Influence of soil structure on contaminant leaching from injected slurry

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ABSTRACT

Animal manure application to agricultural land provides beneficial organic matter and nutrients but can spread harmful contaminants to the environment. Contamination of fresh produce, surface water and shallow groundwater with the manure-borne pollutants can be a critical concern. Leaching and persistence of nitrogen, microorganisms (bacteriophage, *E. coli*, and *Enterococcus*) and a group of steroid hormone (estrogens) were investigated after

injection of swine slurry into either intact (structured) or disturbed (homogeneous repacked) soil. The slurry was injected into hexaplicate soil columns at a rate of 50 t ha⁻¹ and followed with four irrigation events: 3.5-h period at 10 mm h⁻¹ after 1, 2, 3, and 4 weeks. The disturbed columns delayed the leaching of a conservative tracer and microorganisms in the first irrigation event compared to the intact columns due to the effect of disturbed macropore flow paths. The slurry constituents that ended up in or near the macropore flow paths of the intact soil were presumably washed out relatively quickly in the first event. For the last three events the intact soil leached fewer microorganisms than the disturbed soil due to the bypassing effect of water through the macropore flow path in the intact soil. Estrogen leached from the intact soil in the first event only, but for the disturbed soil it was detected in the leachates of last two events also. Leaching from the later events was attributed to higher colloid transport from the disturbed soils. In contrast, NO₃-N leaching from the intact soil was higher for all events except the first event, probably due to a lower nitrification rate in the disturbed soil. A week after the last irrigation event, the redistribution of all slurry constituents except NO₃-N in most of the sections of the soil column was higher for the disturbed soil. Total recovery of *E. coli* was significantly higher from the disturbed soil and total leaching of mineral nitrogen was significantly lower from the disturbed soil. Results demonstrate how manure-borne constituents injected into undisturbed soil columns respond more as expected in the field, in terms of leaching and persistence, than do the same constituents injected into typically constructed columns of disturbed soil.

Keywords: Contaminants fate; Manure management; Microorganisms; Nitrogen; Estrogen; Repacked column

1. Introduction

Soil amendment with animal slurry, a mixture of urine, feces and water, is an economical and viable option for replenishing nutrients and organic matter in cropped soils (Hjorth et al., 2010). It is also a sustaining option for recycling nutrients at the farm level. Application of animal slurry into soil, however, can potentially introduce harmful slurry-borne contaminants into the environment (Lee et al., 2007). The contaminants of land-applied slurry may take different pathways to groundwater and/or surface water bodies via direct runoff, subsurface flow, leaching and percolation depending on the local hydrology (Bech et al., 2014; Kjaer et al., 2007). Inappropriate handling of manure can cause nitrate pollution to groundwater (Mantovi et al., 2006), eutrophication to surface water (Norrington and Jorgensen, 2009), pathogenic contamination of ready-to-eat type crops and untreated water facilities (Franz and van Bruggen, 2008), and aquatic ecology degradation by organic pollutants like steroid hormones (Sumpter and Johnson, 2008).

Identifying the best management practices in relation to animal slurry application to agricultural fields has become very important in the effort to reduce nutrient loading to the stream, contamination of the surrounding environment, and nuisance odor (Hjorth et al., 2010; Lee et al., 2007). Reduced tillage or no-till agriculture has been increasingly practiced worldwide as a soil and water conservation practice. No-till practice reduces the opportunity of incorporation of surface-applied manure with soil, which can augment the runoff loss of nutrient and manure-borne contaminants. Slurry injection on no-till agricultural land is therefore a slurry management technique recognized as a best management practice (Dell et al., 2012). It is important to know whether soil structural condition, disturbed or non-disturbed, prior to slurry injection makes any difference in the leaching and transport susceptibility of different manure-born constituents.

