The Effects of Earthworms on Soil Structure in an Upland Grassland.

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Statement of Originality

I hereby confirm that this research was carried out by the undersigned alone and that all research material has been duly referenced and cited.

Christian Spring **05/12/2003**

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Abstract

As Charles Darwin first noted in 1881, earthworms through their burrowing and casting activities, play an important role in the creation and maintenance of soil structure. Burrowing activity leads to the reorganisation of voids and creation of macropores within the soil. This has implications for aeration and the flow properties of water through soils. Casting activity affects the structural stability of soil through the stabilisation of aggregates. The overall aim of this research project has been to investigate the effects of earthworm activity and diversity on void space and aggregation in an upland soil.

This research has been carried out as part of NERC's Thematic Programme on Soil Biodiversity. The field site was located on the Macaulay Land Use Research Institute's experimental farm at Sourhope in the Scottish Borders. Three experiments were designed to investigate the impact of earthworms on soil fabric, with each experiment representing an increased level of system complexity. The simplest experiment took place in a controlled environment and used an artificial soil and different earthworm treatments. The second level of system complexity used soil from Sourhope which had its structure removed, and then earthworm and liming treatments applied. The most complex experiment also used Sourhope soil and liming and earthworm treatments, except in this case the soil was undisturbed.

The effects of earthworms and liming on void space were characterised using saturated hydraulic conductivity to measure macroporosity, and image analysis to quantify total porosity and void size distribution. Aggregation was assessed through aggregate stability and point counts of earthworm excremental features.

The effect of earthworm inoculation in the simplest experiment led to the reorganisation of voids through increased abundance of voids > 2 mm^2 in area, and

decreases in the proportion of voids with an area < 2 mm^2. No significant effects were observed on aggregate stability. The effect of liming in the experiment using disturbed soil was to increased abundance of voids > 2 mm^2. No significant effects were observed on aggregation due to either liming or earthworm inoculation. In the most complex experiment, neither liming nor earthworm inoculation led to changes in void space or aggregation, except for an increase in saturated hydraulic conductivity and therefore macroporosity due to earthworm inoculation.

The overall conclusions from this research were that as system complexity increased, then the effects of the treatments on void space and aggregation became more difficult to isolate. Nevertheless, it was clear that liming significantly affected void space through increased abundance of earthworms. Out of the two treatments applied to the Sourhope soil, liming had the strongest effect on both earthworm abundance and void space.

Chapter 1: Earthworm Ecology

1.1 Introduction

Earthworms are among of the most important macro-invertebrates found in the soil, and their interactions with soil are outlined in the next two chapters. Charles Darwin understood the importance of earthworms when he stated in his 1881 publication, *The Formation of Vegetable Mould through the Action of Worms,* "It may be doubted whether there are many other animals which have played so important a part in the history of the world, as have these lowly organised creatures" (p148).

Earthworms form part of the phylum Annelida, class Clitellata, sub-class Oligochaeta (worms which have few setae). Oligochaetes inhabit predominantly terrestrial or freshwater environments and in this thesis it is the terrestrial megadriles or earthworms that are being investigated. The key biological features of earthworms are:

- Earthworms are bilaterally symmetrical invertebrate coelomates whose bodies are both internally and externally segmented. Coelomate body structure is composed of two concentric tubes, with the space between filled with coelomic fluid.
- Their bodies are given mechanical strength through a hydrostatic skeleton.
- Earthworms move via peristaltic contractions of circular and longitudinal muscles acting against the coelomic fluid, which fills the space between the gut and the muscles.

- They can burrow through the soil by either ingesting the soil material or by forcing a way through existing voids.
- Earthworms are hermaphroditic and can reproduce either by cross-fertilisation or by parenthogenesis (in some species only). Cocoons (egg cases) are produced at the clitellum (saddle shaped region of glandular epidermal cells) and then deposited in the soil ready for hatching.
- Arranged around earthworm bodies are setae, which are chitinous bristles, used for griping onto the surrounding substrate during locomotion. The number and arrangement of setae varies between species and is used as a diagnostic feature in earthworm identification.

1.2 Ecological Classification of Earthworms

The classification of earthworms relies on the observation of certain morphological features that distinguish different species, such as snout type, number and pattern of setae, number of segments and the presence, distribution and pattern of genitals. The best key produced for the earthworm fauna of the British Isles is that of Sims and Gerard (1985).

One way earthworm species have been grouped together is according to their ecological strategies. The most common way to approach this has been to group species of earthworms according to their morphological, physiological, reproductive and behavioural differences in relation to their vertical stratification (Lee 1985). Lee (1985) also states that earthworms can be classified according to their feeding habits, i.e. detritivores which feed, mainly on plant litter/debris or dung, on or at the soil surface and geophages which feed deeper in the soil, and ingest large quantities of

soil. Most of the early work distinguished between two distinct groups, i) surface dwelling species and ii) soil dwelling species (Evans and Guild 1948). In light of the work of Lee (1959, 1985) on Megascolecidea in New Zealand this system was extended to three distinct groups, 1. Litter species, 2. Topsoil species, 3. Subsoil species. An ecological classification for European Lumbricidae was produced by Bouché(1977). This classification was similar to that of Lee (1985), although there were subtle differences, which can be attributed to the different groups of earthworms studied and the different environmental conditions in the geographical areas used. Bouché's classification has three groups, which are summarised in Table 1.1.

Table 1.1: General diagnostic features of the major ecological groups of European lumbricid earthworms as described by Bouche (1977).

Diagnostic characteristics	Epigeic species	Anecic species	Endogeic species
Food	Decomposing litter on the soil surface; little or no soil ingested	Decomposing litter on the soil surface some of which is pulled into burrows; some soil ingested	Mineral soil with preference for material rich in organic matter
Pigmentation	Heavy, usually both ventrally and dorsally	Medium-heavy, usually only dorsally	Unpigmented or lightly pigmented
Size of adults	Small-medium	Large	Medium
Burrows	None; some burrowing in upper few centimeters of soil by intermediate species	Large, permanent, vertical burrows extending into mineral soil horizon	Continuous, extensive, subhorizontal burrow, usually in the upper 10-15cm of soil
Mobility	Rapid movement in response to disturbance	Rapid withdrawal into burrow but more sluggish than epigeics	Generally sluggish
Longevity	Relatively short lived	Relatively long lived	Intermediate
Generation time	Shorter	Longer	Shorter
Drought survival	survives drought in the cocoon stage	Becomes quiescent during drought	Enters diapause in response to drought
Predation	Very high, particularly from birds, mammals and predatory arthropods	High, especially when they are at the surface; somewhat protected in the burrows	Low; some predation by ground-dwelling birds and predatory arthropods

Examples of British earthworms according to the above classification are (Lee 1985; Edwards and Bohlen 1996):

Epigeic: *Lumbricus rubellus, Dendrobaena octaedra, Lumbricus castaneus, Eisenia fetida*

Anecic: *Aporrectodea longa, Lumbricus terrestris*

Endogeic: *Aporrectodea caliginosa, Aporrectodea rosea, Allolobophora chlorotica, Octoclasion cyaneum*

There are some cases where some earthworm species do vary from Bouché's classification, for example Shaw and Pawluk (1986) suggested that *A. longa* behaved more like an endogeic worm than an anecic worm, in terms of the type of burrows produced. Also several authors have suggested that *L. terrestris* is an intermediate between the epigeic and anecic group, since it feeds on the surface almost exclusively on litter, whereas a true anecic species would be expected to mix the litter with mineral particles, therefore it could be classified as epianecic (Bouché 1972; Jegou *et al* 1998a; Jegou *et al* 1999). Neilson *et al* (2000) investigated $\delta^{13}C$ and $\delta^{15}N$ values in earthworms to test the validity of the functional classification. They found that functional classification of earthworms is site specific, although it was possible to clearly identify those earthworms which were humic formers (litter feeders) and humic feeders (soil organic matter feeders). This suggests that as Edwards and Bohlen (1996) stated, it is virtually impossible to formulate a globally functional ecological classification system.

For this research project, the Bouché (1977) system has been adopted as a basis for the ecological classification of earthworms. This system was chosen because it is the most widely used in the scientific literature, and was created using European lumbricid species, which was relevant to the species present at the field site

1.3 Effect of Environmental Factors on the Spatial and Temporal Distribution of Earthworms

1.3.1 Spatial Distribution

There are approximately 30 species of earthworms in the British Isles, although this figure has been increased by 'alien' invasions from other continents, i.e. those species that are considered to have been non-native (Sims and Gerard 1985). This figure is very low when compared to that of the earthworm inventory of other countries within Europe, for example France has about 180 species of earthworms (Bouché 1972 from Sims and Gerard 1985).

In Scotland, Boag *et al* (1997) found 14 species of earthworms, which is a decrease from the 19 as reported by Sims and Gerard (1985) for the UK. The most prevalent and numerous species found in Scotland by Boag *et al* (1997) were *Aporrectodea longa, Aporrectodea caliginosa* and *Lumbricus terrestris*. All the species recorded had a cosmopolitan distribution apart from *Aporrectodea nocturna* and *Lumbricus festivus*, which were confined to central and southern Scotland.

As well as earthworms having a marked spatial distribution on a regional or national scale, there are also differences between species both horizontally and vertically within soil. Most of the work to date that has investigated the horizontal distribution of earthworms has involved studying the aggregation of earthworm species, populations and associations (Edwards and Bohlen 1996). Strong correlations have been found to exist between the spatial distribution of earthworms and soil moisture content (Cannavacciuolo *et al* 1999). Generally, apart from soil pH and hydromorphy there is a lack of evidence for the influence of physico-chemical properties on the

spatial distribution of earthworms. However, some researchers have shown that food availability in both arable and grassland fields does influence the distribution of earthworms (Boyd 1957, 1958; Knight *et al* 1992). Satchell (1955) also noted that the distribution of the earthworms *Lumbricus castaneus* and *Aporrectodea rosea* showed an aggregated distribution in a pasture field whose habitat conditions were fairly uniform. It was hypothesised that this distribution was caused by a) the earthworms were reproducing faster than the offspring could disperse, and/or b) that the aggregation of earthworms was due to the coming together of adults at specific times of the year to reproduce. Whilst investigating the spatial distribution of earthworms and soil properties in an arable loess soil, Poier and Richter (1992) found that earthworms showed a spatial dependence over a range of 20-50 m. Moreover, they showed that the larger the species of earthworm the greater the heterogeneity there was in their spatial distribution. The same authors also observed correlations between earthworms and soil carbon content and aggregate density. It should be noted that there are still no satisfactory explanations for the distribution of earthworms in the environment, primarily due to the lack of understanding of which factors determine this distribution and the interactions between these factors (Nuutinen *et al* 1998) A further problem when studying the distribution of earthworms *in vivo* is the heterogeneous and variable nature of soil itself, as well as the relatively small scale over which they operate (Lee 1985; Edwards and Bohlen 1996).

Research has also been carried out on the rates and mechanisms for the dispersal of earthworms. The dispersal of earthworms can take two forms, firstly through active dispersal (where the earthworms migrate to other areas), or secondly by passive dispersal (where earthworms are transported by other agents such as cultivation, or

by cocoons being carried on the feet of other animals). Rates of dispersal for earthworms are generally c. 10m yr^{-1}, although this figure can fluctuate according to a) species or b) environmental conditions (Stockdill 1982; Hoogerkamp *et al* 1983; Stein *et al* 1992; Daniel *et al* 1996).

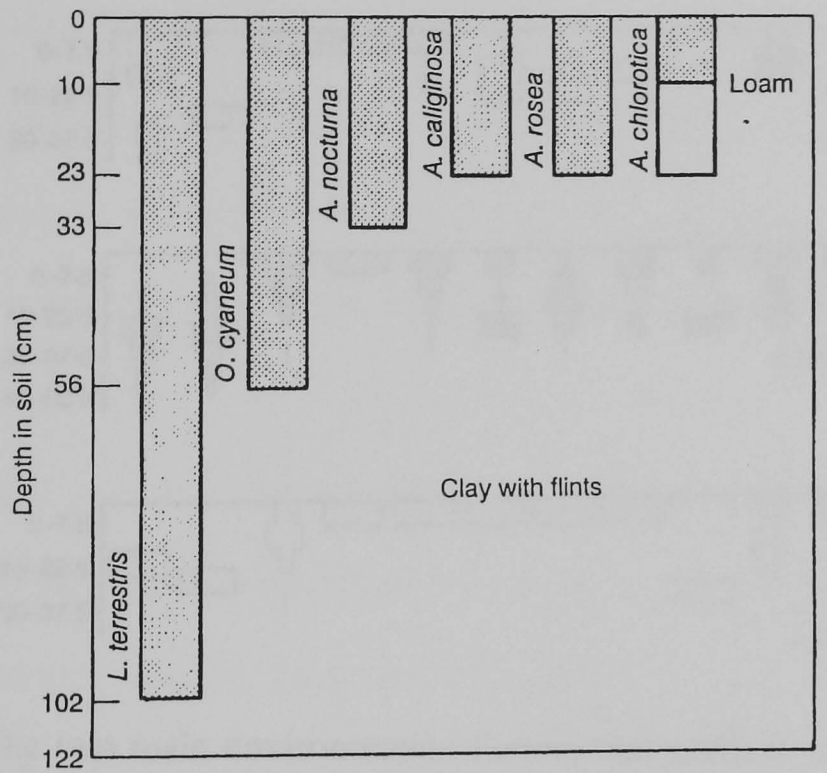


Figure 1.1: Vertical distribution of earthworms in a Rothamsted pasture (adapted from Satchell 1955 in Edwards and Bohlen 1996)

The vertical distribution of earthworms is the other important spatial dimension which has been studied extensively. Different species of lumbricids tend to live at different depths in the soil (Figure 1.1). The vertical distribution of earthworms in the soil is primarily related to their ecological function and strategy (Bouché, 1977). This distribution varies considerably between species at different times of year, i.e. it shows a high degree of seasonality. This seasonal effect was studied in England by Gerard (1967) who found that during cold periods most earthworms were deeper in the soil compared to when the soil was warmer (Figure 1.2).

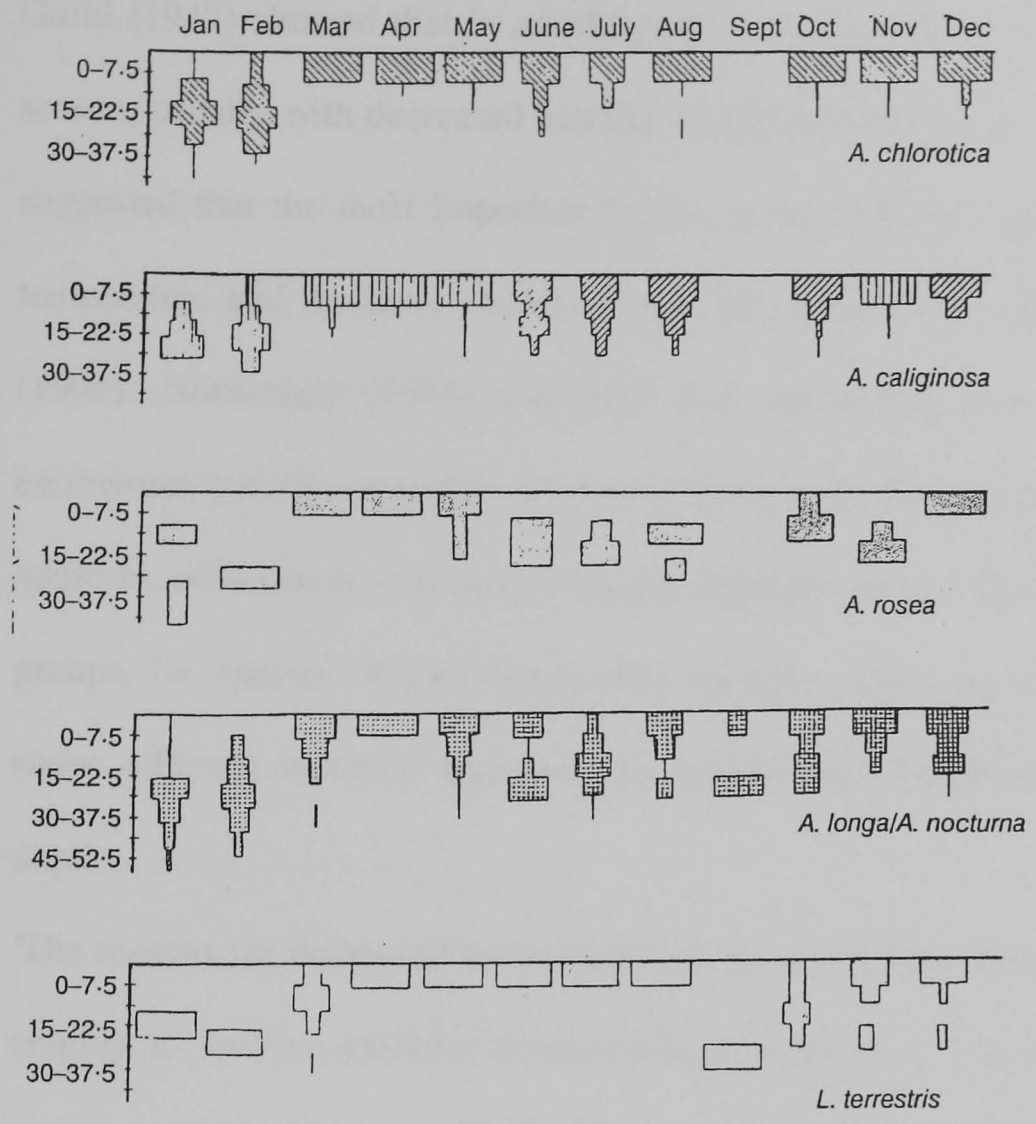


Figure 1.2: The depth of six species of earthworms in monthly soil samples from January to December 1959 (expressed as percentages for each species in each sample) from a pasture field. (Gerard 1967)

The two main environmental factors that seem to influence the vertical distribution of earthworms at various times of the year are soil temperature and soil moisture content (Cannavacciuolo *et al* 1999).

1.3.2 Temporal Distribution

Seasonality has the greatest effect on temporal variations in earthworm populations and activity. In general all earthworms show some sort of seasonality-activity effect. The depth at which earthworms can be found in the soil is affected by the variability in environmental conditions brought about by the different seasons. Seasonality affects the age structure of earthworms populations since it plays a key role in determining how many and when cocoons hatch, and how quickly the hatchlings develop (Evans and Guild 1948; Lee 1985; Edwards and Bohlen 1996). Evans and

Guild (1947) showed that in an old pasture the activity of earthworms varied with season, usually with decreased activity during the summer and winter months. They suggested that the most important factors governing this variation in activity were temperature and moisture content of the soil, which was confirmed by Clapperton (1999). Nordstrom (1975) indicated that seasonality had a major impact on earthworm activity, caused by changes in soil conditions and resource availability. In addition, differences were seen between different species from the three ecological groups, i.e. species of earthworms that are most active on or close to the surface show different seasonal responses to species that predominantly live at greater depths.

The reasons for decreased earthworm activity result from the strategies earthworms employ to survive extreme environmental conditions, (e.g. drying of the soil in summer and the freezing of the soil in winter). Usually when conditions become harsher, earthworms go deeper in the soil where temperature and moisture content remain acceptable for longer. During these adverse periods, earthworms exhibit two kinds of resting or inactive behaviour (aestivation), diapause or quiescence, the definitions of which are taken from Edwards and Bohlen (1996, p61):

'Three states of such inactivity can be distinguished:

Quiescence- where earthworms respond directly to adverse conditions, particularly drought and high temperatures, and becomes active again as soon as conditions become favourable. Bouché (1972) distinguished two types of quiescence: a) anhydrobiosis – a response to dehydrative conditions; and b) hibernation – a response to low soil temperatures. Earthworms coil in small cells below the frozen layers.

Facultative diapause- this is also caused by adverse environmental conditions, but does not terminate until a certain critical time after conditions become favourable.

Obligatory diapause- this occurs at a certain time or times each year, independent of current environmental conditions but usually in response to a certain sequence of environmental changes or to some internal mechanism. These stimuli are usually such that adverse conditions tend to occur during the period of diapause.'

As well as these seasonal effects, many species of earthworms show a diurnal variation. For example, Edwards and Bohlen (1996) found that *Lumbricus terrestris* tended to be active from 6 pm to 6 am although again the exact timing did vary with season. These authors suggested that this diurnal pattern of activity is intrinsic and at least partially independent of temperature and light.

1.3.3 Effects of Soil Moisture on Earthworms

The prevention of water loss is very important for earthworms since between 75 and 90% of their body weight is water (Lee 1985; Edwards and Bohlen 1996). The water balance of earthworms is crucial because of their cutaneous respiratory system, which requires the maintenance of a moist body surface, and excretion of nitrogen as ammonia and urea, which are toxic and therefore need copious losses of hypertonic urine, which means that the earthworms lose large quantities of body water (Lee 1985). However, earthworms can employ a number of strategies to prevent excessive desiccation, although most species can survive quite extreme water loss, e.g. *L. terrestris* and *A. chlorotica* can lose 70% and 75% of their total body water and still survive (Roots 1956).

Not all earthworm species have the same moisture requirements, e.g. *A. caliginosa* can withstand drier conditions than *Octolasion cyaneum*. It has also been shown that the tolerances to different moisture conditions vary within species geographically, e.g. *A. caliginosa* in Europe tends to go into diapause at a soil moisture content below 25-30%, and does not survive below 20% soil moisture, however the same species in the seasonally arid regions of Argentina can remain active with a soil moisture content as low as 15% (Edwards and Bohlen 1996).

Different earthworm species have developed different strategies for surviving in dry soils, some migrate down the soil profile, e.g. *L. terrestris, Aporrectodea longa* and *E. fetida* (Edwards and Bohlen 1996). Another species, *A. caliginosa,* does not really go deeper in the soil but is still capable of surviving fairly dry conditions. Many of the earthworms that make up the *Aporrectodea* spp. are active in the upper zones of the soil (≈10 cm), but when the soil dries out they tend to migrate deeper (≈ >20 cm) where they aestivate tightly curled within spherical mucus-lined cells (Lee 1985).

During extended periods of rain certain species tend to leave the soil and can often be found on the surface, e.g. *Octolasion cyaneum*. Also *L. terrestris* can often be found at the surface at night, following rainfall (this is why this species is often referred to as 'night crawlers' in North America). A large number of earthworm species are able to survive for relatively long periods submerged in aerated water (anything up to between 30-50 weeks) (Edwards and Bohlen 1996). The two limiting factors that determine how long the earthworms could stay submerged are water uptake and the availability of oxygenated water. Even cocoons can hatch and the hatchlings survive for a short time in totally saturated soils (Roots 1956).

1.3.4 Effects of Soil Temperature on Earthworms

Temperature plays an important role in the activity, metabolism, growth, respiration and reproduction of earthworms (Edwards and Bohlen 1996). In fact temperature and moisture are usually inversely related, with high surface temperatures and dry soils having a larger detrimental effect on earthworms than low temperatures and waterlogged soils. However as Lee (1985) suggested, it is difficult to isolate the effect of temperature on earthworm behaviour and population dynamics:

- high temperatures are often associated with drought, and therefore with moisture stress,
- in the absence of moisture stress, earthworms can more easily extract water from soil as temperatures increase, because matric potential become more negative with increasing temperature,
- metabolic rates varies with body temperature (the Q_{10} for earthworms is ≈ 2, i.e. their metabolic rate increases *c.* 2 times for a 10°C increase in body temperature for temperatures ranging between 6°C-15°C Phillipson and Bolton 1976).

As temperature increases then so does the number of cocoons produced (Evans and Guild 1948), the time cocoons take to hatch is reduced and hatchlings grow to sexual maturity more quickly (Evans and Guild 1948). However, Edwards and Bohlen (1996, pp139) stated that 'temperature at which earthworms thrive best and which they prefer is not necessarily the same as that at which they grow fastest or are most active'.

As with soil moisture content, the optimum temperatures for earthworm activity and reproduction vary between species. For example, the optimum temperatures for *A.*

caliginosa and *A. longa* is between 10-15 °C, whilst for *Dendrobaena rubida* and *Lumbricus rubellus* this is 15-20 °C (Daughberger 1988 from Edwards and Bohlen 1996). As a general rule, the optimal temperature for the growth of indigenous Lumbricidae in Europe is between 10 and 15 °C Lee (1985). However, earthworms can become acclimatised to different temperatures, although this process is very gradual (Edwards and Lofty 1977).

1.3.5 Effects of Soil pH on Earthworms

Several authors have shown that pH can have a marked effect on the species diversity found in soils, since it appears that earthworms have particular pH thresholds above and below which they cannot survive. However, Edwards and Bohlen (1996) suggested that it is unclear as to whether it is decreased pH (or rather the increased H^+ concentration) that is directly effecting the earthworms, or whether the earthworms are being effected indirectly through other pH mediated processes (e.g. cation exchange capacity or metal availability). They concluded that a clear cause-effect relationship cannot easily be established between earthworms and pH, due to the influences of other external factors.

Earthworms have been classified according to their ability to withstand acid conditions. Satchell (1955) identified the pH tolerances of a number of British Lumbricidae (Figure 1.3). Piearce (1972) found that in soils from North Wales population density and earthworm biomass both decreased with increasing acidity, with the optimum pH seeming to be c. pH6. In addition, he observed that litter feeders tended to dominate acid sites, whilst topsoil feeding species dominated the other sites.

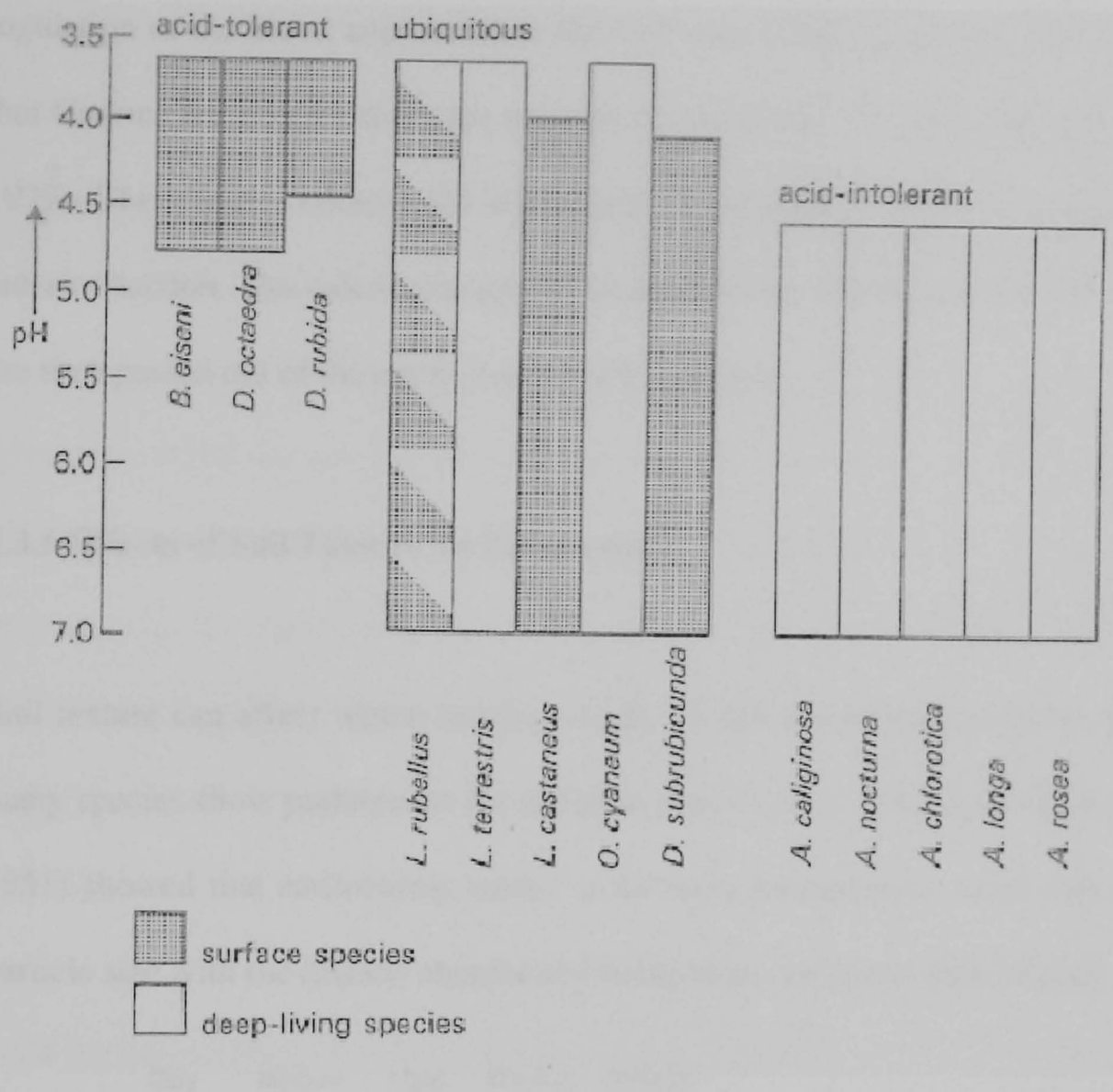


Figure 1.3: Classification of earthworms as a function of the pH of litter (after Satchell 1955)

This classification according to acid tolerance is not always satisfactory, since Lofs-Holmin (1986) found that several species that were classified as acid intolerant could actually survive in an acidic environment, (in this particular case the most notable variation was that of *A. caliginosa*, which was described as acid intolerant by Satchell (1955) but it was surviving in soils of pH less that 4.5). Again, as with soil moisture content and temperature, different species of earthworms have different abilities to survive in what would normally be thought of as non-viable environments for that species.

To ameliorate soil pH lime ($CaCO_3$) is added to the soil to raise pH, which in turn leads to increased earthworm abundance and activity (Edwards and Bohlen 1996). In addition to raising soil pH liming also increases Ca levels in the soil. Calcium is an

important cation for earthworms because it plays a key role in ionic and pH regulation of the blood and coelomic fluid (Prentø 1979). It has also been suggested that Ca ions are important for the removal of metabolic CO_2 from the blood (Prentø 1979). This process takes place in the calciferous glands, which are located in the anterior section. The calciferous glands excrete calcite spherulites into the gut which are then passed out of the earthworm in cast material.

1.3.6 Effects of Soil Texture on Earthworms

Soil texture can affect which and how many earthworm species occur in soil, since many species show preferences for different particle sizes. The work of Guild (1948, 1951) showed that earthworms tended to be more numerous in soils with a smaller particle size with the highest abundances being found in loamy soils (Figure 1.4)

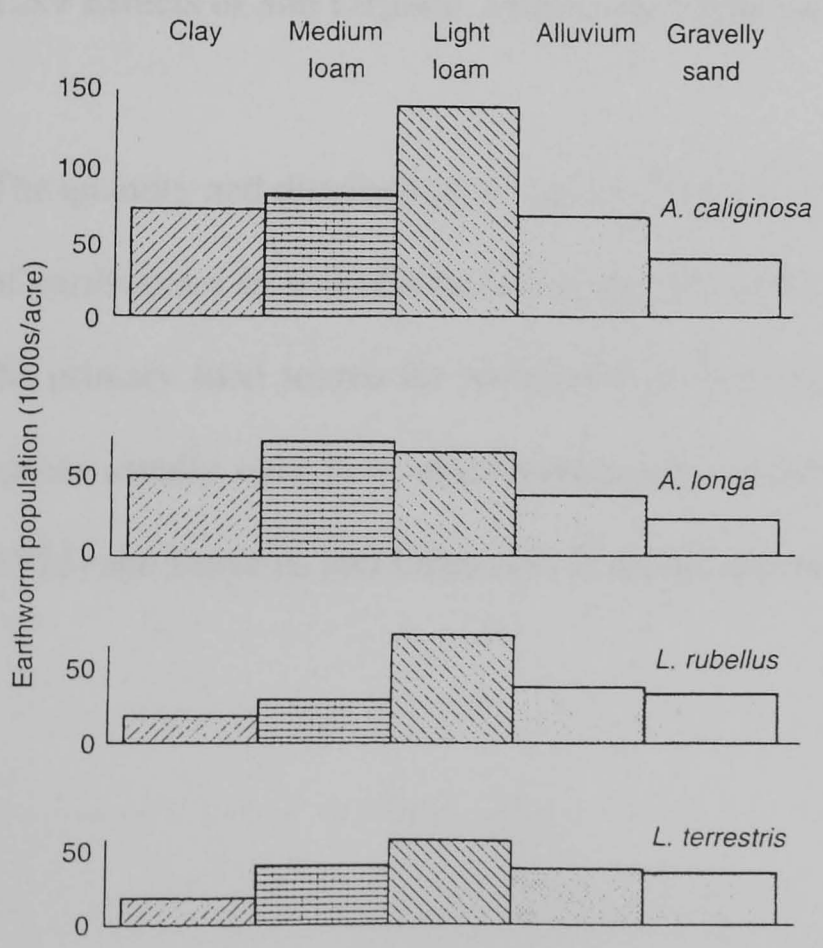


Figure 1.4: Density of earthworm populations (thousands/ha) in various soil types in Scotland (adapted from Guild 1948 in Edwards and Bohlen 1996).

The reason for this association of earthworms with small particle sizes may be due to the fact that coarser textures, such as sandy soils, may be too abrasive for earthworms to move through (Lee 1985), or the fact that geophagous earthworms can only ingest soil particles of a certain size, since they cannot ingest particles larger than their mouth or gut. As well as this, organic matter tends to be associated with the smaller soil fractions (Tisdall and Oades 1982), therefore earthworms will selectively ingest the smaller particle sizes.

Another way that soil texture influences earthworms is by indirect means, in that soil texture plays an important role in determining other soil properties (Edwards and Bohlen 1996). For example soil texture affects soil moisture relationships, nutrient status and cation exchange capacity, all of which would influence earthworm populations.

1.3.7 Effects of Soil Organic Matter on Earthworms

The quantity and distribution of organic matter influence the distribution and number of earthworms in soil (Edwards and Bohlen 1996) since organic matter/litter forms the primary food source for most earthworm species. Soils that are poor in organic matter usually only have small populations of earthworms. Work by both Satchell (1955) and Edwards and Lofty (1977) shows this trend quite clearly (Table 1.2).

Table 1.2: Earthworm populations in plots with and without dung (from Edwards and Bohlen 1996)

Species	1. Grassland. Park Grass, Rothamsted (Satchell 1955)		2. Arable land. Barnfield, Rothamsted (Edwards and Lofty 1977)	
	Unmanured	Dung	Unmanured	Dung
L. terrestris	13.1	22.5	0.23	10.8
L. castaneus	16	59.6	-	-
A. caliginosa	2.9	8	0.8	15.4
A. chlorotica	1.6	-	3.2	44.6
A. rosea	10	21.3	-	0.23
A. longa	-	-	0.46	1.8
A. nocturna	1.3	18.9	-	-
O. cyaneum	6.9	24.5	-	-
Total	51.8	154.8	4.69	72.83

1.3.8 Effects of Resource Quality and Quantity on Earthworms

Earthworms possess the ability to utilise a wide range of food sources, from ingesting plant material/litter, humified soil organic matter, organic exudates, and grazing on micro-organisms present in the soil, although the preference for each food type does vary between species (Lavelle 1988; Edwards and Bohlen 1996) . Several authors have suggested that earthworms will selectively graze on soil bacteria/actinomycetes (Brown 1995) and fungi (Bonkowski *et al* 2000). The type and amount of food available influences not only the size of earthworm populations, but also species diversity, and their rate of growth and fecundity (Edwards and Bohlen 1996). Different species of earthworm have their own food resource utilisation preferences, which generally corresponds with their ecological

classification (where litter tends to be of a higher quality in terms of nutrition than soil Lee 1985):

- Epigeic- Surface dwelling & litter feeder
- Anecic- Soil dwelling & litter and soil feeder
- Endogeic- Soil dwelling & soil feeding

The nutrient status of food resources is also important since earthworms will preferentially consume food of high nutrient status, e.g. fresh leaf litter, over lesser quality food, e.g. soil organic matter (Lavelle 1988). Linked to this is the palatability of the food source, because earthworms will consume more palatable food over that which they find less palatable. For example, certain types of litter are unpalatable to earthworms, e.g. the leaves of larch, spruce, oak and beech which are thought to contain high tannin contents, as compared to other types of litter which have high N contents and low secondary plant metabolite concentration (Lee 1985; Edwards and Bohlen 1996; Bonkowski *et al* 2000).

1.4 Effect of Earthworms on Soil Chemical and Biological Properties

1.4.1 Chemical Effects

The presence and activity of earthworms in soil can have a number of chemical effects. Again these effects are usually caused by the burrowing and casting activities of earthworms and the effect thereof on microbial populations, but other earthworm functions, such as mucus secretion can also affect the soil chemically.

One of the most important chemical effects of earthworms in soil is through their influence over certain key elements of nutrient cycling. The main nutrient cycles

concerned are those for carbon, nitrogen, and phosphorous. In general the amount of carbon in earthworm casts and burrow linings (drilosphere) is usually much higher than those found in the 'undisturbed' or bulk soil (Lee 1985; Lavelle 1988; Binet and Curmi 1992; Zhang and Schrader 1993; Babel and Kretzschmar 1994; Edwards and Bohlen 1996; Schrader and Zhang 1997; Jegou *et al* 1998a; Tiunov and Scheu 1999; Jegou *et al* 2001b). The casts and drilosphere of different earthworm species from differing ecological groups will contain differing concentrations of carbon, for example Schrader and Zhang (1997) found that the casts of *L. terrestris* contained more organic carbon than those of *A. caliginosa*. Not only do earthworm casts and burrow linings differ from the bulk soil in their amount of carbon, but also in the type of carbon present. Edwards and Bohlen (1996) and Zhang and Schrader (1993) showed that casts contained more polysaccharides than uningested soil, and Binet and Curmi (1992) reported that the burrow linings of *L. terrestris* had a large number of orientated litter fragments. The carbon distribution within the soil is also affected by the presence of earthworms. The larger and more active an earthworm community is, the greater is the bioturbation of the soil, therefore the distribution of carbon down the profile is more homogenous (Lee 1985). Jegou *et al* (1998a) found this to be especially true for anecic and endogeic earthworms as opposed to epianecic species such as *L. terrestris*.

Earthworm casts and burrow linings are enriched with nitrogen when compared to surrounding soil (Lee 1985; Edwards and Bohlen 1996; Gorres *et al* 1997; Parkin and Berry 1999; Tiunov and Scheu 1999; Jegou *et al* 2001b). This nitrogen is in the form of both ammonium and nitrate (Edwards and Bohlen 1996; Devliegher and Verstraete 1997; Gorres *et al* 1997; Parkin and Berry 1999). Lee (1985) also reported that earthworms can return N to the soil in four ways:

1. In casts: most of the N in casts is in the form of plant tissue that has gone through the gut with very little chemical change.
2. In urine: is the form that most of the nitrogenous waste products of metabolism are excreted, (the urine is composed mainly of ammonia and urea, and is excreted through the nephridiopores, which are the external openings of the earthworm excretory system).
3. In mucoproteins: secreted onto the body surface to lubricate the earthworm's movement through the soil and to maintain surface moisture which is essential for respiration.
4. In dead earthworm tissue: earthworm tissue is about 60-70% (dry weight) protein and has a N content of about 12%.

The higher concentrations of inorganic N in casts was explained by Edwards and Bohlen (1996), who suggested that this was due not only to excretory products and mucus, but also through the increased rates of mineralization of organic N by micro-organisms in the casts. However, as well as casts having high N mineralization rates they also have a high nitrification rates, and it has been found that there is a simultaneous increase in nitrate and decrease in ammonium as casts age (Edwards and Bohlen 1996; Devliegher and Verstraete 1997). This decline in ammonium and increase in nitrate over time is due to elevated microbial activity in fresh casts (Parle 1963, Syers *et al* 1979). The rates of nitrification and mineralisation show a distinct seasonal pattern, with nitrification rates at their highest during the warmest and lowest during the coldest periods of the year (Syers *et al* 1979). This would have quite serious implications for the leaching of nitrate and therefore N from the soil. Generally the presence and activity of earthworms in soil can increase the rate of N loss from the soil as nitrate and other mobile forms of N (Knight *et al* 1992;

Robinson *et al* 1992; Edwards and Bohlen 1996). Syers and Springett (1983) reported that the walls of earthworm burrows are enriched with nitrate which is then susceptible to being transported through the soil by infiltrating water. The loss of N from soil as a result of earthworm activity is also dependant on the hydrological characteristics of the soil.

The activities of earthworms also affect the distribution and amount of other macro and micronutrients in the soil. For example, earthworm casts tend to have elevated amounts of calcium, magnesium and potassium (Heine and Larink 1993; Edwards and Bohlen 1996). It would also be expected that different ecological groups of earthworms may have different concentrations of differing nutrients in their casts, for example Piearce (1972) showed that the casts of the litter feeding epigeic species *L. rubellus* contained more Ca than the casts of *A. caliginosa*, an endogeic earthworm. Another major element found in the soil that is affected by earthworms is phosphorus, whose available concentration is again higher in casts than bulk soil (Edwards and Bohlen 1996). This is supported by the findings of Lee (1985) who found that the increased amounts of P in earthworm casts are commonly in the order of 5-10 times higher than the surface soil. It has been suggested that this increase in the available P in earthworm casts is due to enhanced phosphatase activity in casts (Satchell and Martin 1984). Not only do casts play an important role in altering soil chemistry, but so does the lining of earthworm burrows. Using electron microprobe microanalysis, Foster (1994) found that the drilosphere of earthworm burrows had higher concentrations of Fe and Mn than the surrounding soil which were fairly evenly distributed, whilst calcium tended to be found in more discrete granules. Judas *et al* (1997) reported that earthworm burrow linings tended to show a shift in

extractable cations from Al through to Ca and Mg when compared to the undisturbed soil.

Another chemical effect that earthworms have on the soil lies in their ability to modify the pH of the soil around them (Judas *et al* 1997). Schrader (1994) showed that earthworms with different pH tolerances could all change the pH of the medium around themselves to suit their particular preferences. All the species used were able to change the pH conditions of their immediate environment at different rates, but with all species changing the high pH environments to more suitable pH most rapidly.

Mixing of soil by earthworms can affect the distribution of heavy metals and radioactive compounds in the soil (Lee 1985; Edwards and Bohlen 1996). Tomlin *et al* (1993) found that the distribution of heavy metals in earthworm burrows is fairly even distributed, leading to a more even distribution throughout the soil. Similarly VandenBygaart *et al* (1998) found that the greater the activity of the earthworm population in a soil, the more even the distribution of ^{137}Cs is down the profile.

Finally, as previously stated, earthworms play an important role in the movement of chemicals through the soil (with particular reference to nitrate leaching). Earthworm burrows may preferentially drain the soil (Lee 1985), which means that any chemicals located in the burrows of earthworms, such as in the drilosphere, may be transported out of the soil. It is in this role that earthworm burrows can be important in the movement of applied chemicals through the soil, as well as facilitating the leaching of nutrients (Tomlin *et al* 1995). Tomlin also suggested that the chemical nature of the burrow lining could influence solute transport since some chemicals, such as herbicides, may be preferentially bound to the organic components located in the wall.

1.4.2 Biological Effects

There are three main biological effects that earthworms have on soil. These are the effects on micro-organisms, meso/macro fauna, and plants. To take the first of these, Edwards and Bohlen (1996, p181) stated that 'earthworms have many complex interrelationships with micro-organisms upon which they depend as their major source of nutrients, they promote microbial activity in decaying organic matter by fragmenting it and introducing micro-organisms into it and finally they have the ability to disperse micro-organisms through out the soil'. The effects of earthworms on soil micro-organisms can be seen in figure 1.5.

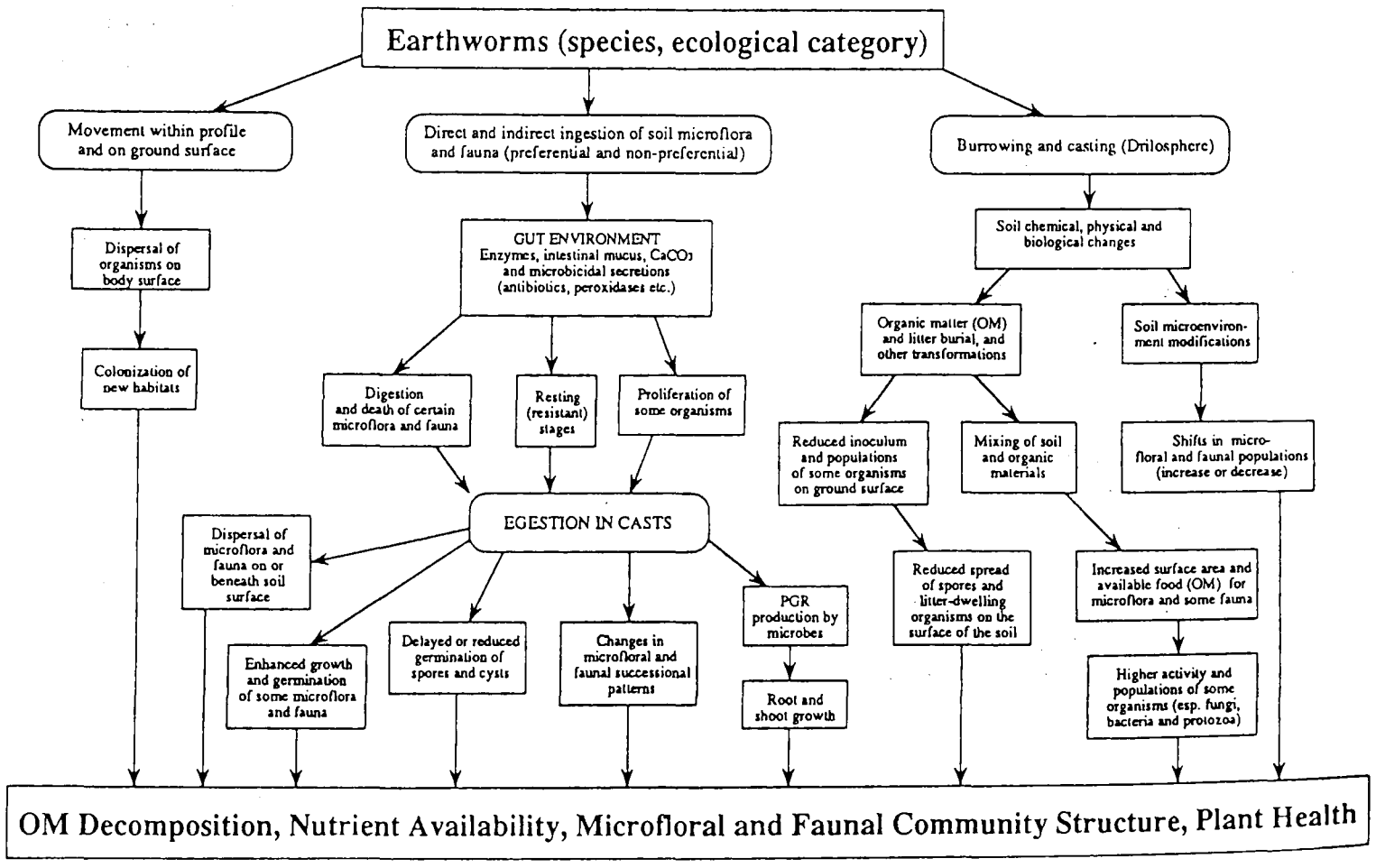


Figure 1.5: The effects of earthworms on soil microflora and fauna, leading to changes in soil properties, processes, microfloral and faunal community structure and plant health (from Brown 1995)

Some early authors have suggested that earthworms have their own characteristic micro-organism communities in their intestines. However, a number of authors, for example Satchell (1967), have shown that this is not the case and that the composition of the micro-organisms in the guts of earthworms is that same as the surrounding soil. Yet, as Brown (1995) highlights, there is still a great deal of controversy about microbial composition and population dynamics in the gut of earthworms.

Earthworms also influence the size of microbial populations in the soil locally through burrowing and casting activity. The numbers of micro-organisms in casts and in the burrow drilosphere are generally higher than the surrounding soil (Lee 1985; Kretzschmar and Monestiez 1992; Martin and Marinissen 1993; Brown 1995; Edwards and Bohlen 1996). The reasons for this increase in microbial populations and activity have been attributed to the more beneficial environment created, e.g. the increase in readily available nutrients such as ammonia in casts and burrow linings (Edwards and Bohlen 1996; Devliegher and Verstraete 1997; Tiunov and Scheu 1999). Some authors have also shown that microbial biomass and activity tends to increase from fresh casts to older casts, although both tend to drop once casts reach a certain age (Edwards and Bohlen 1996). Kretzschmar and Monestiez (1992) showed that the burrowing activity of earthworms can influence microbial activity by allowing more efficient gas exchange in the soil by increasing the diffusivity, thereby allowing the burrow linings to have an increased population which then function at an increased rate.

The effect of earthworms on the mesofauna is less well understood, with little work having been carried out on this subject and often the results show contradicting patterns. However populations of organisms such enchytraeids are generally

detrimentally affected by the earthworm abundance (Hyvönen *et al* 1994; Brown 1995; Yli-Olli and Huhta 2000; Ilieva-Makuleck and Makuleck 2002). Whether this is due to the earthworms out competing these organisms for the food resources or feeding off them, is not known (Brown 1995). However, it seems that the presence of earthworms is beneficial for microarthropods such as Oribatid mites, Acari and Collembola, whose populations seem to increase with the increased presence of earthworms (Brown 1995). This may be because the earthworm channels are opening up more areas of the soil for the microarthropods to inhabit (Brown 1995), but it may also be the case that the microarthropods can preferentially feed on the cast material or the micro-organisms that live in this processed soil (Maraun *et al* 1999). In contrast to the previous findings, McLean and Parkinson (2000) indicated that the activities of the epigeic earthworm *Dendrobaena octaedra* led to decreases in the abundance of orbatid mites and other microarthropods in the FH horizons they sampled. It would therefore seem that earthworms have a mixed effect on the other soil mesofauna.

This section has shown that, earthworms play key roles in determining certain soil chemical and biological properties, which are ultimately brought about through the physical modification of the soil environment. The impact of earthworms on the soil physical environment and in particular soil structure is the main focus of this thesis, and as such will be discussed in detail in the next chapter.

Chapter 2: The Effect of Earthworms on Soil Physical Properties

2.1 Introduction

In chapter 1 the chemical and biological effects of earthworms on soil were outlined along with details of the factors which influence earthworm activity. These are important because they aid in the interpretation of the results obtained during this research. In this chapter the physical impacts of earthworm on soil will be outlined in detail. As early as the 19th century Darwin (1881) noted that earthworms ingest vast quantities of soil, and that there is probably no soil that has not been reworked by earthworms. Indeed it was due to this that Darwin called earthworms "nature's ploughs" for their ability to work the soil.

The two earthworm activities that affect the soil physically are burrowing and casting (the production of faecal material). Burrowing leads to the reorganisation of voids and the creation of macropores within the soil, which has implications for the flow properties of water through soils. Casting affects the structural stability of soil through the stabilisation of soil aggregates. It is these two activities and the physical impacts they have on the soil system, which will be assessed from the literature in this chapter.

2.2 The Effect of Burrowing on Void Space and Water Flow in Soil

Earthworm burrows can be defined as elongated, cylindrical arched voids that have a regular conformation. There are a number of questions about earthworm burrowing activity that this section will aim to answer:

1. How and why do earthworms burrow?
2. Do different earthworm species produce burrows with varying morphologies?
3. What are the factors that influence burrowing activity?
4. What are the effects of earthworm burrows on soil physical properties?

2.2.1 How and Why Do Earthworms Burrow?

There are two mechanisms by which an earthworm can burrow through the soil:

1. A mechanical method whereby earthworms elongate and extend their anterior section into a pre-existing space in the soil. It then expands this part of its body exerting radial pressure which in effect pushes the soil aside allowing the earthworm to move through the soil (Lee and Foster 1991; Edwards and Bohlen 1996).
2. If the soil is too compact then the earthworm can ingest the soil, such as occurs when endogeic species feed. To do this the earthworm everts its pharynx, and if necessary moistens the soil with saliva, fills its pharynx with soil by exerting suction and then retracts its pharynx, at which point the soil is ingested and passed through the gut to be excreted as casts (Lee and Foster 1991; Edwards and Bohlen 1996).

Geophagous earthworms, (i.e. mainly endogeic earthworms), that feed on organic material in the soil utilise both mechanisms at the same time (Keudal and Schrader 1999). The reason for geophagous earthworms to use the mechanical mechanism is so that the soil in front of the earthworm can be broken up allowing the soil to be ingested more easily.

When considering the first mechanism of elongation-insertion-expansion, the pressure that earthworms can exert both radially and axially is very important. Keudal and Schrader (1999) have carried out detailed work on the radial and axial pressures of different species of earthworms. They found that in general the radial pressures exerted by earthworms were greater than those that could be exerted axially. In addition it was found that endogeic earthworm species produced the greatest radial pressures with anecic species exerting the greatest axial pressures. It was concluded that this was because the endogeic species, which are geophagous, have a higher burrowing activity. A number of authors including Keudal and Schrader (1999) also noted that the pressures that could be exerted by earthworms were somewhat smaller than the corresponding pressures exerted by plant roots. This was due to the morphology of the earthworm's body and the fact that earthworms can 'eat' their way through the soil, rather than expending energy to force their way through the soil.

The answer to why do earthworms burrow is fairly straightforward. The reasons for burrowing are to allow earthworms to search for food resources and areas of the soil which have suitable environmental conditions, both of which may be spatially variable due to the heterogeneous nature of soil, in addition to the protection that the soil environment gives earthworms from their predators (Lavelle 1988; Lee and Foster 1991; Cook and Linden 1996; Capowiez 2000).

2.2.2 Earthworm Burrowing Behaviour and Morphology

The burrowing behaviour and the burrow morphology of earthworms vary between species (Joschko *et al* 1991; Jegou *et al* 1998b; Jegou *et al* 1999; Langmaack *et al* 1999; Jegou *et al* 2001a), with most of these variations falling within the three ecological categories. According to Lee (1985) and McKenzie and Dexter (1993) three type of burrows can be identified:

1. More or less permanent burrows which are mostly vertically orientated, open to the surface and often reused (Capowiez 2000). These burrows tend to be produced by species that feed on surface litter, and have smooth linings caused by the compression of the soil and the secretion of mucus by the earthworm. These burrows tend to typify those produced by anecic species.
2. Extensive burrows whose orientation is usually horizontal or sub-vertical with few openings to the surface, which are mostly continuous in nature and tend to decrease in number with depth. The burrow lining is again often smooth, although much thinner than those produced by anecics. Many of these burrows are partly or wholly filled with casts or soil washed down from upper horizons. This type of burrow is produced by geophagous species that forage for food in subsurface horizons and is typical of those burrows produced by endogeic earthworms.
3. More or less vertical, temporary and ephemeral usually without linings and often ending in a mucus lined chamber. This type of burrow is typical of those produced by earthworms that live near the surface as they move deeper into the soil to enter diapause or quiescence, for example epigeic species.

For anecic species, Lee and Foster (1991) found that because their burrows are permanent, the burrow morphology tends to change depending on the stage in the life cycle of the earthworm. This change in burrow morphology is due to the changing requirements of the earthworm as is grows (Lee and Foster 1991).. They also found that anecic earthworms such as *L. terrestris* tend to pull surface litter into their burrows when not actively feeding. The size of earthworm burrows is related to the size of the earthworm, with most burrows being between 1-10 mm in diameter (Lee 1985; Lee and Foster 1991; Edwards and Bohlen 1996). This shows that the burrows of earthworms are among the largest pores found in the soil (Lee and Foster 1991). Obviously, the larger the earthworm then the larger the diameter of the burrow, and in most cases it is the anecic earthworm species which produce the largest burrows since they tend to be the largest earthworms in the soil (for example *L. terrestris* which has a diameter of *c.* 6-10 mm (Sims and Gerard 1985)), followed by the endogeic species and finally the epigeic species. Peres *et al* (1998) added that it is important to consider the growth stages of the different earthworm species when investigating the size and morphology of burrows, since the juvenile of a large earthworm species may produce the same size burrow as an adult of a smaller earthworm species. They used micromorphological and image analysis techniques to investigate the size, shape and nature of burrows, and they found that they could attribute certain size and shaped pores to certain ecological groups and even particular growth stages.

Recently a number of authors have used X-ray computed tomography techniques to investigate burrowing behaviour and activity, burrow morphology and the effect of earthworms on macroporosity. These studies have been carried out using artificially reconstructed cores (Joschko *et al* 1991; Joschko *et al* 1993; Jegou *et al* 1998b;

Jegou *et al* 1999; Jegou *et al* 2001b) and undisturbed cores taken from the field (Capowiez *et al* 1998; Langmaack *et al* 1999; Capowiez *et al* 2000; Pierret *et al* 2002). Most of these studies have been designed to refine the X-ray computed tomographic technique, the results of which show much promise for being able to understand how earthworm burrowing influences porosity in a 3 dimensional way.

The extent of earthworm burrowing varies quite markedly depending on the geographical location, time of the year, species present and on the properties of that soil, since all of these factors will influence the number and activity of earthworms that can be found in the soil. However, in general most of the burrow densities reported are between 50 and 300 burrows/m^2 (Lee 1985; Lee and Foster 1991; Edwards and Bohlen 1996).

2.2.3 Factors Affecting Earthworm Burrowing Activity

Burrowing activity has been determined by a number of authors using different techniques. Burrowing activity has been studied directly by counting the number of burrows in a particular area (e.g. McKenzie and Dexter 1993). The major limitation of this method is that it only gives a snapshot of activity at any one time, and it is generally quite a destructive technique which means replication is an issue. Another way to determine burrowing activity is in the laboratory using cuvettes to study the morphology and rate of burrowing (Haukka 1991; Schrader and Joschko 1991). The major problem associated with this technique is that by placing the earthworms in an 'artificial' environment, their burrowing behaviour may be altered, giving rise to rates of burrowing which are artefacts of the experimental technique. The primary drawbacks of using cuvettes are that the earthworms are placed in a 2D environment,

which alters their burrowing behaviour since it is an unnatural environment. There are also problems with edge effects, where earthworms preferentially burrow along the path of least resistance which is the boundary between the substrate and glass. The final technique used to investigate the rate of burrowing, is deriving burrowing activity indirectly from surface casting, e.g. (Kretzschmar 1991). As with the previous methods, there are a number of limitations associated with this technique. The primary limitation with this method is that it only takes into account surface casting when it has been established that sub-surface casting can often be greater than surface casting (Lee and Foster 1991). Also not all species of earthworms will cast regularly at the surface, so this method is bound to underestimate the actual level of earthworm burrowing activity.

