follows: 5'-ACTTTTTGTTTGTTCACGCCG-3' (forward primer), 5'-AATGTGGTAGTT GCGGTTGAA-3' (reverse primer) and 5'-FAM-ATTTATCTCTTCGTAGCGGCG-BHQ-3' (probe). The results were compiled and analyzed using the QuantStudio software (Life Technologies). After analysis, the data from each plate were exported to an Excel spreadsheet. Mean data for each sample (averaging of replicates) were compiled as aliquot data. These values reflected the number of oocyst genomes in 5  $\mu$ L of each sample. These data were used to calculate the number of oocysts in the entire 30  $\mu$ L sample. The standard deviation for each sample was also computed.

## Statistical Analysis

Results of the transport and retention of *C. parvum* oocysts in soils were expressed as the percentage of *C. parvum* oocysts retrieved in the soil leachates and matrices, which reflect the behavior of *C. parvum* oocysts in soils of different physicochemical properties. All statistical analyses were performed using SAS<sup>®</sup> Studio software. For the statistical analysis of the transport and retention results of *C. parvum* in soils, a *t*-test was applied to determine the least significant difference (LSD) for the percentage of *C. parvum* oocysts retrieved in the soil leachates and matrices of Sparta, Lewiston, Gilford, and Greenson series soils. Statistical significance was accepted at  $p < 0.05$ . Results are expressed in graphs as mean  $\pm$  standard deviation.

## RESULTS

### Soil Physicochemical Characteristics

Physicochemical analyses of the soils used in this study indicate that soils in the Greenson series and Lewiston series are sandy loam soils, while the Sparta and Gilford series were characterized as loamy sand soils (Table 1). The clay contents were highest in the Greenson series soil (19.5%), with Lewiston (12.5%) and Sparta (9.5%) series soils having less clay and the Gilford series soil (7.9%) having the least clay. The organic matter content ranged from 1.28% in Lewiston series to 2.91% in Gilford series soils. The lowest pH values were observed in the Gilford (5.2) and Sparta (6.9) series soils, while the highest pH values were reported in Greenson (7.4) and Lewiston (7.5) series soils. The total calcium concentrations ranged from a low of 9.36 mg·kg<sup>-1</sup> for the Sparta series, to a high of 42.09 mg·kg<sup>-1</sup> for the Greenson series soil. With the addition of artificial rainfall, the highest flow velocity (0.16 cm·h<sup>-1</sup>) was observed in the Lewiston series soil,

while the lowest flow velocity ( $0.10 \text{ cm}\cdot\text{h}^{-1}$ ) was reported in the Greenston series soil (Table 2).

Tracer tests were used to evaluate the hydraulic properties of the soils. The concentration of bromide ions entering the column,  $C_0$ , and in the effluent,  $C$ , were used to calculate BTCs as  $C/C_0$  as a function of PV (Figure 1). The non-reactive chemical tracer added to the *C. parvum* oocysts mixture was measured in every leachate sample as  $\text{mg}\cdot\text{L}^{-1}$  of leachate. No correlation was observed between the number of *C. parvum* oocysts in the leachate and the tracer concentration. Additionally, the BTCs of the tracer and the *C. parvum* oocysts did not display the same behavior. The tracer BTCs in the Sparta, Lewiston, and Gilford experiments presented a monotonic increase, with a  $C/C_0$  value reaching a peak ranging from about 0.13 to 0.20 that occurred at 0.56 to 1 PV, and then a monotonic decrease. Regarding the Greenston series, the tracer BTCs contained two peaks, a first peak at the 0.12 PV and a second peak at 1.5–3.5 PV, possibly indicating a fast and slow convective transport; however this possible dual flow had no effect on the mobility and transport of *C. parvum* oocysts.

Even though the experiments were conducted under unsaturated conditions, with free drainage at the bottom of the columns, the degree of saturation for the replicates of soil columns of all four soil series is high, always above 70% (Figure 2). The effect of such high saturations on *C. parvum* oocysts was not determined, but given the size of these oocysts, it is more than likely that their transport in the soils would have been even slower, and their retention more intense had the degree of saturation been significantly lower.

## Prevalence and Concentration of *C. parvum* Oocysts in Soil Leachates

The leachates from all four soil series were analyzed to assess the prevalence and concentrations of *C. parvum* oocysts. Leachate samples were collected over about 6 PV of each soil column. The numbers of *C. parvum* oocysts in the soil leachates from two replicates for each of the four soils are represented in the breakthrough curves (BTCs) as  $C/C_0$ , where  $C$  is concentration of *C. parvum* oocysts detected in leachates and  $C_0$  is the concentration of *C. parvum* oocysts detected in the input solution

(Figure 1). A visual inspection of the soil columns did not reveal any cracks or holes.

In the Sparta series soil, *C. parvum* oocysts were not detected in the leachates of either of the replicates (Figure 1). The BTCs of the tracer, however, displayed a fast increase and a tailing decrease. The heights of the tracer BTCs were measured as  $C/C_0$  ranging from 0.17 to 0.20. The peaks of the BTC of the tracer occurred at 0.70 and 0.80 PV.