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77 Both intact (non-disturbed monolith-type) and disturbed soil (repacked columns) have been
78 used to study transport of contaminants in soil (Das et al., 2004; Lucas and Jones, 2009).
79 Both methods have some advantages and disadvantages as reviewed by Lewis and Sjöstorm
80 (2010). Disturbed soil is more homogeneous than intact soil. Macropore structure generated
81 through weathering and biogeochemical activities is generally destroyed during the repacking
82 process, altering the transport paths of water, solutes, and suspended colloids (Lewis and
83 Sjöstorm, 2010). Structural changes in the soil can crucially influence the filtering functions
84 that reduce direct groundwater contamination (Robinson et al., 2012). Flow through
85 macropores exerts non-equilibrium solute transport that shifts the sorption/attachment or
86 retardation of contaminants, and, thereby, reduces the filtering effect of the soil and the time
87 available for degradation (Jarvis, 2007; Laegdsmand et al., 2009). Presence of macropore
88 flow under field conditions has been identified to augment manure-borne estrogen transport
89 (Kjær et al., 2007; Laegdsmand et al., 2009). Macropore flows are also known to influence
90 the leaching of microorganisms (Safadoust et al., 2011).

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92 Understanding how the environmental fate of a range of slurry-borne contaminants responds
93 to a change in soil structure is important for soil and manure management options. A leaching
94 experiment using both intact and disturbed soil can show the comparative effects on the
95 environmental fate of different slurry-borne constituents. Soil sampled from a plough layer
96 represents the critical zone of contaminant transport under field conditions because this part
97 of the soil is the most biologically, physically and chemically active. The objective of this
98 study was to determine if disturbing field soil structure, when constructing laboratory soil
99 columns, significantly impacted leaching patterns of common pig slurry constituents. To this
100 end, we quantified the leaching patterns (percent leached, retention in soil and total recovery)

for intact and disturbed soil columns of commonly found contaminants in pig slurry: total organic carbon (TOC), mineral nitrogen (N), *Salmonella enterica* serovar Typhimurium bacteriophage 28B (phage) as a representative organism for viruses, *Escherichia coli* (*E. coli*) and *Enterococcus* spp. as representative organisms for pathogenic bacteria, and the summation of three types of estrogen (estrone, 17 α -estradiol and 17 β -estradiol) to represent steroid hormones.

2. Materials and methods

2.1. Soil and slurry

The study location was at Foulum Experimental Station (56° 29' N, 9° 34' E), Denmark. Fifteen intact soil columns, 20-cm long and 20-cm diameter, were collected from a crop field in a spring barley-winter wheat-spring barley rotation, as described previously in Amin et al. (2013). Soil at location is a Typic Hapludult loamy sand with a visible humic A-horizon, influenced by tillage and ranging as deep as 44 cm. Three repacked columns were prepared using soil from a single field and maintaining a consistent bulk density. Soil moisture content during repacking was at field capacity, and repacking was in accordance with the general guidelines of soil column preparation (Lewis and Sjostrom, 2010). All soil columns were saturated slowly and then drained to a soil water content (SWC) potential of -100 hPa (i.e., field capacity) to achieve uniform soil moisture.

To allow greater application under phosphorus-restricted nutrient management practices, the phosphorous-rich solid part of the raw animal slurry is usually separated out before land application (Hjorth et al., 2010; Liu et al., 2016). Thus, only the liquid fraction of mechanically separated swine slurry was collected from a farm near Åbøl, Denmark and stored at 2°C until applied to the soil columns. *E. coli* and *Enterococcus* spp. in the slurry

acted as model organisms for bacteria. The slurry was spiked with 1.5×10^6 PFU mL⁻¹ of phage and 2 g L⁻¹ of 2,6-difluorobenzoic acid (FBA; CAS RN 385-00-2; Sigma-Aldrich, Germany) before soil application to provide a model organism for pathogenic viruses and a nonreactive tracer, respectively. No significant toxicity effect of FBA on the microorganisms studied was found in a toxicity test before starting the main experiment, which agreed with the findings of McCarthy et al. (2000).

