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By

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ABSTRACT

EFFECTS OF GROWING AND DESICCATED ROOTS
ON *E. COLI* MOVEMENT THROUGH SOIL COLUMNS

By

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Pathogen transport to surface and groundwater is a serious environmental concern when untreated livestock manure is applied to farmland. Artificially drained farmland is particularly at risk because preferential flow pathways can transport contaminants directly to subsurface drains with no opportunity for filtration. A column study was performed to evaluate the effects of the growth and decay of roots on the saturated hydraulic conductivity (Ksat), miscible displacement of a bromide tracer, transport of *E. coli*, and breakthrough of the P22 bacteriophage in loamy sand soil. Compared to bare soil, the initial corn growth decreased the saturated hydraulic conductivity (Ksat) at a rate of -0.5 to -0.75 cm/h-week, but Ksat values increased when the corn plants were killed and the roots decayed. Root regrowth caused a slight decrease in the Ksat (0.06 cm/h-week). There was no detectable difference in the displacement of a bromide tracer through the columns due to root growth/decay. When swine manure was applied there was no significant difference between the initial concentration of *E. coli* in the effluent from bare columns with and without manure. Growing or decaying roots increased the rate of bacterial transport through the soil columns. The recovery of the P22 bacteriophage applied in manure was greater than when applied in deionized water due to a lack of competition for adsorption sites. When applied in manure to a cereal rye cover crop, the bacteriophage broke through and peaked at the same time or slightly before the *E. coli*. The P22 bacteriophage appeared to be a suitable microbial marker for field testing for linking a manure application with water contamination at the field scale.

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CHAPTER 1

1. INTRODUCTION

In Michigan and throughout the Midwest, drainage improvement is a common practice (Fausey et al., 1995; Brown and Ward, 1997). The Great Lakes states include three of the top four states (Illinois, Indiana, Iowa, and Ohio) in the U.S. in the area of land with drainage improvement. Drainage improvement in these states accounts for 37 percent of the total cropland in the region, over 20.6 million hectares of drained land (USDA-ERS, 1987). Much of this land is in animal production areas where animal manure is returned to the land as part of an important nutrient cycling, soil improvement and manure treatment program. Some livestock producers have reported high bacteria levels in drain effluent following manure application even though they followed specific guidelines outlined in comprehensive nutrient management plans (CNMP's). The first rainfall produced the greatest bacterial concentrations irrespective of the application technique or length of time the manure was on the ground (Saini et al., 2001; McMurry et al., 1998).

Contamination of surface and groundwater from the land application of livestock manure has been well documented (Crane et al., 1983; Mawdsley et al., 1994; Pell, 1997). Preferential flow through macropores and soil cracks has been shown to contribute to the movement of manure contaminants to subsurface drains (Fleming and Bradshaw, 1992; Shipitalo and Gibbs, 2000; Jamieson et al., 2002). Macropores are large, continuous openings in the soil formed by plant roots, soil fauna, cracks, fissures

and other natural phenomena. Such pollutants contribute to the eutrophication of surface waters, and water-borne pathogens are an immediate health threat. Shelton et al. (2003) reported that the average velocity of bacteria transport was seven time faster than the average pore velocity of the water, indicating that most of the flow bypassed the soil matrix.

Over the last several years, many farmers have adopted low-disturbance tillage and soil conservation practices that improve profitability and protect the environment. Cropping systems that reduce tillage intensity and increase the use of cover crops improve soil quality and protect the environment in many ways. Tillage can influence the flow of water and transported pollutants in surface and subsurface water by altering the orientation of soil aggregates, disconnecting preferential flow channels and soil cracks, anchoring plant residues within the surface layer, and inducing physical changes in the soil macro- and micro-pore structures. No-till soils often have more continuous flow channels (macropores) than tilled soils (Shipitalo and Protz, 1987; Drees et al., 1994; Pagliai et al., 1995), and this may contribute to the rapid movement of liquid manure to subsurface drains in no-till cropping systems.

When manure was applied to a bare soil surface, near-surface filtration and accumulation of bacteria increased the chance of contaminant transport in the runoff water (Crane et al., 1980). When manure slurry is applied to a vegetative surface, the near-surface zone of high biomass and organic matter can enhance adsorption, straining and filtering of enteric bacteria. Lim et al. (1998) showed complete coliform removal of up to $2x10^7$ colony forming units (cfu) per 100 ml, in passing through a 6.1 m wide, tall fescue filter strip. Coyne et al. (1995) reported 43-74% removal of coliforms, up to 10^8

cfu per 100 ml, in passing through a 9 m wide mixed Kentucky bluegrass and tall fescue filter strip.

While cover crops have been shown to be effective in mitigating overland flow of sediment and bacterial contaminants, little is known regarding the effects of a cover crop on bacterial movement through the soil. In Michigan, liquid manure is often applied after corn grain or corn silage harvest when the corn roots are in decay. Roots create channels through which bacteria may travel and which may reduce the filtering effects of soil particles (Dazzo, 1972), but roots also may preferentially inhabit macropores created by the decayed roots of the previous crop and thus restrict water movement through the soil (Smucker et al., 1995). And, there is evidence that some plants create a rhizosphere environment favorable to the survival of enteric pathogens while others may create a more unfavorable environment. Gagliardi and Karns (2002) reported greater persistence of *E. coli* 0157:H7 on rye roots (47-96 d) and alfalfa roots (92 d) than in bare soil (25-41 d), but the persistence on crimson clover and hairy vetch roots were similar to bare soil.

In livestock cropping systems, the persistence and retention of microbial contaminants in the root zone may be important considerations when selecting cover crops for water quality protection. Where manure land application and sub-surface drainage coexist, a vegetative cover and active root system may provide an important opportunity to mitigate contaminant loss to the environment through sub-surface drains. There is a need to evaluate root systems as mechanisms for remediation of microbial contaminants and enteric pathogens.

The overall goal of this work was to improve the management of soil for water quality protection in livestock-based agro-ecosystems. The specific objectives were to:

1. Evaluate the effects of a growing or decaying root system on the saturated hydraulic conductivity and breakthrough for a bromide tracer in loamy-sand soil columns.
2. Evaluate the effects of a growing or decaying root system on the bacteriological water quality of effluent from soil columns following the application of liquid swine manure and a simulated 25-year storm event.
3. Evaluate the breakthrough of the P22 bacteriophage and *E. coli* from swine manure applied to soil columns as a potential in-tank tracer for linking specific on-farm manure applications with water quality impacts.

CHAPTER 2

2. LITERATURE REVIEW

2.1 Microbial water quality

Water contamination has caused many disease outbreaks in the last several decades, and can cause widespread disease in communities as large populations are served by distribution systems (Guan and Holley, 2003). The increase in outbreaks has been attributed to certain pathogens due to a greater ability to identify specific organisms, and increased surveillance and reporting requirements. *E. coli* O157:H7 was not identified as a human pathogen until 1982 (Kudva et al., 1998). *E. coli* O157:H7 and *Cryptosporidium* are among the most well known causes of waterborne disease outbreaks. Since 1985, there have been 12 documented outbreaks of *Cryptosporidium* in North America (Rose, 1997). One of the largest and deadliest *Cryptosporidium* outbreaks (403,000 cases, 54 deaths) occurred in Milwaukee, WI in 1993 (Auld et al., 2004). Contamination of the Walkerton, ON, Canada municipal water supply by *E. coli* 0157:H7 in May 2000 sickened 2,300 people and resulted in seven deaths (Unc and Goss, 2003; Stratton et al., 2004).

Linking water pollution with specific sources or activities can be difficult. In many cases, agricultural sources have been suspected as the source of the fecal pathogens, but this has rarely been confirmed (Guan and Holley, 2003). Besides the source, there is also a statistically significant relationship between heavy rainfall and the occurrence of disease outbreaks (Auld et al., 2004). In the Milwaukee case, cattle were the suspected source of the *Cryptosporidium* oocysts, which contaminated the water supply (Rose,

1997). The contamination of the Walkerton, ON water supply by *E. coli* O157:H7 and *Campylobacter* occurred when runoff from farmland contaminated a municipal well after several days of heavy rainfall. A rain event of this magnitude was only expected every 60 to 100 years (Auld et al., 2004).

2.1.1 Pathogens

More than 150 zoonotic pathogens have been identified in animal manure (Gerba and Smith, 2005). Zoonotic pathogens are microorganisms that are capable of causing disease in multiple species and are of particular interest in regard to human health when dealing with animal waste. These pathogens include bacteria, protozoa, and viruses. Some of the most well known pathogens are *Escherichia coli* O157:H7, *Salmonella*, *Yersinia*, *Campylobacter*, *Giardia* spp., and *Cryptosporidium parvum* (Pell, 1997; Stratton et al., 2002; Gerba and Smith, 2005).

Pathogens commonly enter the environment through land application of manure from livestock operations. The most commonly applied are dairy, swine, and poultry manures. The concentration of pathogens in swine and poultry manure is generally higher than in cattle manure (Unc and Goss, 2004). Livestock operations have become increasingly large leading to an increased amount of manure to distribute over a smaller land base and farmers generally apply manure close to the source because it is expensive to transport. Environmentally, this practice increases the likelihood of contamination of surface water and groundwater from both nutrients and pathogens. A large part of the cropland in Michigan is tile-drained and managed with no-till practices, thus increasing the number of macropores and preferential flow pathways available. This combination of factors

allows for the rapid transport of water and pollutants directly into the tile lines and to the surface water.

2.1.2 Indicator organisms

Pathogenic organisms present in the environment are often difficult to identify and isolate. In order to monitor these organisms, indicator organisms are used. Fecal indicator organisms are organisms that when present, indicate the possible presence of fecal and enteric pathogenic organisms (USEPA, 2001). There are several characteristics that an organism should have, to be useful as an indicator of water pollution. The organism should: (1) not normally be present in the environment, (2) be easy to detect using simple laboratory methods, (3) have concentrations that are correlated to the pathogenic organism, but in greater concentration, (4) have a longer survival than the pathogenic organism, and (5) not be able to replicate in the environment (USEPA, 2001; Scott et al., 2002).

The most commonly used indicators are total coliforms, fecal coliforms, and *E. coli*. Total coliforms are a group of bacteria found in animal and human feces (but also in soil). This indicator is often used by regulators to assess the safety of drinking water and groundwater that is going out for distribution. They are not as useful for environmental samples as they can be naturally present in the environment. Fecal coliforms are often used as an indicator organism for fecal contamination from warm-blooded animals and the potential presence of human pathogens (Stratton et al., 2004). The fecal coliforms include *E. coli*, which is often used as an indicator because it is naturally found in high numbers in fecal matter (Stratton et al, 2002; Table 2.1).

Table 2.1: Source-specific daily fecal indicator output (USEPA, 2001).

Source	Fecal coliforms Organisms/day	Reference*	Fecal streptococci Organisms/day	Reference*	Total coliforms Organisms/day	Reference*
Human	$2.0x10^{9}$	1	$4.5x10^{8}$	1	---	
Beef cow	$1.0x10^{11}$	2	$1.1x10^{11}$	2	$2.3x10^{11}$	2
Dairy cow	$1.0x10^{11}$	2	$5.9x10^{11}$	2	$7.04x10^{12}$	2
Horse	$4.2x10^{8}$	2	$2.6x10^{11}$	2	$2.2x10^{12}$	2
Chicken	$2.4x10^{8}$ $1.4x10^{8}$	1 2	$6.2x10^{8}$ $2.9x10^{8}$	1 2	$1.98x10^{9}$ ---	2 ---
Pig	$8.9x10^{9}$ $1.1x\ 10^{10}$	1 2	$2.3x10^{11}$ $3.2x10^{11}$	1 2	$2.7x10^{10}$ ---	2 ---
Sheep	$1.8x10^{10}$ $1.2x10^{10}$	1 2	$4.3x10^{10}$ $1.7x10^{10}$	1 2	$5.4x10^{9}$ ---	2 ---

* [1] Metcalf and Eddy, 1991
[2] ASAE, 1998

Enterococci, a sub-group of the fecal streptococci group, is of increasing interest because it seems to better predict the risk of gastrointestinal illness from recreational exposure (USEPA, 2001; Scott et al., 2002). For ambient waters, the EPA recommends the use of *E. coli* and enterococci rather than fecal coliforms, because they show a stronger correlation to gastroenteritis associated with swimming (USEPA, 2001; Table 2.2).

Table 2.2 EPA recommended criteria for indicators (USEPA, 2001).

Designated Use	Indicator	Criteria
Recreation	*E. coli*	Geometric mean of 126 cfu per 100 ml over a 30-day period with no sample exceeding the: 75% Confidence limit (CL) for designated bathing beach 82% CL for moderate use for bathing 90% CL for light use for bathing 95% CL for infrequent use for bathing
	Enterococci	Geometric mean of 33 cfu per 100 ml over a 30-day period with no sample exceeding the 75 to 95% confidence limit based on the designated use as shown in *E. coli* criteria.
	Fecal coliform	Geometric mean of 200 cfu per 100 ml over a 30 day period and no more than 10 percent of the samples exceeding 400 cfu per 100 ml.
Public drinking water sources	*E. coli*	90 % of the daily water samples must be under 100 cfu per 100 ml to remain unfiltered.
	Enterococci	90 % of the daily water samples must be under 20 cfu per 100 ml to remain unfiltered.
	Fecal coliform	10 cfu per 100 ml as an annual average for lakes and reservoirs 50 cfu per 100 ml as an annual average for flowing rivers and streams

Important criteria for indicator organisms are an inability to survive for long periods outside of the host, and an inability to multiply in the natural environment (Stratton et al., 2002). There is evidence that *E. coli* are not reliable indicators under certain conditions in the soil environment. Regrowth of *E. coli* has been documented after a summer manure application (Stratton et al., 2004). Two to three month survival for enteric microorganisms are typical, but some have been documented to survive for as long as five years (Mubiru et al., 2000). *E. coli* and *Enterococcus* spp. from swine has been shown to survive in soil for 40 to 68 days (Unc and Goss, 2004).

2.2 Factors affecting pathogen survival

Pathogens have been shown to have long survival periods in the soil environment and several researchers have reported on the factors associated with the survival of enteric organisms. Long survivals for *E. coli* O157:H7 have been recorded depending on the soil type (Guan and Holley, 2003). Survival of greater than 8 weeks was observed for *E. coli* O157:H7 in moist soil at 25 °C (Mubiru et al., 2000). Survival for up to 90 days was recorded under fluctuating environmental temperatures between -6.5 and 19.6 °C (Guan and Holley, 2003). Some of the most important environmental factors affecting survival include: moisture content, temperature, pH, exposure to sunlight, amount of nutrients and carbon available, organic matter content, soil type, oxygen concentration, age of the manure source, concentration of the source, and antagonistic effects and competition with other soil micro-fauna (Holden and Fierer, 2005; Wang and Mankin, 2001; Dazzo et al., 1973; Reddy et al., 1981; Warnemuende and Kanwar 2002).

2.2.1 Soil moisture and temperature

Soil moisture and temperature, such as cool, moist conditions are known to prolong survival (Jamieson et al., 2002; Sjogren, 1994; Dazzo et al., 1973; Gerba et al., 1975). Under saturated conditions, survival was 2 to 3 times longer at 5 ° and 10 °C than above 20 °C (Sjogren, 1994). Survival at field capacity (15% of saturation) was reduced for all temperatures evaluated (Sjogren, 1994).

Soil texture influences soil moisture. In sand, because of rapid drying, the survival of *Salmonella typhosa* was 4 to 7 days; in moist loamy sand, the organisms survived longer than 42 days (Gerba et al., 1975). Greater soil moisture was correlated with greater numbers of fecal coliforms in the soil (Entry et al., 2000a).

Temperature has also been shown to be key to survival. Reddy et al. (1981) reviewed several studies and reported that die-off of several enteric bacteria doubled with every 10 °C rise in temperature from 5 ° to 30 °C. *E. coli* survived longest at low temperatures -20, 4, and 23 °C (Kudva et al., 1998). The survival was greater at lower temperatures because microbial metabolic rates were slower which enabled stressed organisms to survive longer and adapt to the different environment (Guan and Holley, 2003). However, Jamieson et al. (2002) noted several studies indicating that freezing and thawing cycles were detrimental to bacterial survival. Kibbey et al. (1978) reported that treatments with the most freeze-thaw cycles had the fewest survivors.

2.2.2 Nutrients, carbon, soil texture and organic matter

The proportion of fine soil particles and organic matter have a large influence on moisture retention (Jamieson et al., 2002). Three times greater survival of *E. coli* was observed in soils with fine texture and high organic matter than in coarse textured soil (Wang and Mankin, 2001). Microbial activity is enhanced on silt and clay particles partly due to its ability to concentrate nutrients through adsorption (Dazzo, 1972). Fine textured soils also have the ability to provide refuge from predators (Wang and Mankin, 2001). Soil texture seems to play an important role in the vertical transport of fecal coliforms; they are transported deeper in a sandy loam than a clay loam (Roodsari et al., 2002).

In agricultural production, soil tillage affects the level of nutrients in the soil; fields in no-till cropping systems have greater levels of carbon, nitrogen, and organic matter than conventionally tilled land (Doran, 1980). Holden and Fierer (2005) reported that microbial biomass was greatest near the surface and declined rapidly with depth due to

the decreasing availability of nutrients. The die-off of *E. coli* in soil is negatively related to nutrient availability and the ability to sustain metabolic activities (Sjogren, 1994). Entry et al. (2000b) reported that fecal coliform concentrations were greatest in the top 5 cm of the soil. Gagliardi and Karns (2000) reported that levels of coliform bacteria and *E. coli* O157:H7 were positively correlated with nitrogen (NH_3 and NO_3) in the leachate; perhaps the organisms survived longer in the presence of available N, or were moved by chemotaxis following N sources through the soil. Coliform and *E. coli* levels were not correlated with phosphate content or turbidity, indicating that these organisms did not act as particulates.

2.2.3 pH, sunlight, and oxygen concentration

Most enteric organisms survive best in neutral to alkaline pH environments (Sjogren, 1994; Warnemuende and Kanwar, 2002). Reddy et al. (1981) reported that the survival of enteric bacteria outside the pH range of 5.8 to 8.4 was adversely affected. The survival rate in acidic peat soil (pH 2.9-4.5) was very low (Unc and Goss, 2004). The pH of the soil also affected the adsorptive capacity of the soil; lower soil pH was better for sorption (Marshall, 1971).

Sunlight has a detrimental affect on survival (Gerba et al., 1975; Bell, 1976). It is unclear how much of an effect ultra-violet (UV) light has on the survival of bacteria in surface applied manure. It is impossible to isolate the effects of UV light, high temperatures and low humidity in field data because they occur simultaneously (Bell, 1976). The increased die-off from sunlight appeared to be primarily from an increase in temperature and drying effects rather than the exposure to UV radiation (Dazzo et al., 1973). Pell (1997) noted that bacterial survival was limited by exposure to oxygen.

Enteric bacteria are adapted to the anaerobic conditions of the digestive tract. Anaerobic conditions prolong fecal coliform survival (Wang and Mankin, 2001; Pell 1997). Anaerobic conditions are also most likely to occur when the soil is saturated, increasing the amount of moisture available.

2.2.4 Manure effects on soil and survival of enteric bacteria

The application of manure to soil increased nutrient availability and was shown to extend the survival of enteric bacteria (Dazzo et al., 1973). Stratton et al. (2004) reported that manure significantly increased the number of *E. coli* in the soil. Manure increased both the nutrient level and organic matter content of the soil. Organic matter is important in bacterial survival and retention in several ways. Because of high levels of nitrogen in manure, carbon is the limiting factor to bacterial growth (Unc and Goss, 2004). The soil environment is carbon-limiting, so increasing organic matter increases the amount of carbon available for microbial growth (Buckley and Schmidt, 2002). Organic matter also increases the available water capacity (Lal and Shukla, 2004) thereby increasing survival. Adsorption and retention of enteric bacteria in the soil also increased with increases in organic matter (Marshall, 1971).

The concentration and type of microbial organisms in manure depends on the source animal, animal's state of health and age, and how the manure was stored and treated before use (Gagliardi and Karns, 2000; Crane et al., 1983). Young animals were shown to be more likely to shed *E. coli* O157:H7 (Pell, 1997). *E. coli* O157:H7 remained present in manure much longer after the animals stopped excreting it and this is thought to be a source for reinfection of livestock (Guan and Holley, 2003).

Long-term storage (6 to 30 weeks) of manure decreased the concentration of fecal coliform, total coliform, and fecal streptococci by more than 99%; however, reductions were inconsistent when fresh manure was added to the storage structure (Patni et al., 1985). *E. coli* O157:H7 survived best at temperatures below 23 °C without aeration (Kudva et al., 1998). Survival of more than a year was reported for *E. coli* O157:H7 in non-aerated ovine manure (Guan and Holley, 2003).

The addition of manure to soils can change the physical and chemical properties of the soil. In no-till soils, the presence of manure enhances the survival of *E. coli* probably due to the addition of nitrogen and enhanced microsite habitat (Gagliardi and Karns, 2000). Frequent addition of manure extended the survival of *Salmonella enteritidis* by modifying the soil environment (Dazzo et al., 1973). Finally, at least one study found the soil's ability to strain and filter the microbes can be enhanced by the fibrous organic matter from manure (Unc and Goss, 2004).

2.2.5 Soil fauna

Soil represents a complex web of physical, chemical, and biological interactions. Many microenvironments provide niches supporting great species richness (Buckley and Schmidt, 2002). In agricultural fields, there was greater biological activity near the surface in no-till than with conventional tillage systems, and this provided favorable conditions for the survival of indigenous soil microbes (Unc and Goss, 2004). Protozoa, nematodes, bacteriophage, and *Bdellovibro*, a soil bacterium, prey on enteric bacteria introduced in manure (Jamieson et al., 2002; Unc and Goss, 2004). Competition with other soil microbes for nutrients was reported to be a major factor in the die-off of enteric bacteria in the soil. Enteric organisms do not readily adapt to low nutrient availability.

Some native soil organisms produced antibiotics or toxic substances that inhibited the growth of enteric organisms (Reddy et al., 1981; Warnemuende and Kanwar, 2002). When enteric organisms were added to sterilized soil they exhibited greater persistence and enhanced survival from an increase in available nutrients and a reduction in inhibitory compounds (Guan and Holley, 2003; Jamison et al., 2002). The increase in nutrients from manure application may also increase the predatory population.

2.3 Adsorption, filtering, and transport

The fate and transport of bacteria in the soil matrix are unpredictable because they are subject to adsorption, physical filtration, growth, and death. The retention of bacteria depends on soil chemistry, properties of the microbial cells, the physical configuration of the soil, and the flow characteristics (Unc and Goss, 2004). Soil chemistry and the properties of the microbial cells are the primary influences on bacterial sorption to the soil particles. Flow characteristics and the configuration of the soil particle influence the number of bacteria that are removed through filtration, and transported through water movement.

Stamm et al. (2001) reported that transport processes affected nutrients and bacteria in different ways. Bacteria had more interaction than many nutrients with the soil matrix, because a lot of nutrients were water-soluble and bacteria were large particles subject to filtering and adsorption (Stratton et al., 2004). Physical filtration depends on soil particle size; fine textured soils with smaller pore space were more efficient filters. The removal of bacteria was inversely proportional to the particle size of the soil (Gerba et al., 1975). Significant removal of bacteria was achieved when the bacteria cell size was at least 5% of the size of the soil particles (Warnemuende and Kanwar, 2002). Adsorption appeared

to be the predominate cause of retention within soil, but it was often difficult to separate the adsorption and filtration processes (Reddy et al., 1981). Both living and dead cells appeared to act the same with respect to adsorption and transport (Marshall, 1971).

2.3.1 Filtering

Soil is generally assumed to be an efficient biological and mineral filter for many microbial organisms (Darnault et al., 2004). There was a positive correlation between the size of the bacteria and the fraction retained by the soil (Rockhold et al., 2004). Particle size was important in the transport of *E. coli* under saturated flow in sand columns. The most likely mechanism was physical entrapment based on particle size (Scholl et al., 1990). Wang et al. (2003) reported that 97% of *E. coli* moved through coarse sand, but only 67% moved through fine sand. Smaller pores were responsible for the majority of retention, but particle adsorption capacity was larger for smaller soil particles (Wang et al., 2003). Retention by filtration is likely less permanent and allows remobilization.

2.3.2 Adsorption

The factors influencing adsorption and desorption from surfaces are complex and include van der Waals' forces, electrostatic interactions, hydrophobic effects, and specific adhesion (Rockhold et al., 2004). Two of the most important factors that affect adsorption are the clay and organic matter content (Guber et al., 2005; Mawdsley et al., 1994; Warnemuende and Kanwar, 2002). Increases in either clay or organic matter increase the adsorptive capacity, but clay is the most important because of its large surface area (Ling et al., 2002).

The air-water interface is an important site for bacterial adsorption. Powelson and Mills (2001) reported that bacteria preferentially attach to the triple phase contact point

where air, water, and solid particles meet. Small amounts of air can drastically reduce transport of hydrophobic bacteria (Powelson and Mills, 2001). Bacteria preferentially colonize interfaces within the soil environment (Lehman et al., 2004). Compared to hydrophobic effects, surface charge plays only a minor role in adsorption of bacteria to mineral surfaces (Unc and Goss, 2004). Guber et al. (2005) reported that maximum attachment occurred in the absence of manure. The addition of manure modifies the properties of the soil and microbial surfaces because the presence of ions in the manure alters the adsorption potential (Unc and Goss, 2004). Some bacteria produce extracellular polymeric substances that buffer cells from desiccation and promote adhesion (Rockhold et al., 2004).

2.3.3 Transport

Bacterial transport to subsurface drains is affected by the timing, method and rate of manure application, soil structure, and rainfall intensity (Wang et al., 2001; Saini et al., 2001). Bacteria do not readily move through the soil matrix under unsaturated conditions, mainly due to the filtering and adsorptive properties of the soil and air-water interface (Wang and Mankin, 2001). Gravity and capillary forces dominate unsaturated transport processes in soil near saturation (Mohanty et al., 2001). Guber et al. (2005) found that more *E. coli* were retarded in slower flow columns.

Transport of bacteria is mostly passive. The path followed by water determines the direction of transport of the bacteria and the majority of bacterial movement happens under saturated conditions (Jamieson et al., 2002; Powelson and Mills, 2001). Researchers have reported that bacteria moved through preferential flow pathways (Guber et al., 2005; Mawdsley et al., 1994). Preferential flow occurs through two

mechanisms: 1) redistribution on soil surfaces prior to leaching based on surface elevation differences (some areas will infiltrate more liquid), and 2) movement through macropores (McMurry et al., 1998). Preferential flow pathways mainly consist of macropores; large continuous openings in the soil. Macropores are formed by plant roots, soil fauna such as earthworms, and cracks. Factors that affect the initiation and intensity of macropore flow are pore geometry, distribution, and continuity (Monhanty et al., 2001).

Large, interconnected pores are primarily responsible for bacterial transport (Guber et al., 2005; Scholl et al., 1990). Transport over significant distances requires that the pores are several times larger than the dimensions of the bacterium (Unc and Goss, 2004). During saturated conditions, water flowing through the macropores will bypass the majority of the soil profile, thereby reducing the amount of filtration and adsorption that can occur (McMurry et al., 1998). There can be significant transport through the soil profile even when cracks are not visible and the application rate does not exceed water-holding capacity of the soil (Shipitalo and Gibbs, 2000).

There is substantial proof of bacterial transport by preferential flow. Shelton et al. (2003) reported that the average velocity of bacteria transport was seven times faster than the average pore velocity of the water, indicating that most of the flow bypassed the soil matrix. Even though most macropores have tortuous paths, the velocity of the water flowing through them is much faster than the water that is moving through the bulk soil. McMurry et al. (1998) reported that more than 50 percent of the drainage was collected in less that 20 percent of the soil's cross-sectional area. This indicates that bypass flow is the rule rather than the exception in well-structured soils. There is also a significant

correlation between the percentage of drainage water exiting in an area, and the percentage of the fecal coliforms that were in that water (McMurry et al., 1998). The areas that had the most drainage also had the greatest concentration of coliforms. This reinforces the understanding that bacteria are transported with water movement.

No-till soils often have more continuous macropores than conventionally tilled soils due to the lack of tillage (Shipitalo and Gibbs, 2000; Stratton et al, 2005). Wang et al., (2001) found that disturbing the top 5-10 cm resulted in lower bacteria concentrations in the leachate. Tillage disrupts the structure of the soil and the surface connection of the macropores and retards the movement of bacteria (McMurry et al., 1998).

Timing and intensity of rainfall also influence the movement of bacteria through the soil profile. It has been widely reported that the first rain event is crucial in leaching of bacteria through the soil. The first rainfall produced the greatest bacterial concentrations irrespective of the application technique or length of time the manure was on the ground (Saini et al., 2001; McMurry et al., 1998). In one study, the percent recoveries of *E. coli* ranged from 69.3% to 72.2% for the first rain event (Saini et al., 2001). Increasing the intensity, frequency, or duration of rainfall, lead to greater movement of bacteria in the soil (Saini et al., 2001). Stratton et al. (2005) found that the non-growing season was responsible for more than 60% of the subsurface flow mainly due to the large amount of precipitation and snowmelt during this period. There was also a correlation between flow in the tile lines prior to manure application and the detection of bacteria in the tile lines (Joy et al., 1998). Saturated and near saturated conditions prior to manure application increases the probability of transport.