In the Lewiston series soil, *C. parvum* oocysts were detected in the soil leachates of both replicates (Figure 1). The peaks of the *C. parvum* oocysts BTC were observed at 2.35 PV for a  $C/C_0$  value of 0.006 and at 1.86 PV for a  $C/C_0$  value of 0.005, for the first and second replicates (R1 and R2), respectively. The peak of the tracer BTCs occurred at about 1.00 PV with  $C/C_0$  values of approximately 0.16 to 0.18. These results indicate the transport of *C. parvum* oocysts within the soil matrix and their prevalence in the soil leachates in considerable concentration.

In the Gilford series soil, *C. parvum* oocysts were present in only a few samples (Figure 1), with  $C/C_0$  values of 0.004 at 0.53 PV and 0.008 at 2.03 PV for R1, and 0.011 at 5.2 PV for R2. The peak of the tracer BTCs reached a  $C/C_0$  value of approximately 0.13 at 0.56 PV and 0.69 PV, respectively for replicates 1 and 2.

In the Greenston series soil, *C. parvum* oocysts were present in the leachates of both soil columns (Figure 1). Their peaks reached values of  $C/C_0$  of 0.004 at 0.56 PV and 0.007 at 2.68 PV, respectively, for R1 and R2. Two peaks were observed in each tracer BTC; an early and a late peak occurred in each of the replicates. The BTCs of the tracer displayed early peaks at approximately 0.12 for R1, and 0.20 PV for R2. Late and smaller peaks in the BTCs of the tracer were observed between 1.5 and 2 PV in R2, and at approximately 3.5 PV in R1. The occurrence of two peaks demonstrated the presence of fast and slow infiltration processes in this soil series for the tracer; however this dual-flow phenomenon did not induce two different transport behaviors for *C. parvum* oocysts.

## Spatial Distribution and Concentration of *C. parvum* Oocysts in Soils

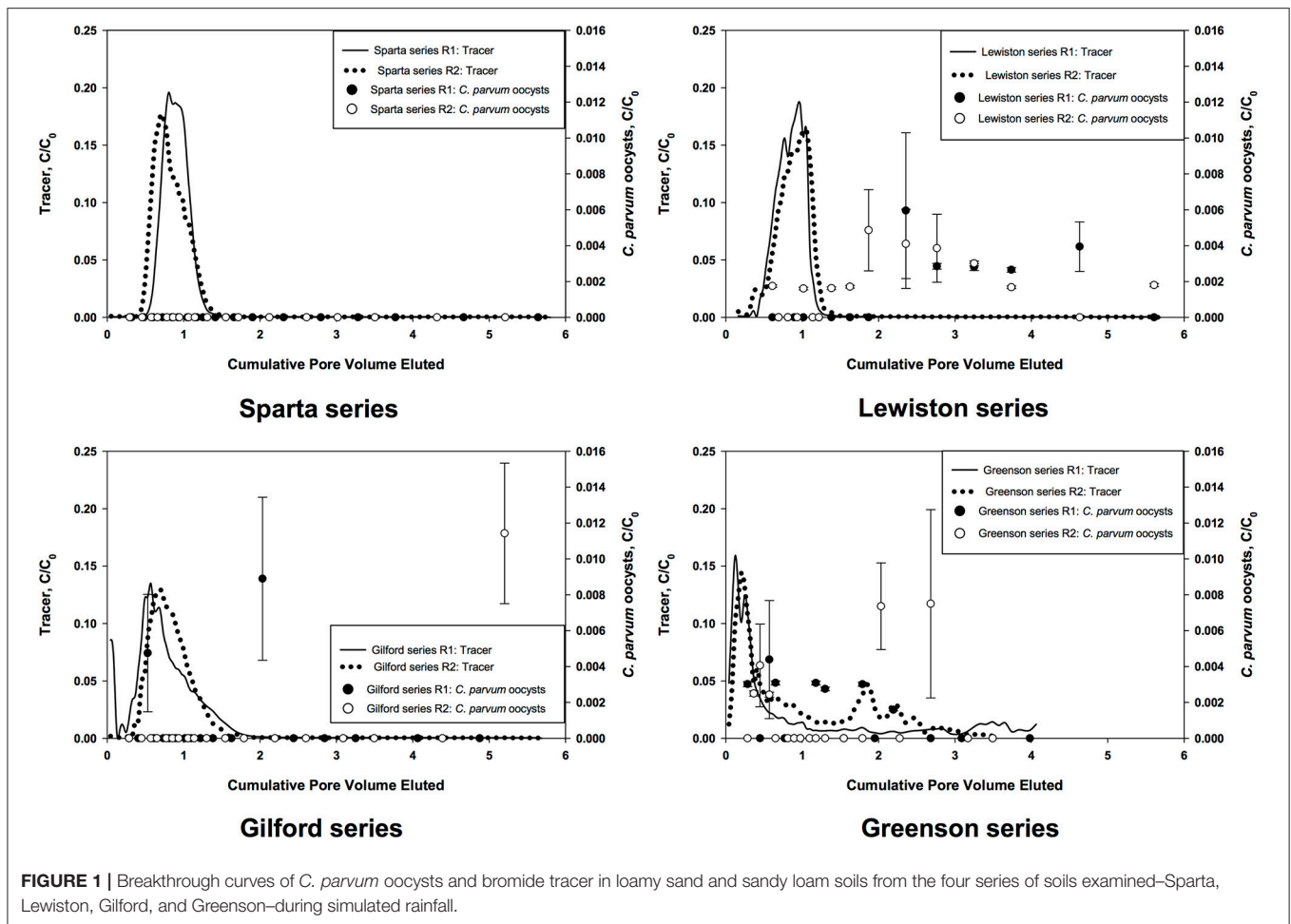
Following the flow and transport experiments in soil, the soil columns were sliced in layers and soil samples were analyzed to establish the spatial distribution and concentration of *C. parvum* oocysts in the soil profile. The numbers of *C. parvum* oocysts in the soil matrices from the two replicates for each of the four soils are presented as number of *C. parvum* oocysts per gram of dry soils (Figure 2).