Those factors mentioned in chapter 1 such as soil temperature and moisture content will have a key role in determining the activity of earthworms which will then impact on the burrowing activity of earthworms (Kretzschmar 1991; McKenzie and Dexter 1993). For example, if soil temperature or moisture content drops below a certain threshold, then earthworm burrowing activity will decrease as the earthworms start to go into diapause or become quiescent (Gerard 1967; Lee 1985). Linked to soil moisture and temperature is the influence of seasonality, since both of these factors will vary with season. It can therefore be expected that burrowing activity will vary throughout the year (Kretzschmar 1991).

A number of more specific factors can be identified as influencing the burrowing activity and behaviour of earthworms. There are complex interactions between earthworms and plant roots, which influence the burrowing behaviour and therefore burrowing activity. It has been found that small endogeic earthworms are associated with plant roots (McKenzie and Dexter 1993; Hirth *et al* 1998), for example Hirth *et*

al (1998) found that *A. rosea* will preferentially burrow towards plant roots. Springett and Gray (1997) found that plant roots modified earthworm numbers and the length and orientation of burrows. They concluded that the interaction between plant roots and earthworms is a two way process, with the quantity and distribution of plant roots tending to determine earthworm burrow distribution, and that earthworm burrows assist plant roots to exploit a larger volume of soil.

The availability of food also influences the burrowing activity of earthworms. Martin (1982) found that the intensity of burrowing activity by geophagous earthworms is related to food supply, in that if food is scarce then geophagous earthworms tend to burrow more than if food is abundantly available. A similar situation is true of *Lumbricus* sp. in that they do not often burrow extensively if there is an adequate food supply, but when food is scarce, then burrowing activity is greatly stimulated (Edwards and Bohlen 1996).

The texture of the soil in which an earthworm is burrowing can affect the rate at which burrowing takes place. Deep-burrowing species, such as *L. terrestris,* can take up to 4-5 times as long to burrow in a clay soil as the same species in a light loam (Edwards and Lofty 1977).

2.2.4 The Effects of Burrowing on Porosity and Water Movement

The physical effect of earthworm burrowing is through the creation and re-organisation of soil voids through the formation of earthworm channels. This influence over soil porosity has some important consequences for the hydrology of soils. A number of authors have found that the presence of earthworms in the soil leads to an increase in soil porosity (Stockdill 1966; Satchell 1967; Ehlers 1975; Lee

1985; Tomlin *et al* 1995; Edwards and Bohlen 1996). Edwards and Bohlen (1996) and Marinissen and Miedema (1994) found that porosity increased by about 5% with the presence of earthworms, which is smaller than some of the earlier authors had estimated. Peres *et al* (1998) found that large pores created by anecic species accounted for about 50% of the total surface porosity, therefore according to the authors, highlighting the importance of anecic species in the dynamics of soils. However, possibly even more importantly than an overall increase in porosity is the effect of earthworms on macroporosity.

Earthworms are some of the largest organisms that are commonly found in many soils and as such their burrows, which can be as large as 2 cm in the case of *L. terrestris*, form some of the largest pores present in the soil (Lee 1985). It has been reported by a number of authors that earthworms increase the macroporosity of soil (Ehlers 1975; Syers and Springett 1983; Lee 1985; Lee and Foster 1991; Binet and Curmi 1992; Knight *et al* 1992; Tomlin *et al* 1995; Edwards and Bohlen 1996; Binet *et al* 1997; Blanchart *et al* 1997; Lachnicht *et al* 1997). In addition, some authors have shown that earthworms do not increase the total porosity but rather lead to the re-organisation of voids with the number of macropores increasing and the number of meso/micropores decreasing (Syers and Springett 1983; Binet and Curmi 1992; Knight *et al* 1992; Binet *et al* 1997; Lachnicht *et al* 1997). This has been attributed to earthworms compacting the soil around their burrows, and has indeed been shown to be the case when visual observations have been made using micromorphological techniques.

Increased macroporosity plays a key role in determining the rate of water infiltration into soil and its flow through the soil. Taking the rate of infiltration, earthworms tend to increase the infiltration rate of soil by allowing more water to enter the soil

(Stockdill 1966; Guild 1952, 1955; Ehlers 1975; Hoogerkamp *et al* 1983; Lee and Foster 1991; Bouché and El-Addan 1997; Francis and Fraser 1998). To give an example of how much quicker the rate of infiltration is, Edwards and Bohlen (1996) state that infiltration is 4-10 times faster in soils with earthworms than in soils without or with low earthworm abundance. The different burrowing behaviour shown by different earthworm species leads to their burrows having contrasting morphologies. This difference in burrow morphology has implications for infiltration rates which will be influenced differently by various earthworm species. The effectiveness of burrow infiltration is dependent on the continuity and shape of the burrow systems present in the soil and its access to the water. The other major soil hydrological effect of earthworm burrows, is the transmission of water through the soil.

The flow of water through soil is determined by hydraulic conductivity, and in particular saturated hydraulic conductivity (i.e. the ability of a soil to transmit water in a saturated state). The ways in which this is measured are discussed in chapter 4. The greater the saturated hydraulic conductivity (K_{sat}), the greater the amount of water, for a given hydraulic gradient, that can pass through the soil whilst saturated. Darcy's Law describes how water flows through a saturated, homogenous and porous medium, and is characterised by the equation below. K_{sat} is also used to determine the degree of macroporosity of a soil, and provides information about the connectivity of the macropores in the soil.

$$q = \frac{K \times \Delta H}{L}$$

q = flux density
K = hydraulic conductivity
ΔH = hydraulic head
L = distance over the which water must flow

Most authors agree that increased earthworm burrowing activity leads to increased K_{sat} due to the increase in the macroporosity (Joschko *et al* 1992; Edwards and Bohlen 1996; Francis and Fraser 1998). The reason for the increased K_{sat} in earthworm worked soil is due to preferential or bypass flow through earthworm burrows (Ehlers 1975; Germann and Beven 1981; Edwards and Bohlen 1996). Anecic species are thought to contribute to bypass flow since their burrows tend to be vertical and regularly open out onto the surface, although Joschko *et al.* (1992) and Schrader (1993) also found that endogeic species can contribute greatly to preferential flow even though their burrows do not open out to the surface as regularly (Edwards and Bohlen 1996). This is due to endogeic earthworm species producing longer and more continuous burrows than anecic species (Schrader 1993). Francis and Fraser (1998) noted that the increase in K_{sat} varies between species due to the variation in the size of earthworm species, the continuity of burrows and the level of burrowing activity. This indicates that preferential flow down burrows will only occur under certain circumstances. Earthworm burrows are of relatively large diameter so will only conduct water when it is in a tension free state, i.e. when water is ponded at the soil surface (Edwards and Bohlen 1996). In addition to this, K_{sat} is determined by the length and continuity of burrows (Tomlin *et al* 1995; Francis and Fraser 1998) since many burrows can be blocked with casts impeding the flow of water. (Edwards and Bohlen 1996). However, it should be noted that the presence of earthworms does not always lead to increased K_{sat}. Boyle *et al* (1997) investigated the effect of earthworms on cut-over peat, and found that bulk density increased and both total porosity and K_{sat} decreased. This was attributed to the blocking of earthworm burrows by cast material. In summary, earthworm burrowing activity has some important effects on certain soil physical properties pertaining to the flow of

water into and through the soil. Generally earthworms increase the rate of water infiltration and the transmission of water though soil.

2.3 The Effects of Earthworm Casts on Soil Aggregation

2.3.1 The Nature of Earthworm Casts

Casts are earthworm faeces, which consist of excreted masses of soil mixed with residues of comminuted and digested plant material (Lee 1985). The amount of plant material found in casts depends on the diet of the earthworms and the efficiency of digestion (Lavelle 1988), for example geophagous earthworms which derive their nutrition from organic matter in the soil will produce casts with a higher mineral content than detritivorous species which only ingest soil in the production of burrows (Lee 1985). The casts that are produced by geophagous species are formed by the shearing and re-moulding of soil particles at low pressures and high water contents in the earthworms gut (Hindell *et al* 1994b).

The location of casts depends on the ecological strategy and feeding behaviour of the earthworm. There are two types of casting behaviour: 1) sub-surface casting where earthworms excrete their casts in existing soil voids or in their channels, and 2) surface casting (Lavelle 1988). In general most geophagous earthworm species produce sub-surface casts, whilst detritivorous species that live in vertical burrows that open out on to the surface produce surface casts (Lee 1985). In the UK there are only three lumbricid species that cast on the surface, *L. terrestris*, *A. longa and A. caliginosa* (Scullion and Ramshaw 1988). In general the size, shape and

composition of surface casts are species specific, with smaller earthworm species producing small casts with a finer structure than larger species (Lavelle 1988).

There are two general forms of casts:

1. Ovoidal or sub-spherical to spherical pellets that range in size from <1 mm to >10 mm in diameter (the size of cast obviously depends on the size of the earthworm).
2. Paste-like slurries that generally form less regular shapes, but still have a rounded form.

However, there are also composite casts that are made up of aggregated masses of both forms. These types of cast are very common (Lee 1985; Lee and Foster 1991).

In soil thin sections earthworm casts can normally be identified due to their rounded forms. They are often enriched with smaller mineral grain size fractions and are darker in colour than the surrounding soil (Marinissen and Miedema 1994). In addition earthworm excrement can be distinguished from other faunal faeces since they contain a higher proportion of soil minerals so that most of their cross section is composed of mineral material, and they lack the densely packed microbial masses that are characteristic of, for example, microarthropod excremental pellets (Lee and Foster 1991).

The composition of casts enables them to be identified from surrounding soil. This is mostly because they have a characteristic particle size and organic matter composition. Taking the particle size distribution in casts first, it has been shown that earthworms tend to preferentially ingest smaller particle sizes (Lee 1985; Lee and Foster 1991). This may be because soil organic matter tends to be associated with the smaller particle sizes, especially the clay fraction (Tisdall and Oades 1982). Alternatively, work by Bolton and Phillipson (1976), as cited in Lee and Foster

(1991), has shown that the maximum size of mineral particles ingested by earthworms is related to earthworm diameter. These authors examined the maximum size of mineral particles found in the posterior gut of three earthworms *A. rosea*, *A. caliginosa* and *O. cyaneum*, which were *c.* 100 μm, 200 μm and 500 μm respectively. These maximum particle sizes were found to be proportional to the relative diameters of the 3 species used. When compared to the surrounding soil earthworm casts tend to contain more clay and silt, and less coarse sand particles (>200 μm) (Lee 1985; Lavelle 1988; Lee and Foster 1991; Zhang and Schrader 1993; Hindell *et al* 1997a; Schrader and Zhang 1997).

Some authors have suggested that earthworms are able to comminute sand-sized mineral particles, thereby leading to only the smaller particle sizes in casts. This seems extremely dubious since the pressures inside the gut are small and the length of residence time is short, both of which would seem to rule out any comminution of larger soil particles either by abrasion or by chemical breakdown (Lee 1985).

Due to the key role earthworms play in carbon cycling in soil, the amount and nature of organic carbon in casts is very important. Earthworms are perhaps the most important invertebrates in the soil in the initial stages of recycling of organic matter (Edwards and Bohlen 1996). Earthworms ingest organic matter which tends to be fairly coarse in nature, which is comminuted and mixed with soil and egested as casts, leading to the reduction of the size fractions of organic matter in casts, which in turn leads to the organic matter having a greater surface area open for microbial decomposition causing increased microbial activity in casts (Parle 1963; Martin and Marinissen 1993; Edwards and Bohlen 1996). Apart from the ingested organic matter being physically comminuted, most of it is not chemically altered (Lee 1985).

Earthworm casts tend to have elevated organic carbon contents compared to surrounding soil and soil aggregates (Lee 1985; Martin and Marinissen 1993; Edwards and Bohlen 1996; Flegel *et al* 1998; Jegou *et al* 1998a). This raised organic carbon contents is due to the selective feeding of earthworms on particular organic materials or fractions within the soil, and the secretion of mucus as the cast material passes through the gut of the earthworm (Lee 1985; Edwards and Bohlen 1996). Parle (1963) found that earthworm casts are enriched with certain organic fractions, in particular polysaccharides, compared to uningested soil.

Lee and Foster (1991) using micromorphological techniques described three main types of plant material that can be identified in casts:

1. Fresh material with intact cell walls that are not humified.
2. Reworked cell debris, i.e. material that has been ingested, excreted and re-ingested, which is characterised by collapsed cells with humified and distorted cell walls, the absence of cytoplasm, and the presence of bacteria or lysis holes caused by bacteria.
3. Highly electron dense humified materials of unknown origin.

This shows that there can be a wide diversity of organic material present in casts, all of which depends on the stage in breakdown that particular organic material is in.

The amount of organic carbon in casts varies with earthworm species due their particular feeding behaviour (Zhang and Schrader 1993; Schrader and Zhang 1997; Flegel *et al* 1998), for example Flegel *et al* (1998) found that the casts of *L. terrestris* had more organic carbon than those of *D. octaedra* and *L. rubellus* whose diets increased in geophagy when food quality was reduced.. Jegou *et al* (1998a) found that the casts of epigeic and epianecic species contained more than 50% litter C,

whilst those of anecic and endogeic species were only about 40% enriched. This shows that increased geophagy reduces the amount of organic carbon in casts.

In general earthworm casts contain higher concentrations of Ca than surrounding soil (Heine and Larink 1993). This is of importance when considering the stability of aggregates. The stability of aggregates will be dealt with in section 2.3.4.

The quantity of casts produced by earthworms show a great deal of variation depending on the earthworm species, location and season. Figure 2.1 shows the seasonal changes in cast production, with cast production greatest during the autumn and the beginning of winter. This could be related to the seasonal activity of earthworms.

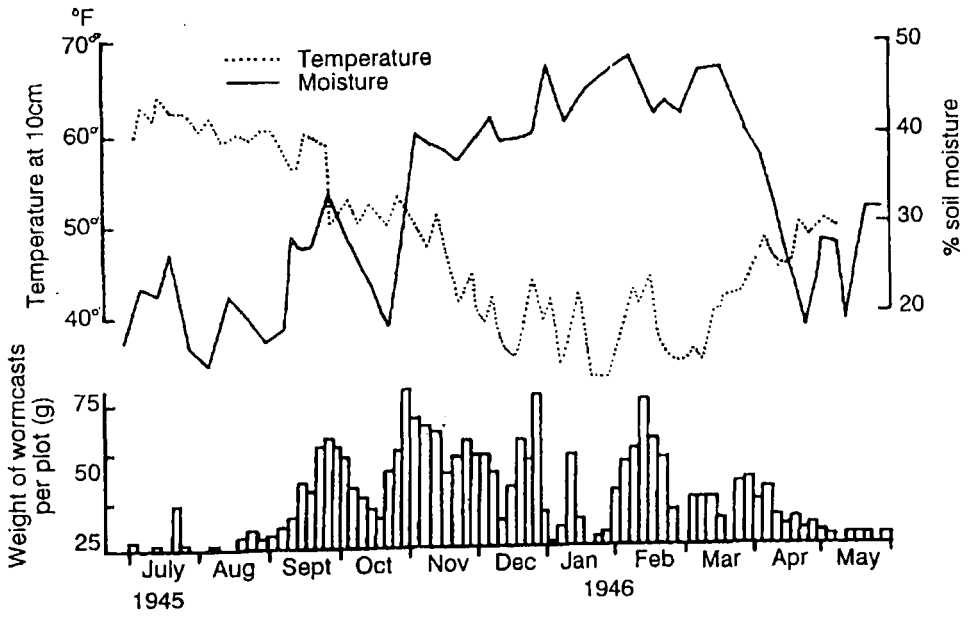


Figure 2.1: The seasonal changes in earthworm cast production in an English pasture (Evans and Guild 1947)

In temperate grasslands and pasture the overall annual production of casts may be as high as 20-30 kg m^{-2} but figures of 1-5 kg m^{-2} are more common (Lavelle 1988). Table 2.1 shows some measurements of the amount of cast produced in temperate environments.

Table 2.1: Annual rate of surface cast production by earthworms (modified from Lee 1985)

Location	Vegetation	Taxa	Wt of casts (kg m^{-2})	Period of cast production	Reference
England	Pasture	Lumbricidae	0.75 - 1.60	Spring - Autumn	Darwin 1881
France	Pasture	Lumbricidae	7	Spring - Autumn	Bouche 1982
Germany	Pasture	Lumbricidae	25.75	Spring - Autumn	Graff 1971
Russia	Grass ley	Lumbricidae	5.2	Spring - Autumn	Ponomareva 1953
New Zealand	Pasture	*A. caliginosa*	2.5 - 3.0	Autumn - Spring	Sharpley and Syers 1976, 1977

Lee and Foster (1991) state that surface casting in temperate soils has been found to be between 2-250 t ha^{-1} yr^{-1}, but with most estimates for temperate pastures and grassland lying between 40-50 t ha^{-1} yr^{-1} which would on average represent a 3-4 mm depth of cast on the surface. However, Lee and Foster (1991) also point out that these figures will underestimate the total number of casts produced since it is very difficult to measure the number of sub-surface casts produced.

2.3.2 Factors Affecting Casting

As with burrowing, most of the factors outlined in chapter 1 that affect the behaviour of earthworms will also affect their casting activity. For example, as figure 2.1 shows, the casting behaviour of earthworms is affected by soil temperature and moisture, both of which fluctuate seasonally.

In addition to these factors there are a number of others that are specific to casting only. These factors are outlined below:

- Soil Compaction: This will influence the ability of earthworms to burrow through the soil and therefore the amount of food ingested which then influences the amount of casts egested (Lee 1985; Scullion and Ramshaw 1988). This would then seem to apply mostly to the geophagous species. The more compact soil is then the harder it is for earthworms that cast subsurface to cast in voids, therefore they are forced to cast at the surface (Lee 1985; Scullion and Ramshaw 1988).
- Food Source and Location: Food source strongly affects the consumption of food which in turn will help to determine cast production (Tomlin *et al* 1995; Flegel *et al* 1998). Scullion and Ramshaw (1988) and Tomlin *et al* (1995) also found that, where the food was situated in relation to particular earthworm species feeding behaviour, will affect the casting behaviour of earthworms.
- Species Effects: It is obvious that different species of earthworm will have different behavioural characteristics, so it follows that speciation effects, through these behavioural characteristics, will affect the casting behaviour and rate of different earthworm species. For example, Scullion and Ramshaw (1988) discovered that surface casting is influenced by species interactions with one species tending to play a dominant role, and that increased surface cast production and the dominant role in casting within a population is partially related to the size of species which is why *L. terrestris* often dominates surface casting. Also Zhang and Schrader (1993) found that the increase in total C and organic C contents of casts followed the order *L. terrestris*, *A. caliginosa* and *A. longa*.
- Seasonality: Many authors have found that there is a marked seasonal effect on earthworm casting (Evans and Guild 1947; Gerard 1967; Scullion and Ramshaw 1988; Bouché and Al-Addan 1997). In a Mediterranean climate, earthworms tend

to cast during winter, spring, and autumn, with no real casting activity during the summer months. In a more temperate climate, casting activity might be reduced over the harshest winter months.

Hindell *et al* (1994a) summarised those physical and chemical soil properties that influence cast production as i) available water and food, ii) soil temperature, iii) soil bulk density and iv) the physiological state of the earthworms. Tomlin *et al* (1995) stated that casting activity is a function of feeding activity and is influenced by:

- Food supply
- Food source and placement
- Earthworm species
- Soil temperature
- Soil pH and Ca concentration

2.3.3 The Effects of Casting

Undoubtedly the most important effect is the formation of aggregates. This process is defined by Edwards and Bohlen (1996, pp204) who stated that "soil aggregates are formed by the adhesion of mineral granules and soil organic matter into composites of various sizes that resist breakdown when exposed to internal and external stress such as wetting, drying, compaction or other physical disturbance". The process of aggregate formation in earthworm casts is shown in figure 2.2.

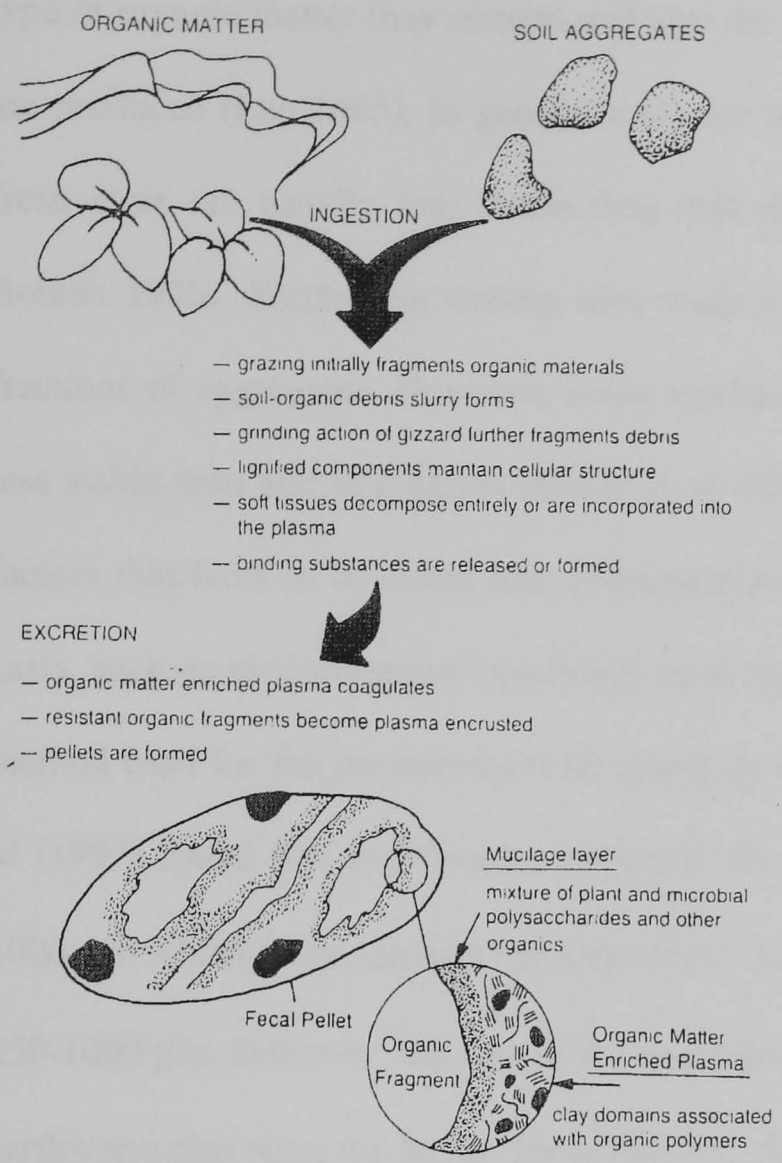


Figure 2.2: A diagrammatic representation of aggregate formation in earthworm casts (Shipitalo and Protz 1989)

Casting is an important aggregate forming process and in particular the formation of stable aggregates. The structural stability of casts is important in order to evaluate a) the potential of soil faunal activity for the formation of soil structure and aggregation patterns, and b) the physical availability of organic matter in excrements as a substrate for soil animals and microbes (Martin and Marinissen 1993). Most workers are agreed that earthworm casts contain more water stable aggregates that the surrounding soil (Swaby 1949; Guild 1955; Parle 1963; Shipitalo and Protz 1988; Marinissen and Dexter 1990; Martin and Marinissen 1993; Hindell *et al* 1994b; Ketterings *et al* 1997; Garvin *et al* 2001). Lee and Foster (1991) state that in certain soils casts often make up the majority of aggregates in the upper horizons. The stability of earthworm casts as aggregates relies to a great extent on the amount and

type of organic matter they contain and also the microbial activity in them after they are produced (Lee 1985). In general casts are more stable than soil aggregates, but fresh casts are usually less stable than soil aggregates (Lee 1985; Edwards and Bohlen 1996). Earthworm casting also tends to affect the stability of certain size fractions of aggregates. However, some workers have found that casts are actually less stable than soil aggregates (Boyle *et al* 1997), although there are a number of factors that have to be taken into consideration when investigating the stability of casts, such as environmental conditions (soil type, moisture conditions etc) and the method used for the measurement of aggregate stability. For example, Ketterings *et al* (1997) found that earthworms increased the stability of aggregates greater than 1000 μm whilst there seems to be little effect on the stability of aggregates between 250-1000 μm. However, the size of the aggregates formed from casts will depend on earthworm size since the larger the earthworm, the larger the casts it produces.

In addition to increasing the proportion of stable aggregates in the soil, casts tend to have a greater tensile strength than similar sized soil aggregates, with tensile strength being a measure of the mechanical strength of casts (McKenzie and Dexter 1987). Lee and Foster (1991) quantified this and found that dried earthworm casts have a tensile strength 2-2.5 times higher than similar sized dried soil aggregates. The mechanisms for increased aggregate stability and tensile strength of casts will be discussed further in section 2.3.4.

One effect of stable aggregates is in the protection of organic matter within the soil (Low 1972; Tisdall and Oades 1982; Chaney and Swift 1984). Earthworm casts aid the protection of soil organic matter (Marinissen and Hillenaar 1997). Some of the organic matter found in stable earthworm casts can be several hundred years old, (Tomlin *et al* 1995). Martin and Marinissen (1993) have suggested reasons for the

reduced rate of organic matter decomposition in casts compared to the surrounding soil:

- The close association between clay and organic matter providing a protective coating of organic particles by clay
- A reduction in oxygen availability within the cast because of the small pore sizes associated with casts in either the outer edge of the casts or in the whole cast due to compaction
- According to Poiseuielle's law, the movement of water into the cast through the small pores would mean that microbes would have limited access to the organic matter within the casts (this is especially true when there is a lack of large pores).

Linked with the organic matter in casts is the microbial community and abundance associated with casts. It is generally accepted that casts tend to have increased numbers of microbes associated with them compared to soil aggregates (Parle 1963). It should be noted that the composition of the microbial community in casts does not differ markedly from the surrounding soil; it is just that the abundance of microbes increases (Parle 1963). This may be because of the beneficial conditions found in the gut of the earthworms and in the cast itself (Martin and Marinissen 1993). The conditions in the gut and cast that may cause an increase in microbial activation are i) an increase in pH, ii) the release of water soluble organic compounds during gut transit, iii) the comminution of organic matter which enhances its availability for microbial decomposition, although this is usually only temporary and decreases with cast age (Martin and Marinissen 1993) and v) casts are a rich in soluble nutrients such as N and P (Foster 1994).

In summary, the effects of earthworm casting activity is to increase the number of stable aggregates in the soil, aid the protection of organic matter and the localised increase in the microbial population associated with casts.

2.3.4 The Stability of Earthworm Casts

As highlighted in section 2.3.3, earthworm casts tend to be more stable than soil aggregates, and that increasing the number of earthworms in soil will lead to an increase in aggregate stability (Martin and Marinissen 1993; Marinissen 1994). A number of authors have put forward mechanisms by which earthworm casts can be stabilised. These are summarised by Tomlin *et al* (1995):

- Stabilisation by internal secretions of earthworms (Dawson 1947)
- Mechanical stabilisation by plant fibres incorporated into casts (Dawson 1947; Lee and Foster 1991)
- Mechanical stabilisation by fungal hyphae (Parle 1963; Marinissen and Dexter 1990; Lee and Foster 1991)
- Stabilisation by bacterial gums (Swaby 1949)
- Stabilisation via the formation of organo-mineral bonds between calcium and humic compounds(Meyer 1943 as cited by Satchell 1967, p294) or mucilage (Dutt 1948)
- Stabilisation due to wetting and drying cycles with (Shipitalo and Protz 1988, 1989) or without organic bonding (Marinissen and Dexter 1990).
- Age-hardening/thixotrophic effects combined with organic bonding (Shipitalo and Protz 1988, 1989)

However, none of the mechanisms for the stabilisation of earthworm casts are mutually exclusive, and under field conditions a combination of mechanisms are probably involved. What is important is whether a particular mechanism is dominant under a given set of circumstances or environmental conditions (Tomlin *et al* 1995). Hindell *et al* (1997b) surmised that the stabilisation mechanisms in age-moist casts are age-hardening and biological processes whilst in dried casts the processes are cohesion and cementation. Each of the three major mechanisms responsible for the stabilisation in earthworm cast, biological processes, age-hardening or thixotrophic effects and wetting and drying cycles are evaluated in greater detail.

There are two groups of microbes responsible for the biological stabilisation of casts, namely fungi and bacteria. Bacterial stabilisation tends to produce microaggregates through the secretion of gums or polysaccharides that form gel coats to which clay particles can adhere (Marinissen and Dexter 1990: Edwards and Bohlen 1996). Micromorphological analysis has confirmed that casts contain large numbers of bacteria and quantities of bacterially produced compounds that could serve to bond mineral particles together (Shaw and Pawluk 1986). Fungi produce hyphae that grow on the surface of casts stabilising the cast surface. It has been suggested that these hyphal nets on the cast surface join the bacterially produced microaggregates together to form stable macroaggregates (Marinissen and Dexter 1990). It appears that the fungal hyphae come from the surrounding soil which explains why fresh casts have very few hyphae, whose number then increases as time progresses (Parle 1963; Marinissen and Dexter 1990; Lee and Foster 1991).

Age or thixotrophic hardening take place under moist conditions. It is a physical process which involves the internal arrangement of clay particles and water films within the cast (Marinissen and Dexter 1990; Marinissen *et al* 1996) to a

configuration of minimal free energy (Hindell *et al* 1997b). This then allows the clay particles and organic matter to come into closer proximity and therefore allows stronger bonds to form between them (Shipitalo and Protz 1988, 1989; Marinissen and Dexter 1990). However the conditions for thixotrophic hardening occur around the plastic limit of soil, so that if the conditions are drier or wetter it becomes less significant as a stabilising mechanism, and as such it is most likely to take place during the first 10 days after cast egestation (Marinissen and Dexter 1990).

Wetting and drying cycles depend primarily on casts becoming drier, since when casts are egested they have a relatively high water content and low moisture potential. The drying of casts by dehydration causes organic and clay particles in casts to become more closely associated through shrinkage (Shipitalo and Protz 1989). It can be seen that there are a number of similarities between thixotrophic hardening and wetting and drying cycles. The wetting and drying mechanism would suggest that the stability of casts is transitory since on wetting the stability of the cast may be reduced, but this depends on the hydrophobic status of the cast.

There are two components to the stabilisation of earthworm casts. These are the clay particles and organic compounds within the cast. It is widely recognised that it is the bonding between clay and organic particles in casts that give them their stability. Indeed it is this bonding mechanism which is responsible for the stability of most aggregates in general. Shipitalo and Protz (1989) put forward a model for the bonding between clays and organic matter (figure 2.3).

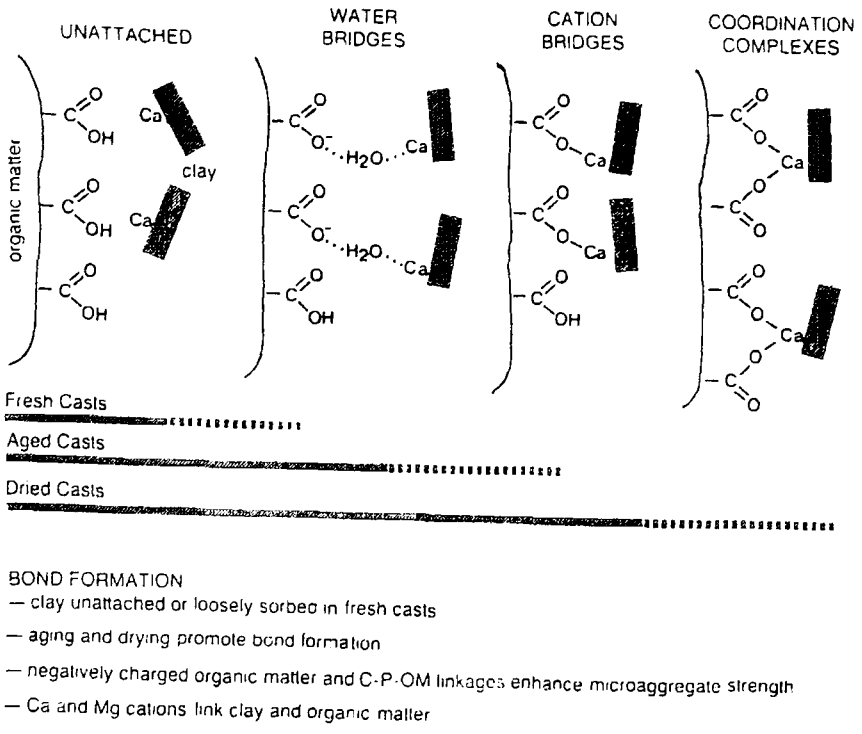


Figure 2.3: The binding mechanisms between clay and organic matter in earthworm casts (Shipitalo and Protz 1989)

The bonding mechanism is a clay-polyvalent cation-organic matter (C-P-OM) bond with the organic matter consisting of plant and microbial polysaccharides. Other authors have found that the distribution and nature of the active organic matter fraction is more important than the total amount present (Shipitalo and Protz 1998; Tomlin *et al* 1995). The polyvalent cations involved are thought to be dominated by Ca^{2+} and to a lesser extent by Mg^{2+} (Shipitalo and Protz 1989; Zhang and Schrader 1993; Schrader and Zhang 1997). This fits in with the finding that casts contain a higher concentration of Ca than the surrounding soil (Zhang and Schrader 1993), which is thought to come from the calciferous glands in the earthworm which secretes Ca granules in to material passing through the gut.

The organic matter in casts may also serve as foci for the formation of aggregates. Studies using micromorphology have shown that some of the ingested organic matter can become highly fragmented and partially humified (Shaw and Pawluk 1986; Shipitalo and Protz 1989; Lee and Foster 1991; Altemuller and Joschko 1992), and it is this fraction of the ingested organic matter that is thought to serve as the aggregation foci (Tomlin *et al* 1995). Shipitalo and Protz (1989) went even further to

suggest that large fragments of organic material could serve as mechanical binding agents to link microaggregates to form pellets and the pellets to form macroaggregates.

The final aspect of the stability of earthworm casts is why are fresh earthworm casts more unstable than soil aggregates, especially in terms of mechanical strength (Hindell *et al* 1997a,b). One possibility is that as the ingested material (soil and organic matter) passes through the gut of the earthworm, mixing occurs leading to the disruption of some of the inter-particle bonds in the cast material leading to destabilisation of the cast when egested (Shipitalo and Protz 1989; Marinissen and Dexter 1990). Linked to this is that the fresh cast material has a very high water content so that the bonding particles are kept apart weakening the bonds, in addition the density of fresh earthworm casts is fairly low since the material has been moulded at pressures as low as 260 Pa in the gut of the earthworm (Marinissen and Dexter 1990). Lee and Foster (1991), using micromorphological techniques, identified that in fresh casts there is a lack of long range binding materials leading to the low mechanical stability of the new casts. These explanations would all lead to reduced stability of fresh casts.

Once the cast has been egested, then the stabilisation mechanisms mentioned previously can start to take effect. Post egestation the water in the cast starts to be lost and the mobile clay particles can move closer to the organic matter leading to stronger bonding. The mobility of the clay particles in moist casts is shown by the relatively high dispersion index that these casts have (Marinissen and Dexter 1990; Hindell *et al* 1994b). In addition, as the casts age the microbial activity increases as does the number of fungal hyphae, further increasing the cast stability.

In summary, aged earthworm casts are generally more stable than soil aggregates although this is not always the case, with fresh casts being less stable than existing soil aggregates. There are a number of mechanism by which casts can be stabilised, but no one mechanism is thought to be mutually exclusive although there does seem to be a progression of processes through time by which casts become more stable. The prime components of casts that contribute to cast stability are clay and organic particles which are linked together by polyvalent cations to form C-P-OM bonds, which are thought to be instrumental in the stabilisation of earthworm casts.

2.4 Summary

What have been discussed in this chapter are the physical impacts of earthworms on soil and their consequences for key soil physical properties. These impacts can be divided into two groups, the first being the effect of earthworm burrowing. When earthworms burrow in soil they tend to cause an increase in the number of macropores and a decrease in the number of meso/micropores in the soil. This has important consequences for the hydrological properties of the soil, with water being able to infiltrate more rapidly into the soil, and then when the soil is in a saturated state, move through the soil at a faster rate due to preferential flow.

The second group involves the effects of earthworm casting activity on the structural stability of the soil. Earthworm casts, when aged, tend to be more stable than normal aggregates in the surrounding soil due to:

- Biological processes: Elevated microbial populations secreting polysaccharides (gums and mucilage) which glue the soil particles together and hyphal nets on the

surface of casts as formed by soil fungi which holds the microaggregates together.

- Chemical processes: The bonding of organic matter and clay particles using polyvalent cation bridges (i.e. where Ca^{2+} or Mg^{2+} forms bonds to both clay particles, which have negatively charged surfaces, and organic matter thereby linking them together).
- Physical processes: Thixotropic age hardening (the physical process whereby clay particles and their films of water arrange themselves into a low energy configuration) and drying bringing organic matter and clay particles closer together allowing stronger bonds to be formed between them, and mechanical stabilisation by large fragments of organic material.

This addition of stable aggregates to the soil through casting has a number of effects from increasing the structural stability of the soil through to the drainage of the soil and the protection of soil organic matter locked up in aggregates.

It should be noted that most of the research to date has focused on the effects of earthworms in low organic matter systems such as agricultural fields and reclaimed soils. This research project investigates the effects of earthworms on void space and aggregation in a highly organic upland soil, which has an inherently high degree of structural stability due to the large organic carbon content. In addition this research project aimed to quantify the effects of earthworm burrowing and casting activity at different spatial scales in an integrated manner. The next chapter outlines the aims, objectives and hypotheses that are being used to evaluate and quantify the effects of burrowing on voids and casting on soil aggregate stability.

Chapter 3: Research Design

3.1 Research Aims and Rationale

The review of the scientific literature in chapter 2 has shown that earthworms have a number of impacts on soil physical properties. Their burrowing activity leads to changes in void space through the creation of macropores and the compaction of the soil around their burrows causing a reduction in microporosity. These changes in the characteristics and size distribution of voids within the soil have a number of important hydrological consequences including the ability of soil to conduct water, especially when saturated. The casting activity of earthworms plays a key role in aggregation and aggregate stability since previous research has found the earthworm casts are some of the most stable aggregates in the soil. This structural stability is vital for the functioning of the soil, for example in the protection of soil organic matter from decomposition and in minimising loss of fine sediment from the soil.

The overall aim of the research described in this thesis is to quantify the effects of earthworm activity and diversity on void space and aggregation in an upland acid grassland soil, which has a high organic matter content. To quantify these effects, measurements of void space and aggregation were made at different spatial scales, from field and laboratory measurements of aggregate stability and saturated hydraulic conductivity to the microscopic analysis on individual earthworm features.

Three experiments were designed to quantify the impacts of earthworms on soil fabric. Each experiment represented an increased level of complexity, i.e. how controlled the experimental conditions were and the degree to which the soil was

exposed to field conditions. Figure 3.1 outlines the three experiments and degree of complexity within each.

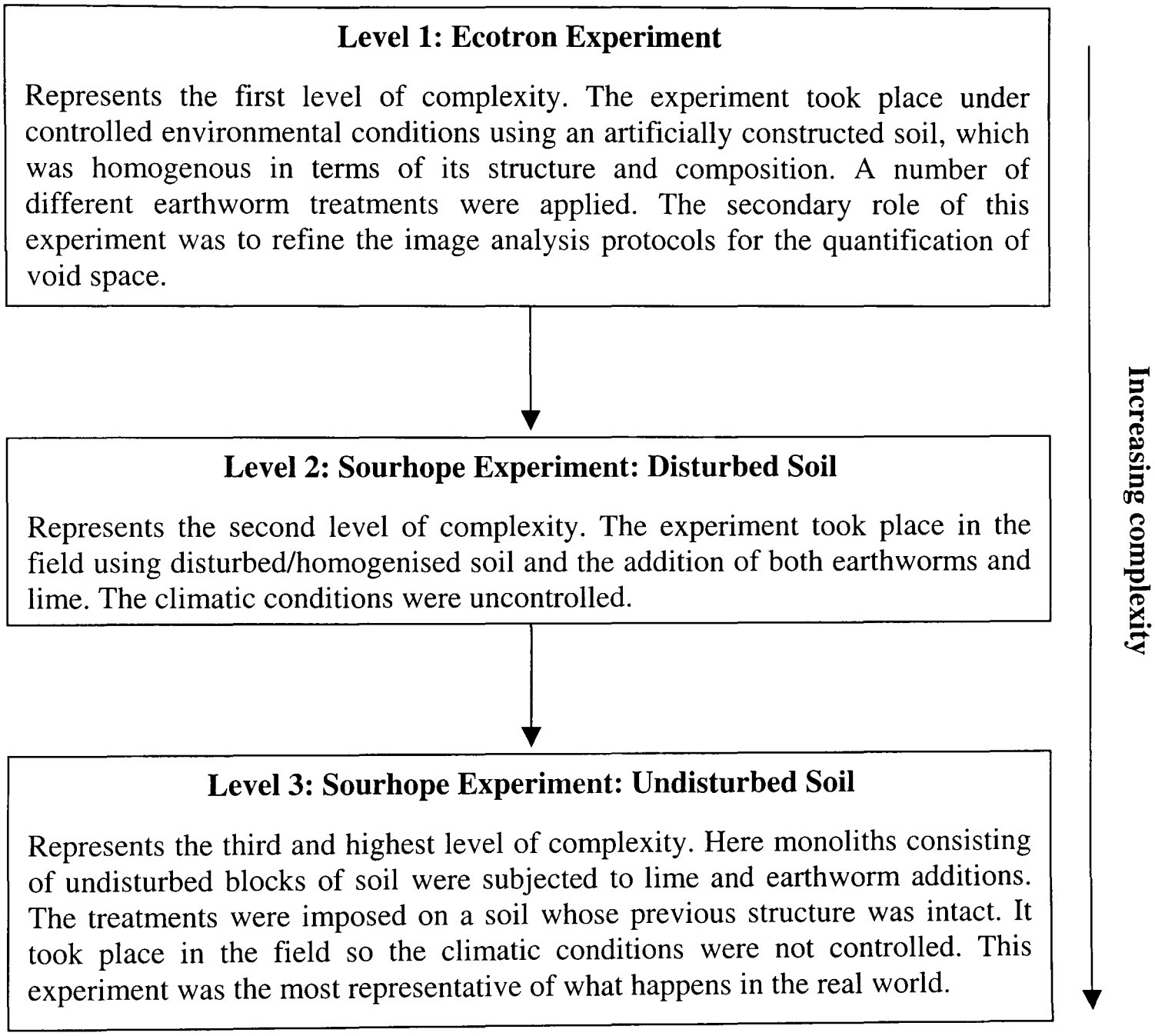


Figure 3.1: Diagram representing the structure of the research in terms of system complexity.

The system complexity in each experiment is determined according to the degree of climatic control, the location of the experiment (laboratory or field based) and the nature of the soil used in terms of its structure. The Ecotron experiment, which represented the first level of complexity, took place under highly controlled conditions in environment chambers using an artificial soil that was a mixture of

sand and organic matter (in the form of washed aquatic peat), and had no pre-existing aggregates or structure. This was a simple experiment where any effects that earthworms had on the soil fabric would be easily identifiable and therefore quantifiable. The other two experiments were more complex in that they both used a real soil and were field based and so exposed to realistic climatic conditions. The soil in the disturbed experiment (level 2) had its structure destroyed as far as possible so that the effects of earthworms could be more clearly observed. This soil was exposed to field conditions, less controlled than in the Ecotron. The final experiment which represented the third level of complexity was most representative of what was happening in the field site, using undisturbed soil blocks which retained their pre-existing structure and associated soil fauna (including earthworms). As a consequence it was expected that the effects of earthworms would be more difficult to identify.

It should be noted that most of the previous investigations into the effects of earthworms on soil physical properties have focused on low organic matter systems such as cultivated and reclaimed soils. The last two experiments in this research project investigated the physical effects of earthworms on an acidic grassland soil which was high in organic matter and located in an upland environment. To date there has been little research into quantifying the effects of earthworms on void space and aggregation in these soils, with most research carried on soils under agricultural land use.

3.2 Overall Hypotheses

Having identified from the literature some effects of earthworms on the soil fabric and how they could be quantified in this research three overall hypotheses were formulated on which the experimental work was based (see below). These hypotheses provided an overall framework for the research, but for each experiment more specific hypotheses were formulated. These experimentally specific hypotheses are stated in chapters 5 to 7.

1. That increased earthworm casting, as expressed as excremental pedo-features, would be associated with increased aggregate stability.
2. That increases in earthworm numbers and activity would lead to increased soil pore space.
3. Pasture improvement through liming will lead to increases in earthworm populations and activity thereby causing changes to soil structure.

The effects of earthworms on void space and aggregation have been discussed previously but the effect of liming has not. It is these three research hypotheses which led to the development of the research design and the techniques used to answer the hypotheses. The remainder of this chapter outlines the design of the experiments whilst chapter 4 details the experimental techniques used during this investigation.

3.3 The Ecotron Experiment

The Ecotron is a controlled environment facility located at Imperial College's Silwood Park campus in Southern England. The Ecotron's primary function is to provide a facility so that terrestrial ecosystems can be studied under controlled conditions. This facility consists of 16 separate chambers, the environmental conditions in which can be controlled (figure 3.2). Some of the environmental parameters that can be adjusted are light regime (both light intensity and light cycles), water inputs (quantity of water, intensity, duration and timing of water addition), temperature and humidity. In addition to the control of the environmental conditions, a wide variety of experimental variables can be altered, e.g. species composition of above ground and/or below ground organisms allowing the interactions between both different organisms and organisms and their environment to be investigated.

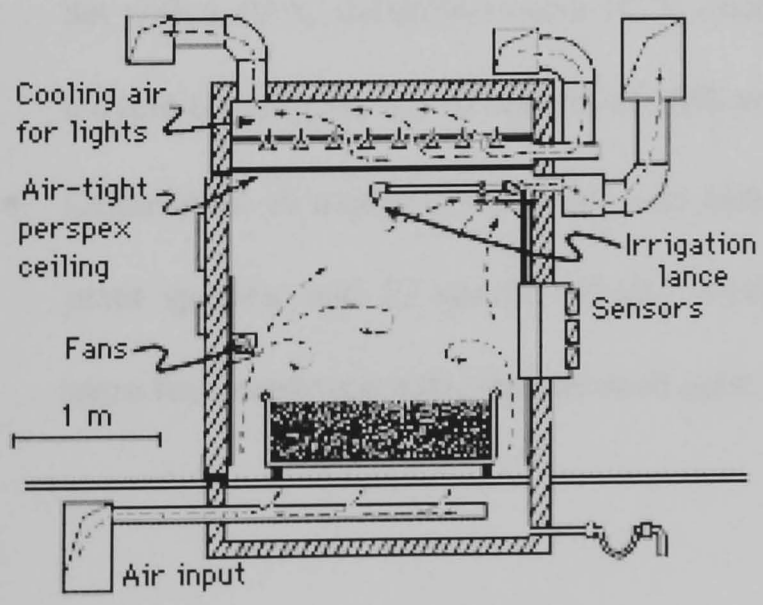


Figure 3.2: A schematic representation of one of the Ecotron chambers. (Ecotron website)

The particular Ecotron experiment that was sampled for this research project was a large multi-collaborator project the aim of which was to investigate the effects of

earthworm species and earthworm functional diversity on ecosystem soil processes and soil fauna diversity. The role of this research project was to identify and quantify the effects of earthworms on aggregate stability and void space, and was one of a number of wide ranging studies.

The design of the Ecotron experiment was:

- Soil: An artificial soil was constructed of a mixture of 1 part sandy loam soil: 1 part aquatic compost: ½ part leaf litter/mulch. This soil mixture was then placed on a bed of washed gravel (approx 10 cm) in large containers (length = 125 cm, width = 80 cm, depth = 45 cm). The gravel was there to provide a barrier to the earthworms whilst allowing water to drain freely from the containers. Before the experiment the soil, gravel and vegetable mulch was fumigated with methyl bromide to ensure no undesired organisms were introduced into the experimental systems.
- Chamber climate: The light cycle was 2 hrs dawn, 14 hrs full light, 2 hrs dusk and 6 hrs full dark. The temperature of the incoming air followed a smooth cycle set with a 10 °C minimum and a 16 °C maximum. The humidity was set to follow a cycle ranging from a minimum of 58% and a maximum of 70%.
- Organisms: A model community was established in each pot consisting of four plant species, and 13 species of soil organisms (other than earthworms). There were four earthworm treatments used each with four replicates:

Treatment	Epigeic Species	Endogeic Species
1 = no earthworms	none	none
2 = 1 functional group (2 x endogeic species)	none	*Allolobophora chlorotica, Aporrectodea tuberculata*
3 = 2 functional groups (1 x epigeic and 1 x endogeic species)	*Eisenia fetida*	*Aporrectodea caliginosa*
4 = 2 functional groups (3 x epigeic and 3 x endogeic species)	*Eisenia fetida, Lumbricus rubellus, Dendrodrilus rubidus*	*Aporrectodea caliginosa, Allolobophora chlorotica, Octolasian cyaneum*

The effect of both functional and species diversity on soil physical properties were studied by comparing treatments 2 and 3 for functional diversity, and treatments 3 and 4 for species diversity. Sampling of the Ecotron chambers took place in October 1999 with only 8 of the chambers being sampled for this study. This was because chambers 1-8 had received a $^{13}CO_2$ pulse therefore to prevent any potential cross contamination of other researchers' samples back in the laboratory at Stirling only chambers 9-16 were sampled. This precaution was taken since several other researchers' were using C^{13} labelling techniques in their investigations. All 16 chambers had full soil descriptions done. Kubiena tins were removed for thin section preparation and loose soil samples were taken for aggregate stability determination (see chapter 4 for methods).

3.4 Sourhope Experiments

This research project is part of NERC's Soil Biodiversity Thematic Programme, with the experimental field site located at the Macaulay Land Use Research Institute's farm at Sourhope in the Scottish borders. The aims of this programme are to achieve an understanding of a) the biological diversity of the soil biota, and b) the functional role played by soil organisms in key ecological processes. This project is linked to two others that were investigating the effects of earthworms on soil microbiological and biogeochemical properties, with all three projects utilising samples from the same soil and with the samples being collected at the same time. The overall aim of the three projects is to investigate the effects of changes in earthworm communities on soil physical, biochemical and microbiological functions in an upland soil. Some data collected by the other two projects (i.e. soil pH and earthworm survey data) were be used to aid the interpretation of the data collected in this research.

3.4.1 Site Location and Information

The experimental site is located at Sourhope in the Scottish borders, about 20 km south of Kelso. The field site is situated at the head of the Bowmont Valley in the Cheviot Hills on the western slope of Cheviot. The field site is at an altitude of around 320 m and has an annual rainfall (10 yr mean) of 952 mm. The site is an example of an upland acid grassland with the vegetation community consisting of a mainly *Agrostis capillaris* based community, NVC class U4d (Kenny 1998). The soils at the site have been characterised as acid brown forest soils (Sourhope series) having developed from locally drift from andesitic lavas of Old Red Sandstone age

(Kenny 1998). The pH of the soils on site was c. pH 4 (Kenny 1998). The soil profiles at Sourhope have roughly the same horizon sequence, i.e. LF, H (not always present), H_{phy} (not always present), A_h (in some cases it can be sub-divided into two separate horizons A_{h1} and A_{h2}) and B. Davidson *et al* (2002) identified the white coloured horizon which was located between the H and A_h as H_{phy} due to its organic nature and the high abundance of phytoliths, which are botanical microfossils formed from opal or calcite that has been deposited in and between plant cells (Mulholland and Rapp 1992).

The site at Sourhope shows evidence of past cultivation in the rigs and furrows that can be seen running down the site (Davidson 2002). To offset any topographic effect on soil properties between the rigs and furrows, only the rigs were sampled.

3.4.2 Field Set-up

At Sourhope the experimental field site was located on a north-facing slope, and was fenced to prevent livestock or deer access to the plots. There were five rows of plots with each row consisting of 6 individual plots, each having a specific treatment imposed on it (figure 3.3). The rows were used as replicates and the whole site was set up using a randomised block structure. The 6 treatments that were used on the field site were control 1, control 2 (to be used by a second phase of projects in the programme), and additions of lime, nitrogen, nitrogen + lime and biocide. The treatments used for this research project were the control and liming. Lime was applied to the treated plots at a rate of 600 g m^{-2} once a year, during late spring or early summer. This lime addition is high in comparison to agronomic additions, and was used to provide a strong perturbation to the system, with the intention of causing

earthworm community changes. The response of the earthworm community to the increase in pH may give some insight as to the resilience of earthworm communities.

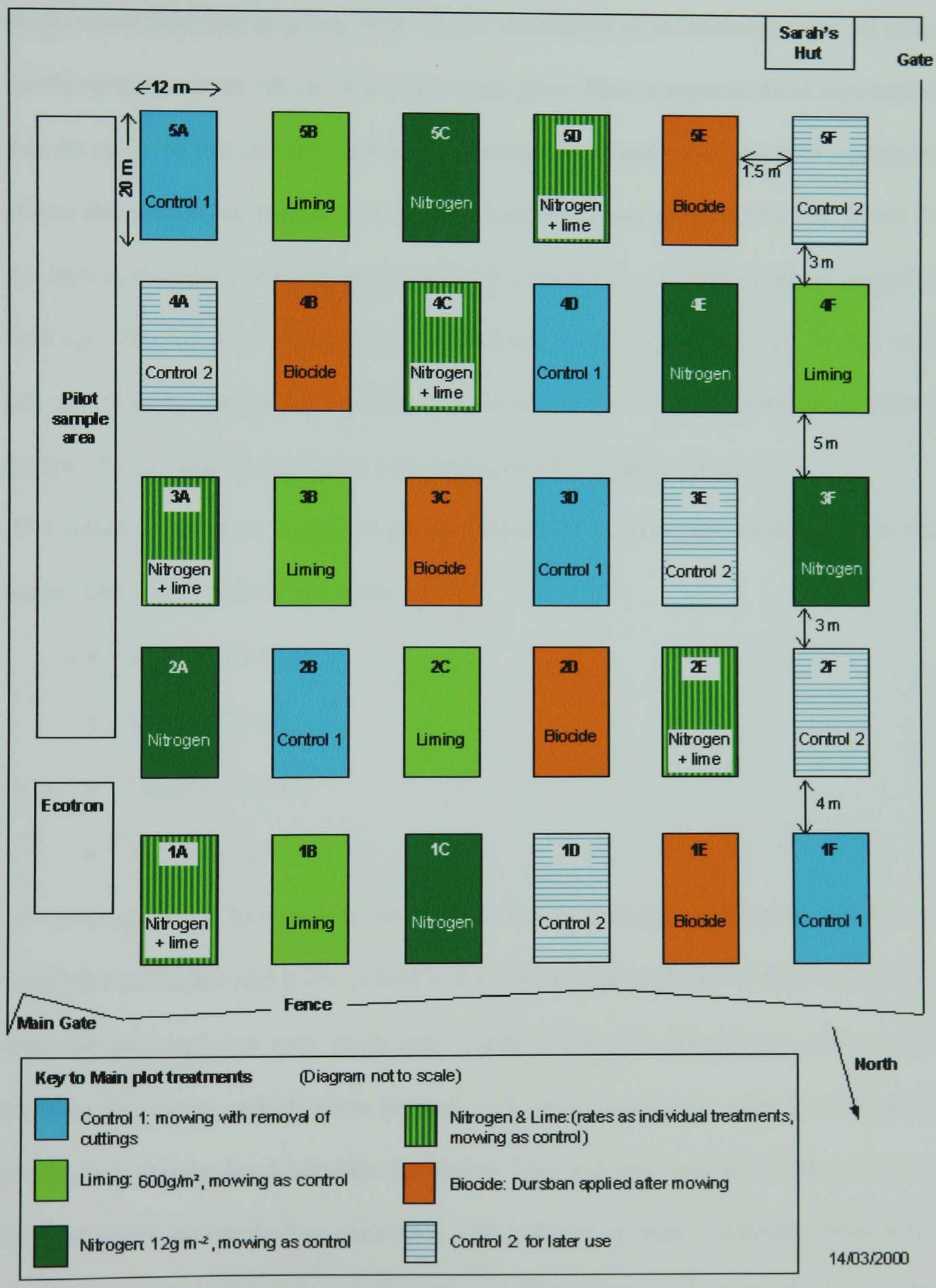


Figure 3.3: Schematic diagram of the plots and treatments used at Sourhope.

It was inappropriate for the experiment to be run on the main Sourhope plots due to the risk of contaminating them with the planned earthworm inoculation. This inoculation may have affected other soil organisms that were being studied by other projects and therefore affecting their results. An animal proof enclosure was set aside for this project, about 100 m west of the main plots. Here a separate field experiment was set up by taking soil from the main Sourhope plots and reburying it in containers at this alternative site named Sweethope. Sweethope was prepared for the burial of the boxes of soil by digging trenches lined with a bed of sand to allow adequate drainage. The layout of Sweethope mirrored that of the main plots (i.e. 5 rows with one set of control boxes and one set of limed boxes in each row), with the individual earthworm and liming treatments arranged randomly within each row.