2.4 Rhizosphere effects

Anderson et al. (1993) described the rhizosphere as a zone of increased microbial activity and biomass at the root-soil interface that is under the influenced of the plant root. The rhizosphere is thought to influence the survival and growth of naturally occurring soil microorganisms (Ibekwe and Grieve, 2004). The presence of plant roots enhances microbial populations. The rhizosphere has a greater number of microorganisms than the surrounding bulk soil because it provides surface area for colonization, and root exudates or excretions are a source of nutrients (Anderson et al., 1993). Roots lose between 40 and 60% of the total photoassimilates, during each growing season (Smucker, 1984). They excrete several forms of soluble organic chemicals and can shed as many as 10,000 cells per day per plant providing many of the nutrients and carbon needed for bacterial growth (Ibekwe and Grieve, 2004; Anderson et al., 1993). An additional benefit of the rhizosphere is that it may provide a refuge from predators (Dazzo, 1972).

Little has been reported on the interactions between the rhizosphere and the fate and transport of enteric organisms. Bacterial communities differ in different root zones. There is continual change at the root-soil interface. These differences are dependent on several factors including soil type, plant species, nutritional status, age, stress, disease, and environmental influences (Yang and Crowley, 2000; Ibekwe and Grieve, 2004). Some plant root systems may create a favorable environment for enteric organisms while others may not (Dazzo, 1972). Gagliardi and Karns (2002) evaluated the persistence of *E. coli* on the roots of various plants in the presence and absence of manure and reported

that rye roots with manure greatly increased the persistence and activity of *E. coli* O157:H7.

There are considerable differences between the root structures of different species. Root systems that exhibit primarily downward growth create root channels through which the bacteria are transported with water movement, reducing the filtering capacity of the soil (Mawdsley et al., 1994). In a lysimeter study, the removal of fecal coliforms was most efficient in bare soil (99.8% removal). Amongst different root systems, millet (99.5 % removal) and sorghum (99.4% removal) were more efficient than oats (76.6% removal) (Dazzo, 1972).

Other root system differences are a function of the amount of branching and surface area. Fibrous grass roots provide a large surface area (Tufekcioglu et al., 1999). Even among grass species, differences in root structure can be seen. Warm season grasses usually have a greater root biomass than cool season grasses (Tufekcioglu et al., 1999). Also, perennial crops have approximately five times more biomass than annuals (Bird et al., 1998). Native grass species are tall, warm-season, sod forming grasses, that have dense fibrous root systems that can extend 5 to 15 feet deep (Conservation Commission of Missouri, 1980). Smucker et al. (1995) evaluated changes in saturated hydraulic conductivity (Ksat) as influenced by plant roots. Corn roots increased the Ksat while ryegrass roots caused a decrease. The die-off and decomposition of both root systems led to large increases in the Ksat. Planting a cover crop of ryegrass after the corn roots had decomposed reduced the Ksat to near pre-decomposition levels (Figure 2.1).

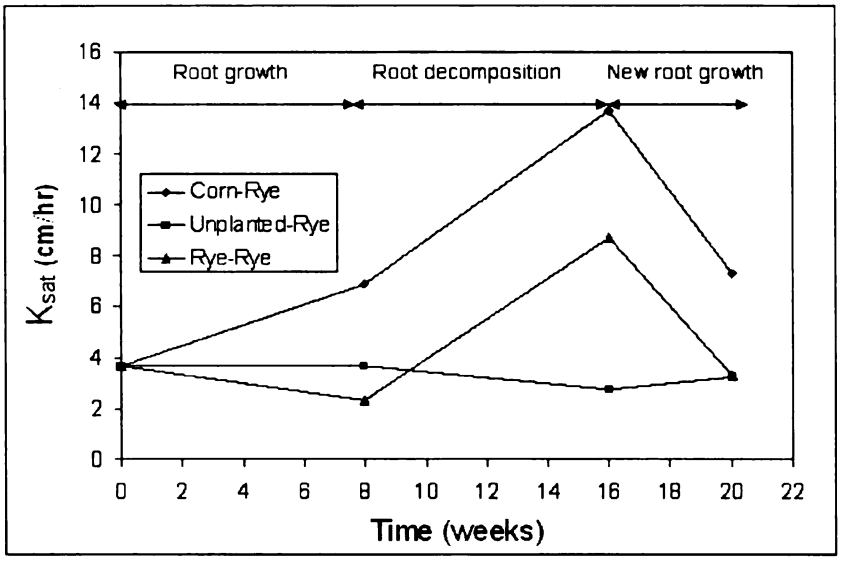

Figure 2.1: Effect of the influence of root growth, die-off and regrowth on saturated hydraulic conductivity (Smucker et al., 1995).

Planting of a cover crop is used to provide a living root system to absorb additional nitrates and plug many of the macropores left by the previous crop (Smucker et al., 1995). The root zone and rhizosphere of sod forming crops are zones of high biological activity, able to utilize the high nutrient loading from a manure application and reduce the likelihood of leaching. The rhizosphere may also prove to be useful in retaining the bacteria present in manure. An increase in the survival of enteric organisms in the soil will not significantly impact water quality if they are retained in the root zone.

2.5 Tracers for hydrologic studies

When testing for the presence of fecal contamination, *E. coli* are often the indicator organism used to gauge the likelihood of pollution of surface and ground waters from animal sources (Crane et al., 1983). *E. coli* has some capacity to multiply in the natural environment and survive for long periods outside a warm-blooded host. Depending upon the environment, it has been shown to reproduce under favorable conditions (Jamieson et al., 2002) and survive for extended periods at low temperatures. This ability to

reproduce, and the presence of multiple sources (e.g. from wildlife), can confound experimental results. Artificial tracers provide an unequivocal way to quantify the hydraulic properties of an aquifer, track the rates and pathways through a hydrologic system, or link specific sites of contamination to discharge points.

Many methods in microbial source-tracking use DNA fingerprints from a known reference library to identify the probable source or sources (Scott et al., 2002). Other methods look for a specific species of virus that is exclusive to the host of interest. An ideal tracer is chemically stable, conservative, inexpensive, and easily detectable; interacts predictably within the system into which it is introduced; and is readily available. Tracers need to be detectable at low concentrations and have low natural, background concentrations. Rhodamine WT ($C_{29}H_{29}O_5N_2Na_2Cl$) and sodium fluorescein ($C_{20}H_{10}O_5Na_2$), referred to as fluorescein dye or simply fluorescein, are fluorescent dyes commonly used in ground water systems. Fluorescent dyes need to be used with caution because they are known to adsorb to subsurface media (Kasnavia et al., 1998; Trudgill, 1987; Omoti and Wild, 1979; Smart and Laidlaw, 1977), and can break down when exposed to sunlight.

In managing animal manure, there is a need for an effective and inexpensive tracer for linking management actions with contamination sites. Studies of fecal contamination and transport often use a bacteriophage along with other tracers and indicators to track the movement of pathogens in the soil environment (McLeod et al. 2001, 2003; Vidales et al., 2003; Nicosia et al., 2001). Bacteriophages are generally safer than bacterial tracers, and they provide a better simulation of enteric virus movement characteristics and lifespan (Sinton and Ching, 1987; Leclerc et al., 2000). Bacteriophage assays are

less expensive and easier to perform than enteric virus detection techniques (Leclerc et al., 2000). A phage is often paired with bromide to compare the phage movement with that of a conservative tracer. McLeod et al. (2001, 2003) found that the host specific *Salmonella* phage moved more rapidly than the bromide tracer. Nicosia et al. (2001) reported that the PRD-1 bacteriophage and a bromide tracer were detected simultaneously. Differences in breakthrough are due in part to the virus removal capacities of the soil. Adsorption capacities increase with clay content, cation exchange capacity, and surface area, and decreased with increased organic content (Nicosia et al., 2001).

The bacteriophage PRD-1 is a virus that infects the bacterium *Salmonella typhimurium* as its host. Several aspects of this organism make it useful as a virus/colloid transport model: 1) its size and transport properties are similar compared to human enteric viruses, 2) detection methodology is relatively inexpensive and easy to perform, 3) it is not commonly found as a natural inhabitant of environmental waters, 4) it is harmless to humans, animals or plants, and 5) it is rather persistent once introduced to groundwater aquifers. PRD-1 has been successfully used as a groundwater tracer in the Florida Keys (Paul et al., 1995).

2.6 Column studies

2.6.1 Intact or undisturbed vs. repacked or disturbed columns

Several researchers have used repacked (Wang et al., 2003; Ling et al., 2002: Gagliardi and Karns, 2000; Powelson and Mills, 2001; Brown et al., 2001) or intact (Warnemuende and Kanwer, 2002; Gagliardi et al., 2001; Guber et al., 2005; Saini et al., 2001) soil columns to evaluate the fate and transport of *E. coli* in the soil environment.

The soil in repacked/disturbed columns is typically air-dried and passed through a sieve, removing stones and other debris. Intact/undisturbed soil columns are whole soil units, extracted by inserting a tube directly into the ground and then excavating around the tube. These columns contain all of the macropores, root channels, and wormholes that were at the location where they were taken.

The benefits of intact or undisturbed columns are that they better represent field conditions and provide better predictive values for natural conditions (Saini et al., 2001; Wang et al., 2001). From an experimental perspective, repacked columns create nearly homogenous conditions which make it possible to isolate key factors of interest that affect solute transport through the soil profile (Saini et al., 2001). A disturbed or repacked column lacks the macropores and aggregate structure found in the natural environment, and is more efficient at removing bacteria than an undisturbed column. Smith et al. (1985) reported that repacked columns removed 93% of *E. coli* added as a solution, whereas intact columns removed from 21 to 78% of the *E. coli*.

2.6.2 Column dimensions

Soil columns of varying dimensions have been reported in experimental work. Column widths ranging from 5 cm (Wang et al., 2003) to 22 cm (Mohanty et al., 2001) and column lengths of 15 cm (Brown et al., 2001) to 80 cm (Mohanty et al., 2001) have been used. Recent intact column studies evaluating bacterial transport through bare soil reported using cores 20 cm in diameter and 30 cm in length (Warnemuende and Kanwar, 2002; Wang et al., 2001; Saini et al., 2001). Gagliardi and Karns (2002) used repacked, 5 cm by 17.5 cm columns in evaluating the effect of plant roots on the rate of die-off of *E. coli* bacteria. The plant roots became constricted after 28 days of growth.

CHAPTER 3

3. METHODS AND MATERIALS

Corn and cereal rye crops were sown in soil columns and liquid swine manure was applied to evaluate the effects of root growth and die-off on the transport of microbial organisms through the soil profile. Key indicators of root-induced changes in the flow regime were saturated hydraulic conductivity (Ksat), *E. coli* concentration of the column effluent following a simulated rain event, and the breakthrough of bromide, *E. coli*, and a bacteriophage marker.

3.1 Soil columns

3.1.1 Soil column construction and filling

Repacked PVC soil columns 14.5 cm in diameter and 40 cm in length were prepared using soil from the 'sandhill' site (42°40'50.28" N, 84°28'00.84" W) on the University Farms in East Lansing, Michigan. The soil was gathered from the surface-to 20-cm depth, air-dried, passed through a 2-mm sieve and mixed to provide a homogenous soil mass. The particle size distribution based on the hydrometer method was 87.2% sand, 8.6% silt, and 4.2% clay (Table A2). The columns were filled to within 2.5 cm of the top using a large funnel to minimize particle size separation and layering. The soil columns were consolidated to an air-dry bulk density of 1.453 g/cm^3 (standard deviation of 0.02 g/cm^3). The base of each column was secured with several layers cheesecloth and a vinyl mesh screen held in place with a steel pipe clamp (Figure C1). Table A1 lists the soil

column physical properties including dry bulk density (g/cm^3), volume (cm^3), porosity (%), and pore volume (cm^3/cm^3).

3.2 Soil column flow regime

Several methods were used to evaluate changes in the soil column flow regime due to root growth and die-off. The saturated hydraulic conductivity was measured at the beginning of the experiment for all columns, and then at the end of every root growth period, after root decomposition, and upon completion of the experiment. Chloride breakthrough was evaluated at the beginning of the trials, and bromide breakthrough was evaluated upon completion of the experiment.

3.2.1 Saturated hydraulic conductivity

Saturated hydraulic conductivity (Ksat) measurements were made with a constant head permeameter to evaluate the water flow rate (cm/hr) through the columns. Soil cores were slowly wetted from the bottom, in a large tank, over three days, to ensure that the soil was completely saturated and without entrapped air (Figure C9). Two soil cores were tested concurrently in an apparatus similar to the one shown in Figure 3.1. A photo of the actual set up is shown in Figure C10. The downward vertical flow method was used, meaning that the water moved vertically through the column from the top to the bottom. A constant head of water was maintained over the soil core to measure the saturated hydraulic conductivity under steady state conditions. The saturated hydraulic conductivity was calculated using a variant of Darcy's Law as:

$$K_{sat} = \frac{V * L}{A * t * (H+L)}$$

where:

K_{sat} = saturated hydraulic conductivity (cm/h)
V = cumulative outflow at the base of the core (cm^3)
A = surface area of the soil core (cm^2)
t = time interval (h)
H = hydraulic head of the free water above soil surface (cm)
L = length of soil core (cm)

Measurements were taken of the outflow, time interval of collection, hydraulic head over the sample, cross-sectional area of the soil core, and length of the soil core, in order to calculate the hydraulic conductivity. Soil characteristics that affect saturated conductivity are total porosity, the distribution of pore sizes, and pore tortuosity.

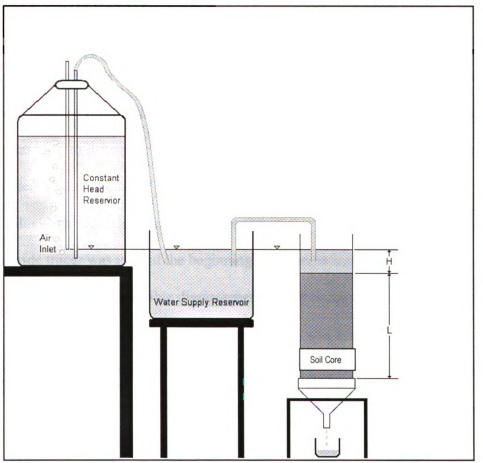

Figure 3.1: Diagram of constant head permeameter for measuring saturated hydraulic conductivity.

3.2.2 Anionic tracers

When a liquid of varying concentration from that in the soil pores is introduced to a soil column, there is a gradual mixing of the two liquids by diffusion and hydrodynamic dispersion. Plots of the outflowing solution's composition versus time or pore volume applied are called breakthrough curves. Ideal breakthrough curves are sigmoidal in shape with the inflection point representing 50% displacement at a cumulative flow of one pore volume if the soil is saturated (Hillel, 1998). Bromide tracers are typically the first choice for an anion tracer due to its lower concentration in the environment. Most studies (Clay et al., 2004; Hatfield et al., 1997; McLeod et al., 2001; McLeod et al., 2003) use bromide as a conservative tracer. In this experiment, both chloride and bromide tracers were used to characterize the flow regime of the columns. Chloride was used first to determine a baseline breakthrough for anion movement, at the end of the experiment bromide was used to determine if there were changes in the breakthrough of anions due to the applied treatments.

3.2.2.1 Chloride tracer

A chloride tracer was used at the beginning of the experiment to provide baseline information regarding the miscible displacement of soil borne solutes. A solution concentration of 200-ppm of potassium chloride (KCl) was used to fall within the range of the ion selective electrode (Thermo Orion, 0 to 200 ppm, ± 1.8 ppm) that was used to quantify the samples. A peristaltic pump was used to apply 250 ml of the 200-ppm KCl solution to the columns at a rate of 15 ml/min, followed by irrigation of tap water at the same rate. This application rate was chosen to simulate a one-hour, 25-year storm event for the region of southwestern Lower Michigan, approximately 5.33 cm/hr. Samples

were collected every 15 minutes until at least 1.25 pore volumes had been applied (3.5 to 4 liters). The applied volume was chosen to ensure that the whole breakthrough curve was collected.

3.2.2.2 Bromide tracer

At the end of the experiment, a bromide tracer was used to evaluate treatment effects on the movement of anions through the soil column. A tracer concentration of 200-ppm sodium bromide (NaBr) was chosen to fall within the range of the ion selective electrode (Cole Parmer, 0.4 ppm to 1000 ppm, ± 2%). Following the procedures established for the chloride tracer, 250 ml of the NaBr solution was applied to the columns, followed by irrigation water at the same rate. This was equivalent to about 4 hours of irrigation. This bromide breakthrough was compared with the chloride breakthrough to identify treatment induced changes in miscible displacement.

3.3 Experimental treatments

Treatments were selected to evaluate the effects of root growth, die-off and regrowth on saturated hydraulic conductivity and transport of *E. coli* bacteria through a soil column. Eight treatments with four replications (32 soil columns) were used to represent four conditions in a natural agro-ecosystem: 1) bare soil with no roots, 2) living root system with no previous crop, 3) a desiccated root system, and 4) regrowth of a living root system (cover crop) following the die-off and decay of the root system of the previous crop. These four conditions were evaluated both with and without manure.

The eight treatments represent a full factorial experimental design with two factors; crop (*bare, rye, desiccated corn* and *desiccated corn replanted with rye*), manure (without and with). Table 3.1 shows the eight treatments.

Table 3.1: Eight treatments

	No Manure	Manure
Bare	1. Bare	2. Bare/manure
Rye	3. Rye	4. Rye/manure
Desiccated Corn	5. D. Corn	6. D. Corn/manure
Desiccated Corn with Rye	7. D. Corn-Rye	8. D. Corn-Rye/manure

Details pertaining to each planting treatment are below.

- Treatments 1 & 2 – Bare soil, with and without manure – The bare soil treatments were the first to receive the manure application and be sampled.
- Treatments 3 & 4 – Rye, with and without manure – Cereal rye was planted and allowed to grow for 10 weeks before the manure application and sampling period.
- Treatments 5 & 6 – Desiccated corn roots, with and without manure – The corn was planted and allowed to grow for 6 weeks, when it reached the 6^{th} to 7^{th} leaf stage. The corn was killed and the roots decomposed for 12 weeks. These treatments received a manure application before the simulated rainfall.
- Treatments 7 & 8 – Rye planted in columns that had previously contained corn, with and without manure – The corn grew for 6 weeks before it was killed and the roots decayed for 10 weeks. Cereal rye was planted and grew for 10 weeks before the manure application and simulated rainfall.

3.4 Instrumentation

Ten of the 32 soil columns were instrumented with three temperature sensors (107, Campbell Scientific, Figure C2; Figure 3.2) and three soil moisture sensors (ECH_2O, 10 cm, Decagon Devices, Figure C3 and C4; Figure 3.2). See Figure C6 for a photo of the instrumented columns. One column within each treatment was instrumented, and one

treatment had three columns instrumented. Additionally, ambient air temperature and battery voltage were monitored and recorded.

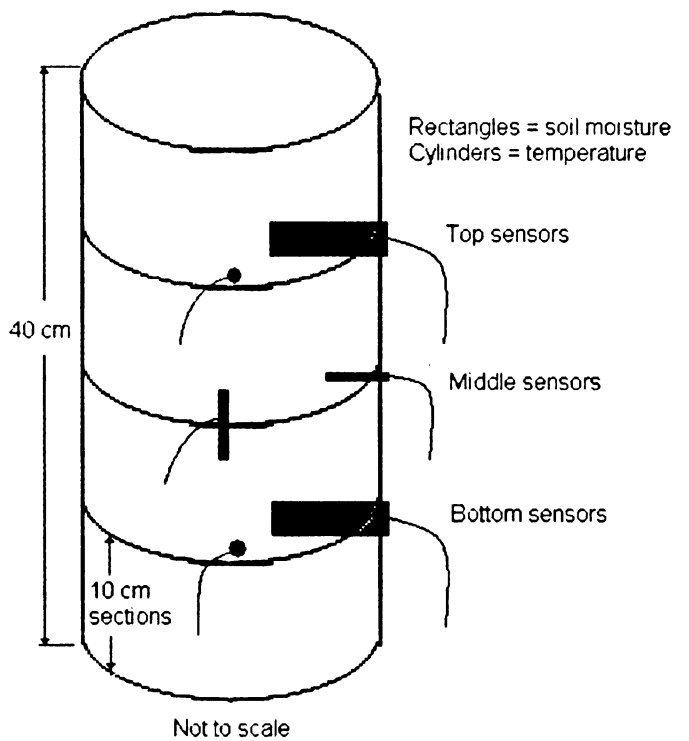

Figure 3.2: Diagram of instrument location within the columns.

A CR10 data logger and an AM416 multiplexer (Campbell Scientific) were used to record the data. Readings were taken every 5 minutes and an average value was recorded every 15 minutes. The program controlling the soil moisture and temperature collection was written by Jason Ritter at Campbell Scientific and is included is appendix E. A wiring diagram for the multiplexer is shown in Figure A1. A photo of the data logging system is shown in Figure C5.

The sensors were connected as single ended inputs to attach the maximum number of sensors to the multiplexer. A maximum of five columns could be attached to the data

logger at a time. This set up was feasible, as the experiment was staged over time to allow for the differing lengths of time needed for the growth of the various treatments.

Several of the ECH_2O soil moisture probes gave false readings at some point during the experiment. They would periodically drop to zero and then return to the previous reading. When this happened, the sensor was replaced.

3.5 Growing conditions

The soil columns were kept in the plant and soil sciences greenhouse on the MSU campus (Figures C7 and C8). Two grow lights mounted above the soil columns were on from 6 am to 10 pm daily. Water was added to the soil columns periodically throughout the experiment to prevent drying and cracking. The columns that contained growing plants were watered daily and nutrient solution was added once a week. The composition of the nutrient solution is listed in Table A4.

The soil temperature data recorded throughout the experiment showed large fluctuations over the course of a 24-hour period. The maximum temperature occurred during the day and the minimum temperature occurred at night and did not fall below 15 °C. The ambient temperature was usually above 20 °C in the greenhouse. Table D1 lists the average daily minimum and maximum soil temperatures, and minimum and maximum daily ambient temperatures (Figures D1-D12).

3.6 Manure application procedure

The manure was collected from the swine nursery two days prior to application. It was sieved through a 2-mm mesh to remove any large pieces. Two days prior to the manure application, the columns were deeply wetted and allowed to drain to bring them to near field capacity when the manure was applied. The amount of manure applied, 93

ml per column was equivalent to 56,122 l/ha (6000 gpa). The same procedure was followed for the treatments without manure except that an equal amount of water was applied instead of manure. The manure characteristics are listed in Table A3.

3.7 Rain events and sample collection

Two days after the manure application, a one-hour, 25-year rain event was simulated in which 5.33 cm of water was applied per column (Figure C11). The irrigation water (source: greenhouse hose) was applied using a peristaltic pump at a rate of 15 ml/min. Chlorine concentrations were not tested at the time of the rain events. However, the background concentrations from the chloride breakthrough portion of the experiment were approximately 25 ppm. Samples were collected every 10 to 15 minutes until flow stopped (Figure C12). The treatments that contained actively growing rye received one and a half hours of irrigation in order to obtain samples (Figures C13 and C14). The samples were kept on ice and were taken to the lab the same day for analysis.

3.8 *E. coli* quantification

The *E. coli* quantification was performed according to section 9222 G of the Standard Methods for the Examination of Water and Wastewater (Clesceri et al., 1998). Water samples were assayed for *E. coli* using the membrane filtration technique (Figure C15). Several different serial dilutions were used to obtain colony counts within the countable range, anywhere from 10^{1} for non-manure column samples, to 10^{-3} for manure column samples. The samples were diluted with sterile phosphate buffered water (PBW). Each dilution was filtered under vacuum through a 0.45 μm pore-size membrane and then plated on to EC Medium with MUG (Difco). Each dilution was run twice to provide a replicate. Sterile filter housings were used for each sample and dilutions were filtered in

these housings from most dilute to most concentrated to reduce contamination between dilutions. A negative control was also run after every sample. A positive control using C-3000 *E. coli* (ATCC 15597) was run with each batch of media. The plates were incubated at 44.5 °C for 24 ± 2 hours before being read under a 365 nm long wave UV light. The positive reaction for *E. coli* colonies fluoresced blue (Figure C16).

3.9 Breakthrough of P22 Bacteriophage

The breakthrough of the P22 bacteriophage and *E. coli* from an application of liquid swine manure were evaluated to assess the potential as a microbial marker in the manure slurry tank for linking specific manure application events with surface water contamination.

3.9.1 Column preparation

Twelve columns were selected and divided into four treatments: 1) bare soil, no manure, 2) bare soil, manure, 3) cereal rye cover, no manure, and 4) cereal rye cover, manure. The bare columns from the previous experiment were reused, and the unused columns were planted with rye. The bare treatments were run first to allow the rye to grow. The rye treatments were run when the rye was 4 to 5 weeks old (approximately 40 to 50 cm tall). The same procedures for manure application and rainfall simulation were used as for the earlier experiment. Two days prior to the manure application, the columns were deeply wetted and allowed to drain to near field capacity. Swine manure from the nursery at the MSU farm was used. The volume of manure applied, 93 ml per column, was equivalent to 56,122 l/ha (6000 gpa). The treatments without manure followed the same procedure except that an equal amount of water was applied instead of manure. Both the manure and the water application contained the bacteriophage P22.

3.9.2 Bacteriophage P22 tracer preparation

The *Salmonella* phage P22 was obtained by Dr. Joan Rose from Dr. Charles Gerba, University of Arizona, and was maintained on the host *Salmonella typhimurium* LT-2 (ATCC 19585). P22 stock were grown by inoculating 100-ml log-phase *S. typhimurium* host with one milliliter of P22 stock (~ 10^{11} pfu/ml) and incubated at 37 °C for approximately 3-5 hours. After incubation, 0.01 g of lysozyme and three milliliters of 0.2 M sterile EDTA were added to the flask and mixed well. The culture was then centrifuged at 4000 rpm for 10-15 min and the supernatant was filter sterilized through a 0.45 μm membrane. P22 stock was prepared by the staff in Dr. Rose's lab and stored at 4 °C until used.

3.9.3 Sample collection

Two days after the manure/water application, tap water was applied using a peristaltic pump at a rate of 15 ml/min. The simulated rain event was 3 hours in length for the bare columns and 4 hours for the rye columns. The longer irrigation period applied one to one and a half pore volumes and was used to ensure that the breakthrough curve for the phage would be collected. This time period was selected based on the simulated rainfall volume and the measured breakthrough of the bromide ion. Nicosia et al., (2001) found that the bacteriophage PRD1 was detected simultaneously or shortly after the bromide. Samples were collected every 10 to 15 minutes until flow stopped. The samples were kept on ice and cultured the same day.

3.9.4 Sample analysis

Water samples were assayed for P22 bacteriophage following the double agar layer procedure described by Adams (1959). Samples were diluted to at least 10^{-3} concentration and between 1 ml and 2 ml of each sample in triplicate were assayed for the phage presence on tryptic soy agar (TSA). The plates were incubated for 24 h at 37 °C. The detection limit of this method is less than one plaque-forming unit per ml.

For the bacteriophage portion of the experiment, the total coliform and *E. coli* were enumerated by using the Colilert®/Quanti-Tray®/2000 test kit (IDEXX Laboratories, Inc., Westbrook, ME). One ml of sample was added to 99ml of phosphate buffered saline (PBS) in a sterile plastic cup with pre-measured IDEXX reagent added. The mixture was mixed well and poured into an IDEXX Quanti-Tray/2000 and incubated for 24 hours at 35 °C. After incubation, a total- coliform-positive reaction turns the medium yellow, and an *E. coli*-positive reaction causes the medium to fluoresce under a long-wave ultraviolet light (366 nm). The results (in MPN/100ml) were calculated using the MPN table provided by IDEXX for both *E. coli* and total coliforms.

3.10 Statistical analysis

3.10.1 Saturated hydraulic conductivity (Ksat)

The saturated hydraulic conductivity of each soil column was measured at the beginning of the experiment. Each column was ranked by Ksat value (cm/h) and assigned to quartile groups. Eight treatment groups of four columns per replication were assembled with one column randomly selected from each of the four Ksat quartile groups. Each group of four columns was then randomly assigned to one of the eight treatments. A two-factor ANOVA with treatment and replication as factors was used to

test for significant differences in Ksat values between treatment groups. There were no significant differences between treatments ($p = 0.188$; Fig. 3.3).

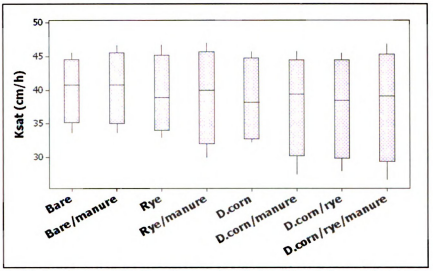

Figure 3.3: Initial saturated hydraulic conductivity (Ksat) for each treatment group.

3.10.2 Anionic tracers

3.10.2.1 Chloride

At the beginning of the experiment, a two-factor ANOVA with treatment and replication as factors was used to evaluate the breakthrough of a chloride tracer. The measures of anion breakthrough included the peak concentration (ppm), the pore volume applied at the peak (cm^3/cm^3), and pore volume applied at 10% and 50% cumulative chloride recovery. An empirical cumulative density function (CDF) graph for each treatment was used to compare the distributions of the 50% chloride breakthrough.

