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Prevalence and spread of pathogenic Escherichia Coli O157 on farms in north Wales

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PREVALENCE AND SPREAD OF PATHOGENIC *ESCHERICHIA COLI* O157 ON FARMS IN NORTH WALES

A thesis submitted to Bangor University by

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SUMMARY

Escherichia coli O157 is a strain of coliform bacteria associated with serious human disease; infection by the strain, particularly in children and the elderly, may lead to death. Although human infection is often associated with food poisoning, other routes of infection have been identified. These include contact with animals, particularly livestock, bathing in infected water, and mere wandering in rural areas. Aside from the human cost in terms of distress to the afflicted and to their families, there is an economic cost.

Although there is extensive literature on *E. coli* O157, much remains uncertain. The present thesis addresses some of these uncertainties. It specifically addresses livestock (cattle and sheep) infection within North Wales. Issues addressed comprise livestock faecal *E. coli* and other coliform bacteria counts within the area; the effects of grazing on the said counts, and differences in land type (upland or lowland) on them; seasonal variations; the effects of physical and chemical soil properties on *E. coli* and other coliform bacteria populations; the differential effects of grazing system and crop type on the bacterial populations; and transmission of *E. coli* O157 to different types of location by yellow dung flies (*Scathophaga stercoraria*). Investigation of these issues comprises field studies and, in the case of transmission of the bacteria by dung flies, laboratory experiments.

The majority of these studies were successful insofar as they suggest robust conclusions. The most important of these are that sheep faeces host more non-pathogenic *E. coli* than does cattle faeces, that *E. coli* populations within the environment vary by season, that lowland farms have higher populations of *E. coli*, that intensive grazing adds to risk of infection, that yellow dung flies can carry substantial populations of *E. coli* O157, and that the flies may transmit the bacteria to the environment, particularly to water and to vegetation (grass).

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ABBREVIATIONS

Abbreviation	Expression
CO_2	Carbon dioxide
N	Nitrogen
N_2O	Nitrate
NH_3	Ammonium
P	Phosphorous
HC	Haemorrhagic colitis
HUS	Haemorrhagic uremic syndrome
TTP	Thrombotic thrombocytopenia purpura
EC	Electrical conductivity
MC	Moisture content
OM	Organic matter
SD	Standard deviation
SE	Standard error
η_p^2	Partial eta squared
PHLS	Public Health Laboratory Service
WHO	World Health Organization
U.S.EPA	U.S. Environmental Protection Agency
UNICEF	United Nations Children's Fund

CHAPTER 1: INTRODUCTION

1.1. Overview

The present thesis has been supported by the Ministry of Higher Education of the Government of Libya. The aim of the thesis is to further our understanding of the bacterium *Escherichia coli* O157; a known human pathogen, responsible for many deaths worldwide each year. In addition to these, often avoidable, deaths, *E. coli* O157 is implicated in serious chronic diseases, some of which involve kidney damage.

E. coli O157 is only one of a number of strains of *E. coli*, the majority of which are harmless to humans and livestock. Their "natural" habitat is the intestinal tracts of animals. However, recent evidence suggests that, not only can the bacteria survive for long periods in soil and other external environments; such external environments may also facilitate the evolution of new, sometimes pathogenic, strains (e.g., Ishii and Sadowsky, 2008). In this regard, humans living in agricultural areas appear most at risk of *E. coli* O157 infection (Jokinen *et al*., 2012). The present study investigates *E. coli* in one largely agricultural area of the UK (North Wales); though many of the results could be extrapolated to other similar areas.

1.2. Aims and objectives

The study has four main aims, namely:

- To determine levels of *E. coli*, including O157, and other coliforms in cattle and sheep faecal samples in North Wales.

- To determine relative levels of *E. coli* and other coliforms in upland versus lowland soils and in soils from grazed versus ungrazed land. The work also aimed to determine how physico-chemical soil properties that might affect such populations.
- To determine the effects of grazing regime (of kale and swede) of sheep on *E. coli*, coliform populations and broader soil properties.
- To determine the transmission of *E. coli*, including O157 and other coliform populations from sheep and cattle faeces by yellow dung flies (*Scathophaga stercoraria*) to the wider environment.

1.3. Organisation of the thesis

The remainder of this thesis is organised as follows. Chapter 2 provides a literature review. It describes a general description of *E. coli* and other coliforms; it notes the threats—particularly those associated with diarrhoeal diseases—posed to human health by pathogenic strains of the bacteria, especially *E. coli* O157; it then discusses populations at risk, and strains of *E. coli* believed to be particularly virulent. The chapter then moves to routes of infection, including contaminated food such as meat. It follows that with discussion of recent UK outbreaks of *E. coli* poisoning, and of reservoirs of pathogenic strains. It next discusses environmental factors possibly relevant to the strains' survival outside the gastro-intestinal tracts of animals. The chapter concludes with discussion of the problems inherent in prevention and control of the pathogenic strains, and of problems in researching such issues.

Chapters 3, 4, and 5 describe field studies. The first of these (Chapter 3) investigated counts of *E. coli* O157, other *E. coli*, and other coliform bacteria in faecal samples from cattle and sheep in North Wales. The study failed to detect any *E. coli* O157 in the faeces; results did suggest, however, that generic *E. coli* comprise the majority of coliform bacteria within the faeces, that livestock faeces is an important source of environmental *E. coli*, and that, in North Wales at least, sheep faeces contains more colonies of the bacteria, on average, than cattle faeces.

The second study (Chapter 4) investigated, via field study of soils, diverse environmental factors pertinent to *E. coli* and other coliform prevalence in the environments. The factors comprised type of land (upland or lowland), grazing, season, and various physical and chemical qualities of the soils. Results suggested that the bacteria were substantially more prevalent in lowland than upland regions, that they were also substantially more prevalent in grazed than ungrazed land, and that they were substantially more prevalent in summer months than winter ones. Results as to the soil physical and chemical properties, however, were indecisive. Overall, the results of the study suggest that, although soil physical and chemical properties might be important in determining the prevalence of coliforms, in North Wales at least, land use, land type, and season are more important.

The third field study (Chapter 5) investigated the impact of intensive grazing over time and different crop types (kale or swede) on soil *E. coli* and other coliform counts and on soil quality. Results suggested that intensive grazing contributes to the bacterial counts but that crop type does not make a substantial difference. Results also suggested that

intensive grazing, in North Wales at least, does not much, if at all, deleteriously affect soil quality.

Chapter 6 describes a field and follow-up experimental study of transmission of *E. coli* O157 from sheep faeces by yellow dung flies. Although no *E. coli* O157 were found in the flies or the faeces, results (after experimental inoculation of the faeces) strongly suggested that the flies can house substantial quantities of pathogenic bacteria and that they may readily transmit the bacteria from the faeces to other areas of the environment, especially to water and to grass. The thesis then finishes with a short general conclusion.

CHAPTER 2: LITERATURE REVIEW

2.1. Introduction

Microbial pollution of the environment from manure has recently become a subject of concern. Point sources of animal waste contamination include animal housing and sites of manure and slurry storage. Point sources can lead to non-point-sources of spread of animal faeces or microbial pathogens by runoff or leaching, and ultimately contamination of the wider environment, including pastures and water sources.

Traditionally, the hilly pastoral systems of Wales are subject to medium to high levels of grazing, with consequent contamination by microbes from animal faeces. The water bodies of the lower part of the catchment in mountainous areas are therefore at risk of water pollution from pathogenic microorganisms.

Most studies on survival of faecal microorganisms and pathogens from animal (mostly bovine) faeces have focused on manure and other forms of organic fertilizer applied to soil (e.g., Ellis *et al.,* 1978; Guan and Holley, 2003; Unc *et al.,* 2004). However, studies on the abundance and survival of bacteria and their responses to soil properties in naturally deposited faeces due to livestock grazing in grassland are few. Reliable data on microbial communities, especially faecal coliforms, in pasture systems, soil and faeces is necessary for microbial risk assessments. Using a wide range of sources, this review will consider the issues associated with faecal contamination due to livestock, and will focus on *E. coli* and *E. coli* O157 in particular.

2.2. Coliform bacteria, including *E. coli*

Coliform bacteria (often termed *coliforms*) are rod-shaped, Gram-negative, non-spore-forming, oxidase-negative bacteria that can ferment lactose at 35°C (Feng *et al.*, 2002). The term coliform has no taxonomic meaning; this is because coliform bacteria are defined by their biochemical characteristics (Figueras *et al.*, 1994; Gleeson and Gray, 1997). Thus the group comprises several different genera of bacteria; these include *Citrobacter*, *Enterobacter*, *Hafnia*, *Klebsiella*, *Serratia*, and *Escherichia*. They are present in human and other vertebrate faeces, but are also found in water, in soil, and on plants (McCoy and Seidler, 1973). *E. coli* are a sub-group of coliforms; however, they can ferment lactose at higher temperatures than other coliforms.

E. coli are Gram-negative, aerobic, facultative anaerobic bacteria with rod-shaped cells (Figure 2.1).

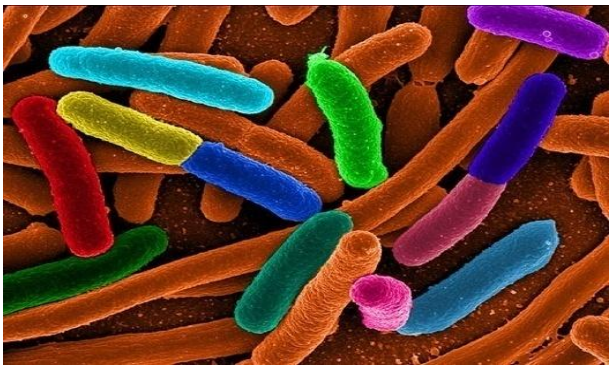

Figure 2.1. *E. coli*

http://www.popsci.com/science/article/2011-01/biostorage-scheme-turns-e-coli-bacteria-data-storing-hard-drives

They are about 2 μm long and 0.5 μm wide (Weiss, 2004), and were first isolated and identified from the faeces of a child in 1885 by the German paediatrician Theodor Escherichia (Robins-Browne, 1987). Table 2.1 shows their biological classification.

Table 2.1. Classification of *E. coli*

Taxonomy	Name
Domain	Bacteria
Kingdom	Bacteria
Phylum	Protobacteria
Class	Gamma protobacteria
Order	Enterobacteriales
Family	Enterobacteriaceae
Genus	Escherichia
Species	Coli

Other genera within the Enterobacteriaceae family include *Salmonella*, *Yersinia*, *Citrobacter*, *Klebsiella*, and *Shigella*. Although *E. coli* derive mainly from the intestines of vertebrate species, some can survive outside the intestines of their hosts for extended periods. One may therefore distinguish between four sub-groups of coliform bacteria: (a) *E. coli*, which, as indicated, differ from other coliforms in their ability to ferment lactose; (b) faecal coliforms, which are present only in faeces; (c) non-faecal coliforms, which are present in the environment outside the intestinal tracts of vertebrates; and (d) non-pathogenic coliforms. These sub-groups overlap. Thus, for example, many *E. coli* are faecal but non-pathogenic; other *E. coli* are faecal and pathogenic (Bermudez and Hazen, 1988).

2.3. *E. coli* and human health

E. coli comprise thousands of distinct strains, most of which are harmless to humans. Indeed, most strains live commensally with their hosts (Park *et al.*, 1997). Non-pathogenic strains of *E. coli* are present as part of the normal intestinal flora of humans and warm-blooded animals. Many are symbionts, and play an important role in maintaining normal gut physiology (Doyle *et al*., 1997). Some studies suggest such symbionts serve a helpful function in the body by synthesizing vitamin K (e.g., Bentley and Meganathan, 1982). It is also possible they kill bacteria ingested with contaminated food and water (Fratamico *et al*., 2002). Thus some strains of *E. coli* might help us in fighting pathogenic strains. In all, *E. coli* comprise about 0.1% of the total microbial population of the intestinal flora of the human beings (Eckburg *et al*., 2005). However, infection by other strains may lead to health complications that range in severity from mild to serious. The most common symptom of pathogenic infection is diarrhoea.

There are three main forms of diarrhoea: acute watery, bloody, and persistent. Acute watery diarrhoea is associated with *V. cholerae, E. coli*, and rotavirus infection (Ahs *et al*., 2010). Bloody diarrhoea is mainly associated with *Shigella* spp. (Bhattacharya and Sur, 2003; UNICEF, 2009), though certain *E. coli* strains also cause it (Kiranmayi *et al.*, 2010). Bloody diarrhoea (often termed *dysentery*) arises when pathogens attack the intestinal lining and associated blood vessels. It has two consequences. First, because intestinal blood vessels are damaged, there may be nutrient loss; people with diarrhoea may fail to get sufficient nourishment even when they eat otherwise good diets. Second, the pathogen may gain access to other parts of the body via the damaged blood vessels and thereby cause

further disease. Bloody diarrhoea may also lead to other conditions. The most important of these are haemorrhagic colitis (HC) and haemorrhagic uraemic syndrome (HUS). HC is inflammation of the large intestine. It is associated with bloody stools, severe abdominal pain, diarrhoea, but little (or even no) fever (Kiranmayi *et al.* 2010). HC causes HUS in some sufferers. HUS symptoms include anaemia, kidney damage, and sometimes kidney failure (Sussman, 1997). The anaemia weakens the individual and may impair breathing.

HUS is a life-threatening condition, and, even if treated, leads to kidney failure in about 10% of sufferers and some form of kidney malfunction in a further 40% of others (Pickering *et al.*, 1994). HUS may also lead to thrombotic thrombocytopenia purpura (TTP). This involves many microscopic thromboses forming within small blood vessels (Moake, 2002). Symptoms of TTP include those of HUS but, in addition, hallucinations, headaches, and purpura (easy bruising, especially of the nasal areas). TPP is rare, and mainly affects children and the elderly. Renal failure is rarer in TTP, however, than in HUS, but some form of neurological disorder is common (Rock *et al.*, 1991). There is also a high risk of stroke, and the condition is more life-threatening than HUS; the mortality rate from TTP can be as high as 50% (Griffin, 1995 as cited by Kiranmayi *et al*., 2010).

HC and HUS are not the only problems associated with verotoxin-producing *E. coli* (VTEC) andshiga-like toxin-producing *E. coli* (STEC). Between them, VTEC and STEC strains, albeit in only a few cases, can also cause infections of the urinary tract, lungs (pneumonia), blood (bacteraemia), and haemolytic anaemia (fragmented red blood cells).

2.3.1. Classification of pathogenic E. coli

Serogroup isolates are distinguished on the basis of three surface antigens (Doyle *et al.,* 1997; Fratamico *et al.,* 2002): the capsular antigens (K), the somatic lipopolysaccharide antigens (O), and the flagellar antigens (H). There are two major pathogenic subgroups of *E. coli*: those that cause intestinal infections, and those that cause urinary tract infections, septicaemia, and neonatal meningitis (Kaper *et al*., 2004).

Of the many of pathogenic strains of *E. coli* (Beutin and Strauch, 2007), those in the enterohemorrhagic (EHEC) group are arguably the most notorious. EHEC strains create two different cytotoxins. These acts like the Shiga toxins made by *Shigella dysenteriae* which may lead to HC and HUS (Sussman, 1997).

Because of the wide number of conditions pathogenic *E. coli* may engender, and in part because of the diverse pathogenic *E. coli* strains, there have arisen a variety of acronyms and initialisms to describe the strains (Table 2. 2).

Table 2.2. Acronyms for different groups of *E. coli* and the associated infections that may occur

E. coli	Acronym/initialism	Infection
Enterotoxigenic	ETEC	Traveller's diarrhoea
Enteropathogenic	EPEC	Children’s diarrhoea
Enteroinvasive	EIEC	Shigella-like dysentery
Enterohemorrhagic	EHEC	HC or HUS
Enteroaggregative	EAggEC	Persistent diarrhoea in children, especially in developing countries
Enteroadherent	EAEC	Childhood diarrhoea and traveller's diarrhoea in Mexico and North Africa

Doyle (1991); Nataro and Kaper (1998); emedicine (2009).

Notice that the acronym EHEC overlaps with VTEC, because VTEC O157 causes HC and HUS. The European Commission's Scientific Committee on Veterinary Measures relating to Public Health (2003) points to another possible source of confusion. The Commission notes an overlap between types of pathogenic *E. coli* O157 and other *E. coli*, as is shown in Figure 2.2.

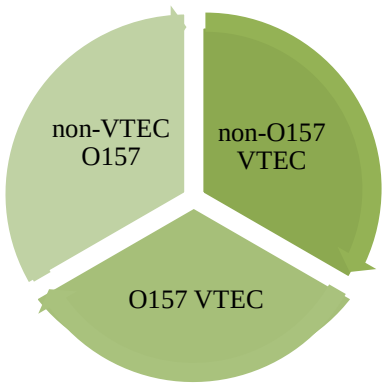

Figure 2.2. Relationship between *E. coli* O157 and VTEC.

Scientific Committee on Veterinary Measures relating to Public Health (2003).

Thus not all HUS-causing bacteria are *E. coli* O157 (HUS may also be caused by *Streptococcus pneumonia*, albeit only rarely—Constantinescu *et al.*, 2004; Copelovitch and Kaplan 2008; Copelovitch and Kaplan 2010), not all pathogenic *E. coli* are VTEC (as indicated, some are STEC), and not all *E. coli* cause HC or HUS—indeed, as indicated, many strains appear symbiotic, not pathogenic.

2.3.2. E. coli O157

The connection between EHEC and human pathologies was first identified in the U.S.A. after two successive outbreaks of HUS and HC were connected to the serotype O157:H7. This was in 1982, and was associated with the eating of undercooked

hamburgers in a restaurant (Riley *et al.,* 1983; Terajima *et al.,* 2000; Neill *et al.,* 2001). *E. coli* O157:H7 is the most extensive of the verocytotoxin-producing *E. coli* and the problems associated with it range from mild diarrhoea HUS, TTP, through to death (Defra, 2008). *E. coli* O157:H7 has been characterised in a number of ways (Table 2.3).

Table 2.3. Characteristics of *E. coli* O157:H7.

Characteristic	
Acid-resistance	The bacteria are more likely to be present in acidic food than less tolerant trains.
Antibiotic resistance	The bacteria now appear to be resistant to most antibiotics.
Thermal inactivation	The bacteria are sensitive to heat. Pasteurization (at 72°C) is sufficient to kill most *E. coli* O157 cells. In addition, many VTEC strains appear unable to grow well—if at all to grow—at temperatures above 44°C. Conversely, they fail to grow at low temperatures, with 8–10°C being minimal temperatures for such activity.
Inability to ferment sorbitol	Most VTEC strains do not ferment sorbitol.
Inability to produce beta-glucoronidase	Most O157 VTEC strains cannot.
Inability to produce gas and indole at 44ºC	This may make detection problematic.
Carriage of a 60-MD plasmid	The plasmid (p O157) contains DNA sequences present in other serotypes associated with HC.
Possession of a high molecular weight	5000–8000 out membrane protein.

Fernandez (2008).

The first recorded outbreak of *E. coli* O157 poisoning occurred in Oregon and Michigan in the U.S.A.in 1982 (Pennington, 2010). After this, and another U.S. outbreak of the same year (Terajima *et al.,* 2000; Neill *et al.,* 2001), it was seen as a serious danger to community health, and the sub-strain *E. coli* O157:H7 was recognized as a major cause of

HUS and HC (Altekruse *et al.,* 1997; Williams *et al.,* 1997; Terajima *et al.,* 2000; Peacock *et al.,* 2001). The 1982 U.S. outbreaks were quickly followed by one outbreak in the U.K., and six in Canada (Griffin and Tauxe, 1991). Outbreaks thereafter spread to Belgium (the first in 1987) (Robaeys *et al.,* 1987), then to Africa (1990) (Effler *et al.,* 2001), then to New Zealand (1993) (Baker *et al.,* 1999).

2.3.3. Other pathogenic strains of E. coli

Although *E. coli* O157 is the most widely studied strain (Pennington, 2010), other scan also be a risk to human health. In the spring of 2011, a new strain—*E. coli* O104:H4—emerged in Germany. By the end of July of the same year it had affected over 4000 people worldwide (almost all lived in or had visited Germany) and had killed 50 of them (WHO, 2011). The strain is a STEC pathogen (Altmann *et al.,* 2011), i.e. it is can produce a Shiga-like toxin *E. coli* (Wray and Woodward, 1997). In the middle of the outbreak (20 June 2011), out of 2518 cases of poisoning, 786 had developed HUS. Altmann *et al.* (2011) describe the outbreak as the largest ever involving STEC poisoning. Research also implicates STEC as a cause of diarrhoea and illness in animals other than human beings, including sheep, goats, cattle, pigs, cats, and dogs (e.g., Willshaw *et al.,*1997; Wray and Woodward, 1997; Zschock *et al.*, 2000).

2.3.4. Conclusion on coliform bacteria and human health

Coliform bacteria, including *E. coli*, are part of the normal flora within the human intestinal tract. They are also present in the intestinal tracts of other species. Most such bacteria are harmless and many, indeed, may be beneficial. However, many strains are

pathogenic to humans and can cause diarrhoea in humans, together with associated ailments. Of these strains, the strain *E. coli* VTEC O157: H7 is among the most virulent. Although this is not the only strain that causes severe health problems in humans (indeed, non-bacterial micro-organisms also cause diarrhoea), it is the most researched.

New strains of *E. coli* are continuously evolving; thus one cannot be sure how the risk to human health from *E. coli* strains will develop in future. Such evidence as exists, however, suggests a need for continuous vigilance. Although deaths from diarrhoeal diseases are relatively rare in the West when compared to rates in poorer countries, they still represent an important public health burden.

2.4. Routes of infection

2.4.1. E. coli O157 *and other pathogens in relation to food poisoning*

Food represents a major pathway for infection with a range of pathogens. The major pathogens associated with food poisoning in the U.K. are *Campylobacter* spp., *Salmonella* spp., *Listeria* spp., *Clostridium perfringens*, *E. coli* (including O157), and viruses (Adak, 2005); though protozoa have also been implicated (Orlandi *et al.*, 2002).

Cattle are a major reservoir of *E. coli* O157, with 1% to 15% of UK cattle herds being infected (Jones, 1999). Excretion by infected animals may contaminate water supplies and pasture land. Faecal excretion of the bacterium varies by season, and this is reflected in human infections. Chapman *et al.* (1997a) suggest the main route of *E. coli* O157 infection to humans is the eating of contaminated foods, unpasteurized dairy products,

dairy products contaminated post pasteurisation, contaminated vegetables, fresh fruit, and water. However, infection via other routes, as is discussed below, is probable.

The commonest bacterial source of food poisoning in the U.K. is *Campylobacter* spp., though, fortunately, symptoms (diarrhoea, etc.) generally clear up by themselves within a week and only a minority require medical intervention (usually, a dose of antibiotics) (Health Protection Agency, 2011a). *Campylobacter* spp. infection is highest in the late spring, after which incidence gradually declines; the reasons for this are not fully understood. The most common sources of infection are raw or undercooked meat (particularly poultry), contaminated (untreated) water, and unpasteurised milk (Health Protection Agency, 2011a).

Salmonella spp. infection is usually mild and clears up by itself. Severe cases, however, may cause death by dehydration (Health Protection Agency, 2011b). Also, there is a vast number of *Salmonella* spp., including *Salmonella enterica*, which causes typhoid fever (Crump *et al.*, 2004); which is often fatal unless treated. *Salmonella* spp. infection is mostly associated with contaminated meat and poultry, eggs and unpasteurised milk. In addition, fish, vegetables, and fruit may become contaminated through contact with manure or sewage; pets (especially reptiles, but also cats, dogs, and rodents) can also become infected and therefore spread it onto human food (Health Protection Agency, 2011b).

Listeria spp. infections are now relatively rare in the U.K., and, when they occur, tend to produce influenza cum gastroenteritis symptoms, sometimes together with eye and skin infections. Some sufferers experience only mild symptoms; however, it can lead to meningitis and septicaemia, each of which is life-threatening (Health Protection Agency,

2011c). It can also pass from mother to unborn child, and thereby cause miscarriage or in other ways harm the foetus. The incubation period varies—sometimes only a few days but sometimes 90 days (average about 30 days). All cases involving septicaemia or meningitis require hospitalisation (Health Protection Agency, 2011c). In this, *L. monocytogenes*, which causes listeriosis, appears especially virulent (Ramaswamy *et al.*, 2007). Pregnant women are at especial risk of literiosis (Ramaswamy *et al.*, 2007; Health Protection Agency, 2011c). *Listeria* spp. are unusual in that they can multiply at low temperatures (5°C or lower—that is, refrigerator temperatures). Thus they may be present in ready-to-eat chilled foods (sandwich packs, pâtés, soft cheese, cold meats, and smoked salmon). They are also unusual in that, although they mainly affect people with a weakened immune system (pregnant women, the elderly, people suffering from HIV/AIDS, alcoholics), they are not a particularly great risk to children (Health Protection Agency, 2011c). Because *Listeria* spp. are found in chilled foods such as soft cheeses, vegetarians may place themselves at especial risk because they often rely on such foods for protein (Health Protection Agency, 2011c).

In the U.K., norovirus is the most common virus associated with food poisoning and non-bacterial gastroenteritis in the U.K. The incubation period is short, usually being one or two days, and symptoms include non-bloody diarrhoea, vomiting, and nausea (Health Protection Agency, 2011d). As well as through ingestion of contaminated food or drink, transmission of this highly infectious organism is by ingestion of human faecal matter, contact with sufferers' vomit or faeces—each sometimes as aerosols.

2.4.2. Outbreaks of food poisoning

2.4.2.1. U.K. AND U.S. OUTBREAKS

Figure 2.3 shows reported outbreaks of food poisoning for the period 1992–2010 by each of the major sources defined above.

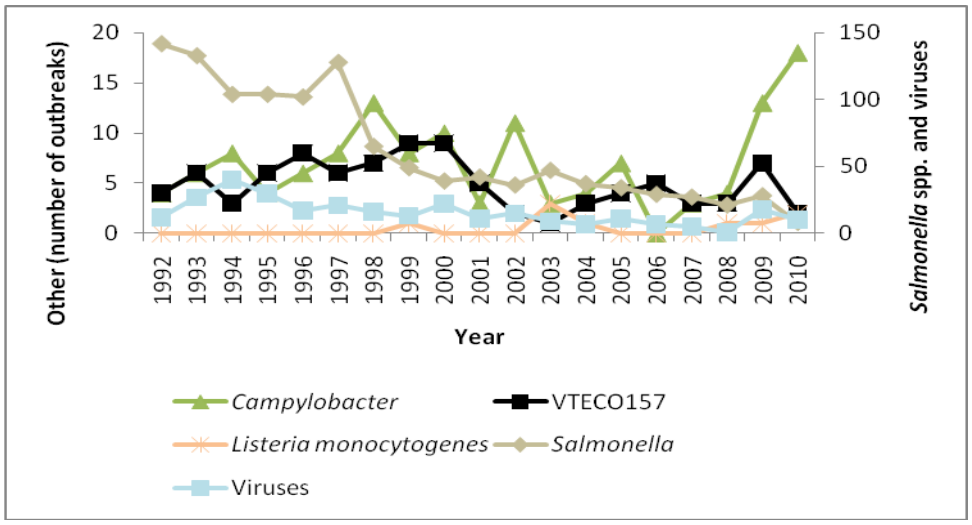

Figure 2.3. Number of food poisoning outbreaks England and Wales, 1992–2010. Health Protection Agency (2011e).

The figure shows that the number of outbreaks due to *Salmonella* spp. and viruses fell during the period but the number of outbreaks due to other sources stayed broadly constant or, especially in the cases of *L. monoytogenes* and *Campylobacter* spp., rose slightly. The number of outbreaks does not show the number of individuals affected. Food poisoning affects tens of thousands of people each year in the U.K. Figure 2.4 shows the number of notified cases of food poisoning for England and Wales for the period 1982–2009.

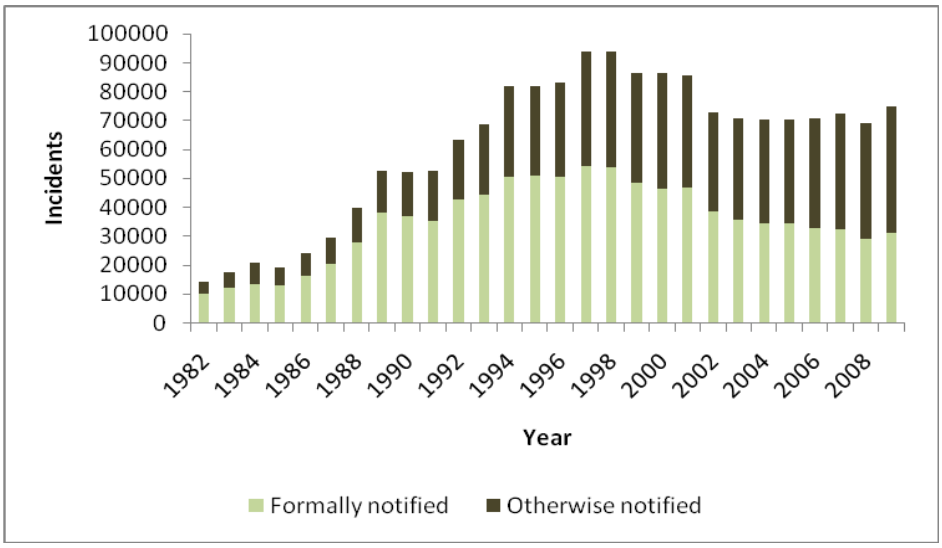

Figure 2.4. Notified cases of food poisoning in England and Wales, 1982–2009.

Formally notified are cases confirmed by laboratory tests. *Otherwise notified* are cases reported to and confirmed by medical authorities (e.g., GPs) but not confirmed by laboratory tests. Health Protection Agency (2010).

The figure suggests a substantial rise in cases since 1982, but a slight fall after 1999; since 2002 incidence appears to have been broadly steady. The slight increases since 2009 may be attributable to improvements in diagnostic techniques and reporting or a degree of expected variation. The current level of notified incidents, however, must indicate only a fraction of the total, for many people affected—especially by mild acute cases—might not visit their GP or in other ways inform medical authorities. Moreover, food poisoning may be misdiagnosed. Thus the actual number of incidents could be much higher than the number of cases reported (Jones, 1999; Dundas and Todd, 2000; Parry and Palmer, 2000).

Most cases of food poisoning are relatively innocuous, albeit distressing to the people experiencing them; therefore disaggregating the number of agents that lead to serious food poisoning, *L. monocytogenes* and VTEC, for the period 1991-2009 is of interest (Figure 2.5).

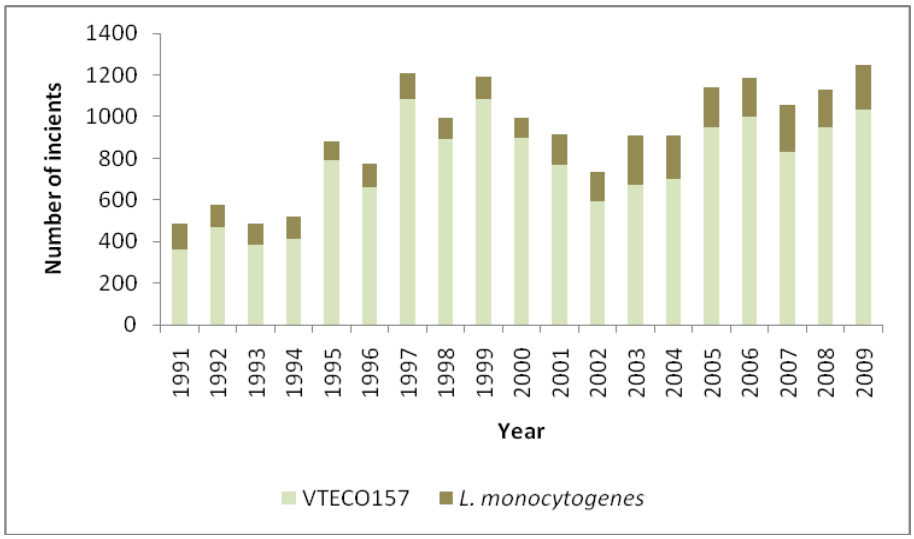

Figure 2.5. Annual incidence of VTEC O157 and *L. monocytogenes* in England and Wales. Health Protection Agency (2011f, 2011g).

Within England and Wales, VTEC infection appears the more problematic: although not as hazardous as *L. monocytogenes* infection, it is much more common. The figure also suggests that incidence of VTEC infection has increased since 2002 and has almost trebled since 1991 (though some of this increase may be due to failure in reporting in earlier years).

Food outlets remain particular sources of VTEC O157 infections. Figure 2.6 shows U.S. sources of outbreak for the period 1982–1993.

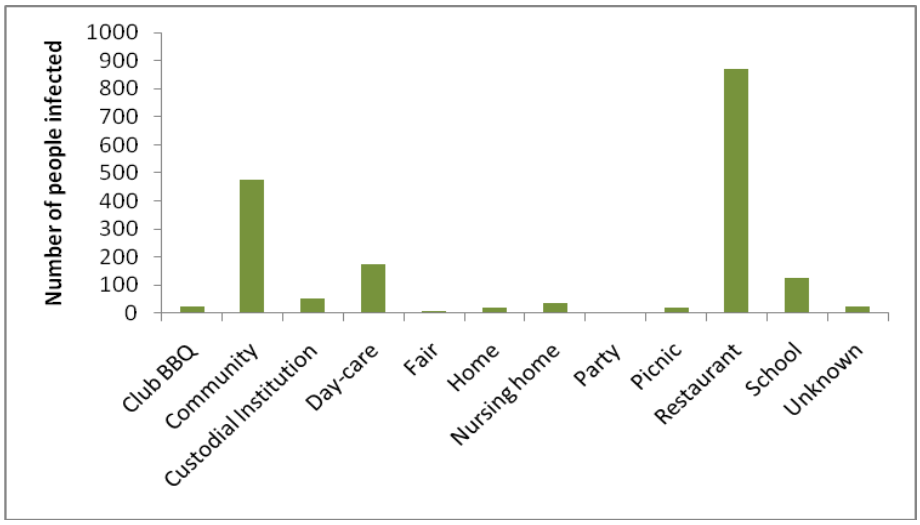

Figure 2.6. Sources of U.S. outbreaks of *E. coli* infection 1982–1993

USDA: APHIS: VS Centers for Epidemiology and Animal Health (1997).

The figure reveals a disproportionate number of restaurant-related outbreaks, suggesting possible low standards of hygiene within the enterprises. However, the figure also reveals high incidence of communal outbreaks, including schools, day-care centres, and prisons; suggesting sources are multifarious, and include person-person transmission. Worldwide statistics point a similar conclusion. Table 2.4 provides example sources of large outbreaks worldwide.

Table2.4. Examples of major outbreaks of *E. coli* poisoning.

Year	Country	Cases	Number infected	HUS	Fatalities	Cause
1985	Canada	Nursing home	73	12	17	Sandwiches*, person to person
1989	U.S.A.	Community	243	2	4	Water*
1992	Swaziland	Community	20,000			Contaminated potable water
1993	U.S.A.	Fast food restaurant	732	55	4	Beef burgers
1993	Wales	Community	17	1		Butcher meat
1996	Japan	Schools, Community	5727	>100	3	White radish sprouted seeds*
1996	Scotland	Community Nursing home	501	27	21	Cooked meats, gravy

* = suspected cause

Scientific Committee on Veterinary Measures relating to Public Health (2003).

2.4.2.2. FOOD TYPES ASSOCIATED WITH OUTBREAKS

Food sources of infection appear diverse. Rangel *et al.* (2005) provide a breakdown of sources of *E. coli* O157 infection in a study of 350 outbreaks across the U.S., involving 8,598 cases and representing 49 states, between 1982 and 2002 (Figure 2.7).

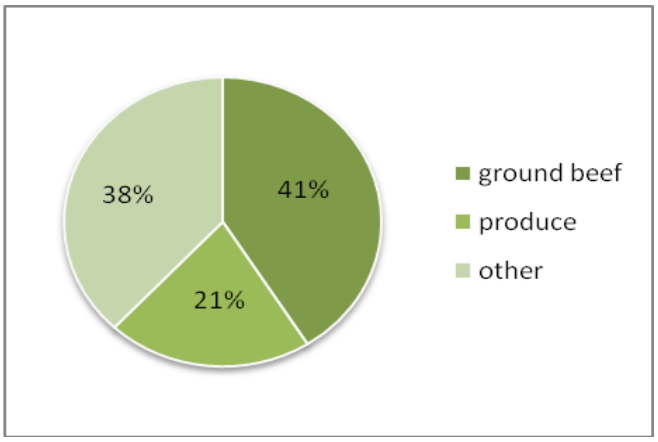

Figure 2.7. Sources of *E. coli* O157 infection in the U.S.A, 1982-2002.

Rangel *et al.* (2005).

The figure shows that, although ground beef is a major source of infection, fresh produce also makes a substantial contribution. Figure 2.8 shows a breakdown of the sources of produce infection.

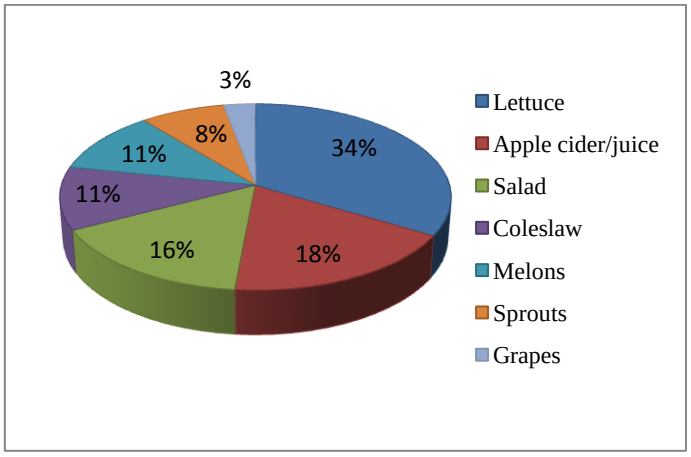

Figure 2.8. Breakdown of sources of U.S. produce *E. coli* O157:H7 infection.

Rangel *et al.* (2005).

Notice from the figure the high incidence of infection arising from acidic produce such as apple juice/cider, coleslaw, and grapes; showing the ability of *E. coli* to survive in acidic environments. Although outside of its natural host, clearly, other such features of the

ecology and physiology of *E. coli* O157 (e.g. its ability to internalise within vegetable produce when they are grown in contaminated soil (Solomon *et al.*, 2002), facilitate its ability to cause human infections via the food-borne pathway.

2.4.3. Direct transmission

Although the WHO (2005) suggests 63–85% of cases of infection arise from exposure to the pathogen through food, this means that at least 15% of cases are caused via other pathways, including person-to-person spread. A variety of pathogens are spread as secondary infections when one household member is already infected; particularly where poor hygiene practises occur (Kagan *et al*., 2002).

Secondary infections may also be prevalent outside the home. Sanderson and Weissier (1992), for example, found evidence of cross-contamination by coliforms between patients and nurses in an orthopaedic hospital. Importantly, incidents of pathogens appeared lower in patients in surgical wards than in patients suffering from spinal injury, though incidence of contamination between nurses and patients was the same for both types of patient; suggesting that greater hygiene in surgical patients may have reduced cross-contamination. Similarly, Ekanem *et al*. (1983), in a study of diarrhoeal outbreaks in U.S. day-care centres, found that centres suffering outbreaks had higher contamination of staff and children's hands and of commonly handled objects (e.g., toys).

2.5. Environmental sources of infection

2.5.1. Reservoirs of E. coli O157:H7

The primary reservoir of *E. coli* O157:H7 is the gastro-intestinal tracts—and therefore faeces—of ruminant livestock; with the organism being detected in several species used in food production but particularly cattle and sheep (see below). Infected animals do not show symptoms of disease and hence cannot be isolated from other animals or separated from slaughter (Fairbrother and Nadeau, 2006). During slaughter of infected animals, *E. coli* O157:H7 may contaminate meat intended for human consumption Ogden *et al.* (2005). Such animals will also excrete contaminated faeces into the environment, where it may lead to infections due to direct human contact (e.g. during recreational activities); and may also serve as a catalyst to wider environment spread.

Numbers of coliform bacteria have long been used as general indicators of water quality and counts of *E. coli* as specific indicators of faecal contamination (Neill, 2004). Faecal contamination of water may be a serious problem. For instance, research on Lake Huron, Canada, suggests the lake is heavily polluted with *E. coli* from farm animals, with cattle and pig manure accounting for 59–62% of the *E. coli* and human faeces accounting for only 1–3% (Kon *et al*., 2009).

Strachan *et al*. (2006) provide evidence that environmental factors may comprise a more substantial risk of *E. coli* O157 infection than eating hamburgers. In a survey of the Grampian region of Scotland, the authors found that 48% of the variance in infection could be explained by cattle density relative to human population density within the area, 33% of the variance by sheep density relative to human population density, and 17% of the

variance by the percentage of the human population having a private water supply. Moreover, the authors found the risk of infection for all age groups other than children aged less than one year was higher for people living in a rural as opposed to urban environments. In the case of 1–4-year-olds, the increased risk was more than three-fold, and in the cases of 15–34-year-olds, and those aged over 65, it was more than double. This suggests environmental factors—possibly, just living near cattle or sheep—comprise a substantial risk. The authors calculate that the risk of infection from eating a single hamburger is 5×10^{-6}, whereas the risk of infection from spending a single day in a field of pasture is 5×10^{-4}—that is, 100 times as great.

2.5.1.1. RUMINANT LIVESTOCK AS A SOURCE OF *E. COLI* O157

Crump *et al.* (2002) and Payne *et al.* (2003) both report that outbreaks of O157:H7 infection followed visits to farms. This suggests that livestock may, directly (through, e.g., tactile contact) or indirectly (through, e.g., faecal contact) transmit infection.

Cattle are a main reservoir of enterohemorrhagic *E. coli* O157:H7 (Beutin *et al.*, 1993; Elder *et al.*, 2000; Caprioli *et al.*, 2005). Reducing the prevalence, contamination and spread of O157 in beef and dairy products across the food chain has therefore been the focus of considerable effort (Riley *et al.*, 1983; Parry *et al.*, 2002). In terms of prevalence within cattle, Rhoades *et al.* (2009) report widely discrepant results. Thus, for example, a national study of the faeces of veal calves in the Netherlands reported only 0.5% incidence of infection. At the other extreme, one Swiss study of three-month-old calves in Western region farms reported 53.4% infection (Busato *et al.*, 1999). The review concluded the

mean prevalence rate to be 6.2% in cattle faeces (Rhoades *et al.,* 2009). Much may depend on the living conditions of the cattle, with densely crowded cattle suffering more than others, possibly to stress (Jones, 1999). Similarly, much may depend on the site in which the cattle are tested. A U.S. study of Midwestern farm calves revealed 7.4% infection (Laegreid *et al.,* 1999), but another of a Midwestern abattoir revealed 27.8% infection (Woerner *et al.,* 2006).Such wide ranges of reported rates of infection may also, of course, reflect variations in infection due to, for example, geography, season, and year. Much may also depend on the manner in which the cattle are tested. Rhoades *et al.* (2009) report the following means and ranges of surveys: faeces, 6.2% (range: 0–57%); hides, 44% (range: 7.3–76%), chilled carcases, 0.3% (range: 0–0.5%), and raw beef products, 1.2% (range: 0–17%). In terms of concentrations excreted, Shere *et al.* (1998) report the prevalence of *E. coli* O157:H7 in bovine faeces ranges from 2.0×10^2 to 8.7×10^4 $CFUg^{-1}$; whilst for calves, Shere *et al.* (2002) report numbers to vary from 60 to 10^5 $CFUg^{-1}$ faeces.