The reburied soil was placed in plastic containers to keep the earthworms in the boxes. The dimensions of the boxes were:

- Depth – 24.5 cm
- Width – 33 cm
- Length – 43 cm
- Volume – 0.035 m^3

To investigate the best way to drain these boxes a small experiment was set up where several boxes had holes drilled in the bottom, and one had its base completely removed and replaced with thick steel mesh. Hole size and density were varied between the boxes, which were packed with handsorted soil, with the degree of compaction standardised between the boxes. The soil was saturated and a constant head of water established in each box. The volume of water draining through the boxes at set intervals was recorded. The optimum arrangement of drainage holes were 50 x ¼ inch holes homogeneously distributed across the base of the box. All the

boxes that were installed at Sweethope had this same pattern of holes drilled into their base. In addition to the holes, the boxes had to be made as earthworm proof as possible to prevent both the inoculated earthworms escaping and alien earthworms from invading the boxes. To do this fine mesh was cut to fit into the bases of the boxes and more mesh cut, to be attached as lids. The lids were stuck onto the rims of the boxes using quick drying silicon sealant, since this was durable yet easily removed during the regular grass cuts and sampling sessions (Figure 3.4).

Figure 3.4: Image showing some of the boxes buried in their trenches with the mesh lids attached. One box had no mesh lid on to see which earthworm species would voluntarily colonise the boxes.

100 boxes of soil were dug out of the Sourhope plots as intact soil monoliths. 50 of these boxes were reburied immediately at Sweethope. These are referred to throughout the rest of the research as the undisturbed soil and form the highest level

of system complexity as outlined in section 3.1. The remaining boxes were brought back to Stirling, where over a period of 1½ months, each of the 50 monoliths were split up by horizon, allowed to become semi-air dried and then crushed by hand to remove any existing soil structure. In addition to removing any structure, it also had the effect of removing any earthworms present, but it would not have removed cocoons. This process was done so as to be sure that any changes seen in the soil structure had been the result of the earthworm inoculum and not the remains of past structural features formed by previous earthworm activity. This forms the intermediate level of system complexity, as outlined earlier in this chapter, and for the rest of the research are referred to as the disturbed soil. After this disturbance, the soil was put back into the boxes in the same horizon sequences and then reburied at Sweethope.

Sweethope was maintained by cutting the grass in the boxes roughly every two weeks during the growing season. This was done to prevent the mesh lids being pushed off by the growth of the grass, and was achieved by cutting the grass by hand using scissors so as to be able to collect the clippings to assess biomass. The grass was cut roughly twice a month during the spring and summer when growth was at its peak. In addition to the grass cuts, lime was added to the boxes whenever lime was applied to the main Sourhope plots, which was done once a year during late spring or early summer.

In addition to the control and lime treatments, a series of earthworm treatments were imposed on the soil. The treatments were:

- No earthworm inoculation
- 1 epigeic species (species A)
- 1 endogeic species (species B)

- 1 anecic species (species C)
- All three functional groups together (ABC)

Only the no earthworms and ABC treatments were sampled for this research project because of the number of samples that had to be collected, prepared and analysed. It was also hoped that these two treatments would have given the greatest contrast therefore making any earthworm effects more evident. Before the species for the inoculation were chosen, a survey of the earthworm community at Sourhope was carried out in September 1999 by taking a soil monolith, 0.035 m^3 in volume from each of the control and lime plots and then hand sorting them. It should be noted that at the time of the survey the preceding weather conditions had been very dry. The summary of the survey data is shown in table 3.1:

Table 3.1: Summary of earthworm survey carried out in September 1999 to establish the existing earthworm community on site showing the species composition of the control and limed plots.

Control Plots		**Limed Plots**	
Species	Mean Earthworm Abundance/ m^2	Species	Mean Earthworm Abundance/ m^2
Allolobophora chlorotica	66	*Allolobophora chlorotica*	40
Dendrobaena octaedra	8	*Dendrobaena octaedra*	8
Dendrodrilus rubidus	14	*Dendrodrilus rubidus*	24
Lumbricus sp*	56	*Lumbricus* sp*	44
Lumbricus festivus	4	*Lumbricus festivus*	1
Dendrobaena/Dendrodrilus sp**	29	*Dendrobaena/Dendrodrilus* sp**	20
Lumbricus rubellus	16	*Lumbricus rubellus*	1
Unidentified juveniles	7	*Lumbricus terrestris*	1
Apporectodea caliginosa	3	Unidentified juveniles	3
Total mean earthworm abundance	202	Total mean earthworm abundance	144

* Includes *Lumbricus* juveniles and those individuals who cannot be identified past their genus

** Individuals who could be of either genus

In addition to the above species, subsequent visits showed that *Octolasion cyaneum* was also present on site, but not been sampled during this initial survey. One possible explanation was that the uncharacteristically dry conditions before the sampling took place had resulted in *O. cyaneum* moving deeper in the soil at the time of sampling.

The data presented in table 3.1 indicates that there were more worms in limed plots than the control plots. The mean abundance of earthworms over the five experimental blocks in the lime plots was 202 individuals m^{-2} as compared to the 144 individuals m^{-2} in the control plots. This can not be due to pH differences alone since pH was only marginally higher in the limed plots than the control plots, with the mean pH of the limed plots at 4.12 compared to 4.06 for the control plots.

The species that were chosen for inoculation had to be already present on site and represent each of the ecological groups. In addition these species had to be available from suppliers in sufficiently large numbers to allow large scale inoculation. The number of earthworms inoculated per box was thought to be sufficient to become dominant over the existing community. This was done because it was impossible to remove the existing earthworm community without serious implications on the structure, biology and chemistry of the soil. The inoculation took place in April 2000 and the species and the number of individuals/box used in the inoculum were:

- Epigeic – *Lumbricus rubellus*, 20 individuals/box
- Endogeic – *Allolobophora chlorotica*, 15 individuals/box
- Anecic – *Lumbricus terrestris*, 4 individuals/box

Over the duration of the field experiment there were five sampling sessions which are outlined below:

1. Initial- December 1999 from the Sourhope main plots. This provided data on the initial conditions of the soil.
2. Intermediate 1- April 2000 form disturbed boxes at Sweethope. This provided data on the initial conditions of the disturbed soil just prior to earthworm inoculation.
3. Intermediate 2- October 2000 from disturbed boxes at Sweethope. This provided data on the effect of earthworms on soil structure six months after inoculation.
4. Intermediate 3- August 2001 from the disturbed boxes at Sweethope. This provided the final data from the disturbed boxes 16 months after earthworm inoculation. This sampling was delayed due to the foot and mouth outbreak.
5. Final- October 2001 from the undisturbed boxes at Sweethope. This provided the final data on the effect of earthworms on soil physical properties in the highest complexity system, the undisturbed soil monoliths.

This chapter has outlined the aims and rationale of the research design, and how the experiment was set-up in the field. In the next chapter the experimental techniques used will be outlined in depth giving the rationale of how the methods evolved and how the data were collected.

Chapter 4: Experimental Methods

A number of different techniques have been used in this research to quantify the effects of earthworms on void space and aggregation. A summary of the key measurements and reasons why those techniques were chosen are given below:

1. % Organic carbon - determined using a modified Walkley-Black wet oxidation to establish whether any changes in organic matter occurred due to the treatments.
2. Saturated hydraulic conductivity - determined using a constant head method in the laboratory. It provided data on the degree of macroporosity in the soil.
3. Aggregate stability – was determined using a laser diffraction particle size analyser. This is a new technique that provides detailed particle size distributions of aggregate samples which are broken down using sonication. Was used as a way of quantifying soil structural stability.
4. Micromorphological analysis of earthworm excrements – quantified by point counting. Every earthworm excrement counted was described in detail (i.e. size, shape, colour, composition etc). This analysis provided data on the effects of earthworm casting on aggregation.
5. Void space – quantified using image analysis of digitally captured images. This technique provided data on total porosity and the size distribution of voids within the soil.

4.1 Organic Carbon Determination

The determination of the percentage organic carbon utilised the same sample of 1-2mm aggregates as in aggregate stability determination. These data were collected

because earthworms tend to lead to the enrichment of casts with organic matter which, as shown in chapter 2, contributes to the stability of soil aggregates. It was therefore important to determine whether the 1-2 mm aggregates were enriched with organic matter and how this related to a) the earthworm treatments and b) aggregate stability.

There are numerous techniques for determining the amount of carbon present in soil, but the two most commonly used are i) loss on ignition and ii) wet oxidation. As their names suggests, loss on ignition involves burning off the organic matter in a furnace at very high temperatures and measuring the subsequent loss in mass, whilst wet oxidation methods are chemical methods whereby organic carbon is oxidised by an excess of strong oxidising agents, such as hydrogen peroxide or potassium dichromate. Loss on ignition is generally considered to be a quick and easy way to determine the organic matter content of the soil. The drawback of this technique is that not only organic matter removed at high temperatures but calcium carbonate will be decomposed producing CO_2 and structural water in clays will be lost, leading to excess weight loss and therefore an overestimation of the amount of organic matter present in a sample (Hesse 1971). Wet oxidation techniques measure soil organic carbon content quite accurately and have no effect on the inorganic carbon (such as $CaCO_3$), but are time consuming and do not completely oxidise all of the organic carbon (Hesse 1971). To convert % organic carbon, as determined by wet oxidation, to % organic matter, the results are multiplied by 1.724 because organic matter generally has a carbon content of 58%. The technique chosen to determine the % organic carbon was the modified Walkley-Black wet oxidation method.

The modified Walkley-Black method (van Lagen 1996) uses acidified potassium dichromate as its oxidising agent and unlike other wet oxidation methods, it does not

require any external heating of the mixture. It works due to the organic carbon being oxidised by the dichromate, which has been added to excess. The volume of dichromate remaining after the finish of the oxidation reaction is calculated by reducing it with ammonium ferrous sulphate in the presence of a redox indicator (in this case barium diphenylamine sulphonate). Therefore having added a known quantity of dichromate in the first place it is possible to calculate how much has been used to oxidise the carbon and therefore the amount of organic carbon in the soil. This method for determining OC oxidises only 70% of the OC in the sample so a correction factor is added to account for this.

4.2 Bulk Density

Bulk density (ρ_b) is defined by the following equation (from Sumner 2000):

$$\rho_b = \frac{M_s}{V}$$

M_s = Mass of solids
V = Volume of solids & voids

Bulk density can be used to calculate total soil porosity. A soil with a high bulk density will have less void space than one with a low bulk density. It is determined by measuring the mass of a known volume of dry soil, which can be achieved in a number of different ways. In this investigation bulk density was calculated from samples for micromorphological analysis and/or saturated hydraulic conductivity cores.

Bulk density was determined from Kubiena tins taken for micromorphological analysis using the following procedure:

1. Kubiena tins of known volume were labelled and weighed.
2. Undisturbed soil samples were taken according to Murphy (1986), wrapped in clingfilm to prevent excess moisture loss and then brought back to the laboratory.
3. In the laboratory the clingfilm and excess soil on the outside of the tins were removed. The tins were weighed to provide the mass of soil and water in the samples.
4. At the same time as the Kubiena tins were taken, loose soil was also collected from the same horizon as the Kubiena tins, for the determination of moisture content.
5. The loose soil was weighed and then placed in an oven at 105 °C for 24 hrs until its mass remained constant. The moisture content was calculated from the mass difference before and after oven drying.
6. The moisture content of the soil in the Kubiena tin was assumed to be the same as that of the loose soil, therefore the mass of dry soil in the Kubiena tins could be calculated.
7. Bulk density was calculated for each Kubiena tin according to the formula presented previously.

Bulk Density was determined from saturated hydraulic conductivity (K_{sat}) cores in the following way:

1. Cores of diameter 68 mm were taken for K_{sat} determination (see 5.3 procedures for the collection of these cores).
2. After K_{sat} had been determined on all the cores, the depth of soil in the cores was noted so that the volume of soil material could be calculated.

3. The soil material was extruded and dried at 105 °C for 24 hrs until a constant mass was obtained. Using these data, dry bulk density was calculated as follows:

$$\rho_b = \frac{M}{L \times SA}$$

M = Mass dry soil
L = Length of core
SA Surface area of core

4.3 Saturated Hydraulic Conductivity (K_{sat})

4.3.1 What is Saturated Hydraulic Conductivity?

Soil is a porous medium through which water can flow both vertically and horizontally. The ability of soil to transmit water is in part determined by the nature and characteristics of the soil pores. Klute and Dirksen (1986) defined the hydraulic conductivity of soil as a measure of its ability to transmit water. The flow of water of through soil follows Darcy's Law, which for vertical water flow through saturated soil states that:

$$q = \frac{K \times \Delta H}{L}$$

q = flux density
K = hydraulic conductivity
ΔH/L = hydraulic gradient

Hillel (1982, pp95) states that "this law indicates the flow of a liquid through a porous medium is in the direction of, and at a rate proportional to the driving force acting on the liquid (i.e. hydraulic gradient) and is also proportional to the property of the property of the conducting medium to transmit the liquid (i.e. its conductivity)". In soil, hydraulic conductivity (K) varies with moisture content and is greatest when the soil is saturated

Having identified what hydraulic conductivity is, saturated hydraulic conductivity (K_{sat}) is defined as the ability of a soil to transmit water when in a saturated state (Klute and Dirksen 1986). When soil is saturated, water will tend to flow through large pores, i.e. macropores, in what is called bypass or preferential flow. This happens because the water is in a tension free state and takes place when ponding occurs at the soil surface (Ehlers 1975). What this means is that by measuring K_{sat} the degree of macroporosity of soil can be inferred because in a saturated state, water will flow through macropores within the soil (Wang *et al* 1994). As has been outlined in chapter 2, earthworms, through their burrowing activity lead to the formation of macropores, therefore by measuring K_{sat} the degree of connected macroporosity of soil and the effect that earthworms have on macroporosity can be determined.

4.3.2 Techniques for Measuring K_{sat}

A number of techniques have been devised for measuring K_{sat} both in the field and in the laboratory. The choice of method depends on factors such as (Klute and Dirksen 1986):

- The equipment available
- The nature of the soil
- The kind of samples available
- The skills and knowledge of the experimenter
- The soil-water suction range to be covered
- The purpose for which the measurements are being made.

Some authors suggest that field measurements may be more reliable than laboratory methods (Soil Survey Manual 1993).

Some examples of the techniques for measuring K_{sat} are outlined below:

Field measurements

- Well/auger methods: these methods rely on the level of the water table. A hole is dug into the soil below the water table so that the soil used is saturated. Some of the water is pumped out with the volume of water that flows back into the hole and the time taken related to the K_{sat} (Burke *et al* 1986 pp48). This method only works on soil situated below the water table and in soils which do not have excessively clayey textures.
- Guelph Permeameter: This is a constant head, in-hole permeameter operating on the Marriott bottle principle (Lilly 1994). Measurements are made of the steady state recharge necessary to maintain a constant depth of water within an unlined well terminating above the water table (Paige and Hillel 1993; Lilly 1994; Bargarello and Giordano 1999).
- Ring infiltrometers: With this technique metal rings are inserted into the ground and water is added to the surface of the soil until a steady infiltration rate is attained at which point K_{sat} can be calculated. It tends to measure only the K_{sat} at the surface and is very sensitive to earthworm and root channels, so it is important to choose a ring size that covers a representative area of the soil (Youngs 1991).

Laboratory methods

There are principally two laboratory methods used to determine K_{sat}, both using undisturbed soil cores and measuring either the time taken for a specific volume of soil to drain through the core, or the volume of water draining from the core over a set time period. The only major difference between the two is in the form of the pressure head in that one uses a constant head of water above the core and the other using a falling hydraulic head.

The constant head method follows Darcy's law and is given by the volume of water passing through a soil in a given time period at a constant pressure head. It is represented by the following equation (Youngs 1991):

$$K = \frac{Q \times L}{A \times \Delta h}$$

K= hydraulic conductivity
Q= flow rate
L= length of column
A= column cross-sectional area
Δh= hydraulic pressure head

The problems associated with the laboratory analysis of K_{sat} using undisturbed soil cores are that a) the cores may not be representative of the actual soil conditions, since Darcy's law assumes a homogeneous material (Burke *et al* 1986; Klute and Dirksen 1986; Youngs 1991), b) the cores have to be undisturbed but the sampling process can lead to disturbance of the soil structure (Burke *et al* 1986), and c) preferential flow may occur down the sides of the core (an edge effect) (Youngs 1991). To ensure that a sample is as representative as possible of the structure of the surrounding soil Klute and Dirksen (1986) suggested that cores should have diameters of between 2-10 cm and lengths of 5-25 cm.

4.3.3 Methodology for K_{sat} Determination

The limitations of working on a relatively isolated site with no readily accessible water supply and the potential disturbance to the soil microbial community through saturation meant that field techniques were not appropriate. Cores of sufficient size (64 mm in diameter) were therefore obtained and transported carefully back to the laboratory for analysis using a constant head method.

K_{sat} was determined on the undisturbed boxes only, because the disturbance process removed all existing soil structure leading to artificial drainage properties. In addition it would have been very difficult to prevent the soil cores from slumping out of the plastic tubes when saturated due to the weakening of the soil structure by the disturbance process. Samples were taken for K_{sat} determination from the Sourhope main plots at the beginning of the research, to provide data on the existing macroporosity of the soil, and from the undisturbed experimental boxes at Sweethope. Three replicate cores were taken from each box.

Cores were taken using plastic drainage pipe (external diameter 68 mm) which had had the outside edges bevelled to produce a thin cutting edge, which would force the pressure created whilst inserting the cores into the soil away from the core, and cause the least disturbance to the core. The LF horizon was removed allowing the H and Ah horizons to be sampled. The cores were 11cm in length. After the pipe had been carefully driven into the soil the cores were cut out of the soil and plastic bags were attached over both ends of the cores to provide support for the soil in the pipe and to prevent excessive water loss, which may have led to some shrinkage of the core. During the final sampling from the Sweethope boxes the cores were driven into the soil to the base of the box since the depth of the soil in the different boxes did vary. It

also proved beneficial in transporting the soil back to Stirling since having the cores still *in situ* protected them from excess disturbance during transportation. These cores were then cut to length using a saw to prevent excessive smearing to the base of the cores.

Before analysis could take place the cores had to be prepared. Thick plastic mesh was attached over the bottom of the core to prevent the core from slumping during saturation. The cores were saturated from the base up by placing them on a metal grid in a box (figure 4.1). Once the cores were ready for saturation water was added to the box, and the water level was raised slowly over several days to allow the complete saturation of the core and to make sure all air in the cores was driven out. After saturation the cores were ready for the Ksat determination. It was noted that during the saturation process a number of worms were driven out of the cores. These were identified and their numbers added to the final earthworm census taken at the end of the experiment from all the experimental boxes at Sweethope.

Figure 4.1: Images showing how the K_{sat} cores were saturated. Some of the cores during the final sampling did not leave enough height above the soil surface for the maintenance of the required matric head, so sections of plastic pipe were cut and attached to the top of the cores using silicon sealant.

To be time efficient in determining K_{sat}, multiple cores were analysed at the same time. To do this a long strip of sturdy metal mesh was cut and raised above the bench top, with 5 clamp stands to hold the Marriott bottles and cores in place. Funnels were then stuck through the mesh allowing the drainage water to be collected beneath the cores. The experimental set up is shown in figure 4.2.

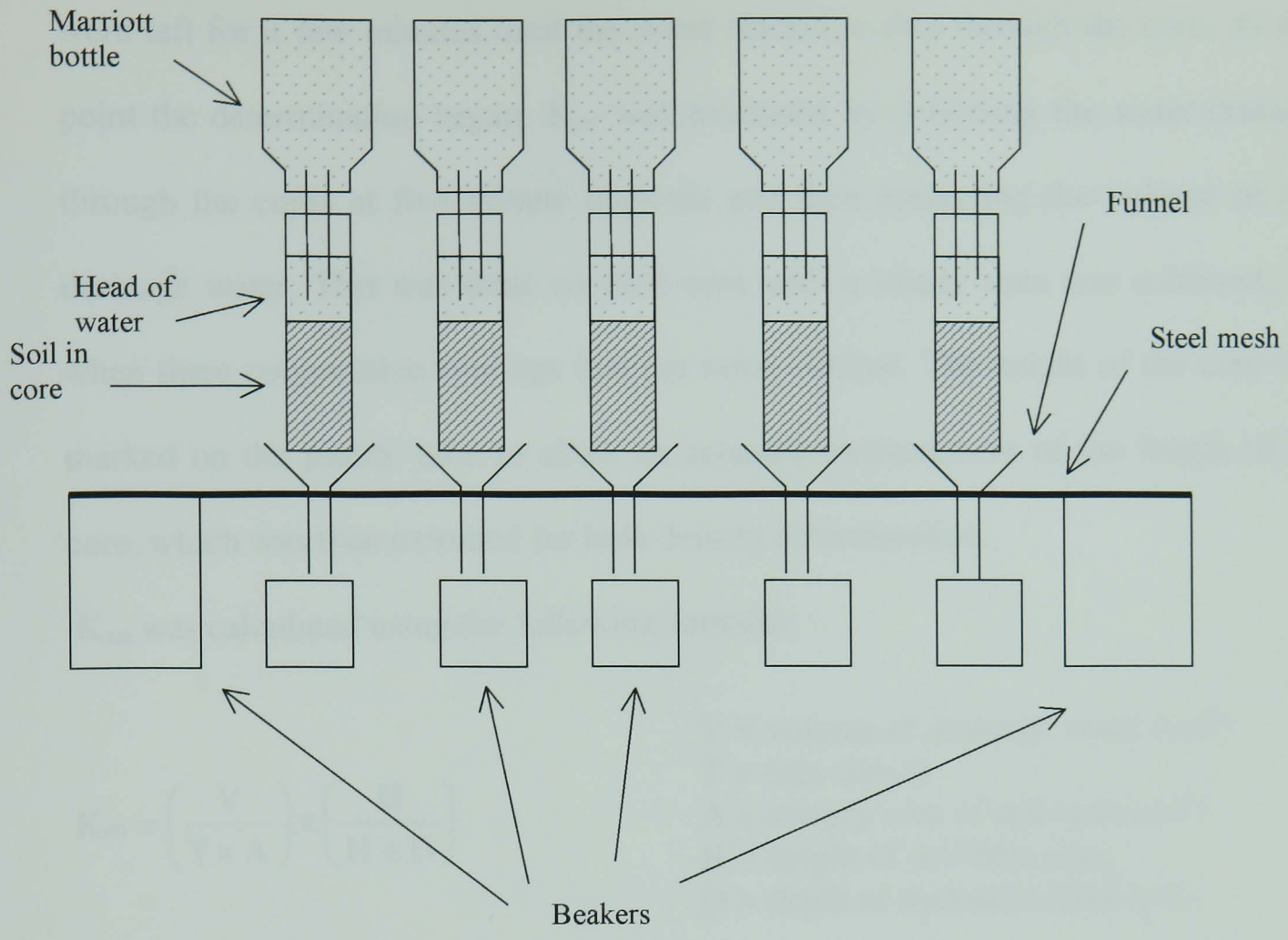


Figure 4.2: Schematic diagram and photograph of the apparatus used to determine K_{sat}. The photo shows only a part of the whole apparatus.

After the cores were installed on the apparatus, a 5 cm head was established on top of the soil surface, which was kept constant using the Marriott bottles. The cores

were left for a few minutes until the water started to drip through the core. At this point the determination began. K_{sat} was measured by collecting the water draining through the cores at five minute intervals and then measuring the volume of this drainage water. This was done on each core until a steady state was achieved, i.e. when three consecutive readings had the same volume. The height of the core was marked on the plastic tube to allow an accurate measurement of the length of the core, which was then extruded for bulk density determination.

K_{sat} was calculated using the following formula:

$$K_{sat} = \left(\frac{V}{T \times A}\right) \times \left(\frac{H}{H + D}\right)$$

V = volume of drainage water (cm^3)
T = time (days)
A = surface area of soil core (cm^2)
H = height of soil core (cm)
D = depth of hydraulic head (cm)

4.4 Aggregate Stability

Soil aggregates are formed through a number of different processes, including the casting activities of earthworms. The stabilisation of soil aggregates is important for the structural stability of soil and is achieved through a number or mechanisms, which in part depend on i) formation processes, ii) environmental conditions and iii) soil characteristics, e.g. soil texture, organic matter content, cation concentration and cation exchange capacity (CEC) (Le Bissonnais 1996).

The formation of stable aggregates is important since aggregation plays a key role in determining soil structure (Low 1972; Chaney and Swift 1984; Emerson and Greenland 1990) as well as protecting the organic matter located within the individual aggregates from decomposition agents such as microbial organisms (Low 1972; Tisdall and Oades 1982; Chaney and Swift 1984). Therefore the proportion of

stable aggregates in the soil and the actual stability of the individual aggregates is important for defining a number of soil properties, e.g. organic matter dynamics, CEC, clay movement, soil hydraulic properties and consequently the erodibility of soil (Le Bissonnais 1996).

Table 4.1: Characteristics of some of the main methods for testing aggregate stability (Le Bissonnais 1996 pp426).

Type of treatment	*Form of sample*	*Expression of the result*	*Authors*
Wet Sieving	3-5 mm	MWD	Yoder (1936)
	< 2mm	% > 200µm	Henin *et al* (1958)
	whole soil	change in MWD	De Leenheer & De Boodt (1959)
	1-2 mm	% > 250µm	Kemper & Rosenau (1986)
	2-3.4mm	MWD	Chruchman & Tate (1987)
	1-2 mm	% > 250µm	Pojasok & Kay (1990)
Raindrops or rainfall	4-5 mm	time to breakdown	Low (1967)
	2-9 mm	MWD	Young (1984)
	5-8 mm	time to breakdown	Farres (1987)
	whole soil	% < 125µm	Loch (1994)
Ultrasonic dispersion	4-5 mm	dispersion rate	Edwards & Bremner (1967)
	4-5 mm	inter-aggregate pore volume	Grieve (1980)
Immersion	3-5mm	qualitative	Emerson (1967)
Dry sieving	<4 mm	MWD	Kemper & Chepil (1965)

A number of different techniques have been used to measure aggregate stability (table 4.1). Most methods make use of either wet sieving or turbidimetry with slight modifications to the protocols to suit the nature of the samples being analysed. The

reason for the diversity of methods used for measuring aggregate stability can be explained by both the existence of several mechanisms of aggregate breakdown, and for methodological reasons Le Bissonnais (1996).

All of these methods for measuring aggregate stability rely on the breakdown of aggregates and then measuring the size distribution of the breakdown products. The four mechanisms of aggregate breakdown, as highlighted by Le Bissonnais (1996), are:

- Slaking, i.e. breakdown caused by the compression of entrapped air during wetting
- Breakdown by differential swelling
- Raindrop impact
- Physico-chemical dispersion due to osmotic stress

The main characteristics of these breakdown mechanisms are shown in table 4.2.

Table 4.2: The characteristics of the main breakdown mechanisms (Le Bissonnais 1996 pp427).

	Mechanism			
Characteristics	*Slaking*	*Breakdown by differential swelling*	*Breakdown by raindrop impact*	*Physico-chemical dispersion*
Type of forces involved	Internal pressure by air entrapment during wetting	Internal pressure by clay differential swelling	External pressure by raindrop impact	Internal attractive forces between colloidal particles
Soil properties controlling the mechanism	Porosity, wettability, internal cohesion	Swelling potential, wetting conditions, cohesion	Wet cohesion (clay, organic matter, oxides)	Ionic status, clay mineralogy
Resulting fragments	Macroaggregates	Macro and micro aggregates	Elementary particles	Elementary particles
Intensity of the disaggregation	Large	Limited	Cumulative	Total

There is a high degree of variability between the results from different methods used to determine aggregate stability (Grieve 1980; Haynes 1993; Le Bissonnais 1996). Haynes (1993) investigated the differences between wet sieving and turbidimetry to measure aggregate stability. This study showed that both methods produced different results for the same soil depending on the soil moisture content and cropping history. It has been suggested that wet sieving measures aggregate stability, whilst turbidimetry is a dispersion test, and that wet sieving measures macroaggregate behaviour and dispersion tests measure microaggregate behaviour (Le Bissonnais 1996). Wet sieving tests tend to combine all of the potential breakdown mechanisms, but tend to overemphasise slaking (Le Bissonnais 1996).

Le Bissonnais (1996) suggests that some standardisation should be used when measuring aggregate stability, particularly in the nature and treatment of the sample, so that aggregate stability of different soils can be directly compared. The two major physical properties of soils that should be standardised are moisture content and structure. Aggregate stability is a transient property in relation to soil moisture conditions, therefore any method for measuring aggregate stability should standardise the moisture content of the aggregates used (Le Bissonnais 1996). The most common way to do this is to air dry the aggregates before any analysis is carried out.

To standardise the structural conditions of an aggregate sample, the samples should be broken down by hand to produce the maximum number of 'natural' soil aggregates (Le Bissonnais 1996). In addition, to allow for comparability of results between soils of different structures, a specific aggregate size fraction should be used, which should be between 2-10 mm in diameter (Le Bissonnais 1996).

Laser diffraction has recently been used to investigate aggregate breakdown and stability in Brazilian Oxisols (Buurman *et al* 1997; Muggler *et al* 1997; Muggler *et al* 1999; Westerhof *et al* 1999). Laser diffraction works according to Fraunhofer Diffraction which states that "when a beam of light falls onto a particle, a diffraction pattern is formed as some of the light is deflected (diffracted) by an amount dependent upon, amongst other things, the size of the particle" (Coulter® 1990). The size of the particles being analysed is inferred from the diffraction pattern produced when the laser beam hits the particles. The instrument used to determine aggregate stability in this research project was a Coulter LS230 Particle Size Analyser, which uses reverse Fourier Optics to measure particle sizes between 0.4 µm-2000 µm. Smaller particles in the size range 0.04 μm-0.4 μm are measured using a different system called PIDS (Polarisation Intensity Differential Scatter. The LS230 uses laser light as well three wavelengths of light which are filtered for polarisation in the vertical and horizontal planes (Coulter® 1999). A combination of 132 detectors is used to give high resolution in the sizing of sub-micron particles. By using the Coulter LS230 a continuous particle size distribution from 0.04 µm-2000 µm can be obtained. The LS230 Particle Size Analyser uses water as a suspension medium into which samples are added. There is a close loop circulatory system, which continually transports the samples into and out of the sample cell where the particle sizing takes place (Figure 4.3). The speed of the water flow and the duration over which the particle size measurements take place are fully controllable so as to produce a representative particle size distribution for the whole sample.

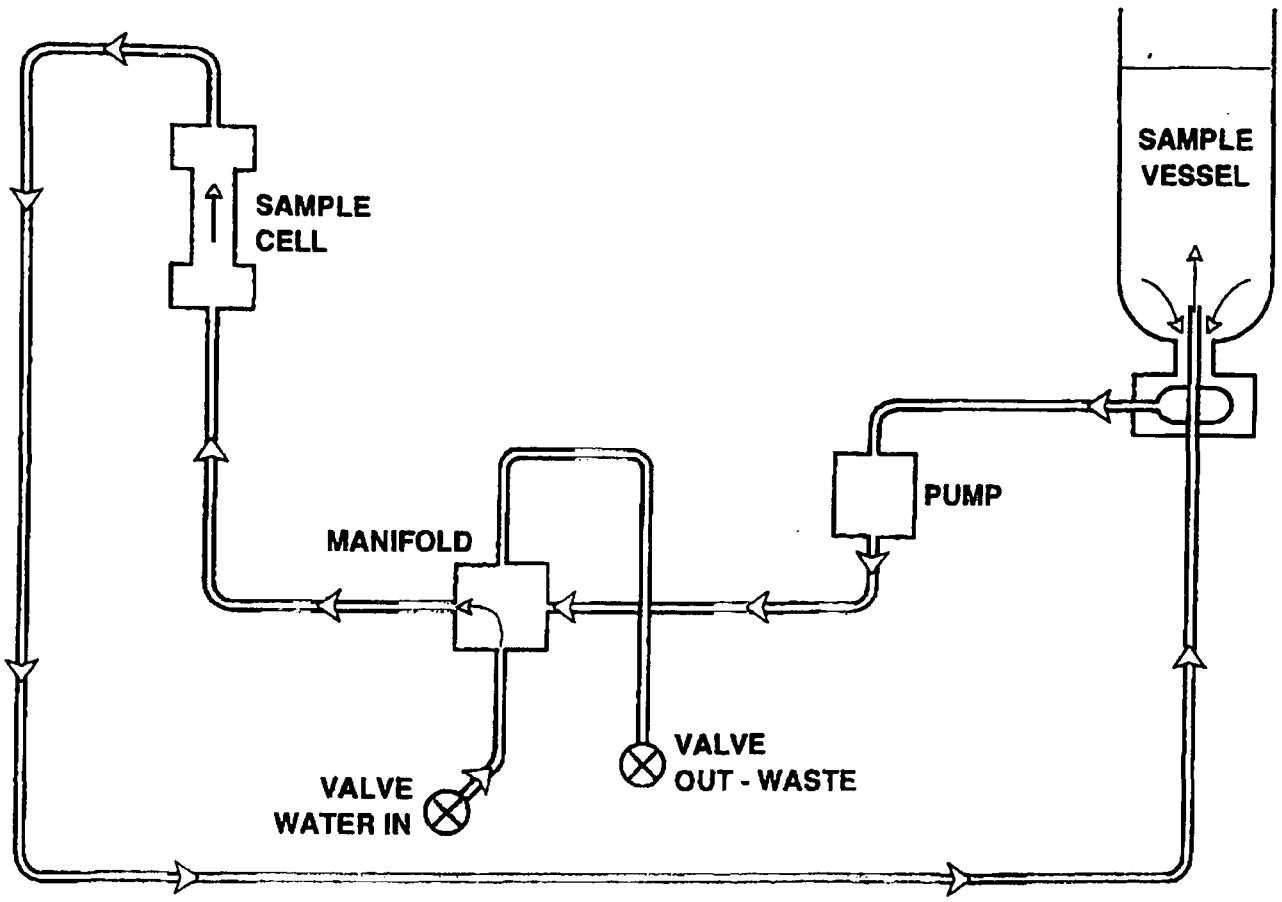


Figure 4.3: Schematic representation of the closed loop circulary system of the LS230 (Coulter® 1999)

There are a number of advantages as well as problems in using laser diffraction techniques in studying aggregate stability:

Advantages

- Using this technique a high degree of reproducibility can be achieved (Buurman *et al* 1997).
- It provides information on the products of aggregate breakdown as well as the rate of breakdown, all of which can be used to investigate aggregate stability.
- Laser diffraction can identify subtle changes in particle sizes in aggregates (Muggler *et al* 1997)

Problems

- One of the fundamental assumptions behind laser diffraction is that the particles being sized are spherical. Obviously this is not true when considering soil particles, especially with soils that have high clay contents, which are plate like in shape and have a propensity to flocculate (Buurman *et al* 1997). They also found that flocculation of the clays also affect the result.
- There are some issues of sample homogeneity and sub-sample representation, but these can mostly be overcome through thorough mixing before addition to the machine (Buurman *et al* 1997).
- Finally finding a suitable optical model to account for the non-spherical nature of soil particles can be difficult (Buurman *et al* 1997).

4.4.1 Aggregate Stability Methodology

Aggregate stability measurements were carried out on samples collected from the Ah horizons of all three experiments. The soil samples were as undisturbed as possible so that the structure of the soil could be maintained until the samples were brought back to the laboratory. In the laboratory the soil samples were set out in foil trays to allow them to air dry. This was achieved by gently breaking the soil apart as it dried so as to have as many natural air-dried aggregates as possible, a process which took anything up to 2 weeks to complete. Once the samples had been air-dried they were sieved to collect the 1-2 mm fraction on an automatic shaker for between 5-15 seconds. The shaking process was kept to a minimum so as to minimise disturbance to the aggregates. The 1-2 mm aggregates were then sealed in containers and stored in a cold room until analysis.

The aggregate stability analysis used 2 g samples of air-dried 1-2 mm aggregates, which had all root material and large stones removed by hand using tweezers. Stones were removed because they would have caused damage to the glass components of the instrument. Three analytical replicates were carried out on each soil sample.

After the preparation of the aggregate samples the analysis was ready to be carried out. The 2 g aggregate sample was added to the sample vessel and exposed to sonication for 120 seconds before the particle sizing took place. This was repeated three more times to produce 4 particle size distributions after 4 periods of sonication which characterised the breakdown of the 1-2 mm aggregates. It is the difference between these four runs which showed the degree of aggregate breakdown occurring and therefore the stability of the aggregates added to the instrument. A typical set of particle size distributions from a sample of 1-2 mm aggregates, highlighting the differences between run 1 and run 4, is shown in figure 4.4.

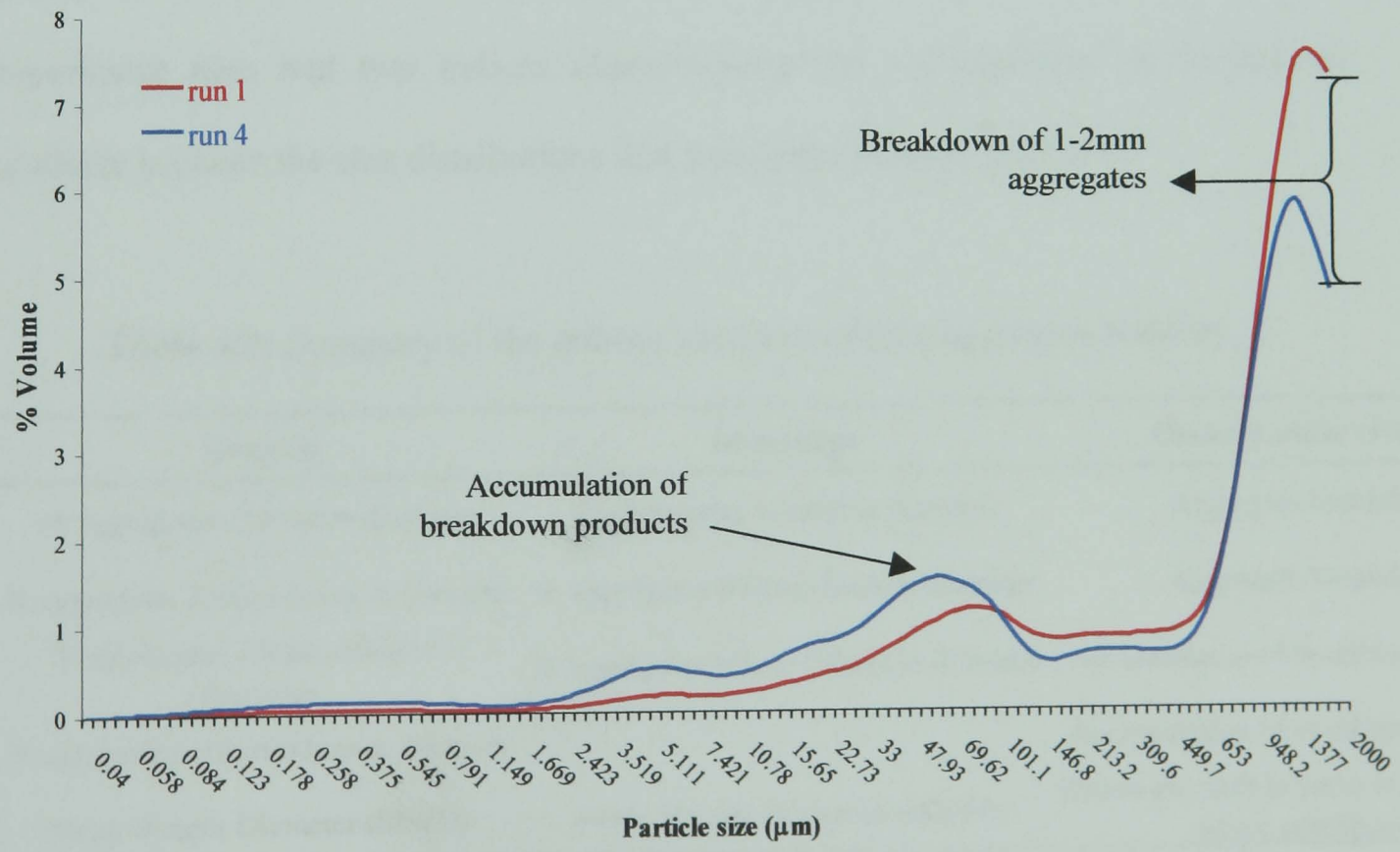


Figure 4.4: Particle size distributions showing the differences between runs 1 and 4 for a typical aggregate sample from the Sourhope research site.

The product from the aggregate stability determinations was a series of particle size distributions showing the breakdown of the 1-2 mm aggregates and the accumulation of small particle sizes, either primary mineral particles or micro-aggregates. To be able to analyse the data quantitatively a number of indices were created which are outlined in table 4.3. These indices can be characterised into 3 groups, 1) those which show the degree of aggregate breakdown, 2) those which quantify the degree of accumulation of breakdown products and 3) those which characterise the shift in the total particle size. Slightly different indices were chosen for the Ecotron and Sweethope experiments due to the different nature of the soils, with the Ecotron soils being dominated by larger sand sized particles and the Sweethope soils having a texture with more silt sized particles. Both the Ecotron and Sweethope soils used two indices of aggregate breakdown. The 1-2 mm index corresponds to the size of aggregates initially added to the instrument, whilst the second index is broader to gather information about any secondary aggregate breakdown. The Ecotron experiment also had two indices characterising the accumulation of breakdown products because the size distributions had two peaks in these fractions.

Table 4.3: Summary of the indices used to evaluate aggregate stability.

Ecotron	Sweethope	Characteristic of Index
% aggregates 1-2mm in diameter	% aggregates 1-2mm in diameter	Aggregate breakdown
% aggregates 800μm-2mm in diameter	% aggregates 600μm-2mm in diameter	Aggregate breakdown
% aggregates 150μm-800μm in diameter	% aggregates 40μm-150μm in diameter	Accumulation of breakdown products
% aggregates 10μm-60μm in diameter		Accumulation of breakdown products
Mean Weight Diameter (MWD)	Mean Weight Diameter (MWD)	Shows the shift in particle size over the whole distribution

4.5 Micromorphological and Image Analysis Techniques

Micromorphology can be defined as "the quantitative and qualitative analysis of undisturbed soils and other unconsolidated materials, usually in thin sections, using microscopic and sub-microscopic techniques" Kooistra (1991, pp315). Micromorphology was pioneered by Kubiena in the 1930s and ever since has played an important role in the investigation of soils (Murphy 1986). Micromorphological analysis is a useful technique because it allows for the visual observation, description and analysis of an inherently opaque material. The samples used are undisturbed so the features present are as they were in the field. Image analysis allows the quantification, such as size and shape, of defined features like void space.

4.5.1 Thin Section Preparation

The first step in micromorphological analysis is the collection of undisturbed soil samples usually using Kubiena tins. Along with impregnation, the collection of soil samples is one of the most critical operations in micromorphology (FitzPatrick 1993). The sampling strategy used when collecting thin section samples relies on the nature of the material being collected and the heterogeneity of the soil (Murphy 1986). For example, very stony soils are usually too complicated to be sampled with a Kubiena tin, so another approach must be used.

To collect a sample the Kubiena tin should be placed at the desired sampling point in the profile. By pressing gently on the tin, whilst at the same time cutting around the tin, it can be gently guided into the soil until full (Murphy 1986). This allows for the least possible disturbance to the soil inside the box. The lid is then be placed on

the tin, and the soil block in the tin carefully removed from the soil by cutting it out (Murphy 1986). Excess soil is removed from the back of with a sharp knife, and a lid attached. The orientation and sampling information are written on the lid of the tin using a waterproof pen (Murphy 1986). Once removed the sample is wrapped up in foil, Clingfilm or placed into a plastic bag to prevent moisture loss from the sample (Murphy 1986). The samples should be placed in a refrigerator as soon as possible so as to inhibit faunal activity.

Once back in the laboratory the water has to be removed from the sample prior to impregnation with resin. This can be done in as number of ways. Freeze-drying can be used although structural deformation can occur as the result of the formation of water crystals (Murphy 1986; FitzPatrick 1993). The preferred method is to use solvent exchange, usually acetone. This can be done in the liquid or vapour phase depending on the nature of the sample. The addition of acetone in the liquid phase is best done in a series of gradual steps where the % v/v of acetone to water increases with time (Tippkotter *et al* 1986). This process can take up to six weeks to complete (Murphy 1986).

Once the soil block has been dehydrated it is then impregnated with resin. The preferred resin is polyester resin although epoxy resins can be used under certain circumstances (Murphy 1986; FitzPatrick 1993). Polyester resins are preferred because they are more readily miscible with acetone than epoxy resins (Kooistra 1991). Both types of resin are hydrophobic so need complete removal of water from the sample (Murphy 1986). In Britain the resin of choice is Crystic resin (Murphy 1986), since this resin is fairly viscous and can be hardened at different rates by varying the amount of catalyst and accelerator used, therefore making it flexible in its use (FitzPatrick 1993).

It is at the impregnation stage of thin section production that stains can be added to aid in the identification of certain features. Conductive voids are stained to obtain a relation between voids observed and soil-physical data, especially hydraulic conductivity (Kooistra 1991). The staining of organic materials is performed in order to produce enough contrast for them to become fully optically visible for identification and/or quantification using image analysis (Kooistra 1991). Methylene blue is a common stain for highlighting void space whilst both non-fluorescent stains as well as flurochromes can be used to highlight biological materials such as microbes (Kooistra 1991; Altemuller and Joschko 1992). A blue dye was used in this research project to highlight void space from the surrounding material.

The next step in thin section preparation is the cutting of cured impregnated blocks. This is done along the entire length exposing the interior (FitzPatrick 1993). After cutting, samples are lapped to remove excess sample, polished and attached to a glass slide using thick crystic resin (FitzPatrick 1993). Once the bonding resin is dry the thin section is ground with a surface grinder and lapped until the sample is only 25-30μm thick (FitzPatrick 1993). The final stages involve the thin section sample being polished and a coverslip attached. This whole process is summarised in figure 4.5.

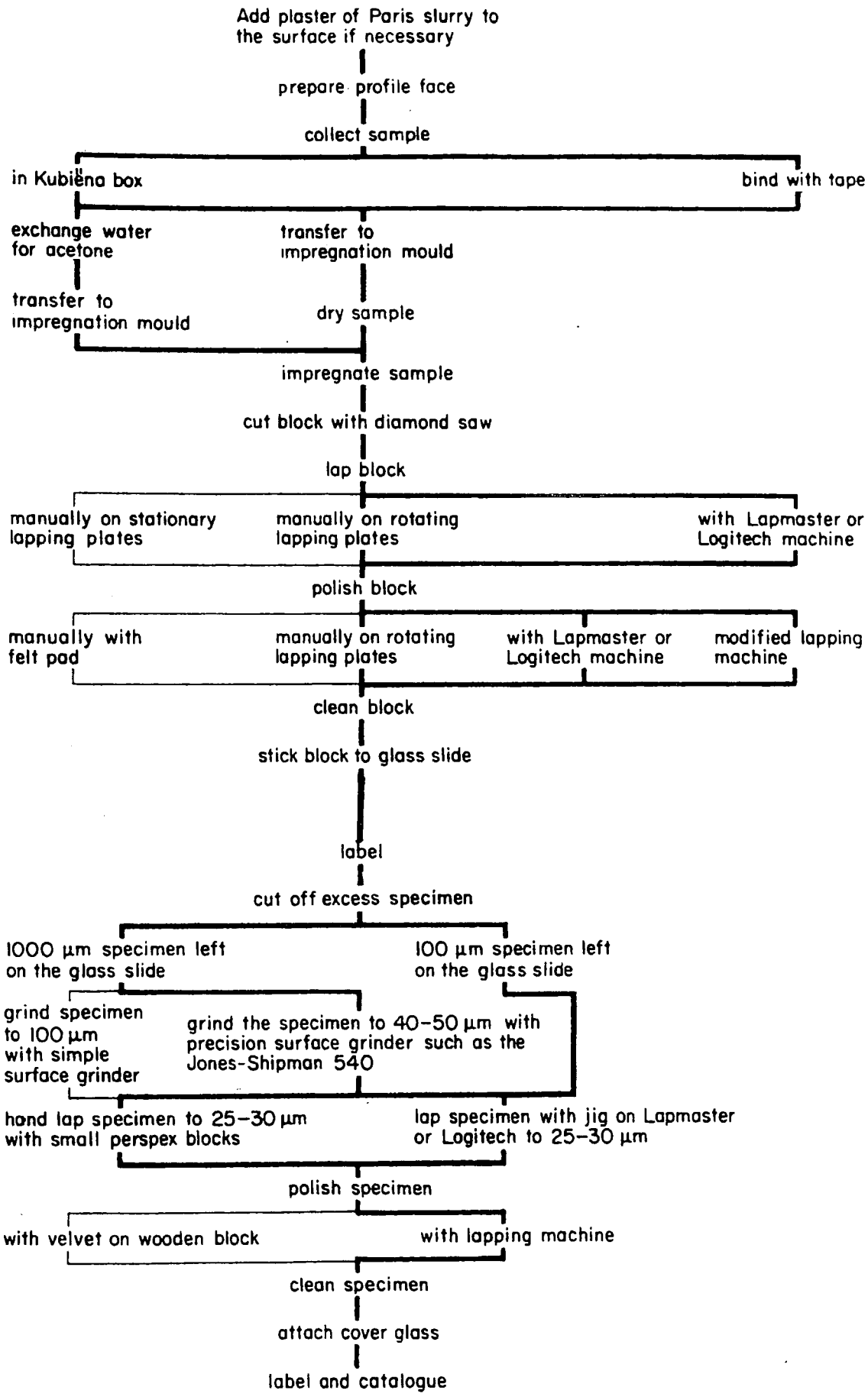


Figure 4.5: Flow chart of the preparation of thin sections. The thick lines give the pathways for the best methods (FitzPatrick 1993 pp7).

The fundamental piece of equipment in micromorphological analysis is the petrological microscope, the components and structure of which can be seen in figure 4.6.

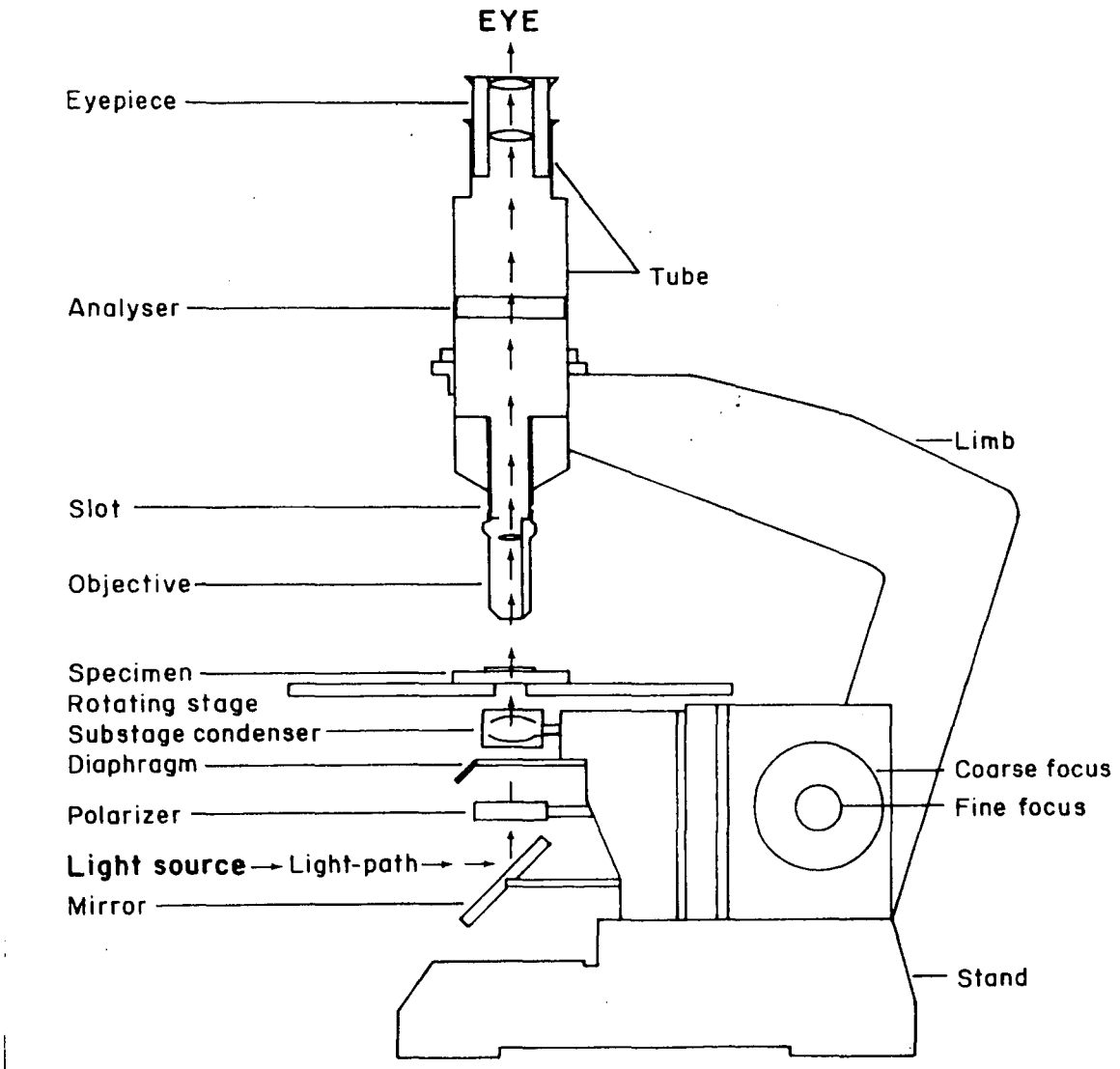


Figure 4.6: The construction of the petrological microscope (FitzPatrick 1993 pp8)

A full description of the fundamental components of a petrological microscope is given by FitzPatrick (1993). The most important feature of a petrological microscope is its ability to alter the polarisation of light. It can produce 3 types of polarised light:

- Plane polarised light
- Cross polarised light
- Circularly polarised light

Under the three different types of illumination, different features of the thin section sample will have different characteristics (for example the colour of soil minerals). This aids their identification and characterisation (FitzPatrick 1993). The main procedures of light and sub-microscopy are shown in table 4.4.

Table 4.4: Overview of the procedure used in light and sub-microscopy (Kooistra 1991)

Procedure light and sub-microscopy		
1. Identifications		
Light microscopy	-optical information	
	-morphological information	
Sub-microscopy	-morpholgical information (SEM)	
	-chemical analyses (EDX)	
2. Properties and occurrences		
	Properties	Occurrences
	-size	-location
	-shape	-orientation
	-composition	-distribution
	-boundaries	-frequency
by light or sub-microscopy		
3. Quantification		
aspects of properties and occurences by light or sub-microscopy or image analysis		
4. Documentation		
presentation of data as:		
	-descriptions	
	-tables	
	-graphs	
	-images	

SEM= Scanning Electron Microscopy; EDX= Energy Dispersive X-ray Analysis

4.5.2 Use of Micromorphology and Image Analysis for Studying Earthworm Pedofeatures

The two earthworm pedofeatures that are of micromorphological importance are casts and earthworm mediated voids, i.e. burrows and channels (Kooistra 1991). A

number of studies have already used micromorphology to investigate earthworm pedofeatures although many of these have not used an integrated approach. Table 4.5 summarises the research literature on earthworm casts and voids:

Table 4.5: Earthworm studies that have used micromorphological techniques (superscript numbers correspond to those numbers shown in the text).

Earthworm studies that have used micromorphological techniques			
Earthworm Casts		***Earthworm Burrows***	
Shaw & Pawluk (1986)	2	Shaw & Pawluk (1986)	3, 5
Shipitalo & Protz (1989)	0	Lee & Foster (1991)	4
Marinissen & Dexter (1990)	0,1	Kretzschmar (1991)	3
Lee & Foster (1991)	0,1	Schrader & Joschko (1991)	4
West *et al* (1991)	1	West *et al* (1991)	4,5
Martin & Marinissen (1993)	0,1	Binet & Curmi (1992)	4
Babel & Krestzschmar (1994)	0,1	Schrader (1993)	3,4,5
Marinissen & Miedema (1994)	1	Babel & Krestzschmar (1994)	3,4
Tomlin *et al* (1995)	0,1	Marinissen & Miedema (1994)	5
Ketterings *et al* (1997)	1	Peres *et al* (1994)	4,5
Hallaire *et al* (2000)	2	Tomlin *et al* (1995)	4,5
Barros *et al* (2001)	1,2	Boyle *et al* (1997)	5
Jongmans *et al* (2001)	2	Binet *et al* (1997)	3.4.5
		Ligthart (1997)	4
		Jegou *et al* (1998)	3,5
		Peres *et al (1998)*	3.4.5
		VandenBygaart *et al* (2000)	5
		Hallaire *et al* (2000)	4,5
		Barros *et al* (2001)	4,5
		Jongmans *et al* (2001)	5

Much of the work done on casts using micromorphology has focused on the mechanisms of stabilisation[0], composition[1] and activity[2]. As for burrows, much of the work has focused on earthworm burrowing behaviour[3], burrow morphology[4] and burrowing activity[5]. In relation to casting not much work has been done on the nature of casts in the soil and their description and quantification through image analysis

due to the inherent problems of being able to isolate the pedofeatures in question from the surrounding soil material.

Areas where micromorphology can provide new information on how earthworms affect soil physical properties are the quantification of earthworm mediated porosity and casts. In addition it would be useful if some species effects could be distinguished in casts and burrows (especially burrow linings) in thin section, even if it is only down to ecological classification (epigeic, anecic, endogeic).

There has been some research on earthworm burrows and burrowing behaviour/activity using sub-microscopic techniques (Joschko *et al* 1991; Joschko *et al* 1993; Capowiez *et al* 1998; Jegou *et al* 1998b; Jegou *et al* 1999; Langmaack *et al* 1999; Capowiez *et al* 2000; Jegou *et al* 2001; Pierret *et al* 2002). Most of this research has used X-ray computed tomography, which used X-rays to look at certain structures in soil samples without the need to destroy the sample and provides a 3 dimensional view of these features. However, so far most of this work has relied on laboratory studies, which may give rise to artefacts such as, the tendency for earthworms to burrow along the edge of containers.

