COMPETITION BETWEEN EARTHWORMS IN HIGH RAINFALL PASTURES IN THE MT. LOFTY RANGES, SOUTH AUSTRALIA

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SUMMARY

Earthworms have been shown to increase pasture production in southern Australia. The most abundant and ubiquitous species of earthworm in high rainfall pastures in this zone are *Aporrectodea caliginosa*, *A. trapezoides*, *A. rosea* and *Microscolex dubius*. Trials have shown that three earthworm species are particularly able to increase pasture growth, *A. trapezoides*, *A. caliginosa* and *A. longa*. *A. longa* is currently rare on mainland Australia but climatic matching suggests that it could have a much wider distribution. There is a research effort aimed at establishing it across broad areas of high rainfall pastures in southern Australia. There is also likely to be an effort to redistribute *A. trapezoides* into areas where it is currently not already present. If these introduction programs are to be successful, an understanding of the limitations to establishment and growth of earthworm populations is required. One limitation may be competitive interactions between *A. trapezoides* or *A. longa* with species already established in pastures. An understanding of these potential interactions and an insight into whether *A. longa* is likely to pose a threat to native habitats is required.

A. caliginosa and *A. trapezoides* are very closely related. *A. trapezoides* is the triploid form of the diploid *A. caliginosa*. They are able to coexist in many high-rainfall pastures, and it would be interesting to know whether there is competition for resources between these two species.

The main objectives of this project were:

1) to determine whether there are competitive interactions between *A. trapezoides* and two other earthworm species found commonly in pastures; *A. caliginosa* and *A. rosea*

2) to investigate whether there are competitive interactions between *A. longa* and three other species common to pastures; *A. caliginosa*, *Microscolex dubius* and *A. trapezoides*

3) to determine the likely impact of *A. longa* on soil fauna, especially the native earthworm *Gemascolex lateralis*, in native ecosystems

Laboratory and glasshouse experiments were mainly conducted in pots containing mixed soil or intact columns of pasture soil. Experiments were also conducted at five different field sites in the Mount Lofty Ranges and were carried out over three field seasons.

The standard experimental design was to have a single species treatment as a control and treatments with the same species plus another species or the same species but at double the density. The performance of earthworms in terms of survival, growth, life-history development and reproduction were then compared between treatments. In some experiments, earthworms were offered a choice of soil types in the presence and absence of another species to determine whether one species was forced into habitat of lower quality by the presence of another species.

The main findings of the project were:

1) Competitive interactions were found between *A. caliginosa* and *A. trapezoides*, *A. longa* and *A. caliginosa* and *A. longa* and *M. dubius*.

2) There are three possible mechanisms for competition between earthworms; scramble competition for food resources, interference competition and consumption of cocoons.

3) *A. longa* does not pose a serious threat to native stringybark forests in the Mount Lofty Ranges, South Australia.

4) Experiments investigating the community ecology of earthworms should be done using small earthworms, in unmixed soil, at a range of different scales, over a number of

generations if possible and at a number of different sites. Competition studies should include a control treatment with only one species. Other species should be added at a range of densities.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or any other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying.

April 1996

Signed

Paul Reginald Dalby

PUBLICATIONS FROM THE THESIS

The following papers have arisen from work done in this thesis:

Journal articles

Dalby, P.R., Baker, G.H. and Smith, S.E. (1995) Glyphosate, 2,4 D-B and Dimethoate: effects on earthworm survival and growth. *Soil Biology and Biochemistry* 27 (12): 1661-1662. **(Results of Chapter 2 - listed in Appendix B)**

Dalby, P.R., Baker, G.H. and Smith, S.E. (1996) "Filter paper method" to remove soil from earthworm intestines and to standardise the water content of earthworm tissue. *Soil Biology and Biochemistry.* In Press. **(Results of Chapter 2 - listed in Appendix B)**

Conference articles

Dalby, P.R. (1994) "Effect of species interactions on growth and survival of three lumbricid earthworms in a pasture soil", Annual Meeting of the Ecological Society of Australia, Alice Springs, 28-30 September (abstract only) p. 37

