INTERACTIONS WITHIN EARTHWORM COMMUNITIES : A LABORATORY-BASED APPROACH WITH POTENTIAL APPLICATIONS FOR SOIL RESTORATION

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ABSTRACT

The role of earthworms in improving soil fertility and structure is widely recognised. As a result earthworms (often single species populations) have been deliberately introduced into pasture and arable land by man in attempts to improve plant production and into degraded land in order to bring about soil amelioration.

The potential of earthworms employed in land restoration programmes may be enhanced by inoculating sites with a combination of species from different ecological groupings which have different roles in soil processes. In order to achieve the type of success envisaged by such projects, detailed information on the ecology and interactions between candidate species is required.

This research investigated inter- and intra-specific interactions in terms of growth, maturation, cocoon production and survival, between five earthworm species (*Allolobophora chlorotica* (Savigny), *Aporrectodea caliginosa* (Savigny), *Aporrectodea longa* (Ude), *Lumbricus rubellus* (Hoffmeister) and *Lumbricus terrestris* (Linnaeus)) under laboratory conditions. Cultures were initially maintained under optimal environmental conditions. Selected environmental variables (*e.g.* food position, food particle size and soil bulk density) were manipulated in order to quantify observed species interactions and subject earthworms to conditions that could be encountered at restored sites.

Results demonstrated that all species could be successfully cultured under laboratory conditions. Techniques developed during the work may have applications in commercial

large-scale rearing of earthworms in addition to production of species cohorts for toxicological testing and further laboratory experimentation.

All experimental species were found to co-exist under the specified laboratory conditions, however both positive and negative inter- and intra-specific interactions were recorded throughout the study. The intensity of negative (competitive) interactions was attributed to the degree of niche overlap between earthworms and was dependant upon the species present, their stage of development and ability to adapt to limiting environmental conditions. Some deep burrowing earthworm species were found to increase the production of smaller conspecifics and other smaller earthworm species. It is suggested that this type of positive (commensal) association arose from smaller individuals feeding on concentrated and easily digestible organic matter present in the castings of larger earthworms.

In addition to the potential applications of culture techniques described earlier, this research has contributed to the knowledge of an important ecological group, provided data for competition theory and should also prove valuable because of its implications for soil restoration.

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CHAPTER 1. INTRODUCTION

1.0 AN INTRODUCTION

Earthworms form a major component of the invertebrate fauna within fertile soils of

temperate regions. Their importance in soil formation has been recognised since the late

nineteenth century (Darwin, 1881). Research since then has demonstrated the physical

role of earthworms in improving the aeration, drainage and water holding capacity of

soils which leads to improved crumb structure. Earthworms are also known to play a

major role in increasing available nutrients for plants and other soil fauna, through the

decomposition of organic matter both within the soil and at its surface. Lavelle *et al.*

(1997) suggest that earthworms play a pivotal role in the soil ecosystem, influencing the

interactions between plant, animal and microbial components of soil biota. They

designated earthworms as soil "ecosystem engineers" (Jones *et al.*, 1994), able to

regulate processes to an extent that overrides organisms in other functional categories.

The irrefutable benefits to the soil gained from the presence of earthworms have resulted

in their deliberate introduction by man. Inoculation projects were initially undertaken to

supplement existing earthworm populations and promote the aboveground plant

production of pastureland and agricultural crops (*e.g.* Stockdill, 1966). Earthworm

inoculations were also undertaken into land devoid of earthworms, most notably by Rhee

(1969a; 1977) who introduced these animals into undeveloped soils of recently reclaimed

polders in the Netherlands. More recently earthworms have been used in restoration trials

of land degraded by man's industrial and mining activities (*e.g.* Rushton, 1986; Scullion

& Mohammed, 1991; Edwards & Bater, 1992). It is recognised that for earthworms to

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form an integral part of large-scale restoration at such sites there may be a need to establish a cost-effective and efficient method of rearing and introducing beneficial earthworm species (*e.g.* Butt *et al.*, 1993; 1995).

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Earthworms can be broadly classified into three ecological groups, anecic, endogeic and epigeic based on horizontal and vertical distributions, species size and feeding regime (Bouché, 1977). Earthworm species are therefore able to occupy different ecological niches allowing communities of up to 15 species to co-exist within the same habitat (Edwards & Bohlen, 1996). The observed physical and behavioural differences between species influences their suitability for use in land amelioration and restoration projects. Anecic and endogeic species, through the creation of vertical and horizontal burrows, are responsible for the majority of physical improvements in soil structure. In contrast, epigeic species live mainly in the surface organic layer and do not generally thrive in mineral soils, and therefore have little role to play in soil amelioration. Several epigeic species (*e.g. Eisenia fetida* and *Eisenia andrei*) are commonly used in commercial vermiculture and waste reduction processes. Research on the intensive culturing of selected epigeic species (*e.g.* Edwards, 1988; Edwards *et al.*, 1998) revealed that they possess high growth and reproductive rates, have wide temperature tolerances and can live in organic wastes with a range of moisture contents. This has led to their (often misguided) use in land restoration projects. In a review of the use of earthworms in land restoration in the UK, Butt (1999) suggested that only epigeic species that fall between litter dwelling (epigeic) and shallow working (endogeic) such as *L. rubellus* should be employed in soil inoculation projects.

In land restoration projects earthworms have been inoculated as single species populations (*e.g.* Craven, 1995) or mixed species populations (*e.g.* Curry & Cotton, 1983) often with little consideration given to the species composition. However, Lee (1995) suggested (supported by preliminary research (*e.g.* Butt *et al.,* 1999)) that the soil ameliorative properties of earthworms may be enhanced by the use of selected species introduced into reclaimed soils as mixed species inocula, in an appropriate manner and at an appropriate stage of restoration. In order to achieve the type of soil amelioration envisaged by mixed species inoculations, the ecology of chosen earthworm species and their interactions needs to be fully understood.

Darwin (1859) considered inter-specific interactions, in particular competitive interactions to evoke strong selection pressures and to be of widespread evolutionary importance in determining the species composition and abundance in many communities, a theory which is now being widely questioned (*e.g.* Hulley *et al.,* 1988a). Abbott (1980) recognised that in spite of this, "research on associations of earthworm species has neglected possible competitive interactions between species in yielding the observed associations". Instead, the composition of individual earthworm communities has been attributed entirely to the prevailing physico-chemical and biological factors. This is reflected in the available literature where, with the exception of Mishra & Dash (1979), Abbott (1980), Dalby *et al.* (1998a) and Butt (1998) there has been no research conducted which has focused primarily on interactions between earthworm species.

The laboratory-based research presented in this thesis will focus on assessing interactions between selected earthworm species, namely *Allolobophora chlorotica, Apporectodea caliginosa, Apporectodea longa, Lumbricus terrestris* and *Lumbricus rubellus* (2

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endogeic, 2 anecic and 1 epigeic species, respectively) All of these species have previously been employed in land restoration projects, indicating that information gained may prove valuable to practitioners in this sphere

1.1 AIMS

This research had four major aims :-

1) To assess competitive and / or beneficial interactions between selected earthworm species assessed in terms of survival, growth rate, maturation and reproduction.

    a) In terms of Competition : To assess the nature of direct interactions between earthworms with similar ecological niches.

    b) In terms of Mutualism : To establish if species pairings can co-exist and assess potentially mutualistic interactions between species with dissimilar ecological niches.

2) To determine the effect of varying selected environmental factors on observed interactions.

3) To provide further information on the life cycle and ecology of the selected species.

4) To suggest appropriate earthworm species combinations for use in land restoration schemes.

Specific objectives related to these aims are presented in appropriate sections of the thesis in association with experimental work.

1.2 THESIS OUTLINE

Chapter 2 reviews selected literature, initially covering, general earthworm ecology, their role in soil structure and fertility and the influence of environmental variables on earthworm behaviour. The major section of the review concentrates on the use of earthworms in land restoration, the development of inoculation techniques and also discusses the suitability of different species for soil amelioration. The final section of Chapter 2 examines the role of inter-specific interactions in determining community structure and provides examples of species interactions from published earthworm research.

Chapter 3 highlights the experimental parameters which influenced the development of experimental design and also describes earthworm culturing techniques which were used to establish laboratory-reared earthworm stocks.

Chapters 4 and 5 relate to specific experimental work undertaken in respect of specified aims (section 1.1). Appropriate objectives are stated at the outset of each set of experiments. The development of experimental design was in part driven by pre-determined goals but also took into account findings from initial and subsequent experiments. Discussion of the results gained from each experiment are initially provided in relation to specific outcomes, but the final discussion (chapter 6) draws together all of the outcomes from the programme and addresses the initial aims and objectives.

CHAPTER 2. LITERATURE REVIEW

2.0 INTRODUCTION

Research relating to the biology and ecology of earthworms is an extremely active and expanding area of scientific study. Darwin (1881), through simple observations, recognised the significant role of earthworms in soil formation, and posed some very fundamental questions. Subsequent research centred on physiological, morphological and behavioural studies, (reviewed by Satchell, 1983), but an increased awareness of the earthworm's role in soil fertility fuelled significant agricultural research (e.g. Evans & Guild, 1948). However, the last 20 years has seen an enormous expansion in earthworm research with the development of potential profit-related applications in vermiculture, organic waste recycling (*e.g.* Edwards & Neuhauser, 1988) and even as an economic source of protein (Edwards, 1985). In recent years applied research has also investigated the role of earthworms in land restoration (*e.g.* Scullion, 1992; Judd & Mason, 1995; Butt *et al.*, 1997), ecotoxicology (*e.g.* Edwards, 1983; Sturzenbaum *et al.*, 1998a) and environmental monitoring (*e.g.* Scott-Fordsmand *et al.*, 1998). Despite these undoubted advances in earthworm research many questions posed in Darwin's era still remain unanswered.

2.1 EARTHWORM ECOLOGICAL GROUPINGS

World-wide, some 3000 earthworm species have been described, of which only 28, excluding exotic introductions, are found in Britain (Sims & Gerard, 1985). Earthworm communities are separated into ecological niches by various vertical and horizontal distributions, alimentary specialisation and species size (Lavelle, 1983). It is therefore

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possible to sub-divide earthworm species into categories based upon these ecological and physical characteristics. Lee (1959) and Bouché (1971; 1972) independently recognised 3 main ecological groupings among New Zealand Megascolecidae and European Lumbricidae respectively. The latter employed three terms that are now commonly accepted: -

1. *Epigeic*: These earthworms are; small in size, have red colouration, show a high reproduction rate, are highly mobile and live above the mineral soil within the organic matter, *e.g. Eisenia fetida* (the brandling or tiger worm).

2. *Endogeic*: These species are; moderate to small in size, unpigmented, live permanently within the soil in horizontal burrows gaining nutrition from organic matter ingested with the soil (geophagous), have low reproduction rate, *e.g. Allolobophora chlorotica* (the green or stubby worm).

3. *Anecic*: These earthworms are; moderate to large in size, are normally coloured brown to black, highly contractile, soil dwelling, producing vertical burrows which open onto the soil surface where, usually at night, they feed on surface litter, *e.g. Lumbricus terrestris* (the lob worm or nightcrawler).

Bouché (1977) recognised that there were no sharp boundaries between these 3 ecological extremes and that many intermediate categories also existed. For example, Lavelle (1983) divided earthworms into 5 ecological categories: epigeic; anecic; oligo-; meso- or poly-humic endogeic).

It has been suggested that earthworm behaviour is not stable, populations are able to adapt to changing environmental conditions by displaying a plasticity in terms of their activities, fecundity, growth rate and mortality (Kretzschmar, 1998). Therefore as a population adapts to local environmental conditions it may acquire morphological and behavioural characteristics that differ from other populations of the same species. This ecological plasticity means that it is possible for populations of the same species to belong to different ecological categories. For example, in the Northern Hemisphere *Aporrectodea caliginosa* is a strictly endogeic species, making short disconnected burrows whereas in the temperate soils of the Southern Hemisphere the same species makes long vertical burrows characteristic of anecic species (Kretzschmar, 1998).

2.2 ENVIRONMENTAL FACTORS AFFECTING EARTHWORM DISTRIBUTION

Soil type, climate and organic resources in addition to historical land use and soil disturbance can influence the diversity of an earthworm community at a given location (Edwards & Bohlen, 1996) and in extreme circumstances may prevent earthworm establishment within a given habitat.

2.2.1 Temperature and Soil Moisture

Temperature and moisture conditions of soils are considered the most important environmental factors determining earthworm activity and distribution (*e.g.* Gerard, 1967). In the field, earthworm activity is strongly correlated with seasonal variations in both of these factors. In temperate regions surface cast production reaches a maximum in spring and autumn and may cease at other times of the year when soil temperature and moisture content are at the extremes of their range (Satchell, 1967). Ireland (1983) states

that in temperate climates, the maximum activity of soil dwelling earthworms occurs when the soil is between 4 and 11 °C and moisture content is high.

Water constitutes 75-90 % of earthworm body weight, a considerable part of this consisting of coelomic fluid and blood (Edwards & Bohlen, 1996), and is vital for homeostasis and maintenance of the hydraulic skeleton. Earthworms are very susceptible to dehydration, the cuticle is thin and permeable and species show no physiological ability to maintain a constant internal water content (*e.g.* Kretzschmar & Bruchou, 1991). Edwards & Bohlen (1996) suggested that *A. chlorotica* could lose 75 % of its total body water and still survive and concluded that most lumbricids can sustain water loss of 50 % with no ill effects. In adverse soil moisture (and temperature) conditions earthworms have developed a range of survival strategies. Certain species (e.g. *A. longa*) are able to enter a resting phase (diapause). During such periods the earthworm stops feeding and lines a small cell in the soil with mucus, rolls into a ball and enters an inactive state. This is an obligatory function; individuals enter this phase in May and cannot be aroused until the end of September / start of October (in temperate conditions). Other species are able to enter into a less permanent quiescent state (such as *A. chlorotica*, *A. caligionsa* and *A. rosea* (Evans & Guild, 1947)) in response to adverse conditions and become active once conditions become more favourable. Species may also migrate either horizontally or vertically to more favourable conditions and exhibit behavioural changes in response to drought. Satchell (1967) reported that *L. terrestris* reduced casting and surface activity and decreased cocoon production in response to drought. Parmelee & Crossley (1988) suggested that cocoons could act as the main survival stage during drought for some earthworm species such as *L. rubellus*, as all adults and juveniles may well die.

The impact of soil moisture on earthworm growth and reproduction is not governed by the amount of water present, but by the force with which the available water is held by the soil, often determined by soil type and texture. Evans & Guild (1948), demonstrated this by placing *A. chlorotica* in clays and gravely soils with a range of soil moisture contents. In the clay soil, maximum cocoon production was achieved at 28 % soil moisture whilst in the gravely soil maximum cocoon production was at 33 and 42 % moisture. This might seem anomalous but when these moisture contents were converted to pF values (a measure of the matric potential of a soil, which relates to the energy required to extract water from the soil) the figures were virtually identical.

Optimum soil moisture content can vary between species (as shown below by Daugbjerg, 1988) and even between spatially separated populations of the same species. For example, in Europe, *A. caliginosa* goes into diapause at a soil moisture content of 25-30 %, however in seasonally arid regions of Argentina this species is still active in soils with a moisture content of 15 % (reviewed by Edwards & Bohlen, 1996). In general earthworms are most active at soil water potentials approaching field capacity (-10 kPa), and activity declines rapidly as water potential exceeds 100 kPa and ceases for most species below the permanent wilting point (1500 kPa) (reviewed by Curry, 1998). Kretzschmar & Bruchou (1991) assessed the ability of *A. longa* to tolerate different soil water potentials over a 17 day period and identified certain critical thresholds for this species. At water potentials less than 60 kPa there was no effect on earthworm mass but above 620 kPa, *A. longa* entered into diapause and lost 40 % of original mass. Between these two extremes earthworm mass was closely governed by water potential with exchange of water between the soil and the earthworm reaching a maximum at 167 kPa. Using soil columns with continuous moisture gradients from 6 % at the top to 30 % at its

base, Daugbjerg (1988) determined the soil moisture preferences of *A. caliginosa, A. longa* and *L. terrestris*. Adult *A. caliginosa* exhibited a narrow range of preferred soil moisture levels from 18 – 22 % while *L. terrestris* spread out across the whole range showing preference for soil moisture content of 20 %. Daugbjerg suggests that the reasons for observed differences are due to the different activity patterns of the 2 species. *L. terrestris* is subject to dryer soil conditions as it feeds at the soil surface and does not enter a dormant state in adverse conditions unlike the geophagous *A. caliginosa*. Therefore *L. terrestris* is able to tolerate lower soil moisture contents than the endogeic *A. caliginosa*. The effect of soil moisture content is also dependent on the age structure of a population. In periods of high drought, populations may suffer heavy mortality, particularly if there are a large number of juveniles present which are unable to burrow deep within the soil and enter into a dormant state (Curry, 1998).

Lee (1985) suggested that temperatures in the range 10 – 15 °C were optimal for natural populations of Lumbricidae in Europe. In adverse temperature conditions earthworms have developed several survival strategies, as described previously. In high temperatures, earthworms are also able to maintain a lower body temperature by the evaporation of water from the body surface which in soils with a low moisture content can lead to desiccation. Daugbjerg (1988) observed soil temperature preferences for adults and juveniles of *A. caliginosa, A. longa* and *L. terrestris*. Earthworms were introduced at 10 °C into a continuous soil column with a stepwise temperature gradient which ranged from 0 to 20 °C. Approximately 40 % of *A. longa* and *A. caliginosa* adults preferred soil temperatures of 10-15 °C while 60 % of *A. longa* juveniles and adult *L. terrestris* preferred 10 °C (which concurs with the results obtained by Satchell, 1967). The differences in temperature preferences between adult and juvenile *A. longa* were seen as

an adaptation to the positions they inhabit within the soil profile. Juvenile *A. longa* live nearer to the surface and so are more vulnerable to drought (associated with higher temperatures) than adults (Daugbjerg, 1988).

The temperatures at which earthworms thrive and which they seem to prefer are not necessarily the same as those at which they either grow most rapidly or are most active (Edwards & Bohlen, 1996). The seminal paper of Evans & Guild (1948) showed experimentally, that changes in temperature can influence growth rates, maturation time, cocoon production and incubation times in earthworm species. Since the publication of this paper, the influence of temperature on earthworm activity has been widely studied. This research has become of more importance in the last 30 years due to implications in the intensive breeding of earthworms for waste management (*e.g.* Hartenstein, 1984; Edwards, 1988; Gestel *et al.*, 1992) and land restoration (*e.g.* Butt 1991). Optimum temperatures for growth, maturation and reproduction have been determined in several species (*e.g.* Löfs-Holmin, 1983; Daniel *et al.*, 1996). Butt *et al.* (1992) proposed that different life stages had different optimal temperatures. These researchers determined that the optimal environmental conditions for the intensive rearing of *L. terrestris* were different for each of three life stages: cocoon production (15 °C), cocoon incubation (20 °C) and hatchling growth (15 - 20 °C). The maintenance of earthworms at constant temperatures, above which they would normally encounter, can have negative effects on cocoon production and survival. Earthworms kept under such conditions can suffer from reproductive fatigue, experience high death rates and loss in body mass compared to earthworms kept under fluctuating temperature (Evans & Guild, 1948; Butt, 1991, Uvarov, 1995).

2.2.2 Organic Matter

Earthworm abundance is strongly affected by the availability of food resources, and the main source of organic matter available to earthworms in terrestrial ecosystems is litter from above ground plants. However, dead roots and rhizodeposition are also important (Curry, 1998). Baylis *et al.* (1986) also observed earthworms ingesting living roots. Within farming systems animal dung may also form an important, but localised, food source for several (usually epigeic) species and result in their aggregation under dung pats (Haukka, 1987; Hendrikson, 1991). Intensive livestock production techniques result in the production of large quantities of semi-liquid animal slurry. Slurry is initially toxic to earthworms (Curry, 1976), however once treated (allowing ammonia volatilisation) it is highly palatable to earthworms (*e.g.* Butt *et al.*, 1992). Earthworms are able to utilise organic matter in varying states of decomposition. Piearce (1978) analysed the gut contents of six lumbricid species obtained from permanent pasture in North Wales. *Lumbricus castaneus* and *L. rubellus* (epigeic species) were described as consumers of material rich in relatively undecomposed plant remains. *A. caliginosa* and *A. chlorotica* (endogeic species) were found to feed on well decomposed detritus while *A. longa* (anecic) and *Dendrobaena mammalis* [*Satchellius mammalis*] (epigeic) were intermediate in diet. The occurrence of root fragments in the diet of *A. chlorotica* and *L. rubellus* supports the view that some species may be root browsers.

Earthworm populations have often been shown to increase after the addition of organic matter to the soil. Curry (1976) conducted field experiments on the effect of various animal manures on earthworms in grassland. Single, annual applications of pig, poultry and cattle slurry were applied to experimental plots. After one year earthworm

populations were found to have increased by 52, 40 and 41 % respectively, compared to control plots. Löfs-Holmin (1983) demonstrated that earthworms, especially *A. caliginosa* and *A. rosea*, grown with a limited food supply, matured slowly and became dwarf adults, never attaining the same mass as regularly fed worms. Daniel *et al.* (1996) determined that earthworms deprived of food resources reduced their energy expenditure to maintenance costs to minimise weight loss and maximise survival time. Differences in maturation time of *L. terrestris*, between field and laboratory studies (more than 1 year and less than 6 months respectively at 10 °C) were attributed to differences in food resource availability. However, it is suggested that quality rather than quantity of a food resource most often limits population size (Satchell, 1967). *L. terrestris* have been observed to preferentially draw specific leaves into their burrows. Protein rich litter (high nitrogen content) was more readily taken than leaves containing less protein. The nitrogen content of a food resource is seen as a useful indicator of food quality when comparing widely different litter-types (Curry, 1998). Nitrogen-rich food resources have been shown to increase growth and reproduction in several species (*e.g.* Boström, 1988). The addition of nitrogen rich, spent brewery yeast to solid paper mill residues (a potential earthworm food source utilising waste materials) by Butt (1993), resulted in rapid growth rates of *L. terrestris* compared to earthworms fed on paper residues alone. However a high nitrogen content does not always ensure increased production rates. Boström & Löfs-Holmin (1986) observed the growth rate of *A. caliginosa* fed on meadow fescue (*Festuca pratensis* L.) and barley (*Hordeum distichum* L.) roots. Despite having a similar protein and crude fibre content, earthworm growth was eleven times greater when fed barley compared to meadow fescue roots. It was suggested that fescue roots might contain compounds that retard growth (*e.g.* polyphenols). Therefore organic matter may require an initial weathering period before it becomes an acceptable food source. Plant

litter may contain high concentrations of distasteful polyphenolic substances including catechin which is a known earthworm repellent (Satchell, 1967). Micro-organisms that are able to oxidise phenols and also increase the nutritive value of the food source (Cooke & Luxton, 1980) can assist the process of weathering.

Food particle size has also been shown to affect both species growth and reproduction (*e.g.* Boström & Löfs-Holmin, 1986; Boström, 1988). Boyle (1990) demonstrated experimentally that the extent to which earthworm growth was influenced by food particle size was also species specific. Boyle reared juvenile *A. caliginosa* and *L. terrestris* in a mixed culture of a peat/mineral soil with either chopped (8 mm pieces) or milled (< 1 mm) ryegrass. *A. caliginosa* achieved higher growth rates on the milled ryegrass, whereas *L. terrestris* showed no significant differences for growth, between particle size treatments.

The location of food within the soil profile can also affect species abundance, growth rates and behaviour. Boyle (1990) observed that both *L. terrestris* and *A. caliginosa* grew more rapidly when food was applied at the soil surface of experimental cultures compared with intimate mixing in the soil profile. It was proposed that surface applied food represented a more easily located and concentrated source even for the endogeic species, *A. caliginosa*. Agricultural tillage practices will influence the location of residue-food (Staricka *et al.,* 1991). Cook & Linden (1996) mimicked 3 tillage practices within the laboratory: - 1) no-till; most of the residue is present on the soil surface, 2) Mold-board ploughing; this buries the surface residue near the bottom of the plough layer and 3) Disking; the residue is uniformly mixed within the plough layer. The effect of food placement on the burrowing pattern and behaviour of *Aporrectodea tuberculata* may

influence the preferential transport of water and chemicals within the agricultural ecosystem. Random burrowing was observed until the feed resource was located at which point burrowing then centred around the food resource.

Organic matter, within and upon the soil surface, therefore has a marked influence on earthworm distribution and abundance. However the quality, quantity, position in the soil profile and particle size and combinations of these factors (possibly in further combination with others) ultimately determine the earthworm species present.

2.2.3 Soil Type

Several authors (*e.g.* Nordström & Rundgren, 1974; Al-Yousuf & Shoreit, 1992; Hendrix *et al.*, 1992; Baker *et al.*, 1998) have shown a strong correlation between earthworm abundance and soil texture. In all cases, increases in earthworm populations were positively correlated with soil clay content. Baker *et al.* (1998) surveyed 163 pastures in Western Australia for the presence of the introduced lumbricid species *Aporrectodea trapezoides*. They also established a field trial in South Australia in which varying amounts of clay were added to a non-wetting sandy soil beneath pasture. The clay content of soils positively influenced the abundance and biomass of *A. trapezoides*. It was proposed that observed increases in biomass were a function of improved water holding ability and cation exchange capacity exhibited in soils with a higher clay content and not just the soil *per se*. This corroborates work in Egypt by El-Duweini & Ghabbour (1965) who observed that the abundance of *A. trapezoides* decreased with increasing amounts of gravel and sand in soils, but that this decrease could be off-set by increasing soil water content.

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In terms of responses to soil pH, earthworms species can be classified as acid intolerant, acid tolerant or ubiquitous (*i.e.* those that can tolerate a wide pH range) (Satchell, 1955). Bouché (1972) related the distribution of 67 taxa of Lumbricidae in France to pH. He concluded that most species of European Lumbricids exhibited a wide range of tolerance for varying soil pH but preferred soil with a pH around neutral. Some 26 (acid tolerant), species were, however, found in soils with a pH below 4.0 and four (acid intolerant) species were found only in soils with a pH above 6.6. Soil pH is related to, and a function of other soil factors such as clay content and cation exchange capacity (Edwards & Bohlen, 1996). It is therefore difficult to attribute differences in population size and diversity with soil pH, except where populations are inhibited by extremely high or extremely low hydrogen ion concentrations. Earthworm species also exhibit differences in the pH of their mucus secretions (Schrader, 1994). These mucus secretions have been shown to change the local pH of the substrate in which earthworms were placed.

Increasing the bulk density of a soil causes an increase in soil strength and decreases in both air permeability and hydraulic conductivity. The continued compaction of soil affects its ability to absorb high-intensity rainfall and can lead to anaerobic conditions (Whalley *et al.,* 1995). Increasing the bulk density of soils has been shown experimentally to affect burrowing ability and cast production in earthworms (Rushton, 1986; Joschko *et al.,* 1989; Kretzschmar, 1991). Cast production is known to be positively correlated with bulk density until soil strength limits burrowing activity. Kretzschmar (1991) suggested for *A. longa*, that increased cast production is a function of changes in burrowing behaviour. At low bulk densities *A. longa* is able to burrow into the soil by pushing soil particles aside. However, at higher bulk densities burrowing is achieved by ingesting the soil, resulting in increased cast production to a point where

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earthworms are constrained by the mechanical strength of the soil. Rushton (1986) determined that *L. terrestris* was able to burrow into soils up to a bulk density of 1.6 g dry mass cm^{-3} although Wendt (1988) found that *L. terrestris* was able to burrow in soils up to 1.73 g dry mass cm^{-3}. In compacted soils (as with soil texture and pH) it is the combination of several factors, in this case probably bulk density, water content and oxygen supply, that affect earthworm activity (Whalley *et al.*, 1995).

2.3 THE EFFECT OF EARTHWORMS ON SOIL STRUCTURE AND FERTILITY

The activity of any organism influences the environment in which it lives. Lavelle *et al.* (1997) described earthworms, based on their ability to move through the soil and build organo-mineral structures, as soil "ecosystem engineers" (Jones *et al.*, 1994). They proposed that structures created by these macrofaunal engineers promote and constrain soil processes on a range of spatial and temporal scales through: -

1) Nutrient mineralisation

2) Physical stabilisation

3) Stabilisation of organic matter, nutrient conservation and ultimately pedogenesis.

There is little doubt that through these processes earthworms can improve soil structure and fertility (Lee 1985; Edwards & Bohlen, 1996). The nature and extent of such changes is however dependant on the species present and how they function ecologically (*e.g.* Springett, 1983; Shaw & Pawluk, 1986). This is a very active research area with implications for both agriculture and land rehabilitation.

2.3.1 Soil Aeration and Drainage

Earthworm burrows (biopores) are roughly cylindrical in shape with lightly compacted walls and / or at least several coatings of secreted mucopolysaccharides (Kretzschmar, 1998). Lee (1985) described three principal forms of earthworm burrow: -

1) Vertical burrows (formed mainly by anecic species) sometimes with branching near the surface.

2) Burrows of geophagous species in the sub-surface horizons that are predominantly horizontal with some surface openings.

3) More or less vertical burrows made by surface living (epigeic) earthworms as a retreat during cold or dry seasons.

Burrow formation, cocoon deposition and quiescent chambers within the soil are known to increase pore space (Satchell, 1967), thus increasing soil aeration. The extent to which burrowing influences the pore space is dependent on soil type and environmental conditions. Earthworm burrows are consolidated structures; they can stay open when soil moisture content is high and when clay swelling has closed down most of the cracks (air filled porosity at its lowest). In such conditions it is estimated that burrows might represent approximately 20 % of air filled space (Kretszchmar, 1998)

Burrows can play a major role in improving water infiltration in soils (Joschko *et al.*, 1992; Knight *et al.*, 1992). Pitkänen & Nuutinen (1998) investigated the contribution that earthworm burrows made on infiltration rates and surface run-off in a 15 year old tillage experiment. Reduced tillage practices were found to enhance earthworm activity (especially deep burrowing *L. terrestris*) which led to an increased number of continuous

macropores in the soil profile, enhancing infiltration by bypassing the soil matrix and reducing surface run-off in the silty clay loam soil. In long-term, no-till watersheds at Coshocton, U.S.A., Edwards *et al.* (1988) estimated (from burrow characterisation data) that there were 1.6×10^6 *L. terrestris* burrows ha^{-1}. These estimates alongside water sampling equipment established in burrows, allowed for the accurate measurement of water quality and quantity flowing into *L. terrestris* burrows. During 12 summer storms in 1987 it was estimated that 4 % of the total rainfall and 711 g NO_3-N ha^{-1} were transported into earthworm burrows. The degree at which water will flow down burrows depends on soil moisture status, rainfall intensity, soil type, residue cover and micro-relief (Edwards & Bohlen, 1996). Under certain conditions a single macropore (burrow) can dominate water movement (Edwards & Shipitalo, 1998). Hoogerkamp *et al.* (1983) observed large increases in water infiltration in Dutch Polders reclaimed from the sea, 8 - 10 years after the introduction of earthworms. Water infiltration rates measured over a 24 hour period were up to 136 times greater in earthworm inoculated plots, than in control plots without earthworms. Edwards & Bohlen (1996) suggested that this is an extreme case resulting from the unstructured soils present on the recently reclaimed land.

It is also suggested that earthworm burrows facilitate increased root growth by providing channels within the soil along which roots are able to grow with minimal resistance (Springett, 1985). These channels often have a lining rich in organic matter and available plant nutrients providing ideal conditions for root growth (Edwards & Shipitalo, 1998). However, there is no evidence that roots grow preferentially towards burrows or entered holes filled with earthworm casts (Hirth *et al.*, 1997; Kretszchmar, 1998).

2.3.2 Incorporation of Organic Matter Within the Soil

The role of earthworms in incorporation of organic matter into the soil system is related to their size and ecological grouping (section 2.1). In general terms earthworms enhance incorporation of organic matter into the soil and its turnover. This is achieved by either physically dragging plant litter from the soil surface via burrows into the soil, or by the production of casts containing high levels of comminuted organic matter within the soil or at its surface. The action of burrowing within the soil also assists in mixing of soil layers. Springett (1983) studied the casting and burrowing of 5 species of Lumbricidae within the laboratory and determined the diurnal pattern of surface burrow production, the length of time burrows persist and the mixing of lime within the soil profile. *A. longa* (anecic) was found to mix the soil vertically while *A. caliginosa* (endogeic) and *L. rubellus* (epigeic) mixed the soil horizontally and showed a marked degree of burrow site tenacity, which is unusual for these species which are thought to only inhabit temporary burrows. Shaw & Pawluk (1986) proposed that anecic species such as *L. terrestris* have a primary role in structural development of the soil by initiating contact between organic and inorganic constituents. This anecic species was shown to enhance the production of organic constituents that firmly bind soil components together, resulting in the fusion of the soil matrix. These changes are often localised to the linings of a single permanent burrow and as deposits (castings) on the soil surface. Endogeic species are active throughout the upper layers of the soil, causing homogenisation of the substrate, but have little contact with surface litter and are seen as having only a secondary role in structural development. Species position within the soil profile and their behaviour also influence the structure of their casts. Fifty percent of the total carbon measured by Jégou *et al.* (1998) in surface casts of epigeic and epianecic species was derived from surface litter,

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compared to forty percent in anecic and endogeic species. Litter carbon enrichment of burrow linings was high and constant whatever the depth; 43.3 % for *L. terrestris* (considered to be epianecic) whereas it was lower and tended to decrease with depth with *A. caliginosa* (endogeic) and *Aporrectodea giardi* (anecic).

2.3.3 Formation of Water Stable Aggregates (WSA)

Soil aggregates are formed by the adhesion of mineral granules and soil organic matter into structures of varying size, which resist breakdown when exposed to internal or external stress such as wetting. Soil particle aggregation forms a granular, friable soil improving water infiltration, water holding capacity, porosity and aeration (Edwards & Bohlen, 1996). Earthworms are not essential for formation of well-aggregated soils, but through casting and lining of burrows earthworms are known to contribute to the proportion of water stable aggregates within the soil. The influence of earthworms is dependent on species present (Shaw & Pawluk, 1986), soil type (Schrader & Zhang, 1997) and organic matter quality (Shipitalo & Protz, 1988).

Flegel *et al.* (1998) conducted laboratory experiments involving *L. terrestris, L. rubellus* and *Dendrobaena octaedra*. These three species were offered dandelion, lupin, rye, alder, beech and larch leaves in order to determine the effects of different food sources on physical and chemical cast properties. Cast production was strongly influenced by plant species, and only cast production by the anecic *L. terrestris* remained relatively constant. For *D. octaedra* cast production was negatively correlated with the C:N ratio of the food source. A deteoriation in food source quality resulted in higher soil consumption (to obtain necessary nutrients) and was followed by higher cast production. It was suggested that the nutrient content of casts influenced the proportion of water stable aggregates. In

this experiment water stable aggregates were highest in casts of earthworms fed dandelion leaves and lowest when fed alder. The proportion of water stable aggregates present in the casts of the three species was also different. Casts of *D. octaedra* had the highest percentage of water stable aggregates while *L. terrestris* had the lowest. The proportion of aggregates present in the casts was positively correlated to the carbon content however, the influence of micro-organisms during passage through the gut is also thought to be important and species specific. Micro-organisms are believed to play a key role in stabilising aggregates (Coleman & Crossley, 1996) therefore increased levels of micro-organisms will increase aggregate levels. It is suggested that species specific intestinal conditions may cause different development of microflora within the earthworm guts and so result in observed differences in the percentage aggregates present in different species casts.

The role of earthworms in the maintenance of soil structure under field conditions was investigated by Blanchart *et al.* (1997). The study was conducted in a shrub savannah in the Côte d'Ivoire where four treatments were established:- 1) natural earthworm community (control), 2) presence of *Millsonia anomala* (large species) only, (3) presence of an eudrilid earthworm species only, 4) absence of earthworms. Aggregate stability, size distribution and soil porosity were measured after 6, 13 and 28 months. After 28 months significant differences in all 3 measurements were detected between the four treatments. In the *M. anomala* addition treatment, there was a rapid increase in the proportion of coarse aggregates (> 5 mm) in the upper 15 cm of soil, compared with the control. In the Eudrilidae addition and no earthworm treatments, coarse and very coarse aggregates (> 5 mm) had been destroyed. There was a significant increase in intermediate aggregates (0.63 – 5 mm) in the Eudrilidae treatment and of small aggregates (< 0.5 mm)

in the no earthworm treatment. Soil porosity and structural porosity also increased. *M. anomala* were responsible for the formation of macro-aggregates and large macro-pores, their casts were strongly compacted and resulted in low porosity in soils when abundant. However, the smaller eudrilid earthworms favoured the destruction of the larger species casts, shortening cast life span and produced finer less compacted casts. In this environment it would therefore appear that soil structure was most improved in soils with a range of earthworm species. Shaw & Pawluk (1986) also observed the relationship between earthworm species in the improvement of soil structure. These workers observed a synergistic effect upon soil structural development in laboratory experiments, initiated by *L. terrestris* (anecic) and enhanced by geophagous species. Withdrawal of litter from the soil surface by *L. terrestris* increased the availability of organic matter to the geophagous species, which ingested this extra food source and transported it into the bulk of the soil.

2.3.4 Nutrient Mineralisation: Relationships With Micro-organisms

Interactions between earthworms and micro-organisms are of major importance in degradation of organic matter and release of mineral nutrients into the soil (Edwards & Fletcher, 1988). The exact nature of the relationship is not fully understood and it has been suggested that earthworms depend on micro-organisms solely as feed. However it is more likely that earthworms and micro-organisms also exhibit a mutualistic relationship involving the breakdown of organic matter and the releases of nutrients either in the earthworm digestive system or within cast material and burrow linings. Mutualistic digestive systems have been described in several endogeic species from both tropical and temperate biomes (Lavelle *et al.*, 1997). In the anterior region of the gut the pH is neutral, water content is high and there are large quantities of secreted intestinal mucus which can

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be readily utilised by free living micro-organisms. In the middle intestine mucus secretion stops and mucus that has not been degraded is reabsorbed. In the earthworm gut the degradation of soil organic matter by micro-organisms occurs at a much greater rate (30 fold) than in the field at the same temperature. Organic matter excreted in the casts is composed of fully comminuted, but otherwise negligibly altered plant residues (Ziegler & Zech, 1992). This provides a greater surface area for degradation and subsequent release of nutrients by micro-organisms which are present in significantly higher numbers compared with the surrounding soil. During the progress of ingested substances through the earthworm digestive system there is a dramatic increase (up to 1000 fold) in the number of micro-organisms (Edwards & Fletcher, 1988). Edwards & Lofty (1977) and Lee (1985) have documented the enrichment of earthworm casts with available nutrients compared with the surrounding soil. Allen *et al.,* (1998) assessed the microbial uptake and turnover of carbon and nitrogen in the middens of *L. terrestris*. Their results suggested that the microbial pool in midden soil incorporated and respired carbon more quickly than the non-midden microbial pool.

2.3.5 Influence on Plant Production

The introduction of earthworms into pastureland in New Zealand has resulted in considerable increases in plant production (*e.g.* Stockdill, 1982; Springett, 1985), brought about by incorporation of surface organic matter, lime, fertilisers and insecticides and the improvement of soil structure allowing greater root development. Rhee (1977) studied the effect of earthworm inoculation into apple orchards located on worm free polders in the Netherlands. Increases in fruit yield in inoculated plots were small (2.5 %) compared to control plots, while the density of thin and thick roots was significantly higher in inoculated plots. As in the pastures of New Zealand, improvements in plant production

were associated with increased incorporation of organic matter and increased aggregate stability. Brown *et al.,* (1998) conducted 16 experiments (in 6 countries) investigating the effects of earthworm inoculations on plant production both at greenhouse and field scale over a 7 year period. Plant growth stimulation was observed in 72 % of all cases. The mechanisms involved ranged from large-scale effects on soil physical properties to the microsite level, where earthworms enhanced microbial activity, nutrient availability and rhizosphere processes.

The effect of earthworms on plant growth has also been shown to be species specific. Derouard *et al.* (1997) studied the effect of 3 selected earthworm species (*Millsonia anomala, Chuniodrilus zielae* and *Hyperiodrilus africanus*) on the growth of 3 food crops (rice, peanuts and maize). The peanut plants showed no response, whereas maize had increased above ground production and reduced root production, and rice produced more roots in the presence of earthworms. Earthworm species combinations were also shown to exhibit different effects on plant growth. The pairing of *M. anomala* and

C. zielae provided the largest improvements in plant production whereas either *C. zielae* or *H. africanus* alone had little effect. Such species specific responses have also been shown in the growth of birch seedlings by Haimi *et al.,* (1992) and Haimi & Einbork (1992). The presence of the epigeic species *L. rubellus* caused an increase in above ground biomass and a 2 fold increase in the concentration of nitrogen in the leaves. In the presence of the endogeic *A. caliginosa* the seedlings again showed increased above ground plant production but there was no increase in the nitrogen concentration of tissues. The production of casts has been shown to directly effect root growth by Springett & Syers (1979). Casts of *L. rubellus* have been demonstrated to override the natural geotropism of ryegrass seedling roots, which grew upwards into casts on the soil

surface. This result was not seen when seedlings were grown in the presence of *A. caliginosa*, but the mean shoot length of seedlings grown in the presence of casts on the soil surface was greater than that of plants grown alone for both species treatments. The authors suggested that the observed root responses were a result of the presence of an auxin-like substance or a substance that reacts with natural auxins of the plant. Plant-growth substances have been detected and identified as indole compounds by Nielson (1965) in extracts from *A. caliginosa, A. longa, L. rubellus, L. terrestris, E. fetida* and *D. rubida*. It was determined that the active component was different for each species.

2.4 THE ROLE OF EARTHWORMS IN SOIL RESTORATION

The beneficial effect of earthworms on the physical and chemical nature of soils is well established (*e.g.* Edwards & Bohlen, 1996). Through their burrowing activities, anecic and endogeic species increase rainfall infiltration rates, improve soil aeration and allow greater root development. Casting increases the proportion of water stable aggregates, improving soil water holding capacity and developing more friable topsoil. The incorporation of organic matter through casting and surface removal of litter, and its mixing with the mineral soil also leads to enhanced nutrient availability. The role of earthworms in soil fertility has led to their introduction by man, with varying degrees of success, into areas of land often lacking earthworms in attempts to increase plant production in agricultural land, and enhance soil amelioration in degraded land.

In New Zealand pasture improvement associated with earthworm activity has been recognised since the 1940's (reviewed by Stockdill, 1982). The majority of the 192 species described by Lee (1959) are native to New Zealand and are associated mostly with native vegetation with the exception of 14 introduced species of the Lumbricidae.

However, several of these native species are found in sown pastures but they afford no beneficial effects to pasture production (Stockdill, 1966). It is the introduced lumbricids, in particular *A. caliginosa,* which have been responsible for recorded improvements in soil structure and plant production.

The introduction of beneficial species was initially achieved by gathering earthworms and placing them directly onto the soil (broadcast method). Later turves were cut from sites with large established populations and dug into the experimental sites. Further trials led to turves being placed onto the soil surface at fixed distances to achieve a uniform cover with lime applied to ensure calcium levels were adequate for establishment. As the turves dried out the earthworms migrated into the moister soil beneath. This led to the development of a machine that was able to cut turves and apply lime, enabling introductions to occur on a large scale. In unpopulated pastures it was estimated that an increase in carrying capacity of 2.5 stock units ha^{-1} could be achieved 6 - 7 years after earthworm inoculation and that pasture production was increased by over 70 % (Stockdill, 1982).

Springett (1985) recognised that soil structure and plant production could be increased further by the introduction of *A. longa.* In adverse soil moisture conditions plant production was often reduced as their roots were restricted to the top 10 cm of soil, the activity zone of earthworm species like *A. caliginosa.* The deeper burrowing *A. longa* was introduced into experimental pasture plots and was found to increase surface infiltration rates, total soil porosity at 10 - 15 cm and root biomass at 15 - 20 cm soil depth. This species was also responsible for increased surface casting and greater mixing of surface applied lime. In Southern Australia *A. longa* is currently being considered for

widespread inoculation into agricultural areas of high rainfall because of its stated beneficial effects in pasture production (Dalby *et al*, 1998b). However, there are concerns about the impact of this exotic species on native woodland ecosystems. Initial experiments by Dalby *et al* (1998b) suggest that *A. longa* prefers pastureland and had no significant effects on the reproduction growth and survival of the native species *Gemascolex lateralis*. Projects are also underway to restore populations of beneficial populations in agricultural land where they have been greatly reduced due to agricultural practices. Kladivko *et al* (1998) found that row cropped fields in the corn belt of the United States were devoid of *L. terrestris*, a species now common in pastures and undisturbed land in the region. In the past these fields were regularly moldboard ploughed, which is believed to have reduced the populations of this deep burrowing species. Many farmers have now changed to a no-till policy and *L. terrestris* have been introduced into these fields and are establishing viable populations.

Stockdill (1982) also recognised undesirable effects of introduced earthworm's activity in pasture. The removal of the surface organic mat and increased casting on the soil surface resulted in an increased soil intake by livestock increasing teeth wear. Increased infiltration rates can also lead to increased leaching. Stockdill (1966) also recorded that after many years of earthworm activity pH and calcium levels were lowered necessitating increased levels of liming.

In the Netherlands large scale earthworm inoculation by Rhee (1969a), in undeveloped soils of newly reclaimed polders in Eastern Flevoland were an attempt to assess the effect of earthworm activity on soil productivity. In an initial trial *A. caliginosa* and *L. terrestris* were inoculated into apple orchards. This site was chosen due to the lack of

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disturbance in orchards and the expected increase in root growth and yield associated with soil improvement (*e.g.* Rhee, 1969a; 1977). In earthworm inoculated plots aggregate soil stability increased by 70 % and root density was also increased. In an earlier inoculation *A. caliginosa* and *A. chlorotica* were inoculated in grassland (Rhee, 1969b). In this study the expansion, dispersal and natural immigration of earthworms was also established. *A. caliginosa* was found to have a higher expansion and more rapid dispersion (6 m year) than *A. chlorotica* (4 m year) with *L. rubellus* the first species to naturally colonise the site.

Within the last 20 years increased attention has been focused upon the efficient and cost-effective restoration of degraded land caused by large-scale man-made changes in the landscape. Such activities include surface mining and civil engineering works. These two processes often result in the removal, storage and reinstatement of high-grade agricultural land (Samuel, 1990). It is therefore important that land be restored to its pre-working quality. Scullion (1992) indicated that to prevent reinstated disturbed land deteoriating the restoration of soil biological functions should be a primary objective. Initial mechanical disturbance of topsoils during removal and its subsequent storage (often on site) for between 2 and 10 years, results in structural damage and the build up of anaerobic conditions (*e.g.* Hunter & Currie, 1956; Scullion *et al.,* 1988) which can seriously deplete earthworm populations. On reinstatement, such populations that exist can be further reduced by cultivation and ripping operations aimed at alleviating compaction (caused by earthmoving machinery) and the removal of large stones and other obstructions (Scullion, 1992). Therefore the development of viable earthworm populations relies on; reproduction by individuals that have survived reclamation, natural colonisation of the site from the surrounding areas, or deliberate introduction by man.

Rushton (1986) studied the earthworm populations on pastureland reclaimed from open-cast mining in Northumberland, UK within 15 years of reclamation. On a site one year after reclamation no earthworm populations were found. In subsequent annual monitoring, populations of *L. rubellus* and *A. chlorotica* were detected but at densities far lower than those found at the 15 year old site and unmined control sites. At this older site populations were dominated by *A. longa* and *A. chlorotica* at densities higher than control sites suggesting that earthworm populations are able to recover quite rapidly after disturbance. One explanation for this rapid recovery is the application of high levels of organic material. The addition of organic fertiliser into agricultural soils has been shown to increase earthworm populations (Noble *et al.*, 1970; Edwards & Lofty, 1978) and subsoiling of semi-organic fertiliser (Scullion & Mohammed, 1991) resulted in increased *L. rubellus* populations and accelerated structural rehabilitation at depth soils restored from opencast mining.

The establishment of viable earthworm populations at restored sites is to a great extent dependent on cost and proposed after-use. Many sites are restored lacking topsoil; the resulting cover is deficient in both organic matter and a resident fauna. In the case of landfill sites the material may also be deliberately compacted (Butt *et al.*, 1997) while at other industrially reclaimed sites the land may be contaminated with substances toxic to earthworms resulting in the death of introduced populations (Bain *et al.*, 1999). At such sites natural colonisation may be very slow (Judd & Mason, 1995) and the inoculation of earthworms at an appropriate stage of restoration may enhance soil amelioration. Satchell & Stone (1977) monitored the survival of *L. terrestris*, *A. longa* and *A. chlorotica* in Pulverised Fuel Ash (PFA) a by-product of coal-fired electricity generating power stations which is dumped on the land and restored to agricultural use. They determined

there to be a high mortality of earthworms in both fresh and recently deposited (up to 7 years old) PFA. Survivorship comparable with soil, was only established in PFA after 20 years indicating the importance of timing in earthworm inoculation.

The method of inoculation is also important in determining the survivorship and successful colonisation of introduced populations. Most inoculation projects (*e.g.* Marfleet, 1985) employed the broadcast method of inoculation in which earthworms, generally field collected, are spread (by hand) over the soil surface. Therefore at the point of inoculation earthworms are vulnerable to predation by birds and the prevailing environmental conditions, both of which may severely affect the chances of establishing a viable earthworm population at the inoculation site. Butt *et al.* (1995) developed an inoculation technique designed to ensure effective long-term colonisation, particularly in hostile soil environments. Four mature *A. longa* were cultivated in cylindrical plastic bags filled with 2 litres of topsoil and 150 - 200 g of separated cattle solids as a feed source. In the spring of 1992 eighty eight of these earthworm inoculation units (E.I.U.) were inoculated at a partially restored landfill site in Buckinghamshire. The bottom of the bags was split and the intact contents were manually placed into drilled holes within the cap. This method ensures that initial populations are provided with a "safe haven" from the surrounding hostile conditions and therefore maximises the chances of colonisation. Subsequent sampling utilising a variety of methods has shown that *A. longa* has established viable populations and individual numbers have at least doubled (Butt *et al.*, 1999). However, due to the compacted nature of the cap, spread has been slow, 0.5 m yr.$^{-1}$, compared with natural immigration rates of approximately 5 m yr.$^{-1}$ observed by Brockman *et al.* (1980) and predicted by Marinissen & Van den Bosch (1992) in a simple dispersal model.

2.4.1 Species Associations: Suitability For Regenerating Sites

Certain earthworm species tend to be associated with one another. Usually, such associations result from some characteristic of the habitat (section 2.2) (Edwards & Bohlen, 1996). For example *A. chlorotica* and *Eiseniella tetraedra* are very resistant to disturbance and may occur in wet or waterlogged soils (Sims & Gerard, 1985) and are therefore often found on poorly drained restoration sites (Satchell & Stone, 1977; Brockman *et al.*, 1980; Butt *et al.*, 1999).

The review by Tamis & Udo de Haes (1995) (Appendix II & III) indicates common species associations in agricultural land, natural grasslands and restored sites. Species diversity is greatest in natural grassland while epigeic species along with *A. chlorotica* and *A. caliginosa* dominate restored sites. Such species are considered to be pioneers and are therefore important in recolonising restored land (Rhee, 1969a; Rushton, 1986; Judd & Mason, 1995). A species predisposition to soil environmental conditions, rate of dispersal and fecundity will determine which species are initially dominant. Dunger (1969) observed species succession on restored coal mining dumps in Germany, where colonisation was closely linked to vegetation succession. Initial colonisation by pioneer species such as *A. caliginosa* coincided with the development of a herb layer and the formation of a litter layer. As a shrub layer formed and the litter layer developed large populations of epigeic species (*e.g. L. rubellus*) were recorded. Anecic populations only became established once there was tree cover and further immigration of species occurred as woodland developed, 20 - 25 years after the initial restoration.

The late appearance of anecic species in disturbed and degraded land (especially *L. terrestris*) has been observed by several authors (Standen *et al.*, 1982; Rushton, 1986; Judd & Mason, 1995). Luff & Hutson (1977) proposed that adverse soil conditions at depth, prevented colonisation of reclaimed land by these deep burrowing species. However they are also sensitive to soil disturbance and display k-selected characteristics (Satchell, 1980), such as low growth rates and fecundity that predisposes these species to more stable environments. Epigeic and endogeic species exhibit far greater dispersal rates and fecundity (r-selected) enabling them to adapt to more unstable habitats. Certain species (*e.g. A. caliginosa*) are also able to enter into a quiescent state if environmental conditions become limiting and so are present within the soil when conditions improve. Lee (1985) stated that a lack of understanding of earthworm ecology has led to the widespread introduction of the easily reared epigeic species *E. fetida* as a soil improver in New Zealand agricultural and pastoral land. Despite their ability to colonise unstable environments epigeic species play only a small role in soil formation and fertility. It is to a great extent the soil dwelling species and in particular the deep burrowers that are influential in soil amelioration and is the reason for their use, with varying degrees of success, in many land restoration projects (*e.g.* Rhee, 1969a). If adverse environmental conditions exist within degraded soils, inhibiting earthworm establishment, then it is possible to alter the environment to facilitate earthworm survival. The addition of lime counteracts low soil pH, and has been used in Scandinavia to increase the pH in coniferous forests exposed to acid rain (Rundgren, 1994). However, in large-scale restoration projects liming may prove prohibitively expensive (Judd & Mason, 1995) and the development of extensive plant cover and an associated root mat may prove more beneficial. Addition of organic matter where vegetation is sparse will provide a food supply for earthworms and also helps to stabilise soils (Scullion & Mohammed, 1991).

The quantity and quality of available earthworms limits the scale of inoculation projects. The majority of introductions have relied on field collection of earthworms, which has drastically limited its potential. Brun *et al.* (1987) realised that for the role of earthworms in land restoration to progress there was a need for the intensive production of appropriate soil dwelling species. The research into vermiculture had previously centred on epigeic species and their role in the breakdown of organic wastes (*e.g.* Edwards, 1988), little was known about the intensive production of soil dwelling species (with the exception of *e.g.* Löfs-Holmin, 1983; Butt *et al.*, 1992). Butt *et al.* (1992) looked at the potential of intensively cultivating *L. terrestris* for use in soil amelioration by manipulating all stages of the life cycle. These authors concluded that continuous production of this species was possible and at levels much higher than recorded in the field. Further studies investigated the laboratory production of *A. longa* and *Octolasion cyaneum* (Butt, 1993) and *A. chlorotica* (Butt, 1997) which allowed for the introduction of mixed species inocula, able it is suggested, to enhance ameliorative properties (Butt *et al.*, 1999). Further research into the intensive cultivation of soil dwelling species is required if it is to become economically viable.

2.5 SPECIES INTERACTIONS

Inter-specific interactions are important in determining species composition and abundance in many communities (*e.g.* MacArthur, 1972). All populations interact in some way with populations of other species (Abrams, 1987). Interactions occur whenever properties or actions exhibited by individuals of one population alter some characteristic within another population. The outcome of interactions is not fixed and will vary as environmental conditions change, populations vary in structure and individuals within a population vary in the expression of traits. Interactions can be described in general terms, based on the mechanism involved, as antagonism, commensalism or mutualism (Thompson, 1988).