3.10.2.2 Bromide

The breakthrough of a bromide tracer was evaluated for each treatment group at the end of the experiment. The procedures used were the same as for the chloride tracer. A two-factor ANOVA with treatment and replication as factors was used to evaluate peak concentration (ppm), pore volume applied at the peak (cm^3/cm^3), and pore volume

applied at the 10%, 50%, and 90% cumulative bromide recovery. A CDF graph was used to compare the distributions of the 50% bromide breakthrough.

3.10.3 *E. coli*

E. coli concentrations were evaluated in the four manured columns and the *bare/no manure* soil columns. The *E. coli* data were fit to a normal distribution using the natural log of the measured *E. coli* concentration. A two-factor ANOVA was used to evaluate the cumulative number of *E. coli* eluted and the percent retention of *E. coli* for each treatment. The cumulative number of E. coli collected for each sample were calculated by taking the concentration of the E. coli in each sample (cpu/ml) and timing that by the total volume of sample collected (ml). Then the cumulative E. coli for each sample were summed for all of the samples taken for each column.

An ANOVA was run on the manured and the *bare* treatments using the General Linear Model (GLM) in Minitab (Minitab Inc., 2003) with crop (*Bare, Rye, D. Corn, and D. Corn/Rye*), manure (manure and no manure), and replication (1-4) as factors. The equation for the GLM was:

$$y = \mu + Crop + Manure + Rep + Crop*Rep$$

where y is the natural log of the *E. coli* concentration, μ is the overall mean, *Crop* is the crop main effect, *Manure* is the manure main effect, *Rep* is the replication main effect, and *Crop*Rep* is the interaction term.

The null hypothesis of no difference between treatments was tested at $\alpha = 0.05$. Pairwise comparisons between crop, manure, replication, and crop x replication were conducted using Tukey's multiple comparison tests with a family error rate of 0.05.

CHAPTER 4

4. RESULTS AND DISCUSSION

Winter cover crops can temporarily immobilize nutrients, especially N, prevent NO_3-N leaching losses and reduce winter soil erosion. When manure is applied to a vegetative surface, the near-surface zone of high biomass and organic matter could enhance adsorption, straining and filtering of microbial organisms. However, below the soil surface, roots create channels through which bacteria may travel and reduce the filtering effects of soil particles, but in a managed agro-ecosystem, the growing roots of a cover crop may fill the channels left by dessicated roots from the previous crop and inhibit preferential flow to subsurface drains or shallow ground water. This work evaluated the effects of root growth, die-off, and regrowth on saturated flow, the breakthrough of anionic tracers, and the movement of microbial organisms through recompacted soil columns.

4.1 Measurement of the column flow regime

4.1.1 Saturated hydraulic conductivity

The saturated hydraulic conductivity (Ksat) was measured at the beginning of the experiment for all columns, and then at the end of every root growth period, after root decomposition, and upon completion of the experiment (Figure 4.1 and Table A5).

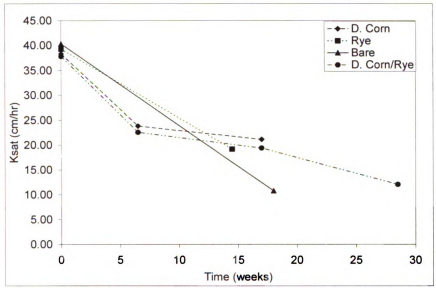

Figure 4.1: Average measured saturated hydraulic conductivity of the four crop treatments.

The measured Ksat of the bare columns decreased from near 40 cm/h at the beginning of the experiment to between 10 and 15 cm/h at the end, presumably from soil settling. Because the Ksat values changed over time, the measured treatments were normalized based on the bare column Ksat values. The N(Ksat) was a measure of the change in saturated hydraulic conductivity of each treatment relative to the Ksat of the bare columns (Figure 4.2).

The treatment Ksat values were normalized as:

$$N(Ksat) = (Ksat_{treatment} - Ksat_{bare})$$

Where:

N(Ksat) is the normalized saturated hydraulic conductivity, cm/h.

$Ksat_{treatment}$ is the average measured saturated hydraulic conductivity for the treatment of interest, cm/h.

$Ksat_{bare}$ is the saturated hydraulic conductivity of the bare soil columns, cm/h.

The Ksat of the bare columns was measured twice, at the beginning of the experiment and after 18 weeks. Because the greatest amount of settling likely occurred early in the trial, an exponential decay function was used to estimate the change in bare Ksat values over time.

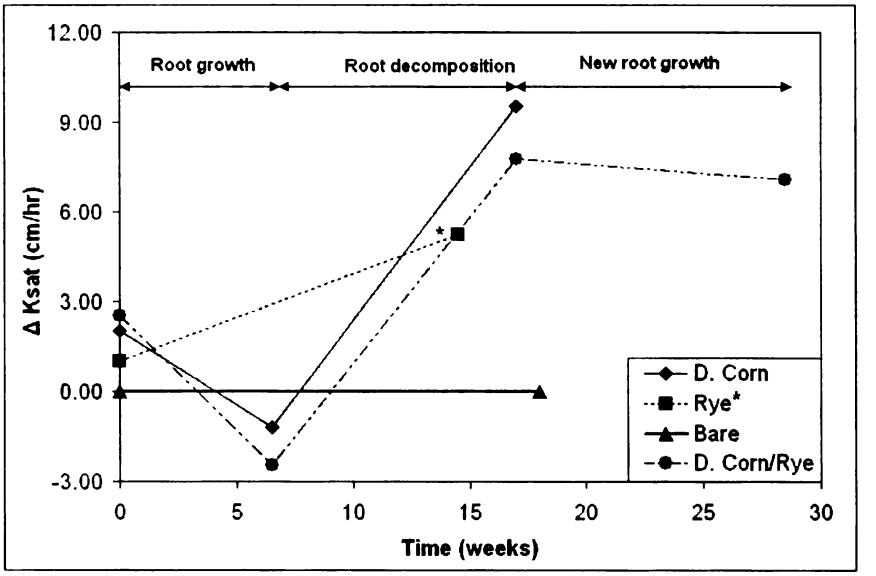

Figure 4.2: Normalized Ksat values. *Rye line reflects only the growth period.

The initial corn growth in the *d. corn* and the *d. corn/rye* treatments lowered the saturated hydraulic conductivity by compressing the soil and reducing the pore space. The rate of change in the Ksat during this initial growth ranged from -0.5 cm/h-week (*d. corn*) to approximately -0.75 cm/h-week (*d. corn/rye*; Figure 4.2). In contrast, the rye growth caused an increase in the rate of change of 0.3 cm/h-week. Because the rye roots were smaller in diameter than the corn roots there may have been less lateral expansion and soil compression with the rye.

The Ksat values increased when the corn plants were killed and the roots began to decay. Root decay increased the Ksat likely by creating macropores in the root channels that enhanced the rate of water movement through the soil. The rate of increase in the Ksat during the decay period was 0.5 to 0.8 cm/h-week, similar to the rate of decrease

observed during the initial root growth. Regrowth of the rye roots in the *d. corn/rye* treatment caused a slight decrease in the Ksat (0.06 cm/h-week) as the growing rye roots filled the root channels from the previous corn crop.

4.1.2 Breakthrough of anionic tracers

4.1.2.1 Chloride

The breakthrough of the chloride tracer was quite consistent across the treatment groups at the beginning of the experiment. Chloride began to break through after the application of approximately 0.45 pore volumes (PV) of water (Figure 4.3). The peak concentration occurred at approximately 0.75 PV, and then returned to the background level at approximately 1.0 PV. The total recovery of the tracer ranged from 73.6 to 130.5%. Descriptive statistics for each column are listed in Table A6.

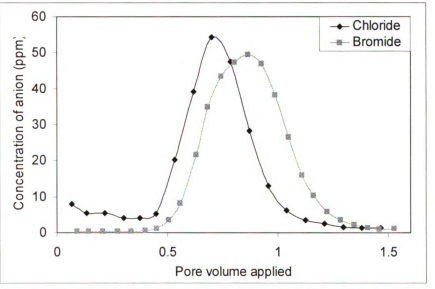

Figure 4.3: Representative chloride and bromide breakthrough curves.

There was no significant difference between treatment groups in peak chloride concentration ($p = 0.196$) or pore volume applied at the time of peak concentration ($p = 0.704$; Tables B2 and B3). Additionally, there was no significant difference between

treatment groups in pore volume applied at the time of 10% ($p = 0.473$) and 50% ($p = 0.838$) cumulative recovery of the chloride (Tables 4.1, B4-B5).

Table 4.1. Chloride and bromide breakthrough by crop treatment.

Treatment	Chloride Breakthrough		Bromide Breakthrough		
	10% * PV	50% PV	10% PV	50% PV	90% PV
Bare	0.455 a	0.757 a	0.636 a	0.853 a	1.072 a
Cereal rye	0.404 a	0.723 a	0.553 a	0.747 a	0.989 a
D. corn	0.417 a	0.750 a	0.539 a	0.749 a	1.015 a
D. corn/rye	0.452 a	0.761 a	0.561 a	0.769 a	0.971 a

* abc letters within columns indicate values not significantly different by Tukey's procedure (α=0.05)

Figure 4.4 shows a cumulative density function fitted to the empirical chloride breakthrough data. Fifty percent chloride breakthrough was expected in 50% of the columns with a water application of 0.71 to 0.75 PV. There was little difference between treatment columns at the beginning of the experiment.

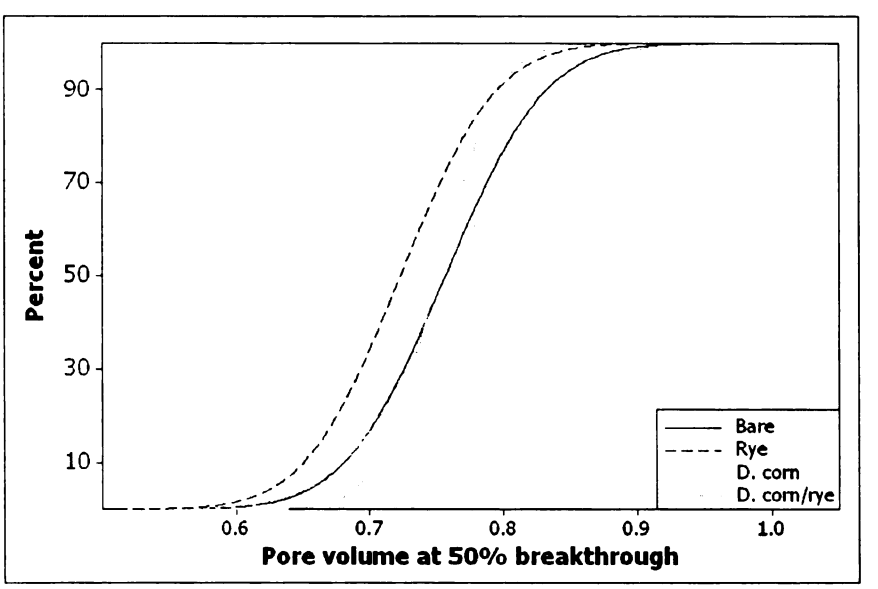

Figure 4.4: Fitted cumulative distribution function of the chloride breakthrough measured in the pre-treatment columns.

4.1.2.2 Bromide

At the end of the experiment, the breakthrough of the bromide tracer was quite consistent across the treatment groups. Breakthrough began after approximately 0.58

pore volumes (PV) of water, the peak concentration was at about 0.78 PV, and the concentration returned to the background level at approximately 1.1 PV (Figure 4.3). There was little difference in the column flow regime due to treatment effects. There was no significant difference in peak bromide concentration ($p = 0.783$) or pore volume applied at the time of peak concentration ($p = 0.296$; Table A7 and B6-B7). Additionally, there was no significant difference between treatment groups in pore volume applied at the time of 10% ($p = 0.598$), 50% ($p = 0.475$), and 90% ($p = 0.697$) cumulative recovery of the bromide (Tables B8-B10). Recovery of the tracer ranged from 95.1 to 123.3%.

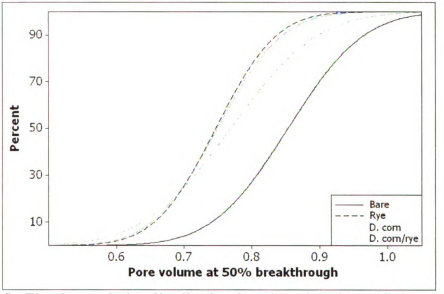

Figure 4.5: Fitted cumulative distribution function of the bromide breakthrough measured in the different crop treatment columns.

Fifty percent breakthrough occurred in 50% of the planted columns at about 0.75 PV, but that level of breakthrough was delayed until about 0.85 PV in the bare columns (Figure 4.5). Presumably, the delay was caused by soil settling during the experiment. This delay was not statistically significant. Descriptive statistics for the bromide break through of each column are listed in Table A7.

There was little change between the Cl and Br tracer, in the pore volume applied at the 50% breakthrough for the columns that had plants. The bare columns show an apparent difference with a later breakthrough with the bromide tracer. An empirical CDF, Figure 4.6, shows the difference between the 50% chloride breakthrough and the 50% bromide breakthrough.

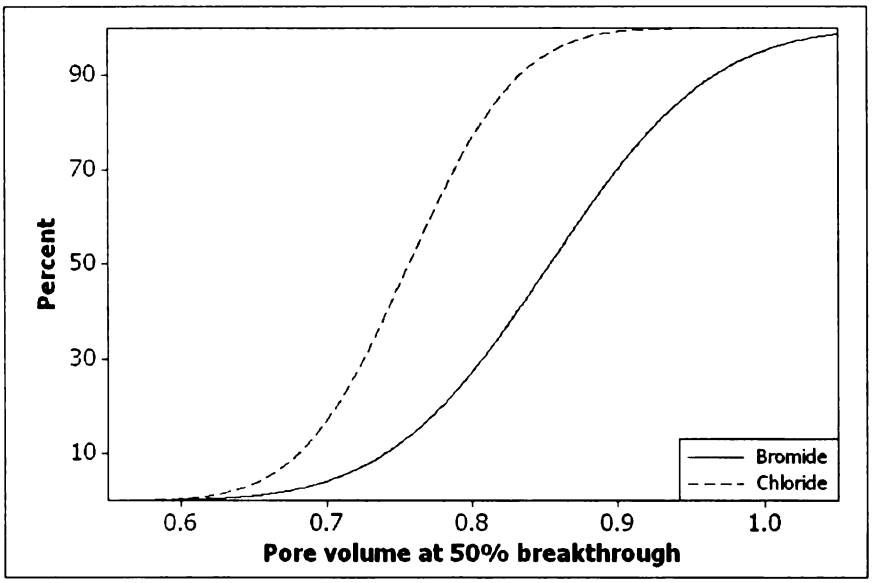

Figure 4.6: Fitted cumulative distribution function of the chloride and bromide breakthrough measured in the bare columns.

4.2 Simulated storm event: *E. coli*

4.2.1 *E. coli* recovery

The manured treatments received a volumetric equivalent of 56,000 L/ha (6,000 gpa) of liquid swine manure as a surface application. The manure was applied 48 hours after a deep wetting and draining of gravitational water so that the soil was near field capacity at the time of manure application. Forty-eight hours after the manure application, a simulated rainfall event with a 25-year frequency was applied and the column effluent was collected and analyzed for *E. coli* concentration.

Factors that may have affected *E. coli* elution and retention were filtration; sorption; preferential flow through root channels, soil crack and fissures; and die-off/regrowth in the soil environment. Generally, *E. coli* elution increased and retention decreased with the number of root growth/die-off cycles (Table 4.2). The concentration of *E. coli* eluted from the *bare* and *bare/manure* columns was not significantly different ($p = 0.9766$; Table B16). The soil was effective in filtering and retaining the *E. coli* in a 25-year rain event in the absence of plant roots, desiccated root channels and other preferential flow paths. The *E. coli* concentration in the column effluent was significantly greater in the treatments with growing or desiccated roots. *Rye/manure* was significantly greater than *bare/manure* ($p = 0.0000$; Table B16), as was *d. corn/manure* and *d. corn-rye/manure* ($p = 0.0000$, $p = 0.0000$; Table B16). There was no detectible difference between *rye/manure* and *d. corn/manure* ($p = 0.4538$; Table B16) or *d. corn-rye/manure* ($p = 0.8816$; Table B16). Similarly, there was no detectible difference between *d. corn/manure* and *d. corn-rye/manure* ($p = 0.8978$; Table B16).

The cumulative *E. coli* collected in the simulated rainfall represents the volume of effluent in each sample (ml) times the concentration of *E. coli* (*E. coli*/100 ml) in the sample. No livestock manure had been applied at the collection site for at least one year, but all of the columns contained detectable levels of *E. coli* (Table A9).

An analysis of variance was performed on the transformed *E. coli* data using the General Linear Model in Minitab (Table B15). There was considerable variability in the cumulative *E. coli* collected in the effluent from each column. Because of the column variability we were not able to detect statistically significant differences between treatments (Table 4.2).

Table 4.2: Elution and retention of the *E. coli*.

Treatments	Column #	*E. coli* applied, cfu/93 ml	Effluent *E. coli* concentration, cfu/100ml	Average *E. coli* concentration, cfu/100ml	Cumulative eluted, cfu	Average cumulative elution, cfu	Column retention, %	Treatment retention, %
Bare	16	-	6,017	5,826 a	22,957	26,622 a	-	-
	40		15,575		75,793			
	2		1,273		6,042			
	14		438		1,695			
Bare/manure	38	6.35E+07	21,120	5,595 a	564,876	143,190 a	99.1107	99.7746 a
	10		228		1,183		99.9981	
	15		15		98		99.9998	
	21		1,016		6,603		99.9896	
Rye/manure	35	2.54E+07	26,755	192,706 b	48,159	237,325 a	99.8103	99.0653 a
	36		402,000		285,420		98.8758	
	42		15,817		28,470		99.8879	
	33		326,250		587,250		97.6870	
D. corn/manure	25	1.07E+08	47,398	87,632 b	249,691	355,544 a	99.7665	99.6676 a
	6		4,396		31,057		99.9710	
	1		248,583		937,858		99.1231	
	39		50,150		203,570		99.8097	
D. corn-rye/manure	43	2.31E+06	64,242	221,094 b	314,430	738,147 a	86.3671	67.9957 b
	19		3,257		17,284		99.2506	
	18		404,375		1,091,125		52.6914	
	7		412,500		1,529,750		33.6737	

* abc letters within columns represent values significantly different by Tukey's HSD procedure ($\alpha = 0.05$; Tables B12, B14, B16).

The coefficient of variation (CV, %) ranged from 94% for *d. corn-rye/manure* to 196% for the *bare/manure* treatment. The *E. coli* elution from the *bare* treatment (26,622 cfu) with the least *E. coli* in the effluent was not significantly different from the *d. corn-rye/manure* treatment with the greatest amount of *E. coli* (738,147 cfu; $p = 0.312$; Table B16); however, there was a tendency to elute a greater quantity from the columns with plant growth. Compared to the *rye/manure* treatment, there was a greater amount of *E. coli* in the *d. corn/manure* effluent. This is consistent with the hypothesis that the root channels left by the corn roots facilitate bacterial movement compared to actively growing, turbid rye roots. The greatest cumulative *E. coli* was recovered from the *d. corn-rye/manure* treatment (Figure 4.7). Presumably, rather than obstructing water and bacterial movement through the desiccated root channels, the rye roots created additional channels for bacterial movement, or were ineffective in obstructing flow through the desiccated root channels, or both.

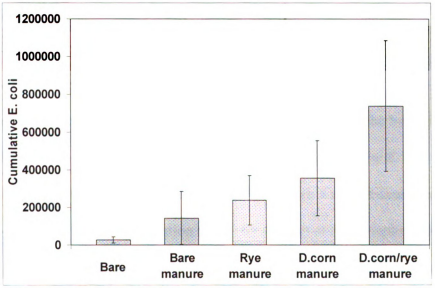

Figure 4.7: Cumulative *E. coli* eluted by treatment. Standard error bars indicate the dispersion in sample data.

E. coli retention in the manured columns was calculated as a percentage of the organisms recovered in the treatment effluent relative to the amount applied in the manure application. A goal in evaluating the initial flush of *E. coli* through the soil column was to highlight the effects of root growth and die-off on the preferential movement of water and bacteria through the column. The percent retention for the *bare*, *rye*, and *desiccated corn* treatments was greater than 99% indicating that little of the *E. coli* was moving through the column by macropore flow. The *E. coli* retention for the *d. corn-rye/manure* treatment, however, was significantly lower (68%) than either of the other manured treatments ($p = 0.036$; Tables B17, B18).

4.3 P22 bacteriophage

The P22 bacteriophage was evaluated as a potential in-tank marker for linking manure land application with specific land management practices. The bacteriophage was added to water or swine manure and applied in a volumetric equivalent of 56,000 l/ha (6,000 gpa) on bare soil and rye growth columns. A simulated rainfall with intensity of a 25-year storm was used to elute *E. coli* and the bacteriophage.

4.3.1 Breakthrough curves

No *E. coli* were detected in the non-manured treatments. When the bacteriophage was applied with swine manure on bare soil, the bacteriophage and the *E. coli* broke through differently with the bacteriophage breaking through 5 to 10 minutes earlier (Figure 4.8-4.9). The peak concentration (1.2E+05 pfu/ml) of bacteriophage in the column effluent was recorded at about 185 minutes from the start of the simulated rainfall in the *bare/manure* treatment (Figure 4.8). The peak concentration of *E. coli* (27 cfu/ml) occurred at about the same time as the peak concentration of the bacteriophage. The

bacteriophage concentration declined at a greater rate than the *E. coli* concentration, and neither the P22 nor the *E. coli* were tailing off at the end of the rain event. Thus, the full breakthrough curve was not obtained due to an inadequate duration of sampling (sampled up to 280 min).

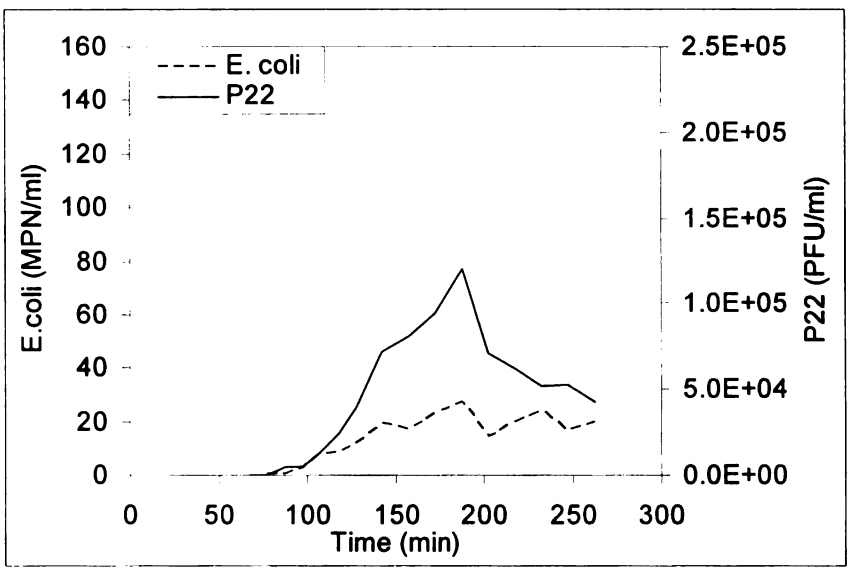

Figure 4.8: Breakthrough concentration of P22 bacteriophage and *E. coli* in the *bare/manure* treatments.

When the bacteriophage was applied with swine manure on growing rye (*rye/manure*) it began to break through about 80 minutes after the start of the rainfall (Figure 4.9). The peak concentration (2.3E+05 pfu/ml) was recorded about 115 minutes after the start of rainfall and the concentration dropped off rapidly to about 5.0E+04 pfu/ml after 180 minutes. The P22 concentration approached background levels after about 300 minutes of rainfall.

The *E. coli* began to breakthrough about 80 minutes after the beginning of rainfall, similar to the bacteriophage. Compared to P22, the peak concentration of *E. coli* (150 cfu/ml) was delayed about 30 minutes and was recorded about 140 minutes after the beginning of rainfall. The *E. coli* concentration then declined rapidly to about 90 cfu/ml

at 160 minutes and remained nearly constant rate until the end of the rainfall event. The *bare/manure* treatments had fewer *E. coli* in the effluent than the *rye/manure* treatments, probably due to the filtering ability of the soil.

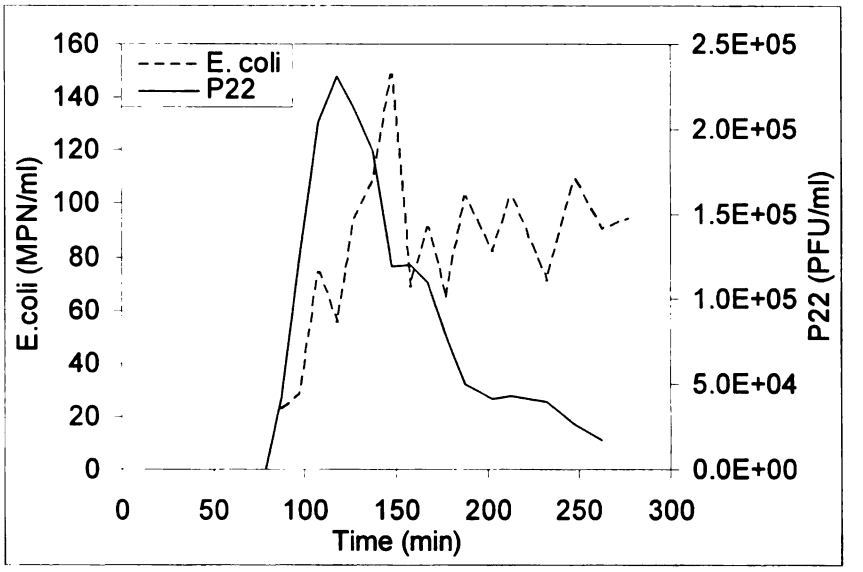

Figure 4.9: Breakthrough concentration of P22 bacteriophage and *E. coli* in the *rye/manure* treatments.

The *bare/manure* P22 breakthrough curve (BTC) was compared with a bromide BTC representative of the bare columns (Figure 4.10). The peak bromide concentration occurred earlier than the peak P22 concentration (140 versus 180 minutes), and the bromide returned to near the background level at 250 minutes while the P22 was still tailing off. The bromide BTC was not a suitable model for predicting the movement of the P22 bacteriophage through the bare soil columns.

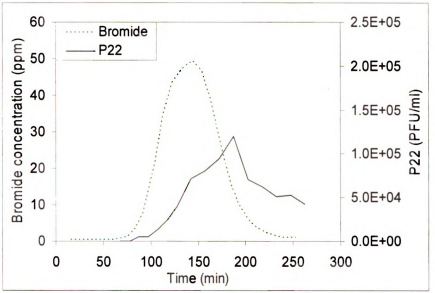

Figure 4.10: Breakthrough curves of bromide and P22 for the *bare/manure* treatment.

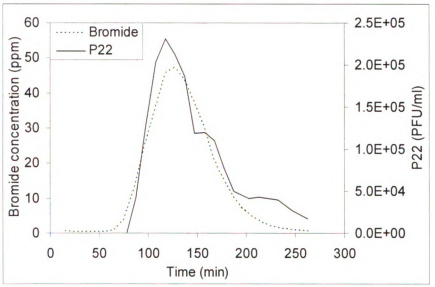

Figure 4.11: Breakthrough curves of bromide and P22 for the *rye/manure* treatment.

The P22 BTC (*rye/manure*) was compared with a representative bromide BTC for the rye growth columns. The bromide BTC was a suitable model for predicting the P22 BTC (Figure 4.11). The bromide began to break through at about the same time as the P22 (60 minutes for the bromide versus 75 minutes for the P22). The peak concentration was recorded at 115 minutes for the bromide and at 125 minutes for the P22. Both the

bromide and P22 concentrations decreased at a similar rate and approached the background levels at the end of the rainfall event.

4.3.2 Phage/manure vs. phage/water

The host for the P22 bacteriophage is *Salmonella typhimurium*. The initial concentration applied to the columns ranged from 2.0E+09 pfu/ml on the *bare/water* treatment to 5.3E+07 pfu/ml on the *bare/manure* treatment (Table 4.3). When the phage was applied in deionized water, the concentration in the column effluent was about 1.0E+03 pfu/ml (Figure 4.12-4.13). When the phage was applied in swine manure, the concentration in the column effluent was about 1.0E+05 pfu/ml. Presumably, the two-log reduction in phage recovery from the non-manured columns, was caused by more of the phage being adsorbed in the soil matrix. There was not competition with the manure particle for the adsorbtion sites that occurred in the manured treatment.