Dalby, P.R. (1993) "The ecology of earthworms in agricultural soils", Ecological Society of Australia Open Forum and Symposium Conference in Canberra, 26th September - 1st October 1993 (abstract only)

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Chapter 1 INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

The research presented in this thesis is a part of a larger research program which aims to identify species of earthworms that have the potential to increase pasture production in southern Australia (Baker, 1991). These species, once identified, would then be introduced into areas where they are not already present (Baker, 1989, Kingston and Temple-Smith, 1989, Baker *et al.*, 1994b). If this redistribution is to be successful, an understanding of what factors limit earthworm survival, reproduction and establishment need to be understood (Baker *et al.*, 1994b). An estimate of the potential risk of invasion from pastures into native areas is also required before these earthworms are redistributed on a large scale (Baker *et al.*, 1994b). There is already some understanding of the effect of factors such as climate, soil type and management on earthworm abundance in southern Australia (Tisdall, 1978, 1985; Buckerfield, 1992; 1993a,b; Baker *et al.*, 1992a,b, 1993a,b,c, 1994a; Doube *et al.*, 1994a, 1995). What is not well understood are the effects of biotic interactions within the community; factors such as competition, predation, parasitism and food relations (Curry, 1994). The work described in this thesis aims to determine whether there are competitive interactions between earthworms in pasture soils in southern Australia and to assess the potential impact of introducing an exotic lumbricid, *Aporrectodea longa* (Ude), on native habitats adjacent to these soils.

The amount of literature on earthworms is vast and it is beyond the scope of this review to cover it all. Rather, this review will give an overview of the basic biology of earthworms, put forward the proposition that earthworms are keystone species in soil decomposer communities and describe how earthworms have been introduced into various agricultural regions around the world to increase plant productivity. The review then illustrates some of the impediments that might arise when trying to introduce earthworm species into new environments, concentrating particularly on how

competition with other earthworms might affect introduction programs. Because of the study location, examples from temperate areas and particularly southern Australia are used wherever possible to illustrate points.

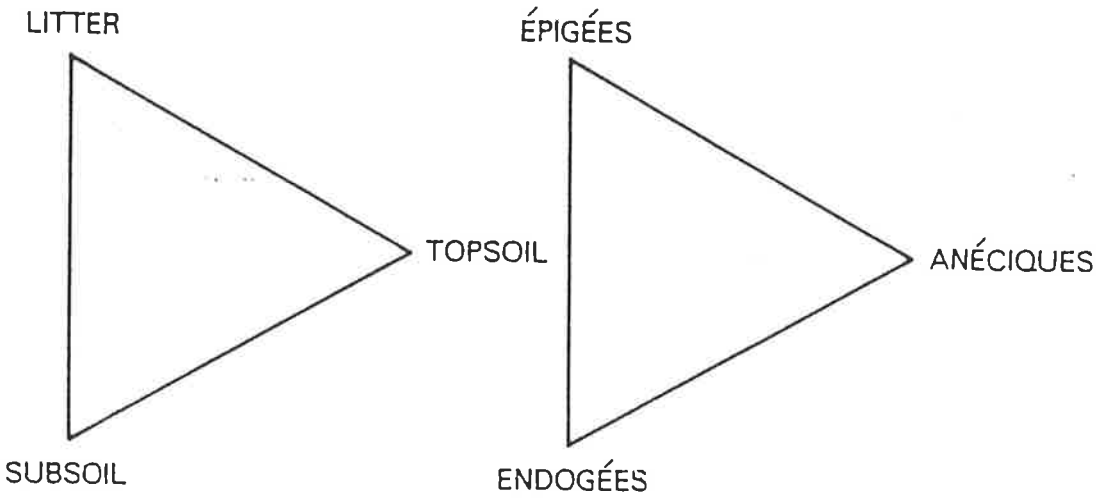
1.2. Basic biology of earthworms

1.2.1 Ecological groupings

Earthworms have been classified into groups by Lee (1959) and Bouché (1977) that relate to their ecological characteristics (Lee, 1985). Epigeic earthworms live predominantly in the litter layers consuming mostly organic materials and are darkly pigmented. Anecic earthworms live in the mineral soil layers, create deep, permanent burrows, come to the surface to feed on decaying organic matter and are darkly pigmented. Endogeic earthworms inhabit and feed on mineral soil and organic material mixed with that soil in the top 10 to 15 cm of soil and are unpigmented.