Darwin (1859) first introduced the idea of inter-specific competition to explain the increase of one species linked to the decline of another related species resulting in competitive exclusion. He considered inter-specific competition to be a strong selection pressure and of widespread evolutionary importance (Hulley *et al.*, 1988b). Since then competition theory has been developed and has become virtually a unifying principle explaining the structure of ecological communities. Differences between species were looked upon as an evolutionary response to inter-specific competition allowing co-existence. The concept of resource partitioning (MacArthur, 1958; Hutchinson, 1959; Schoener, 1965) in which species sub-divide resources within a community, so that each is limited by a different factor, was seen as an evolutionary response to the selection pressures generated by inter-specific competition. However, the importance of inter-specific competition in ecology and evolution is now being questioned. Observed species characteristics, regardless of why they evolved, were upheld with little or no justification

as features that lessen the impact of competition (Walter, 1991). Hulley *et al.* (1988a) suggest that inter-specific competition is a weak, localised or sporadic ecological process unlikely to generate strong selection processes. The likelihood of co-evolution occurring between two competing species depends on the similarity of their resource requirements and how often they meet. Therefore co-evolution is only likely to occur if species are from the same trophic level or in communities with low species diversity and stable species composition. Connell (1980) predicted that competition was not of primary importance in community structure and that adaptations already possessed by the species at the time of meeting were the principle determinants of co-existence.

Studies of inter-specific interactions have often concentrated on the amount of resource available to consumers. Heard (1994) recognised that the quality or condition of a resource may also affect consumer population dynamics. A single resource may pass through a sequence of changes (*e.g.*, decomposition of organic detritus). If species utilise this resource in different conditions then their dynamics may be linked. This is an indirect interaction as the resource is not simultaneously available and in certain circumstances forms a processing chain. Nearly all the relevant literature (reviewed by Heard, 1994) suggests a positive effect of the primary consumer ('upstream') on the secondary consumer ('downstream'). This type of commensal interaction may be quite common in nature especially in the decomposer systems.

Earthworm communities may compromise up to 15 species (Edwards & Bohlen, 1996). Between species there is a large degree of niche separation (Hutchinson, 1959) suggesting ecological adaptation to allow species co-existence. Lavelle (1983) indicates that the main separation factors characterising earthworm ecological niches are spatio-

temporal and that within these niches species occupy different trophic niches defined by alimentary specialisation and species size. Phillipson *et al.* (1976) observed differences in food preference and evidence for ecological separation in time and space among 10 earthworm species of an English Beechwood. Lavelle (1983) stated that competition for resources was greatest in temperate regions where litter is the primary food resource and earthworms are concentrated near to the soil surface. This restricts species size range and limits behavioural specialisation leading to greater food specialisation and wider niche separation. Competition between species need not be continuous (Curry, 1998), and may only be a limiting factor when resources are scarce at critical stages of the life cycle.

Inter-specific competition is thought to play a major role in determining the structure of certain earthworm communities (Bouché, 1983; Lavelle, 1983). The decline of native megascolecid species in pastureland in Australia, New Zealand and South Africa has been attributed to the accidental introduction of non-indigenous lumbricid species. It was assumed that the lumbricid species were out competing the native species, however it is more likely that removal of native vegetation and inability of native species to adapt is the more likely explanation (Lee, 1961). This explanation was further corroborated in experiments conducted by Dalby *et al.* (1998b) in South Australia who assessed the effect of *A. longa* (non-native) on the growth, survival and reproduction of the native species *G. lateralis* (reported in section 2.4). Several authors have however observed competitive interactions between earthworm species. Butt (1998) compared growth, maturation and fecundity between monocultures and paired species combinations of 6 species. In all species growth rates and fecundity were higher within monocultures suggesting there to be a negative association within paired treatments. It was proposed that the extent to which species competed for resources was dependent on the degree of

niche overlap exhibited by paired species. Negative interactions were observed between *L. terrestris* and *A. longa* (anecic species) which was also observed in the field by Edwards & Lofty (1982). Rushton (1986) suggested that, in pasture land recently reclaimed from open-cast mining *L. terrestris* is restricted from colonising the site from the surrounding area as it is out competed by the earlier colonising *A. longa*. Butt (1998) also found that the epigeic *L. rubellus* and *Dendrobaena veneta* had a significantly negative effect on anecic species and suggested that these ecological groupings are competing for feed at the soil surface. Hamilton *et al.* (1988) also observed this negative interaction between epigeic and anecic species using *E. fetida* and *L. terrestris* in an experimental sewage sludge system. Dalby *et al.* (1998a) also observed a suite of negative interactions between earthworm species. They suggested 3 possible mechanisms for the observed interactions: 1) scramble competition for food resources, 2) interference competition and 3) the consumption of cocoons.

Abbott (1980) assessed interactions between *E. fetida, A. trapezoides* and *Microscolex dubius* in pot based experiments. Changes in biomass were used as a measure of interaction between species pairings. Abbott detected evidence of persistent inter-specific competition between *E. fetida* and *M. dubius* but not between *E. fetida* and *A. trapezoides*. It was suggested that differences were attributed to a toxic interaction or differences in the efficiency of nutrient extraction. Both Neuhauser *et al.* (1980) and Rouelle *et al.* (1987) reported the negative effect of toxic excretory compounds on the growth of other species. Rouelle *et al.* (1987) found a thiamine destroying factor in the faeces of *E. andrei* and *Dendrobaena* species which when ingested by other species had a significantly negative effect on growth. Mishra & Dash (1979) also reported negative inter-specific interactions between four species in terms of respiration rates. Respiration

rates were significantly reduced in all paired combinations compared to monocultures. This type of interaction in which 2 populations interfere with one another while striving for something not in short supply is mutual inhibition competition. The authors suggest that secreted chemical substances are responsible for the observed results.

Within the literature there are also examples of positive interactions between earthworm species. Commensal associations have been recorded within the field. Phillipson *et al.* (1976) observed *Dendrodrilus rubidus* living within the decomposing leaf bundles drawn into the burrows of *L. terrestris*. It is proposed that this concentration of organic matter provided a favourable microsite for this epigeic species. Saussey (1957) reported the presence of *D. mammalis* [*Satchellius mammalis*] present in the burrows of both *L. terrestris* and *A. longa*. Lukose (1960) observed a closer relationship between *Drawida grandis* and a megascolecid earthworm species. The megascolecid was found crawling over the surface of the much larger *Drawida grandis* and when removed always returned to its host, but was not believed to be feeding directly upon the larger earthworm. Positive associations between species have also been recorded under experimental conditions. The growth and reproduction of epigeic species has been studied in detail due to their potential in the vermicomposting of organic waste. Elvira *et al.* (1996) observed the growth and reproduction of *L. rubellus* and *Dendrobaena rubida* [*Dendrodrilus rubidus*] in cow manure and their possible interactions with *E. andrei*. In mixed cultures the presence of *L. rubellus* and *D. rubida* increased the growth rates of *E. andrei* compared to monocultures. However the growth rate of *L. rubellus* and *D. rubida* was decreased slightly. Butt *et al.* (1997) cultured the anecic *A. longa* and the endogeic *A. chlorotica* in single and paired species culture under optimal conditions for growth and survival. The presence of the two species in a single container enhanced individual production of

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cocoons and hatchlings when compared to single species cultures in an equivalent soil volume indicating there to be a mutualistic interaction between the 2 species. These authors suggested that *A. longa* may be making more of the surface applied feed available to *A. chlorotica* by covering it with casts. Evidence of this positive interaction has also been detected following field inoculations with this species combination at a restoration site (Butt *et al.*, 1999). The site was sampled 5 years after the initial introduction of earthworms, and digging plus handsorting indicated that numbers of *A. chlorotica* were significantly higher ($p<0.05$) in plots where both species had been inoculated compared with plots where *A. chlorotica* had been introduced in isolation.

The relevance of inter-specific interactions, observed under laboratory conditions as, compared with field populations, needs to be questioned. Abbott (1980) concluded that his results should not be extrapolated to field populations of earthworms and that the results obtained only applied to conditions of soil fertility and moisture found within his laboratory experiments. It is also important to note that in the field, populations are naturally controlled by a combination of factors such as predation (Judas, 1989) and disease. Inter-specific interactions form only a part of such natural control systems. However, in favourable conditions, such as those created within many experimental systems, earthworm populations escape this natural control and ultimately will be controlled by competition acting in a strongly density dependant manner (Curry, 1998). In the reviewed experimental studies only interactions between two species at any given time are considered. However, few populations will interact with only one species, and the interaction between 2 species can affect how these species interact with yet another species producing correlated outcomes (Thompson, 1988). Within soil faunal

communities earthworms also interact with other functional groups (*e.g.* Bonkowski *et al.*, 1998).

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CHAPTER 3. DEVELOPMENT OF EXPERIMENTAL DESIGN

3.0 INTRODUCTION

It is essential when undertaking any research project that the questions posed are balanced against what is achievable given the available resources. This may lead to simplification of experimental design in order that valid and repeatable results may be obtained. This chapter describes initial practical and logistical considerations which influenced and constrained experimental design in the following chapters.

3.1 LOCATION : FIELD- VERSUS LABORATORY-BASED RESEARCH

Conducting experiments under laboratory conditions enables the investigator to hold environmental variables constant or vary them in a systematic manner in order to answer a specific question (Hairston, 1989). Experiments conducted under such controlled conditions are easily repeatable. However, such experiments may be questioned as to their applicability to natural conditions. In contrast, if experiments are conducted in the field it is not possible to exercise control over most variables and as a result the questions that can be asked are less precise than those asked in the laboratory. Under field conditions it is assumed that environmental variables affect each treatment within an experiment equally over the selected time period. This assumption and the inherent variability places uncertainty into the interpretation of results and in the ability to accurately repeat the experiment.

Survival, growth and fecundity of earthworms are largely determined by the environmental conditions to which the animals are subjected, in particular soil moisture,

temperature and food availability (section 2.2). In contrast it is proposed that the influence of inter- and intra-specific interactions on earthworm production may be subtle in nature and as a result, easily masked by environmental factors. Therefore, it was vital, in order to confidently attribute differences in earthworm production to the influence of inter- or intra-specific interactions and also to allow for accurate repetition of experiments that all environmental variables were set at fixed levels and prevented from unmonitored fluctuations. Consequently all experimentation was conducted under controlled laboratory conditions with the exception of one field-related study (appendix 7).

3.2 SPECIES SELECTION

Initial earthworm stocks were obtained from pasture at Valentine House Farm, Preston (Nat. Grid Ref. SD 505315) in November 1995 using formalin expulsion (Raw, 1959) and handsorting of cut turves (appendix 4). Six species were collected from the field site: *A. chlorotica* (plate 3.2.4), *A. caliginosa* (plate 3.2.3), *A. longa* (plate 3.2.2), and *L. rubellus* (plate 3.2.5), which were locally abundant, plus *L. terrestris* (plate 3.2.1) and *O. cyaneum* (which were seemingly less common) descriptions of which are given in table 3.2.1. Species identification was undertaken in the laboratory with reference to Sims & Gerard (1985).

Plate 3.2.1 *Lumbricus terrestris*

Plate 3.2.2 *Aporrectodea longa*

Plate 3.2.3 *Aporrectodea caliginosa*

Plate 3.2.4 *Allolobophora chlorotica*

Plate 3.2.5 *Lumbricus rubellus*

Note. Pictures are not to scale

O. cyaneum is obligatory parthenogenetic and although locally common is never abundant (Sims & Gerard, 1985) as a result this species has little influence on soil formation and therefore was not used experimentally. The five other species were selected to form the basis of laboratory cultures for experimental use. These species are found throughout the British Isles and provide representatives of the three main ecological groupings enabling a full range of proposed interactions (see section 1.1) to be investigated. These species have been used in many laboratory-based earthworm studies (*e.g.* Evans & Guild, 1948; Löfs-Holmin, 1983; Elvira *et al.*, 1996; Butt, 1997) and also in soil inoculation trials (*e.g.* Rhee, 1969b; Springett, 1985; Butt *et al.*, 1997) allowing comparisons to be drawn between current research and the established literature.

Sufficient numbers of *L. terrestris* required to establish initial stocks were difficult to obtain from the field site, however, this species is collected as fishing bait and stocks were readily purchased from a commercial supplier (Ecology Earthworms).

3.2.1 Morphological Variation within Species

A. caliginosa is a highly plastic species with many morphological variants (Sims & Gerard, 1985). Several authors have classified the four recognised morphs as separate species (*e.g.* Gates, 1972), however the variations that occur between the morphs are largely phenotypic. For the purpose of this study mature *A. caliginosa* were sub-divided into two groups according to body size and only the smaller individuals were employed experimentally (with the exception of early experimentation). Two morphs of *A. chlorotica* (pink and green) also exist and differences in their distribution have been recorded (*e.g.* Satchell, 1967). The two morphs generally form separate populations, the

Table 3.2.1 Species Description (adapted from Sims and Gerard (1985))

Species	Classification	Reproduction	Habitat	Soil Type	British Distribution
Allolobophora chlorotica (Savigny) 2 morphs : pink and green	Endoge	Obligatory Biparental - copulation within the soil	Often numerically co-dominant with *A. caliginosa* usually found in the top 60 cm	Found in a variety of soil types *e.g.* sand, clay and peaty soils pH 4.5 - 8.2	Widespread
Aporrectodea caliginosa (Savigny) 4 morphs : *caliginosa, tuberculata, nocturna* and *trapezoides*	Endoge	Obligatory Biparental - copulation mainly in the soil	Dominant in most cultivated land, also occurs in the banks of streams	Present in alkaline soils pH 5.9 - 11.1	Widespread
Aporrectodea longa (Ude)	Anecic	Obligatory Biparental - copulation in the soil	Cultivated soils, pastures and woodland, also in the banks of rivers or lakes	Prefers loamy or chalky soils pH 6.7 - 9.4	Widespread
Lumbricus rubellus (Hoffmeister)	Epige	Obligatory Biparental - copulation in soil or litter layer	Found in a wide range of habitats usually moist with high organic content	Recorded from soils pH 3.5 - 8.4	Widespread
Lumbricus terrestris (Linnaeus)	Anecic	Obligatory Biparental - copulation on the soil surface	Undisturbed terrestrial habitats - most numerous in grasslands and orchards	Especially abundant in clay pH 6.2 - 10.0	Widespread
Octolasion cyaneum (Savigny)	Anecic	Obligatory Parthenogenetic	Prefers moist habitats, in gardens, pastures, arable land, woodland and caves	Prefers limnic soils pH 3.5 - 8.2	Widespread

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green type in wet sites and the pink type in drier sites. Both morphs were recorded at the Valentine House Farm site, however as the green morph was more locally abundant it was selected for experimental use.

3.3 QUANTIFYING THE EFFECT OF EARTHWORM INTERACTIONS

It is proposed that earthworm interactions, both negative and beneficial, may influence the energy available for growth (tissue production) and reproduction (cocoon production). Negative interactions may result in the reduction of available resources or increase the energy required for locomotion (*e.g.* resulting from avoidance responses) and in doing so decrease the energy available for production. However, beneficial interactions may result in increased availability of resources and therefore increase the energy available for production.

The scientific literature contains numerous studies relating to the influence of environmental variables on earthworms (section 2.2). In general, the effect of these has been quantified by measuring:-

   a) earthworm survival rates at the population level in the field

or

   b) growth, maturation and reproductive rates at the individual level in

   laboratory-based studies.

The latter was adopted by Butt (1998) in order to quantify earthworm interactions and will also be employed in this study. By monitoring growth, maturation and reproduction rates, the influence of inter- and intra-specific interactions may be assessed throughout earthworm life stages.

3.3.1 Growth

This was monitored by recording live earthworm masses. Before mass determination, worms were washed in distilled water to remove any soil adhering to the body surface and carefully blotted dry, as suggested by Phillipson & Bolton (1977). Care was taken in handling earthworms to prevent the exudation of coelomic fluid (an escape response), which would have affected mass measurement. In order to increase the accuracy of live mass measurements, several workers have induced earthworms to void their gut contents prior to weighing (*e.g.* Hartenstein & Amico, 1983) by placing earthworms on moistened filter paper. This procedure was impractical in this study due to the time constraints (8 hours at 25 °C for *L. terrestris* (Hartenstein & Amico, 1983)).

3.3.2 Maturation

This was assessed by a simple visual assessment based on the presence or absence of a swollen clitellum.

3.3.3 Cocoon Production

Reproduction was determined by collection of cocoons from experimental soil in which adult earthworms had been maintained. Cocoons were separated from the worked soil by wet-sieving (described by Butt, 1990) through a series of graded sieves (mesh size : 6.0, 3.35 and 2.0 mm). The three sieves were stacked in descending mesh size and the soil (approximately one litre at a time) placed into the top sieve. As the soil was removed from each sieve the larger fragments (including cocoons) left in each sieve (see plate 3.3.1) were carefully examined for cocoons which were removed using forceps and placed in distilled water. Due to their colouration and the adherence of soil to their

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fibrous outer-coating, cocoons of both *L. rubellus* and *L. terrestris* were initially difficult to distinguish from soil particles. However, with experience cocoons of the 5 species were readily located and identified from their size, colour and shape (using the identification guide of Sims & Gerard, 1985) with the exception of cocoons produced by *A. chlorotica* and *A. caligionsa* which were difficult to distinguish from one another. In general, due to their size, cocoons of *A. chlorotica, A. caliginosa* and *L. rubellus* were collected from the smallest mesh size sieve (2.0 mm) while the two anecic species were restricted to the 3.35 mm sieve. A description of cocoons produced by the five species is given in table 3.3.1.

Table 3.3.1 Cocoon Descriptions (adapted from Sims and Gerard (1985))

Species	Cocoon diameter (mm)	Shape	Colour	Surface texture
A. chlorotica	2.1 - 3.3	Spherical	Pale yellow with brownish poles (opaque)	Smooth
A. caliginosa	2.4 - 5.5	Lemon shaped, cylindrical or spherical	Pale yellow often with brown stippling (opaque)	Smooth
A. longa	3.3 - 5.2	Cylindrical	Pale yellow darkening to green or brown at poles (opaque)	Smooth
L. rubellus	1.6 - 3.3	Spherical	Dirty olive brown (opaque)	Surface mat covered by fibrous material
L. terrestris	3.9 - 5.7	Spherical	Medium brown with a greenish hue (opaque)	Fibrous surface composed of several layers.

Plate 3.3.1. Wet Sieving for Cocoons : Sieve Containing Soil and a _L. terrestris_ Cocoon
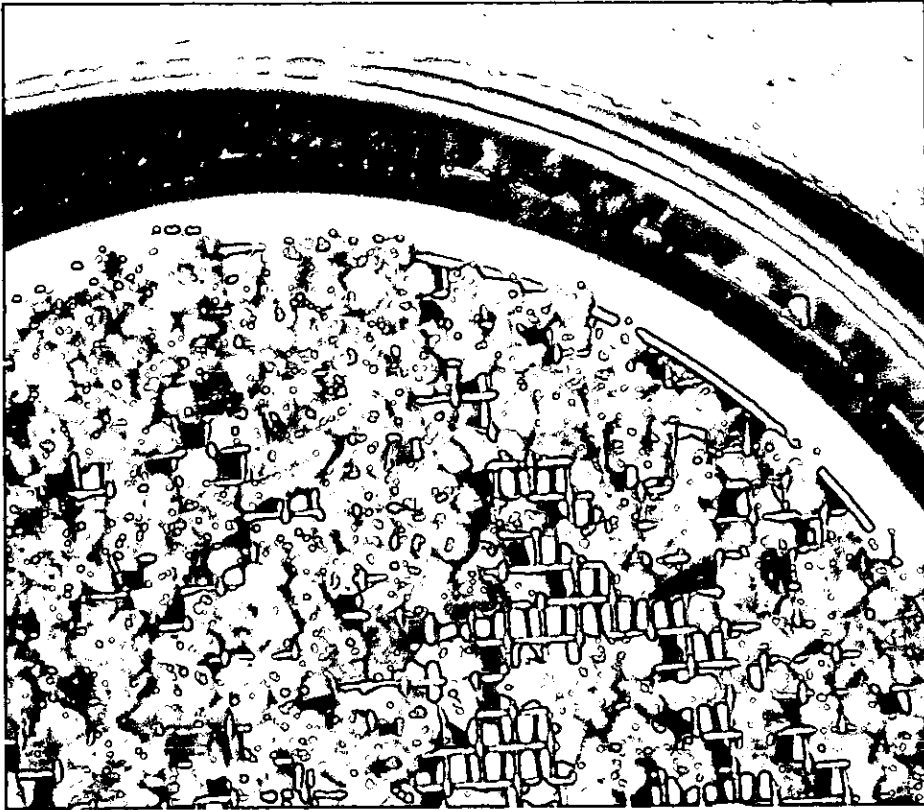
Plate 3.4.1 Experimental Vessels

3.3.4 Mortality

This was recorded by comparing the number of individuals present at any given sampling with the number at the previous sampling. The replacement of earthworms in treatments where mortality occurred was considered impractical as substitute animals would not have been maintained under the same conditions as experimental treatments. However, when mortality occurred in two or more replicates of the same treatment, the surviving earthworms were combined to reform as many replicates as possible at the specified earthworm density .

3.4 EXPERIMENTAL VESSEL SELECTION

All major experiments during this research were conducted in darkness in temperature controlled (Gallenkamp) incubators (internal dimensions: h 1110, w 445 and d 410 mm). Therefore the complexity and the number of experiments which could be conducted concurrently was restricted by:-

a) the basic scientific requirement for adequate replication

and

b) the size of experimental vessels.

Selection of vessels used in experiments was a function of practical manipulations, earthworm density and reference to field conditions, and not simply based on the number that could be fitted into a fixed volume.

Löfs-Holmin (1983) recommended that " small vessels should be preferred to large ones for ease of handling and sampling". For this research it was also important that vessels

were re-usable, easily stacked to maximise available space and had sealable lids to prevent excess loss of soil moisture.

Increased earthworm biomass and density in laboratory cultures has been shown to have significant negative effects on both species growth and reproduction, *e.g.* with *Eisenia fetida* (Reinecke & Viljoen, 1993). In the current work it was essential, as with other abiotic factors, that earthworm density was prevented from becoming a limiting factor, potentially negating the influence of any inter- or intra-specific interactions. Therefore, optimal earthworm densities / biomass, were assessed in terms of growth rates and reproduction, and experimental vessels were selected accordingly. As adults of the five selected earthworm species varied greatly in biomass, vessels were selected on the basis of satisfying the requirements of the species with the greatest individual mass (*L. terrestris*). However, since Butt *et al.* (1992) had shown that *L. terrestris* could be cultured successfully in pots of 2 l and possibly as small as 0.6 l, larger vessels were not thought to be necessary.

Experimental vessels were also required to enable representative behaviour of the five species, as if under something approaching field conditions. Adult *L. terrestris* and *A. longa* naturally construct semi-permanent vertical burrows opening to the soil surface into which they are able to retract themselves (Edwards & Bohlen, 1996). Therefore a minimum vessel depth was required that corresponded to the body length of these two species. It was also desirable that the influence of "edge effects" (contact between earthworms and the vessel walls) was minimised by reducing the surface area to volume ratio.

Three vessel sizes were selected for use throughout the research: -

1) **600 ml** (surface area 0.013 m^2, depth 50 mm) used exclusively in the interaction

between laboratory-reared hatchlings experiment

2) **1 litre** (surface area 0.011 m^2, depth 140 mm) used in the majority of experiments

3) **2 litre** (surface area 0.02 m^2, depth 170 mm) used in experiments which employed

adult earthworms from the outset.

All three vessel types had air-tight, sealable lids. The straight-sided 2 litre vessels had four 2 mm diameter holes (one in each corner of the lid) made with a heated needle. The 2 smaller vessels had ten 1 mm holes punctured in the lids with a mounted needle, allowing air circulation within the varying containers (see plate 3.4.1). All vessels were obtained from Gregory & Co. Ltd, London.

3.4.1 Equating Species Biomass

Individuals of the five selected earthworm species differed significantly in mass (see table 3.4.1). In assessing the effects of several species on the production of a single species it was necessary that their biomasses were comparable or any observed differences may be attributed to differences in the total biomass of each treatment and not to the species present. It was determined that individual numbers of each species would be assigned to experimental treatments according to a fixed ratio.

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Table 3.4.1 Masses of Clitellate Earthworms Collected From Valentine House Farm

(March 1996)

Species	Mean Individual Mass (g)
L. terrestris	5.23
A. longa	3.42
L. rubellus	1.01
A. caliginosa	0.95
A. chlorotica	0.42

N.B. The figure for *L. terrestris* was calculated from earthworms purchased from Ecology Earthworms.

The calculated ratio finally determined was:

2 *L. terrestris* : 3 *A. longa* : 4 *L. rubellus* : 6 *A. caliginosa* : 8 *A. chlorotica*

This was not simply a function of species individual mean biomass, but also took account of earthworm numbers in the field and also that all five species were obligatory bi-parental in reproduction (Sims & Gerard, 1985).

3.5 FOOD

Animal dung is a recognised earthworm food source. The preference of dung over other organic materials has been demonstrated in a number of earthworm species (*e.g.* Guild, 1955; Barley, 1959) and has been used experimentally as a source of feed for earthworms by several authors since the pioneering work by Evans & Guild (1948).

The intensification of livestock production has led to large volumes of animal dung and urine (slurry) being produced. Slurry is often stored in tanks prior to disposal by spreading onto the land. Slurry has been shown to be highly toxic to plant and animal life

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as it contains benzoic acid and phenols (from the breakdown of urine) and ammonia, methane and sulphide from the fermentation of faeces (Curry, 1976). However, after simple treatment procedures, cattle slurry in the form of separated cattle solids (SCS) provides a consistent and abundant earthworm food source and has been employed successfully in earthworm culture (*e.g.* Edwards & Burrows, 1988; Butt, 1998). SCS were readily available from Myerscough College Farm, Lancashire and were selected as the food source in this research. Passing the slurry through a separating machine attached to the slurry tank at the farm removed the majority of the liquor. The solid fraction was oven dried in the laboratory at 105 °C for 12 hours to cause the volatilisation of ammonia. Drying the SCS also allowed for storage without microbial degradation, further assisting production of a consistent and reliable food source. In preparation for experiments the dried SCS were rewetted. The species selected for research represent all three earthworm ecological groupings and their feeding habits varied accordingly. Both the anecic and epigeic species feed primarily at the soil surface, whereas the two endogeic species are restricted to feeding within the soil. It was important in experiment design that a food source was made available to each species. Therefore in all mixed species experiments SCS were both mixed into the soil profile and also placed on the soil surface. Carbon and nitrogen analysis (appendix 2) revealed a C : N ratio of 16 : 1, similar to results obtained by Butt (1990). Forage fibre analysis (appendix 3) was also conducted following a slightly modified (Knight, 1987) van Soest method (Goering and van Soest, 1970). The results of which are shown in table 3.5.1.

Table 3.5.1 Forage Fibre Results of SCS From Myerscough Agricultural College.

Cell Soluble Material %	Hemicellulose %	Cellulose %	Lignin %	Ash %	Other %
25	25	25	20	2	3

3.6 SOIL TYPE

Soil type and texture are known to influence the distribution of earthworm species (Edwards & Bohlen, 1996). In general earthworms are thought to prefer soils with a neutral pH (*e.g.* Bouché, 1972) low gravel and sand content (El-Duweini & Ghabbour, 1965) and a relatively high clay content (Nordström & Rundgren, 1974). For the purpose of this research, a consistent soil source was required which satisfied the former requirements and was devoid of a resident soil fauna.

Initially a 3 tonne batch of soil was purchased from a commercial supplier, D. F. & M. Hargreaves, Blackpool. This followed an inspection of stock, from which samples were taken for laboratory analysis. The selected soil was classified as a sandy clay loam (MAFF, 1984) had a pH of 7.3 and a C : N ratio of 8 : 1. Prior to use any large stones were removed by passing the soil through a 7 mm sieve. Heating to 75 °C for 45 minutes (in a Camplex Soil Steriliser) killed any living material present (Meinhardt, 1974) and any potential predators (M[c]Leod, 1954). As the research developed this soil treatment process became impractical due to increased requirements and the initial experimental soil was replaced by a pre-sterilised and sieved loamy soil (pH 6.4 and organic content 5 %) obtained from Boughton Loam Ltd, Kettering, UK. This type of soil has been previously used by earthworm researchers (*e.g.* Butt *et al.,* 1994).

As experiments were conducted using two soil types during the research period, a simple comparative trial was set up to assess the potential influence of soil type using *L. terrestris* as an experimental animal.

3.6.1 An Experiment to Assess Growth and Survival of *L. terrestris* in Two Soil Types [Kettering Loam and a Sandy Clay Loam]

3.6.1.1 Introduction

An experiment was conducted to determine if differences existed in *L. terrestris* growth and survival when cultured in the two experimental soils (Kettering loam and a sandy clay loam) used throughout the research.

3.6.1.2 Materials and Methods

Six replicates of each of the two soil types were set up in 1 litre vessels to which 30 g of rewetted separated cattle solids (SCS) were applied on the soil surface. Two laboratory-produced *L. terrestris* hatchlings (mean individual mass 70 mg) were placed in each vessel. The vessels were kept in darkness and at fluctuating temperatures in an outside storeroom (temperature 8 - 16 °C). The experiment was sampled every 4 weeks, at which point earthworm survival, mass and reproductive condition was recorded and feed replenished. The experiment was terminated after 20 weeks.

3.6.1.3 Results and Discussion

There was 100 % survival in both soil treatments. Statistical analysis (one-way ANOVA) showed there to be no significant difference ($p > 0.05$) between *L. terrestris* growth rates in the 2 soils at any of the 4 weekly samplings (figure 3.6.1). After 20 weeks *L. terrestris* mean masses were 2.88 and 2.81 g for the sandy clay loam and the Kettering loam respectively. From these results it was concluded that meaningful comparisons could be drawn between experiments employing the two different soils

Figure 3.6.1. Growth of *L. terrestris* in Two Experimental Soil Types
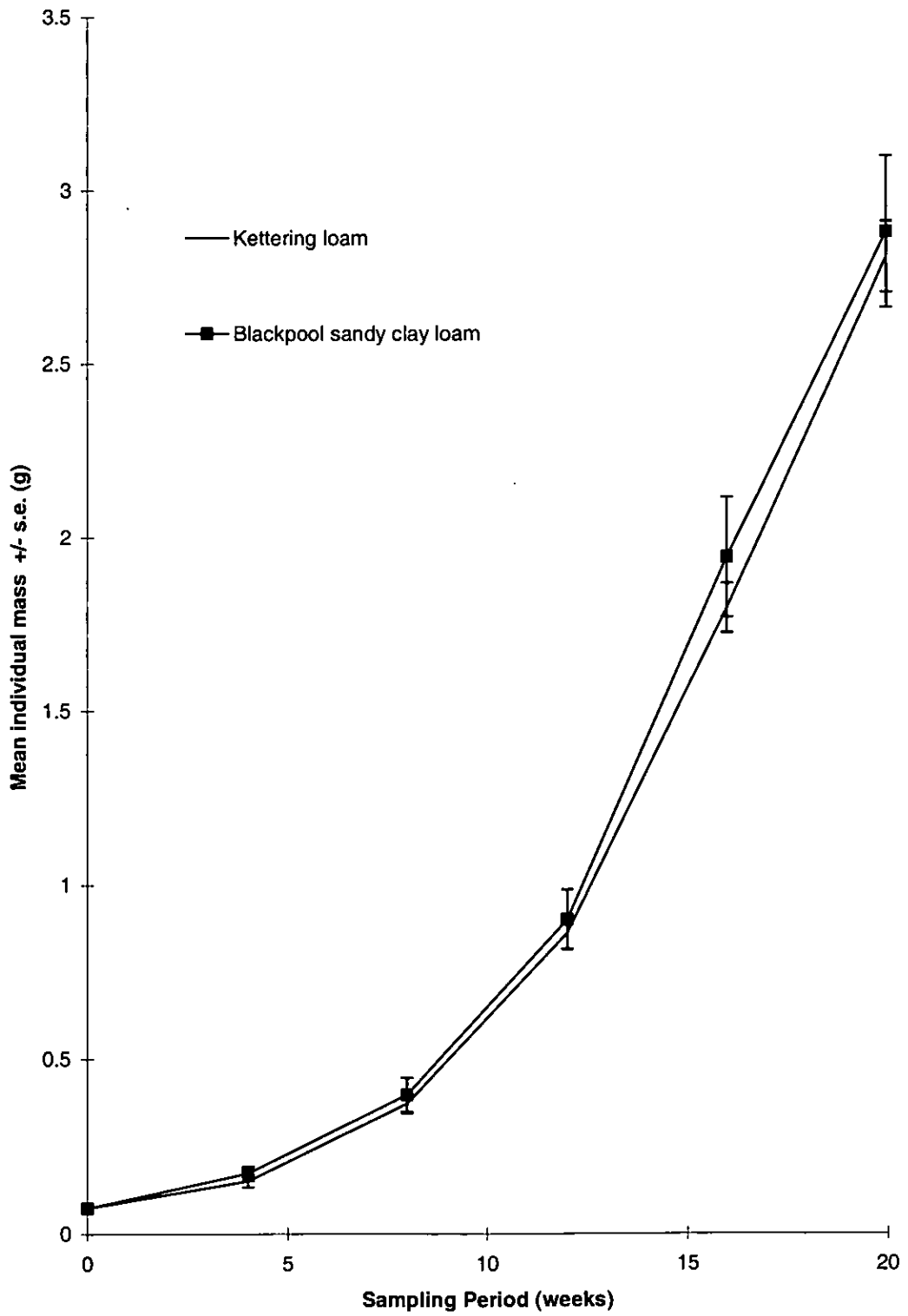
3.7 ESTABLISHMENT AND MAINTENANCE OF EARTHWORM

LABORATORY CULTURES

The age and physical condition of earthworms was identified as a potential source of experimental error. Reliable comparisons between different experimental treatments would only be possible, by ensuring that individuals of a species were of the same age and in similar physical condition. This could only be achieved by culturing the 5 experimental species in the laboratory. Design of several experiments required large numbers of earthworms, specifically the smaller endogeic species (*e.g.* 320 *A. chlorotica* hatchlings were required in assessing the influence of mature earthworms on the growth of hatchlings). Producing a cohort of a sufficient number involved the manipulation of life stages and the careful planning of an experimental timetable (appendix 1).

3.7.1 Hatchling Production

Cocoons collected by wet sieving of worked soil (section 3.3.3) were placed in 90 mm diameter Petri dishes containing filter paper which was covered with distilled water. The dishes were labelled with the species type, date and cocoon number and placed in a refrigerator at 4 ± 1 °C . Temperature has been shown by several authors (*e.g.* Gerard, 1967; Butt *et al.*, 1992) to influence the time required for earthworm embryonic development before cocoons hatch. Holmstrup *et al.* (1991) demonstrated that the incubation time of *A. chlorotica* cocoons increased significantly with decreasing temperature (34 - 38 days at 15 °C and 400 days at 5 °C). Therefore by maintaining cocoons at 4 ± 1 °C it was possible, over time, to amass large numbers of cocoons. During refridgeration it was necessary to change the filter paper (due to bacterial growth) and replenish the water (lost due to evaporation) every 3 - 4 weeks. Attempts were made

to limit fungal and bacterial growth by employing a cocoon sterilisation method as used by Nuutinen *et al.* (1991). Prior to placing the cocoons in the Petri dishes unhatched cocoons were surface sterilised in 1 % sodium hypochlorite for 3 minutes and then rinsed with sterilised water. However, laboratory trials indicated that the pre-treatment of cocoons had little influence on bacterial and fungal growth within dishes. It was proposed that infection rates were primarily associated with the frequency that the Petri dishes were exposed to the laboratory atmosphere.

When hatchlings were required for experimentation cocoons were moved from the refrigerator to an incubator at 15 ± 1 °C which stimulated hatching. Once placed at this higher temperature, a large proportion of the cocoons hatched over an initial two week period producing a cohort of individuals. On hatching the filter paper initially served as a source of food. Spent cocoons were removed from the dishes as these were thought to be a source of bacterial infection. Hatchlings not required for experimentation or development were placed in 1 litre containers three quarters filled with a mixture of soil and separated cattle solids (SCS). These containers were then placed at 4 ± 1 °C. At this temperature hatchlings became inactive and growth was retarded. This allowed for large numbers of hatchlings (of all species) to be maintained in small volumes of soil (up to 100 worms l^{-1}) enabling the establishment of large stocks of hatchlings. It was possible to maintain earthworms in this state for months, but it was thought that this period of inactivity might influence earthworm growth and maturation once conditions became favourable for development. Therefore a small scale trial was set up to compare the growth of hatchlings maintained in this quiescent state with newly hatched earthworms.

3.7.2 An Experiment to Investigate the Influence of Enforced Quiescence on the Growth of *L. terrestris*

3.7.2.1 Introduction

The effect of enforced quiescence on hatchling growth rate was addressed by comparing *L. terrestris* hatchlings maintained at 4 ± 1 °C for 12 months, with recently hatched individuals of the same species.

3.7.2.2 Materials and Methods

Two treatments were established employing *L. terrestris* hatchlings from two different sources: -

a) Non-quiescent: Newly hatched individuals which were obtained from cocoons maintained at 15 ± 1 °C in Petri dishes. These individuals had all hatched in the 2 weeks prior to the start of the experiment and were kept in the Petri dishes until required (mean individual mass 61 mg).

b) Quiescent : Hatchlings that had been kept at 4 ± 1 °C in a mixture of Kettering loam and SCS for 12 months (mean individual mass 76 mg).

Two hatchlings from each source were assigned to 1 litre vessels filled to a depth of 0.11 m with Kettering loam (moisture content 25 %). Excess, rewetted SCS were applied to the soil surface. Five replicates of each treatment were maintained in darkness at

15 ± 1 °C. Earthworm condition and mass were recorded every 4 weeks at which point food was replenished. The experiment was terminated after 16 weeks

3.7.2.3 Results and Discussion

During the experiment there was 100 % survival within both treatments. No significant difference ($p > 0.05$) was recorded in growth rates up to week 12. However, at week 16 the growth of the non-quiescent worms was significantly ($p < 0.01$) greater than quiescent worms (mean individual masses 2.9 and 2.3 g respectively) (figure 3.7.1). The results indicate that maintaining hatchlings in a dormant state can negatively affect growth rates. It was therefore important that all hatchlings used in experiments had been kept under the same conditions and were comparable in terms of age, or if this was not possible, that individuals were randomly assigned within treatments.

Figure 3.7.1. Growth of Hatchling *L. terrestris* a) Maintained at 4 +/- 1 Degrees Celcius For 12 Months (Quiescent) b) Recently Hatched (Non-Quiescent)
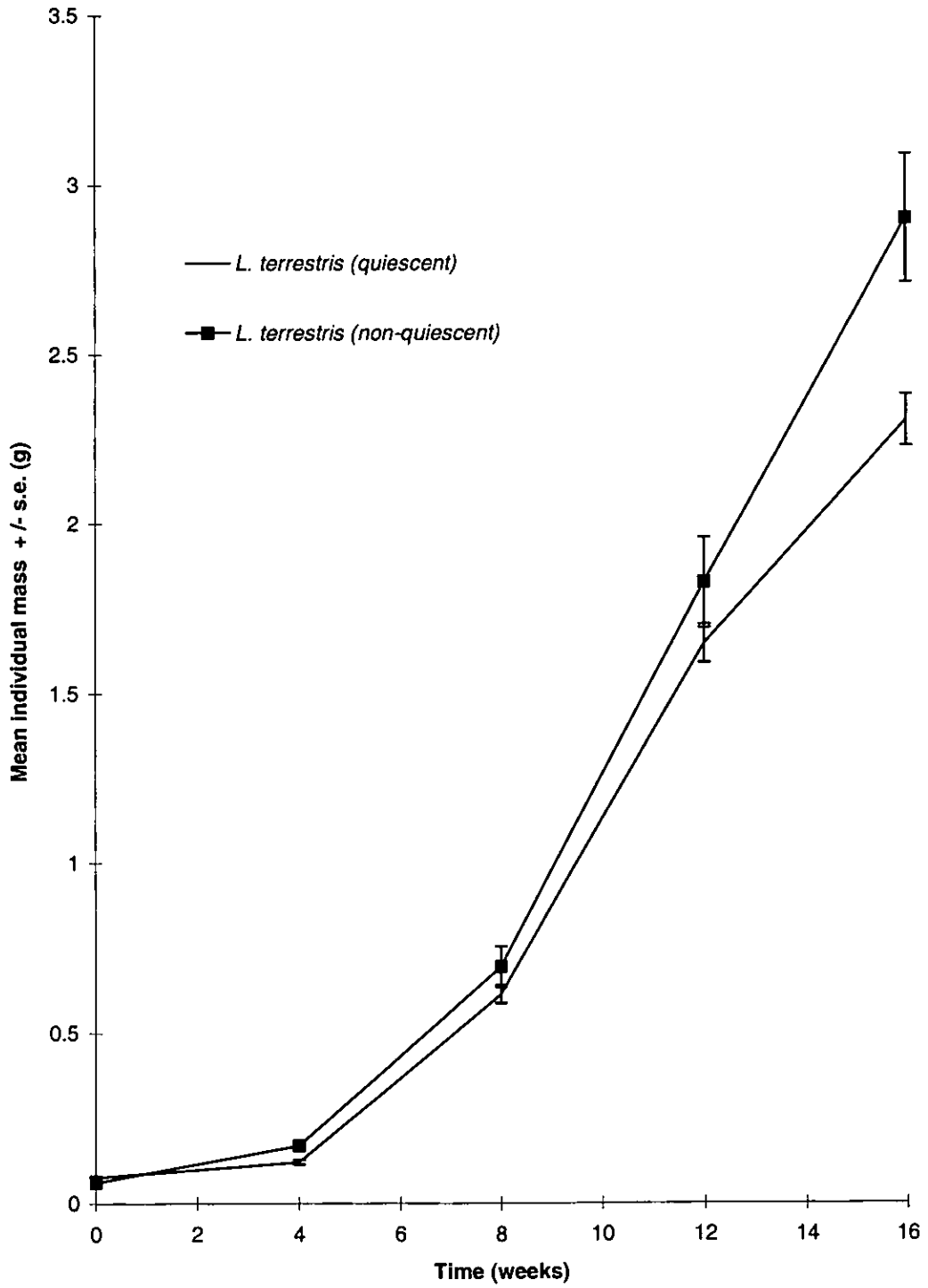
3.7.3 Maintenance of Juvenile and Adult Stocks

A low maintenance system of keeping earthworms was required from which individuals of known history could be taken (with minimal disturbance) for use in experimentation. The development of this system was made more complex by the varying environmental needs of the 5 selected species.

Epigeic and endogeic species were maintained in 2 litre vessels, while anecic species were kept in 25 litre sealed containers (surface area 0.08 m^2 and depth 0.28 m). These larger containers were lined with a plastic sack allowing for simple removal of contents without excessive disturbance to the soil. Vessels containing epigeic and anecic species had excess SCS applied to the soil surface while the two endogeic species were supplied with excess SCS mixed into the soil profile. Stocking densities were established in part by trial and error, but were also based on previous work (*e.g.* Löfs-Holmin, 1983; Butt *et al.*, 1994), with the intention of establishing sustainable populations. In general earthworms were maintained at the following densities: - *L. terrestris* 2 worms l^{-1}, *A. longa* 3 worms l^{-1}, *L. rubellus* 5 worm l^{-1}, *A. caliginosa* 7 worms l^{-1} and *A. chlorotica* 10 worms l^{-1}.

Earthworm stocks were maintained in an unheated storeroom. Temperatures ranged from 2 to 20 °C depending on the time of year. During the winter months stocks required little maintenance as the earthworms were largely inactive. However, as temperatures increased it was necessary to refeed and water containers on a monthly basis. For the endogeic species this involved the removal of earthworms from the soil, allowing SCS to be mixed into the soil.

3.8 ANALYSIS OF EXPERIMENTAL DATA

Statistical analyses were performed on results using the statistical computer package Minitab (registered trademark of Minitab Inc.) for Windows 98. One-way analysis of variance (ANOVA) was used to compare differences between treatment means (for example growth of *L. terrestris* in 2 experimental soil types). However, in experiments with several treatments ANOVA was inappropriate in comparing the means of all pairs of treatments and was only useful as a test for the equality of all treatment means due to the problem of multiple testing. This problem was overcome by employing a multiple comparison procedure. Several procedures of this type exist, the most appropriate for the data obtained from this research was the Tukey-Kramer method (also called Tukey's and Tukey's HSD) (Sokal & Rohlf, 1995). The General Linear Model (GLM) was applied to enable the calculation of interaction terms between factors with uneven data sets.

3.9 INITIAL EXPERIMENTS : DEVELOPMENT OF OPTIMAL CONDITIONS

Laboratory-based research by numerous workers has demonstrated, the powerful effects that environmental factors such as temperature (Daniel *et al.*, 1996), moisture (Kretschmar & Bruchou, 1991), food quality (Boström, 1988) and quantity (Löfs-Holmin, 1983) can have on earthworm production. In initial experiments (Chapter 4) it was essential for environmental variables to be maintained at fixed 'optimal levels' for earthworm production. This was to permit any observed differences in earthworm survival, growth and fecundity to be attributed to earthworm interactions, and not result from a limiting environment.

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Optimal environmental conditions for growth, maturation and reproduction have been proposed by several authors in a number of earthworm species. This information provided the basis from which initial experiments were designed.

3.9.1 Soil Temperature and Moisture Conditions

In the field, earthworm activity is strongly correlated with seasonal variations in both soil temperature and moisture. Scheu (1987) demonstrated that these two factors can act synergistically to influence earthworm activity. He showed that an increase in temperature from 10 to 15 °C at a soil moisture content of 60 % doubled the number of casts produced by *A. caliginosa,* whereas in soil with a moisture content of 48 % cast production was increased by only 20 %.

If soil becomes too dry, too cold or too warm many earthworm species migrate deeper into the soil and become comparatively inactive. Three states of inactivity (quiescence, facultative diapause and obligatory diapause) have been distinguished in earthworm species (section 2.2.1). Laboratory experiments have suggested that by manipulating environmental conditions diapause in many species may be prevented. Michon (1954) proposed that *A. chlorotica, A. caliginosa* and *A longa* could be prevented from entering diapause if these species were maintained at 9 °C and given excess feed. Butt (1993) observed cocoon production in *A. longa* over a 12 month period at a temperature of 20 °C with surface fed separated cattle solids. He observed a drop in cocoon production during months 6 - 8, but individuals did not enter into obligatory diapause.

3.9.1.1 Temperature

Table 3.9.1 summarises temperature preferences and optimal temperatures for growth and cocoon production established by previous workers for the 5 selected earthworm species. It is evident from the table that the preferred temperature of a species does not correspond with the temperatures which are optimal in terms of production. Ireland (1983) suggested that in temperate climates, maximum activity of soil dwelling earthworms occurs when the soil temperature is between 4 and 11 °C. However, in experiments where temperature is maintained in excess of field conditions, earthworm production has been greatly enhanced (*e.g.* Löfs-Holmin, 1983). But maintenance of earthworms at artificially high temperatures has been shown to result in reproductive fatigue (section 2.2.1).

Ideally experiments would have been conducted at controlled fluctuating temperatures covering the thermal range of the 5 experimental species. However practical considerations also had to be accounted for. The available temperature controlled incubators allowed only the maintenance (± 1 °C) of a fixed temperature, space within these incubators was limited, as was the time available for research. Therefore in order to complete the research programme the duration of individual experiments was minimised. This was partially achieved by selecting an experimental temperature which was optimal in terms of earthworm production and also within the acceptable range of all 5 species. Based on these requirements, available published data and temperatures used in other earthworm production experiments (*e.g.* Löfs-Holmin, 1983; Dalby *et al.*, 1998a) all major experiments were maintained at 15 °C.

Table 3.9.1 Summary of Optimal and Preferred Temperature Ranges Recorded in the Literature For the Five Selected Species

Species	Temperature Preference °C		Optimal Temperature °C		State of Inactivity
	Juvenile	Adult	Growth	Cocoon Production	
A. chlorotica (green)	Unknown	15 - Graff, 1953	20 - Butt, 1997	20 - Butt, 1997	Facultative Diapause Morgan & Winters, 1991
A. caliginosa	Unknown	10 - 15 - Daugbjerg, 1988 10 - 23.2 - Grant, 1955	Unknown	Unknown	Facultative Diapause Morgan & Winters, 1991
A. longa	10 - Daugbjerg, 1988	10 - 15 - Daugbjerg, 1988 10 - 12 - Nordström, 1975	Unknown	Unknown	Obligatory Diapause Morgan & Winters, 1991
L. rubellus	Unknown	15 - 18 - Graff, 1953	Unknown	Unknown	Quiescence
L. terrestris	Unknown	10 - Daugberg, 1988 10.5 - Satchell, 1967	20 - Butt et al., 1992	15 - Butt, 1991	Quiescence

3.9.1.2 Soil Moisture

Soil type and texture is known to strongly influence the effect of soil moisture on earthworm production as these factors determine the force with which available water is held by the soil (section 2.2.1). Therefore reported percentage soil moisture preferences for the five experimental species were of limited use in determining optimal moisture conditions for the soils used. However, a soil moisture content of 25 - 30 % has been suggested as being optimal for lumbricid species (Tomlin, 1979; Löfs-Holmin, 1983). For this research it was essential that moisture content was maintained at a level above which earthworms became inactive, but below which the soil became waterlogged.

Butt (1990) cultured *L. terrestris* in soil with a moisture level at which the soil was wet enough not to adhere to worms' bodies, but dry enough not to coat the worms with mud, a protocol which was adopted for this study. The soil moisture content to which this definition corresponded was determined to be approximately 25 % for both experimental soils. Sealable lids on experimental vessels limited evaporation of soil moisture between sampling periods. However, in experiments where soil was not replaced at each sampling, it was still found necessary to replace soil moisture lost through evaporation, by spraying water onto the soil surface within each vessel.

3.9.2 Food Quantity

In order to prevent bias between experimental treatments it was essential that equal quantities of food (SCS) was applied to each treatment within an experiment and also that this fixed quantity was sufficient to prevent food from becoming limiting between sampling (3 weeks in the experiment assessing interactions between field-collected adults, 4 weeks in all other experiments). The size of vessels was specific to each experiment and related directly to the earthworm life stages that were being investigated. Therefore the quantity of SCS used was also specific to each individual experiment. The exact quantities of SCS used were initially developed through trial and error and subsequent experimental designs were developed accordingly. In early experiments the removal of surface applied SCS was monitored within treatments containing the larger surface feeding anecic species. If between samplings all food was removed from the soil surface, then the quantity of SCS applied to all the treatments in a single experiment was increased.

3.9.3 Light

In order to relate laboratory-based research to natural conditions the exposure of experiments to light should have followed a fixed diurnal pattern. However, this was not feasible as all experiments were required to be maintained in temperature controlled incubators which were not all equipped with a variable light facility. Therefore between sampling periods experiments were kept in darkness. Under field conditions surface-feeding species (*e.g. L. terrestris* and *A. longa*) only come to the surface to feed at night or in very low light intensities during the day (Lee, 1985). It is also known that sub-surface feeding species exhibit strong negative phototropism which is believed to be

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correlated to their lack of pigmentation. It was therefore proposed that placing earthworms under a fixed darkness regime would be optimal in terms of growth and reproduction. It has also been suggested that ultraviolet light may be harmful to earthworms (Merker & Braunig, 1927), as a result during sampling of experiments steps were taken to prevent exposure of earthworms to direct sunlight.

3.9.4 Earthworm Density

Earthworm culture techniques have improved significantly since the seminal work of Evans & Guild (1948). As a result recommended optimal earthworm densities for growth and reproduction have also increased. As all five experimental earthworm species varied considerably in mass, optimal densities were determined using the largest species, *L. terrestris*. Evans & Guild (1948)suggested that three *L. terrestris* in a 5 pint jar (1 per litre of soil) provided good results, while Löfs-Holmin (1983) recommended that *L. terrestris* densities of 1 to 2 per litre of soil was optimal for cocoon production. However, more recently Butt *et al.* (1994) suggested that an optimum density for *L. terrestris* growth and reproduction was 15 - 22 live g litre $^{-1}$ (3 - 4 individuals) a maximum stocking density that was employed throughout this research.

CHAPTER 4. ASSESSING INTERACTIONS BETWEEN SELECTED

EARTHWORM SPECIES

4.0 INTRODUCTION

This chapter presents findings from laboratory-based experiments, which were undertaken to investigate the first of the major research aims *i.e.* : -

> To reveal competitive and / or beneficial interactions between five selected earthworm species assessed in terms of survival, growth rate, maturation and reproduction.

4.1 EXPERIMENTAL DESIGN

Three experiments were set up, following the same basic design. Single species monocultures were employed as controls, compared with treatments containing all possible paired species combinations. Monoculture treatments were also established which contained twice the number of individuals used in controls. This design allowed the assessment of both inter- and intra-specific interactions.

The first two experiments investigated interactions between 2 anecic species (*A. longa* and *L. terrestris*) and 2 endogeic species (*A. caliginosa* and *A. chlorotica*). These experiments used earthworms of the same age class, the first employed field-collected, adult worms and the second used laboratory-reared hatchlings. The third experiment monitored growth from hatchling to maturity, of *L. terrestris*, *A. longa*, *A. chlorotica* and *L. rubellus*, in the presence of clitellate earthworms of these four species and also of

A. caliginosa.

The experiments were designed around two major assertions :-

     1a) Negative (competitive) interactions are likely to occur between individuals

     from the same species or between species from the same ecological

     grouping.

Conversely

     1b) Positive (mutualistic) interactions are more likely to occur between

     species from different ecological groupings.

     2) The age and reproductive condition of earthworms would influence

     observed species interactions.

Experiments are presented in chronological order, highlighting the development of experimental design which occurred during this initial research period.

4.2 AN EXPERIMENT TO ASSESS INTERACTIONS BETWEEN FOUR SELECTED EARTHWORM SPECIES UNDER OPTIMAL CONDITIONS (FIELD-COLLECTED MATURE ADULTS)

4.2.1 Introduction

This preliminary study assessed interactions (in terms of changes in biomass and reproductive output) between mature individuals of four earthworm species

(*A. chlorotica, A. caliginosa* (endogeic), *A. longa* and *L. terrestris* (anecic)).

4.2.2 Materials and Methods

Clitellate *A. chlorotica, A. caliginosa,* and *A. longa* were field-collected from pasture land at Valentine House Farm, Preston (SD 505315). Clitellate *L. terrestris* (not abundant at the field site) were purchased from a commercial earthworm supplier (Ecology Earthworms). Prior to use in experimentation the earthworms were maintained under fluctuating temperature conditions in an outside storeroom (section 3.7.2) for a period of 6 weeks. Treatments (table 4.2.1) comprised, a monoculture of each species, all possible paired combinations plus a 'double density' monoculture of each species (X2). Earthworm numbers were assigned to the treatments in accordance with a pre-determined species ratio (section 3.4.1), the X2 monocultures having twice the pre-determined number of earthworms per vessel.

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Table 4.2.1 Treatments Employed in Two Earthworm Interaction Experiments

Using : i) Field-Collected Adults and ii) Laboratory-Reared Hatchlings

Species combinations	No. of individuals of each species present
L. terrestris	2
L. terrestris (X2)	4
A. longa	3
A. longa (X2)	6
A. caliginosa	6
A. caliginosa (X2)	12
A. chlorotica	8
A. chlorotica (X2)	16
L. terrestris + *A. longa*	2 + 3
L. terrestris + *A. caliginosa*	2 + 6
L. terrestris + *A. chlorotica*	2 + 8
A. longa + *A. caliginosa*	3 + 6
A. longa + *A. chlorotica*	3 + 8
A. caliginosa + *A. chlorotica*	6 + 8

Five replicates of the 14 treatments were set up in 2 litre culture vessels. The vessels were filled to a depth of 0.14 m with a 7 : 1 mixture of a pre-sterilised sandy-clay loam (pH 7.3) and separated cattle solids (SCS), with an initial moisture content of 25 %. Excess SCS were added to the substrate surface to prevent food from becoming a limiting factor between sampling periods. Treatments were maintained in darkness at

15 ± 1 °C within temperature-controlled incubators.