Sheep are the second most important source of *E. coli* O157 infection in humans (Barlow *et al.*, 2006; Chapman *et al.*, 1996, 2001). Ogden *et al.* (2005) proposed that sheep can act as a vehicle for the spread of *E. coli* O157 in agricultural environments and that counts within sheep faeces may reach 10^4 $CFUg^{-1}$. More recently, Roberto *et al.* (2009) report goats and sheep are among the main sources of human disease caused by *E. coli* O157. This may be through the eating of sheep or goat meat, unpasteurized milk products (mainly cheese), and the petting of farmyard animals. Mainil (1999) reports the prevalence of *E. coli* O157:H7 is higher in sheep and goats than in cattle. Sheep are also reservoirs of non-O157 serogroups (counting O26, O91, O130 O115, and O128) (Djordjevic *et al.*, 2001;

Cookson *et al.,* 2002a, 2006; Blanco *et al.,* 2003; Aktan *et al.,* 2004; Kalchayan *et al.,* 2007).

Many studies have looked at the prevalence of O157 within sheep faeces. Oporto *et al.* (2008) report the prevalence of sheep and goat faeces containing *E. coli* O157:H7 in Spain was 8.7% positive, with approximately 7.3% of the animals within infected flocks producing it.

Studies from other countries provide similar results. Franco *et al.* (2009), for example, in a study of Italian sheep at slaughter, found 7.1% of the animals had VTEC O157 and 2.1% had virulent strains in colonies ranging in size from 1×10^3 to 6×10^5 $CFUg^{-1}$ of faeces. Similarly, Chapman *et al*. (2001) reported that, in the Netherlands, *E. coli* O157:H7 were isolated from 3.8% of sheep faeces and 4.1% of lamb faeces. They also report that the incidence of VTEC O157 in sheep in the U.S.A. appears transient, and ranges from 31% of sheep in June to none in November (Kudva *et al*., 1996). In the U.K., the pathogen was recovered from 1.4% of 7200 sheep and was more frequently isolated from lamb (2.9%) than from beef products 1.1% (Chapman *et al.,* 2000). Lenahan *et al.* (2007) report the presence *E. coli* O157:H7 in abattoirs after slaughter of Irish lambs. The authors confirmed the virulence profiles of these isolates.

2.5.1.2. OTHER ANIMALS

Many studies have isolated *E. coli* O157:H7 in non-ruminant animals, including dogs, horses, birds, and flies. *E. coli* O157:H7 strains have been sporadically detected in wild birds such as seagulls and pigeons (Wallace *et al*., 1997; Kobayashi *et al*., 2002;

Foster *et al*., 2006), but do not appear to be as prevalent or as persistent in these animals as in ruminants (Chalmers *et al.,* 1997; Wallace *et al.,* 1997; Hancock *et al.,* 1998; Shere *et al.,* 1998). Similarly, Doyle and Schoeni (1987) report the presence of VTEC O157:H7 in meat sold in shops, not only in beef and, lamb, but also isolation from chicken and pork.

Both Rios *et al.* (1999) and Nakazawa and Akiba (1999) report the isolation of *E. coli* O157:H7 from pigs. Beery *et al.* (1985) report the presence *E. coli* O157:H7 in the intestines of poultry (and see Kobayashi *et al*., 2002), and that it may be excreted in their faeces for a number of months. In this connection, Griffin and Tauxe (1991) and Ruggenenti *et al.* (2001) report that *E. coli* may transmit from cattle faeces to non-ruminant species described previously.

Rabbits are also a reservoir. An outbreak of HUS and hemorrhagic diarrhoea was attributed to Dutch Belted rabbits infected with *E. coli* O153:H7 (Garcia *et al.,* 2002; Garcia and Fox, 2003). Other reservoir species include deer, camels, and water buffalo (Pritchard *et al*., 2001; Eriksson *et al*., 2003; Galiero *et al*., 2005; Dipineto *et al*., 2006; Foster *et al*., 2006; Garcia-Sanchez *et al*., 2007; Cornick and Vukhac, 2008; Hajian *et al.*, 2011). *E. coli* O157:H7 has also been reported in horses (Chalmers *et al*., 1997; Scaife *et al*., 2006). However, although the bacteria were detected in such animals, it was not always clear whether the organism had originated in the animal, whether it was contracted from cattle faeces or possibly due to cross-contamination with other carcasses when sampled at an abattoir or in raw meat products.

2.5.2. Conclusion on sources of E. coli infection

Sources of *E. coli* infection appear multifarious. Although contaminated beef is commonly cited, this may in part reflect a U.S. bias: Americans tend to eat (much) more beef than lamb. It is also known that livestock can pose a source of infection through excretion of contaminated faeces into the environment, to be ingested thereafter (e.g. in lake water). There is uncertainty as to the role of feral animals in human *E. coli* infection. Numerous studies attest to a range of fauna housing pathogenic *E. coli*. However, whether these pose a substantial risk to humans is uncertain.

2.6. Factors affecting transmission of *E. coli* O157

2.6.1. Seasonal and climatic factors

Incidence of *E. coli* O157 infection in humans appears most common in the summer months and least common in the winter months. Defra (2008) reports that, in 2007 for example, in England and Wales there were fewer than 0.2 incidents per 100,000 of the population during the months January through April, but that there were more than 0.6 incidents per 100,000 of the population during the months May through to October. Incidence peaked in July and August, with incidence rates of over 0.8 per 100,000 of the population. Defra reports similar proportionate increases during the summer months in Scotland and Northern Ireland. The Defra statistics are consistent with the observation, noted above, that *E. coli* O157:H7 do not grow at low temperatures (Fernandez, 2008). The Advisory Committee on Gastrointestinal Diseases (2000) also reports seasonal variation in incidence in infection throughout the U.K.

The effects of seasonal variations on bacterial counts have been reported by Van-Donsel *et al*. (1967), who found that a 90% decrease in faecal coliform numbers took from 3.3 days in the summer but 13.4 days in the autumn. However, faecal coliform counts in the soil leach often decreased to non-detectable levels within two months of fertilizer application; faecal coliform concentration in the soil decreased to background levels within 180 days after spring manure applications and within 60 days after manure applications in autumn (Stoddard *et al.,* 1998).

2.6.2. Environmental conditions

In a study of *E. coli* O157:H7 survival on a range of materials under different environmental conditions, Williams *et al*. (2005) found the bacteria thrived most on wooden as opposed to metal environments, and that they thrived least in hot, arid environments. The results suggest that *E. coli* may persist in the environment for long periods (over 28 days), but only if environmental conditions are favourable to them. Materials that may be contaminated in such way include water and feed troughs. A similar response to environmental conditions has been seen in feedlot pens and manure (Hancock *et al*., 2001; McGee *et al*., 2004; Scott *et al*., 2006).

2.6.3. Geographical distribution

Incidence of *E. coli* O157 infection varies according to area. Figure 2.9 shows incidence rates per 100,000 of the population for each of the four major regions within the U.K. for the period 1992–1997.

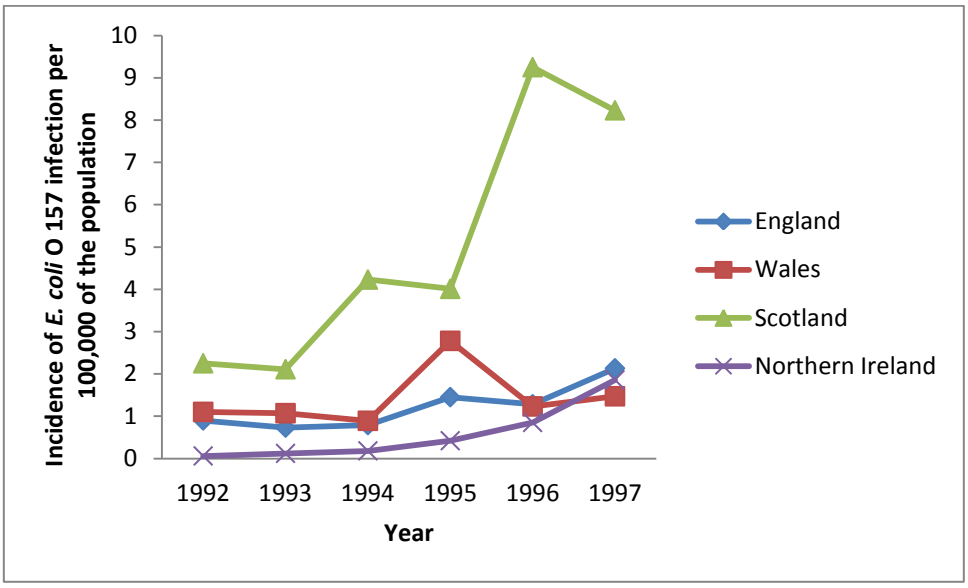

Figure 2.9. Incidence of *E. coli* O157 infections within the U.K.

Subcommittee of the PHLS Advisory Committee on Gastrointestinal Diseases (2000).

Even within Scotland, rates of infection vary. In 1997, for example, there was a 12-fold difference between infection within the region with the highest incidence (Borders—40.5 per 100,000 of the population) and the region with the lowest incidence (Fife—3.15 per 100,000 of the population); though it is acknowledged that one large outbreak could have a considerable effect on such values. The same appears true of England, with incidence in the Trent region having the highest incidence and the South Thames region having the lowest (Figure 2.10).

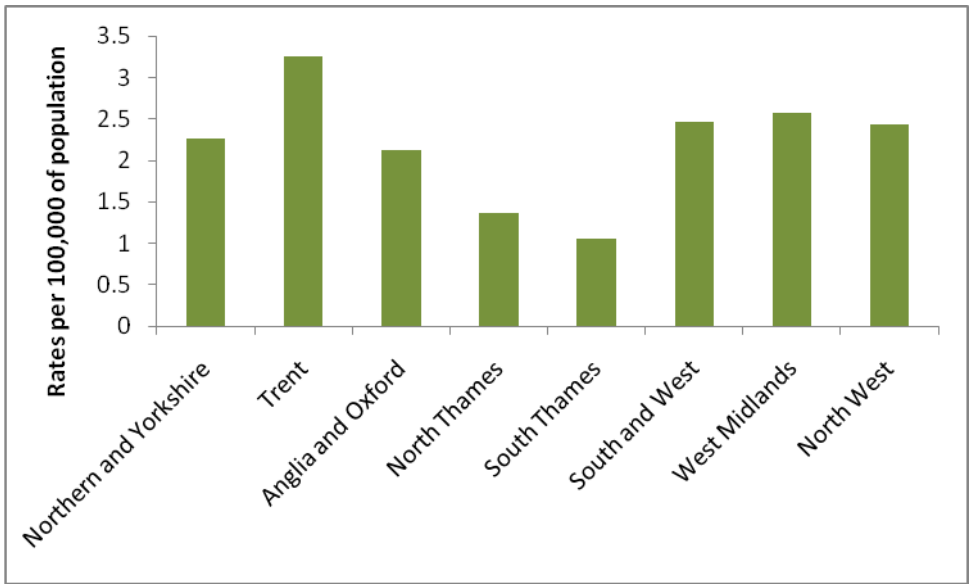

Figure 2.10. Incidence of *E. coli* infections within England during 1997.

Subcommittee of the PHLS Advisory Committee on Gastrointestinal Diseases (2000).

Reasons for this regional variation may be multifarious. Differing practices in animal husbandry, different cultures within the UK (e.g., standards of hygiene, preference for specific food types or cooking methods), different climatic conditions, different levels of livestock, soil types, terrain, and a host of other factors may all play a role. Strachan *et al.* (2011), in a comparison of public knowledge of *E. coli* O157 in Scotland (Grampian region) and North Wales found that such factors as contact with livestock, farm visits, and having a private water supply were associated with cases of infection (the same study found that high levels of infection were associated with greater public knowledge of *E. coli* O157, though even in areas of highest infection public knowledge was poor).

2.6.4. Random factors

Incidence varies widely according to year and outbreak. Statistics for the period 1982–1993 in U.S.A, for instance, show markedly high variance in size of outbreak (Figure 2.11).

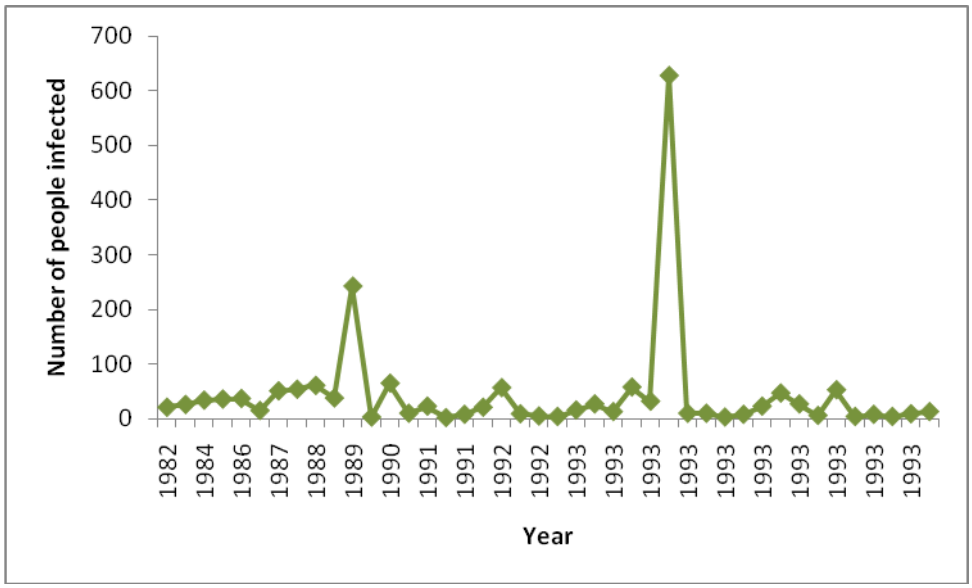

Figure 2.11. Sizes of U.S. outbreaks of *E. coli* poisoning 1982–1993.

U.S.DA: APHIS: VS Centers for Epidemiology and Animal Health (1997).

The figure reveals a remarkably large outbreak in 1993, affecting over 600 individuals. In the U.S.A., typical sizes of outbreak vary according to source of outbreak (see below), but are rarely more than 100 (Rangel *et al*., 2005).

2.6.5. Conclusion on transmission factors

Research suggests a wide variety of factors affect survival of *E. coli* within the environment; including environmental conditions and the weather. There are also very substantial regional variations, with incidence of infection in Scotland being by far the highest in the U.K. and some regions within Scotland having especially high rates. Random

factors are also implicated in incidence rates, suggesting that numerous factors contribute to transmission. The relatively high incidence of transmission within Scotland, together with national differences in infection within the U.K, suggests geographical factors may be important.

2.7. Survival of *E. coli* in the environment

2.7.1. Survival of E. coli outside the intestinal tract

E. coli O157:H7 can survive in range of environments, and may survive even if changes in such variables as pH, availability of nutrients, moisture, and temperature are rapid (Farrell and Finkel, 2003). Survival of *E. coli* O157:H7 in the wider environment can be predicted by extrapolating findings from studies conducted with food. For instance, it is known that the bacterium can survive acidic environments as it was shown to persist for up to 31 days in apple juice of pH < 4.0 (Zhao *et al.*, 1993). Similarly, Clavero and Beuchat (1996) report *E. coli* O157:H7 can survive in salt and hard salami through high levels of nitrites and nitrates.

However, given the importance of the environmental pathway in human infections, studies of the organism in the environment have also been conducted. Under certain conditions, *E. coli* O157:H7 can survive in cattle faeces for extensive periods and maintain the ability to produce shiga toxin (Fukushima *et al*., 1999). Kudva *et al*. (1998) collected cattle and sheep faeces samples, inoculated them with *E. coli* O157:H7, and then exposed the samples to climatic changes. They found the *E. coli* survived for 47 days. Similarly, Wang and Doyle (1996) found survival times of *E. coli* O157:H7 at 5ºC, 22ºC, and 37°C,

with an initial inoculum of 10^5 $CFUg^{-1}$, were 70, 56, and 49 days respectively. There are some reports that *E. coli* O157:H7 outbreaks are linked to drinking and recreational bathing (Swerdlow *et al*., 1992; Bopp *et al*., 2003). Survival of enteric bacteria is known to vary considerably in environmental waters; however, under favourable conditions, pathogenic bacteria may also multiply (Loehr, 1979). Lance and Gerba (1984) report that bacteria can easily be adsorbed on the surface of clay particles; however, and the survival time of bacteria is generally less in sandy soil than in clay soil. When such soils are sterile, the bacteria may survive for around 234 and 179 days respectively (Duffitt *et al*., 2011); indicating the importance of competitive and antagonistic micro-organisms.

2.7.2. Effects of grazing on soil microbial populations

Grazing livestock deposit faeces directly onto pasture, soil, and the surrounding areas. Gray*et al*. (1983) compared the bacterial densities in nearby stream water where cattle were grazing with adjacent ungrazed grassland. They found significantly higher bacterial counts where 150 cattle were grazing but that counts dropped to a level similar to ungrazed pasture when the number of cattle was only 40. Similar findings have been reported by Howell *et al*. (1995) and Bohn and Buckhouse (1981). Doran and Linn (1979) report a 5–10 times higher concentration of faecal coliform in the runoff from grazed areas than from ungrazed areas. Conflicting results were found by some researchers, however. Buckhouse and Gifford (1976) suggest that, in semiarid rangeland, there was no difference in the indicator bacterial counts in the soils of grazed and ungrazed grasslands. Also, some investigators have found little differences between grazed and ungrazed grassland (Moore *et al*., 1989).

There may be much variation in bacterial populations of grazed grassland because of differences in, for example, pedo-climatic conditions, grazing intensity, and management policy. In a study in the Pacific Northwest, Jawson *et al*. (1982) suggested that the effect of grazing on the bacteriological quality of surface runoff water can persist for more than one year after removal of the animals from the grazed land. Kress and Gifford (1984) report that faecal coliform counts in a rangeland stream increased after cattle grazing activities; moreover, the bacterial counts remained high for three months after the cattle were removed.

Underground water table and the runoff water from the grazing pasture can easily contaminate adjacent lakes and rivers, particularly in hilly areas. High levels of faecal coliform bacteria in water bodies indicate contamination by animal faeces, with subsequent risk of infection for people using the polluted water (Jones and Roworth, 1996; Ackman *et al*., 1997) with a range of pathogens, including *E. coli* O157:H7 (Jones and Roworth, 1996; Gugnani, 1999; Licence *et al*., 2001).

2.7.3. Factors affecting the survival and transport of soil microorganisms

Soil is known to play a key role in the cycling of *E. coli* O157 in the environment as it can lead to contamination of water and food such as salad crops (Jones, 1999). The survival of pathogenic micro-organisms in soil depends on a multitude of hydro-environmental factors related to interactions between soil, water, microorganisms, and the surrounding environment, as detailed below.

2.7.3.1. SOIL HYDROLOGICAL PROPERTIES

The contamination of the underground water table due to surface-applied bovine manure and/or faecal deposition from grazing animals is of concern. Available information indicates that agricultural lands are the major contributor of non-point source pollution in both surface and ground water (U.S.EPA, 1994). Generally, bacteria can transport rapidly through saturated soil conditions; however, their survival is favoured in unsaturated soil (Bitton and Harvey, 1992). During a series of field experiments, Gerba *et al.* (1975) found that the horizontal movement of coliform bacteria in soil ranged from 1-450 min different soils. They also found that bacterial contaminated ground water can move laterally from 1-830 m through soil.

2.7.3.2. SOIL PHYSICAL PROPERTIES

Soil properties, especially physical properties, regulate the movement of coliform and other microorganism in soil. As indicated, bacteria and various pathogenic microorganisms can survive in soil from weeks to months, depending on environmental conditions. Relevant soil physical-chemical properties include soil particle size distribution, soil pH, electrical conductivity, and climatic conditions such as temperature and moisture (Crane and Moore, 1984; Yates and Yates, 1988). Soil microbes are adsorbed on the surface of mineral particles.

Ngole *et al*. (2006) found that changes in the soil properties such as pH, water retention capacity, moisture content, phosphorus, and texture played important roles in determining the survival of *E. coli* in the different soil/sludge mixtures, and that increases

of clay content, organic matter content, and water holding capacity allow for enhanced bacterial survival.

2.7.3.3. VEGETATIVE COVER AND SEDIMENT TRANSPORT

For open waters in particular, the presence of stream-side vegetation can affect survival and transport of bacteria from soil. Vegetative filter strips are sometimes used to control the transport of sediment and nutrients and to consequently reduce the movement of pathogens. Wilson (1967) found that Bermuda grass can trap sand, silt, and clay particles in an area treated with organic manure. Coyne *et al.* (1995) and Crane *et al*. (1983) suggested that filter strips can effectively control the movement of pathogens from overland flow. Moore *et al.* (1988) recommended that effective vegetative filter strips should be at least three metres wide and have a slope of 0 to 15%.

Tall vegetation can also reduce the transport and deposition of sediments by reducing the surface runoff and increasing infiltration (Li and Shen, 1973). However, a conflicting result of no reduction in concentration of soil microorganism due to grass filter strips was reported by Entry *et al*. (2000).

2.7.3.4. LAND TYPE

Land type, use, and topography (e.g. lowland versus upland; pasture versus arable; slope) have profound influence on transport of soil microorganisms. The degree of slope can affect the total surface runoff of soil particles, with runoff volume increasing logarithmically with increasing slope of 1–28% (Haggard *et al*., 2002). The rate of infiltration affects the vertical movement of coliform bacteria and is determined by factors

such as grazing, rainfall, slope, soil cover and soil type. Nassif and Wilson (1975) found a 54% decrease in infiltration in grassland soil due to increase in slope after 15 minutes of rainfall.

2.8. Detection and prevention of *E. coli* O157

2.8.1. Detection

To be able to reduce and detect *E. coli* O157 infections and sources, there is a need for reliable systems of detection. However, there are many issues to consider with regards to the detection of pathogenic bacteria.

2.8.1.1. THE IDEAL

Ideally, a detection method should have five characteristics: it should be *accurate* (i.e., it should reliably indicate the number of a given bacterial cells of a given strain); it should be *specific* (i.e., it should count only those bacteria in the given strain); it should be *quick*; it should be *inexpensive*; and it should be *practicable* (i.e., it should be so easy to use that interested parties could easily learn how to use it). Unfortunately, no single technique, or combination of techniques, meets this ideal (Yousef, 2008). All present techniques face a number of fundamental problems.

2.8.1.2. FUNDAMENTAL PROBLEMS

False positives versus false negatives: Any technique that accurately detects the presence of all colonies of a target strain is likely to also detect members of similar, but

non-target, strains, and thereby produce false positives. False positives produce over-estimates of target populations. Conversely, any technique designed to detect only members of a target strain is likely to fail to identify all members of the target strain, and thereby produce false negatives. False negatives produce under-estimates of the target strain. In practice, therefore, use of any technique involves a trade-off between getting false positives and false negatives.

Time: Perez *et al.* (2001) describean amperometric culture-based method for the rapid detection of *E. coli* in water. In it, the bacteria are recovered by filtration and incubation in lauryl sulphate broth (LSB) supplemented with the substrate 4-aminophenyl-b-d-galactopyranoside (4-APGal) at 44.5°C. The time necessary for completion of the test appears inversely proportion to the initial *E. coli* concentration of the environmental samples. Small initial concentrations may require up to 10 hours. Even large concentrations may require two hours or longer.

In general, those techniques that are the most accurate and specific tend to be the most time-consuming (Yousef, 2008). In clinical situations, where speedy identification of a specific strain may involve life and death decisions, clinicians may lack the time for full identification of the target strain.

Problems of detection within the food industry are illustrative. Rapid detection and control of pathogenic organisms within the industry is obviously important (Vunrcrzant and Pllustoesser, 1987). The detection and control must have four aims: (a) to offer direct information on the probable presence of pathogenic microbial organisms in uncooked material and expired products; (b) to give an indication of the presence of the number of

pathogenic organisms in the food, so as to provide an indication of the infection dose; (c) to monitor process, control, and cleaning during manufacture; and (d) to decrease human error, to keep time, and to reduce the cost of labour. Realising these aims involves compromises between time, cost, accuracy, and a host of other factors.

Economics: The same considerations apply here as to time. The most accurate and specific techniques tend to be the most expensive. Abubakar *et al*. (2007), for example, report that polymerase chain reaction (PCR) tests are highly accurate. Quilliam *et al*. (2011), note that for detection of water borne *E. coli* O157, culture methods, while relatively easy, are time consuming and produce false positives; conversely, molecular and biosensor methods, though accurate, are usually prohibitively expensive and require expertise that may not always be found in researchers.

Sampling: The number of colonies in a given area (e.g., a field) may vary greatly according to site; with one sampling location perhaps yielding values that do not reflect typical values (e.g. manure "hotspots"). This is a particular problem with environmental sampling; there is a danger of sampling error biasing estimates of target species population unless large numbers of replicates are taken, adding to time and expense.

The matrix: pertains to the medium of the sample in which the search for bacteria is conducted; it is highly variable. A sample of tap water, for instance, may contain few bacteria, and this may make detection, and species isolation, easier. A sample of faeces, by contrast, may contain as many as 10^{10} $CFUg^{-1}$, comprising many different species (Yousef, 2008) (for soil samples, the figure is 100–200 $CFUg^{-1}$). Such vast numbers of bacteria within a sample may make detection of a single target strain difficult.

Colonies versus cells: Many techniques in use at present at some stage demand, in practice, amplification of target strain cell numbers (Yousef, 2008) and counting of subsequent colonies. However, it is impossible to determine whether any of the colonies so-formed resulted from a pre-existing colony or a single bacterium. This may lead to under-estimates of target strain numbers.

Mutation: Bacteria, including pathogenic strains of *E. coli*, mutate rapidly. Thus techniques that are appropriate for known virulent strains may prove inappropriate for equally virulent, but hitherto non-existent cum unknown strains. Hence the problems in detection, as indicated, of *E. coli* O104:H4 in Germany in 2011 (Altmann *et al.*, 2011). Techniques that are appropriate today may fail to be so in the future.

Bacterial morphology: Detection of pathogenic strains of bacteria cannot be done by visual inspection. Bacteria are morphologically similar, with only a few basic variations (Gram-positive versus Gram-negative; rod or cocci; strepto of non-streptococci; flagellum, and type of flagellum, or no flagellum). Pathogenic *E. coli* may be visually identical to beneficent bacteria. The most that visual inspection, via electron microscope, may do is eliminating certain strains from being members of a target strain (Yousef, 2008). For this reason, all techniques for detecting pathogenic bacteria are indirect.

2.8.2. Prevention

The WHO (2005) advises that prevention of *E. coli* infection must occur at all stages of food production, from "agricultural production on the farm to processing, manufacturing and preparation of foods, in both commercial establishments and the domestic environment". It specifically mentions the importance of screening beef cattle for

E. coli prior to slaughter, high levels of hygiene within abattoirs and farms, and preventing animal wastes entering recreational waters.

This last consideration may be especially important on a worldwide scale. The WHO (2005) reiterated the point that data concerning *E. coli* infections mainly come from the developed world. However, it is plausible that such are also common in the developing world. Figure 2.12 shows that, in many African countries, over one-third of people lack access to good drinking water.

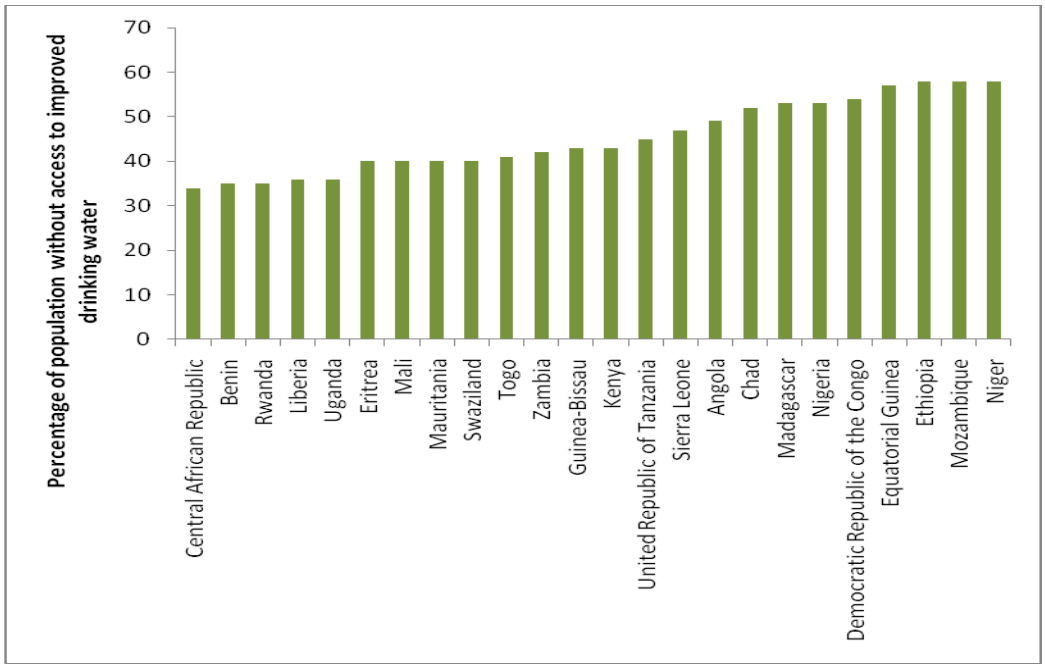

Figure 2.12. African countries that lack access to improved drinking water.

WHO (2009a). All data are 2006 estimates.

Contact with livestock and other animal species, as outlined earlier, may also constitute a risk. Person-to-person infections highlight the need for improved hygiene, education and effective communication of risk during disease outbreaks (Strachan *et al*., 2006; Kon *et al*., 2009). As regards general hygiene, hand washing is known to reduce the

risk of infection to visitors (Payne *et al*., 2003). For all risk pathways, children appear to be especially vulnerable.

The food-borne pathway represents the primary route of *E. coli* O157 infections, and the WHO (2009b) (2005) highlight five key considerations regarding food handling and preparation (Table 2.5).

Table 2.5.WHO five key considerations for safer food.

No	Key
1	Keep clean
2	Separate raw and cooked foods
3	Cook thoroughly
4	Keep food at safe temperatures
5	Use safe water and raw materials

WHO (2009b).

The importance of hygiene is paramount. One study of U.S. infections suggests that improved hygiene (adequate hand-washing) by food preparers would have prevented 34% of food-borne *E. coli* O157:H7 infections in the U.S. (Mead *et al*., 1999). The importance of proper cooking should also be noted. The WHO (2005) states that cooking should involve all areas of food, including the centre, to reach at least 70°C. This, as indicated (Fernandez, 2008), should suffice to kill *E. coli* O157:H7 bacteria; whereas undercooking (particularly of beef) have led to large and serious outbreaks of infection (O'Brien *et al*., 1993). Restaurants, pubs and other area open to the public should be particularly vigilant of effective cooking procedures.

On a worldwide scale, the expedients of improving the quality of drinking water and improving knowledge of hygiene and food preparation would vastly reduce the number of deaths from *E. coli* infections. Besides, improving the quality of drinking water would carry benefits other than reducing the number of diarrhoeal deaths (from *E. coli* or other sources). It is clear though that infrastructure and resources may be limited in developing countries to ensure that such changes happen widely.

In developed countries however, improving the quality of drinking water would be insufficient to make large reduction in number of O157 infections as most people already have access too clean, treated drinking water. Other measures, particularly related to food, may need to be implemented for significant reductions in infections. Such options include irradiating foods, which kills many pathogenic bacteria, including pathogenic *E. coli* (FDA, 2008) and carries the benefit of preserving foods for longer. Steam pasteurisation may also be utilised.

Pre-slaughter control strategies include developing quick analytical tests, a vaccine for use in cattle, and agents involved in the reduction of *E. coli* O157 in the bovine intestine (Jordan *et al.,* 1999). Other than vaccination, there are diverse methods used in farm livestock to control VTEC. These include the use of novel antimicrobials, including bacteriocins, chloral hydrate, bacteriophage, and substances that disrupt quorum sensing. Callaway *et al.* (2004) and Sargeant *et al*. (2007) report another option, particularly as regards *E. coli* O157 in cattle, is to increase herd resistance to infection. Methods include the use of antimicrobials, probiotics, bacteriophages, and sodium chlorate. Such methods may be appropriate to livestock other than cattle. Edrington *et al*. (2003) report that chlorate

product, managed in the feed, was effective in reducing levels of *E. coli* O157:H7 from the lower gut of sheep, as evidenced by lower cecal and rectal but not ruminal concentrations. Feeding chlorate may thus be an effective method to decrease *E. coli* O157:H7 populations in ruminant animals prior to slaughter.

Changes in animal husbandry may also be important. Given that *E. coli* infection of cattle and sheep appears inevitable, changing feeding practices may be important. There is evidence that feeding cattle meat products increases risk of infection (Herriot *et al*., 1998); this is now illegal in the E.U. In the U.K., efforts may be needed to understand more about the transmission and re-cycling of the organism in feeds such as silage, given the possibility that it may be contaminated. For instance, *E. coli* O157 infection appears higher in cattle fed silage than in cattle not so fed (Herriot *et al*., 1998). This may be attributed to poor silage handling prior to feeding. Unless silage is stored properly so as to produce sufficient fermentation and hence sufficient heat and acidity to destroy *E. coli*, the bacteria may survive (Fenlon *et al*., 2000; Fenlon and Wilson, 2000).

The issue of acidity is also pertinent to types of food fed to cattle. Concentrated diets, especially grain fed to calves, appear to increase fermentation and therefore acidity in the rumen. This may lead to the development of acid-tolerant *E. coli*. This may also encourage faecal shedding of the acid-resistant *E. coli* (Tkalcic *et al*., 2000); the same may also apply to sheep (Lema *et al*., 2002).

There is also the question of reducing *E. coli* infection of land, and thereby direct infection of vegetation and indirect infection of livestock. As indicated, such infection may be reduced by improved use of vegetation (Crane *et al*., 1983; Moore *et al*., 1988; Coyne *et*

al., 1995). Similarly, changes in practices concerning use of slurry may lead to reduced soil contamination. Slurry embedded in soil, for example, as opposed to merely being spread on it, appears to reduce contamination (Avery *et al*., 2004).

Thus there appear a range of methods to control *E. coli* infections at source (animal reservoir) and in the wider environment and food. These appear to be of three sorts: (a) to prevent entry of pathogenic *E. coli* into the environment (by, e.g., vaccinating cattle); (b) killing pathogenic *E. coli* before they enter the food chain (by, e.g., irradiating food); and (c) improving general hygiene. These methods are not mutually exclusive; and there is some overlap between them.

Two general considerations are relevant. First, given the degree of uncertainty in the area—*E. coli* persistence in the soil, the importance of grazing, the relative contribution of cattle, and so forth—it is important to collect data. Second, the best solution in terms of reducing *E. coli* presence in the environment may not be the "best" overall. One needs a cost-benefit analysis of control mechanisms, and also an assessment of what is practicable, economically viable, and likely to result in the greatest reductions for least effort.

Control measures may be resisted by farmers if the cost cannot be passed on to the retailer or consumer. Thus, for example, vast changes in feeding strategies, land management, screening of livestock, may require both an army of regulators and, initially at least, great cost to farmers. Other measures might be resisted by the general public. Irradiation of foods, for instance, may be opposed, however ill-founded, on fears of radioactive contamination. Others may conflict with other environmental policies. In many poor countries, for instance, manure is used both as fertiliser and as fuel. Any attempts to

reduce manure in the environment may so increase pressure on using wood for fuel, at cost to trees within the countries. It is also notable that improving access to good drinking water does not necessarily involve cleaning rivers: as Lomborg (2001) argues, many rich countries (e.g., Australia, Japan, and the U.S.A.) have high numbers of *E. coli* in some rivers, but still have access to good drinking water because of access to underground sources.

Other control methods may prove counter-productive. Any efforts to reduce *E. coli* in the environment—either in soil or in the intestinal tracts of ruminants—for instance, may also kill those beneficent *E. coli* that harm pathogenic *E. coli* (Fratamico *et al*., 2002), thereby, on balance, increasing the numbers of *E. coli*. Thus, at present, the best strategy, or combination of strategies, for reducing pathogenic *E. coli* within the environment is unclear. These considerations suggest a need for continuous monitoring of how well, or badly, use of a strategy fares. Finally on this issue, one may note that what may be good for one country need not be good for another. As indicated, the priority in poor countries is almost certainly to improve quality of drinking water; it is not, as might be the case in the U.K., to improve the quality of cattle feed.

2.9. General conclusion

Although part of the normal gastro-intestinal tracts of humans and other animals, some *E. coli* constitutes a substantial risk to the health of human beings. Of these, pathogenic *E. coli* O157:H7 strains appear especially damaging.

The major sources of *E. coli* O157:H7 appear to be the faeces of cattle, sheep and goats. Over 5% of sheep and cattle faeces appear to be contaminated, but the precise

amount is uncertain, and, in any event, varies both by season and geographical area. Other animals may also act as reservoirs.

Several transmission routes are possible between animal faeces and human beings. The main ones appear to comprise eating undercooked infected meat, eating produce contaminated with animal faeces, eating dairy products (especially cheeses) produced by infected animals, and swimming in contaminated water, touching contaminated animals, and, possibly distance to infected animals. It is plausible that risk of contamination varies according to cultural differences in human populations, such as diet.

Because of uncertainty as to major causes of risk, and the variety of means of infection (through eating various types of food, contact with infected animals, and so on), there does not appear a single means of curtailing infection. Instead, a variety of measures may be taken, mostly in tandem. These include improved hygiene in all stages of food production, both commercial and at home; improved hygiene in all areas of life but especially in food production and when coming into contact with animals; improved methods of water monitoring and improved standards of water quality; and monitoring infection rates of animals, especially cattle and sheep.

CHAPTER 3: COUNTS OF *E. COLI*, *E. COLI* O157, AND COLIFORMS IN SHEEP AND CATTLE FAECES

3.1. Introduction

As indicated in Chapter 2, the faeces of ruminant animals is a major source of *E. coli* O157, and hence a major source of gastrointestinal and related illness in humans. Most of the research has focussed on cattle faeces, with much less research conducted on sheep. Research in Scotland suggests some 40% of flocks may contain infected sheep and that about 6.5% of faecal samples may contain *E. coli* O157 (Ogden *et al*., 2004). Similar results, though with higher incidence of infected faecal samples, has recently been reported for flocks in the Grampian region (Solecki *et al.*, 2009). Similarly, Omisakin *et al*. (2003), in a study of cattle at time of slaughter in a Scottish abattoir over a nine week period report individual incidence of 7.5% and group prevalence of 40.4% (all samples faecal, taken from the dead cows' rectums); 9% of the cattle were high shedders (> 10^4 CFU g^{-1}), and these cattle produced more than 96% of the *E. coli* O157 of the total produced by all cattle. For comparison, from examination of sheep carcases, Paiba *et al*. (2002) estimate that about 2% of UK sheep carry *E. coli* O157. Research in Idaho, USA has suggested slightly higher levels of *E. coli* O157 in sheep than in cattle (Weaver *et al.*, 2005). However, prevalence rates are known to vary considerably by geographical location, as discussed previously.

The results imply that there may be substantial regional differences in carriage rates, and sheep might carry as much *E. coli* as cattle. Knowing the prevalence rates within ruminant livestock is important to design risk-based mitigation strategies. This is particularly important given the association of *E. coli* O157 infections with direct or

indirect contact with their faeces. We hypothesized that sheep faeces contain substantial quantity of *E. coli* O157 like cattle faeces and thus be crucial for sheep grazing areas like North Wales. The aim of this study was to determine numbers of coliforms, *E. coli*, and *E. coli* O157 bacteria in fresh cattle and sheep faeces from farms in North Wales. This would allow a comparison to be made of livestock in this area relative to the other areas; which would be useful when devising effective risk mitigation strategies (Strachan *et al.*, 2011).

3.2. Materials and methods:

3.2.1. Sample collection

Between 10 and 15 samples of sheep and cattle faecal material were collected from fresh droppings in the field, each month from September 2010 through August 2011 (total n = 150 for sheep and 150 for cattle). Samples were collected from 25 farms, chosen randomly, all within a 60 mile radius of Bangor, Gwynedd. Some farms reared sheep only (n = 10); others reared cattle only (n = 10); others reared sheep and cattle (n = 5). Each sample weighed approximately 40–50 g. All samples were collected fresh, to avoid cross-contamination by different species.

Upon collection, each sample was immediately placed in a sterile plastic bag, which was then labelled and sealed, and then transported to the laboratory within 2 hours of collection. In the laboratory, the samples were stored in a refrigerator at 4ºC until they were analysed within 24 hours of collection.

For purposes of analysis, monthly data were pooled into seasonal data as follows:

Autumn: September through November

Winter: December through February

Spring: March through May

Summer: June through August

3.2.2. Enumeration of total coliform and E. coli

First, 5 g of each faecal sample were placed in 45 ml of ¼-strength Ringer's solution (Oxoid BR0052G; Oxoid Ltd., Hampshire, UK). The mixture was then stomached for 30 sec in a Lab Blender 400C (Seward Ltd., Sussex, UK) and incubated in an orbital incubator shaker (Stuart Scientific Plc S150, Staffordshire, UK) at 25ºC for 15 min with shaking at 200 rev min^{-1}. Next, four serial dilutions were prepared from the homogenised samples; 1 ml of the dilution was then plated on a Petrifilm *E. coli* and coliform Count Plate (3M Microbiology, St. Paul, MN, USA). The plated dilution was then incubated at 37ºC for 24 hours in a Model 280 LMS cooled incubator (LMS Ltd., Kent, UK).

The number of coliforms and *E. coli* per gram was estimated by counting the pink and purple colonies using a colony counter (Stuart Scientific). The number of coliform and *E. coli* bacteria were estimated by counting the number of purple and blue colonies, respectively. Mean values were calculated from three replicates.

3.2.3. Isolation and enumeration of E. coli O157

Samples were analysed for *E. coli* O157 by enrichment followed by immunomagnetic separation (IMS) (Ogden *et al*., 2001). Five g of each faecal sample were homogenised in Modified Tryptone Soy Broth (mTSB) (Oxoid, CM 0989), containing VCC supplemented (Oxoid). Broths were then incubated at 37ºC for 6 hours with shaking at 150 rev min^{-1} in the orbital incubator. To determine the presence or absence of *E. coli* O157, 1ml of the enriched sample was analysed by Dynamag™2 IMS

(Invitrogen Dynal, A.S., Oslo, Norway) with 0.02 ml of Captivate™ O157 immunomagnetic beads (Lab M Ltd., Lancashire, UK) and incubated again in the orbital incubator at 25ºC for 30 min. After the IMS, the beads were washed three times using phosphate buffered saline + 0.05% Tween 20 as a washing buffer, resuspended in 0.1 ml of the same buffer, spread equally on a CT-SMAC plate (sorbitol MacConkey agar plates, SMAC; Oxoid CM813) supplemented with cefixime (0.05 mgl^{-1}) and potassium telluride (2.5 mg l^{-1}), and incubated at 37°C for 18-24 hours. Presumptive *E. coli* O157 colonies (non-sorbitol fermenting) were confirmed by agglutination with a latex test kit (DR0620, Oxoid). As before, counts were taken from the mean of three replicates.