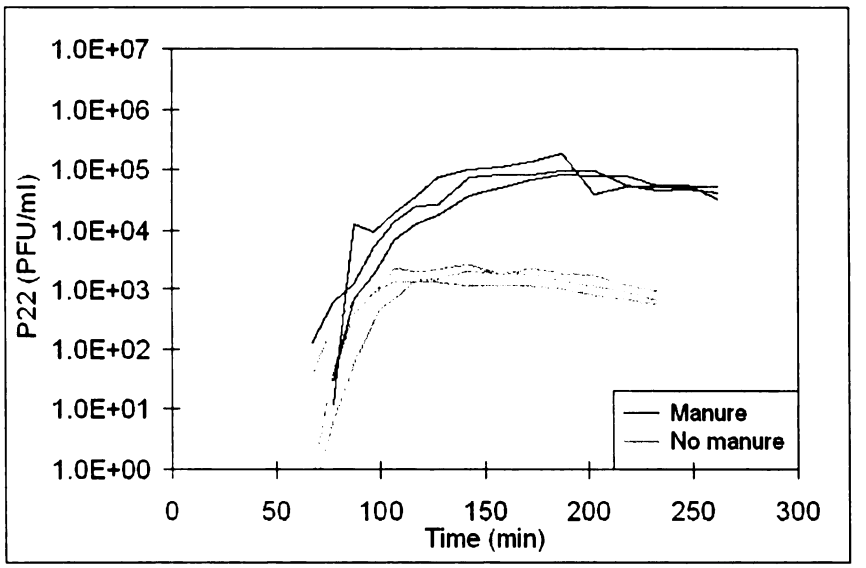

Figure 4.12: Concentration of the P22 bacteriophage in each of the three replicates for the *bare/manure* and *bare/water* treatments.

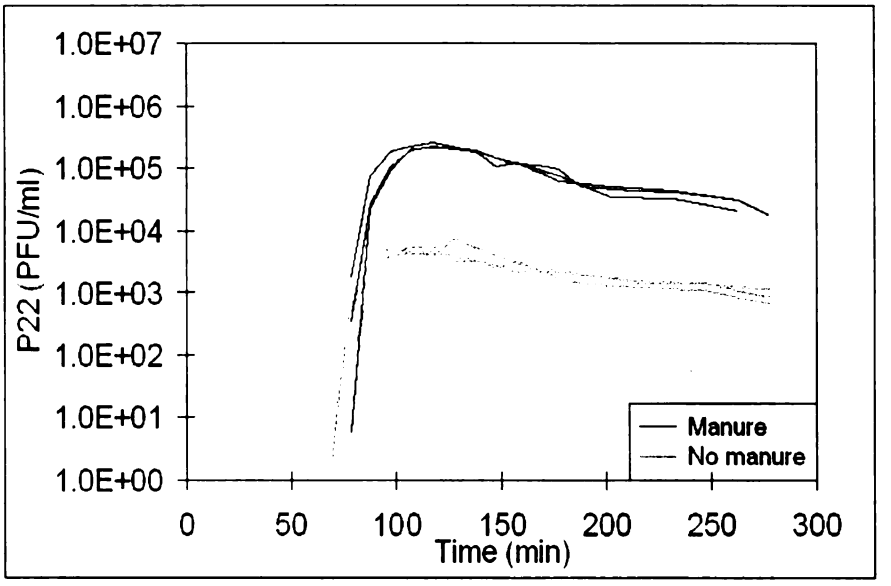

Figure 4.13: Concentration of the P22 bacteriophage in each of the three replicates for the *rye/manure* and *rye/water* treatments.

4.3.3 Cumulative recovery

Factors that may have affected P22 retention were filtration; preferential flow through root channels, soil cracks and fissures; and die-off of the bacteriophage. There was considerable variability in the P22 recovered in the column effluent. The recovery from the *bare/water* and *rye/water* columns was less than 2% of the initial application (Table 4.3). This low recovery indicates a higher adsorption of the bacteriophage in the absence of manure. The manure competed for the adsorption sites in the soil and thus few phage were retained in the column. When the P22 was applied in swine manure to the *bare/manure* and *rye/manure* treatments, the P22 recovery in the column effluent ranged from about 59% with the *rye/manure* treatment to greater than 100% with the *bare/manure* treatment (Table 4.3).

It is not uncommon to have variability in microbial counts. There was variability in this case because the host stock was prepared separately for each treatment. The concentration of the inoculate was measured on day one, and the column samples were

assayed on day 3 and day 4. It was common to a see a two-fold difference in the recovery (50 to 200%).

Table 4.3: Cumulative P22 bacteriophage recovery

Treatments	Column #	Cumulative P22 applied, pfu/ml	Cumulative P22 recovered, pfu/ml	P22 recovery, %	Average recovery, %
Bare/water	2	2.0E+09	1.8E+06	0.092	0.1173
	14		2.2E+06	0.11	
	16		3.1E+06	0.15	
Bare/manure	10	5.3E+07	1.3E+08	240	175
	15		8.6E+07	163	
	21		6.5E+07	122	
Rye/water	8	5.1E+08	7.0E+06	1.4	1.3
	24		6.7E+06	1.3	
	30		6.3E+06	1.2	
Rye/manure	12	4.2E+08	2.6E+08	61.0	58.87
	27		2.5E+08	58.6	
	32		2.4E+08	57.0	

4.4 Discussion

4.4.1 Soil moisture and temperature

Soil moisture and temperatures for the week leading up to each sample event are shown in Figures D13 to D32. The deep wetting prior to each rain event occurred on the afternoon of day 1, the application of the manure or water occurred on day 4 and the simulated rain events and collection of samples took place on day 6. There was a large decrease in the soil moisture during the days prior to sampling the *rye/manure* treatment (Figures D20, D22, and D24), likely from an increase in evapotranspiration. The soil temperature sensors that week recorded daily highs of 35 to 40 °C whereas the week of the previous treatment the maximum soil temperatures were less than 30 °C (Figures D17, D19, D21, and D23). There was a difference in the amount of sample that was collected from treatments with actively growing plants and treatments that did not have growing plants (Table 4.4). The columns with growing plants had less than 27% of the

applied water exit the column verses more than 45% from the *bare* and *d. corn* columns. This lower moisture content is probably due to uptake of water by the growing plants.

Table 4.4: Amount of water applied and recovered during each treatment.

Treatment	Amount irrigation applied per column (ml)	Average amount of leachate collected (ml)	% of application Recovered
Bare	900	420	46.7
Bare/manure	900	425	47.2
Rye	930	120	12.9
Rye/manure	1350	145	10.7
D. corn	900	410	45.6
D. corn/manure	900	495	55.0
D. corn-rye	1200	225	18.8
D. corn-rye/manure	1200	320	26.7

For the treatments that had rye growing at the time of sampling, the rain event was extended in order to collect enough sample to assay. The *rye/manure* treatment required a longer rain event due to the high temperatures that week and increased moisture loss from evapotranspiration.

4.4.2 Difficulties in examining the results

4.4.2.1 Soil problems

The soil used in this experiment had a high percentage of sand (87%) and may not have held structure well. The macropores and root channels that were created by the plants may have collapsed, contributing to the lack of significance between the treatments when examining the anionic tracers. The settling of the soil also contributed to difficulties in examining the data.

4.4.2.2 *E. coli* variability

There was a lot of variability in the number of *E. coli* found in these columns. All of the non-manured columns had some level of *E. coli* in them and several sample had extremely high counts. The columns could have been contaminated by birds or rodents

while in the greenhouse. The same can be true of field sites; outside sources of bacteria from wildlife can add variability to both manured and non-manured plots. Aside from the outside sources of *E. coli,* there was considerable variability between the columns and samples of different treatments (Figure A4).

4.4.3 *E. coli* reduction

Because the concentration of *E. coli* in manure is so high, the allowable limits in waters of the state are so low, extremely high filtration is needed to achieve current water quality standards. The limit for *E. coli* in recreational waters is a geometric mean of 126 cfu per 100 ml (USEPA, 2001). To achieve this level after a manure application with ~ 5.0×10^7 cfu of *E. coli* per 100 ml, the best management practices in place must provide a 99.9997% reduction in the *E. coli* concentration. This is greater than a 5-log reduction.

4.4.4 Broader application of bacteriophage markers

It is important to be able to determine if a manure application is the source of *E. coli* contamination. The P22 bacteriophage shows promise as an inexpensive method for linking specific manure applications with water contamination. The phage and *E. coli* had similar breakthrough curves when applied to a growing crop. The die-off of the phage was rapid in the absence of a host organism. It is not known how long the phage will survive in the presence of a host in a terrestrial environment with unsaturated soil. The phage level began to decline before the *E. coli* (Figure 4.9); in the field, the P22 concentration may drop below detectable levels while *E. coli* is still present.

CHAPTER 5

5. CONCLUSIONS

1. The initial corn growth in loamy sand decreased the saturated hydraulic conductivity (Ksat) at a rate of -0.5 cm/h-week to -0.75 cm/h-week. The Ksat values increased when the corn plants were killed and the roots began to decay. The rate of increase was 0.5 to 0.8 cm/h-week, similar to the rate of decrease observed during the initial root growth. Regrowth of cereal rye roots in soil with decomposed corn roots caused a slight decrease in the Ksat (0.06 cm/h-week) as the growing rye roots filled the root channels from the previous corn crop.
2. Cereal rye root growth caused an increase in the Ksat of 0.3 cm/h-week compared to bare soil.
3. There was no detectable difference in the miscible displacement of a bromide tracer through loamy sand soil columns due to root growth and decay.
4. When swine manure was applied at a volumetric equivalent of 56,000L/ha (6,000 gpa) there was no significant difference between the initial concentration of *E. coli* in the effluent from bare columns and bare columns with manure. Growing or decaying roots increased the rate of bacterial transport through the soil columns. The initial concentration of *E. coli* in the effluent from soil columns with growing or decaying plant roots with manure were significantly greater than either of the bare column treatments.

5. The recovery of the P22 bacteriophage applied with manure was greater than when applied in deionized water presumably because there was a lack of competition with the manure for the adsorption sites.
6. When applied with manure to the cereal rye, the bacteriophage broke through at the same time or slightly before the *E. coli* and reached the peak concentration at about the same time. However, the bacteriophage tailed off before the *E. coli*. The bromide tracer was a suitable model for the P22 transport in the cereal rye column but was not suitable for the bare column.
7. The P22 bacteriophage appeared to be a suitable microbial marker for linking a manure application with water contamination at the field scale.

CHAPTER 6

6. RECOMMENDATIONS

Future column studies for evaluating the movement of bacteria from a manure application can be improved in several ways. Intact or undisturbed columns will better replicate field conditions and perhaps reduce the amount of soil settling and change in Ksat that occurred in this experiment. If repacked columns are used again, soil with a structurally stable sand, silt and clay content should be used to insure that root channels from decayed roots remain intact during Ksat analysis.

Evaluation of the soil Ksat was difficult because the columns were in use for several months and settling occurred. More frequent measurements may provide a more accurate representation of changes that occur from root growth and die-off.

The dark gray PVC soil columns absorbed a lot of the solar radiation in the greenhouse and contributed to wider fluctuations in soil temperature than would be expected in a natural environment. Future column studies should use white columns to reduce soil temperature fluctuations due to solar radiation. Perhaps additional protection from solar radiation could also be provided.

The soil moisture sensors (ECH_2O-10 probes, Decagon Devices) measured the dielectric constant of the soil and provided a volumetric water content. They tended to be unreliable for measuring the moisture content of dry soil. TDR probes might be more reliable.

E. coli were detected in all of the non-manured treatments. This could have been from residual *E. coli* in the soil at the time of collection, contamination while in the greenhouse by birds or rodents, or both. One way to reduce the difficulty in linking the *E. coli* in the column effluent with the manure application would be to use a labeled strain of *E. coli* in the manure application.

Another challenge in evaluating the breakthrough of the *E. coli* was the small number of samples that were collected. Because membrane filtration was time consuming and the samples needed to be assayed within 24 hours, we were not able to evaluate the entire breakthrough event for multiple columns; however, a longer simulated rain and/or multiple rain events would have provided a better picture of the survival and movement of *E. coli* through the soil columns. A more rapid assay method would increase the number of samples that could be evaluated within the 24 hour time limit.

The bacteriophage P22 showed promise as a microbial marker for modeling the movement of *E. coli* through vegetative soil. The technique needs to be evaluated at the field scale for linking a manure application with manure contamination of tile effluent. The persistence and survivability of P22 in a terrestrial agro-ecosystem needs to be evaluated.

APPENDIX A

Table A1: Column soil properties.

Column number	*Dry Bulk Density* (g/cm^3)	*Volume* (cm^3)	*Porosity*	*Pore Volume* (cm^3)
2	1.50	6109.8	0.43	2622.5
14	1.50	5862.1	0.43	2533.8
16	1.50	6076.8	0.44	2646.6
40	1.50	6010.7	0.43	2605.1
10	1.47	6043.8	0.44	2689.0
15	1.49	6093.3	0.44	2665.0
21	1.48	5994.2	0.44	2641.4
38	1.48	6208.9	0.44	2733.4
17	1.49	6126.3	0.44	2675.4
22	1.46	6175.9	0.45	2777.6
31	1.49	6142.8	0.44	2678.7
41	1.51	5994.2	0.43	2586.7
33	1.49	6126.3	0.44	2688.6
35	1.50	6208.9	0.43	2691.9
36	1.49	6076.8	0.44	2654.1
42	1.47	6159.3	0.44	2740.5
11	1.50	6076.8	0.43	2633.4
20	1.45	6192.4	0.45	2813.1
26	1.52	6093.3	0.43	2600.8
37	1.52	6159.3	0.43	2636.7
1	1.50	6060.3	0.43	2622.5
6	1.48	6192.4	0.44	2735.8
25	1.52	6060.3	0.43	2579.1
39	1.49	5994.2	0.44	2616.9
5	1.52	5895.1	0.43	2523.4
13	1.46	6208.9	0.45	2778.7
29	1.51	6076.8	0.43	2614.5
34	1.53	6043.8	0.42	2553.2
7	1.49	6027.2	0.44	2640.5
18	1.49	6060.3	0.44	2660.3
19	1.47	6208.9	0.45	2773.0
43	1.52	5878.6	0.43	2516.4
Minimum	1.45	5862.1	0.42	2516.4
Maximum	1.53	6208.9	0.45	2813.1
Spread	0.08	346.8	0.03	296.7
Average	1.453	5915.9	0.425	2587.3
Standard Deviation	0.020	94.8	0.007	75.7

		Sensor type	label	Wire color		Sensor type	label	Wire color
	19 H	107	Top 4	Red	18 L	107		Black
	19 L	107		Black	18 H	107	Top 3	Red
	20 H	107	Top 5	Red	17 L	107		Black
	20 L	107		Black	17 H	107	Top 2	Red
	21 H	107	Middle 1	Red	16 L	107		Black
	21 L	107		Black	16 H	107	Top 1	Red
	22 H	107	Middle 2	Red	15 L	ECHO		White
	22 L	107		Black	15 H	ECHO	Bottom 5	Red
	23 H	107	Middle 3	Red	14 L	ECHO		White
	23 L	107		Black	14 H	ECHO	Bottom 4	Red
	24 H	107	Middle 4	Red	13 L	ECHO		White
	24 L	107		Black	13 H	ECHO	Bottom 3	Red
	25 H	107	Middle 5	Red	12 L	ECHO		White
	25 L	107		Black	12 H	ECHO	Bottom 2	Red
	26 H				11 L	ECHO		White
	26 L				11 H	ECHO	Bottom 1	Red
COM	LO	connected to the CR10 E1 channel			10 L	ECHO		White
	HI	connected to the CR10 H1 channel			10 H	ECHO	Middle 5	Red
	27 H	107	Bottom 1	Red	9 L	ECHO		White
	27 L	107		Black	9 H	ECHO	Middle 4	Red
	28 H	107	Bottom 2	Red	8 L	ECHO		White
	28 L	107		Black	8 H	ECHO	Middle 3	Red
	29 H	107	Bottom 3	Red	7 L	ECHO		White
	29 L	107		Black	7 H	ECHO	Middle 2	Red
	30 H	107	Bottom 4	Red	6 L	ECHO		White
	30 L	107		Black	6 H	ECHO	Middle 1	Red
	31 H	107	Bottom 5	Red	5 L	ECHO		White
	31 L	107		Black	5 H	ECHO	Top 5	Red
	32 H				4 L	ECHO		White
	32 L				4 H	ECHO	Top 4	Red
	RES	connected to the CR10 C1 channel			3 L	ECHO		White
	CLK	connected to the CR10 C2 channel			3 H	ECHO	Top 3	Red
	12V				2 L	ECHO		White
	GND				2 H	ECHO	Top 2	Red
					1 L	ECHO		White
					1 H	ECHO	Top 1	Red

All Purple, clear and bare wires to a common ground
The common grounding strip connected to AG on CR10

Figure A1: Wiring diagram for Campbell Scientific AM32 Multiplexer.

Table A2: Soil chemical properties.

	Unit	Soil test 1 1/18/2006	Soil test 2 1/18/2006
Soil Nutrient Levels			
Soil pH		7.3	7.3
Phosphorous (P)	Ppm	96	96
Potassium (K)	Ppm	59	63
Magnesium (Mg)	Ppm	94	96
Calcium (Ca)	Ppm	496	530
CEC	Meq/100g	3.4	3.6
% of Exchangeable Bases			
Potassium (K)	%	4.4	4.5
Magnesium (Mg)	%	22.9	22.2
Calcium (Ca)	%	72.6	73.4
% Organic Matter	%	0.6	0.6
Soil Texture			
Sand	%	87.7	86.7
Silt	%	8.6	8.6
Clay	%	3.7	4.7

Table A3: Manure characteristics for *E. coli* experiment.

	Unit	Bare 4/14/2006	Rye 6/2/2006	Corn 8/10/2006	Corn/Rye 9/22/2006
E. coli concentration (in manure prior to application)	cfu/100ml	6.83E+7	2.73E+7	1.15E+8	2.48E+6
Moisture	%	95.28	96.50	99.46	98.29
Solids	%	4.72	3.50	0.54	1.71
Nitrogen, Total (N)	%	0.483	0.261	0.057	0.259
Nitrogen, Ammonium (NH_4-N)	%	0.290	0.178	0.036	0.201
Nitrogen, Organic (N)	%	0.193	0.083	0.021	0.058
Phosphorous (P)	%	0.196	0.117	0.012	0.037
Potassium (K)	%	0.172	0.153	0.034	0.238
Sulfur (S)	%	0.04	0.03	0.01	0.02
Magnesium (Mg)	%	0.08	0.05	0.01	0.03
Calcium (Ca)	%	0.21	0.18	0.03	0.03
Sodium (Na)	%	0.04	0.03	0.01	0.06
Aluminum (Al)	ppm	40	35	5	5
Boron (B)	ppm	3	3	1	3
Copper (Cu)	ppm	7	14	6	9
Iron (Fe)	ppm	223	198	29	45
Manganese (Mn)	ppm	18	12	2	4
Zinc (Zn)	ppm	152	166	2	49

Table A4: Formula for the NSF corn nutrient solution.

Solution	ml of solution per 1 L nutrient solution
1 M KH_2PO_4	0.6
1 M KNO_3	2.5
1 M $Ca(NO_3)_2$	2.5
1 M $MgSO_4$	1.2
Micronutrients	1.0
1.43 g H_3BO_3 per L	
0.04 $CuSO_4$ * $5H_2O$ per L	

Table A5: Saturated hydraulic conductivity values at various stages in the experiment.

Treatment	Column number	Initial Ksat, cm/h	Growing Ksat, cm/h	Desiccated Ksat, cm/h	Final Ksat, cm/h
1 – Bare	16	33.65			6.72
	40	39.76			13.48
	2	41.70			12.08
	14	45.57			6.79
2 – Bare Manure	38	33.65			15.83
	10	39.07			9.77
	15	42.40			11.54
	21	46.64			9.99
3 – Rye	31	32.92	18.71		
	17	37.13	18.49		
	22	40.60	21.45		
	41	46.72	12.25		
4 – Rye Manure	35	29.93	18.99		
	36	38.07	22.45		
	42	41.82	21.32		
	33	47.04	19.79		
5 – Desiccated	26	32.17	17.62	17.99	
Corn	37	34.35	15.56	15.21	
	11	42.03	26.12	23.47	
	20	45.71	37.53	29.85	
6 – Desiccated	25	27.43	17.44	15.68	
Corn Manure	6	38.31	24.78	23.95	
	1	40.43	20.60	21.16	
	39	45.83	30.91	21.82	
7 – Corn Rye	34	27.97	16.17	10.64	11.44
	29	35.30	20.79	17.85	9.12
	5	41.68	16.59	22.46	9.73
	13	45.49	28.11	27.52	12.00
8 – Corn Rye	43	26.65	18.86	20.72	14.53
Manure	19	37.32	29.67	12.48	10.85
	18	40.91	24.82	16.35	13.50
	7	46.88	25.47	26.93	15.36

Table A6: Chloride breakthrough curve statistics.

Treatment	Rep	Column	Background	Tracer concentration	Peak Cl concentration	PV applied at peak	PV at 10% recovery	PV at 50% recovery	PV at 90% recovery	Total % recovery
1. Bare No Manure	1	16	23.6	221	85	0.73	0.280	0.699	0.883	98.03
	2	40	29.8	272	110	0.73	0.624	0.794	-	73.58
	3	2	28.4	265	100	0.71	0.175	0.643	0.805	123.31
	4	14	27.7	258	100	0.83	0.259	0.823	1.027	101.94
2. Bare Manure	1	38	20.2	173	60	0.70	0.611	0.805	-	79.67
	2	10	27.0	247	88	0.72	0.569	0.767	-	82.08
	3	15	27.3	255	105	0.79	0.536	0.765	-	86.24
	4	21	28.3	258	110	0.74	0.589	0.758	-	87.36
3. Rye No Manure	1	31	23.5	209	72	0.76	0.518	0.758	0.996	98.48
	2	17	20.4	195	85	0.72	0.476	0.722	0.873	118.49
	3	22	20.7	197	75	0.70	0.319	0.667	0.822	130.54
	4	41	28.3	264	78	0.79	0.343	0.767	1.223	93.64
4. Rye Manure	1	35	20.8	182	56	0.78	0.248	0.692	0.930	105.55
	2	36	23.0	202	65	0.74	0.316	0.654	0.866	108.51
	3	42	19.8	173	62	0.71	0.423	0.702	0.949	100.82
	4	33	29.4	229	72	0.76	0.587	0.823	-	83.94
5. D. Corn No Manure	1	26	19.2	175	65	0.82	0.366	0.757	0.968	99.46
	2	37	24.6	235	70	0.89	0.311	0.759	1.000	114.30
	3	11	20.5	162	75	0.70	0.573	0.735	0.946	96.59
	4	20	25.0	211	105	0.65	0.579	0.706	1.091	92.02
6. D. Corn Manure	1	25	26.4	258	110	0.76	0.327	0.792	1.011	98.37
	2	6	22.5	190	82	0.78	0.539	0.712	0.853	114.16
	3	1	24.4	206	90	0.82	0.194	0.810	0.965	103.56
	4	39	24.3	232	65	0.84	0.436	0.737	0.995	109.04
7. D. Corn-Rye No Manure	1	34	28.3	270	62	0.75	0.416	0.804	-	88.84
	2	29	20.5	187	60	0.73	0.514	0.773	1.174	92.00
	3	5	18.8	189	60	0.74	0.600	0.832	1.196	93.03
	4	13	40.3	346	115	0.78	0.221	0.679	0.866	104.53
8. D. Corn-Rye Manure	1	43	20.3	181	56	0.78	0.523	0.825	-	84.66
	2	19	27.0	264	100	0.71	0.280	0.666	0.828	107.59
	3	18	21.5	215	95	0.70	0.562	0.732	1.098	92.42
	4	7	24.2	259	72	0.72	0.498	0.779	-	86.74

Table A7: Bromide breakthrough curve statistics.

Treatment	Rep	Column	Background	Tracer concentration	Peak Br concentration	PV applied at peak	PV at 10% recovery	PV at 50% recovery	PV at 90% recovery	Total % recovery
1. Bare No Manure	1	16	0.157	197.8	40.9	0.88	0.663	0.928	1.248	95.11
	2	40	0.231	194.3	44.4	0.91	0.471	0.790	0.985	107.39
	3	2	0.275	205.1	60.3	0.64	0.544	0.714	1.027	110.73
	4	14	0.157	197.8	57.9	1.06	0.792	0.983	1.129	98.97
2. Bare Manure	1	38	0.231	194.3	41.5	0.91	0.632	0.923	1.189	98.16
	2	10	0.275	205.1	61.9	0.76	0.642	0.797	1.006	106.88
	3	15	0.157	197.8	66.4	0.86	0.695	0.850	0.982	108.54
	4	21	0.157	197.8	54.9	0.87	0.647	0.841	1.009	110.75
3. Rye No Manure	1	31	0.278	203.9	47.9	0.77	0.469	0.658	0.868	105.11
	2	17	0.278	203.9	71.8	0.78	0.626	0.772	0.907	113.56
	3	22	0.278	203.9	43.5	0.77	0.595	0.790	1.043	99.57
	4	41	0.250	203.3	34.2	0.50	0.459	0.733	1.164	101.36
4. Rye Manure	1	35	0.250	203.3	44.1	0.73	0.597	0.805	1.150	95.20
	2	36	0.250	203.3	49.6	0.82	0.630	0.832	1.046	101.74
	3	42	0.250	203.3	53.4	0.74	0.563	0.755	0.940	101.69
	4	33	0.278	203.9	69.2	0.65	0.484	0.629	0.790	102.60
5. D. Corn No Manure	1	26	0.242	201.6	38.7	0.60	0.487	0.737	1.016	113.91
	2	37	0.231	194.3	42.9	0.55	0.441	0.635	0.997	105.48
	3	11	0.275	205.1	50.1	0.75	0.572	0.760	1.006	101.40
	4	20	0.242	201.6	39.8	0.70	0.579	0.807	1.063	107.16
6. D. Corn Manure	1	25	0.242	201.6	50.4	0.67	0.560	0.754	1.018	99.58
	2	6	0.275	205.1	64.6	0.86	0.687	0.845	0.996	106.77
	3	1	0.242	201.6	40.7	0.77	0.560	0.809	1.066	105.07
	4	39	0.231	194.3	43.2	0.62	0.429	0.645	0.955	114.42
7. D. Corn-Rye No Manure	1	34	0.259	193.5	40.3	0.66	0.418	0.640	0.880	123.34
	2	29	0.211	186.9	52.4	0.82	0.607	0.795	0.950	122.32
	3	5	0.211	186.9	47.6	0.76	0.589	0.782	1.023	116.71
	4	13	0.211	186.9	55.4	0.85	0.695	0.869	1.028	116.34
8. D. Corn-Rye Manure	1	43	0.259	193.5	31.8	0.49	0.360	0.629	0.983	121.56
	2	19	0.211	186.9	43.4	0.94	0.613	0.894	1.084	122.13
	3	18	0.259	193.5	62.8	0.90	0.654	0.838	0.978	118.51
	4	7	0.259	193.5	65.8	0.70	0.549	0.702	0.839	121.32

Table A8: Manure characteristics for bacteriophage experiment.

	Unit	Bare 12/1/2006	Rye 12/15/2006
Moisture	%	96.69	98.01
Solids	%	3.31	1.99
Nitrogen, Total (N)	%	0.259	0.220
Nitrogen, Ammonium (NH_4-N)	%	0.089	0.159
Nitrogen, Organic (N)	%	0.170	0.061
Phosphorous (P)	%	0.113	0.043
Potassium (K)	%	0.084	0.251
Sulfur (S)	%	0.03	0.03
Magnesium (Mg)	%	0.05	0.02
Calcium (Ca)	%	0.19	0.06
Sodium (Na)	%	0.02	0.05
Aluminum (Al)	ppm	38	15
Boron (B)	ppm	2	3
Copper (Cu)	ppm	26	16
Iron (Fe)	ppm	151	60
Manganese (Mn)	ppm	10	8
Zinc (Zn)	ppm	132	59

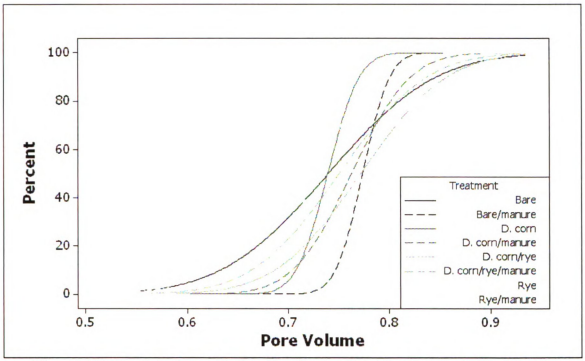

Figure A2: Empirical cumulative distribution function for the 50% chloride breakthrough for each of the treatment groups.

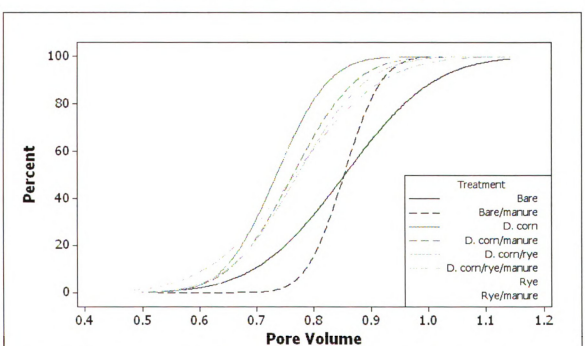

Figure A3: Empirical cumulative distribution function for the 50% bromide breakthrough for each of the treatment groups.

Table A9: Cumulative *E. coli* recovered from each column.