The total biomass for each species within each vessel was recorded at the outset. Thereafter the condition and mass of the earthworms was recorded every 3 weeks, at which point fresh food was mixed into the soil and applied to the surface. After 12 weeks the experiment was terminated and the culture medium was wet-sieved (section 3.3.3) to locate any cocoons produced by the earthworms.

4.2.3 Results

Figure 4.2.1 (a-d) illustrates the cumulative change in biomass recorded for *L. terrestris*, *A. longa*, *A. chlorotica* and *A. caliginosa* respectively in single, and paired, species treatments. In the majority of treatments 100 % survival was recorded over the 12 week experimental period. However, in treatments involving *L. terrestris*, mortality rates were high particularly in monoculture and X2 treatments resulting in no data being recorded at final sampling in the two former treatments. (see figure 4.2.1 (a)). Cocoons of all four species were recorded in related experimental treatments (see table 4.2.2).

4.2.3.1 *L. terrestris*

Average *L. terrestris* biomass, in all relevant treatments, decreased over the 12 week experimental period, except in the pairing with *A. caliginosa* (figure 4.2.1(a)). In the latter, *L. terrestris* initially lost mass (at week 3) but by week 12 had achieved an average biomass almost identical to that at the outset of the experiment. The mortality of earthworms in the monoculture and X2 treatments prevented direct comparisons from being drawn between the five treatments (after week 9) and may also have affected the loss in biomass experienced in the two single species cultures. Of the three remaining treatments the presence of *A. longa* resulted in the greatest loss in *L. terrestris* biomass.

4.2.3.2 *A. longa*

The changes in biomass recorded for *A. longa* (figure 4.2.1 (b)) followed similar trends to those observed for *L. terrestris*. After 3 and 6 weeks *A. longa* decreased in biomass within all treatments. However, by week 9, *A. longa*, in the pairings with both

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A. chlorotica and *L. terrestris*, began to increase in biomass and by week 12 had attained a mean biomass greater than at the outset of the experiment. At week 12 the greatest loss in *A. longa* biomass was recorded in the X2 treatment.

4.2.3.3 *A. chlorotica*

Figure 4.2.1(c) illustrates the average change in biomass of *A. chlorotica* in all relevant treatments over the experimental period. At week 3, all treatments (except X2) exhibited increases in *A. chlorotica* biomass, which was maintained throughout the 12 week period in the pairings with *L. terrestris* and *A. longa*. In the pairing with *A. caliginosa*, the average biomass of *A. chlorotica* decreased between weeks 3 and 6 but after this point biomass increased and by week 12 *A. chlorotica* exhibited an increase in mean biomass in this treatment.

4.2.3.4 *A. caliginosa*

In contrast with the other experimental species, *A. caliginosa* (in all treatments) exhibited an increase in the mean biomass by week 12. This was greatest in the pairing with

A. longa. In contrast the X2 treatment and the pairing with *A. chlorotica* led to no net gain in mass over 12 weeks.

Figure 4.2.1 Cumulative Mean Change in Pot Biomass of Adult a) *L. terrestris,* b) *A. longa,* c) *A. chlorotica* and d) *A. caliginosa* in Monoculture, Double Density Monoculture (X2) and All Paired Species Combinations After 12 Weeks

(a) *L. terrestris*

Legend:
- Plus A. longa
- Plus A. caliginosa
- Plus A. chlorotica
- monoculture
- X2 monoculture

Y-axis: Change in biomass compared with time zero (g)

X-axis: Time (weeks)
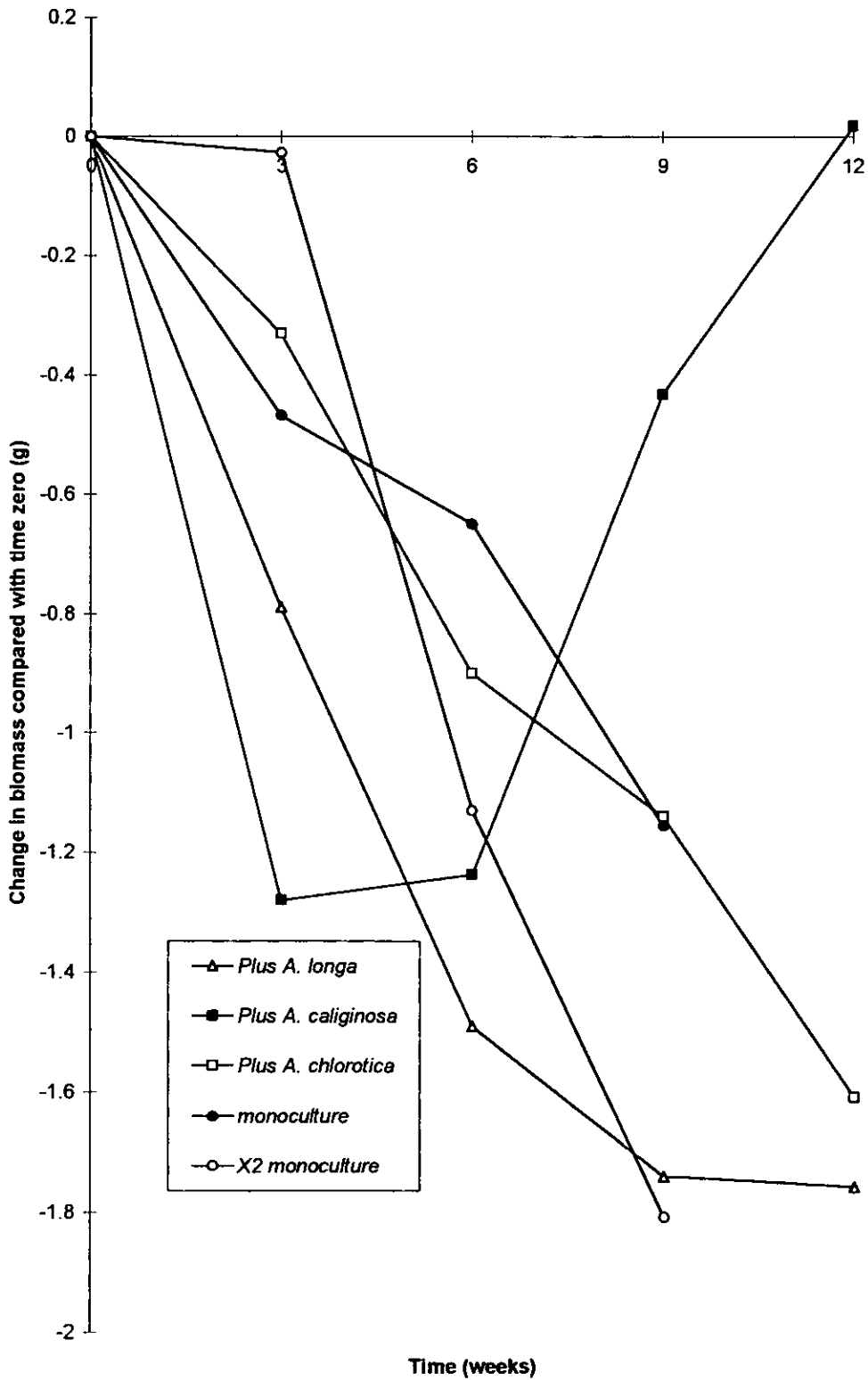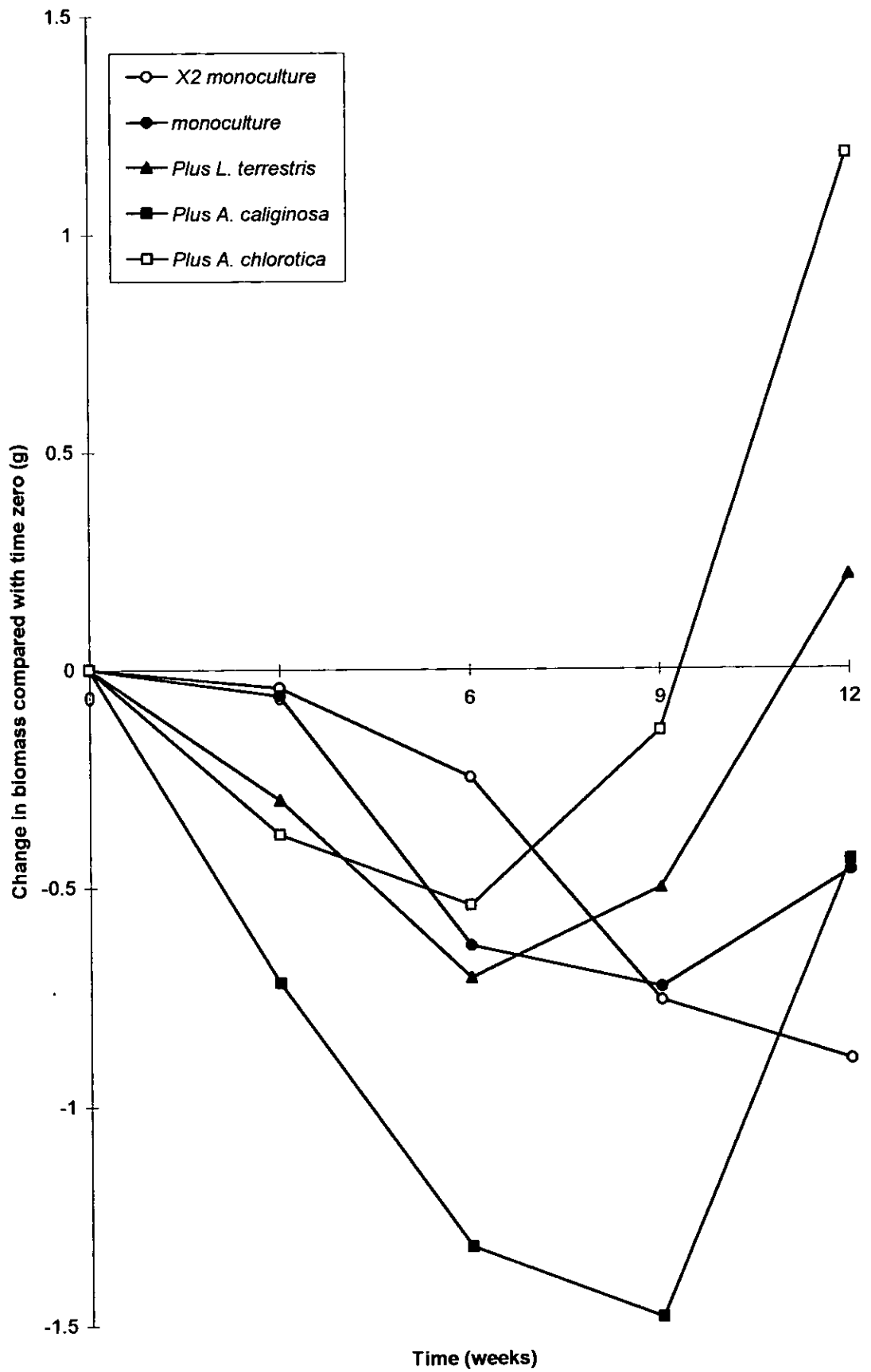
b) *A. longa*

(c) _A. chlorotica_

(d) *A. caliginosa*

Time (weeks)
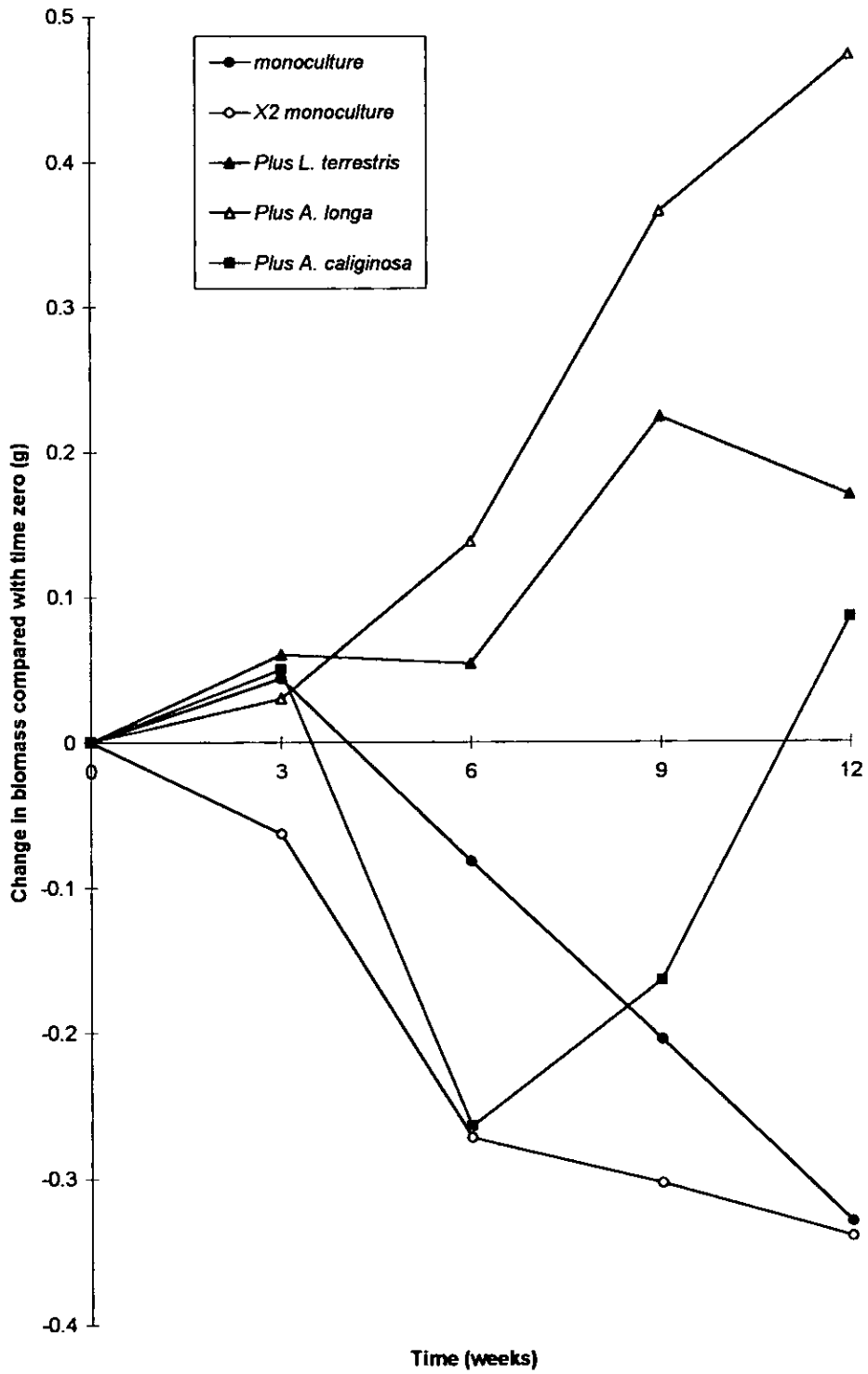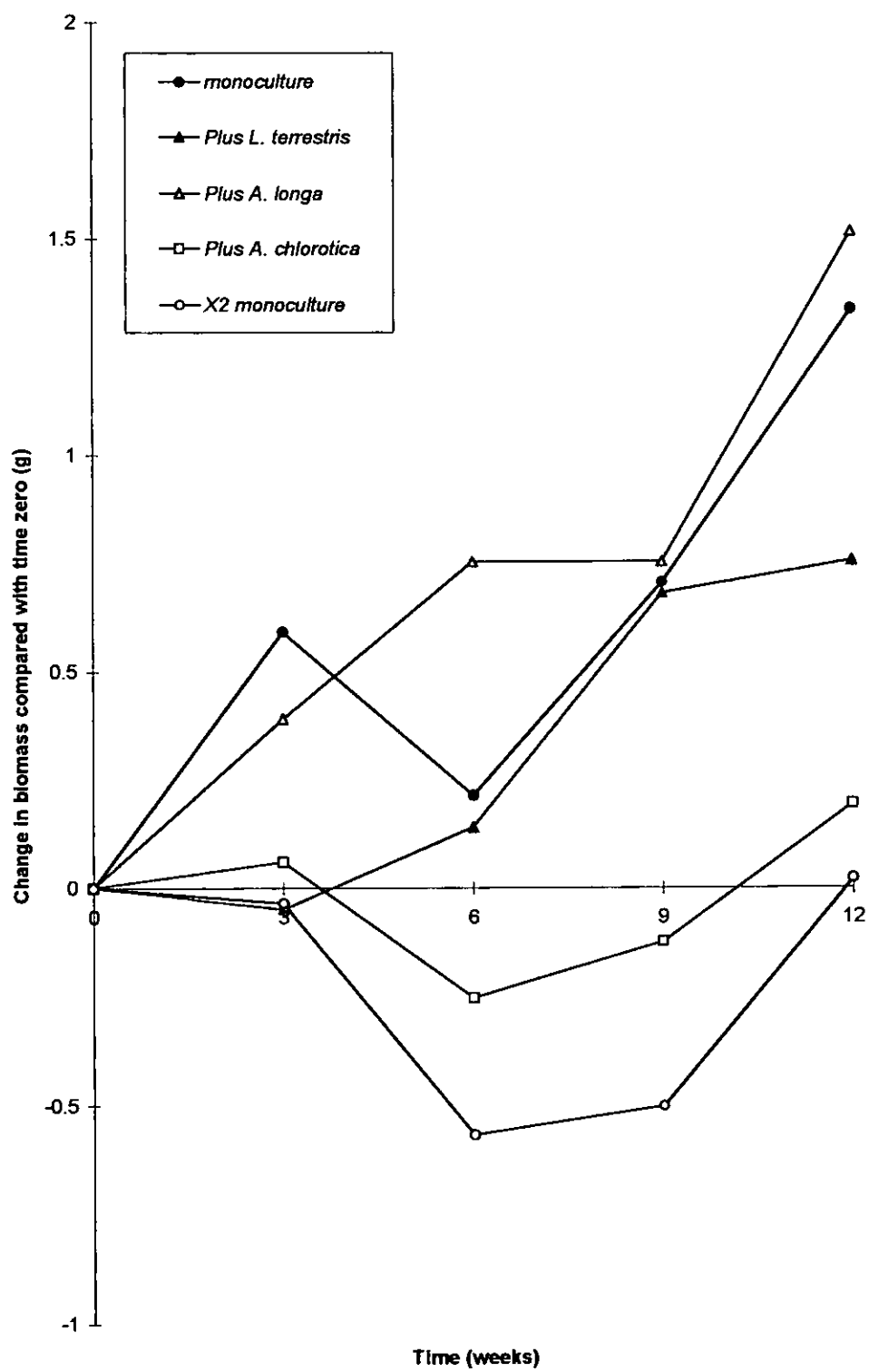
Table 4.2.2 Mean Cocoon Production by Field-Collected Adult *A. chlorotica*, *A. caliginosa*, *A. longa* and *L. terrestris* in Monoculture, Double Density Monoculture (X2) and All Paired Species Combinations After 12 Weeks

Species	Treatment (Coc. production worm^{-1} month^{-1})					
	Monoculture Control	Monoculture X2	Plus A. chlorotica	Plus A. caliginosa	Plus A. longa	Plus L. terrestris
A. chlorotica	3.0	3.1	■	no data	2.6	3.0
A. caliginosa	5.7	3.7	no data	■	3.9	5.3
A. longa	3.3	4.7	2.9	2.0	■	3.5
L. terrestris	no data	no data	3.8	3.4	2.9	■

4.2.4 Discussion

This experiment was established as a preliminary investigation into earthworm interactions under optimally controlled laboratory conditions. As such the study yielded some useful results, but also highlighted areas of experimental design which required improvement if future experiments were to provide reliable results.

4.2.4.1 Suitability of Laboratory Conditions

During the experimental period the survival rate of the three field collected species was 100 % in the majority of treatments and all four species produced cocoons (table 4.2.2). Results obtained for cocoon production were comparable with those recorded by other researchers (*e.g.* Evans & Guild, 1948; Löfs-Holmin, 1983; Butt, 1993; Butt, 1997) who employed the same species in laboratory cultures. These observations indicated that the environmental conditions used in the experiment were acceptable for culturing the 4 species.

4.2.4.2 Evidence of Species Interactions

Results demonstrated that adult earthworms of all four species were able to co-exist in paired species culture under the specified laboratory conditions. There was also a suggestion that negative inter-specific interactions were distinguishable in observations made concerning species biomass and that these differences were related to a species ecological grouping. In comparing only paired species treatments it was evident that both anecic species exhibited a greater increase in biomass when cultured in treatments with either of the endogeic species, than when paired with each other. This relationship was also detected in the biomass results of the 2 endogeic species. These results complement

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field observations by Edwards & Lofty (1982) who reported negative correlations between *L. terrestris* and *A. longa* and also between *A. chlorotica* and *A. caliginosa* populations. Observed differences in biomass may therefore be attributed to the degree of niche overlap experienced by these species. Anecic and endogeic species are separated in terms of vertical distribution within the soil, feeding behaviour and size. Therefore competition for resources between species from these two groupings would be minimal. Contrastingly competition between species from the same ecological grouping would be more intense as these earthworms have very similar requirements. This theory was in part substantiated by the results obtained for the double density monoculture treatments which exhibited the lowest biomass records of all species, except *A. longa*. In these treatments competition for resources would be most intense as conspecifics occupy identical niches. However, if the degree of niche overlap was the sole factor influencing results one may have expected that the greatest increase in biomass for each species would have been recorded in individual species monocultures (control). In these treatments, individual species density was at its lowest and therefore in comparison with other treatments competition for resources would have been minimised.

The trends observed in recorded species biomass between treatments were not reflected in cocoon production (table 4.2.2). Results (although incomplete) indicated that there was very little difference in individual species reproductive output between treatments.

4.2.4.3 Refinements to Experimental Design

Earthworm growth rates are known to vary with age, for example Lakhani & Satchell (1970) demonstrated that *L. terrestris* grew rapidly in short seasonal pauses (when conditions were favourable) for about 3 years, and after this period individual mass

changed little in the next 4 years. Growth of *A. caligionsa* was followed in field cultures by Nowak (1975), she recognised that after attaining reproductive condition, these earthworms entered a phase of steadily decreasing growth. Butt *et al.* (1994) monitored cocoon production by *L. terrestris* over a three year period during which mean rates of cocoon production decreased, indicating that this is also influenced by age. It was therefore suggested that the variation in the age and condition of field-collected species and the unknown history of the commercially purchased *L. terrestris* employed in this experiment had major influences on observed biomass and cocoon production results. To redress this problem, subsequent experiments only employed laboratory-bred earthworms of known age.

Distinguishing between cocoons of *A. chlorotica* and *A. caliginosa* which are very similar in shape colour and size also provided (at this stage) a source of experimental error. As hatchlings of *A. chlorotica* are readily identified by a distinct white dorsal band anterior to the crop (Sims & Gerard, 1985), it was decided that cocoons of these two species would be incubated and identification confirmed on hatching. Cocoons were placed on filter paper in Petri dishes and covered with distilled water, and kept at 15 ± 1 °C in a temperature-controlled incubator. As cocoons hatched, earthworms were identified and removed, however before all the cocoons had hatched the dishes became infected with a fungal growth preventing complete cocoon production data for these two species from being determined.

4.3 AN EXPERIMENT TO ASSESS INTERACTIONS BETWEEN FOUR EARTHWORM SPECIES UNDER OPTIMAL CONDITIONS (LABORATORY-REARED HATCHLINGS)

4.3.1 Introduction

This experiment employed laboratory-reared hatchlings of *A. chlorotica, A. caliginosa, A. longa* and *L. terrestris* following the same regime of treatments used in section 4.2. Earthworm growth rates were recorded allowing both inter- and intra-specific interactions to be assessed and comparisons drawn with earlier work.

4.3.2 Materials and Methods

A. chlorotica, A. caliginosa, A. longa and *L. terrestris* (mean individual mass 0.09, 0.06, 0.17 and 0.08 g, respectively) were obtained from cocoons produced by laboratory-reared earthworms (section 3.7). All earthworms had hatched in the 4 weeks prior to the onset of experimentation (section 3.7.1). The treatments and individual number of each species employed were identical to those used in section 4.2 (table 4.2.1). Five replicates of each of the 14 treatments were set up 0.6 litre culture vessels, filled to a depth of 0.04 m with sandy-clay loam soil mixed with 20 g of SCS. A further 20 g of SCS were placed on the soil surface. The total biomass for each species per vessel was recorded at the outset. Thereafter the mass and reproductive condition of the earthworms was recorded every 4 weeks, at which point fresh food was applied. The experiment was terminated after 20 weeks.

Differences in the masses of each of the four species across treatments was analysed statistically using the Tukey-Kramer multi-comparison one way analysis of variance (ANOVA).

4.3.3 Results

Figure 4.3.1(a-d) illustrates growth of the four earthworm species over the 20 week experimental period, during which all exhibited 100 % survival and showed an increase in mass.

Monocultures of all 4 species achieved the highest mean biomass-per-pot, compared (species for species) with mixed cultures (*e.g.* growth rates of *A. chlorotica*, *A. caliginosa*, *A. longa* and *L. terrestris* in monoculture were 0.014, 0.029, 0.067 and 0.15 g worm^{-1} week^{-1}, respectively).

Growth curves for *L. terrestris* (figure 4.3.1(a)) and *A. longa* (figure 4.3.1(b)) exhibited similar trends in all paired combinations. At the end of the experimental period *A. caliginosa* exhibited the least negative effect on growth rates as there was no significant difference ($p > 0.05$) between the biomass of the 2 anecic species in this pairing and in single species monoculture. Lowest growth rates were recorded in the treatment which paired *A. longa* with *L. terrestris* (0.085 and 0.044 g worm^{-1} week^{-1} respectively) with biomasses in monoculture of each species being significantly greater ($p < 0.01$) than those obtained from this pairing.

A. chlorotica growth rates (figure 4.3.1 (c)) were most negatively affected in the pairing with *A. caliginosa*. Biomass of *A. chlorotica* in this pairing was significantly less ($p < 0.01$)

compared with conspecifics in monoculture or in pairings with either anecic species (at week 20). There was no significant difference (p>0.05) in the final biomass of *A. caliginosa* (figure 4.3.1(d)) in monoculture and paired species treatments.

For all four species, doubling the initial hatchling number greatly reduced growth to a level comparable with the most negative species pairings. For *A. caliginosa* growth rates of the X2 treatment were very similar to those in pairing with *L. terrestris* (0.019 and 0.018 g worm^{-1} month^{-1}, respectively).

Table 4.3.1 Statistical Analysis of Differences in Species Biomasses Between Treatments in the Laboratory-Reared Hatchling Experiment at Week 20

a) L. terrestris

	Monoculture	+ *A. longa*	+ *A. caliginosa*
+ *A. longa*	**		
+ *A. caliginosa*	ns	**	
+ *A. chlorotica*	**	ns	**

b) *A. longa*

	Monoculture	+ *L. terrestris*	+ *A. caliginosa*
+ *L. terrestris*	**		
+ *A. caliginosa*	ns	ns	
+ *A. chlorotica*	*	ns	ns

c) *A. caliginosa*

	Monoculture	+ *A. longa*	+ *L. terrestris*
+ *A. longa*	ns		
+ *L. terrestris*	**	ns	
+ *A. chlorotica*	ns	ns	ns

d) *A. chlorotica*

	Monoculture	+ *A. longa*	+ *L. terrestris*
+ *A. longa*	ns		
+ *L. terrestris*	ns	ns	
+ *A. caliginosa*	**	**	**

(**ns** no significant difference, ** significant at the 1 % level, * significant at 5 % level)

Figure 4.3.1 Mean Increase in Biomass (± s.e.) of Hatchling a) *L. terrestris,*
b) *A. longa,* **c)** *A. chlorotica* **and d)** *A. caliginosa* **in Monoculture,**
Double Density Monoculture (X2) and in All Paired Species
Combinations After 20 Weeks

(a) *L. terrestris*

(b) *A. longa*

Legend:
- monoculture
- plus *L. terrestris*
- plus *A. caliginosa*
- plus *A. chlorotica*
- X2 monoculture

Y-axis: Mean pot biomass +/- s.e. (g)
X-axis: Time (weeks)

(c) *A. chlorotica*

(d) *A. caliginosa*

Figure legend:
- ● monoculture
- ▲ plus L. terrestris
- △ plus A. longa
- □ plus A. chlorotica
- ○ X2 monoculture

Y-axis: Mean pot biomass +/- s.e. (g)
X-axis: Time (weeks)
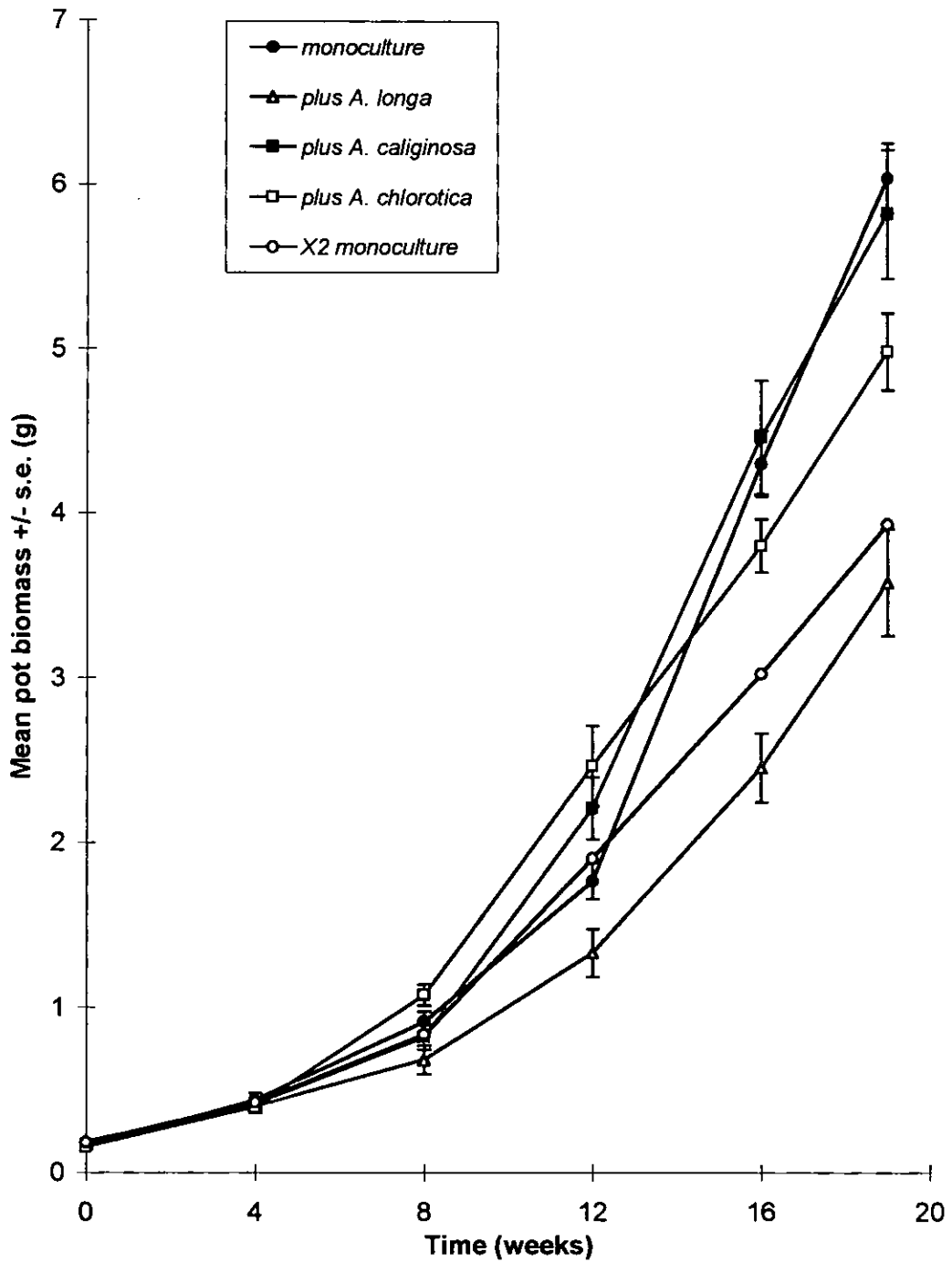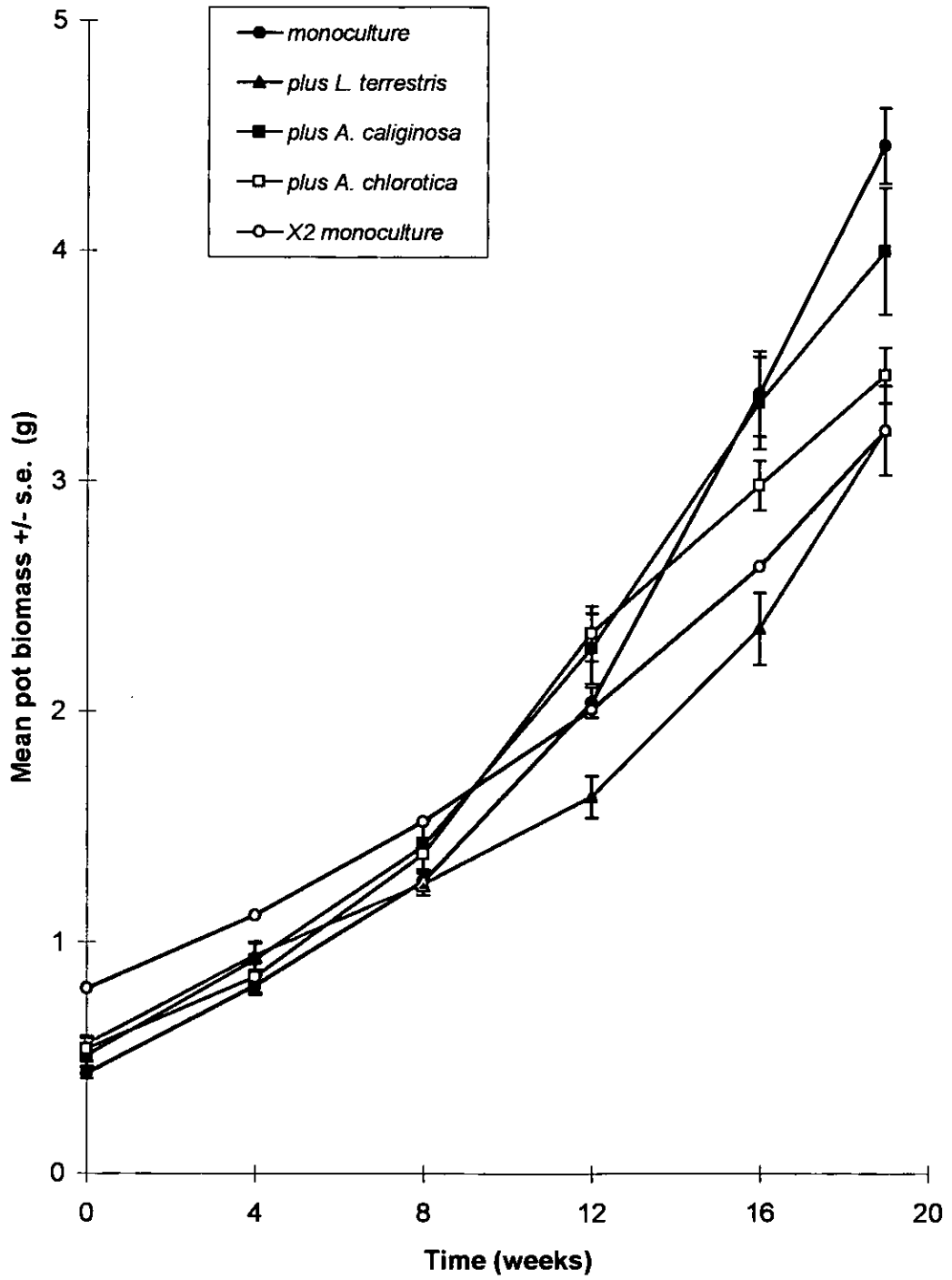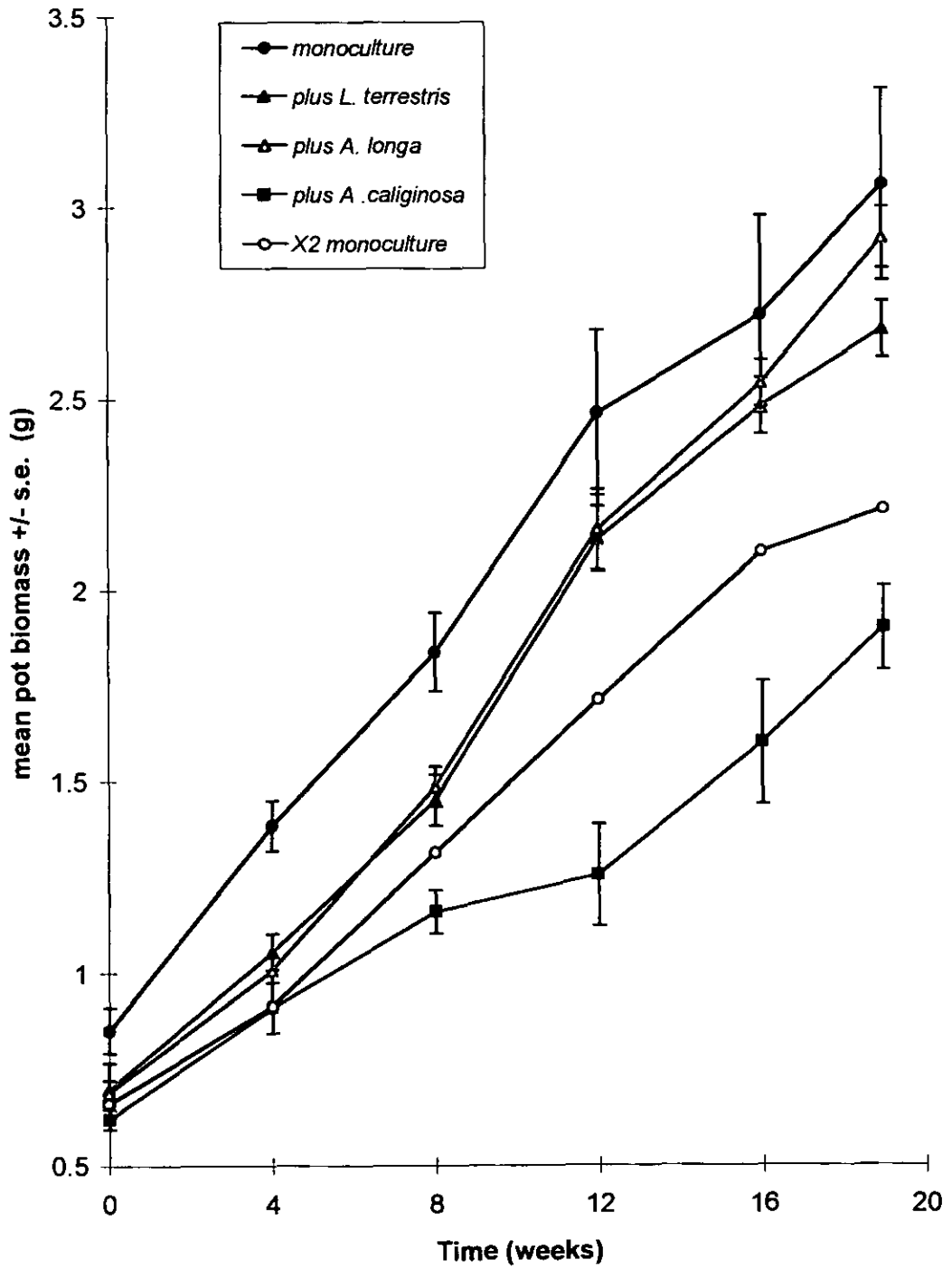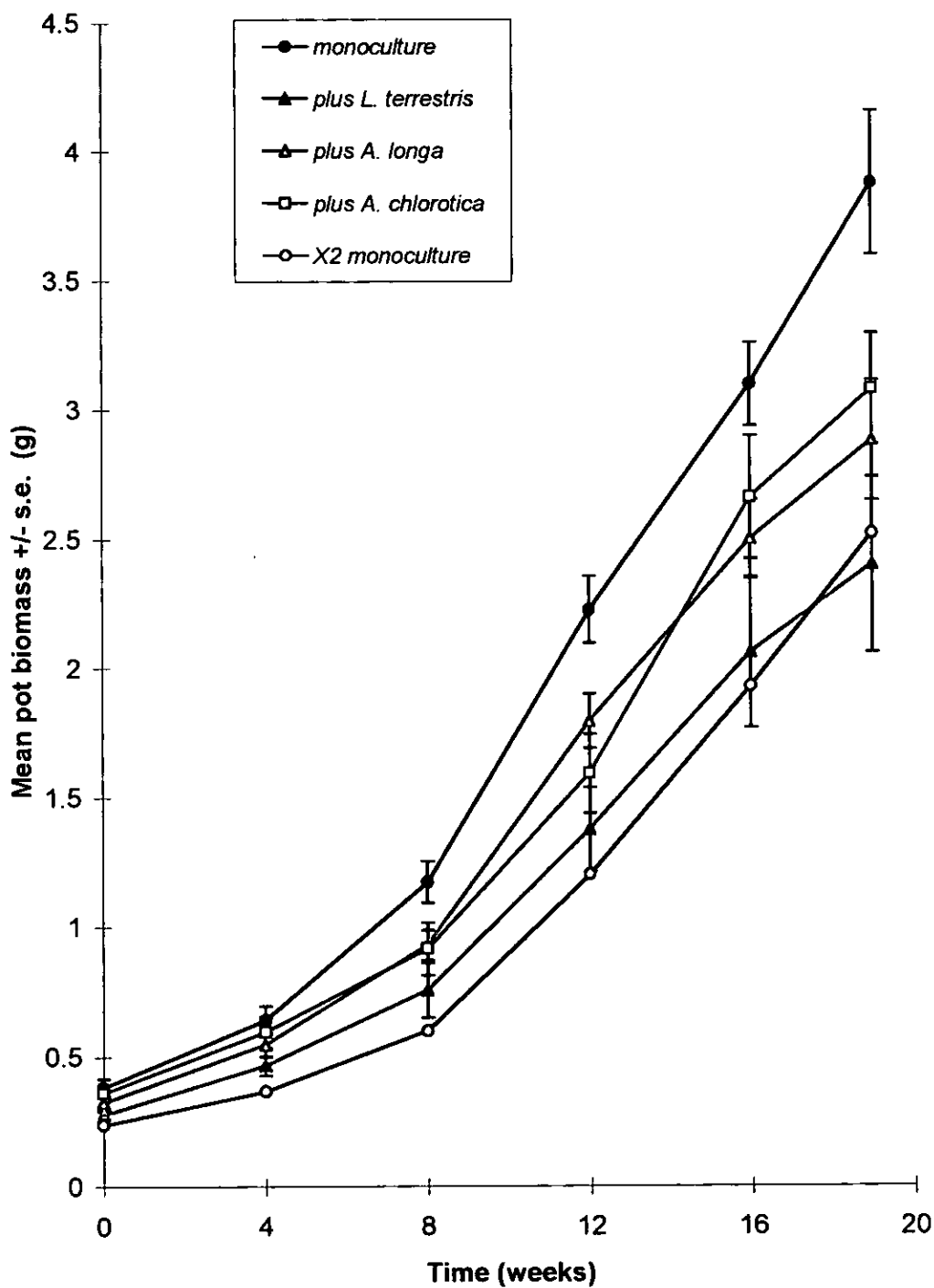
N.B. The graphs are colour coded with reference to earthworm ecological groupings : pairings with the two anecic species are coloured red, while pairings with the two endogeic species are coloured blue. Furthermore to enable comparisons with other treatments total pot biomass for the X2 treatments have been halved.

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4.3.4 Discussion

Results suggested that growth rates of *A. chlorotica*, *A. longa* and *L. terrestris* were significantly (p<0.05) reduced in presence of other species in comparison with species monocultures (controls) under the optimally controlled environmental conditions imposed by the experiment. Furthermore results indicated that the extent to which individual species growth rates were influenced by these negative inter-specific interactions was species specific and associated with ecological grouping. For example, the growth rates of the 2 anecic species (*L. terrestris* and *A. longa*) were more negatively influenced when paired together than when paired with either of the endogeic species (*A. chlorotica* and *A. caliginosa*). Results also showed intra-specific interactions (represented by comparison of monocultures at 2 densities) negatively influenced all four species. Growth rates under X2 treatments were reduced to levels comparable with those exhibited in pairings between species from the same ecological grouping. In contrast to the interactions experiment (section 4.2) using field-collected adults, and almost certainly as a result of improvements made in experimental design, the highest growth rates for all species were recorded in the monoculture controls.

Results substantiate the assertions made at the outset of this chapter : -

a) Competitive inter- and intra-specific interactions between earthworms are detectable under laboratory conditions.

b) The degree to which individual earthworms interact may, in many cases, be dependant on the extent to which their ecological niches overlap.

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It was suggested that as earthworms grew and biomass increased within the culture vessels then competition for both the fixed amount of food and space would have increased. This type of resource competition would have been at its most intense in treatments where earthworms shared identical (X2 monocultures) or almost identical (2 anecic species) requirements. Furthermore, in comparison with treatments containing an anecic and an endogeic species which have different niches within the soil, individual encounter rates within the former treatments would be at their most frequent. It was suggested that encountering another earthworm might result in an avoidance response diverting time and energy away from feeding and so negatively influence growth rates.

4.3.4.1 Further Refinements to Experimental Design

Results indicated that *A. caliginosa* was not significantly (p>0.05) affected by the presence of other species. However, differences in *A. caliginosa* biomass could have been hidden by experimental error arising from individual variances in mass *A. caliginosa* is a highly plastic species (section 3.2.1), individuals of which may vary greatly in mass. Hatchlings for this experiment were obtained from the cocoons of field collected earthworms which had not been selected according to size (section 3.2.1). As hatchlings gained mass over the course of the experiment it became apparent that treatments containing *A. caliginosa* contained individuals of varying size. This was not borne out by the data as individual earthworm masses were not recorded. In addition to highlighting this type of experimental error, the recording of individual masses in future experiments would allow for statistical comparisons to include the X2 treatments and also increase their reliability.

4.4 AN EXPERIMENT TO ASSESS THE INFLUENCE OF MATURE

EARTHWORMS ON THE GROWTH AND MATURATION

OF HATCHLINGS

4.4.1 General Introduction

The final experiment of this chapter investigated both inter- and intra-specific interactions between adult and hatchling earthworms. It was envisaged that the presence of adult worms in the enclosed experimental system would greatly influence hatchling growth and behaviour due initially, to the large differences in biomass, resource acquisition and movement within the soil, which would therefore highlight interactions between and within species. It was also proposed that the pairing of adult and hatchling earthworms would provide a more realistic impression of interactions occurring within natural communities which are composed of several age classes.

This experiment used *A. chlorotica, A. caliginosa, A. longa* and *L. terrestris* as before but also incorporated the epigeic species *L. rubellus*, allowing interactions between all 3 ecological groupings to be observed.

4.4.2 Materials and Methods

All earthworms used in the experiment were obtained from laboratory-reared stocks. Hatchling *L. terrestris*, *A. longa* and *A. chlorotica* (individual mean mass 0.06, 0.05, 0.001 g, respectively) were cultured in species monoculture, double density monoculture (X2) and in species pairings with mature *L. terrestris*, *A. longa*, *A. chlorotica*, *L. rubellus* and *A. caliginosa*. *L. rubellus* hatchlings (individual mean mass 0.006 g) were maintained in identical treatments as above except for the *L. rubellus* (hatchling) and *A. longa* (mature) treatment which was omitted as there was insufficient stocks of mature *A. longa*.

Five replicates of each treatment were set up in 1 litre culture vessels filled to a depth of 0.11 m with a mixture of pre-sterilised Kettering loam and 30 g of SCS (combined moisture content 25 %), with a further 30 g of SCS placed on the soil surface. For the duration of the experiments the culture vessels were kept in darkness at 15 ± 1 °C in temperature-controlled incubators. At the outset of the experiment, earthworms of each species (hatchlings and adults) had masses determined individually and were assigned to treatments according to the regime outlined in table 4.4.1. This regime was developed from the pre-determined ratios (section 3.4.1). However, the number of *A. longa* and *L. terrestris* used were identical reflecting the similarity in hatchling mass of these 2 species (table 4.4.2). At week zero there was no significant difference ($p > 0.05$) in individual species hatchling masses between treatments.

Table 4.4.1 Earthworm Numbers Employed in Experimental Treatments

Investigating the Effect of Mature Earthworms on the

Growth and Maturation of Hatchlings

Treatments	No. of hatchlings used (per pot)			
	L. terrestris	*A. longa*	*A. chlorotica*	*L. rubellus*
Monoculture	2	2	8	4
X2 Monoculture	4	4	16	8
+ 1 mature *L. terrestris*	2	2	8	4
+ 1 mature *A. longa*	2	2	8	0
+ 2 mature *L. rubellus*	2	2	8	4
+ 3 mature *A. caliginosa*	2	2	8	4
+ 4 mature *A. chlorotica*	2	2	8	4

Earthworm survival, individual mass and reproductive condition were recorded every 4 weeks, at which point both soil and food were replaced. However, due to the initial small size of hatchling *A. chlorotica* and *L. rubellus*, and the associated time required to locate these species in experimental cultures, initial sampling of treatments containing hatchlings of these two species took place after 8 weeks. The treatments containing *L. terrestris* were terminated after 36 weeks and the treatments containing the other 3 species terminated after 24 weeks reflecting the differences in species growth rates and time to maturation. On termination of the experiment, soil from culture vessels containing *A. chlorotica* and *L. rubellus* was wet-sieved for cocoons.

Differences in the mean individual mass of each of the four species across treatments was statistically analysed using the Tukey-Kramer multi-comparison analysis of variance (ANOVA)

Table 4.4.2 Mean Hatchling Mass and Total Number of Each Species Employed in Determining the Effect of Mature Earthworms on the Growth and Maturation of Hatchlings

Species	Individual mean hatchling mass (g) at week 0	No. used in experiment
L. terrestris	0.067	80
A. longa	0.0528	80
A. chlorotica	0.01	320
L. rubellus	0.005	180

4.4.3 Results

For all four species 90 to 100 % survival was recorded during the experimental period, with all survivors exhibiting an increase in mass.