3.2.4. Statistical analysis

All statistical calculations were performed using IBM SPSS Statistics (Version 19). Statistical analysis comprised two between measures 2 × 4 ANOVAs. The DVs were *E. coli* and coliform counts. The IVs were livestock type (cattle or sheep) and season (autumn, winter, spring, or summer). A within measures design was not employed for the season variable because samples came from different farms within each season. Two independent ANOVAs were used ANOVA (Field, 2009).

Because two related tests were used, alpha was set at $p = 0.025$ for all main effects; this was a Bonferroni adjustment to cater for Type I errors. For post-hoc tests, alpha was set at $p = 0.05$ (Tukey's HSD). This was because, in the event of an overall significant main effect, corrections for the Type I error rate within post-hoc tests (in this case, Tukey's HSD) are automatic.

Because of severe violations of parametric assumptions (again, see below), two precautions measures were taken. First, where possible, results were cross-checked

using equivalent non-parametric tests. Second, data were logarithmically transformed (base 10) and the ANOVAs re-run using the transformed data. The logarithmic transformation did nothing to render the data more amenable to parametric analysis, and the results of the ANOVAs using the transformed data were effectively the same as those using the raw data.

3.3. Results

3.3.1. Coliform bacteria

3.3.1.1. DESCRIPTIVE STATISTICS ON COLIFORM COUNTS

Figure 3.1 shows the log (base 10) of the mean faeces coliform bacteria for sheep and cattle for the seasons (autumn, winter, spring and summer). Data are from all samples taken in 2010 and 2011.

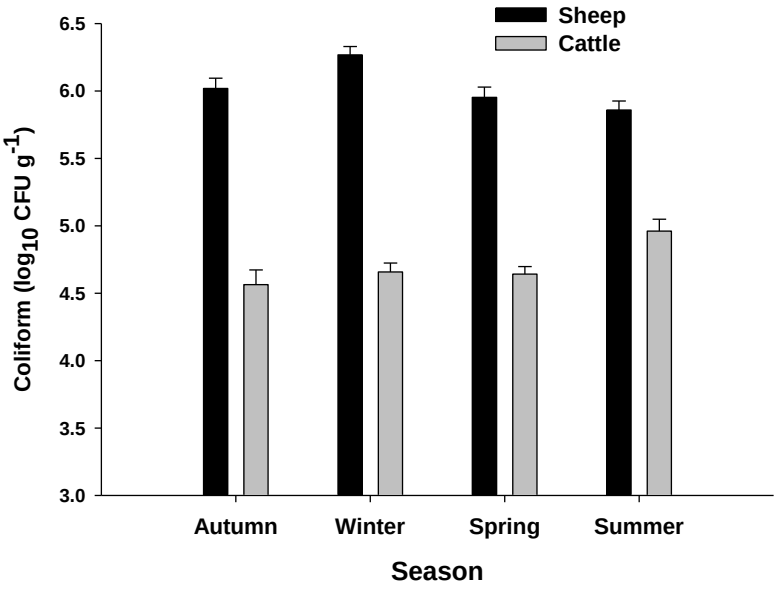

Figure 3.1. Mean faecal coliform concentrations in sheep and cattle faeces (log DW). Error bars denote standard error.

The figure suggests higher numbers of coliform in sheep faeces, and slightly higher numbers in faeces of sheep and cattle during the winter months. It also suggests the possibility of interaction in that sheep levels were highest in winter but cattle levels were highest in summer.

3.3.1.2. INFERENTIAL STATISTICS

The 2-way 2 × 4 between measures ANOVA on coliform counts revealed a significant main effect of livestock type, F (1, 292) = 145.215, $p < 0.0005$; the effect size was substantial, $\eta_p^2 = 0.332$, suggesting about 30% of the variance in coliforms was explained by livestock type. The mean coliform count for sheep was 6.03 $\log_{10}$ CFU g^{-1}; that for cattle was 4.70 $\log_{10}$ CFU g^{-1}. Thus coliform counts were significantly higher in sheep.

The same ANOVA revealed a significant main effect of season, F (3, 292) = 4.631, $p = 0.004$; the effect size was small, $\eta_p^2 = 0.045$, suggesting less than 5% of the variance in coliforms was explained by season.

Mean coliform counts were highest in winter (mean: 5.52 $\log_{10}$ CFU g^{-1}), followed by summer (mean: 5.44 $\log_{10}$ CFU g^{-1}), autumn (mean: 5.34 $\log_{10}$ CFU g^{-1}), and spring (mean: 5.17 $\log_{10}$ CFU g^{-1}). Post-hoc tests (Tukey's HSD) revealed the difference between winter and (spring, autumn) was significant ($p < 0.0005$), as was the difference between spring and summer ($p = 0.005$); no other differences were significant.

The ANOVA also revealed a significant interaction between livestock type and season, F (3, 292) = 5.382, $p = 0.001$; the effect size was small, $\eta_p^2 = 0.052$, suggesting less than 5% of the variance in coliforms was explained by the interaction.

Figure 3.1 suggests coliform populations varied seasonally more in sheep than in cattle. Overall, the ANOVA explained over 30% of the variance in coliform counts, $R^2 = 0.386$; $R^2_{adjusted} = 0.372$.

Data grossly violated parametric assumptions. Shapiro–Wilk tests on all samples yielded significant results ($p < 0.0005$ in all cases; results of Kolmogorov–Smirnov tests were similar); graphical output suggested extreme positive skew and copious outliers. Levene's test revealed variances were unequal ($p < 0.0005$). A Mann–Whitney U test corroborated the main effect of livestock ($p < 0.0005$), but a Kruskal–Wallis test failed to corroborate the effect of season; however, the result approached significance ($p = 0.08$).

Given the insignificance of the result of the Kruskal–Wallis test, the small effect sizes for season and the interaction, and the gross violations of ANOVA assumptions, the existence of seasonal differences, much less an interaction, in coliform counts appears problematic. The difference between sheep and cattle, however, appears real.

3.3.2. E. coli

3.3.2.1. DESCRIPTIVE STATISTICS OF *E. COLI* COUNTS

Figure 3.2 shows the log (base 10) of the mean faeces *E. coli* bacteria for sheep and cattle for the season (autumn, winter, spring and summer. Data are from all samples taken in 2010 and 2011.

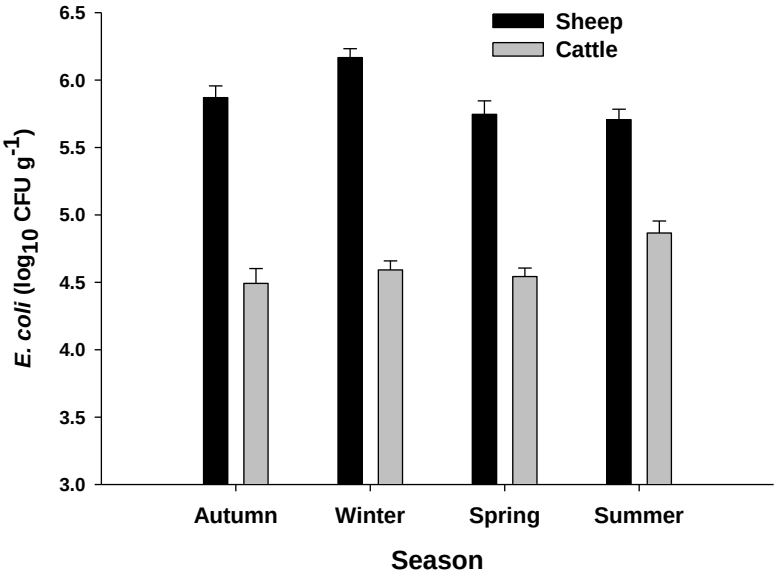

Figure 3.2. Mean faecal *E. coli* concentrations in sheep and cattle faeces (log DW).

Error bars denote standard error.

Again, the figure suggests higher numbers of *E. coli* in sheep faeces and slightly higher numbers during the winter months. It also suggests the possibility of interactions.

3.3.2.2. INFERENTIAL STATISTICS

The 2-way 2 × 4 between measures ANOVA on *E. coli* counts revealed a significant main effect of livestock type, F (1, 292) = 124.066, $p < 0.0005$; the effect size was large, $\eta_p^2 = 0.298$, suggesting about 25% of the variance in *E. coli* was explained by livestock type. The mean *E. coli* count for sheep was 5.89 $\log_{10}$ CFU g^{-1}; that for cattle was 4.62 $\log_{10}$ CFU g^{-1}. Thus *E. coli* counts were substantially higher in sheep.

The same ANOVA revealed a significant main effect of season, F (3, 292) = 4.551, $p = 0.004$; the effect size was small, $\eta_p^2 = 0.045$, suggesting less than 5% of the variance in *E. coli* was explained by season. Counts were highest in winter (mean: 5.43

$\log_{10}$ CFU g^{-1}), next highest in summer (mean: 5.31 $\log_{10}$ CFU g^{-1}), next highest in autumn (mean: 5.23 $\log_{10}$ CFU g^{-1}), and next highest in spring (mean: 5.02 $\log_{10}$ CFU g^{-1}). Post-hoc tests (Tukey's HSD) revealed the difference between winter and (spring, autumn) was significant ($p < 0.0005$), as was the difference between winter and summer ($p = 0.007$); no other differences were significant.

The ANOVA also revealed a significant interaction between livestock type and season, F (3, 292) = 5.176, $p = 0.002$; the effect size was small, $\eta_p^2 = 0.050$, suggesting less than 5% of the variance in *E. coli* s was explained by the interaction.

Figure 3.2 suggests *E. coli* populations varied seasonally more in sheep than in cattle. Overall, the ANOVA explained about 30% of the variance in *E. coli* counts, $R^2 = 0.356$; $R^2_{\text{adjusted}} = 0.340$.

Data grossly violated parametric assumptions. Shapiro–Wilk tests on all samples yielded significant results ($p < 0.0005$ in all cases); graphical output suggested extreme positive skew and copious outliers. Levene's test revealed variances were unequal ($p < 0.0005$). A Mann–Whitney U test corroborated the main effect of livestock ($p < 0.0005$); a Kruskal–Wallis test corroborated the effect of season; however the result was barely significant ($p = 0.019$), suggesting, in line with the ANOVA result, that the effect size was, most likely, trivial.

It therefore appears that there are genuine seasonal differences in *E. coli* populations, and a genuine difference between cattle and sheep. Whether there is an interaction between season and livestock type, however, appears problematic. In any event, neither seasonal factors nor interaction factors, if they produce real differences, appear very important.

3.3.3. E. coli O157

No *E. coli* O157 were detected by enrichment in any of the samples.

3.4. Discussion

The results of this study show that coliform and *E. coli* counts to be substantially higher in sheep faeces than in cattle faeces; which is consistent with results of Wilcock (2006) and Geldreich *et al.* (1962). Although counts of coliform and *E. coli* were slightly higher in winter than in spring and summer months, statistical analyses shows that this difference to be insignificant. The effect sizes as provided by the ANOVAs appear trivial (less than 5%), and the Kruskal–Wallis tests as regards seasonal differences were insignificant or barely significant—suggesting trivial effect sizes. Thus, for the data collected, there appears to be no seasonal differences in counts of coliforms or *E. coli* in livestock faeces. This negative finding as regards seasonal factors is at variance with some of the literature (e.g., Van-Donsel *et al*., 1967), and with the observation that incidence of *E. coli* O157 infection is substantially more common in the summer months (e.g., Defra 2008). However, research cited in Chapter 2 (e.g., Kress and Gifford, 1984) suggests that *E. coli* in the environment are sensitive to a range of environmental conditions and the present study investigated only *E. coli* from fresh animal faeces. However, such research mainly concerns survival and propagation of the bacteria after faecal shedding.

The present study failed to detect *E. coli* O157 in faecal samples from either cattle or sheep. The literature shows the degree of carriage and excretion of the organism by livestock varies according to study. Rhoades *et al.* (2009) report a mean incidence rate of 6.2% but a range of 0–57% for cattle (and see Fernandez, 2008). In

their recent Scottish (Grampian region) study, Soleckiet *al.* (2009) found of a total of 390 sheep faecal samples taken from 14 farms, 41 were infected with *E. coli* O157 (from six farms). The prevalence of *E. coli* O157 infections is markedly higher in Scotland than in the rest of the UK, so it is plausible that prevalence of the strain is lower in livestock in North Wales than found in Scotland by Solecki *et al*. (2009); even though the sample sizes of both studies were broadly similar. The present study took 150 sheep samples and 150 cattle samples, yielding a total of 300 samples. If sheep and cattle in North Wales were no different from those in Grampian as regards faecal *E. coli* O157, about 30 faecal samples should have tested positive for the strain. Even allowing for the higher infection rate in Scotland, the sample size in the present study appears sufficiently large enough to have detected *E. coli* O157, if present. Even if one takes the incidence rate of infection reported by Paiba *et al*. (2002), of 2%, some six infected samples should have been detected. Thus the failure to detect the strain suggests that its incidence within the livestock population in North Wales is low. Reasons for the low incidence in North Wales need identification, but could relate to animal husbandry, diet, and climatic effects (Jones, 1999).

Generic *E. coli* numbers were approximately ten-fold greater in sheep faeces as compared with cattle faeces. This compares with the results of Weaver *et al*. (2005), who reported a difference of only about 30% (with counts in sheep faeces being the higher). Moreover, the literature (e.g., Griffin and Tauxe, 1991; Chapman *et al*., 1996, 2001; Elder *et al.*, 2000; Barlow *et al.*, 2006) suggests *E. coli* O157 infection of humans stems more from cattle than from sheep. However, if proportions of *E. coli* O157 in livestock manure reflect the proportions of other coliforms—a ten-fold difference as found in the present study, one would expect much greater prevalence of the pathogenic

strain in sheep than in cattle. Therefore there should, all things being equal, be far more cases of human *E. coli* O157 infections originating from sheep than from cattle. However, the UK population eat more beef than lamb (consumption of beef and lamb in 2010 were 114g and 44g per person per week, respectively (Defra, 2012) and beef is often cooked to a lower temperature than lamb.

It is unclear as to why coliform counts are higher in sheep than in cattle and is a question for future research. The reason may lie in differences between ovine and bovine physiology; it may also lie in the different types of animal feed used for the two species of livestock (however, empirical studies of the effects of livestock diet on intestinal microbes produce conflicting results).

Of course, results of the present study cannot be generalised to areas other than North Wales. On the face of it, they suggest that sheep faeces present a greater threat to human health than do cattle faeces. However, this may not necessarily be the case as greater numbers of *E. coli* may not directly lead to greater risk of infection due to the lower volume of faeces produced by sheep, the fact that the infectivity of cells is also a factor of cell metabolic activity, etc. Such areas could therefore be the subject for future research.

3.5. Conclusion

Results of the present study indicate substantial numbers of coliform bacteria, including *E. coli* exist in sheep and cattle faeces in North Wales; especially so in sheep faeces. There appears to be little seasonal differences in counts. It is also apparent that sheep and cattle from North Wales are largely free of *E. coli* O157. Why this is the case is a subject for future research.

CHAPTER 4: EFFECT OF GRAZING ON SOIL IN THE UPLANDS AND LOWLANDS

4.1. Introduction

As a porous medium, soil is suitable for microbial conductance of many bacteria responsible for environmental health hazards. Soils of grasslands are particularly important for water contamination because of grazing. Direct deposition of faeces from grazing animals is a major source of zoonotic organisms (Acha and Szyfres, 1994). During rainfall, the overland flows of water from grazed pastures can contain a considerable number of faecal microbes; these can enrich the surface water with microbes (Collins and Rutherford, 2004).

Studies with animal faeces in grassland often focus on uptake and cycling of plant nutrients and applications of manure when spread on the agricultural field as organic fertilizers (rather than direct deposition by grazing animals) (Hutchison *et al*., 2005; Oliver *et al*., 2007). To understand the faecal reservoir in grazed pasture, it is necessary to estimate the size of the soil reservoir of faecal microbes. This is a prerequisite to initiate any mitigation measures to reduce the losses of faecal microbes from grassland to surface water reservoirs.

Research suggests the most important factor for the regulation of the entry of bacteria into soil-water systems is the survival of the organisms during their residence in soils (Jamieson *et al.*, 2002). Parsons *et al*. (1975) and Morrison and Martin (1977) reviewed the survival time of different pathogens in soil-water systems and showed that the period ranges from 30 min to several years. Other factors that influence the persistence of faecal microbes in soils are soil moisture, temperature, sunlight, pH,

competitive organisms, availability of nutrients, and soil type. Another important factor is the retention of microbes on the charged surface of the clay particle; this affects the movement of microorganisms in soil water systems.

Snowdonia comprises both hilly grassland and adjacent low lying plain lands. Both are traditionally grazed by sheep (Bardgett *et al*., 1997), though much more intensely in the lowland areas. Many advocate the reduction of livestock numbers as a way to decrease faecal contamination of soils and microbial pollution of catchments. Adoption of agri-environment schemes in Wales has led to considerable reduction in the numbers of livestock, especially so in the upland areas. However, little is known about the actual changes in soil microbial levels following removal of livestock. As livestock grazing and land topography have potential impacts on abundance of coliform, it was hypothesized the soils of upland and lowland areas differ in coliform population after recession of grazing. The objective of the present study was to ascertain the changes in coliform populations within soils following changes in grazed systems in the uplands and lowlands of North Wales.

4.2. Materials and methods

4.2.1. Study sites

Two contrasting physiographic locations were selected for the study: (a) specific areas of the hilly mountain areas of Snowdonia National Park in the Llanberis area, namely the sites of Pen y Pass, Llyn Llydaw, and Cwm Idwal (upland site) and (b) low lying areas at Henfaes Research Station located in the village of Abergwyngregyn, Gwynedd (lowland site; grid reference: 53º14'N; 4º01'W). Figure 4.1 shows an upland site (Pen y Pass). Figure 4.2 shows the lowland site (Henfaes Research Station).

Figure 4.1. An upland site (Pen y Pass).

Figure 4.2. The lowland site (Henfaes Research Station).

The climate within North Wales is hyperoceanic; with an average annual rainfall of 1000 mm. The rainfall in the uplands usually exceeds 1500 mm, rising to over 3000 mm in parts of Snowdonia (Jones and Taylor, 1983). The average annual rainfall in lowland (Henfaes Research Station) areas is around 1000 mm (Hoosbeek *et al*., 2010).

Sampling plots were fenced in 1957 within Snowdonia National Park to study the ecological impacts of grazing by excluding livestock. These sites measured approximately 20 × 15 m. There are some variations in the vegetation of grazed and ungrazed plots. The dominant plant species in the grazed grassland are heather (*Calluna*

vulgaris) and common bilberry (*Vaccinium myrtillis*); in contrast, the ungrazed area is dominated by tall densely growing grasses and perennial herbs (Keiller *et al*., 1995). The general landform and dominant vegetation are described in the Table 4.1.

At Henfaes, grassland plots were fenced in 1992 as part of a silvopastoral agroforestry experiment. The unfenced area is density grazed by sheep; it comprises a mixture of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*). The soil is a fine loamy brown earth over gravel (Rheidol series). Each of these sites measured approximately 20 × 20 m.

4.2.2. Collection of soil samples and sample preparation

Samples were collected in October, November, April, and May of the years 2010 and 2011 from grazed and ungrazed plots at the upland and lowland sites (n = 6 of each grazing regime for both upland and lowland). Each plot contained five sampling sites, providing a total of 60 samples for each sampling occasion therefore 240 samples for both upland and lowland over the study period.

At each sampling occasion, 50 g of soil was collected from the top 15 cm of the five sites within each grazed and ungrazed plot. The auger was sprayed with 70% ethanol before each sampling to prevent cross-contamination. All samples were kept in sterile plastic bags and transported to the laboratory immediately, where they were stored at 4°C until further analysis.

Table 4.1. Physiography and vegetation of study sites in Snowdonia.

Location	National Grid Reference	Vegetation		Altitude(m)	Slope (◦)	Aspect (◦)	Soil type
		Grass	Shrubs				
Pen y pass	SH 646 556 & SH 646 555	*Nardus stricta*, *Danthonia decumbens*, *Narthecium ossifragum*, and*Festuca ovina*.	ericoid shrubs such as *Calluna vulgaris*, *Ulex gallii*, *Calluna vulgaris*, *Erica cinerea*	396	16°–20	100°–105	Peaty Podzol and brown earth- gley
Llyn Llydaw	SH 638 548 & SH 637551	*Festuca ovina* and *Nardus stricta*.	*Calluna vulgaris*, *Vaccinium myrtillus*, and other ericoid Shrubs	426	18°–25	183°–305	Peaty Podzol and brown earth
Cwm Idwal	SH 643 595 & SH 642 592	*Festuca ovina*, *Agrostis capillaries*, and *pleurocarpous mosses*.	*Erica cinerea* and other shrubs.	365	3˚–10	13°–90	Peaty Podzol and brown earth

Hill *et al*. (1992).

4. 2.3.Analysis of physico-chemical properties of soils

Analysis of soil physico-chemical properties was through standard soil science methods. This was as follows:

4.2.3.1. MOISTURE CONTENT

Percent moisture content (MC) was determined gravimetrically after drying the sample in an oven at 105°C for 24 hours. Equation 4.1was used for calculating the difference between the weight of the soil before and after drying (Standards Association of Australia, 1977).

$$MC\% = \frac{W_2 - W_3}{W_3 - W_1} \times 100 \qquad \text{Equation 4.1}$$

whereW_1 = weight of crucible empty; W_2 = weight of fresh soil plus crucible; W_3 = weight of oven dried soil plus crucible.

4.2.3.2. MAXIMUM WATER HOLDING CAPACITY (MWHC)

MWHC was determined by saturating the soils for 24 hours in the laboratory. After saturation, the samples were allowed to drain the excess water for several hours until no water was leached. The moisture content in soil at this condition was determined by oven drying at 105°C for 24 hours using Equation 4.2.

$$MWHC\% = \frac{W_2 - W_1}{W_2 - W_3} \times 100 \qquad \text{Equation 4.2}$$

Where, W_1 = weight of empty crucible; W_2 = weight of fresh soil plus crucible; W_3 = weight of oven dry soil plus crucible. MWHC analysis was conducted only once because it is unlikely to change much over short periods.

4.2.3.3. SOIL PH AND ELECTRICAL CONDUCTIVITY (EC)

First, soil samples were prepared by sieving with a 2 mm sieve. Next, 10 g of fresh soil was added to 25 ml of distilled water; this was in the ratio 1:2.5 by w/v (weight/volume). The suspension was then mixed by shaking at 200 rev min^{-1} for 30 min on a horizontal shaker, and left to stand for 30 min. Each sample's pH and EC were measured using glass electrodes. Soil pH was measured by a HANNA pH 209 meter (HANNA Instruments, RI, U.S.A) Soil EC was measured by a Jenway 4520 conductivity meter (Jenway, Dunmow, U.K.).

4.2.3.4. NITRATE (NO_3^-) AND AMMONIUM (NH_4^+)

To determine the NO_3^- and NH_4^+ in the soil samples, 10 g soil was mixed with 25 ml of 0.5M K_2SO_4. The soil solution was shaken for 30 min in a SM25 shaker (Edmund Bühler GmbH, Hechingen, Germany) and then centrifuged in an Eppendorf 5810 R centrifuge (Eppendorf, Cambridge, U.K.) at 4000 rev min^{-1} for 5 min. Filter paper (125 mm) (Fisher Brand FB 59249, Fisher Scientific) was used for filtration. The clean extract was collected and stored at 4°C in a refrigerator before analysis. The NO_3^- and NH_4^+ concentrations in the soil extract were analysed as follows:

Nitrate (NO_3^-) was determined colorimetrically using a PowerWave XS microplate spectrometer (Bio-Teck Instuments Inc, Miami, FL, U.S.A.). This reduced the NO_3^- to NO_2^-

and was followed by reaction with N-(1-napthyl) ethylenediamine to produce chromophore. Absorbance was read at 540 nm.

Ammonium (NH_4^+) was determined colorimetrically by the salicylate-nitroprusside method using a PowerWave XS Spectrometer using a microplate. Absorbance was read at 667 nm (Mulvaney, 1996). For determining NO_3^- and NH_4^+ concentrations, samples were only collected from each plot during November and May, representing winter and summer.

4.2.3.5. ORGANIC MATTER AND TOTAL CARBON IN SOIL

Soil organic matter (OM) was determined by the loss on ignition (LOI) method. In this method, oven dry soil (105°C) was ignited at 450°C in a muffle furnace overnight and the soil carbon concentration ($C_{c\ \%}$) was determined by using Equation 4.3 (Guo and Gifford, 2002).

$$C_c\ \% = 0.58 \times OM\ \% \qquad \text{Equation 4.3}$$

Note that this method yields two perfectly correlated sets of data; so, in terms of inferential statistics, only one measure need be used. For soil OM, samples were only collected from each plot during November and May, representing winter and summer.

4.2.4. Microbial analysis of soils

The most widely recognized indicator of bacterial pollution is coliform counts (American Public Health Association, 1971). The enumeration of bacteria followed the procedure used by Frampton *et al.* (1988). Faecal coliform and *E. coli* were enumerated (CFU g^{-1}) with the OXOID CM 0956 Brilliance™ *E. coli*/coliform medium (Oxoid Ltd., Hampshire, U.K.).

For enumeration of total coliforms and *E. coli*, 5 g of each soil sample were placed in 20 ml of ¼-strength Ringer's solution (Oxoid), stomached for 30 second in a Lab Blender 400C, as described in Chapter 3, and incubated at 25°C for 15 min with shaking at 200 rev min^{-1}. Serial dilutions were then prepared from homogenised samples: 1 ml of each dilution was plated and spread over the surface with a plastic spreader on a CM 0956 Brilliance™ *E. coli*/coliform (Oxoid) medium plate and incubated at 37°C for 24 hours. The number of coliforms and *E. coli* per gram was estimated by counting the pink and purple colonies, respectively, using a colony counter (Stuart Scientific, Chelmsford, UK).

4.2.4.1. STATISTICAL ANALYSIS

Statistical analysis comprised three main parts: ANOVA, correlation, and regression, using IBM SPSS (version 19).

4.2.4.1.1. ANOVA

A series of five 2 × 2 × 4 between measures ANOVAs were performed to test for differences in five of the measures. The dependent variables (DVs) were pH, EC, MC, *E. coli*, and coliform. The independent variables (IVs) were land type (lowland and upland), land use (grazed and ungrazed), and season (winter, 240; summer, 240) Tukey's HSD was used for post-hoc tests of differences between months. Prior to analysis, Kolmogorov–Smirnov and Shapiro–Wilk tests were run for all IVs for all DV to test for normal distribution. Both tests of normality were used because the number of observations for each DV was neither very large nor very small (Field, 2009).

A second series of two 2 × 2 × 2 between measures ANOVAs were performed to tests for differences in two other measures. The DVs were NO_3^- and NH_4^+. The IVs were land type, land use, and season (winter, n = 24, v. summer, n = 24).

Finally, 2 × 2 × 2 between measures ANOVA was performed using OM as the DV. The IVs were land type, land use, and season. Total carbon was not analysed because it correlated perfectly with OM.

Prior to analysis, Shapiro–Wilk tests were run for all IVs for each DV in the last three ANOVAs to test for normal distribution. Only Shapiro–Wilk was used because the number of observations for each DV was small (Field, 2009).

4.2.4.1.2. Correlation

Correlations were computed for all DVs used in the above ANOVAs. The IVs were not included in the analysis because (a) their relative importance had already been established by the ANOVAs, and (b) it proved impossible to include them in regression models (see below).

Pearson and Spearman correlations were calculated; the latter because data violated assumption of normal distribution of residuals. Attempts to rectify this by logarithmic transformation failed. The only correlations of major interest were those concerning *E. coli* and the seven other variables. Accordingly, alpha was set at p = 0.007—again, a Bonferroni adjustment to control for Type I errors.

4.2.4.1.3. Regression

First, from the correlation matrix for all DVs as described above between *E. coli* and all other DVs used in the eight ANOVAs, all significant predictors were identified, except those for NO_3^- and NH_4^+. These two variables were not initially considered because there were too few observations for each (n = 48 for each), and regression requires at least 15 observations per predictor variable; so, for example, with 6 predictor variables one needs at least 90 observations (Field, 2009).

It had been hoped to include the IVs used in the ANOVAs, together with interaction terms, in the regression models. However, preliminary analysis revealed unacceptably high variance inflation factors (VIFs) for each categorical variable/interaction term (25 < > 10); such high VIFs are unacceptable by any standards (Field, 2009). The variables and the interaction terms were therefore excluded from consideration.

Using *E. coli* as the outcome variable, curve estimations, using linear, logarithmic, and quadratic models, were established using each of the other significant correlates of *E. coli* in the above described correlations. This was to determine, in line with regression assumptions, that all relationships between predictor variables and the outcome variable were broadly linear. In this, the value of R^2_{adjusted} and the F value of each model (linear, logarithmic, and quadratic) were compared. A relationship was deemed linear if (a) the linear relationship explained the most variance, as judged by R^2_{adjusted}, and was the more significant, as judged by the F value; or (b) the values of R^2_{adjusted} and F for the linear model were broadly the same as those of the better other model.

Prior analysis also revealed violations of regression assumptions. These mainly concerned non-normal distributions of standardized residuals, but also, in some instances, violation of homoscedasticity and independence of errors. Attempts to rectify these by logarithmic transformation of data again failed. Moreover, graphical output suggested that the distributions of the standardised residuals, if not normal, were broadly bell-shaped—the main problem seemed to be outliers, with resultant platykurtosis, skew, or both. There was also, in some instance, slight suggestion of bi- or multi-modality. Given therefore that transformation of data did little to improve the data, that the relationships between outcome variable (*E. coli*) and predictors were broadly linear, and that the deviations from normality using non-transformed data did not appear exceptionally severe, raw data (not transformed data) were entered into regression modelling.

The above procedure allowed for four regression models. Model 1 is shown in Equation 4.4.

$$E.\ coli = \beta_0 + \beta_{1ph} + \beta_{2EC} + \beta_{3MC} + \beta_{4OM} + \beta_{5coliform} + \varepsilon \qquad \text{Equation 4.4}$$

This regression had 144 observations (the relatively small number was due to the relatively small number of OM counts).

However, *E. coli* are coliform bacteria. So it should be expected the coliform would be a highly significant predictor of *E. coli*. Accordingly, Model 2 was the same as Model 1, save that it omitted coliform as a predictor variable. This is shown in Equation 4.5.

$$E.\ coli = \beta_0 + \beta_{1ph} + \beta_{2EC} + \beta_{3MC} + \beta_{4OM} + \varepsilon \qquad \text{Equation 4.5}$$

However, Models 1 and 2, because they failed to use all observations, failed to optimally utilize the data. The problem lay with the small number of observations for OM. This was rectified in Model 3, which is shown in Equation 4.6.

$$E.\ coli = \beta_0 + \beta_{1ph} + \beta_{2EC} + \beta_{3MC} + \beta_{4coliform} + \varepsilon \quad \text{Equation 4.6}$$

This allowed for 480 observations. The same logic, however, that led to the exclusion of coliform from Model 2, could also be used to exclude it from Model 3. This led to Model 4, as shown in Equation 4.7.

$$E.\ coli = \beta_0 + \beta_{1ph} + \beta_{2EC} + \beta_{3MC} + \varepsilon \quad \text{Equation 4.7}$$

Finally, because of difficulty with including OM and NO_3^- in a regression model (see above), various attempts were made to include OM and NO_3^- into a model such that (a) only three predictor variables were included in the model, and (b) there were no serious violations (e.g., high VIFs) of regression assumptions. This procedure was unsatisfactory, but there seemed no alternative. After some trial and error, one arrived at Model 5, as shown in Equation 4.8.

$$E.\ coli = \beta_0 + \beta_{1MC} + \beta_{2NO3^-} + \beta_{3OM} + \varepsilon \quad \text{Equation 4.8}$$

Forced entry was used for all regressions.

The assumptions of regression were checked as follows:

Linearity—by curve analysis, as described above (also, partial plots as provided by SPSS). Normality—Kolmagorov–Smirnov and Shapiro–Wilk tests of standardized residuals (also, normal distribution plots as provided by SPSS).

Independence of errors—Durbin-Watson statistic (value in the range 1–3 are acceptable). Homoscedasticity—plot of standardized residuals against standardized predicted values should appear random; a funnel shape indicates heteroscedasticity.

Co-linearity—correlations between predictor variables should be no higher than 7 and no VIF should be higher than 10; the mean VIF should be lower than 2, and all VIFs should, ideally, be close to 1.

Alpha was set at $p = 0.01$ (a Bonferroni adjustment to allow for five separate tests) for each overall regression significance. Alpha was set at $p = 0.05$ for individual predictors within each model because its purpose was only to assess the relative importance of each significant predictor. This was assessed by observing by taking the square of the partial correlation ($r_{partial}^2$) for each predictor. This gives a rough (generally overestimate) of the variance explained by the predictor. If the regression model is valid, the sum of each significant $r_{partial}^2$ should broadly equal R^2 (Field, 2009). Finally, if a regression model is valid, R^2 should be broadly equal to $R_{adjusted}^2$ (Field, 2009).

4.3. Results

4.3.1. ANOVAs

4.3.1.1. SOIL PH

4.3.1.1.1. Descriptive statistics

Table 4.2 shows the mean soil pH for lowland and upland land types for grazing and ungrazed land use for the months October, November, April, and May. Data are from all samples taken in 2010 and 2011.

Table 4.2. Soil pH in lowland and upland under ungrazed and grazed conditions.

	Lowland		**Upland**	
Month	Grazed	Ungrazed	Grazed	Ungrazed
October	5.57 (± 0.05)	5.48 (± 0.06)	4.82 (± 0.11)	4.66 (± 0.09)
November	5.89 (± 0.05)	5.78 (± 0.06)	4.76 (± 0.11)	4.73 (± 0.08)
April	5.91 (± 0.03)	5.83 (± 0.07)	4.79 (± 0.08)	4.72 (± 0.12)
May	5.99 (± 0.03)	5.78 (± 0.06)	4.78 (± 0.07)	4.74 (± 0.12)

4.3.1.1.2. Inferential statistics

The 2 × 2 × 4 between measures ANOVA that tested influences on soil pH revealed a significant effect of land type, F (1, 464) = 662.06, $p < 0.0005$. The effect size was substantial, $\eta^2_p = 0.588$, suggesting that land type explained over 55% of the variance in pH. The ANOVA also revealed a significant effect of month, F (3, 464) = 5.173, $p = 0.002$.

The effect size, however, was small, $\eta^2_p = 0.032$, suggesting that month explained at most about 3% of the variance in pH. The ANOVA failed to detect a significant difference in land use; however, the result was close to significance, F (1, 464) = 6.121, $p = 0.014$. The effect size, if real, was very small, $\eta^2_p = 0.013$, suggesting that land use explained at most about 1% of the variance in pH.

Post-hoc tests (Tukey's HSD) revealed the only significant differences lay between October and November ($p = 0.025$), October and April ($p = 0.007$), and October and May ($p = 0.004$). The tests also revealed, surprisingly in view of the October differences, nothing close to a significant difference between November and the summer months (for April, $p = 0.973$; for May, $p = 0.928$), suggesting that, if anything, November pH was not significantly different from that of the summer months. Inspection of Table 4.2 shows that mean pH was lower in October than in the other three months.

The ANOVA revealed only one significant interaction, that between land type and month, F (3, 464) = 4.241, $p = 0.006$. The effect size, however, was small, $\eta^2_p = 0.027$, suggesting that the interaction explained only about 2% of the variance in pH. Figure 4.3 shows the interaction.

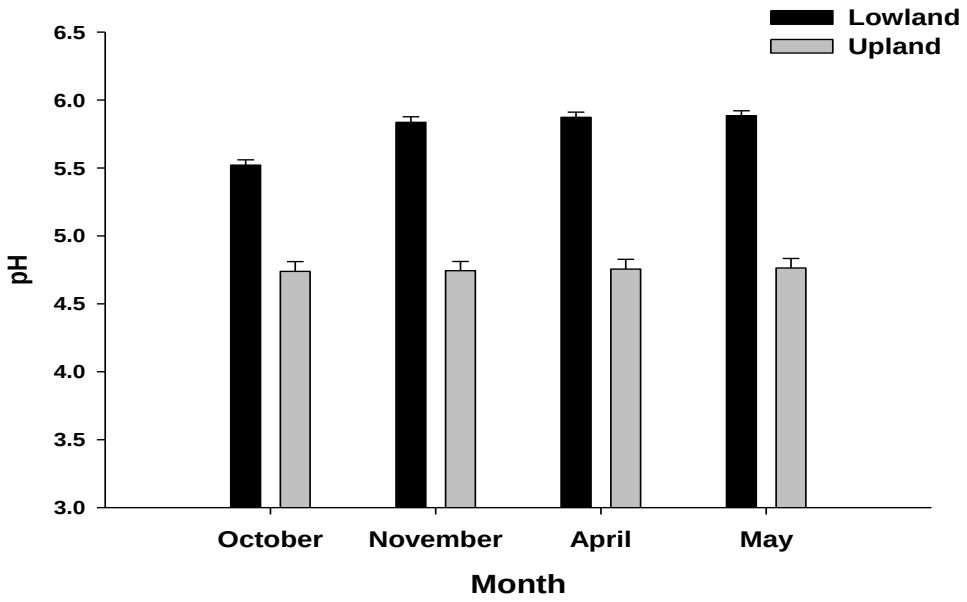

Figure 4.3. Interaction between land type and month for soil pH. Error bars denote standard error.

Overall, the ANOVA explained almost 60% of the variance in pH, $R^2 = 0.601$, $R^2_{adjusted} = 0.588$. The ANOVA violated assumption of equality of error variances (Levene's test, $p < 0.0005$). The pH distributions appeared normal, other than those for grazed (Shapiro–Wilk, $p = 0.002$) and ungrazed land (Shapiro–Wilk, $p = 0.001$; Kolmogorov results near-identical).

4.3.1.2. ELECTRICAL CONDUCTIVITY (EC)

4.3.1.2.1. Descriptive statistics

Table 4.3 shows the mean soil EC for lowland and upland land types for grazing and ungrazed land use for the months October, November, April, and May. Data are from all samples taken in 2010 and 2011.

Table 4.3. Soil EC (μS cm^{-1}) in lowland and upland under ungrazed and grazed conditions.

	Lowland		**Upland**	
Month	Grazed	Ungrazed	Grazed	Ungrazed
October	124.76 (± 4.68)	114.11 (± 3.51)	98.17 (± 3.79)	121.24 (± 4.84)
November	50.52 (± 1.93)	36.3 (± 2.12)	49.23 (±5.78)	53.8 (± 5.37)
April	36.68 (± 1.0)	36.2 (± 1.05)	23.77 (± 1.11)	35.07 (± 1.65)
May	37.01 (± 1.08)	36.55(± 1.01)	24.91 (± 0.87)	37.1 (± 1.62)

4.3.1.2.2. Inferential statistics

The 2 × 2 × 4 between measures ANOVA that tested influences on soil EC revealed a significant effect of month, F (3, 464) = 624.594, $p < 0.0005$. The effect size was substantial, $\eta^2_p = 0.804$, suggesting that month explained about 80% of the variance in EC. The ANOVA failed to detect a significant effect of land type; however, the result was close to significance, F (1, 464) = 5.430, $p = 0.02$. The effect size, if real, was small, $\eta^2_p = 0.014$, suggesting that land type explained around 1% of the variance in EC. The ANOVA failed to detect a significant difference in land use, but again the result was close to significance, F (1, 464) = 4.126, $p = 0.043$. The effect size, again if real, was very small, $\eta^2_p = 0.009$, suggesting that land use explained less than 1% of the variance in EC.

Post-hoc tests (Tukey's HSD) revealed the significant differences between October and November ($p < 0.0005$), October and April ($p < 0.0005$), October and May ($p < 0.0005$), November and April ($p < 0.0005$), and November and May, $p < 0.0005$).

Inspection of Table 4.3 shows that mean EC was highest in October, substantially lower in November, and lower still in April and May.

The ANOVA revealed a significant interaction between land type and land use, F (1, 464) = 38.245, $p < 0.0005$. The effect size was moderate, $\eta^2_p = 0.072$, suggesting that the interaction explained about 7% of the variance in EC. Figure 4.4 shows the interaction.

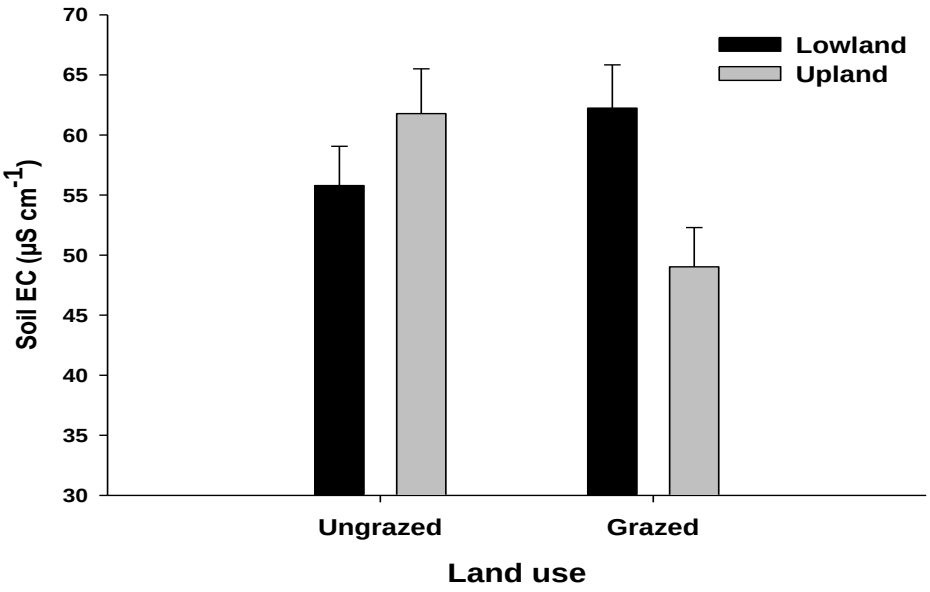

Figure 4.4 Interaction between land type and land use for EC. Error bars denote standard error.

The ANOVA revealed a significant interaction between land type and month, F (3, 464) = 6.605, $p < 0.0005$. The effect size was small, $\eta^2_p = 0.042$, suggesting that the interaction explained about 4% of the variance in EC. Figure 4.5 shows the interaction.