2.2. Experiment procedure

The experiment was conducted in a climate-controlled room at 10°C. Each soil column was placed on a glass filter disc of 60–100 µm pore size and 1.6 cm thickness (ROBU, Glassfiltergerate GMBH, Germany), on top of a stainless steel plate (Fig. 1). Suction of -12.5 hPa was maintained on each soil column's lower boundary by a water-filled space between the glass filter disc and stainless steel plate and by a hanging water column in a hypodermic needle attached to the stainless steel plate (Fig. 1) (Laegdsmand et al., 2005). The filtering effect due to the combination of filter disc, steel plate and hypodermic needle was tested, and the differences in contaminant concentrations entering and leaving the below-column setup were insignificant.

The mechanically separated liquid slurry fraction was added to hexaplicate intact and triplicate disturbed soil (repacked) columns by subsurface injection at a rate of 50 t ha⁻¹ (145 kg ha⁻¹ mineral N and 215 kg ha⁻¹ total N) as described by Amin et al. (2013). A slit was created at the center of the column surface to inject the slurry and then backfilled with soil loosely after slurry application, resulting in an injection shape, depth, and closure similar to a shallow-disk field coulter (Fig. 1). A rainfall simulator was calibrated to apply artificial rainwater (0.1mM NaCl, 0.01mM CaCl₂ (2H₂O), and 0.01mM MgCl₂ (6H₂O)); supplied by

VWR, Denmark) to the columns at a uniform intensity of 10 mm h^{-1} (Laegdsmand et al., 2009). Four 3.5-h long separate irrigation events were applied to each of the slurry injected columns 1, 2, 3 and 4 weeks after slurry application. Triplicate soil columns without slurry injection, as control, were also exposed to similar irrigation events. The control columns were undisturbed to study the background concentration of contaminants. We used acid-washed and sterilized equipment to avoid any contamination during experiment period.

Leachates were collected in sterilized blue cap laboratory glass bottles until percolation from each irrigation event had ended. Subsamples of leachates of the first irrigation event were collected at 15, 30, 60, 120, 180, 240 min for tracer breakthrough analysis. One week after the final (the fourth) irrigation event, soil columns were slowly pressed out from the stainless steel casing and sectioned into four subsamples—the original slurry hotspot (S1) and three other subsamples (S2, S3 and S4) (Fig. 1). All samples were prepared immediately after collection and analyzed for the selected contaminants within 24 hours.

2.3. Analytical methods

The gravimetric SWC was determined by drying (105°C for 24 h), and volatile solids (VS) content by combustion of dried soil at 550°C for three hours (Broadbent, 1965). A Sentron 3001 pH-meter (Roden, The Netherlands) was used for pH and a Radiometer conductivity-meter (Copenhagen, Denmark) for electrical conductivity (EC) measurement for both soil samples and leachates. A HACH 2100 AN turbidimeter (Hach, Loreland, CO) equipped with an EPA filter measuring at wavelengths 400–600 nm measured turbidity in the leachates. Concentration of TOC in the leachates was measured on a total organic carbon analyzer (TOC-V_{CPH}, Shimadzu, Duisburg, Germany). The leachates were analyzed for FBA concentrations by a LC-MS/MS technique following the method of Juhler and Mortensen

(2002). Both $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ concentration in soil extracts and leachates were analyzed on an Auto-analyzer III Digital Colorimeter (Bran & Luebbe, Germany). Soil subsamples extracted in 1 M KCl were filtered (GA55; Advantec, Japan) for this analysis.

A double-agar layer method (Adams, 1959) was followed for phage enumeration. The host strain *Salmonella enterica* serovar Typhimurium Type 5 was grown in Nutrient Broth (Oxoid) at 37°C for 4 hours. Next, two grams of soil were added to 18 mL Maximum Recovery Diluent (MRD, Oxoid) and sonicated for 30 s. One mL 10-fold diluted sample was mixed with 1 mL broth culture of the host strain and 3 mL soft agar, a mixture of 70% Blood agar base (Oxoid) and 30% Nutrient Broth (Oxoid). The mixture was spread on a well-dried Blood agar base plate (Oxoid) and incubated at 37°C for 18 h. Clear zones were counted as plaques forming units (PFUs). The error associated with the method is $\pm 20\%$ (Chu et al., 2001). The detection limit for the phage analysis was 10 PFU g^{-1} for soil and 1 PFU mL^{-1} for leachates.