4.4.3.1 Growth and Maturation of *L. terrestris*

Figure 4.4.2 illustrates the growth of *L. terrestris* in the seven treatments over the 36 week experimental period. After 8 weeks the mass of hatchlings in the pairing with mature *L. terrestris* was significantly greater ($p < 0.05$) than in the other six treatments. Hatchlings in this treatment had an individual mean mass of 0.65 g compared with hatchlings in pairings with mature *A. chlorotica*, *A. caliginosa*, *A. longa*, *L. rubellus* and single and X2 monocultures which had mean individual masses of 0.32, 0.45, 0.42, 0.51, 0.36 and 0.39 g, respectively. By week 20 this situation had changed and *L. terrestris* in the monoculture treatment then had a significantly greater ($p < 0.01$) mean mass than all

Figure 4.4.1 Growth of _L. terrestris_ in Monoculture, Double Density Monoculture (X2) and Paired Combinations With Mature Earthworms, Over a 12 Week Period
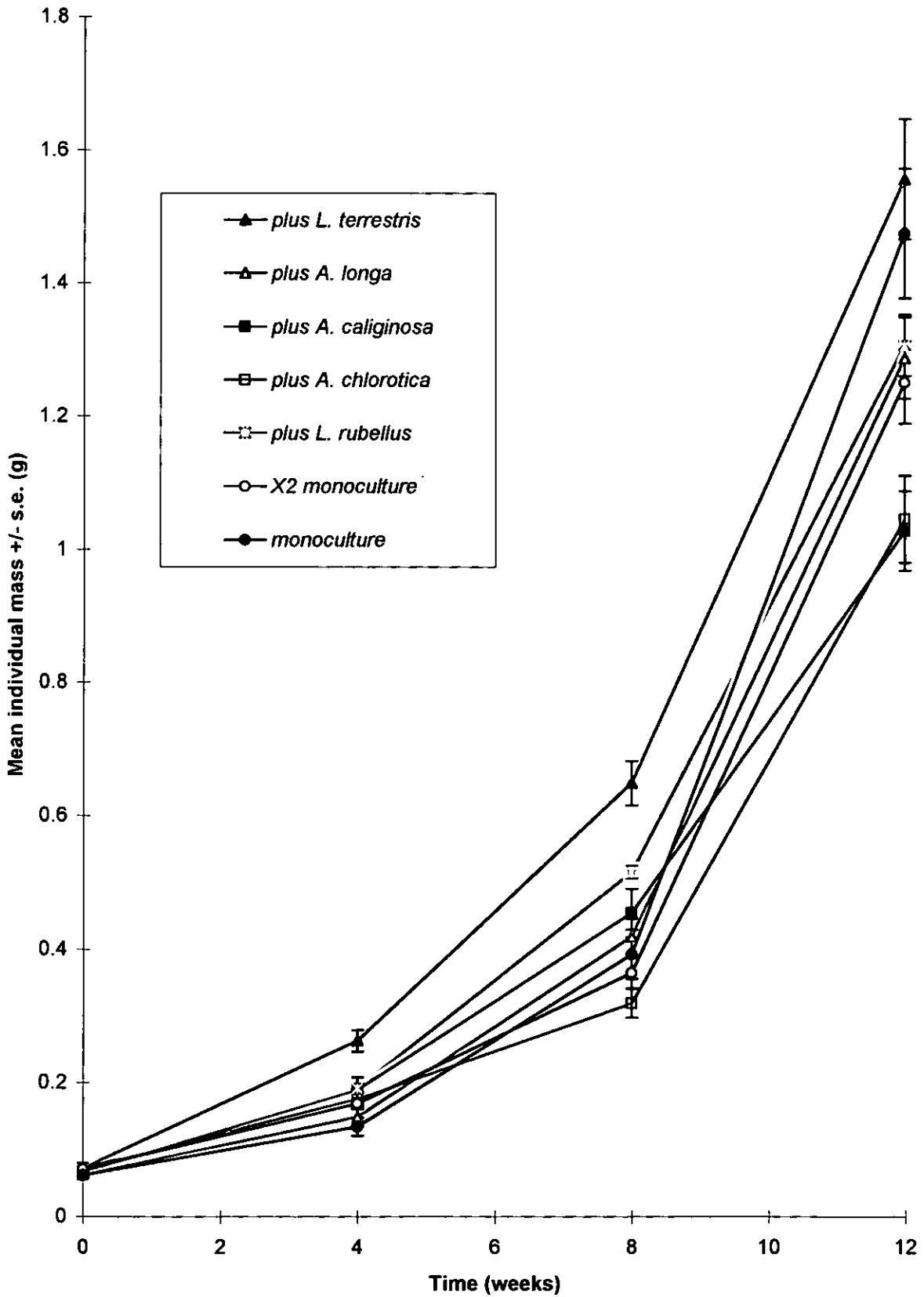
Figure 4.4.2 Growth of _L. terrestris_ in Monoculture, Double Density Monoculture (X2) and Paired Combinations With Mature Earthworms, Over a 36 Week Period
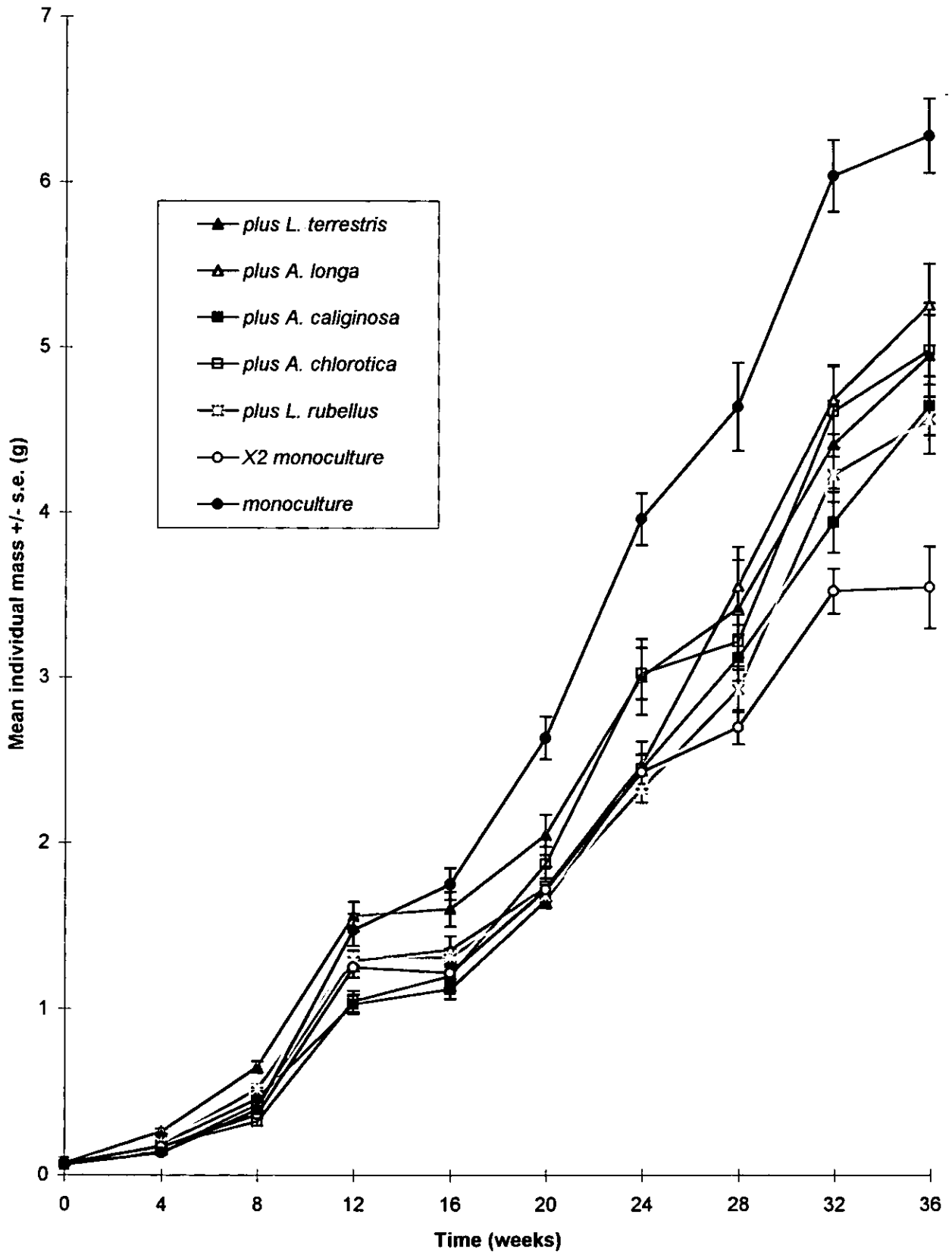
Figure 4.4.3 Percentage Maturation of Individual *L. terrestris,* Weeks 28-36 (Treatments Refer to the Mature Earthworm Species Cultured With Hatchling *L. terrestris*)
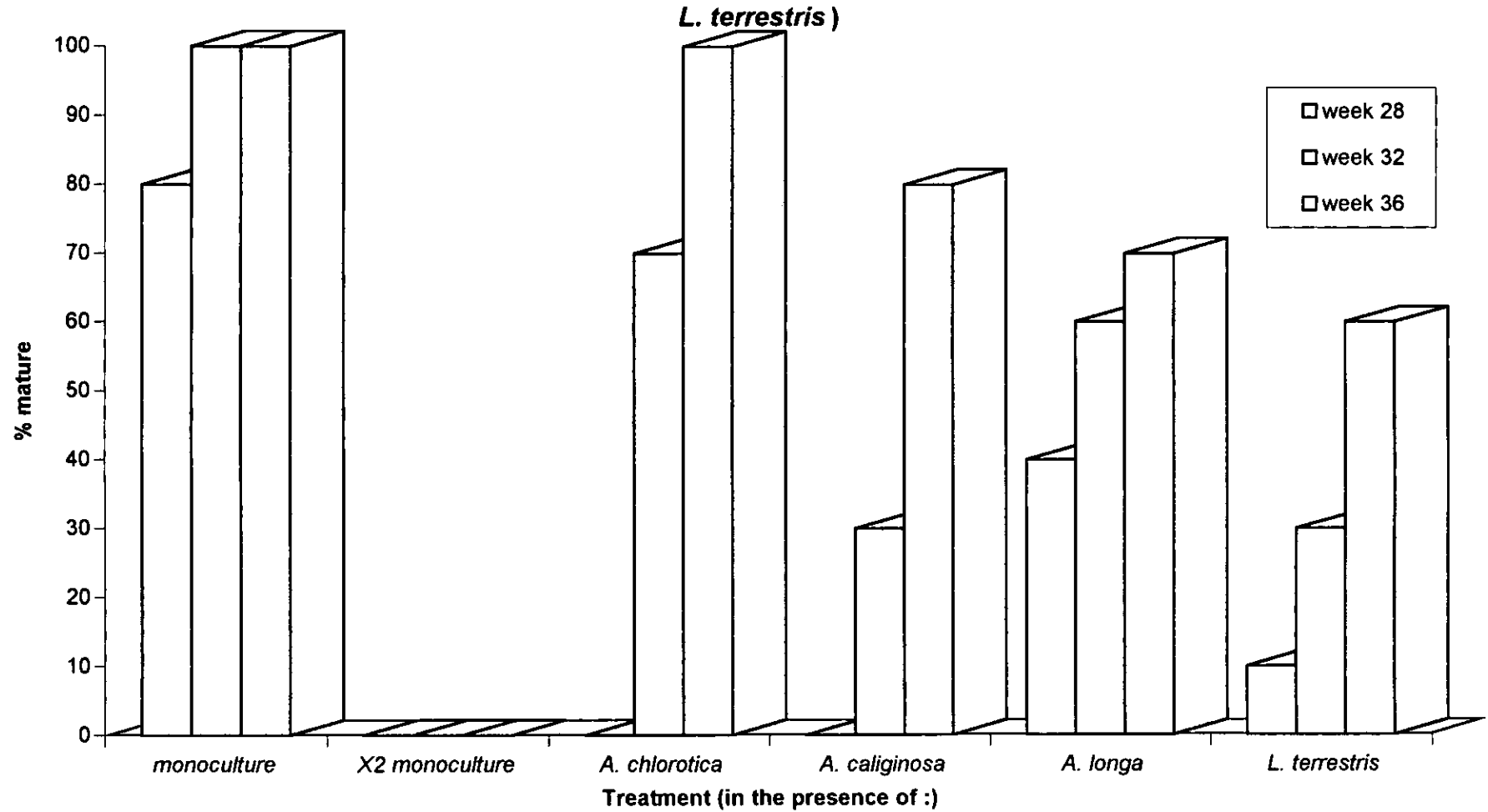
other treatments, between which there was no significant difference (p>0.05). After 36 weeks the individual mean mass of *L. terrestris* in the monoculture treatment (6.014 g) remained significantly greater (p<0.05) than in all other treatments, of which, the lowest individual mean mass (3.52 g) was attained in the X2 treatment. In paired treatments there remained no significant difference in the mean individual mass of *L. terrestris*. In spite of the recorded parity in the individual mass of *L. terrestris* in paired species treatments, figure 4.4.3 suggested that differences existed in *L. terrestris* maturation (as shown by clitellum development) between these treatments during weeks 28 to 36. Maturation was most rapid in *L. terrestris* monoculture and reproductive condition was attained by 100 % of individuals by week 32, in contrast to the X2 treatment where no clitellum development was recorded. In paired treatments, *L. terrestris* maturation was least developed in the presence of *L. rubellus* (20 % of individuals clitellate after week 36). Clitellum development in treatments containing mature *L. terrestris* and *A. longa* was observed at week 28 but by week 36 only 60 and 72 % of individuals, respectively, were clitellate compared with 100 % in the pairing with *A. chlorotica*.

4.4.3.2 Growth and Maturation of *A. longa*

Figure 4.4.4 illustrates the growth of *A. longa* in the seven experimental treatments over a 24 week period. After 12 weeks the individual mean mass (1.67 g) of *A. longa* in monoculture was significantly greater (p<0.01) than in all other treatments. At the end of the experiment the individual mean mass of *A. longa* (1.22 g) in the pairing with mature *A. longa* was significantly lower (p<0.01) compared with the other 6 treatments. The greatest mean mass was still found in monoculture (2.71 g) but was not significantly different (p>0.05) to mean masses in the pairings with *A. caliginosa*, *A. chlorotica*, *L. rubellus* and *L. terrestris* (2.2, 2.45, 2.29 and 2.48 g, respectively) but was still

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Figure 4.4.4 Growth of *A. longa* in Monoculture, Double Density Monoculture (X2) and Paired Combinations With Mature Earthworms, Over a 24 Week Period
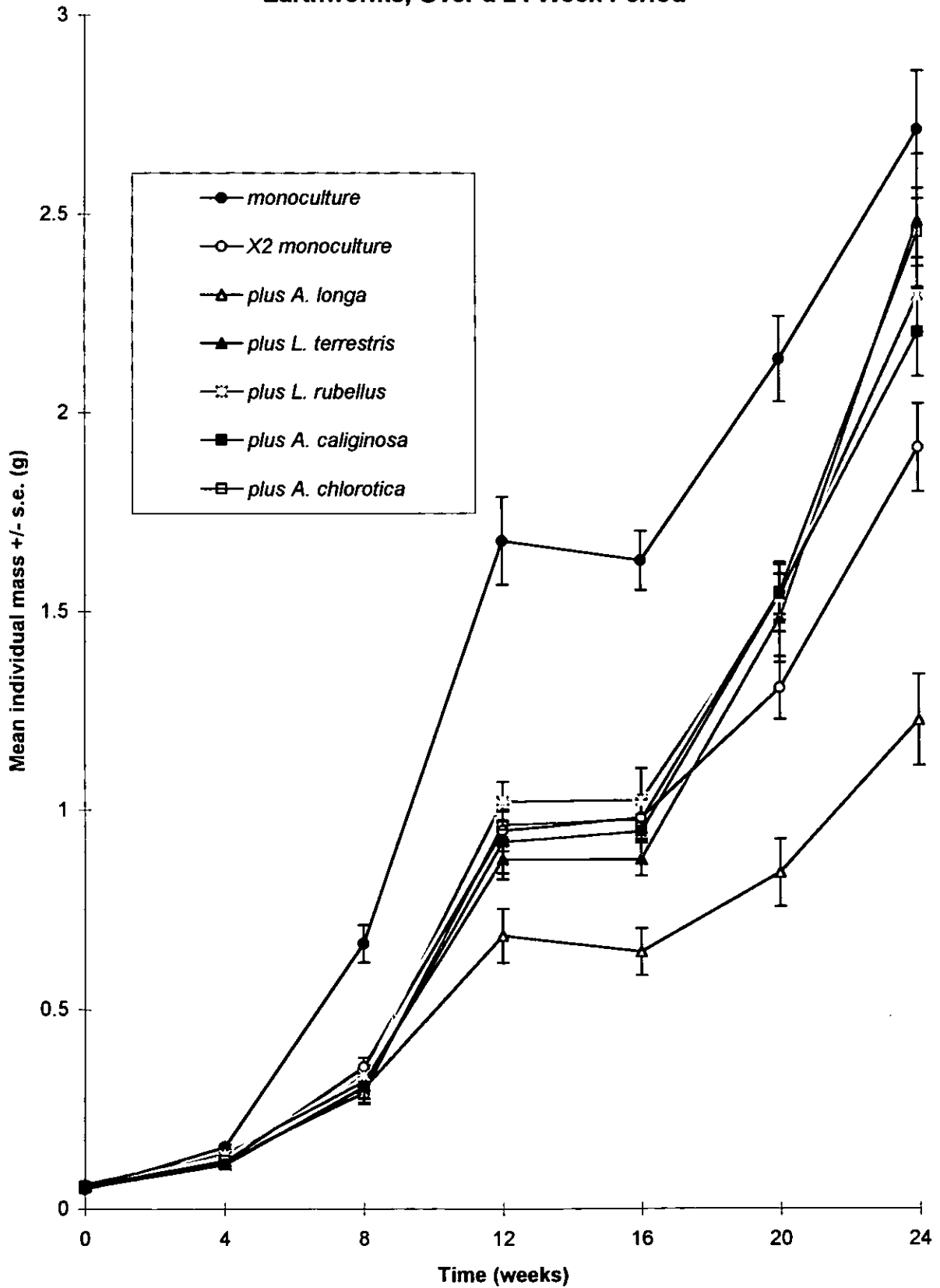
Figure 4.4.5 Percentage Maturation of Individual *A. longa,* Weeks 20-24 (Treatments Refer to the Mature Earthworm Species Cultured With Hatchling *A. longa*)
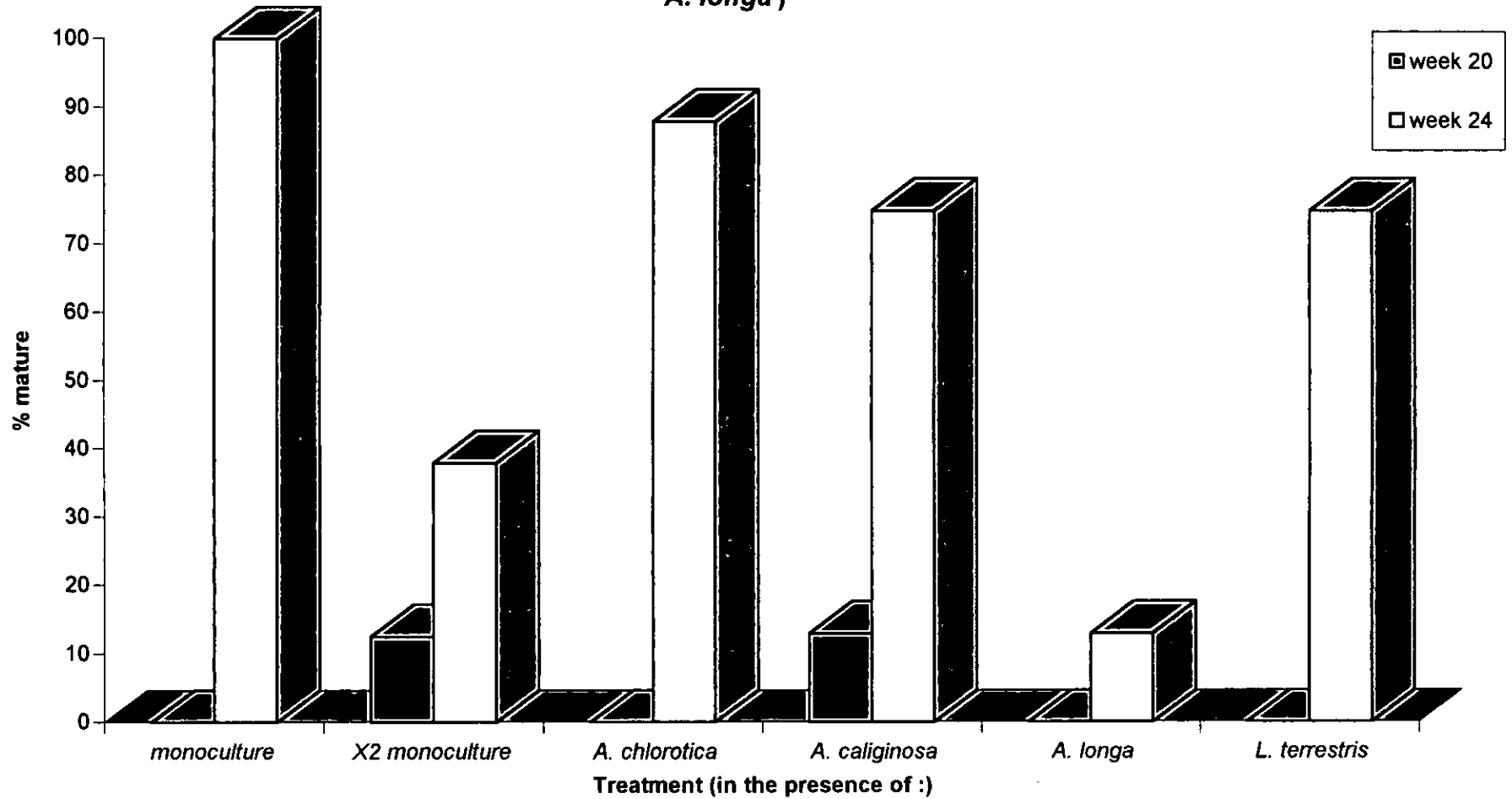
significantly greater (p<0.05) than the X2 treatment (1.91 g). As for *L. terrestris* (section 4.4.3.1), these growth results are mirrored in the percentage of clitellate *A. longa* recorded in treatments (figure 4.4.5); 100 % of individuals in the monoculture were clitellate at week 24 compared with 40 and 15 % in the X2 and mature *A. longa* pairing, respectively.

4.4.3.3 Growth, Maturation and Fecundity of *A. chlorotica*

As with the previous 2 species, differences in the mean mass of earthworms between treatments varied over the experimental period (figure 4.4.6). At week 12 the lowest hatchling growth was exhibited in pairings with *L. terrestris* and *L. rubellus* (individual mean mass of *A. chlorotica* in both treatments 0.113 g). This value was significantly (p<0.01) lower than that observed in the other 5 treatments, with the monoculture yielding a mean mass of 0.27 g. At the end of the experiment this had increased to 0.43 g, when the largest negative influence on *A. chlorotica* growth (individual mean mass 0.28 g) was recorded from the pairing with adult *A. chlorotica*. In the other 5 treatments there were no significant differences (p>0.05) in *A. chlorotica* mass.

The first clitellate individuals (Figure 4.4.7) were recorded after 12 weeks in all treatments except pairings with *L. terrestris* and *L. rubellus*. After 16 weeks, 90 % of the individuals in the monoculture were clitellate compared with 42 % in the double density treatment. In species pairings at week 16, greatest number of clitellate *A. chlorotica* (62 %) was recorded in the treatment with *A. longa* followed by *A. caliginosa* (58 %), *A. chlorotica* (26 %), *L. terrestris* (4 %) and *L. rubellus* (0 %), a trend that was continued in week 20 and was also observed in cocoon production (figure 4.4.8). At week 24 over 90 % of *A. chlorotica* were clitellate in all treatments.

Figure 4.4.6 Growth of *A. chlorotica* in Monoculture, Double Density Monoculture (X2) and Paired Combinations With Mature Earthworms, Over a 24 Week Period

Legend:
- monoculture
- X2 monoculture
- plus a. chlorotica
- plus L. terrestris
- plus A. longa
- plus L. rubellus
- plus A. caliginosa

Y-axis: Mean individual mass +/- s.e. (g)
X-axis: Time (weeks)
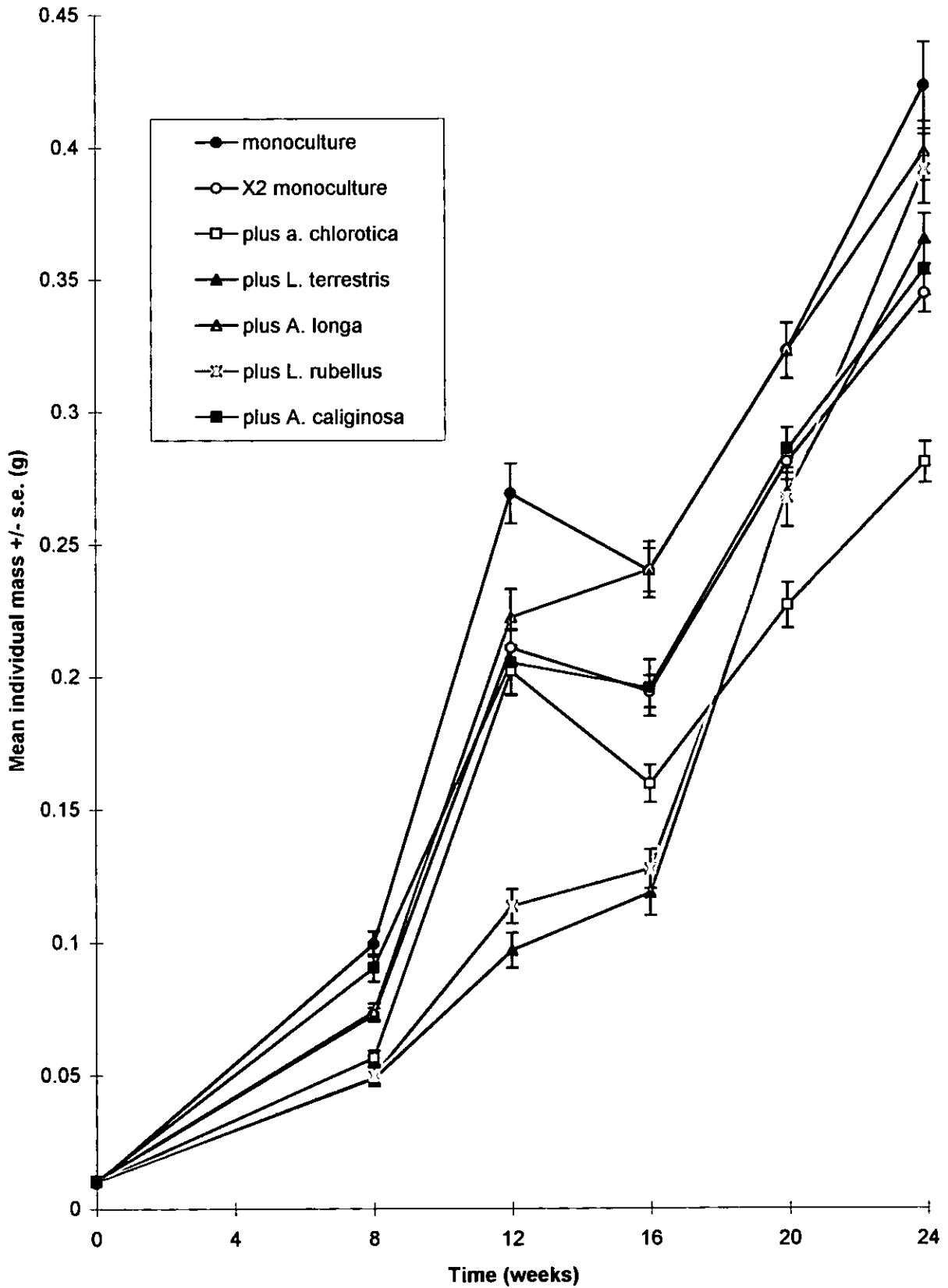
Figure 4.4.7 Percentage Maturation of Individual *A. chlorotica*, Weeks 12-24 (Treatments refer to the Mature Earthworm Species Cultured With Hatchling *A. chlorotica*)
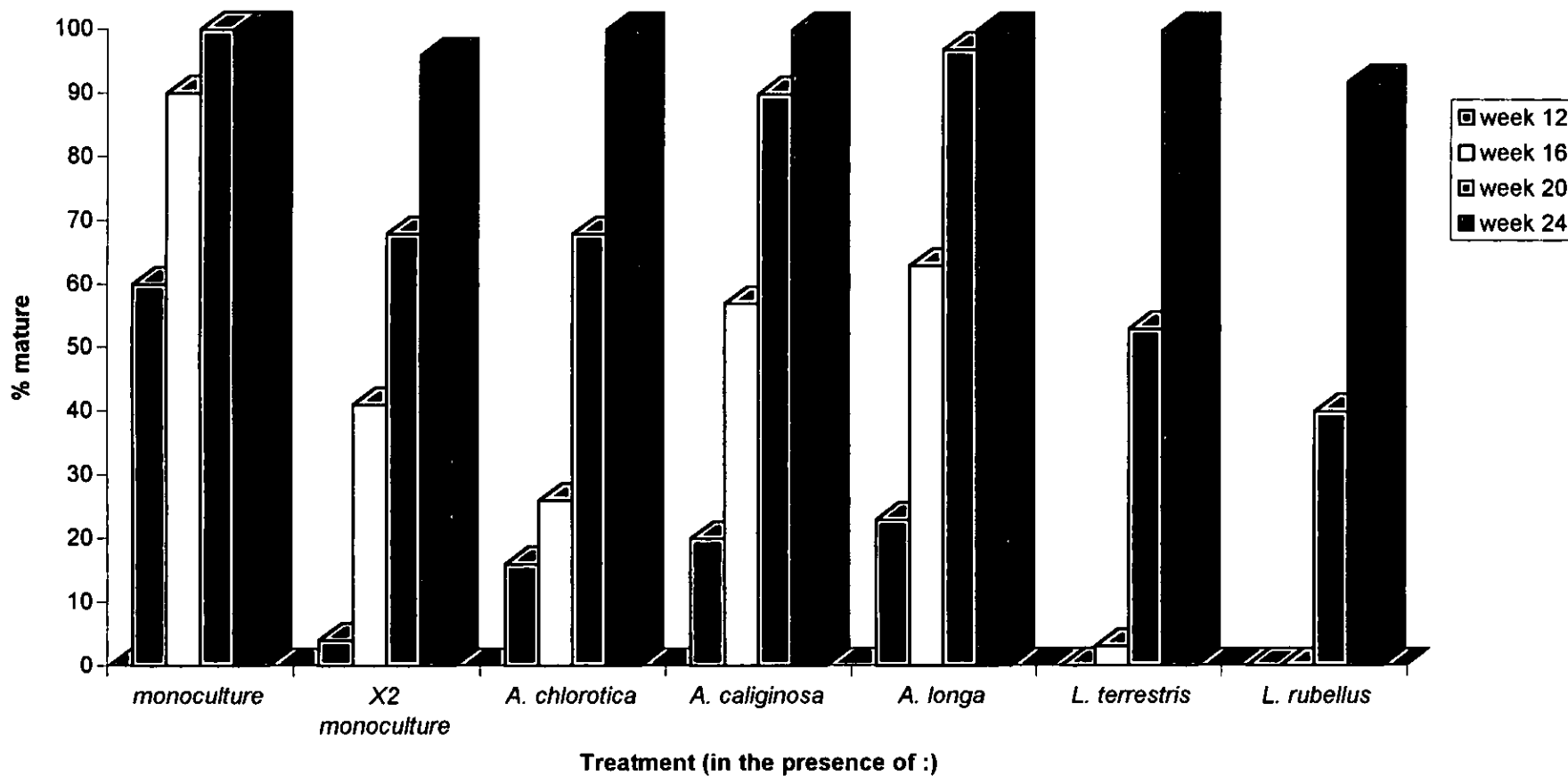
Figure 4.4.8 Cocoon Production by *A. chlorotica,* Week 28 (Treatments Refer to the Mature Earthworm Species Combined With *A. chlorotica*)
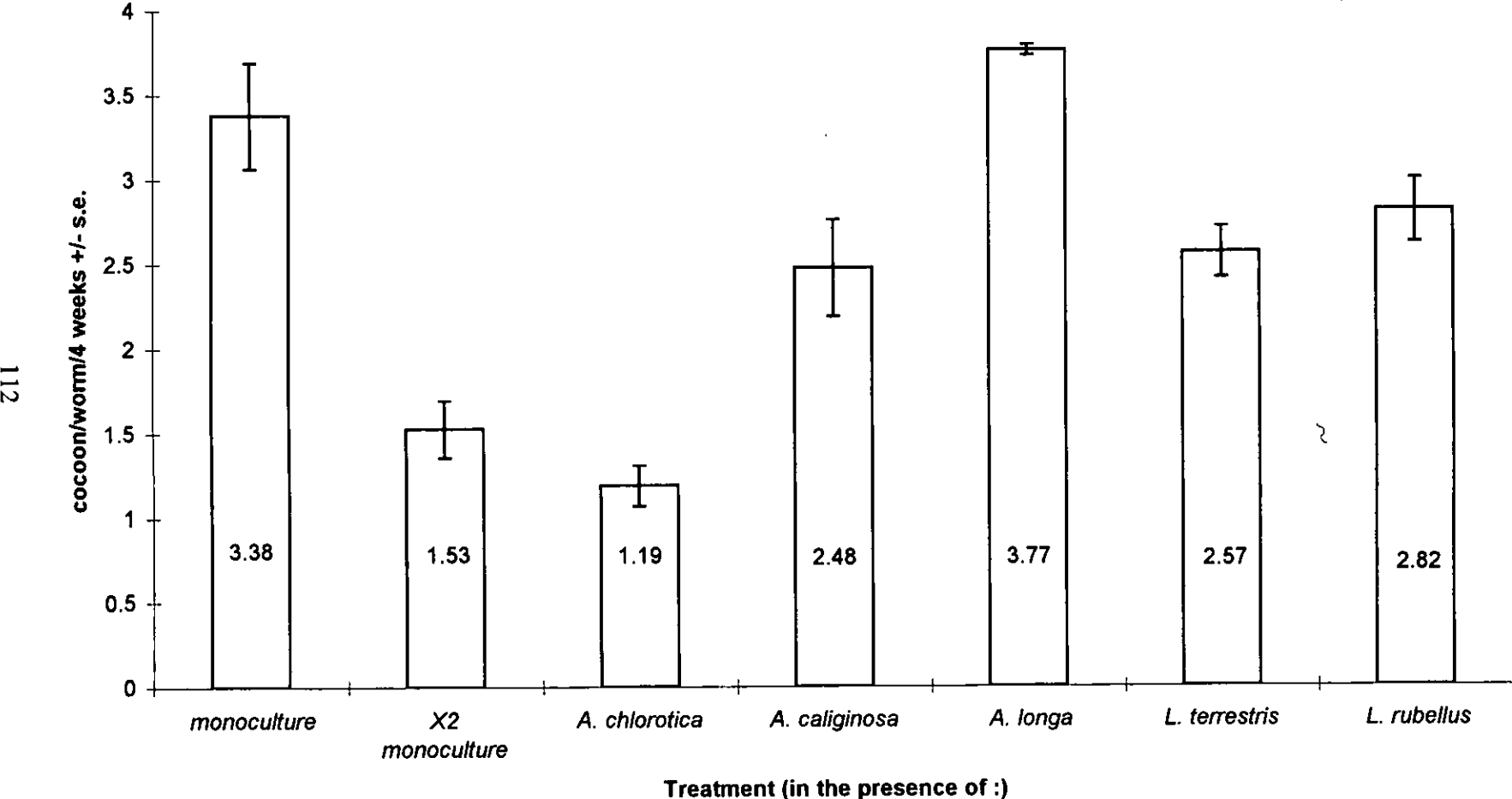
4.4.3.4 Growth, Maturation and Fecundity of *L. rubellus*

At week 12 the monoculture exhibited the largest individual mean mass (0.663 g), significantly greater (p<0.01) than all other treatments, of which the pairing with mature *L. rubellus* had the lowest mean mass (0.154 g). At week 24 this treatment still had the lowest mean mass (0.874 g), however this was comparable with the X2 treatment (0.9178 g). Both of which were significantly (p<0.01) lower than the other 4 treatments (figure 4.4.9). These trends were also observed in maturation and cocoon production of *L. rubellus* across treatments (figures 4.4.10 & 4.4.11). At week 20 over 90 % of individuals in monoculture and in pairings with *A. caliginosa*, *A. chlorotica* and *L. terrestris* were clitellate, compared with 65 % in the X2 treatment and 0 % in the pairing with mature *L. rubellus*. The two latter treatments also exhibited lowest cocoon production (4.69 and 3.19 cocoons worm $^{-1}$ 4 weeks $^{-1}$, respectively) less than half of that achieved by earthworms in monoculture (11.83 cocoons worm $^{-1}$ 4 weeks $^{-1}$).

Figure 4.4.9 Growth of *L. rubellus* in Monoculture, Double Density Monoculture (X2) and Paired Combinations with Mature Earthworms, Over a 24 Week Period
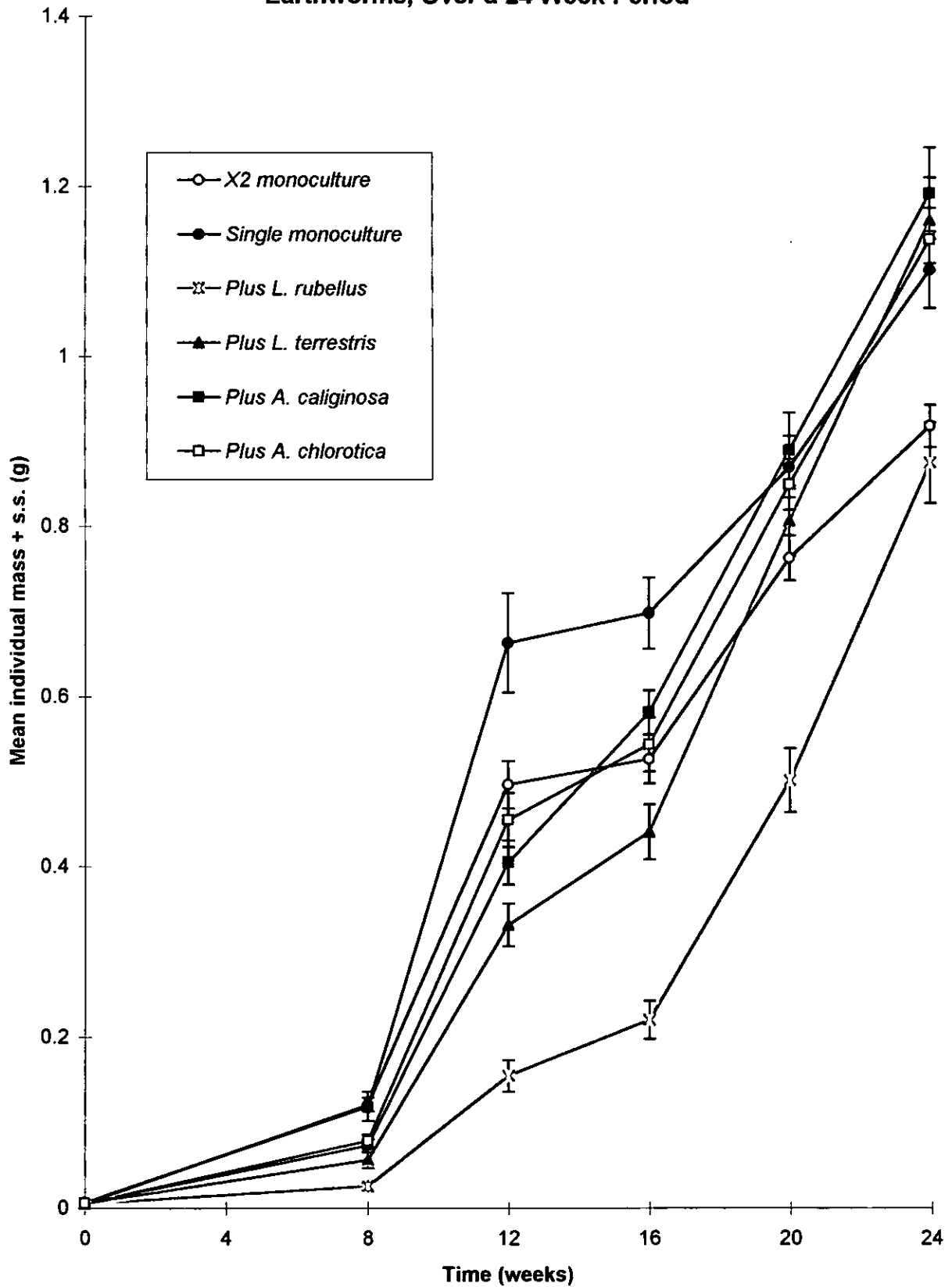
Figure 4.4.10 Percentage Maturation of Individual *L. rubellus*, Weeks 16-24 (Treatments Refer to the Mature Earthworm Species Cultured With Hatchling *L. rubellus*)
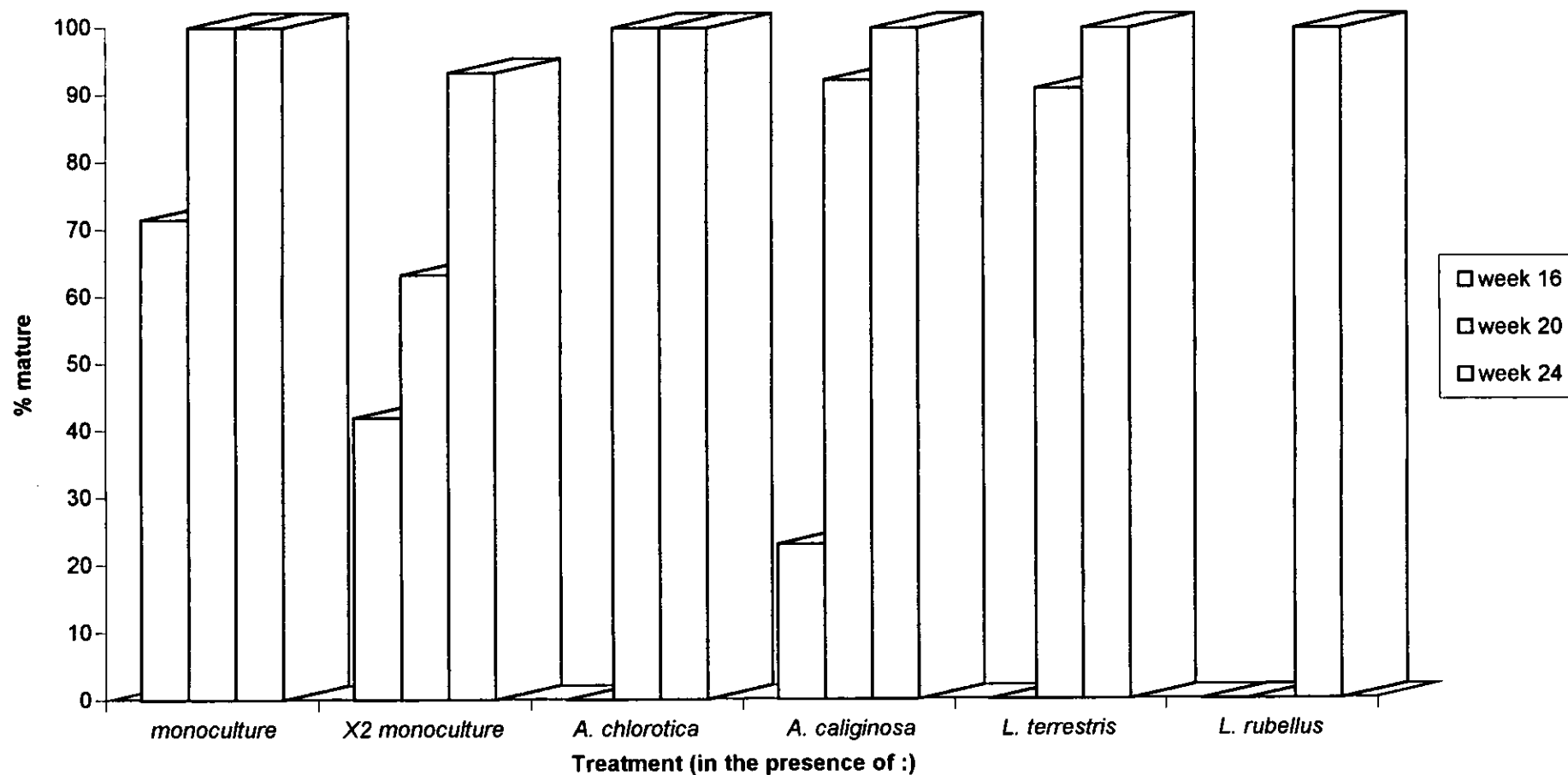
Figure 4.4.11 Cocoon Production by *L. rubellus*, Week 28 (Treatments Refer to the Mature Earthworm Species Combined With *L. rubellus*)
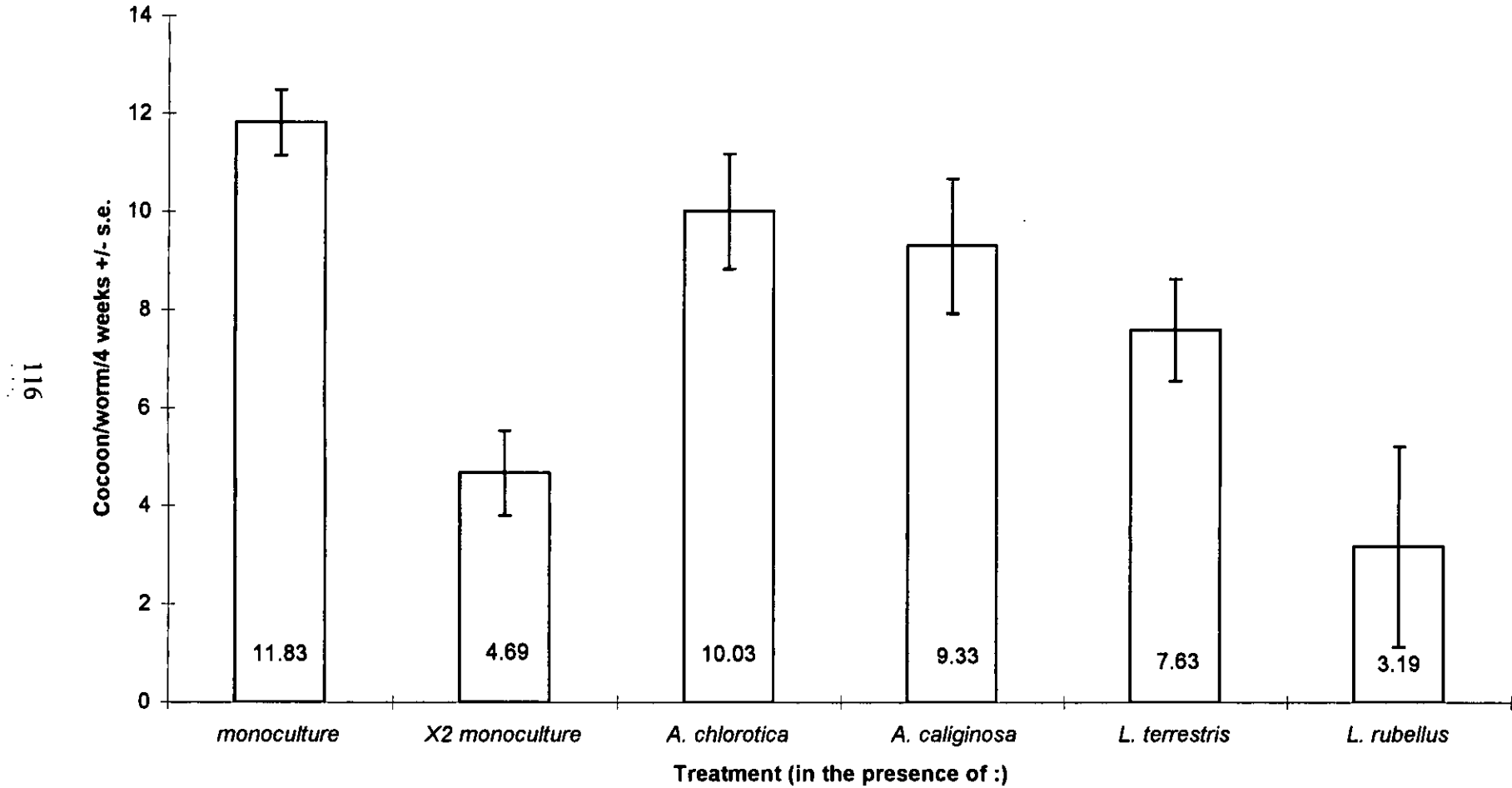
4.4.4 Discussion

For the purposes of clarity the results relating to each of the four species are discussed separately.

4.4.4.1 *L. terrestris*

After 36 weeks mean individual mass of *L. terrestris* in pairings with all other species fell between the control monoculture which achieved the greatest mass and the X2 monoculture. These results agree with previous results that the 'intensity' of observed interactions between earthworms are determined by the degree of niche overlap which they exhibit. With this in mind, one may also have considered that intra-specific interactions in the treatment with adult *L. terrestris* would have significantly reduced juvenile growth rates in comparison with juveniles grown in the presence of adults of other species. However, at week 36 there was no significant difference ($p > 0.05$) in the mean mass of *L. terrestris* in pairings with adults of other species and the pairing with adult *L. terrestris*. This unexpected result may be attributed to the higher growth rates of *L. terrestris* juveniles in the latter in comparison with other treatments in the early stages of the experiment (see figure 4.4.1). At the third sampling (12 weeks) juveniles in the pairing with mature *L. terrestris* exhibited significantly ($p < 0.05$) higher growth rates than in all other treatments including the control monoculture. It is proposed that at this stage of the experiment adult and juvenile *L. terrestris* may have been exhibiting a form of commensalistic association, with juveniles benefiting from the surface casting of the larger adults. Particles of organic matter ingested by earthworms may become fragmented during their passage through the gut and as a result the organic matter present in casting is of a much finer particle size than that ingested. It was suggested that as the diameter of

the mouth aperture of juveniles was related to body size the organic matter present in the casts of adult *L. terrestris* may have provided juveniles (mass < 1 g) with a concentrated and more readily digestible source of feed in comparison with the larger particle sizes of undigested SCS in other treatments. This hypothesis was supported by field observations by Butt (pers. comm.) who has recorded the presence of juvenile *L. terrestris* in the middens of adults.

During the initial phase of the experiment (up to 12 weeks) the influence of the two endogeic species on *L. terrestris* growth also conflicted with the results obtained from earlier experiments (figure 4.4.1). In these experiments the presence of endogeic species in cultures with *L. terrestris* had little influence on growth rates. In contrast, after the first three samplings in the current experiment *L. terrestris* growth rates were lowest in treatments containing *A. chlorotica* and *A. caliginosa*. After 12 weeks the growth rate of *L. terrestris* in these two treatments increased and by week 24 *L. terrestris*, in the treatment with *A. chlorotica*, had a greater mean mass than in any other species pairing. It is suggested that this increase in mass is linked to a change in the behaviour of *L. terrestris* juveniles. Observations made during the first three samplings indicated that when small, juvenile *L. terrestris* were limited in their movement within the soil as they were only recorded in the upper soil layers (to a depth of 3 - 4 cm). Therefore, juvenile *L. terrestris* would initially have occupied the same position within the soil profile as the endogiec species and were also of similar mass and size. This would have increased encounter rates (interference competition) and may also have resulted in direct competition for food within the upper soil layers. As juveniles gained in mass (after 12 weeks) they began to adopt anecic characteristics, forming single vertical burrows, some of which extended to the bottom of the culture vessel. As a result of behavioural changes

interactions between *L. terrestris* juveniles and adults of the two endogeic species were also reduced and *L. terrestris* growth rates in these treatments increased.

In contrast to growth there were very marked differences in the percentage of mature *L. terrestris* recorded in treatments with other species (100, 80, 72, 60 and 20 % in pairings with *A. chlorotica*, *A. caliginosa*, *A. longa*, *L. terrestris* and *L. rubellus* respectively). It is suggested that both inter- and intra-specific competition between earthworms may have reduced the energy available for the development of secondary sexual characteristics and influenced the recorded differences in *L. terrestris* maturation rates across paired species treatments. Fewest *L. terrestris* matured in the pairing with *L. rubellus* and when it did occur was delayed until week 36. *L. rubellus* is a highly mobile, pioneer earthworm species with rapid growth rates and as an epigeic species tends to live and feed in the surface organic layer. Therefore in the enclosed culture vessels *L. rubellus* may be outcompeting the more sedentary *L. terrestris* for food and encounter rates between the two species at the surface would be frequent. *A. longa* like *L. terrestris* is an anecic species and occasionally feeds at the soil surface. Therefore one may expect that the negative influence of *A. longa* on *L. terrestris* maturation rates would be greater than that of *L. rubellus*. However *A. longa* is not as mobile as *L. rubellus* and as a result encounter rates with *L. terrestris* may have been reduced and competition for organic matter may not have been as intense. The greatest number of mature *L. terrestris* (except for the control monoculture) were achieved in pairings with the two endogeic species. Interactions between these two species and *L. terrestris* would have been minimised as they are separated both spatially, and in terms of feeding behaviour. In contrast no *L. terrestris* in the X2 monoculture reached maturity during the experimental period. Given this, one may also have expected *L. terrestris* maturation rates in the pairing with

adult *L. terrestris* to have been reduced to a level similar to that in the X2 monoculture. However, at the end of the experiment maturation rates in pairing with adult *L. terrestris* were comparable with those in the pairing with *A. longa* and probably resulted from the initial (up to week 12) high growth rates, for juvenile *L. terrestris* in this pairing.

4.4.4.2 *A. longa*

At the end of the experimental period (24 weeks) the growth curves for *A. longa* followed a similar pattern to those observed for *L. terrestris*. Greatest mean mass of *A. longa* was recorded in the control monoculture, with paired species treatments intermediate in mass between this and the X2 monoculture. However, the lowest *A. longa* mass in all treatments was recorded in the pairing with adult *A. longa*. Observations of juveniles in the soil profile, made during sampling, were similar to those for *L. terrestris*, as until week 12, juvenile *A. longa* were restricted to the upper soil layers and only started to form vertical burrows after this time, as individuals gained in mass. Unlike *L. terrestris*, growth rates of juvenile *A. longa* were not enhanced (in comparison with other treatments) by the presence of adults during the initial 12 week period. After 12 weeks, adult *A. longa* significantly (p<0.05) reduced the growth rate of juveniles when compared with all other treatments including the X2 monoculture. It was suggested that competition (intra-specific) for both food and space would have been at its most intense in the pairing with adult *A. longa* as individuals possessed exactly the same requirements. This was also the case in the X2 monoculture but during the early part of the experiment the total biomass in this treatment was substantially lower than in the pairing with adult *A. longa*. Therefore competition for space and food would have been initially lower in the X2 monoculture. It was proposed that the negative influence of adults on juveniles observed in enclosed laboratory conditions where earthworms are in close proximity may, under

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field conditions, serve to encourage species dispersal. This would increase the rate of habitat colonisation, limit the competition between individuals of the same species for common resources as well as reducing inbreeding.

4.4.4.3 *A. chlorotica*

Recorded growth, maturation and cocoon production rates for *A. chlorotica* further substantiated the proposal that the intensity of competitive interactions between earthworms was dependant upon the degree to which their resource requirements overlapped. Furthermore, results suggested that although inter-specific interactions may be influenced greatly by the ecological grouping to which species belong, the influence that two species from the same group have on a third species may vary substantially. *L. terrestris* and *A. longa* are both anecic species and therefore one may have expected the growth, maturation and reproductive rates of *A. chlorotica* grown in the presence of these two species to have been similar. Results suggested that the presence of *L. terrestris* negatively influenced *A. chlorotica* growth, maturation and cocoon production rates in comparison with the control monoculture. However, *A. chlorotica* cultured with *A. longa* exhibited growth and maturation rates which were comparable to those recorded for the control monoculture and cocoon production in the presence of *A. longa* was greater than that achieved in the control monoculture (3.8 and 3.4 cocoon worm^{-1} month^{-1}). This result suggested that the presence of *A. longa* may enhance the production of *A. chlorotica*, a situation that was also recognised by Butt *et al.* (1997). These workers cultured *A. longa* and *A. chlorotica* in single and paired species cultures for inoculation at a partially restored landfill site. Results obtained for earthworm production indicated that the presence of the two species in a single container enhanced individual production of cocoons and hatchlings in comparison with single species

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cultures in an equivalent soil volume. This positive inter-specific interaction was also recorded at the inoculation site (Butt *et al.*, 1999). Five years after the inoculation of mixed and single species cultures onto the site the recorded numbers of *A. chlorotica* were significantly higher (p<0.05) in plots where mixed species had been inoculated in comparison with plots inoculated with single species cultures. A mechanism for this beneficial inter-specific interaction was also put forward by these workers. It was suggested that *A. longa* may have been making surface applied food more available to *A. chlorotica* by covering it with casts. However, if this were the case, then why in the current study did *A. chlorotica* not benefit from the presence of the surface casting *L. terrestris*. Clearly this is an area which requires further research.

4.4.4.4 *L. rubellus*

Results relating to the growth of *L. rubellus* demonstrated that unlike the other three species there was no significant difference (p>0.05) between the control monoculture and the pairings with *A. chlorotica, A. caliginosa* and *L. terrestris*. There was also no substantial difference in maturation rates and cocoon production of *L. rubellus* between these paired species treatments. This suggested that the presence of both the anecic and endogeic species had little influence on the growth and fecundity of *L. rubellus*. In contrast *L. rubellus* negatively influenced the growth and maturation of the other three species. Most epigeic species exist almost entirely in the surface organic layer. However, in this experiment *L. rubellus* was recorded throughout the soil profile concurring with habitat descriptions made for this species by Sims & Gerard (1985). The presence of *L. rubellus* in the soil profile indicated that this species may also be feeding on the soil incorporated organic matter. Therefore *L. rubellus* may have been in direct competition with anecic species for food at the surface and with the endogeic species for food mixed

into the soil. *L. rubellus* has both high dispersal and reproductive rates and as a result is often the first species to colonise unstable habitats (see section 2.4). Therefore it was suggested that *L. rubellus*, under the given conditions of this experiment, outcompeted the anecic (corroborating results obtained by Butt, 1998) and endogeic species which are more suited to undisturbed stable habitats (*e.g.* permanent pasture).

In summary results suggested, that both inter and intra-specific interactions negatively influenced the growth maturation and fecundity of the selected earthworm species in an enclosed laboratory system. Also, in certain species, the extent to which these interactions influenced earthworm production was found to be not only determined by the species present but also by individual earthworm mass and state of development.

CHAPTER 5. THE EFFECT OF SELECTED ENVIRONMENTAL FACTORS ON RECORDED EARTHWORM SPECIES INTERACTIONS

5.0 INTRODUCTION

This chapter presents findings from experiments undertaken to determine the influence of selected environmental variables on earthworm interactions and to further investigate findings from the previous chapter. The latter demonstrated the negative influence of intra- and inter-specific interactions on earthworm growth, maturation and cocoon production of all experimental species. Those experiments were conducted under controlled laboratory conditions considered to be optimal for earthworm survival and growth.

Experiments described in this chapter investigated the effects of manipulating three experimental variables; food placement, food particle size and soil bulk density on species interactions. These 3 factors have been shown to have a significant influence on earthworm community structure within different habitats (section 2.2). It was therefore proposed that manipulation of such variables within experimental cultures might affect the nature and relative intensity of earthworm interactions, and also provide a more realistic insight into species interactions under field conditions, especially those which might be encountered at land restoration sites.