Treatments	Column #	Cumulative *E. coli* per column	Average cumulative *E. coli* per treatment
1 – Bare	16	22,957	26,622
	40	75,793	
	2	6,042	
	14	1,695	
2 – Bare, manure	38	564,876	143,190
	10	1,183	
	15	98	
	21	6,603	
3 – Rye	31	NA	346
	17	642	
	22	306	
	41	90	
4 – Rye, manure	35	48,159	237,325
	36	285,420	
	42	28,470	
	33	587,250	
5 – Desiccated corn	26	1,781	3,158
	37	191	
	11	1,556	
	20	9,103	
6 – Desiccated corn, manure	25	249,691	355,544
	6	31,057	
	1	937,858	
	39	203,570	
7 – D. corn/rye	34	60	268,434
	29	NA	
	5	660,143	
	13	145,100	
8 – D. corn/rye, manure	43	314,430	738,147
	19	17,284	
	18	1,091,125	
	7	1,529,750	

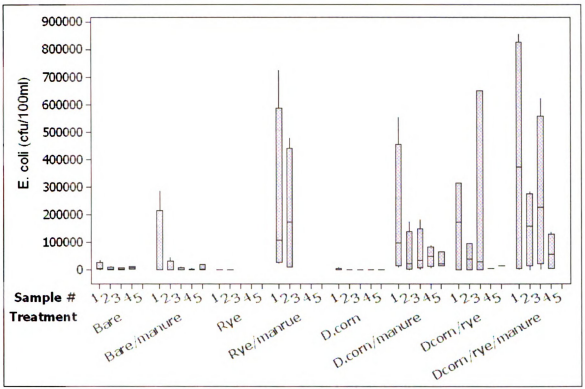

Figure A4: Box plot of *E. coli* concentration by treatment and sample number.

Table A10: *E. coli* concentrations for treatment 1, *bare with no manure.*

Treatment 1 – Bare, no manure		
Column	Sample #	*E. coli* concentration (cfu/100 ml)
16	1	7,050
	2	5,250
	3	5,750
40	1	32,950
	2	11,500
	3	6,300
	4	11,550
2	1	2,238
	2	748
	3	563
	4	1,543
14	1	930
	2	384
	3	0

Table A11: *E. coli* concentrations for treatment 2, *bare with manure.*

Treatment 2 – Bare, manure		
Column	Sample #	*E. coli* concentration (cfu/100 ml)
38	1	285,000
	2	43,250
	3	8,967
	4	4,400
	5	20,483
10	1	80
	2	10
	3	235
	4	585
15	1	15
	2	5
	3	10
	4	10
	5	35
21	1	148
	2	195
	3	1,669
	4	1,032
	5	2,035

Table A12: *E. coli* concentrations for treatment 3, *rye with no manure.*

Treatment 3 – Rye, no manure		
Column	Sample #	*E. coli* concentration (cfu/100 ml)
31	1	Error in sample
	2	Error in sample
17	1	724
	2	210
22	1	344
	2	250
41	1	100
	2	0

Table A13: *E. coli* concentrations for treatment 4, *rye with manure.*

Treatment 4 – Rye, manure		
Column	Sample #	*E. coli* concentration (cfu/100 ml)
35	1	38,683
	2	14,827
36	1	72,500
	2	329,500
42	1	23,133
	2	8,500
33	1	173,500
	2	479,000

Table A14: *E. coli* concentrations for treatment 5, *desiccated corn with no manure.*

Treatment 5 – Desiccated Corn, no manure		
Column	Sample #	*E. coli* concentration (cfu/100 ml)
26	1	1,123
	2	150
	3	0
	4	78
	5	75
37	1	113
	2	33
	3	0
	4	10
	5	0
11	1	930
	2	215
	3	133
	4	0
	5	250
20	1	6,325
	2	580
	3	392
	4	348
	5	618

Table A15: *E. coli* concentrations for treatment 6, *desiccated corn with manure.*

Treatment 6 – Desiccated Corn, manure		
Column	Sample #	*E. coli* concentration (cfu/100 ml)
25	1	166,667
	2	4,075
	3	16,750
	4	27,000
	5	22,500
6	1	9,033
	2	995
	3	2,650
	4	7,750
	5	15,500
1	1	552,500
	2	172,500
	3	181,333
	4	88,000
39	1	27,500
	2	39,250
	3	49,000
	4	70,000
	5	65,000

Table A16: *E. coli* concentrations for treatment 7, *desiccated corn/rye with no manure.*

Treatment 7 – D. Corn/Rye, no manure		
Column	Sample #	*E. coli* concentration (cfu/100 ml)
34	1	15
	2	40
	3	45
29*	1	3,000,000*
	2	950,000*
	3	300,000*
5	1	315,000
	2	96,250
	3	650,000
	4	4,550
	5	14,900
13	1	173,750
	2	37,750
	3	30,333

* Possible contamination to column, this replicate was not used in any analysis.

Table A17: *E. coli* concentrations for treatment 8, *desiccated corn/rye with manure.*

Treatment 8 – Corn/Rye, manure		
Column	Sample #	*E. coli* concentration (cfu/100 ml)
43	1	7,717
	2	61,250
	3	85,000
	4	103,000
19	1	3,317
	2	610
	3	2,050
	4	7,050
18	1	855,000
	2	257,500
	3	367,500
	4	137,500
7	1	740,000
	2	282,500
	3	622,500
	4	5,000

APPENDIX B

Table B1: Two-Factor ANOVA: Ksat values by treatment and replication.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Treatment	31.17	7	4.453	1.609	0.1877
Replication	1058.12	3	352.707	127.410	1.24E-13
Error	58.13	21	2.768		
Total	1147.42	31			

Table B2: Two-Factor ANOVA: Peak chloride concentration by treatment and replication.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Treatment	3209.5	7	458.496	1.58	0.196
Replication	1472.8	3	490.948	1.69	0.199
Error	6093.4	21	290.162		
Total	10775.7	31			

Table B3: Two-Factor ANOVA: Pore volume applied at chloride peak by treatment and replication.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Treatment	0.0136	7	0.001943	0.66	0.704
Replication	0.0043	3	0.001425	0.48	0.697
Error	0.0619	21	0.002949		
Total	0.0798	31			

Table B4: Two-Factor ANOVA: Pore volume at 10 % recovery of applied chloride by treatment and replication.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Treatment	0.1492	7	0.02131	0.98	0.473
Replication	0.0083	3	0.00277	0.13	0.943
Error	0.4582	21	0.02182		
Total	0.6156	31			

Table B5: Two-Factor ANOVA: Pore volume at 50 % recovery of applied chloride by treatment and replication.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Treatment	0.0116	7	0.00166	0.48	0.838
Replication	0.0073	3	0.00242	0.70	0.562
Error	0.0725	21	0.00345		
Total	0.0914	31			

Table B6: Two-Factor ANOVA: Peak bromide concentration by treatment and replication.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Treatment	430.94	7	61.563	0.56	0.783
Replication	763.09	3	254.365	2.29	0.107
Error	2329.07	21	110.908		
Total	3523.11	31			

Table B7: Two-Factor ANOVA: Pore volume applied at bromide peak by treatment and replication.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Treatment	0.1502	7	0.02156	1.31	0.296
Replication	0.0369	3	0.01230	0.75	0.536
Error	0.3453	21	0.01644		
Total	0.5325	31			

Table B8: Two-Factor ANOVA: Pore volume at 10 % recovery of applied bromide by treatment and replication.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Treatment	0.0549	7	0.00784	0.80	0.598
Replication	0.0267	3	0.00891	0.91	0.455
Error	0.2066	21	0.00984		
Total	0.2882	31			

Table B9: Two-Factor ANOVA: Pore volume at 50 % recovery of applied bromide by treatment and replication.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Treatment	0.0629	7	0.00899	0.97	0.475
Replication	0.0057	3	0.00191	0.21	0.890
Error	0.1936	21	0.00922		
Total	0.2622	31			

Table B10: Two-Factor ANOVA: Pore volume at 90 % recovery of applied bromide by treatment and replication.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Treatment	0.0527	7	0.00752	0.67	0.697
Replication	0.0120	3	0.00401	0.36	0.785
Error	0.2365	21	0.01126		
Total	0.3012	31			

Table B11: General Linear Model (GLM): Ln (*E. coli*) vs. Crop, Manure, and Replication.

Source of Variation	*Sequence SS*	*Adjusted SS*	*df*	*Adjusted MS*	*F*	*P-value*
Crop	311.11	295.06	3	98.35	31.62	0.000
Manure	17.81	20.84	1	20.84	6.70	0.012
Replication	37.42	12.78	3	4.26	1.37	0.261
Crop*Replication	193.91	193.91	9	21.55	6.93	0.000
Error	183.50	183.50	59	3.11		
Total	743.75		75			

Table B12: P-values for pairwise comparisons of the main effects of crop using Tukey. Groupings are on the diagonal. Ln (*E. coli*) is the response variable.

Crop	Bare	Bare manure	Rye manure	D. Corn manure	D. Corn/Rye manure
Bare	**a**	0.9766	0.0000	0.0000	0.0000
Bare manure	0.9766	**a**	0.0000	0.0000	0.0000
Rye manure	0.0000	0.0000	**B**	0.4538	0.8816
D. Corn Manure	0.0000	0.0000	0.4538	**b**	0.8978
D. Corn/Rye Manure	0.0000	0.0000	0.8816	0.8978	**b**

Table B13: P-values for pairwise comparisons of the main effects of manure using Tukey. Groupings are on the diagonal. Ln (*E. coli*) is the response variable.

Crop	No Manure	Manure
No Manure	**a**	0.0121
Manure	0.0121	**b**

Table B14: P-values for pairwise comparisons of the main effects of crop using Tukey. Groupings are on the diagonal. Ln (*E. coli*) is the response variable.

Replication	1	2	3	4
1	**a**	0.3622	0.9235	0.9974
2	0.3622	**a**	0.7465	0.2663
3	0.9235	0.7465	**a**	0.8461
4	0.9974	0.2663	0.8461	**a**

Table B15: General Linear Model (GLM): Ln (Cumulative *E. coli*) by treatment and replication.

Source of Variation	*Sequence SS*	*Adjusted SS*	*df*	*Adjusted MS*	*F*	*P-value*
Treatment	55.45	55.45	4	13.863	2.63	0.087
Replication	9.41	9.41	3	3.136	0.60	0.630
Error	63.18	63.18	12	5.265		
Total	128.04		19			

Table B16: P-values for pairwise comparisons of the main effects of crop using Tukey. Groupings are on the diagonal. Ln (Cumulative *E. coli*) is the response variable.

Crop	Bare	Bare manure	Rye manure	D. Corn manure	D. Corn/Rye manure
Bare	**a**	0.9766	0.6053	0.4448	0.3116
Bare manure	0.9766	**A**	0.3088	0.2046	0.1327
Rye manure	0.6053	0.3088	**A**	0.9983	0.9776
D. Corn Manure	0.4448	0.2046	0.9983	**a**	0.9985
D. Corn/Rye Manure	0.3116	0.1327	0.9776	0.9985	**a**

Table B17: General Linear Model (GLM): Cumulative *E. coli* percent retention by treatment and replication.

Source of Variation	*Sequence SS*	*Adjusted SS*	*df*	*Adjusted MS*	*F*	*P-value*
Treatment	2979.2	2979.2	3	993.07	4.41	0.036
Replication	702.6	702.6	3	234.20	1.04	0.421
Error	2028.2	2028.2	9	225.35		
Total	5710.0		15			

Table B18: P-values for pairwise comparisons of the main effects of crop using Tukey. Groupings are on the diagonal. Cumulative *E. coli* percent retention is the response variable. ($\alpha = 0.10$)

Crop	Bare	Rye	D. Corn	D. Corn/Rye
Bare	**a**	0.9999	1.0000	0.0606
Rye	0.9999	**a**	0.9999	0.0670
D. Corn	1.0000	0.9999	**a**	0.0616
D. Corn/Rye	0.0606	0.0670	0.0616	**b**

APPENDIX C

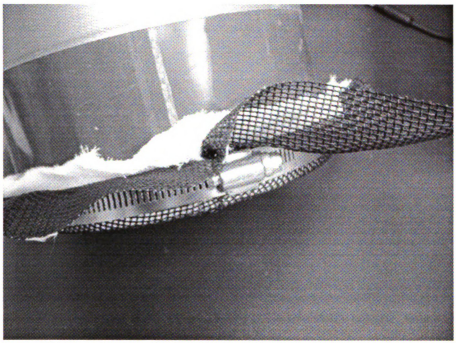

Figure C1: Construction of the bottom of the column.

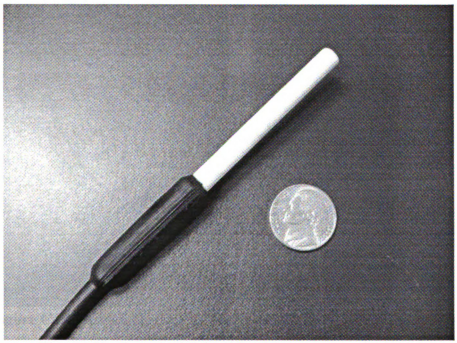

Figure C2: Campbell Scientific 107 temperature sensor.

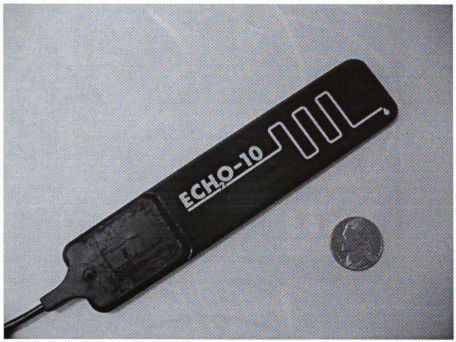

Figure C3: Decagon Devices ECH_2O probe moisture sensor, side view.

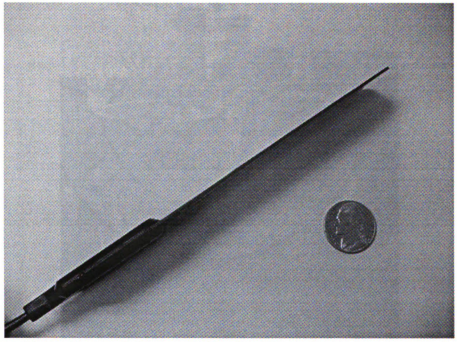

Figure C4: Decagon Devices ECH_2O probe moisture sensor, top view.

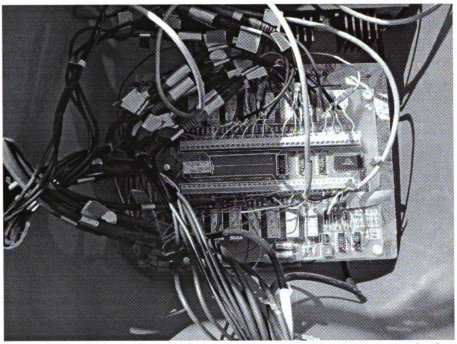

Figure C5: Campbell AM32 multiplexer with sensors attached.

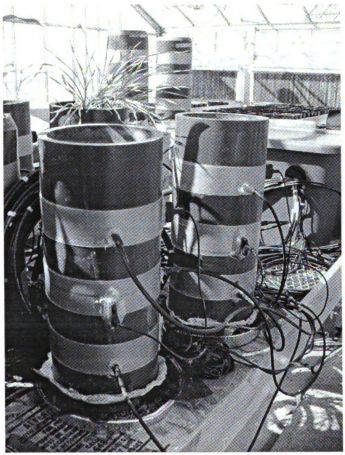

Figure C6: Instrumented columns.

Figure C7: Overview of columns in greenhouse.

Figure C8: Another overview of columns in greenhouse.

Figure C9: Saturation tank for saturated hydraulic conductivity.

Figure C10: Saturated hydraulic conductivity set up.

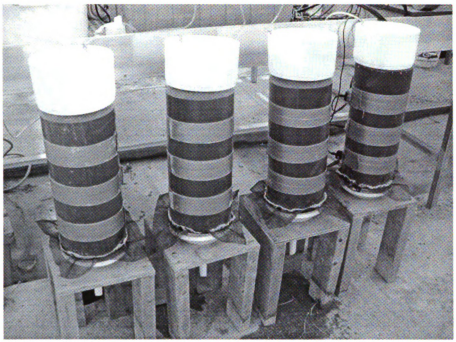

Figure C11: Set up of a rain event.

Figure C12: Sample collection during a rain event.

Figure C13: Rain event sample collection, *d. corn/rye manure* treatment.

Figure C14: View into column after rain event.

Figure C15: Membrane filtration lab set up.

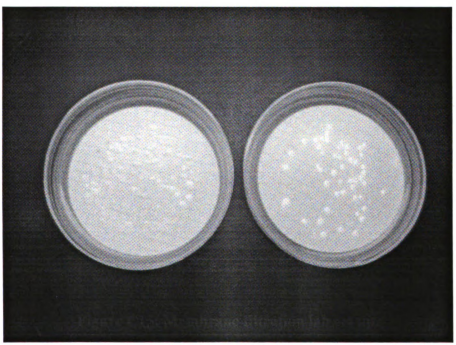

Figure C16: An example of blue fluorescing *E. coli* colonies under UV light.

APPENDIX D

Table D1: Average daily soil and air temperature highs and lows.

Date	Average daily soil temperature minimum	Average daily soil temperature maximum	Daily air temperature minimum	Daily air temperature maximum
9-Feb	23.79	28.00		
10-Feb	22.83	26.34		
11-Feb	22.91	26.30		
12-Feb	21.73	29.46		
13-Feb	21.82	25.68		
14-Feb	23.44	28.15		
15-Feb	23.80	26.72		
16-Feb	21.32	24.11		
17-Feb	19.46	28.57		
18-Feb	14.30	26.16		
19-Feb	16.06	27.14		
20-Feb	19.04	27.09		
21-Feb	20.64	29.45		
22-Feb	23.16	31.19		
23-Feb	22.84	29.56		
24-Feb	21.38	29.14		
25-Feb	22.40	29.54		
26-Feb	19.36	28.61		
27-Feb	22.22	26.86		
28-Feb	20.58	29.87		
1-Mar	22.94	28.20		
2-Mar	23.10	25.68		
3-Mar	21.75	27.31		
4-Mar	20.41	31.96		
5-Mar	22.92	27.91		
6-Mar	23.18	28.03	Air temperature sensor was not hooked up yet.	
7-Mar	22.03	32.26		
8-Mar	22.56	29.91		
9-Mar	23.49	26.19		
10-Mar	23.53	27.37		
11-Mar	23.44	30.95		
12-Mar	24.10	28.44		
13-Mar	23.32	31.23		
14-Mar	21.75	25.86		
15-Mar	20.67	31.33		
16-Mar	23.32	28.33		
17-Mar	21.58	31.55		
18-Mar	20.19	33.13		
19-Mar	20.89	33.83		
20-Mar	21.73	31.40		
21-Mar	21.32	29.43		
22-Mar	22.38	26.25		
23-Mar	22.94	28.34		
24-Mar	23.13	28.51		
25-Mar	22.79	27.63		
26-Mar	22.88	29.16		
27-Mar	22.96	33.14		
28-Mar	23.28	26.71		
29-Mar	22.92	27.68		
30-Mar	23.19	34.38		
31-Mar	23.68	30.64		

Table D1 (cont'd)

Date	Average daily soil temperature minimum	Average daily soil temperature maximum	Daily air temperature minimum	Daily air temperature maximum
1-Apr	23.81	27.88		
2-Apr	23.10	29.51		
3-Apr	23.62	26.32		
4-Apr	22.03	29.52		
5-Apr	22.83	34.01		
6-Apr	23.55	30.29		
7-Apr	23.63	25.93	25.06	26.14
8-Apr	20.61	33.74	21.66	29.66
9-Apr	22.65	34.19	23.97	30.12
10-Apr	23.50	34.28	25.03	30.99
11-Apr	23.14	34.00	24.10	32.19
12-Apr	24.40	28.57	25.66	29.53
13-Apr	24.11	35.28	25.58	38.75
14-Apr	24.35	36.45	24.92	38.54
15-Apr	24.19	37.76	24.63	36.66
16-Apr	23.72	28.82	24.23	28.49
17-Apr	23.50	35.69	24.14	29.15
18-Apr	24.02	37.21	24.45	30.85
19-Apr	24.08	37.14	24.60	32.13
20-Apr	24.19	35.56	24.64	32.17
21-Apr	24.29	37.13	24.98	33.81
22-Apr	24.26	32.52	24.81	31.73
23-Apr	23.89	27.76	24.29	28.40
24-Apr	24.13	36.29	24.59	35.06
25-Apr	23.94	32.45	24.17	30.03
26-Apr	23.11	36.97	23.33	34.53
27-Apr	23.90	35.87	24.34	32.05
28-Apr	23.91	36.29	23.95	33.36
29-Apr	24.29	31.11	24.85	30.67
30-Apr	24.02	27.91	25.06	27.96
1-May	24.17	31.24	24.91	30.17
2-May	23.97	27.40	24.81	27.18
3-May	24.15	35.30	25.00	33.06
4-May	24.56	33.76	24.79	32.93
5-May	24.41	30.73	24.91	30.28
6-May	23.87	31.90	24.26	29.76
7-May	24.03	33.94	24.99	31.8
8-May	24.38	34.41	25.02	33.00
9-May	24.48	34.67	25.09	32.85
10-May	24.80	30.98	25.46	30.58
11-May	23.87	27.24	23.62	27.48
12-May	22.72	26.42	23.24	26.58
13-May	23.27	26.70	23.71	26.95
14-May	23.25	27.45	23.87	27.51
15-May	23.39	26.96	23.9	26.94
16-May	23.47	30.22	23.62	29.90
17-May	23.74	32.06	23.32	31.13
18-May	23.25	26.96	23.24	27.34
19-May	22.89	28.84	23.61	28.59
20-May	23.19	32.77	23.59	30.66
21-May	23.55	29.65	23.02	27.15

Table D1 (cont'd)

Date	Average daily soil temperature minimum	Average daily soil temperature maximum	Daily air temperature minimum	Daily air temperature maximum
22-May	22.82	31.10	23.01	28.34
23-May	23.72	34.69	23.8	32.74
24-May	24.20	35.33	24.48	33.8
25-May	24.38	33.95	24.49	34.94
26-May	24.45	32.62	24.3	32.66
27-May	24.47	38.73	24.1	37.98
28-May	24.91	41.29	24.62	40.48
29-May	25.68	42.80	25.11	42.78
30-May	25.38	38.17	24.83	40.11
31-May	24.36	36.57	24.06	37.59
1-Jun	24.68	34.37	24.46	34.73
2-Jun	23.54	34.21	23.81	33.54
3-Jun	23.72	33.81	23.59	34.11
4-Jun	23.98	36.48	24.36	34.79
5-Jun	24.24	38.91	23.47	37.09
6-Jun	24.52	35.45	24.53	35.14
7-Jun	24.26	30.86	23.82	31.20
8-Jun	24.37	33.77	23.90	33.89
9-Jun	24.11	32.05	24.48	32.16
10-Jun	23.95	32.47	24.21	31.37
11-Jun	23.66	32.66	23.2	31.57
12-Jun	23.87	32.19	22.91	31.66
13-Jun	24.12	31.13	24.19	31.06
14-Jun	24.22	34.11	24.23	32.59
15-Jun	24.32	34.49	23.65	32.69
16-Jun	24.46	37.02	24.53	35.36
17-Jun	24.90	38.68	24.51	37.54
18-Jun	25.25	30.41	24.79	31.36
19-Jun	24.55	35.99	24.67	34.86
20-Jun	24.40	34.32	23.16	32.64
21-Jun	24.37	30.25	24.45	31.45
22-Jun	24.46	32.58	24.51	32.27
23-Jun			23.96	30.74
24-Jun			23.62	31.33
25-Jun			24.24	32.78
26-Jun			23.95	32.34
27-Jun			23.98	32.31
28-Jun			24.04	32.2
29-Jun			23.67	32.02
30-Jun			23.30	32.12
1-Jul			24.17	35.59
2-Jul	Period between treatments, no columns were hooked up.		25.04	32.88
3-Jul			24.42	32.00
4-Jul			24.17	33.26
5-Jul			23.26	29.86
6-Jul			23.14	30.22
7-Jul			23.64	32.20
8-Jul			23.99	32.19
9-Jul			24.56	33.70
10-Jul			24.55	31.59
11-Jul			24.82	30.58

Table D1 (cont'd)

Date	Average daily soil temperature minimum	Average daily soil temperature maximum	Daily air temperature minimum	Daily air temperature maximum
12-Jul			24.87	30.59
13-Jul			24.50	35.14
14-Jul			24.45	34.46
15-Jul			24.62	39.61
16-Jul			24.64	41.34
17-Jul			25.19	41.29
18-Jul			25.30	37.43
19-Jul			24.46	37.47
20-Jul			24.91	35.38
21-Jul	25.82	31.51	24.88	32.90
22-Jul	24.24	35.34	24.60	33.82
23-Jul	22.12	33.07	24.27	35.06
24-Jul	24.30	39.56	24.32	38.94
25-Jul	25.36	36.46	25.59	36.24
26-Jul	25.34	31.26	24.72	33.00
27-Jul	24.56	38.07	24.60	38.27
28-Jul	25.03	37.66	24.80	39.16
29-Jul	25.47	39.89	25.67	40.09
30-Jul	25.18	33.90	24.68	36.04
31-Jul	24.93	41.26	24.63	42.09
1-Aug	27.07	43.05	26.29	42.97
2-Aug	27.22	42.99	25.41	42.05
3-Aug	25.31	30.56	24.87	30.86
4-Aug	24.01	37.92	24.46	38.76
5-Aug	24.43	36.84	24.54	35.79
6-Aug	24.29	35.89	24.76	34.97
7-Aug	24.57	39.14	24.03	38.06
8-Aug	24.12	35.36	24.14	35.85
9-Aug	24.10	36.31	24.51	36.21
10-Aug	25.04	33.42	23.98	31.70
11-Aug	22.20	32.77	21.54	30.89
12-Aug	20.88	34.62	19.91	32.43
13-Aug	21.79	35.16	21.06	32.77
14-Aug	23.31	30.91	23.34	30.56
15-Aug	22.64	34.67	22.67	33.19
16-Aug	21.68	35.62	21.21	33.24
17-Aug	23.47	35.37	23.13	34.82
18-Aug	24.05	31.34	24.22	31.80
19-Aug	23.69	30.03	23.61	29.98
20-Aug	22.32	34.14	21.62	31.72
21-Aug	21.95	34.67	21.15	33.75
22-Aug	22.91	35.73	22.69	34.26
23-Aug	22.36	34.24	21.74	33.74
24-Aug	23.50	29.76	23.33	30.38
25-Aug	23.88	33.78	24.17	32.45
26-Aug	23.91	34.98	23.84	34.64
27-Aug	24.05	28.62	24.05	28.75
28-Aug	23.51	28.78	23.52	28.04
29-Aug	23.10	30.36	23.19	30.06
30-Aug	23.27	32.17	21.59	31.55
31-Aug	20.46	33.26	20.05	31.29

Table D1 (cont'd)

Date	Average daily soil temperature minimum	Average daily soil temperature maximum	Daily air temperature minimum	Daily air temperature maximum
1-Sep	21.82	30.00	20.89	31.91
2-Sep	21.82	29.69	21.26	28.37
3-Sep	20.87	32.64	20.50	30.20
4-Sep	21.91	33.81	21.29	32.77
5-Sep	22.27	32.24	21.71	32.15
6-Sep	22.72	34.64	22.52	34.86
7-Sep	22.56	33.55	22.50	33.17
8-Sep	22.65	35.52	22.56	34.79
9-Sep	22.04	25.80	21.31	25.18
10-Sep	21.29	25.44	20.57	24.91
11-Sep	21.79	26.04	21.60	25.50
12-Sep	22.38	26.10	22.53	25.97
13-Sep	23.20	26.73	22.96	26.23
14-Sep	22.25	27.91	22.28	27.22
15-Sep	22.27	33.53	21.85	34.02
16-Sep	21.12	32.59	20.30	33.79
17-Sep	22.09	34.29	21.87	33.89
18-Sep	23.34	26.82	22.03	26.00
19-Sep	20.55	26.23	17.98	25.08
20-Sep	21.60	27.61	21.62	25.90
21-Sep	21.48	30.81	21.43	28.16
22-Sep	21.94	25.50	21.71	24.74
23-Sep	22.65	27.17	22.39	26.58
24-Sep	22.20	26.76	21.05	25.51
25-Sep	21.27	30.21	21.35	28.64
26-Sep	21.68	31.63	21.23	27.33
27-Sep	21.96	27.50	22.27	27.75
28-Sep	21.46	27.87	21.51	24.81
29-Sep	21.25	28.17	20.73	24.74
30-Sep	21.01	25.21	21.72	23.70
1-Oct	20.69	27.92	21.12	28.22
2-Oct	21.01	25.73	21.89	26.91
3-Oct	21.49	32.75	21.25	29.64
4-Oct	22.80	25.89	21.62	25.80
5-Oct	21.20	29.40	20.96	26.73
6-Oct	20.92	31.85	20.73	27.15
7-Oct	21.34	33.01	21.16	28.96
8-Oct	21.36	35.67	21.57	32.56
9-Oct	21.45	31.65	21.8	29.46
10-Oct	21.12	28.30	21.11	26.69
11-Oct	23.22	26.19	23.51	26.32
12-Oct	22.27	26.69	22.05	25.63
13-Oct	21.10	26.14	21.52	25.15
14-Oct	22.71	29.58	22.41	27.92
15-Oct	22.91	31.77	23.15	29.15
16-Oct	23.36	25.84	24.08	25.63
17-Oct	23.53	26.18	24.24	25.70
18-Oct	23.83	27.38	24.03	27.34
19-Oct	23.78	25.55	23.53	25.09
20-Oct	23.57	27.27	22.92	25.76
21-Oct	23.22	25.21	23.70	25.08