Image analysis is a technique in which images are scanned or captured using high-resolution digital/CCD cameras and certain features identified, recorded and measured automatically using specialised image analysis software (FitzPatrick 1993). Images are captured using a camera and then digitised to produce a pixel map of the image. To analyse single features, black and white images are adequate, since for the computer to store red, blue and green pixels takes up substantially more memory (FitzPatrick 1993). However when analysing multiple or complex features, digitising the image in colour is essential. Once an image is captured it can be manipulated and certain features measured. For example it is possible to calculate the size, shape,

orientation, number and volume of soil pores in the soil. The key feature of image analysis is that it reduces the subjectivity of the quantification process. There are relatively few studies which have used image analysis to quantify the effect of earthworms on soil porosity, especially in temperate ecosystems (Binet *et al* 1997; Peres *et al* 1998; Hallaire *et al* 2000; VandenBygaart *et al* 2000; Barros *et al* 2001; Jongmans *et al* 2001).

In summary, micromorphology is the study of soil thin sections using light and sub-microscopic techniques. It can be used to observe and analyse microscopic features within the soil, which is why it lends itself well to the study of earthworm excrement and channels. In itself micromorphology is mostly a qualitative or at best semi-quantitative technique, but when used in conjunction with image analysis, quantitative information can be gathered for statistical analysis.

4.5.3 Micromorphological Analysis

In this project samples were taken for micromorphological and image analysis from all three experiments. The thin sections were prepared as shown in the previous section by acetone replacement, impregnation with Crystic resin and cutting and lapping to produce a thin section approximately 30 μm thick. A blue dye was added at the impregnation stage so as to make identification of voids easier for the image analysis process, (better contrast between voids and the surrounding soil material).

4.5.4 Micromorphological Assessment of Earthworm Excremental Features

Preliminary trials showed that it was very difficult to identify earthworm excrement reliably, as it was similar to the surrounding soil material. Point counting was used to quantify excrement in thin sections. The rationale for using this technique was that a human operator would be able to more accurately identify what was earthworm in origin. This was because the identification of excrements was not only determined by key diagnostic features, but also by the context of the pedofeature within the soil. At present computerised systems are not able to perform these contextual analyses. There are a number of limitations with using this technique:

1. Produces a more limited range of data as compared to image analysis techniques.
2. Point counting is a very time consuming technique, therefore imposing limitations on the number of samples that can be analysed.
3. Potential for operator error/bias- this was countered by firstly building up a large knowledge base and a great deal of experience on the identification of earthworm excrement, and bias was countered for by only one operator performing all the counts.
4. The resolution of the point count, i.e. how many points have to be counted before a representative data set is collected?

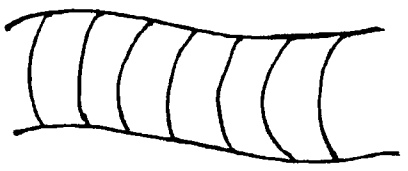
Firstly a classification system for the identification of earthworm excrements was designed. This was done by searching the existing literature on this subject and using the guidelines set out in the Handbook for Soil Thin Section Description (Bullock *et al* 1985), and by observing numerous slides from a variety of sites both in the UK and from around the world to identify what earthworm excrement looks like in thin

section. This earthworm excrement classification had five classes of excremental features, which are outlined below:

1. Mammilate: formed primarily through the coalescence of individual excrements until part or all of their boundaries have merged. The mammilate shape can be defined as consisting of mutually interfering spheroidal faces, which have a characteristic 'blobby' look.

2. Bacillo-cylinder: are individual excrements, which are characterised by two parallel sides, rounded ends and distinct boundaries. These excrements often tend to be clustered together.

3. Channel Form: are formed when earthworms infill their burrows with cast material. When sectioned transversely they are roughly spherical but when longitudinally sectioned they appear as a line of excrement with parallel sides, often with some cracks, which are no greater than the width of the excrement. A sub-class of this excrement type are vermiform excrements which again form linear excremental features with parallel sides, but also have cresentic laminations running through them.



4. Undifferentiated Excrement: features which are possibly earthworm in origin but lack definitive features to be placed into one of the other categories, e.g. a large excremental feature which has a vastly different composition to the surrounding soil material but lacks any diagnostic features.

Having identified the characteristics which defined each of the excrement classes the protocols for the point counting procedure were laid down. The primary data that came out of this procedure was the abundance of earthworm excrement in a whole thin section. This was achieved by using a mechanical stage which had 1 mm graduations on both the x and y-axis. The slide was placed on the stage in such a way as to ensure the largest area of the slide would be included in the count as possible, i.e. the traverse of the slide began at the soil material and not at the boundary of the glass slide. The stage was moved at 5 mm intervals on both axes and the feature under the crosshairs of the graticule was recorded at each point (figure 4.7). This 5mm interval was chosen to cover as much of the slide as possible. Earthworm excremental features are also large and a 5 mm interval was considered to be of sufficient size to accurately assess the occurrence of this excrement. The interval had to be of large enough to allow all of the thin sections to be analysed (a smaller interval would lead to more points therefore more time point counting). The features recorded were:

- Earthworm Excrement
- Other Faunal Excrement
- Stone Fragments
- Plant Fragments/Organic Matter
- Voids
- Undifferentiated Organo-Mineral Material
- Production artefact (i.e. features which were a by product of the production process such as air bubbles, cracks or areas of the thin section where the material is too thin).

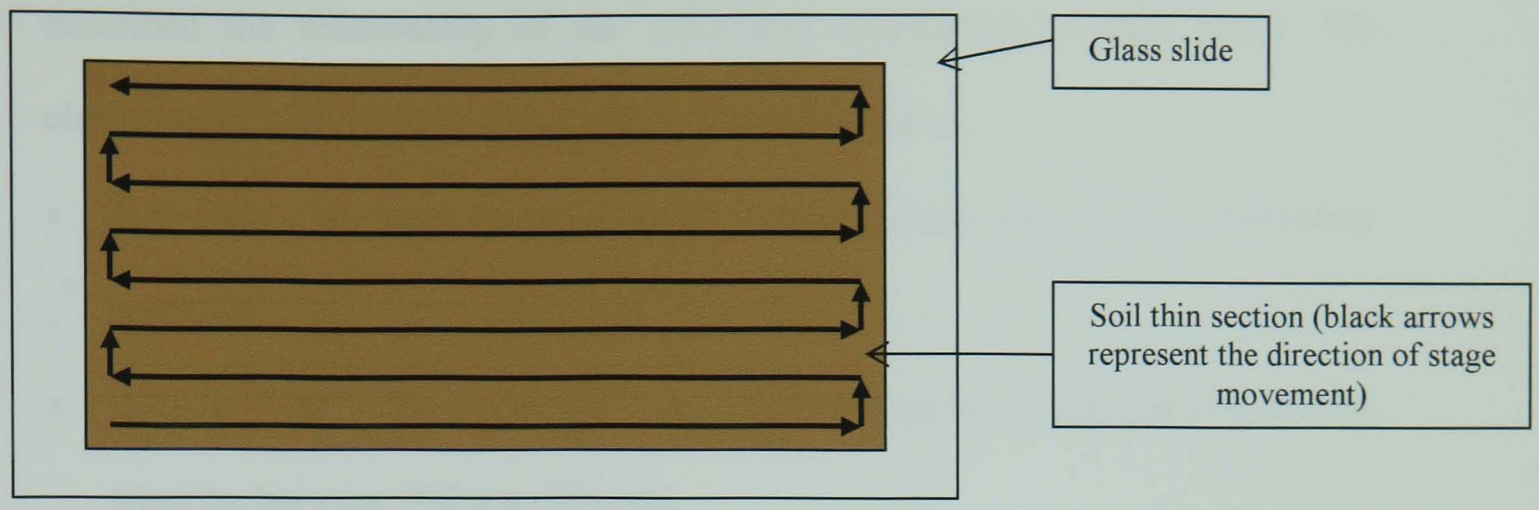


Figure 4.7: Diagram showing how the stage was moved during the point counting process. This led to between 154-176 separate observations per slide depending on the size of the thin section.

All the features were recorded according to their presence or absence along with a note as to the horizon they were found in. However, if the feature in question was an earthworm excrement, then a series of measurements and observations were taken to provide more information as to the nature and characteristics of the excremental features present. The sequence of what was measured is outlined below:

1. **Excrement morphology**: i.e. was the excrement mammilate, bacillo-cylinder, channel form, vermiform or undifferentiated in form.
2. **Excrement composition**: this described the nature of the excremental feature in terms of whether it was composed predominantly of mineral or organic material, or a combination of the two (i.e. what was termed organo-mineral). In addition to this the relationship of the excrement to the surrounding material was recorded, i.e. was it composed of similar material (an ortho-excrement) or was its composition different to that of the material around it (a meta-excrement).
3. **Degree of clustering**: clustering referred only to bacillo-cylinder excrements because these were individual features with their boundaries still visible and

described the relationship of the individual excrements to each other. The clustering of individual bacillo-cylinders was defined as:

- *Individual*- No other excrements of the same type were in the immediate vascinity.
- *Weakly clustered*- Excrements were in a cluster and were separated from each other by between 300 μm-500 μm.
- *Moderately clustered*- Excrements were in a cluster and were separated from each other by between 50 μm-300 μm.
- *Strongly clustered*- Excrements were in a cluster with the separation between excrements <50 μm. If the excrements were touching then their entire boundaries had to be visible otherwise they would be classified as mammilate.

4. **Degree of coalescence**: this referred only to mammilated excrements, since mammilates will have formed through the coalescence of individual excrements. This descriptor related the amount of void space within the excrements to the level of coalescence, and was defined as such:
   - *Weakly coalesced*- A large amount of void space present in the excrement.
   - *Moderately coalesced*- Some void space present within the excrement.
   - *Strongly coalesced*- Few to no voids present within the excrement.
5. **Degree of degradation**: this was used for all excrement types, and describes the extent to which the morphology of the excrements present had been modified by ageing (i.e. decomposition and fragmentation) and/or the reworking by other soil organisms. For bacillo-cylinder and mammilate types this refers to the degradation of their boundaries, whilst for channel form/vermiform excrements

this relates to the degree of fragmentation. The four classes of degradation used were:

- *Intact excrements*: No degradation of the excrement.
- *Slight degradation*: A small amount of degradation had occurred with the boundaries of the excrement still clear or little fragmentation of the excrement.
- *Moderate degradation*: Some degradation to the excrement with the boundaries still visible or some fragmentation to the excrement.
- *Strongly degraded*: A large amount of degradation had occurred with the boundaries of the excrement poorly visible or the excrement had suffered a great deal of fragmentation.

6. **Excrement size**: both the length and the width of excrements were noted, with length defined as the maximum length that could be measured in a straight line and width defined as that measurement which was the most representative of the overall width of the excrement (could be viewed as representing mean width). The size classes used were taken from Bullock *et al* (1985) and are shown in table 4.6.

Table 4.6: Size classification of pedofeatures in soil thin sections (in this case earthworm excrements) from Bullock *et al* (1985).

Length			Width	
Micro	Fine	<2μm		
	Medium	2-20μm		
	Coarse	20-50μm	Extremely thin	<50μm
Meso	Fine	50-100μm	Very thin	20-100μm
	Medium	100-200μm	Moderately thin	100-200μm
	Coarse	200-500μm	Thin	200-500μm
Macro	Fine	500-1000μm	Moderately broad	500-1000μm
	Medium	1-2mm	Broad	1-2mm
	Coarse	2-5mm	Very broad	2-5mm
Mega	Fine	5-10mm		
	Medium	10-20mm		
	Coarse	20-50mm		

7. **Excrement colour**: colour can only be described on a slide by slide basis because colour as seen through a microscope depends not only on the intensity of the lighting used but also on the thickness of the slide, and since slide thickness will vary slightly between slides, slide comparisons cannot be made. The colour of the excremental features was described relative to the dominant colour of the surrounding material, so that excrements with a different coloration were described as either being redder or browner and lighter or darker than the material around them.

4.5.5 Determination of Soil Porosity and the Characterisation of Voids Using Image Analysis

As indicated in section 4.5.2, image analysis uses digital images to characterise features present in the soil. Image analysis was used to quantify the porosity of the soil and to quantitatively investigate how earthworms influence this porosity. Of

major importance was the effect of earthworms on macroporosity, so voids present within the soil were classified and quantified according to their size, in terms of their area (mm^2). There were 51 classes going up in increments of 0.5 mm^2 from 0 to 20 mm^2, then in increments of 1 mm^2 up to 51 mm^2 with the final class consisting of voids >51 mm^2. The reason for the change in increment size was that there were more voids in the smaller size ranges so a smaller resolution of class was needed to be able to distinguish any subtle changes happening to voids of this size.

The image analysis system used consists of an Olympus polarising microscope with a three chip CCD video camera attached. A frame grabber card is fitted to the control computer, which is used to capture images as well as driving the motorised stage. This system offers high resolution imaging and image capture but when large scale pedofeatures are being investigated, the area that can be captured is limited, even with the ability of this system to capture multiple images and mesh them together with the image analysis software. The image analysis software called analySIS 3.0 (Soft Imaging Systems GmbH, Germany). AnalySIS offers the ability to measure a number of characteristics of the features of interest, such as variety of size and shape parameters. The exact measurements carried out using this software are outlined later in this section.

However, this system was not used here because the features under investigation were relatively large in size and were not likely to be evenly distributed throughout the thin sections, so since the system outlined above can only capture images over a relatively small area a different approach was taken. This alternative approach was to scan the whole thin sections using a flat bed scanner (Epson GT-10000) and Adobe Photoshop 5.5 at a resolution of 1200 dpi which produced images with a pixel resolution of 21.86 μm. Once the images from each sampling campaign had been

scanned they were then cropped to standardise their size and to include only soil material. Examples of some of the scanned thin sections are shown in Figure 4.8. After the images had been acquired, they were saved as TIFF or Tagged Image Format Files ready for the image analysis process. This file type was chosen because 1) they are not compressed so no data is lost during the compression process which is common when using file types such as JPEG, and 2) a header or tag is included in the file which provides image information such as magnification. The one major drawback of using the TIFF format is their large file size, e.g. the average file size of one of the cropped thin section images was around 19 Mb.

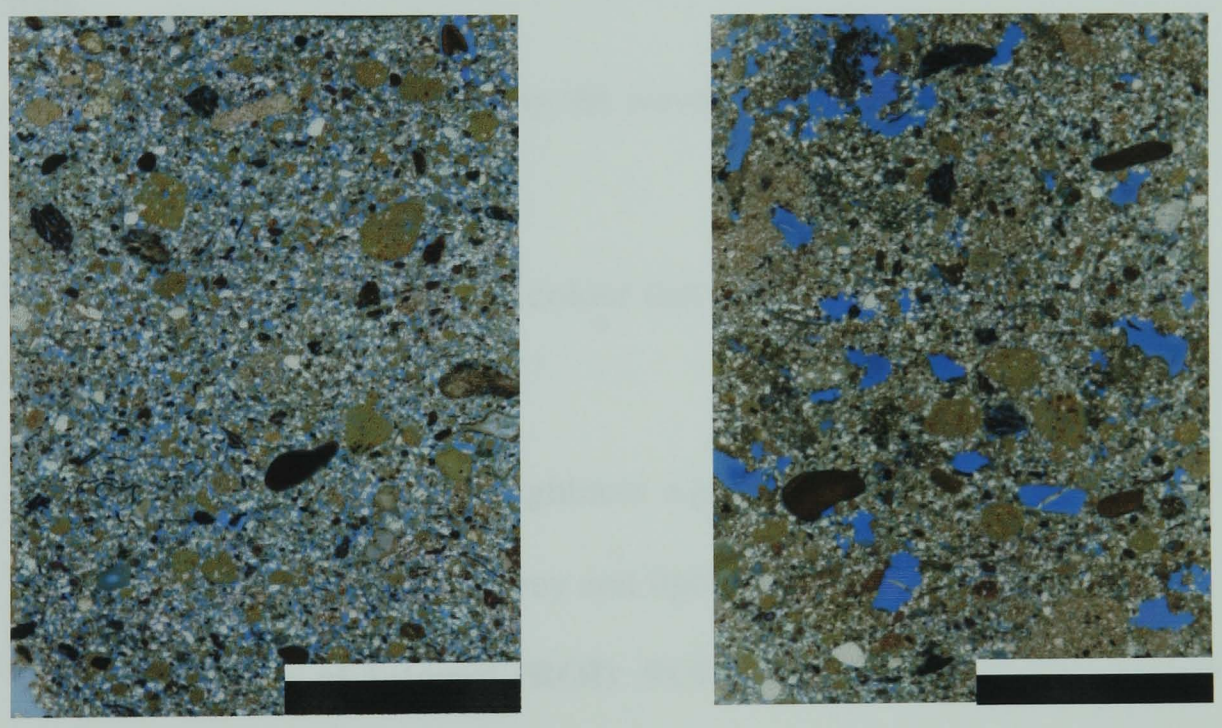


Figure 4.8: Examples of scanned thin sections used in the image analysis process. Scale bars represent 20 mm.

Once the images had been prepared then they could be run through the image analysis software to produce the required measurements. The protocol for the image analysis process is outlined below:

1. **Colour Thresholding**: The software was told which pixels were to be quantified according to their colour. The colour of interest was blue since the voids had been stained blue during the impregnation of the soil with resin. The thresholding was achieved by altering the HSI colour histograms until the range of pixel colours representing void space were correct. HSI (Hue, Saturation, Intensity) is a 3D colour model, like the RGB model (Red, Green, Blue), which is used to describe the characteristics and composition of colour, for example using the RGB model yellow is composed of different proportions of red (very little), green and blue (major components). The three components of the HSI model are define thus:

   - Hue – colour as described by the wavelength, e.g. the distinction between red and yellow.
   - Saturation – the amount of colour that is present, e.g. the distinction between red and pink.
   - Intensity – the degree of lightness e.g. the distinction between dark red and light red or between dark grey and light grey.

2. **Drawing Region of Interest (ROI)**: ROI's are the user defined areas that were to be analysed by the image analysis software. The ROI was usually defined as the whole image area, but in several slides there were anomalies present at the edges, so the ROI was draw around them.
3. **Measurements**: These were the morphological measurements determined on the voids, i.e. size, shape, orientation etc. Three groups of measurements were made 1) those on the individual voids, 2) those on each class of void (i.e. mean values for each class) and 3) those on the whole ROI (i.e. mean values for the image). Many measurements were made on each individual void or class of void because

it was easier and more time efficient to have data redundancy rather than having to go back an reanalyse all the images to include any that were needed. Only measurements used in the final analysis was the area fraction which was defined as the percentage area of all voids of a given size class relative to the area of the ROI

4. **Detection**: During this phase of the process the software identified those pixels which represented voids and carried out the defined measurements. Those voids which had been identified and measured were overlaid over the original image.
5. **Operator Checks**: This is the final stage of the data acquisition from the image analysis process. It involved visually checking the image and void detection overlay to make sure that those pixels which had been detected as voids were voids. Sometimes, due to uneven dying, voids were not detected properly so they were deleted from the detection and drawn in manually using the mouse to outline their boundaries. This was automatically changed in the data spreadsheets.

The image analysis produced vast quantities of data, which then had to be distilled to produce summary data. The class data provided information on the size distribution of the voids present in the thin sections, whereas the ROI data provided total porosity for the thin sections.

4.6 Data Analysis

During the analysis of the data collected in this research project a number of statistical tests have been used depending primarily on the distribution of the data.

All of the tests either compare the mean or median of two populations to see if they are statistically different. Minitab Statistical Software v13.32 was used to perform the statistical analysis. Wheater and Cook (2000) provided general statistical information and the details of non-parametric tests used in this research. If the data were normally distributed and had equal variances the either one way or two ANOVA was performed. Whilst carrying out ANOVA, if significant differences were observed then multiple comparisons were used to identify which treatment combinations were the cause of the significant result. Tukeys Pairwise Comparisons were used for one way ANOVA whilst Bonferroni Pairwise Comparisons were performed for two way ANOVA. If the data were not normally distributed then attempts were made to transform it. If these transformations were successful then ANOVA was used. For normally distributed data standard errors were used as a measure of variability since usually more than one mean was being presented. For transformed data back transformed means and 95% confidence limits were used.

If the data were not normally distributed and resisted transformation then either Kruskal-Wallis or Mood's Median tests were used. Mood's Median test was used in situations where outliers were present within the data, because this test is more resistant than the Kruskal-Wallis test although it is less powerful (Wheater and Cook 2000). In some instances a type of non-parametric two way ANOVA was performed by carrying out a normal two way ANOVA on ranked data and then calculating a new test statistic H which was compared the χ^2 distribution (Wheater and Cook 2000).

What has been detailed in this chapter is that a number of different techniques have been applied at different spatial scales to investigate the impacts of earthworm

burrowing and casting on soil structure. The effects of earthworms on soil porosity and structural stability have been determined on a laboratory scale through bulk density, K_{sat} and aggregate stability measurements, whilst on the micro-scale by point counting earthworm excrements and image analysis of void space.

The next three chapters of this thesis analyse and discuss the data produced from the various techniques outlined here. The three experiments, which represent increasing levels of system complexity will be dealt with individually and a concluding chapter at the end draws together the conclusions of the individual experiments in order to answer the research questions posed in chapter 3.

Chapter 5: Ecotron Experiment: Results & Discussion

This chapter presents the results, discussion and conclusions of the effects of earthworm activity on aggregate stability and the re-organisation of voids in the highly controlled environment of the Ecotron.

5.1 Aggregate Stability

Aggregate stability was determined on loose soil samples collected from chambers 9-16 only. The methodology used for the determination has been described in section 4.4.

5.1.1 Hypotheses

The specific hypotheses that were under investigation in the experiment were concerned with effects of earthworms on aggregation, and the influence of earthworm functional and species diversity on aggregate stability. These two hypotheses were that:

1. Inoculation of earthworms into soil devoid of earthworm features will lead to increased aggregate stability due to intimate mixing of organic and mineral material, and the promotion of microbial activity associated with their casting activity.

2. Changes to earthworm species and functional diversity will lead to increases in aggregate stability by altering casting dynamics, i.e. through the differential ingestion of organic matte and the location of casting.

5.1.2 Results

The raw output from the aggregate stability determination was a series of particle size distributions held in Excel spreadsheets. Each individual sample had four distributions, each representing one consecutive run, i.e. the resultant particle size distribution after one pulse of sonication lasting 120 s. To be able to quantitatively assess the differences between these distributions a series of indices were chosen, as outlined in section 4.4.1. An example of a typical set of the particle size distributions produced from one of the samples is shown in figure 5.1.

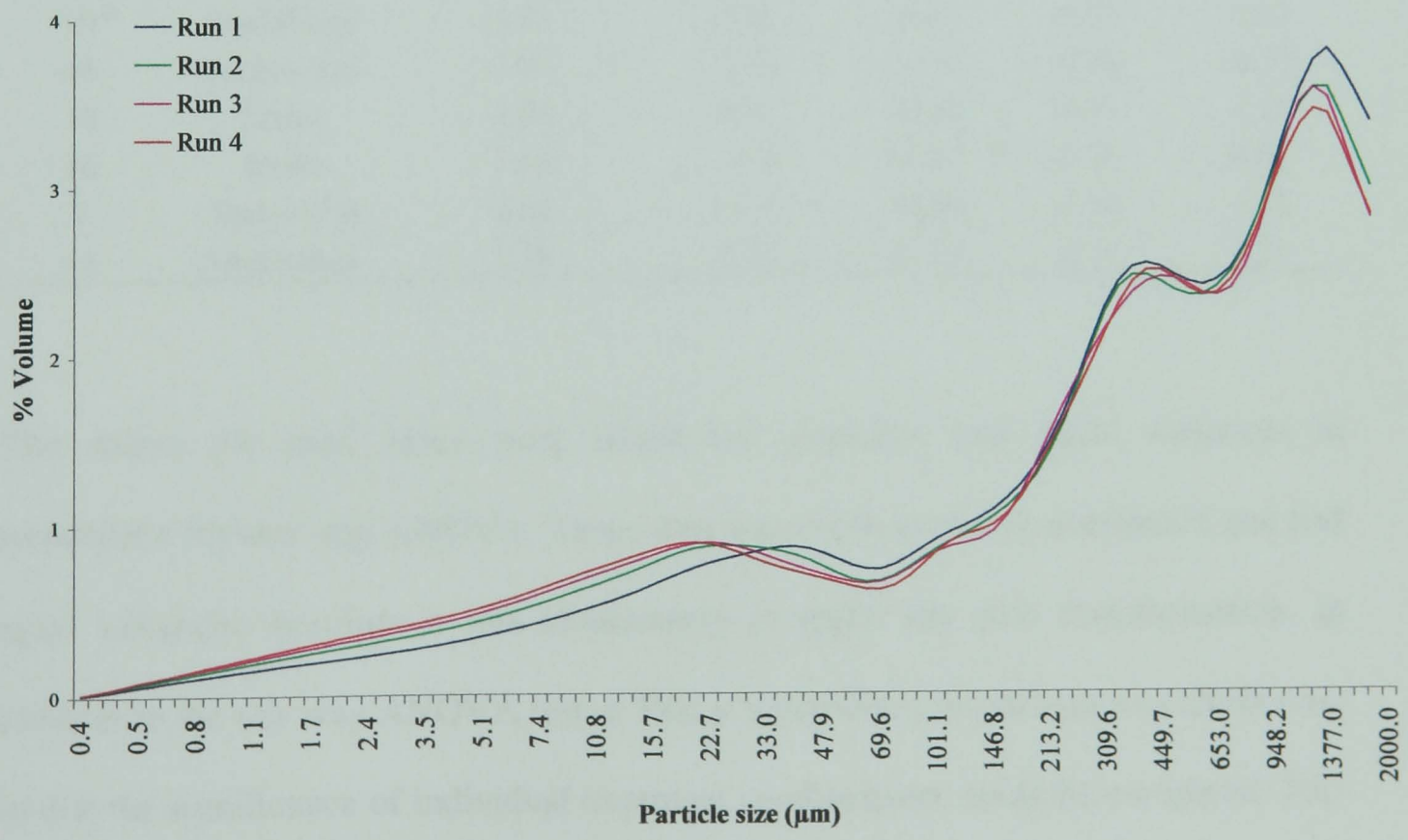


Figure 5.1: Particle size distributions of four runs of an aggregate sample from chamber 9 (3 endogeic + 3 epigeic earthworm species).

The determination of aggregate stability was carried out on three analytical replicates from each of the chambers sampled. From these replicates a mean value was calculated for each run for the five aggregate stability indices. The indices themselves were calculated as the difference between run 1 and run 4. They were calculated in such a way that aggregate breakdown produced negative values whilst the accumulation of breakdown products gave positive values (Table 5.1). These mean values were then entered into Minitab Statistical Software v13.32 ready for analysis.

Table 5.1: Summary of mean aggregate stability indices for each of the chambers sampled. (endo = endogeic, epi = epigeic, MWD = mean weight diameter). No. of replicates = 2.

		Aggregate Stability Indices				
Chamber	**Treatment**	**1000-2000mm**	**800μm-2mm**	**MWD**	**150-800μm**	**10-60μm**
11	No Worms	-3.98	-4.33	-61.78	-0.17	0.62
13	No Worms	-3.04	-3.36	-50.64	-0.45	0.10
10	1endo+1epi	-2.85	-2.91	-46.83	-0.47	0.63
15	1endo+1epi	-1.74	-2.06	-31.42	-0.58	-0.07
12	2endo	-2.88	-3.37	-48.89	-0.77	1.13
16	2endo	-3.66	-4.15	-60.94	-0.76	0.91
9	3endo+3epi	-2.66	-2.97	-48.06	-1.34	1.14
14	3endo+3epi	-2.11	-2.23	-37.45	-0.77	1.21

The means for each index were tested for normality and equal variances in preparation for one way ANOVA. These data were both normally distributed and had equal variances therefore it was unnecessary to apply any data transformation. In addition to the one way ANOVA test, a Tukey's pairwise comparison was carried out so that the significance of individual treatment combinations could be compared. This was to investigate whether functional or species diversity had a significant effect on aggregate stability.

The mean values of each aggregate stability index for each treatment and the p values obtained from the one way ANOVA are shown in table 5.2. The standard error was calculated instead of standard deviation for these means because more than one mean was being compared (Wheater and Cook 2000). No statistically significant differences at the 95% confidence level were observed between the means of the aggregate stability indices and the different earthworm treatments. In addition the results of Tukey's pairwise comparison showed that no significant differences were observed between treatments with varying functional and species diversity.

Table 5.2: Table showing the mean values of the aggregate stability indices for each treatment and the resultant p values from the one way ANOVA at the 95% confidence level. Standard errors are shown in brackets. No. of replicates = 2.

	Aggregate Stability Indices				
Treatment	1-2 mm	800-2000 μm	MWD	150-800 μm	10-60 μm
No worms	-3.51 (±0.47)	-3.85 (±0.49)	-56.211 (±5.57)	-0.31 (±0.14)	0.36 (±0.26)
1 endo+1 epi	-2.30 (±0.56)	-2.48 (±0.43)	-39.127 (±7.71)	-0.52 (±0.05)	0.28 (±0.35)
2 endo	-3.27 (±0.39)	-3.76 (±0.39)	-54.914 (±6.03)	-0.77 (±0.00)	1.02 (±0.11)
3 endo+3 epi	-2.39 (±0.27)	-2.60 (±0.37)	-42.757 (±5.30)	-1.06 (±0.28)	1.18 (±0.04)
P value	**0.26**	**0.16**	**0.27**	**0.11**	**0.10**

Table 5.3: Matrices showing the confidence intervals produced by Tukey's pairwise comparisons. A result is significant if the interval does *not* contain zero. No. of replicates = 2.

1-2 mm			
Treatments	1 endo+1 epi	2 endo	3 endo+3 epi
2 endo	-1.53 to 3.48		
3 endo+3 epi	-2.42 to 2.62	-3.39 to 1.62	
No worms	-1.29 to 3.72	-2.27 to 2.75	-1.38 to 3.63
800-2000 μm			
Treatments	1 endo+1 epi	2 endo	3 endo+3 epi
2 endo	-1.15 to 3.70		
3 endo+3 epi	-2.31 to 2.54	-3.58 to 1.26	
No worms	-1.06 to 3.78	-2.34 to 2.51	-1.18 to 3.67
MWD			
Treatments	1 endo+1 epi	2 endo	3 endo+3 epi
2 endo	-20.05 to 51.62		
3 endo+3 epi	-32.30 to 39.46	-47.99 to 23.68	
No worms	-18.75 to 52.92	-34.54 to 37.13	-22.38 to 49.29
150-800 μm			
Treatments	1 endo+1 epi	2 endo	3 endo+3 epi
2 endo	-0.68 to 1.17		
3 endo+3 epi	-0.39 to 1.46	-0.64 to 1.21	
No worms	-1.14 to 0.71	-1.38 to 0.47	-1.67 to 0.18
10-60 μm			
Treatments	1 endo+1 epi	2 endo	3 endo+3 epi
2 endo	-2.02 to 0.55		
3 endo+3 epi	-2.18 to 0.39	-1.45 to 1.13	
No worms	-1.37 to 1.21	-0.63 to 1.95	-0.47 to 2.10

5.1.3 Discussion and Conclusion

The first hypothesis, which stated that if earthworms were inoculated into a virgin soil which had no past history of earthworm activity then aggregate stability would be increased as a result of casting activity. The data presented in tables 5.2 and 5.3 has shown that no statistically significant differences were observed between the four

treatments. This indicated that earthworms did not affect aggregate stability either by increasing or decreasing the level of aggregate breakdown. This was surprising since it has been reported that earthworm casting activity leads to increased aggregate stability because when dry, earthworm casts tend to be more stable than surrounding aggregates (Shipitalo and Protz 1988; Marinissen and Dexter 1990; Martin and Marinissen 1993; Hindell *et al* 1994b; Ketterings *et al* 1997; Garvin *et al* 2001). Several authors have also reported that earthworms can also destabilise aggregates (Boyle *et al* 1997; Schrader and Zhang 1997; Shuster *et al* 2000).

There are several potential reasons as to why earthworms did not influence aggregate stability or that this influence may have been masked. The first is that the soil material used in this experiment had a coarse texture, i.e. it was very sandy with only very small amounts of clay and organic matter present. Indeed the only organic matter in the experimental systems came from washed aquatic peat which was mixed into the soil to provide a source of organic matter for the earthworms. The coarse texture would physically prevent what little clay and organic matter that was present in the soil from being in close contact. It is these soil components that bind aggregates together (Shipitalo and Protz 1988), therefore the aggregates formed in this experiment would be less stable than those formed in systems with higher clay and organic matter contents. The important property of clay particles in terms of aggregation is their negative surface charge onto which polyvalent cations can bond and join the clay particles together or with fragments of organic matter (Emerson and Greenland 1990). This combination of a coarse texture and low clay/organic matter content, especially fresh residues, led to less stable aggregates which could have masked any earthworm effects. Other studies that have investigated the effects of earthworms on aggregate stability have tended to focus on cultivated or reclaimed

soils with low organic matter contents (Hoogerkamp *et al* 1993; Marinissen 1994; Binet *et al* 1997; Ketterings *et al* 1997; Jongmans *et al* 2001).

A second explanation was that the sample size in this experiment was very small (sample size of 8 comprising 4 treatments with a replication factor of 2). Associated with this small sample size were the high levels of sample variability within the treatments, as show by the mean values in table 5.1 and the standard errors in table 5.3. Both the small sample size and high variability would have masked any earthworm effects on aggregate stability, especially if they were subtle in nature.

Apart from using a higher replication factor and therefore a larger sample size, the determination of aggregate stability could have been improved by using larger aggregates. 1 – 2 mm aggregates were used in this determination because a) it is a size range into which earthworm casts fit (Ketterings *et al* 1997) and b) the instrumentation had an upper size limit of 2 mm. If larger aggregates could have been analysed this potentially would have been more representative of the cast material present in the soil.

The second hypothesis concerned whether earthworm functional or species diversity influenced aggregate stability. The mean aggregate stability indices values that are presented in table 5.2 indicate that functional diversity (i.e. treatments 1 endogeic + 1 epigeic species Vs 2 endogeic species) had no statistically significant effect on the stability of soil aggregates. There was a slight trend for the treatment with 2 functional groups to have slightly less aggregate breakdown and therefore more stable aggregates than the treatment with only one functional group. This was also true when the comparison was made between those treatments with epigeic species and those without. It should be stressed that although there was a trend, it was not statistically significant. This effect may have been seen because epigeic earthworms would cast in

different areas of the soil to endogeic species since they tend to live higher in the soil profile. The results were due to the different casting dynamics of those treatments with and without epigeic earthworm species. No statistically significant differences were observed when the effect of earthworm species diversity on aggregate stability was investigated, (1 endogeic species + 1 epigeic species Vs 3 endogeic species + 3 epigeic species).

The conclusions from this experiment, in terms of the effects of earthworms on aggregate stability were that:

1. Earthworms, through their casting activity, did not have a statistically significant effect on aggregate stability because the soil had a very coarse texture and low organic matter content.
2. Functional and species diversity of an earthworm community did not appear to affect the stability of soil aggregates although there was a non-significant trend for there to be more stable aggregates in those soils with two functional groups as compare with just one.

5.2 Re-organisation of Void Space

The generally accepted view has been that earthworm activity increases the number of voids in soil, but several authors have shown that earthworms have a re-organisational effect on voids (Syers and Springett 1983; Binet and Curmi 1992; Knight *et al* 1992; Binet *et al* 1997; Lachnicht *et al* 1997). This means that earthworm burrowing activity neither increases nor decreases overall soil porosity but rather alters the size distribution of voids. The aim of this section of the research project was

to see if this re-organisational effect could be identified and then quantified. This was achieved using image analysis techniques on digital images of whole soil thin sections where the voids had been dyed blue, (the protocol for this has been outlined in section 4.5.5). Thin section samples were taken from chambers 9 – 16 for this analysis and were prepared according to the methodology outlined in section 4.5.1.

5.2.1. Hypotheses

To investigate the effects of earthworms on soil void space, three hypotheses were proposed. They provided the framework for investigating the effects of earthworm burrowing activity on total soil porosity, the size distribution of voids within soil and to identify whether the functional or species diversity of an earthworm community impacted on void space. The three hypotheses were that:

1. Burrowing activity of earthworms leads to an increase in the number of voids within soil, therefore increasing total soil porosity.
2. The burrowing activity of earthworms causes the re-organisation of void space by reducing the number of small voids whilst increasing the number of large voids.
3. Changes in the functional and species diversity of earthworm communities will lead to changes in void space since earthworm species of varying functional groups will affect void space differently.

5.2.2 Results

The output from the image analysis process consisted of three groups of data, the first representing data on the whole slide, the second was class data for the defined size

classes outlined in section 4.5.5, whilst the final data set was for each individual void that the software had quantified. It was the first two groups that were of interest in this research. As outlined in chapter 4 the key measurement used to identify the effect of earthworms on void space was void area as expressed as a percentage of the area of the whole thin section. For the first group of data this represented the total porosity of the whole thin section whilst for the second data group it represented the size distribution of the voids in the thin section.

The mean total porosity values for each of the treatments are shown in figure 5.2. Differences appeared to exist between the earthworm inoculated treatments and the earthworm free treatment. However, when the data were analysed using One way ANOVA no statistically significant differences existed at the 95% confidence level (P= 0.105, F= 2.55, df= 3).

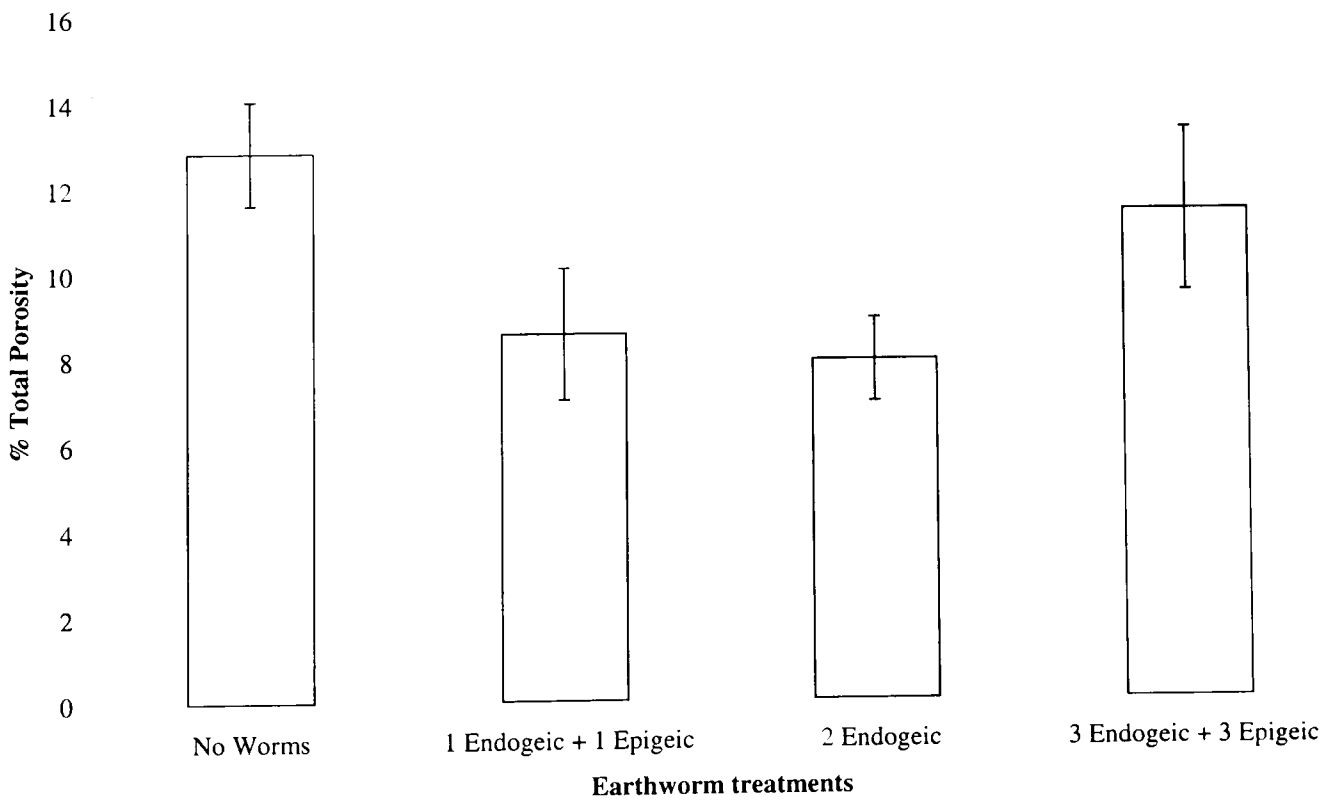
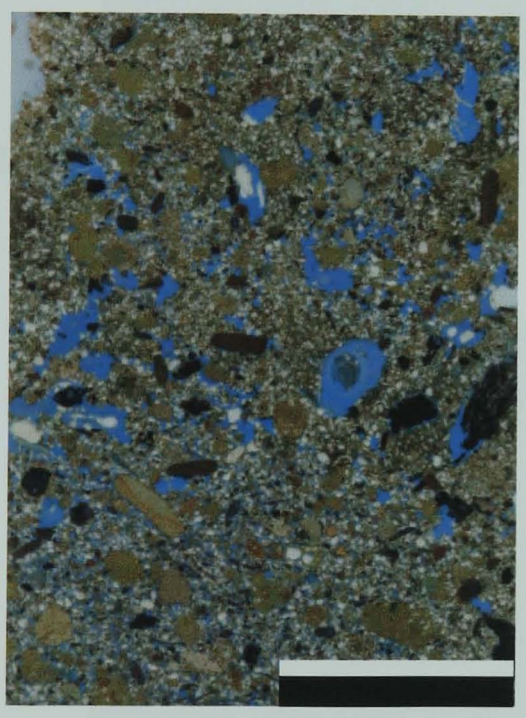


Figure 5.2: Mean total porosity of the four earthworm treatments. Error bars show standard errors. No. of replicates = 2.

The size distribution of voids in the thin sections were analysed using a void area of 2 mm^2 as a threshold. Figure 5.3 shows images of the thin sections that were used for the image analysis protocol, whilst figures 5.4 and 5.5 show enlarged areas of two contrasting slides. These images indicate clearly the effects that earthworm burrowing activity has on the re-organisation of void space. When the images of the three earthworm inoculated treatments were compared to the treatment where none, there was a marked increase in the number of large voids (channels) and a reduction in the number of small voids. The data for these size classes had to be transformed because it was either not normally distributed or did not have equal variances. The less than 2 mm^2 class was log transformed, whilst the greater than 2 mm^2 class was arcsine transformed. The mean values used were back transformed so as to present them in their original units, i.e. as a percentage area of the whole slide. Back transformed 95% confidence limits were used as a measure of variability for the means presented (Wheater and Cook 2000). These were calculated following the protocol laid out in Wheater and Cook (2000) which involved calculating the 95% confidence limits of the transformed data and then back transforming these in exactly the same way as was done for the means. These confidence limits are asymmetrical because the data from which they were derived were not normally distributed.

No worms

1 endogeic + 1 epigeic



2 endogeic

3 endogeic + 3 epigeic

Figure 5.3: Examples of scanned full-sized thin sections as used for image analysis. Scale bars represent 20 mm. Blue features in the images correspond to the blue dyed voids.

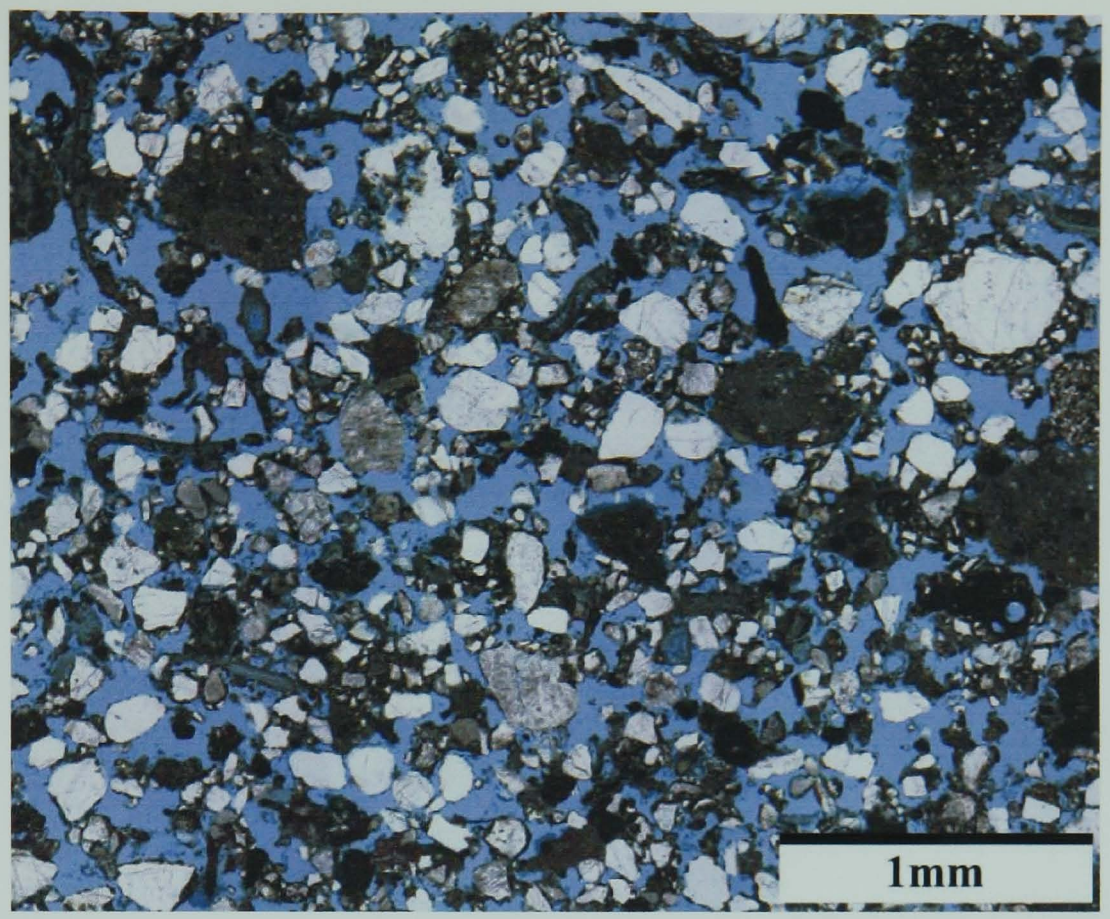


Figure 5.4: High magnification image from a soil thin section showing the effect of the no worm treatment on porosity.

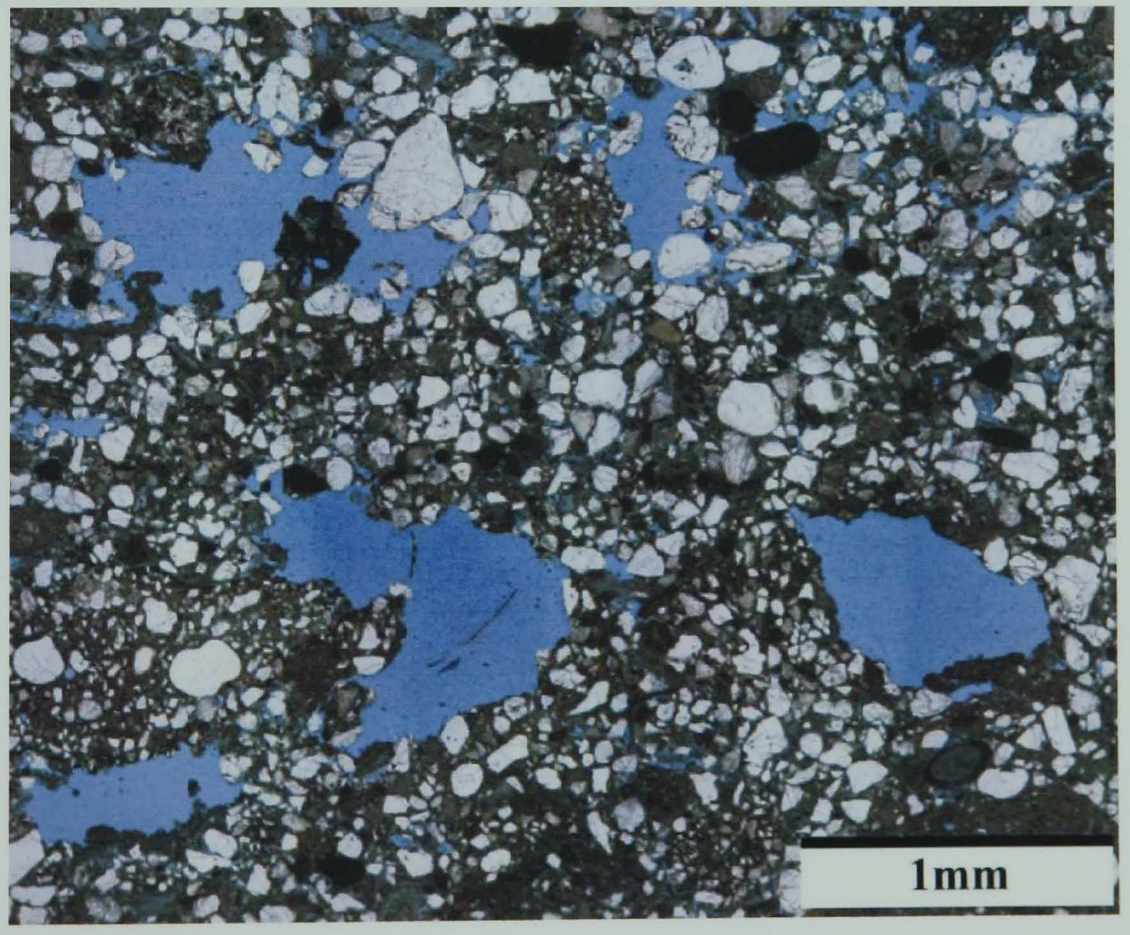


Figure 5.5: High magnification image from a soil thin section showing the effect of the 1 endogeic + 1 epigeic treatment on porosity.

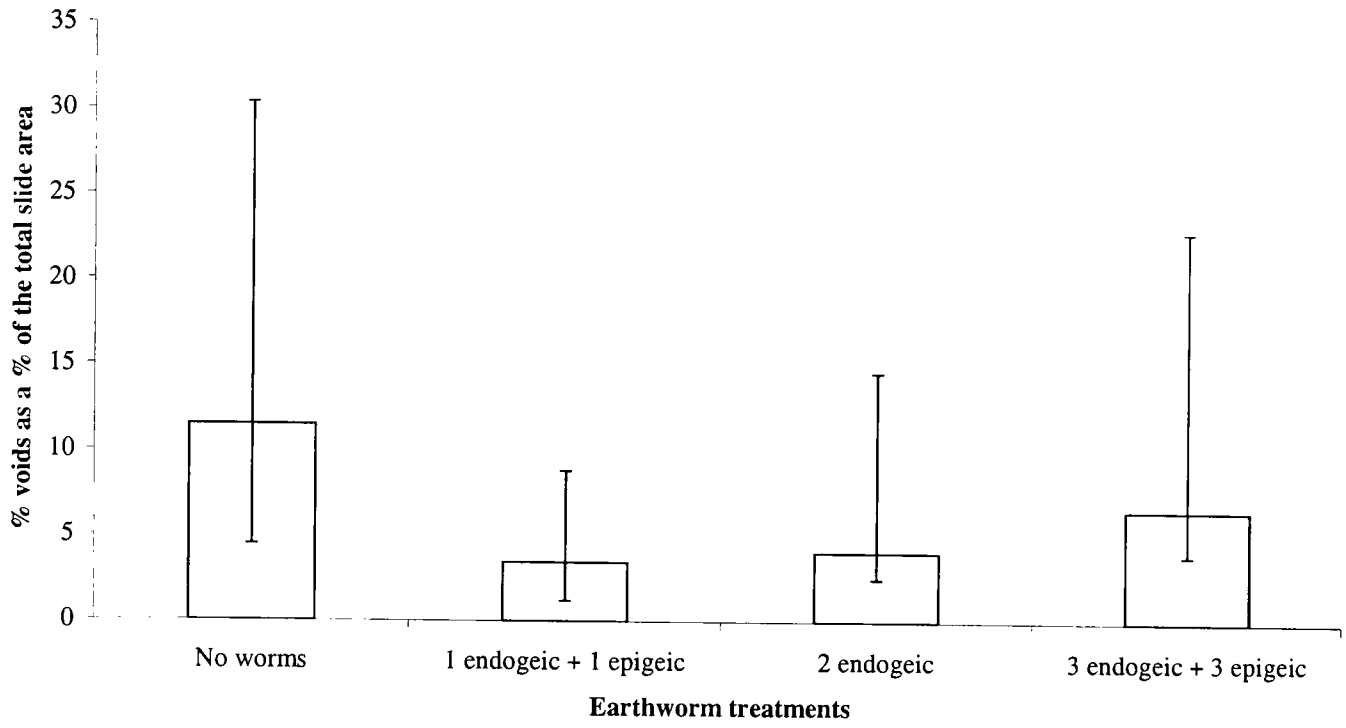


Figure 5.6: Mean proportion of voids expressed as percentage of the total slide area for the < 2 mm² size fractions. Error bars represent back transformed 95% confidence limits. No. of replicates = 2.

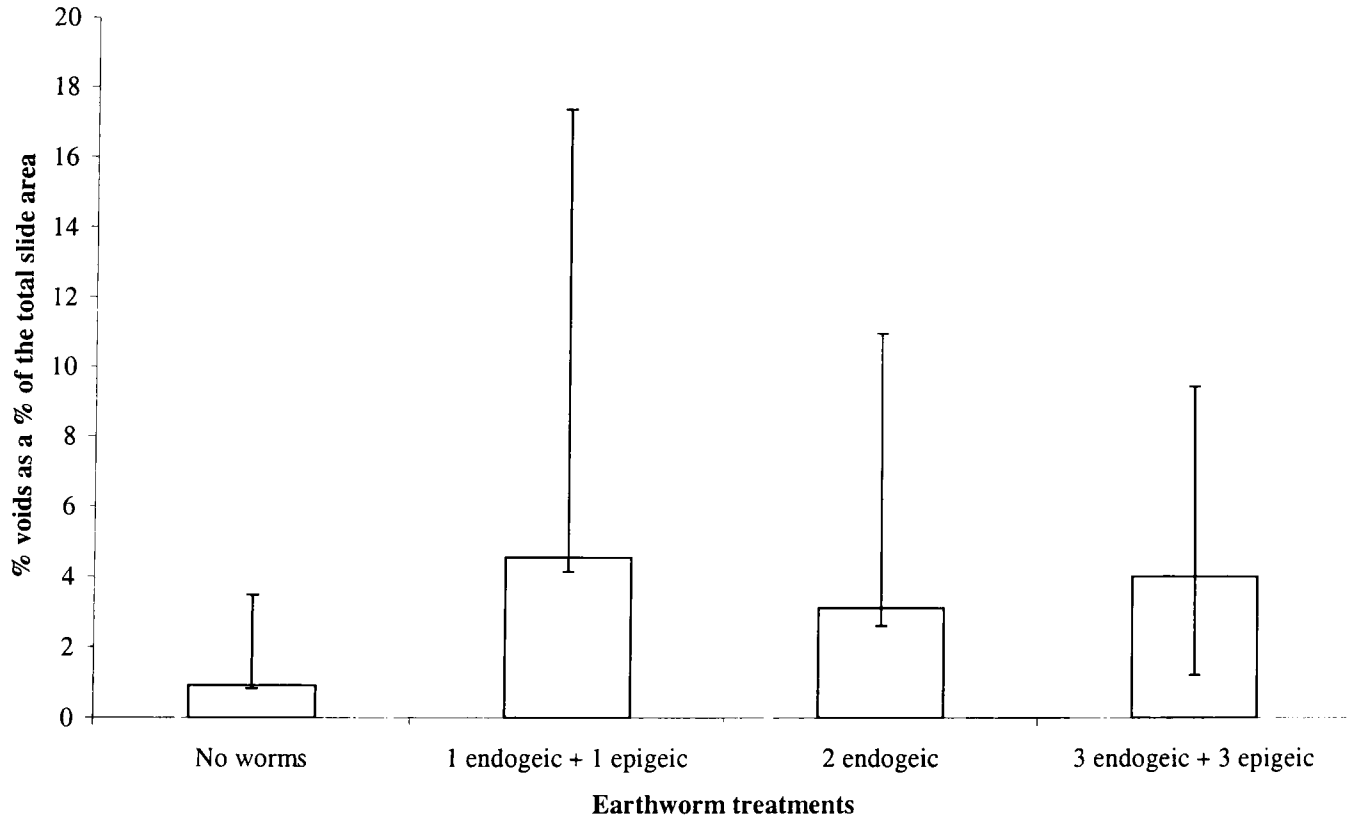


Figure 5.7: Mean proportion of voids expressed as percentage of the total slide area for the > 2 mm² size fractions. Error bars represent back transformed 95% confidence limits. No. of replicates = 2.

Figures 5.6 and 5.7 show the mean proportion of voids, expressed as a percentage of the total slide area, for the less than and greater than 2 mm² void size fractions. Statistically significant differences were found to exist between the less than size fraction (< 2 mm² **P= 0.05**, F= 7.28, df= 3). On examination of the confidence intervals produced from the Tukey's pairwise comparisons (table 5.4), it became clear that the significant differences occurred between the no worms and 1 endogeic + 1 epigeic and 2 endogeic treatments. The effects of these two treatments, as compared to the no worm treatment, were to significantly reduce the number of small voids.

Table 5.4: Confidence intervals produced from Tukey's pairwise comparisons for the < 2 mm^2 and > 2 mm² size fractions. The results that are statistically significant at the 95% confidence level are shown in bold italics. No. of replicates = 2.