Not all earthworms fit neatly into these ecological classifications. Bouché (1977) argues that the groupings represent the extreme points on a triangular space (Fig. 1.1). On this figure, *Gemascolex lateralis* (Spencer) which is an earthworm species used experimentally in this thesis, may fall between epigeic and endogeic, because it mostly inhabits the litter layers and is darkly pigmented, but occasionally burrows into the soil (Lawson, 1993). Briones (1993) classified *Aporrectodea trapezoides* (Dugès) as an anecic earthworm and *Aporrectodea caliginosa* (Savigny) as an endogeic. Like other anecic species such as *A. longa*, *A. trapezoides* is heavily pigmented and comes to the surface to feed and cast more often than endogeic species such as *Aporrectodea rosea* (Savigny) (Baker *et al.*, 1994a). However, *A. trapezoides* is observed to consume large amounts of mineral soil (P.R. Dalby, unpublished observation) and is active at soil depths typical of endogeic species (Baker *et al.*, 1992a, 1993a) and so *A. trapezoides* probably falls in between endogeic and anecic.

Fig 1.1 Major ecological groupings of of earthworms developed by Lee (1959) and Bouche (1977)



from Lee 1985

1.2.2 Population biology

Earthworms may be dioecious or parthenogenetic (Sbordoni *et al.*, 1987). As an example, the species complex of *A. caliginosa* includes a diploid dioecious form (*A. caliginosa caliginosa*) and a triploid parthenogenetic form (*A. caliginosa trapezoides*) which are morphologically very similar but have quite different genetic (Sbordoni *et al.*, 1987) and ecological characteristics (Briones, 1993).

The incubation time of cocoons for anecic and endogeic species varies from 3 weeks to 9 months and is strongly dependent on temperature and moisture conditions (Holmstrup *et al.*, 1990, 1991; Butt *et al.*, 1992; Butt, 1993). It is generally accepted that one juvenile usually hatches successfully from each cocoon, although the number may vary from species to species (Lee, 1985). Hatchlings take a few weeks to more than a year to mature (Lee, 1985; Hamoui, 1991; Butt *et al.*, 1994) and adults may reproduce for a few weeks or many years (Lee, 1985). Sexual organs may regress during periods of stress such as water-stress (McCredie *et al.*, 1992).

Cocoon production is dependent on ecological factors such as earthworm density (Reinecke and Reinecke, 1995) and environmental conditions such as temperature, moisture (Senapati and Dash, 1984; Christensen and Mather, 1990; Edwards and Bater, 1992; Hallatt *et al.*, 1992; James, 1992) and the quality and quantity of food available to individual earthworms (Evans and Guild, 1948; Boström, 1988). For example, the fecundity of *A. rosea* was 3 cocoons individual^{-1} year^{-1} in an English woodland (Phillipson and Bolton, 1977), and 1.0 individual^{-1} week^{-1} in laboratory culture under optimal conditions of temperature and moisture and with excess food (Lofs-Holmin, 1982). In the temperate and mediterranean regions of southern Australia where the summers are hot and dry and the winters cool and wet, the density of cocoons in the soil peaks at 63 m^{-2} during winter when the soil moisture is highest (McCredie *et al.*, 1992).

1.2.3 Growth

Earthworm growth and development is dependent on moisture, temperature and food availability in much the same way as cocoon production (Abbott and Parker, 1981; Lofs-Holmin, 1982; Martin, 1982b; Hartenstein and Amico, 1983; Boström, 1987; Baker *et al.*, 1992a,b; Daniel, 1992; McCredie *et al.*, 1992; James, 1992; Baker *et al*, 1993a,b; Michalis and Panidis, 1993; Kaushal and Bisht, 1994). The food requirements of earthworms will be discussed in more detail in section 1.5.2.3 *Food*. Earthworm growth varies with the stage of development of the individual earthworm. Meinhardt (1974) found that *Lumbricus terrestris* (Linnaeus) grew to 1.1 g in the ninety days from hatching, and then grew another 2.6 g in the next forty days. Hartenstein and Amico (1983) and Butt *et al.* (1994) found that earthworm growth rates could be reduced at high population densities.