Table D1 (cont'd)

Date	Average daily soil temperature minimum	Average daily soil temperature maximum	Daily air temperature minimum	Daily air temperature maximum
22-Oct	22.88	24.31	23.21	24.83
23-Oct	22.71	24.60	22.58	24.79
24-Oct	22.24	26.29	22.98	25.05
25-Oct	23.08	29.38	22.70	29.40
26-Oct	22.90	29.51	22.83	26.93
27-Oct	23.19	24.99	23.69	25.15
28-Oct	23.16	27.37	23.53	25.59
29-Oct	22.75	31.30	22.88	28.56
30-Oct	23.27	32.49	23.47	34.10
31-Oct	23.82	30.09	23.48	29.99
1-Nov	23.12	29.53	23.06	26.6
2-Nov	22.46	25.84	22.35	25.78
3-Nov	22.12	25.63	23.11	25.12
4-Nov	22.53	27.48	23.55	25.62
5-Nov	23.29	31.22	23.99	31.06
6-Nov	20.18	26.18	24.18	30.81
7-Nov				
8-Nov				
9-Nov				
10-Nov				
11-Nov				
12-Nov				
13-Nov	23.37	24.99	23.39	24.61
14-Nov	23.00	25.27	23.38	24.76
15-Nov	23.13	26.48	23.21	24.57
16-Nov	21.93	23.73	21.37	23.80
17-Nov	22.41	25.91	23.10	24.52
18-Nov	23.05	26.40	23.33	25.06
19-Nov	22.88	26.03	22.85	24.70
20-Nov	22.69	28.11	23.15	25.21
21-Nov	22.64	29.23	22.23	25.93
22-Nov	22.86	30.29	22.33	29.76
23-Nov	23.02	30.25	21.73	29.91
24-Nov	23.18	30.79	22.22	30.10
25-Nov	23.56	27.62	23.07	26.42
26-Nov	23.86	28.68	23.55	28.17
27-Nov	23.80	27.92	23.6	27.33
28-Nov	23.93	30.41	23.03	31.29
29-Nov	24.00	26.03	23.84	25.62
30-Nov	23.01	24.86	22.91	25.05
1-Dec	18.93	23.16	16.76	24.02
2-Dec	20.90	26.79	21.74	24.69
3-Dec	20.64	26.12	20.24	24.31
4-Dec	18.89	24.63	19.39	23.95
5-Dec	19.85	26.51	20.23	25.02
6-Dec	20.14	24.55	20.12	24.37
7-Dec	17.21	26.02	17.91	26.23
8-Dec	17.67	26.56	18.67	24.91
9-Dec	17.46	27.00	18.49	26.13
10-Dec	20.80	27.75	21.37	28.32
11-Dec	23.33	27.11	23.24	25.91

Table D1 (cont'd)

Date	Average daily soil temperature minimum	Average daily soil temperature maximum	Daily air temperature minimum	Daily air temperature maximum
12-Dec	23.36	24.87	23.58	24.83
13-Dec	23.06	25.15	23.18	24.78
14-Dec	22.92	26.13	23.00	24.66
15-Dec	22.81	25.05	23.58	24.71
16-Dec	23.00	26.98	23.41	25.63
17-Dec	23.55	27.45	23.03	25.25
18-Dec	23.00	27.37	22.89	26.14
19-Dec	22.54	27.89	21.54	27.29
20-Dec	21.86	26.92	22.61	25.66
21-Dec	22.88	25.29	23.58	24.73
22-Dec	23.06	24.79	23.47	24.85
23-Dec	22.53	23.90	23.26	24.55
24-Dec	22.41	26.91	22.59	27.00
25-Dec	22.78	25.40	23.49	24.77
26-Dec	22.18	24.22	22.92	24.65
27-Dec	22.17	25.89	22.83	24.43
28-Dec	22.82	26.45	23.05	25.04
29-Dec	22.98	26.25	22.67	24.94
30-Dec	22.47	26.62	22.31	26.67
31-Dec	22.78	24.35	23.99	24.97
1-Jan	22.55	24.59	23.03	25.12
2-Jan	22.17	27.03	22.44	27.72
3-Jan	21.06	26.27	22.29	26.77
4-Jan	22.45	25.74	22.99	25.85
5-Jan	22.91	25.01	24.08	25.09
6-Jan	22.73	24.79	23.67	25.02
7-Jan	22.34	24.71	23.69	25.12
8-Jan	21.13	25.63	21.72	26.44
9-Jan	20.60	24.62	20.51	24.75
10-Jan	17.92	25.93	18.72	24.13
11-Jan	19.77	25.49	20.48	24.59
12-Jan	22.42	23.52	23.07	24.59

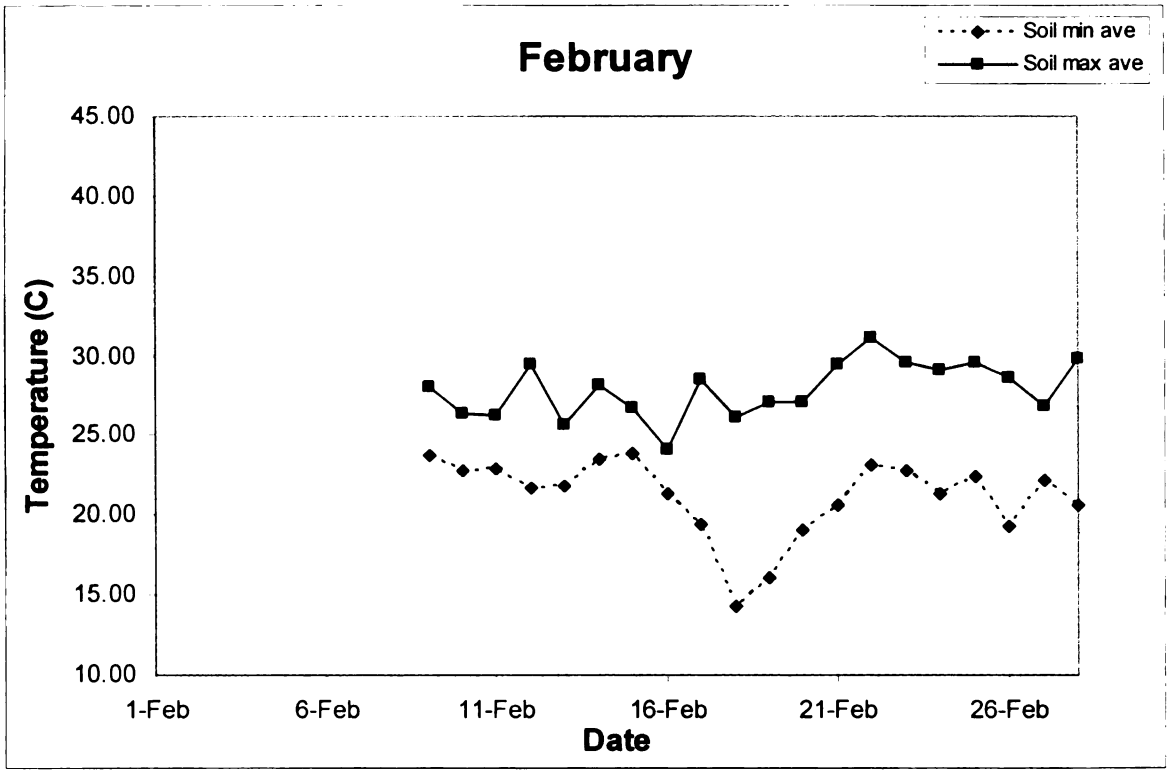

Figure D1: February temperature data.

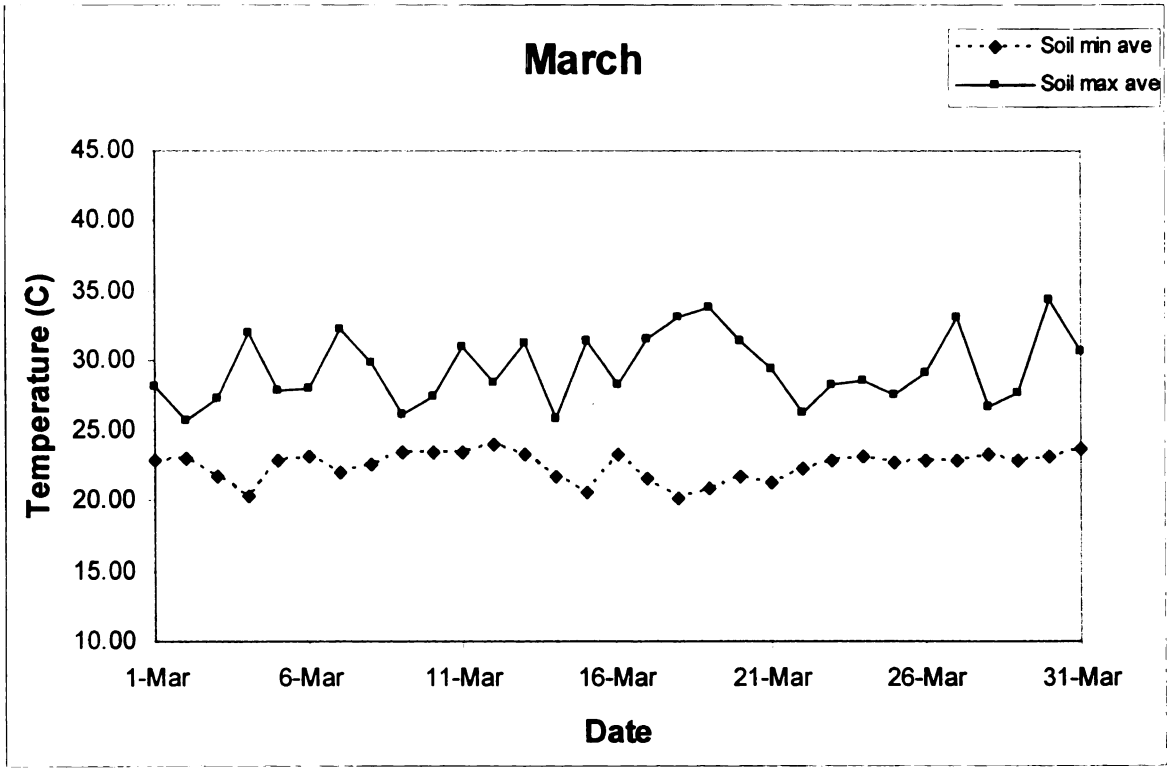

Figure D2: March temperature data.

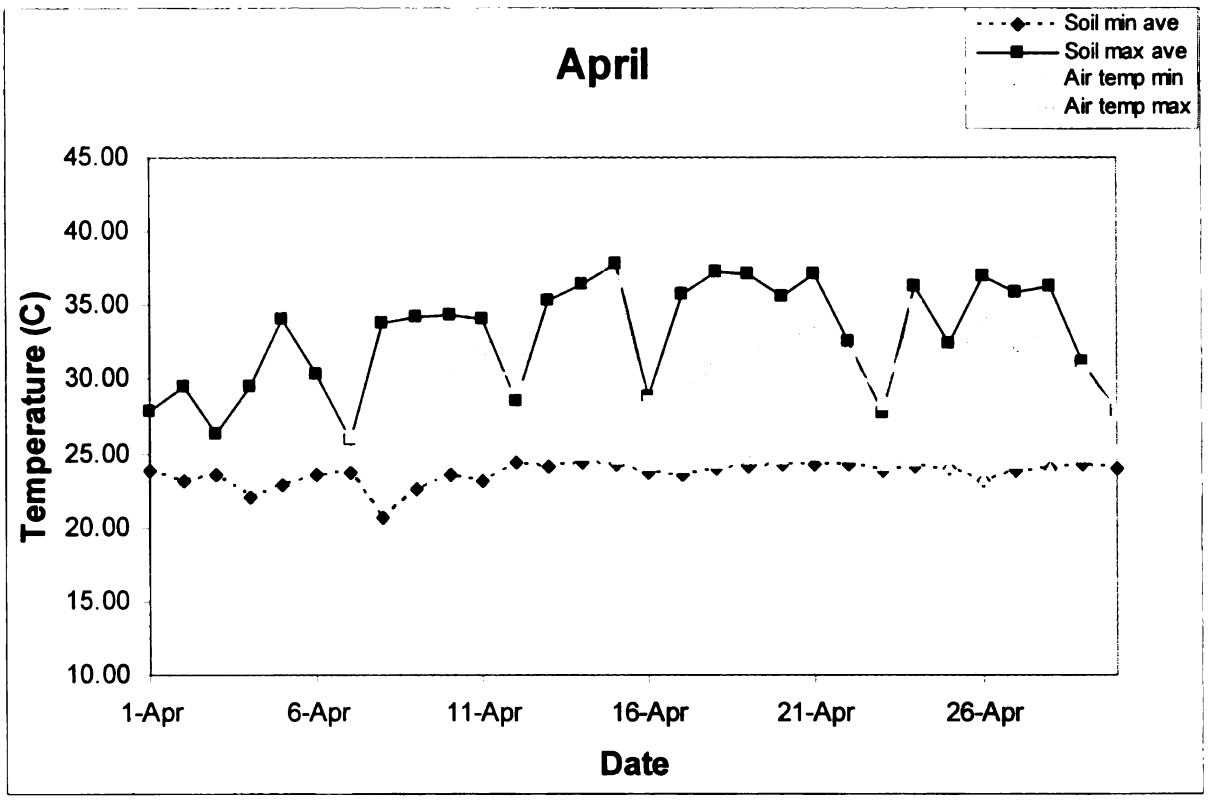

Figure D3: April temperature data.

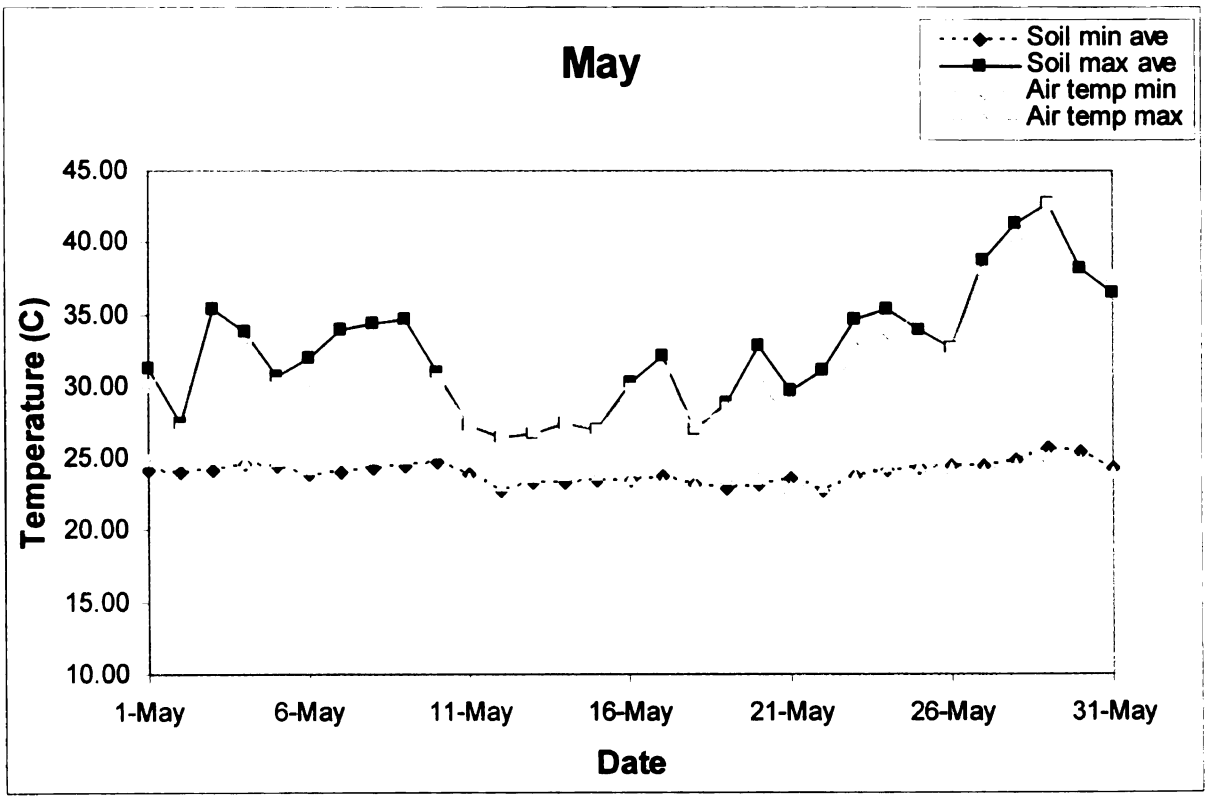

Figure D4: May temperature data.

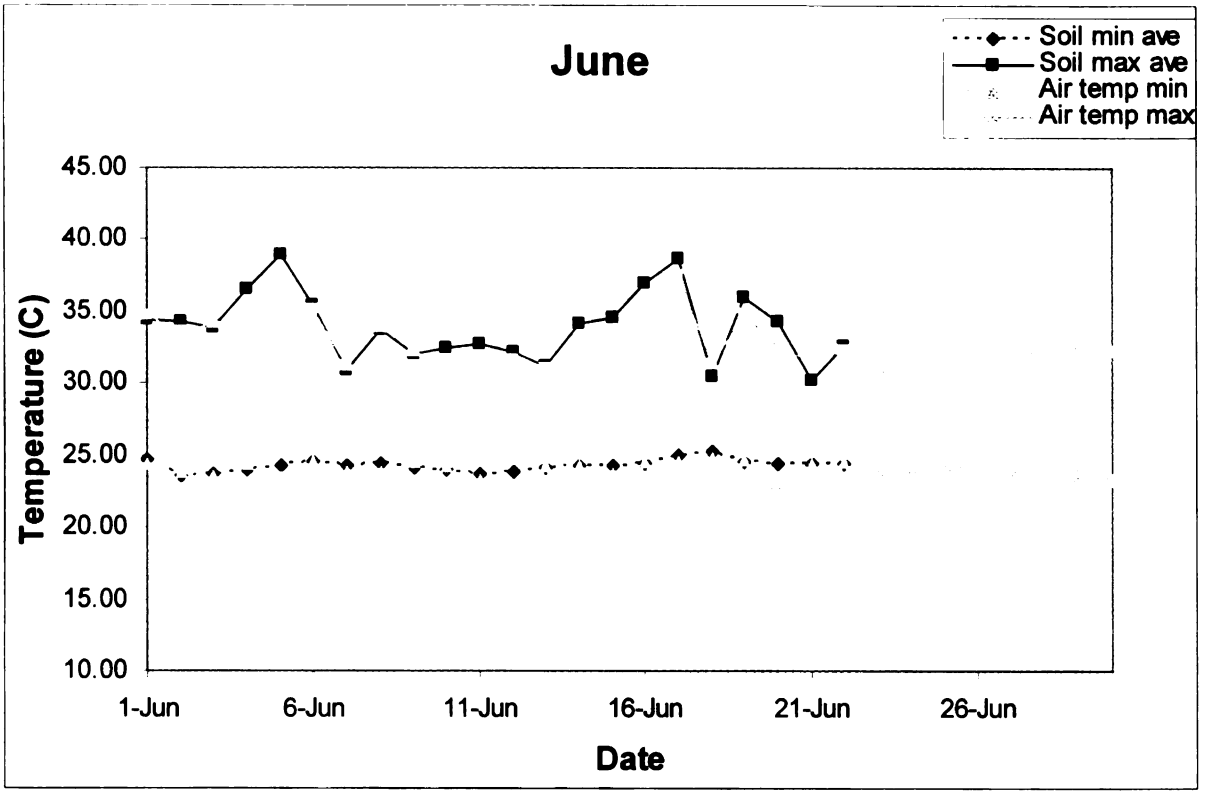

Figure D5: June temperature data.

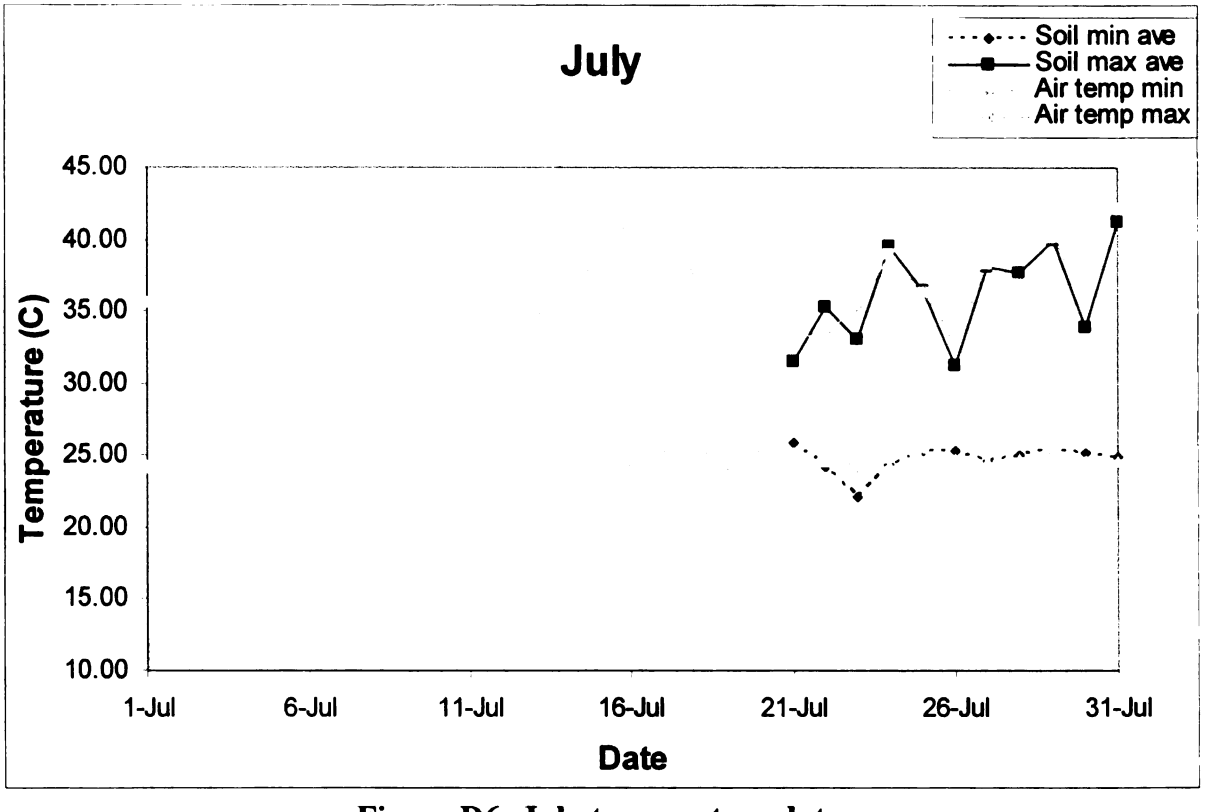

Figure D6: July temperature data.

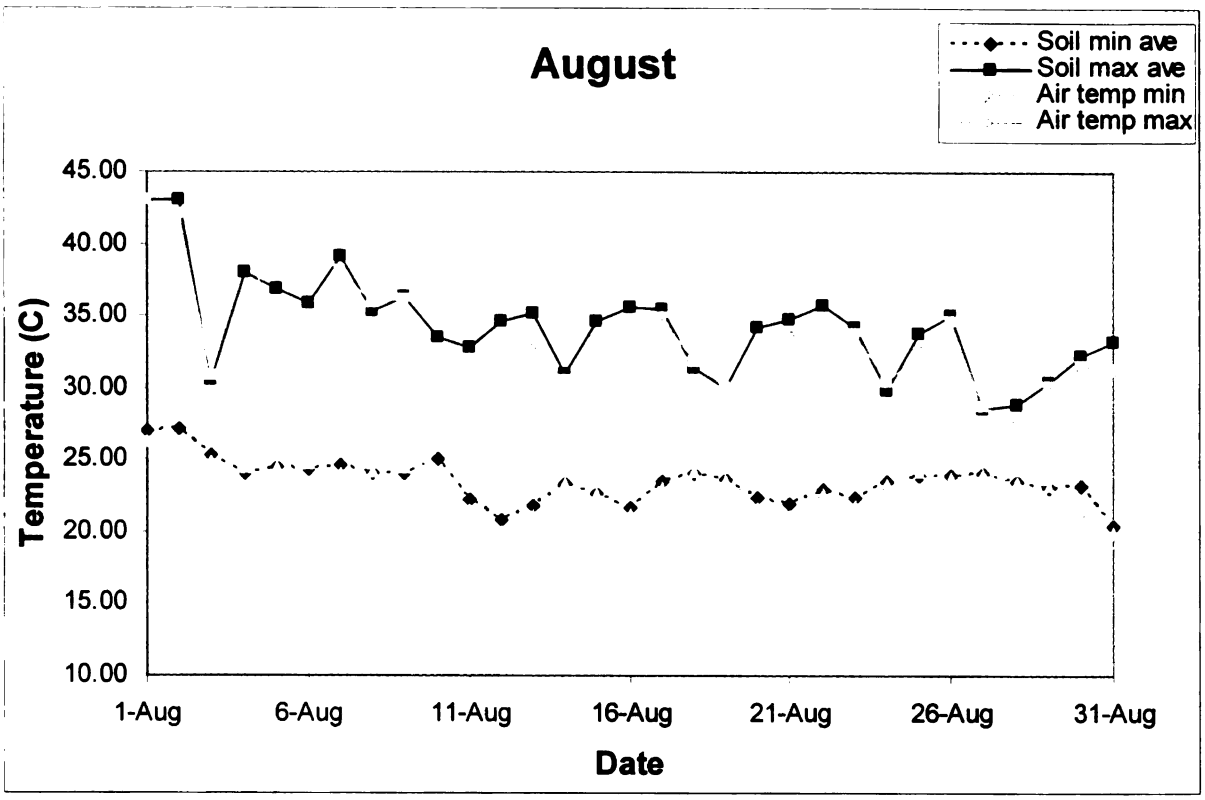

Figure D7: August temperature data.

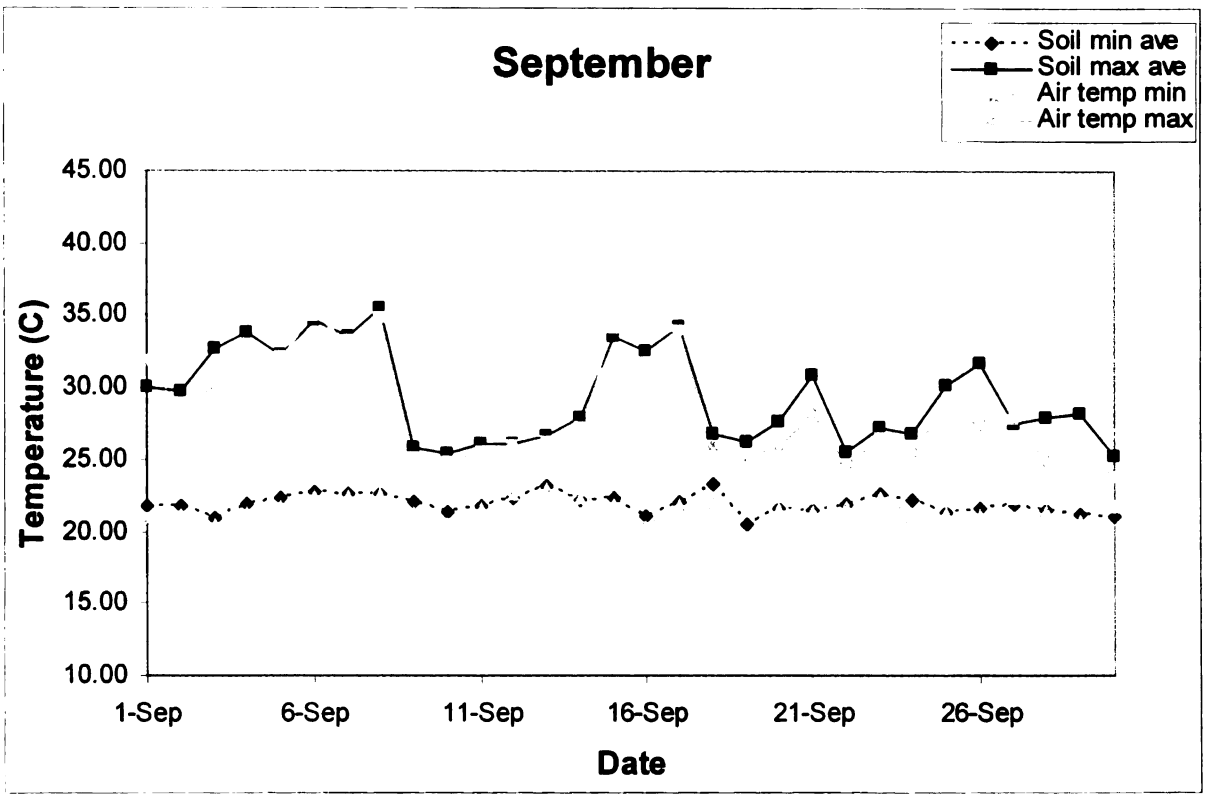

Figure D8: September temperature data.