< 2 mm²			
Treatments	1 endo+1 epi	2 endo	3 endo+3 epi
2 endo	-0.44 - 0.29		
3 endo+3 epi	-0.65 - 0.08	-0.57 - 0.16	
No worms	***-0.89 - -0.16***	***-0.81 - -0.08***	-0.60 - 0.13
> 2 mm²			
Treatments	1 endo+1 epi	2 endo	3 endo+3 epi
2 endo	-0.08 - 0.15		
3 endo+3 epi	-0.10 - 0.13	-0.14 - 0.90	
No worms	***0.01 - 0.23***	-0.03 - 0.20	-0.01 - 0.22

Statistically significant differences existed for the > 2 mm² size fraction (**P= 0.037**, F= 3.91, DF= 3). Tukey's pairwise comparisons showed that this significant relationship was between the no worm and 1 endogeic + 1 epigeic treatments. In this instance the inoculation of the soil with this treatment gave rise to a significant increase in the number of voids > 2 mm² in area.

5.2.3 Discussion and Conclusions

The first hypothesis stated that 'earthworm burrowing activity leads to an increase in the number of voids within soil, therefore increasing total soil porosity'. The results have shown that earthworms did not significantly affect total soil porosity, i.e. that the net effect of earthworms was to neither increase or decrease soil porosity (P= 0.105, F= 2.55, df= 3). This was expected since recent studies have found that earthworms do not affect total soil porosity, but rather caused changes to void size distribution by decreasing the proportion of small voids whilst increasing the proportion of large voids, i.e. production of earthworm channels (Syers and Springett 1983; Binet and Curmi 1992; Knight *et al* 1992; Binet *et al* 1997; Lachnicht *et al* 1997).

The effects of the different treatments on porosity can be clearly seen in figures 5.3 – 5.5. The data from the less than and greater than 2 mm^2 void size fractions indicated that earthworms significantly reduced the proportion of small voids in the thin section (< 2 mm^2 **P= 0.05**, F= 7.28, df= 3). However, Tukey's pairwise comparisons showed that these significant differences occurred in the 1 endogeic + 1 epigeic and 2 endogeic treatments as compared to the treatment without earthworms (see table 5.4), indicating that earthworms significantly reduced the proportion of small voids in these two treatments. This compaction of the soil was probably due to a) the mechanical compaction of the soil surrounding channels and b) the reworking/remoulding of the soil as it passed through the earthworm's gut and was egested. It was unclear as to why no significant differences were observed in the 3 endogeic + 3 epigeic treatment. Statistically significant differences were found for the > 2 mm^2 fraction (> 2 mm^2 **P= 0.037**, F= 3.91, df= 3). This indicated that earthworm burrowing activity significantly increased the proportion of voids > 2 mm^2 in area. The Tukey's pairwise comparisons

indicated that the 1 endogeic + 1 epigeic treatment was the cause of this statistically significant difference.

In terms of the degree to which earthworm functional and species diversity affect soil porosity, the results of the multiple comparison test showed that no significant differences existed. In other words the confidence intervals produced by Tukey's pairwise comparison all contained zero and were therefore not statistically significant. This was expected since both different earthworm species and functional groups will inhabit and burrow through different areas of the soil (Edwards and Bohlen 1996), so it was anticipated that some differences would be observed. One reason why differences were not observed was that this experiment was very simple in terms of habitat complexity and resource availability (the system was very resource poor with only aquatic peat being included as a source of subsurface carbon), therefore earthworm behaviour would not have been the same as in an *in vivo* environment. This change in behaviour would probably have influenced burrowing activity and therefore the observed burrow patterns.

The effects or earthworms on soil porosity are:

1. Earthworms did not have any statistically significant impact on total soil porosity, i.e. overall there was no net increase in the proportion of voids quantified.
2. The presence of earthworms in a soil as compared to one without earthworms present, did significantly alter the size distribution of the voids in the soil. Earthworms tended to reduce the number of small voids present through compaction and the reworking of the soil fabric and in some treatments to significantly increase the proportion of voids larger than 2 mm^2 in area.

5.3 Conclusions from the Ecotron Experiment

The Ecotron experiment was a simple experiment carried out in a highly controlled environment, and as such represented the first level of complexity (i.e. it was the simplest and most controlled experiment). Two measures were used to assess the impacts of earthworms on the fabric of the artificial soil used, these were aggregate stability as determined using laser diffraction particle sizing and the image analysis of blue dyed voids in thin section.

The effects that earthworms had on aggregate stability in this experiment were that:

1. The casting activity of earthworms did not significantly effect the stability of soil aggregates.
2. Earthworm functional and species diversity did not influence aggregate stability.

The effects that earthworms had on soil porosity were threefold:

1. The presence of earthworms led to no significant changes in total soil porosity for all three earthworm treatments.
2. Earthworms tended to reorganise void space by reducing the proportion of small voids, as seen in the 1 endogeic + 1 epigeic and 2 endogeic treatments, and increase the proportion of voids > 2 mm^2 in area in the 1 endogeic + 1 epigeic treatment.
3. Earthworm functional and species diversity did not appear to affect the size distribution of voids found in the soil

Having identified the effects of earthworms on the soil fabric in this simplistic system, the next chapter outlines the effects of earthworms of soil fabric in an *in vivo* field based experiment that again used soil which had its structure removed. This

represented the second level of complexity because although the soil had its structure removed through the homogenisation of undisturbed soil monoliths on a horizon by horizon basis, and it took place as part of a field based experiment so therefore was exposed to natural environmental conditions.

Chapter 6: Sourhope Experiment – Disturbed Soil: Results and Discussion

6.1 Introduction

In this chapter the results of an experiment investigating the effects of earthworms on aggregation and void space in a structureless soil are presented. This experiment represents the second level of system complexity, as outlined in chapter 3, and took place at the Sourhope research station. It consisted of 50 boxes of disturbed soil that had been buried in the ground at Sweethope (chapter 3). The disturbance process was achieved by splitting soil monoliths on a horizon by horizon basis, with the horizons partially dried, then broken down by hand and replaced in the boxes in their original order. This was done to remove any existing structural features from the soil, thereby making the effects of earthworms on aggregation and void space more easily observed. Liming and earthworm treatments were then applied to these boxes (details of these treatments are shown in chapter 3).

Aggregate stability and thin section samples were taken from these boxes three times during the experiment:

1. Intermediate 1 (April 2000)
2. Intermediate 2 (October 2000)
3. Intermediate 3 (August 2001)

These were intermediate samples because they were taken in between the initial and final samples from the undisturbed boxes. The results presented are based on samples taken at intermediate 1 (just before earthworm inoculation) and intermediate 3 (16

months after earthworm inoculation). Lime was applied to the soil on two occasions, 1) summer 2000 and 2) summer 2001. The first three sets of data presented are soil pH, earthworm data as sampled at the end of the whole field experiment (October 2001), and % organic carbon of 1-2 mm aggregates. These data are included because they help to explain the results of the effects of earthworms on the soil fabric. For each soil property measured during the experiment, the initial state of the soil at intermediate 1 and the effects of time were examined. Both the initial soil state and effects of time were assessed to make sure that any significant effects observed were due to the treatments and not soil variability. It was important to assess the effects of time on the soils so that any changes that occurred between the beginning and end of the experiment could be taken into account during data interpretation. If time effects were not significant, then it could be assumed that the intermediate 3 unlimed + no worms treatment was representative of the initial soil state. The next section presents the results and interpretation of soil pH as measured in the LFH and A_h horizons at sampling times intermediate 1 and intermediate 3.

6.2 Soil pH

The raw data for this section were provided by Pawlett (2003). Soil pH was measured using a pH meter and soil:1M KCl suspensions at both intermediate 1 and 3 (the ratio of soil:KCl was 1:2.5). pH was determined on samples from the LFH (combined sample containing all three horizons) and A_h horizons. The two key questions concerning soil pH were:

1. What was the effect of liming on soil pH?
2. How did pH vary over time, i.e. between intermediate 1 and 3, for each horizon?

The median pH values for each horizon from each of the liming treatments for both intermediate 1 and intermediate 3 are shown in figure 6.1 and table 6.1. The median values were chosen because when the data were analysed they were not normally distributed and there were outliers present. Mood's Median Test was used to analyse the data (chapter 4 gives an outline of the statistical tests used in this research).

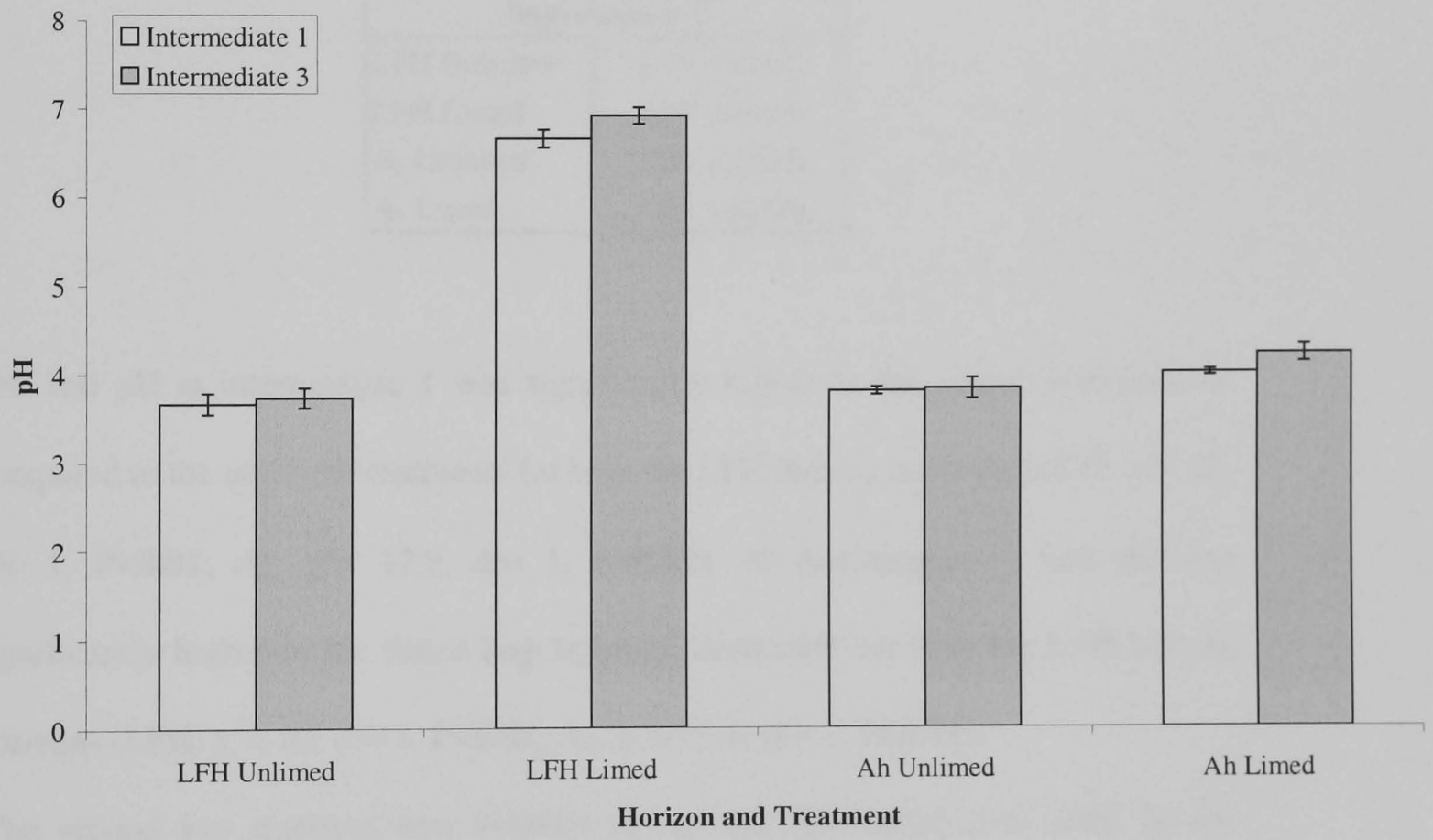


Figure 6.1: Median soil pH values of the LFH and A_h horizons for both liming treatments from intermediate sampling 1 and 3. Error bars show quartile deviations i.e. $\frac{Q_3 - Q_1}{2}$. No. of replicates = 5.

Table 6.1: Median soil pH values of the LFH and A_h horizons for both liming treatments from intermediate sampling 1 and 3. Values in brackets are the quartile deviations. No. of replicates = 5.

Horizon and Treatment	Median
Intermediate 1	
LFH Unlimed	3.67 (±0.12)
LFH Limed	6.67 (±0.10)
A_h Unlimed	3.82 (±0.05)
A_h Limed	4.03 (±0.04)
Intermediate 3	
LFH Unlimed	3.74 (±0.12)
LFH Limed	6.93 (±0.10)
A_h Unlimed	3.85 (±0.12)
A_h Limed	4.25 (±0.10)

The soil pH at intermediate 1 was significantly higher in the limed treatments as compared to the unlimed treatments for both the LFH and A_h horizons (LFH: χ^2= 20, df= 1, **P<0.01**; A_h: χ^2= 12.8, df= 1, **P<0.01**). At intermediate 3 soil pH was significantly higher in the limed than unlimed treatments for both the LFH and A_h horizons (LFH: χ^2= 20, df= 1, **P<0.01**; A_h: χ^2= 12.8, df= 1, **P<0.01**).

The second key question was whether or not soil pH varied over time. In the unlimed treatments no significant differences were found between intermediate 1 and intermediate 3 for the LFH and A_h horizons (LFH: χ^2= 3.2, df= 1, P= 0.07; A_h: χ^2= 3.2, df= 1, P= 0.07). In the limed treatments pH was significantly higher in both the LFH and A_h horizons at intermediate 3 compared to intermediate 1 (LFH: χ^2= 7.2, df= 1, **P= 0.01**; A_h: χ^2= 12.8, df= 1, **P<0.01**).

Two conclusions can be drawn the pH data:

1. The effect of liming was to increase the pH significantly in both the LFH and A_h horizons as compared to the unlimed treatment, but the increase was more marked in the LFH.
2. Soil pH was significantly increased in the limed treatment after 16 months, i.e. between intermediates 1 and 3.

6.3 Earthworm Abundance and Community Composition

At the end of the experiment, all the boxes were removed from the Sweethope site and brought back to Stirling so that an earthworm inventory could be taken of each box. This was achieved by handsorting the boxes and removing all earthworms found. The data presented here are only for the disturbed boxes that were sampled at the intermediate sampling points. Bishop (2003) provided the raw earthworm data presented in this section. The first section deals with earthworm abundance and the effects that the treatments had.

6.3.1 Earthworm Abundance

The raw earthworm abundance data were not normally distributed, but after log transformation the data were normally distributed and had equal variances. The data were analysed using a 2 way General Linear Model along with Bonferroni Pairwise Comparisons. Table 6.2 shows the back transformed mean earthworm abundance for each of the treatments and for the combined liming and earthworm treatments. Two questions had to be answered about earthworm abundance and they were:

1. What was the effect of liming on earthworm abundance?
2. What was the effect of earthworm addition on abundance?

Table 6.2: Back transformed mean abundance and confidence limits for each individual treatment combination and for the combined liming and earthworm treatments as sampled at the end of the experiment. No. of replicates = 5.

Treatment	Mean Abundance m^{-2}	95% CL_{lower}	95% CL_{upper}
Unlimed + no worms	44.1	11.4	169.8
Unlimed + worms	130.9	79.3	216.3
Limed + no worms	188.8	113.5	314.1
Limed + worms	787.0	473.2	1309.2
Unlimed	76.0	38.2	151.4
Limed	385.5	210.9	704.7
No worms	91.2	41.8	119.1
Worms	320.6	154.5	665.3

Liming of the disturbed soil significantly increased the mean abundance of earthworms from 76.0 to 385.5 m^{-2}, a 407% increase, (F= 31.32, df= 1, **P<0.01**). This was a direct effect of the increase in soil pH after liming, making the soil more habitable for earthworms (Piearce 1972). Bonferroni Pairwise Comparisons showed that the liming treatments were statistically significant in both the worm-amended and no-worm treatments (Unlimed + no worms Vs Limed + no worms: T= -3.55, **P= 0.02** and Unlimed + worms Vs Limed + worms: T= -4.369, **P= 0.03**).

The inoculation of earthworms into disturbed soil significantly increased the mean abundance of earthworms at the end of the experiment, no worms $\bar{x}$ = 91.2 and worms $\bar{x}$ = 320.6 (F= 18.77, df= 1, **P<0.01**). This was expected since the addition of earthworms would have bolstered any existing earthworm population. The Bonferroni Pairwise Comparisons indicated that the only significant difference

amongst the individual treatments was between the Limed + no worms ($\bar{x}$ = 188.8) and Limed + worms ($\bar{x}$ = 787.1) treatments (T= -3.48, **P= 0.02**).

The effect of the disturbance process on the earthworm population, as a comparison of total median abundance can be seen in figure 6.2. The disturbance process did have a statistically significant effect on total earthworm abundance (H= 4.45, df= 1, **P= 0.035**). This process significantly reduced the earthworm population as compared to that found in the undisturbed soil (Disturbed median abundance = 166 ±154; Undisturbed median abundance = 428 ±576). The difference was principally due to the disturbance process wiping out the existing population which remained in the undisturbed soil. However, in the disturbed soils which were not inoculated, an earthworm population still developed as a result of recruitment from cocoons and invasion of the boxes by earthworms from outside.

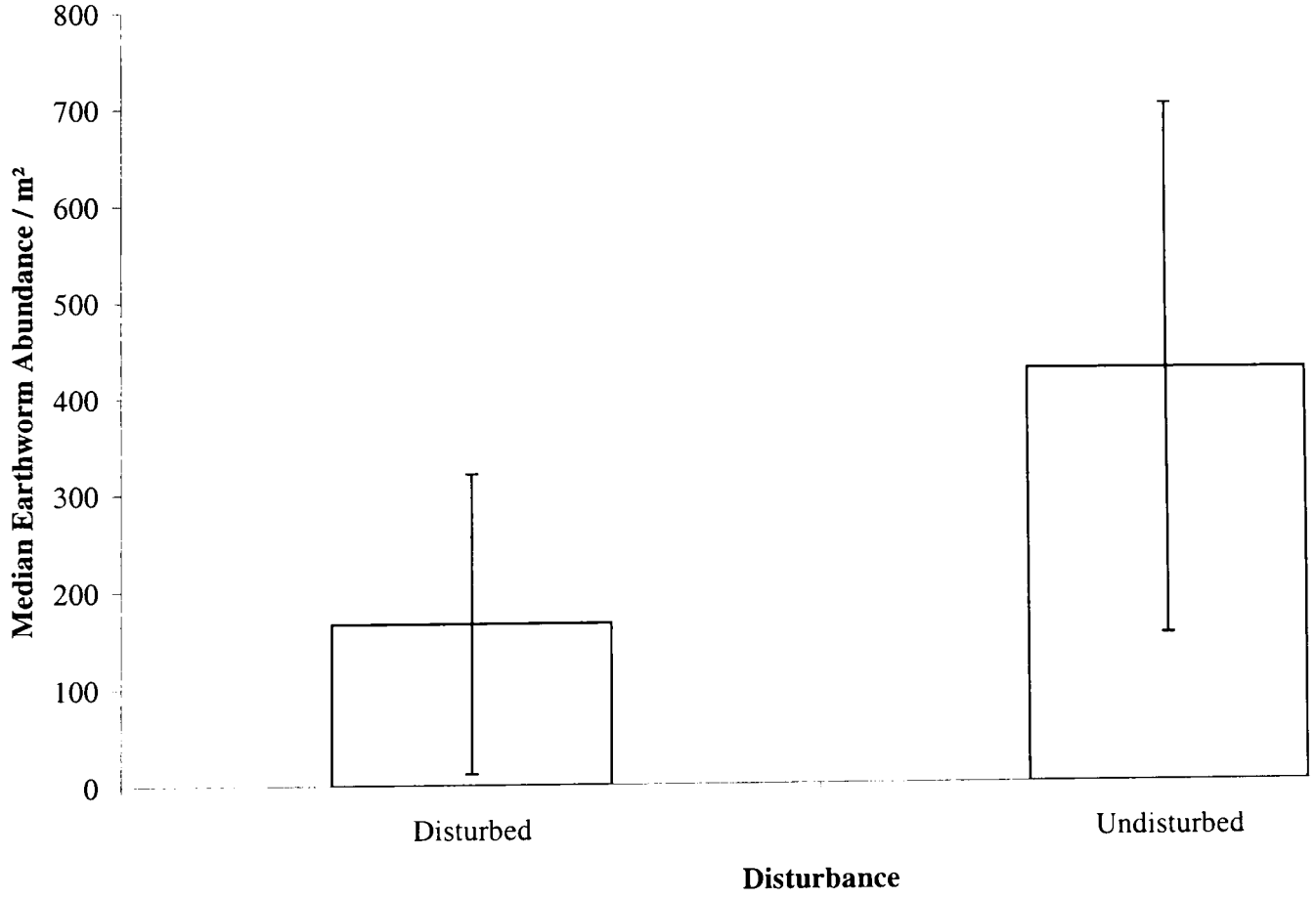


Figure 6.2: The effects of disturbance on median earthworm abundance. No. of replicates = 5.

6.3.2 Earthworm Community Composition

The composition of the earthworm community present at the end of the experiment was important because it showed how well the inoculated treatments had taken effect, and what the functional diversity of the earthworm community was. Figures 6.3, 6.4 and table 6.3 show the species composition for the individual treatments whilst table 6.4 gives the species composition of the unlimed and limed treatments.

Figure 6.3: Earthworm community composition for the individual unlimed treatments at the end of the experiment (no worms = no added worms). No. of replicates = 5.

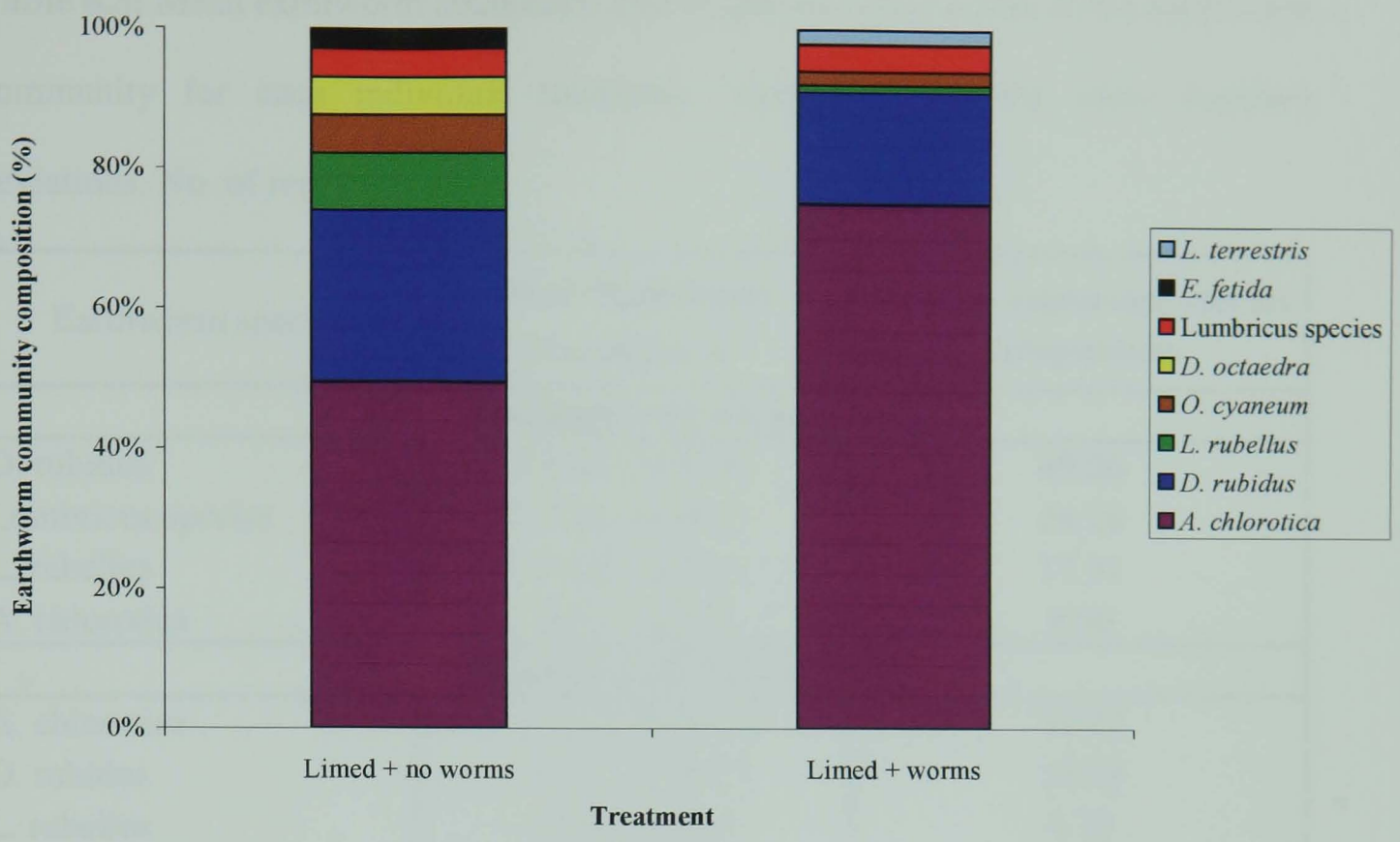


Figure 6.4: Earthworm community composition for the individual limed treatments at the end of the experiment. No. of replicates = 5.

Table 6.3: Mean earthworm abundance and % species composition of the earthworm community for each individual treatment. Values in brackets show standard deviations. No. of replicates = 5.

Earthworm species	Mean Earthworm Abundance m^{-2}	Mean % earthworm species composition
Unlimed + no worms		
D. rubidus	48.44 (± 23.4)	49.26
Lumbricus species	23.86 (± 20.3)	24.26
L. rubellus	17.22 (± 0.0)	17.51
A. chlorotica	8.81 (± 0.0)	8.96
Limed + no worms		
A. chlorotica	155.67 (± 50.2)	49.47
D. rubidus	77.37 (± 44.7)	24.59
L. rubellus	25.83 (± 0.0)	8.21
O. cyaneum	17.15 (± 0.1)	5.45
D. octaedra	17.10 (± 0.2)	5.43
Lumbricus species	12.91 (± 4.9)	4.10
E. fetida	8.64 (± 0.0)	2.75
Unlimed + worms		
L. rubellus	98.03 (± 49.7)	36.87
D. rubidus	68.73 (± 75.1)	25.85
Lumbricus species	38.63 (±6.6)	14.53
O. cyaneum	17.32 (± 0.0)	6.51
D. octaedra	16.98 (± 0.0)	6.39
L. terrestris	8.77 (± 0.2)	3.30
A. chlorotica	8.77 (± 0.2)	3.30
Lumbricus adult	8.63 (± 0.0)	3.25
Limed + worms		
A. chlorotica	655.19 (± 222.6)	75.24
D. rubidus	138.55 (± 136.4)	15.91
Lumbricus species	33.34 (± 28.3)	3.83
L. terrestris	17.44 (± 0.0)	2.00
O. cyaneum	17.41 (± 8.3)	2.00
L. rubellus	8.89 (± 0.2)	1.02

Table 6.4: Mean % species composition of the earthworm community for the liming treatments only. No. of replicates = 5.

Earthworm species	Mean % earthworm species composition
Unlimed	
D. rubidus	53.20
L. rubellus	21.11
Lumbricus species	17.10
A. chlorotica	2.61
L. terrestris	1.74
O. cyaneum	1.71
D. octaedra	1.68
Lumbricus adult	0.85
Limed	
A. chlorotica	72.01
D. rubidus	20.77
Lumbricus species	3.56
O. cyaneum	1.66
L. rubellus	0.84
D. octaedra	0.66
L. terrestris	0.34
E. fetida	0.17

The earthworm community composition data showed that the inoculated treatments did not work well, since many of the species found were not part of the inoculum and *L. terrestris* was mostly missing or at insignificant abundances in all the earthworm treatments. The poor survival of *L. terrestris* was not surprising considering the depth of the boxes (around 25 cm). This lack of *L. terrestris* is likely to have had a significant effect on the results of this experiment because without any anecic earthworms, which form semi-permanent burrows (Bouché 1977), the effects of earthworms on porosity will be reduced. An additional complication to the lack of anecic earthworms was the presence of earthworms in the no worms treatments. It was hoped that the earthworm populations in these treatments would be minimal as a result of the disturbance process (handsorting and breakdown of the partially dried

soil). These populations will be due to either recruitment from cocoons or by earthworm invasion through the worm proofing.

The earthworm inoculum was not very successful in terms of the survival of the selected earthworm species. In the unlimed treatments the epigeic species *D. rubidus* (53%) and *L. rubellus* were dominant, whilst in the limed treatments the dominant species were *A. chlorotica* (72%) and *D. rubidus* (21%), endogeic and epigeic respectively.

Both earthworm inoculation and liming had effects on the dominant species found in the treatments. In the unlimed soils which were inoculated with earthworms, the community composition changed from being dominated by *D. rubidus* (Unlimed + no worms = 49%, Unlimed + worms = 25%) to *L. rubellus* (Unlimed + no worms = 18%, Unlimed + worms = 37%). A possible explanation is that both of these species are epigeic and are therefore likely to utilise the same habitats, so when *L. rubellus* was inoculated it out competed *D. rubidus*. In the limed treatments the earthworm inoculation had the effect of increasing the abundance of *A. chlorotica* from 49% to 75%, whilst *D. rubidus* abundance decreased from 25% to 16%. This is probably due to liming raising soil pH to a level that is more suitable for *A. chlorotica* since it has been described as being acid intolerant (Satchell 1955).

The liming of the disturbed soil led to statistically significant rises in pH that resulted in *A. chlorotica* becoming dominant in the limed treatments at the expense of *D. rubidus* and *L. rubellus*. The abundance of *A. chlorotica* rose from 3% in the unlimed treatments to 72% in limed soil, whilst the abundance of *D. rubidus* and *L. rubellus* dropped from 53% and 21% respectively in unlimed soil to 21% (*D. rubidus*) and 1% (*L. rubellus*).

There were some differences in the dominant earthworm species between the undisturbed and disturbed soils. In the disturbed soils *D. rubidus, L. rubellus* and *A. chlorotica* tended to dominate whilst in the undisturbed soils the dominant species were *A. chlorotica, D. rubidus* and *O. cyaneum*. The major differences occurred in the unlimed treatments where *L. rubellus* was more prevalent in the disturbed soil as compared to the undisturbed soil. Overall these differences probably would not have been overly significant since the dominant species composition tended to be functionally quite similar

6.3.3 Conclusions

The conclusions that can be drawn from the earthworm inventory can be summarised as follows:

1. Both liming and earthworm inoculation increased earthworm abundance.
2. The success of the earthworm inoculations was limited by the occurrence of small numbers of worms in the no-worm treatments and by the lack of success of the anecic species added and the consequent dominance by epigeic worms.
3. The dominant earthworm species in the unlimed soil were *D. rubidus* and *L. rubellus* whilst in the limed soil this was *A. chlorotica* and *D. rubidus*.
4. Both liming and earthworm inoculation led to changes in the dominant earthworm species found in the disturbed soil.
5. The total earthworm abundance in the undisturbed soil was significantly higher than in the disturbed soil.

6.4 % Organic Carbon

The % organic carbon (%OC) was determined on sub-samples of the 1-2 mm aggregates used in the aggregate stability determination. The method used for the determination was the Walkley-Black wet oxidation method. Figures 6.5 - 6.6 show the comparisons used to evaluate the initial state of the soil.

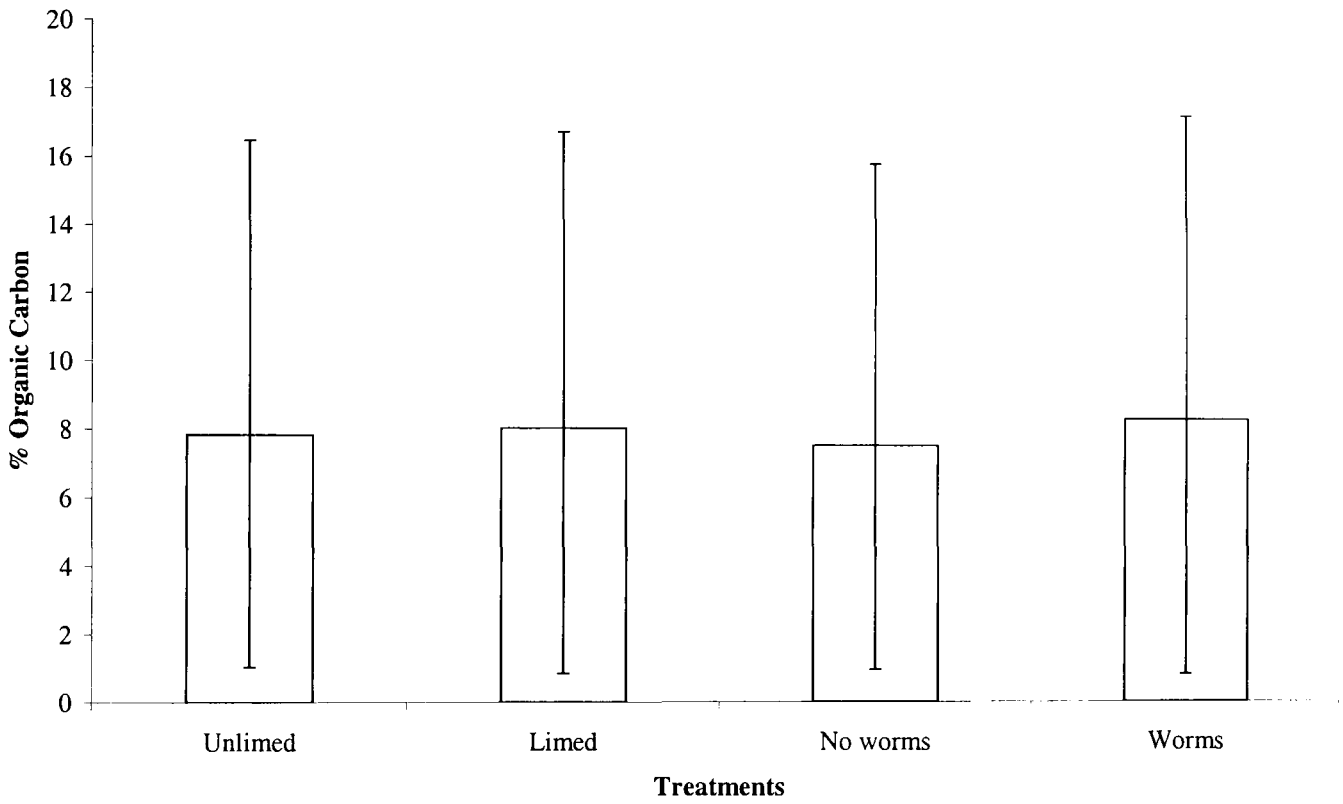


Figure 6.5: Intermediate 1 back transformed %OC means for the liming and no worms treatments. Error bars represent back transformed 95% confidence limits. No. of replicates = 5.

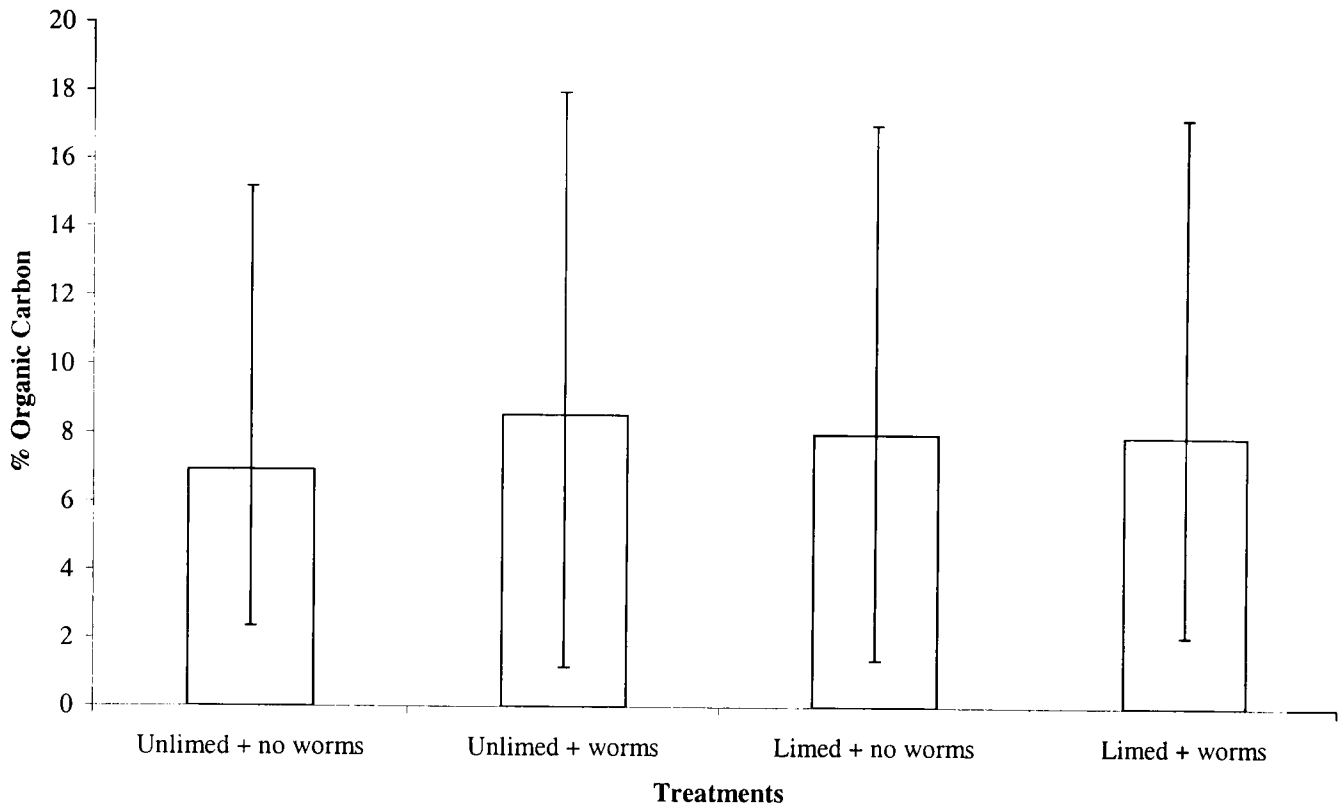


Figure 6.6: Intermediate 1 back transformed mean %OC for the individual treatment combinations. Error bars represent back transformed 95% confidence limits. No. of replicates = 5.

After carrying out a x^3 transformation the intermediate 1 %OC data were analysed using a 2 way General Linear Model and Bonferroni Pairwise Comparisons to investigate the differences between the individual treatments. The results show that there were no significant differences between the liming (F= 0.17, df= 1, P= 0.686) and worm treatments (F= 2.53, df= 1, P= 0.131) and no statistically significant differences were found for the individual treatments. This was expected since all the soils had been subjected to the same disturbance process and they were sampled before the earthworm inoculation took place.

Time effects were analysed by comparing both unlimed + no worms (unlimed effects) and limed + no worms (liming effects) between intermediate 1 and intermediate 3. Figures 6.7 and 6.8 show these differences for the unlimed and limed time effects. The unlimed comparison was carried out using the Kruskall-Wallis test

since the data was not normally distributed, and was not improved through data transformation. The limed comparison was carried out using one way ANOVA. No statistically significant differences were observed for either the unlimed and limed comparisons (unlimed: H= 0.27, df= 1, P= 0.602 and limed: F= 4.74, df= 1, P= 0.06). This meant that the intermediate 3 unlimed and limed + no worms treatments could be used as representative control treatments since time had no significant effect on these soils.

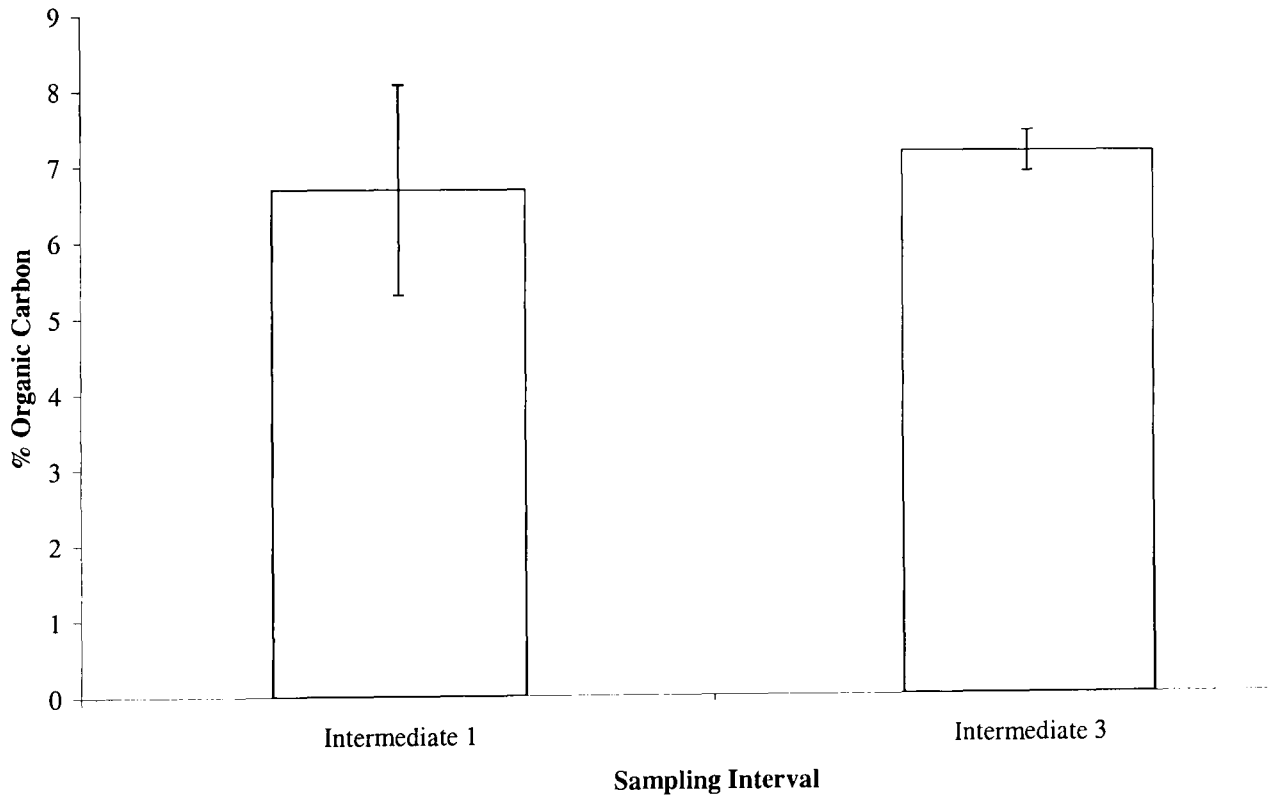


Figure 6.7: Differences in median %OC between intermediates 1 and 3 for the unlimed + no worms treatment (unlimed time effect). Error bars show the quartile deviation. No. of replicates = 5.

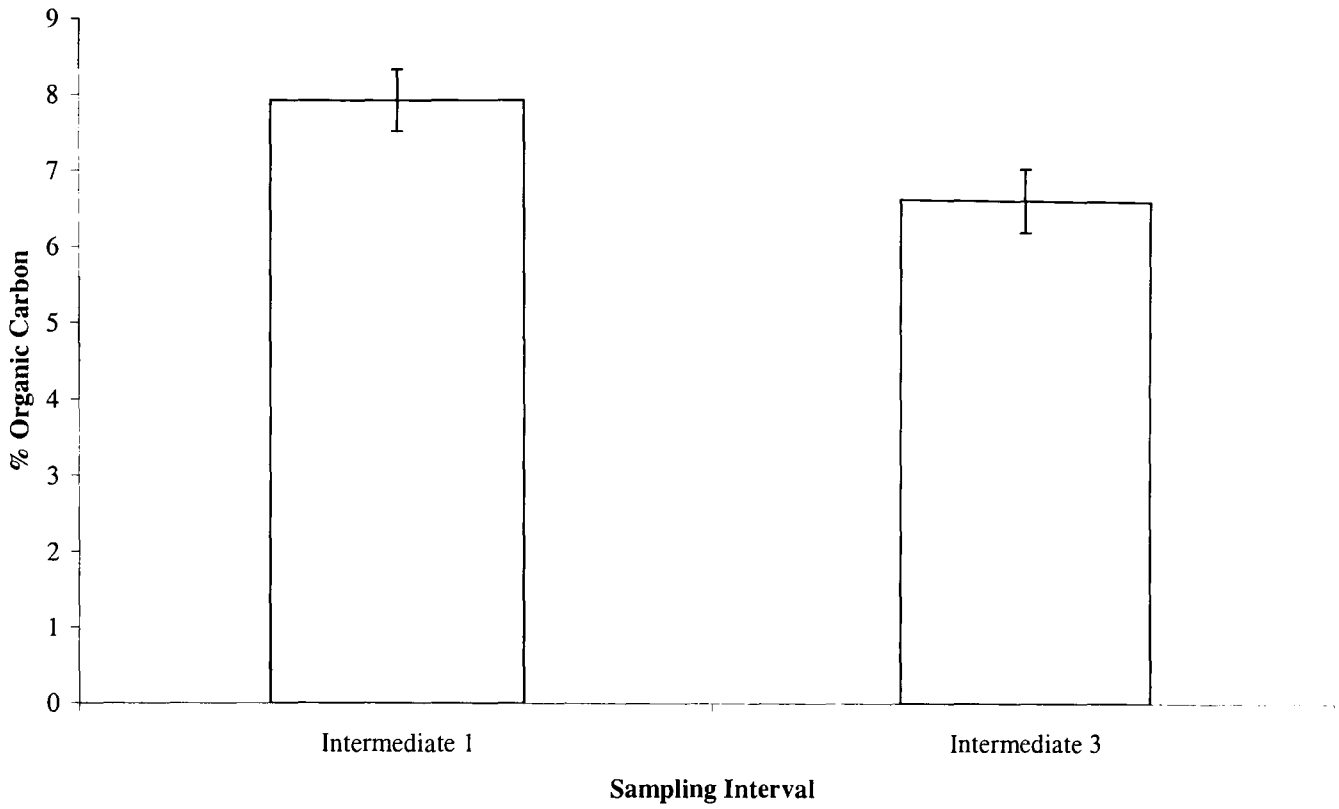


Figure 6.8: Differences in median %OC between intermediates 1 and 3 for the limed + no worms treatment (limed time effect). Error bars show the quartile deviation. No. of replicates = 5.

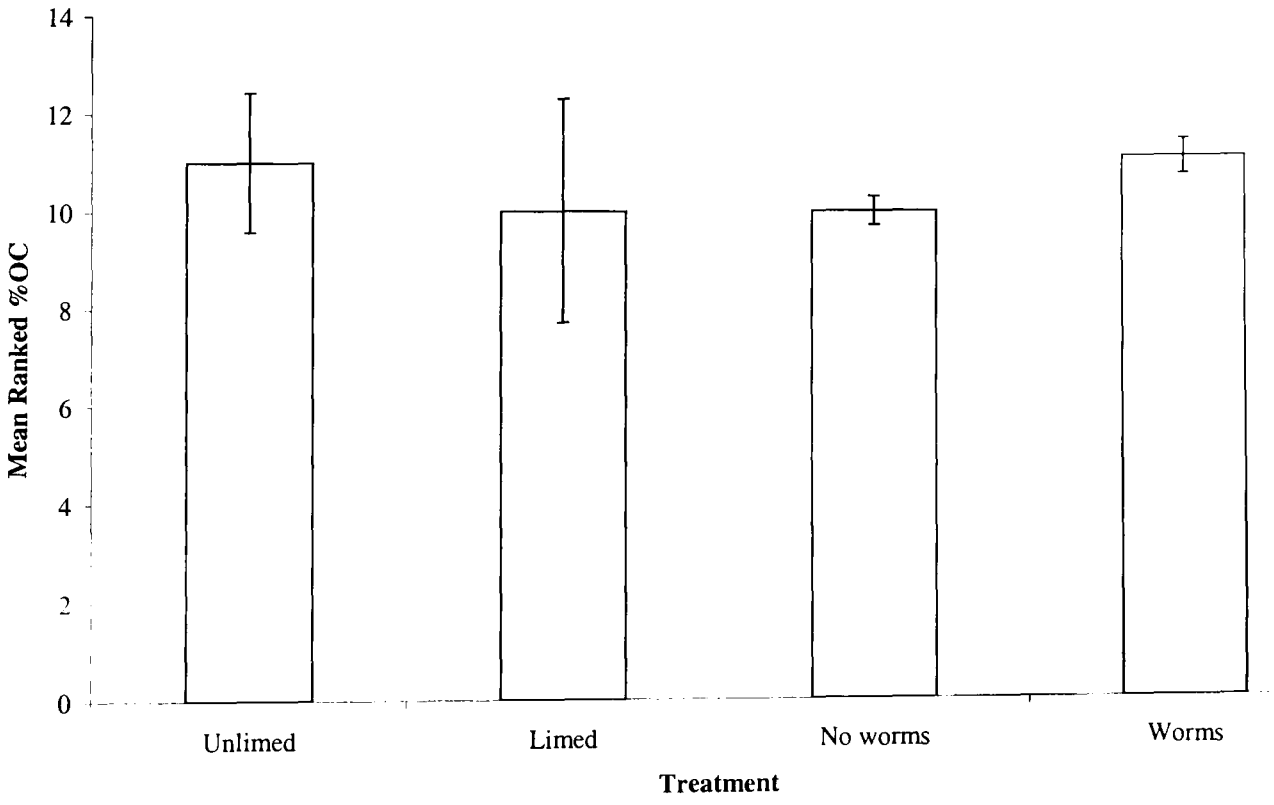


Figure 6.9: Mean ranked %OC values comparing the effects of earthworm inoculation and liming. Error bars show standard errors. No. of replicates = 5.

The data used for the analysis of the earthworm and liming effects on %OC was not normally distributed and was resistant to transformation, therefore a type of non-parametric two way ANOVA was carried out (see chapter 4 for details of this test). Figure 6.9 shows the mean ranked %OC data for the liming and earthworm treatments. No significant differences were found between the unlimed and limed treatments ($\bar{x}$ = 10, SE= 0.29 and $\bar{x}$ = 11.1, SE= 0.36 respectively). In addition there were no significant differences between the no added worms and worms treatments at the 95% confidence level. This indicated that in the disturbed soils earthworm inoculation and liming had no statistically significant effect on the %OC of the 1-2 mm soil aggregates.

6.5 Aggregate Stability

Aggregate stability was determined on the 1-2 mm samples collected from the A_h horizon of the disturbed boxes at all three intermediate sampling times, although for data analysis, only intermediate 1 and 3 were used. The methodology for the determination of aggregate stability has been outlined in chapter 4. 2 g samples of aggregates were disrupted with four periods of sonication for 120 seconds each, and aggregate size distribution was measured after each sonication. The difference between the first and last aggregate size distribution gave an indication as to the amount of aggregate breakdown that had taken place and therefore the stability of the aggregates. To summarise the aggregate size distributions, a series of stability indices were calculated; as outlined in chapter 4.4.1.

6.5.1 Hypotheses

Two hypotheses were under investigation in this experiment concerning the effects of both earthworm casting and lime addition on aggregate stability. The hypotheses are outlined below:

1. That the inoculation of earthworms into a structureless soil, which is devoid of earthworms, will lead to increased aggregate stability through the production of cast material.
2. That the liming of soil will increase aggregate stability by increasing earthworm casting activity.

The next two sections present the data used to investigate these hypotheses followed by discussion and interpretation of results.

6.5.2 Results

The first data that were analysed were from the intermediate 1 samples, which provided information on the initial state of the soil before any earthworms were inoculated. This analysis was in the form of two way General Linear Model ANOVAs. No statistically significant differences were observed for the aggregate stability indices between treatments from the intermediate 1 sampling (table 6.5 shows the mean values of the indices). This was the expected outcome.

Table 6.5: Mean values for aggregate stability indices from intermediate 1, which represented the initial soil state (value for the 40 μm-150 μm index is the back transformed mean). Values in brackets are standard errors except for the 40 μm-150 μm index which are back transformed 95% confidence limits. No. of replicates = 5.

	Aggregate Stability Indices			
Treatment	1-2 mm	600 μm-2000 μm	MWD	40 μm-150 μm
Unlimed	-11.93 (±0.42)	-12.53 (±0.50)	-189.43 (±6.04)	4.20 (3.25, 4.85)
Limed	-11.48 (±0.73)	-11.70 (±0.72)	-179.20 (±10.30)	3.80 (2.89, 4.40)
No worms	-11.47 (±0.69)	-11.82 (±0.74)	-181.30 (±10.00)	3.74 (2.81, 4.36)
Worms	-11.94 (±0.48)	-12.40 (±0.50)	-187.39 (±6.81)	4.24 (3.34, 4.87)

Table 6.6: Medians of the aggregate stability indices for the unlimed + no worms treatment for the intermediate 1 and intermediate 3 sampling sessions. The P values are from the Kruskall-Wallis Tests (significant values are shown in bold). Values in the brackets represent quartile deviation. No. of replicates = 5.

Sampling Session	Aggregate Stability Indices			
	1-2 mm	600 μm-2000 μm	MWD	40 μm-150 μm
Intermediate 1	-11.49 (±0.96)	-11.90 (±1.09)	-179.70 (±16.56)	3.93 (±0.94)
Intermediate 3	-11.73 (±1.08)	-13.78 (±1.81)	-198.1 (±16.72)	4.23 (±1.75)
P value	0.602	**0.047**	**0.047**	0.347

The differences between the aggregate stability indices for the intermediate 1 and intermediate 3 unlimed + no worm treatment are shown in table 6.6. The data were not normally distributed so were analysed using a Kruskall-Wallis Test. Statistically significant differences between the medians were observed for the 600 μm-2000 μm and MWD indices, with the aggregates in the intermediate 3 samples being less stable than those from intermediate 1. No significant differences existed for the 1-2 mm aggregates, therefore it was likely that it was aggregates in the size range 600 μm-1000 μm that were less stable which probably represented the breakdown of

secondary aggregates. The decrease in aggregate stability was not surprising considering the soil was highly organic and therefore would have been inherently stable (Tisdall and Oades 1982; Chaney and Swift 1984), so after an extensive disturbance process the aggregates were bound to loose some stability. It should also be noted that over time an earthworm community developed in these treatments and it has been reported that earthworms can have a destabilising effect on aggregate (Schrader and Zhang 1997; Shuster *et al* 2000), so these decreases in aggregate stability could also be due to earthworm activity.

Table 6.7: Mean aggregate stability values for the limed + no worms treatment for the intermediate 1 and intermediate 3 sampling sessions. The P values are the result of one way ANOVA (significant values are shown in bold). Values in the brackets represent standard errors. No. of replicates = 5.

Sampling Session	Aggregate Stability Indices			
	1-2 mm	600 μm-2000 μm	MWD	40 μm-150 μm
Intermediate 1	-11.36 (±1.36)	-11.41 (±1.37)	-177.66 (±19.14)	2.62 (±1.08)
Intermediate 3	-13.09 (±1.34)	-15.99 (±1.25)	-227.35 (±17.42)	5.64 (±0.82)
P value	0.393	**0.039**	0.091	0.057

One way ANOVA showed that no statistically significant differences existed for the aggregate stability indices between intermediate sample 1 and 3 for the limed + no worms treatment except for the 600-2000 μm aggregates (table 6.7). The aggregates in this size range tended to be less stable at the end of the experiment (intermediate 3) than they were at the beginning. Again, as was the case for the unlimed + no worms treatment, no significant differences were found for the 1-2 mm aggregates, so this probably represented the breakdown of secondary aggregates. The decrease in aggregate stability was probable due the initial disruption not destroyed all of the

stable aggregates formed by worms and other fauna, and it was these which lost their stability as the system adjusted to the new conditions, so the destabilisation was a function of the disturbance process.

Table 6.8: Means of ranked aggregate stability data for the liming and earthworm treatments. Standard errors are shown in brackets. No. of replicates = 5.

Treatment	Aggregate Stability Indices			
	1-2 mm	600μm-2000μm	MWD	40μm-150μm
Unlimed	11.2 (±1.33)	10.9 (±1.59)	11.6 (±1.20)	9.3 (±1.20)
Limed	9.9 (±2.35)	10.2 (±2.19)	9.5 (±2.39)	11.7 (±2.39)
No worms	10.7 (±1.82)	10.8 (±1.81)	10.8 (±1.78)	10.3 (±1.78)
Worms	10.4 (±2.02)	10.2 (±2.02	10.3 (±2.05)	10.7 (±2.05)

The effects of earthworms and liming on aggregate stability were assessed by comparing the differences in aggregate stability indices between the earthworm treatments and the liming treatments for the intermediate 3 sampling session. This was justified even though there were some statistically significant differences in aggregate stability between intermediate 1 and 3, because changes in structural stability were expected after such a disruptive disturbance process. The data were not normally distributed and did not lend themselves to transformation, so a non-parametric two way ANOVA was carried out (Wheater and Cook 2000). The means of the ranked data for the earthworm and liming treatments are shown in table 6.8 (the means of the ranked means had to be presented since there was no way to back transform the data to get it back to its original measurement units). No statistically significant differences were observed for either the liming or earthworm treatments (table 6.9), indicating that neither earthworms nor liming had a statistically significant effect on aggregate stability in disturbed soil.

Table 6.9: Statistical output from the non-parametric two way ANOVA test for the effects of liming and earthworms on aggregate stability. No. of replicates = 5.

Treatment	Aggregate Stability Indices			
	1-2 mm	600 μm-2000	MWD	40 μm-150 μm
Liming	H= 0.043, df= 1, P > 0.05	H= 0.071, df= 1, P > 0.05	H= 0.632, df= 1, P > 0.05	H= 0.824, df= 1, P > 0.05
Earthworms	H= 0.014, df= 1, P > 0.05	H= 0.051, df= 1, P > 0.05	H= 0.037, df= 1, P > 0.05	H= 0.023, df= 1, P > 0.05

6.5.3 Discussion and Conclusions

The first hypothesis put forward concerned the effect of earthworm casting activity on aggregate stability. Earthworm casts have been reported as being some of the most stable aggregates found in soil (Shipitalo and Protz 1988; Marinissen and Dexter 1990; Ketterings *et al* 1997; Garvin *et al* 2001), therefore it was expected that after inoculating the disturbed soil, (which was supposedly earthworm free), aggregate stability would be significantly increased in the inoculated soil as compared to the uninoculated soil. As the data has shown in tables 6.8 and 6.9, no statistically significant differences at the 95% confidence level were observed between the earthworm treatments.