In the Sparta series soil (Figure 2), *C. parvum* oocysts were detected in the top layers of the soil profiles of each of the replicates and in the deepest layer of the second replicate (R2). The highest concentration of *C. parvum* oocysts was detected in the top layer of the first replicate (R1). Although *C. parvum* oocysts were not detected in the soil leachates, their transport through the soil matrices of Sparta series was established, as they were detected and enumerated in the topmost soil layers of R1 and R2, and the deepest soil layer of R2.

In the Lewiston series soil (Figure 2), *C. parvum* oocysts were detected in the soil matrices of both of the R1 and R2 soil column

**TABLE 2** | Flow velocities from soil columns packed with the four series of soils examined—Sparta, Lewiston, Gilford, and Greenston—resulting from application of rainfall treatments.

Soil series	Flow Velocity cm/hour	
	Soil column replicates with artificial rainfall	
	R1	R2
Sparta	0.141	0.145
Lewiston	0.163	0.160
Gilford	0.125	0.137
Greenston	0.094	0.109



**FIGURE 1** | Breakthrough curves of *C. parvum* oocysts and bromide tracer in loamy sand and sandy loam soils from the four series of soils examined—Sparta, Lewiston, Gilford, and Greenson—during simulated rainfall.

replicates. The highest number of *C. parvum* oocysts in the soil column of replicate 1 was detected in the top layer, with about 2 *C. parvum* oocysts per gram of dry soil. In contrast, the highest number of *C. parvum* oocysts in the soil column of R2 was found in the deepest layer, with about 215 oocysts per gram of dry soil. Our results demonstrated the transport of *C. parvum* oocysts throughout the soil matrix of the Lewiston series soil.

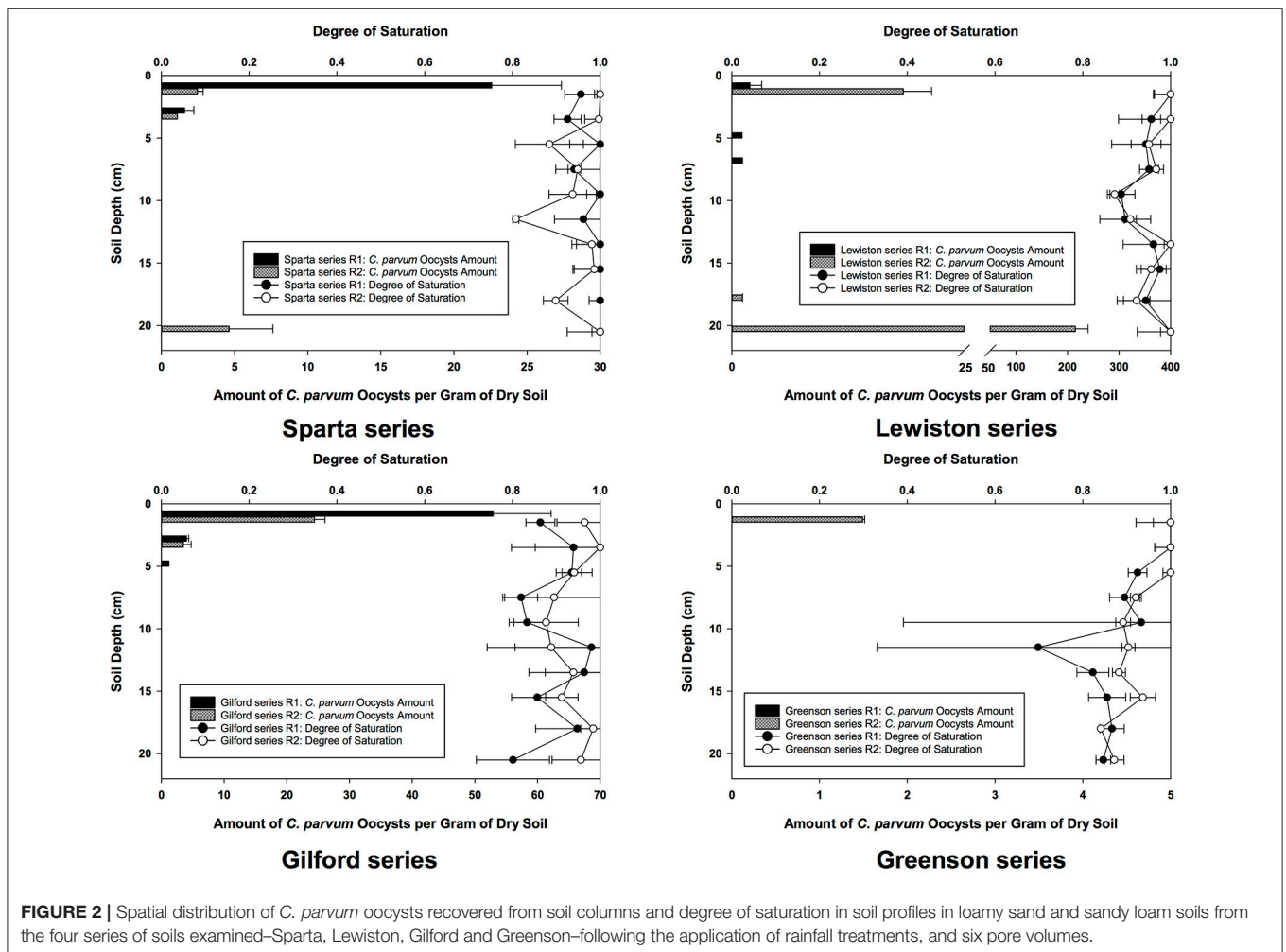
In the Gilford series soil (Figure 2), *C. parvum* oocysts were found only in the top layers of the soil column in both replicates, whereas in the Greenson series soil (Figure 2), no *C. parvum* oocysts were detected in the soil column from R1, while only a few oocysts detected in the top layer the soil column from R2.