5.1 SPECIES SELECTION

Experimental design was restricted by constraints as described in Chapter 3. Previous experiments investigated interactions between five selected species. In these experiments, species growth, maturation and cocoon production, in all species pairings were compared with species monocultures (controls) under optimally maintained environmental conditions. This design was relatively simple and therefore it was possible, to employ the whole suite of species in a single experiment with adequate treatment replication. However, the design of several experiments described in this chapter was more complex. Inter- and intra-specific interactions were assessed in experimental treatments in which specific levels of an environmental factor were manipulated (*e.g.* compacted or uncompacted soil). Due to this added complexity the number of species used was reduced. Of the five species described in the previous chapter only three:- *A. chlorotica, L. rubellus* and *L. terrestris* were retained for use (with the exception of *A. longa* employed in the first food position experiment (section 5.2)). Species selection was based upon several factors :-

1) A major aim of this research was to establish if positive (mutualistic) interactions could be detected between earthworm species. [The three selected species represent each of the three main ecological groupings (Bouché, 1971). Results from Chapter 4 suggested that the intensity of negative interactions was directly related to the degree of niche overlap between earthworms. It was therefore proposed that beneficial (mutualistic) interactions were more likely to occur between species from different ecological groupings where niche separation was greatest.]

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2) *A. caliginosa* was not used as it is a highly plastic species known to exist in several morphs (section 3.2.1) This variation was thought to have influenced recorded interactions with other species in experiments of Chapter 4. Therefore *A. chlorotica* was selected to represent the endogeic grouping.

5.2 AN EXPERIMENT TO INVESTIGATE THE EFFECT OF FOOD PLACEMENT ON THE GROWTH AND BEHAVIOUR OF TWO ANECIC EARTHWORMS

5.2.1 Introduction

The previous experiment indicated that *L. terrestris* displayed two distinct foraging and burrow formation behaviours during growth to maturity. It was proposed that *L. terrestris* initially behaved as an endogeic species; feeding within the top soil layer and creating non-permanent horizontal burrows. However, as *L. terrestris* gained in mass, individuals started to form single, permanent, vertical burrows opening to the soil surface (at which they also fed). It was suggested that these changes in feeding behaviour might be characteristic of the anecic earthworm grouping. This experiment was therefore set up to investigate this further and also determine the effect of food placement regimes on the growth of *L. terrestris* and *A. longa* another anecic species.

5.2.2 Materials and Methods

Hatchling *A. longa* and *L. terrestris* (mean individual masses of 0.07 and 0.06 g, respectively) were obtained from cocoons produced by laboratory-reared earthworms (see section 3.7.1). Monocultures (containing 2 individuals) of each species were established with two food regimes provided with :-

1) 30 g of rewetted SCS applied to the soil surface (SUR)

2) 30 g of rewetted SCS incorporated into the soil profile (MIX)

Five replicates of the four treatments were set up in 1 litre culture vessels filled to a depth of 0.11 m with pre-sterilised Kettering loam (moisture content 25 %) or a mixture of this and SCS (MIX) as appropriate. These treatments were maintained in darkness at 15 ± 1 °C, within temperature controlled incubators. Individual earthworm masses were recorded at the outset, and at 4 weekly intervals thereafter. At sampling, culture vessels were inverted and carefully removed, leaving the soil contents intact. These were then sorted in layers and the location of earthworms and burrowing activity recorded. After sampling earthworms were replaced in identical vessels containing fresh soil and food as per treatment. The experiment was terminated after 28 weeks. One-way analysis of variance (ANOVA) was used to statistically analyse differences in earthworm masses of each species between SUR and MIX treatments.

5.2.3 Results

5.2.3.1 *L. terrestris*

Figure 5.2.1 illustrates the growth of *L. terrestris* in both SUR and MIX treatments. Within both treatments earthworms exhibited 100 % survival. At the outset of the experiment there was no significant difference (p>0.05) in individual *L. terrestris* masses between the two food treatments. However, after 4 weeks individuals in the SUR treatment were significantly (p>0.001) heavier than in the MIX treatment (0.11 and 0.055 g, respectively). This trend was observed at each subsequent sampling and by week 28 *L. terrestris* in SUR treatments had obtained a mass of 3.06 g compared with 1.22 g in the MIX treatment

Figure 5.2.1 Growth of *L. terrestris* Provided With Separated Cattle Solids (SCS) as Food in Two Treatments: Surface Applied (SUR) or Incorporated Into the Soil Profile (MIX)
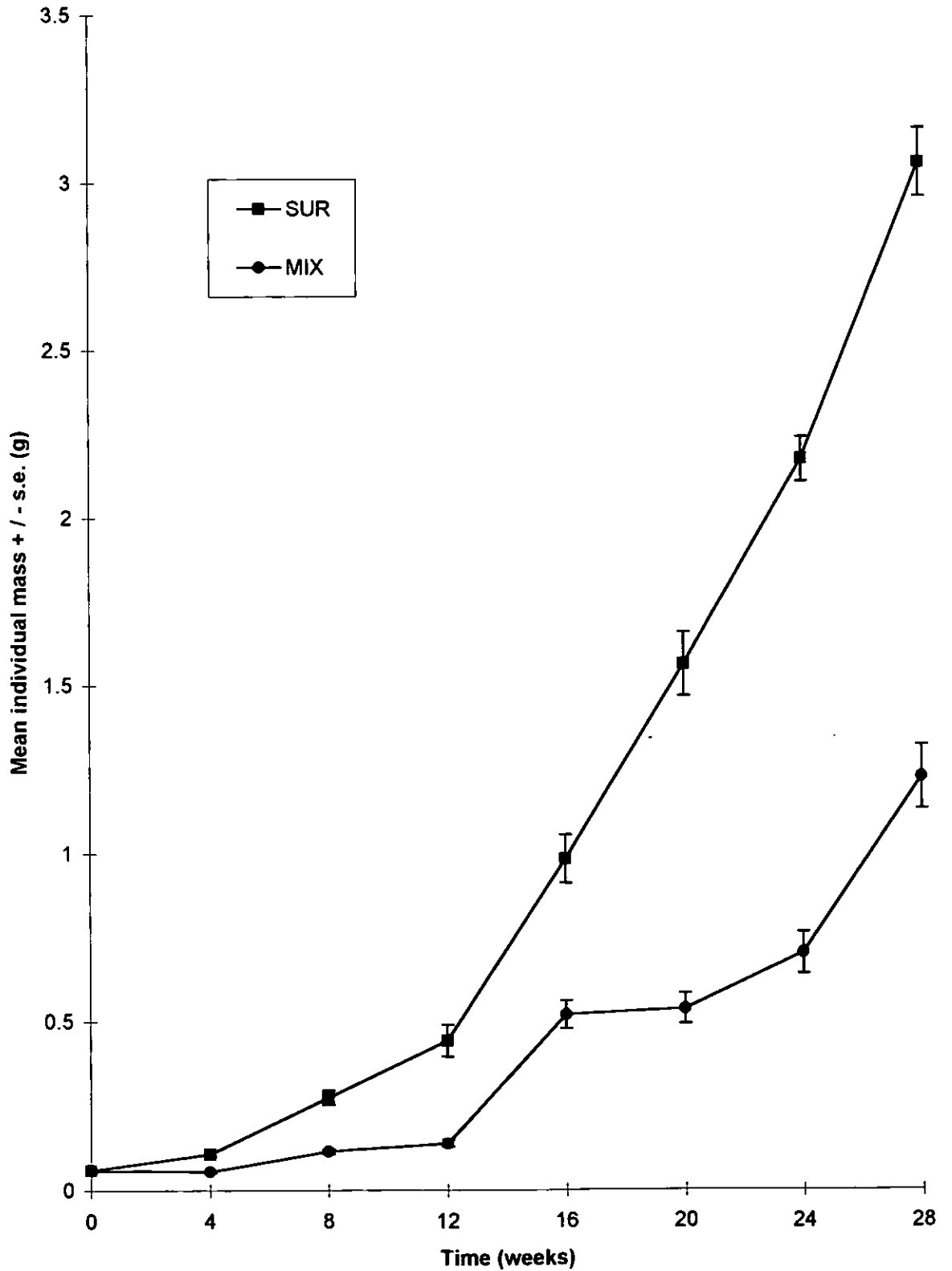
5.2.3.2 A. *longa*

Figure 5.2.2 illustrates the growth of *A. longa* in SUR and MIX treatments. During the experimental period there was 100 and 90 % survival in SUR and MIX treatments, respectively. At the outset of the experiment there was no significant difference (p>0.05) in individual *A. longa* masses between feed treatments. Thereafter *A. longa* in SUR treatments had a greater individual mean mass than in MIX treatments. At week 28 the individual mean mass of worms in the SUR treatment was 24.5 % greater than that achieved in the MIX treatment (1.60 and 1.23 g, respectively).

5.2.3.3 Burrow Formation

Observations on earthworm position within the soil profile and burrow formation were comparable for both species. During the first two samplings (week 4 and 8) juveniles were found in the top 0.05 m of soil in all replicates. Below this depth there was no evidence of earthworm activity (*i.e.* no burrows were present). At the third sampling (week 12) juveniles were found throughout the soil profile often within single burrows which extended vertically (often to the base of the vessel) from the surface in both SUR and MIX treatments. This behaviour was observed thereafter until final sampling.

Figure 5.2.2 Growth of *A. longa* Provided With Separated Cattle
Solids (SCS) as Food in Two Treatments: Surface Applied
(SUR) and Incorporated Into the Soil Profile (MIX)
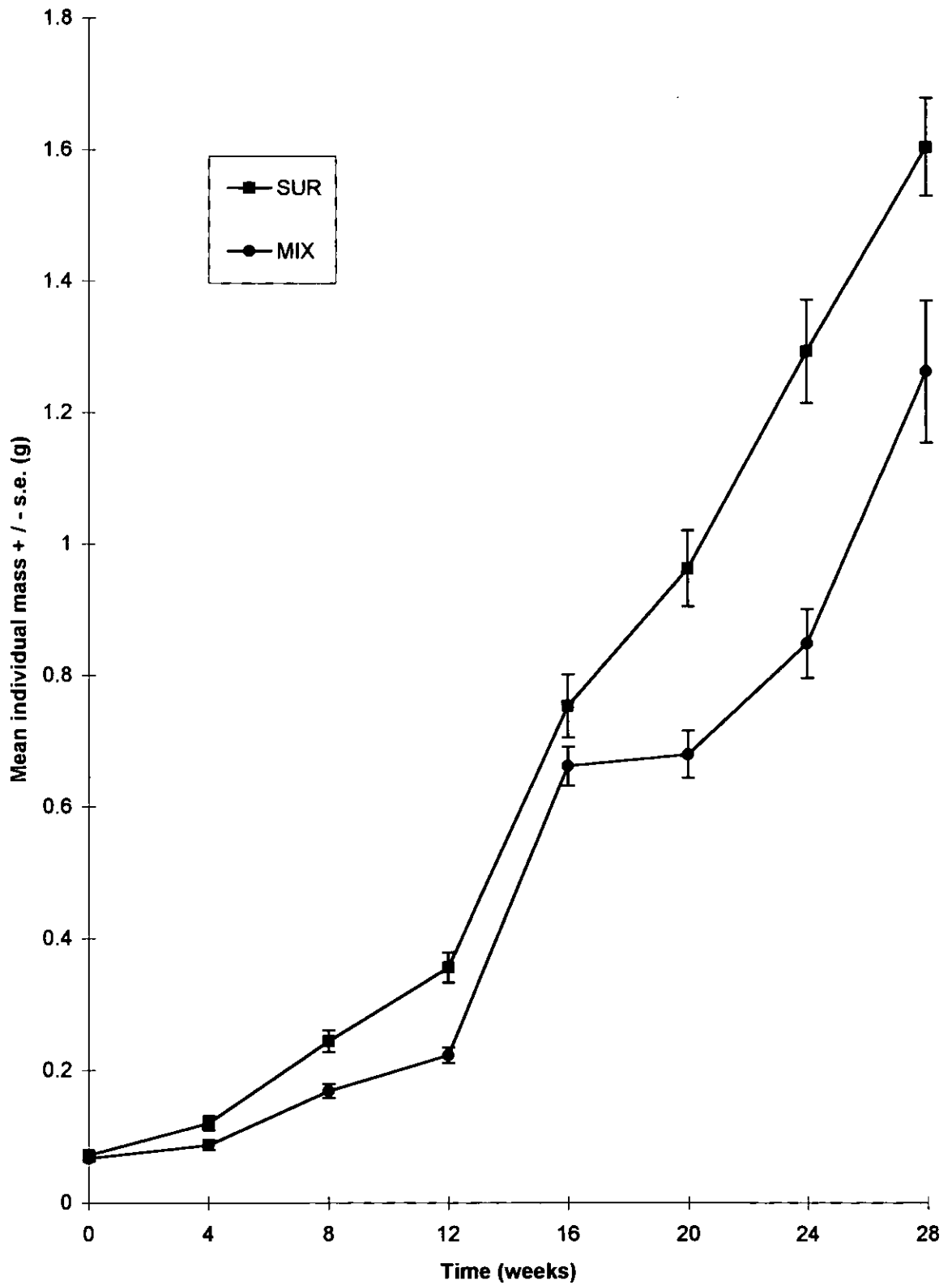
5.2.4 Discussion

5.2.4.1 Feeding Behaviour

Results demonstrated that both experimental species attained significantly greater masses in surface fed treatments (SUR) at every sampling (except week 16 for *A. longa*) suggesting that even in the early stages of development these species preferred to feed at the soil surface. In contrast with the MIX treatment, earthworms in the SUR treatment were presented with a concentrated food source at the soil surface. Hatchlings of both species were initially confined to the upper soil layers. Therefore food applied to the soil surface would have been more accessible than food mixed into the soil profile and probably resulted in the higher growth rates found in the SUR treatment. Boyle (1990) reared juvenile *L. terrestris* and *A. caliginosa* in mixed culture (one of each species together) in 1 litre culture vessels containing a peat/mineral soil medium at 15 ° C for 18-19 weeks. Organic matter (ryegrass - *Lolium perenne*) was applied chopped (8 mm pieces) or milled (< 1 mm) and either surface fed or mixed within the profile. As in this current experiment *L. terrestris* growth rates were greatest when food was applied to the soil surface. Boyle suggested that earthworms benefited (in terms of growth rate) from organic matter being concentrated in one location reducing the energy expended in locomotion.

Results also suggested that differences existed between the two species in their abilities to forage within the MIX treatment. *A. longa* was shown to be more capable of foraging within the soil profile. This result may have implications for the treatment of degraded land prior to earthworm inoculation and the choice of species employed in such inoculation projects. For example, restoration sites often lack plant cover and there is

little organic matter at the soil surface. These results would suggest that the success of inoculating *L. terrestris* at such sites may depend on the surface application of organic matter and that *A. longa* would be a more suitable alternative if organic matter was present mainly in the soil profile. This hypothesis was supported in part by results obtained by Butt *et al.* (1997) on the inoculation of earthworms into a partially restored landfill site where *A. longa* out-performed *L. terrestris*.

5.2.4.2 Burrow Formation

At the initial sampling of both food treatments, juveniles of *L. terrestris* and *A. longa* were confined to the upper soil layers. However as earthworms gained in mass individuals began to form single vertical burrows, a recognised innate behaviour of these two species. In uncompacted soils both species are capable of burrowing into the soil by pushing soil particles aside. This is achieved by increasing the hydrostatic pressure in the coelomic cavity and stiffening the longitudinal muscles of the body wall forcing the anterior segments forwards (Edwards & Bohlen, 1996). The pressure that can be exerted depends on the hydrostatic pressure that can be generated in the coelomic cavity. When small in size, it is possible that these species are incapable of exerting sufficient hydrostatic pressure in order to form the characteristic vertical permanent burrows produced by adults. However, the formation of deep burrows allows earthworms to avoid predation from birds and mammals and also avoid unfavourable environmental conditions.

5.3 AN EXPERIMENT TO INVESTIGATE THE EFFECT OF FOOD PLACEMENT WITHIN THE SOIL PROFILE ON THE GROWTH OF *LUMBRICUS TERRESTRIS*

5.3.1 Introduction

In the previous experiment several hypotheses were put forward relating to the influence of food position on earthworm growth rates and the confinement of juvenile anecic earthworms to the upper soil layers. This experiment was designed to investigate these hypotheses by culturing *L. terrestris* hatchlings in vessels in which organic matter (SCS) was banded at different depths.

5.3.2 Materials and Methods

Hatchling *L. terrestris* (mean individual mass 0.082 g) were obtained from cocoons produced by laboratory-reared earthworms (section 3.7.1). Three food treatments were set up (see figure 5.3.1) in 1 litre culture vessels filled to a depth of 0.11 m using pre-sterilised Kettering loam (moisture content 25 %) :-

1) 30 g of SCS applied to soil surface (SUR) (as in the previous experiment).

2) 30 g of SCS placed in an upper band within the soil profile approximately

0.03 m below the soil surface (UPP).

3) 30 g of SCS placed in a lower band within the soil profile approximately

0.08 m below the soil surface (LOW).

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Four replicates of the three food treatments were established, each replicate containing 2 *L. terrestris* hatchlings. The experiment was maintained in darkness at 15 ± 1 °C in temperature controlled incubators. The mass of each individual was recorded at the outset and at subsequent 4 weekly samplings, when the contents of each culture vessel was sorted in layers and position of earthworms recorded. After each sampling earthworms were replaced in fresh cultures in accordance with the experimental design. The experiment was terminated after 24 weeks. Differences between food treatments in individual masses of *L. terrestris* were statistically analysed using the Tukey-Kramer multi-comparison one way analysis of variance (ANOVA).

Figure 5.3.1 Illustration of the Three Feed Treatments Employed in Determining the Effect of Food Placement on *L. terrestris* Growth

0.03 m

0.14 m

0.08 m

1. SUR   2. UPP   3. LOW
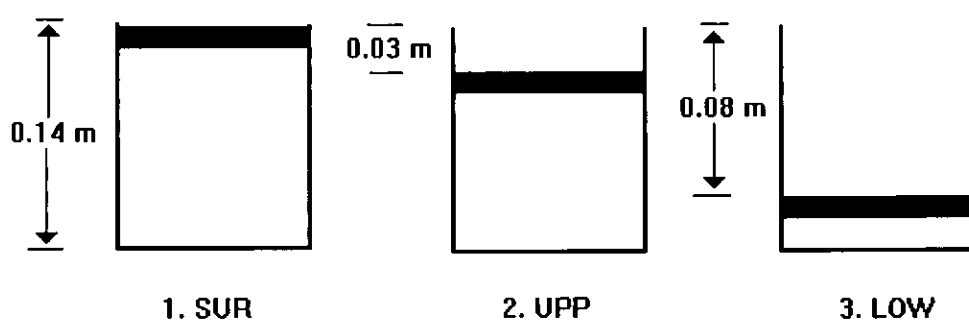
5.3.3 Results

5.3.3.1 Growth

Figure 5.3.2 illustrates the growth of *L. terrestris* within the three food treatments. At the outset of the experiment there were no significant differences (p>0.05) in earthworm masses between the three food treatments. However, at each subsequent sampling the

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mass of individuals in the SUR treatment was greater than those in either of the other treatments. Earthworms in the SUR culture attained significantly ($p<0.01$) greater masses than those in the LOW treatment at each sampling (table 5.3.1) and at the end of the experiment (week 24) mean individual masses were 3.1 and 1.6 g, respectively. In comparison earthworm mass in the UPP treatment (at each sampling) was not significantly ($p>0.05$) different to the SUR treatment, achieving a final mean individual mass of 2.2 g. Also the mass of individuals in the UPP treatment did not differ significantly ($p>0.0.5$) from the LOW treatment except at the initial sampling (after 4 weeks) where masses were significantly different ($p<0.01$).

5.3.3.2 Earthworm Position Within the Soil Profile

In the SUR and UPP treatments earthworms were (after weeks 4 & 8) found in the upper 0.05 m of the soil profile with individuals in the UPP cultures located within the band of SCS. After 8 weeks earthworms in the SUR treatment were found throughout the soil profile forming vertical burrows which opened onto the soil surface. In these subsequent samplings, earthworms in the UPP cultures were generally located within the band of SCS, however individuals were also found throughout the soil profile. At weeks 4 and 8, earthworms in the LOW treatments were located near to the base of the soil profile within the band of SCS. Again as these individuals gained in mass they tended to be found within vertical burrows throughout the soil profile.

Figure 5.3.2 Growth of *L. terrestris* Provided With Separated Cattle Solids (SCS) in Three Food Treatments: Surface Applied (SUR), in an Upper Band in the Soil Profile (UPP) and in a Lower Band in the Soil Profile (LOW)
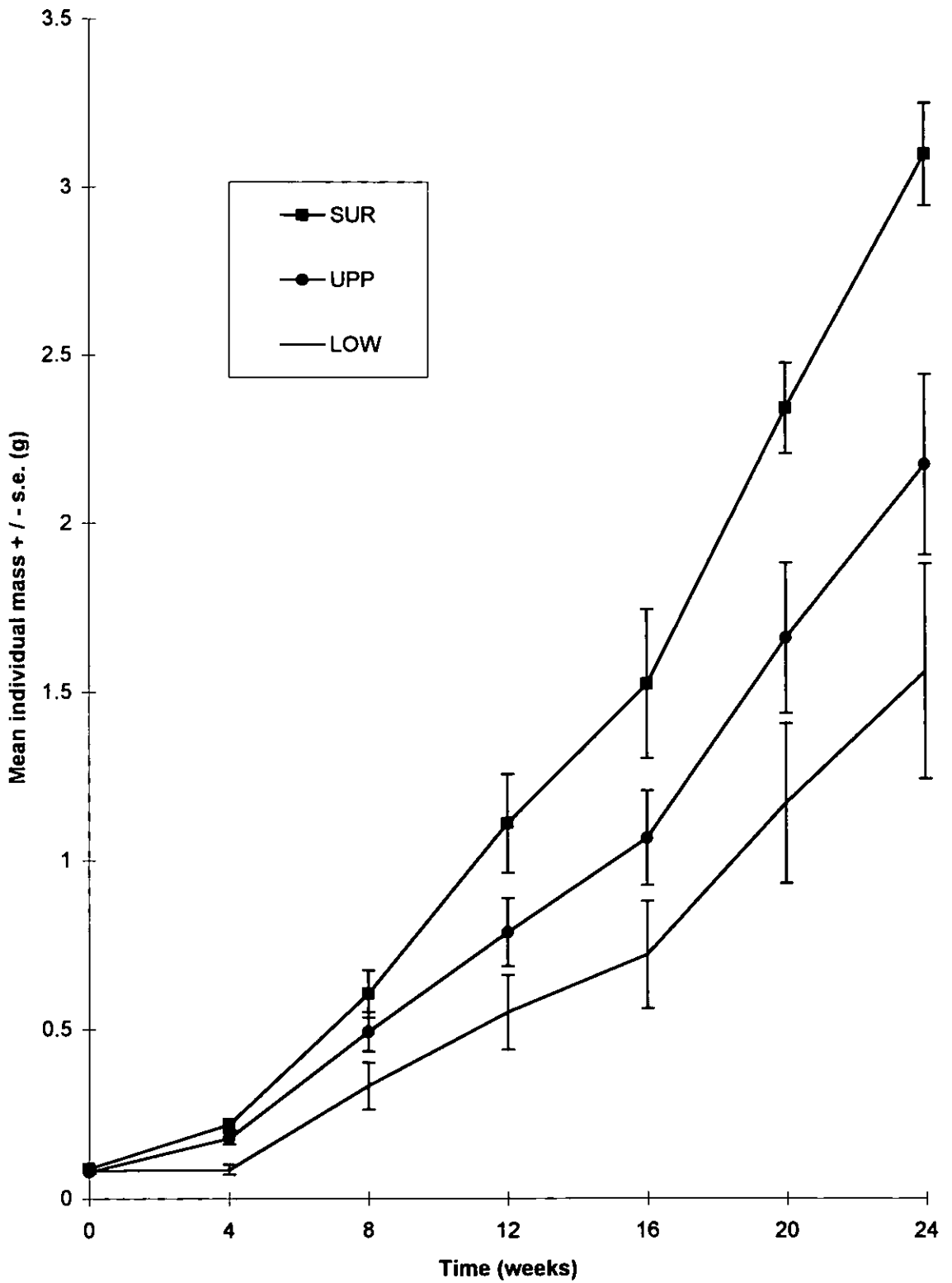
Table 5.3.1 Summary of the Statistical Analysis of Mean Individual *L. terrestris*

Mass in the Three Food Placement Treatments

Selected Samplings	Treatment	SUR	UPP
Week 0	UPP	ns	
	LOW	ns	ns
Week 4	UPP	ns	
	LOW	**	**
Week 8	UPP	ns	
	LOW	*	ns
Week 16	UPP	ns	
	LOW	*	ns
Week 24	UPP	ns	
	LOW	**	ns

(ns no significant difference, * significant at the 5 % level, ** significant at 1 % level)

5.3.4 Discussion

Results suggested (as in section 5.2) that the growth rate of *L. terrestris* was influenced by food position. *L. terrestris* cultured in experimental vessels where food was applied to the soil surface achieved greater growth rates than those grown in cultures where food was incorporated into the soil. In the previous experiment, after 24 weeks, *L. terrestris* cultured in the SUR treatment (food positioned on the soil surface) attained masses 230 % greater than in the MIX treatment (where food was mixed throughout the soil profile). Here, in contrast, *L. terrestris* mass in SUR treatments was only 42 % and 99 % greater than in UPP and LOW treatments, respectively. This suggested that concentrating the food in distinct layers (UPP and LOW) within the soil profile reduced the negative effect on *L. terrestris* growth rates in comparison with evenly dispersing organic matter (SCS) throughout the soil profile (MIX). It was proposed that when the food was concentrated within a single layer earthworms expended less energy on the locomotive activity of foraging. Therefore this extra energy could be used for tissue production.

At week 4 individuals in UPP cultures had a significantly greater (p<0.01) mass than earthworms in LOW cultures. This trend was maintained at each subsequent sampling, although differences in mass were not significant (p>0.05). In contrast with the previous experiment (section 5.2), where initially juvenile *L. terrestris* were located only in the upper layers, individuals in the LOW culture were found within the organic matter layer near the base of the vessel. This would indicate that when small in size (<0.1 g) this species is still capable of burrowing at depth within the soil in order to locate food. However, it was proposed that this extra locomotive activity diverted energy away from tissue production causing a reduction in growth rate in comparison with earthworms in

SUR and UPP treatments. These results concur with the observations of Evans (1947) who indicated that often earthworms of the genus *Lumbricus* do not burrow extensively, as long as an adequate food supply is present on, or close to the surface, but when food is scarce their burrowing activity is stimulated greatly.

In the banded treatments, juvenile *L.* terrestris aggregated within the organic matter layer. This observed aggregation of earthworms in relation to organic matter has been recorded by several authors. In the field earthworms (especially epigeic species) have been observed aggregating under animal manure on farms (Haukka, 1987; Hendrikson, 1991). A field experiment was conducted in pastureland in South Australia by Hughes *et al.* (1994) to assess the effects of additional organic matter on earthworm populations. Dried sheep manure was added in one of two forms (whole pellets or finely milled) in one of three locations:- 1) on the soil surface, 2) in a layer 5-10 cm under the surface and 3) evenly dispersed within the soil profile. A control treatment in which no manure was applied was also established. Each treatment was seeded with 2 adults and 3 juvenile *A. trapezoides*. Four earthworm species were recorded from the bags, *M. dubius*, *A. rosea*, *A. caliginosa* and *A. trapezoides*. Results showed that significantly higher numbers of each species were found in bags containing manure compared to the control. It was also observed that both numbers and biomass of earthworms were positively correlated with the food position. Cook & Linden (1996) studied the burrowing characteristics of *A. tuberculata*, in response to food placement and source within two-dimensional microcosms (Evans boxes (Evans, 1947)). Four food placement treatments were established : - 1) band - food placed in a band halfway down the soil profile, 2) incorporated - food dispersed throughout the soil profile, 3) pocket - food placed in a central pocket in the soil profile and 4) control - no food. Two food types were used

either ground corn or 'Magicworm' food (a mixture of high protein grains). Results suggested that although new burrow generation was random, once a food source was located burrowing / feeding behaviour centred around the food source.

5.4 AN EXPERIMENT TO ASSESS THE INFLUENCE OF FOOD PLACEMENT ON INTER-SPECIFIC INTERACTIONS BETWEEN EARTHWORMS OF THE THREE ECOLOGICAL GROUPINGS

5.4.1 Introduction

The aim of this experiment was to further investigate inter-specific interactions between *A. chlorotica*, *L. rubellus*, and *L. terrestris*. It was proposed that manipulating food position in experimental cultures would effect the growth and fecundity of the three species and therefore influence inter-specific interactions. In this experiment *A. chlorotica*, *L. rubellus*, and *L. terrestris* were cultured using three food treatments in single, paired and three species combinations.

5.4.2 Materials and Methods

Juvenile *A. chlorotica*, *L. rubellus* and *L. terrestris* (initial mean masses: 0.09, 0.33 and 0.91 g, respectively) were obtained from laboratory-reared and maintained stocks (section 3.7). Monocultures of the 3 species plus all paired combinations and a 3 species combination were established for each of three food treatments. These 3 treatments used SCS as a food source. This was presented : -

1) as 60 g applied to the soil surface (SUR);

2) as 60 g incorporated within the soil profile (MIX);

3) as 30 g applied to the soil surface and 30 g incorporated within the soil profile (surface and mixed = SAM).

Treatments were set up in 2 litre culture vessels filled with a pre-sterilised sandy-clay loam (moisture content 25 %) to a depth of 0.13 m. Numbers of each species were assigned to treatments according to pre-determined species ratio (section 3.4.1). All treatment combinations are summarised in table 5.4.1. Four replicates of each of the 21 treatments were set up. Earthworm mass and reproductive condition were both recorded at the outset, and at each monthly sampling thereafter, when earthworms were transferred to fresh soil. Fresh food was applied in accordance with the experimental design. At the fourth, and at subsequent samplings, the total amount of food applied to each vessel was increased to 80 g in order to prevent food quantity from becoming a limiting factor as species biomass increased. Termination of this experiment occurred after 24 weeks. After the 4th, 5th and 6th sampling periods the spent experimental soil was wet-sieved (section 3.3.3) and the number of cocoons produced by the three species (which are easily distinguished (Sims & Gerard, 1985)) in each treatment recorded.

The Tukey-Kramer multicomparison analysis of variance test was used to analyse observed differences in the growth of *A. chlorotica*, *L. rubellus* and *L. terrestris* and also cocoon production in the three treatments. The General Linear model for analysis of variance was also employed to determine the possible effect of interactions between the presence of other species and food position on cocoon production for both *A. chlorotica* and *L. rubellus*.

Table 5.4.1 Treatments Employed in Determining the Effect of Food Placement

on Interactions between Earthworms of the Three Ecological

Groupings

Species Combinations in of each the 3 Food Treatments	Number of Individuals of each species
1. *A. chlorotica* monoculture	8
2. *L. rubellus* monoculture	4
3. *L. terrestris* monoculture	2
4. *A. chlorotica* & *L. rubellus*	8 & 4
5. *A. chlorotica* & *L. terrestris*	8 & 2
6. *L. rubellus* & *L. terrestris*	4 & 2
7. *A. chlorotica*, *L. rubellus* & *L. terrestris* (3 species culture)	8, 4 & 2

5.4.3 Results for *L. terrestris*

Figures 5.4.1 (a - c) illustrate the growth of *L. terrestris* in monoculture, paired with

A. chlorotica and *L. rubellus* and in 3 species culture in SUR, MIX and SAM food

treatments.

5.4.3.1 Growth in the Surface Applied Food Treatment (SUR)

At the outset of the experiment there was no significant difference ($p > 0.05$) in individual

L. terrestris mass, across the four species combinations. This was also found at each

subsequent sampling. However, the individual mean mass of *L. terrestris* in monoculture

and in the pairing with *A. chlorotica* were greater at each sampling than that achieved in

the pairing with *L. rubellus* or in 3 species culture, the latter having the lowest mean

Figure 5.4.1 (a) Growth of *L. terrestris* Provided With a
Surface Application (SUR) of Separated Cattle Solids (SCS)
in Monoculture, Paired and Three Species Treatments With
A. chlorotica and *L. rubellus,* Over a 24 Week Period
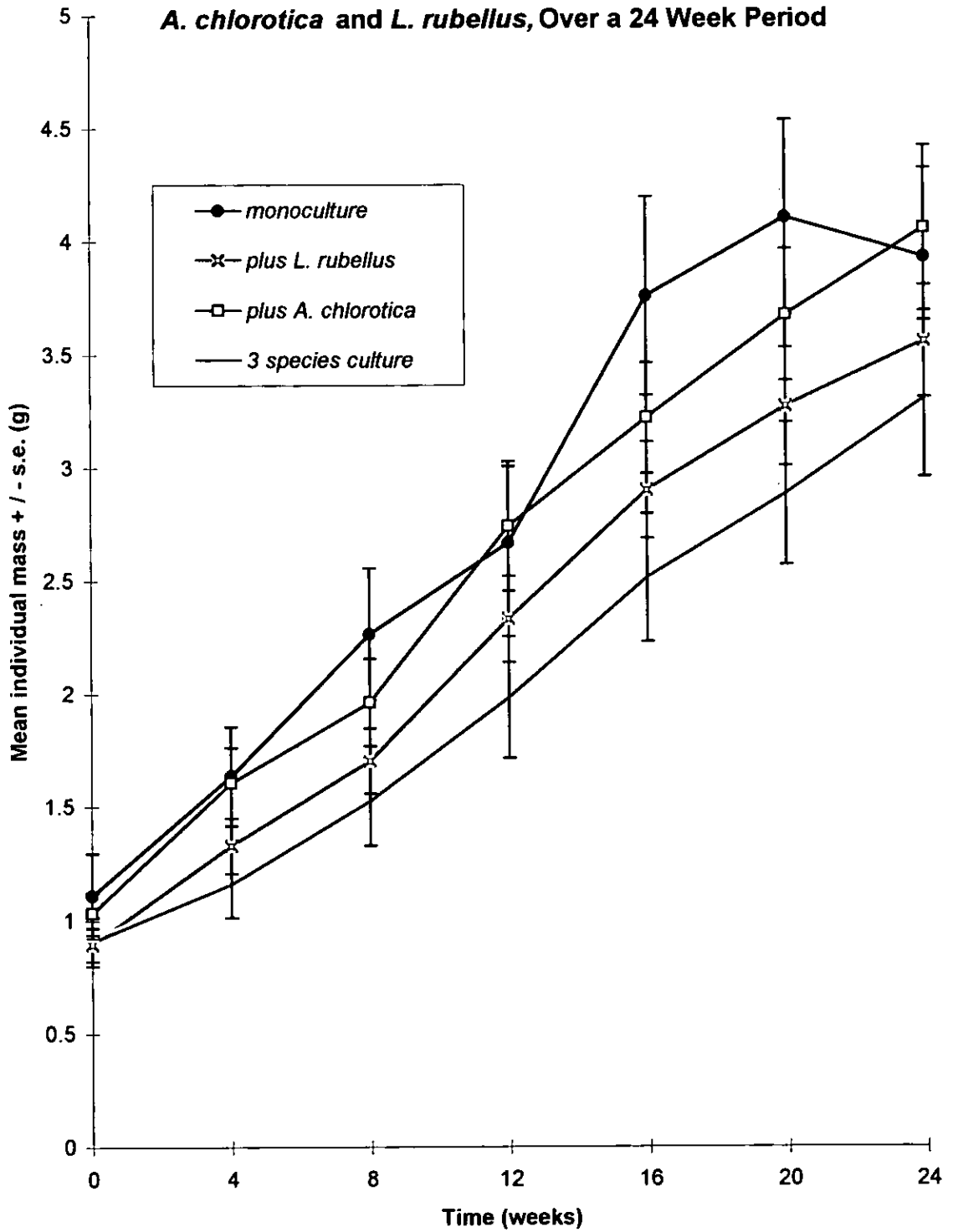
mass at each sampling. At week 24, individual mean mass of *L. terrestris* was 3.9, 4.0, 3.5 and 3.2 g in the monoculture, pairing with *A. chlorotica*, pairing with *L. rubellus* and 3 species culture, respectively.

5.4.3.2 Growth in the Incorporated Food Treatment (MIX)

There was no significant difference (p>0.05) in individual mass between the four species combinations at the outset. However, at subsequent samplings significant differences (p<0.05) in *L. terrestris* mass between these cultures was recorded. At week 24 the mass of *L. terrestris* in the monoculture and the pairing with *A. chlorotica* (3.8 and 3.7 g, respectively) was significantly (p<0.01) greater than that achieved in the 3 species culture (2.1 g) and the pairing with *L. rubellus* (3.0 g) was intermediate in mass between these combinations (see table 5.4.2).

Table 5.4.2 Summary of the Statistical Analysis of *L. terrestris* Growth in the Incorporated Food (MIX) Treatment After 24 Weeks of Experimentation

MIX treatment	monoculture	plus *L. rubellus*	plus *A. chlorotica*
plus *L. rubellus*	*		
plus *A. chlorotica*	ns	ns	
3 species culture	**	*	**

(ns no significant difference, * significant at the 5 % level, ** significant at the 1 % level)

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Figure 5.4.1 (b) Growth of *L. terrestris* Provided With Separated Cattle Solids (SCS) Incorporated Into the Soil Profile (MIX) in Monoculture, Paired and Three Species Treatments With *A. chlorotica* and *L. rubellus*, Over a 24 Week Period
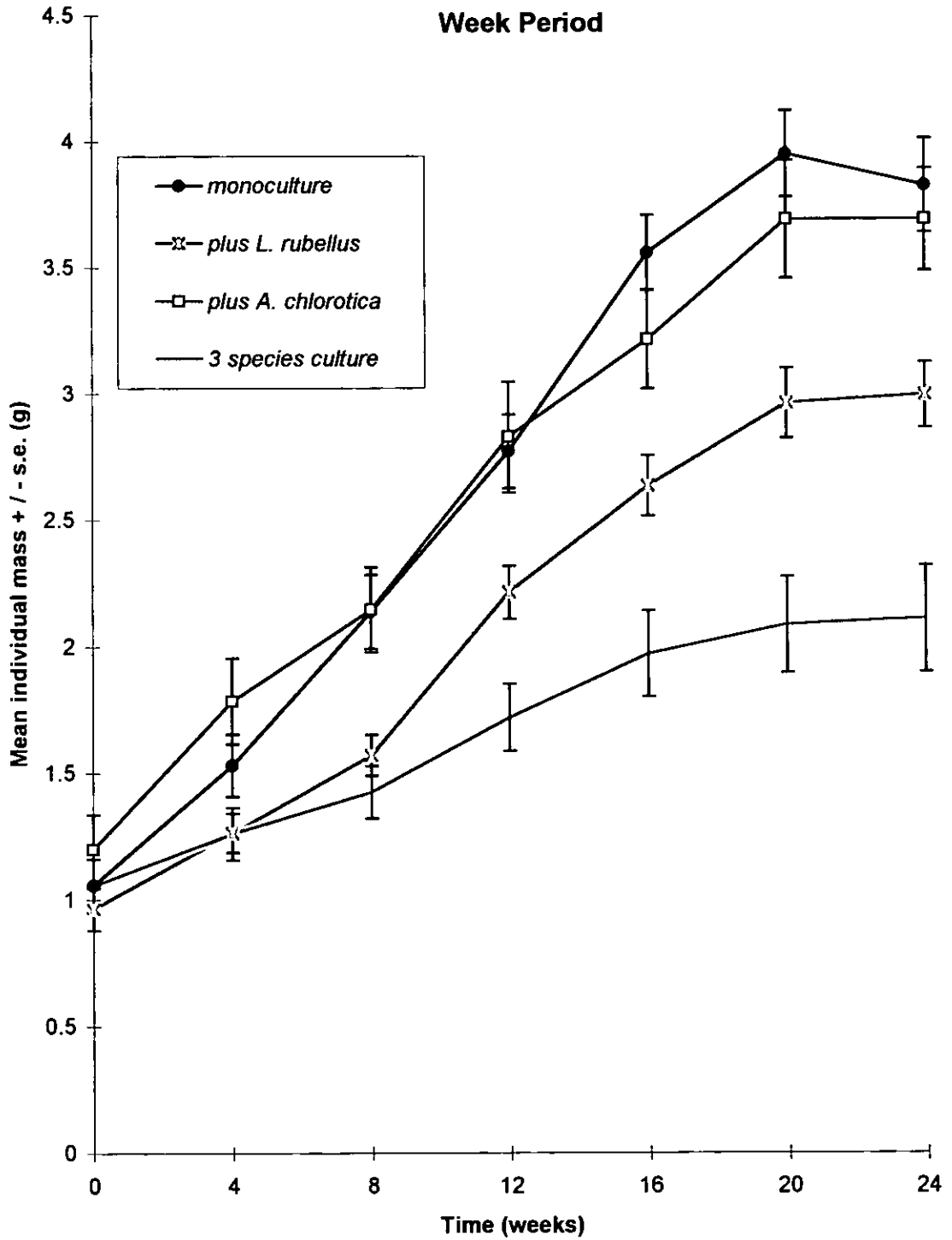
5.4.3.3 Growth in the Surface and Incorporated Food Treatment (SAM)

At the outset there was no significant difference (p>0.05) in *L. terrestris* mean individual mass between species combination. At week 24, *L. terrestris* mean individual mass in monoculture (3.6 g) and pairing with *A. chlorotica* (4.2 g) were significantly (p<0.0.5) greater than in 3 species culture (1.8 g) with the pairing with *L. rubellus* (2.9 g) intermediate (see table 5.4.3).

Table 5.4.3 Summary of the Statistical Analysis of *L. terrestris* Growth in the Surface Applied and Incorporated Food (SAM) Treatment After 24 Weeks of Experimentation

SAM treatment	monoculture	plus *L. rubellus*	plus *A. chlorotica*
plus *L. rubellus*	ns		
plus *A. chlorotica*	ns	**	
3 species culture	**	**	**

(ns no significant difference, * significant at the 5 % level, ** significant at the 1 % level.)

5.4.3.4 The Effect of Food Placement on *L. terrestris* Growth

Statistical analysis showed no significant (p>0.05) difference in the individual mass of *L. terrestris* (at any sampling point) between SUR , MIX and SAM food treatments in species monoculture, the pairing with *A. chlorotica* and the pairing with *L. rubellus*. However, in 3 species culture, food position had a significant (p<0.05) influence on *L. terrestris* mass by the end of the experiment. *L. terrestris* in the SUR treatment

Figure 5.4.1 (c) Growth of *L. terrestris* Provided Separated Cattle Solids (SCS) Incorporated Into the Soil Profile and Surface Applied (SAM) in Monoculture, Paired and Three Species Treatments With *A. chlorotica* and *L. rubellus*, Over a 24 Week Period

Legend:
- monoculture
- plus *L. rubellus*
- plus *A. chlorotica*
- 3 species culture

Y-axis: Mean individual mass + / - s.e. (g)

X-axis: Time (weeks)
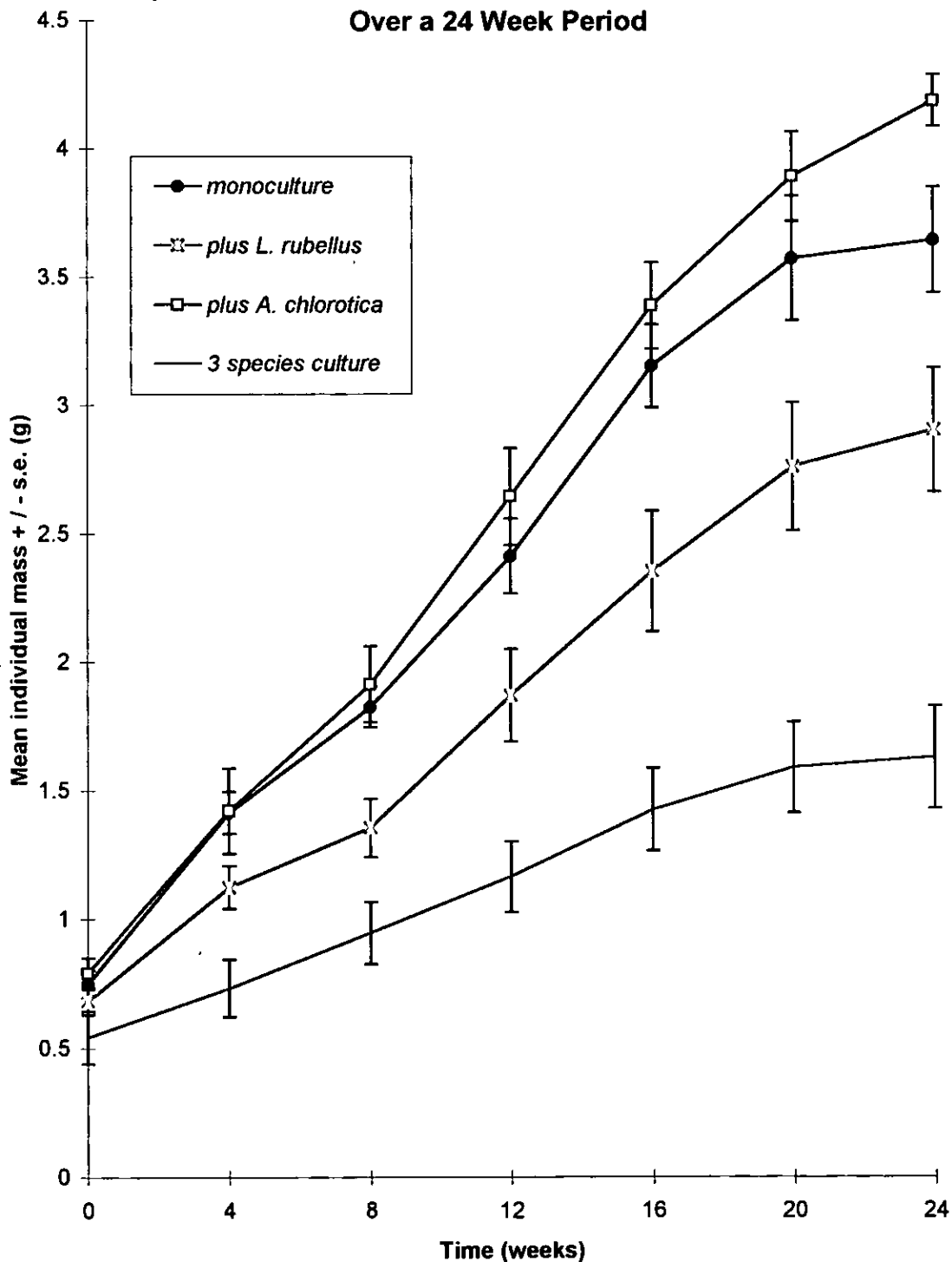
(individual mean mass 2.9 g) exhibiting significantly greater masses than those in both the MIX and SAM treatments (individual mean mass 2.1 and 1.7 g, respectively).

5.4.3.5 The Effect of Other Species and Food Position on Maturation Rates

Figure 5.4.2 illustrates the percentage of clitellate *L. terrestris* at week 24 across all treatments. Differences observed in the growth of *L. terrestris* in species combinations are mirrored in the percentage of clitellate individuals. The largest number of clitellate individuals were found in monocultures and the pairing with *A. chlorotica*. No clitellate individuals were found in 3 species cultures and in the pairing with *L. rubellus*, clitellate individuals were only present in the SUR food treatment.

Table 5.4.4 ANOVA Table Showing the Effect of Experimental Food Position and Species Combinations on *L. terrestris* Growth

Sources of Variation	Degrees of Freedom	Mean Squares	F	P	significance
Food Regime	2	1.1705	2.46	0.094	ns
Species Combination	2	2.1220	4.46	0.016	*
Interaction	4	0.6785	1.43	0.237	ns
Error	57	0.4755			

(ns no significant difference, ** significant at the 5 % level)

Note: the 3 species treatment is not included in this analysis.

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Figure 5.4.2 Percentage of Mature *L. terrestris* Recorded After 24 weeks (in All Relevant Treatments), Provided With SCS in Three Locations : Surface Applied (SUR), Incorporated Into the Soil Profile (MIX) and Both Incorporated and Surface Applied (SAM)
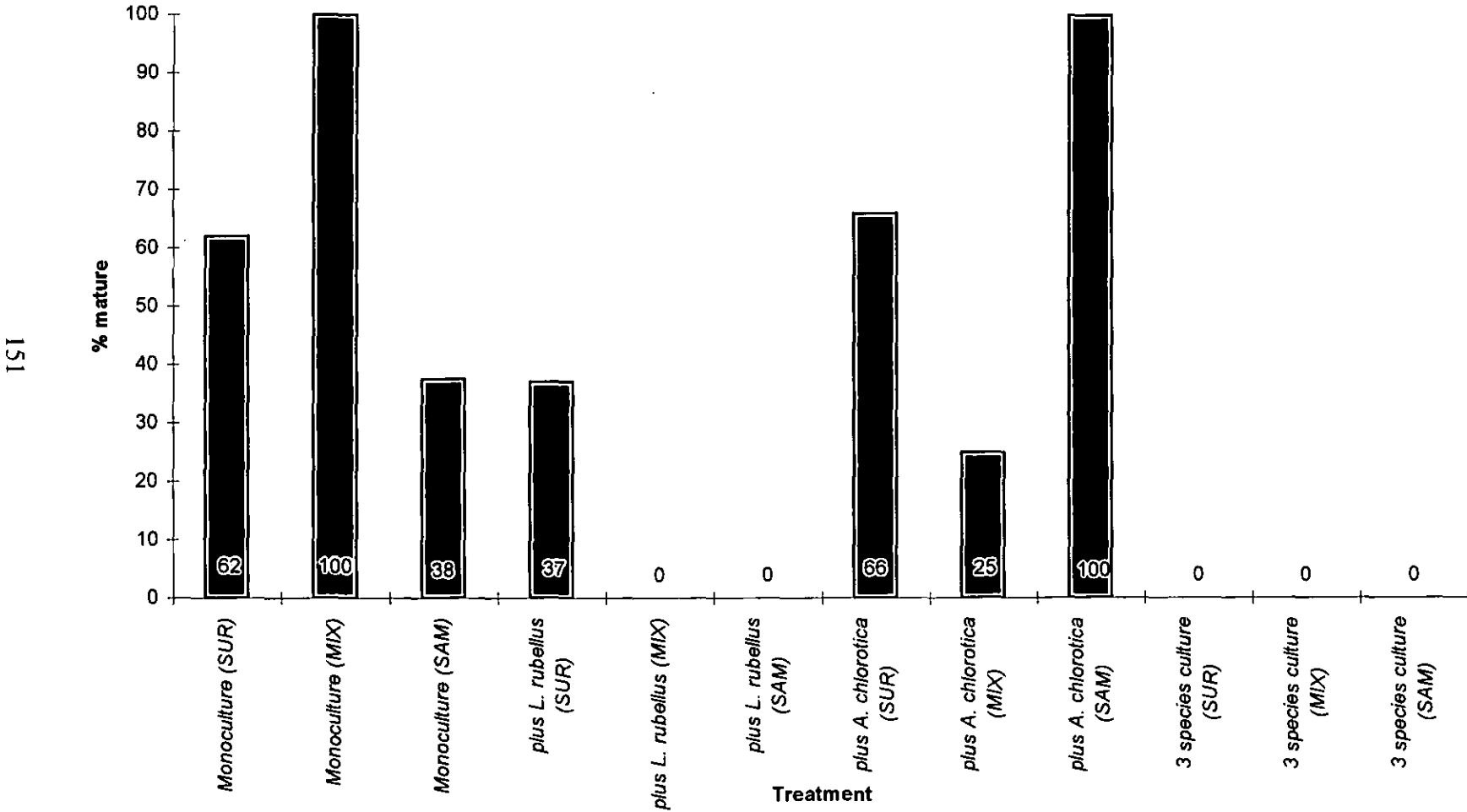
ANOVA results presented in table 5.4.4 indicated that food treatment had no significant effect ($p > 0.05$) on *L. terrestris* growth. However, the presence of other species significantly affected growth ($p < 0.05$) while the interaction between these two factors was not significant ($p > 0.05$).

During the experimental period, no *L. terrestris* cocoons were recorded from any experimental cultures.

5.4.4 Results for *A. chlorotica*

Figures 5.4.3 (a - c) illustrates the growth of *A. chlorotica* in monoculture, paired with *L. terrestris*, paired with *L. rubellus* and in 3 species culture in SUR, MIX and SAM food treatments. *A. chlorotica* gained in mass in all three food treatments with maximum mass records at week 16 sampling. After this time the mean mass declined until the experiment was terminated at week 24.

5.4.4.1 Growth in the Surface Applied Food treatment (SUR)

No significant difference ($p > 0.05$) in mean individual masses was recorded at the outset of the experiment (mean individual mass across treatments was 0.093 g) or at any subsequent sampling.