Some values for rates of growth of earthworm species are given in Table 1.1. The maximum growth rates are higher for larger species such as *L. terrestris* , *Octolasion cyaneum* (Savigny) and *A. longa* and less for smaller species such as *A. rosea* and *A. caliginosa*, although smaller species usually take less time to reach maturity (Fig. 1.2). Most of the growth rates shown in Table 1.1 have been measured from laboratory cultures, often with optimal moisture and temperature conditions and surplus food (e.g. Boström, 1987, 1988; Butt, 1993). Growth rates measured in the field are much lower than in culture. For example, the growth rate of *A. caliginosa* measured in a reclaimed, cutaway peat bog ranged from 1.5 to 2.1 mg individual^{-1} day^{-1} (Curry and Boyle, 1987), but was as high as 21 mg individual^{-1} day^{-1} under optimal conditions in the laboratory (Boström, 1988).

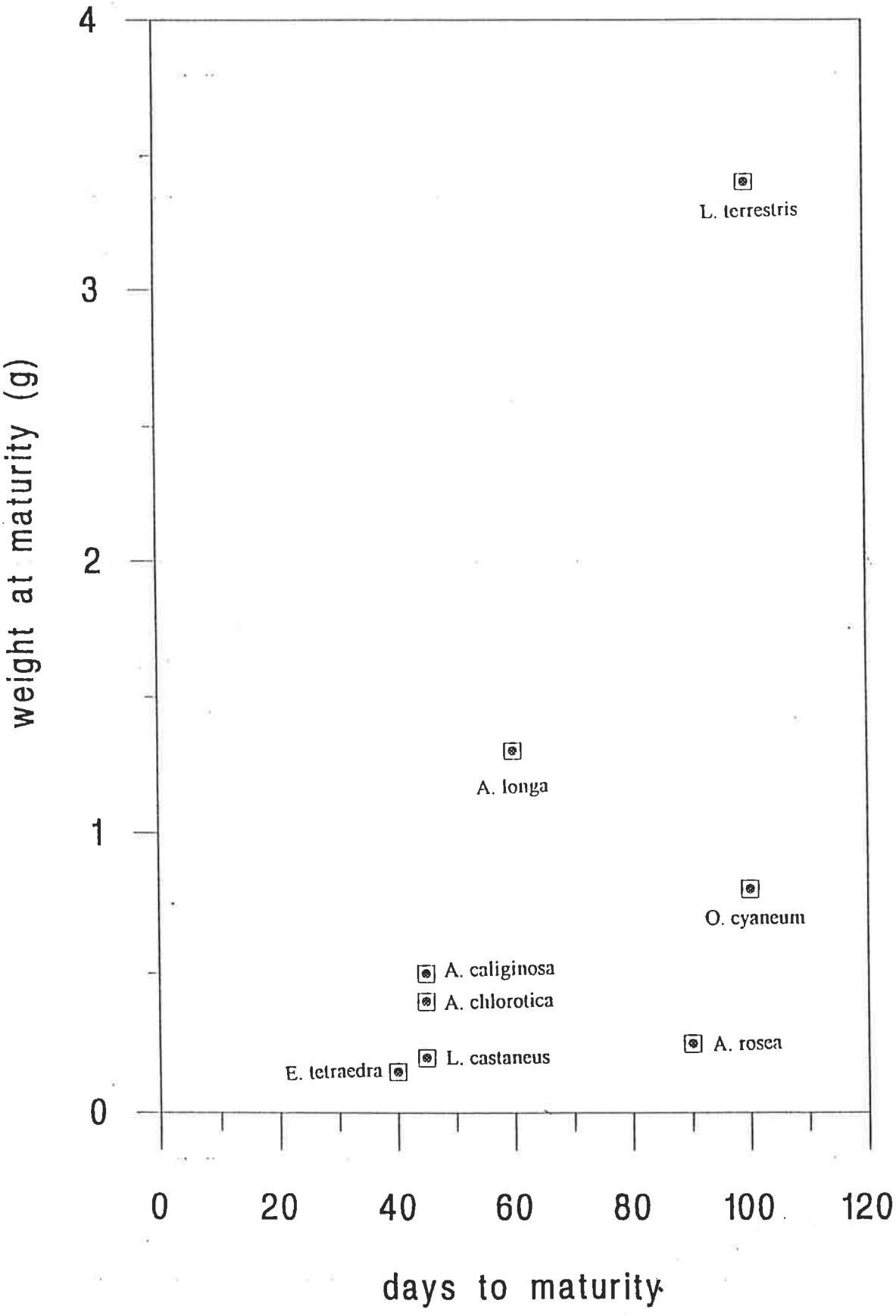
1.2.4 Dispersal

Earthworms actively disperse by moving through the soil (Marinissen, 1991) or across the soil surface (Schwert, 1980), or they can be passively transported as cocoons through the action of other agents, for example by animals, machinery or by stream drift

Table 1.1 Growth rates of some earthworm species (mg individual^{-1} day^{-1})

Earthworm species	**Growth rate** (mg ind.$^{-1}$ day^{-1})	**Reference**
Aporrectodea caliginosa	approx. 11	Lofs-Holmin 1982
	1.5 to 2.1	Curry and Boyle 1987
	1.0 to 3.8	Boström 1987
	13 to 21	Boström 1988
Aporrectodea longa	approx. 28	Lofs-Holmin 1982
	approx. 20	Butt 1993
Aporrectodea rosea	-0.06 to 0.57	Phillipson and Bolton 1977,
	approx. 3	Lofs-Holmin 1982
		Bostrom and Lofs-Holmin 1986
Lumbricus rubellus	-5.6 to 15.7	Shipitato *et al* 1988
Lumbricus terrestris	65.0	Meinhardt 1974
	approx. 30	Lofs-Holmin 1982
	6 to12	Curry and Boyle 1987
	-16.7 to 54.2	Shipitato *et al* 1988
	approx. 13	Butt 1993