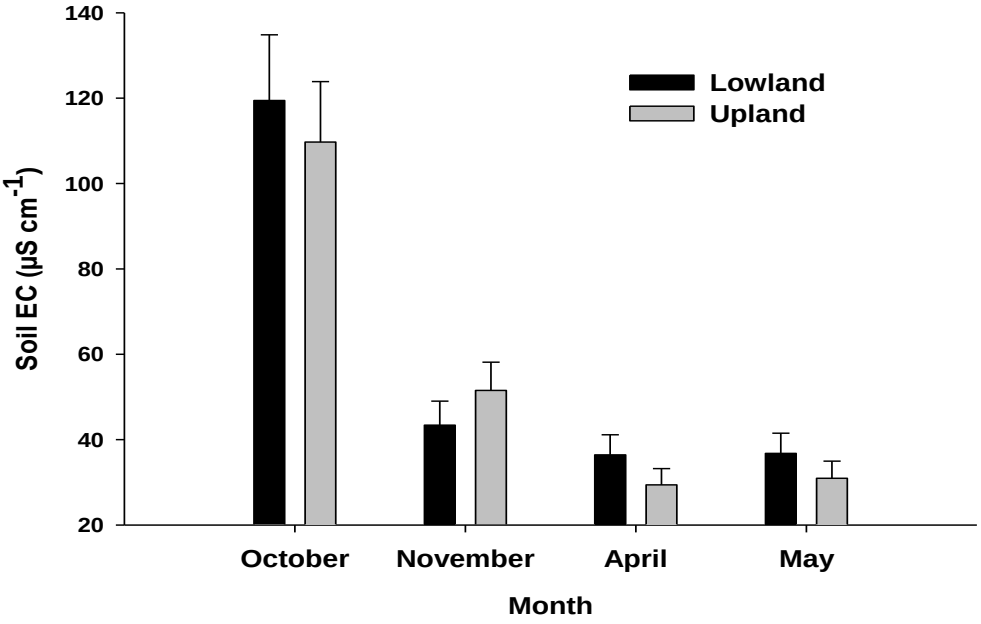

Figure 4.5. Interaction between land type and month for soil EC. Error bars denote standard error.

Both other tests of interaction were close to significant. That for land use and month provided F (3, 464) = 2.942, p = 0.033. The effect size was small, η^2_p = 0.019, suggesting that the interaction explained less than 2% of the variance in EC. That for land type, land use, and month provided F (3, 464) = 2.680, p = 0.046. The effect size was small, η^2_p = 0.017, suggesting that the interaction explained less than 2% of the variance in EC.

Overall, the ANOVA explained about 80% of the variance in EC, R^2 = 0.808, R^2_{adjusted} = 0.802. The ANOVA violated assumption of equality of error variances (Levene's test, $p < 0.0005$). The EC distributions appeared normal, other than those for grazing (Shapiro–Wilk, p = 0.004) and for the months November and May (Shapiro–Wilk, p = 0.001 in each case; Kolmogorov results near-identical).

4.3.1.3. SOIL MOISTURE CONTENT (MC).

4.3.1.3.1. Descriptive statistics

Table 4.4 shows the mean soil MC for lowland and upland land types for grazed and ungrazed land use for the months October, November, April, and May. Data are from all samples taken in 2010 and 2011.

Table 4.4. Soil MC in lowland and upland under ungrazed and grazed conditions

	Lowland		**Upland**	
Month	Grazed	Ungrazed	Grazed	Ungrazed
October	27.86 (± 0.39)	26.95 (± 0.27)	31.35 (± 0.41)	31.81 (± 0.44)
November	27.49 (± 0.31)	26.65 (± 0.28)	31.77 (± 0.38)	31.74 (± 0.33)
April	25.75 (± 0.52)	22.84 (± 0.32)	31.22 (± 0.31)	31.95 (± 0.46)
May	25.87 (± 0.51)	23.88 (± 0.29)	31.94 (± 0.31)	31.46 (± 0.35)

4.3.1.3.2. Inferential statistics

The 2 × 2 × 4 between measures ANOVA that tested influences on soil MC revealed a significant effect of land type, F (1, 464) = 936.081, $p < 0.0005$. The effect size was substantial, $\eta^2_p = 0.669$, suggesting that land type explained about 65% of the variance in MC. MC was higher in uplands than in lowlands (respective means: 28.78 and 25.91). The ANOVA revealed a significant effect of land use, F (1, 464) = 15.799, $p < 0.0005$. The effect size was small, $\eta^2_p = 0.033$, suggesting that land use explained about 3% of the variance in MC. The ANOVA revealed a significant effect of month, F (3, 464) = 17.539, p

< 0.0005. The effect size was moderate, $\eta^2_p = 0.102$, suggesting that month explained about 10% of the variance in MC.

Post-hoc tests (Tukey's HSD) revealed significant differences between October and April ($p < 0.0005$), October and May ($p < 0.0005$), November and April ($p < 0.0005$), and November, and May, $p < 0.0005$).

The ANOVA revealed a significant interaction between land type and land use, F (1, 464) = 23.790, $p < 0.0005$. The effect size small, $\eta^2_p = 0.049$, suggesting that the interaction explained less than 5% of the variance in MC. Figure 4.6 shows the interaction.

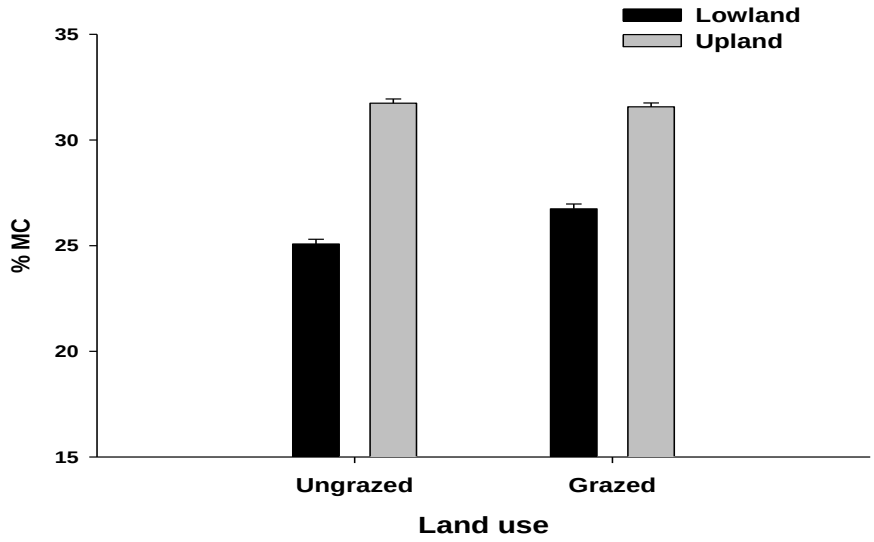

Figure 4.6. Interaction between land type and land use for soil MC. Error bars denote standard error.

The ANOVA revealed a significant interaction between land type and month, F (3, 464) = 16.934, $p < 0.0005$. The effect size was moderate, $\eta^2_p = 0.099$, suggesting that the interaction explained almost 10% of the variance in MC. Figure 4.7 shows the interaction.

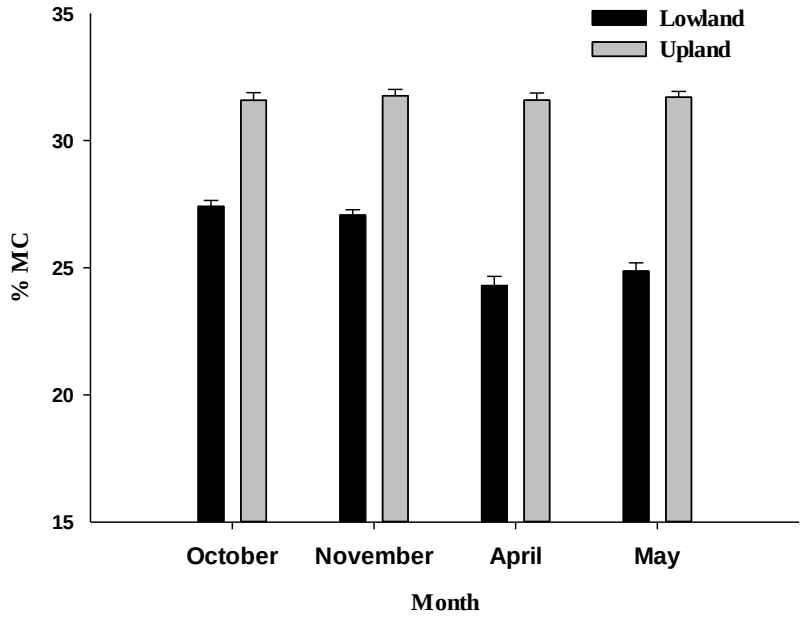

Figure 4.7. Interaction between land type and month for soil MC. Error bars denote standard error.

Both other tests of interaction were insignificant. However, that for land type, land use, and month was close to significance, F (3, 464) = 2.746, p = 0.043. The effect size, if real, was small, η^2_p = 0.017, suggesting that the interaction explained less than 2% of the variance in MC.

Overall, the ANOVA explained almost 70% of the variance in EC, R^2 = 0.702, R^2_{adjusted} = 0.692. The ANOVA violated assumption of equality of error variances (Levene's test, $p < 0.0005$). The MC distributions appeared normal.

4.3.1.4. *E. COLI*

4.3.1.4.1. Descriptive statistics

Figure 4.8 shows the log (base 10) of the mean soil *E. coli* CFU g^{-1} for lowland and upland land types for grazed and ungrazed land for the months October, November, April, and May. Data are from all samples taken in 2010 and 2011.

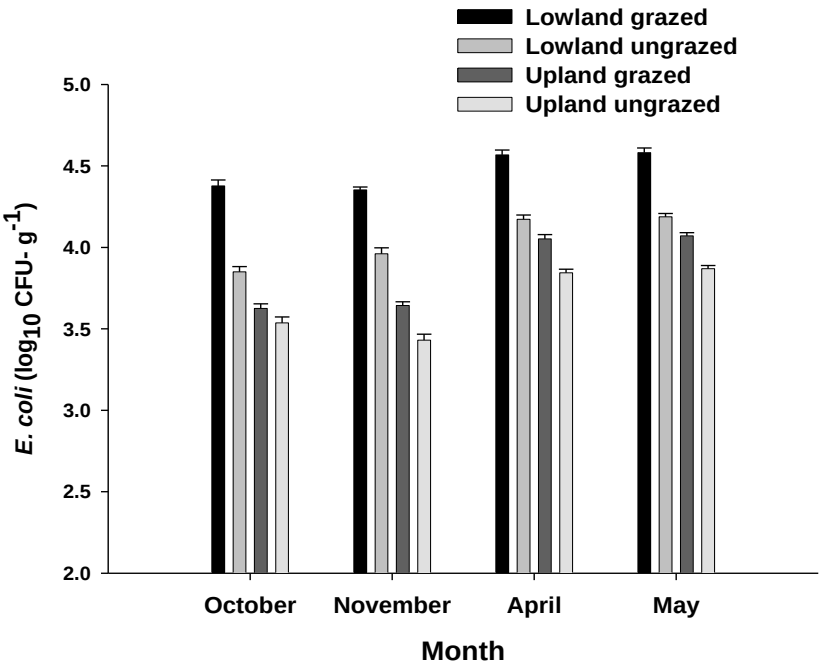

Figure 4.8. Mean soil *E. coli* concentrations (log DW). Error bars denote standard error.

For grazed lands, the mean number of colonies was 4.2 $\log_{10}$ CFU g^{-1} dry soil; for ungrazed lands it was 3.9 $\log_{10}$ CFU g^{-1} dry soil. For lowlands the mean number was 4.3 $\log_{10}$ CFU g^{-1} dry soil; for uplands it was 3.8 $\log_{10}$ CFU g^{-1} dry soil. The overall mean number of colonies for all land types and land uses was 4.0 $\log_{10}$ CFU g^{-1} dry soil. These figures suggest not only high numbers of *E. coli* colonies within the soils sampled, but also considerable variation according to land type and land use.

4.3.1.4.2. Inferential statistics

The 2 × 2 × 4 between measures ANOVA that tested influences on soil *E. coli* revealed a significant effect of land type, F (1, 464) = 709.929, $p < 0.0005$. The effect size was substantial, $\eta^2_p = 0.605$, suggesting that land type explained about 60% of the variance in *E. coli*. The ANOVA revealed a significant effect of land use, F (1, 464) = 380.288, $p < 0.0005$. The effect size was substantial, $\eta^2_p = 0.450$, suggesting that land use explained over 40% of the variance in *E. coli*. The ANOVA revealed a significant effect of month, F (1, 464) = 76.969, $p < 0.0005$. The effect size was substantial, $\eta^2_p = 0.332$, suggesting that over 30% of the variance in *E. coli* was explained by month. Inspection of the figure reveals that *E. coli* were, as indicated, more common in grazed than in ungrazed land, during the summer months, and in lowland than in upland.

Post-hoc tests (Tukey's HSD) revealed significant differences between October and April ($p < 0.0005$), October and May ($p < 0.0005$), November and April ($p < 0.0005$), and November and May, $p < 0.0005$). Inspection of the figure shows that mean *E. coli* was lowest in October and November, and highest in April and May.

The ANOVA revealed a significant interaction between land type and land use, F (1, 464) = 214.194, $p < 0.0005$. The effect size was substantial, $\eta^2_p = 0.316$, suggesting that the interaction explained about 30% of the variance in *E. coli* (Figure 4.9).

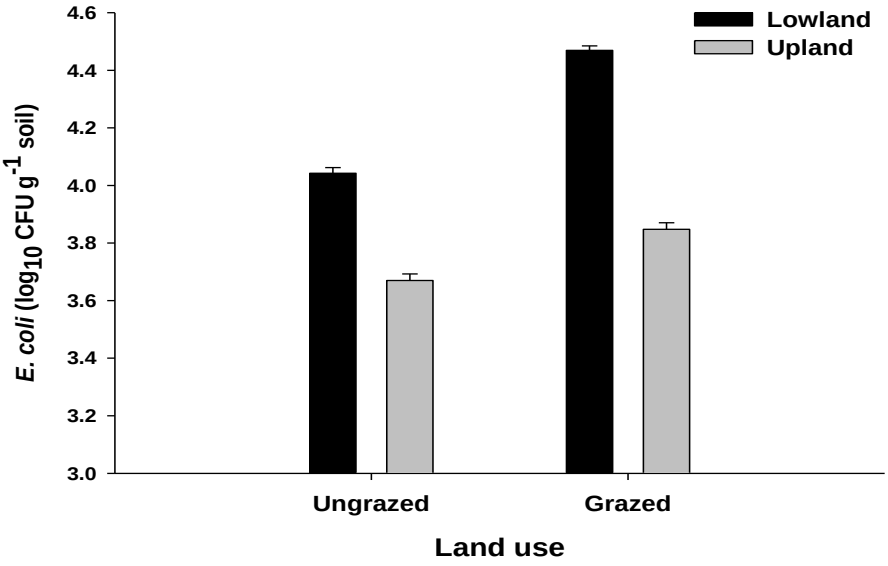

Figure 4.9. Interaction between land type and land use for *E. coli*. Error bars denote standard error.

The ANOVA revealed a significant interaction between land type and month, F (3, 464) = 9.258, $p < 0.0005$. The effect size was small, $\eta^2_p = 0.056$, suggesting that the interaction explained about 5% of the variance in *E. coli* (Figure 4.10).

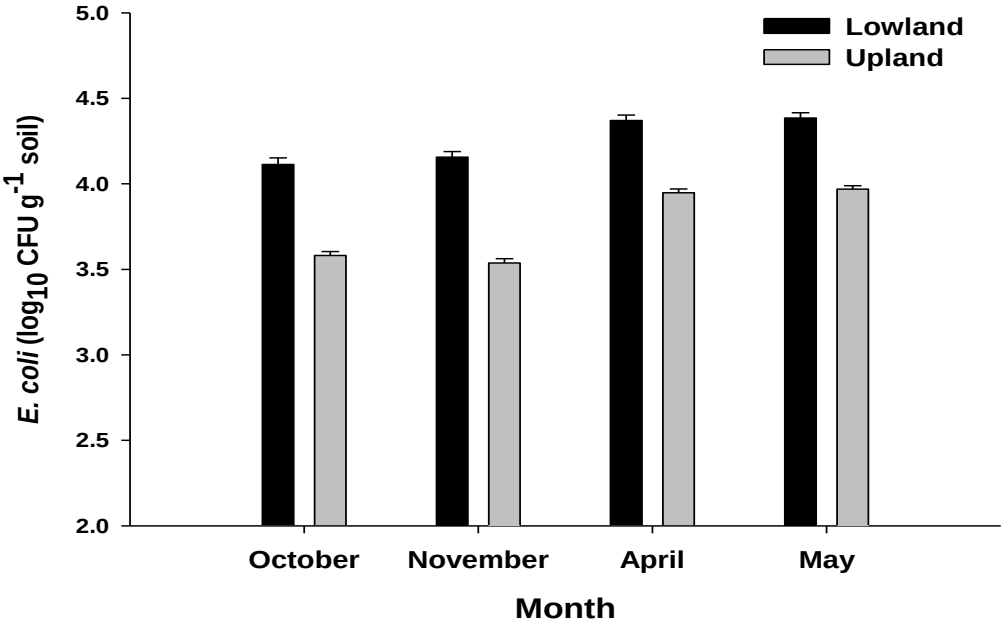

Figure 4.10. Interaction between land type and month for *E. coli*. Error bars denote standard error.

The ANOVA revealed a significant interaction for land use and month, F (3, 464) = 11.02, p <0.0005. The effect size was small, η^2_p = 0.067, suggesting that the interaction explained about 6% of the variance in *E. coli* (Figure 4.11).

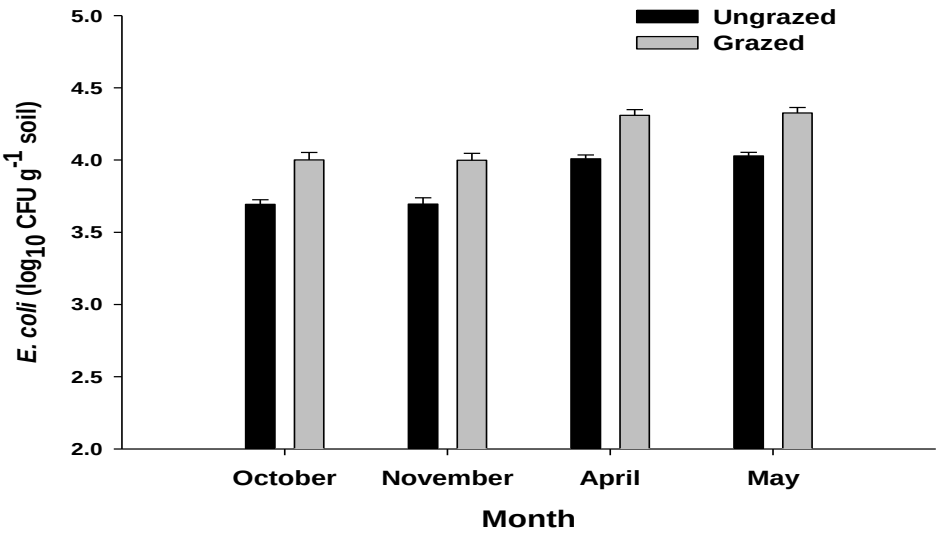

Figure 4.11. Interaction between land use and month for *E. coli*. Error bars denote standard error.

The ANOVA failed to reveal a significant interaction between land type, land use, and month; however, the result was close to significance, F (3, 464) = 2.986, p = 0.031. The effect size, if real, was small, η^2_p = 0.067, suggesting that the interaction explained less than 2% of the variance in *E. coli*.

Overall, the ANOVA explained about 75% of the variance in *E. coli*, R^2 = 0.776, R^2_{adjusted} = 0.769. The ANOVA violated assumption of equality of error variances (Levene's test, $p < 0.0005$). The *E. coli* distributions appeared normal, save for upland and November (Shapiro–Wilk, p = 0.001 in each case; Kolmogorov–Smirnov results were similar).

4.3.1.5. COLIFORM BACTERIA

4.3.1.5.1. Descriptive statistics

Figure 4.12 shows the log (base 10) of the mean soil coliform bacteria CFU g^{-1} for lowland and upland land types for grazed and ungrazed land for the months October, November, April, and May. Data are from all samples taken in 2010 and 2011.

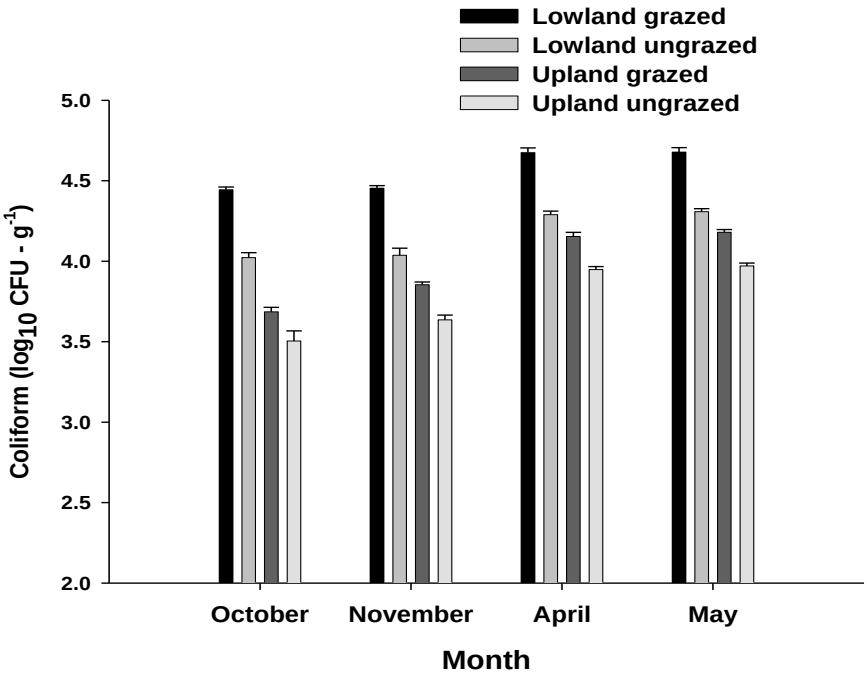

Figure 4.12. Mean soil coliform bacteria concentrations. Error bars denote standard error.

For grazed lands, the mean number of colonies was 4.3 $\log_{10}$ CFU g^{-1} dry soil; for ungrazed lands it was 4.0 $\log_{10}$ CFU g^{-1} dry soil. For lowlands the mean number was 4.4 $\log_{10}$ CFU g^{-1} dry soil; for uplands it was 3.9 $\log_{10}$ CFU g^{-1} dry soil. The overall mean number of colonies for all land types and land uses was 4.1 $\log_{10}$ CFU g^{-1} dry soil. These figures suggest, not only high numbers of coliforms within the soils sampled, but also, as with *E. coli*, considerable variation according to land type and land use.

From these figures, coupled with *E. coli* colony counts, it is possible to estimate the percentage of *E. coli* within coliform colonies. Table 4.5 shows the percentage of *E. coli* within coliforms for all types of land type and land use.

Table 4.5. Percentage of *E. coli* colonies within coliform colonies.

	Grazed (%)	**Ungrazed (%)**	**Total (%)**
Lowland	80.5	76.3	79.3
Upland	77.0	80.3	78.3
Total (%)	79.8	77.5	79.0

As is clear from the table, coliform colonies within North Wales comprise just short of 80% *E. coli* colonies, and this percentage does not substantially differ, regardless of land type and land use.

4.3.1.5.2. Inferential statistics

The 2 × 2 × 4 between measures ANOVA that tested influences on soil coliform bacteria revealed a significant effect of land type, F (1, 464) = 707.279, $p < 0.0005$. The effect size was substantial, $\eta^2_p = 0.604$, suggesting that land type explained about 60% of the variance in coliform bacteria. The ANOVA revealed a significant effect of land use, F (1, 464) = 360.885, $p < 0.0005$. The effect size was substantial, $\eta^2_p = 0.437$, suggesting that land use explained over 40% of the variance in coliform bacteria. The ANOVA revealed a significant effect of month, use, F (1, 464) = 81.509, $p < 0.0005$. The effect size was substantial, $\eta^2_p = 0.345$, suggesting that over 30% of the variance in coliform bacteria was explained by month. Inspection of figure reveals that coliform bacteria were, as indicated,

more common in grazed than in ungrazed land, during the summer months, and in lowland than in upland.

Post-hoc tests (Tukey's HSD) revealed significant differences between October and April ($p < 0.0005$), October and May ($p < 0.0005$), November and April ($p < 0.0005$), and November and May ($p < 0.0005$). Inspection of the figure shows that mean coliform bacteria was lowest in October, November and highest in April and May.

The ANOVA revealed a significant interaction between land type and land use, $F(1, 464) = 183.990$, $p < 0.0005$. The effect size was substantial, $\eta^2_p = 0.284$, suggesting that the interaction explained over 25% of the variance in coliform bacteria (Figure 4.13).

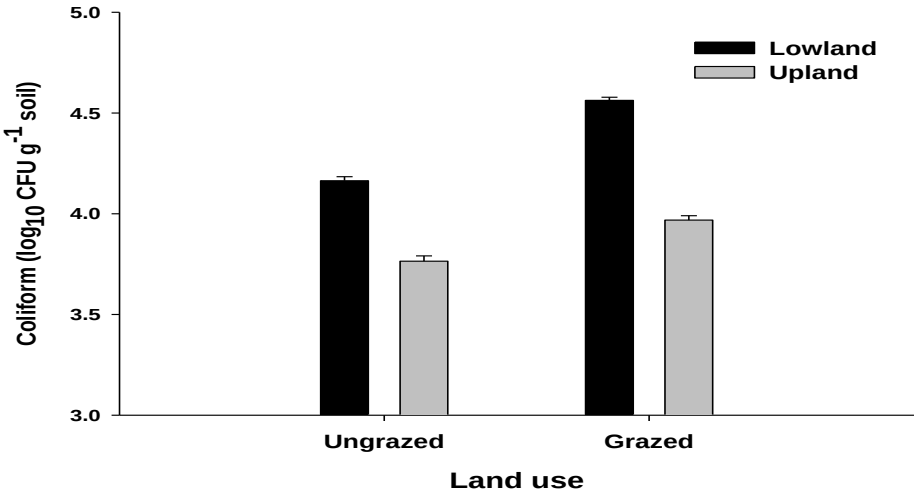

Figure 4.13. Interaction between land use and land type for coliform bacteria. Error bars denote standard error.

The figure suggests that the difference between lowland and upland areas was greater in grazed than in ungrazed lands.

The ANOVA revealed a significant interaction between land type and month, F (3, 464) = 11.308, $p < 0.0005$. The effect size was small, $\eta^2_p = 0.068$, suggesting that the interaction explained about 6% of the variance in coliform bacteria (Figure 4.14).

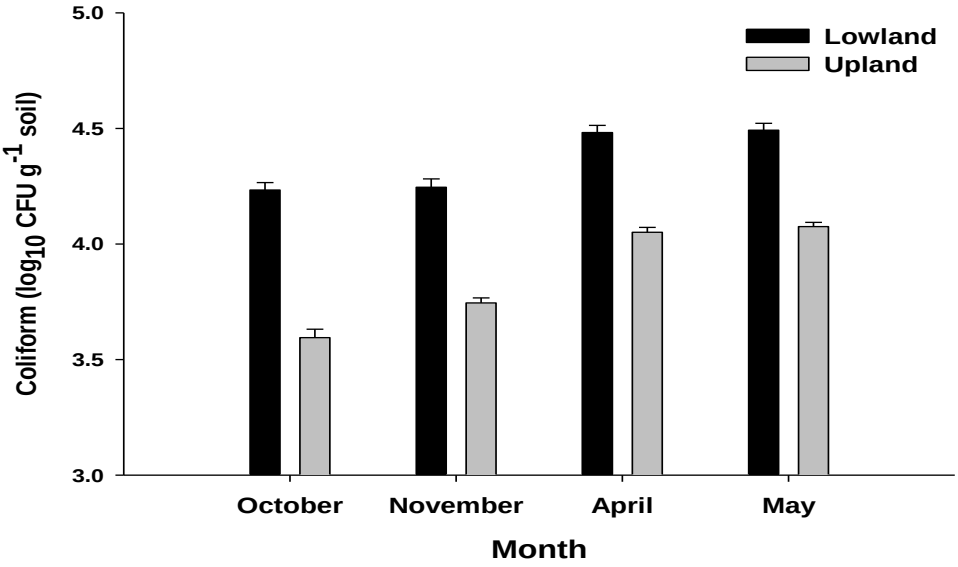

Figure 4.14. Interaction between land type and month for coliform bacteria. Error bars denote standard error.

The figure suggests the difference between lowland and upland counts of coliform bacteria was greatest during the summer months.

The ANOVA revealed a significant interaction for land use and month F (3, 464) = 11.02, $p < 0.0005$. The effect size was small, $\eta^2_p = 0.067$, suggesting that the interaction explained about 6% of the variance in coliform bacteria (Figure 4.15).

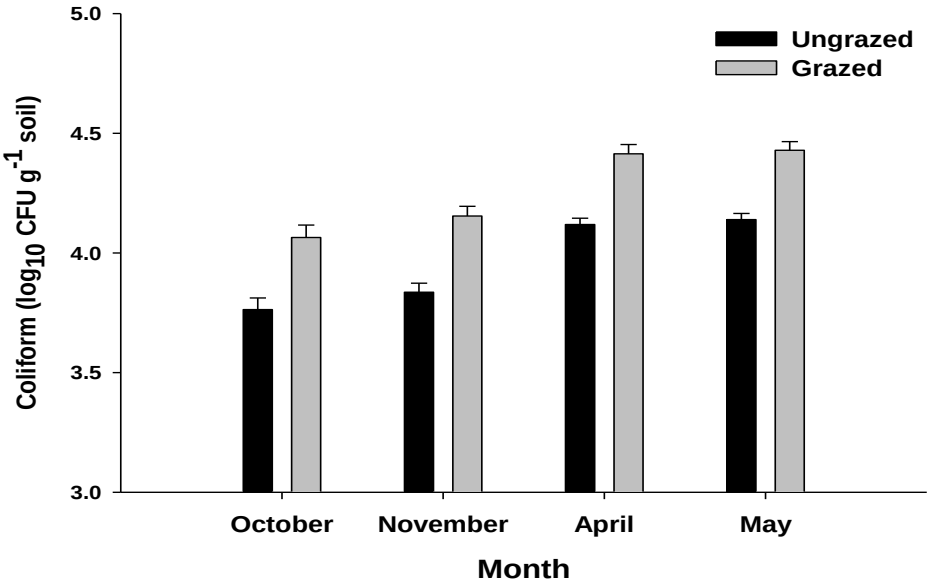

Figure 4.15. Interaction between land use and month for coliform bacteria. Error bars denote standard error.

The ANOVA failed to reveal a significant interaction between land type, land use, and month; however, the result was close to significance, F (3, 464) = 3.716, p = 0.012. The effect size, if real, was small, η^2_p = 0.023, suggesting that the interaction explained about 2% of the variance in coliform bacteria.

Overall, the ANOVA explained about 75% of the variance in coliform bacteria, R^2 = 0.773, R^2_{adjusted} = 0.765. The ANOVA violated assumption of equality of error variances (Levene's test, $p < 0.0005$). The coliform bacteria distributions appeared normal, save for upland (Shapiro–Wilk, p = 0.001), ungrazed (Shapiro–Wilk, p = 0.022), and November (Shapiro–Wilk, $p < 0.0005$) (Kolmogorov–Smirnov results were similar).

4.3.1.6. SOIL NITRATE

4.3.1.6.1. Descriptive statistics

Figure 4.16 shows the mean soil nitrate for lowland and upland land types for grazed and ungrazed land for winter and summer. Data are from all samples taken in 2010 and 2011.

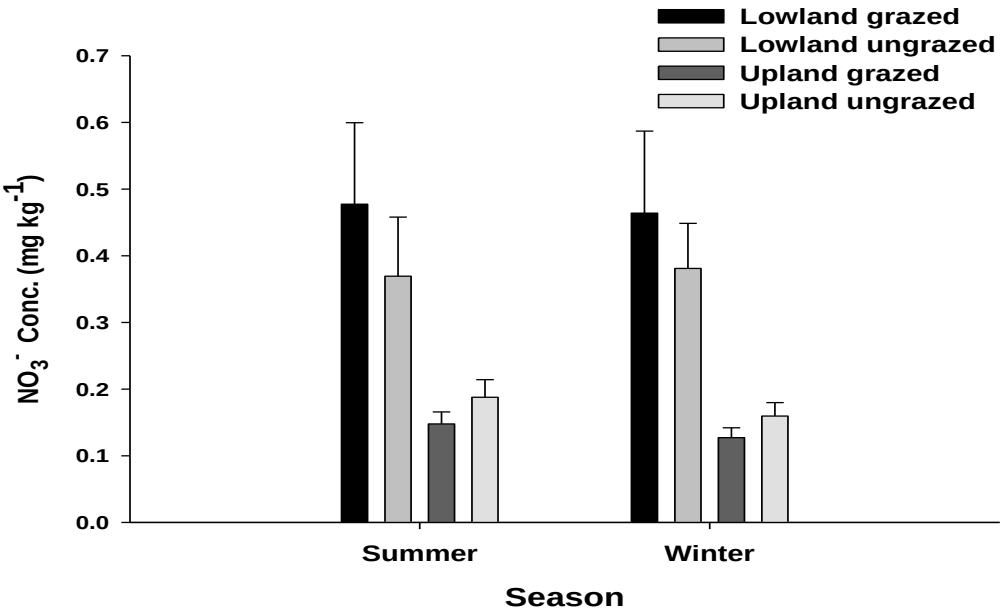

Figure 4.16. Mean soil NO_3^- concentrations. Error bars denote standard error.

4.3.1.6.2. Inferential statistics

The 2 × 2 × 2 between measures ANOVA that tested influences on soil nitrates revealed a significant effect of land type, F (1, 40) = 25.914, $p < 0.0005$. The effect size was substantial, $\eta^2_p = 0.393$, suggesting that land type explained more than 35% of the variance in nitrates. Thus lowland genuinely appears to have higher levels of nitrates than

has upland. The ANOVA revealed no other significant differences and no significant interactions.

Overall, the ANOVA explained about 30% of the variance in nitrates, $R^2 = 0.411$, $R^2_{\text{adjusted}} = 0.308$. The ANOVA violated assumption of equality of error variances (Levene's test, $p = 0.001$). The nitrate distributions appeared non-normal (lowland, Shapiro–Wilk: $p = 0.015$; grazing and no grazing, Shapiro–Wilk: each $p < 0.0005$; summer and winter: Shapiro–Wilk: each $p < 0.0005$), save for upland. The large discrepancy between R^2 and R^2_{adjusted} also suggests data seriously violated ANOVA assumptions.

4.3.1.7. AMMONIUM

4.3.1.7.1. Descriptive statistics

Figure 4.17 shows the mean soil ammonium for lowland and upland land types for grazed and ungrazed land for winter and summer. Data are from all samples taken in 2010 and 2011.

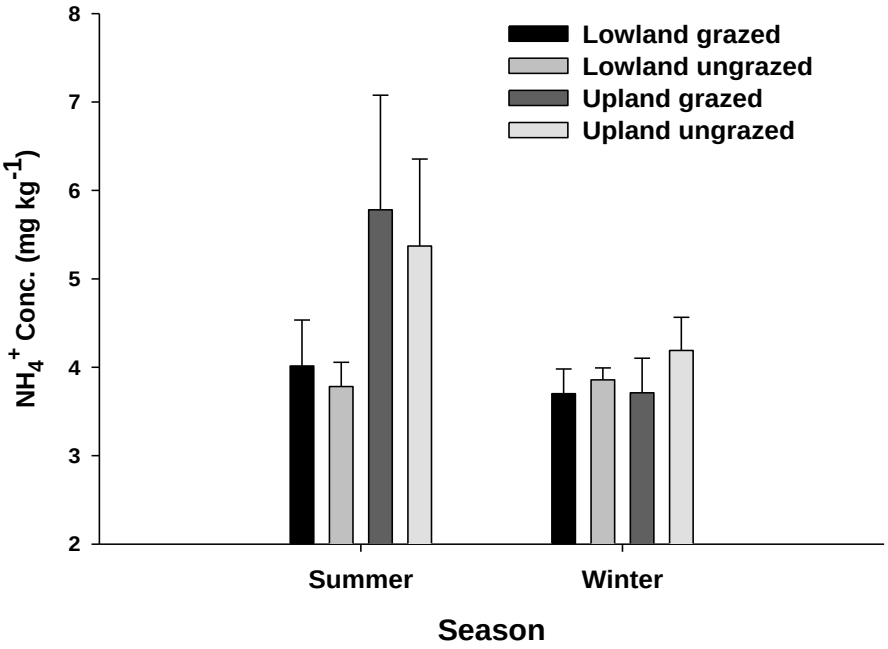

Figure 4.17. Mean soil NH_4^+ concentrations. Error bars denote standard error.

4.3.1.7.2. Inferential statistics

The 2 × 2 × 2 between measures ANOVA that tested influences on soil NH_4^+revealed no significant differences and no significant interactions. The ANOVA violated assumption of equality of error variances (Levene's test, p = 0.003). The Ammonium distributions appeared non-normal (upland, Shapiro–Wilk: $p < 0.0005$; grazing and no grazing, Shapiro–Wilk: each $p < 0.0005$; summer: Shapiro–Wilk: p = 0.001), save for lowland and winter.

4.3.1.8. SOIL ORGANIC MATTER AND TOTAL CARBON CONTENT IN SOIL

4.3.1.8.1. Descriptive statistics

Figure 4.18 shows the mean soil OM for lowland and upland land types for grazed and ungrazed land for winter and summer. Data are from all samples taken in 2010 and 2011.

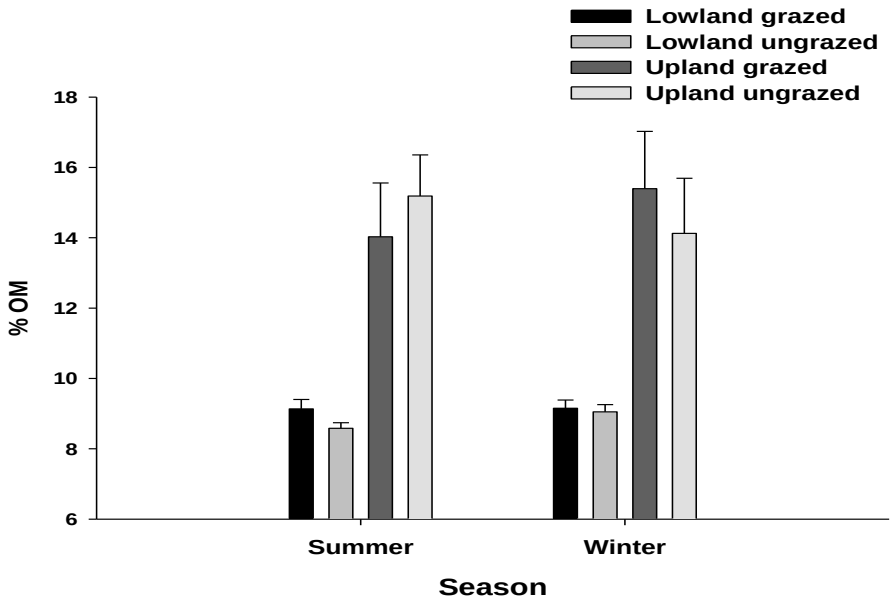

Figure 4.18. Mean soil OM concentrations. Error bars denote one standard error.

4.3.1.8.2. Inferential statistics

The 2 × 2 × 2 between measures ANOVA that tested influences on soil OM revealed a significant effect of land type, F (1, 136) = 119.630, $p < 0.0005$. The effect size was substantial, $\eta^2_p = 0.468$, suggesting that land type explained more than 45% of the variance in soil OM. The ANOVA revealed no other significant differences and no significant interactions.

Overall, the ANOVA explained more than 40% of the variance in soil OM, R^2 = 0.473, $R^2_{adjusted}$ = 0.446. The ANOVA violated assumption of equality of error variances (Levene's test, $p < 0.0005$). The OM land use and season distributions appeared non-normal (grazing and ungrazed, Shapiro–Wilk: each $p < 0.0005$; winter and summer, Shapiro–Wilk: each $p < 0.0005$).

4.3.1.9. MAXIMUM WATER HOLDING CAPACITY (MWHC)

The mean soil MWHC was higher in upland than in lowland regions. For upland regions it was 72%; for lowland regions it was 39%. Results of the present study suggest the soil OM was higher in upland than in lowland areas. Also sandy soils have a low water-holding capacity due to the lack of small pore space.

4.3.1.10. SUMMARY

Table 4.6 summarizes the significant results of the ANOVAs.

Table 4.6. Summary of ANOVA results.

ANOVA	Land type (sig/effect size)		Land use (sig/effect size)		Month/season (sig/effect size)		Land type × land use (sig/effect size)		Land type × month/season (sig/effect size)		Land use × month/season (sig/effect size)		Comments
pH	Sig.	> 50%			Sig.	< 5%			Sig.	< 5%			pH lower in uplands
EC					Sig.	> 70%	Sig.	5–10%	Sig.	< 5%			EC very high in October. Low in summer; intermediate in November.
MC	Sig.	> 60%	Sig.	< 5%	Sig.	5–10%	Sig.	< 5%	Sig.	5–10%			Greater MC in upland
E. coli	Sig.	> 50%	Sig.	> 40%	Sig.	20–30%	Sig.	< 5%	Sig.	5–10%			More *E. coli* in lowland than upland, in grazed than ungrazed, and summer than winter.
Coliform	Sig.	> 50%	Sig.	> 40%	Sig.	> 30%	Sig. 20–30%		Sig.	5–10%	Sig.	5–10%	More coliform bacteria in lowland than upland, in grazed than ungrazed lands, and summer than winter.
NO_3^-	Sig.	> 30%											More NO_3^- in lowland than upland.
OM	Sig.	> 40%											More OM in upland than lowland.

As regards the main subject of the present study, prevalence of *E. coli*, the ANOVA results are instructive. All three IVs not only had significant main effects; they also had large associated effect sizes. The same was true for the ANOVA on coliform bacteria.

All other DVs studied by the ANOVAs, with the exception of NH_4^+, also showed at least one significant main effect, and in each case at least one effect size was substantial. This leaves open the question of whether the differences in *E. coli* and other coliform bacteria populations were due to land type differences, land use differences, seasonal differences, as suggested by the two ANOVAs on the bacterial populations, or whether such differences reflect differences in the physical and chemical properties of the soils, as reflected by the three IVs. This question cannot be answered by ANOVA. It can be answered, but only in part, by correlations.

4.3.2. Correlation

Table 4.7 shows the Summary of correlation (Pearson) matrix for all DVs in the ANOVA models.

Table 4.7. Pearson correlation matrix.