### Cumulative Recovery of *C. parvum* Oocysts in Leachates and Soils

Throughout the transport experiments, higher numbers of *C. parvum* oocysts were detected in the soil matrices than in the soil leachates of all four soil series. Although the relative proportions of *C. parvum* oocysts in the soil matrices and soil leachates from the different soil series varied, the trends of total numbers of *C. parvum* oocysts recovered from each of the replicates of each soil series differed (Table 3). The highest recovery of the number of *C. parvum* oocysts in soil matrices

and leachates was 2.718% in the soil column of replicate 2 of the Lewiston series soil. The majority of *C. parvum* oocysts recovered were from the soil matrices in all of our experiments, except in the first replicate of the Greenson series soil, where *C. parvum* oocysts were not detected in the soil matrices. The distribution of percentage recovery between soil matrices and leachates in this case (Greenson first replicate) was attributed to soil leachate only. Similarly, no *C. parvum* oocysts were detected in the leachates of either replicate of the Sparta series soil, which resulted in *C. parvum* oocysts being recovered only from the soil matrices.

The recovery of *C. parvum* oocysts in the leachates was very low, ranging from 0 to 0.006% (Table 3). *C. parvum* oocysts were detected in the leachates of three out of four soil types. Overall, the recovery of *C. parvum* oocysts in leachates was impacted by the soil type. Significance differences ( $p < 0.05$ ) were detected among the percentages of *C. parvum* oocysts retrieved in the soil leachates of the Sparta, Lewiston, Gilford, and Greenson series (Figure 3). In sandy loam soils, the highest *C. parvum* oocyst recovery was observed in leachates from the Lewiston series soil (0.004–0.006%). In loamy sand soils, the highest recovery of *C. parvum* oocysts was detected in the leachates from the Gilford series soil (0.002–0.004%).



*Cryptosporidium parvum* oocysts were detected in the soil of all replicates of all four soils, except for the soil from the first replicate of the Greenson series soil. Overall, the *C. parvum* oocyst recoveries in the four different soils were not significantly different. No significant differences ( $p < 0.05$ ) were observed among the percentages of *C. parvum* oocysts retrieved in the soil matrices for the Sparta, Lewiston, Gilford, and Greenson series (Figure 4). *C. parvum* oocysts recovery in soils ranged from 0 to 2.718%. The highest *C. parvum* oocyst recovery from sandy loam soils was reported for the Lewiston series soil (0.060–2.718%). For the loamy sand soils, the Sparta series and Gilford series, soils showed *C. parvum* oocyst recoveries ranging from 0.092 to 0.165% and 0.345 to 0.919%, respectively.

## DISCUSSION

The fate and transport of *C. parvum* oocysts in soil were investigated in a series of laboratory soil column experiments subject to simulated artificial rainfall events in order to induce unsaturated flow conditions. The transport of *C. parvum* oocysts with infiltrating water through the vadose zone was demonstrated in all soils. However, differences in the degree of

the transport of *C. parvum* oocysts were observed. While *C. parvum* oocysts were detected in the leachates of the Lewiston, Gilford, and Greenson series, they were not detected in the leachates of the Sparta series. More *C. parvum* oocyst transport and breakthrough occurred in sandy loam soils (Greenson and Lewiston series) than in loamy sand soils (Sparta and Gilford series).

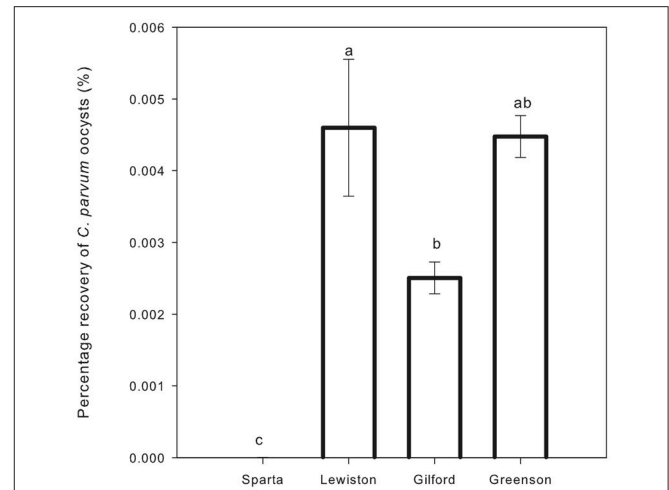
*Cryptosporidium parvum* oocysts were detected in the soil matrices of all four soil types, except in the first replicate of the Greenson series soil, and were usually retained in the topmost layer in considerably larger quantity than that seen in the rest of the soil profile. The retention of microorganisms in upper soil layers has also been reported in a previous study (Gerba et al., 1975), and in particular with *C. parvum* oocysts (Mawdsley et al., 1996a). However, in several instances, additional *C. parvum* oocysts were detected in the deepest soil layer, as considerable transport had occurred. These findings indicate that the physicochemical properties of the soils have primary and direct control over the behavior of *C. parvum* oocysts.