Viable *E. coli* in each soil section and leachates were determined on *E. coli* Petrifilms (3M a/s, Denmark). Two grams of soil were mixed with 18 mL of 0.01 M phosphate buffer in a glass tube followed by sonication for 20 s (Vail et al., 2003). Ten-fold diluted soil and leachate samples were plated in triplicate and then incubated at 37°C for 24 hours. Characteristic blue colonies were counted as *E. coli* (CFU). The detection limit for *E. coli* was 10 CFU g^{-1} for soil and 1 CFU mL^{-1} for leachates.

Both soil and leachate samples were diluted in MRD as described for phage dilution. One mL diluted sample was spread on Slanetz and Bartley Medium (Oxoid, Denmark). The number of *Enterococcus* spp. was determined as typical red-maroon colonies on the Slanetz and Bartley

Medium following incubation at 44°C for 48 ± 4 h (DS 2401, 1999). The detection limit for *Enterococcus* spp. was 10 CFU g⁻¹ for soil and 1 CFU mL⁻¹ for leachates. Since concentrations of microorganisms were determined from triplicate diluted samples, average concentrations reported can be less than 10 CFU/PFU g⁻¹ soil or 1 CFU/PFU mL⁻¹ leachate.

Estrogens (estrone, 17 α -estradiol and 17 β -estradiol) in water samples were measured by a GC-MS/MS technique. Water samples were treated according to Laegdsmand et al. (2009) before analysis. Analysis was performed on a GC (CP-3800 GC, Varian), coupled to an ion trap MS (Saturn 2000, Varian). The GC was fitted with a DB-5MS column (30 m $\times$ 0.25 mm $\times$ 0.25 μ m, Agilent Technologies, Denmark) and temperature programmed as in Laegdsmand et al. (2009). Analytes were ionized by electron ionization (70 eV) and non-resonant collision induced dissociation was applied for further fragmentation of selected precursor ions.

2.4. Statistical analysis

The statistical software R was used for statistical analyses (R Development Core Team, 2009). Analysis of variance (ANOVA) of the data was conducted at the 95% confidence level to evaluate differences in leaching of the contaminants between treatments.

3. Results and discussion

3.1. Leachates and soils

Physicochemical properties of soil and slurry are shown in Table 1. The initial N content in soil was very low and microorganisms and estrogens were not detected. In contrast, the concentrations of the constituents in slurry (total N 4.3 g kg⁻¹, total solids 3.14%, VS 1.97%, *E. coli* 2.6×10^4 CFU mL⁻¹, and *Enterococcus* sp. 3.2×10^4 CFU mL⁻¹) were high enough to act as model indicators for this leaching study.

One week after the final irrigation event, SWC in all sections except S3 of the disturbed soil (repacked columns) was significantly greater than those of the intact columns (Fig. 2). Mixing and disturbance of soil aggregates presumably increased water holding capacity by decreasing both the number of larger macropores and very fine micropores and increasing the number of capillary pores (Schjonning et al., 1998). It also increased matrix flow by reducing any possible preferential flow path. The disturbed soil had higher pH in all sections than did the intact soil, probably due to the effect of the higher redistribution of slurry constituents in disturbed soil by the end of the final irrigation event (Fig. 2). Correspondingly, leachate pH from the disturbed soil columns was consistently less than from the intact soil throughout the experiment, but increased steadily from the first through the fourth irrigation events while electrical conductivity decreased steadily (Fig. 3).

Soil section S1 (the slurry-applied slit), in both the intact and disturbed columns, maintained a higher SWC than the other sections one week after the last irrigation event, whereas SWC was similar in soil sections S2–S4 of all treatments (Fig. 2). The organic matter of slurry retained in S1 possibly increased the residual SWC as described by other studies (Amin et al., 2014; Petersen et al., 2003). Volatile solids content can be used as an indicator of organic matter. Values of VS, pH, and EC were greater in S1 for both column types as a result of the effect of residual slurry constituents.