5.4.4.2 Growth in the Incorporated Food Treatment (MIX)

The individual mean mass of *A. chlorotica* in the pairing with *L. terrestris* (0.044 g) was significantly lower ($p < 0.05$) at the start of the experiment than that in the pairing with *L. rubellus* (0.098 g) and the 3 species culture (0.095). This resulted from error in the experimental set up and prevented direct comparisons of *A. chlorotica* masses in the

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Figure 5.4.3 (a) Growth of *A. chlorotica* Provided With a Surface Application (SUR) of Separated Cattle Solids (SCS) in Monoculture, Paired and Three Species Treatments With *L. rubellus* and *L. terrestris*, Over a 24 Week Period
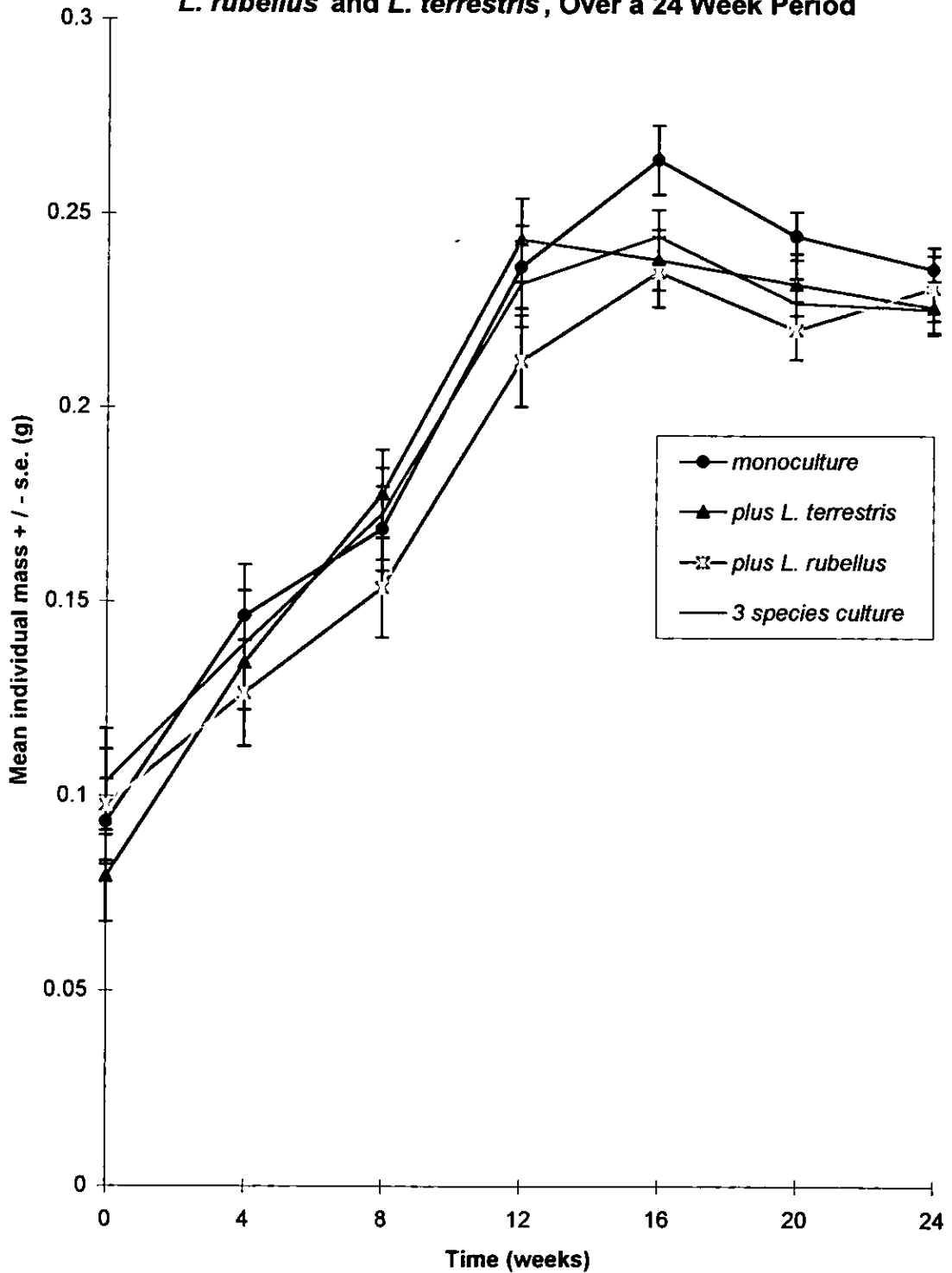
Figure 5.4.3 (b) Growth of *A. chlorotica* Provided With Separated Cattle Solids (SCS) Incorporated Into the Soil Profile (MIX) in Monoculture, Paired and Three Species Treatments With L. rubellus and *L. terrestris*, Over a 24 Week Period

Legend:
- monoculture
- plus L. terrestris
- plus L. rubellus
- 3 species culture

Y-axis: Mean individual mass + / - s.e. (g)
X-axis: Time (weeks)
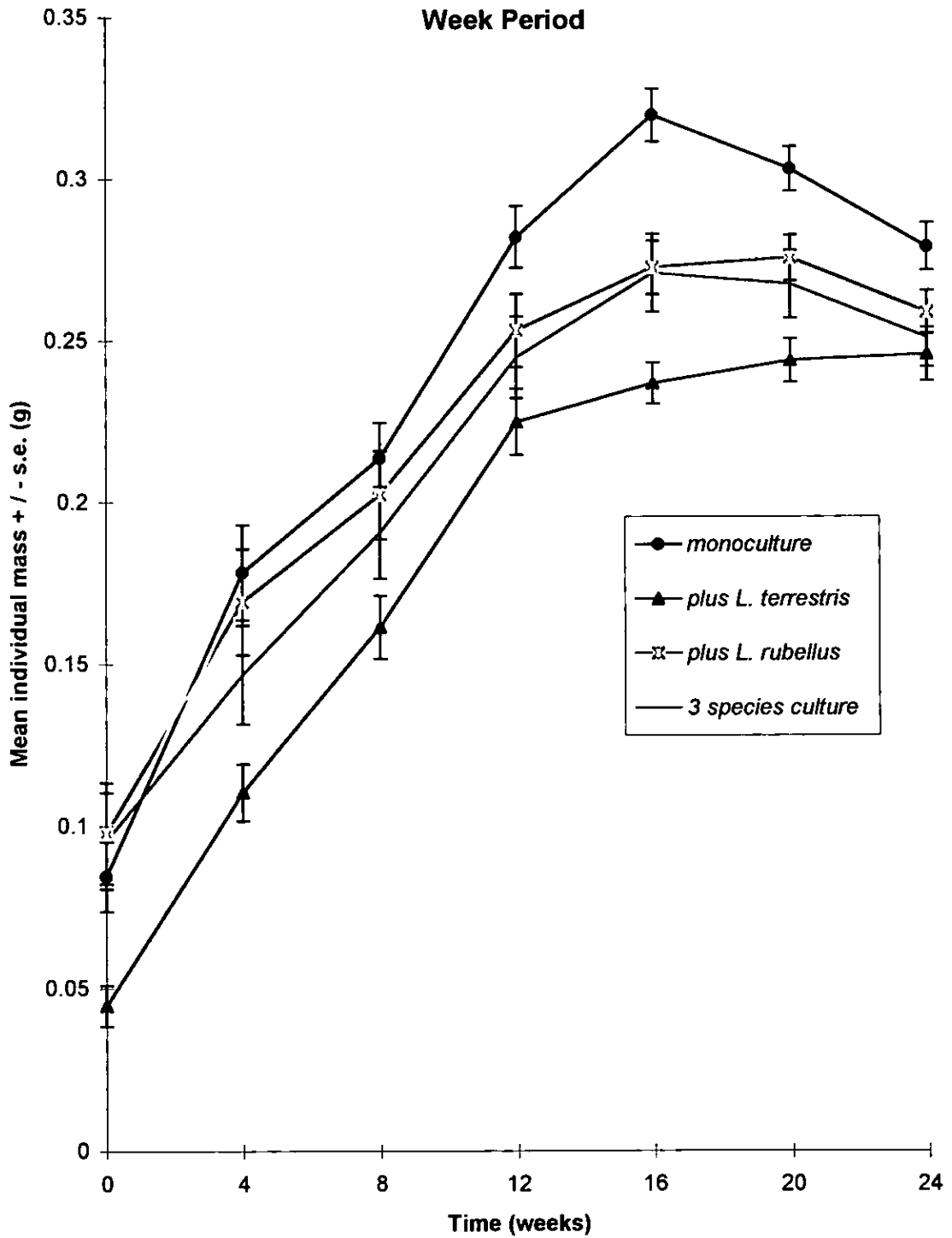
pairing with *L. terrestris* at further samplings with the other combinations. At each sampling the monoculture provided the greatest individual *A. chlorotica* mass in comparison with the pairing with *L. rubellus* and 3 species culture. These differences in mass were significant ($p<0.05$) at weeks 16 and 20.

5.4.4.3 Growth in the Surface and Incorporated Food Treatment (SAM)

The experimental error experienced in the MIX treatment was also present in the SAM treatment leading to the omission of the *A. chlorotica* pairing with *L. terrestris* from statistical comparisons with other species combinations. Within the other species combinations there was no significant ($p>0.05$) difference in individual *A. chlorotica* mass at any sampling. However, *A. chlorotica* mass in the monoculture was greater than that achieved in any other species combination (except at week 24).

5.4.4.4 The Effect of Food Placement on *A. chlorotica* Growth

The greatest individual *A. chlorotica* mass in monoculture, the pairing with *L. rubellus* and 3 species culture was recorded in the MIX treatment. In the monoculture *A. chlorotica* mass was significantly ($p<0.05$) greater in the MIX, compared with SUR and SAM treatments. This result was also seen at week 20 in the pairing with *L. rubellus* and the 3 species culture. Results from the pairing with *L. terrestris* were omitted as at the start of the experiment there were significant differences ($p<0.05$) in masses between food treatments.

5.4.4.5 Cocoon Production

In all treatments *A. chlorotica* were clitellate by week 16. The mean number of cocoons worm^{-1} 4 weeks^{-1} over the 12 week period (weeks 12 - 24) for the four species treatments

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Figure 5.4.3 (c) Growth of *A. chlorotica* Provided Separated Cattle Solids (SCS) Incorporated Into the Soil Profile and Surface Applied (SAM) in Monoculture, Paired and Three Species Treatments With *L. rubellus* and *L. terrestris*, Over a 24 Week Period
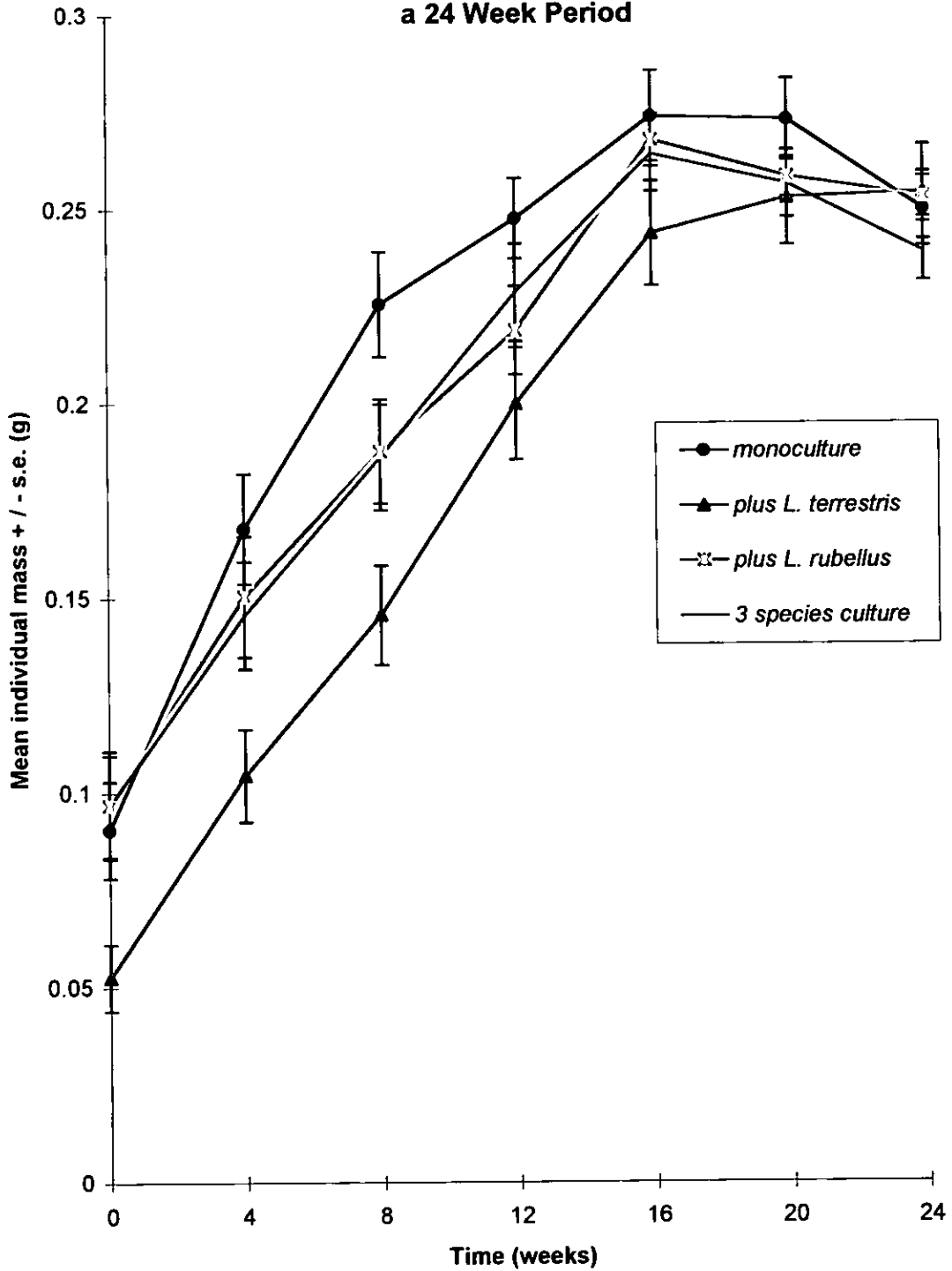
in SUR, MIX and SAM food treatments is shown in figure 5.4.4. In all 3 food treatments the greatest number of *A. chlorotica* cocoons were produced in monocultures. In SUR and MIX treatments the monoculture produced significantly ($p<0.0.1$) more cocoons worm^{-1} 4 weeks^{-1} than in either the pairing with *L. rubellus* or 3 species culture. Whereas in the SAM treatment this difference was only significant ($p<0.01$) when compared with 3 species culture. Overall the greatest cocoon production (2.48 cocoons worm^{-1} 4 weeks^{-1}) occurred in the MIX treatment monoculture.

Table 5.4.5 ANOVA Table Showing the Effect of Experimental Food Position and Species Combinations on *A. chlorotica* Cocoon Production

Sources of Variation	Degrees of Freedom	Mean Squares	F	P	significance
Food Regime	2	1.7837	5.40	0.006	**
Species Combination	2	8.2405	24.93	0.0001	***
Interaction	4	1.0477	3.17	0.017	*
Error	93	0.3305			

(*** significant at the 0.1 % level, ** significant at the 1 % level, * significant at the 5 % level)

Note: the 3 species treatment is not included in the analysis.

Table 5.4.5 indicates that:- food regime, species combinations and the interaction of the two had a significant effect ($p<0.05$) on *A. chlorotica* cocoon production.

Figure 5.4.4 Mean Cocoon Production by *A. chlorotica* (in Relevant Species Combinations) Provided With SCS in Three Locations: Surface Applied (SUR), Incorporated in the Soil Profile (MIX) and Both Incorporated and Surface Applied (SAM)
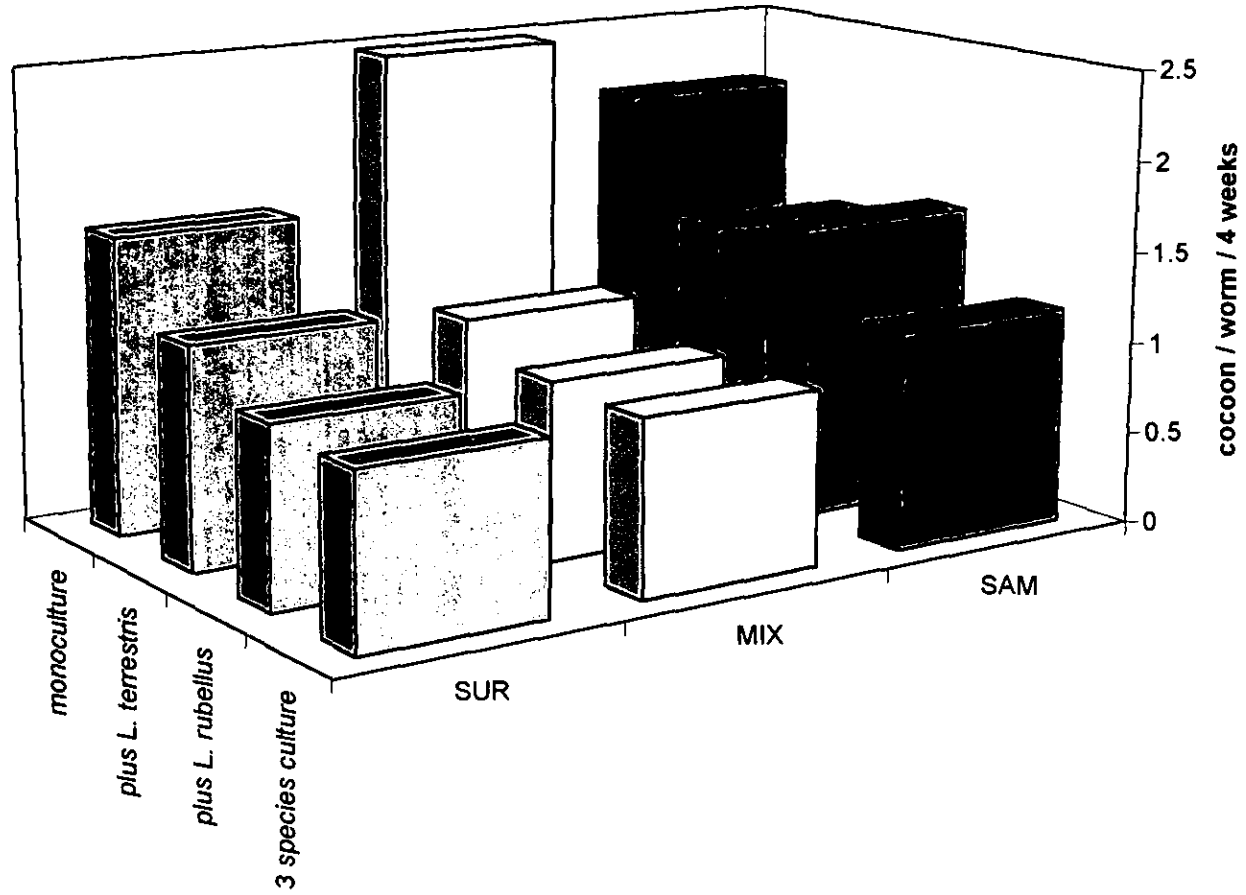
5.4.5 Results for *L. rubellus*

5.4.5.1 Growth

Figures 5.4.5 (a - c) illustrate growth of *L. rubellus* in monoculture, paired with *L. terrestris*; *A. chlorotica*; and in 3 species culture in SUR, MIX and SAM food treatments. *L. rubellus* gained in mass in all treatments over the experimental period. There was no significant ($p > 0.05$) difference in the mass of *L. rubellus* between the four species combinations in the three food treatments.

5.4.5.2 Food Placement

This had no significant ($p > 0.05$) effect on *L. rubellus* growth in any of the treatments.

5.4.5.3 Cocoon Production

In all treatments all *L. rubellus* were clitellate by week 16. Figure 5.4.6 show the mean cocoon production (cocoons worm^{-1} 4 weeks^{-1}) over the final 12 week period (weeks 12 - 24) in SUR, MIX and SAM food treatments.

5.4.5.3a Surface Applied Food Treatment (SUR)

The highest record of cocoons was in monoculture (6 cocoons worm^{-1} 4 weeks^{-1}), which was not significantly different compared with pairings containing either *L. terrestris* or *A. chlorotica* (5.5 and 5.5 cocoons worm^{-1} 4 weeks^{-1}, respectively). Lowest cocoon production was in 3 species culture (3.5 cocoons worm^{-1} 4 weeks^{-1}) and this figure was significantly ($p < 0.01$) less than in all other species combinations.

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Figure 5.4.5 (a) Growth of *L. rubellus* Provided With a Surface Application (SUR) of Separated Cattle Solids (SCS) in Monoculture, Paired and Three Species Treatments With *A. chlorotica* and *L. terrestris*, Over a 24 Week Period

Legend:
- monoculture
- plus *L. terrestris*
- plus *A. chlorotica*
- 3 species culture

Y-axis: Mean individual mass + / - s.e. (g)

X-axis: Time (weeks)
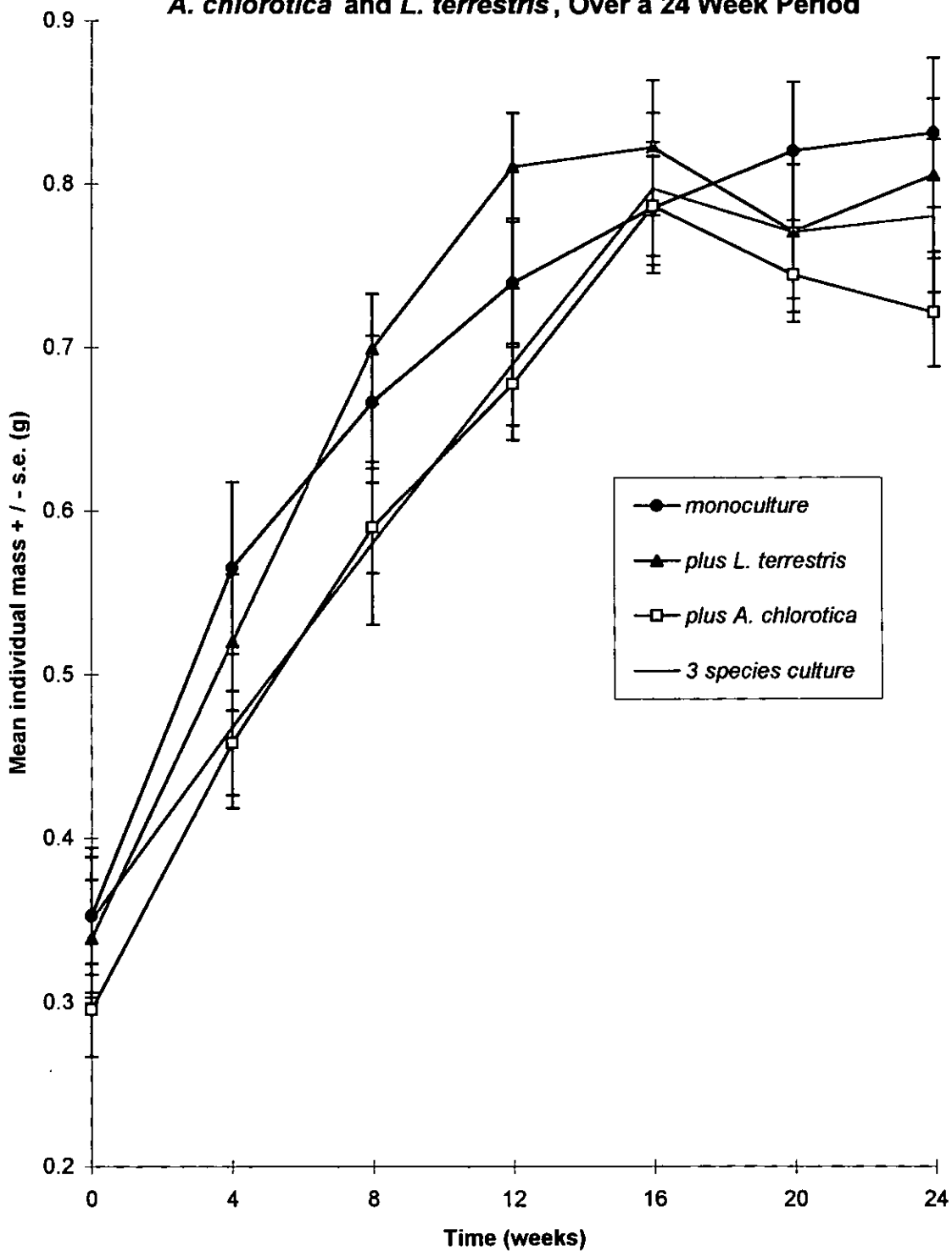
5.4.5.3b Incorporated Food Treatment (MIX)

The greatest cocoon production was in the monoculture (9.6 cocoons worm^{-1} 4 weeks^{-1}), which was significantly ($p<0.01$) higher than in other species combinations. Lowest cocoon production was recorded in 3 species culture (2.2 cocoons worm^{-1} 4 weeks^{-1}).

5.4.5.3c Surface and Incorporated Food Treatment (SAM)

L. rubellus monoculture had the highest reproductive output (7.7 cocoons worm^{-1} 4 weeks^{-1}) which was significantly ($p<0.05$) greater than cocoon production in other species combinations. However, there was little difference in cocoon production in 3 species culture (5.0 cocoons worm^{-1} 4 weeks^{-1}) compared with pairings containing either *L. terrestris* or *A. chlorotica* (5.3 and 5.1 cocoons worm^{-1} 4 weeks^{-1}, respectively).

Figure 5.4.5 (b) Growth of *L. rubellus* Provided With Separated Cattle Solids (SCS) Incorporated Into the Soil Profile (MIX) in Monoculture, Paired and Three Species Treatments With *A. chlorotica* and *L. terrestris*, Over a 24 Week Period

Legend:
- monoculture
- plus *L. terrestris*
- plus *A. chlorotica*
- 3 species culture

Y-axis: Mean individual mass + / - s. e. (g)

X-axis: Time (weeks)
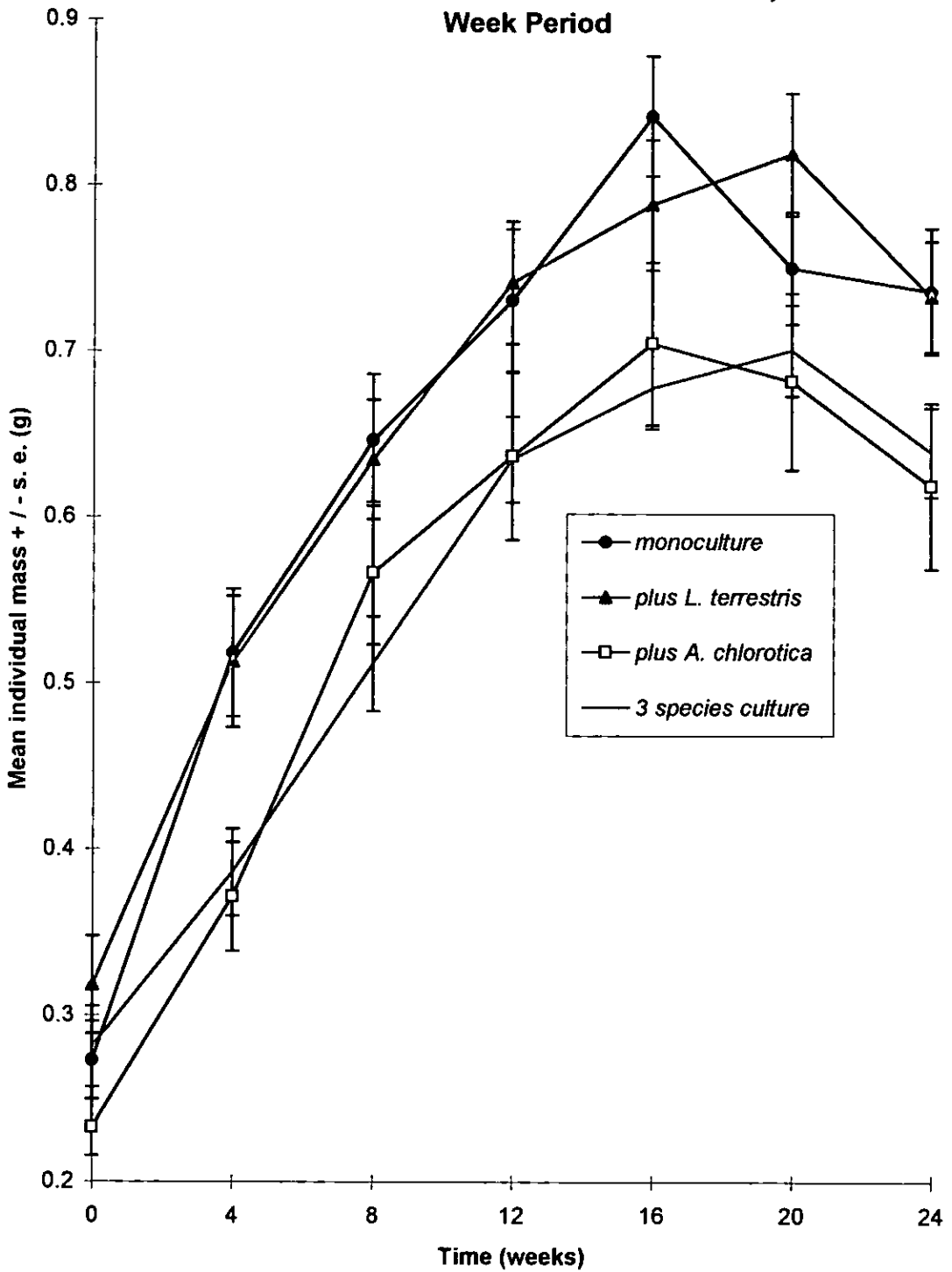
Figure 5.4.5 (c) Growth of *L. rubellus* Provided Separated Cattle Solids (SCS) Incorporated Into the Soil Profile and Surface Applied (SAM) in Monoculture, Paired and Three Species Treatments With *A. chlorotica* and *L. terrestris*, Over a 24 Week period
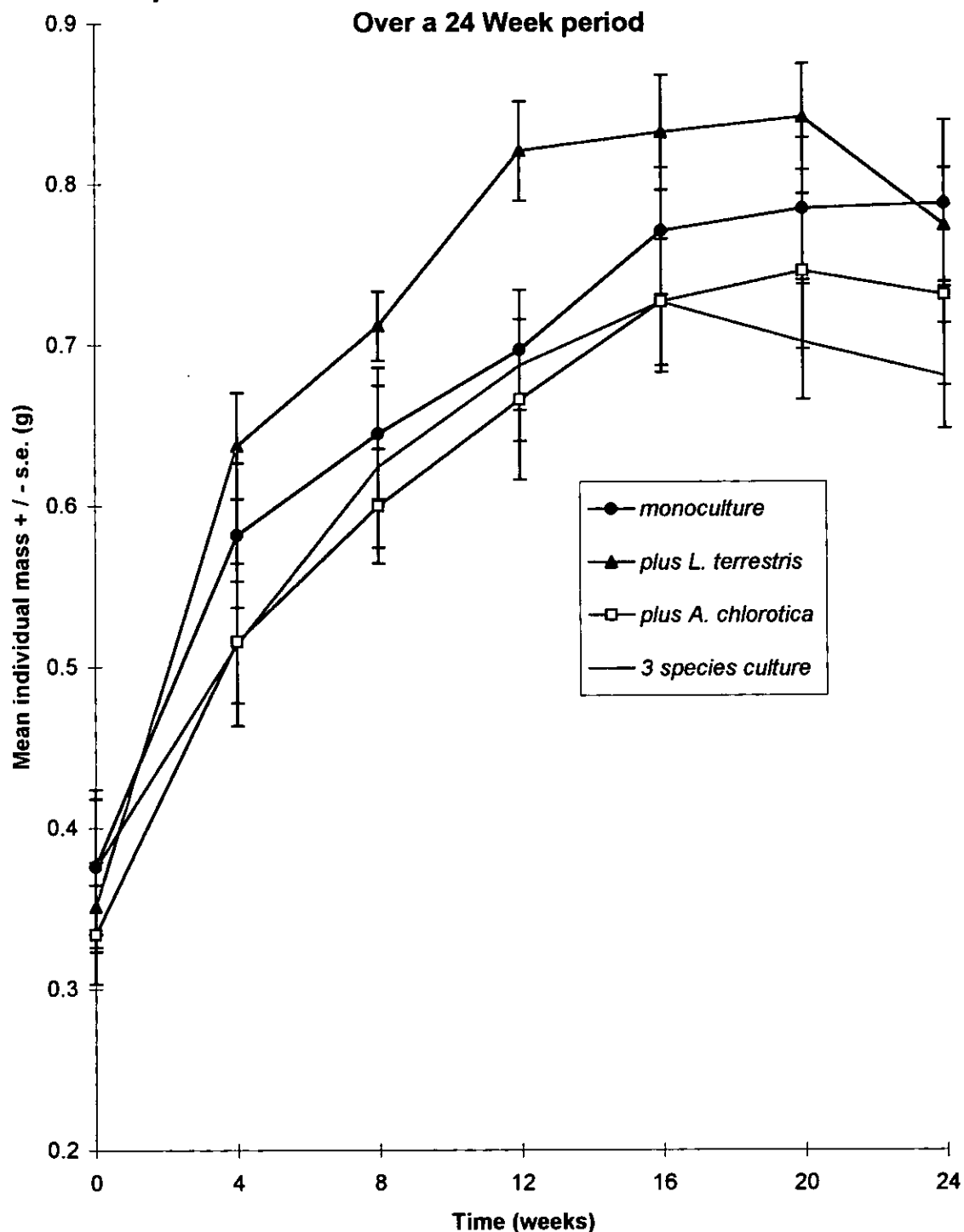
Table 5.4.6 ANOVA Tables Showing the Effect of Experimental Food Position and Species Combinations on _L. rubellus_ Growth and Cocoon Production

a) Growth

Sources of Variation	Degrees of Freedom	Mean Squares	F	P	significance
Food Regime	2	0.02685	0.91	0.406	ns
Species Combination	2	0.02169	0.73	0.482	ns
Interaction	4	0.00383	0.13	0.971	ns
Error	119	0.02956			

(ns no significant difference)

b) Cocoon Production

Sources of Variation	Degrees of Freedom	Mean Squares	F	P	significance
Food Regime	2	2.643	0.53	0.591	ns
Species Combination	2	87.874	17.58	0.0001	***
Interaction	4	25.005	5.00	0.0001	***
Error	96	4.997			

(ns no significant difference, *** significant at the 0.1 % level)

Note: the 3 species treatment is not included in the analysis.

Table 5.4.6 (a & b) indicated that food placement, the presence of other earthworm species and interaction between the two had no significant effect on _L. rubellus_ growth. However, the presence of other species and interaction between food regime and species combination had a significant (p<0.001) effect on cocoon production.

Figure 5.4.6 Mean Cocoon Production by *L. rubellus* (in Relevant Species Combinations) Provided With SCS in Three Locations: Surface Applied (SUR), Incorporated in the Soil Profile (MIX) and Both Incorporated and Surface Applied (SAM)
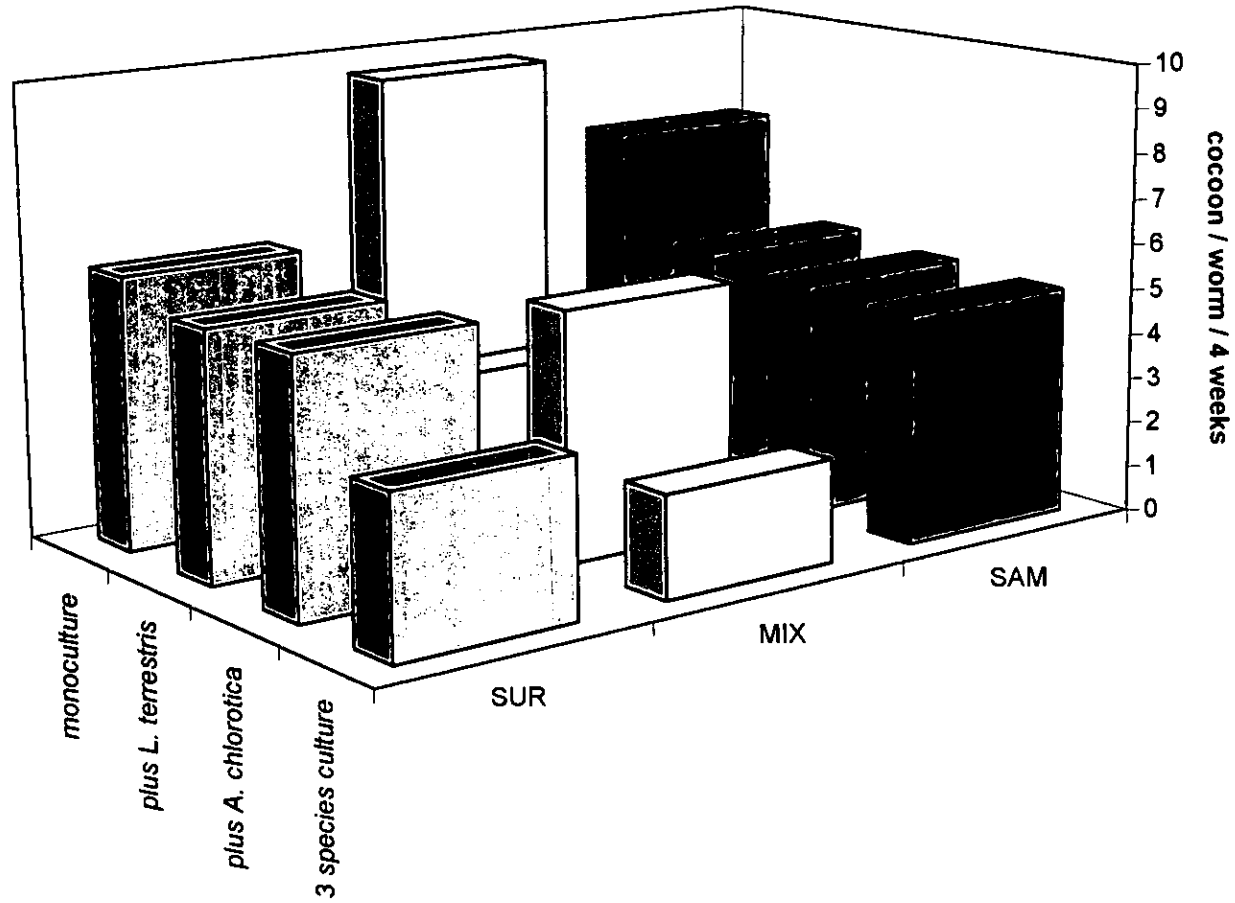
5.4.6 Discussion

5.4.6.1 *L. terrestris*

Results suggested that the growth of *L. terrestris* in single, paired and 3 species cultures followed a distinct pattern across all feed treatments. At each sampling within food treatments the mean individual mass of *L. terrestris* in monoculture and in pairing with *A. chlorotica* was greater than that recorded in either the pairing with *L. rubellus* or 3 species culture. The latter, exhibited lowest mean individual *L. terrestris* mass of the four species treatments.

The recorded differences in *L. terrestris* mass in species pairings with *A. chlorotica* and *L. rubellus* were consistent with results from the experiment assessing the effect of mature earthworms on hatchlings detailed in section 4.4 and were considered a function of the degree of niche overlap that was experienced for these three species. *L. terrestris* (above 1 g (see previous food position experiments)) form single, permanent burrows opening onto the soil surface from where they pull down organic matter. Therefore encounter rates with *A. chlorotica*, which is thought to remain exclusively within the upper soil profile, would be minimal and restricted to occasions when *A. chlorotica*, when foraging, encounters a *L. terrestris* burrow. However, it was proposed that encounters with *L. rubellus* would occur regularly as this species feeds at the soil surface and is in direct competition with *L. terrestris* for organic matter present there. It was suggested that such encounters may result in an avoidance response from *L. terrestris*, with individuals retreating into their burrows. This would decrease the time available for feeding and possibly have resulted in the differences in mass recorded in this pairing in comparison with both the monoculture and the pairing with *A. chlorotica*. The lowest

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L. terrestris mass was recorded in the cultures containing three species in all three food treatments. Earthworm density and biomass was highest in these cultures and was thought to have increased the negative effects of resource competition and individual encounter rates. Differences in *L. terrestris* growth rates in the four species combinations are further substantiated by recorded maturation rates as the highest percentage of clitellate *L. terrestris* occurred in the monoculture and in the pairing with *A. chlorotica*.

Results also suggested that food placement may have influenced *L. terrestris* growth and maturation and that these differences varied with species combinations. The differences in *L. terrestris* mass between the four species combinations were consistent across the three food treatments. However, significant differences in *L. terrestris* masses were only recorded in the MIX and SAM treatments. In the SUR treatment the negative influence of being paired with *L. rubellus* or placed in 3 species culture on *L. terrestris* growth was reduced to non-significant levels when compared to the monoculture or pairing with *A. chlorotica*. In this treatment organic matter was placed exclusively on the soil surface. Whereas in the MIX treatment *L. terrestris* would have been forced to feed within the soil in direct competition with *L. rubellus* and *A. chlorotica*. While in the SAM treatment, although food was placed on the soil surface the amount was reduced by 50 % in comparison with the SUR treatment, and this, it was suggested, resulted in increased competition between *L. rubellus* and *L. terrestris*.

ANOVA results presented in table 5.4.4 indicated that overall food placement and its interaction with species combination did not significantly ($p > 0.05$) influence *L. terrestris* growth. However, the presence of other species did significantly ($p < 0.05$) reduce growth rates and it was suggested that *L. rubellus* was the major influence in this observation.

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5.4.6.2 A. chlorotica

Results suggested that the presence of other species had a negative effect on *A. chlorotica* growth. In all food treatments the highest growth rates for *A. chlorotica* were recorded in monoculture. However, the presence of both *L. rubellus* and *L. terrestris* in 3 species culture did not decrease *A. chlorotica* growth rates in comparison with the *L. rubellus* pairing. It can be inferred from this (in spite of the omission of the *A. chlorotica* pairing with *L. terrestris*) that *L. terrestris* may have had little influence on *A. chlorotica* growth.

In the establishment of experimental cultures individuals of a species were assigned to one treatment at a time, selected at random from the total experimental population for each species. In selecting individuals for cultures larger worms could have been selected first (although unintentionally), as these are more easily picked out from the population This procedure built into the design a potential source of experimental error which was realised in the pairing of *A. chlorotica* with *L. terrestris*. Individuals of *A. chlorotica* were assigned to these cultures last and this resulted in the average individual mass of this species being significantly lower in the MIX and SAM cultures of this pairing than in any other experimental culture. This prevented viable comparisons of *A. chlorotica* mass to be made between these and other cultures throughout the experimental period. To prevent this from happening in future experiments individuals of each species were assigned one at a time to each of the experimental cultures.

Results also suggested that the influence of food position had a major influence on *A. chlorotica* growth. In all species combinations the greatest mean individual *A. chlorotica* mass was recorded in the MIX treatment. In this treatment (as described

previously) SCS was incorporated in excess within the soil profile and therefore suited the endogeic feeding behaviour of *A. chlorotica.*

In all cultures the greatest *A. chlorotica* mass was achieved at the week 16 sampling. After this point biomass declined until the experiment was terminated at week 24. By week 16 all *A. chlorotica* were clitellate and cocoons of this species were recorded in each of the relevant cultures. It is suggested that loss in mass recorded after week 16 was due to the increased energy demands of cocoon production.

Trends recorded for *A. chlorotica* growth rates were also recorded in cocoon production. In each food treatment, monoculture yielded the greatest number of *A. chlorotica* cocoons. It is proposed that the recorded differences in cocoon production were a function of disturbance during mating and deposition of cocoons. These factors were reduced in the monoculture as the number of earthworms and total biomass were lower than in other species combinations and consequently individual encounter rates were also at their lowest within these cultures. Earthworms (*L. terrestris*) have been shown to interfere with mating of conspecific pairs (Nuutinen & Butt, 1997) and this may also occur between species. The position of food also influenced cocoon production. In monocultures the greatest cocoon production was recorded in the MIX treatment. In this treatment organic matter was more readily available than in either the SAM or SUR treatments due to differences in food position and quantity. It can therefore be inferred that increased consumption of organic matter by *A. chlorotica* in the MIX treatment resulted in greater energy availability for cocoon production, in comparison with the other two food treatments. Overall, statistical analysis of cocoon production (table 5.4.5) indicated that both food position, the presence of other species and the interaction

between these two factors had significant ($p<0.001$, $p<0.01$ and $p<0.05$, respectively) effects on *A. chlorotica* cocoon production.

5.4.6.3 *L. rubellus*

Growth of *L. rubellus* was not affected significantly ($p>0.05$) by food position or the presence of other species in experimental cultures (see table 5.4.6). However, *L. rubellus* has been shown to negatively influence the growth rates of both *L. terrestris* and *A. chlorotica*. In comparison with the other two species, *L. rubellus* has greater mobility and growth rates. It is proposed that these factors provided this species with a competitive advantage, negatively influencing the growth of other more sedentary species.

In contrast with growth rates, cocoon production by *L. rubellus* was significantly ($p<0.001$) influenced by the presence of other species. As for *A. chlorotica*, greatest cocoon production in all three food treatments was recorded in monoculture and was lowest in 3 species culture, with greatest cocoon production achieved in the MIX monoculture. The reduction in cocoon production in mixed, compared with monocultures is attributed (as for *A. chlorotica*) to differences in biomass, density and encounter rates. These results supported the findings of Reinecke & Viljoen (1993) who studied the influence of earthworm density on growth and cocoon production in *Eudrilus eugeniae*. These workers cultured this epigeic species at densities of 4, 8, 12, 16 and 20 worms in 500 ml flasks. Results showed that at higher densities (12,16 and 20 worms) and biomass earthworms displayed similar growth rates while cocoon production was reduced markedly.

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Statistical analysis showed that (unlike growth) although food position alone did not significantly influence cocoon production the interaction of both food and species combination did have a significant (p<0.001) effect. This suggested that the influence of species interactions may be more easily observed by recording cocoon production rather than growth rates.

5.5 AN EXPERIMENT INVESTIGATING THE EFFECT OF FOOD PARTICLE SIZE ON INTER-SPECIFIC INTERACTIONS BETWEEN *A. CHLOROTICA* AND *L. TERRESTRIS* : ASSESSED IN TERMS OF EARTHWORM GROWTH AND MATURATION

5.5.1 Introduction

Earthworm growth rates and fecundity are inversely related to the particle size of organic matter utilised as a food resource and the maximum particle size ingested by individual species is directly related to body size (Piearce, 1978). It has also been suggested that earthworms, both hatchlings (section 4.4), and adults of smaller species (Butt *et al.*, 1997) may benefit (in terms of growth rates and cocoon production) from the faecal casts produced by adults of larger species. It was thought that this casting presented a concentrated and more palatable source (due to decreased particle size) of organic matter. The primary aim of this experiment was to determine the effect of feed particle size on interactions between *A. chlorotica* and *L. terrestris*.

5.5.2 Materials and Methods

As in previous experiments all earthworms were obtained from laboratory-reared stocks. Hatchlings and mature individuals of *A. chlorotica* and *L. terrestris* were used, enabling the influence of casting (by the larger anecic *L. terrestris*) on hatchling growth (both species) and cocoon production (by *A. chlorotica*) to be investigated. Two food treatments were set up in 1 litre culture vessels, filled to a depth of 0.11 m with pre-sterilised Kettering loam (moisture content 25 %) :-

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1) Unground, SCS applied at the soil surface and incorporated into the soil profile (60 g in each location)

2) Ground SCS applied at the soil surface and incorporated into the soil profile (60 g in each location)

Five replicates of 6 earthworm combinations were established in accordance with pre-determined species ratios (section 3.4.1) in the two food regimes as shown in table 5.5.1 All treatments were maintained in darkness at 15 ± 1 °C.

Table 5.5.1 Treatments Employed in Determining the Effect of Food Particle Size on Interactions Between *A. chlorotica* and *L. terrestris*

Species Combinations in the two feed regimes	Number of individuals of each species
L. terrestris (h) monoculture	2
A. chlorotica (h) monoculture	8
L. terrestris (h) + *A. chlorotica* (h)	2 + 8
L. terrestris (h) + *L. terrestris* (m)	2 + 1
A. chlorotica (h) + *L. terrestris* (m)	8 + 1
A. chlorotica (h) + *A. chlorotica* (m)	8 + 4

h = hatchling, m = mature

Individual earthworm masses were recorded at the outset of the experiment and at each subsequent sampling, when reproductive condition was also recorded. Both SCS and soil were replaced with fresh at every sampling. The first sampling took place after 6 weeks, allowing *A. chlorotica* hatchlings to gain sufficient mass so the time taken to find earthworms in cultures was minimised. Thereafter sampling took place every 4 weeks until the experiment was terminated after 18 weeks.

The Tukey-Kramer multi-comparison ANOVA was used to statistically analyse differences in the recorded mass of both species between treatments at each sampling. The General Linear model for analysis of variance was also employed to determine the possible effect of interactions between the presence of other species and food particle size on the growth of *A. chlorotica* and *L. terrestris*.

5.5.2.1 Feed Preparation

Ground SCS were prepared by milling oven-dried SCS in a sample mill (Kinifetec 1095) for 5 seconds. This produced a relatively uniform substrate with 90 % of particles less than 1mm (determined by sifting through a series of sieves 2 mm - 150 μm (appendix 5)).

In previous experiments oven dried SCS was soaked in water, manual pressure was applied to remove excess, and the required mass placed in cultures. In order that viable comparisons could be made between the unground and ground food treatments it was essential that equal quantities of food were applied to both. However, preparing ground SCS in the manner described would have provided cultures with a food containing a higher organic matter to water ratio than the unground food. This was overcome by determining the ratio of dry mass (g) of SCS to water in known masses of rewetted unground food and using this ratio in the establishment of ground food treatments. In a preliminary experiment 80, 40, 20 and 10 g of unground, rewetted SCS had water content determined gravimetrically. From these results the ratio of organic matter to water for required masses of rewetted ground SCS could be determined (see appendix 5).

5.5.3 Results for *A. chlorotica*

5.5.3.1 Growth

Figure 5.5.1 illustrates growth of *A. chlorotica* in all relevant experimental treatments, where, in all cases, gains in mass were recorded throughout the experimental period. At the outset of the experiment there was no significant ($p>0.05$) difference in mean individual *A. chlorotica* hatchling mass between treatments (overall mean individual mass 0.0064 g). At each subsequent sampling the mass of hatchlings in all ground food treatments was greater than that recorded in unground food treatments. At week 18, mean individual mass of *A. chlorotica* in ground food treatments (0.37 g) was 147 % greater than in unground food treatments (0.15 g). Within similar food regimes there were also significant differences in *A. chlorotica* mass between species combinations. In the ground food regime, individual mean mass of *A. chlorotica* was significantly lower ($p<0.01$) at each sampling in the pairing with mature *A. chlorotica* than in any other combination. In the other species treatments *A. chlorotica* exhibited significantly ($p<0.01$) higher growth rates at weeks 6 and 10 in the pairing with mature *L. terrestris*. However, after week 10 there was no significant ($p>0.05$) difference in growth rates between these three treatments.

In contrast, in unground food treatments clear differences in *A. chlorotica* masses were recorded between all four species combinations at week 18. The lowest mean individual mass (0.081 g) was recorded in the pairing with mature *A. chlorotica*, and was significantly ($p<0.01$) less than that recorded for other species combinations. Greatest mean individual *A. chlorotica* mass (0.21 g) was recorded in the pairing with mature

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Figure 5.5.1 Growth of Hatchling *A. chlorotica* in All Relevant Species Combinations, Provided With Either Ground (Open Symbols) or Unground (Closed Symbols) Separated Cattle Solids (SCS) as Feed (m - With Mature Animals; h - With Hatchling Animals)

Legend:
- monoculture - h
- monoculture - h
- plus L. terrestris - m
- plus L. terrestris - h
- plus L. terrestris - h
- plus L. terrestris - m
- plus A. chlorotica - m
- plus A. chlorotica - m

Y-axis: Mean individual mass +/- s. e. (g)
X-axis: Time (weeks)
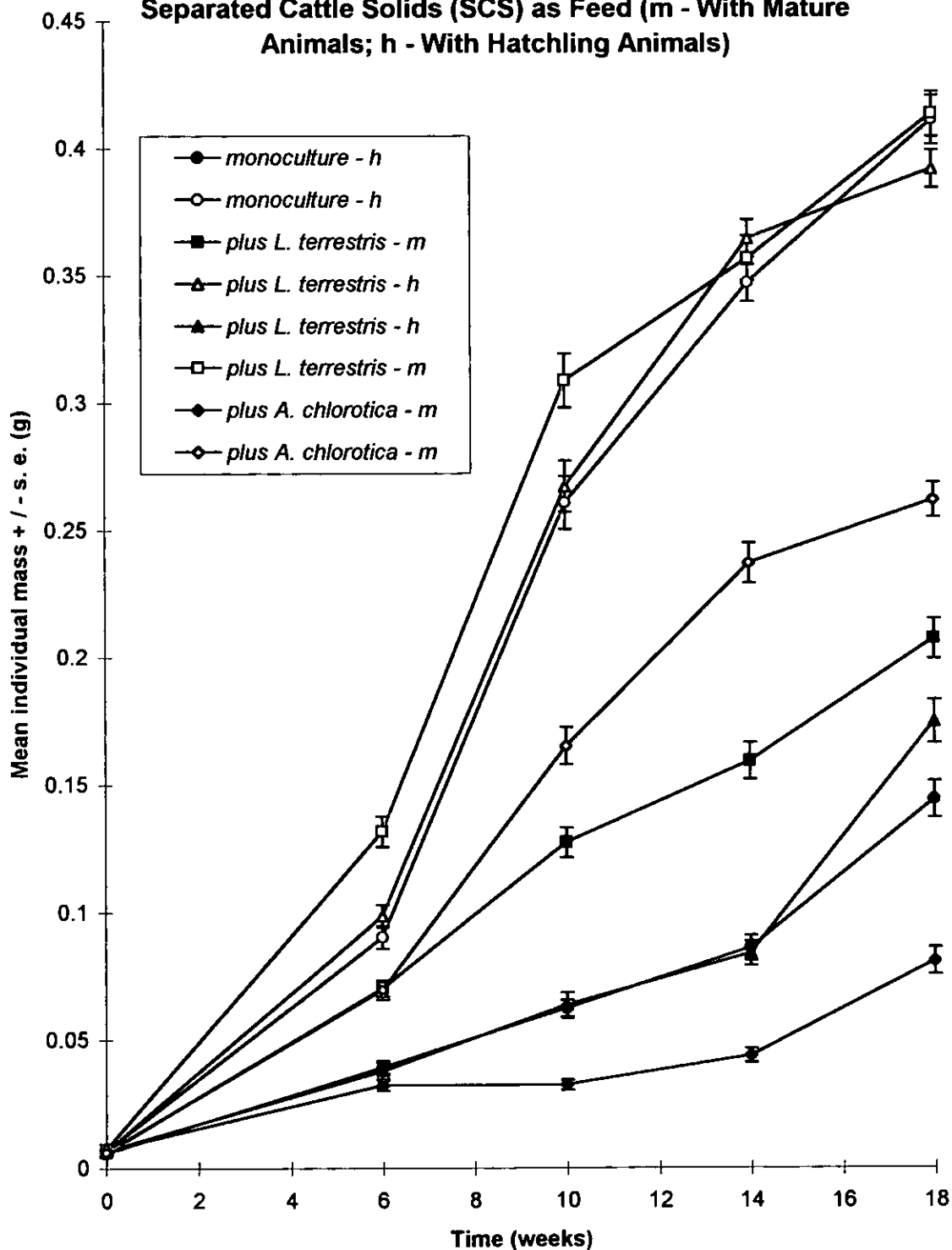
L. terrestris, which was significantly (p<0.01) different compared with the monoculture

(0.143 g) but not significantly different to the pairing with hatchling *L. terrestris* (0.17 g).