		E. coli	coliform	pH	EC	MC	NO_3^-	NH_4^+	OM
E. coli	Pearson Correlation	1	0.984**	0.505**	–0.169**	–0.419**	0.598**	–0.032	–0.349**
	Sig. (2-tailed)		0.000	0.000	0.000	0.000	0.000	0.830	0.000
	n	480	480	480	480	480	48	48	144
Coliform	Pearson Correlation	0.984**	1	0.508**	–0.195**	–0.430**	0.550**	–0.031	–0.348**
	Sig. (2-tailed)	0.000		0.000	0.000	0.000	0.000	0.834	0.000
	n	480	480	480	480	480	48	48	144
pH	Pearson Correlation	0.505**	0.508**	1	–0.158**	–0.630**	0.581**	–0.343*	–0.622**
	Sig. (2-tailed)	0.000	0.000		0.001	0.000	0.000	0.017	0.000
	n	480	480	480	480	480	48	48	144
EC	Pearson Correlation	–0.169**	–0.195**	–0.158**	1	0.085	0.352*	–0.277	–0.074
	Sig. (2-tailed)	0.000	0.000	0.001		0.063	0.014	0.057	0.377
	n	480	480	480	480	480	48	48	144
MC	Pearson Correlation	–0.419**	–0.430**	–0.630**	0.085	1	–0.584**	0.179	0.583**
	Sig. (2-tailed)	0.000	0.000	0.000	0.063		0.000	0.222	0.000
	n	480	480	480	480	480	48	48	144

NO_3^-	Pearson Correlation	0.598**	0.550**	0.581**	0.352*	–0.584**	1	–0.016	–0.673**
	Sig. (2-tailed)	0.000	0.000	0.000	0.014	0.000		0.912	0.002
	n	48	48	48	48	48	49	48	18
NH_4^+	Pearson Correlation	–0.032	–0.031	–0.343*	–0.277	0.179	–0.016	1	0.214
	Sig. (2-tailed)	0.830	0.834	0.017	0.057	0.222	0.912		0.394
	n	48	48	48	48	48	48	48	18
OM	Pearson Correlation	–0.349**	–0.348**	–0.622**	–0.074	0.583**	–0.673**	0.214	1
	Sig. (2-tailed)	0.000	0.000	0.000	0.377	0.000	0.002	0.394	
	n	144	144	144	144	144	18	18	144

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 4.8 shows the Summary of equivalent Spearman correlations.

Table 4.8. Spearman correlation matrix.

			E. coli	coliform	pH	EC	MC	NO_3^-	NH_4^+	OM
Spearman's rho	*E. coli*	Correlation Coefficient	1.000	0.971**	0.568**	–0.172**	–0.517**	0.593**	0.038	–0.442**
		Sig. (2–tailed)	.	0.000	0.000	0.000	0.000	0.000	0.796	0.000
		n	480	480	480	480	480	48	48	144
	coliform	Correlation Coefficient	0.971**	1.000	0.576**	–0.189**	–0.533**	0.503**	0.060	–0.446**
		Sig. (2–tailed)	0.000	.	0.000	0.000	0.000	0.000	0.688	0.000
		n	480	480	480	480	480	48	48	144
	pH	Correlation Coefficient	0.568**	0.576**	1.000	–0.151**	–0.627**	0.568**	–0.257	–0.604**
		Sig. (2–tailed)	0.000	0.000	.	0.001	0.000	0.000	0.077	0.000
		n	480	480	480	480	480	48	48	144
	EC	Correlation Coefficient	–0.172**	–0.189**	–0.151**	1.000	–0.044	0.445**	–0.200	–0.149
		Sig. (2–tailed)	0.000	0.000	0.001	.	0.333	0.002	0.173	0.075
		n	480	480	480	480	480	48	48	144
	MC	Correlation Coefficient	–0.517**	–0.533**	–0.627**	–0.044	1.000	–0.612**	0.113	0.637**
		Sig. (2–tailed)	0.000	0.000	0.000	0.333	.	0.000	0.443	0.000
		n	480	480	480	480	480	48	48	144

NO_3^-	Correlation Coefficient	0.593**	0.503**	0.568**	0.445**	–0.612**	1.000	0.162	–0.767**
	Sig. (2–tailed)	0.000	0.000	0.000	0.002	0.000	.	0.272	0.000
	n	48	48	48	48	48	49	48	18
NH_4^+	Correlation Coefficient	0.038	0.060	–0.257	–0.200	0.113	0.162	1.000	0.104
	Sig. (2–tailed)	0.796	0.688	0.077	0.173	0.443	0.272	.	0.681
	n	48	48	48	48	48	48	48	18
OM	Correlation Coefficient	–0.442**	–0.446**	–0.604**	–0.149	0.637**	–0.767**	0.104	1.000
	Sig. (2–tailed)	0.000	0.000	0.000	0.075	0.000	0.000	0.681	.
	n	144	144	144	144	144	18	18	144

Comparison of the two tables shows the correlations are broadly the same, lending some confidence to the Pearson correlations. The most important part of Table 4.7 concerns the correlates with *E. coli*. All variables other than NH_4^+ correlated highly significantly ($p <$ 0.0005) with *E. coli*. In the cases of EC, MC, and OM, the significant correlations were negative. In all other cases, they were positive. The correlation between *E. coli* and coliform is near perfect.

Very few of the correlations in the matrix are insignificant. The only insignificant ones are all those regarding NH_4^+, and EC regarding MC, NO_3^-, and OM. However, with the exception of that between *E. coli* and coliform, no correlation is higher than 0.7. Nonetheless, two of the variables, pH and NO_3^-, were very highly correlated (absolute $r >$. 5) with most other variables, and, with the exception of EC, most other significant correlation coefficients were modest to high (r: 5 < > 3). This suggests non-trivial effect sizes.

Many of the correlations in the matrix are negative. The mixture of negative and positive correlations, coupled with the large number of significant relationships and the non-trivial effect sizes, suggests complex soil chemistry in the regions examined in the present study.

4.3.3. Regression

4.3.3.1. MODEL 1

The model was highly significant, F (5, 138) = 1206.51, $p < 0.0005$, and explained over 95% of the variance in *E. coli*, $R^2 = 0.978$, $R^2_{\text{adjusted}} = 0.977$. Errors appeared

independent (Durbin–Watson = 1.115). There was little evidence of co-linearity; all correlations between predictor variables were less than 7; the VIFs for predictor variables were pH, 2.489; EC, 1.215; coliform, 1.561; MC, 1.960).

Only two predictor variables were significantly associated with *E. coli*: MC (p = 0.009) and coliform ($p < 0.0005$). The respective partial correlations were -0.220, yielding an effect size of 0.048, or less than 5%, and 0.983, yielding an effect size of 0.966, or greater than 90%.

There was violation of some regression assumptions. Standardized residuals were not normally distributed (Kolmogorov–Smirnov, p = 0.015; Shapiro–Wilk, $p < 0.0005$). Graphical output suggested slight bi-modality and heteroscedasticity. There were two outliers, but each was less than five *SD*s from the mean. Broadly, however, the output suggested roughly symmetrical distribution; the distribution did not therefore appear grossly non-normal.

Overall, therefore, although the data violated three of regression's assumptions, in no instance did the violations appear particularly serious. This conclusion is reinforced by comparison of the values of R^2 and R^2_{adjusted}, which are nearly identical. The explained variance from the partial correlations also broadly matches the values of R^2 and R^2_{adjusted}, lending further confidence in the model.

4.3.3.2. MODEL 2

The model was highly significant, F (4, 139) = 17,567 $p < 0.0005$, and explained about 30% of the variance in *E. coli*, R^2 = 0.336, R^2_{adjusted} = 0.317. Errors appeared related

(Durbin–Watson = 0.579). There was little evidence of co-linearity; all correlations between predictor variables were less than 7; the VIFs for predictor variables were pH, 2.199; EC, 1.063; MC, 1916; OM 1.847.

Only two predictor variables were significantly associated with *E. coli*: pH (p = 0.001) and EC ($p < 0.0005$). The respective partial correlations were 0.289, yielding an effect size of 0.084, or around 8%, and –0.324, yielding an effect size of 0.103, or around 10%.

There was violation of some regression assumptions. Standardized residuals were not normally distributed (Kolmogorov–Smirnov, $p < 0.0005$; Shapiro–Wilk, $p < 0.0005$). Graphical output suggested slight multi-modality. There were four outliers, but each was less than five *SD*s from the mean. Broadly, however, the output suggested roughly symmetrical distribution; the distribution did not therefore appear grossly non-normal.

Comparison of the values of R^2 and R^2_{adjusted}, which are close, suggests the model is sound. However, the explained variance from the partial correlations is grossly different from that explained by R^2. This, coupled with the violation of independence of errors, casts doubt on Model 2.

4.3.3.3. MODEL 3

The model was highly significant, F (4, 476) = 3802.78, $p < 0.0005$, and explained over 95% of the variance in *E. coli*, R^2 = 0.970, R^2_{adjusted} = 0.969. Errors appeared independent (Durbin–Watson = 1.60). There was little evidence of co-linearity; all

correlations between predictor variables were less than 7; the VIFs for predictor variables were pH, 2.88; EC, 1.04; coliform, 1.41; MC, 1.7-8.

Only two predictor variables were significantly associated with *E. coli*: EC (p = 0.002) and coliform ($p < 0.0005$). The respective partial correlations were .139, yielding an effect size of 0.019, or less than 2%, and 0.979, yielding an effect size of 0.958, or greater than 90%.

There was violation of some regression assumptions. Standardized residuals were not normally distributed (Kolmogorov–Smirnov, $p = 0.003$; Shapiro–Wilk, $p < 0.0005$). Graphical output suggested possible heteroscedasticity. There were nine outliers, two of which were greater than four *SD*s from the mean and one of which was greater than eight. Broadly, however, the output suggested roughly symmetrical distribution; the distribution did not therefore appear grossly non-normal.

The model seemed broadly valid. Comparison of the values of R^2 and R^2_{adjusted}, were very close. Also, the explained variance from the partial correlations accorded with that explained by R^2.

4.3.3.4. MODEL 4

The model was highly significant, F (3, 476) = 61.754 $p < 0.0005$, and explained over 25% of the variance in *E. coli*, $R^2 = 0.280$, $R^2_{\text{adjusted}} = 0.276$. Errors appeared related (Durbin–Watson = 0.398.). There was little evidence of co-linearity; all correlations between predictor variables were less than 0.7; the VIFs for predictor variables were pH, 1.689; EC, 1.026; MC, 1.649.

All predictor variables were significantly associated with *E. coli*: pH ($p < .0005$); EC ($p = 0.017$); MC ($p = 0.001$). The respective partial correlations were 0.328, yielding an effect size of 0.108, or around 10%, –0.109, yielding an effect size of .011, or around 1%, and –0.153, yielding an effect size of 0.023, or around 2%.

There was violation of some regression assumptions. Standardized residuals were not normally distributed (Kolmogorov–Smirnov, $p < 0.0005$; Shapiro–Wilk, $p < 0.0005$). Graphical output suggested multi-modality and heteroscadasticity. There were 13 outliers, but each was less than five *SD*s from the mean.

Comparison of the values of R^2 and $R^2_{adjusted}$, which are close, suggests the model is sound. However, the explained variance from the partial correlations is grossly different from that explained by R^2. This, coupled with the violation of independence of errors, evidence of multi-modality, and heteroscadasticity, casts doubt on Model 4.

4.3.3.5. MODEL 5

The model was highly significant, F (3, 14) = 11.402, $p < 0.0005$, and explained over 60% of the variance in *E. coli*, $R^2 = 0.710$, $R^2_{adjusted} = 0.647$. Errors appeared independent (Durbin–Watson = 1.626). There was some evidence of co-linearity; although all correlations between predictor variables were less than 7; the VIFs for predictor variables were MC, 2.244; NO_3^-, 1.957; OM, 2.542.

The only predictor variable significantly associated with *E. coli* was OM ($p = 0.043$). The respective partial correlation was –0.510, yielding an effect size of 0.2601, or around 25%.

There was violation of regression assumptions. Standardized residuals were not normally distributed (Shapiro–Wilk, $p < 0.0005$). Graphical output suggested slight bi-modality. There were no outliers.

Comparison of the values of R^2 and R^2_{adjusted}, which are close, suggests the model is sound. However, the explained variance from the partial correlations is grossly different from that explained by R^2. This casts doubt on Model 5.

4.4. Discussion

The present study had two main objectives: (a) to relate coliform bacteria and *E. coli* soil populations to differences in land type, land use, and season, together with different chemical properties of the soil; and (b) to determine which factors to be the best predictors of *E. coli* populations. Realising the first of the objectives was attempted by using ANOVAs, the second by correlation and multiple regression.

4.4.1. ANOVA results

Factor 1: pH: Johnston *et al*. (1971), in a Canadian study, report increased grazing led to an increase in soil pH from 5.7 to 6.2. Against this, Dormaar and Williams (1998), in an analysis of soil samples from the foothills of south-western Alberta, found that soil pH increased minimally, from 5.7 to 5.8, when grazing increased from light to moderate. The authors speculate that the reason for this is the higher levels of ammonium in grazing animal faeces and urine—a conclusion shared by Kamaruzaman (1988).

As regards the results of the present study, the low pH of upland is expected, given the results of the higher levels of rainfall in Welsh mountains. Rainwater is acidic, and the

rainwater over Snowdonia, like that of other mountainous region of the UK, typically has a pH of less than 5.5 (Martin and Barber, 1978; and see Helliwell *et al*., 2007). Furthermore, the soil type found in the uplands is known to be more acidic than the soil type found in the lowlands (Helliwell *et al*., 2007).

It is of note here that, although uplands were more acidic than lowlands, neither land type was especially acidic the difference between a pH of 5.26 and 5.78 might be statistically significant, but may be small in terms of *E. coli* and other coliform bacteria survival. *E. coli* have been shown to survive at much lower pH than those found in the present study; such as in apple cider (pH < 4) (Zhao *et al.*, 1993; and see Rangel *et al*., 2005) and salami (pH 4.6) (Clavero and Beuchat, 1996). Therefore, although Estrada *et al*. (2004) argue that soil of pH 6 < > 8 tend to have negative effects on bacteria, such effects may not pertain to *E. coli*.

Factor 2 EC: The lack of any significant difference as regards EC and land use, as found in the present study, accords with the literature. Lavado and Alconada's (1994) Argentinean study, for example, found no differences in EC between grazed and ungrazed lands.

Factor 3 MC: Land use made a trivial (about 3% of the explained variance)—though nonetheless significant—impact on MC, with grazed land having slightly higher MC than had ungrazed land. This result is inconsistent with that of Al-Seekh *et al*. (2009), who, in a Palestinian study, found higher MC in ungrazed land. The result that soil MC was higher in upland areas than in lowland may be explained by the higher precipitation over

upland areas and by the peaty soil characteristics of the upland sites, with the greater levels of organic matter helping to retain moisture.

Factor 4 E. coli and coliforms: The higher population of *E. coli* in grazed land is consistent with results from other studies (Bardgett *et al*., 1993, 1997, 2001). Bardgett *et al*. (2001) also found that incidence of bacteria in soils was reduced when sheep were removed from the lands. This is likely to be explained by the fact that sheep faeces contain copious bacteria; therefore faecal shedding may increase soil populations of bacteria.

The result that *E. coli* are more populous in lowlands than in uplands is open to two obvious explanations. First, as indicated, all the uplands in the present study were sloped. The slopes may have facilitated drainage from the slopes into lowland regions (see, Haggard *et al*., 2002). There is also, of course, higher livestock densities in lowland versus upland areas.

The result that *E. coli* populations are higher in the summer months is consistent with the findings of Fernandez (2008) and Van-Donsel *et al*. (1967), that faecal coliform contamination is (much) greater in summer than in autumn.

The only substantial finding as regards coliform bacteria colonies was the high percentage of *E. coli* colonies within them. This appeared to be just under 80%, regardless of land type and land use and indicates that in North Wales soils, *E. coli* are by far the most populous species within these coliform colonies.

Factor 6 Soil nitrates: Results suggested that the only factor that affects soil nitrate levels is whether the land is grazed or ungrazed, with grazed land having higher levels. This result is counter to that of some other research. Lavado *et al*. (1996), for instance, found

grazed land was associated with lower levels of nitrates. However, other research suggests little impact of grazing on nitrate levels. Al-Seekh *et al*. (2009), for instance, found no impact of grazing. Indeed, their results suggested considerable variation by year. Also note that animal urine and faeces contain nitrates, so it is possible that grazing animals contribute to nitrate levels.

Factor 7 Ammonium: Analyses of data suggested no differences in ammonium levels according to land type, land use, or season. This result accords with that of Al-Seekh *et al*. (2009), who found that yearly changes due to grazing regime were more important than differences in land use.

Factor 8 Soil OM: The only significant factor as regards soil OM was land type, with soil OM being higher (almost double) in upland than in lowland. Other research suggests that soil OM generally increases with higher clay content (Nichols, 1984), with lower mean annual temperature (Jenny, 1980), and higher mean annual rainfall (Burke *et al.*, 1989). Other research also shows that soil OM is generally more prevalent in uplands than in lowlands (e.g., Bot and Benites, 2005). Some other research also suggests that soil OM is more prevalent in ungrazed than grazed lands (e.g., Johnston *et al*., 1971; Dormaar *et al*., 1977; Naeth *et al*., 1990), yet the present study failed to detect any influence of grazing on soil OM. Note that the present study also found that the mean soil MWHC was higher in upland areas than in lowland ones (respective values 72% and 39%). Soil texture and organic matter are the key components in the determination of soil water holding capacity, and, as indicated, soil OM improves MWHC (Leu *et al.*, 2010).

The depletion in soil OM associated with grazing reported in other studies may arise, not from grazing per se, but from overgrazing (Bot and Benites, 2005). Sheep numbers and stocking densities have reduced considerably over the last decade in Wales, with overgrazing by far less common (AP Williams, personal communication). Against this, some evidence suggests that even light grazing has a negative impact on soil OM. Smoliak (1965), for example, found light grazing negatively impacted on the dry matter surface and underground plant matter. A possible reason for the discrepancy between results of the present study and others lies in the different soil types and rainfall patterns, in North Wales.

4.4.2. Correlation and regression

The negative correlation between *E. coli* and soil OM ($r = –0.349$) appears curious, as one would expect the bacteria to thrive in environments rich in organic matter. However, Fratamico *et al*. (2002) argued that non-pathogenic *E. coli* may act as symbionts in that they may compete with pathogenic *E. coli* for resources. Regression analyses suggest that the survival of *E. coli* within soil broadly follows that of other coliform bacteria. This may be useful in predicting *E. coli* populations from simple counts of coliform populations.

4.4.3. Limitations and suggestions for future research

There are two obvious limitations to the present study. First, due to logistical issues and the large number of soil samples that would need to be collected, the study did not directly investigate pathogenic *E. coli*. Thus, for example, although it is plausible that pathogenic *E. coli* are more common in lowlands than in uplands, and that they are more common in soils rich in other forms of coliform bacteria, the present study has not

demonstrated this. Second, the study was restricted to North Wales. How much its results may be generalised to other locations is therefore uncertain, due to the effects of soil type, climate, and other variables that might affect coliform populations in soil. Such questions may be answered by larger studies that also include more soil types, grazing patterns, and diverse climatic factors.

4.5. Conclusion

Active grazing leads to elevated levels of *E. coli* and coliforms populations in soils. However, the study has shown that even when livestock are omitted from grazing for extended periods of time, can still be found within soil. Further research is needed to identify the physico-chemical properties of soils that govern the persistence of *E. coli* populations.

CHAPTER 5: EFFECTS OF GRAZING REGIME ON SOIL PROPERTIES

5.1. Introduction

In the UK, a considerable proportion of agricultural land is managed for livestock (cattle and sheep) grazing. The grazing of animals on pasture can affect the soil, water, and general environment of the grassland either positively or negatively (Núñez *et al.*, 2007; Hubbard *et al.*, 2012). The most obvious effects of grazing management are on forage production; it follows that grazing systems should be designed to optimally satisfy the food demands of livestock. Thus one of the challenges of agricultural management is to design a grazing system that allows for maximal utilisation of animal resources yet has minimal adverse effects (e.g., soil compaction, microbial contamination) on the environment (Núñez *et al.*, 2007).

5.1.1. Grass versus forage crops for grazing

The approach, style, and success of a grazing system depend on many factors. These include land configuration, type of livestock, capital resources, and the producer's goals (Thornel *et al.*, 2007). In an effort to reduce costs and improve returns from gazing, an increasing amount of farmers are moving from continuous grazing on grassland towards growing forage crops for grazing. This system is also being adopted the numerous farmers that have joined agri-environment schemes (see below).

Grazing animals experience an annual cycle of body conditions. These conditions depend on the availability of forage. During winter in temperate regions, weight loss by gazing animals is associated with the dormancy period of plant foodstuffs (Wallis-DeVries, 1998). During this period, intensive sheep grazing on

winter forage crops of the brassica family is common (Houlbrooke *et al.*, 2009). Kale (*Brassica oleracea*) and swede (*Brassica napus*) are two common winter forage crops; they are suitable for animals when there is shortage of pasture. Kale is cold-tolerant, highly palatable, high in protein content (15–17%); and is suitable for rotational grazing as late season forage. Swede is a slow growing, long-season plant; it is especially suitable for grazing during late autumn (Lemus, 2009). Fodder beet (*mangel beet*) and stubble turnip (*Brassica rapa*) are also used, but to a lesser extent than swede and kale.

Brassica crops are particularly suitable due to their high production per unit area allowing for a dense stocking density therefore greater productivity (Figure 5.1). Rotational grazing includes short-term winter forage cropping practices that follow a return to pasture as part of an on-going pasture renewal program (Pottinger *et al.*, 1993).

Figure 5.1. Sheep grazing swede and kale during the winter.

http://www.fao.org/ag/AGP/AGPC/doc/Counprof/Ireland/plates/plate8.htm

5.1.4. Effects of grazing management system on soil quality

The short- and long-term impacts of grazing with winter forage crops on soil quality, along with the effects of livestock type, have not, to date, been much studied (Houlbrooke *et al.*, 2009). Research suggests grazing animals affect a soil's physical properties, including its bulk density, compaction, and infiltration, and that grazing animals contribute to soil erosion through hoof actions (Bell, 2010); as evidenced in a rotational grazing system in New Zealand with a high stocking densities (200–500 sheep per hectare) (Greenwood and McNamara, 1992). Treading displaces top soil and affects surface runoff and water infiltration capacity. Such processes increase the potential for increased water runoff and sediment contamination (Lambert *et al.*, 1985). One study (Bakker, 1989) found that, in two sandy soils under different grazing intensities, soil compaction in heavily grazed areas was higher (11.5 ± 2.3 kg cm^{-2}) than in lightly grazed areas (5.5 ± 0.7 kg cm^{-2}).

Nitrogen is arguably the single most important element in soil. Grazing management practice may affect N losses from surface soil to subsurface water (e.g., Madramootoo *et al.*, 1992; Saner *et al.*, 2000; Stout *et al.*, 2000). Research also suggests vegetative filter strips may control soil erosion, and filter nutrient elements, sediments, and other pollutants by reducing surface runoff (Gharahaghi *et al.*, 2001, Koelsch *et al.*, 2006). The impact of animal grazing on the bacteriological quality of soils can be assessed by measuring faecal coliforms (Hubbard *et al.*, 2004); it is known that changes in grazing management can substantially modify the abundance, diversity, and spatial distribution of soil micro-flora (Hutchinson and King, 1980). Also, sheep grazing can influence the fungal biomass of surface soils with consequent impact on the microbial decomposition of organic matter and on nutrient dynamics (Bardgett, 1991). Grazing

can also be a source of faecal organisms such as *E. coli*; however, the extent of contamination in different grazing systems used for sheep is not clear. This was hypothesized that grazing regime especially intensive grazing system affect soil physical and chemical characteristics in addition to soil microbial property.

The present study therefore investigates the effects of intensive grazing system with two different crop types (swede and kale) on the physical, chemical, and microbial properties of soil on a farm in North Wales.

5.2. Materials and methods

5.2.1. Experimental site

The experimental site was Henfaes Research Station of Bangor University, as described in the previous chapter. The landscape comprises plain lands between the mountain range of the Snowdonia National Park to the East and the Irish Sea to the West. The site includes silvo-pastural land, semi-natural managed grassland, and agricultural experimental plots. The soil type is clay–loam.

The main experimental site was one hectare in area. For the purposes of the present study, it was divided into two equal half hectare areas. Each half hectare area had four control plots, each measuring 2 × 2 m and fenced to restrict grazing by livestock. In one half hectare all plots were planted with swede; in the other all were planted with kale (Figure 5.2).

Figure 5.2. Control area with plots (swede and kale)

Sheep were free to graze between swede and kale areas (i.e., the two areas were not fenced off from each other).

5.2.2. Setup of grazing regime

Both forage cops were first planted on 14th July, 2011. Grazing within the one hectare area commenced on 15th February 2012. The crops were grazed by 160 sheep (all mature Welsh Mountain ewes), equal to a stocking density of 160 / ha. One month later (from 15th March, 2012), the sheep were also allowed access to graze grass within an adjacent one hectare area; thus reducing grazing intensity.

5.2.3. Collection of soil samples

For coliform and *E. coli* counts and measurements of soil NH_4^+, NO_3^-, P, pH, MC, soil respiration, and EC, the procedure was as follows.

Soil samples were taken on 1st, 15th and 30th of March 2012. Three samples were collected from each plot within the one hectare area; three samples were also collected

from the area adjacent to each plot. Thus for each sampling date, 8 × 3 × 2 = 48 samples were taken, 24 from the plots and 24 from the areas adjacent to them. All samples from outside each plot came from the one hectare area as shown in Figure 5.2. Each sample (30-40 g) was taken to a depth of approximately 5 cm (as measured by a spatula). Core samples (0–5 cm) were used for analysis of bulk density, air filled porosity, and MC. These were collected at the same dates and sites as those for coliform and *E. coli* counts and measurements of soil NH_4^+, NO_3^-, pH, MC, and EC, with three replicates; this rendered the total number of core samples as 144 (48 × 3). Note here that the core samples, unlike the other samples, were not combined.

Each sample was immediately placed in a plastic bag, labelled, and sealed; each was transferred to the laboratory within three hours of collection and placed in a refrigerator (4°C) until further analysis (and see below: Section 5.2.6. soil analytical procedures).

5.2.4. Collection of faeces samples

Faecal samples were collected on 24th March, 2012. Twenty four samples were taken from the one hectare crop grazing area and a further 24 samples were taken from the adjacent one hectare grassland site which sheep had also been grazing.

Each sample comprised about 15 g of fresh faeces. Samples were taken with a small spoon and immediately placed in a plastic bag and labelled and sealed. All samples were then taken to the laboratory, where their ash content was measured (see below: Section 5.2.7.1).

5.2.5. Collection of samples for soil erosion

Samples were collected on 17th April, 2012. One sample was measurement from each of the experimental plots (kale and swede) and one sample was collected from an adjacent area (approx. 2 m) to each of them, rendering a total of 4 × 2 × 2 = 16 samples. The procedure for each was as follows:

First, a pit was dug in the soil approximating to the size of a plastic bowl (maximum radius approx. 5 cm, tapering to approx. 3 cm at base; depth approx. 5 cm). Care was taken to place the bowl such that there was no interference with the crop growing within the plot; hence, depending of the location of the crop within the plot, some pits were dug at the edges of the plot, and some in the middle. The bowl was placed in the pit such that its rim was level with the top of the surrounding soil surface. The bowl was left for 24 hours to allow the runoff water to collect during rainfall (data from the weather station at Henfaes Research Station showed that average precipitation for March–April was 0.8 mm per day). The collected runoff water was then transferred into a screw-top plastic bottle (250 ml) and immediately sealed (with the screw-top), then transferred to the laboratory within 2 hours and placed in a refrigerator (4ºC).

5.2.6. Soil analytical procedures

Soil analytical procedures for chemical and physical properties (pH, EC, MC, NH_4^+, and NO_3^-) and microbial counts (*E. coli* and coliform) were the same as those described in Chapter 4 (Section 4.2.3).

5.2.6.1. SOIL PHOSPHORUS

P in the soil samples was determined by colorimetrically using ascorbic acid. According to this method, 5 g soil was mixed with 25 ml of 0.5M of acetic acid

(CH_3CO_2H). Only 48 (16 × 3) samples were available. The soil solution was shaken for 30 min in a horizontal shaker (SM25) at 200 rev min^{-1} and then centrifuged (Eppendorf 5810 R) at 4000 rev min^{-1} for 5 min. Filtrate was collected using 125 mm filter paper (Fisher Brand FB 59249). The clean extract was collected and stored at 4°C in a refrigerator before analysis. The P concentration in the soil extract was analysed using a spectrometer (Zen5, BioTek).

5.2.6.2. BULK DENSITY

The bulk density of the soil was determined using a stainless steel core sampler (volume: 100 cm^{-3}). Samples were collected from undisturbed level ground using the core sampler. After removing extra soil and protruding roots, the content was transferred to a pre-weighed aluminium tray and dried at 105ºC in an oven to a constant weight; the oven dry mass of each sample was then recorded. The volume of the core was measured accordingly.

Because the soils contained some stones (> 2 mm diameter), the soil was washed on a 2 mm sieve. All stones were collected and their total mass was recorded after drying. Soil bulk density was calculated after correction for stones, using the formula in Equation 5.1.

$$BD\left(\frac{g}{cm^{-3}}\right) = \frac{Ms}{Vc} = \frac{Ms + r - Mr}{Vs + r - Vr} \qquad \text{Equation 5.1}$$

Where

BD = Bulk density of the soil (g m^{-3})

Ms = Oven dry Mass of only soil taken with core sampler (g)

Vc = Volume of the only soil in the core (cm^{-3})

M s+r = Dry Mass of the soil sample with rocks taken with core sampler (g)

V s+r = Volume the core (soil with rocks) (g)

Mr = Mass of the rocks (g)

Vr = Volume of the rocks

5.2.6.3. AIR FILLED POROSITY

The air-filled porosity of each soil sample was calculated from the soil's bulk density and the volume of soil moisture, using the formula in Equation 5.2 (Wollersheim *et al.*, 1987).

$$fa = \left(1 - \frac{Pb}{Pp}\right) - Ov \qquad \text{Equation 5.2}$$

Where

fa = air filled porosity.

Pb = Bulk density of soil.

Pp = Particle density of soil (most mineral soil it is 2.65).

Ov = Volume of moisture in soil.

5.2.6.4. INFILTRATION

Measurements were taken for 3 times on 4th and 21st March and 2nd of April, 2012. Infiltration rates were determined by using a single ring infiltrometer. This included a heavy duty (3 mm thickness of ring wall) cylindrical plastic ring (length: 15 cm; diameter: 5 cm). The cylinder was carefully inserted into the soil to a depth of 5 cm, using a wooden hammer; this was to ensure that no water was lost laterally. Exactly 150 ml of water was poured into the cylinder and the volume of the water measured at zero time. After 10 minutes the depth of water (cm) in the ring was recorded using a plastic

scale. The infiltration rate was calculated from the amount of water leached through the soil.

5.2.6.5. SOIL PENETRATION RESISTANCE

Soil penetration measurements were done on 18th April, 2012. Five replicate measurements were taken from inside (ungrazed) and outside (grazed) of both kale and swede plots. This rendered a total of 80 measurements (4 × 2 × 5 × 2). Soil penetration was measured using a hand penetrometer (Eijelkamp, Gliesback, Netherlands). Cone Number 1 (base area: 1 cm^2; diameter: 11.28 mm; rejection diameter: 11.00 mm) was used for each measurement; this allows for testing to a depth of 50 cm without augering.

Care was exercised when taking measurements to avoid "forcing" the penetrometer through stones or other objects that might damage the instrument or in other ways provide inaccurate records. When stones or similar materials were encountered, an adjacent spot was used as an alternative.

The penetrometer provides a manometer reading from a dial positioned at its apex. Cone resistance was then calculated in terms of cone resistance, as measured by Newton cm^{-2}, using Equation 5.3.

$$Coneresistance = \frac{manometerreading}{baseareaofcone} \qquad \text{Equation 5.3}$$

5.2.6.6. SOIL EROSION

To determine soil erosion, runoff samples were collected from the field in plastic bottles as described earlier (Section 5.2.5). The volume and weight of the runoff water in the bottle was measured. The bottle was placed on a horizontal shaker (SM25) for 30 min at 200 rev min^{-1}.The sample was then left to stand at room temperature for

approximately 15 min. Soil erosion was estimated by measuring turbidity, dry solid content, volume of water, and soil nutrient elements (P, NO_3^-, and NH_4^+) as described previously.

5.2.6.7. TURBIDITY

The turbidity of the sample was determined using a portable turbidity meter (TN-100/T-100—Oakton Instruments, IL, USA/Eutech Instruments Pte Ltd., Singapore). The meter was calibrated using four calibration standard solution, provided by the manufacturer with turbidity of 800, 100, 20.0 and 0.02 Nephelometric Turbidity Units(NTU). Continuous measurement of turbidity was done following the standard procedure (Oakton, 2003); which basically compares the scattering of light.

5.2.6.8. DRY SOLIDS

Measurement used a porcelain crucible (approx. radius: 2.5 cm; approx. depth: 3.cm). The empty crucible was first weighed; then the soil and water mixture was placed within it. The filled crucible was then weighed again. The crucible, complete with soil and water mixture, was then placed in an oven and dried overnight at 105ºC. The crucible, complete with dried soil sample, was then weighed again.

The weight of the dry solids was known, as were the weight of the empty bottle and the weight of the bottle filled with the sample (solids and water). Therefore the mass of the water was that shown in Equation 5.4.

$$Ww = Wfb - Web - Ws \qquad \text{Equation 5.4}$$

Where

Ww = weight of water

Wfb = weight of filled bottle

Web = weight of empty bottle

Ws = weight of dry solids

From this, one calculated the volume of water using Equation 5.5.

$$Vw = \frac{Mw}{Dw} \qquad \text{Equation 5.5}$$

Where

Vw = volume of water

Mw = Ww (on Earth mass and density are, for practical purposes, the same).

Dw = density of water at room temperature (20ºC) = 0.998203 gcm^{-3}

5.2.6.9. AMMONIUM AND NITRATES

The bottle, with contents, was placed in an SM25 shaker and shaken for 30 min at 200 rev min^{-1}. The sample was then left to stand at room temperature for approximately10 min. A 1.5 ml aliquot was then centrifuged in an Eppendorf 5810 R centrifuge at 18,000*g* for 5 min. Thereafter, the procedure followed that described in Chapter 4 (Section 4.2.3.4).

5.2.6.10. SOIL RESPIRATION

Soil respiration was determined by placing the sample in an automated multichannel infrared gas analyser in the laboratory (PP-systems Ltd., Hitchin, UK) for 24 hours. In this method, 10 g of sieved (2 mm) soil sample was put into a clear 50 ml centrifuge tube and connected with channel plugs. The analyser works continuously in 12-stage cycles, each stage pertaining to a chamber in the analyser. The 12^{th} stage is the most accurate, therefore only the recording of the last (after the 24 hours period)

completed 12 cycle was used, Soil respiration was expressed in nmole h^{-1} and calculated by using Equation 5.6.

$$SR = \frac{CR \times 69.12 \times 360}{10^6}$$ Equation 5.6

Where

SR = soil respiration/nanomolecule/chamber h^{-1}.

CR = CO_2 respiration/molecule/chamber S^{-1}.

5.2.7. Faeces analytic procedure

5.2.7.1. ASH CONTENT

Faecal samples were dried at 80ºC overnight in an electric oven. The ash content of the dried faeces was determined by ashing the samples at 600°C for 48 hours in a muffle furnace (Carbolite, Sheffield, UK) (Curll and Wilkins, 1983) and using the formula in Equation 5.7.

$$Ash\% = \frac{AW}{DW} \times 100$$ Equation 5.7

Where

AW = Ash weight

DW= dry weight

5.3. Statistical analysis

All statistical calculations were performed using IBM SPSS Statistics (Version 19). Analyses first comprised a series of 12 × 2 × 2 mixed measures ANOVAs. The IVs comprised date of sampling (within measures: three dates), crop type (between measures: two crops, kale or swede), and ground type (between measures: two ground

types, grazed or ungrazed). The DVs were soil coliform and soil *E. coli* counts; soil levels of NH_4^+, NO_3^-, pH, P, MC, and EC; and soil levels of bulk density, air filled porosity, infiltration, and soil respiration. The DVs were correlated and their variances were grossly unequal, a serious violation of MANOVA assumptions. Therefore ANOVAs were used in this experiment.

Data for many tests violated assumptions of ANOVA (see Results, below). Attempts to rectify this by logarithmic transformation failed. Accordingly, raw data were used for all 12 ANOVAs. In cases in which parametric assumptions were violated, results were checked, where possible, using equivalent non-parametric tests. In cases in which sphericity was violated, multivariate analysis was used.

Because 12 related tests were used, alpha was set at $p = 0.004$ for all main effects; this was a Bonferroni adjustment to cater for Type I errors. Without the adjustment, the probability of making at least one Type I error would be 0.5367 ($1 - 0\ 95^{15}$)—that is, greater than 50%.

For post-hoc tests (for within measures), alpha was set at $p = 0.05$; SPSS (for within measures) may automatically use a Bonferroni adjustment and, if this option is selected (which it was), the reported probability is "straight" (e.g., if, as in this study, three comparisons are necessary, it reports a probability of 0.0167 as 0.05, thereby "reversing" the adjustment).

Significant 2-way interactions between date of sampling and forage type and between date of sampling and land use were investigated first by viewing graphical output, and second by using post-hoc*t*-tests. Because three such tests were necessary for each interaction, alpha was set at $p = 0.0167$. This was a Bonferroni adjustment to control for Type I errors (note that it was not set lower because the significance of the

interaction had already been established). In cases in which data violated the assumption of equal variances (by Levene's test), the violation was noted and the result for unequal variances reported. In problematic cases, the result of the *t*-tests was checked using non-parametric equivalent tests (Mann–Whitney U).

Significant 3-way interactions were checked following the protocol advised by Field (2009), namely:

- A mixed 2-way 3 × 2 mixed post-hoc ANOVA (IVs: sampling date and forage type.
- A mixed 2-way mixed 3 × 2 post-hoc ANOVA (IVs: sampling date and land use).
- Six post-hoc *t*-tests (three each for forage type and land use).
- A between measures 2-way 2 × 2 post-hoc ANOVA (IVs: forage type and land use).

Because three post-hoc ANOVAs were necessary, alpha for each was set at $p = 0.0167$. Because six post-hoc *t*-tests were used, alpha for each was set at $p = 0.0083$. For all tests of ANOVA assumptions, alpha was set at $p = 0.05$.

A single simple independent samples *t*-test was used to determine whether there was a difference in the faecal ash content between the samples collected in the forage area and the samples collected in the grassland. The DV was faecal ash content; the IV was type of gazing (grassland or crop). Because only one test was used for this analysis, alpha was set at $p = 0.05$.

Analyses next examined soil erosion. This comprised a series of seven 2 × 2 between measures ANOVAs. The IVs comprised crop type (two crops, kale or swede) and ground type (between measures: two ground types, grazed or ungrazed). The DVs

comprised measures of water volume, turbidity, solids, soil penetration, NO_3^-, NH_4^+, and phosphorous. As before, MANOVA was not used—this because the DV's variances were grossly unequal.

Data for some tests violated assumptions of ANOVA (see Results, below). Attempts to rectify this by logarithmic transformation failed. Accordingly, raw data were used for all seven ANOVAs. In cases in which parametric assumptions were violated, results were checked, where possible, using equivalent non-parametric tests (Mann–Whitney U).

Because seven related tests were used, alpha was set at $p = 0.007$ for all main effects; this was a Bonferroni adjustment to cater for Type I errors. Without the adjustment, the probability of making at least one Type I error is would be 0.3017 ($1 – 0\ 95^7$)—that is, about 30%.

5.4. Results

5.4.1. Mixed ANOVAs

Table 5.1 shows descriptive statistics (mean and standard error) for the three sampling dates for pH, EC, MC, infiltration, BD, and air filled porosity. Table 5.2 shows summary descriptive (mean and standard error) for the variables shown in Table 5.1.

Table 5.1. Values represent means and SEs: physical and chemical soil properties over time.

	15 Days				**30 Days**				**45 Days**			
	Kale		Swede		Kale		Swede		Kale		Swede	
Parameter	Grazed	Ungrazed	Grazed	Ungrazed	Grazed	Ungrazed	Grazed	Ungrazed	Grazed	Ungrazed	Grazed	Ungrazed
pH	6.49 ± 0.02	6.44 ± 0.05	6.46 ± 0.05	6.41 ± 0.03	5.82 ± 0.08	6.25 ± 0.03	5.75 ± 0.06	6.03 ± 0.02	5.42 ± 0.03	5.99 ± 0.08	5.81 ± 0.10	6.13 ± 0.10
EC(µS cm^{-1})	141.68 ± 65.37	45.08 ± 11.51	131.80 ± 15.66	52.65 ± 4.21	233.10 ± 41.93	43.90 ± 3.39	268.83 ± 30.85	65.80 ± 2.57	281.50 ± 31.12	52.10 ± 13.54	363.25 ± 56.09	63.35 ± 14.70
Moisture (%)	31.30 ± 0.55	31.67 ± 1.20	29.88 ± 0.58	32.42 ± 0.26	26.92 ± 0.43	25.34 ± 0.87	26.98 ± 0.20	26.33 ± 1.25	17.14 ± 0.83	18.36 ± 0.72	16.96 ± 1.15	18.15 ± 0.67
Infiltration (cm min^{-1})	0.07 ± 0.02	8.67 ± 0.68	0.16 ±0.06	7.46 ± 1.57	0.14 ± 0.08	7.71 ± 0.43	0.17 ± 0.05	7.21 ± 0.34	3.33 ± 0.95	9.99 ± 0.01	1.05 ± 0.16	9.67 ± 0.12
Bulk density (g cm^{-3})	1.16 ± 0.03	0.94 ± 0.03	1.16 ± 0.02	0.87 ± 0.04	1.20 ± 0.03	0.95 ± 0.03	1.18 ± 0.01	0.91 ± 0.02	1.10 ± 0.01	0.95 ± 0.01	1.14 ± 0.02	0.89 ± 0.01
Air filled porosity (%)	18.63 ± 2.06	33.50 ± 2.69	20.68 ± 0.92	37.16 ± 2.49	21.08 ± 1.46	37.68 ± 1.69	21.01 ± 0.71	40.20 ± 1.62	36.98 ± 0.61	45.59 ± 0.67	33.63 ± 0.73	47.20 ± 1.00

Table 5.2. Means and SEs of physical and chemical soil properties.