There are several reasons why earthworms did not have a significant effect on aggregate stability in this experiment or how these effects could have been masked. The first issue to highlight is that after the earthworm inventory at the end of the experiment, the desired inoculum of *L. rubellus*, *A. chlorotica* and *L. terrestris* had only limited success in establishing, since *L. terrestris* was either absent or present in only very small numbers, and *A. chlorotica* was scarce in the unlimed soil. In the unlimed treatments most of the earthworms found were epigeic species. (i.e. *D.*

rubidus and *L. rubellus*), so would not have likely casted in the more mineral horizons such as A_h, which is where the aggregate stability samples originated. This was therefore bound to have a significant affect.

Another major cause for the lack of earthworm effects may be due to the presence of relatively high earthworm populations in the no worm treatments, (the mean abundance for the unlimed and limed no worm treatments were 44.1 m^{-2} and 188.8 m^{-2} respectively). These earthworm populations in the no worm treatments could have come from either recruitment from cocoons which survived the disturbance process and/or from earthworms finding a way through the worm proofing of the boxes (very fine mesh over the drainage holes in the bases of the boxes and the same very fine mesh as lids which were attached with silicon sealant). One possible route of entry for the earthworms would have been through the holes created in the sides of the boxes during the intermediate sampling. Holes had to be cut into the sides of the boxes so as to remove soil samples for the aggregate stability determination and thin section samples for micromorphological analysis, as shown in figure 6.10. These holes were sealed after every sampling by replacing the plastic which was cut away and sticking it in place with very strong industrial gaffer tape. However it may have been possible that over time the adhesion of the tape to the box surface may have diminished allowing gaps to develop. This was minimised as much as possible by regular checks on the worm proofing of these holes during sampling periods and site maintenance visits. The presence of earthworms in the no worm treatments meant that there was background noise in the data because the aggregate stability results from the earthworm inoculated treatments were not able to be compared against a soil whose aggregate stability was devoid of earthworm influences. This may have

had the effect of masking any earthworm effects on aggregate stability, especially if they were subtle in nature.

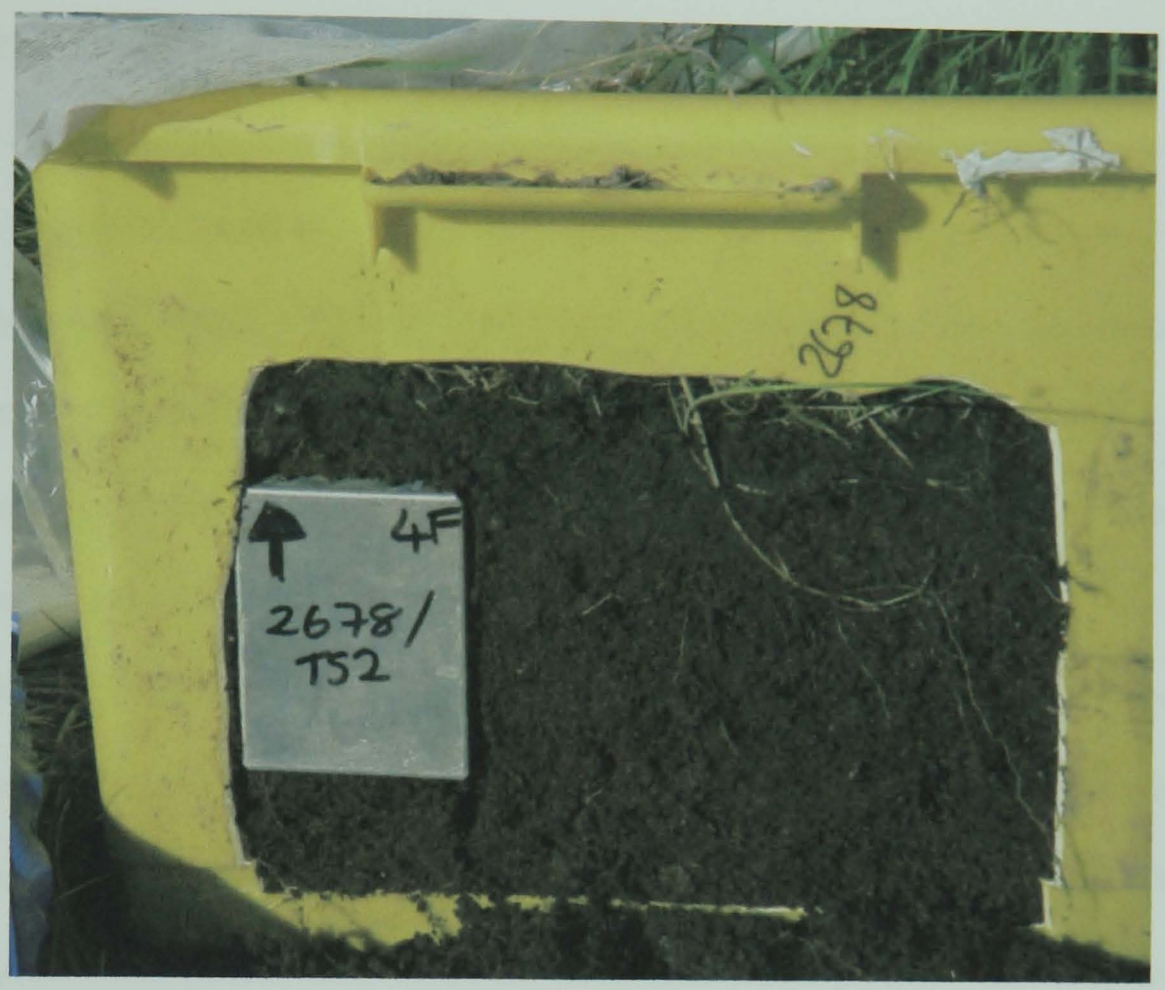


Figure 6.10: Image showing how sampling was carried out during the intermediate sampling sessions from holes cut into the sides of the boxes.

The second hypothesis was concerned with the effects of liming on aggregate stability through the stimulation of the earthworm community in terms of species composition, abundance and activity. The effect of liming on the soil was to raise soil pH in both the LFH and A_h horizons which in turn led to increased earthworm abundance. The increase in pH due to liming was most pronounced in the LFH horizon. Liming also had the effect of reducing the abundance of epigeic species (*D. rubidus* and *L. rubellus*) and increasing the abundance of endogeic species (*A. chlorotica*). This change in community composition would more than likely have caused an increase in casting in the mineral horizons, i.e. the A_h horizon which was

the horizon that was sampled for aggregate stability determination. Despite liming leading to changes in the earthworm community, no statistically significant differences were observed in aggregate stability at the 95% confidence level, which was surprising. An explanation for this is that again earthworms were found in the no worm treatment, therefore these treatments were not behaving as controls. As with the earthworm effects, this may well have led to noise in the data that could have masked any liming effects.

In terms of aggregate stability, this experiment could have been improved in a number of ways:

1. Better control treatments: Make sure that the no worm treatments were earthworm free. This would be a very difficult task but the best way would be to carry out a disturbance process like the one used in this experiment (i.e. handsorting) except with complete removal of all cocoons. The removal of all cocoons was not possible due to a) the large quantities of soil processed and b) the time available to complete the disturbance process before the soil had to be reburied at the field site, thus it was likely that some cocoons survived soil processing. Two possible alternatives would have been to 1) irradiate all the soil (problems with finding sources strong enough and safe enough to guarantee that all earthworms and cocoons were killed), and 2) to start with an artificial 'clean' soil (then this would not be realistic to the true soil conditions).
2. Larger aggregate sizes: It would have been useful to carry out the aggregate stability determinations on a variety of difference aggregate sizes. This would have helped to include more earthworm cast material in the analytical samples, but in this experiment the instrumentation placed an upper size limit of 2 mm on the size of the aggregates used.

3. Sampling from the H horizon: The high abundance of epigeic earthworms in the treatments meant that the H horizon would have had a high proportion of cast material, therefore aggregate samples from this horizon would have been useful.

There are two main conclusions that can be drawn from the aggregate stability data presented here. These conclusions are that:

1. The inoculation of the disturbed soil with earthworms did not significantly affect aggregate stability
2. The liming of the disturbed soil significantly increased earthworm abundance and therefore casting activity, but this increase in earthworm abundance and activity did not significantly increase or decrease the stability of soil aggregates.

6.6 Earthworm Excremental Features

A detailed outline of the protocols used in the description and quantification of earthworm excremental features is given in chapter 4.5.4. By way of summary, thin section samples were taken from the A_h horizon for each disturbed box for all three intermediate sampling sessions. The thin sections were then point counted to quantify the amount of earthworm excrement present in the disturbed soil. A full description of how earthworm excrements were identified is given in chapter 4.

6.6.1 Hypotheses

Two hypotheses were formulated to identify what the effects of earthworms on the abundance of excremental features were and how the application of lime affected the abundance of earthworm excrement. The hypotheses were that:

1. The inoculation of earthworms into a soil devoid of any earthworm artifacts will lead to an increase in the abundance of earthworm excremental features.
2. The application of lime to soil will lead to an increase in earthworm excremental pedofeatures as a result of increased earthworm abundance and/or casting activity.

6.6.2 Results

The initial state of the disturbed soil in terms of the abundance of earthworm excrement is shown in figure 6.11. This was the comparison of the liming and earthworm treatments for the intermediate 1 data only. A ranked two way ANOVA was used to test the differences between the treatments (non-parametric two way ANOVA). The results of this analysis showed that there were no statistically significant differences for the liming treatments ($P > 0.05$) but there were significant differences for the earthworm treatments ($P < 0.05$). There was significantly more earthworm excrement in the earthworm treatment ($\bar{x} = 13.61$, SE= 1.66) when compared to the no worms treatment ($\bar{x} = 6.75$, SE= 1.66). These samples were taken before the earthworm inoculation took place, showing that there was an earthworm population present even before the inoculum was added. These earthworms had either hatched from cocoons or had found a way into the boxes of soil from outside. The latter is more probable because the time period from when the disturbed boxes were reburied at the Sweethope site to sampling was only two months. This was probably too soon for the cocoons to have hatched and for the juvenile earthworms to have grown sufficiently large for their excrements to become identifiable.

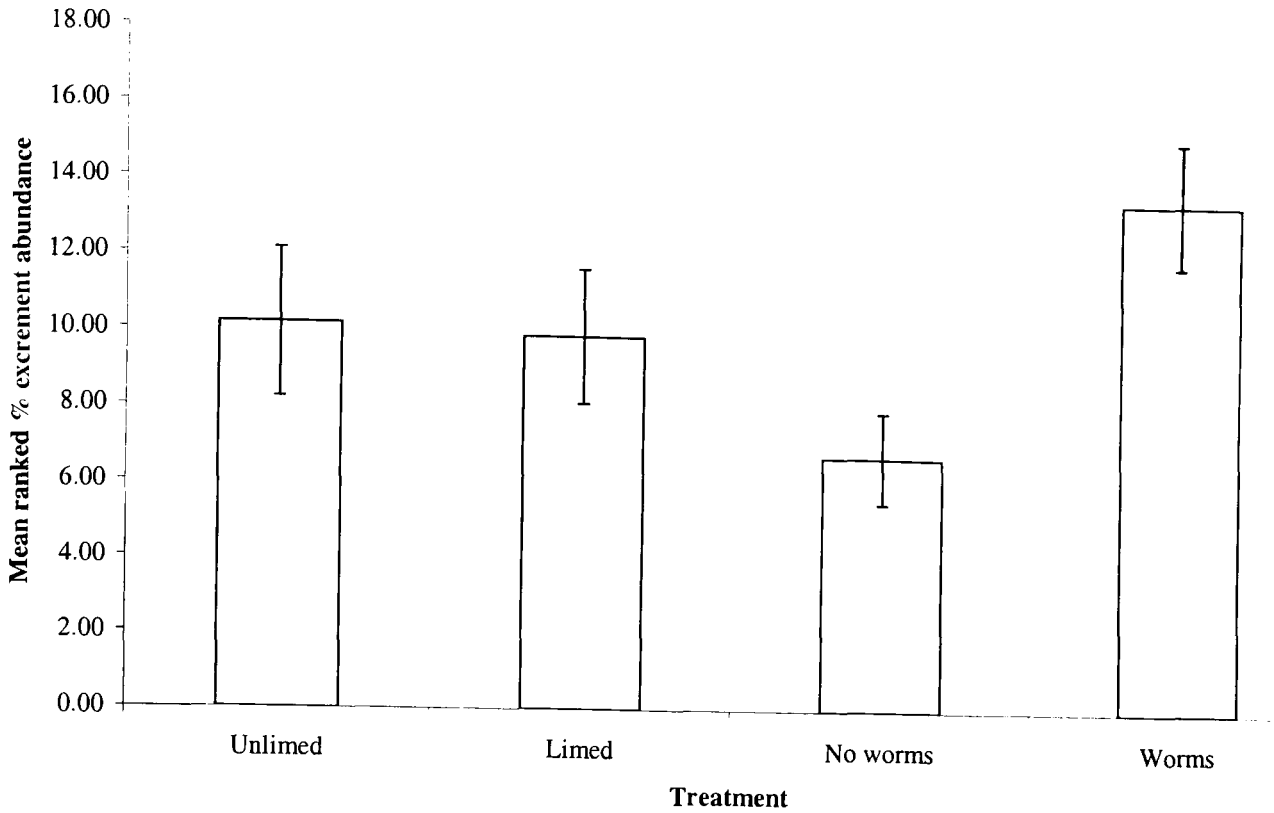


Figure 6.11: Mean ranked earthworm excrement abundance for the intermediate 1 thin sections only, expressed as a % of the whole slide (i.e. sampled before earthworm inoculation). The error bars show standard errors. No. of replicates = 5.

The differences in the no worms treatment between intermediate 1 and intermediate 3 for both unlimed and limed soil show any changes that have occurred to the soil over time (table 6.10). A Kruskall-Wallis Test was used to compare the unlimed + no worm treatments between intermediates 1 and 3. The same comparison but for the lime + no worms treatment was carried out using one way ANOVA. The results of the statistical analysis showed that for both the unlimed and limed treatments there was significantly more earthworm excrement present in the intermediate 3 sample than in the intermediate one samples, i.e. there was more earthworm excrement at the end than at the beginning for these treatments. This was borne out by the earthworm abundance data collected in the earthworm inventory at the end of the experiment where the total mean abundance for the unlimed + no worms treatment was 98.3 m^{-2} and 314.7 m^{-2} for the limed + no worms treatment. This again shows that an

earthworm population developed over time in treatments where there should have been very few to no earthworms.

Table 6.10: Median (unlimed) and Mean (limed) data for the comparison of % earthworm excrement present in thin section at the beginning and end of the experiment. Medians are shown with quartile deviations, whilst for the mean data standard errors are presented. No. of replicates = 5.

Sampling Session	Unlimed Median	Limed Mean
Intermediate 1	0.00 (±0.85)	0.62 (±0.28)
Intermediate 3	7.43 (±3.94)	1.90 (±0.38)
P value	**0.026**	**0.025**

The effects of the liming and earthworm treatments on the abundance of earthworm excrement present in the intermediate 3 thin sections are shown in figure 6.12. The data were analysed using a two way General Linear Model after being arcsine transformed. The results of the statistical analysis showed that neither liming ($F= 0.01$, $df= 1$, $P= 0.929$) nor earthworms ($F= 4.15$, $df= 1$, $P= 0.058$) significantly affected the abundance of earthworm excrement in the disturbed soil.

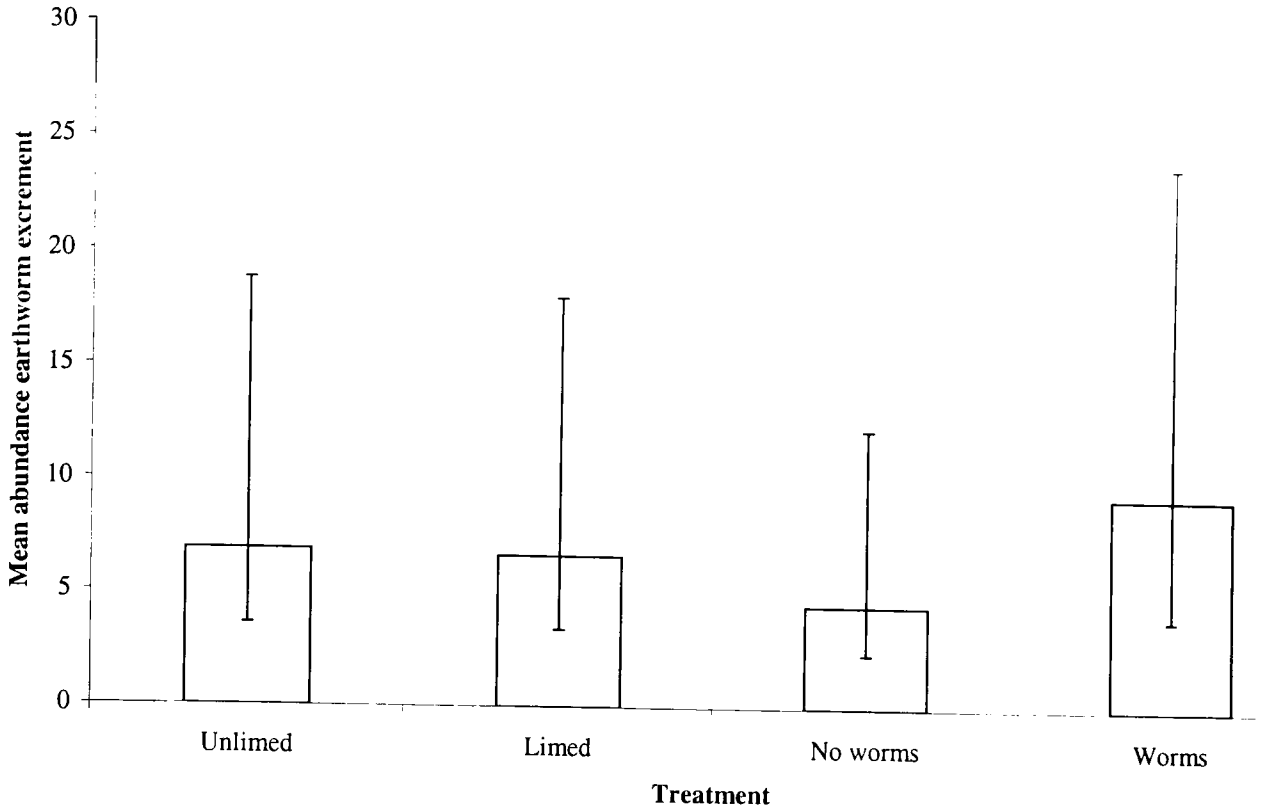


Figure 6.12: Back transformed means of the abundance of earthworm excrement in the intermediate 3 thin sections. The error bars show the back transformed 95% confidence limits. No. of replicates = 5.

6.6.3 Discussion and Conclusions

It was expected that both the earthworm and liming treatments would increase the amount of earthworm excremental features found in the intermediate 3 thin sections because both treatments led to increases in the total abundance of earthworms (table 6.2) and more earthworms would mean more excrement produced. However this was not the case, neither the earthworm nor liming treatments gave rise to significantly more earthworm excrement despite increased earthworm abundance. There are a number of possible reasons to explain this.

The presence of earthworms in the no worms treatments, as shown by both the earthworm inventory at the end of the experiment and through the visual observation of earthworm excrements in the thin sections, may have masked both the liming and

earthworm effects. This was because these treatments would not have acted as true controls since there were earthworm effects already present in them.

From thin section observations, much of the soil had been worked and reworked by soil organisms and with many being coprophagus, i.e. feeding off the excreta of other organisms, the earthworm features observed showed high levels of degradation. Figure 6.13 highlights this degenerative effect on earthworm excrements. One of the key organisms involved in this degradation were the enchytraeid worms Davidson *et al* 2002), the droppings of which were often seen scattered around or actually within earthworm excrements. This biological degeneration would have a) quickly reworked fresh earthworm features into other types of faunal excrement and b) rendered some of the earthworm excremental features unidentifiable, both of which would have led to underestimation of the quantities of earthworm excrement present in the soil.

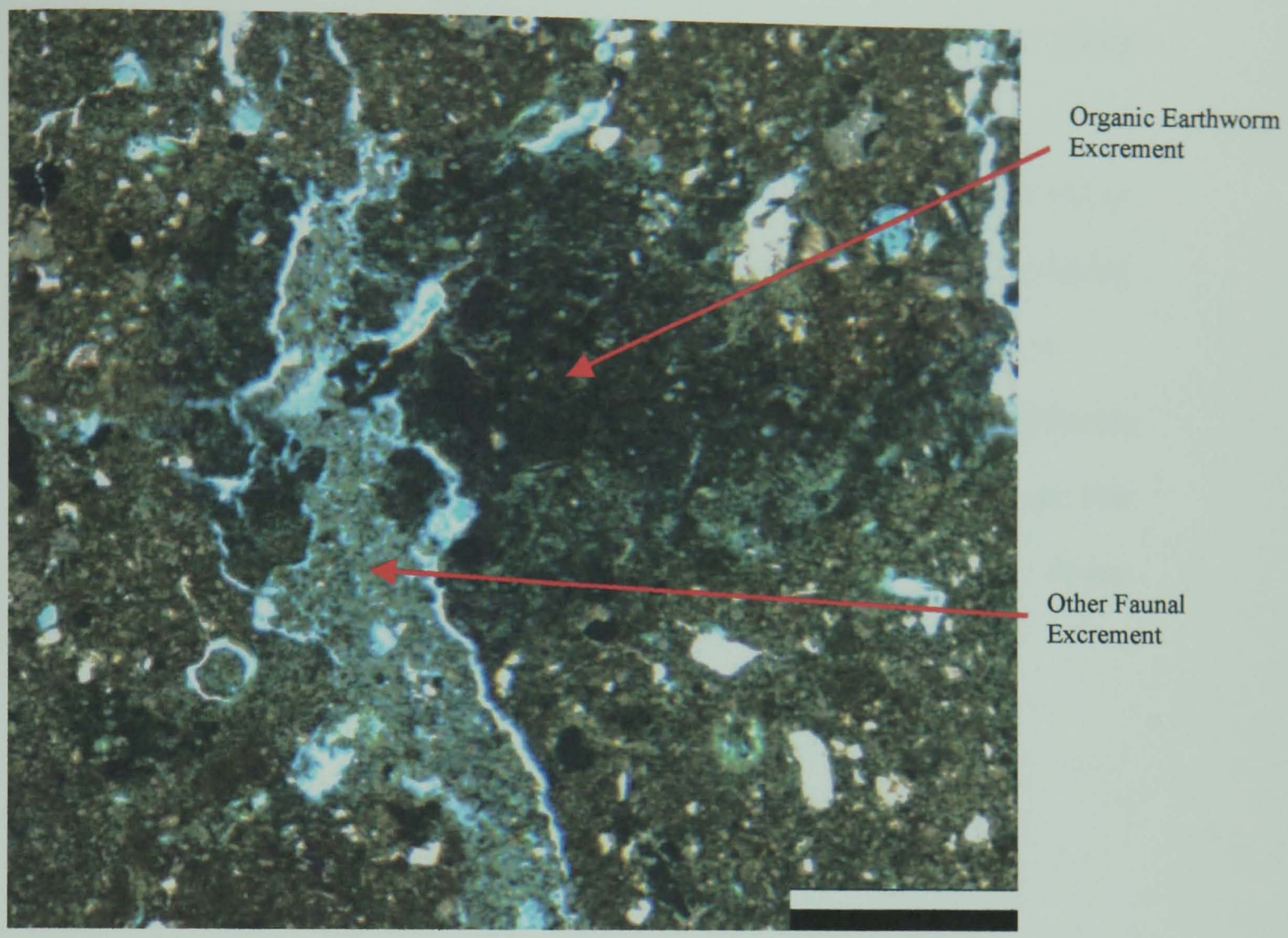


Figure 6.13: Image showing an example of the degrading effect of other soil organisms on earthworm excrement. The large dark object in the centre of the frame is an organic earthworm excrement and running through it is a line of much finer textured faunal excrement. Scale bar represents 20 mm.

A final explanation is that the thin section samples were taken from the A_h horizon only, but since many of the earthworm species were epigeic, it would be expected that the uppermost organic horizons such as the H would be full of earthworm excrements. Indeed from the examination of thin sections containing H horizon material from the Sourhope main plots, as collected for another research project, the H horizon was almost entirely constructed from faunal excrements. Again this would then have led to the underestimation of the amount of earthworm excrement in the disturbed soils.

The conclusions that can be drawn from the effects of the earthworm and liming treatments on the abundance of excremental features are that:

1. The inoculation of earthworms into a soil that turned out not to be devoid of earthworm features led to no statistically significant differences in the abundance of earthworm excremental features found between the earthworm treatments.
2. The application of lime to soil did not lead to an increase in earthworm excremental pedofeatures as a result of increased earthworm abundance and that no statistically significant difference were observed between the liming treatments.

6.7 Re-organisation of Void Space

The background literature review on the effects that earthworms have on soil porosity is outlined in chapter 2, whilst a detailed outline of the accepted methodology is given in chapter 4. The effects of earthworms on void space were quantified using image analysis techniques, the product of which were large quantities of data. Of interest to this research was the effects of earthworms on total soil porosity and the size distribution of voids < 2 mm^2 and > 2 mm^2 in area in the disturbed soil.

6.7.1 Hypotheses

Three hypotheses were constructed on the effects of both liming and earthworms on total soil porosity and void size distribution. These hypotheses were that:

1. The inoculation with earthworms of a soil devoid of earthworm features will result in an increase in total soil porosity through the creation of earthworm channels.
2. Earthworms will change the void size distribution by re-organising void space, reducing the number of small voids and increasing the number of large voids.
3. Liming of soil will lead to increased earthworm abundance and activity, resulting in changes to soil porosity.

6.7.2 Results

Taking total soil porosity first, figure 6.14 shows how mean total porosity varied between the earthworm and liming treatments for the intermediate 1 samples. The data were analysed using two way ANOVA after being normalised using an arcsine transformation. Both the liming and earthworm treatments had no statistically significant effects on total soil porosity (Liming: $F= 1.89$, $df= 1$, $P= 0.190$. Worms: $F= 1.29$, $df= 1$, $P= 0.275$). This indicated that total soil porosity was the same for all the disturbed soils at the beginning of the experiment.

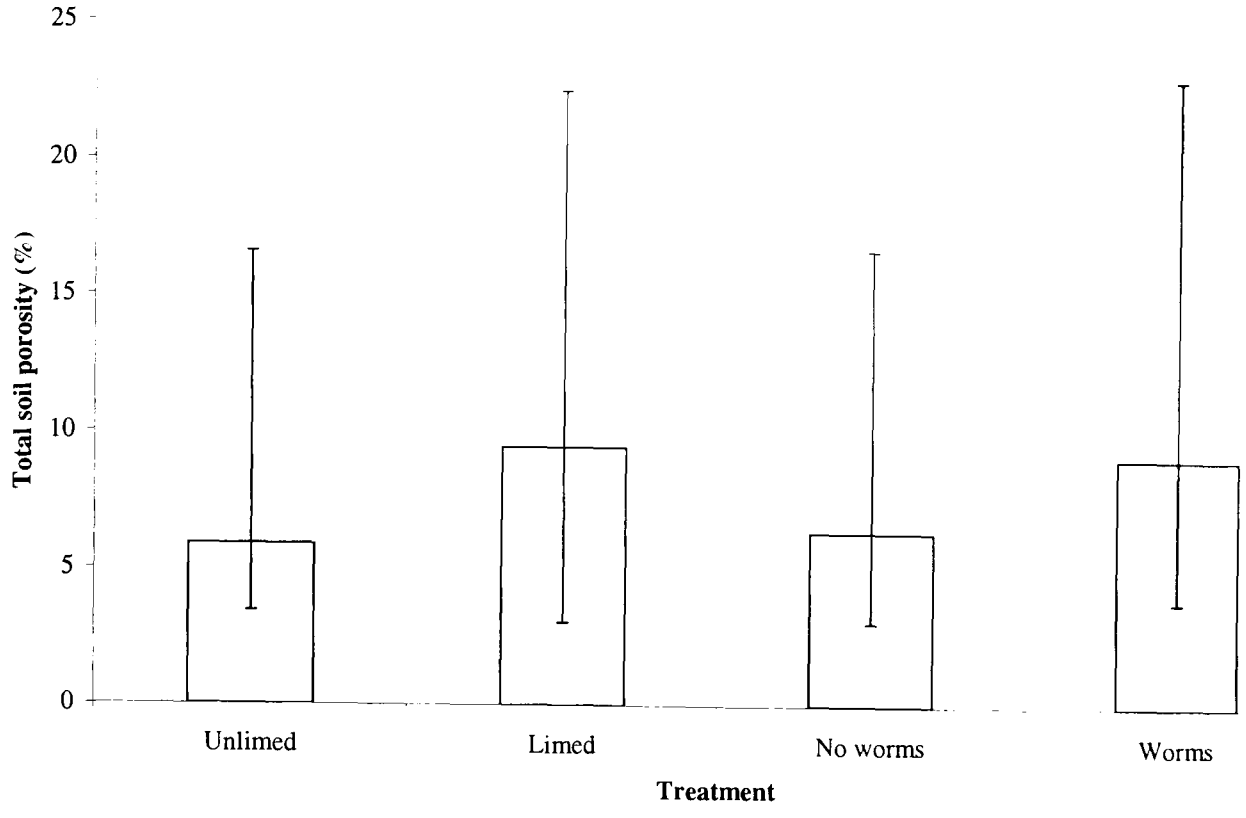


Figure 6.14: Back transformed mean total soil porosity for intermediate 1 samples showing how total porosity varied between the treatments at the beginning of the experiment. Error bars show back transformed 95% confidence limits. No. of replicates = 5.

Comparison of the mean total soil porosity between intermediate 1 and intermediate 3 indicated the effect of time on the soil; i.e. did the total soil porosity change over time? The data again had a skewed distribution, so was log transformed for the unlimed comparison and square root transformed for the limed comparison. Figure 6.15 shows how mean total porosity varied between intermediates 1 and 3. One way ANOVA showed that there were no significant differences between the two intermediate treatments for the unlimed ($F= 2.94$, $df= 1$, $P= 0.125$) and limed soil ($F= 0.01$, $df= 1$, $P= 0.935$), i.e. that time had no effect on total porosity for the disturbed soils.

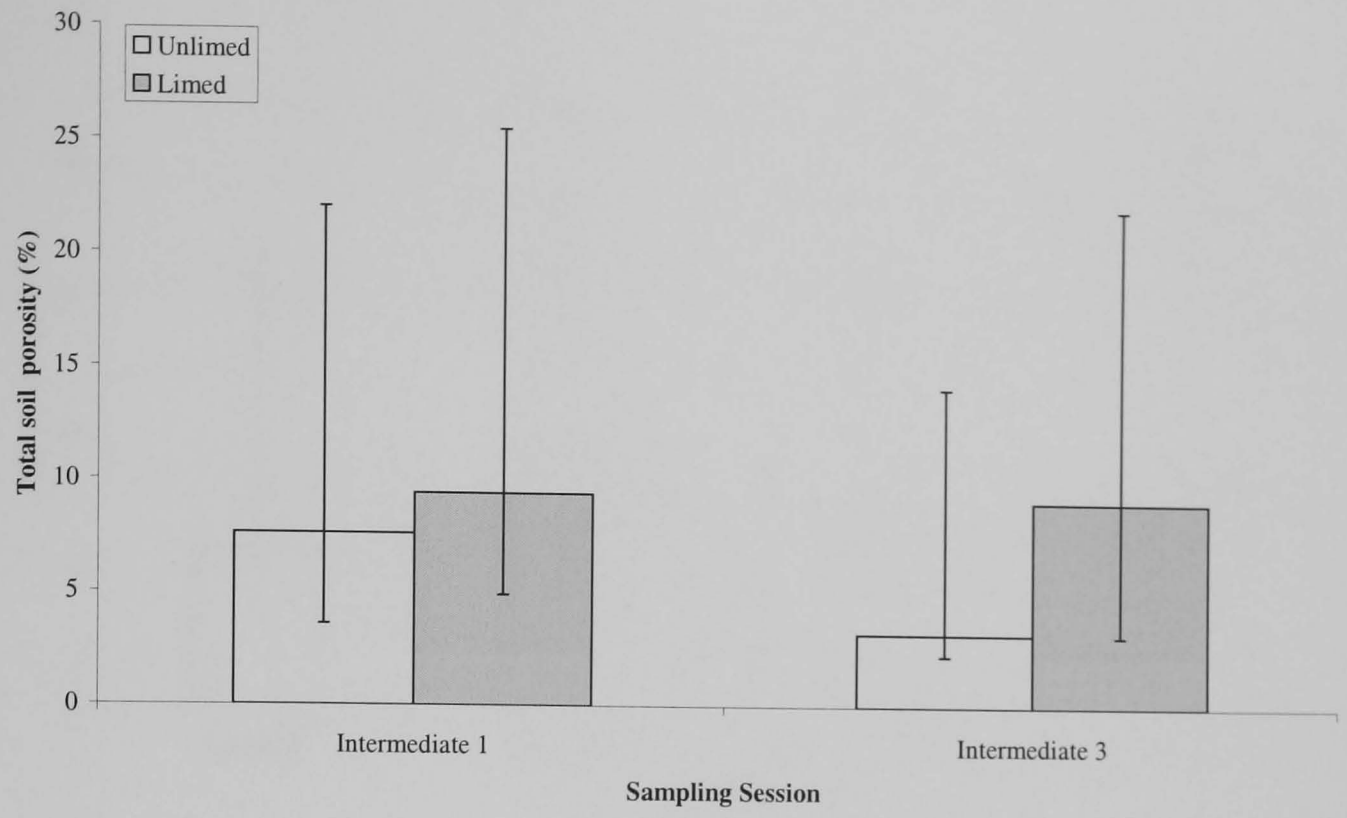


Figure 6.15: Back transformed mean total porosity of unlimed and limed soil for the two intermediate sampling points. Error bars show back transformed 95% confidence limits. No. of replicates = 5.

To assess the effects of the earthworm and liming treatments on the total mean porosity data for the intermediate 3 sampling session was compared (figure 6.16). The data were not normally distributed, so were transformed using a square root transformation, and then analysed with a two way ANOVA. Neither liming nor earthworm inoculation had a significant effect on the total soil porosity for the disturbed soil (Liming effect: F= 0.206, df= 1, P= 0.171. Earthworm effect: F= 1.59, df= 1, P= 0.225). This showed that total soil porosity neither increased nor decreased by earthworm activity.

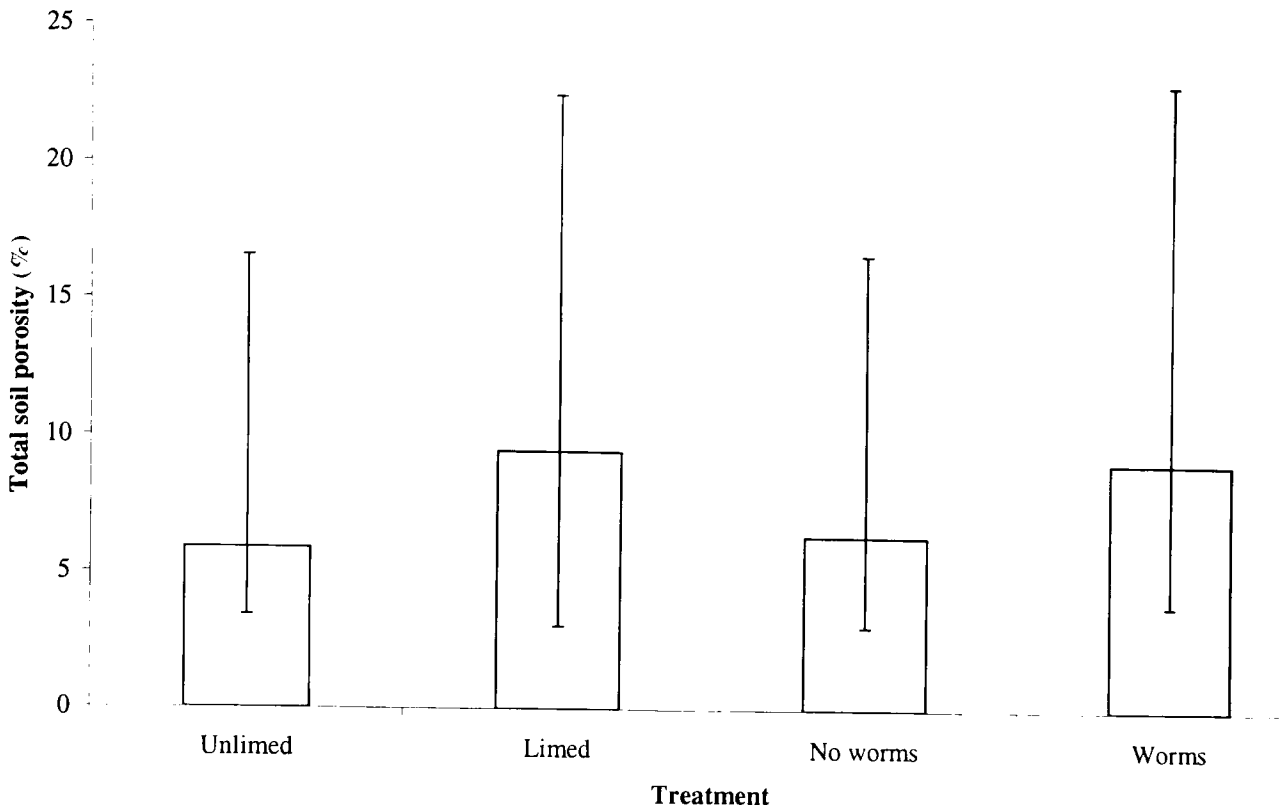


Figure 6.16: Mean total porosity of the intermediate 3 thin sections. Error bars show back transformed 95% confidence limits. No. of replicates = 5.

The second hypothesis was concerned with the effects of earthworms on re-organising void space. To be able to assess these effects, two void size classes were chosen from those outlined in chapter 4. The size thresholds chosen here were the proportion of voids with an area > 2 mm^2 or < 2 mm^2, as expressed as a percentage of the whole thin section area. All voids with an area < 2 mm^2 were classified as small and those > 2 mm^2 were classified as large voids or macropores. These size thresholds were chosen because they best represented the typical sizes of the earthworm channels found in these soils.

The size distribution of voids found in the disturbed soil before the earthworm inoculations took place was assessed to make sure that there were no significant differences at the beginning of the experiment. The statistical test used was a two way General Linear Model. Figures 6.17 and 6.18 show the mean porosity values for the earthworm and liming treatment for both void size thresholds. For the < 2 mm^2

size threshold, no statistically significant differences were observed in mean porosity for the liming (F= 0.09, df= 1, P= 0.763) and earthworm treatments (F= 0.02, df= 1, P= 0.892). The data for the > 2 mm² threshold was not normally distributed, so was square root transformed. As for the < 2 mm² threshold no statistically significant differences in mean porosity were found for the liming (F= 0.30, df= 1, P= 0.591) and earthworm treatments (F= 0.00, df= 1, P= 0.997). This meant that the mean porosity for the < 2 mm^2 and > 2 mm² voids did not significantly vary between the treatments at the beginning to the experiment.

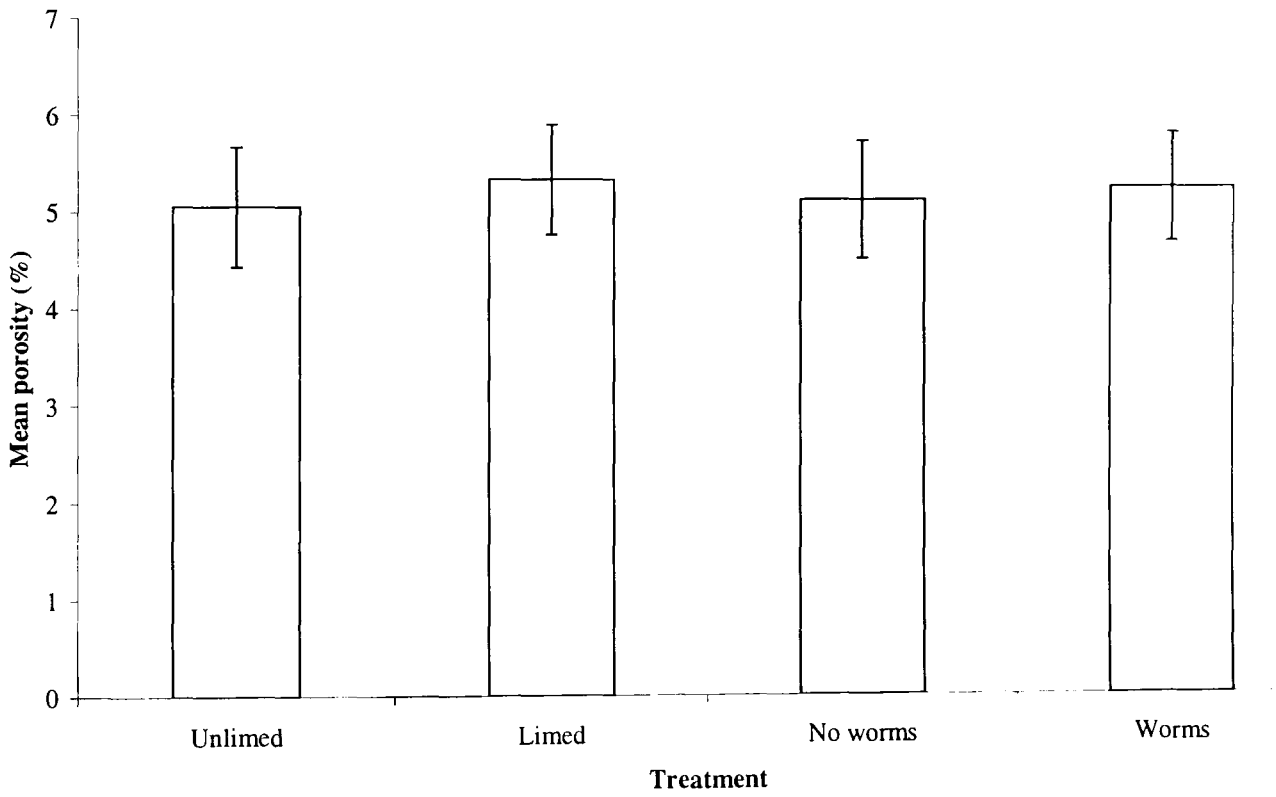


Figure 6.17: Mean porosity for voids < 2 mm² in area for the intermediate 1 samples. Error bars represent standard errors. No. of replicates = 5.

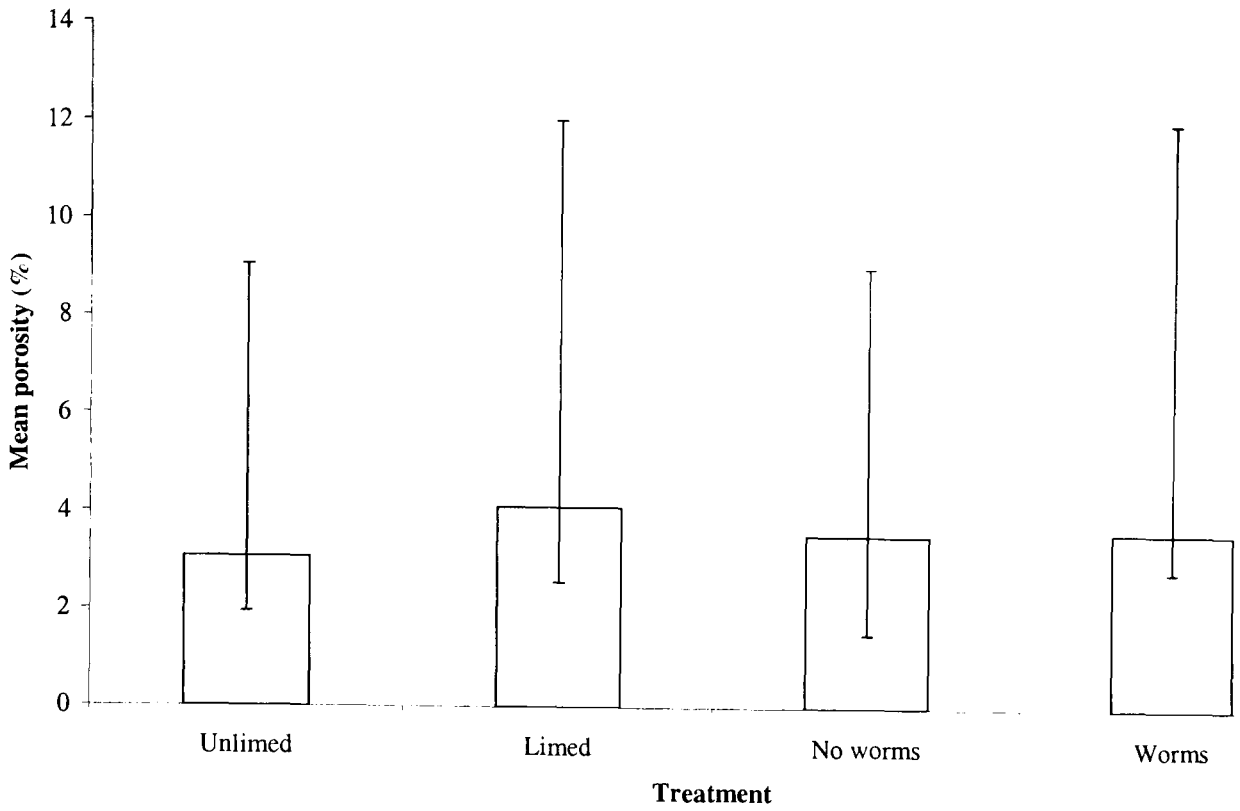


Figure 6.18: Back transformed means for voids > 2 mm² in area for the intermediate 1 samples. Error bars represent back transformed 95% confidence limits. No. of replicates = 5.

The effects of time on soil porosity for the control treatments (unlimed and limed + no worms) was compared for the < 2 mm^2 and > 2 mm² size thresholds. The median soil porosity values are shown in table 6.11, along with the P values from the Kruskall-Wallis Tests used to assess the effects of time on the size distribution of voids. Time had no statistically significant effects on the size distribution of voids in the unlimed and limed treatments except for the > 2 mm² voids in the unlimed treatments. This result was due to the presence of earthworm populations in the no worms treatments, which was revealed both at the end of the experiment when the earthworm inventory was completed and by visual observation of the slides during the earthworm excrement point counting. The presence of worms in this treatment meant that the earthworms would have been burrowing and therefore creating channels that were > 2 mm² in area.

Table 6.11: Median % soil porosity values for voids < 2 mm^2 and > 2 mm^2 in area in unlimed and limed + no worms treatments. No. of replicates = 5.

Sampling Session	< 2 mm²		> 2 mm²	
	Unlimed	Limed	Unlimed	Limed
Intermediate 1	5.30 (±1.52)	6.66 (±2.11)	4.49 (±1.66)	3.22 (±2.78)
Intermediate 3	2.65 (±2.95)	5.57 (±1.18)	0.32 (±0.92)	3.19 (±1.72)
P Value	0.175	0.754	**0.028**	0.602

The effects of earthworms and liming on the porosity of voids < 2 mm^2 in area are shown in figure 6.19. These data were analysed using a two way General Linear Model. Neither liming (F= 0.03, df= 1, P= 0.872) nor earthworm inoculation (F= 0.02, df= 1, P= 0.877) had a significant effect on the porosity of < 2 mm^2 voids and therefore the size distribution of small voids. However when the same analysis was carried out on the data for voids > 2 mm^2, statistically significant differences were observed for the liming treatments (F= 4.97, df= 1, **P= 0.04**) but not the earthworm treatments (F= 3.70, df= 1, P= 0.07). Figure 6.20 shows the mean values for the mean porosity of voids > 2 mm^2 for the liming and earthworm treatments.

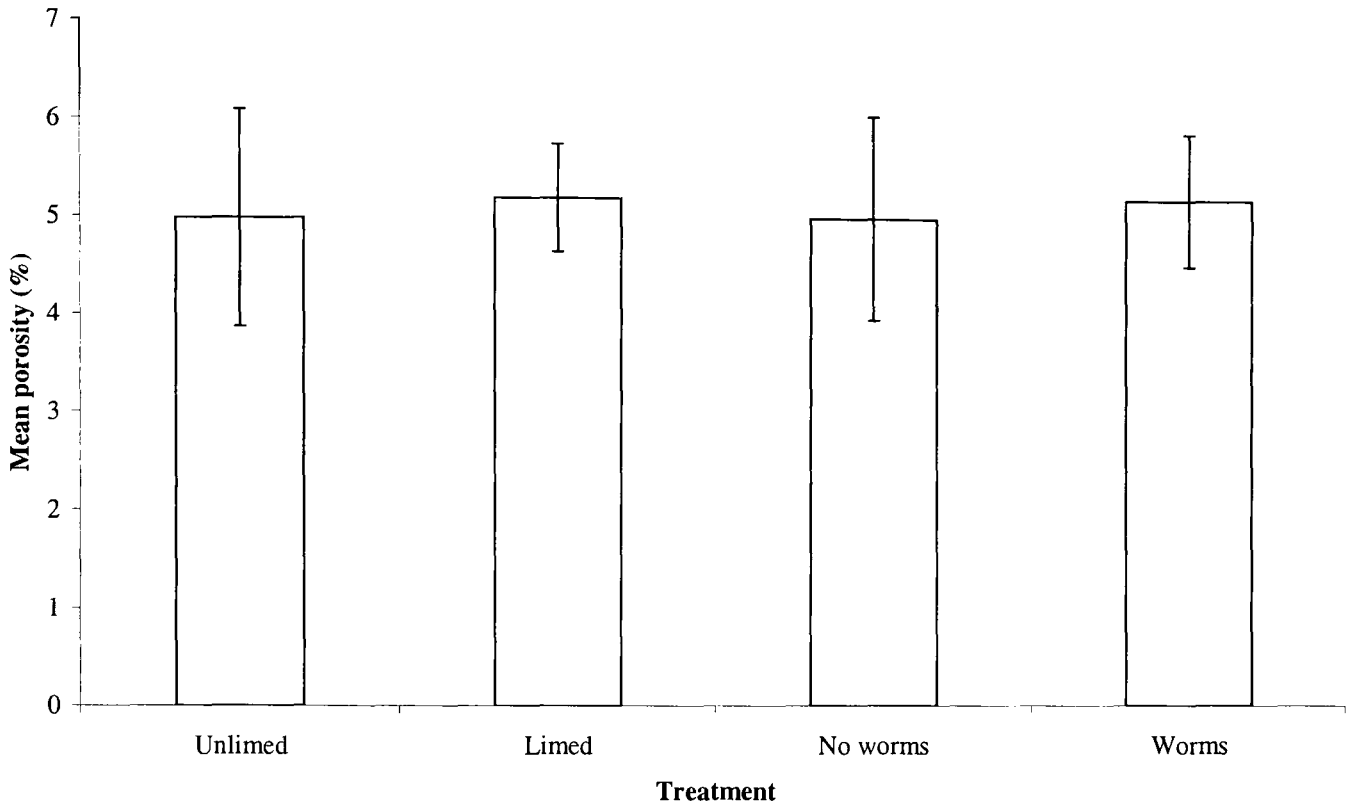


Figure 6.19: Mean porosity of voids < 2 mm² for the liming and earthworm treatments from intermediate 3 samples. Error bars represent standard errors. No. of replicates = 5.

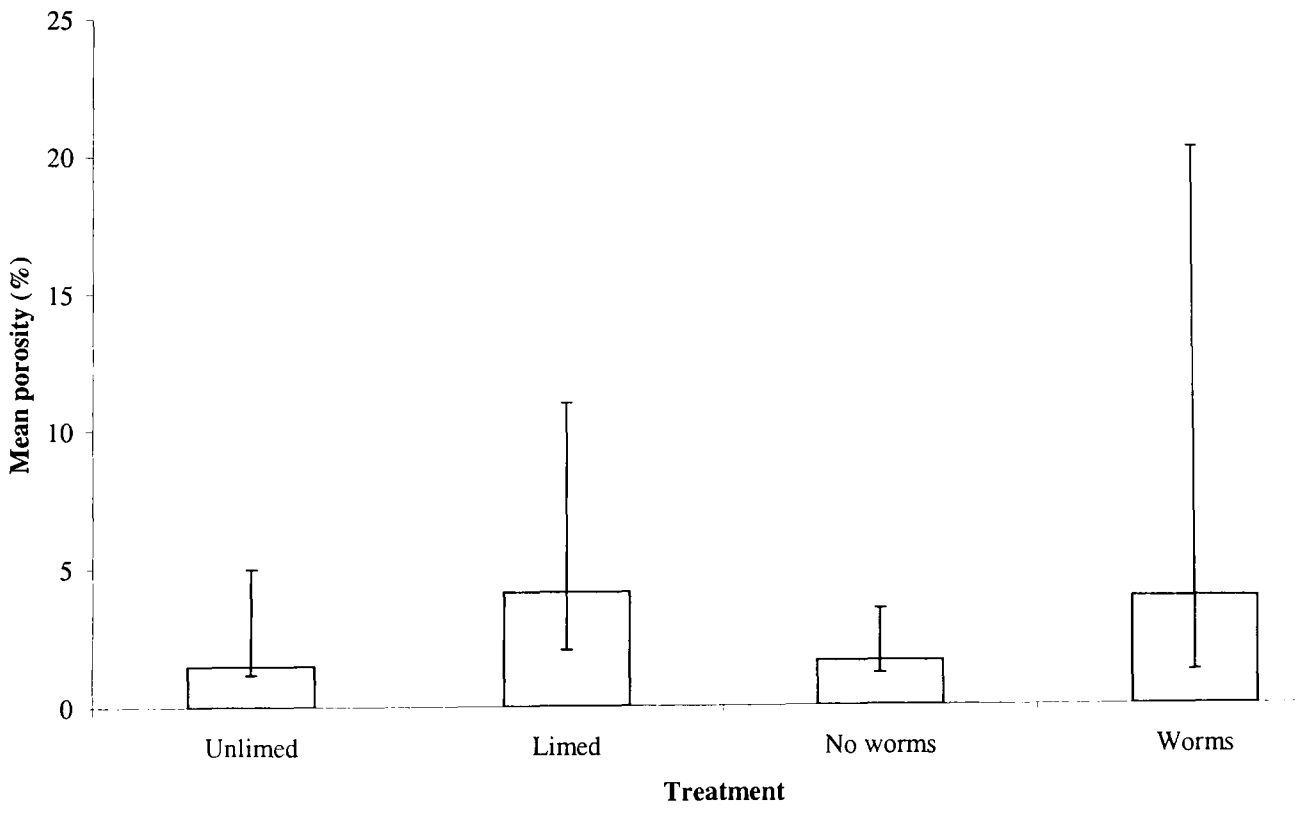


Figure 6.20: Back transformed soil porosity means for voids > 2 mm² in area for the liming and earthworm treatments from intermediate sampling 3. Error bars represent back transformed 95% confidence limits. No. of replicates = 5.

6.7.3 Discussion and Conclusions

Total soil porosity was not significantly affected by either the addition of earthworms or lime, i.e. neither treatment caused any statistically significant change. It was expected that by increasing the abundance of earthworms in disturbed soil, by either added more individuals or through the stimulatory effect of soil pH increases due to liming, that the increased burrowing activity would result in changes in the total amount of voids present in the soil. Early work on the effects of earthworm burrowing activity on porosity found that it increased with increased earthworm abundance (Stockdill 1966; Satchel 1967; Ehlers 1975; Lee 1985; Marinissen and Miedema 1994; Tomlin *et al* 1995). However, more recent studies have shown that earthworms do not increase or decrease the total porosity of soil but rather re-organise the voids by reducing the number of small voids and increasing the number of large voids (Syers and Springett 1983; Binet and Curmi 1992; Knight *et al* 1992; Binet *et al* 1997; Lachnicht *et al* 1997). It was this effect that was quantified by looking at the void size distribution in the disturbed soil for voids < 2 mm^2 and > 2 mm^2 in area.

For the percentage of voids < 2 mm^2, the liming and earthworm treatments did not have a statistically significant effect, i.e. no significant changes to the proportion of voids < 2 mm^2 were detected. However the addition of lime did significantly increase the proportion of voids > 2 mm^2 in area (F= 4.97, df= 1, **P= 0.04**). This increase in the proportion of voids > 2 mm^2 was due the significant increases in earthworm abundance caused by increased soil pH due to liming. In addition, liming also dramatically changed the species composition of the earthworm community. The proportion of *A. chlorotica* significantly increased in the liming treatments, and since

this earthworm species is endogeic and would spend much of its time burrowing through the mineral horizons (which were sampled in this experiment), then its increased abundance in this treatment would have had an effect on void space. Due to *A. chlorotica* being endogeic in nature it would have cast into its burrows partially or fully blocking them which explains why there were no distinctive earthworm channels observed, unlike in the Ecotron experiment. No statistically significant differences in the proportion of voids > 2 mm^2 were observed in the earthworm treatments ($F= 3.70$, $df= 1$, $P= 0.07$). It was expected that by adding earthworms, the consequential increases in earthworm numbers would have led to significant increases in the number of large voids but despite the increased earthworm abundance in soil where earthworms had been inoculated no changes were observed in the proportion of voids > 2 mm^2. The explanation for this is that the presence of earthworms in the no worms treatments led to the differences between the earthworm treatments to be masked.

The absence of *L. terrestris* in the treatments has had a significant effect on void space. This anecic species would have created semi-permanent burrow systems, which would have been clearly identifiable in the thin sections, so its absence has led to less earthworm channels being observed and therefore less large voids (> 2 mm^2) being quantified in the disturbed soil. It is the anecic species of earthworms that tend to have the most significant effect on the re-organisation of void space (Binet & Curmi 1992), therefore the absence of any representatives from this ecological group has significantly altered the void system observed in the disturbed soil.