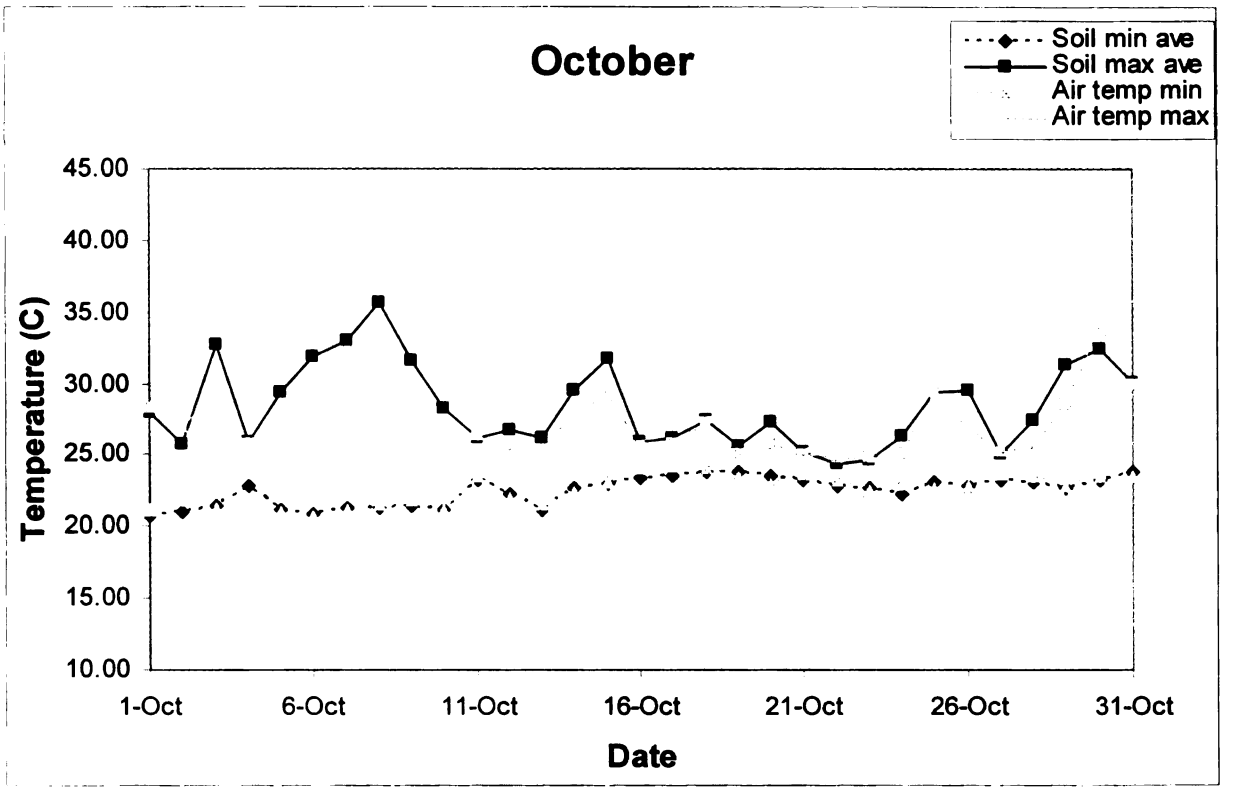

Figure D9: October temperature data.

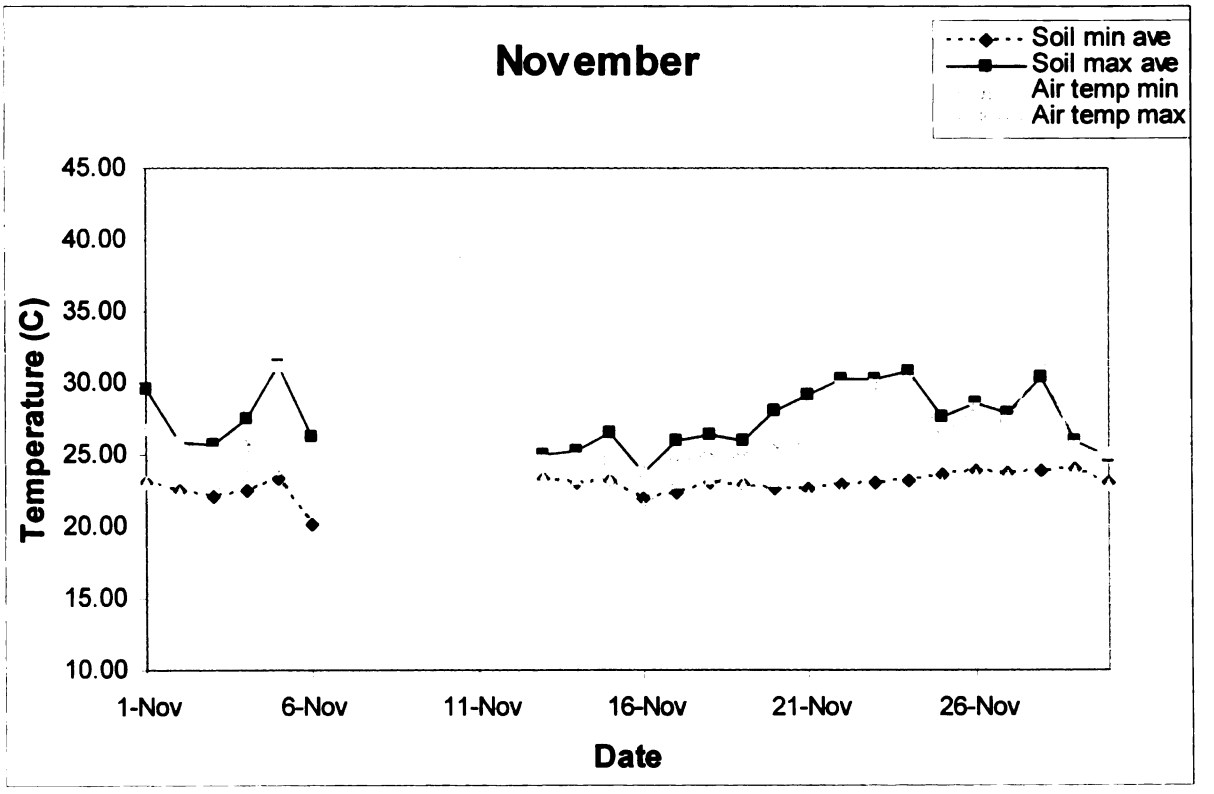

Figure D10: November temperature data.

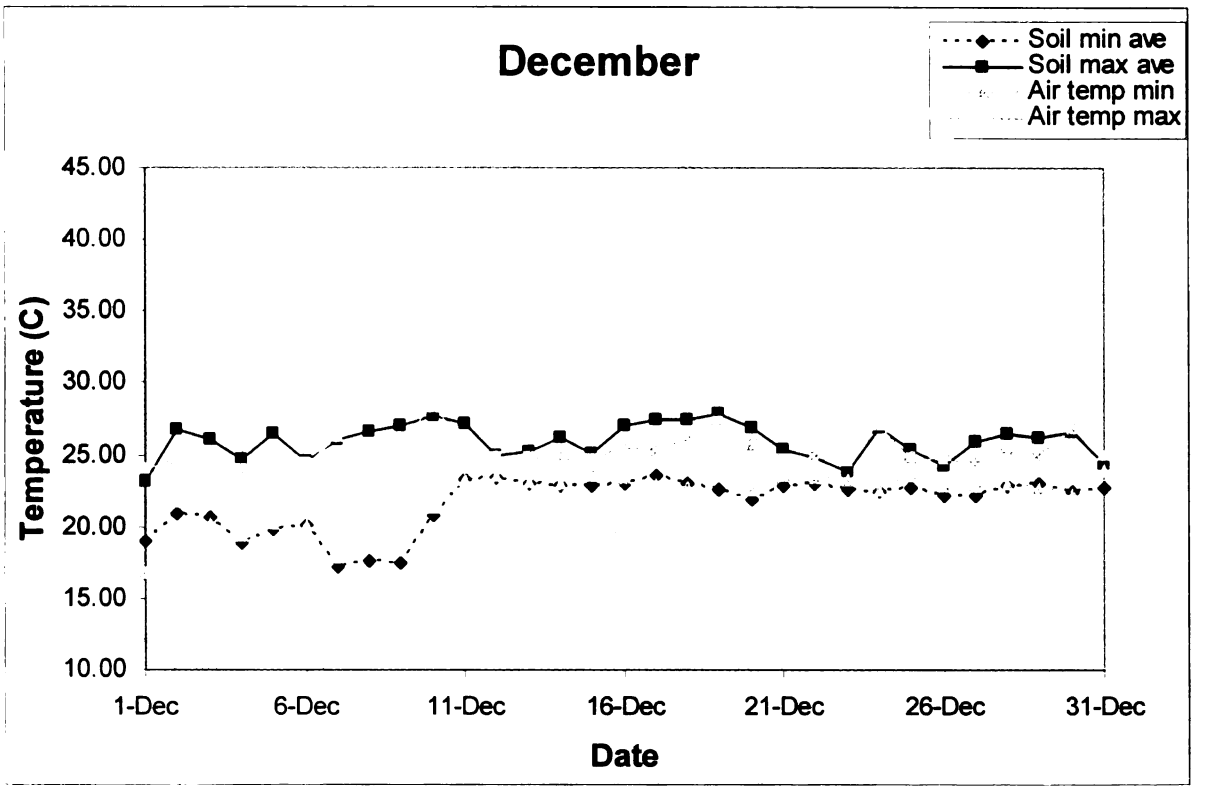

Figure D11: December temperature data.

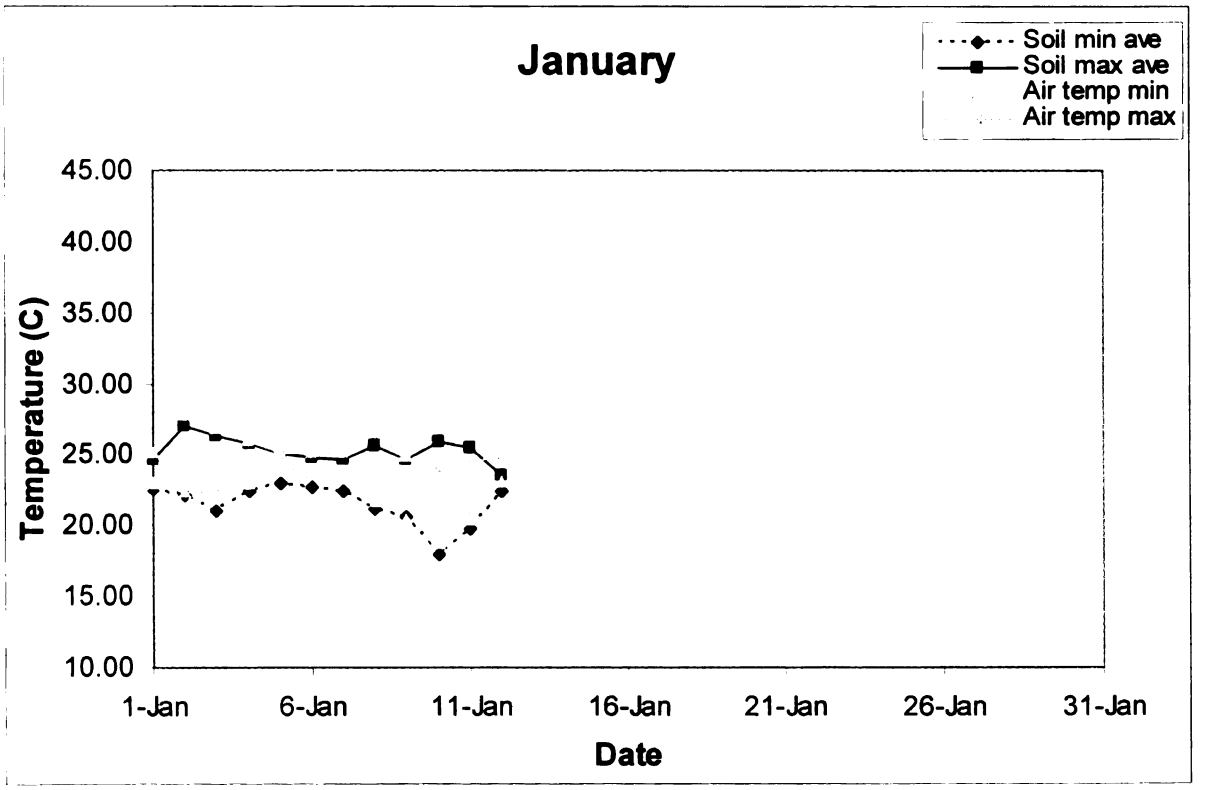

Figure D12: January temperature data.

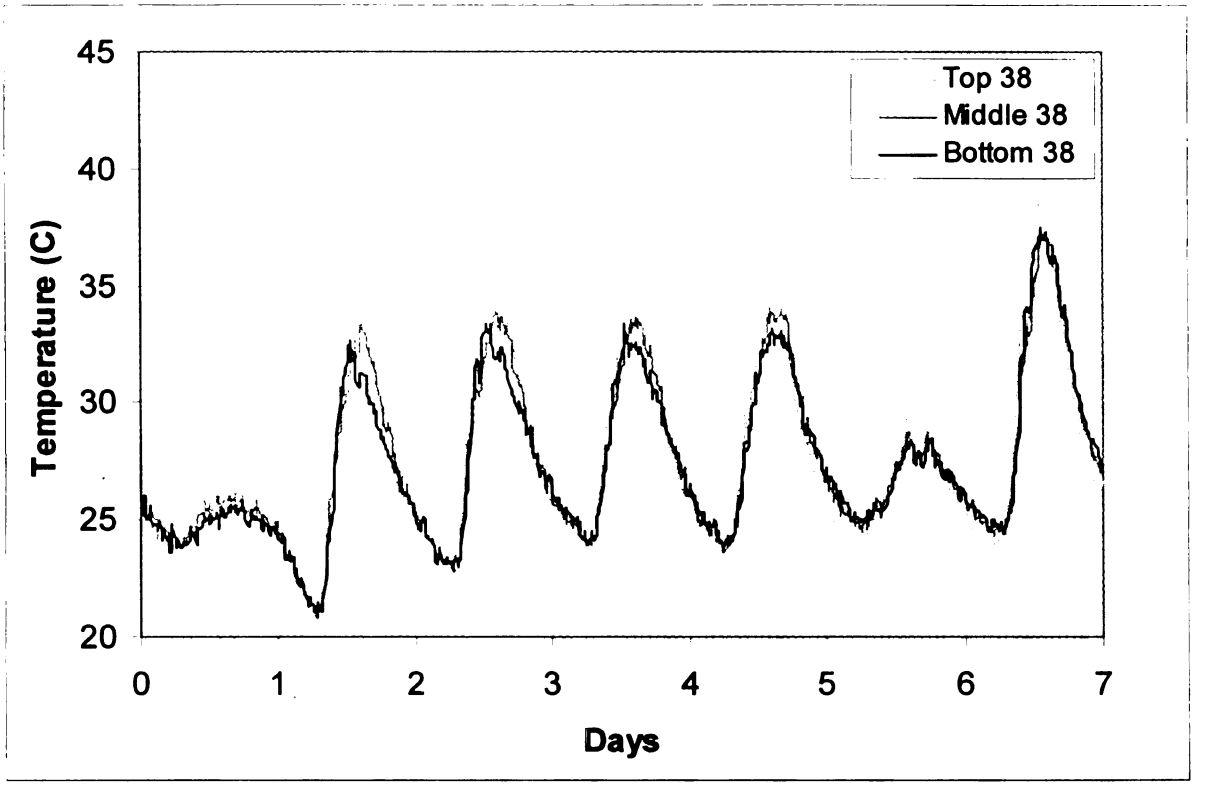

Figure D13: Soil temperature during the week before the *bare, no manure* simulated rain event.

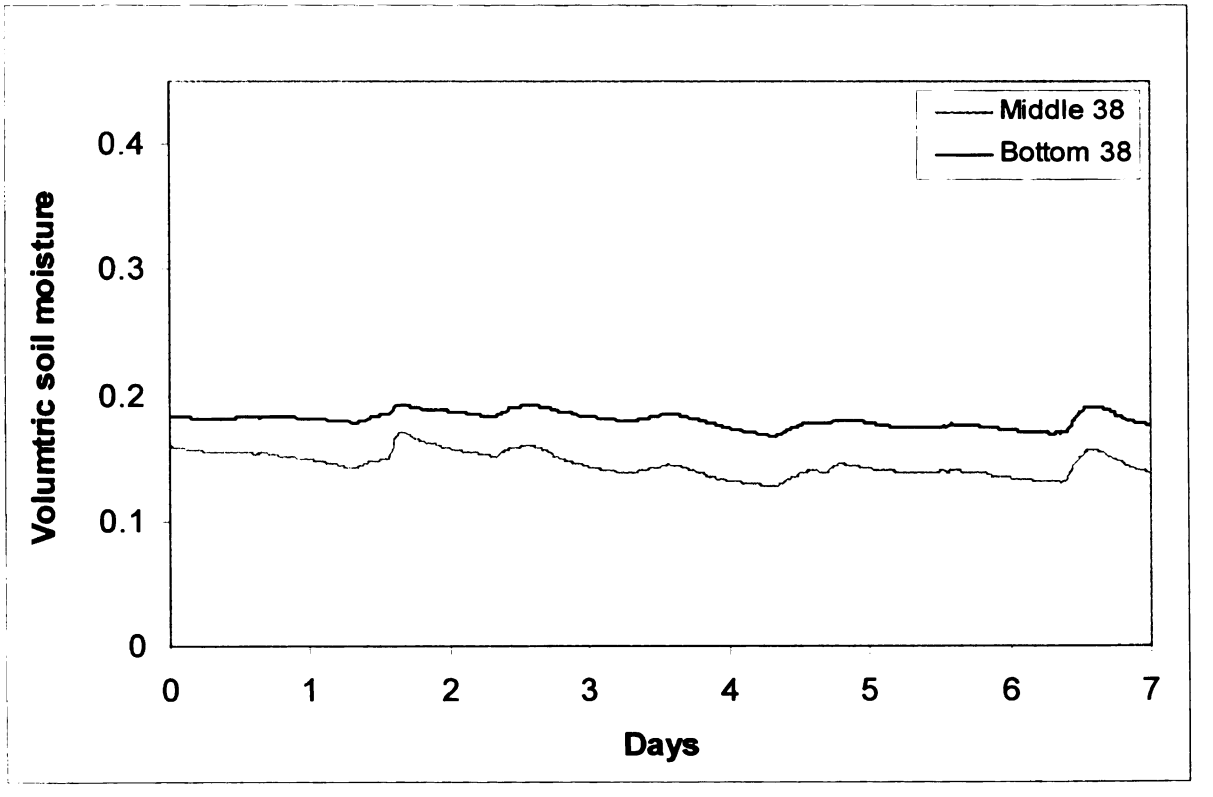

Figure D14: Soil moisture during the week before the *bare, no manure* simulated rain event.

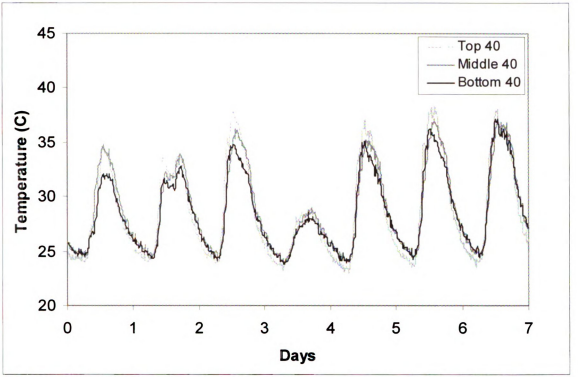

Figure D15: Soil temperature during the week before the *bare, manure* simulated rain event.

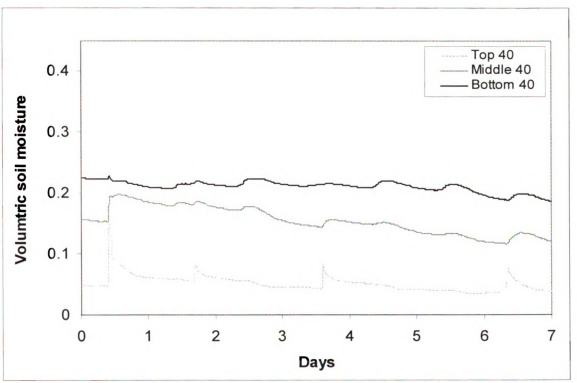

Figure D16: Soil moisture during the week before the *bare, manure* simulated rain event.

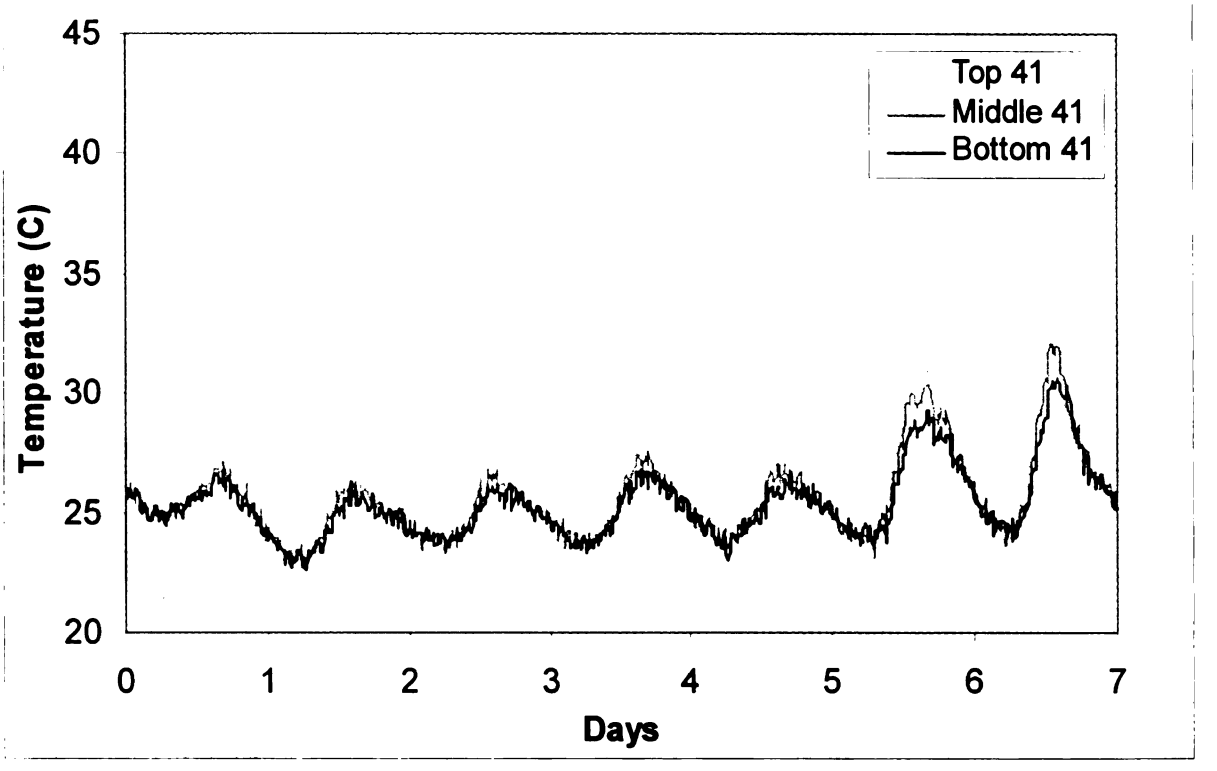

Figure D17: Soil temperature during the week before the *rye, no manure* simulated rain event.

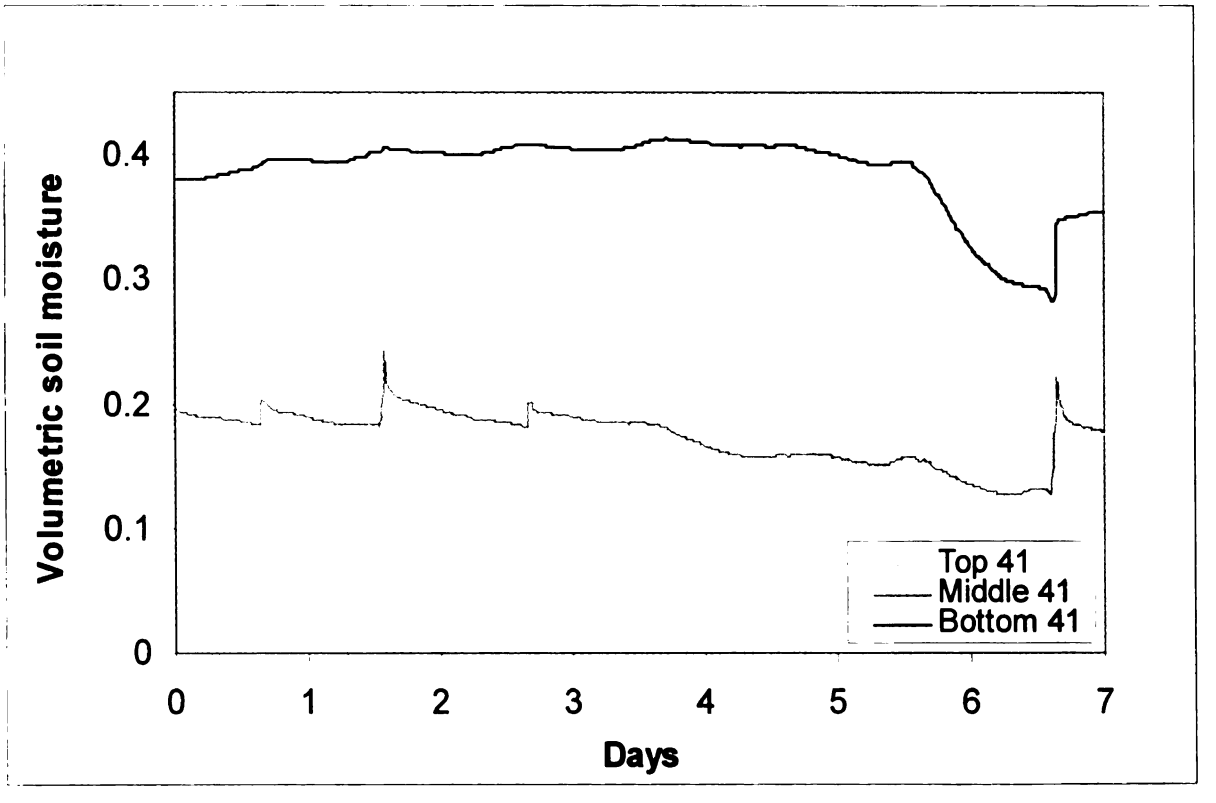

Figure D18: Soil moisture during the week before the *rye, no manure* simulated rain event.

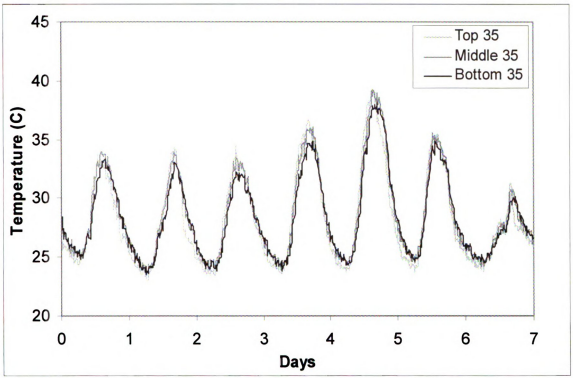

Figure D19: Soil temperature in column 35 during the week before the *rye, manure* simulated rain event.

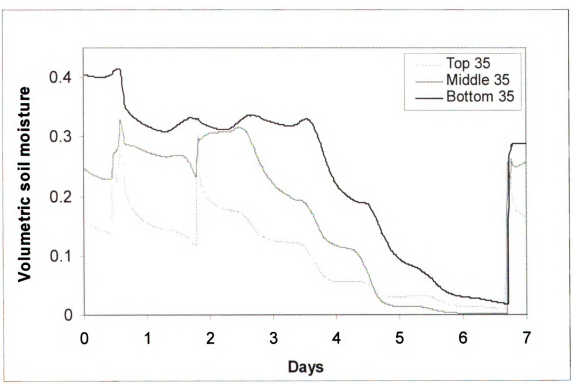

Figure D20: Soil moisture in column 35 during the week before the *rye, manure* simulated rain event.

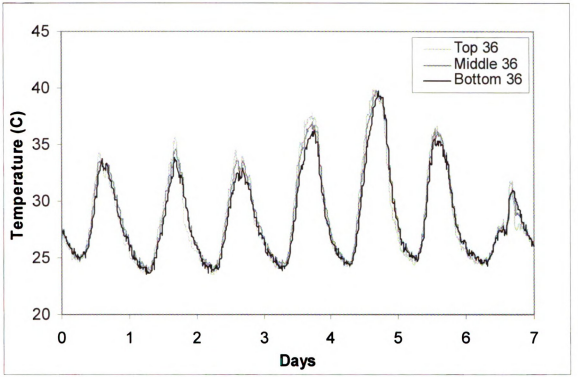

Figure D21: Soil temperature in column 36 during the week before the *rye, manure* simulated rain event.