	Kale		**Swede**		**Kale and Swede**	
Parameter	Grazed	Ungrazed	Grazed	Ungrazed	Grazed	Ungrazed
pH	5.91 ± 0.14	6.23 ± 0.06	6.01 ± 0.10	6.19 ± 0.06	5.96 ± 0.08	6.21 ± 0.04
EC (µS cm^{-1})	218.76 ± 30.69	47.03 ± 5.56	254.63 ± 34.87	60.60 ± 4.98	236.69 ± 23.02	53.81 ± 3.92
Moisture (%)	25.12 ± 1.81	24.65 ± 1.11	25.07 ± 1.88	25.63 ± 1.80	25.10 ± 1.15	25.14 ± 1.28
Infiltration (cm min^{-1})	1.18 ± 0.54	8.79 ± 0.37	0.46 ± 0.14	8.11 ± 0.59	0.82 ± 0.28	8.45 ± 0.35
Bulk density (g cm^{-3})	1.15 ± 0.02	0.95 ± 0.01	1.16 ± 0.02	0.89 ± 0.01	1.16 ± 0.01	0.92 ± 0.01
Air filled porosity (%)	254 ± 25.56	38.93 ± 1.73	25.11 ± 1.85	41.52 ± 1.51	25.33 ± 1.54	40.22 ± 1.16

5.4.2. Impact of grazing systems on soil physical properties

5.4.2.1. ANOVA: SOIL MOISTURE CONTENT

Within measures results suggested a significant effect for sampling date, F (2, 24) = 768.173, $p < 0.0005$; the effect size was substantial, $\eta_p^2 = 0.985$, suggesting that sampling date explained over 90% of the variance in soil MC. Contrast analysis suggested that the relationship between date of sampling and soil MC was broadly linear (linear: $F = 1402.055$, $p < 0.0005$, $\eta_p^2 = 0.992$; quadratic: $F = 41.784$, $p < 0.0005$, $\eta_p^2 = 0.777$).

The respective mean of total for days 15, 30, and 45 samples were 31.3%, 26.4%, and 17.7%, respectively (see Table Appendix 5). Post-hoc tests (Bonferroni) suggested that all differences between sampling dates were significant ($p < 0.0005$ in each case).

Within measures results suggested a close to significant interaction between sampling date land use, F (2, 24) = 6.789, $p = 0.005$; the effect size, if real, was substantial, $\eta_p^2 = 0.361$, suggesting that the interaction explained about 30% of the variance in soil MC. Results suggested a more precipitous decline in MC in ungrazed land than in grazed land.

Between measures results suggested no significant main effects and no significant interaction. Results of Shapiro–Wilk tests suggested that all distributions were broadly normal. Results of Levene's test suggested all variances were roughly equal.

5.4.2.2. ANOVA: BULK DENSITY

Results of Mauchly's test suggested no violation of sphericity. Within measures results suggested no significant differences between dates of sampling and no significant interactions between dates of sampling and the other IVs.

Between measures results suggested a significant effect of land use, F (1, 12) = 523.73, $p < 0.0005$; the effect size was substantial, $\eta_p^2 = 0.978$, suggesting that land use explained 90% or more of the variance in bulk density. Inspection of Table 5.2 shows that grazed land had a higher bulk density (mean: 1.16 gcm^{-3}) than had ungrazed land (mean: 0.92 gcm^{-3}).

Between measures results revealed no other significant effects. However, the result for forage type was close to significant, F (1, 12) = 5.700, $p = 0.034$; the effect size, if real, was substantial, $\eta_p^2 = 0.322$, suggesting that forage type explained about 30% of the variance in bulk density. The mean for kale was 1.05 gcm^{-3}; that for swede was 1.03 gcm^{-3}.

Shapiro–Wilk tests suggested all distributions were normal other than those for swede samples for day 30 ($p = 0.032$) and for day 45 ($p = 0.041$). Levene's test suggested all variances were equal.

Because of the slight violation of parametric assumptions, the significant effect of land use was checked using a Mann–Whitney U test; this corroborated the between measures result: there was a significant difference in land use ($p < 0.0005$). The possibly significant effect of forage type was checked using a Mann–Whitney U test; this test proved insignificant. The "significant" effect in this regard provided by the ANOVA therefore appeared spurious.

5.4.2.3. ANOVA: AIR FILLED POROSITY

Within measures results revealed a significant effect of date of sampling, F (2, 24) = 106.427, $p < 0.0005$; the effect size was substantial, $\eta_p^2 = 0.899$, suggesting that date of sampling accounted for over 80% of the variance in air filled porosity. Contrast analysis suggested that the relationship between date of sampling and air filled porosity was broadly linear (linear: $F = 177.408$, $p < 0.0005$, $\eta_p^2 = 0.937$; quadratic: $F = 26.179$, $p < 0.0005$, $\eta_p^2 = 0.686$).

The respective means for air filled porosity for day 15, 30, and 45 samples were 27.5%, 30.0%, and 40.9%, suggesting the trend was up. Results of post-hoc tests (Bonferroni) suggested significant differences ($p < 0.0005$) between samples from day 15 and day 45 and between samples from day 30 and day 45.

Within measures results also revealed a close to significant interaction between date of sampling and land use, F (2, 24) = 6.358, $p = 0.006$; the effect size, if real, was substantial, $\eta_p^2 = 0.346$, suggesting that the interaction accounted for over 20% of the variance in air filled porosity. Within measures results suggested no interaction between forage type and date of sampling and no three-way interaction.

Between measures results suggested a significant effect of land use, F (1, 12) = 403.800, $p < .0005$; the effect size was substantial, $\eta_p^2 = 0.971$, suggesting that land use explained 90% or more of the variance in air filled porosity. Inspection of Table 5.2 shows that ungrazed land had a higher air filled porosity (mean: 40.2%) than had grazed land (mean: 25.3%). Between measures results revealed no other significant effects.

Shapiro–Wilk tests suggested all distributions were normal other than those for swede samples for day 30 ($p = 0.033$) and for day 45 ($p = 0.033$). Levene's test suggested all variances were equal.

Because of the slight violation of parametric assumptions, the significant effect of land use was checked using a Mann–Whitney U test; this corroborated the between measures result: there was a significant difference in land use ($p < 0.0005$). The within measures result was corroborated using a Friedman test; there was a significant effect of date of sampling ($p < 0.0005$).

5.4.2.4. ANOVA SOIL INFILTRATION

Results of Mauchly's test suggested no violation of sphericity. Within measures results suggested a significant effect for measurement date, F (2, 24) = 17.290, $p < 0.0005$; the effect size was substantial, $\eta_p^2 = 0.590$, suggesting that measurement date explained about 50% of the variance in soil infiltration. Contrast analysis suggested that the relationship between date of measurement and soil infiltration was broadly quadratic, though a linear model also provided a close fit (linear: $F = 16.180$, $p = 0.002$, $\eta_p^2 = 0.574$; quadratic: $F = 19.719$, $p = 0.001$, $\eta_p^2 = 0.622$).

Inspection of Table Appendix 9 shows that the respective means for day 15, day 30, and day 45 samples were 4.09, 3.81, and 6.01 cm min^{-1}, suggesting U-shaped levels of soil infiltration during the period of study. Post-hoc tests (Bonferroni) suggested that the difference between day 15 and day 45 samples was significant ($p = 0.005$) and the difference between day 30 and day 45 samples was significant ($p < 0.0005$). Within measures results suggested no significant interactions.

Between measures results suggested a significant effect of land use, F (1, 12) = 461.386, $p < 0.0005$; the effect size was substantial, $\eta_p^2 = 0.975$, suggesting that land use explained over 90% of the variance in soil infiltration. Inspection of Table 5.2

shows that the mean for grazed land (0.82 cm min^{-1}) was lower than that for ungrazed land (8.45 cm min^{-1}).

Results of Shapiro–Wilk tests suggested that all distributions save those for grazed and ungrazed land on day 30 were non-normal. Results of Levene's test suggested all variances were unequal ($p = 0.012$, $p = 0.012$, and $p < 0.0005$ for day 15, day 30, and day 45 respectively. A Mann–Whitney U test corroborated the difference in land use ($p < 0.0005$). A Friedman test corroborated the difference in measurement date ($p < 0.0005$).

5.4.2.5. ANOVA: PENETRATION

Results of the ANOVA suggested a significant effect of land use, F (1, 12) = 15.244, $p = 0.002$; the effect size was substantial, $\eta_p^2 = 0.560$, suggesting that land use explained over 50% of the variance in penetration. Overall, however, the ANOVA explained just under 50% of the variance, $R^2 = 59.2$, $R^2_{adjusted} = 0.490$. Inspection of Table 5.4 shows that penetration was higher for grazed (mean: 336.8 Ncm^{-2}) than for ungrazed land (mean: 259.5 Ncm^{-2}). Results suggested no other significant effects.

Results of Shapiro–Wilk tests suggested data were broadly normally distributed. Results of Levene's test suggested variances were approximately equal.

5.4.3. ANOVA: Effects of grazing systems on soil erosion

To estimate soil erosion the physical and chemical characteristics of runoff water was determined. The analytical results have been presented in table5.4.

5.4.3.1. ANOVA: WATER VOLUME, TURBIDITY & SOLIDS

Table 5.3 shows descriptive statistics. Results of the ANOVA suggested no significant main effects and no significant interaction.

Table 5.3. Means and SEs of soil erosion analyses.

	Kale		**Swede**		**Kale and Swede**	
Parameter	Grazed	Ungrazed	Grazed	Ungrazed	Grazed	Ungrazed
Turbidity (NTU ml^{-1})	336.5 ±57.0	199.4 ± 78.2	307.8 ± 79.4	174.9 ± 53.6	322.13 ± 45.59	187.18 ± 44.14
Dry solids (mg ml^{-1})	5.15±0.85	2.88 ± 1.11	6.12 ± 1.28	5.94 ± 2.09	5.63 ± 0.74	4.41 ± 1.24
Water volume (cm min^{-1})	37.06 ± 0.70	39.33 ± 3.57	43.66 ± 4.62	43.66 ± 3.59	40.36 ± 2.50	41.50 ± 2.48
Penetration (Newton cm^{-2})	339.50 ± 20.52	239.00 ± 12.50	334.00 ± 25.18	280.00 ± 18.83	336.75 ± 15.07	259.50 ± 13.02
P (mg kg^{-1})	0.49 ± 0.03	1.27 ± 0.55	0.82 ± 0.26	0.90 ± 0.20	0.65 ± 0.13	1.08 ± 0.28
NO_3^- (mg kg^{-1})	2.32 ± 0.84	0.91 ± 0.21	1.94 ± 0.40	0.81 ± 0.10	2.13 ± 0.44	0.86 ± 0.11
NH_4^+(mg kg^{-1})	1.48 ± 0.23	2.61 ± 1.03	2.23 ± 0.41	1.98 ± 0.33	1.85 ± 0.26	2.30 ± 0.52

5.4.3.4. ANOVA: NITRATES, AMMONIUM & PHOSPHORUS

Table 5.4 shows descriptive statistics. Results of the ANOVA suggested no significant main effects for Nitrate. However, the result for land use was close to significance, F (1, 12) = 7.128, p = 0.020; the effect size, if real, was substantial, η_p^2 = 0.373, suggesting that land use explained over 30% of the variance in NO_3^-. Overall, however, the ANOVA explained just over 20% of the variance, R^2 = 0.383, R^2_{adjusted} = 0.229. Results of Shapiro–Wilk tests suggested data were broadly normally distributed, save for kale data (p = 0.015). Results of Levene's test suggested variances were unequal (p = 0.006). Results of a Mann–Whitney U test suggested a significant effect of land use (p = 0.006). His level of significance is substantially greater than that of the ANOVA—this despite non-parametric tests having less power than have parametric tests. Accordingly, particularly given the violations of parametric assumptions, the non-parametric result is taken as valid.

Results of the ANOVA suggested no significant main effects and no significant interaction for either ammonium or phosphorus (Table 5.4).

5.4.4. Faecal ash content

The *t*-test failed to detect a significant difference between faecal ash content in grassland as opposed to crop grazed areas.

5.4.5. Impacts of grazing systems on soil chemical properties

5.4.5.1. ANOVA: pH

Results of Mauchly's test suggested no violation of sphericity. Within measures results revealed a significant effect of date of sampling, F (2, 24) = 97.823, $p < 0.0005$; the effect size was substantial, $\eta_p^2 = 0.891$, suggesting that date of sampling accounted for over 80% of the variance in pH. Contrast analysis suggested that the relationship between date of sampling and pH^- was broadly linear (linear: $F = 166.949$, $p < 0.0005$, $\eta_p^2 = 0.933$; quadratic: $F = 21.170$, $p = 0.001$, $\eta_p^2 = 0.638$).

Our result shows that the respective means for day 15, day30, and day 45 samples were 6.45, 5.96, and 5.84, suggesting that the trend was down (Appendix table7). Results of post-hoc tests (Bonferroni) suggested significant differences ($p < 0.0005$) between samples from day 15 and day 30 and between samples from day 15 and day 45.

Within measures results also revealed a significant interaction between date of sampling and land use F (2, 24) = 16.645, $p < 0.0005$; the effect size was substantial, $\eta_p^2 = 0.581$, suggesting that the interaction accounted for about 50% of the variance in pH. Figure 5.3 shows the interaction.

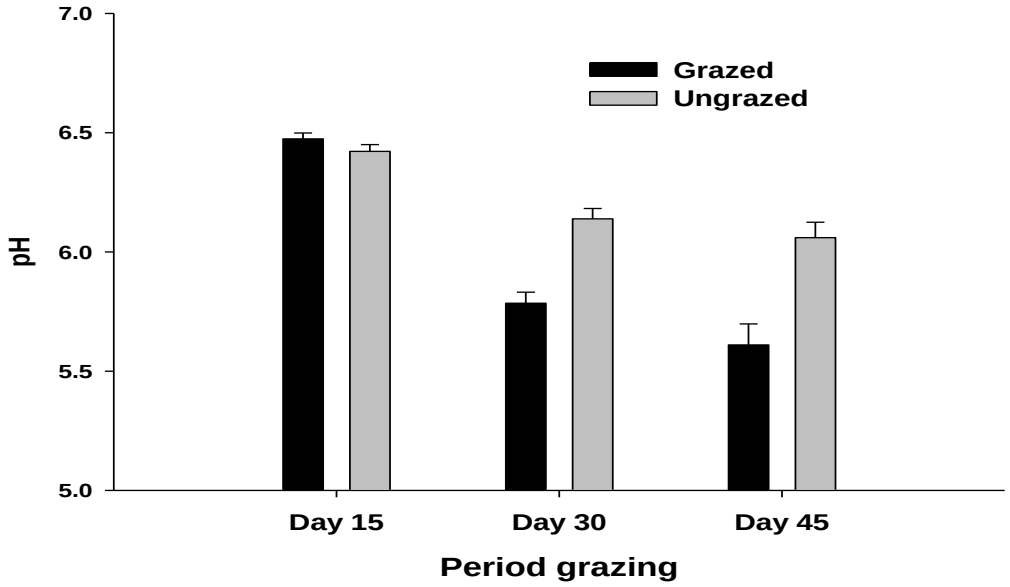

Figure 5.3. Interaction between land use and date of sampling for soil pH. Error bars denote SE.

A series of three post-hoc *t*-tests corroborated the impression provided by the figure. The difference between day 15 samples was insignificant, but the between day 30 samples was significant, t (14) = –5.580, $p < 0.0005$, as was the difference between day 45 samples, t (14) = –4.125, $p = 0.001$.

Within measures results also suggested an interaction between date of sampling and forage type, F (2, 24) = 10.041, $p = 0.001$; the effect size was substantial, $\eta_p^2 = .456$, suggesting that the interaction accounted for about 40% of the variance in pH. Figure 5.4 shows the interaction.

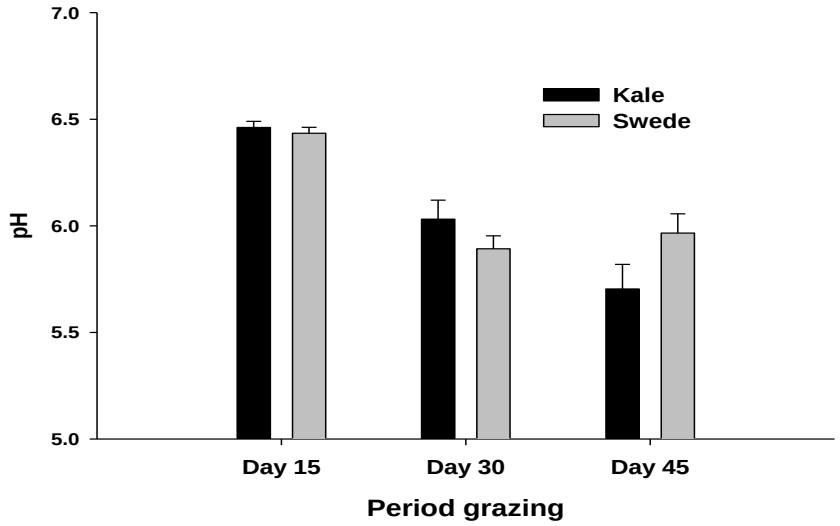

Figure 5.4. Interaction between forage type and date of sampling for soil pH. Error bars denote SE.

A series of three post-hoc *t*-tests failed to reveal any differences between forage types on any of the three dates of sampling. The three-way interaction was insignificant.

5.4.5.2. ANOVA: EC

Results can be seen in appendix Table 8. Results of Mauchly's test suggested no violation of sphericity. Within measures results revealed a significant effect of date of sampling, F (2, 24) = 15.951, $p < 0.0005$; the effect size was substantial, $\eta_p^2 = 0.571$, suggesting that date of sampling accounted for about 50% of the variance in EC. Contrast analysis suggested that the relationship between date of sampling and EC was broadly linear (linear: $F = 24.652$, $p < 0.0005$, $\eta_p^2 = 0.673$; quadratic: $F = 0.798$, $p = 0.389$, $\eta_p^2 = 0.062$).

Results of post-hoc tests (Bonferroni) suggested significant differences between samples from day 15 and day 30 ($p = 0.019$) and between samples from day 15 and day 45 ($p = 0.001$). The difference between samples from day 30 and day 45 was close to

significant (p = 0.058). Thus, although in broad terms EC rose over time, the question of true linearity remains open.

Within measures results also revealed a significant interaction between date of sampling and land use F (2, 24) = 13.153, p < 0.0005; the effect size was substantial, η_p^2 = 0.523, suggesting that the interaction accounted for over 40% of the variance in EC. Figure 5.5 shows the interaction.

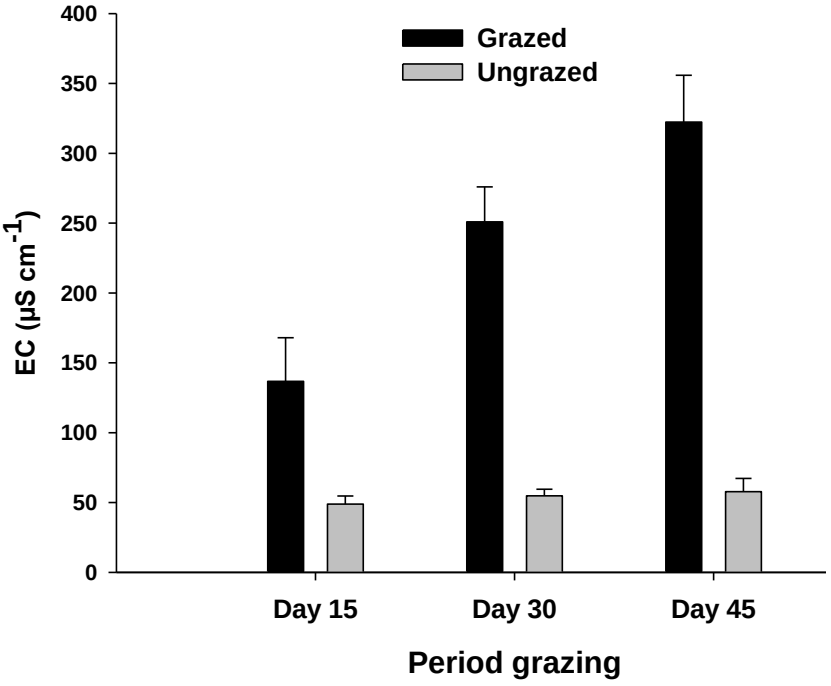

Figure 5.5. Interaction between land use and date of sampling for soil EC. Error bars denote SE.

A series of three post-hoc t-tests revealed significant differences between samples for each of the three dates of sampling. That for day 15 was t (14) = 2.771, p = 0.015; that for day 30 was t (7.469) = 7.708, p < 0.0005 (unequal variances); that for day 45 was t (8.119) = 7.607, p < 0.0005 (unequal variances). Within measures results suggested no other interactions

Between measures results suggested a significant effect of land use, F (1, 12) = 56.697, p < 0.0005; the effect size was substantial, η_p^2 = 0.825, suggesting that land use

explained over 70% of the variance in EC. Inspection of Table 5.2 shows that grazed land had a higher EC (mean: 236.7 µS cm^{-1}) than had ungrazed land (mean: 53.8 µS cm^{-1}). Between measures results suggested no other significant effects.

Shapiro–Wilk tests suggested all distributions were normal save those for kale for day 15 and day 30 samples, those for the swede for day 30 sample, and grazed land samples for day 30. Levene's test suggested all variances were unequal ($p = 0.020$, $p < 0.0005$, and $p = 0.027$ for day 15, day 30, and day 45 respectively).

Because of the violations of parametric assumptions, the significant effect of land use was checked using a Mann–Whitney U test; this corroborated the result of the between measures ANOVA; the difference in land use was significant ($p < 0.0005$). The within measures result was corroborated using a Friedman test; there was a significant effect of date of sampling ($p < 0.0005$).

5.4.5.3. ANOVA: NITRATES

Figure 5.6 and Appendix Table 6 provides summary descriptives.

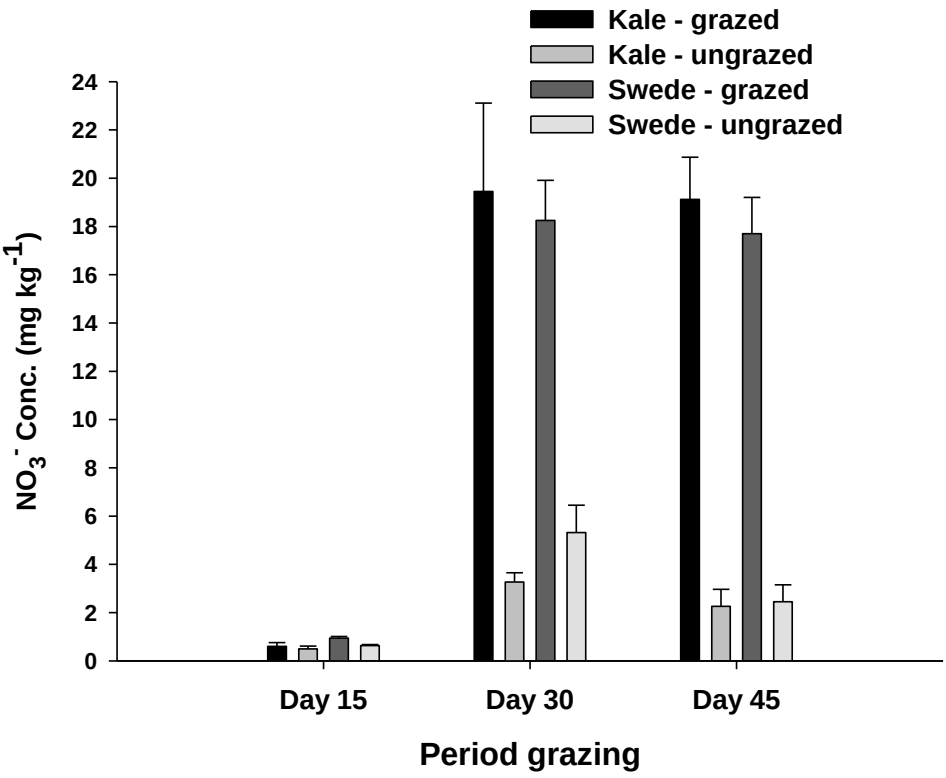

Figure 5.6. Summary statistics for NO_3^-. Error bars denote SE.

Results of Mauchly's test suggested no violation of sphericity. Within measures results revealed a significant effect of date of sampling, F (2, 24) = 83.697, $p < 0.0005$; the effect size was substantial, $\eta_p^2 = 0.875$, suggesting that date of sampling accounted for over 80% of the variance in NO_3^-. Contrast analysis suggested that the relationship between date of sampling and NO_3^- was broadly linear (linear: $F = 234.089$, $p < 0.0005$, $\eta_p^2 = 0.951$; quadratic: $F = 37.309$, $p < 0.0005$, $\eta_p^2 = 0.757$).

Results of post-hoc tests (Bonferroni) suggested significant differences ($p < 0.0005$) between samples from day 15 and day 30 and between samples from day 15 and day 45.

Within measures results also revealed a significant interaction between date of sampling and land use, F (2, 24) = 44.791, $p < 0.0005$; the effect size was substantial, $\eta_p^2 = 0.789$, suggesting that the interaction accounted for over 70% of the variance in NO_3^-. Figure 5.7 shows the interaction.

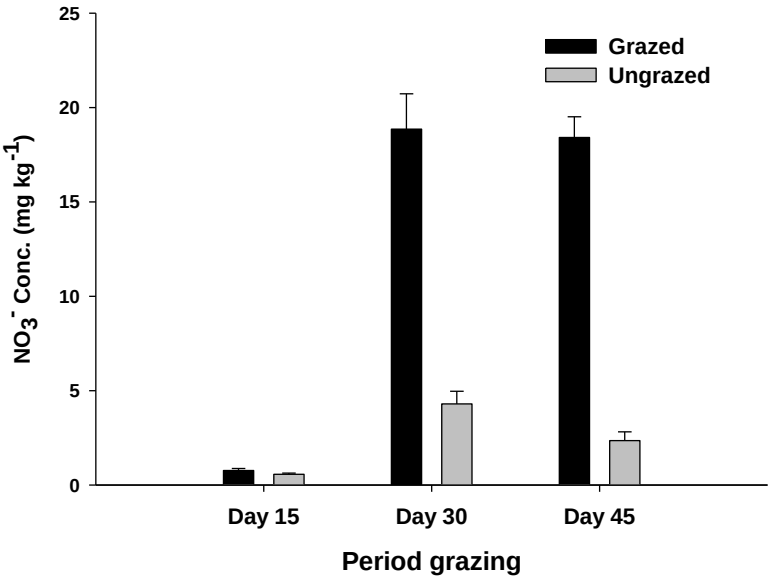

Figure 5.7. Interaction between land use and date of sampling for NO_3^-. Error bars denote SE.

A series of three post-hoc *t*-tests corroborated the impression provided by the figure. There was no significant difference between grazed and ungrazed sample mean for day 15, but the difference for day 30 samples was significant, t (14) = 7.312, $p < 0.0005$, as was the difference for day 45 samples, t (14) = 13.462, $p < 0.0005$. Within measures results suggested no interaction between forage type and date of sampling and no three-way interaction.

Between measures results suggested a significant effect of land use, F (1, 12) = 124.909, $p < .0005$; the effect size was substantial, $\eta_p^2 = 0.912$, suggesting that land use explained over 80% of the variance in NO_3^-. Grazed land had a higher levels of NO_3^-

(mean: 18.4 mg kg^{-1}) than had ungrazed land (mean: 2.4 mg kg^{-1}), (Appendixtable 6). Between measures results revealed no other significant effects.

Shapiro–Wilk tests suggested all distributions were normal other than those for kale samples for day 30 ($p = 0.033$). Levene's test suggested all variances were equal save for those for day 30 samples ($p < 0.0005$). Because of the slight violation of parametric assumptions, the significant effect of land use was checked using a Mann–Whitney U test; this corroborated the result of the between measures ANOVA: there was a significant difference in land use ($p < 0.0005$). The within measures result was corroborated using a Friedman test; there was a significant effect of date of sampling ($p < 0.0005$).

5.4.5.4. ANOVA: AMMONIUM

Figure 5.8 provides summary descriptives.

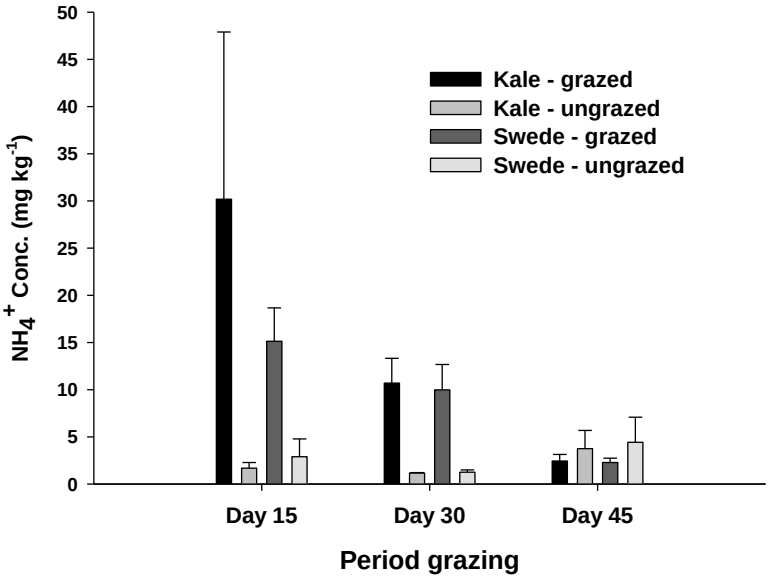

Figure 5.8. Summary statistics for NH_4^+. Error bars denote SE.

Results of Mauchly's test suggested severe violation of sphericity ($p < 0.0005$). Accordingly, multivariate results (Pillau's trace) are reported for within measures analyses.

Within measures results revealed no significant effect of date of sampling and a significant interaction between date of sampling and land use, F (2, 11) = 10.336, p = 0.003; the effect size was substantial, η_p^2 = 0.653, suggesting that the interaction accounted for about 60% of the variance in NH_4^+. Figure 5.9 shows the interaction.

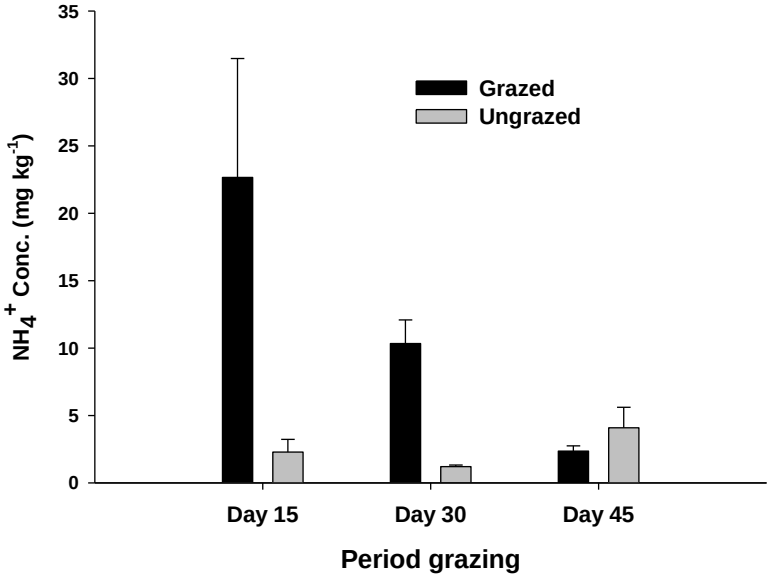

Figure 5.9. Interaction between land use and date of sampling for NH_4^+. Error bars denote SE.

The figure suggests that levels of NH_4^+ were higher in grazed land than ungrazed on 15 and 30 days after grazing however, after 45 days it was higher in ungrazed land compare to grazed land.

A series of three post-hoc *t*-tests corroborated the impression provided by the figure. There was no significant difference between grazed and ungrazed sample mean

for day 15 and days 45 samples. On the other hand, the difference in day 30 samples was significant, t (7.060) = 75.223, $p < 0.001$.

Within measures results suggested no interaction between forage type and date of sampling and no three-way interaction. There was no significant difference between forage types within our measurements. But the result for land use was close to significant, F (1, 12) = 7.210, $p < 0.020$ however, the overall difference was not statistically significant. The effect size, if real, was substantial, $\eta_p^2 = 0.37$, suggesting that land use explained over 30% of the variance in NH_4^+. Grazed land had a higher levels of NH_4^+ (mean: 11.8 mg kg^{-1}) than had ungrazed land (mean: 2.5 mg kg^{-1}). Between measures results revealed no other significant effects.

Shapiro–Wilk tests suggested all distributions were non-normal other than those for swede samples for day 15 and for grazed land for day 30. Levene's test suggested variances were unequal for day 15 and day 30 samples ($p = 0.008$ and $p = 0.014$ respectively).

Because of the violation of parametric assumptions, the significant effect of land use was checked using a Mann–Whitney U test; this corroborated the result of the between measures ANOVA: there was a significant difference in land use ($p < 0.0005$).

5.4.5.5. ANOVA: SOIL PHOSPHOROUS

Figure 5.10 shows the descriptive statistics.

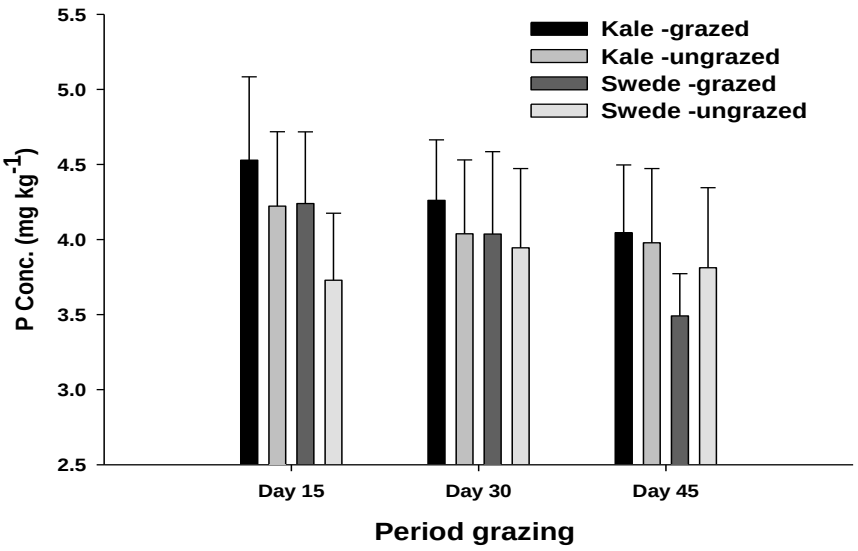

Figure 5.10. Descriptive statistics for soil phosphorous. Error bars denote SE.

Results of Mauchly's test suggested no violation of sphericity. Within measures results suggested no significant effects. However, the result for sampling date was close to significant, F (2, 24) = 3.520, p = 0.046; the effect size, if real, was substantial, η_p^2 = 0.227, suggesting that forage type explained about 20% of the variance in soil phosphorous. Non-parametric analysis also suggested a near significant effect (p = 0.028). Contrast analysis suggested that the relationship between date of sampling and soil phosphorous was broadly linear (linear: F = 7.418, p = 0.018, η_p^2 = 0.382; quadratic: F = 0.287, p = 0.607, η_p^2 = 0.023).

The respective means for day 15, day 30, and day 45 samples were 4.2 mg kg^{-1}, 4.1 mg kg^{-1}, and 3.8 mg kg^{-1}, suggesting the trend was down (Appendix table 10). Post-hoc tests (Bonferroni) failed to detect any significant difference, though that between day 15 and day 45 samples was close to significance (p= 0.055).

Between measures results suggested no significant main effects and no significant interaction. Results of Shapiro–Wilk tests suggested that all distributions

were broadly normal. Results of Levene's test suggested all variances were roughly equal.

5.4.6. Impacts of grazing systems on soil bacterial population

5.4.6.1. COLIFORMS

Figure 5.11 shows the descriptive statistics. Because of the number of the colonies; means and SEs are shown as $\log_{10}$ CFUg^{-1}.

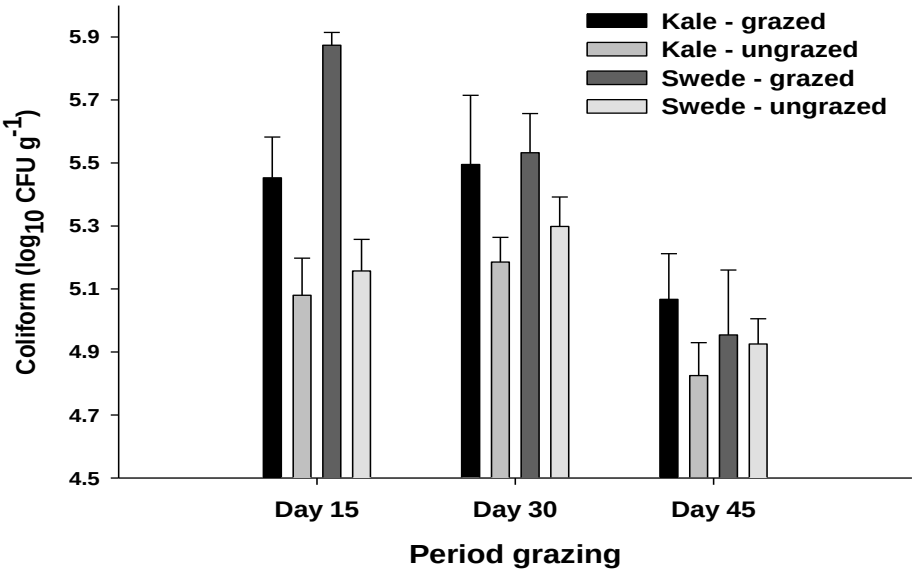

Figure 5.11. Descriptive statistics for coliform counts. Error bars denote SE.

Within measures results revealed a significant effect of date of sampling, F (2, 24) = 15.495, $p < 0.0005$; the effect size was substantial, $\eta_p^2 = 0.564$, suggesting that date of sampling accounted for about 50% of the variance in coliform bacteria. Contrast analysis suggested that the relationship between date of sampling and coliform counts was broadly linear (linear: $F = 68.235$, $p < 0.0005$, $\eta_p^2 = 0.850$; quadratic: $F = 2.664$, $p = 0.129$, $\eta_p^2 = 0.182$).

The respective mean counts for day 15, day 30, and day 45 samples were 5.54, 5.49, and 5.04 $\log_{10}$ CFU g^{-1}, suggesting that the trend was down. Results of post-hoc tests (Bonferroni) suggested significant differences ($p < 0.0005$) between samples from day 15 and day 45 and between samples from day 30 and day 45.

Within measures results also revealed a significant interaction between date of sampling and land use F (2, 24) = 7.077, p = 0.004; the effect size was substantial, η_p^2 = 0.371, suggesting that the interaction accounted for over 30% of the variance in coliforms. Figure 5.12 shows the interaction.

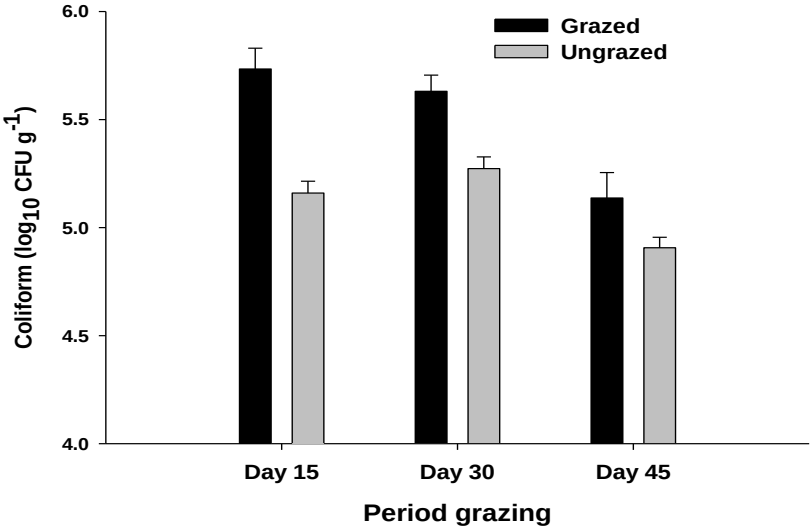

Figure 5.12. Interaction between land use and date of sampling for coliforms. Error bars denote SE.

A series of three post-hoc t-tests revealed that only the difference between day 15 samples was significant, t (7.721) = 3.878, p = 0.005 (unequal variances).

Within measures results suggested no interaction between forage type and sampling date and no three-way interaction. However, each result was close to significance. That for forage type was F (2, 24) = 4.047, p = 0.023; the effect size, if real, was substantial, η_p^2 = 0.269, suggesting that the interaction accounted for over 20%

of the variance in coliforms. That for the three-way interaction was F (2, 24) = 5.103, p = .014; the effect size, if real, was substantial, η_p^2 = 0.298, suggesting that the interaction accounted for over 20% of the variance in coliforms.

Grazed land had a higher levels of coliforms (mean: 5.57 $\log_{10}$ CFU g^{-1}) than had ungrazed land (mean 5.14: $\log_{10}$ CFU g^{-1}); however between measures results suggested no significant effects.

Shapiro–Wilk tests suggested all distributions were non-normal other than those for swede samples for day 15 and day 30 and for grazed and ungrazed land for day 15 and for ungrazed land for day 45. Levene's test suggested variances were unequal save for day 30 samples ($p < 0.033$).

Because of the violations of parametric assumptions, the significant effect of land use was checked using a Mann–Whitney U test; this corroborated the result of the between measures ANOVA: there was a significant difference in land use ($p < 0.0005$). The within measures result was corroborated using a Friedman test; there was a significant effect of date of sampling ($p < 0.0005$).

5.4.6.2. ANOVA: *E. COLI*

Figure 5.13 shows the descriptive statistics.

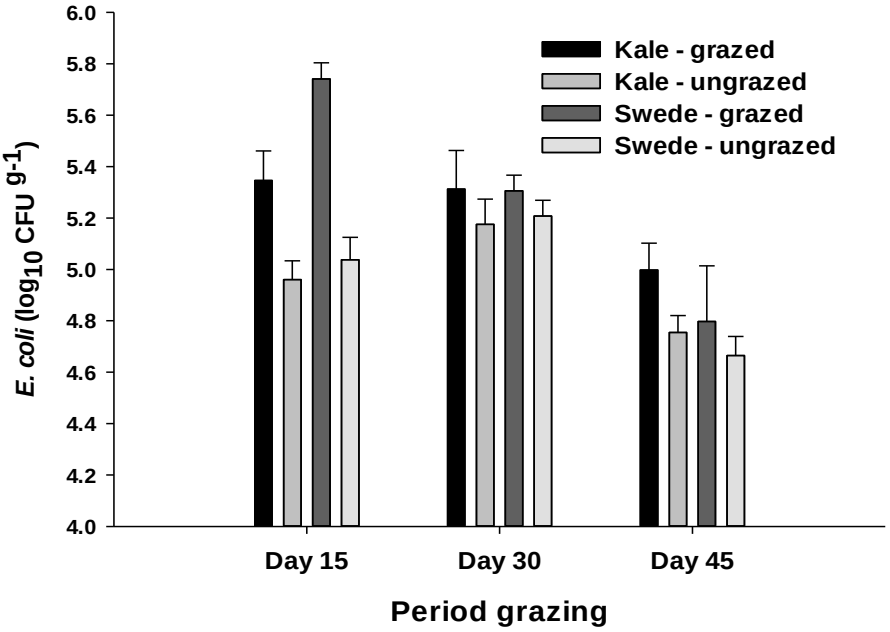

Figure 5.13. Descriptive statistics for *E. coli* counts. Error bars denote SE.