In conclusion, this experiment into the effects of earthworms and liming on soil porosity has shown that:

1. Neither liming nor earthworm inoculation had a significant effect on total soil porosity.
2. Neither liming nor earthworm inoculation had a significant effect on the proportion of voids with an area < 2 mm^2.
3. Earthworm inoculation did not significantly affect the proportion of voids with an area > 2 mm^2.
4. The addition of lime to the soil led to significantly more voids > 2 mm^2 in area. This was due to liming significantly increasing the earthworm population.

6.8 Conclusions from the Sourhope Experiment: Disturbed Soil

This experiment into the effects of earthworms and liming on the soil fabric of a disturbed soil represented the second level of system complexity, because it was an *in vivo* experiment that used a non-artificial soil and was less highly controlled due to being field based and therefore exposed to real world environmental conditions. Three measures were used to evaluate the effects of earthworms and liming of soil fabric. These were a) aggregate stability, b) quantification of earthworm excrement and c) quantification of void space.

The data presented in this chapter have shown that earthworms had the following effects on soil fabric in a disturbed soil:

1. Earthworm casting activity did not significantly affect changes in aggregate stability.
2. The inoculation of earthworms into a soil where all evidence of earthworm activity was removed did not significantly increase the abundance of excremental

features. This indicated that earthworm inoculation did not significantly affect casting activity.

3. Earthworm burrowing activity had no statistically significant effect on total soil porosity and the re-organisation of void size distribution, i.e. there was no effect on the proportion of voids less than or greater 2 mm^2 in area.

The addition of lime to the disturbed soil had the following effects on soil fabric:

1. Liming did not significant affect aggregate stability despite increasing earthworm abundance.
2. The addition of lime to the disturbed soil did not significantly affect the abundance of earthworm excremental features and therefore casting activity.
3. Liming of the disturbed soil did not significantly effect total soil porosity and the abundance of voids with an area < 2 mm^2.
4. Liming did have a significant affect on the abundance of voids > 2 mm^2 in area because liming increased earthworm abundance and therefore burrowing activity.

There were several reasons as to why liming and earthworm inoculation had very little effect on the fabric of the disturbed soil. Firstly, the inoculated earthworm treatments had only limited success as was shown in the earthworm species inventory at the end of the experiment. This showed that there were few to no *L. terrestris* present in the earthworm inoculated soil and that a number of other species were found in the treatments, the dominant species of which was *D. rubidus*. Secondly, and possible more importantly was the presence of earthworm populations in the no worms treatments. This meant that those treatments which were acting as

controls were not really controls because earthworms were present when there should not have been any. The effect of this would have been to add noise to the data masking any subtle earthworm effects.

Chapter: 7 Sourhope Experiment – Undisturbed Soil: Results and Discussion

The final experiment investigated the effects of earthworms and liming on soil fabric in undisturbed monoliths of the Sourhope soil. A detailed outline of the experimental design was given in chapter 3 but in summary, 50 intact soil monoliths were taken from the Sourhope main plots, installed in boxes and reburied at the Sweethope experimental site. These boxes were inoculated with earthworms and left for 18 months before the soils were sampled at the end of the experiment. The initial state of the soils was determined from samples taken from the Sourhope main plots at the same time as the monoliths were removed. The effects of time on the soil were analysed by comparing the unlimed soil at the beginning of the experiment and the unlimed + no worms at the end. At both sampling dates, saturated hydraulic conductivity was determined on undisturbed cores, thin sections were made from undisturbed blocks to quantify earthworm excrement features and void space and disturbed samples were taken for aggregate stability, pH and organic C. Overall this experiment represented the third and final level of system complexity in that it took place as a field experiment, and the soil itself was undisturbed, therefore representing as far as possible what was happening in the soil on the main Sourhope plots. The first three sections present pH and % organic carbon (%OC) data as well as the earthworm inventory for all the undisturbed boxes sampled. These data provide the essential background to understanding the results from the analyses of soil structural properties. A summary of the statistical techniques used is given in chapter 4.

7.1 Soil pH

pH values were again kindly provided by Mark Pawlett (Pawlett 2003). At the beginning of the experiment pH was measured in the LFH horizons only, with the median pH greater for the limed soil (6.19 ±0.275) than unlimed soil (3.97 ±0.125); ± values represent quartile deviations. The data were not normally distributed and were therefore analysed using a Kruskall-Wallis test. The test showed that the differences between the unlimed and limed soil were statistically significant (H= 6.82, df= 1, **P= 0.009**). This difference was expected since an application of lime had already been made to the soil and would have been present in the uppermost horizons. These data represented the initial state of the soil. The effects of time and further lime application were assessed for the LFH horizon only. The median pH values of the LFH horizon in the unlimed soil at the beginning (3.97 ±0.125) and end of the experiment (3.89 ±0.105) were similar. The data were analysed using a Mood's Median test. The differences in median pH were not statistically significant (χ^2= 3.60, df= 1, P= 0.058). However when the median pH data for the limed soil at the beginning and end of the experiment were analysed using a Kruskall-Wallis test, statistically significant differences were observed (Beginning 6.19 ±0.225 and End 6.99 ±0.275. H= 5.77, df= 1, **P= 0.016**). These statistically significant differences were due to further applications of lime being added to the soil.

Soil pH was measured at the end of the experiment for both the LFH and A_h horizons (figure 7.1). The data were not normally distributed, so were analysed using the Kruskall-Wallis Test which found that for both the LFH and A_h, pH was significantly greater in the limed soil compared to the unlimed (LFH: H= 14.29, df= 1, **P < 0.001**; A_h: H= 13.47, df= 1, **P < 0.001**). This rise in pH was most marked in

the LFH horizons which is not surprising since the lime was applied to the soil surface on a number of occasions and for a long while afterwards could be seen on or near the surface, but in addition these data showed that liming also had an effect lower down the profile. This finding is supported by the work of Dampney (1985) who found that even after 3 years lime applied to the soil surface had no significant effect on pH below a depth of 5 cm. The conclusion is therefore that liming did have a statistically significant effect on soil pH by causing elevated pH values in the liming treatments as compared to the unlimed treatments.

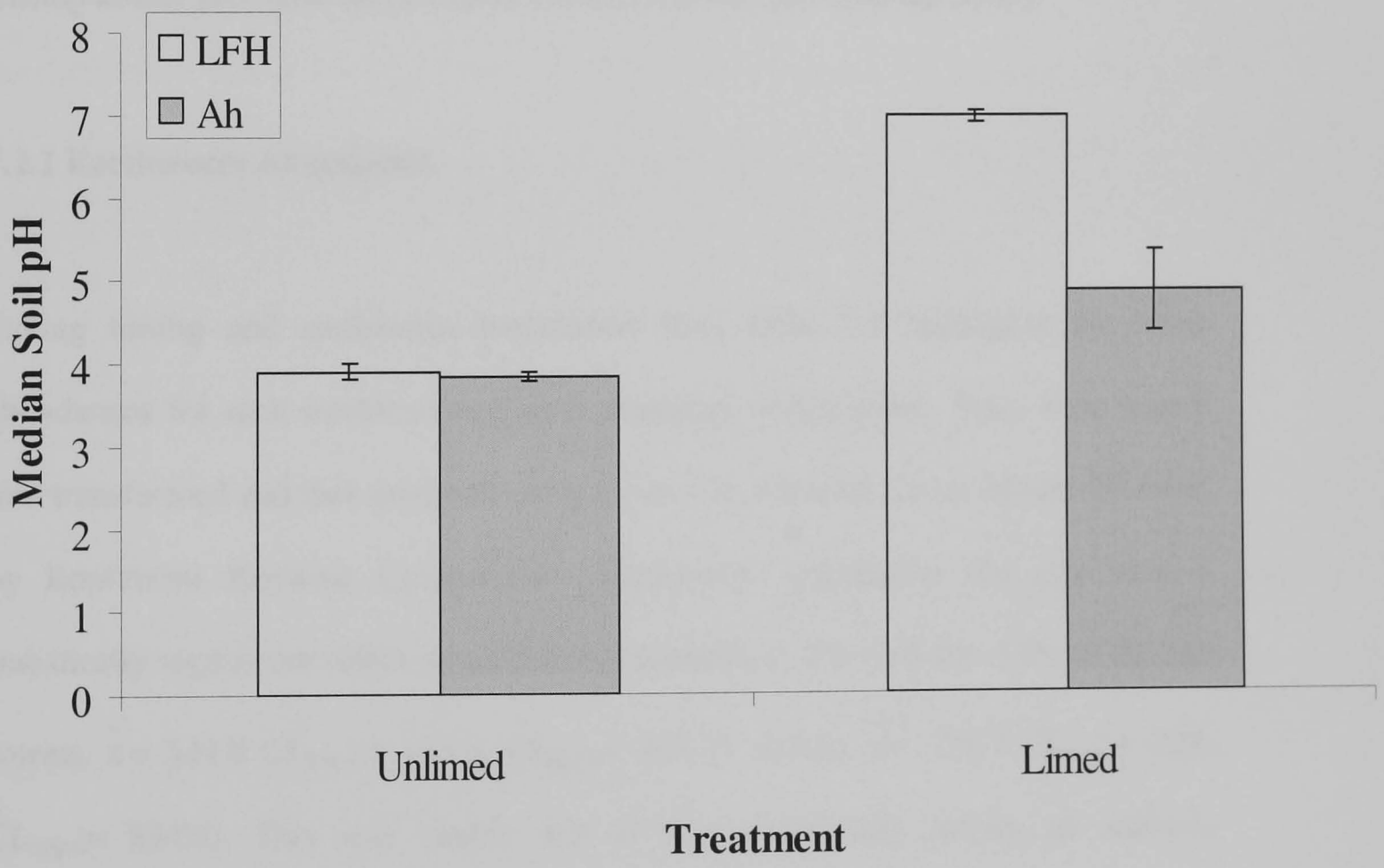


Figure 7.1: Median soil pH of both the LFH and A_h horizons from the final sampling of the undisturbed boxes. Error bars show quartile deviations. No. of replicates = 5.

7.2 Earthworm Abundance and Community Composition

At the end of the whole experiment all the boxes were removed from the Sweethope site and brought back to Stirling so that an earthworm inventory could be taken of each box. This was achieved by handsorting soil from all of the boxes and removing all the earthworms found. Soil samples for the various analyses were taken both in the field and in the laboratory before the handsorting took place. The inventory data were for the undisturbed boxes that were sampled at the end sampling point. Hannah Bishop kindly provided the raw earthworm inventory data (Bishop 2003).

7.2.1 Earthworm Abundance

Taking liming and earthworm inoculation first, table 7.1 highlights the mean abundances for each treatment and each treatment combination. They were square root transformed and then analysed using a two way General Linear Model followed by Bonferroni Pairwise Comparisons. Earthworm inoculation did not have a statistically significant effect on earthworm abundance, F= 1.95 df= 1 P= 0.182 (no worms $\bar{x}$= 344.8 CL_{lower}= 143.2, CL_{upper}= 961.7; worms $\bar{x}$= 538.7 CL_{ower}= 278, CL_{upper}= 884.9). This was largely due to the soil already having an intrinsic earthworm population, so when more earthworms were added in the form of the inoculum, they were absorbed into the existing community. This led to the differences between the treatments not being large enough to be statistically significant.

In contrast liming did have a significant effect on earthworm abundance with the addition of lime increasing abundance F= 11.03, df=1, **P= 0.004** (Unlimed $\bar{x}$= 235.9

CL_{lower}= 86.9, CL_{upper}= 457.9; Limed $\bar{x}$= 698 CL_{lower}= 476.5, CL_{upper}= 961.7). However on further investigation with Bonferroni Pairwise Comparisons, it was unclear as to which treatment combinations were the cause (Unlimed + no worms Vs Limed + no worms P= 0.448; Unlimed + worms Vs Limed + worms P= 0.079). The fact the liming caused an increase in earthworm abundance was due to liming significantly increasing soil pH which made the soil environment more habitable for earthworms (Piearce 1972).

Table 7.1: Back transformed mean abundance values and 95% confidence limits for the individual and combined liming and earthworm treatments. No. of replicates = 5.

Treatment	Mean Abundance m^{-2}	95% CL_{lower}	95% CL_{upper}
Unlimed + no worms	198.2	0.1	778.7
Unlimed + worms	277.2	90.5	565.7
Limed + no worms	531.8	318.3	799.7
Limed + worms	886.8	451.9	1467.1
Unlimed	235.9	86.9	457.9
Limed	698.0	476.5	961.7
No worms	344.8	143.2	633.8
Worms	538.7	278.0	884.9

7.2.2 Earthworm Community Composition

The data in tables 7.2 and 7.3 and figures 7.2 and 7.3 show that the dominant earthworm species in the undisturbed soil were *A. chlorotica*, *D. rubidus*, *O. cyaneum*. The effect of earthworm inoculation in the unlimed soil was to reduce the proportion of *D. rubidus* whilst increasing the proportion of a number of the less abundant species. In the limed soil the proportion of *A. chlorotica* was increased from 42% to 66% whilst *O. cyaneum* was reduced from 28% to 7%. This increase in

the proportion of *A. chlorotica* in the limed soil was due to it being one of the species in the inoculum.

Table 7.2: Mean % species composition of the earthworm community in the undisturbed soil showing the effects of liming. No. of replicates = 5.

Earthworm Species	Mean % earthworm species composition
Unlimed	
D. rubidus	50.61
A. chlorotica	27.46
O. cyaneum	9.33
L. rubellus	4.84
Lumbricus species	4.70
D. octaedra	1.54
L. castaneus	0.90
L. terrestris	0.62
Limed	
A. chlorotica	59.27
D. rubidus	22.75
O. cyaneum	15.42
L. rubellus	1.16
Lumbricus species	0.76
D. octaedra	0.51
L. festivus	0.13

Table 7.3: Mean earthworm abundance and % species composition of the earthworm community in the undisturbed soil for each of the individual treatments. Values in brackets represent standard deviations. No. of replicates = 5.

Earthworm species	Mean Earthworm Abundance m^{-2}	Mean % earthworm species composition
Unlimed + no worms		
D. rubidus	273.28 (± 150.8)	44.04
A. chlorotica	254.28 (± 0.0)	40.98
Lumbricus species	37.96 (± 1.7)	6.12
O. cyaneum	27.01 (± 1.7)	4.35
L. rubellus	18.82 (± 0.0)	3.03
Lumbricus terrestris	9.19 (± 0.0)	1.48
Limed + no worms		
A. chlorotica	274.82 (± 279.7)	41.53
O. cyaneum	186.09 (± 156.9)	28.12
D. rubidus	148.67 (± 206.8)	22.46
L. rubellus	19.09 (± 0.6)	2.88
Lumbricus species	18.93 (± 0.9)	2.86
D. octaedra	14.21 (± 6.3)	2.15
Unlimed + worms		
A. chlorotica	158.10 (± 185.5)	35.84
D. rubidus	140.08 (± 118.7)	31.75
O. cyaneum	56.52 (± 55.5)	12.81
L. rubellus	31.63 (± 17.1)	7.17
Lumbricus species	16.33 (± 9.4)	3.70
D. octaedra	15.43 (± 5.1)	3.50
L. castaneus	13.50 (± 6.3)	3.06
L. terrestris	9.58 (± 0.0)	2.17
Limed + worms		
A. chlorotica	651.64 (± 391.4)	66.07
D. rubidus	215.52 (± 205.6)	21.85
O. cyaneum	67.86 (± 60.6)	6.88
L. rubellus	23.59 (± 20.3)	2.39
L. festivus	9.29 (± 0.0)	0.94
D. octaedra	9.22 (± 9.22)	0.93
Lumbricus species	9.11 (± 0.2)	0.92

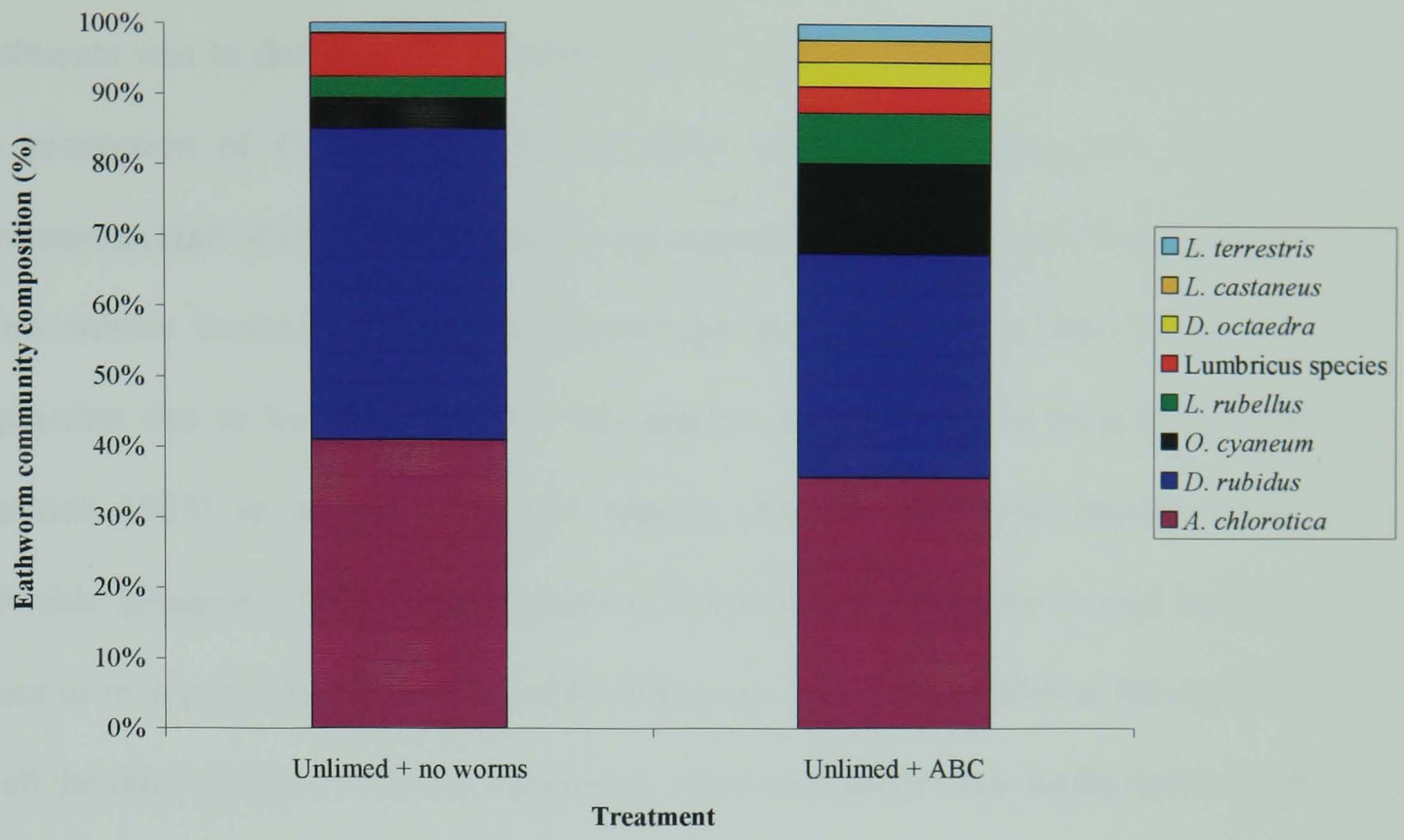


Figure 7.2: Mean species composition for the unlimed soil (ABC = + worms). No. of replicates = 5.

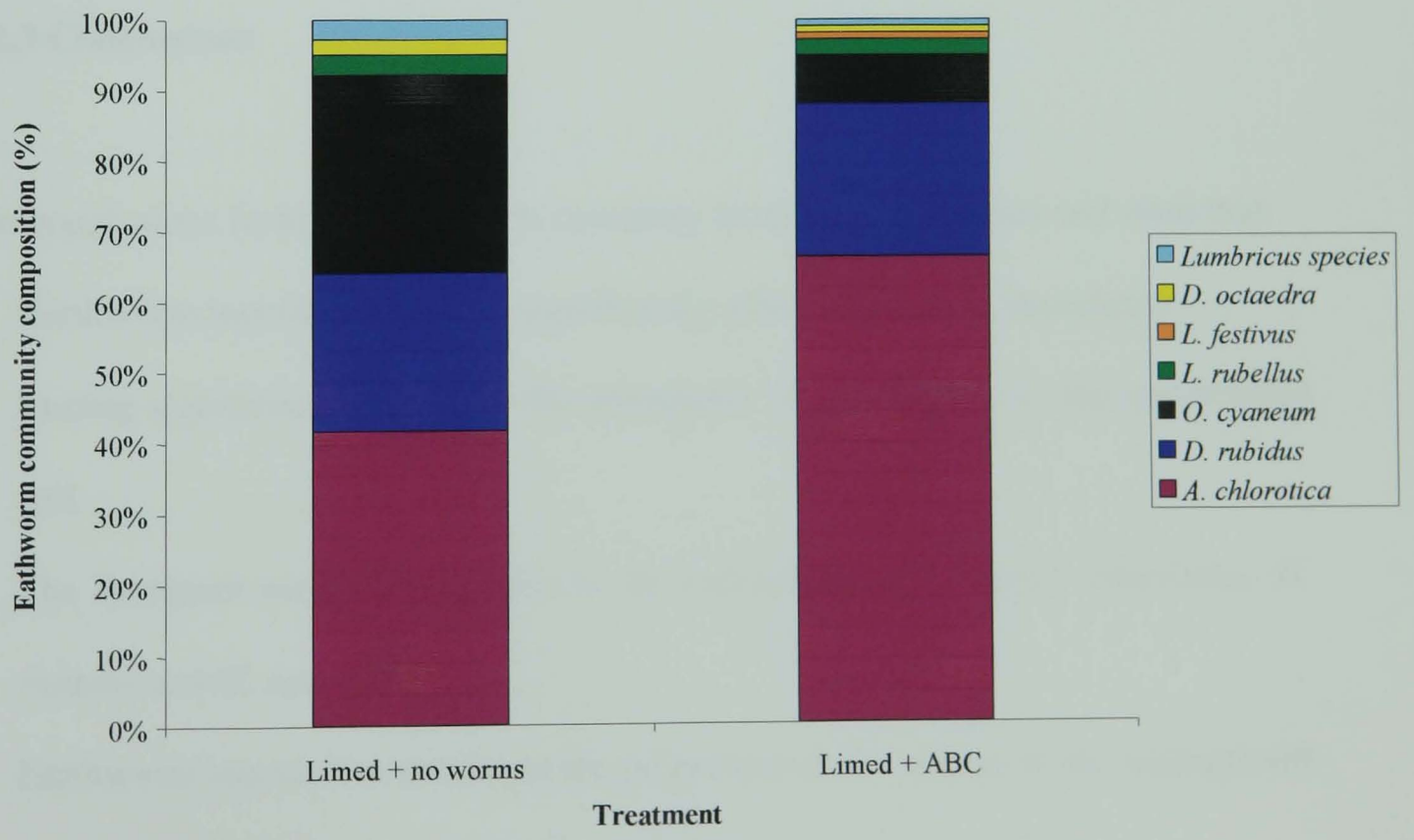


Figure 7.3: Mean species composition for the limed soil (ABC = + worms). No. of replicates = 5.

The effect of liming on the earthworm species composition in the no worms treatments was to decrease the proportion of *D. rubidus* (22% to 7%) and increase the proportion of *O.cyaneum* (13% to 28%), although *A. chlorotica* was still dominant overall (41% in the Unlimed + no worms treatment and 42% in the Limed + no worms treatment). The explanation for the reduction in the *D. rubidus* population due to liming is because this species is considered to be acid tolerant (Satchell 1955) so as pH increased, then so the soil conditions became more habitable (Piearce 1972). In the earthworm inoculated treatments liming had the effect of increasing the dominance of *A. chlorotica* from 41% to 66% at the expense of all the other species. This was because *A. chlorotica* prefers less acidic soils, so as soil pH was increased then its population also increased due to the more habitable soil conditions (Satchell 1955; Piearce 1972).

7.2.3 Conclusions

The conclusions from the earthworm inventory from the undisturbed soil were that:

1. Earthworm inoculation did not significantly affect earthworm abundance.
2. Liming significantly increased the abundance of earthworms in the undisturbed soil.
3. The dominant earthworm species in the undisturbed soil were *A. chlorotica, D. rubidus* and *O. cyaneum.*
4. Earthworm inoculation decreased the proportion of *D. rubidus* in the unlimed soil whilst increased the population of *A. chlorotica* in the limed soil.

5. Liming reduced the *D. rubidus* and increased the *O. cyaneum* populations in the no worms treatments, whilst the proportion of *A. chlorotica* increased in the limed + worms treatments.
6. Earthworm community composition was fairly similar between the undisturbed and disturbed soils although the species composition was affected less by the treatments in the undisturbed soil.

7.3 Soil Organic Carbon

Soil organic carbon content was determined on sub-samples of the 1-2 mm aggregates used for the aggregate stability determination.

The data from this analysis were not normally distributed, so to assess the differences in %OC at the beginning and between the beginning and end of the experiment Kruskall-Wallis tests were used.

Figure 7.4 presents the median %OC data for both the unlimed and limed treatments at the beginning of the experiment. There were no statistically significant differences between these treatments (H= 1.84, df= 1, P= 0.175), indicating that liming had not had any significant effect on %OC at the beginning of the experiment. The analysis of the median %OC between the unlimed + no worms treatment at the beginning and end of the experiment, (figure 7.5), found that no statistically significant differences occurred (H= 1.84, df=1, P= 0.175). This meant that time had no significant effect on soil organic carbon in the unlimed treatments, which was the expected outcome.

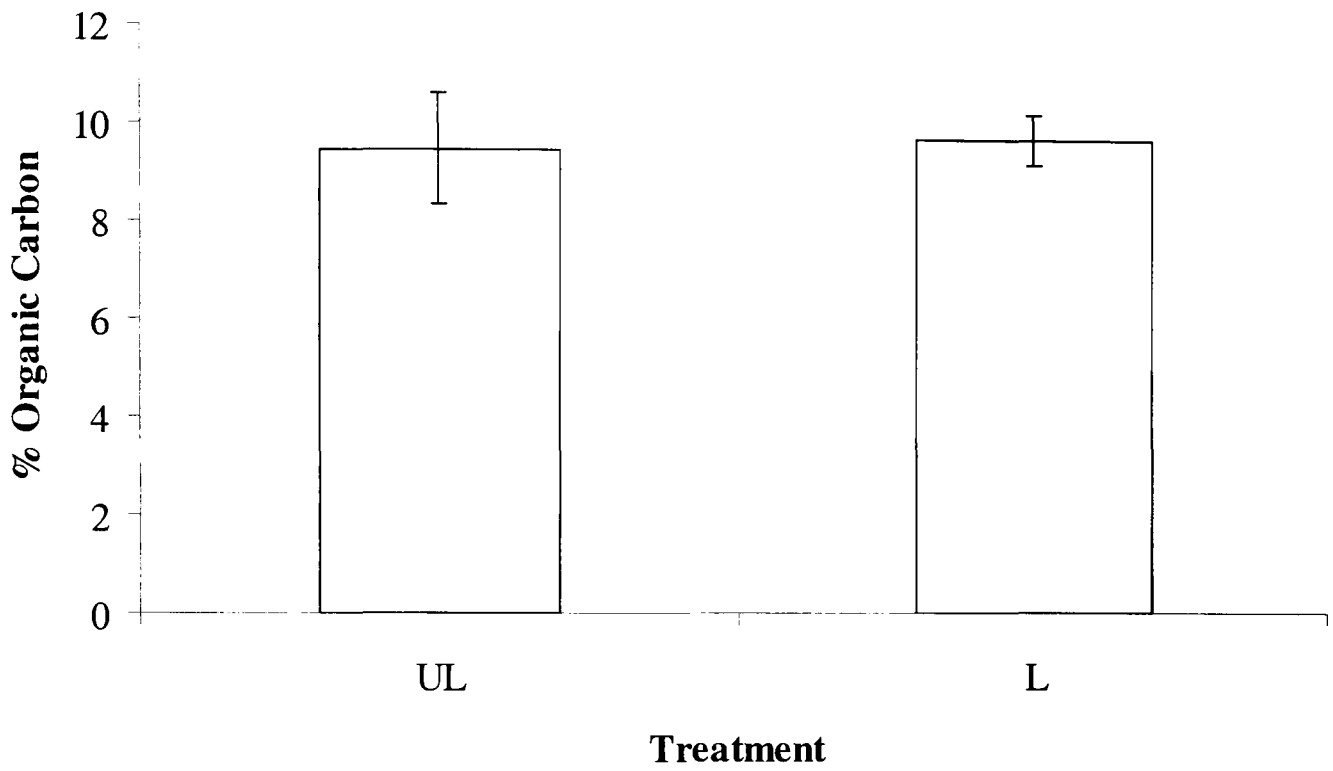


Figure 7.4: Median % OC for the unlimed (UL) and limed (L) treatments at the beginning of the experiment, and before earthworm inoculation. Error bars represent quartile deviation. No. of replicates = 5.

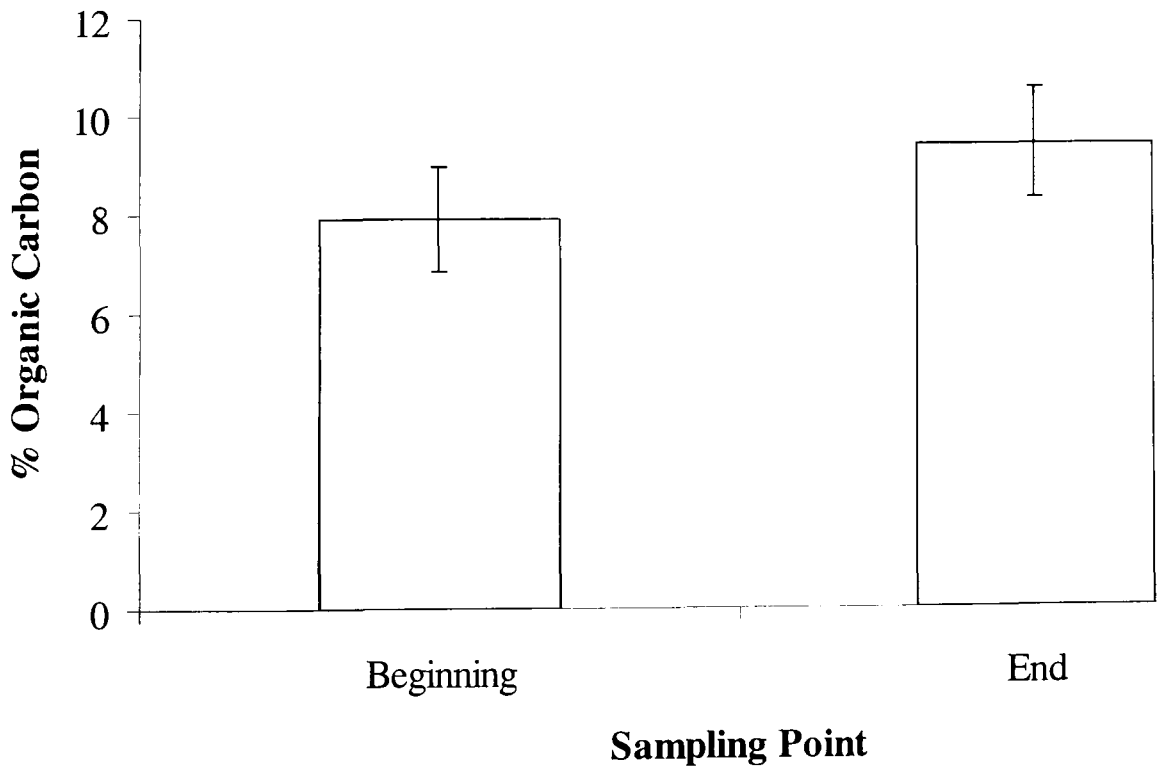


Figure 7.5: Median % OC for the unlimed + no worms treatments at the beginning and end of the experiment. Error bars represent quartile deviations. No. of replicates = 5.

The effects of earthworms and liming on soil organic carbon were assessed on the differences between the treatments at the end of the experiment. The data were not normally distributed so were transformed using a cubed transformation. This transformation was used because in this instance it was the most effective at producing normally distributed data. The back transformed means and 95% confidence limits are presented in figure 7.6. No statistically significant differences were observed for either the earthworm or liming treatments (Liming: F= 0.01, df= 1, P= 0.908 and Earthworms: F= 0.02, df= 1, P= 0.880). This indicates that neither earthworms nor liming had a significant effect on the %OC content of the undisturbed soil. There was a high degree of variability among the replicates as shown by the large back transformed 95% confidence limits.

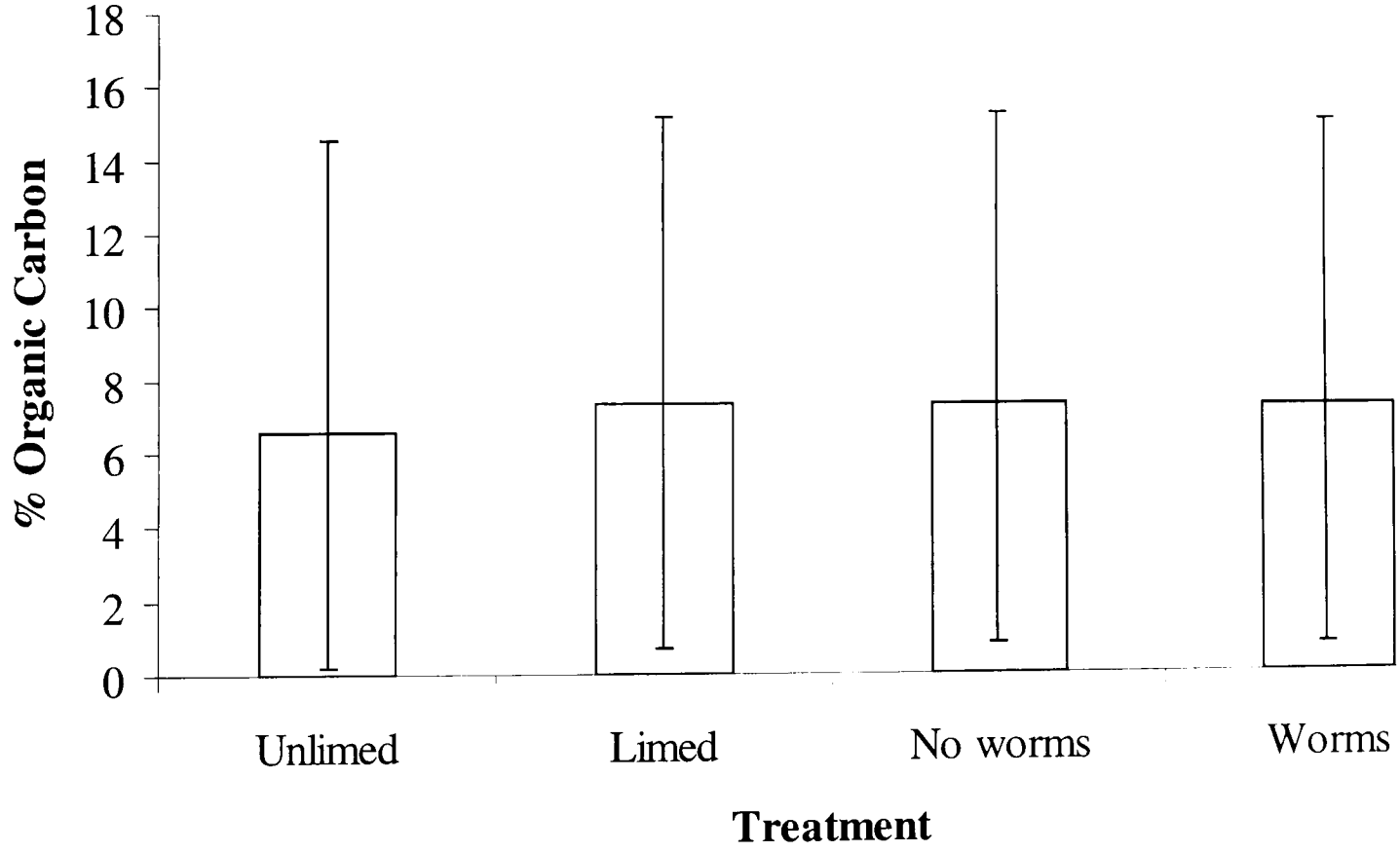


Figure 7.6: Back transformed mean % OC for the liming and earthworm addition treatments. Error bars represent back transformed 95% confidence limits. No. of replicates = 5.

7.4 Aggregate Stability

Aggregate stability was determined on the 1-2 mm aggregate samples collected from A_h horizon of the undisturbed boxes at the beginning and end of the experiment, with the beginning samples being collected from the main Sourhope plots. The methodology for the determination of aggregate stability was outlined in chapter 4 but as a brief summary, 2 g aggregate samples were exposed to four periods of sonication for 120 seconds and after each sonication the particle size distribution was measured. The difference between the first and last particle size distribution gave an indication as to the amount of aggregate breakdown that had taken place and therefore the stability of the aggregates. To help in the interpretation of the particle size distributions, a series of stability indices were calculated. These indices were outlined in chapter 4.4.1.

7.4.1 Hypotheses

Two hypotheses were tested in this experiment concerning the effects of both earthworm casting and lime addition on aggregate stability. The hypotheses are outlined below:

1. The inoculation of undisturbed soil monoliths with earthworms leads to more stable aggregates by increasing the amount of cast material produced as a result of higher earthworm abundance.
2. The liming of an undisturbed soil leads to more stable aggregates through increased earthworm casting activity.

7.4.2 Results

The initial state of the soil at the beginning of the experiment, in terms of aggregate stability is shown in table 7.4. The data for all of the indices except the 1-2 mm aggregates were not normally distributed so were analysed with non-parametric tests. Mood's Median Test was used for the 600-2000 μm aggregates, whilst Kruskall-Wallis test were carried out for the others. The results of the statistical analysis found that there were no significant differences between the unlimed and limed treatments for all four aggregate stability indices. This indicated that aggregates at the beginning of the experiment had the same stability irrespective of whether they were from the unlimed or limed treatments.

Table 7.4: Differences between the unlimed and limed treatments for the four aggregate stability indices and the results of the statistical analyses at the 95% confidence level. The values in brackets for the mean data are standard errors, whilst for the median data these are quartile deviations. No. of replicates = 5.

Treatment	Aggregate Stability Indices			
	1-2 mm (Mean)	600-2000 μm (Median)	MWD (Median)	40-150 μ (Media
Unlimed	-12.16 (±0.73)	-13.85 (±1.58)	-207 (±28.60)	6.20 (±5.
Limed	-10.95 (±1.08)	-13.26 (±2.37)	-192 (±19.80)	5.40 (±1.
Statistical Test	1-way ANOVA	Mood's Median Test	Kruskall-Wallis Test	Kruskall-W Test
Statistical Results	F= 0.86, df= 1, P= 0.381	χ^2= 0.40, df= 1, P= 0.381	H= 0.27, df= 1, P= 0.602	H= 1.84, d P= 0.17

The comparison between the aggregate stability indices for the unlimed + no worms treatments at the beginning and end of the experiment (table 7.5) highlighted the effect of time. The data for the individual indices were analysed using one way ANOVA, the results of which are in table 7.5. For 1-2 mm aggregates there were no statistically significant differences observed, which was not the case for the three remaining indices. The mean values for the 600-2000 μm and MWD indices all indicated that aggregate stability decreased over time in the unlimed + no worms treatment. This was because at the end of the experiment, these values were significantly more negative at the end indicating that more breakdown had occurred and therefore the aggregates were less stable. For the 40-150 μm particle size range, which represented the accumulation of breakdown products, the mean values were greater at the end of the experiment showing that there had been more aggregate breakdown and therefore the aggregates were less stable.

Table 7.5: Differences in mean aggregate stability between the beginning and end of the experiment (standard errors are shown in brackets). The P values in bold show those results of one way ANOVA that were statistically significant at the 95% confidence level. No. of replicates = 5.

Sampling Point	Aggregate Stability Indices			
	1-2 mm	600-2000 μm	MWD	40-150 μm
Beginning	-10.95 (±1.08)	-12.76 (±1.46)	-186.81 (±16.43)	3.896 (±1.21
End	-13.06 (±1.01)	-18.61 (±1.38)	-242.09 (±14.27)	9.72 (±1.78)
Statistical Results	F= 2.01, df= 1, P= 0.191	F= 8.48, df=1, **P=0.020**	F= 6.46, df= 1, **P= 0.035**	F= 7.35, df= 0 **P= 0.027**

These differences in aggregate stability between the beginning and end were not expected. Several explanations exist which could account for these differences:

1. The secondary breakdown of micro-aggregates formed by the breakdown of the original 1-2 mm aggregates. This could be possible since no significant differences were observed between the beginning and the end sampling for the original 1-2 mm aggregates.
2. Changes caused by the disturbance to soil when it was removed from the Sourhope main plots and reburied, e.g. by reburying the boxes of undisturbed soil on beds of sand would have changed the soils drainage characteristics, thereby allowing if to drain more freely.
3. A number of studies have found that earthworms can have a destabilising effect on aggregates (Schrader and Zhang 1997; Shuster *et al* 2000). These undisturbed soils had an existing population of earthworms and it could have been their activity that has led to this decrease in aggregate stability.

The effects of the liming and earthworm treatments on the four aggregate stability indices at the end of the experiment are highlighted in table 7.6. The data were normally distributed for all the indices except for 40-150 μm size range. The normally distributed data were analysed using two way ANOVAs, whilst the 40-150 μm data were analysed using a ranked two way. No statistically significant differences existed between the treatments for any of the indices (Table 7.7). This indicated that neither liming nor earthworm inoculation led to significant changes in aggregate stability in the undisturbed soil.

Table 7.6: Mean aggregate stability values for the liming and earthworm treatments at the end of the experiment. Standard errors are shown in brackets except for the 40-150 μm index where back transformed 95% confidence limits are used instead. No. of replicates = 5.

Treatment	Aggregate Stability Indices			
	1-2 mm (Mean)	600-2000 μm (Mean)	MWD (Mean)	40-150 μm (Ra Mean)
Unlimed	-13.92 (±1.15)	-19.22 (±1.14)	-250.10 (±14.10)	10.41 (8.27, 11
Limed	-11.58 (±2.14)	-16.03 (±2.14)	-218.70 (±28.60)	8.77 (4.43, 10.
No worms	-12.07 (±1.39)	-16.91 (±1.52)	-225.60 (±18.90)	9.59 (6.58, 11.
Worms	-13.42 (±2.04)	-18.34 (±2.01)	-243.6 (±26.50)	9.74 (6.83, 11.

Table 7.7: The effects of liming and earthworm inoculation on aggregate stability at the end of the experiment. No. of replicates = 5.

Aggreage Stability indices	Results of Statistical analysis	
	Effect of liming	Effect of earthworm inoculation
1-2mm	F= 0.84, df= 1, P= 0.373	F= 0.28, df= 1, P= 0.603
600-2000μm	F= 1.57, df= 1, P= 0.229	F= 0.31, df= 1, P= 0.584
MWD	F= 0.86, df= 1, P= 0.363	F= 0.28, df= 1, P= 0.605
40-150μm	F= 1.47, df= 1, P= 0.243	F= 0.02, df= 1, P= 0.899

7.4.3 Discussion and Conclusion

The first hypothesis stated that 'the inoculation of undisturbed soil monoliths with earthworms will lead to more stable aggregates by increasing the amount of cast material produced as a result of higher earthworm abundance'. The results have not shown that the inoculation of the undisturbed soil significantly influenced aggregate stability, which was unexpected since several authors have found that dry earthworm cast material form some of the most stable aggregates in soil (Shipitalo and Protz 1988; Marinissen and Dexter 1990; Ketterings *et al* 1997; Garvin *et al* 2001). There are several possible explanations for this. Firstly, most of the research to date on the effect of earthworms on aggregate stability has used either cultivated or reclaimed soils with low organic carbon contents. However, the soil in this investigation was highly organic and as such would be inherently more stable (Tisdall and Oades 1982; Chaney and Swift 1984), therefore any changes to aggregate stability by earthworms may have been masked by this.

The earthworm inventory at the end of the experiment showed that the abundance of earthworms in the inoculated treatments was not significantly different to those which were not inoculated, F= 1.95 df= 1 P= 0.182 (no worms $\bar{x}$= 344.8 CL_{lower}= 143.2, CL_{upper}= 961.7; worms $\bar{x}$= 538.7 CL_{ower}= 278, CL_{upper}= 884.9). This meant that there would not have been significantly more cast material in the inoculated soil as compared to the uninoculated and therefore no significant differences in the aggregate stability between the two. This was supported by the earthworm excrement count data presented in the next section, which showed no significant differences in the abundance of earthworm excrement between the earthworm treatments. Associated with the earthworm population data was the fact that in the no worm

treatments there was an intrinsic earthworm community that was present in the soil when the monoliths were taken. By comparing the inoculated soil with the uninoculated, any changes in aggregate stability due to the inoculation treatment would probably have been masked. Associated with the excremental point count data was the observation in thin section that the soil showed signs of a great deal of bioturbation with a large number of earthworm excrements showed signs of degradation by other soil organisms. Davidson *et al* (2002) investigated the effects of soil organisms on the Sourhope main plots, and they found that a large proportion of the soil had been ingested by soil organisms especially enchytraeids and earthworms. This breakdown of the earthworm cast material would have led to a large proportion of the excremental features not contributing towards the stability of the soil aggregates. A final reason for the lack of significant differences between the earthworm treatments could be due to the significant differences in aggregate stability observed in the unlimed + no worm treatments between the beginning and end of the experiment. These differences indicated that the aggregates at the end were less stable than at the beginning. This would have had a masking effect on any earthworm effects especially if they were quite subtle.

The second hypothesis stated that 'the liming of an undisturbed soil will lead to more stable aggregates through increased earthworm casting activity'. No statistically significant differences in aggregate stability were observed between the liming treatments. This was surprising because the earthworm inventory showed clearly that liming significantly increased earthworm abundance. With this increase in abundance it was anticipated that there would have been an increase in the amount of cast material present in the soil, which would have led to more stable aggregates. Evidence from the thin sections showed that there was not significantly more cast

material in the limed soil as compared to that which was unlimed, and again there was a high level of degradation of the excrements by other soil organisms. These two pieces of evidence indicate that the degradation of earthworm excrements in the soil by other organisms could have played a key role in influencing aggregate stability in the limed soil.

The conclusions from this experiment in terms of aggregate stability were:

1. Significant differences were observed in three of the four aggregate stability indices between the beginning and end of the experiment in the unlimed + no worms treatment. This indicated that aggregate stability was affected by factors other than the treatment imposed on the soil.
2. Earthworm inoculation had no significant effect on aggregate stability.
3. The liming of the undisturbed soil did not significantly affect aggregate stability despite significantly increasing earthworm abundance.
4. That other soil organisms may have had an important role in determining aggregate stability through the degradation of earthworm excrements.

The next section presents the results of the micromorphological analysis and quantification of earthworm excremental features. As has been shown this analysis has provided information on not only the abundance of cast material in the soil but also its nature and degree of degradation.

7.5 Earthworm Excremental Features

A detailed outline of the protocols for the description and quantification of earthworm excremental features was given in chapter 4.5.4. In summary, thin section samples were taken from the A_h horizon of the Sourhope main plots as the beginning of the experiment, to give an idea as to the state of the soil before any earthworm inoculations had taken place. Thin sections were also taken the end of the experiment, 18 months after earthworms were inoculated into the soil. The thin sections were then point counted to quantify the amount of earthworm excrement present in the disturbed soil. A full description of how earthworm excrements were identified was given in chapter 4.

7.5.1 Hypotheses

Two hypotheses were formulated to aid in identifying what the effects of earthworm inoculation and liming were on the abundance of earthworm excrements in the soil. The hypotheses were that:

1. The inoculation of undisturbed soil monoliths with earthworms will lead to the increased abundance of earthworm excrements as a product of a larger earthworm population.
2. The liming of an undisturbed soil will lead to a larger earthworm population and therefore an increased abundance of earthworm excrements.

7.5.2 Results

The initial abundance of earthworm excremental features in the undisturbed soil is highlighted in table 7.8. The data were analysed using one way ANOVA. No statistically significant differences were found for the abundance of earthworm excrement between the unlimed and limed treatments at the beginning of the experiment. This suggested that earthworm casting activity was similar in both treatments.

Table 7.8: Mean abundance of earthworm excremental features in the thin sections from the beginning of the experiment. No. of replicates = 5.

Treatment	Mean % abundance	Standard Error
Unlimed	8.40	± 1.86
Limed	5.40	± 1.26
Statistical Output	F= 1.76, df= 1, P= 0.222	

The differences for the unlimed + no worms treatments between the beginning and end of the experiment are shown in table 7.9. The data were not normally distributed and were also characterised by the presence of outliers. After checking that these outliers were not errors in the data recording and were actual *bona fide* measurements, a Mood's Median test was used. The result of this analysis indicated that there were no statistically significant differences in the abundance of earthworm excrement between the beginning and end of the experiment.

Table 7.9: Median abundance of earthworm excremental features for the unlimed + no worms soil between the beginning and end of the experiment. No. of replicates = 5.

Treatment	Median % abundance	Quartile Deviation
Beginning	6.00	± 2.75
End	2.00	± 4.75
Statistical Output	F= 1.76, df= 1, P= 0.222	

The data for the end sampling point were used to assess the effects of earthworms and liming on the abundance of earthworm excrements. These data were analysed using a ranked two way ANOVA. The differences between the ranked means for the four treatments can be seen in figure 7.7. The results of the statistical analysis showed that there were no significant differences at the 95% confidence level for either the liming (F= 0.27, df= 1, P= 0.608) or earthworm treatments (F= 2.01, df= 1, P= 0.175). This indicated that neither earthworm inoculation nor liming significantly affected the quantity of earthworm cast material in the undisturbed thin sections. However, although no statistically significant differences were observed there were some trends in these data suggesting that both liming and earthworm inoculation led to increased earthworm excrement abundance (figure 7.7).

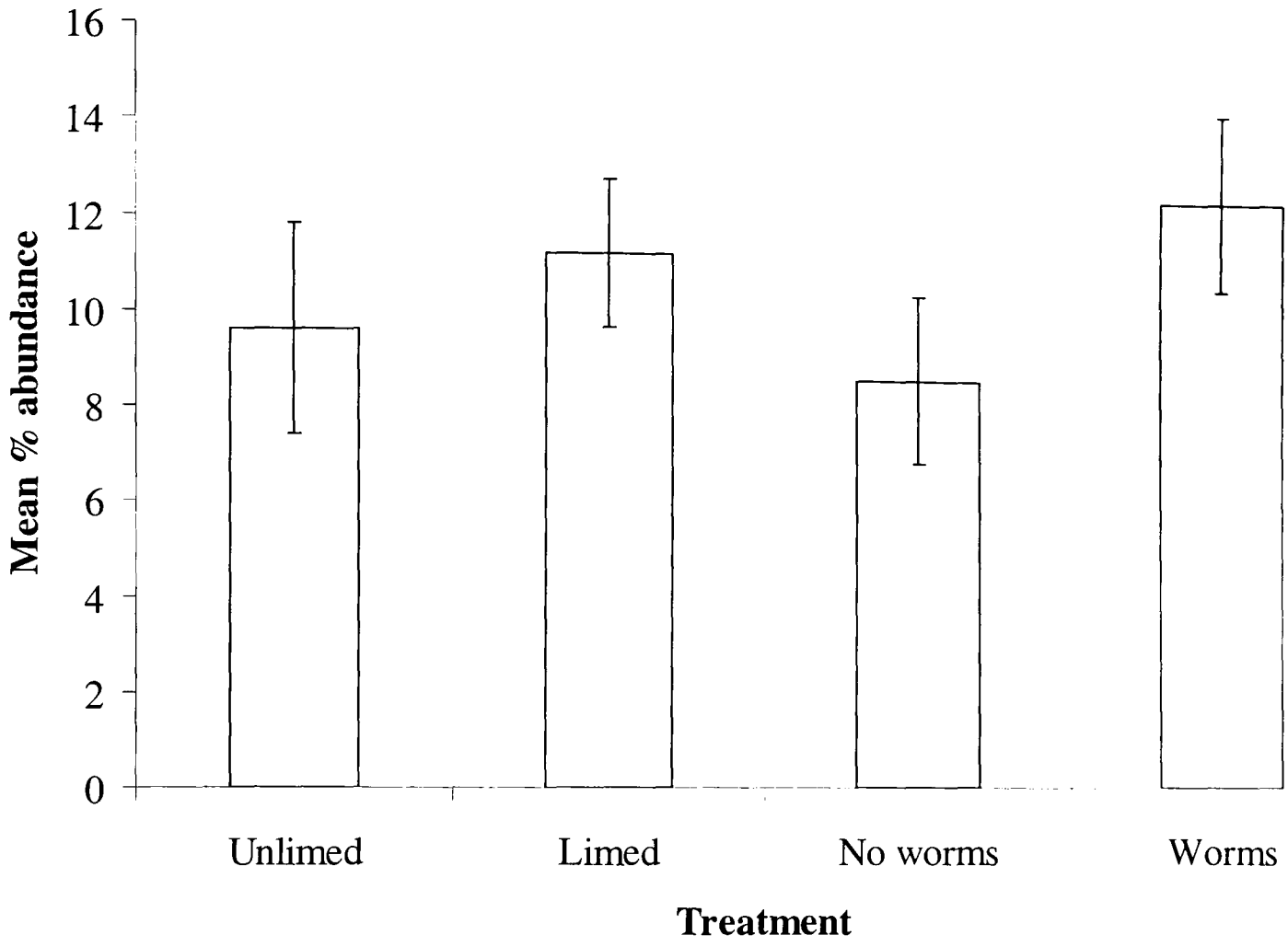


Figure 7.7: Ranked mean excrement abundance for the liming and earthworm treatments. Error bars represent standard errors. No. of replicates = 5.

7.5.3 Discussion and Conclusion

The point count data from the end sampling point indicated that neither earthworm inoculation nor liming had a statistically significant effect on the amount of earthworm excrement observed in the thin sections. The earthworm abundance data from the inventory carried out at the end of the experiment showed that the differences in earthworm abundance between the no worms and worms treatments were not significant. If both of these treatments had similar populations of earthworms, then under the same environmental conditions it could be assumed that they would produce similar amounts of excrement. However, this was not the case in

the liming treatments where the application of lime raised both soil pH and earthworm abundance, but no significant differences were observed in the quantities of excrement produced. This was not expected since it was assumed that the larger the earthworm population, the more excrement they would produce.

The only explanation for this was that there were high levels of bioturbation and ingestion by other soil organisms in all the treatments. As in the disturbed soil, this high level of bioturbation was observed to have the effect of severely degrading the earthworm excrements due to the coprophagous feeding activity of the other soil organisms which is supported by the findings of Davidson *et al* (2002). This breakdown of the cast material would have led to it having a small residence time in the soil before it was reworked by other soil organisms, so differences in the abundance of earthworm excrements was an underestimate of the true level of earthworm activity.

Another explanation for no differences being observed in either treatment was that because the soil was removed in the form of undisturbed soil monoliths, it would have had an intrinsic earthworm population even before any of the treatments were imposed. This existing population made it more difficult to pick out the treatment effects over the historical earthworm features.

The conclusions that can be drawn from the excremental point count were that:

1. The inoculation of undisturbed soil monoliths with earthworms led to no significant differences in the abundance of earthworm excremental features observed.
2. The liming of an undisturbed soil did lead to higher earthworm populations but this did not translate into more earthworm excremental features.

3. The high bioturbation of the soil by soil organisms led to the degradation of the earthworm excrements present in the undisturbed soil.

Having dealt with the structural effects of earthworms on the fabric of an undisturbed soil, the effects of earthworms on void space are examined in the final sections of this chapter. Firstly the effects of earthworms on soil macroporosity are evaluated by determining the saturated hydraulic conductivity on undisturbed soil cores.

7.6 Saturated Hydraulic Conductivity (K_{sat})

Saturated hydraulic conductivity was used as a measure of the soil macroporosity, since when soil is saturated water tends to preferentially flow through larger voids because it is in a tension free state. The greater the value of K_{sat} the more macroporosity there is within the soil. K_{sat} was determined on undisturbed soil cores from the A_h horizon taken at the beginning and end of the experiment. The detailed protocol for its determination was outlined in chapter 4.

7.6.1 Hypotheses

Two hypotheses were formulated to investigate the effects of earthworm inoculation and liming on soil macroporosity that:

1. The inoculation of undisturbed soil with earthworms will lead to increased burrowing activity and macropore creation, and therefore higher K_{sat}.

2. The liming of undisturbed soil will cause increased earthworm abundance and burrowing activity leading to greater K_{sat}.

7.6.2 Results

The initial K_{sat} of the undisturbed soil was determined from cores taken at the beginning of the experiment from the Sourhope main plots. The data were analysed using Mood's Median Test. The median K_{sat} values and the output of the statistical analysis are shown in table 7.10. The results of the analysis indicated that there were no statistically significant differences in K_{sat} at the beginning of the experiment between the unlimed and limed treatments. The quartile deviation indicates that there is a high variability within the data, especially from the limed treatments.

Table 7.10: Median K_{sat} values of the soil cores taken at the beginning of the experiment. No. of replicates = 5.

Treatment	Median ($cm^3\ d^{-1}$)	Quartile Deviation
Unlimed	66	± 30
Limed	73	± 237
Statistical Output	χ^2= 0.40, df= 1, P= 0.527	

The differences between K_{sat} values in the unlimed + no worms treatment at the beginning and end of the experiment indicate how K_{sat} varied over time (table 7.11). Again the data was not normally distributed, so was analysed using Mood's Median test. The results of this analysis showed that there were significant differences in K_{sat} between the beginning and end of the experiment (P> 0.05). Median K_{sat} was higher

at the end of the experiment despite being unlimed and with no earthworms inoculated. Again this data was characterised by high levels of variation as indicated by the quartile deviations.