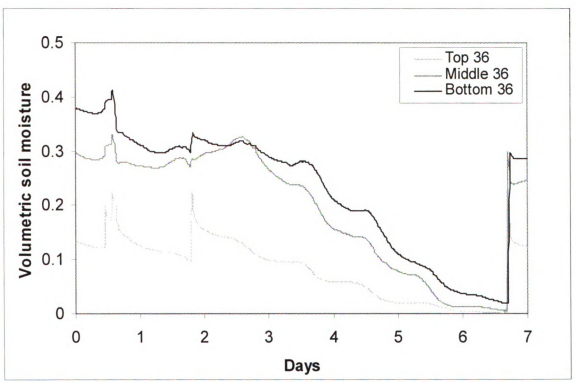

Figure D22: Soil moisture in column 36 during the week before the *rye, manure* simulated rain event.

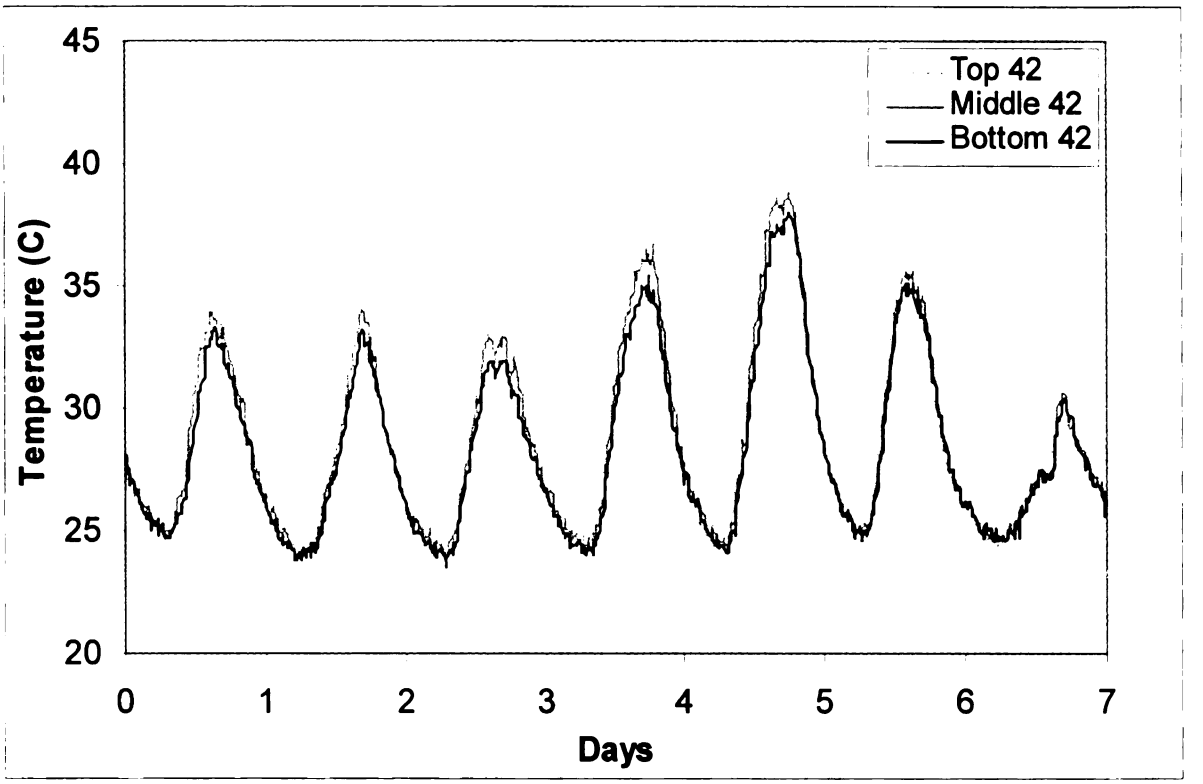

Figure D23: Soil temperature in column 42 during the week before the *rye, manure* simulated rain event.

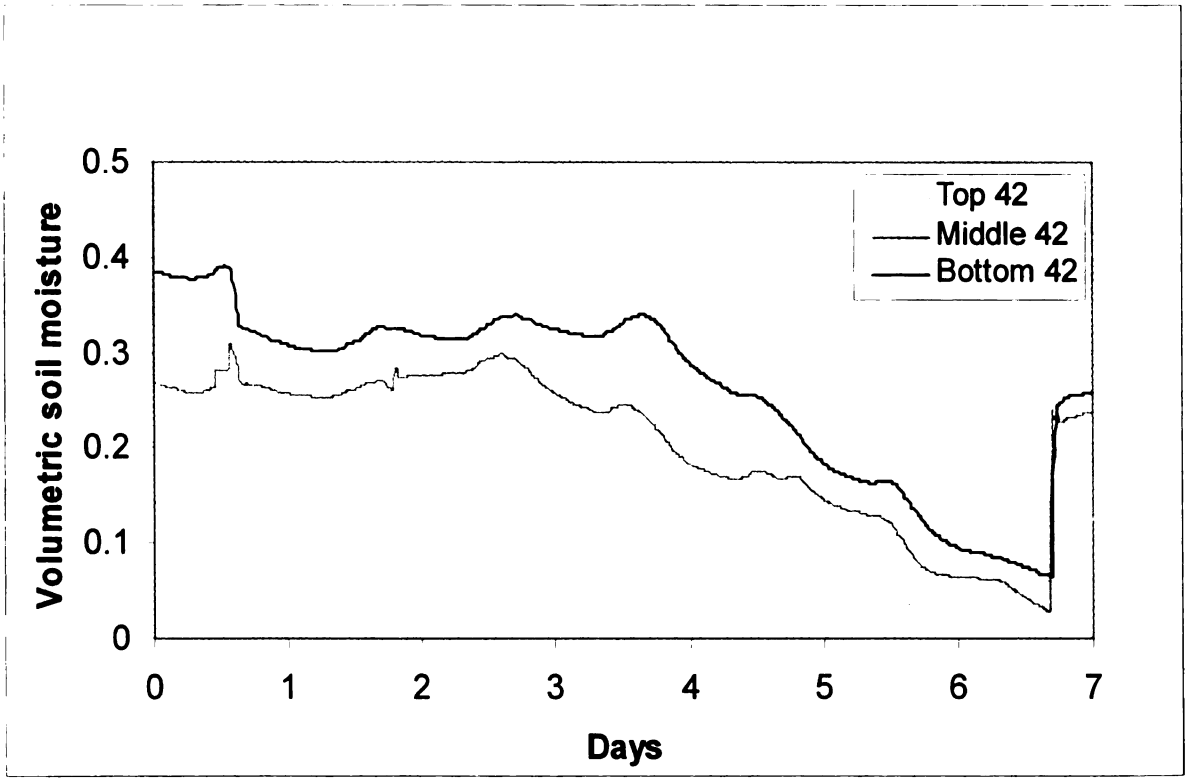

Figure D24: Soil moisture in column 42 during the week before the *rye, manure* simulated rain event.

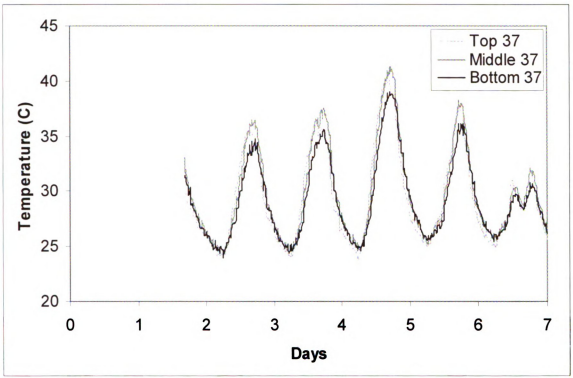

Figure D25: Soil temperature during the week before the *desiccated corn, no manure* simulated rain event.

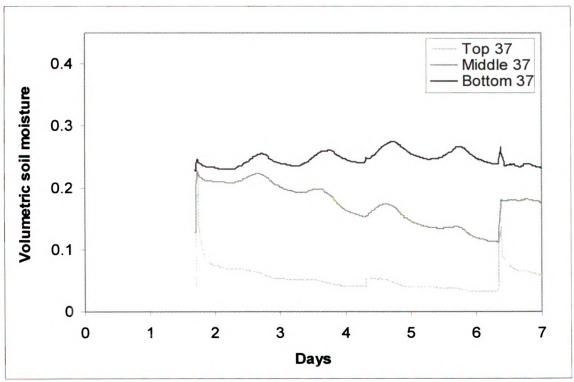

Figure D26: Soil moisture during the week before the *desiccated corn, no manure* simulated rain event.

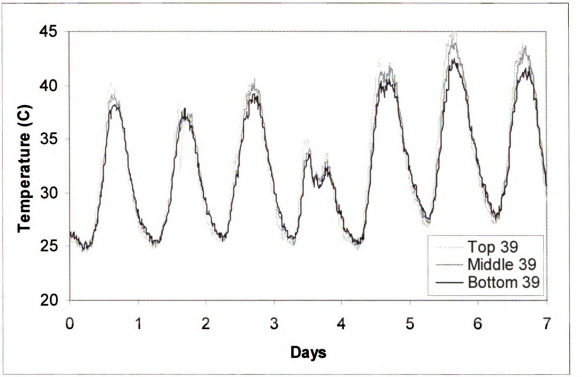

Figure D27: Soil temperature during the week before the *desiccated corn, manure* simulated rain event.

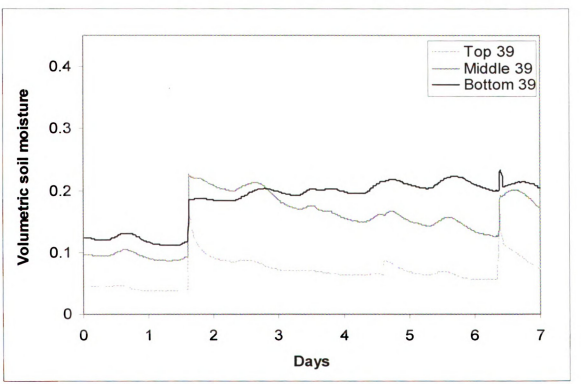

Figure D28: Soil moisture during the week before the *desiccated corn, manure* simulated rain event.

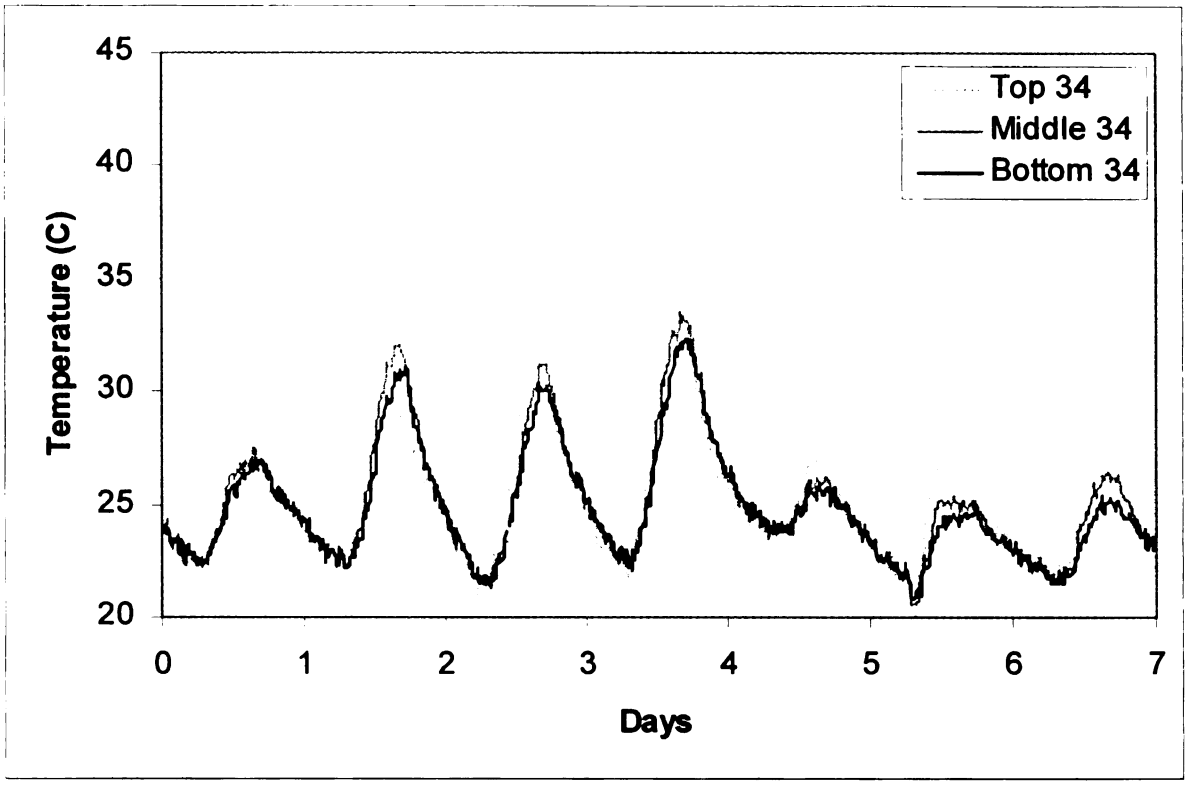

Figure D29: Soil temperature during the week before the *desiccated corn/rye, no manure* simulated rain event.

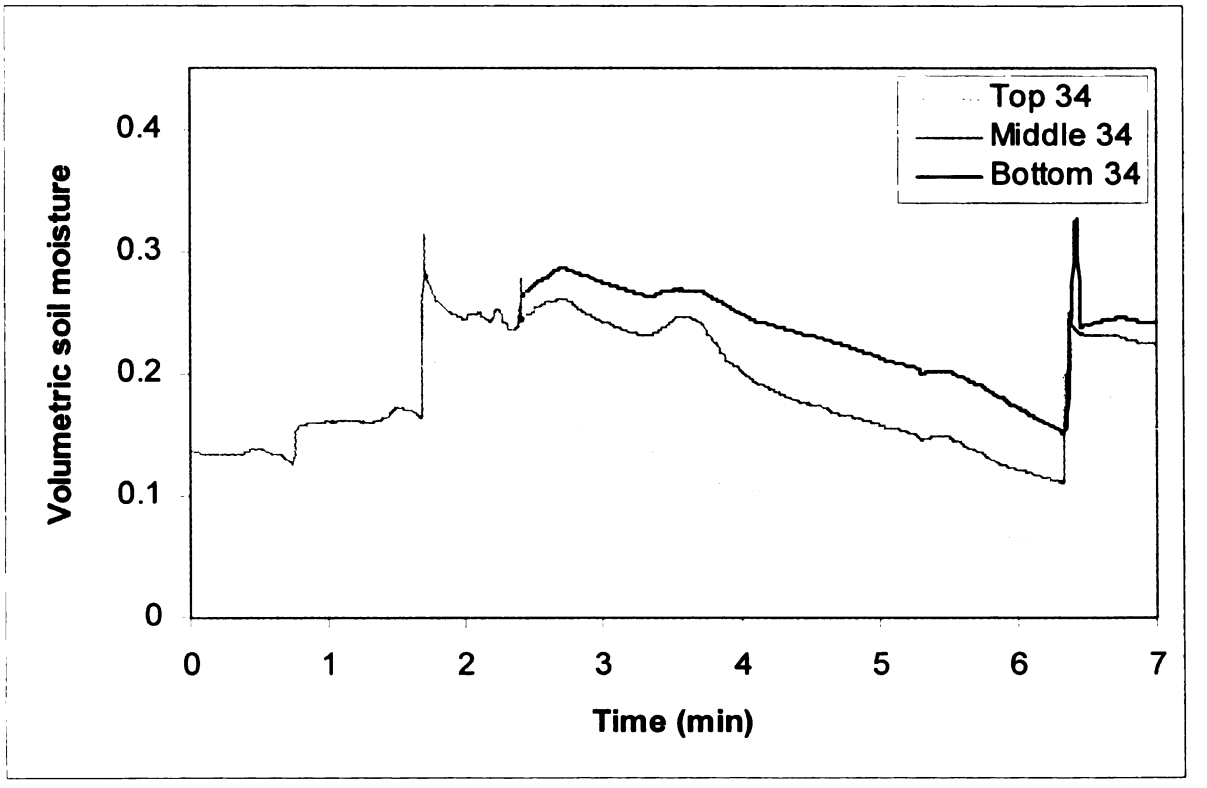

Figure D30: Soil moisture during the week before the *desiccated corn/rye, no manure* simulated rain event.

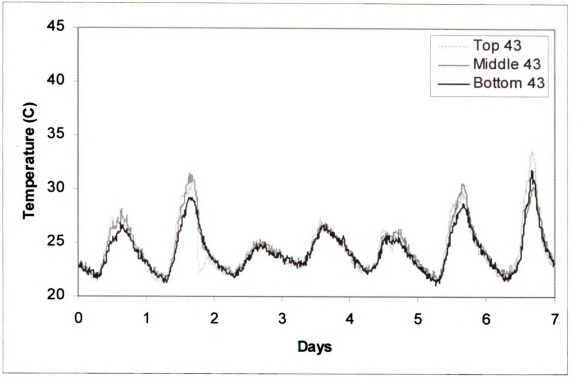

Figure D31: Soil temperature during the week before the *desiccated corn/rye, manure* simulated rain event.

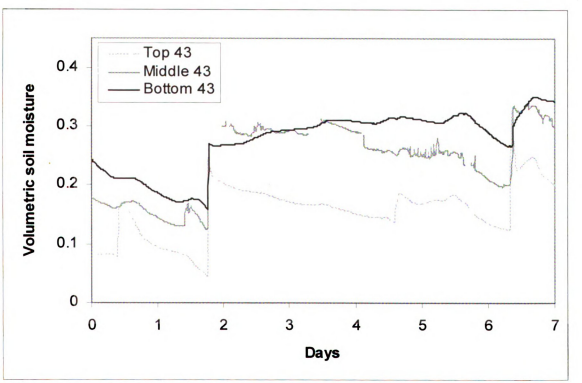

Figure D32: Soil moisture during the week before the *desiccated corn/rye, manure* simulated rain event.

APPENDIX E

CSC File for CR-10 datalogger with AM32 multiplexer

```
;{CR10X}
;-Wiring for CR10-

; AM32 Multiplexer
;   1H: COM HI
;   E1: COM LO
;   C2: CLK
;   C1: RES
;   G: GND
;   12V: 12V

;-Wiring for AM32 Multiplexer-

;ECHO Top (1)
;   1L: Red
;   1H: Black

 ;ECHO Top (2)
;   2L: Red
;   2H: Black

 ;ECHO Top (3)
;   3L: Red
;   3H: Black

 ;ECHO Top (4)
;   4L: Red
;   4H: Black

 ;ECHO Top (5)
;   5L: Red
;   5H: Black

 ;ECHO Middle (1)
;   6L: Red
;   6H: Black

 ;ECHO Middle (2)
;   7L: Red
;   7H: Black

 ;ECHO Middle (3)
;   8L: Red
```

; 8H: Black

;ECHO Middle (4)
; 9L: Red
; 9H: Black

;ECHO Middle (5)
; 10L: Red
; 10H: Black

;ECHO Bottom (1)
; 11L: Red
; 11H: Black

;ECHO Bottom (2)
; 12L: Red
; 12H: Black

;ECHO Bottom (3)
; 13L: Red
; 13H: Black

;ECHO Bottom (4)
; 14L: Red
; 14H: Black

;ECHO Bottom (5)
; 15L: Red
; 15H: Black

;107 Top (1)
; 16L: Red
; 16H: Black

;107 Top (2)
; 17L: Red
; 17H: Black

;107 Top (3)
; 18L: Red
; 18H: Black

;107 Top (4)
; 19H: Black
; 19L: Red

```
;107 Top (5)
; 20H: Black
; 20L: Red

;107 Middle (1)
; 21H: Black
; 21L: Red

;107 Middle (2)
; 22H: Black
; 22L: Red

;107 Middle (3)
; 23H: Black
; 23L: Red

;107 Middle (4)
; 24H: Black
; 24L: Red

;107 Middle (5)
; 25H: Black
; 25L: Red

;107 Bottom (1)
;  26H: Black
;  26L: Red

;107 Bottom (2)
;  27H: Black
;  27L: Red

;107 Bottom (3)
;  28H: Black
;  28L: Red

;107 Bottom (4)
;  29H: Black
;  29L: Red

;107 Bottom (5)
;  30H: Black
;  30L: Red

; 107 Air Temperature
;  31H: Black
```

```
;  31L: Red

;  32H:
;  32L:


*Table 1 Program
  01: 60.0000   Execution Interval (seconds)

1: Batt Voltage (P10)
 1: 1       Loc [ Batt_Volt ]

2: If time is (P92)
 1: 0       Minutes (Seconds --) into a
 2: 5       Interval (same units as above)
 3: 30      Then Do

    3: Do (P86)
     1: 41      Set Port 1 High

    4: Beginning of Loop (P87)
     1: 0       Delay
     2: 15      Loop Count

        5: Do (P86)
         1: 72      Pulse Port 2

        6: Excitation with Delay (P22)
         1: 1       Ex Channel
         2: 0       Delay W/Ex (0.01 sec units)
         3: 1       Delay After Ex (0.01 sec units)
         4: 0       mV Excitation

;Measure EC10 probes
        7: Excite-Delay (SE) (P4)
         1: 1       Reps
         2: 25      5000 mV, 60 Hz Reject, Fast Range (Delay must be 0)
         3: 1       SE Channel
         4: 1       Excite all reps w/Exchan 1
         5: 1       Delay (0.01 sec units)
         6: 2500    mV Excitation
         7: 2    -- Loc [ EC_Top_1  ]
         8: .0936   Multiplier
         9: -37     Offset

;Check for negative reading and convert to m3/m3 water content
```

```
  8:  If (X<=>F) (P89)
   1: 2     -- X Loc [ EC_Top_1  ]
   2: 4       <
   3: 0       F
   4: 30      Then Do

     9:  Z=F x 10^n (P30)
      1: 0       F
      2: 0       n, Exponent of 10
      3: 2     -- Z Loc [ EC_Top_1  ]

    10:  End (P95)

    11:  Z=X*F (P37)
     1: 2     -- X Loc [ EC_Top_1  ]
     2: .01     F
     3: 2     -- Z Loc [ EC_Top_1  ]

12:  End (P95)

13:  Beginning of Loop (P87)
 1: 0       Delay
 2: 15      Loop Count

    14:  Do (P86)
     1: 72      Pulse Port 2

    15:  Excitation with Delay (P22)
     1: 1       Ex Channel
     2: 0       Delay W/Ex (0.01 sec units)
     3: 1       Delay After Ex (0.01 sec units)
     4: 0       mV Excitation

    16:  Temp (107) (P11)
     1: 1       Reps
     2: 1       SE Channel
     3: 1       Excite all reps w/Exchan 1
     4: 17    -- Loc [ ST_Top_1  ]
     5: 1.0     Mult
     6: 0.0     Offset

17:  End (P95)

18:  Do (P86)
 1: 72      Pulse Port 2
```

```
   19:  Temp (107) (P11)
    1: 1        Reps
    2: 1        SE Channel
    3: 1        Excite all reps w/Exchan 1
    4: 32       Loc [ Tair_C    ]
    5: 1.0      Mult
    6: 0.0      Offset

   20:  Do (P86)
    1: 51       Set Port 1 Low

21:  End (P95)

22:  If time is (P92)
 1: 0        Minutes (Seconds --) into a
 2: 15       Interval (same units as above)
 3: 10       Set Output Flag High

23:  Set Active Storage Area (P80)^24532
 1: 1        Final Storage Area 1
 2: 15       Array ID

24:  Real Time (P77)^13165
 1: 1220     Year,Day,Hour/Minute (midnight = 2400)

25:  Minimum (P74)^26642
 1: 1        Reps
 2: 0        Value Only
 3: 1        Loc [ Batt_Volt ]

26:  Average (P71)^16767
 1: 31       Reps
 2: 2        Loc [ EC_Top_1  ]

*Table 2 Program
  01: 10       Execution Interval (seconds)

1:  Serial Out (P96)
 1: 71       Storage Module

*Table 3 Subroutines

End Program
```

```
-Input Locations-
1 Batt_Volt 1 1 1
2 EC_Top_1  7 3 3
3 EC_Top_2  3 1 0
4 EC_Top_3  3 1 0
5 EC_Top_4  11 1 0
6 EC_Top_5  19 1 0
7 EC_Mid_1  7 1 0
8 EC_Mid_2  11 1 0
9 EC_Mid_3  11 1 0
10 EC_Mid_4  11 1 0
11 EC_Mid_5  19 1 0
12 EC_Bot_1  7 1 0
13 EC_Bot_2  11 1 0
14 EC_Bot_3  11 1 0
15 EC_Bot_4  11 1 0
16 EC_Bot_5  19 1 0
17 ST_Top_1  7 1 1
18 ST_Top_2  11 1 0
19 ST_Top_3  11 1 0
20 ST_Top_4  11 1 0
21 ST_Top_5  19 1 0
22 ST_Mid_1  7 1 0
23 ST_Mid_2  11 1 0
24 ST_Mid_3  11 1 0
25 ST_Mid_4  11 1 0
26 ST_Mid_5  19 1 0
27 ST_Bot_1  7 1 0
28 ST_Bot_2  11 1 0
29 ST_Bot_3  11 1 0
30 ST_Bot_4  11 1 0
31 ST_Bot_5  19 1 0
32 Tair_C    1 1 1
-Program Security-
0000
0000
0000
-Mode 4-
-Final Storage Area 2-
0
-CR10X ID-
0
-CR10X Power Up-
3
-CR10X Compile Setting-
```

3
-CR10X RS-232 Setting-
-1
-DLD File Labels-
0
-Final Storage Labels-
0,15,24532
1,Year_RTM,13165
1,Day_RTM
1,Hour_Minute_RTM
2,Batt_Volt_MIN~1,26642
3,EC_Top_1_AVG~2,16767
3,EC_Top_2_AVG~3
3,EC_Top_3_AVG~4
3,EC_Top_4_AVG~5
3,EC_Top_5_AVG~6
3,EC_Mid_1_AVG~7
3,EC_Mid_2_AVG~8
3,EC_Mid_3_AVG~9
3,EC_Mid_4_AVG~10
3,EC_Mid_5_AVG~11
3,EC_Bot_1_AVG~12
3,EC_Bot_2_AVG~13
3,EC_Bot_3_AVG~14
3,EC_Bot_4_AVG~15
3,EC_Bot_5_AVG~16
3,ST_Top_1_AVG~17
3,ST_Top_2_AVG~18
3,ST_Top_3_AVG~19
3,ST_Top_4_AVG~20
3,ST_Top_5_AVG~21
3,ST_Mid_1_AVG~22
3,ST_Mid_2_AVG~23
3,ST_Mid_3_AVG~24
3,ST_Mid_4_AVG~25
3,ST_Mid_5_AVG~26
3,ST_Bot_1_AVG~27
3,ST_Bot_2_AVG~28
3,ST_Bot_3_AVG~29
3,ST_Bot_4_AVG~30
3,ST_Bot_5_AVG~31
3,Tair_C_AVG~32

DLD File for CR-10 datalogger with AM32 multiplexer

```
};CR10X
;5COL_10X.DLD
;$
;:Batt_Volt:EC_Top_1 :EC_Top_2 :EC_Top_3 :EC_Top_4
;:EC_Top_5 :EC_Mid_1 :EC_Mid_2 :EC_Mid_3 :EC_Mid_4
;:EC_Mid_5 :EC_Bot_1 :EC_Bot_2 :EC_Bot_3 :EC_Bot_4
;:EC_Bot_5 :ST_Top_1 :ST_Top_2 :ST_Top_3 :ST_Top_4
;:ST_Top_5 :ST_Mid_1 :ST_Mid_2 :ST_Mid_3 :ST_Mid_4
;:ST_Mid_5 :ST_Bot_1 :ST_Bot_2 :ST_Bot_3 :ST_Bot_4
;:ST_Bot_5 :Tair_C
;$

MODE 1
SCAN RATE 60.0000

1:P10
1:1

2:P92
1:0
2:5
3:30

3:P86
1:41

4:P87
1:0
2:15

5:P86
1:72

6:P22
1:1
2:0
3:1
4:0

7:P4
1:1
2:25
3:1
4:1
```

5:1
6:2500
7:2--
8:.0936
9:-37

8:P89
1:2--
2:4
3:0
4:30

9:P30
1:0
2:0
3:2--

10:P95

11:P37
1:2--
2:.01
3:2--

12:P95

13:P87
1:0
2:15

14:P86
1:72

15:P22
1:1
2:0
3:1
4:0

16:P11
1:1
2:1
3:1
4:17--
5:1.0
6:0.0

17:P95

18:P86
1:72

19:P11
1:1
2:1
3:1
4:32
5:1.0
6:0.0

20:P86
1:51

21:P95

22:P92
1:0
2:15
3:10

23:P80
1:1
2:15

24:P77
1:1220

25:P74
1:1
2:0
3:1

26:P71
1:31
2:2

MODE 2
SCAN RATE 10

1:P96
1:71

MODE 3

MODE 10
1:32
2:64
3:0

MODE 12
1:0000
2:0000
3:0000

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