Results of Mauchly's test suggested no violation of sphericity. Within measures results revealed a significant effect of date of sampling, F (2, 24) = 27.292, $p < 0.0005$; the effect size was substantial, $\eta_p^2 = 0.695$, suggesting that date of sampling accounted for over 60% of the variance in *E. coli*. Contrast analysis suggested that the relationship between date of sampling and *E. coli* counts was broadly linear (linear: $F = 39.643$, $p < 0.0005$, $\eta_p^2 = 0.768$; quadratic: $F = 2.673$, $p = 0.128$, $\eta_p^2 = 0.182$). Results of post-hoc tests (Bonferroni) suggested significant differences ($p < 0.0005$) between samples from day 15 and day 45 and between samples from day 30 and day 45.

Within measures results also revealed a significant interaction between date of sampling and land use F (2, 24) = 16.656, $p < 0.0005$; the effect size was substantial, η_p^2

= 0.581, suggesting that the interaction accounted for over 50% of the variance in *E. coli*. Figure 5.14 shows the interaction.

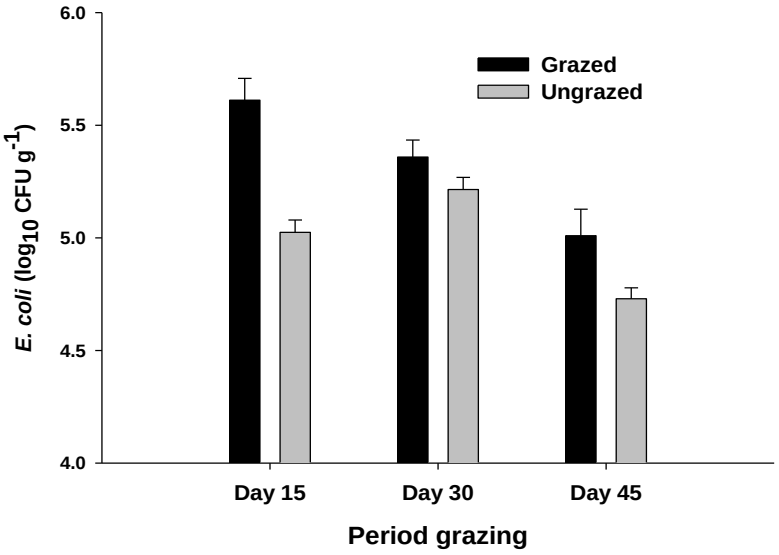

Figure 5.14. Interaction between land use and date of sampling for *E. coli*. Error bars denote SE.

A series of three post-hoc *t*-tests revealed that only the difference between day 15 samples was significant, t (7.445) = 3.796, p = 0.006 (unequal variances).

Within measures results also suggested an interaction between date of sampling and forage type, F (2, 24) = 9.302, p = 0.001; the effect size was substantial, η_p^2 = 0.437, suggesting that the interaction accounted for about 40% of the variance in *E. coli*. Figure 5.15 shows the interaction.

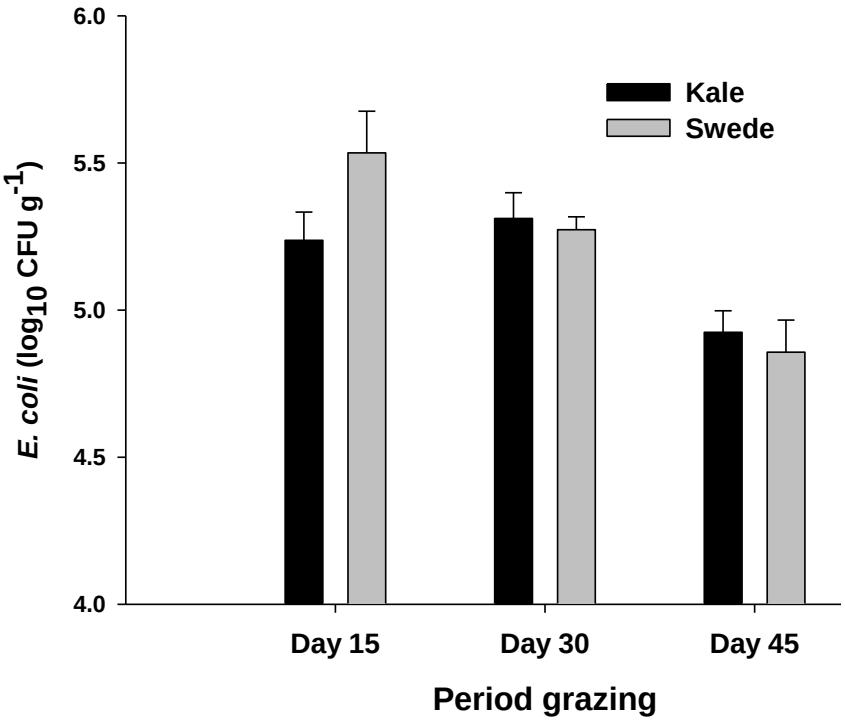

Figure 5.15. Interaction between forage type and date of sampling for *E. coli*. Error bars denote SE.

A series of three post-hoc *t*-tests failed to reveal any differences between forage types on any of the three dates of sampling. Between measures results suggested a significant effect of land use, F (1, 12) = 12.824, p = 0.004; the effect size was substantial, $\eta_p^2 = 0.517$, suggesting that land use explained over 40% of the variance in *E. coli*. Grazed land had a higher counts of *E. coli* (mean: 5.39 $\log_{10}$ CFU g^{-1}) than had ungrazed land (mean: 5.03 $\log_{10}$ CFU g^{-1}).

Shapiro–Wilk tests suggested all kale distributions were non-normal, as were the swede distribution for day 45, the ungrazed distribution for day 15, and the grazed distribution for day 30.Levene's test suggested variances were equal save for day 45 samples ($p < 0.045$).

Because of the violations of parametric assumptions, the significant effect of land use was checked using a Mann–Whitney U test; this failed to corroborate the result

of the between measures ANOVA; however, the result was close to significance (p = 0.005). The within measures result was corroborated using a Friedman test; there was a significant effect of date of sampling ($p < 0.0005$).

5.4.7. ANOVA: Soil respiration

Figure 5.16 shows the descriptive statistics.

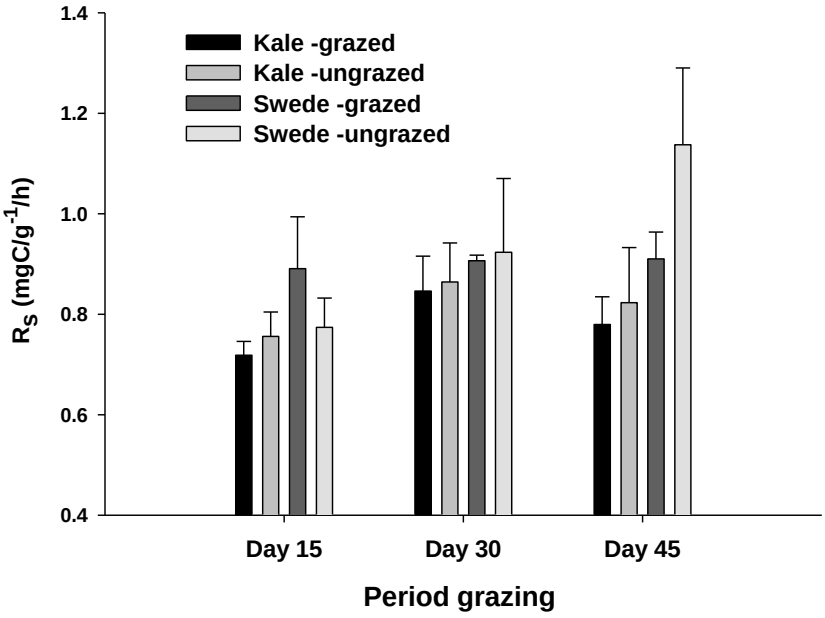

Figure 5.16. Descriptive statistics for soil respiration. Error bars denote SE.

Results of Mauchly's test suggested no violation of sphericity. Within measures results suggested no significant effects as regards soil respiration. Between measures results failed to find any effects. However, that for forage type was close to significant, F (1, 12) = 4.955, p = 0.046; the effect size, if real, was substantial, η_p^2 = 0.292, suggesting that forage type explained over 20% of the variance in soil respiration. Kale samples had a lower soil respiration (mean: 0.8 mg C $g^{-1}h^{-1}$) than had swede samples (mean: 0.9 mg C $g^{-1}h^{-1}$).

Shapiro–Wilk tests suggested all distributions were normal save those for swede for day 30 and day 45 samples. Levene's test suggested all variances were equal save those for day 30 samples ($p = 0.047$).

Because of the violations of parametric assumptions, the close to significant effect of forage type was checked using a Mann–Whitney U test; this failed detect a significant effect, or anything close to one.

5.4.8. Summary of mixed ANOVAs

Table 5.4. Summary of mixed ANOVA results.

	Date (Trend: direction)	Forage type (higher value)	Land use (higher value)	Date§Forage	Date§Land	Effect size within	Effect size (forage)	Effect size between (land)
BD	NS	§Kale	Grazed	NS	NS	N/A	N/A	90%
AFP	Linear: Up	NS	Ungrazed	NS	§20%	80%	N/A	90%
Nitrates	Linear: Up	NS	Grazed	NS	70%	80%	N/A	80%
Ammonium	NS	NS	§Grazed	NS	60%	N/A	N/A	§30
Coliform	Linear: Down	NS	§Grazed	20%	30%	50%	§20	§30%
E. coli	Linear: Down	NS	Grazed	40%	50%	60%	§20	40%?
pH	Linear: Down	NS	Ungrazed	40%	50%	80%	§20	80%
EC	Linear: Up	NS	Grazed	NS	40%	50%	N/A	70%
RES	NS	§Kale	NS	§20%	NS	N/A	§20	N/A
P	§Linear: Down	NS	NS	NS	NS	§20%	N/A	N/A
MC	Linear: Down	NS	NS	NS	NS	90%	N/A	N/A
SI	Quadratic	NS	Ungrazed	NS	NS	50%	N/A	90%

§ = result is only close to significance, result is significant only from non-parametric tests or both. NS = result is not significant.

Linear: up = the trend is upwards over time.

Linear down = the trend is downwards over time.

Quadratic = the trend is significant but non-linear.

Effect size = rounding down η_p^2 to the nearest plausible multiple of 10, and expressing the result as the percentage explained variance.

? = result is problematic because of disparity between parametric and non-parametric results.

5.5. Discussion

The present study investigated the effects of grazing systems with two winter crops on the physical, chemical, and microbial properties of soils in managed grasslands.

5.5.1. Soil physical properties and grazing systems

Results of previous studies suggest soil moisture content (MC), bulk density (BD), air filled porosity (AFP), infiltration, penetration and magnitude of soil erosion have are affected by land use, forage types and also the interaction of the both. These findings are generally agreed with many previous studies (Abdel-Magid *et al.*, 1987; Singleton and Addson, 1999; Drewry and Paton, 2005).

In our study, soil MC decreased over time; the obvious explanation of this is that rainfall decreased during the period of sampling (daily rainfall fell from 9.2 mm to 6.2 mm) while temperature increased. The decline in soil MC appeared less marked in grazed than in ungrazed land. The effect could be explained by the ground becoming more compacted over time because of its being trampled by sheep (Donkor *et al.*, 2002). Likewise, grazed land had a higher bulk density than had ungrazed land. This might be because of land that is trampled (by sheep) should have a higher soil mass and volume

ration than land that is not trampled (Donkor *et al.,* 2002). Soil air filled porosity (or soil macroporosity) is often related to soil compaction which reduces the soil porosity and air movement (Drewry, 2008). Grazing animal such as sheep and cattle create high forces onto the soil, causing lower AFP in grazed land compare to ungrazed grassland (Drewry and Paton , 2005).

Soil infiltration was reduced where grazing occurred, as reported by McCalla *et al*. (1984) using different grazing intensity in Edwards Plateau of Texas. Reduced infiltration rate in grazed soil might be attributed to soil compaction, loss of vegetative cover and increased bulk density (Blackburn *et al.,* 1982). Results suggested that grazing also substantially affected soil penetration. In grazed land, animal hooves can create pressure as high as 200 kPa, causing soil compaction and consequently reduction of soil porosity (Donkor *et al*., (2002; Du-Toit *et al., 2*009). However, contrasting result of no increasing trend in penetration resistance due to compaction was reported by Ingram (2003) in a long term grazing experiment.

Results suggested no effects of grazing or of crop type on water volume, turbidity; solid particles, NH_4^+, or P in the runoff water. Soil erosion was estimated by considering the characteristics of runoff water from both grazed and ungrazed lands. Although the variations were not statistically significant, the mean values of most of the parameters were slightly higher in the grazed land. Blackburn (1984) reported that grazing with heavy stocking rates causes sediment loss, especially in continuous grazing. The present study, however, as indicated, used rotational grazing; its result, therefore, is consistent with that of the Savory (1983), who found that rotational grazing reduces soil erosion.

Faecal ash content generally used to estimate the amount of soil ingested by the grazing animals. Results of the present study indicated no detectable difference in faecal ash content between grassland grazing and crop grazing. Although some investigators reported that the quantity of soil ingested by animals has been related to plant and soils (Mayland *et al.,* 1975). However some of studies have been reported the influence of factors such as management (Healy 1973), season (Healy and Ludwig 1965), and individual livestock behaviour (Healy 1968b). This suggests that sheep grazing forage crops digest no more soil than do sheep grazing grassland. This might be because of sheep consumed mostly the above ground portion of root crops and its vegetative part.

5.5.2. Soil chemical properties and grazing systems

pH values between day 15 and day 45 were significantly different, suggesting that pH was less reduced with less intensive grazing. Generally, grazing increase soil pH (Dormaar and Williams, 1998); however, in the present study, the soil in the ungrazed plots had the higher pH. The results suggest that grazing increases EC; possibly because sheep excreta contain ions which increase soil EC (Mapfumo *et al.,* 2000).

The increase in soil nitrate levels throughout the sampling period might be because of microbial transformation of NH_4^+ to NO_3^- over time (Firestone and Davidson, 1989). In nitrification process in aerobic condition, NH_4^+ is oxidized to NO_3^-. The association of increased NO_3^- with animal grazing is reinforced by other results. Mapfumo *et al*. (2000) suggested that both mineral nitrogen and NO_3^- level were high in heavy grazing compared to medium and low grazing intensities because of dung and urine depositions. Contrasting results of higher NO_3^- in ungrazed grassland compare to graze was reported by Lavado *et al*. (1996) due to higher vegetation cover in ungrazed

condition. However in our studies the rotational grazing scheme was designed with root crops, without any grass cover hence, ungrazed plots was lower in NO_3^- content.

Ammonium levels decreased with time, although not significantly so. This may be because during the nitrification process, ammonium rapidly converts to nitrate especially in agricultural soils when nitrate is the dominant forms of nitrogen. Another possible fate of NH_4 is assimilated by soil microorganisms in agricultural soils (Robertson 1997). Similarly, there were no significant changes in soil P between grazed and ungrazed plots, although it was tended to decrease in our rotation grazing system. This result is consistent with the findings of Garcia *et al*. (2011) who reported that solubilising activities of phosphorus was higher in ungrazed soils system compare to rotational grazing, however it was not statistically significant.

5.5.3. Soil biological properties and grazing systems

The coliform results corroborate with the bacterial counts of soil samples collected from grazed and ungrazed lands of same location (Chapter 4) where the bacteria was appeared more numerous in grazed lands. Results for *E. coli* broadly mirrored those of other coliforms, as expected. The fall in *E. coli* counts was associated with the less intensive grazing during the period from day 30 to day 45. However, there are others factors that influence the reduction of *E. coli* levels in soil, such as temperature, livestock diets, soil type, UV radiation, rainfall, physiological status of the microbial and strain variability and competition among different microorganisms (Avery *et al.,* 2004). Counts of *E. coli* were higher in swede grazing areas than in kale grazing areas, but this appeared true only of day 15. This is likely to be due to the sheep preferentially grazing swede before kale, hence contributing to the soil reserve of

coliforms and *E. coli*. In this regard, future research might try a greater variety of forage crops.

Although grazing system can change the physical and chemical properties of soil which in general can affect soil respiration, our results showed no significant changes in soil respiration rate. Similar result of no significant effect of grazing on soil respiration was observed by Jia *et al*. (2007). This might be because of smaller quantities of organic matter decomposing especially cellulose decomposing bacteria and the fungal biomass in intensive grazed soils, as reported by Jiang *et al*. (1995).

5.5.5. Conclusions

In line with results reported in Chapter 4, results suggested that grazing substantially increases numbers of soil *E. coli* and other coliforms. Results of the study also suggested that the effects of grazing sheep into ungrazed land, albeit to a relatively minor extent. Forage type, by contrast, appears to have little impact. Surprisingly, there seemed little evidence of grazing-induced soil erosion. The only major findings were that (a), in line with the AFP and SI results, grazing increases soil density and (b), in line with results from Chapter 4, grazing increases levels of soil NO_3^-.

CHAPTER 6: ENVIRONMENTAL TRANSMISSION OF *E. COLI* O157 BY YELLOW DUNG FLIES (*SCATHOPHAGA STERCORARIA*)

6.1. Introduction

Much literature implicates flies in the ecology of *E. coli* O157. Hancock *et al*. (1998), for example, isolated the strain in 1.1% to 6.1% of samples of cattle faeces, 1.1% of equine faeces, 3.1% of canine faeces, 0.5% of bird faeces, and 3.3% of fly samples. Similarly, in a study in the USA, Alam and Zurek (2004) found 2.9% of houseflies (*Musca domestica*) captured in cattle feed bunks tested positive for *E. coli* O157, and 1.4% of the flies captured in cattle feed storage sheds tested positive. Counts within the flies were high, with many flies having 1.5×10^5 CFU within them. These results suggest, as Alam and Zurek argue, that houseflies may play a role in the dissemination of *E. coli* O157 in cattle farms. Similarly, Keen *et al*. (2006), in a study of US animal fairs, isolated STEC O157:H7 in 11.4% of cattle, 1.2% of swine, 3.6% of sheep and goats, and 5.2% of flies. Similar results were obtained by Sanderson *et al*. (2006), though here the percentage of cattle faeces harbouring the strain was very high, at 28% while that of fly samples was relatively low, at 3.4%. Collectively, the results imply that flies may be frequent carriers of pathogenic *E. coli*. Due to their feeding and roaming nature, flies are therefore likely vectors of transmission and may transmit infection between feral and domestic animal populations, and from species to species (Environmental Protection Agency, 2005). Related to this, Ahmad *et al*. (2007) experimentally demonstrated cattle to cattle transmission of *E. coli* O157:H7 by houseflies. However, although the numbers of houseflies (around 400 per cow) were environmentally realistic, virtually all the flies in the experiment carried the strain, so,

as the authors note, the extent of transmission on farms, in which relatively few flies are infected, is unclear.

It has long been recognized that flies are important vectors of microbial pathogens. Ostrolenk and Welch (1942), for instance, state that houseflies were implicated in the typhoid outbreaks during the Boer War and the Spanish–American War, and that the flies were also implicated in the 1901 outbreak of diarrhoea in Southend-on-Sea, UK. It is, of course, intuitively obvious that flies could spread pathogenic bacteria from animal faeces to human food. However, the flies may also be important reservoirs of the pathogenic bacteria in their own right. Flies defecating on food could therefore be an important source of human infection, though fly regurgitation on food may also be important, as may simple contact between fly legs and food. Here note that Sanderson *et al*. (2006) observe that the ecology of pathogenic *E. coli* is complex, and is not yet fully understood.

Much research has centred on house flies. However, the same logic implicates *S. stercoraria*, of which there are several species and of which the yellow dung fly (*S. stercoraria*) is common throughout Europe, including the UK. The species is easily recognized because of its wasp-like appearance. It is also easily sexed: male flies of the species are larger than the females, and have hairier front legs and differ quite starkly in colour (Kraushaar and Blanckenhorn 2002). In contrast to house flies, little to no research appears to have been performed on the role of *S. stercoraria* in the transmission of disease. As a considerable proportion of their lives is spent on cattle dung, a proportion of which is known to harbour *E. coli* O157, it may be hypothesised that *S. stercoraria* are also potential vectors of the pathogen.

The life cycle of the species varies according to ambient conditions, but, typically during summer, it is 20–30 days. When raised in laboratory conditions of 20ºC and 70% humidity and 14 hours of daylight, eggs typically hatch after two days, after which emerge larvae (maggots). After five days the maggots pupate. Mature flies emerge after about 17 days, after which they live for a further 5–10 days (Blanckenhorn *et al.*, 2010).

6.2. Objectives

The objectives of the present study were two-fold:

(i) To determine relative concentrations of *E. coli* O157 and other coliforms within male and female *S. stercoraria* collected from sheep and cattle farm.

(ii) To determine the concentration of *E. coli* O157 transmission by *S. stercoraria* from farmland to water, solid material, vegetation, and soil. The following two hypotheses were agreed for the experiment:

(i) *S. stercoraria* contain significant number of *E. coli* O157 in their body organs and differ over sex.

(ii) *S. stercoraria* play an important role in transmission of *E. coli* O157 to surrounding environment.

6.3. Materials and methods

6.3.1. Sample collection from fields

The first part of the study was to screen fly and faecal samples collected in the field for the named micro-organisms. Samples comprised sheep faeces, cattle faeces, and *S. stercoraria*. All sheep (faeces and *S. stercoraria*) samples were collected from a field within Henfaes Research Station, during June and July 2012. All cattle (faeces and *S. stercoraria*) samples were collected from a cattle farm adjacent to Henfaes Research Station, alsoduring June and July 2012.

Several hundred *S. stercoraria* were collected from the farms by use of a sweep net; the flies were potted in a small autoclaved jar and transported to the laboratory within 2 hours of collection. Of those collected, a total of 500 (250 each from the sheep and cattle farms) were randomly (stochastically) selected for analysis.

Fresh sheep and cattle faeces were collected from both farms (n = 50 of each), each weighing approximately 20 g. Upon collection, each sample was immediately placed in a sterile plastic bag; labelled and sealed, and transported to the laboratory within two hours of collection. In the laboratory, samples were stored in a refrigerator at 4ºC until analysis (maximum of 24 hours of collection). They were then screened for *E. coli* O157, *E. coli* and coliforms as detailed in (Section 6.3.5).

6.3.2. Preparation of sheep faeces and flies in the laboratory

The second part of the study investigated the potential transfer of the above pathogens from *S. stercoraria* into the surrounding environment via a laboratory study. Sheep faecal samples were collected as described above, with approximately 20 g from ten samples pooled to generate a sample of 200 g. This was repeated so that there were

six × 200 g portions (three controls, three experimental); each of which was placed in a box (dimensions 50 cm × 40 cm × 40 cm). The purpose was to allow time for any fly eggs within the faeces to hatch. In the experimental boxes, the faeces were inoculated with *E. coli* O157 (see below). Each box was placed in a growth cabinet (Sanyo, Gallenkamp Plc., UK) (set at 20ºC, humidity of 70%, and a 14 hours photo-period (Figure 6.1).

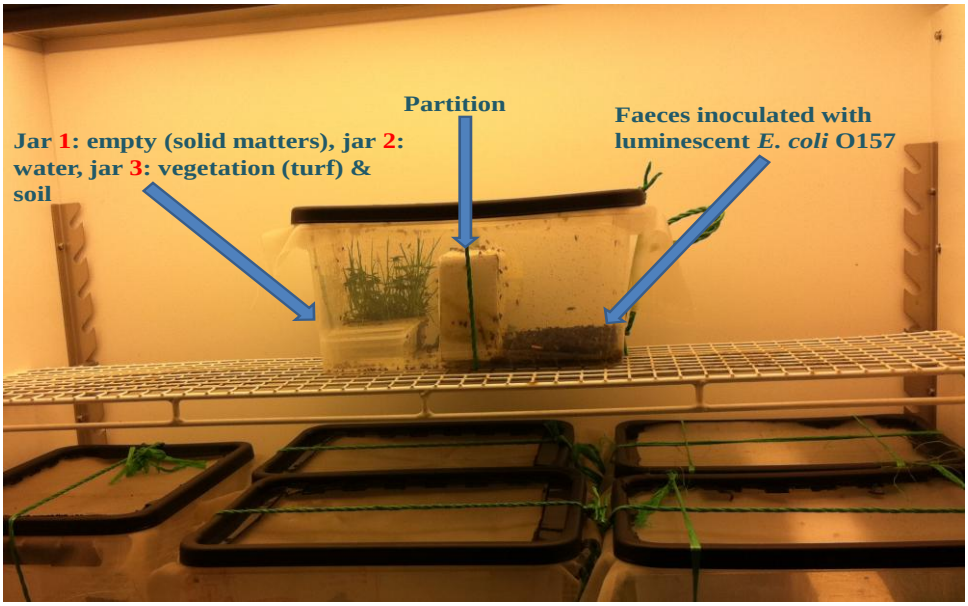

Figure 6.1. Experimental layout.

Each box was divided in two by a partial barrier, with the sheep dung (200 g) placed on one side of the barrier. On the other side were three jars (each of 5cm diameter and 5cm depth). One jar was empty; one contained sterile distilled water (45ml); and one contained vegetation (turf) and soil; the vegetation filled the jar to its top. When the eggs within the dung hatched, maggots could climb over the barrier and, later, when flies emerged they could climb or fly over the barrier. It was anticipated that water might evaporate from the jars and that, if so, each jar was to be topped up with water; however, such evaporation did not occur. The faecal sample within each box was mist-sprayed daily to keep it moist. If flies were visible on the grass in the turf sample,

the grass was harvested and immediately analysed. Boxes were re-randomised daily. The boxes remained in the growth cabinet (26 days).

6.3.3. Preparation of E. coli O157 *and inoculation of sheep faecal samples*

E. coli O157 were prepared by culturing a non-toxigenic strain in Luria Bertani broth (Difco, Oxford, UK) for 18 hours at 37ºC (150 rev min^{-1}). The strain (no. 3704) has been shown to lack both toxin activity and toxin genes (Campbell *et al*., 2001), but to resemble other O157 strains in all important respects (Ritchie *et al*., 2003). Sterile ¼-strength Ringer's solution was then used to wash the bacteria three times. The bacteria were then concentrated by centrifugation using the procedure described by Avery *et al*. (2005). Two ml of the innoculum were added to each 200 g portion of experimental dung at a concentration of approximately 8.25 $\log_{10}$ CFU g^{-1}. Care was taken to smear the suspension over the entire surface of each sample.

6.3.4. Collection of laboratory samples

Samples comprised water, solid (empty jars), vegetation, and soil from the second side of each experimental box. Samples were collected on day 26; five days after flies had first been visible on the second side on the boxes. Water samples were transferred to a sterile 25 ml test tube. Grass samples were cut from the top 1 cm of grass above the turf, weighed and placed in a stomacher bag, and shaken in a Lab Blender 400C, as described in Chapter 3. Soil samples were collected from the top surface of turf; each weighed 5 g.

All solid samples (empty jars) were collected by adding 5 ml ¼-strength Ringer's solution to each jar. Each jar was then closed and hand shaken. Each sample was then transferred to a sterile 25 ml test tube.

6.3.5. Bacterial testing

6.3.5.1. FIELD SAMPLES TESTING

6.3.5.1.1. Enumeration of coliforms and E. coli from fly samples

The sex of each fly was noted (by, as indicated, size, colour, and hairiness of front legs). Samples were then analysed in two stages: (a) *E. coli* and other coliforms on the fly's body (wings, legs, etc.) (*before crushing*), and (b) inside and on the fly's body (mouth parts, intestines, etc.) (*after crushing*).

For counts of the bacteria on each fly's body, the fly was placed in a 2 ml tube together with 1 ml of ¼-strength Ringer's solution (Oxoid). The tube was sealed and placed on a Vortex machine (Vortex-Genie 2, NY, USA) for 30 seconds. Thereafter, serial dilutions were prepared and 1 ml then placed on a Petrifilm plate and incubated overnight at 37ºC. For counts of the bacteria inside and on each fly's body the procedure was the same, save that the fly was crushed when added to the ¼-strength Ringer's solution and 1.5 ml of the solution was used. These counts were individual, so each fly had an external count of the bacteria and an internal and external count.

6.3.5.1.2. Isolation of E. coli O157 *from field samples*

Flies and field faecal samples were analysed for *E. coli* O157 by enrichment followed by immunomagnetic separation (IMS) (Ogden *et al*., 2001). Each sample was placed in a sterile 25 ml test tube; 5 ml of mTSB was then added to the test tube and the sample crushed with a glass rod. Thereafter, treatment was enrichment followed by IMS, as described in Chapter 3.

6.3.5.2. TESTING OF LABORATORY SAMPLES

6.3.5.2.1. Isolation and enumeration of E. coli O157

Water samples: The procedure was direct prepared serial dilutions using ¼-strength Ringer's solution in a 1:9 ratio. The number of *E. coli* O157 was determined by the drop-plate method; 0.1 ml of the samples was spread onto CT-SMAC plates (Oxoid); these were then incubated at 37°C for 18 to 24 hours. Presumptive *E. coli* O157 colonies (non-sorbitol fermenting) were confirmed by agglutination with a latex test kit (DR0620, Oxoid)

Grass samples: The grass was weighed and added to a ¼-strength Ringer's solution in a 1:5 ratio, then shaken using a Vortex machine for 30 seconds. Thereafter, serial dilutions were prepared then plated and incubated as described for water.

Soil samples: The soil samples were weighed and added to a ¼-strength Ringer's solution in a 1:5 ratio, then shaken using a Vortex machine for 30 seconds. Serial dilutions were then prepared, plated and incubated as described for water.

Solid matter samples: The procedure was the same as that for water samples save that 5 ml of ¼-strength Ringer's solution was placed within the jar, a sterile lid was placed on the jar, and the jar then shaken by hand. The samples werepreparedserial dilutions using a ¼-strength Ringer's solution in 1: 9 ratio; the numbers of *E. coli* O157 were determined by the drop-plate method, as described for water.

Enumeration of E. coli: Samples were taken from the three control boxes and the three experimental boxes. The procedure was the same as that described for enumeration of *E. coli* O157, save that the samples were plated on a Petrifilm *E. coli* and coliform Count Plate (3M Microbiology, MN, USA). The plates were then

incubated at 37ºC for 24 hours in a Model 280 LMS cooled incubator (LMS Ltd., Kent, UK).

6.3.5.2.2. Metabolic activity (RLU)

The luminescence of *E. coli* O157 was measured from water, grass, soil, and solid matter samples from the control and experimental samples; 300 µl from each sample was placed on the microplate using a Tecan Infinite 200® PROluminometer (Tecan Austria GmbH, Gröig, Austria), which displayed results in RLU (relative light units).

6.3.6. Statistical analysis

All statistical analyses were performed using SPSS Statistics. Alpha was set at $p = 0.05$. Analysis comprised three parts. The first investigated coliform and *E. coli* CFU/fly. Counts were categorised by sex of fly and whether the count was made before or after crushing. All data were first logarithmically transformed (base 10).

The first part of the analysis used a mixed 2 × 2 × 2 ANOVA. The DV was type of bacteria (coliform counts and *E. coli* counts) (within measures); the IVs were sex (male or female) and crushing (before or after) (between measures).Data were checked for homogeneity of variance (Levene's test) and normal distribution (Shapiro–Wilk test). Results of the ANOVAs were then cross checked using non-parametric tests.

The second part of the analysis used a mixed 2 × 4 ANOVA. The DV (within measures) was type of *E. coli* (*E. coli* or *E. coli* O157). The IVs was location (water, solid, vegetation, and soil). Data were checked for violation of ANOVA assumptions in the same manner as that of the first part of the analysis.

The third part investigated the metabolic activity (RLU) within the four locations (soil, water, grass, and solid material). It used a 1 × 4 between measures ANOVA. The DV was RLU; the IV was location. Data were checked first for violation of parametric assumptions in the same manner as that described above.

6.4. Results

6.4.1. First ANOVA

There were 1000 data entries, 500 before crushing and 500 after crushing. Each entry noted *E. coli* and total coliform counts and the gender of each fly; as indicated, 250 flies were male and 250 female. Table 6.1 shows summary statistics.

The within measures test revealed a significant effect of type of bacteria, F (1, 996) = 0 367.33, $p < 0.0005$; the effect size was large, $\eta_p^2 = 0.27$, suggesting that type of bacteria explained about 25% of the variance. There were no significant interactions.

Between measures tests revealed a main effect of crushing, F (1, 996) = 1023.71, $p < 0.0005$; the effect size was large, η_p^2 =0.51, suggesting that crushing explained almost 50% of the variance. There was no effect of sex. However there was a significant interaction between sex and crushing, F (1, 996) = 5.98, $p = 0.006$; the effect size, however, was trivial, $\eta_p^2$0.008, suggesting that the interaction explained less than 1% of the variance.

Data seriously violated assumptions of ANOVA. Levene's tests suggested that variances for *E. coli* and coliforms were unequal ($p = 0.026$ and $p = 0.004$, respectively); Kolmogorov–Smirnov tests suggested that all distributions were non-normal ($p < 0.0005$ in each case; results of Shapiro–Wilk tests were similar).

Table 6.1. Mean numbers of fecal coliforms associated with *S. stercoraria* collected from sheep and cattle farms (CFU/fly)

	Faecal coliform count per fly						*E. coli* count per fly					
	Mean	Minimum	Maximum	Range	Median	*n*	Mean	Minimum	Maximum	Range	Median	*n*
Female (external)	3.29	0.95	4.93	3.98	3.38	250	3.18	0.85	4.88	4.04	3.25	250
Male (external)	3.24	1.48	4.64	3.17	3.25	250	3.13	1.30	4.57	3.27	3.15	250
Female (internal)	4.44	2.78	5.63	2.85	4.58	250	4.33	2.65	5.51	2.86	4.43	250
Male (internal)	4.61	2.88	6.53	3.65	4.62	250	4.50	2.78	6.48	3.70	4.50	250

A Wilcoxon's signed ranks test corroborated the within measures result of the ANOVA: the medians of *E. coli* and coliforms were different, $p < 0.0005$. Two independent samples median tests corroborated the between measures result: the medians of before crushing and after crushing were different for *E. coli* and for coliforms ($p < 0.0005$ in each case). Two independent samples median tests revealed no significant differences in sex; however, the results were close to significance ($p = 0.077$ for *E. coli; p* = 0.058 for coliforms).

Because of the serious violations of ANOVA assumptions, only non-parametric results are reported henceforth. Figure 6.2 shows the median values for CFU of coliforms and *E. coli* per fly before and after crushing.

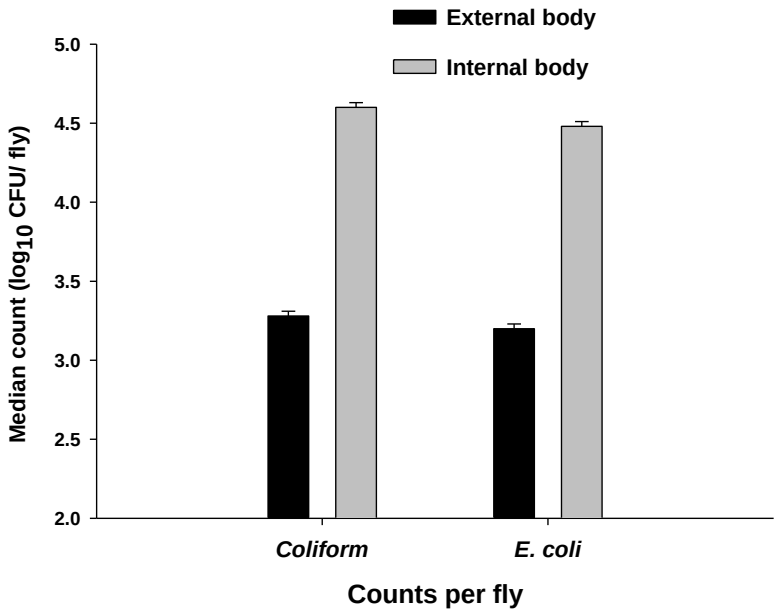

Figure 6.2. Median *E. coli* and coliform counts per fly before and after crushing.

The ranges were large. For *E. coli* before crushing the range was 0.85–4.88 CFU/fly; for after it was 2.65–6.48 CFU/fly. For coliforms before crushing the range was 0.95–4.93 CFU/fly; for after it was 2.78–6.53 CFU/fly.

The logarithmic results translate, as judged by median values, to about 80% of the coliform bacteria being *E. coli*, and about 95% of the bacteria residing in the internal organs of the flies. The results also suggest substantial differences in colony counts between individual flies, with some flies having 1000s more colonies than others.

6.4.2. Second ANOVA

There were 36 data entries. The within measures test revealed a significant effect of type of *E. coli*, F (1, 32) = 21.67, $p < 0.0005$; the effect size was large, η_p^2 =0.40, suggesting that type of *E. coli* explained almost 40% of the variance. The ANOVA also revealed a significant interaction between type of *E. coli* and location, F (3, 32) = 8.06, $p < 0.0005$; the effect size was large, η_p^2 =0.43, suggesting that type of the interaction explained about 40% of the variance.

The between measures test revealed a significant effect of location, F (3, 32) = 31.20, $p < 0.0005$; the effect size was large, η_p^2 =0.75, suggesting that location explained about 70% of the variance. Post-hoc tests (Tukey's HSD) revealed all locations were significantly different except water and grass. All other comparisons were highly significant: solid and soil, $p = 0.007$; grass and soil, $p = 0.002$; all other comparisons, $p < 0.0005$.

Results of Levene's tests suggested variances were approximately equal. However, results of Shapiro–Wilk tests suggested that *E. coli* data for solid material and for grass were non-normal ($p = 0.007$ and $p = 0.004$ respectively), as were *E. coli* O157 data for grass. Because of these slight violations of ANOVA assumptions, estimates of effect size and of means for solid material and grass should be viewed cautiously. Figure 6.3 shows environmental transmission of *E. coli* and *E. coli* O157 by *S. stercoraria*.

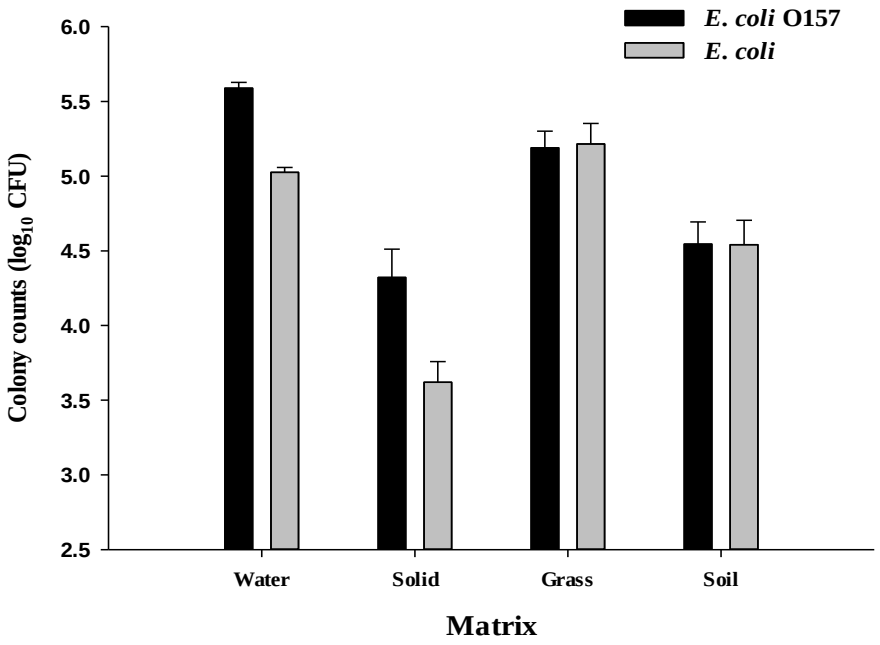

Figure 6.3. Environmental transfer of *E. coli* and *E. coli* O157 by *S. stercoraria*.

Measures: water in $\log_{10}$ CFU ml^{-1}; solid material in $\log_{10}$ CFU cm^3; grass and soil in $\log_{10}$ CFU g^{-1}. (Error bars denote SE).

The figure shows that bacteriacolonies were most common in water and on grass and least common on solid material; they were also relatively low in the soil. For grass and soil numbers of colonies of *E. coli* O157 and other *E. coli* were approximately equal, but numbers of *E. coli* O157 were substantially higher for water and for grass; this was the interaction.

6.4.3. Third ANOVA

There were three observations for each of the four locations. The ANOVA revealed a significant effect of location, F (3, 8) = 101.14, $p < 0.0005$; the effect size was large, η_p^2 =0.97, suggesting that location explained over 90% of the variance. Post-hoc tests (Tukey's HSD) revealed all locations were significantly different. All comparisons were highly significant: solid and soil, $p = 0.011$; grass and soil, $p = 0.003$;

water and grass, $p = 0.001$; all other comparisons, $p < 0.0005$. Figure 6.4 shows functional metabolic activity.

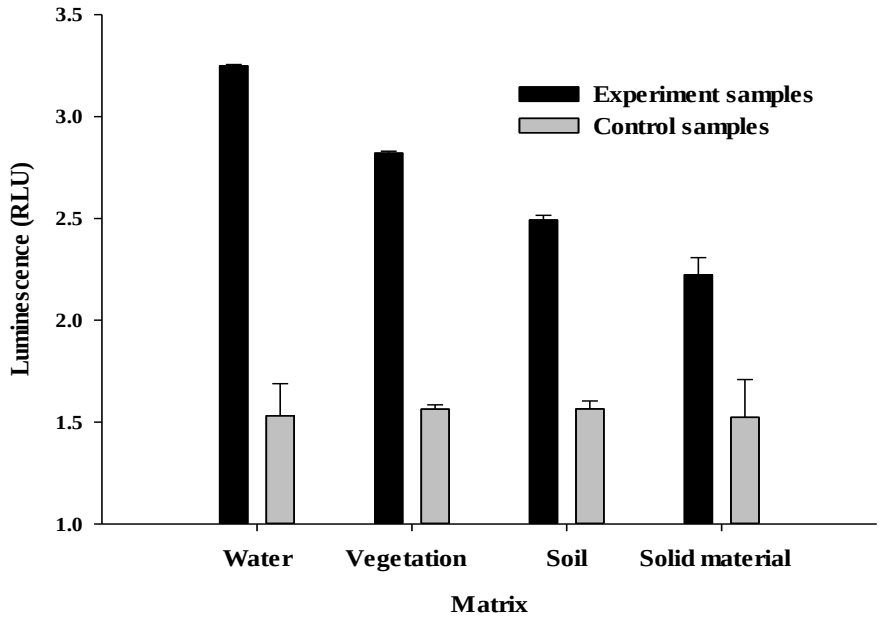

Figure 6.4. Functional metabolic activity of *E. coli* O157. Error bars denote SE.

The figure shows that bacterial metabolic activity mirrors numbers of colonies as revealed by the previous ANOVA. Metabolic activity was highest in water and lowest on solid material; it was also relatively low in soil. The only major difference was that metabolic activity was substantially lower in grass than in water.