Table 7.11: Median K_{sat} values of the soil cores taken at the beginning and end of the experiment. No. of replicates = 5.

Treatment	Median (cm^3 d^{-1})	Quartile Deviation
Beginning	66	± 30
End	408	± 888
Statistical Output	χ^2= 10, df= 1, P= **0.002**	

The K_{sat} values for the soil cores taken at the end of the experiment were used to assess the effects of liming and earthworm inoculation on soil macroporosity (figure 7.8). The data were normalised using a square root transformation and analysed with a two way General Linear Model. No statistically significant differences were observed between the liming treatments (F= 0.03, df= 1, P= 0.871). However significant differences were observed between the earthworm treatments (F= 5.98, df= 1, P= **0.026**), with earthworm inoculation leading to higher K_{sat} values and therefore macroporosity. Bonferroni Pairwise Comparisons were carried out to identify if these significant differences could be observed between the individual treatments. This test showed that these differences did not exist at the individual treatment level (unlimed + no worms Vs unlimed + worms: P= 0.254 and limed + no worms Vs limed + worms: P= 1.00). This analysis indicated that although there were significant differences, they were not associated with any of the individual treatments.

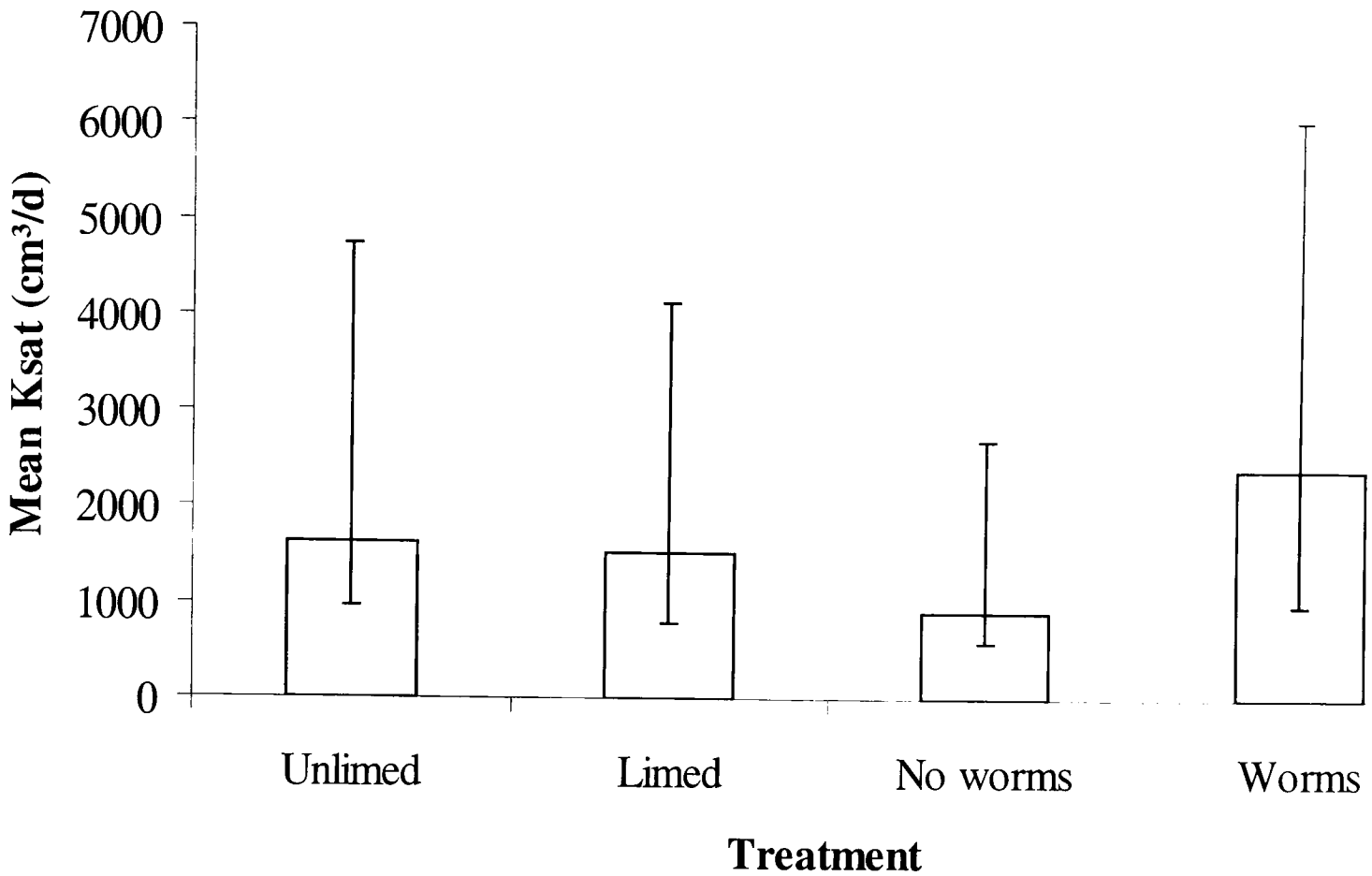


Figure 7.8: Back transformed mean K_{sat} values for the liming and earthworm treatments at the end of the experiment. Error bars represent 95% back transformed confidence limits. No. of replicates = 5.

7.6.3 Discussion and Conclusion.

Tables 7.10, 7.11 and figure 7.8 indicated that the data were characterised by high variability and the presence of outliers. This high variability may account for the unexpected results obtained in this experiment, i.e. that there were statistically significant differences in K_{sat} between the earthworm treatments whilst no significant differences in earthworm abundance were found. In addition, the liming treatments did show significant differences in earthworm abundances, whilst no significant differences were observed in K_{sat} between the liming and no lime treatments. It was hypothesised that K_{sat} would increase in those treatments where earthworm

abundance was elevated, since it was expected that more earthworms would have led to greater burrowing activity and therefore more macropores. It is suggested that it was the high variability within the data and the presence of outliers, which may have led to these results.

There are several reasons that would explain the high variability within the K_{sat} data:

1. The cores had to be transported over 130 km from the field site to the laboratory, so could have been disturbed in transit. This would have caused disruption to the soil void space, leading to alterations in the drainage properties of the soil when saturated. The effect of the disturbance during the transportation of the samples was minimised by the careful packing of the undisturbed cores into appropriately padded containers. Further evidence of disturbance but this time from storage prior to K_{sat} determination was the fact that several cores were desiccated to such an extent that they were freely moving within the cores.

2. During the saturation process a number of earthworms were observed to have been present in the cores. These earthworms would have altered the hydraulic conductivity of the cores by a) creating preferential flow paths in the soil which were not representative of the soil in the field, especially when they burrowed around the edges of the plastic cylinders, b) blocking their burrows hindering the flow of water down the core, and c) infilling their burrows with cast material (this would have been especially true for the endogeic species). Both of these would have had a marked effect of K_{sat} and led to high levels of variability.

The conclusions that were drawn from the K_{sat} data were that:

1. The inoculation of undisturbed soil with earthworms led to higher K_{sat} values, although no differences in earthworm abundance were observed between the treatments.
2. Liming led to elevated earthworm abundance in the soil but no statistically significant differences were found to exist between the treatments.
3. The data was characterised by high variability and the presence of outliers that may have led to the unexpected results.

7.7 Re-organisation of Void Space.

The methodology for the assessment of void space was outlined in chapter 4. In brief, voids were dyed blue during the thin section preparation phase so as to contrast them from the surrounding material. These thin sections were scanned and then analysed using the AnalySIS image analysis software. Two sets of data were collected:

1. Total porosity- the total area of voids expressed as a percentage of the total slide area.
2. The proportion of voids in the thin section with an area less than and greater than 2 mm^2.

7.7.1 Hypotheses

Three hypotheses were formulated to help assess the effects of both earthworm inoculation and liming on total porosity and the re-organisation of void space:

1. The inoculation of soil with earthworms will lead to increased porosity through their burrowing activity.
2. The addition of lime to the soil will lead to elevated earthworm populations, which through their burrowing activity, will increase total soil porosity.
3. Both the addition of lime and the inoculation of undisturbed soil with earthworms will lead to increased earthworm populations which will cause the re-organisation of void space by creating macropores through their burrowing activity.

The results and discussion of the total porosity data are presented in the next section followed by the analysis of the effects of earthworms on the re-organisation of void space.

7.7.2 Total Soil Porosity- Results and Discussion

An assessment of the total void space characteristics at the beginning of the experiment was carried out to identify what the initial state of the soil was. This was done by comparing the unlimed and limed treatments at the start of the experiment (figure 7.9). The data were analysed using one way ANOVA. There were no statistically significant differences between the mean total porosity at the beginning of the experiment for the unlimed and limed soil ($F= 0.09$, $df= 1$, $P= 0.778$). This

indicated that the unlimed and limed soils had similar proportions of void space at the start of the experiment.

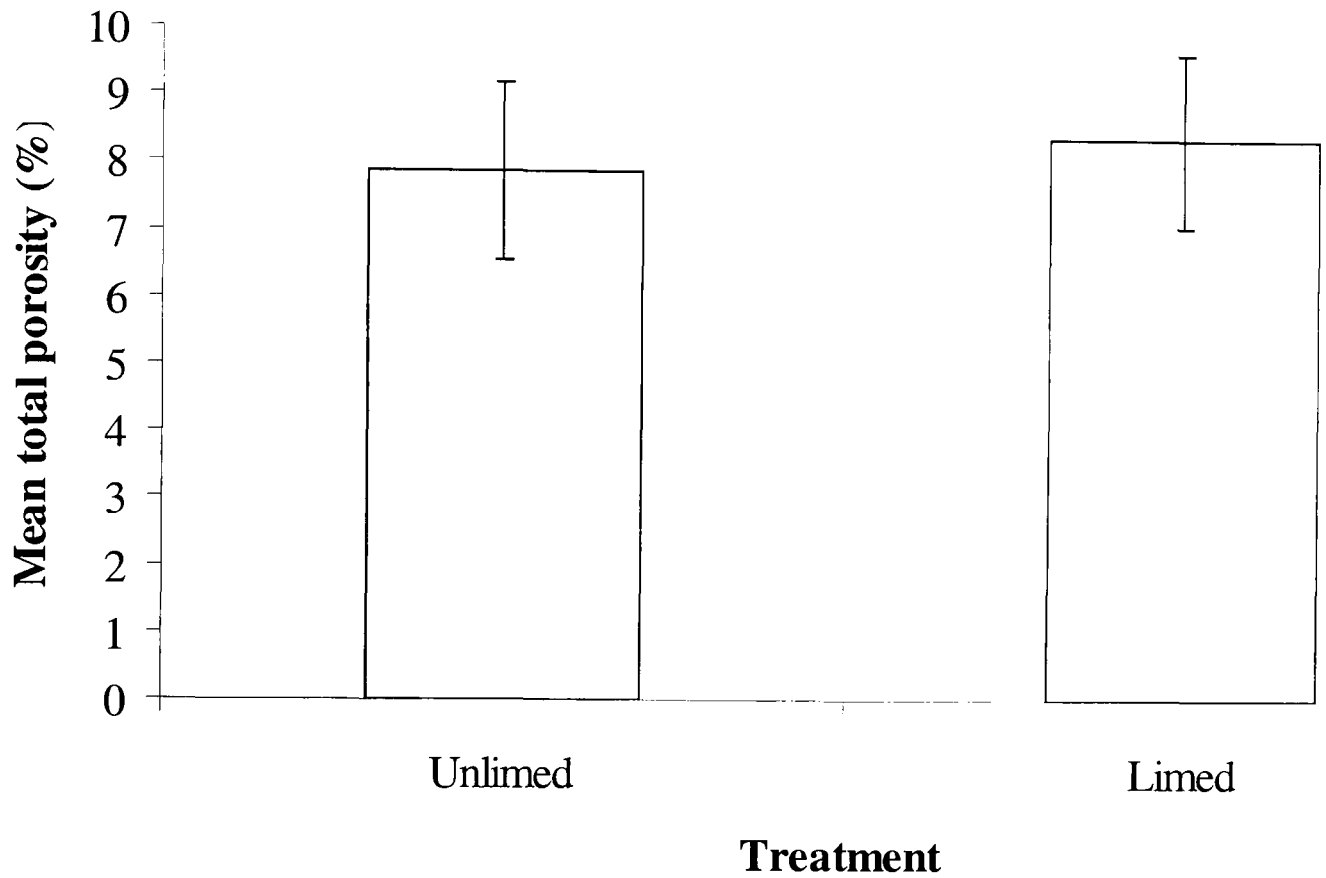


Figure 7.9: Mean total porosity for the unlimed and limed soil at the beginning of the experiment. Error bars represent standard errors. No. of replicates = 5.

The effect of time on total void space change over time was assessed by analysing the unlimed + no worms treatments at the beginning and end of the experiment (figure 7.10). The data were square root transformed and then analysed using one way ANOVA. This analysis showed that there were no statistically significant differences in total soil porosity between the beginning and end of the experiment in the unlimed + no worms treatments ($F= 0.01$, $df= 1$, $P= 0.933$).

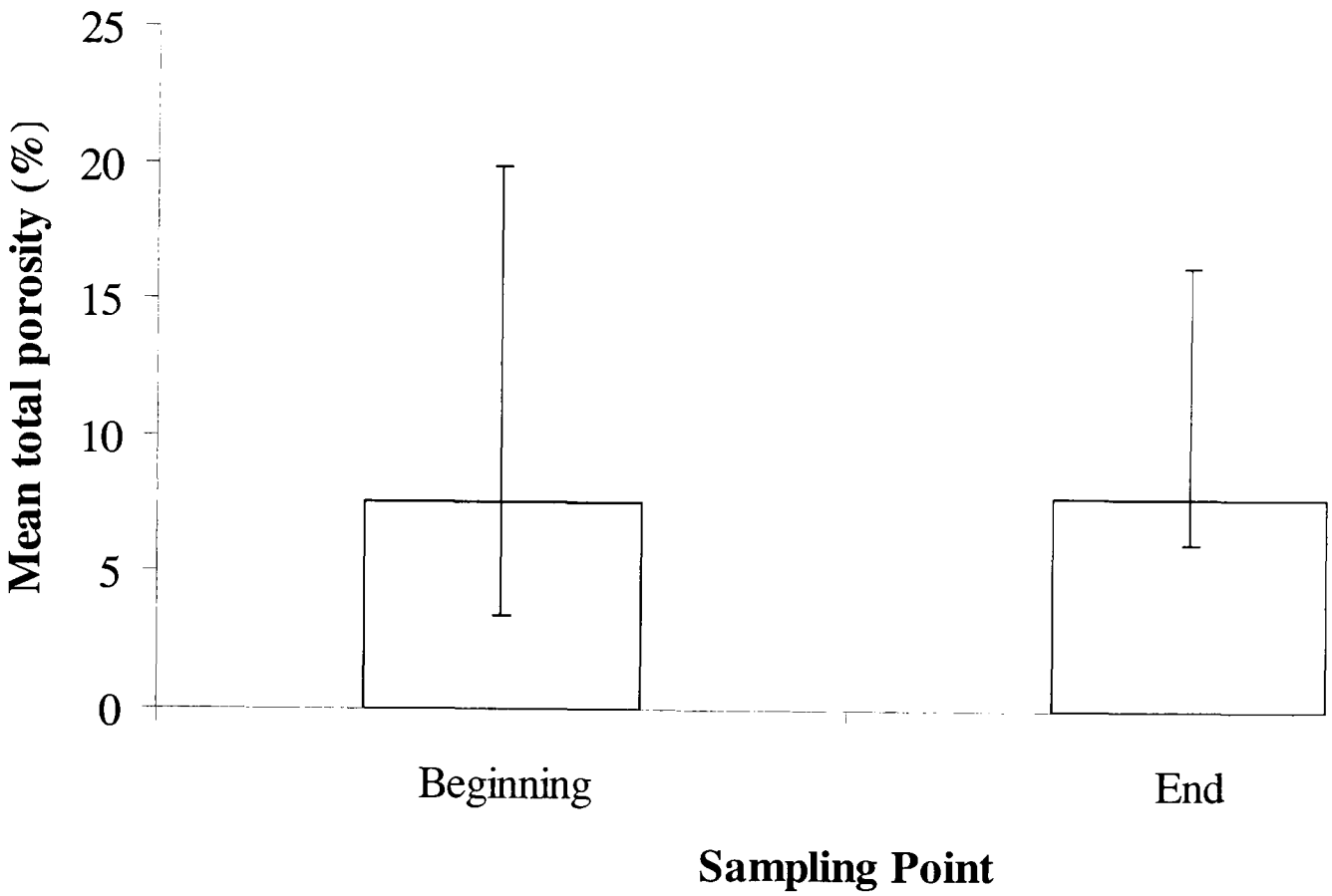


Figure 7.10: Back transformed mean total porosity for the unlimed + no worms treatments at the beginning and end of the experiment. Error bars represent back transformed 95% confidence limits. No. of replicates = 5.

The effects of both earthworm inoculation and lime addition were assessed by analysing the data collected at the end of the experiment (figure 7.11). These data were log transformed and then analysed using a two way General Linear Model. No statistically significant differences were found for either the liming or earthworm treatments (Liming: F= 0.01, df= 1, P= 0.457, Earthworm inoculation: F= 1.72, df= 1, P= 0.208). This indicated that neither earthworm inouclation nor liming significantly affected amount of voids present in the soil.

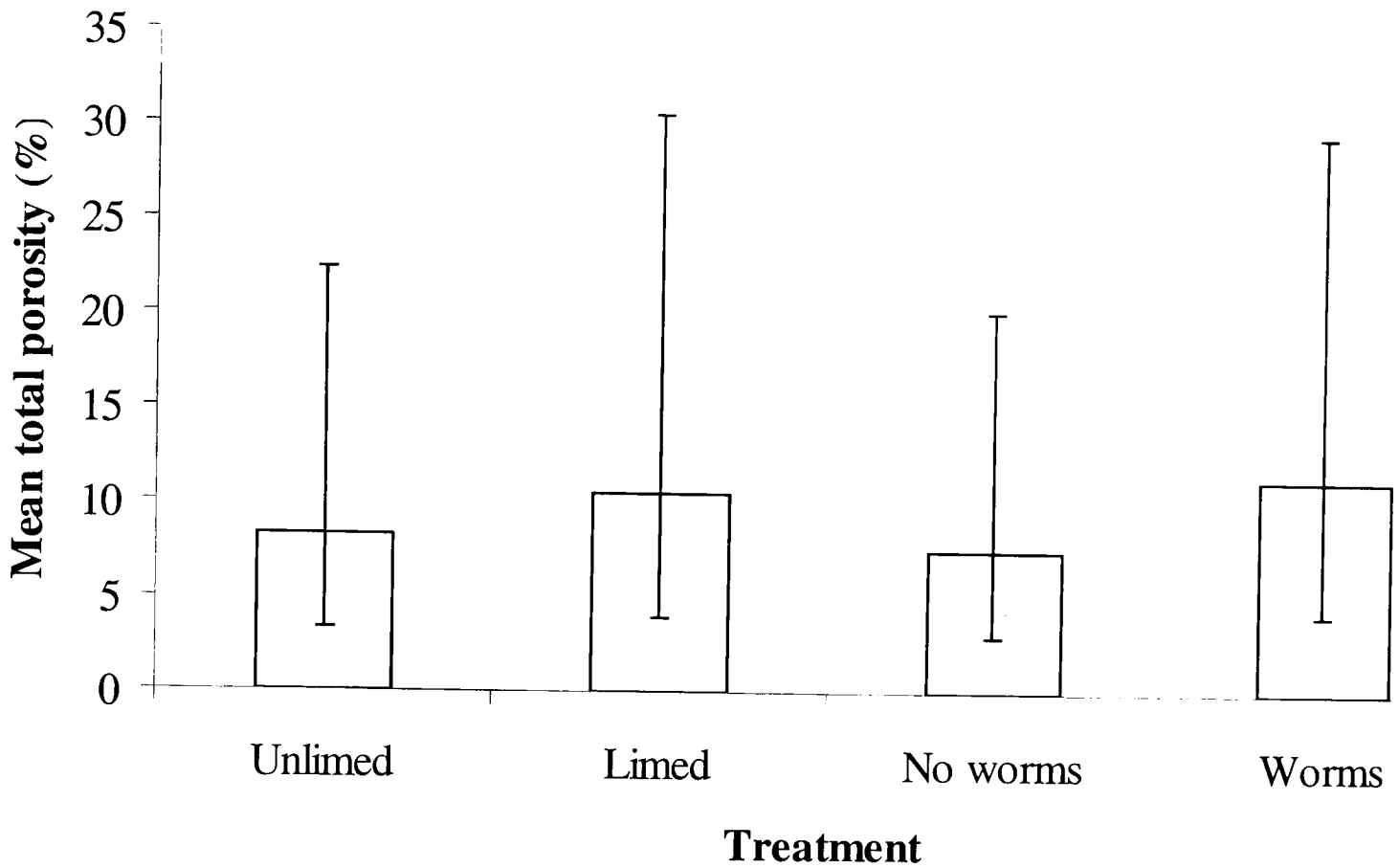


Figure 7.11: Back transformed mean total porosity for the liming and earthworm treatments at the end of the experiment. Error bars show back transformed 95% confidence limits. No. of replicates = 5.

The first two hypotheses presented in section 7.8.1 stated that increased earthworm abundance through either earthworm addition or liming would significantly increase total soil porosity through the creation of voids in the form of burrows. The data indicated that neither earthworm inoculation nor liming significantly increased the amount of voids present in the soil. It should be noted that earthworm inoculation did not significantly increase earthworm abundance although liming did. Due the increased abundance of earthworms in limed soil it was expected that total porosity would also increase (Stockdill 1966; Satchel 1967; Ehlers 1975; Lee 1985; Marinissen and Miedema 1994; Tomlin *et al* 1995). Several studies have shown that rather than increasing total porosity, earthworms lead to a re-organisation of the

voids present by reducing the proportion of small voids and increasing the number of large voids (Syers and Springett 1983; Binet and Curmi 1992; Knight *et al* 1992; Binet *et al* 1997; Lachnicht *et al* 1997). It was this finding that was outlined in the third hypothesis and was investigated in the next section.

7.7.3 Void Size Distribution- Results and Discussion

The effects of earthworm inoculation and liming on void size distribution were assessed using a 2 mm^2 threshold. The proportion of voids less than and greater than 2 mm^2, as expressed as a percentage of the total slide area, were calculated. The initial state of the soil at the beginning of the experiment was assessed in terms of void size distribution by comparing the data for the unlimed and limed treatments (figure 7.12). The data were analysed using one way analysis of variance, with the > 2 mm^2 class arcsine transformed. No statistically significant differences were found for either the < 2 mm^2 or > 2 mm^2 classes for the unlimed and limed soil (< 2 mm^2: $F= 0.23$, $df= 1$, $P= 0.646$ > 2 mm^2: $F= 0.04$, $df= 1$, $P= 0.851$). This indicated that the size distribution of voids were similar for both the soils at the beginning of the experiment.

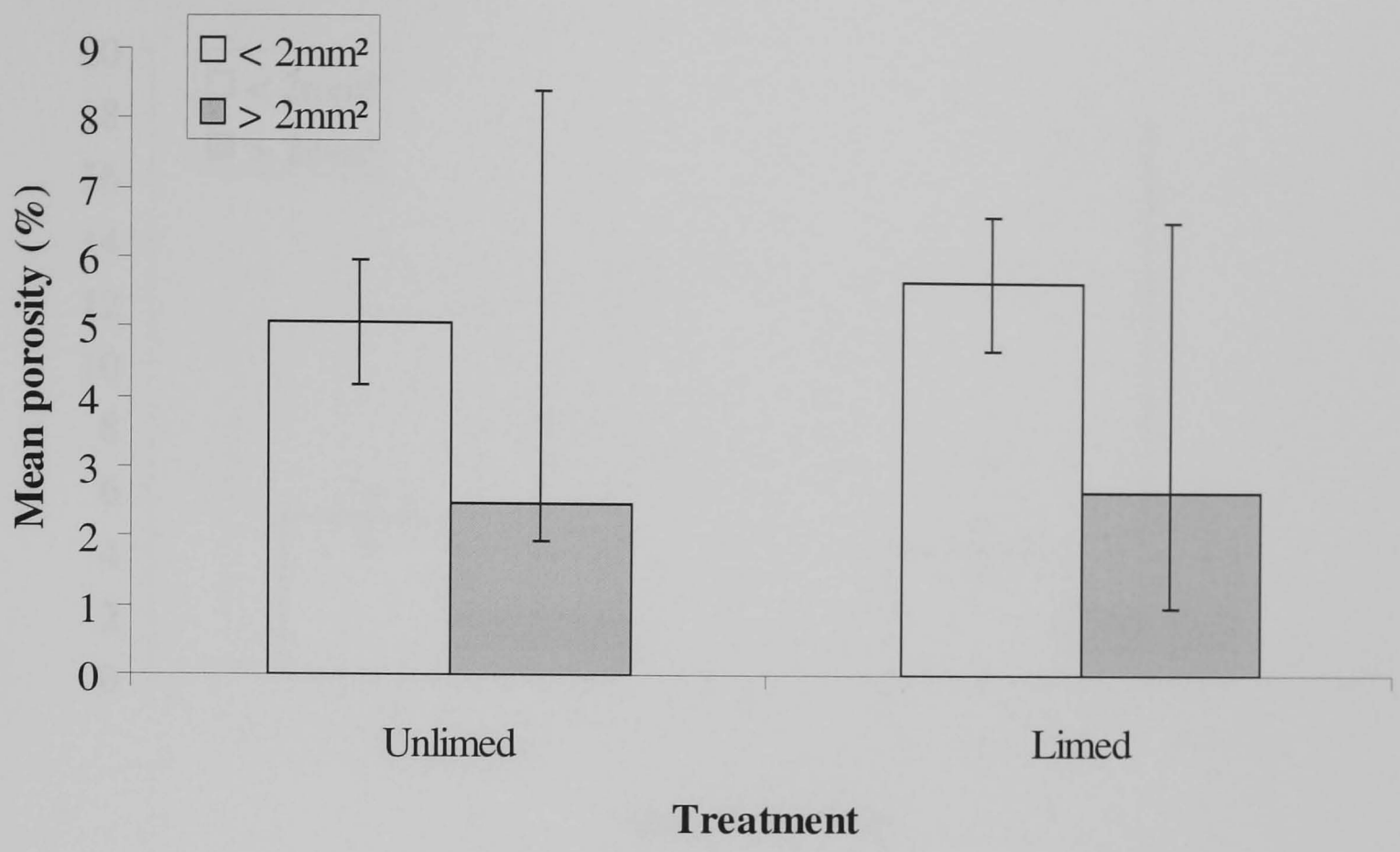


Figure 7.12: Mean porosity for the < 2 mm² and > 2 mm² size classes at the beginning of the experiment for the unlimed and limed soil. The mean values for the > 2 mm² size class are back transformed means. The error bars for the < 2 mm ² size class are standard errors, whilst for the > 2 mm² size class they represent back transformed 95% confidence limits. No. of replicates = 5.

The effects of time on void size were evaluated by comparing the data for the < 2 mm² and > 2 mm² classes for the unlimed + no worms treatments at the beginnig and end of the experiment (figure 7.13). Again one way ANOVA was used to analyse the data, with the > 2 mm² data arcsine transformed. No statistically significant differences in the proportion of voids < 2 mm² and > 2 mm² were observed between the beginning and end of the experiment for the unlimed + no worms treatment (< 2 mm²: F= 0.68, df= 1, P= 0.435. > 2 mm²: F= 0.01, df= 1, P= 0.934). The data indicated that time did not have a statistically significant effect on void size distribution in soil which was not inoculated or limed.

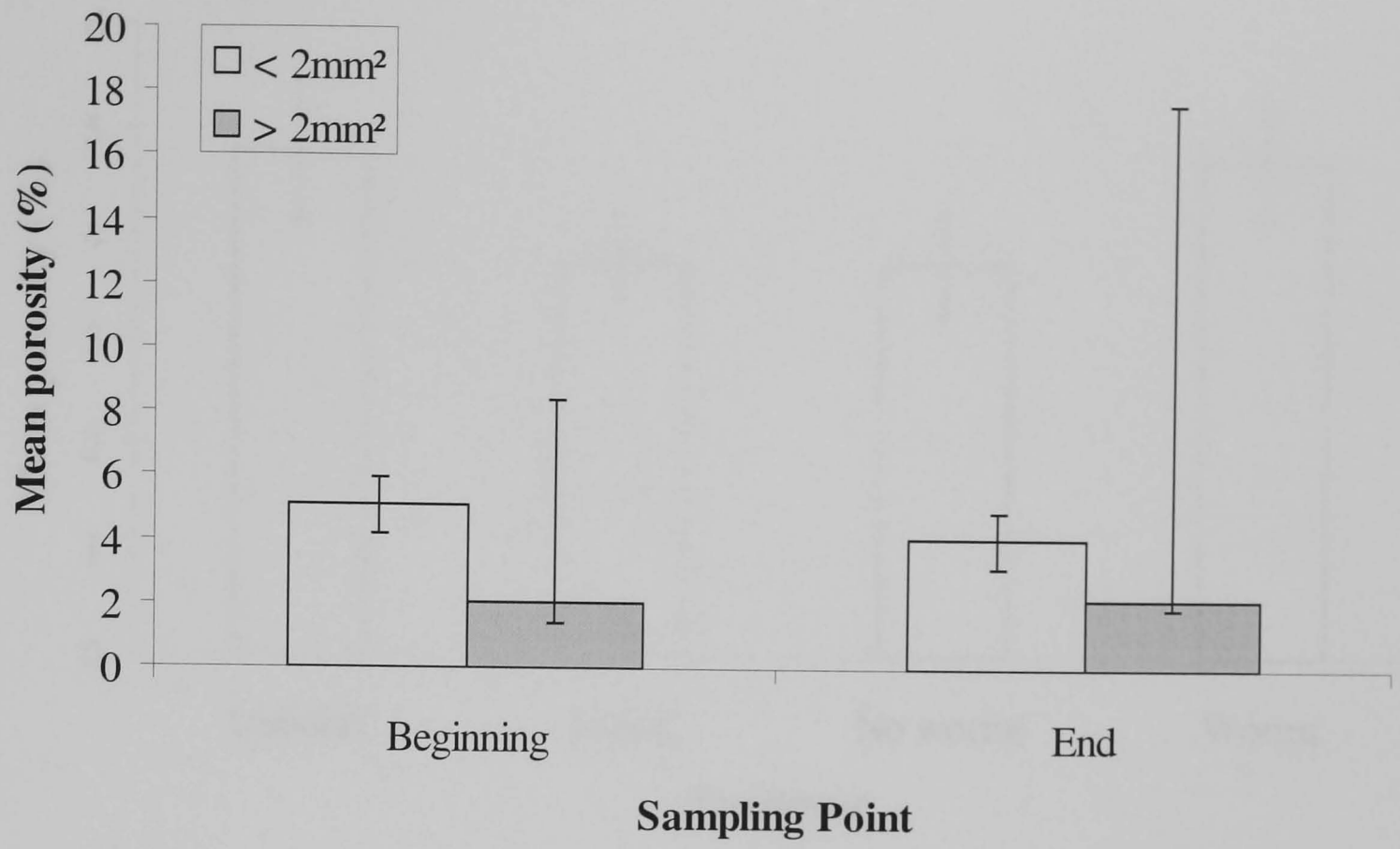


Figure 7.13: Mean porosity for the < 2 mm² and > 2 mm² size classes at the beginning and end of the experiment for the unlimed + no worms treatment. The mean values for the > 2 mm² size class are back transformed means. The error bars for the < 2 mm ² size class are standard errors, whilst for the > 2 mm² size class they represent back transformed 95% confidence limits. No. of replicates = 5.

The effects of liming and earthworm inoculation on the void size distribution were assessed by comparing the treatments at the end of the experiment. The data for both the < 2 mm² and > 2 mm² were analysed using two way General Linear Models. The > 2 mm² size class data were normalised using a square root transformation, so the mean values presented are back transformed means. No statistically significant differences existed for the proportion of voids < 2 mm² between either the liming (F= 1.95, df= 1, P= 0.182) or earthworm treatments (F= 1.82, df= 1, P= 0.196) at the end of the experiment (figure 7.14). This indicated that neither liming nor earthworm inoculation had a statistically significant effect on the proportion of voids < 2 mm².

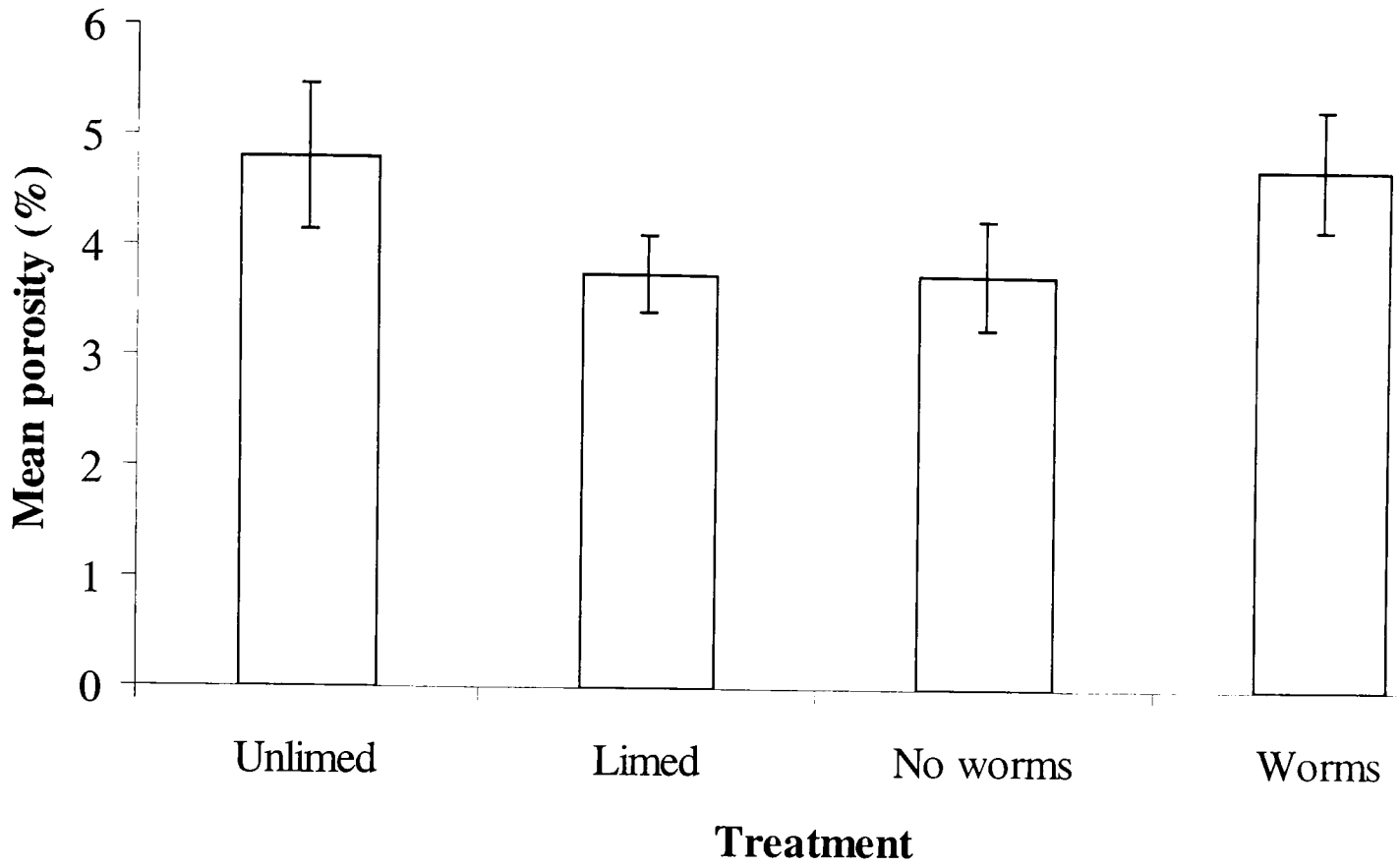


Figure 7.14: Mean soil porosity of voids < 2 mm² for the liming and earthworm treatments at the end of the experiment. Errors bars represent standard errors. No. of replicates = 5.

No statistically significant differences in the proportion of voids > 2 mm² were found for the liming (F= 0.160, df= 1, P= 0.224) and earthworm treatments (F= 0.80, df= 1, P= 0.386) in the undisturbed soil at the end of the experiment (figure 7.15). This indicated that neither lime addition no earthworm inoculation had a statistically significant effect on the proportion of voids > 2 mm² at the end of the experiment.

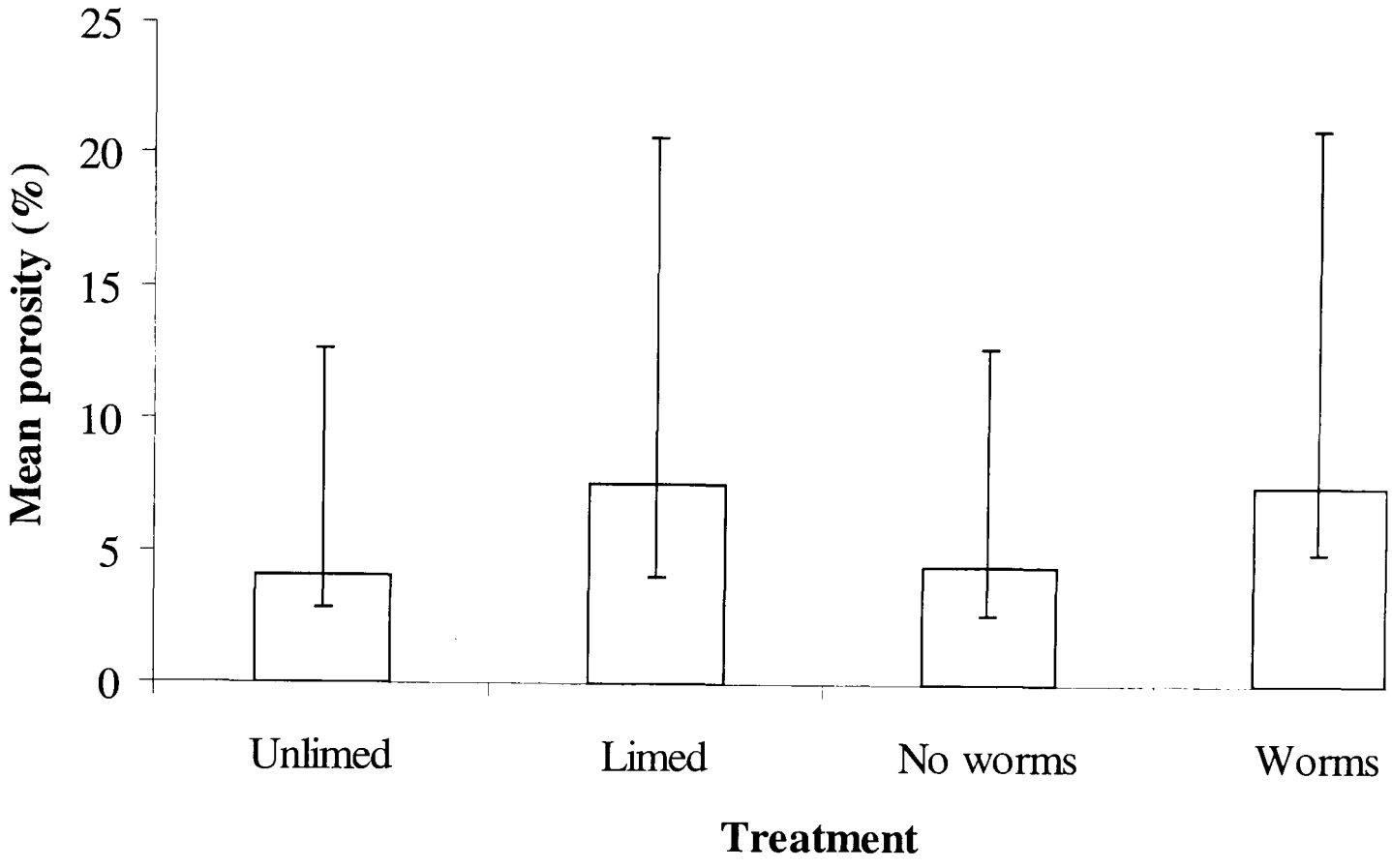


Figure 7.15: Back transformed mean soil porosity of voids > 2 mm² for the liming and earthworm treatments at the end of the experiment. Errors bars represent back transformed 95% confidence limits. No. of replicates = 5.

It was expected that both the inoculation of earthworms and liming of the undisturbed soil would have increased the proportion of large voids in the soil (Syers and Springett 1983; Binet and Curmi 1992; Knight *et al* 1992; Binet *et al* 1997; Lachnicht *et al* 1997), because they both should have led to larger earthworm populations. This was not the case for the earthworm inoculated treatments since earhtworm abundance was not significantly different between those which were and were not inoculated. This meant that there would have been no difference in burrowing activity and therefore void formation in these treatments.

Liming did significantly increase earthworm abundance, as shown in section 7.2.1, so it was suprising that no changes in the size distribution of the voids were observed. This could have been because there were little or no anecic earthworm

species in any of the treatments, with the dominant species being *D. rubidus* (epigeic) and *A. chlorotica* (endogeic). The lack of this ecological group, which form semi-permanent burrows (Bouché 1977), and the prevalence of the epigeic and endgoeic species would have led to dramatic changes to the void size distribution. The presence of an anecic species would have led to distinct and long lasting earthworm burrows being observed in the thin sections and therefore altering the void size disribution. Neither the epigeic nor endogeic earthworm species would have extensively contributed to the production of voids because:

1. Epigeic earthworms would have been unlikely to have burrowed in the more mineral horizons (Lee 1985), from which the thin sections were taken.
2. Endogeic species often cast in their burrows infilling them (Edwards and Bohlen 1996), thereby destroying any voids they may have created in the mineral horizons.

7.7.4 Conclusions

Three conclusions can be drawn from this experiment on the effects of earthworm inoculation and liming on both total soil porosity and void space re-organisation:

1. Earthworm inoculation and liming did not have a significant effect on total porosity indicating that overall, despite liming leading to increased earthworm abundance, there was no net creation of void space.
2. Neither earthworm inoculation nor liming significantly affected the proportion of voids < 2 mm^2 or > 2 mm^2 in area, which was most likely due to the lack of anecic earthworm species in the community.

3. That significantly increased earthworm abundance, caused by raises in soil pH as a result of liming, had no statistically significant effect on void space.

7.8 Conclusions from the Sourhope Experiment: Undisturbed Soil

This experiment into the effects of earthworm inoculation and liming on the soil fabric of an undisturbed soil represented the third and final level of system complexity, since it was an *in vivo* field experiment, which used undisturbed soil monoliths that still had their original structure. It was located in the field so was exposed to real world environmental conditions. Four parameters were used to evaluate the effects of earthworms and liming on the soil fabric. These were a) aggregate stability, b) quantification of earthworm excrement, c) K_{sat} determination and d) quantification of voids space.

The data in this chapter have shown that the results of earthworm inoculation on the fabric of an undisturbed soil were:

1. No statistically significant differences were found in aggregate stability between the earthworm treatments.
2. There were no statistically significant differences in the amount of earthworm excrement in the undisturbed soil thin sections between the earthworm treatments.
3. Earthworm inoculation significantly increased K_{sat}, but the data were characterised by very high variability and no significant differences in earthworm abundance were observed between the treatments, therefore it has to be concluded that inoculation had no significant effect of K_{sat}.

4. No statistically significant differences were observed in total porosity and void size between the earthworm treatments.

The primary reason why no significant differences were found between the two earthworm treatments was because the differences in total earthworm abundance for both these treatments were not statistically significant (No worms $\bar{x}$= 344.8 m^{-2} 95% CL_{lower} 143.2, 95% CL_{upper} 633.8. Worms $\bar{x}$= 538.7 m^{-2} 95% CL_{lower} 278.0, 95% CL_{upper} 884.9). It was expected that inoculating the undisturbed soil with earthworms would have increased their abundance leading to increased activity and therefore changes to the soil fabric. Inoculation did not lead to increased earthworm abundance, so it was not surprising that no significant changes to the soil fabric were found. In addition the high degradation of earthworm excremental features by other soil organisms led to differences in aggregate stability and the amount of excrement between the treatments being masked to some extent. From thin section observation the soil was highly bioturbed therefore the impact of soil organisms on excremental degradation was an important mediating factor.

The data in this chapter have shown that liming of the undisturbed soil has had the following effects on the soil fabric of an undisturbed soil:

1. No statistically significant differences existed between the liming treatments for aggregate stability.
2. There were no statistically significant differences in the amount of earthworm excrement in the undisturbed soil thin sections between the liming treatments.
3. No statistically significant differences were found in K_{sat} between the liming treatments, despite liming leading to increased earthworm abundance.

4. No statistically significant differences were observed in total porosity and void size between the liming treatments.

It was surprising that liming did not lead to significant differences in the four parameters measured, since the addition of lime led to significantly higher earthworm abundances (No lime $\bar{x} = 235.9$ m^{-2} 95% CL_{lower} 86.9, 95% CL_{upper} 457.9. Lime $\bar{x} = 698.0$ m^{-2} 95% CL_{lower} 476.5, 95% CL_{upper} 961.7). Again it was expected that increased earthworm abundance would have led to changes in the soil fabric. In terms of aggregate stability and earthworm excremental features, this could be explained by the high levels of degradation caused by other soil organisms, as highlighted earlier. The lack of any anecic earthworms would have had a dramatic impact on void space since it is these earthworms which produce semi-permanent burrows which form long-lasting large voids within the soil (Lee 1985). Their absences meant that very few earthworm channels and therefore macropores were found.

Both the inoculation and liming treatments caused changes to the composition of the earthworm community, with endogeic earthworm species becoming more dominant over epigeic species (i.e. the proportions of *A. chlorotica and O. cyaneum* generally increased whereas *D. rubidus*, the dominant epigeic species, decreased). At the same time the functioning of the soil was maintained as expressed by the lack of changes in soil physical properties, caused either by the treatments or the effect of time. The lack of changes to the functioning of the soil despite, the shift in species composition could be seen as an example of redundancy. Redundancy is the ecological concept where "different species perform the same functional role in ecosystems so that changes in species diversity does not affect ecosystem functioning" (Loreau 2004).

This tends to indicate that it is not necessarily the functional role of different earthworm species that is important for ecosystem functioning but rather that they are present and active within the soil.

Within ecology the concept of redundancy it a 'hot topic' with many authors arguing over its importance (Wolters 2001). The view that has begun to emerge in the debate over redundancy vs. biodiversity is that both are important in maintaining ecosystem stability (Bengtsson 2002; Giller and O'Donovan 2002), and that redundancy does exist in nature but only operates over small temporal and spatial scales (Loreau 2004). To be able to identify how important redundancy is in this soil the experiment would have had to run for a longer period of time to monitor whether there was no loss in the functioning of the soil in term of its physical characteristics.

This experiment as discussed in this chapter represented the final and highest level of system complexity. This complexity would have had an influence over the results obtained because firstly, the experiment was carried out under real world environmental conditions that would have introduced greater variability into the data. Secondly the soil had an existing earthworm community and its original soil structure, so isolating the effects of the treatments imposed was more difficult. Indeed the undisturbed nature of the soil could well have caused higher levels of background noise within the data than was the case with the simpler and less complex experiments.

8. Overview of Earthworm Effects in an Upland Soil

The overall aim of this research was to quantify the effects of earthworms on soil structure. Experimental work was carried out at a range of scales to stimulate earthworm activity through liming and earthworm inoculation, and then to investigate impacts on void space and aggregation in an upland soil.

8.1 Effects of Disturbance, Liming and Inoculation on the Earthworm Community.

As expected, the liming of both the disturbed and undisturbed soil significantly increased earthworm abundance, due to large pH rises in the organic horizons. The species composition of the earthworm community was also affected by liming since the addition of lime changed the dominant earthworm species from *D. rubidus* to *A. chlorotica*.

Inoculation of earthworms led to increased earthworm abundance in the disturbed soil only. This was due to the earthworm community being completely destroyed at the beginning of the experiment by the disturbance process. Additionally, earthworm inoculation caused no significant changes to the community composition in either the disturbed or undisturbed soil. What was evident from the results of the earthworm survey was that liming had a far greater impact on the earthworm community than did inoculation.

The effects of the experimental treatments were however difficult to isolate due to the effects of physical disturbance on the earthworm community. During the drying

and structural disruption of the soil, all the earthworms died due to desiccation. However, an earthworm population did develop in the disturbed soil due to recruitment from cocoons and invasion of worms from outside the experimental boxes.

8.2 Effects of Earthworm Inoculation and Liming on Void Space and Aggregation

The Ecotron experiment was the simplest in terms of system complexity since it took place in a controlled environment, and used an artificial soil and earthworm inoculation treatments. The impact of the earthworms on void space was to significantly increase the proportion of large voids (> 2 mm^2 in area) whilst decreasing the abundance of small voids (< 2 mm^2). The earthworm inoculation had no effect on aggregate stability due to the very coarse texture and low organic matter content of the soil.

The disturbed experiment used Sourhope soil that had its structure removed and represented the second level of system complexity. Liming had a significant effect on void space by increasing the proportion of large voids due to elevated earthworm abundance and activity. No significant effects were observed in aggregation due to the treatments, although aggregate stability did decrease over time in the unlimed + no worms treatment. This destabilisation with time was because the soil was highly organic and therefore inherently stable, but after the disturbance process the aggregates in the soil adjusted to the new conditions, thereby losing stability.

The undisturbed experiment was the most complex because the soil had a pre-existing structure and earthworm community before any of the treatments were

imposed. Inoculation of earthworms led to significantly higher K_{sat} and therefore increased connection of macropores. Apart from this impact on $K_{sat,}$ liming and earthworm inoculation did not cause any significant effects on voids space or aggregation. As in the disturbed experiment, aggregate stability was significantly reduced over time. This was attributed to the activity of the pre-existing earthworm population since several authors have reported that earthworm activity can destabilise aggregates.

It was anticipated that liming may have had a direct impact on soil aggregation through the flocculation of clay particles. This was not the case since when the comparisons were made in aggregate stability between the unlimed and limed + no added worms treatments no significant relationships were found that could be attributed to flocculation. Liming would have increased the concentration of Ca^{2+} in the soil therefore playing a key role in stimulating flocculation by bonding to the negatively charged surface of clay particles thereby joining them together. There are several reasons why there was lack of evidence for flocculation influencing aggregation. Firstly, all the samples taken from both the disturbed and undisturbed soils were from the A_h horizon and pH data clearly highlighted that the effect of the lime was localised to the LFH horizons only. This would have limited the supply of Ca^{2+} ions available to flocculate any clay present. Secondly, the soil was inherently stable due to it high organic matter content which meant that a large proportion of the clay in the soil would have been unavailable for flocculation.

This three-level experimental approach, each representing increased system complexity, was relatively successful because the greatest number of changes were observed in the simplest system and the least in the most complex. In addition the

results of the research project were generally consistent across all three levels of complexity. Both of these findings vindicate the overall research design. By using this research design the problems of performing experiments in complex and realistic systems have been highlighted. The difficulties that were encountered whilst trying to interpret the data from the most complex experiment were primarily a result of a) the high levels of variability within not only the data but also the soil itself, and b) that the Sourhope soil was a dynamic system where changes were constantly taking place irrespective of the imposed treatments.

In order to evaluate the extent to which the original three research hypotheses were substantiated, these are restated below along with responses based on the results of all three experiments:

1. Increased earthworm casting, as expressed as excremental pedofeatures, would be associated with increased aggregate stability – Inoculation of the soil with earthworms did not lead to increased casting as quantified by point counting earthworm excremental features, and consequently no increases in aggregate stability were observed. Indeed there is some evidence in the data from the undisturbed experiment that earthworms overall had a destabilising effect.
2. Increases in earthworm abundance and activity would lead to increased soil pore space – Increases in earthworm abundance did not increase soil pore space but did change its size distribution by increasing the abundance of pores > 2 mm^2 in area. These changes were observed only in the Ecotron and disturbed experiments which represented complexity levels 1 and 2.
3. Pasture improvement through liming would lead to increases in earthworm populations and activity, thereby causing changes to soil structure – Liming did

lead to significant increases in earthworm abundance (in some instances increases of up to 407%) by raising soil pH. However, increased abundance only had an effect on void space but not on aggregation. Of all the perturbations used, liming had the most significant effect on both the earthworm communities and void space.

8.3 Ecological Implications of the Undisturbed and Disturbed Experiments.

There are two key ecological concepts that are of relevance to the undisturbed and disturbed experiments. The first is that of redundancy, where by the species or functional composition of a community changes and yet the soil continues to function. This was evident from the data presented in chapter 7, where the earthworm community shifted from one in which epigeic earthworms played an important role to one where endogeic earthworms dominated, and yet despite the change in the functional diversity the soil continued to function as before. Redundancy has an important role to play in maintaining ecosystem resilience, since species or functional groups can be lost and the soil will still be able to function normally. However, whether the soil could continue to function under these conditions is a question the only long-term monitoring experiments could answer.

The other ecological concept of relevance in this research is that of resilience. This can be defined as the speed or ability of an ecosystem to recover from a perturbation, and is an important factor in determining ecosystem stability. This ability to recover or adapt to a perturbation was shown in both experiments. In the undisturbed soil the imposed earthworm and liming treatments had no significant effect on soil physical

properties and therefore on functioning. Whilst resilience was evident in the disturbed soil because it recovered relatively rapidly from the severe physical disruption process used to remove its structure.

The causes of resilience in both soils are similar and are related to the history of the soil and the resultant physical, chemical and biological properties. Initially the soil was cultivated resulting in the ridge and furrow system which was still visible. It was then turned to pasture and the resultant grassland community that developed helped create a soil which is highly organic in nature with regular inputs of organic materials, and is structurally stable. Combined with a well developed and diverse biological community this has led to a relatively stable ecosystem, which when disturbed will return to an equilibrium state rapidly. This is in stark contrast to a low resilience system, such a reclaimed polder soil in the Netherlands. These soils were recovered from the sea and are relatively young in age. Polder ecosystems tend to be low resilience systems because they are dominated by finely textured soils that are relatively poor in organic matter, are weakly structured and initially have a paucity of soil organisms. These factors mean that these young soils are more susceptible to disturbance and recover from perturbations more slowly than the older, more established and structurally stable soils found at Sourhope.

8.4 Improvements to Research Design

On the basis of the research results and reflection on the research methodology, several improvements can be proposed:

1. Minimisation of variability and better control over the experimental variables. The data collected during this research project are characterised by relatively

high levels of variability which have undoubtedly played a major role in masking some of the earthworm effects. If experimental variability was minimised, then any earthworm effects caused by the treatments would have been more obvious. An example where better experimental control would have been beneficial was in the disturbed experiment, where earthworm populations had developed in the no worms treatments. It was hoped that the disturbance process would have minimised any population in these treatments. As a result of these populations, the contrast between the non- inoculated and inoculated treatments was reduced.

2. The use of larger boxes for holding the soil and containing the earthworm populations. It was the limited size of the boxes which primarily caused no *L. terrestris* to survive the inoculation, since this anecic earthworm would normally have burrowed to considerable depths (Edwards and Bohlen 1996). If the inoculation treatments had been more successful in terms of survival of the three species used, then more functional effects may have been distinguishable.
3. To run the experiment over a longer period with regular sampling to better quantify the longer term effects of earthworms on soil structure. By running the experiment for longer more of the soil would be utilised by the earthworms which in turn would make their impacts on soil more distinct.
4. The use of a bigger and/or different perturbation to the soil system. The pH data showed that the effect of liming on soil pH was most marked in the organic horizons; indeed for some time after application lime was still seen on or near the surface of the soil. This was due to the lime being applied as a powder, however, if it was applied in solution then its effects would most likely have been found at greater depth.

8.5 Future Research

A number of areas for further research have been identified on the basis of this research. Three of these suggestions relate to better understanding of earthworm effects below the soil surface. These suggestions are:

1. Integration of two-dimensional and three-dimensional void space characterisation. Recently X-ray tomography studies have been used to investigate the three dimensional effects of earthworm burrowing activity on void space (Capowiez *et al* 1998; Jegou *et al* 1998b; Jegou *et al* 1999; Langmaack *et al* 1999; Capowiez *et al* 2000; Jegou *et al* 2001; Pierret *et al* 2002; Bastardie *et al* 2003). However, this technique has mainly been used on re-packed cores under laboratory conditions. By combining this technique with micromorphological observations and image analysis on undisturbed soils, void space could be better characterised both in two and three dimensions.
2. Investigation of the impacts of liming and earthworm activity on Ca distribution in soil using electron microprobe analysis. Electron microprobe analysis is able to produce high-resolution element maps for relatively low elemental concentrations, as is quite often the case in soil. This technique could be used to investigate how earthworm activity and liming influence the distribution of Ca within the soil. Calcium is an important element for earthworms and plays a key role in aggregate bonding and stability, therefore by using this technique it would be possible to examine the link between earthworm casts, aggregate stability and Ca distribution. A major advantage of electron microprobe analysis is that the instrumentation uses normal thin sections thereby allowing micromorphological observations and image analysis to be performed on the same samples.

3. Determination of the residence time of earthworm excrements in the soil. A great deal of work has been carried out on earthworm casting activity and calculating the amount of earthworm casts produced per year using surface casts. There has been very little work on rates of sub-surface casting and the residence time of cast material within the soil. This research has shown there are high levels of bioturbation and degradation of earthworm excrement, and it is important to quantify the amount and rate earthworm cast break down. This type of investigation could make use not only of micromorphology but also isotopic labelling techniques to trace cast material in the soil and determine rates of decomposition.
4. Further development of the novel approach used in this research project to determine aggregate stability. The determination of aggregate stability using laser diffraction has only been used by a handful of authors (Buurman *et al* 1997; Muggler *et al* 1997; Muggler *et al* 1999; Westerhof *et al* 1999), and this technique provides a very neat way of characterising aggregate breakdown. This technique requires some further development in terms of analysing different sized aggregates.

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