The mobility behavior of *C. parvum* oocysts in soils was monitored in natural repacked and homogeneous soil columns that allowed investigation of the effects of soil physicochemical

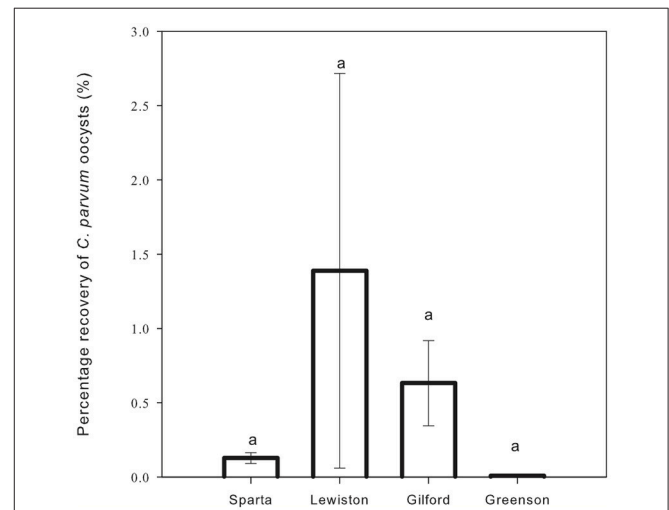


**TABLE 3** | Recovery of *C. parvum* oocysts in soil matrix and leachate of loamy sand and sandy loam soils from four different series—Sparta, Lewiston, Gilford, and Greenson—following the application of rainfall treatments, at an approximate six-pore volume.

Soil series	Soil column and treatment	C. parvum oocysts in soil matrix				C. parvum oocysts in soil leachates				Total	Percentage distribution		
		Number of C. parvum oocysts detected in soil column	Percentage of C. parvum oocysts retrieved in soil column (%)	Number of C. parvum oocysts detected in soil leachate	Percentage of C. parvum oocysts retrieved in soil leachate (%)	Total number of C. parvum oocysts detected in soil matrix and leachate	Total percentage of C. parvum oocysts retrieved in soil matrix and leachate (%)	Percentage of C. parvum oocysts detected in soil matrix to total detected (%)	Percentage of C. parvum oocysts detected in soil leachates to total detected (%)				
Sparta	R1	2,405,010	0.165	0	0.000	3,965	0.165	100,000	0.000	3,965	0.165	100,000	0.000
	R2	2,027,976	0.092	0	0.000	1,864	0.092	100,000	0.000	1,864	0.092	100,000	0.000
Lewiston	R1	1,342,821	0.060	49	0.004	854	0.064	94,272	5.728	854	0.064	94,272	5.728
	R2	2,237,160	2.718	124	0.006	60,922	2.723	99,796	0.204	60,922	2.723	99,796	0.204
Gilford	R1	1,045,941	0.919	29	0.003	9,641	0.922	99,704	0.296	9,641	0.922	99,704	0.296
	R2	1,352,535	0.345	31	0.002	4,697	0.347	99,342	0.658	4,697	0.347	99,342	0.658
Greenson	R1	1,124,925	0.000	47	0.004	47	0.004	0.000	100,000	47	0.004	0.000	100,000
	R2	1,383,567	0.017	66	0.005	305	0.022	78,386	21.614	305	0.022	78,386	21.614



**FIGURE 3** | Percentage recovery of *C. parvum* oocysts in soil leachates from loamy sand and sandy loam soils from the four series of soils examined—Sparta, Lewiston, Gilford and Greenson—following the application of rainfall treatments. Significance levels are  $p < 0.05$ .



**FIGURE 4** | Percentage recovery of *C. parvum* oocysts in soil columns from loamy sand and sandy loam soils the four series of soils examined—Sparta, Lewiston, Gilford and Greenson—following application of rainfall treatments. Significance levels are  $p < 0.05$ .

properties. Repacked soil columns provided a homogeneous environment to study the impacts of soil parameters and soil water chemistry on the mobility of *C. parvum* oocysts. A similar approach was used previously to study the fate and transport of other microorganisms (Trevors et al., 1990; Gannon et al., 1991a,b; van Elsas et al., 1991). Soil water chemistry, water content, and flow of water through soil have been reported as major factors affecting the extent of mobility of microorganisms in soils (Kuikman et al., 1990; Trevors et al., 1990; van Elsas et al., 1991; Balthazard-Accou et al., 2014). Mawdsley et al. (1996a) established the possible transport of *C. parvum* oocysts through

three different and intact soil cores obtained from clay loam, silty loam, and loamy sand soils. Transport studies simulating the aquifer flow also examined the deposition processes and associated breakthrough of *C. parvum* oocysts in saturated porous media (Harter et al., 2000). Transport experiments in unsaturated flow porous media (Darnault et al., 2003, 2004) demonstrated that preferential flow in the form of fingered flow and macroporous flow in soils allowed the fast transport of *C. parvum* oocysts with breakthrough concentrations above infection doses. Therefore, the results presented here are consistent with previous research that also demonstrated the significant transport of *C. parvum* oocysts in soil.