6.5. Discussion

6.5.1. First ANOVA

Results of the first ANOVA and subsequent non-parametric tests corroborate the results of Chapter 3, which suggested that about 80% of sheep and cattle faecal coliform bacteria were *E. coli*. Given that *S. stercoraria* live on animal faeces, it is unsurprising that the proportion of *E. coli* to other coliforms found in and on them was virtually the

same. However, results of the present study suggest a lower proportion of *E. coli* relative to other coliforms than do other studies. Generic *E. coli* appear widespread throughout the animal kingdom, comprising 90% to 100% of all coliforms in faeces of eight domestic animal species, and approximately 97% of the total coliforms in human faeces (Health and Welfare Canada 1992; Neill 2004).

Results also suggest substantially higher numbers of *E. coli* and other coliforms residing in the internal organs of the *S. stercoraria* than on their external organs: about 95% of the bacteria, as judged by median values, are internal. This result is consistent with Kobayashi *et al*.'s (1999) suggestion that transmission of *E. coli* O157 is bioenhanced. If, as seems plausible, *S. stercoraria*, like house flies, carry *E. coli* O157 (Sasaki *et al*., 2000), they may be active carriers of them.

Results of the present study suggest no substantial differences in coliform counts, including *E. coli*, between the sexes of the *S. stercoraria*. True, the ANOVA suggested a significant interaction between sex and internal versus external counts (with female flies having higher external counts). However, as indicated, the ANOVA was flawed and, in any event, the associated effect size was trivial (less than 1%). Moreover, neither the ANOVA nor the non-parametric test suggested any overall difference between the sexes.

Overall, the results suggest that *S. stercoraria* carry substantial reservoirs of pathogenic *E. coli*. Whether they do is a subject for future research. How much they are a threat to human health also needs to be determined. On the one hand, because, other than agricultural workers and similar people, most people plausibly have less contact with *S. stercoraria* than with house flies, so *S. stercoraria* may represent less of a menace. On the other hand, because of their eating habits, and because, plausibly, they

carry more pathogenic bacteria, they may represent more of a menace. Moreover, they may also transmit the pathogens to livestock and diverse areas of the environment—water and soil, for instance. In short, *S. stercoraria* may be important agents in the transmission of pathogenic bacteria, though this has yet to be determined experimentally.

6.5.2. Second ANOVA

The ANOVA revealed a significant difference between type of bacteria, with *E. coli* O157 being roughly twice as prevalent, as judged by means, as other coliforms. This is unsurprising in that the faecal matter was heavily inoculated with *E. coli* O157. However, it does demonstrate that yellow *S. stercoraria* are capable of transmitting pathogenic *E. coli*.

The location results are more important. Transmission was most evident in water and grass. The *E. coli* O157 were roughly 20 times more prevalent in water than on solid material and roughly 7 times more prevalent in grass than in solid material. The *E. coli* O157 were also relatively uncommon in soil, with colony counts being only about 50% higher in soil than on solid matter. The relatively low transmission to soil is slightly surprising; as indicated, the grass was repeatedly watered, so one would expect some transmission via seepage; relatively little such transmission, evidently, occurred.

The high level of transmission to water warrants explanations. The flies may have entered the water in order to drink, and, if they are similar to house flies and have large reservoirs of *E. coli* O157 in their mouth parts (Kobayashi *et al*., 1999), such transmission may easily have occurred orally; it is also possible that *E. coli* O157 on the exterior organs of the flies may have become easily dislodged in water. In any event, the result suggests that *S. stercoraria* may transmit pathogenic bacteria to water sources. In

this regard, the dispersal range of the flies may be important. Evidence from house flies (Broce, 1993) suggests that this may be large; normally house flies have a dispersal range of 0.5 to 2 miles, but this may sometimes be as great as 20 miles. If *S. stercoraria* are similar to house flies, such would suggest that they could infect drinking troughs, lakes, puddles, and similar over vast areas. Further, such infected water sources could pass the pathogens to livestock, wildlife, and humans and in so doing magnify the transmission. Such direct and indirect transmission is a subject for future research. The same be said of *S. stercoraria* transmission of pathogenic bacteria to vegetation. Results of the present experiment suggest that the flies frequently land on vegetation, not on solid surfaces. As indicated, *E. coli* O157 were seemingly more readily transmitted to water and solid material than the other *E. coli*. Determining the reason for this is a matter for future research.

Finally, one may question the ecological validity of the present study. The faecal samples were heavily dosed with *E. coli* O157, and this dose may have been substantially higher than that which occurs naturally. Also, the flies had only four options as to which location they were to land; in the wild they may prefer to deposit themselves on other types of habitat (e.g., animal skin, rotting meat, milk), and the results, even if ecologically valid, pertain only to *S. stercoraria*, not all species of *S. stercoraria*. Nonetheless, results of the experiment strongly suggest, as does the immediately previous study (First ANOVA), that *S. stercoraria* are important transmitters of pathogenic bacteria.

6.5.3. Third ANOVA

Results substantially corroborated those of the second ANOVA. Microbial metabolic activity was over 10 times greater in water than on solid material. It was also

more than double in water than on grass and over five times higher in water than in soil. To an extent, this is unsurprising in that metabolism requires water. Nonetheless, with the partial exception of grass (for which microbial metabolic activity was significantly lower than water), the results mirrors that of the second ANOVA, and again suggests that *S. stercoraria* may transmit bacteria, pathogenic or other, through water supplies. Results also suggest that the bacteria are most active, and therefore reproduce most, when, if in the external environment, they are in water.

6.6. Conclusion

Results of the three studies reported in the present chapter suggest that *S. stercoraria* might carry, as hosts, substantial reservoirs of *E. coli* O157, that the flies might easily transmit the bacteria to the environment—especially to water and to vegetation—and that the bacteria may thrive in environments outside the internal organs of arthropods and other animals.

Of course, non-pathogenic *E. coli* are not necessarily the same as pathogenic strains, grass is not the same as other vegetation, laboratory conditions are not the same as field conditions, and so forth. Even so, the results reported suggest a need to research into the plausible threats to human well-being from *S. stercoraria* as they may be a neglected vector of human disease.

CHAPTER 7: OVERALL DISCUSSION AND CONCLUSIONS

Two caveats to what follows are necessary. First, the field studies described in previous chapters were all conducted within a small number of farms within a small area of North Wales. How much can therefore be generalised to other areas is a matter for speculation; future research is necessary. Here note that North Wales is atypical of much of the UK: it has high levels of rainfall, is lightly populated, has a landscape varying from the mountainous Snowdonia National Park through the relatively flat Isle of Anglesey (Ynys Môn); sheep farming within the area also predominates over cattle farming. The second caveat is that the study's findings, as regards practical import, are largely theoretical. This is because, despite repeated efforts, no *E. coli* O157 were detected in North Wales cattle or sheep faeces; neither were any detected in North Wales soil; and neither were any detected in North Wales *S. stercoraria*. This does not suggest, of course, that *E. coli* O157 are entirely absent from North Wales livestock, or that other pathogenic strains are similarly absent, but it does suggest, *prima facie*, that the pathogen is relatively rare in the area.

These caveats aside, results reported in the present thesis suggest areas in need for further research. The first of these is that generic *E. coli* appears more prevalent in sheep faeces than in cattle faeces; accounting for 25% of the variance in counts. This suggests that, if *E. coli* counts can be used as a proxy measure of danger of *E. coli* O157 infection, then sheep represent a more serious threat to human health than has hitherto been thought. In this regard, in its discussion of sources of food poisoning outbreak, Chapter 2 noted the large number of outbreaks attributed to people eating contaminated beef. Beef is often eaten rare (undercooked), and this may contribute to number of outbreaks. However, it is now becoming fashionable in the UK to eat lamb rare. This

change in culinary practice may constitute a risk to health. In any event, the threats posed by sheep—either in the form of live ones shedding pathogenic bacteria in the countryside, or in the form of partially cooked sheep meat transmitting the bacteria directly to human beings—requires further investigation.

Another finding suggestive of further research is the effect of land type on soil coliform counts. Counts were much lower in upland than in lowland. Moreover, this difference is not easily explained by differences in grazing intensity; this is because of the interactions between land type, land use, and season. Land use and seasonal factors made much more difference in lowland areas than in upland ones. This suggests, again if *E. coli* counts are an accurate proxy of *E. coli* O157, that lowland sheep farming constitutes a higher risk factor than upland sheep farming. Such findings should be borne in mind in devising prophylactic measures to help protect the public from infection. The research failed to detect substantial and unequivocal effects of soil chemistry and soil physical properties on soil *E. coli* populations. Because of problems with the data (violations of test assumptions), it is impossible to rule out such chemical and physical influences. These findings need to be corroborated in areas other than North Wales. The study didn't detect substantial soil erosion in fields used for grazing forage crops. The only impacts of grazing were increased soil density—unsurprisingly; the soil became more compacted—increased levels of NO_3^-, and increased counts of *E. coli* and other coliforms. The reason for this lack of substantial negative impact is unclear, but may lie in soil qualities, climatic conditions, and other variables peculiar to North Wales.

The final study of the research—the one that combined field work with laboratory experiment—demonstrated that *S. stercoraria* are hosts to large numbers of

E. coli and other coliforms; moreover the majority of these bacteria reside in the internal organs of the flies. Determining which internal organs these are is a subject for future research—if the flies are like house flies one should expect the bacteria to be present especially in the mouth parts and intestines. Further research should also determine whether the flies are passive carriers of the bacteria or, as seems possible, are important reservoirs in the own right. The experimental part of the study demonstrated that flies are capable of transmitting *E. coli* O157 to diverse areas of the environment (Figure 7.1), but especially to water and grass (and by implication, vegetation). The ecological validity of the study has to be corroborated by further research. In the laboratory, the animal faeces was heavily inoculated with *E. coli* O157; given the studies' repeated failure to detect *E. coli* O157 in the field, the flies' ability to play host to and transmit pathogenic strains should be tested in areas that have high infection rates of the bacteria.

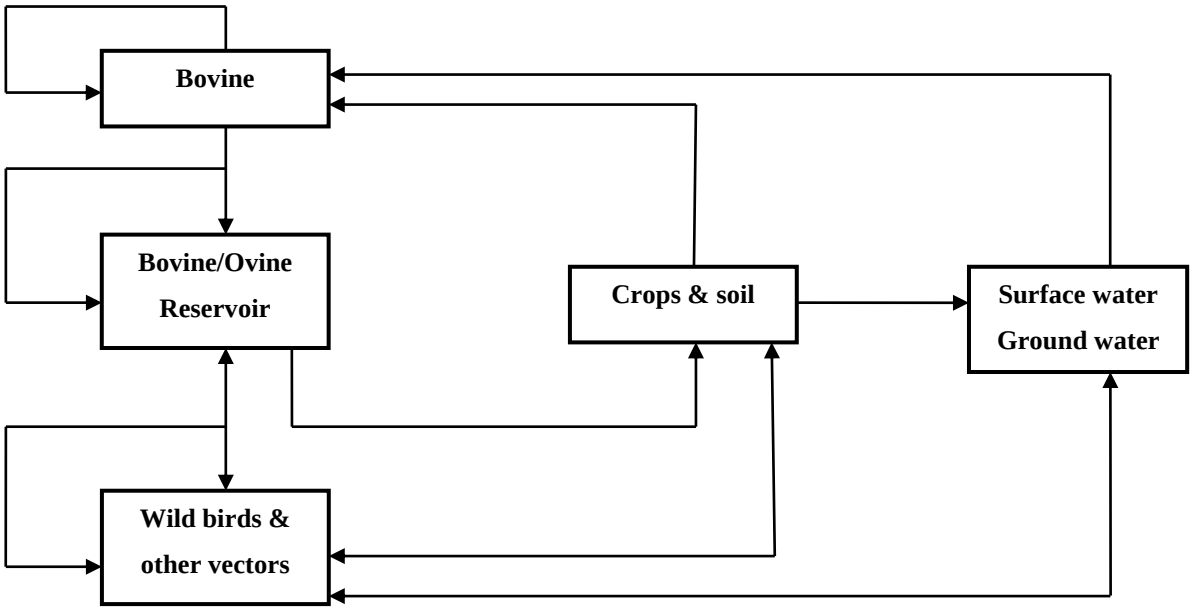

Figure 7. 1. Possible ecological cycle of *E. coli* O157 in the farm environment

Some of the results reported in this thesis are unsurprising, and merely support what is already known—for instance that grazing leads to increased soil *E. coli* and other coliform counts, or that it leads to greater soil density. Other results, however, are relatively novel. The most important of these are that sheep may be as important in the transmission of *E. coli* as cattle—indeed, they may be more important (which suggests a need to determine whether they are important transmitters of pathogenic *E. coli*); that grazing regime affects the degree of soil contamination and that coliforms can reside in soil many years following the removal of livestock; that *S. stercoraria* are hosts to large numbers of coliform bacteria, the majority of which are *E. coli*; and that the flies seem capable of transmitting pathogenic strains to the environment, especially to water and to grass. These results may form the basis for much future research.

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APPENDIX

Table A 1. Mean count of coliforms in sheep and cattle faeces.

Sex	Month	Season	Mean	*SD*	*SE*	*n*
Sheep	September	Autumn	5.92	0.47	0.09	30
		Total	5.92	0.47	0.09	30
	October	Autumn	5.81	0.48	0.07	45
		Total	5.81	0.48	0.07	45
	November	Autumn	6.29	0.40	0.06	45
		Total	6.29	0.40	0.06	45
	December	Winter	6.71	0.05	0.01	30
		Total	6.71	0.05	0.01	30
	January	Winter	6.17	0.36	0.05	45
		Total	6.17	0.36	0.05	45
	February	Winter	6.07	0.33	0.05	45
		Total	6.07	0.33	0.05	45
	March	Spring	6.18	0.27	0.05	30
		Total	6.18	0.27	0.05	30
	April	Spring	5.95	0.40	0.07	30
		Total	5.95	0.40	0.07	30
	May	Spring	5.72	0.49	0.09	30
		Total	5.72	0.49	0.09	30
	June	Summer	5.79	0.35	0.05	45
		Total	5.79	0.35	0.05	45
	July	Summer	5.98	0.40	0.07	30
		Total	5.98	0.40	0.07	30

	August	Summer	5.84	0.56	0.08	45
		Total	5.84	0.56	0.08	45
	Total	Autumn	6.02	0.50	0.05	120
		Winter	6.27	0.40	0.04	120
		Spring	5.95	0.44	0.05	90
		Summer	5.86	0.46	0.04	120
		Total	6.03	0.47	0.02	450
Cattle	September	Autumn	4.45	0.56	0.08	45
		Total	4.45	0.56	0.08	45
	October	Autumn	5.09	0.41	0.08	30
		Total	5.09	0.41	0.08	30
	November	Autumn	4.21	0.74	0.14	30
		Total	4.21	0.74	0.14	30
	December	Winter	4.80	0.38	0.06	45
		Total	4.80	0.38	0.06	45
	January	Winter	4.57	0.44	0.08	30
		Total	4.57	0.44	0.08	30
	February	Winter	4.54	0.33	0.06	30
		Total	4.54	0.33	0.06	30
	March	Spring	4.62	0.29	0.04	45
		Total	4.62	0.29	0.04	45
	April	Spring	4.56	0.36	0.05	45
		Total	4.56	0.36	0.05	45
	May	Spring	4.75	0.49	0.07	45
		Total	4.75	0.49	0.07	45
	June	Summer	5.15	0.45	0.08	30

		Total	5.15	0.45	0.08	30
	July	Summer	5.20	0.40	0.06	45
		Total	5.20	0.40	0.06	45
	August	Summer	4.42	0.33	0.06	30
		Total	4.42	0.33	0.06	30
	Total	Autumn	4.56	0.67	0.07	105
		Winter	4.66	0.40	0.04	105
		Spring	4.64	0.39	0.03	135
		Summer	4.96	0.53	0.05	105
		Total	4.70	0.52	0.02	450
Total	September	Autumn	5.04	0.89	0.10	75
		Total	5.04	0.89	0.10	75
	October	Autumn	5.52	0.57	0.07	75
		Total	5.52	0.57	0.07	75
	November	Autumn	5.46	1.17	0.14	75
		Total	5.46	1.17	0.14	75
	December	Winter	5.56	0.99	0.11	75
		Total	5.56	0.99	0.11	75
	January	Winter	5.53	0.88	0.10	75
		Total	5.53	0.88	0.10	75
	February	Winter	5.45	0.82	0.09	75
		Total	5.45	0.82	0.09	75
	March	Spring	5.24	0.82	0.09	75
		Total	5.24	0.82	0.09	75
	April	Spring	5.11	0.78	0.09	75
		Total	5.11	0.78	0.09	75

	May	Spring	5.14	0.68	0.08	75
		Total	5.14	0.68	0.08	75
	June	Summer	5.53	0.51	0.06	75
		Total	5.53	0.51	0.06	75
	July	Summer	5.51	0.55	0.06	75
		Total	5.51	0.55	0.06	75
	August	Summer	5.27	0.85	0.10	75
		Total	5.27	0.85	0.10	75
	Total	Autumn	5.34	0.93	0.06	225
		Winter	5.52	0.90	0.06	225
		Spring	5.17	0.76	0.05	225
		Summer	5.44	0.66	0.04	225
		Total	5.37	0.83	0.03	900

Table A 2. Mean count of *E. coli* in sheep and cattle faeces.

Sex	Month	Season	Mean	*SD*	*SE*	*n*
Sheep	September	Autumn	5.81	0.52	0.09	30
		Total	5.81	0.52	0.09	30
	October	Autumn	5.60	0.60	0.09	45
		Total	5.60	0.60	0.09	45
	November	Autumn	6.18	0.42	0.06	45
		Total	6.18	0.42	0.06	45
	December	Winter	6.62	0.05	0.01	30
		Total	6.62	0.05	0.01	30
	January	Winter	6.08	0.40	0.06	45

		Total	6.08	0.40	0.06	45
	February	Winter	5.96	0.34	0.05	45
		Total	5.96	0.34	0.05	45
	March	Spring	6.11	0.29	0.05	30
		Total	6.11	0.29	0.05	30
	April	Spring	5.87	0.42	0.08	30
		Total	5.87	0.42	0.08	30
	May	Spring	5.26	0.58	0.11	30
		Total	5.26	0.58	0.11	30
	June	Summer	5.64	0.43	0.06	45
		Total	5.64	0.43	0.06	45
	July	Summer	5.87	0.42	0.08	30
		Total	5.87	0.42	0.08	30
	August	Summer	5.66	0.64	0.10	45
		Total	5.66	0.64	0.10	45
	Total	Autumn	5.87	0.57	0.05	120
		Winter	6.17	0.42	0.04	120
		Spring	5.75	0.57	0.06	90
		Summer	5.71	0.52	0.05	120
		Total	5.88	0.55	0.03	450
Cattle	September	Autumn	4.37	0.59	0.09	45
		Total	4.37	0.59	0.09	45
	October	Autumn	5.02	0.43	0.08	30
		Total	5.02	0.43	0.08	30
	November	Autumn	4.14	0.75	0.14	30
		Total	4.14	0.75	0.14	30

	December	Winter	4.73	0.40	0.06	45
		Total	4.73	0.40	0.06	45
	January	Winter	4.51	0.46	0.08	30
		Total	4.51	0.46	0.08	30
	February	Winter	4.47	0.33	0.06	30
		Total	4.47	0.33	0.06	30
	March	Spring	4.53	0.30	0.04	45
		Total	4.53	0.30	0.04	45
	April	Spring	4.41	0.45	0.07	45
		Total	4.41	0.45	0.07	45
	May	Spring	4.68	0.50	0.07	45
		Total	4.68	0.50	0.07	45
	June	Summer	5.09	0.47	0.09	30
		Total	5.09	0.47	0.09	30
	July	Summer	5.05	0.46	0.07	45
		Total	5.05	0.46	0.07	45
	August	Summer	4.35	0.34	0.06	30
		Total	4.35	0.34	0.06	30
	Total	Autumn	4.49	0.69	0.07	105
		Winter	4.59	0.41	0.04	105
		Spring	4.54	0.44	0.04	135
		Summer	4.87	0.54	0.05	105
		Total	4.62	0.54	0.03	450
Total	September	Autumn	4.95	0.90	0.10	75
		Total	4.95	0.90	0.10	75
	October	Autumn	5.37	0.61	0.07	75

	Total	5.37	0.61	0.07	75
November	Autumn	5.36	1.16	0.13	75
	Total	5.36	1.16	0.13	75
December	Winter	5.48	0.98	0.11	75
	Total	5.48	0.98	0.11	75
January	Winter	5.45	0.88	0.10	75
	Total	5.45	0.88	0.10	75
February	Winter	5.36	0.81	0.09	75
	Total	5.36	0.81	0.09	75
March	Spring	5.16	0.83	0.10	75
	Total	5.16	0.83	0.10	75
April	Spring	5.00	0.84	0.10	75
	Total	5.00	0.84	0.10	75
May	Spring	4.91	0.60	0.07	75
	Total	4.91	0.60	0.07	75
June	Summer	5.42	0.52	0.06	75
	Total	5.42	0.52	0.06	75
July	Summer	5.38	0.60	0.07	75
	Total	5.38	0.60	0.07	75
August	Summer	5.14	0.84	0.10	75
	Total	5.14	0.84	0.10	75
Total	Autumn	5.23	0.93	0.06	225
	Winter	5.43	0.89	0.06	225
	Spring	5.02	0.77	0.05	225
	Summer	5.31	0.68	0.05	225
	Total	5.25	0.84	0.03	900

Table A 3. Mean count of coliform in soil under different conditions.

Land type	Land use	Month	Season	Mean	*SD*	*SE*	*n*
Lowland	Grazed	October	Winter	4.44	0.13	0.02	30
			Total	4.44	0.13	0.02	30
		November	Winter	4.44	0.17	0.03	30
			Total	4.44	0.17	0.03	30
		April	Summer	4.67	0.16	0.03	30
			Total	4.67	0.16	0.03	30
		May	Summer	4.68	0.16	0.03	30
			Total	4.68	0.16	0.03	30
		Total	Winter	4.44	0.15	0.02	60
			Summer	4.68	0.16	0.02	60
			Total	4.56	0.19	0.02	120
	Ungrazed	October	Winter	4.02	0.20	0.04	30
			Total	4.02	0.20	0.04	30
		November	Winter	4.04	0.27	0.05	30
			Total	4.04	0.27	0.05	30
		April	Summer	4.29	0.12	0.02	30
			Total	4.29	0.12	0.02	30
		May	Summer	4.31	0.10	0.02	30
			Total	4.31	0.10	0.02	30
		Total	Winter	4.03	0.23	0.03	60
			Summer	4.30	0.11	0.01	60
			Total	4.16	0.23	0.02	120
	Total	October	Winter	4.23	0.27	0.04	60
			Total	4.23	0.27	0.04	60

		November	Winter	4.24	0.30	0.04	60
			Total	4.24	0.30	0.04	60
		April	Summer	4.48	0.24	0.03	60
			Total	4.48	0.24	0.03	60
		May	Summer	4.49	0.23	0.03	60
			Total	4.49	0.23	0.03	60
		Total	Winter	4.23	0.28	0.03	120
			Summer	4.49	0.23	0.02	120
			Total	4.36	0.29	0.02	240
Upland	Grazed	October	Winter	3.69	0.28	0.05	30
			Total	3.69	0.28	0.05	30
		November	Winter	3.85	0.16	0.03	30
			Total	3.85	0.16	0.03	30
		April	Summer	4.15	0.19	0.04	30
			Total	4.15	0.19	0.04	30
		May	Summer	4.18	0.18	0.03	30
			Total	4.18	0.18	0.03	30
		Total	Winter	3.77	0.24	0.03	60
			Summer	4.17	0.18	0.02	60
			Total	3.97	0.29	0.03	120
	Ungrazed	October	Winter	3.50	0.43	0.08	30
			Total	3.50	0.43	0.08	30
		November	Winter	3.64	0.31	0.06	30
			Total	3.64	0.31	0.06	30
		April	Summer	3.95	0.20	0.04	30
			Total	3.95	0.20	0.04	30

		May	Summer	3.97	0.21	0.04	30
			Total	3.97	0.21	0.04	30
		Total	Winter	3.57	0.38	0.05	60
			Summer	3.96	0.20	0.03	60
			Total	3.76	0.36	0.03	120
	Total	October	Winter	3.59	0.37	0.05	60
			Total	3.59	0.37	0.05	60
		November	Winter	3.75	0.27	0.03	60
			Total	3.75	0.27	0.03	60
		April	Summer	4.05	0.22	0.03	60
			Total	4.05	0.22	0.03	60
		May	Summer	4.08	0.22	0.03	60
			Total	4.08	0.22	0.03	60
		Total	Winter	3.67	0.33	0.03	120
			Summer	4.06	0.22	0.02	120
			Total	3.87	0.34	0.02	240
Total	Grazed	October	Winter	4.07	0.44	0.06	60
			Total	4.07	0.44	0.06	60
		November	Winter	4.14	0.33	0.04	60
			Total	4.14	0.33	0.04	60
		April	Summer	4.41	0.32	0.04	60
			Total	4.41	0.32	0.04	60
		May	Summer	4.43	0.30	0.04	60
			Total	4.43	0.30	0.04	60
		Total	Winter	4.11	0.39	0.04	120
			Summer	4.42	0.31	0.03	120

		Total	4.26	0.38	0.02	240
Ungrazed	October	Winter	3.76	0.43	0.06	60
		Total	3.76	0.43	0.06	60
	November	Winter	3.84	0.35	0.05	60
		Total	3.84	0.35	0.05	60
	April	Summer	4.12	0.24	0.03	60
		Total	4.12	0.24	0.03	60
	May	Summer	4.14	0.24	0.03	60
		Total	4.14	0.24	0.03	60
	Total	Winter	3.80	0.39	0.04	120
		Summer	4.13	0.24	0.02	120
		Total	3.96	0.36	0.02	240
Total	October	Winter	3.91	0.46	0.04	120
		Total	3.91	0.46	0.04	120
	November	Winter	3.99	0.37	0.03	120
		Total	3.99	0.37	0.03	120
	April	Summer	4.27	0.32	0.03	120
		Total	4.27	0.32	0.03	120
	May	Summer	4.28	0.31	0.03	120
		Total	4.28	0.31	0.03	120
	Total	Winter	3.95	0.42	0.03	240
		Summer	4.28	0.31	0.02	240
		Total	4.11	0.40	0.02	480

Table A 4. Mean count of *E. coli* in soil under different conditions.

Land type	Land use	Month	Season	Mean	*SD*	*SE*	*n*
Lowland	Grazed	October	Winter	4.38	0.20	0.04	30
			Total	4.38	0.20	0.04	30
		November	Winter	4.35	0.17	0.03	30
			Total	4.35	0.17	0.03	30
		April	Summer	4.57	0.16	0.03	30
			Total	4.57	0.16	0.03	30
		May	Summer	4.58	0.16	0.03	30
			Total	4.58	0.16	0.03	30
		Total	Winter	4.37	0.18	0.02	60
			Summer	4.57	0.16	0.02	60
			Total	4.47	0.20	0.02	120
	Ungrazed	October	Winter	3.85	0.20	0.04	30
			Total	3.85	0.20	0.04	30
		November	Winter	3.96	0.24	0.04	30
			Total	3.96	0.24	0.04	30
		April	Summer	4.17	0.14	0.03	30
			Total	4.17	0.14	0.03	30
		May	Summer	4.19	0.15	0.03	30
			Total	4.19	0.15	0.03	30
		Total	Winter	3.90	0.22	0.03	60
			Summer	4.18	0.14	0.02	60
			Total	4.04	0.23	0.02	120
	Total	October	Winter	4.12	0.34	0.04	60

			Total	4.12	0.34	0.04	60
		November	Winter	4.15	0.28	0.04	60
			Total	4.15	0.28	0.04	60
		April	Summer	4.37	0.25	0.03	60
			Total	4.37	0.25	0.03	60
		May	Summer	4.38	0.25	0.03	60
			Total	4.38	0.25	0.03	60
		Total	Winter	4.13	0.31	0.03	120
			Summer	4.38	0.25	0.02	120
			Total	4.26	0.31	0.02	240
Upland	Grazed	October	Winter	3.63	0.21	0.04	30
			Total	3.63	0.21	0.04	30
		November	Winter	3.64	0.25	0.05	30
			Total	3.64	0.25	0.05	30
		April	Summer	4.05	0.19	0.04	30
			Total	4.05	0.19	0.04	30
		May	Summer	4.07	0.19	0.04	30
			Total	4.07	0.19	0.04	30
		Total	Winter	3.63	0.23	0.03	60
			Summer	4.06	0.19	0.02	60
			Total	3.85	0.30	0.03	120
	Ungrazed	October	Winter	3.54	0.40	0.07	30
			Total	3.54	0.40	0.07	30
		November	Winter	3.43	0.34	0.06	30
			Total	3.43	0.34	0.06	30
		April	Summer	3.84	0.20	0.04	30

			Total	3.84	0.20	0.04	30
		May	Summer	3.87	0.22	0.04	30
			Total	3.87	0.22	0.04	30
		Total	Winter	3.48	0.37	0.05	60
			Summer	3.86	0.21	0.03	60
			Total	3.67	0.35	0.03	120
	Total	October	Winter	3.58	0.32	0.04	60
			Total	3.58	0.32	0.04	60
		November	Winter	3.54	0.32	0.04	60
			Total	3.54	0.32	0.04	60
		April	Summer	3.95	0.22	0.03	60
			Total	3.95	0.22	0.03	60
		May	Summer	3.97	0.23	0.03	60
			Total	3.97	0.23	0.03	60
		Total	Winter	3.56	0.32	0.03	120
			Summer	3.96	0.23	0.02	120
			Total	3.76	0.34	0.02	240
Total	Grazed	October	Winter	4.00	0.43	0.06	60
			Total	4.00	0.43	0.06	60
		November	Winter	4.00	0.42	0.05	60
			Total	4.00	0.42	0.05	60
		April	Summer	4.31	0.31	0.04	60
			Total	4.31	0.31	0.04	60
		May	Summer	4.33	0.31	0.04	60
			Total	4.33	0.31	0.04	60
		Total	Winter	4.00	0.42	0.04	120

		Summer	4.32	0.31	0.03	120
		Total	4.16	0.40	0.03	240
Ungrazed	October	Winter	3.69	0.35	0.05	60
		Total	3.69	0.35	0.05	60
	November	Winter	3.69	0.39	0.05	60
		Total	3.69	0.39	0.05	60
	April	Summer	4.01	0.24	0.03	60
		Total	4.01	0.24	0.03	60
	May	Summer	4.03	0.24	0.03	60
		Total	4.03	0.24	0.03	60
	Total	Winter	3.69	0.37	0.03	120
		Summer	4.02	0.24	0.02	120
		Total	3.86	0.35	0.02	240
Total	October	Winter	3.85	0.42	0.04	120
		Total	3.85	0.42	0.04	120
	November	Winter	3.85	0.43	0.04	120
		Total	3.85	0.43	0.04	120
	April	Summer	4.16	0.32	0.03	120
		Total	4.16	0.32	0.03	120
	May	Summer	4.18	0.32	0.03	120
		Total	4.18	0.32	0.03	120
	Total	Winter	3.85	0.43	0.03	240
		Summer	4.17	0.32	0.02	240
		Total	4.01	0.41	0.02	480

Table A 5. Mean of MCin soil under different conditions.

Cropes type	Land use	Time	Mean	*SD*	*SE*	*n*
Kale	Grazed	01-Mar	31.30	0.95	0.24	4
		15-Mar	26.92	0.74	0.18	4
		30-Mar	17.14	1.44	0.36	4
		Total	25.12	6.26	0.52	12
	Ungrazed	01-Mar	31.67	2.08	0.52	4
		15-Mar	25.34	1.50	0.38	4
		30-Mar	16.96	2.00	0.50	4
		Total	24.65	6.52	0.54	12
	Total	01-Mar	31.48	1.51	0.19	8
		15-Mar	26.13	1.38	0.17	8
		30-Mar	17.05	1.62	0.20	8
		Total	24.89	6.25	0.26	24
Swede	Grazed	01-Mar	29.88	1.00	0.25	4
		15-Mar	26.98	0.35	0.09	4
		30-Mar	18.36	1.25	0.31	4
		Total	25.07	5.18	0.43	12
	Ungrazed	01-Mar	32.42	0.45	0.11	4
		15-Mar	26.33	2.16	0.54	4
		30-Mar	18.15	1.17	0.29	4
		Total	25.63	6.25	0.52	12
	Total	01-Mar	31.15	1.54	0.19	8
		15-Mar	26.66	1.48	0.18	8
		30-Mar	18.25	1.13	0.14	8

		Total	25.35	5.62	0.23	24
Total	Grazed	01-Mar	30.59	1.18	0.15	8
		15-Mar	26.95	0.54	0.07	8
		30-Mar	17.75	1.41	0.18	8
		Total	25.10	5.62	0.23	24
	Ungrazed	01-Mar	32.04	1.45	0.18	8
		15-Mar	25.84	1.80	0.23	8
		30-Mar	17.55	1.64	0.21	8
		Total	25.14	6.26	0.26	24
	Total	01-Mar	31.32	1.48	0.09	16
		15-Mar	26.39	1.41	0.09	16
		30-Mar	17.65	1.48	0.09	16
		Total	25.12	5.89	0.12	48

Table A 6. Mean of NO_3^- in soil under different conditions.

Cropes type	Land use	Time	Mean	*SD*	*SE*	*n*
Kale	Grazed	01-Mar	0.60	0.33	0.16	4
		15-Mar	19.45	7.32	3.66	4
		30-Mar	19.13	3.48	1.74	4
		Total	13.06	10.13	2.93	12
	Ungrazed	01-Mar	0.50	0.25	0.12	4
		15-Mar	3.27	0.77	0.38	4
		30-Mar	2.26	1.41	0.71	4
		Total	2.01	1.47	0.42	12

	Total	01-Mar	0.55	0.27	0.10	8
		15-Mar	11.36	9.90	3.50	8
		30-Mar	10.69	9.34	3.30	8
		Total	7.53	9.05	1.85	24
Swede	Grazed	01-Mar	0.95	0.13	0.06	4
		15-Mar	18.25	3.31	1.66	4
		30-Mar	17.70	3.02	1.51	4
		Total	12.30	8.71	2.51	12
	Ungrazed	01-Mar	0.64	0.07	0.04	4
		15-Mar	5.32	2.27	1.13	4
		30-Mar	2.45	1.40	0.70	4
		Total	2.80	2.45	0.71	12
	Total	01-Mar	0.79	0.19	0.07	8
		15-Mar	11.79	7.40	2.62	8
		30-Mar	10.08	8.44	2.98	8
		Total	7.55	7.92	1.62	24
Total	Grazed	01-Mar	0.77	0.30	0.10	8
		15-Mar	18.85	5.30	1.87	8
		30-Mar	18.41	3.11	1.10	8
		Total	12.68	9.25	1.89	24
	Ungrazed	01-Mar	0.57	0.18	0.07	8
		15-Mar	4.29	1.91	0.68	8
		30-Mar	2.36	1.30	0.46	8
		Total	2.41	2.01	0.41	24
	Total	01-Mar	0.67	0.26	0.07	16
		15-Mar	11.57	8.45	2.11	16

	30-Mar	10.38	8.61	2.15	16
	Total	7.54	8.41	1.21	48

Table A 7. Mean of pH in soil under different conditions.

Cropes type	Land use	Time	Mean	*SD*	*SE*	*n*
Kale	Grazed	01-Mar	6.49	0.04	0.02	4
		15-Mar	5.82	0.15	0.08	4
		30-Mar	5.42	0.05	0.03	4
		Total	5.91	0.47	0.14	12
	Ungrazed	01-Mar	6.44	0.11	0.05	4
		15-Mar	6.25	0.06	0.03	4
		30-Mar	5.99	0.15	0.08	4
		Total	6.23	0.22	0.06	12
	Total	01-Mar	6.46	0.08	0.03	8
		15-Mar	6.03	0.25	0.09	8
		30-Mar	5.70	0.33	0.12	8
		Total	6.07	0.39	0.08	24
Swede	Grazed	01-Mar	6.46	0.10	0.05	4
		15-Mar	5.75	0.12	0.06	4
		30-Mar	5.81	0.20	0.10	4
		Total	6.01	0.36	0.10	12
	Ungrazed	01-Mar	6.40	0.07	0.03	4
		15-Mar	6.03	0.05	0.02	4
		30-Mar	6.13	0.20	0.10	4

		Total	6.18	0.20	0.06	12
	Total	01-Mar	6.43	0.09	0.03	8
		15-Mar	5.89	0.17	0.06	8
		30-Mar	5.97	0.26	0.09	8
		Total	6.10	0.30	0.06	24
Total	Grazed	01-Mar	6.47	0.07	0.03	8
		15-Mar	5.79	0.13	0.05	8
		30-Mar	5.61	0.25	0.09	8
		Total	5.96	0.41	0.08	24
	Ungrazed	01-Mar	6.42	0.09	0.03	8
		15-Mar	6.14	0.13	0.04	8
		30-Mar	6.06	0.18	0.06	8
		Total	6.20	0.20	0.04	24
	Total	01-Mar	6.45	0.08	0.02	16
		15-Mar	5.96	0.22	0.05	16
		30-Mar	5.84	0.31	0.08	16
		Total	6.08	0.35	0.05	48

Table A8. Mean of EC in soil under different conditions.

Cropes type	Land use	Time	Mean	*SD*	*SE*	*n*
Kale	Grazed	01-Mar	141.68	130.73	65.37	4
		15-Mar	233.10	83.86	41.93	4
		30-Mar	281.50	62.23	31.12	4
		Total	218.76	106.31	30.69	12

	Ungrazed	01-Mar	45.08	23.03	11.51	4
		15-Mar	43.90	6.79	3.39	4
		30-Mar	52.10	27.08	13.54	4
		Total	47.03	19.28	5.56	12
	Total	01-Mar	93.38	101.08	35.74	8
		15-Mar	138.50	115.16	40.71	8
		30-Mar	166.80	130.42	46.11	8
		Total	132.89	115.23	23.52	24
Swede	Grazed	01-Mar	131.80	31.32	15.66	4
		15-Mar	268.83	61.70	30.85	4
		30-Mar	363.25	112.18	56.09	4
		Total	254.63	120.78	34.87	12
	Ungrazed	01-Mar	52.65	8.43	4.21	4
		15-Mar	65.80	5.15	2.57	4
		30-Mar	63.35	29.40	14.70	4
		Total	60.60	17.26	4.98	12
	Total	01-Mar	92.23	47.34	16.74	8
		15-Mar	167.31	115.84	40.96	8
		30-Mar	213.30	177.37	62.71	8
		Total	157.61	130.15	26.57	24
Total	Grazed	01-Mar	136.74	88.16	31.17	8
		15-Mar	250.96	70.78	25.03	8
		30-Mar	322.38	94.67	33.47	8
		Total	236.69	112.77	23.02	24
	Ungrazed	01-Mar	48.86	16.56	5.85	8
		15-Mar	54.85	12.97	4.58	8

		30-Mar	57.73	26.85	9.49	8
		Total	53.81	19.19	3.92	24
	Total	01-Mar	92.80	76.25	19.06	16
		15-Mar	152.91	112.57	28.14	16
		30-Mar	190.05	152.30	38.08	16
		Total	145.25	122.24	17.64	48

Table A 9. Mean of measurements soil Infiltration.

Cropes type	Land use	Time	Mean	*SD*	*SE*	*n*
Kale	Grazed	01-Mar	0.07	0.03	0.02	4
		15-Mar	0.14	0.15	0.08	4
		30-Mar	3.33	1.90	0.95	4
		Total	1.18	1.88	0.54	12
	Ungrazed	01-Mar	8.67	1.36	0.68	4
		15-Mar	7.71	0.85	0.43	4
		30-Mar	10.00	0.00	0.00	4
		Total	8.79	1.29	0.37	12
	Total	01-Mar	4.37	4.68	1.66	8
		15-Mar	3.93	4.08	1.44	8
		30-Mar	6.67	3.77	1.33	8
		Total	4.99	4.19	0.86	24
Swede	Grazed	01-Mar	0.16	0.11	0.06	4
		15-Mar	0.17	0.10	0.05	4
		30-Mar	1.05	0.31	0.16	4

		Total	0.46	0.47	0.14	12
	Ungrazed	01-Mar	7.46	3.14	1.57	4
		15-Mar	7.21	0.69	0.34	4
		30-Mar	9.67	0.24	0.12	4
		Total	8.11	2.04	0.59	12
	Total	01-Mar	3.81	4.41	1.56	8
		15-Mar	3.69	3.79	1.34	8
		30-Mar	5.36	4.62	1.63	8
		Total	4.28	4.17	0.85	24
Total	Grazed	01-Mar	0.11	0.09	0.03	8
		15-Mar	0.15	0.12	0.04	8
		30-Mar	2.19	1.76	0.62	8
		Total	0.82	1.39	0.28	24
	Ungrazed	01-Mar	8.06	2.33	0.82	8
		15-Mar	7.46	0.77	0.27	8
		30-Mar	9.83	0.24	0.08	8
		Total	8.45	1.71	0.35	24
	Total	01-Mar	4.09	4.40	1.10	16
		15-Mar	3.81	3.81	0.95	16
		30-Mar	6.01	4.13	1.03	16
		Total	4.64	4.15	0.60	48

Table A 10. Mean of Phosphorus in soil under different conditions.

crops type	Land use	Time	Mean	*SD*	*SE*	*n*
Kale	Grazed	01-Mar	4.53	1.11	0.56	4
		15-Mar	4.26	0.81	0.40	4
		30-Mar	4.04	0.90	0.45	4
		Total	4.28	0.88	0.25	12
	Ungrazed	01-Mar	4.22	0.99	0.50	4
		15-Mar	4.04	0.98	0.49	4
		30-Mar	3.98	0.99	0.50	4
		Total	4.08	0.90	0.26	12
	Total	01-Mar	4.38	0.99	0.35	8
		15-Mar	4.15	0.84	0.30	8
		30-Mar	4.01	0.88	0.31	8
		Total	4.18	0.88	0.18	24
Swede	Grazed	01-Mar	4.24	0.96	0.48	4
		15-Mar	4.04	1.10	0.55	4
		30-Mar	3.49	0.56	0.28	4
		Total	3.92	0.88	0.25	12
	Ungrazed	01-Mar	3.73	0.89	0.45	4
		15-Mar	3.94	1.06	0.53	4
		30-Mar	3.81	1.07	0.53	4
		Total	3.83	0.92	0.26	12
	Total	01-Mar	3.98	0.90	0.32	8
		15-Mar	3.99	1.00	0.35	8
		30-Mar	3.65	0.81	0.29	8

		Total	3.88	0.88	0.18	24
Total	Grazed	01-Mar	4.38	0.97	0.34	8
		15-Mar	4.15	0.90	0.32	8
		30-Mar	3.77	0.76	0.27	8
		Total	4.10	0.88	0.18	24
	Ungrazed	01-Mar	3.98	0.91	0.32	8
		15-Mar	3.99	0.95	0.33	8
		30-Mar	3.89	0.96	0.34	8
		Total	3.95	0.90	0.18	24
	Total	01-Mar	4.18	0.93	0.23	16
		15-Mar	4.07	0.90	0.22	16
		30-Mar	3.83	0.84	0.21	16
		Total	4.03	0.88	0.13	48