Table 5.5.2 ANOVA Table Showing the Effect of Food Particle Size and Species

Combination on *A. chlorotica* Growth

Sources of Variation	Degrees of Freedom	Mean Squares	F	P	significance
Particle size	1	2.92352	1287.43	0.0001	***
Species Combination	3	87.874	17.58	0.0001	***
Interaction	3	25.005	5.00	0.0001	***
Error	96	4.997			

(*** significant at the 0.1 % level)

5.5.3.2 Maturation

In ground food treatments, clitellate *A. chlorotica* were first recorded at week 10 and at

all subsequent samplings (figure 5.5.2). Differences observed in the growth of

A. chlorotica between species combinations in the ground feed regime were mirrored in

maturation rates. At week 14 all *A. chlorotica* in monoculture, and in pairings with

hatchling and mature *L. terrestris* were clitellate compared with 62 % of *A. chlorotica* in

the pairing with mature conspecifics. In the unground food regime, clitellate

A. chlorotica were only recorded at week 18 in the pairings with hatchling and mature

L. terrestris (both at a figure of 70 %).

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Figure 5.5.2 Percentage of Mature *A. chlorotica* Present in All Relevant Species Combinations, Provided Either Ground (g) or Unground Separated Cattle solids (SCS) as Feed (m -With Mature Animals; h - With Hatchling Animals)

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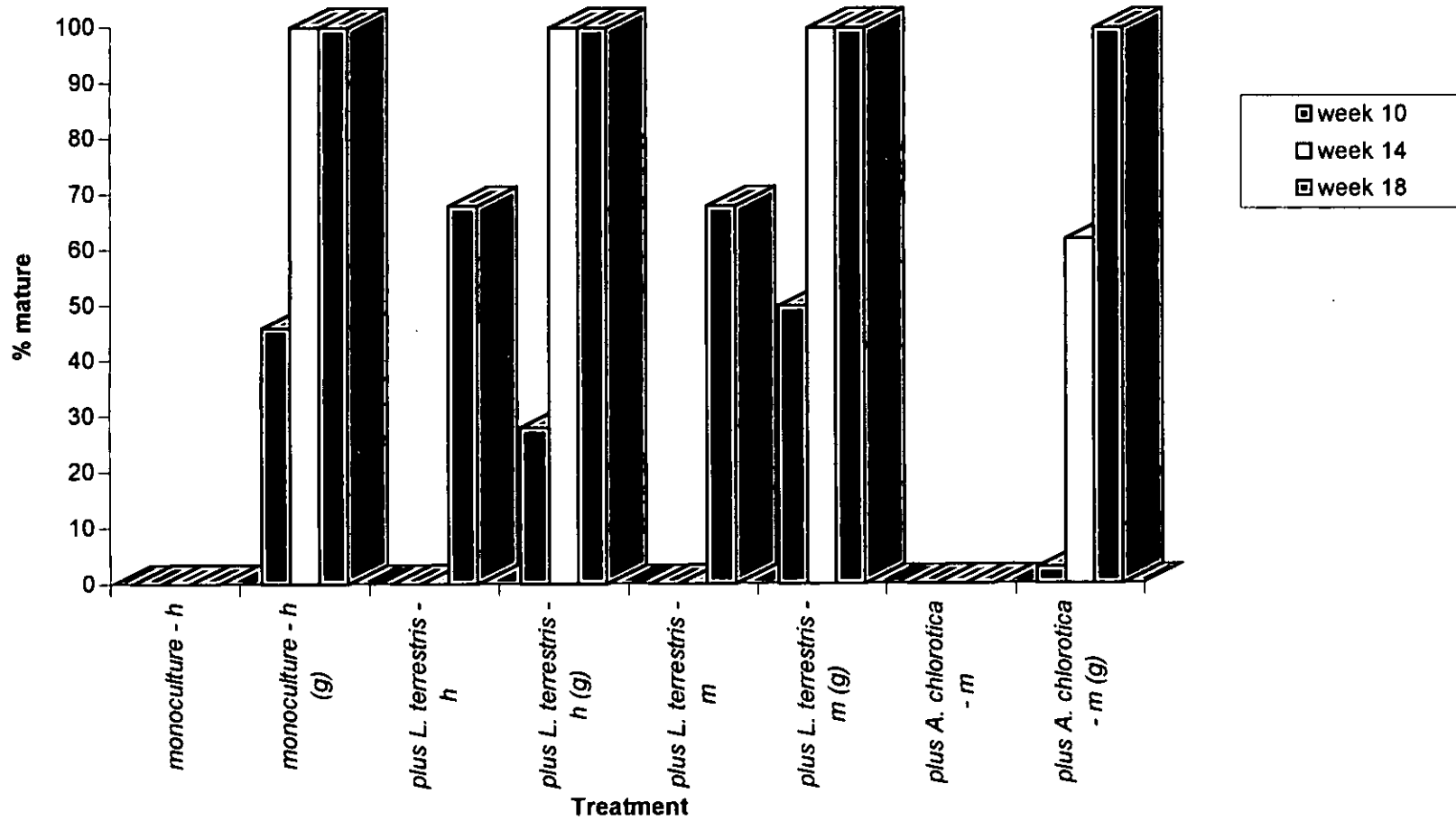
5.5.4 Results for *L. terrestris*

5.5.4.1 Growth

L. terrestris gained in mass in all treatments over the 18 week period (see figure 5.5.3) and at the outset of the experiment there was no significant (p>0.05) difference in the mass of *L. terrestris* within relevant treatments (overall mean individual mass 0.058 g). The average mass of *L. terrestris* (at week 18) in ground food treatments was 49.4 % greater than in the unground treatments. At week 18, *L. terrestris* in the ground food monoculture had an individual mean mass of 3.23 g. This was significantly (p<0.01) greater than *L. terrestris* mass in the pairings with hatchling *A. chlorotica* and mature *L. terrestris* (individual mean mass 1.95 and 2.00 g, respectively). In the latter two treatments there was no significant (p>0.05) difference in *L. terrestris* mass. At week 18 in the unground food treatments, *L. terrestris* mass was greatest in the monoculture (individual mean mass 2.10 g). This figure was not significantly (p>0.0.5) different from that recorded in the pairing with hatchling *A. chlorotica* (1.66 g) but was significantly (p<0.01) greater than in the pairing with mature *L. terrestris* (1.04 g).

Figure 5.5.3 Growth of Hatchling *L. terrestris* in All Relevant Species Combinations, Provided With Either Ground (Open Symbols) or Unground (Closed Symbols) Separated Cattle Solids (SCS) as Feed (m - With Mature Animals; h - With Hatchling Animals)

Legend:
- monoculture - *h* (closed circle)
- monoculture - *h* (open circle)
- plus *A. chlorotica* - *h* (closed triangle)
- plus *A. chlorotica* - *h* (open triangle)
- plus *L. terrestris* - *m* (closed square)
- plus *L. terrestris* - *m* (open square)

Y-axis: Mean individual mass + / - s.e. (g)
X-axis: Time (weeks)
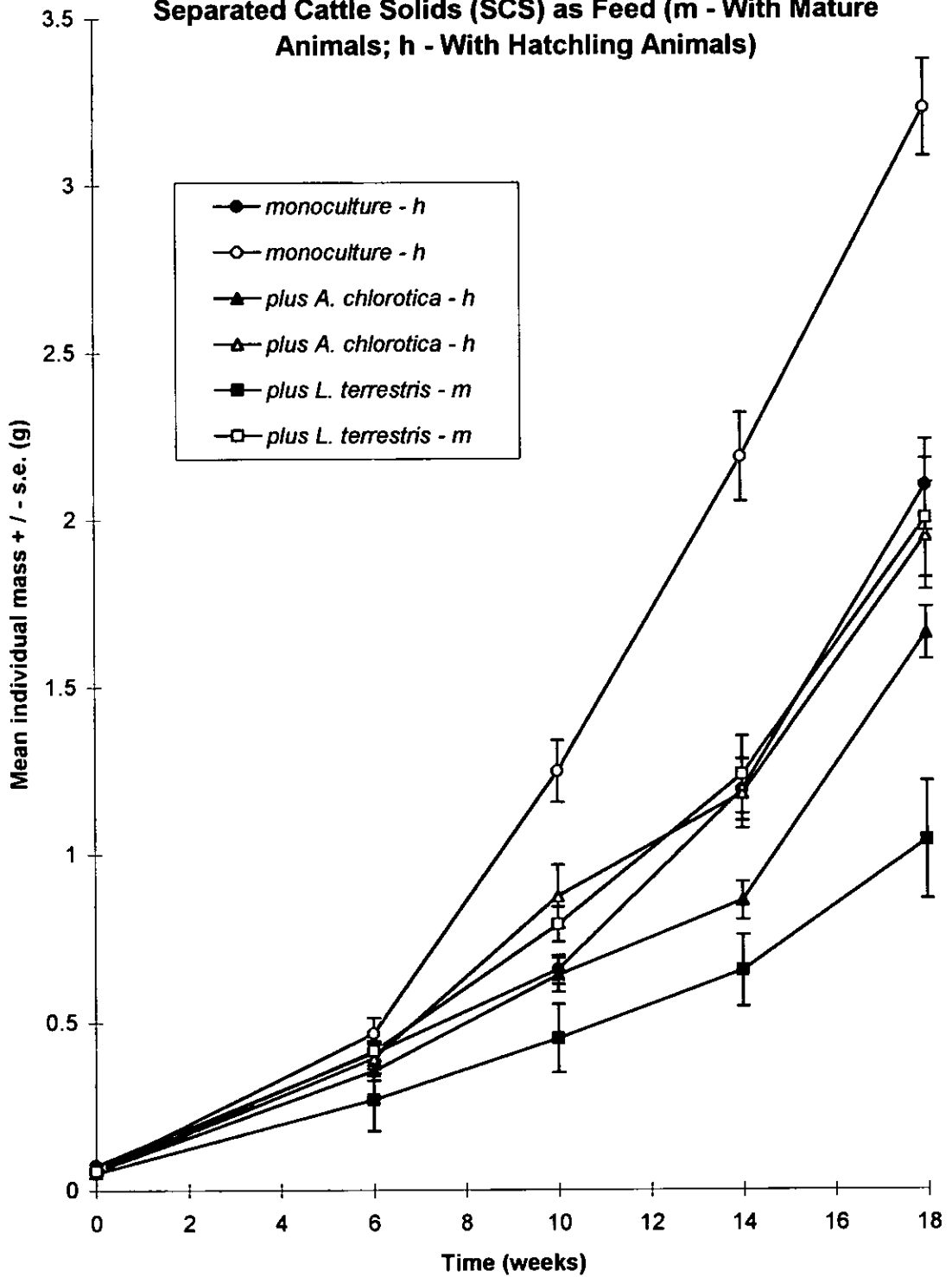
Table 5.5.3 ANOVA Table Showing the Effect of Food Particle Size and Species

Combination on *L. terrestris* Growth

Sources of Variation	Degrees of Freedom	Mean Squares	F	P	significance
Particle Size	1	7.5871	44.44	0.0001	***
Species Combination	2	11.1025	32.52	0.0001	***
Interaction	2	0.8883	5.20	0.009	***
Error	43	0.1707			

(*** significant at the 0.1 % level)

5.5.5 Discussion

5.5.5.1 A. chlorotica

Results suggested (table 5.5.2) that growth rates recorded for *A. chlorotica* in this experiment were significantly ($p < 0.001$) influenced by both food particle size and the presence of other species. In ground food treatments, *A. chlorotica* exhibited higher growth and maturation rates than in the unground food treatments. In species monoculture, *A. chlorotica* mass at week 18 in the ground food regime was 185 % greater than in the unground food regime. This result supported previous work by other authors (*e.g.* Boström, 1988) which indicated an inverse relationship between food particle size and growth rates in various earthworm species.

As in all previously related experiments the presence of other earthworms in culture negatively influenced growth rates. This negative influence was greatest (in both food regimes) in pairings with mature *A. chlorotica*. It was suggested that this negative influence was a direct result of intra-specific competition for both space and food resources. Results also suggested that food particle size influenced recorded interactions between earthworms. In ground food treatments, there was no significant ($p > 0.05$) difference in the growth rates of *A. chlorotica* in monoculture compared with pairings with hatchling and mature *L. terrestris* after 18 weeks. However, in unground food treatments mean *A. chlorotica* mass in the pairing with *L. terrestris* hatchlings was significantly ($p < 0.05$) greater than the monoculture, at each sampling. This result was also recorded at week 18 in the pairing with mature *L. terrestris*, which suggested that when SCS were unground *A. chlorotica* growth was promoted in the presence of *L. terrestris*, a benefit not found when the food was ground (particle size reduced). It was

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proposed that *L. terrestris*, through the consumption, digestion and egestion of SCS reduced the size of food particles in its casting in comparison with the surrounding food resource. This presented *A. chlorotica*, in these treatments, with a more easily ingested and more palatable food resource than in monoculture. As recorded in sections 4.4. and 5.2 hatchling *L. terrestris* tend to be located in the upper soil layers and it was likely that casting by this species also occurred within the soil at this location. This observation could have accounted for the differences in growth rates between *A. chlorotica* in pairings with hatchling and mature *L. terrestris*. As endogeic earthworms, *A. chlorotica* would, it was suggested, have initially benefited from the sub-surface casting of juvenile *L. terrestris* in the hatchling pairing. However, in the pairing with adult *L. terrestris* the initial benefit of sub-soil casting on *A. chlorotica* growth would have been diminished as adult *L. terrestris* cast on the soil surface. Casting in this location may initially have been out of reach of hatchling *A. chlorotica*, however, as individuals gained in mass this more palatable food source may have become more accessible, resulting in a difference in mass between this pairing and the monoculture at week 18. This type of positive interaction between endogeic and anecic species has also been recorded between *A. chlorotica* and *A. longa* in experimental cultures (Butt *et al.*, 1997) and has been inferred from field inoculation trials (Butt *et al.*, 1999) (see section 2.5).

5.5.5.2 *L. terrestris*

As demonstrated by *A. chlorotica*, results showed that the growth of *L. terrestris* was influenced by food particle size and the presence of other earthworms in experimental cultures (see table 5.5.3). However, the positive influence of smaller food particle size on growth rates was not as great as that experienced by *A. chlorotica*. At week 18, in ground food monoculture *L. terrestris* recorded an individual mean mass, only 54 %

higher than in unground food monoculture. It was proposed that the influence of food particle size on earthworm growth was dependant on individual size. Piearce (1978) quantitatively examined the gut contents of six lumbricid species taken from permanent pasture soil in North Wales. He determined the maximum size of particles in the crop and gizzard of the six species and used these measurements as a crude measure of the limit of size of particle ingested. In general the maximum size of particle ingested was directly related to body size. For example, *A. chlorotica* was found to ingest organic fragments of mean width 0.37 mm while the larger anecic *A. longa* ingested fragments of mean width 0.70 mm. Therefore, as shown in this experiment, the reduction of food particle size would have greater benefit on the growth rates of smaller species in comparison with larger species which are able to ingest a greater range of particle size.

In both food treatments *L. terrestris* growth rates were highest in monoculture. However, results indicated (see interaction term (table 5.5.3)) that food particle size was influencing the degree to which mature *L. terrestris* and *A. chlorotica* influenced hatchling growth rates. In ground food treatments the growth of *L. terrestris* in these two pairings was approximately equal, whereas in the unground food regime the presence of mature *L. terrestris* markedly reduced *L. terrestris* growth rates in comparison with the pairing with mature *A. chlorotica*. It was suggested that either :-

a) the influence of intra-specific competition and increased food particle size had a cumulative effect upon *L. terrestris* growth

or

b) decreased food particle size reduced the negative influence of intra-specific interactions in the ground feed regime.

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5.6 AN EXPERIMENT INVESTIGATING THE EFFECT OF SOIL BULK DENSITY ON INTER-SPECIFIC INTERACTIONS BETWEEN *A. CHLOROTICA*, *L. RUBELLUS* AND *L. TERRESTRIS* : ASSESSED IN TERMS OF COCOON PRODUCTION

5.6.1 Introduction

Several authors (*e.g.* Rushton, 1986) have demonstrated experimentally that increased soil bulk density affects the burrowing ability, behaviour and cast production of earthworms (section 2.2.3). The three species used here display different spatial distributions and burrowing abilities, in keeping with their ecological classification. It was therefore proposed that increased soil bulk density might effect the three species differently and in so doing influence inter-specific interactions. Monocultures and all paired combinations of mature *A. chlorotica*, *L. rubellus* and *L. terrestris* were established under 2 soil bulk density regimes. The influence of soil bulk density and inter-specific interactions were assessed in terms of cocoon production.

5.6.2 Materials and Methods

Mature *A. chlorotica*, *L. rubellus* and *L. terrestris* (mean individual masses 0.318, 1.135 and 5.82 g, respectively) were obtained from laboratory-reared and maintained populations (see section 3.7). Individuals of each species were of comparable age, sexual and general condition. Two soil bulk density treatments were established in 1 litre culture vessels. These were:

1) compacted soil (bulk density 1.52 $g\,cm^{-3}$)

and

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2) uncompacted soil (bulk density 1.37 g cm^{-3}).

The compacted soil treatment was set up using standard Proctor soil compaction test equipment (see appendix 6). In the uncompacted soil treatment culture vessels were filled to the top with pre-sterilised Kettering loam (moisture content 25 %). The vessels were then tapped firmly on the laboratory bench in order to achieve a uniform bulk density and at the same time creating space for the addition of SCS. An excess (80 g) of SCS were applied to the soil surface of each treatment ensuring that adequate was available between sampling.

Monocultures and all paired combinations of the three species were employed in both soil compaction treatments (table 5.6.1). Individual numbers of each species were introduced to relevant treatments in accordance with pre-determined species ratios (section 3.4.1). Four replicates of each of the 12 treatments were set up. The experimental cultures were maintained in darkness within temperature controlled incubators at 15 ± 1 °C. The mass and reproductive condition of individual earthworms was recorded at the outset of the experiment and at subsequent 4 weekly samplings, at which point earthworms were transferred to freshly prepared soil and excess SCS was reapplied. After sampling, the earthworm-worked soil was wet sieved and the number of cocoons produced by each species in each treatment recorded. The experiment was terminated after 12 weeks.

Table 5.6.1 Treatments Employed in Determining the Effect of Soil Bulk Density

on Interactions Between *A. chlorotica*, *L. rubellus* and *L. terrestris*

Species combinations in both soil compaction treatments	Number of individuals of each species
L. terrestris monoculture	2
L. rubellus monoculture	4
A. chlorotica monoculture	8
L. terrestris + *L. rubellus*	2 + 4
L. terrestris + *A. chlorotica*	2 + 8
L. rubellus + *A. chlorotica*	4 + 8

Differences in species cocoon production within treatments was statistically analysed using the Tukey-Kramer method multicomparison ANOVA. No statistical analysis was applied to mass change as the masses of adults were not comparable at the start of the experiment. The General Linear model for analysis of variance was also employed to determine the possible effect of interactions between the presence of other species and soil bulk density on cocoon production.

5.6.3 Results for *A. chlorotica*

In all relevant treatments, survival rates of *A. chlorotica* were between 90 and 100 % . The mean individual mass of *A. chlorotica* in all treatments decreased over the 12 week experimental period (see table 5.6.2 below).

Table 5.6.2 Individual Mean Masses of *A. chlorotica* in Treatments From the Soil

Bulk Density Experiment

a) Uncompacted

Time (weeks)	Monoculture ind. mean mass (g)	+ *L. rubellus* ind. mean mass (g)	+ *L. terrestris* ind. mean mass (g)
0	0.303	0.323	0.337
4	0.254	0.235	0.277
8	0.271	0.228	0.286
12	0.230	0.205	0.247

b) Compacted

Time (weeks)	Monoculture ind. mean mass (g)	+ *L. rubellus* ind. mean mass (g)	+ *L. terrestris* ind. mean mass (g)
0	0.305	0.315	0.324
4	0.263	0.243	0.228
8	0.246	0.212	0.243
12	0.220	0.203	0.210

In compacted soil treatments, *A. chlorotica* were only found in the surface soil layers, while in uncompacted treatments individuals were found throughout the soil profile.

Figure 5.6.1 illustrates the percentage of mature *A. chlorotica* in all experimental treatments at each sampling. Over the 12 week period some individuals in all treatments lost reproductive condition. In all three species combinations (monoculture, pairing with *L. terrestris* and pairing with *L. rubellus*) reproductive condition was lost most rapidly in

the compacted soil treatments. In both the compacted and uncompacted soil regimes differences between species combinations follow a similar pattern. *A. chlorotica* reproductive condition was lost most rapidly in the pairing with *L. rubellus* with monocultures retaining the greatest percentage of mature individuals.

5.6.3.1 Cocoon Production

Cocoon production results (figure 5.6.2) were adjusted to account for the recorded losses in reproductive condition experienced within the various treatments. In all treatments cocoon production decreased over the twelve week period and in all treatments fell by at least 50 % after the first sampling. This decrease with time was greatest in species combinations from the compacted soil regime. No *A. chlorotica* cocoons were recorded in pairings with *L. terrestris* and *L. rubellus* at week 8. Although reproductive output decreased rapidly over the 12 week period, certain trends in production are evident from the first sampling (week 4). Greatest cocoon production, in all species combinations was achieved in uncompacted soil. At week 4, cocoon production in monocultures was reduced by half in compacted compared with uncompacted soils (0.5 and 1.0 cocoons worm^{-1} 4 weeks^{-1}). In compacted treatments, *A. chlorotica* cocoon production was greatest in the pairing with *L. terrestris* (0.7 cocoons worm^{-1} week^{-1}) whilst production in monoculture and in the pairing with *L. rubellus* were very similar. In uncompacted treatments, the presence of *L. rubellus* reduced *A. chlorotica* cocoon production (0.5 cocoons worm^{-1} 4 weeks^{-1}) in relation to both the monoculture and the pairing with *L. rubellus*.

Figure 5.6.1 Percentage of Mature *A. chlorotica* Present in Species Combination Treatments, in Either Compacted (c) or Uncompacted (u) Soils

Figure 5.6.2 Cocoon Production by *A. chlorotica* in Species Combination Treatments, in Either Compacted (c) or Uncompacted (u) Soils
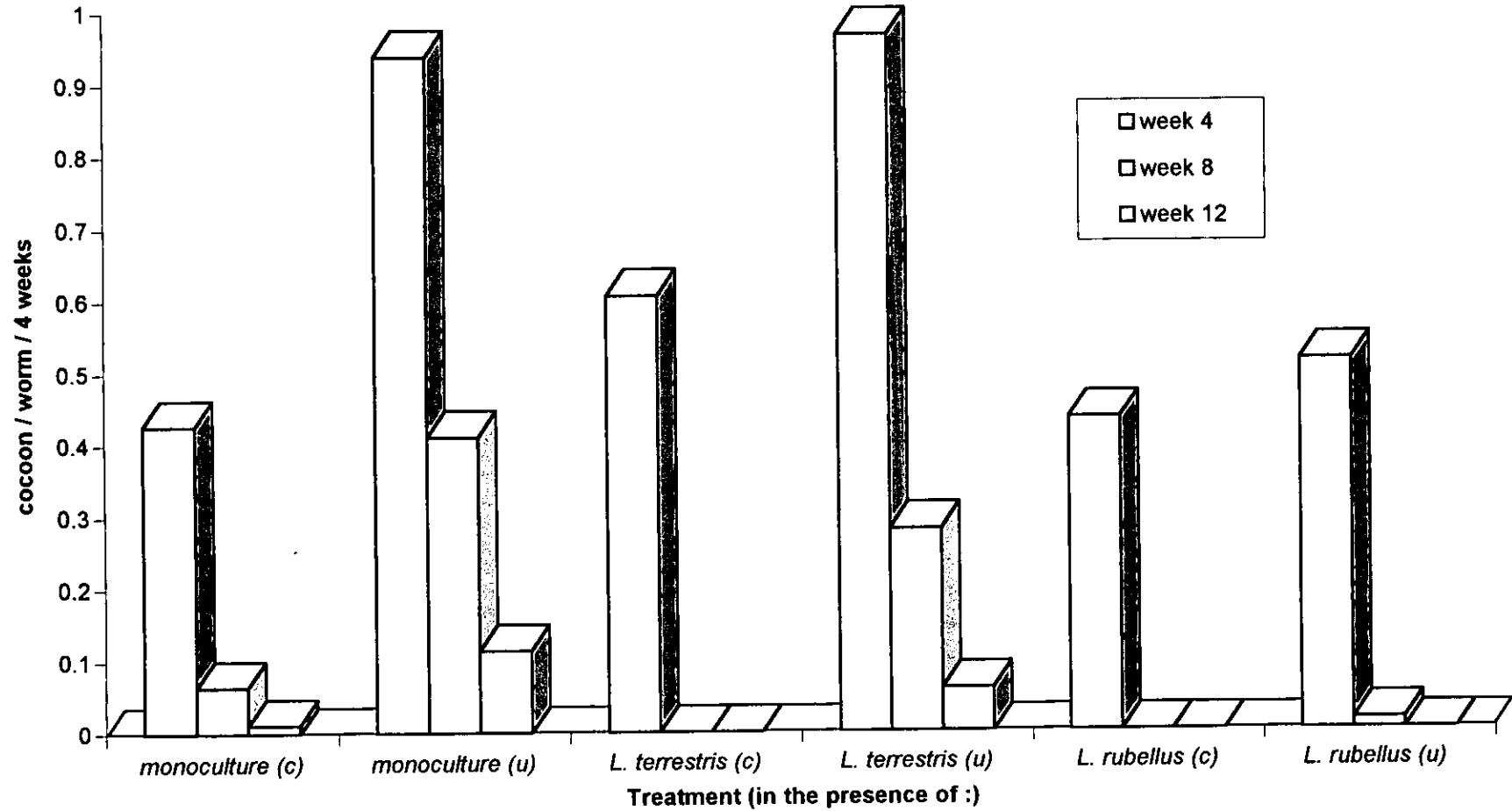
Table 5.6.3 ANOVA Table Showing the Effect of Soil Bulk Density and Species Combination on *A. chlorotica* Cocoon Production After 4 Weeks of Experimentation

Sources of Variation	Degrees of Freedom	Mean Squares	F	P	significance
Soil Bulk Density	1	0.375	4.28	0.053	ns
Species Combination	2	0.23893	2.73	0.092	ns
Interaction	2	0.11133	1.27	0.305	ns
Error	18	0.08767			

(**ns** no significant difference)

5.6.4 Results for *L. rubellus*

L. rubellus exhibited 100 % survival in all treatments and all individuals maintained reproductive condition throughout the experimental period. However, within these treatments mean individual mass of *L. rubellus* decreased with time (table 5.6.4).

Table 5.6.4 Individual Mean Mass of *L. rubellus* in Experimental Soil Bulk Density Treatments

a) Uncompacted

Time (weeks)	Monoculture ind. mean mass (g)	+ *A. chlorotica* ind. mean mass (g)	+ *L. terrestris* ind. mean mass (g)
0	1.195	1.163	1.118
4	1.017	0.942	1.036
8	0.927	0.906	0.951
12	0.852	0.865	0.864

b) Compacted

Time (weeks)	Monoculture ind. mean mass (g)	+ A. chlorotica ind. mean mass (g)	+ L. terrestris ind. mean mass (g)
0	1.161	1.142	1.032
4	0.860	0.918	0.811
8	0.848	0.873	0.777
12	0.809	0.797	0.768

In compacted soil treatments *L. rubellus* were only recorded within the organic matter at the soil surface while in uncompacted treatments individuals were recorded throughout the soil profile.

5.6.4.1 Cocoon Production

Figure 5.6.3 illustrates *L. rubellus* cocoon production in compacted and uncompacted soil over the experimental period. *L. rubellus* cocoons were recorded in all treatments at each sampling. In both uncompacted and compacted soil treatments there was no significant difference ($p>0.05$) in *L. rubellus* cocoon production between the monoculture, the pairing with *A. chlorotica* and the pairing with *L. terrestris* at each sampling. However, lowest cocoon production at each sampling was recorded in compacted soil pairing with *L. terrestris* (1.25, 1.25 and 0.70 cocoons worm^{-1} 4 weeks^{-1} at weeks 4, 8 and 12, respectively). Comparisons of mean cocoon production over the 12 week period showed that *L. rubellus*, in this pairing, produced significantly ($p<0.0.5$) fewer cocoons than in the pairing with *A. chlorotica* or the monoculture in compacted soil.

Figure 5.6.3 Cocoon Production by *L. rubellus* in Species Combination Treatments, in Either Compacted (c) or Uncompacted (u) Soils
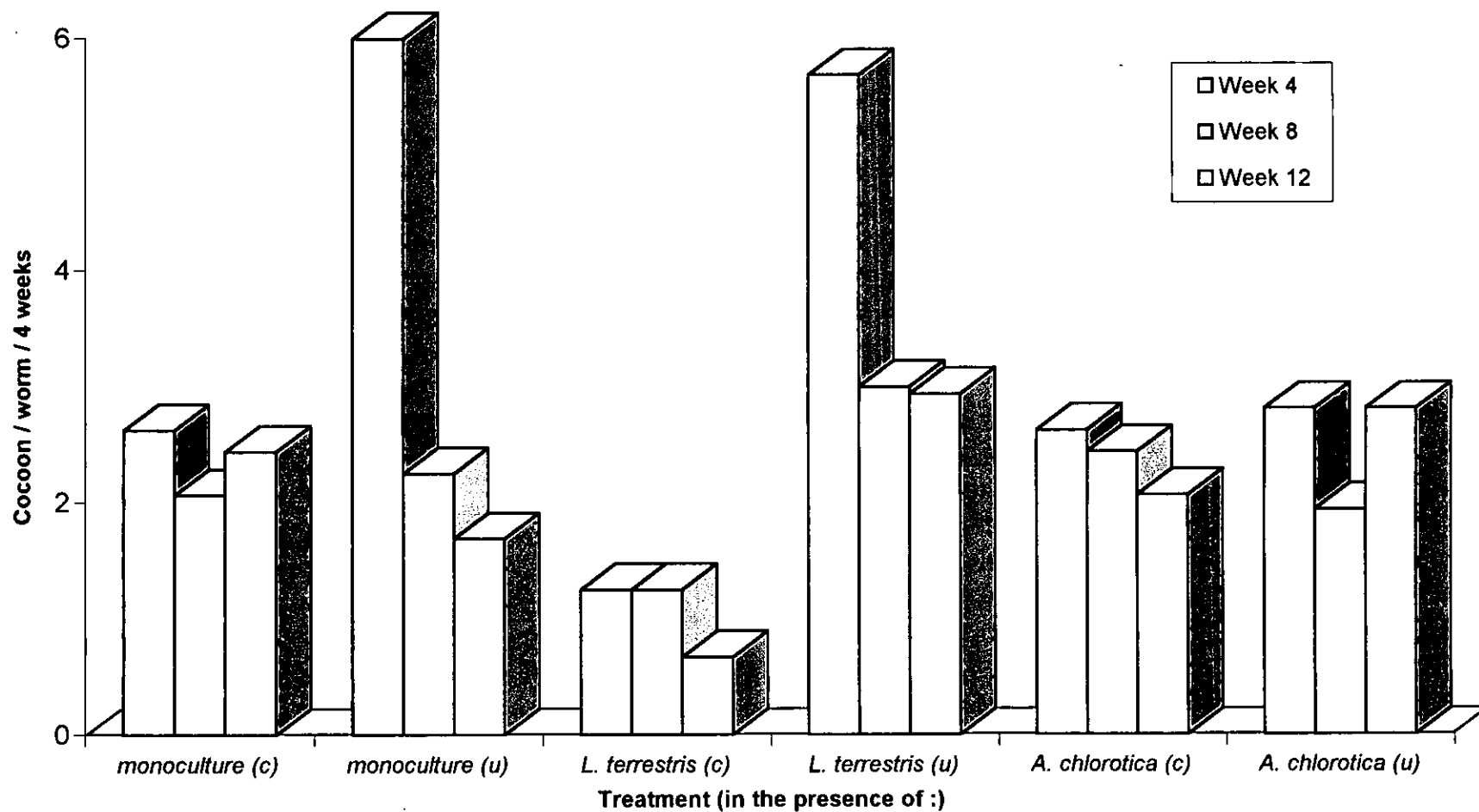
At week 4, *L. rubellus* cocoon production was significantly (p<0.05) greater in the uncompacted monoculture and the pairing with *L. terrestris* (6.00 and 5.70 cocoons worm^{-1} 4 weeks^{-1}, respectively) compared with the same species combinations in compacted soils (2.62 and 1.25 cocoons worm^{-1} 4 weeks^{-1}, respectively). At weeks 8 and 12 there were no significant (p>0.05) differences in *L. rubellus* cocoon production between compacted and uncompacted soil treatments in any species combination

Table 5.6.5 ANOVA Table Showing the Effect of Soil Bulk Density and Species Combination on *L. rubellus* Cocoon Production After 4 Weeks of Experimentation

Sources of Variation	Degrees of Freedom	Mean Squares	F	P	significance
Soil Bulk Density	1	42.667	18.08	0.0001	***
Species Combination	2	5.086	2.16	0.145	ns
Interaction	2	9.784	4.15	0.033	*
Error	18	2.359			

(ns no significant difference, * significant at the 5 % level, *** significant at the 0.1 % level)

5.6.5 Results for *L. terrestris*

In all relevant treatments *L. terrestris* exhibited 100 % survival and maintained reproductive condition throughout the 12 week experimental period. Within these treatments mean individual masses of *L. terrestris* decreased with time.

Table 5.6.6 Individual Mean Mass of *L. terrestris* in Experimental Soil Bulk

Density Treatments

a) Uncompacted

Time (weeks)	Monoculture ind. mean mass (g)	+ *A. chlorotica* ind. mean mass (g)	+ *L. rubellus* ind. mean mass (g)
0	6.211	5.195	5.844
4	5.320	4.693	4.967
8	5.228	4.559	4.934
12	5.421	4.335	4.690

b) Compacted

Time (weeks)	Monoculture ind. mean mass (g)	+ *A. chlorotica* ind. mean mass (g)	+ *L. rubellus* ind. mean mass (g)
0	5.928	6.093	5.653
4	5.321	5.047	4.844
8	4.885	4.967	4.782
12	5.032	4.916	4.315

At sampling of compacted soil treatments *L. terrestris* were often found on the soil surface unattached to a burrow. Any burrows present were limited to the edges of the vessel. In uncompacted treatments individuals were recorded in vertical burrows within the soil profile.

5.6.5.1 Cocoon Production

Cocoon production by *L. terrestris* was recorded in all treatments throughout the 12 week period (figure 5.6.4). In both soil compaction regimes there was no significant ($p>0.05$) difference in *L. terrestris* cocoon production between the monoculture, the pairing with *A. chlorotica* and the pairing with *L. rubellus* at weeks 4, 8 and 12. At each sampling *L. terrestris* cocoon production in the uncompacted soil monoculture (1.75, 2.00 and 3.40 cocoons worm^{-1} 4 weeks^{-1} at 4, 8 and 12 weeks, respectively) was greater than that recorded in any other treatment and was significantly ($p<0.05$) greater than in the compacted monoculture at week 12.

Table 5.6.7 ANOVA Table Showing the Effect of Soil Bulk Density and Species Combination on *L. terrestris* Cocoon Production After 4 Weeks of Experimentation

Sources of Variation	Degrees of Freedom	Mean Squares	F	P	significance
Soil Bulk Density	1	2.3437	2.97	0.102	ns
Species Combination	2	0.4479	0.57	0.576	ns
Interaction	2	0.0312	0.04	0.961	ns
Error	18	0.7882			

(ns no significant difference)
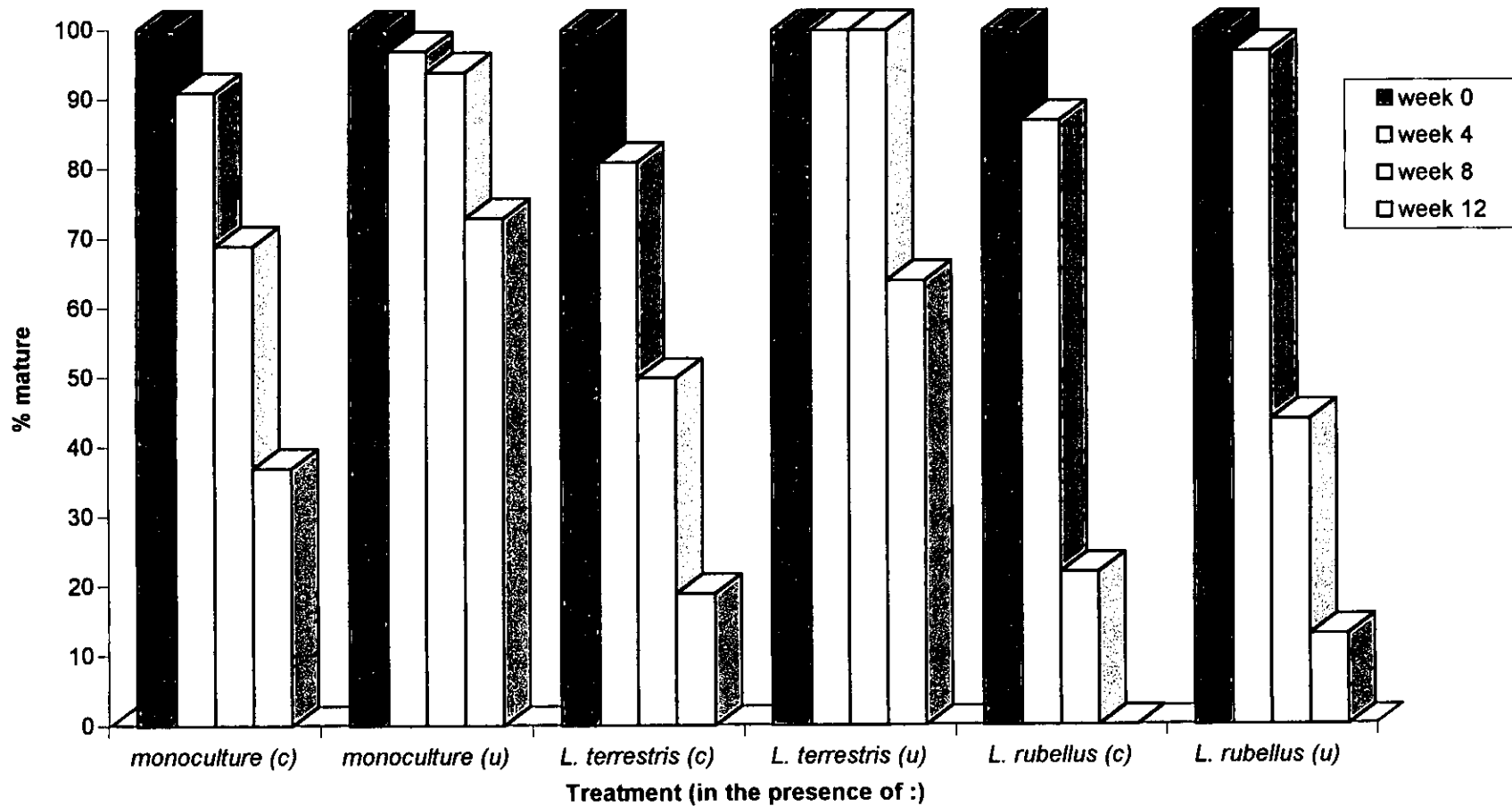
Figure 5.6.4 Cocoon Production by *L. terrestris* in Species Combination Treatments, in Either Compacted (c) or Uncompacted (u) Soils
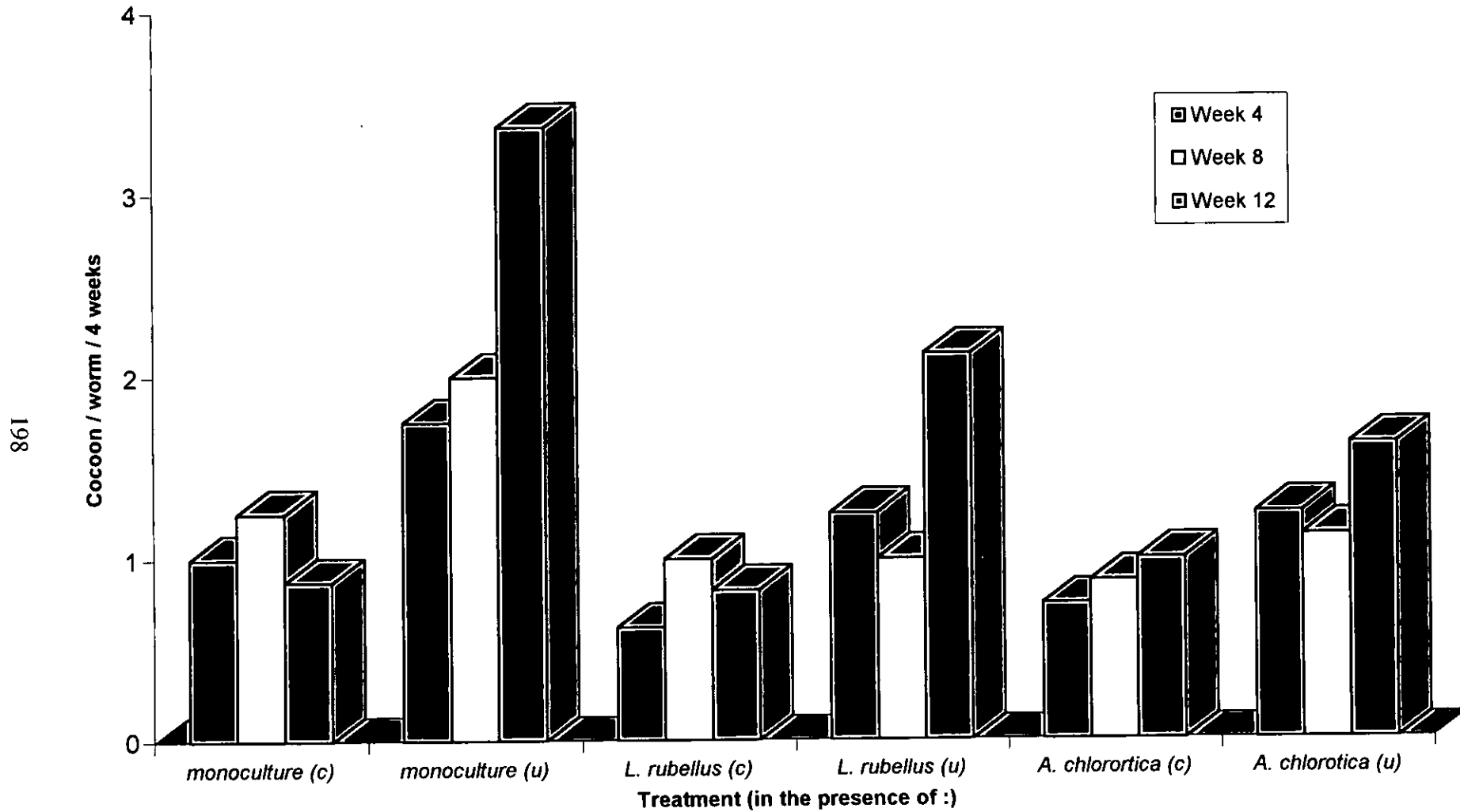
5.6.6 Discussion

5.6.6.1 A. chlorotica

Results (although often not significant) suggested that *A. chlorotica* cocoon production was influenced by the bulk density of the soil and interactions with other species. Increasing the bulk density of soil was shown to decrease *A. chlorotica* cocoon production. *A. chlorotica* is an endogiec species and lives exclusively within the soil. This species is geophagous gaining most of its nutrition by ingesting organic matter along with the soil and in so doing forming non-permanent burrows of horizontal orientation. It was proposed that increasing soil bulk density increased the energy required by this species for burrow formation and in so doing diverted energy from cocoon production. This was also reflected in the loss of reproductive condition, which occurred more rapidly in compacted soil treatments. It must also be noted that SCS were only applied to the soil surface of experimental treatments and therefore restricted the amount of organic matter available to this endogeic species which may have further decreased reproductive output and condition.

In uncompacted soils the influence of the other two species on cocoon production was consistent with results for *A. chlorotica* from previous experiments. Cocoon production by *A. chlorotica* in the presence of *L. terrestris* was very similar to that recorded in monoculture. In comparison the presence of *L. rubellus* negatively influenced *A. chlorotica* cocoon production. This result was attributed to differences in the spatial distribution and behaviour of the three species. As discussed in section 5.5.5, encounters between *L. terrestris* and *A. chlorotica* may have been minimised due to differences in feeding behaviour. However, *L. rubellus* feeds both at the surface and in the upper soil

layers, increasing the chance of individual encounters with both of the other two species. It has previously been proposed (see section 5.4.4.2) that encounters with other species may result in disturbance during mating or the deposition of cocoons which may negatively influence a species reproductive output. In compacted treatments (at week 4) the highest *A. chlorotica* cocoon production was found in the presence of *L. terrestris* with production in monoculture and in the presence of *L. rubellus* reduced to similar levels. Observations of burrow formation indicated that *L. terrestris* was burrowing into the compacted soil whilst *L. rubellus* remained at it's surface, within the layer of SCS. It was suggested that in comparison with the monoculture and the pairing with *L. rubellus*, burrowing by *L. terrestris* may have locally reduced soil bulk density allowing *A. chlorotica* to move more easily within the soil and access the surface applied SCS. This proposed mutualistic interaction between anecic and endogeic species has also been suggested by Butt *et al.* (1999) in compacted soils at a recently restored landfill site.

5.6.6.2 *L. rubellus*

In the initial sampling (week 4) *L. rubellus* cocoon production was significantly (p<0.05) higher in uncompacted soil in both the monoculture and in the presence of *L. terrestris* compared with corresponding compacted treatments. However, after this sampling soil compaction did not significantly effect cocoon production in any of the three species combinations. In compacted treatments *L. rubellus* were found exclusively in the SCS layer and so would have been largely unaffected by soil compaction, providing adequate organic matter was present to prevent desiccation. However, the restricted movement of *L. rubellus* may have attributed to the recorded cocoon production in the presence of *L. terrestris* in the compacted soil treatment. At every sampling lowest *L. rubellus* cocoon production was recorded in this treatment. It was suggested that this

resulted from increased encounter rates with *L. terrestris* which was also largely restricted to the SCS layer.

5.6.6.3 *L. terrestris*

Increased soil compaction significantly ($p<0.05$) reduced cocoon production in *L. terrestris* monocultures. Increasing soil bulk density changed the behaviour of this species. In compacted soil treatments individuals were often found in the organic matter on the soil surface and any signs of burrowing were restricted to the edges of the culture vessels (in spite of attempts in experimental design to prevent earthworms utilising the gap between soil and vessel). In the field this type of behaviour would have increased the risk of predation (from birds and mammals) and desiccation whereas, in the experimental cultures these factors did not influence earthworm survival. It was suggested that the restriction of *L. terrestris* to the organic layer negatively influenced cocoon production as cocoons are usually deposited within the soil. It was also suggested that the negative influence of compaction on *L. terrestris* cocoon production was further enhanced in the presence of *L. rubellus* due to increased encounter rates.

CHAPTER 6. DISCUSSION

6.0 INTRODUCTION

This is the first detailed, laboratory-based study of earthworm interactions. Experiments were designed to assess earthworm interactions at different life stages and under different environmental conditions in order to determine the potential of different species combinations for use in soil restoration. Initial sections in this chapter propose mechanisms for both negative and beneficial interactions recorded between species employed in this research. The effects from manipulation of environmental factors on earthworm behaviour and production are also discussed and comparisons are drawn with relevant published literature. Later sections detail the implications of this research for laboratory culture of earthworms and soil restoration, before making proposals on the future direction of such research.

6.1 EARTHWORM INTERACTIONS

This research has demonstrated that, under given laboratory conditions (specified in chapter 3), experimental species were capable of co-existence in paired species culture over a variety of time periods (3 to 9 months). Such results may have been expected as species were originally recorded and collected from the same location (Valentine House Farm) and in addition, field studies by other researchers (reviewed by Edwards & Bohlen, 1996) have indicated that *A. caliginosa*, *A. longa* and *L. terrestris* are characteristic species of English pastures and are commonly associated with *A. chlorotica* and *L. rubellus* in temperate regions.

However, results have also suggested that, both inter- and intra-specific interactions were detectable between these 5 species in terms of growth, maturation and reproduction under laboratory conditions. Furthermore, results indicated that the nature and intensity of interaction was both species specific and dependant upon the life stage of the earthworms involved.

Recorded interactions are sub-divided in the following two sections into negative (competitive) and positive (commensal) and mechanisms for these interactions are suggested with reference to niche theory. Comparisons are also drawn between the current work and previous earthworm-related studies (see section 2.5) which were, in part or entirely, concerned with this research area.

6.1.1 Negative Interactions

6.1.1.1 Inter-specific Interactions

Results obtained from section 4.2 indicated that the growth of hatchling *A. caliginosa*, *A. chlorotica*, *A. longa* and *L. terrestris* was negatively influenced by the presence of other species in experimental cultures, in comparison with monocultures. It is suggested that these differences may result from inter-specific exploitation competition (in which there is no direct interaction) (Begon *et al.*, 1996), each individual being affected by the amount of resource (in this case space and food (SCS)) that remains after exploitation by others. The intensity of recorded negative interactions was specific to species pairings and, it is suggested, related to the ecological grouping (section 2.1) to which individual species belong. The influence of resource competition was most intense in treatments which paired together either two anecic (*A. longa* and *L. terrestris*) or two endogeic

(*A. chlorotica* and *A. caliginosa*) species. Earthworms in these two pairings exhibit similar resource requirements. There is considerable niche overlap in terms of location within the soil profile and feeding behaviour. In contrast, in pairings between earthworms from different ecological groupings, individual species occupied different positions in the soil profile and differed in their feeding behaviour, weakening the intensity of resource competition.

Negative interactions between species from the same ecological grouping were also recorded in the latter stages of an experiment involving hatchling and adult earthworms (section 4.4). However, initial results contradicted earlier findings (section 4.3) and demonstrated that the life stage of individuals can influence the outcome of interactions. Early results indicated that the growth of *L. terrestris* was inhibited more by the presence of adult *A. chlorotica* than by the presence of *A. longa*. Observations relating to the position of juvenile *L. terrestris* in the soil profile during this experiment (and also in section 5.2) suggested that when small in size (less than 1 g), *L. terrestris* behaved in an endogeic fashion. Therefore the resource requirements of hatchling *L. terrestris* may be more similar to adult *A. chlorotica* than adult *A. longa* at this stage of development.

The experiment described in section 4.4 also used the epigeic species *L. rubellus*. Growth of this species was not significantly affected by either anecic or endogeic species, however, the presence of *L. rubellus* reduced the growth rates of *A. chlorotica*, *A. longa* and *L. terrestris*. The interactions between *L. rubellus* and the latter three species can be described as asymmetrical; where one species is markedly influenced by the presence of the other and the latter is little affected (Begon *et al.*, 1996). In contrast the competitive interaction observed between the two anecic species (section 4.3) can be described as

symmetrical in its outcome, as growth of both species was negatively affected. The asymmetrical interaction between *L. rubellus* and the other four experimental species is documented throughout the research and has also been recorded by Butt (1998), for *L. rubellus* and *D. veneta* with endogeic and anecic species. As previously described (section 4.4.4.4) *L. rubellus* has high dispersal and reproductive rates (r-selected) and unlike the majority of epigeic species, forages for food in the soil profile and at its surface. Therefore in experimental cultures this species may severely reduce the amount of food available to endogeic and anecic species and, in comparison with monocultures, limits the amount of energy available for tissue production and reproduction.

This is an over simplification of the processes involved, one may not assume that all species from one ecological grouping have the same negative effect on other species. For example, the presence of adult *L. terrestris* negatively influenced *A. chlorotica* growth, maturation and cocoon production. However in the presence of *A. longa*, growth and maturation rates of *A. chlorotica* were comparable with that in monoculture and cocoon production was greater than that achieved in monoculture.

Several authors (*e.g.* Abbott, 1980; Butt, 1998 and Dalby *et al.*, 1998a) have recorded competitive interactions between earthworm species in both field and laboratory experiments. Abbott (1980) assessed interactions between *M. dubius* and *E. fetida* and between *E. fetida* and *A. trapezoides*, by measuring changes in earthworm biomass in single and paired species treatments in the laboratory. Abbott observed persistent interspecific interactions between *E. fetida* and *M. dubius* but not between *E. fetida* and *A. trapezoides*. He suggested two possible mechanisms to explain these observed differences :-

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1. Coelomic fluid exuded by *E. fetida* may be toxic to *M. dubius* but not to

A. trapezoides.

2. *E. fetida* and *A. trapezoides* may possess certain digestive enzymes lacking in

M. dubius allowing the former species to extract nutrients from the soil more efficiently

or extract a greater range of nutrients than *M. dubius*.

Dalby *et al.* (1998a) investigated interactions between *A. longa, A. caliginosa* and

A. trapezoides in a field experiment and also investigated the influence of *A. longa* on the

survival, growth and cocoon production of *M. dubius* in a pot experiment. These workers

established that competition between *A. longa* and *A. caliginosa* was weak and no

competitive effects were measured between *A. longa* and *A. trapezoides*. However, the

presence of *A. longa* significantly reduced the reproductive output of *M. dubius* and

three mechanisms were put forward to account for these observed interactions;-

1. Scramble competition for resources, deemed to be food (litter and dung).

2. Interference competition

3. Consumption of cocoons

In this research, albeit with different species, there is no evidence of the types of direct

interference competition that have been proposed by Abbott, (1980) and Dalby *et al.*

(1998a) and several other authors (*e.g.* Rouelle *et al.,* 1987 (section 2.5)). However, it is

proposed that the frequency with which earthworms encounter one another may have

influenced the intensity of observed negative interactions. For example both *L. rubellus*

and *L. terrestris* feed on organic matter at the soil surface which may increase the

frequency with which these two species encounter one another (in comparison, for

example, with the pairing of *L. terrestris* and *A. chlorotica*). It is suggested that on

encountering another species at the soil surface *L. terrestris* may retreat inside its' burrow (an avoidance response to both avian and mammalian predators) therefore reducing the foraging time of this species at the soil surface and in turn inhibiting growth and maturation rates. It is also suggested that the frequency with which earthworms encounter one another may influence reproduction rates as a direct result of disturbance during mating, something which has been observed in the mating of *L. terrestris* by Nuutinen & Butt (1997).

6.1.1.2 Intra-specific Interactions

Competitive intra-specific interactions were recorded between adult pairings, juvenile pairings (of all experimental species) and also in adult and juvenile pairings of *A. chlorotica*, *A. longa* and *L. rubellus*. In general results suggested that these competitive intra-specific interactions were more intense than inter-specific interactions, reducing species growth, maturation and cocoon production (in given experiments) to their lowest recorded levels. This general observation may have been expected as conspecifics share identical resource requirements (their niches are identical) and therefore, in single species cultures (double density monocultures) individuals would act symmetrically, competing for the same space in the soil profile and food in the same location. It has also been suggested that the negative influence of adults on juveniles (recorded for *A. chlorotica*, *A. longa* and *L. rubellus* in section 4.4) may serve to enhance the dispersal of species by forcing offspring away from adults.

It is important to recognise that intra-specific competition does not always produce the strongest negative exploitation interaction between animals, as conspecifics can differ in their stage of development (section 6.1.2.2) and physical condition which can influence

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interactions. Also individuals of another species may be more efficient in depleting resources than individuals of the same species. For example, a rabbit in a field may be more effectively deprived of food by a sheep than by another rabbit (Begon *et al.*, 1996). This type of association was also recorded in the current research, growth of *L. terrestris* being more negatively influenced when cultured with *L. rubellus* than when cultured with *L. terrestris* (section 4.4).

6.1.2 Positive Interactions

One of the aims of this research was to try and identify mutualistic interactions between experimental earthworm species. This type of beneficial interaction in which both organisms experience a net gain (+ +) was not observed during the course of this research. However, results suggested that commensal interactions [associations], where one organism experienced a net gain and the other was unaffected (+ 0) were occurring both within and between species under certain conditions.

6.1.2.1 Inter-specific Interactions

The presence of *A. longa* enhanced cocoon production by *A. chlorotica* in comparison with a monoculture of this species (section 4.4). This type of commensal association between anecic and endogeic species was also recorded from a food particle size experiment (section 5.5). Here the presence of adult *L. terrestris*, in an unground food treatment significantly (p<0.001) increased the growth of hatchling *A. chlorotica* in comparison with a monoculture of this species, although this positive interaction was not recorded in the corresponding ground food treatment. Several suggestions regarding the mechanism of this positive interaction have been put forward :-

1. Anecic species draw organic matter from the soil surface (at which they feed) into their burrows. This activity may increase the amount of organic matter available to the endogeic *A. chlorotica* which is known to feed exclusively within the soil profile. However it is likely that organic matter, drawn into the anecic burrows, would not be easily accessible to endogeic species due to the almost continuous anecic presence within the burrow.

2. It may be more likely that endogeic species are benefiting from feeding upon the casts of anecic species deposited within the soil profile. As described previously (section 4.3) casts of anecic species may contain a more concentrated and easily ingested food source (in terms of particle size) than SCS present in the soil profile. This is supported by results obtained from the particle size experiment (section 5.5). The positive interaction between *L. terrestris* and *A. chlorotica* was not recorded in the ground food treatment where the particle size of SCS present in the soil profile was reduced in comparison to unground treatments. Therefore *A. chlorotica*, present in ground food treatments were not disadvantaged by feeding on organic matter in the soil profile in comparison with organic matter present in the castings of anecic species.

This type of commensal interaction between anecic and endogeic species has also been recorded in initial sampling of an inoculation trial at a partially restored landfill site by Butt *et al.* (1999). *A. chlorotica* and *A. longa* were inoculated into the site as mixed or single species treatments. Sampling, five years after inoculation, indicated that the number of *A. chlorotica* present in mixed species plots was significantly higher ($p < 0.05$) than in plots where *A. chlorotica* had been introduced in isolation.