The deposition processes of *C. parvum* oocysts have been investigated in model granular media—sand and glass beads—(Dai and Hozalski, 2002; Kim et al., 2010), and in natural media (Harter et al., 2000; Hijnen et al., 2005). In their analysis of the effect of mineral charges in the deposition of *C. parvum* oocysts in soils Harter et al. (2000) observed that the transport and retention of *C. parvum* oocysts in saturated porous media followed the first order attachment theory. Also experiments undertaken to investigate the fate and transport of *C. parvum* oocysts during infiltration processes demonstrated that they are subject to advection-dispersion and reversible adsorption processes (Brush et al., 1999). Additionally, Darnault et al. (2004) emphasized the effects of gas-water interfaces on the capture and retention of *C. parvum* oocysts during unsaturated flow.

Our discussion attempts to provide an understanding of the different environmental processes that govern the mobility of *C. parvum* oocysts in soils, as illustrated with the quantitative results from our different experimental systems. Our study clearly demonstrates that the transport and retention of *C. parvum* oocysts in natural soils varies by soil types. *C. parvum* oocysts have a negative surface charge under typical environmental conditions (Thomas et al., 2001; Hsu and Huang, 2002) due to carboxylate, carboxylic and phosphate groups on the surface of oocysts (Karaman et al., 1999). The glycocalyx that is located at the surface of the outer wall of *C. parvum* oocysts impacts the interactions between *C. parvum* oocysts and surfaces by inducing a steric repulsion (Nanduri et al., 1999; Jenkins et al., 2010; Liu et al., 2010; Dumètre et al., 2012), which may hinder adsorption. In our experimental system, the presence of clay particles and organic matter, as well as dissolved ions in the soil water solution and soil matrices, are likely to have also impacted the surface properties of the *C. parvum* oocysts.

## Effects of Soil Types on Transport and Retention of *C. parvum* Oocysts in Natural Soils

Although numerous studies have been undertaken to investigate the effects of soil type on the mobility and transport of microorganisms (i.e., bacteria and viruses) in soil, research on the fate and transport of protozoan cysts is scarce (Gerba et al., 1975; Bitton and Harvey, 1992). In soil, the mobility of bacteria is controlled by adsorption, straining, and sedimentation processes (Reddy et al., 1981; Gannon et al., 1991a,b; Tan et al., 1992), whereas in the case of viruses, mobility is governed mainly by

adsorption as a result of their small size (Tan et al., 1992). For protozoa and their cysts, such as *C. parvum* oocysts, which have a diameter of 4–6  $\mu\text{m}$ , adsorption, straining, and sedimentation are expected to impact their mobility in a manner similar to that of bacteria (Mawdsley et al., 1996b).

Soil type is a critical parameter that affects the mobility of microorganisms (Bitton et al., 1974; Bashan and Levany, 1988; Tan et al., 1991; Huysman and Verstraete, 1993; Mawdsley et al., 1996a). The presence of clay and organic matter, in particular, affects the adsorption processes of bacteria and viruses as a result of their large surface area and negative charge (Reddy et al., 1981).

This study has demonstrated that leaching of *C. parvum* downward through the soil profile and the vadose zone does occur, although the extent of their transport is affected by the soil type. The soil type was a determinant in the transport of *C. parvum* oocysts through soil and into leachate, as larger quantities with concentrations of *C. parvum* oocysts found in sandy loam soils (i.e., Lewiston and Greenson series) as opposed to loamy sand soils (i.e., Sparta and Gilford series).

## Interactions at Interfaces between *C. parvum* Oocysts and Soil Components: Interplay of Clay Particles, Organic Matter, and Calcium Ions

Potential interaction of the *C. parvum* oocysts with surfaces and interfaces they encounter, particularly the surfaces of soil particles, during the soil infiltration process is possible. Among the soil components, clay particles have the largest surface areas for potential interactions. Consequently, soil with high clay content provides the highest probability and potential for *C. parvum* oocysts to interact with clay surfaces, which may result in greater adsorption of *C. parvum* oocysts to soil clay particles (Balthazard-Accou et al., 2014). In soil, most of the organic matter (OM) is generally combined with minerals that consist of clay and silt particles (Cheshire et al., 2000). Therefore, since most of the OM in soil is bound to the clay and silt surfaces, we can postulate that clay-OM and silt-OM complexes may also facilitate the interplay between *C. parvum* oocysts and OM, as *C. parvum* oocysts collide with clay surfaces that are coated with OM. Soil that contains high clay and silt contents may enhance and promote a higher degree of interaction between OM present at the surface of minerals and *C. parvum* oocysts that encounter these surfaces during their transport through soil. Interactions of OM and surfaces of *C. parvum* oocysts is a complex phenomenon, and previous studies have suggested the influence of OM on the fate and transport of *C. parvum* oocysts in porous media. In particular, an increase in OM concentration has been found to induce an increase in the breakthrough of *C. parvum* oocysts in sand and a decrease in the collision efficiency (Abudalo et al., 2010). The adsorption of OM on the surface of *C. parvum* oocysts has been postulated to be mediated by the presence of the polyvalent cations  $\text{Ca}^{2+}$  (Dai and Hozalski, 2003).