Octolasion cyaneum	approx. 40	Butt 1993
	approx. 5	Lofs-Holmin 1982
Pontoscolex corethrurus	approx. 2.0	Hamoui 1991

Fig 1.2 The time taken to reach maturity for earthworm species of different sizes (data from Lofs-Holmin 1982)



(Schwert, 1980; Marinissen and Bosch, 1992). The total dispersal rate of *A. caliginosa* in reclaimed polders in the Netherlands was measured at 5 m yr^{-1} while the active dispersal rate was measured at 2 m yr^{-1} indicating that both active and passive dispersal were important in this system (Marinissen, 1991).

The rate of dispersal depends on the environment and the species. In a dispersal model developed by Marinissen and Bosch (1992), the influential factors were reproductive rate, active dispersal rate and in what part of the soil profile earthworms were most active in. Under optimum environmental conditions, the model predicted that epigeics would disperse faster because they had a high reproduction rate, and spent more time at the soil surface and so had a greater chance of being passively dispersed. However under adverse conditions, the model predicted that endogeic species would disperse at a higher rate because they had a higher survival due to their ability to aestivate, and could remain active in the soil when the surface conditions were unsuitable for epigeic activity. An epigeic species, *Lumbricus rubellus* (Hoffmeister), dispersed at a higher rate (10 m yr^{-1}) than an endogeic species, *A. caliginosa* (2.5 m yr^{-1}), in an Irish cutover peat bog (Curry and Boyle, 1987). This pattern was repeated in grasslands in the Netherlands where *L. rubellus* dispersed at an average rate of 14.1 m yr^{-1} compared to 7.0 m yr^{-1} for *A. caliginosa* (Marinissen and Bosch, 1992). However the rate of dispersal was the same for both species (8 m yr^{-1}) in grasslands in newly reclaimed polders in the Netherlands (Hoogerkamp *et al.*, 1983).