Butt *et al.* (1997) suggested that, under laboratory conditions, the presence of *A. chlorotica* and *A. longa* in mixed culture enhanced the reproductive performance of both species in comparison with monocultures in a given volume of soil, thus providing evidence of a mutualistic interaction between two earthworm species. However, this statement was based upon a comparison of the total production for two mixed culture units with one monoculture unit of each species. Therefore, effectively comparing species cocoon production in monocultures with twice the density of the same species in mixed cultures. If monocultures and mixed cultures are compared one-to-one (as in the current research), so that species densities are equal, results indicate that the presence of both *A. longa* and *A. chlorotica* in mixed culture led to a significant decrease in cocoon production in both species when compared to monocultures.

6.1.2.2 Intra-specific Interaction

Hatchling *L. terrestris* were cultured with clitellate adults of all five experimental species (section 4.4). Initial results (to week 12) indicated that the presence of adult *L. terrestris* significantly ($p<0.05$) increased the growth rate of hatchling *L. terrestris* in comparison with a monoculture and all other treatments. It is proposed that juvenile *L. terrestris*, which were initially located within the upper soil layers, benefited from feeding on organic matter present in the middens of adult *L. terrestris*. This organic matter may have presented juveniles with a more concentrated and easily digested food source than the surrounding SCS (section 2.3.2). However, results for *A. longa* did not support the positive interaction recorded for *L. terrestris*. Growth of hatchling *A. longa* was significantly ($p<0.05$) reduced in the presence of adult *A. longa* in comparison with all other treatments.

6.1.3 The Effect of Manipulating Environmental Factors on Species Interactions

It is proposed that the influence of varying selected environmental factors (Chapter 5) produced species specific effects on earthworms, which were dependant on their innate behavioural and physiological characteristics and as a result altered inter-specific interactions accordingly. For example, in a food placement experiment (section 5.4) there was no significant difference in *L. terrestris* growth in all treatments where SCS was exclusively applied at the soil surface. However, in treatments where food was exclusively incorporated into the soil profile *L. terrestris* growth was significantly ($p<0.05$) reduced in the presence of *L. rubellus* compared with a monoculture of this species. It is suggested that in treatments where food is incorporated into the soil profile *L. terrestris* is forced to change its normal feeding behaviour, and forage within the soil profile where it is then less competitive than the more flexible (in terms of foraging behaviour) epigeic *L. rubellus*.

6.2 EFFECT OF SELECTED ENVIRONMENTAL VARIABLES ON EARTHWORM BEHAVIOUR AND PRODUCTION

6.2.1 Food Position

This research demonstrated that the position of food within the soil profile (section 5.2 and 5.3) had a marked effect on the behaviour and production of the two anecic species. Both attained a significantly greater mass in treatments where food was surface applied compared with treatments where food was incorporated into the soil profile, in keeping with their anecic classification and corroborating results for *L. terrestris* recorded by Boyle (1990). In treatments where food was incorporated throughout the soil profile

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juveniles of both species (less than 1 g) were initially located solely in the upper soil layers forming burrows of horizontal orientation (in endogeic fashion). However, if the food was concentrated into bands at various depths in the soil profile (section 5.3) juvenile *L. terrestris* (of less than 1 g) were located in and around this concentrated food source. This suggested that juveniles of this species were capable of burrowing throughout the experimental soil profile (0.14 m in depth) in search of food. Results also indicated that banding food in the soil profile increased *L. terrestris* growth rates in comparison with treatments in which food was incorporated throughout the soil. These results supported research conducted by Cook & Linden (1996), who positioned organic matter in various locations within Evans' box microcosms, filled with a moist loam soil and recorded the burrowing patterns of *A. tuberculata*. It was determined that burrowing was random until food resources were encountered, at which point burrowing then centred around the food resource.

In contrast with the two anecic species, *A. chlorotica* achieved greatest mass, and cocoon production in treatments where food was incorporated into the soil compared with surface applied food treatments. This was not unexpected as *A. chlorotica* lives solely within the upper soil profile. It is suggested that, like juveniles of the two anecic species, *A. chlorotica* may benefit from the banding of organic matter within the soil profile by reducing the energy expended in foraging for food.

Growth and cocoon production of *L. rubellus* was not significantly (p>0.05) influenced by either incorporating food within the soil profile or its surface-application. This result further emphasised this opportunist species' ability to adapt to environmental conditions

and hence its' reported role as a pioneer species in many disturbed habitats (*e.g.* Judd & Mason, 1995).

6.2.2 Food Particle Size

Results from a food particle size experiment (section 5.5) indicated that growth of *A. chlorotica* and *L. terrestris* may be significantly enhanced by decreasing the particle size of supplied organic matter, supporting results obtained for *A. caliginosa* production by Boström & Löfs-Holmin (1986) and Boström (1988). It was also demonstrated that the extent to which decreasing food particle size influenced growth rates was species specific. Growth of the smaller endogeic species supplied with ground SCS was increased by 147 % over the experimental period in comparison with individuals of the same species supplied with unground SCS. However the growth of *L. terrestris* in corresponding treatments was only increased by 49 % (these results support the findings of Boyle, 1990). It is proposed that these differences are linked to individual mouth diameter, with larger earthworms able to ingest larger size particles more easily than smaller species.

6.2.3 Soil Bulk Density

Increasing experimental soil bulk density reduced cocoon production and restricted the distribution and movements of all three species employed experimentally (section 5.6). Observations made at each sampling indicated that in compacted soil treatments, *L. rubellus* was restricted to a surface-applied organic matter layer. *L. terrestris* was also frequently observed on the soil surface, and any burrows present were located beside the edge of the vessel. It is suggested that the location of these species at the soil surface is a direct response to increased soil bulk density and specified laboratory conditions. Field

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observations have shown that *L. terrestris* (unless taking advantage of wet conditions to migrate over the soil surface) is very rarely found on the soil surface unattached to its burrow, into which it is able to retract as an escape response during feeding or mating. Therefore, under field conditions, the chances of earthworm survival on the soil surface with no means of escape (*e.g.* a burrow) would be greatly reduced. However, in laboratory cultures earthworms were free from predators and were also maintained in continual darkness (except at sampling). Given a sufficient covering of organic matter to prevent desiccation both *L. rubellus* and *L. terrestris* may be conserving energy by not burrowing directly into experimentally compacted soil (research by Rushton (1986) and Wendt (1988) indicated that *L. terrestris* was capable of burrowing into soil with bulk densities greater than those employed here). Both of these species deposit their cocoons within the soil profile and their reluctance to burrow into compacted soil treatments may have resulted in the observed decreased cocoon production in comparison with earthworms in uncompacted soil treatments.

A. chlorotica, in contrast with the previously discussed species, was found within the soil profile in both compacted and uncompacted experimental soils. This species is geophagous in its feeding behaviour, living exclusively within the upper soil layers and gaining nutrition by ingesting organic matter in combination with mineral soil. In this experiment, food was applied solely at the soil surface and may not therefore have been accessible to this species, which could have accounted for loss of reproductive condition in all treatments over the course of the experiment. However, clitellum regression, and as a result cocoon production, was most reduced in the compacted soil treatment. It is proposed that in compacted soils this species would have expended more energy burrowing (ingesting) through the soil in search of organic matter than in uncompacted

soils where soil particles were less tightly packed. As organic matter (SCS) had not been incorporated into the soil profile, earthworms in both bulk density treatments were gaining very little nutrition from the soil alone. However, the increased energy required to forage in the compacted soils may have led to the more rapid loss of reproductive condition experienced by *A. chlorotica* in this treatment.

6.3 CONCLUSIONS

From the experimental work described a limited number of conclusions have been drawn:-

1. All experimental species were successfully cultured under specified laboratory conditions, providing base line data (and adding to published work) on individual species behaviour, growth and reproduction rates.

2. Both positive and negative interactions were recorded between experimental species under laboratory conditions. The effect of interactions on earthworm growth, maturation and reproduction was often subtle in nature and may be related to field conditions.

i. Inter-specific interactions : The intensity of negative inter-specific interactions was dependant upon the degree of niche overlap experienced between species in terms of feeding behaviour and space (both of which were limited within culture vessels). This type of exploitation competition was most intense between species from the same ecological grouping (*e.g.* anecic and anecic) and also between ecological groupings where, due to experimental conditions (*e.g.* anecic and epige) and the stage of development (*e.g.* juvenile *L. terrestris* and mature *A. chlorotica*), earthworms competed

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for the same limited resource. Positive (commensal) inter-specific interactions were also recorded between anecic and endogeic species. Casting and feeding behaviour of anecic species may increase the organic matter available to endoges resulting in increased growth and reproduction rates of the latter.

ii. Intra-specific interactions : Competitive (exploitation) intra-specific interactions were, in general, more intense than inter-specific interactions which was attributed to individuals sharing identical resource requirements (niches). However, it was also shown that the outcome of intra-specific interactions between two individuals was influenced by their stage of development and the species studied. The presence of adult *L. terrestris* had a positive (commensal) effect on hatchling growth, arising, it was suggested, from hatchlings feeding on a more concentrated and easily digested organic matter in the middens of adults. However, growth of juvenile *A. chlorotica*, *A. longa* and *L. rubellus* was reduced significantly in the presence of conspecific adults and it is suggested that this negative intra-specific interaction may serve to improve species dispersal in stable habitats.

3. Manipulation of environmental factors (*e.g.* food placement / particle size and soil bulk density) influenced earthworm species growth, maturation and reproduction. The extent to which earthworms are able to adapt to alterations in the level of an environmental variable is species specific, dependant on inherent behavioural and physiological characteristics. These enforced adaptations alter a species' "realised" niche and inter-specific interactions are influenced accordingly.

6.4 IMPLICATIONS FOR SOIL RESTORATION

The effects of inter- and intra-specific interactions on earthworm production may be very subtle (Chapter 4). However it is suggested that when environmental conditions become limiting (Chapter 5), as at many soil restoration sites, species interactions may play an important role in determining earthworm community structure and size. Therefore, if as envisaged, mixed species inocula are to enhance the role of earthworms in soil restoration, it is essential that negative interactions between selected species are minimised. This research has indicated that this type of mixed species inocula may be realised by employing earthworms from the endogeic and anecic ecological groupings. Competition between experimental species from these two groups was minimal and both play an important role in soil formation processes (section 2.3). In contrast, epigeic species strongly inhibited growth of species from the other two groups and are also believed to play little part in the amelioration of soil. These proposals support the assertions of Elvira *et al.* (1996) that mixed species cultures would be of more use in agrosystems management, soil restoration and soil amelioration when the selected species belong to different ecological categories and thus competition for the same food resources could be avoided.

Lee (1995) proposed several criteria, that he believed need to be met, in order to increase the success of earthworm introduction programs in soil amelioration. He recognised the need for a minimum target earthworm community, and suggested that this might comprise one or more anecic species (that make vertical burrows opening onto the soil surface at which they feed and deposit casts and in so doing remove or bury plant litter) and one or more endogeic species (feeding on dead roots and subsurface organic matter

and make horizontally orientated burrows). Lee (1995) also recognised that information was needed concerning the behaviour of candidate species and also on culture methods capable of yielding sufficient numbers for inoculation programmes. Results presented in this thesis support these proposals and may form the basis for future research in this area (section 6.6).

6.5 IMPLICATIONS FOR THE LABORATORY CULTURE OF
EARTHWORMS

This research has shown, through the development of earthworm maintenance and culturing techniques, that it is possible to successfully culture a range of experimental species under laboratory conditions for experimental use. In manipulating earthworm development it has also proved possible to produce cohorts of individual species of comparable age and condition allowing reliable comparisons to be drawn between experimental treatments, relating to earthworm growth, maturation and reproduction rates. These culturing techniques may allow for detailed studies to be made concerning the ecology of a range of earthworm species and also provide extensive life cycle data. Furthermore, the ability to produce large numbers of earthworms of the same age and physical condition may prove useful in toxicological studies where earthworms are employed as bio-indicators in addition to intensive culturing of earthworms for use in soil restoration.

In the past earthworms used in both laboratory and field-based research were often of unknown origin, age and reproductive state, leading to uncertainty when quantifying outcomes. This was highlighted at the outset of this work, where use of field-collected and commercially supplied earthworms (section 4.2) concealed differences in species

growth and cocoon production, revealed in later experiments using cultured (laboratory-produced) animals.

6.6 FUTURE RESEARCH

This area of earthworm research is really still in its infancy and there is scope for a wealth of future research. Such areas are outlined below.

1. Field-Based Research : Extensive field trials need to be conducted employing a range of species and at a number of different locations (including soil restoration sites). [As part of this work a preliminary field trial was established to test observed laboratory interactions between *L. terrestris* and *A. chlorotica* in the field. However, due to the high levels of toxic elements present within the soil (resulting from prolonged spreading of sewage sludge) all the inoculated earthworms died preventing comparisons with laboratory results (appendix 7)]. The success of field trials could be enhanced by inoculating earthworms into soils from which the resident earthworm fauna had been totally removed or by the "tagging" of introduced species into sites where earthworms are already present.

Comparing the influence of suggested beneficial mixed species combinations with respect to their influence on soil conditions and plant production may be achieved by establishment of long-term field experiments at sites of soil restoration.

2. Laboratory-Based Research : There is scope for a wide range of research involving the manipulation of:- species combinations; numbers and density; soil type; food type and other environmental variables such as temperature.

This may lead to the development of laboratory-based mesocosms, which more accurately replicate field conditions, allowing for a range of interactions to be assessed between earthworm species and other flora and fauna. This would develop upon previous research, such as that conducted by Thompson *et al.* (1993) in the "Ecotron" at the NERC Centre for Population Biology at Silwood Park.

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APPENDIX 1. EXPERIMENTAL TIMETABLE

Figure A illustrates the duration and the time at which experiments were conducted during the period of the Ph.D. The order in which experiments are shown relates to their appearance in the thesis.

Figure A

□ Expt. in progress

Effect of soil type on L. terrestris growth

Effect of enforced quiescence on L. terrestris growth

Interactions between adults

Interactions between juveniles

Effect of adults on hatchling growth

Effect of food placement on growth of 2 anecic species

Effect of food placement on L. terrestris growth

Effect of food placement on species interactions

Effect of food particle size on species interactions

Effect of soil compaction on species interactions

Field study

0   5   10   15   20   25   30   35   40

Months (Oct. 95 - Sept. 98)

a

APPENDIX 2. CARBON AND NITROGEN ANALYSIS

INTRODUCTION

C : N ratios for both sandy clay loam soil obtained from D. F. & M Hargreaves of Blackpool and separated cattle solids obtained from Myerscough College Farm, Lancs. were established using Kjeldahl and Walkley and Black analyses modified from procedures detailed by van Reeuwijk (1993).

MODIFIED WALKLEY & BLACK METHOD FOR DETERMINING THE ORGANIC CARBON CONTENT OF SOILS

Principle

The oxidation of samples is carried out in potassium dichromate solution at the temperature of the heat of dilution of sulphuric acid. The reduced chromic acid produced is read colormetrically at 620 nm.

Reagents

Potassium dichromate 0.17 M : 49 g potassium dichromate in 1 l distilled water.

Barium chloride 0.4 % : 20 g barium chloride in 5 l distilled water.

Conc. sulphuric acid SG 1.84.

Stock Standards :

1. Dry sucrose at 105 °C for 2 hours, don't let temperature rise above this.

2. Accurately weigh 29.68 g of dry cooled sucrose and dissolve in distilled water.

3. Transfer into 250 ml volumetric flask and make up to the mark with distilled water. This solution contains 50 mg C / ml.

b

Working Standards:

Pipette 5, 10, 15, 20 and 25 ml o the standard sucrose solution into 100 ml volumetric flasks, make up to the mark with distilled water, mix well. Two ml of each of these standards will contain 5, 10, 15, 20 and 25 mg of Carbon (store frozen).

Procedure

1. Place 2 ml of each standard solution into a 250 ml conical flask, dry off in an oven at 105 °C, allow to cool.

2. Do a blank, to be used as reference zero.

3. Weigh out accurately sufficient of the sample (<0.5 mm) to contain about 10 -15 mg C into a 250 ml conical flask (try 0.5 g).

To both standards and samples

4. Add 10 ml of potassium dichromate reagent

5. Completely wet sample by swirling, take care not to deposit fine particles of soil up walls of flask.

6. In fume cupboard add 250 ml of conc. sulphuric acid with a zipette, swirl to mix. Leave to cool.

7. Remove from fume cupboard and add 100 ml of barium chloride solution by zipette.

8. Mix well by swirling and leave over night to settle.

9. Measure the absorbance in a 4 cm optical cell at 620 nm of the clear supernatant solutions (draw off supernatant using pipette).

10. Conduct a linear regression (mg carbon vs. absorbance) to calculate the amount of readily oxidisable carbon in samples and hence % content.

$$\% \text{ red. ox. } C = \frac{\text{red. ox. C in sample}}{\text{sample weight (g)}} \cdot \frac{1}{10}$$

c

Results

i) Sandy Clay Loam Soil (D.F. & M Hargreaves)

Table A

Standard (mg C)	Absorbance at 620 nm
5	0.064
10	0.139
15	0.201
20	0.299
25	0.325

Table B

Sample	Mass (g)	Absorbance	Adjusted Absorbance (absorbance - blank)
1	0.51	0.12	0.117
2	0.509	0.121	0.118
3	0.51	0.12	0.117
4	0.507	0.12	0.117
5	0.502	0.119	0.116
6	0.511	0.122	0.119
7	0.509	0.13	0.127
8	0.498	0.117	0.114
9	0.501	0.122	0.119
Average	0.5063	0.121	0.118
Blank		0.003	

Regression equation: $C1 = 0.209 + 73.5\ C2$

$C1 = 0.209 + 73.5\ (0.118)$

8.82 mg C

% red. ox. C = 1.74

ii) Separated Cattle Solids (SCS)

Table C

Standard (mg C)	Absorbance at 620 nm
5	0.065
10	0.133
15	0.193
20	0.254
25	0.33

Table D

Sample	Mass (g)	Absorbance	Adjusted Absorbance (absorbance - blank)
1	0.102	0.043	0.041
2	0.098	0.045	0.043
3	0.098	0.042	0.040
4	0.1	0.044	0.042
5	0.099	0.041	0.039
6	0.103	0.044	0.042
7	0.099	0.043	0.041
8	0.0.098	error	error
9	0.102	0.041	0.039
Average	0.0999	0.0429	0.0409
Blank		0.002	

Note: samples were diluted by a factor of 10.

Regression equation: $C1 = 0.023 + 76.8\ C2$

$C1 = 0.023 + 76.8\ (0.040875)$

3.3158 mg C

X 10 dilution   33.158 mg C

% red. ox. C = 33.1948

e

MODIFIED KJELDAHL METHOD FOR THE ESTIMATION OF TOTAL ORGANIC NITROGEN IN SOIL AND SCS

Principle

The sample is digested by boiling with conc. sulphuric acid in the presence of sodium sulphate (which raises the boiling point) and copper and/or selenium catalyst (these are both components of Kjeldahl catalyst tablets). Digestion converts all of the organic nitrogen to ammonia which is trapped in the solution as ammonium sulphate.

Completion of the digestion stage is usually recognised by the formation of a clear solution. Trapped ammonia is then released by the addition of excess sodium hydroxide and removed by steam distillation in a Kjeltech still. It is collected in boric acid indicator (a mixture of boric acid and methyl red / bromocresol green indicator) and is titrated with hydrochloric acid.

Reagents

1 Conc. sulphuric acid (ammonia free grade)

2) Kjeltec catalyst tablets

3) HCl M/140 (1 ml = 0.1 mg NH_4+-N)

4) Boric acid indicator solution.

5) Nitrogen standard (1ml = 0.1 mg NH_4-N)

f

Method

The method uses the Kjeltec- system apparatus, the accuracy of which can be tested using nitrogen standards and adjusted accordingly. No prior extraction is required.

1) Grind the sample, oven dry for 24 hours at 40 °C.

2) Weigh the sample (approx. 0.25g for organic rich soils and 0.50 to 1.0g for mineral soils with low organic content) into the digestion tubes.

3) In a fume cupboard, into each tube add 15 ml of H_2SO_4 and 2 catalyst tablets.

4) Place the stand with the samples into the preheated digestion unit (420 °C) and place the exhaust system on top of the tubes. Heat the samples until the solutions become clear (approx. 1 hour).

5) Remove the stand with the exhaust system and allow to cool.

6) Dilute each sample with 50 ml of distilled water.

7) Connect a sample digestion tube into the 1030 analyser.

8) Close the safety door. Distillation, titration and calculation are performed fully automatically in approx. 2 minutes.

9) The result is presented on a digital display or printed (in % Protein, %N or ml titrant)

g

Calculation

Using 0.1M HCl = T vol (mls)   Using M/140 HCl = T vol (mls)

1 mole HCl = 1 mole NH_{4+} (unknown   greater titrant vol = better accuracy

alkali)

1 litre (1000 mls) M HCl contains 1 mole

10,000 mls 0.1 M HCl contains 1 mole

T/10000 = moles NH_{4+} T/140000 = moles NH_{4+}

Since 1 mole contains 14g of N

T/10,000 x 14 = g of N   T/140000 x 14 = g of N

$$\%N = (g \text{ of } N / wt \text{ of sample}) \times 100$$

% N = T/g x 0.14 x (extract vol/aliquot vol) = T/g x $1/10^2$ x (extract vol/ aliquot vol)

Extract vol = vol of sample = 15 ml H_2SO_4 + 50 ml H_20 = 65 ml

Aliquot vol = vol of sample placed in the machine = 65 ml

Extract and aliquot values cancel each other out.

Results

Table E Soil Analysis

Replicate No.	Mass of Soil (g)	mls HCl	Adjusted mls HCl (- blank)
1	0.513	0.813	0.774
2	0.517	0.860	0.821
3	0.519	0.797	0.758
4	0.512	0.977	0.938
Blank		0.039	
Average	0.5153	0.86175	0.8228

Soil Calculation

$0.8228 / 10{,}000 = 8.228^{-05}$

$8.228^{-05} \times 14 = 1.15192^{-03}$ g of N

% N $= 1.15192^{-03} / 0.5153 \times 100$

$= 0.2235$ %

Table F Feed Analysis

Replicate No.	Feed 1 Mass (g)	mls HCl	Adjusted mls HCl (- blank)	Feed 2 Mass (g)	mls HCl	Adjusted mls HCl (- blank)
	0.049	0.836	0.797	0.103	1.655	1.616
	0.051	0.852	0.813	0.110	1.587	1.548
	0.053	0.821	0.782	0.103	1.806	1.767
Blank		0.039			0.039	
Average	0.051	0.8363	0.7973	0.1053	1.683	1.647

Feed Calculation

1) % N $= 1.11622^{-03} / 0.051 \times 100$

$= 2.189$ %

2) % N $= 2.3058^{-03} / 0.1053 \times 100$

$= 2.190$ %

Table G C : N ratios

Sandy Clay Loam Soil	8 :1
Separated Cattle Solids (SCS(	15 : 1

APPENDIX 3. FORAGE FIBRE ANALYSIS OF SEPARATED CATTLE

SOLIDS (SCS)

INTRODUCTION

The van Soest method was followed for fibre analysis as described in Goering & van

Soest (1970), but slight modifications were made (Knight 1987).

ANALYSIS

All samples referred to were oven dried and ground to pass a 1 mm mesh.

Neutral Detergent Fibre (NDF)

i. 100 ml of 30 % (30 g / l) sodium lauryl (dodecyl) sulphate solution. Buffered to a pH

of 7.0 (found not to be necessary). Add two - three drops of silicone antifoaming agent.

ii. Boil 0.5 g of sample SCS for one hour in a 250 ml round-bottomed flask fitted with a

reflux condenser (setting 6 after coming to the boil).

iii. Filter through a tared, sintered glass crucible using a vacuum pump. Rinsed twice

with hot (90 - 100 °C) water, then twice with acetone, breaking up the fibrous mat with a

glass rod. Oven-dry for eight hours or overnight.

iv. Weigh crucible after cooling in a dessicator.

v. Ash residue in the crucible for three hours at 500 °C in furnace. Cool in dessicator and

reweigh.

vi. Report findings of recovered NDF as % of sample, = cell wall constituents

(hemicellulose, cellulose and lignin).

vii. Estimate cell soluble material by subtracting this value from 100. Ash content = ash insoluble in neutral detergent.

Results for Neutral Detergent fibre (NDF)

0.5 gm of SCS sample added to each crucible

Table H

Replicate No.	Sample + Crucible Mass (g)	Oven Dry Mass (g)	Ash (500 °C) Mass (g)
1	35.734	35.598	35.251
2	33.619	33.507	33.148
3	38.744	38.621	38.262

Table I

Replicate No.	Recovered NDF %	Cell Soluble Material %	Ash Insoluble in Neutral Detergent %
1	72.8	27.2	3.4
2	77.6	22.4	5.8
3	75.4	24.6	3.6
Average	75.27	24.73	4.27

Acid Detergent Fibre (ADF)

i. 100 ml of sulphuric acid-cetyltrimethylammonium bromide (CTAB) solution and two-three drops of octan-2-ol in 250 ml flask. CTAB solution made by dissolving 28 ml of conc. sulphuric acid in 1 litre of water (= 0.5 M), then dissolve 10 g of CTAB in this. No filtering necessary.

ii. Reflux, as for NDF, for 1 hour. octan-2-ol prevents excessive foaming.

iii. Filter through a tared, sinterred glass crucible, as for NDF, using a vacuum pump. Rinse twice with hot (90 - 100 °C) water, then twice with acetone, breaking up the fibrous mat with a glass rod. Oven-dry for eight hours or overnight.

iv. Weigh when cool, then calculate ADF.

v. Retain the contents of the crucible for further analysis.

vi. Hemicellulose = NDF - ADF

Results from ADF

Table J

Replicate No.	Sample + Crucible Mass (g)	Oven Dry Mass (g)	Acid Detergent Fibre %
4	34.89	34.63	48
5	35.51	35.25	47.2
6	38.86	38.59	54.0
Average	36.42	36.16	49.7

Hemicellulose = 25.537%

Cellulose

i. Determined by treating the residue from ADF with 72 % sulphuric acid. Cover the contents of the crucible with cooled (15 °C) H_2SO_4 and stir with a glass rod to a smooth paste, breaking all the lumps. Fill crucible about half full and stir. Refill with acid at hourly intervals as acid drains away (a 50 ml beaker is a useful drainage vessel). Crucibles do not need to be kept full at all times. Three additions suffice.

ii. After three hours, filter off as much acid as possible with a vacuum pump, then wash contents with hot water until free from acid. Take care to rinse stirring rod.

iii. oven-dry crucible and weigh. Loss is an estimate of cellulose content.

iv. Retain residue.

Results for Cellulose

Table K

Replicate No.	Oven Dry after H_2SO_4 Mass (g)	Cellulose %
4	34.52	23.0
5	35.12	25.4
6	38.49	26.8
Average	36.04	25.07

Lignin & Ash (silica)

i. Ignite crucibles, from cellulose (above) in a muffle furnace at 500 °C for three hours. Allow to cool, then reweigh

ii. Loss is an estimate of acid detergent lignin content. Residue is acid insoluble ash (mainly silica).

Table L

Replicate No.	Furnace (500 °C) Mass of crucible + sample (g)	Lignin Content %	Silica Content %
4	34.41	21.4	3.6
5	35.02	20.6	1.2
6	38.37	18.6	0.4
Average	35.93	20.2	1.73

APPENDIX 4. FIELD COLLECTION OF EARTHWORMS

INTRODUCTION

There have been various methods developed (reviewed by Edwards & Bohlen (1996)) for extracting earthworms from the soil. In this study two methods were employed in the field collection of earthworms. The first used a combination of digging and the application of a vermifuge and in the second electrical stimulus was used to bring earthworms to the soil surface.

DIGGING AND FORMALIN APPLICATION

Digging and handsorting soil for earthworms is the simplest method of earthworm collection. It is a very efficient means of obtaining both epigeic and endogeic species which are present in the upper soil layers, but unless dug to a depth of several metres adult anecic species may be missed. This problem may be overcome by combining digging with the application of a 0.4 % solution of formalin (formaldehyde), a standard vermifuge (Raw, 1959) used in research literature.

Methods

Where a quantitative assessment of earthworm populations was required soil was dug out to a depth of 0.15 m within a fixed quadrat area of either 0.1 m^2 or 0.25 m^2 (see plate A). This soil was handsorted in the field for the presence of earthworms. All animals located were preserved in 4 % formaldehyde for identification in the laboratory. Dilute 0.4 % formaldehyde was poured into the hole (using a watering can) created by soil removal

n

(see plate B). Any earthworms emerging from deeper burrows were added to those collected from handsorting.

When earthworms were required for use in experiments dilute 0.4 % formaldehyde was sprayed directly onto the soil surface (see plate C). Any earthworms that were brought to the surface were immediately washed in water to remove the formaldehyde.

Formalin is very toxic and a known carcinogen. In some regions were ground water quality is of concern, formalin cannot be used on the soil (Fox, 1997). These factors have led to the development of non-toxic vermifuges such as mustard suspension (Gunn, 1992).

ELECTRICAL STIMULUS : OCTET APPARATUS

Passing an electrical current through the soil stimulates earthworms and drives them to the surface. This method of earthworm collection is both non-destructive and non-toxic, allowing sampling at ecologically sensitive sites as well as in public areas, where damage to the land (*e.g.* golf courses) and the spreading of vermifuges (*e.g.* parks) was prohibited.

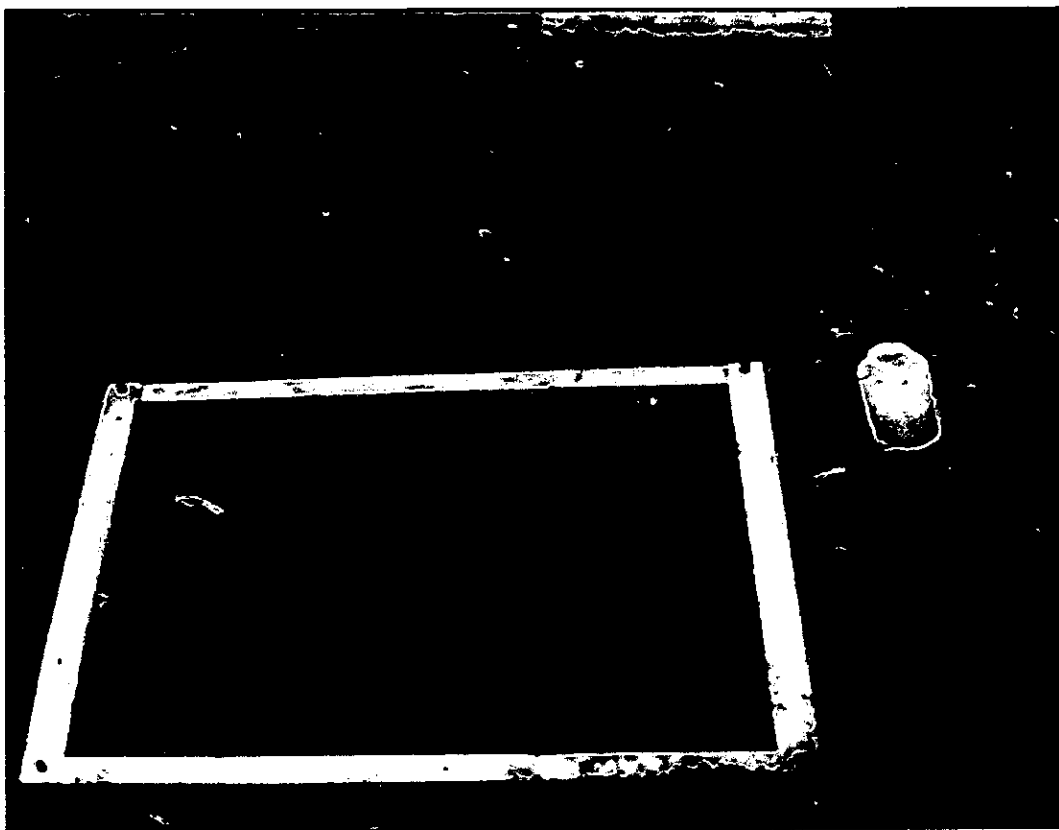**Plate A Digging for Earthworms (Within a Fixed Area)**

Plate B Addition of Formalin (0.4 %) in to Holes

p

Plate C Spraying Formalin (0.4 %) Directly on to the Soil Surface

Plate D Expelling Earthworms Using the Octet Apparatus

q

Method

The Octet method developed by Thielemann (1986), employs 8 steel electrodes pushed into the soil in a regular circular pattern within an area of 0.2 m^2 attached to a 12 volt battery (see plate D). A control unit allows manipulation of both the voltage and the switching frequency. Before turning on, any fallen leaves and overgrown vegetation were removed from within the demarcated area to enable any earthworms that were brought up to the surface to be easily located. In estimating earthworm populations it was essential that earthworms were only collected from inside the electrode ring and that the time of sampling (20 - 30 minutes) and range of voltages and switching frequencies were constant between replicates.

BUWAL (1996) and Butt & Kostecka (in press) compared the efficiency and suitability of different earthworm collection techniques including the two used in this study. Both groups indicated that the efficiency of different methods could not be directly compared and that technique selection should be determined by environmental restrictions at the site and available resources. Butt & Kostecka (in press) also recommended that whenever possible a range of extraction methods should be used.

APPENDIX 5. PRELIMINARY FOOD PARTICLE SIZE TESTS

INTRODUCTION

In a preliminary experiment 80, 40, 20 and 10 g of unground, rewetted SCS had water content determined gravimetrically by drying samples at 105 ° C for 24 hours. From these results the ratio of organic matter to water for required masses of rewetted ground SCS could be extrapolated for use in investigation of the effect of food particle size on earthworm interactions (section 5.5) .

Table M

Unground SCS sample	Mass of tray (g)	Mass of SCS + tray (g) wet	Mass of SCS + tray (g) dry	Mass of SCS (dry)
80 g	1.777	81.777	18.364	16.587
40 g	1.851	42.251	10.173	8.322
20 g	1.427	21.527	5.652	4.225
10 g	1.371	11.471	3.554	2.183

60 g of rewetted ground SCS was required for relevant treatments in experiment 5.5. It was calculated that this was equivalent to 12.45 g dry mass of SCS plus 47.55 ml of water (1 ml H_2O = 1 g).

Figure B

Graph Showing the Relationship Between Wet and Dry Mass of
Separated Cattle Solids (SCS)

t

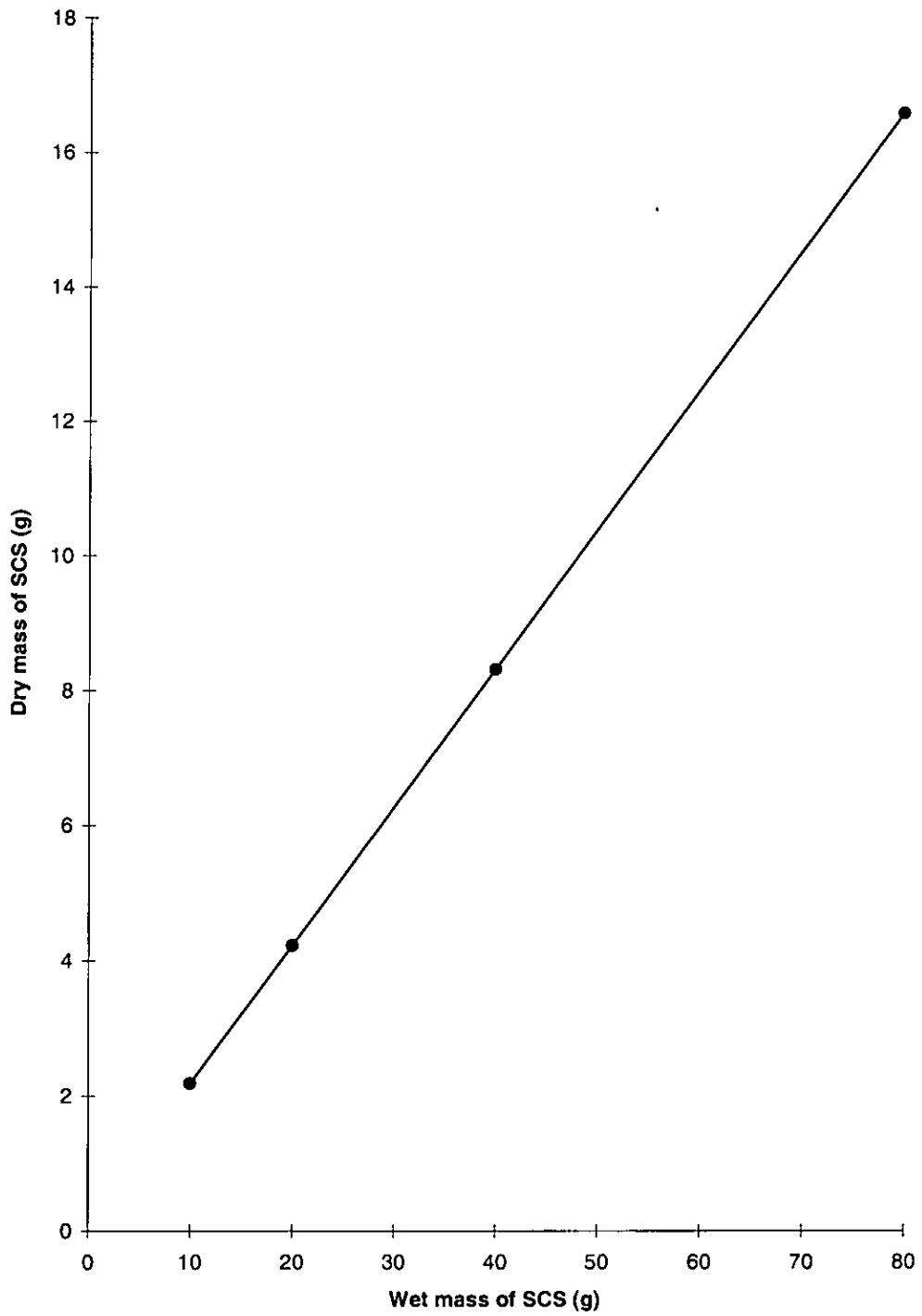**Table N Results Obtained From Milling Separated Cattle Solids**

Particle Size	Percentage of Sample (%)
> 2 mm	0.7
1 mm - 2 mm	10
710 μm - 1 mm	8
300 μm - 710 μm	41.5
150 μm - 300 μm	2.7
< 150 μm	14.2

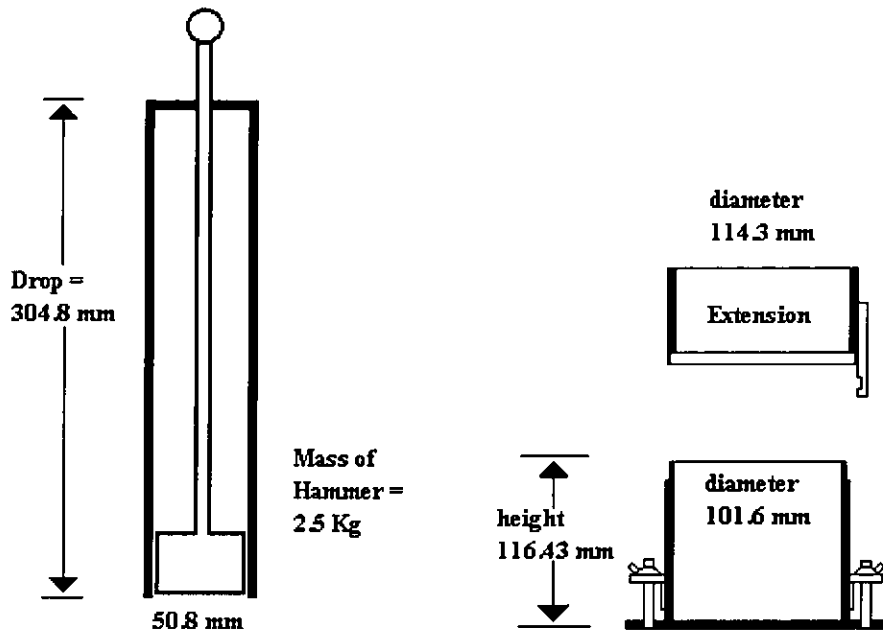# APPENDIX 6. PRELIMINARY SOIL BULK DENSITY TESTS

INTRODUCTION

In section 5.6 a compacted soil treatment was required that allowed earthworms to burrow into the soil but also offered increased resistance to earthworm penetration in comparison with uncompacted soil. This was achieved by a system of trial and error in which the ability of earthworms to burrow into a range of soil bulk densities was assessed.

Method

Soil was compacted using Standard Proctor test equipment illustrated in figure C. The experimental soil, a pre-sterilised Kettering loam (moisture content 25 %) was compacted in three equal layers (to a depth of approximately 120 mm) within one litre culture vessels (mass 52.2 g) held securely within the test equipment mould. Each layer was compacted by dropping the hammer (2.5 kg) directly onto an aluminium disc which covered the soil surface and dissipated the force of the hammer. Different levels of soil compaction were established by varying both the frequency and height from which the hammer was dropped. In this manner 2 replicates of four different levels of compaction were established : -

1) 1.77 g cm^{-3} - Hammer dropped twice from the maximum height allowed by

       the equipment (305 mm).

2) 1.69 g cm^{-3} - Hammer dropped once from 305 mm.

3) 1.61 g cm^{-3} - Hammer dropped once from 150 mm

4) 1.52 g cm^{-3} - Hammer dropped once from 75 mm

Figure C Standard Proctor Test Equipment

diameter
114.3 mm

Drop =
304.8 mm

Extension

Mass of
Hammer =
2.5 Kg

height
116.43 mm

diameter
101.6 mm

50.8 mm

a) Hammer

b) Mould

adapted from Head (1980)

The mass and volume of soil in each replicate was established and the wet soil bulk density calculated using the following simple equation:-

$$\text{Bulk Density} = \frac{\text{mass of soil (g)}}{\text{volume of soil (cm}^3)} \qquad \text{g cm}^{-3}$$

Bulk densities in each treatment were averaged over the 2 replicates.

3 mature *A. chlorotica* were placed onto the soil surface in both replicates of each treatment and the time taken to burrow into the soil recorded.

Results

Table O

Wet Soil Bulk Density g cm^{-3}	Mean time taken for all three *A. chlorotica* to burrow into the soil
1.77	16 minutes
1.69	7 minutes
1.614	5 minutes
1.52	2 minutes

From these results it was decided to employ the fourth soil bulk density treatment (1.52 g cm^{-3}) as the compacted soil treatment in section 5.6.

A Note on Observed Earthworm Burrowing Behaviour

In early tests earthworms were recorded moving between the edge of the vessel and the soil. Kretzschmar (1991) studied the burrowing ability of *A. longa* in relation to soil compaction and water potential. In experimental soil columns earthworms were prevented from utilising the space between soil and container by lining the inside of the vessels with sealing varnish and sharp fine sand. In this study earthworms were initially deterred from utilising this space by pushing the soil adjacent to the space firmly against the vessel wall.

APPENDIX 7. STOKE BARDOLPH FIELD STUDY

INTRODUCTION

This appendix describes the establishment and monitoring of a field experiment, primarily designed to test the findings of laboratory-based experimentation and also to provide information on survival of earthworms in agricultural land heavily treated with sewage sludge.

Experiments described in Chapters 4 and 5 demonstrated that both negative inter- and intraspecific interactions between earthworms (quantified in terms of growth rates and fecundity) were detectable under controlled laboratory conditions. Furthermore, results from selected experiments indicated that under certain conditions, mutualistic (beneficial) interactions occurred between anecic and endogeic species. Such beneficial interactions, if verified in the field, would support greater use of combinations of these two ecological groupings, in land restoration schemes.

THE STOKE BARDOLPH ESTATE FIELD EXPERIMENT

This was set up in September 1997 at Stoke Bardolph in Nottinghamshire (Nat. Grid Ref. SK 637418). Adult, laboratory-reared *L. terrestris* and adult, laboratory-reared and also field collected *A. chlorotica* were inoculated into a fallow, agricultural field (see plate E) (lacking earthworms) in a number of treatments. Inoculated earthworms were monitored after 6 months and after one year.

Site Description

The estate is situated within the Trent valley to the east of the city of Nottingham. Part of the estate boundary is formed by the river Trent itself. The estate, owned by Severn Trent Water Ltd, comprises 630 ha of farmland used for cereal production and dairy cattle (herd of 400). Since 1900 the fields have received applications of sewage sludge from the adjacent sewage treatment works. Soil survey and chemical analysis of the estate soils (*e.g.* Hemming *et al.,* 1991; Heaven & Delve, 1997) indicated that the spreading of sewage sludge had resulted in a build up of elements potentially toxic to earthworms. The distribution of heavy metals was heterogeneous resulting in localised 'hot spots' of individual elements.

Earthworm Survey

Earthworm survey work at the estate was conducted in March 1997 (Butt & Lowe, 1999) on behalf of Severn Trent Water Ltd. Sampling was conducted in a number of fields along two transects across the estate. In each field soil was removed to a depth of 0.15 m within 3 randomly located 0.1 m^2 quadrats. The soil was handsorted in the field and all recorded earthworms preserved in 4 % formaldehyde for identification in the laboratory. Dilute (0.4 %) formaldehyde (Raw, 1959) (see appendix 4) was poured into the holes created by soil removal in order to expel earthworms from deeper burrows and these were added to those collected from handsorting. During this sampling 7 species were identified representing all three ecological groupings. Results indicated that earthworm distribution across the estate was not uniform. In several fields no earthworms were recorded during sampling, whilst in one field an earthworm density of 315 per m^2 was recorded.

Plate E Stoke Bardolph Field Site
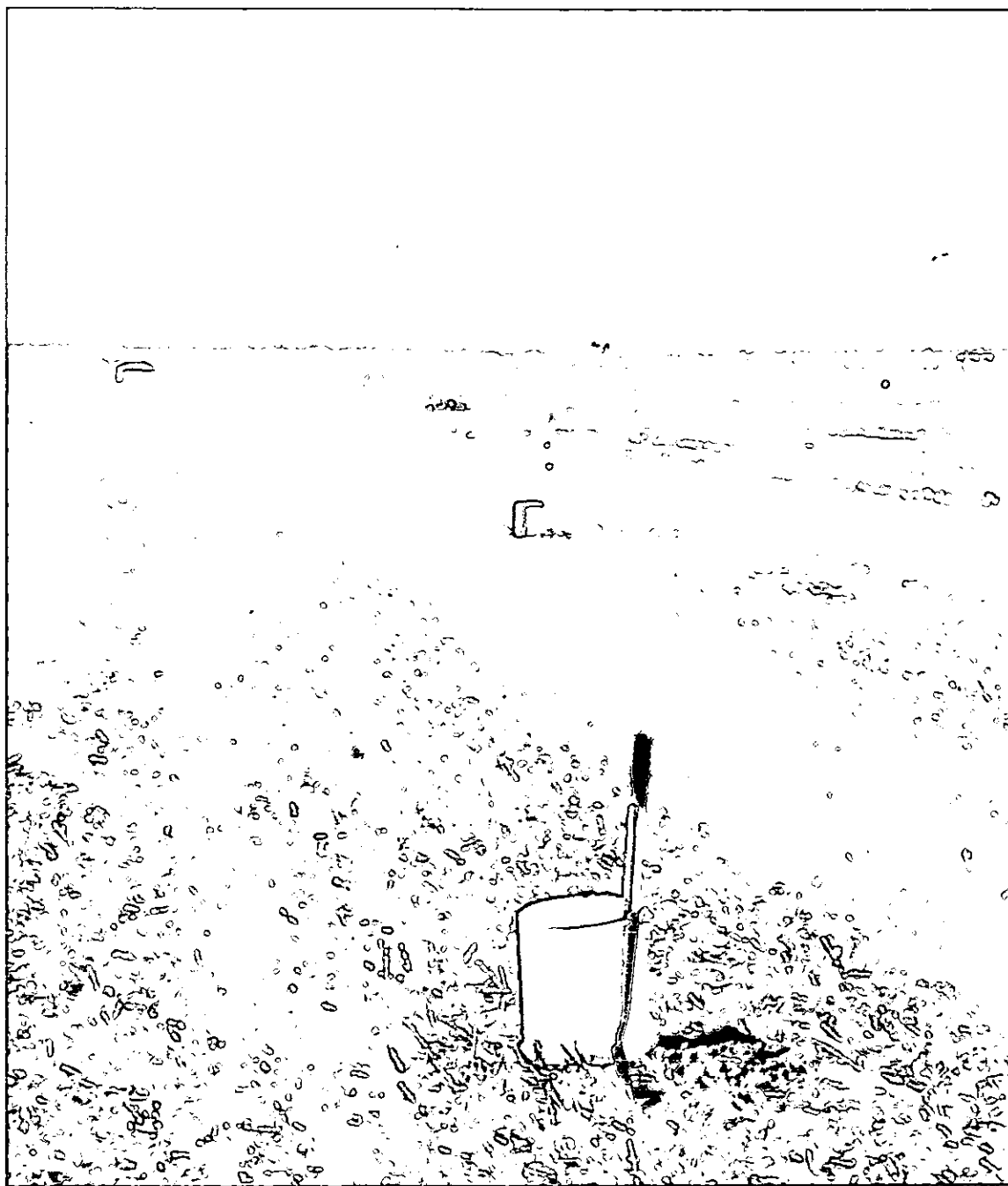
Soil samples were taken from several fields along the transects. Single hatchling *L. terrestris* and 2 mature *A. chlorotica* (both species laboratory-reared) were placed in individual samples of the field collected soil in one litre culture vessels in darkness at 15 ± 1 °C. The mass and survival of *L. terrestris* and the survival and reproduction of *A. chlorotica* were monitored over a 12 week period. Results suggested that earthworms were capable of survival, growth and reproduction in the soil from the estate.

From the initial survey and associated laboratory work, it was recognised that fields without earthworms might provide suitable sites for an earthworm inoculation experiment.

ESTABLISHMENT OF THE FIELD TRIAL - SEPTEMBER 1997

Method

Inoculation

A field on the estate (29.5 ha) due to be left fallow for a year after previously being planted with maize was selected for this experiment. Mature, laboratory-produced *L. terrestris* and *A. chlorotica* were taken to the site for inoculation. *A. chlorotica* were also collected from a nearby field, which had been identified during the survey work of March 1997 as having a high density of earthworms. This was to enable comparisons to be drawn between locally collected and laboratory-reared earthworms.

Five treatments were replicated three times within the experimental field along a transect at intervals of 20 m. These were additions of:

a) 100 laboratory-reared *A. chlorotica* (LabAC)

b) 100 field-collected *A. chlorotica*   (FdAC)

c) 20 laboratory-reared *L. terrestris*   (LabLT)

d) 20 LabLT and 100 FdAC   (LT+AC)

e) No earthworms   (control)

In each case treatments were randomly assigned to points along the transect. Earthworms were inoculated into the soil within an area of 0.25 m^2 where the soil had been broken up using a garden fork.

MONITORING (MARCH AND SEPTEMBER 1998)

After 6 months (March 1998) the field site was revisited and an initial assessment of the inoculated earthworms was undertaken. The transect line was re-laid and earthworm treatments located. Beside each inoculation point (within 1 m) a single (0.5 m^2) hole was dug and soil removed was handsorted for earthworms. Other signs of earthworm presence including burrows, cocoons and casting was also sought. The soil and any recorded earthworms were replaced.

One year after field inoculation (September 1998) a more thorough investigation of the site was undertaken utilising two sampling methods:

1) As in March 1998 each inoculation point was relocated. Beside each point a hole was dug (within 1m) of area 0.25 m^2 to a depth of 0.15 m. The soil removed from the hole was handsorted for the presence of earthworms and a standard vermifuge applied (Raw, 1959) (see appendix 4).

2) An electrical apparatus developed by Thielmann (1986) (see appendix 4) was also used at each inoculation point. This was placed within 1 m of each inoculation point parallel to the hole dug in (1). The apparatus passes an electrical charge into the soil which drives earthworms to the surface. This technique was applied for 20 - 30 minutes at each site (employing a range of voltages and switching frequencies) and the number of earthworms brought to the surface during this period recorded.

Results

After 6 months (March 1998) the following were noted in the five experimental treatments:

a) LabAC   No earthworms seen

b) FdAC   Adults and one cocoon of *A. chlorotica* seen

c) LabLT   An adult *L. terrestris* was seen along with numerous large burrows

d) LT+AC   Adults of *A. chlorotica* (living and necrotic) and signs of *L. terrestris* burrows seen.

e) Control   No earthworms seen

In September 1998 (after 12 months) sampling using both the electrical apparatus and handsorting followed by the application of a vermifuge revealed no signs of earthworms.

Discussion

During the first sampling period (after 6 months), all recorded earthworms were identified as *A. chlorotica* or *L. terrestris* (the two inoculated species) providing further indication that the field was devoid of a resident earthworm population prior to inoculation. Numbers observed during sampling were very low suggesting that the inoculated earthworms had failed to become established at the site. Indeed, 6 months later, a more detailed survey indicated that all of the introduced earthworms and any of their offspring had died.

Earthworm mortality was attributed to two major factors:

1) The chronic bioaccumulation of toxic elements (in particular heavy metals) which were present within the soil as a direct result of sewage sludge application onto the fields over the last century.

2) The regular disturbance of the site (every 2 months) caused by sub-soil injection of further liquid sewage sludge.

In laboratory culture trials, the influence of toxic elements on earthworm survival was not observed. It was suggested that the length of the experiment (12 weeks) had not been sufficient for the uptake of toxic elements by the earthworms to reach lethal levels.

The mortality of the experimental earthworms in the inoculation trial prevented interactions between the two selected species from being assessed under field conditions,

highlighting the problems associated with field experimentation (see section 3.1). Under laboratory conditions it had been possible to accurately monitor earthworm growth, fecundity and survival over a short time-scale. However, under field conditions and with the time available for the field study such accurate and repeated monitoring of inoculated earthworms was not feasible.

Observations made at the first sampling, 6 months after inoculation indicated that the laboratory-reared *A. chlorotica* had failed to survive, with no evidence of their presence recorded in the LabAC treatments. However, field collected *A. chlorotica* were recorded in both the FdAC and LT+AC treatments. These observations suggested that

A. chlorotica collected from the estate may be more tolerant to soil chemical conditions at the inoculation site than laboratory-reared earthworms of the same species.

A reduction in the levels of heavy metals in the soil at the donor site in comparison with the inoculation site (Heavan & Delve, 1997) may have permitted earthworm populations at the former to become established. However, levels of heavy metals at the donor site were still above normal levels found in agricultural soils. Sturzenbaum *et al.* (1998a) stated that some earthworm populations can tolerate heavy metal concentrations well above the critical concentration known to induce lethal effects in more typical populations. The increased tolerance of these populations was shown to be linked to the up-regulation of certain genes. The presence of heavy metals is known to induce the production of the stress related proteins Metallothioneins. These proteins sequester heavy metals and are crucial in reducing their toxic effects (Landis & Yu, 1995). Two isoforms of this protein have been isolated and sequenced in the earthworm species *L. rubellus* (Sturzenbaum *et al.*, 1998b). Therefore, it was proposed that earthworms from the donor site, which had been pre-exposed to heavy metals, may have been better adapted to the

high levels of heavy metals at the inoculation site compared with the laboratory-reared earthworms. These observations may have important implications concerning the origin of earthworms used in land restoration projects. It was suggested that the establishment of viable earthworm populations in degraded land contaminated with heavy metals may be enhanced by: a) utilising earthworms which had been obtained from the same location as the restoration site, or b) exposing earthworms to soil conditions similar to those experienced at the site prior to inoculation.