As observed in transport experiments in the Greenson series soil, the highest combination of clay, organic matter, and  $\text{Ca}^{2+}$  resulted in the highest transport through the soil matrix and the highest release of *C. parvum* oocysts in leachates (Tables 1, 3 and

**Figure 1**). These results demonstrate the role that clay particles, organic matter, and  $\text{Ca}^{2+}$  play in the transport of *C. parvum* oocysts. These results and proposed description of an enhanced transport process of *C. parvum* oocysts provided by adsorption of organic matter at the oocysts surface mediated by  $\text{Ca}^{2+}$  and promoted by the presence of clay are in agreement with similar proposed enhanced-transport phenomena in other contexts. In our study, sandy loam soils have a higher content of clay (12.5 and 19.5% for Lewiston and Greenon series, respectively) than loamy sand soils (9.5 and 7.5% for Sparta and Gilford series, respectively). Consequently, a soil that contains higher concentrations of clay and silt particles offers larger surface areas for *C. parvum* oocysts to potentially encounter and a higher probability of *C. parvum* oocysts to interact with these surfaces as they are transported through the soil. The largest CEC values were observed for sandy loam soils Lewiston ( $115 \text{ mmol}_c \cdot \text{kg}^{-1}$ ) and Greenon ( $175 \text{ mmol}_c \cdot \text{kg}^{-1}$ ) compared to loamy sand soils Sparta ( $86 \text{ mmol}_c \cdot \text{kg}^{-1}$ ) and Gilford ( $840 \text{ mmol}_c \cdot \text{kg}^{-1}$ ). In our experiments, a larger number of *C. parvum* oocysts were observed in leachates from soils with high clay content (0.004–0.006% oocysts recovered in water in Lewiston and Greenon series, with clay content of 12.5 and 19.5%, respectively) than in leachates from soils with low clay content (0–0.003% oocysts recovered in water in Sparta and Gilford series, with clay content of 9.5 and 7.5%, respectively). Indeed, direct and indirect effects of clay particles, as well as interplay effects of other soil parameters have been identified to significantly govern the fate and transport of *C. parvum* oocysts. Since clay and silt surfaces are the sites where most of the OM resides and the probability of interaction of *C. parvum* oocysts with OM increases as the percentage of clay and silt particles increases. As previously reported, when *C. parvum* oocysts are placed in the presence of elements from natural OM, OM may adsorb onto their surface and increase their negative charges and hydrophobicity which enhance the electrostatic repulsion between the OM-coated *C. parvum* oocysts and the negatively charged soil particle surfaces that they encountered, and therefore increase their mobility and impact their transport (Mawdsley et al., 1996a; Ongerth and Pecoraro, 1996; Considine et al., 2002; Dai and Hozalski, 2002, 2003; Mohanram et al., 2010, 2012). These phenomena may result in enhanced transport, as observed in sandy loam soils. Adsorption of organic matter on the surface of *C. parvum* oocysts induces an increase in zeta potential that counteracts the negative charge.

In addition, the presence of  $\text{Ca}^{2+}$  has been postulated as possible mediator in the coating of *C. parvum* oocysts by OM (Dai and Hozalski, 2003). Soils that had the highest concentration of  $\text{Ca}^{2+}$ , Greenon series ( $42.09 \text{ mg} \cdot \text{kg}^{-1}$ ) and Lewiston series ( $37.10 \text{ mg} \cdot \text{kg}^{-1}$ ), and the highest clay content (12.5 and 19.5% for Lewiston and Greenon series, respectively) had the greatest breakthrough of *C. parvum* oocysts, as corroborated by the *C. parvum* oocysts BTC results and the analyses of the physicochemical properties of the soils. Our findings support and demonstrate the critical role of  $\text{Ca}^{2+}$  in mediating the transfer of OM from the surface of minerals to the surface of *C. parvum* oocysts. In particular, the Lewiston and Greenon soil series that had the highest soil  $\text{Ca}^{2+}$  concentration, the highest clay

content, and the highest silt content resulted in the highest breakthrough of *C. parvum* oocysts among all soils, although the OM concentrations of all soils were similar (3.4, 0.8, 4.20, and 3.80% OM for Sparta, Lewiston, Gilford, and Greenon, respectively) (Tables 1, 3 and Figure 1). We can then postulate that the interplay of clay (and silt to some extent), OM, and  $\text{Ca}^{2+}$  facilitated and mediated the transfer of OM to *C. parvum* oocysts surface, which resulted in the breakthrough of *C. parvum* oocysts through the soil. Based on our findings, this interplay between these biogeochemical compounds and processes—clay and silt content, presence of OM,  $\text{Ca}^{2+}$  content, and  $\text{Ca}^{2+}$  mediated transfer of OM from mineral surfaces to *C. parvum* oocysts—is critical in controlling the mobility and transport of *C. parvum* oocysts in soils. Our observations are also in agreement with previous research findings by Ferguson et al. (2003) and Peng et al. (2008) that also highlighted the role of soil texture and soil physico-chemical properties on the fate and transport of *C. parvum* oocysts in soil.