The movement of humans has facilitated the dispersal of some earthworm species on a global scale. There were few native North American earthworms in Canada prior to European colonisation, because the large ice sheet that covered the area during the pleistocene era made the area too cold for earthworm activity (Fender, 1995). However several European and Asian species now flourish in Canada after being introduced with European settlement from the ballast of ships, potted plants and by fishermen (Reynolds, 1977; Fender, 1995; Alban and Berry, 1994). Introduced lumbricids of European origin

colonised agricultural and urban areas in New Zealand (Lee, 1961) and Australia (Baker *et al.*, 1994b). Their introduction was thought to be attributed to the importation of English plants and dumping of soil from England used in ship ballast (Smith in Lee, 1961).

1.3. Earthworms as keystone species in agricultural soils

Keystone species are species that have a disproportionate effect on the survival of many other species within a community or assemblage (Paine, 1966, 1969, 1971; Bond, 1994). An example of a keystone species is the red-naped sapsucker *Sphyrapicus nuchalis* (Daily, 1992; Daily *et al.*, 1992). This woodpecker drills wells into shrubs and trees and feeds on the sap that flows from them. These holes act as nest holes for tree and violet-green swallows (*Tachycineta bicolor* and *T. thalassina*). The woodpeckers cause heavy mortality among willows and also supply sugary sap to a wide range of vertebrates and invertebrates that feed from the wells. The woodpecker is a keystone species because it's activity determines the survival of many other species within it's community and it's absence would completely alter the structure of that community.

Collembola from the genera *Folsomia* and *Tomocerus* are examples of key-stone species in decomposer communities. The collembolans are able to detoxify phenols from European oak leaves by filling their intestines with inorganic clay particles from the A horizon before migrating to the litter layer in the evenings to feed on and shred the oak leaves. This promotes bacteria which would otherwise die on contact with the phenols to become active in the litter. Subsequently, nutrients are more available and cycle faster, and so the oak forests are able to sustain far more biomass on clay soils than on sandy soils due to the activities of these two collembolan species (Touchot *et al.*, 1983 in Moldenke *et al.*, 1994).

Earthworms are keystone species in certain environments due to their generalist feeding behaviour and their ability to alter the structure of soil profiles and soil communities.

Under the classification developed by Bond (1994), earthworms belong to a group of keystone species named the Earth-movers which includes species such as rabbits, gophers and termites. They affect communities by altering the structure of soil, which changes the habitat for decomposers and plants and has flow on effects through the food web.

1.3.1 Changing soil structure

Earthworms modify soil structure by creating burrows and depositing casts (as well as mixing litter and top soil with soil further down the profile as was discussed in the previous section). Excellent reviews on the effects of earthworms on soil structure have been written by Lee (1985), Lee and Foster (1991), Lee and Smettem (1995), Oades (1994) and Tomlin *et al.* (1995).

The physical action of earthworms moving through the soil creates burrows which are channels for air and water movement (Hoogerkamp *et al.*, 1983; Trojan and Linden, 1992; Springett *et al.*, 1992; Springett *et al.*, 1994) and are utilised by roots (Edwards and Lofty, 1978, 1980; Lodgson and Linden, 1992). When earthworms are abundant their burrows are large enough to dominate the macroporosity in soils and play a major role in water infiltration and gaseous exchange (Oades, 1994). In orchard plots where earthworm numbers were high, the soil macroporosity was 1.5 times higher than where earthworm populations were low (Tisdall, 1978, 1985). Water infiltration was significantly increased in a brown sandy loam in Western Australia in the presence of *A. trapezoides* compared to similar soil where earthworms were absent (McCredie and Parker, 1992).

Different ecological groups have different effects on macropore development. Epigeic earthworms live above the mineral soil layers and do not burrow into the soil. Endogeic species live below the soil surface, making extensive burrow systems with predominantly horizontal orientations that rarely open to the surface (Lee and Smettem, 1995). The

burrows are not always continuous and are often filled with cast material. Anecic species create permanent burrows that open at the surface, are usually vertical in orientation and may penetrate to a depth of over 1m (Lee and Foster, 1991). When anecics and endogeics are present in the same soil, the combination of vertical and horizontal burrows provides pathways for large amounts of ponded water to infiltrate into the soil (Kretzschmar, 1987).