3.2. Non-reactive tracer and total organic carbon (TOC)

The breakthrough curves of the tracer from two soil structures differed (Fig. 3). For the intact soil, the highest concentration of FBA in leachate was found in the 1st irrigation event; after the 2nd irrigation event the amount of FBA leached decreased linearly. In contrast, the leachate concentration of the tracer for the disturbed soil was much lower in the 1st event,

peaked in the 2nd event, and then started to decrease steadily. The difference in the 1st event was attributed to the macropore flow pattern in the intact soil, which was largely non-existent in the disturbed soil. Macropore flow, often observed in intact soils, is a mechanism leading to preferential flow, and it is reflected in the early arrival of tracer. The disturbed soil produced higher leaching of FBA in 2nd through 4th irrigation events than the intact soil.

The leaching pattern of TOC was similar to that of the nonreactive tracer. The hydrophilic parts of organic matter are transported in solution as a non-reactive tracer. However, the hydrophobic constituents of organic matter could be attached to soil particles (Dunnivant et al., 1992), explaining why the difference in TOC leachate between the two column types during the 2th–4th irrigation events was smaller than the difference in non-reactive tracer FBA (Fig. 3). Although the leaching patterns for the two soil column types were somewhat different, the total amount of TOC leached over the four irrigation events from each was similar, 216±13 and 217±25 mg for intact and disturbed soil, respectively.

3.3. Mineral nitrogen (N)

Total mineral N leached from the disturbed columns was lower than that of the intact columns during the experimental events and percentage retained in the disturbed columns was higher, but the total recovery was similar (Table 2). Pradoa et al. (2011) conducted a nitrate fate study using both intact and disturbed soil and found that water bypassed 27% of pore space in intact column because of preferential flow pathways. They observed that nitrate fate in the soil profile was affected by preferential flow in intact soil. This process increased the NO₃-N mobility, thus reducing the resident solute time and increasing the risk for groundwater contamination.

Due to matrix-flow dominated conditions in the disturbed soil, $\text{NH}_4\text{-N}$ came into contact with more sorption sites than in the intact soil (Abdulgawad et al., 2008). Accordingly, sections S1–S3 of the disturbed columns retained more $\text{NH}_4\text{-N}$ a week after the final irrigation event than those of the intact columns (Fig. 2). Also, in the first three irrigation events, the disturbed columns released less $\text{NH}_4\text{-N}$ than the intact columns (Fig. 3).

The leaching pattern of $\text{NO}_3\text{-N}$ was markedly different for the two soil conditions. Leaching of $\text{NO}_3\text{-N}$ decreased from the first through the third irrigation events for the disturbed soil while it increased linearly for the intact soil (Fig. 3). Leaching of $\text{NO}_3\text{-N}$ from the disturbed soil in the 1st irrigation event was even higher than that of the intact soil. The nitrification rate in the disturbed soil during the time between slurry injection and the 1st irrigation event was probably higher, allowing $\text{NO}_3\text{-N}$, the more mobile form of mineral N, to accumulate sufficiently for leaching in the first irrigation event. This higher initial nitrification before the irrigation event in the disturbed soil can be attributed to the higher initial redistribution of slurry in soil (Petersen et al., 2003) and soil aeration during repacking. After the 1st irrigation event, SWC was comparatively higher in the disturbed soil as discussed earlier, which could reduce nitrification after the 1st irrigation event and reduce $\text{NO}_3\text{-N}$ leaching from 2nd irrigation event.

3.4. Microorganisms

Leaching of microorganisms as affected at the temporal scale by the soil structures was similar for all species studied (Fig. 3). In the 1st irrigation event, microorganisms leached from the disturbed soil were either equal (bacteria) or lower (phage) than those from the intact soil, but for the rest of the irrigation events the disturbed soil resulted in higher leaching (Fig. 3). This trend was also shown by the non-reactive tracer and TOC. However,

these differences were not significant for *Enterococcus* spp. In addition to the effects of soil structure, microorganism leaching generally decreases with time because of its natural die-off. These results are in general agreement with other findings of microbial transport (Mawdsley et al., 1995). Safadoust et al. (2012) reported that earlier peaks in the breakthrough curves of *E. coli* were observed for weathered soil columns compared to those for disturbed soil.