## Recovery of *C. parvum* Oocysts in Natural Soil and Water Samples

Compared with the total number of *C. parvum* oocysts applied to the soil columns surface, a highest oocyst recovery rate reported for soil samples (2.718%) was considerably greater than the highest oocyst recovery rate reported for leachate samples (0.006%). These recovery results are in agreement with those from Petersen et al. (2012), with the highest recovery rate of oocysts at 9.8% for soil samples and 0.03% for leachate samples. The median of the efficiency of *C. parvum* oocysts recovery from the different soil matrices in our experiment was calculated as 0.128%. This is similar to the previously reported efficiency of *C. parvum* oocyst recovery from soils in other studies (Mawdsley et al., 1996a; Petersen et al., 2012). Mawdsley et al. (1996a) investigated the effect of incubation time on the number of *C. parvum* oocysts extracted from soil initially inoculated with *C. parvum* oocysts. Mawdsley et al. (1996a) found that the efficiency of *C. parvum* oocyst recovery from soil decreases rapidly as time passes, from 61.6% for an incubation time of 15 min to 0.3% for an incubation time of 7 days. A substantially higher number of oocysts were recovered from the sandy loam soils (Lewiston and Greenon series) than the loamy sand soils (Sparta and Gilford series), as shown in Table 3. However, the distributions of *C. parvum* oocysts within the soil profiles were similar. Most of the *C. parvum* oocysts were encountered in the soil top layers (0–5 cm) and in the bottom layers (17.5–20 cm). Our *C. parvum* oocyst recoveries are in agreement with previously published results (Mawdsley et al., 1996a; Petersen et al., 2012). Also, our time of inoculation of *C. parvum* oocysts in soils and the volume of soil samples treated for *C. parvum* extraction are higher than in previously reported studies (Mawdsley et al., 1996a; Petersen et al., 2012).

## CONCLUSION

Given the intrinsic difficulties involved in the completion of experiments relative to the transport of *C. parvum* oocysts in soil

columns, and especially given the onerous nature of analytical measurements that are a requisite of these experiments, the research described here had from the outset a modest objective. Succinctly, this objective was the creation of a screening test to determine the expected range of transport patterns in the field under conditions that are the least favorable to oocyst transport (since the soils exhibit no preferential pathways) but are nevertheless likely to be found routinely in the field.

Experimental results we obtained demonstrate that *C. parvum* oocysts can be transported in the soil matrix. Although the number of *C. parvum* oocysts that broke through the soil matrix accounted for only a small percentage of the initial inputs at the soil surface, they still pose a significant threat to human health, especially in groundwater systems where the water table of the aquifer is near the soil surface, as a small number of *C. parvum* oocysts can lead to the waterborne disease cryptosporidiosis. In the context of climate change and extreme hydrological events, the risk of transport of *C. parvum* oocysts and contamination of aquifers augment as matrix flow increases under wetter conditions. Under these wetter climate conditions, extreme precipitation events may result in a large amount of water infiltrating the soil, and higher than normal frequency of extreme precipitation events may result in more continuously wet soil conditions. Such wet soil conditions would lead to higher soil moisture and soil hydraulic conductivity that would favor the mobility and transport of *C. parvum* oocysts, particularly through the soil matrix.

The soils used in the experiments vary simultaneously according to a number of parameters, whose influence cannot be teased apart via simple screening tests. Nevertheless, experimental results suggest the interplay of clay particles, organic matter, and calcium ions on the mechanisms controlling the transport and retention of *C. parvum* oocysts in natural soils. In this context, our work makes clear the urgent need to conduct research so as to simultaneously analyze the interaction of various processes that can affect both the mobility and transport of *C. parvum* oocysts in the subsurface environment in natural complex soil systems. Although the interactions of these processes increase the difficulty of directly identifying and ranking the contribution of the hydrologic and biogeochemical processes that influence the transport of *C. parvum* oocysts, hypotheses can be developed from results of realistic experimental conditions identical, or similar, to conditions in either the field or in natural systems. Findings

from such studies, resulting from experiments conducted in soils and enabling the interaction of various processes, may be coupled with findings from experiments designed to investigate the influence of these processes separately and to establish a fundamental understanding of how these individual processes influence *C. parvum* oocysts behavior in the environment. Therefore, a more coherent picture of the interaction and influence of these processes concerning the transport of *C. parvum* oocysts may emerge from individual process-based experiments that either do or do not support hypotheses formulated from experiments involving various interacting processes.

An extension of the current study is necessary to characterize the surface properties of *C. parvum* oocysts upon which depend the interactions of *C. parvum* oocyst with surfaces and therefore their mobility in the natural environment, in particular soil environment systems. An extension of this work must also include the study of the effects of climate change and extreme hydrologic events on the fate and transport of *C. parvum* oocysts in soil. Among the many possible climate scenarios, the increase in soil wettability, an increase in the frequency of extreme precipitation events, and changes in biogeochemical cycles are likely to induce changes in the physical and biochemical properties of soil, as well as changes in the soil infiltration process, soil moisture, and hydraulic properties. Such climate change therefore will have direct and indirect effects on the risk of contamination of groundwater resources by *C. parvum* oocysts.

## AUTHOR CONTRIBUTIONS

The authors CD, AJ, and PB conceived and designed the experiment; CD, ZP, CY, and PB performed the experiment; CD, BL, ZP, AJ, and PB analyzed the data; and CD and PB wrote the paper.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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