The surfaces of some soils are almost entirely composed of earthworm casts (Kubiena, 1953; Lavelle, 1978; Lawson, 1993). In temperate pasture soils up to 50% of the aggregates in surface layers are recognizable earthworm casts (Lee and Foster, 1991). Many studies have shown that earthworm casts have greater tensile strength (McKenzie and Dexter, 1987), and are more stable (van Rhee, 1977; Lal and Akinremi, 1983; De Vleescheuwer and Lal, 1981; Mulongoy and Bedoret, 1989) than soil aggregates (although see Zhang and Schrader, 1993). In all of these studies, the casts were dried before measurements were made. The drying process stabilises the cast material, and fresh casts which are not dried may be less stable than soil aggregates (Marinissen and Dexter, 1990; Hindell *et al.*, 1994; Oades, 1994). The casting rate of earthworms and factors which affect casting rate are discussed in section 1.5.2.3 *Food*.

1.3.2 Modifying decomposer community structure

Populations of organisms that pass through the intestines can either increase, remain constant or decrease in abundance (Day, 1950; Parle, 1963a,b; Atlavinyte and Pociene, 1973; Mulongoy and Bedoret, 1989; Kristufek *et al.*, 1992b; Pederson and Hendrikson, 1993; Karsten and Drake, 1995; Heijnen and Marinissen, 1995). Communities of decomposer organisms that pass through the intestines of an earthworm can change in overall diversity (Tiwari and Mishra, 1993; Russom *et al.*, 1993; Brown, 1995) and species dominance (Day, 1950; Parle, 1963b; Senapati, 1992; Kristufek *et al.*, 1992a; Moody *et al.*, 1995). Earthworms may reduce or increase plant diseases by consuming the disease organism, affecting the ability of the plant to resist disease attack, and

repressing or promoting microorganisms in the soil which attack the disease organism (Raw, 1962; Hoffman and Purdy, 1964; Melouk and Horner, 1976; Hampson and Coombes, 1989; Niklas and Kennel, 1981; Stephens *et al.*, 1994a).

Earthworms act as vectors of soil organisms because they live in the upper layers of the soil profile and pass large volumes of soil through their bodies (Lee, 1985). Increasing the ability of an organism to disperse improves the relative fitness of that organism in the community (Tilman, 1982). Organisms that have been shown to be transported via earthworm activity include fungi (Hampson and Coombes, 1989), nematodes (Shapiro *et al.*, 1993), *Rhizobium* bacteria (Doube *et al.*, 1994c; Stephens *et al.*, 1994b) and biocontrol agents of disease (Doube *et al.*, 1994b). It has been suggested that mycorrhizal fungi are transported by earthworms as spores but all that has been shown to date is that spores can survive passage through the intestines of an earthworm (Rabatin and Stinner, 1989; Reddell and Spain, 1991; Gange, 1993; Harinikumar and Bagyaraj, 1994) and evidence for transport of mycorrhizal spores by earthworms has yet to be presented.

Earthworms can affect soil communities indirectly by modifying the soil environment (Yeates, 1981; Thompson *et al.*, 1993; Hyvonen *et al.*, 1994). Marinissen and Bok (1988) found that the species composition of populations of collembolans was different in soils containing earthworms compared to earthworm-free soil. Large collembolans made up a significantly higher proportion of the population in the presence of earthworms where the average pore size was larger and food was more readily available. However Marinissen and Bok hypothesised that collembolans were relatively larger in soil with earthworms present because the architecture of the soil allowed freer movement of the collembolans to lower soil layers to escape adverse conditions. Similarly, Yeates (1987) found that the presence of earthworms shifted the community of nematodes of Mononchoidea from being dominated by the small *Clarkus*

propapillatus (Clark) species to being dominated by the larger *Iotonchus stockdilli* (Yeates) species.

1.3.3 Altering energy and nutrient flows

Earthworm casts are sites of increased microbial respiration (Parle, 1963a; Scheu, 1987a; Ruz Jerez *et al.*, 1988; Daniel and Anderson, 1992; Smith and Steenkamp, 1992) which results in increased organic matter mineralisation. The reason for the increased microbial respiration is not fully understood, but is probably due to the mechanical mixing and moistening of the soil (Lunt and Jacobson, 1944) which exposes previously inactive microorganisms to organic matter (Hassink, 1992).