Although the temporal leaching pattern of phage and *E. coli* differed across the two soil conditions, no significant difference in total leached amount was observed for any microorganism (Table 2). Total retention and recovery for the two soil conditions were also similar except for *E. coli* (Table 2). *E. coli* retained, and recovered, in soil after the final irrigation event was significantly higher in the disturbed columns compared to the intact columns.

Concentrations of *E. coli* across the four soil sections were higher in the disturbed soil compared to the intact soil (Fig. 2), suggesting that the redistribution and/or survival of *E. coli* was higher in the disturbed soil. Relatively higher SWC and medium pore size in the disturbed soil presumably helped *E. coli* survive longer, facilitating higher leaching of viable *E. coli* in 2nd–4th irrigation events. Redistribution levels and survival rates of *E. coli* were proportional, as observed by other studies (Amin et al., 2014). Better redistribution of *E. coli* towards relatively smaller pores in the disturbed soil may have provided physical protection against predation (Kim and Corapcioglu, 2002; Vargas and Hattori, 1986). In contrast, the concentrations of phage and *Enterococcus* spp. in different soil sections were not affected by the soil structures (Fig. 2).

The redistribution of phage was higher due to its smaller size compared to bacteria. *Enterococcus* spp. was not detected in S4 of the disturbed columns and was detected at very low concentrations in S4 of the intact columns while the concentrations in S1 were still much higher (1000 CFU g⁻¹) for both soil conditions (Fig. 2), suggesting a slow transport of these bacteria. *Enterococcus* spp. cells are smaller than *E. coli*, but the chain organization of cells may have caused lower mobility in soil (Kokkinos et al., 1998; Pachepsky et al., 2006).

3.5. Estrogen

Estrogen was detected in leachates of the intact columns in the 1st irrigation event only, but it leached during the last two events also for the disturbed columns (Fig. 3). Estrogen sorption to slurry organic matter is very high (Lucas and Jones, 2009). Sorption of estrogen to any sorbent material increases with its TOC content. The solid–liquid partition coefficients of estrogen with slurry particles were 10 to 30 times higher than those of soils (Amin et al., 2012), so any release of estrogens from the slurry slit (S1) to the surrounding soil was possibly very low. When some estrogen released with the liquid phase of the slurry, its movement in soil was retarded because it readily sorbs to the soil particles (Das et al., 2004). Estrogen also degrades quickly in soil (Lee et al., 2007). However, colloid-mediated as well as preferential flow-facilitated transport of estrogen up to one meter soil depth is reported in literature (Kjær et al., 2007; Laegdsmand et al., 2009). The detection of estrogen in the last two irrigation events of the disturbed soil was attributed to the slurry-colloid mediated transport. The turbidity data of the disturbed soil supports this argument. de Jonge et al. (2004) suggested that colloids that are mobilized (dispersed) and stable in suspension are able to carry strongly bound contaminants downward and can increase the contamination of soil and groundwater.

3.6. *Implications and limitations*

Pattern differences in leaching of the nonreactive tracer between the disturbed and intact soil during the first irrigation event, as also supported by the leaching patterns of phage and TOC, suggest that there were influences of preferential flow paths in the intact soil columns. Slurry constituents/ contaminants that ended up close to and/or in the preferential flow path were transported with infiltrating water relatively quickly in the 1st irrigation event. The remaining slurry constituents in the soil matrix received less interaction with infiltrated water because some of water bypassed via the preferential flow path. In the homogeneous disturbed soil, slurry constituents interacted more with infiltrated water in the matrix-flow dominated condition. Leaching of all contaminants/ microorganisms except mineral nitrogen during 2nd–4th irrigation events was thus higher for the disturbed columns compared to the intact columns.

Preferential flow phenomena were apparently not dominant in this study because clay content (8%) was low. In structured soil with high clay content, preferential flow can have pronounced effect on the transport of contaminants (Horn, 1990; Jarvis, 2007). The method of introducing contaminants to the soil environment may interact with soil structure to influence leaching pattern and persistence, e.g. contaminants with surface applied versus injected slurry (Forslund et al., 2011) and application on ploughed versus no-till soils (Saini et al., 2003). In this study, contaminants in a single-injection slurry scenario were studied, but the effect of soil structure would be more pronounced in a scenario for which contaminants were introduced over multiple irrigation events (i.e., Safadoust et al., 2011). In the latter scenario contaminants can bypass the majority of the soil by moving through the active macropore flow path in each event. More tortuous, finer pores within soil aggregates in the

bulk portion of the soil can slow down the water and chemical transport as well as retard the chemical exchange processes (Horn, 1990).

4. Conclusions

Changes in soil structure before manure injection significantly impacted the leaching pattern of slurry-borne contaminants/constituents. Particularly for biologically and chemically active manure-borne compounds, disturbing the soil adversely influenced the results as compared to the expected field response, which was represented by the intact soil columns. The findings should be considered when drawing conclusions from leaching experiments with only disturbed columns. These findings will be useful in assessing the manure, tillage and soil management practices for mitigation and regulatory strategies relative to nutrient loss reduction and fecal bacteria contamination of soil and water. Similar studies with different textured soils and different methods of introducing contaminants to the soil environment broaden the applicability of the knowledge gained by this study.

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536 Table 1

537 Selected physicochemical properties of soil and animal slurry used.

Properties	Soil	Slurry
Bulk density/ density (g cm^{-3})	1.53	1.01
Porosity (%)	41	NA ^a
Texture: clay, silt, sand (%)	8, 13, 79	NA
EC ^a (mS cm^{-1})	0.047	15.8
pH	6.3	7.6
NH ₄ -N (g kg^{-1})	0.0005	2.9
Total N (g kg^{-1})	0.012	4.3
<i>E. coli</i> (CFU mL^{-1})	ND ^a	2.6×10^4
<i>Enterococcus</i> sp. (CFU mL^{-1})	ND	3.2×10^4
TOC ^a (%)	2	1.15
K ^a (mm h^{-1})	61.1	NA

538 ^aND indicates not detected, NA not applicable, EC electrical conductivity, TOC total organic
 539 carbon, and K hydraulic conductivity.

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Table 2

Average ($\pm$ standard deviation) percentage leached, retained, and recovered, of total applied mineral N, phage, *E. coli*, and *Enterococcus* spp. for different treatments. Means within a constituent and single column that are followed by different letters are significantly different at $P < 0.05$.

Constituents	Soil	Leached (%)	Retained (%)	Recovery (%)
Mineral N	Intact	22 ± 2 a	59 ± 5 a	81 ± 6
	Disturbed	17 ± 1 b	69 ± 4 b	86 ± 3
Phage	Intact	15.9 ± 8.4	3.1 ± 1.3	19 ± 8.4
	Disturbed	9.3 ± 3.6	4.7 ± 2.3	14 ± 4.7
<i>E. coli</i>	Intact	0.61 ± 0.34	11.4 ± 9.5 a	12 ± 9.3 a
	Disturbed	0.82 ± 0.10	60.2 ± 3.5 b	61 ± 3.5 b
<i>Ent. spp.</i>	Intact	0.17 ± 0.17	17.0 ± 11.0	17.2 ± 10.9
	Disturbed	0.12 ± 0.01	15.3 ± 1.1	15.4 ± 1.1

Fig. 1. Setup of the leaching experiment (S1, slurry slit; S2 and S3, surrounding soil; and S4, bottom section of the column).

Fig. 2. Within-soil measurements of pH, electrical conductivity (EC), volatile solids (VS), soil water content (SWC), $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, Phage, *E. coli*, and *Enterococcus* species, taken at different soil positions (S1, slurry slit; S2 and S3, surrounding soil; and S4, bottom section of the column) of the intact and disturbed soil columns one week after the final irrigation event. The error bars are the standard error.

Fig. 3. Leachate measurements of total organic carbon (TOC), FBA (a conservative tracer), $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, estrogen, phage, *E. coli*, *Enterococcus* species, pH, electrical conductivity (EC), and turbidity during each of the four irrigation events, for both intact and disturbed soil conditions. The error bars are the standard error.

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