The action of mixing organic matter with mineral soil by earthworms is often associated with higher rates of nitrogen cycling. Nitrogen is transformed from an organically bound form to ammonium due to the combined digestion of the earthworm and microorganisms. This is followed by a rapid transformation to nitrate ions that are slowly oxidised by denitrifying bacteria. Scheu (1987b) found that nitrogen mineralisation was proportional to the size of the earthworm species. Syers *et al.* (1979) determined that the presence of *L. rubellus* in pasture soil was associated with an extra 2.6 kilograms of ammonium-nitrogen hectare^{-1} and 0.9 kilograms of nitrate-nitrogen hectare^{-1} present in the soil after one season. In a New Zealand pasture, the presence of *A. caliginosa* was associated with an extra 109 - 147 kg N ha^{-1} yr^{-1}, representing about 20% of the total quantity mineralised from organic matter annually (Keogh, 1979 in Curry, 1989). The presence of earthworms can also affect the rate of denitrification (Elliot *et al.*, 1990; Svensson *et al.*, 1986; Parkin and Berry, 1995) because respiration is often higher in casts, depleting oxygen levels and resulting in more anaerobic conditions (Svensson *et al.*, 1986).

By mixing plant material with soil and increasing the rate of mineralisation, earthworms accelerate the rate of phosphorus (P) cycling in pastures (Sharpley and Syers, 1977).

Approximately 9 kg ha^{-1} of inorganic forms of P and 13 kg ha^{-1} of organic P were accumulated in earthworm casts over the period of one year in a New Zealand pasture (Sharpley and Syers, 1977). Release of inorganic P to solution from casts was approximately four times greater than that from surface soil per volume of material (Sharpley and Syers, 1976). Satchell and Martin (1984) found that phosphatase activity was higher in earthworm casts than soil for four earthworm species, due mostly to the increase in microbial acid-phosphatase activity.

1.3.4 Effects on the compositioin of plant communities

Earthworms can increase the total primary productivity of a soil through their effects on soil structure, nutrient dynamics and soil organisms (see reviews in Lee, 1985; Curry, 1994). Earthworms can also change the composition of plant communities by altering the soil environment (van Rhee, 1965) or by preferentially burying seeds of certain plant species (McRill and Sagar, 1973; McRill, 1974; Grant, 1983; Thompson *et al.*, 1994; Shumway and Koide, 1994; Willems and Huijsmans, 1994; Piearce *et al.*, 1994). In a simple, experimental community, the presence of earthworms increased the proportion of *Trifolium dubium* in the community by increasing available soil P, increasing root nodulation of *T. dubium* and preferentially burying the seeds of the other two plant species (*Poa annua* and *Senecio vulgaris*), reducing their seedling establishment (Thompson *et al.*, 1993).

1.4. Manipulating earthworm communities for agriculture

1.4.1 Irish peat bogs, reclaimed polders in the Netherlands and New Zealand pastures

As earthworms become recognised as keystone species in certain agricultural ecosystems, there is increasing interest in utilising earthworm activity to carry out certain important functions such as developing soil structure, altering energy flows and increasing Net Primary Production (Stockdill, 1982; Hoogerkamp *et al.*, 1983; Rovira *et al.*, 1987; Curry and Boyle, 1987; Baker, 1992; Marinissen, 1991; Butt *et al.*, 1992; Springett *et al.*, 1992; Edwards and Bater, 1992; Lee and Smettem, 1995). This section

describes three habitats (Irish peat bogs, reclaimed polders in the Netherlands and New Zealand pastures) where earthworms have been successfully introduced, resulting in increased Net Primary Production and improved soil properties. The second section will describe attempts to introduce earthworms species into high-rainfall pastures in southern Australia.

When Irish peat bogs are reclaimed for agriculture, they are drained and the peat is cut away leaving about 50cm of peat above the mineral soil. The peat is then mechanically mixed with the mineral soil, limed to pH 7, and sown with a grass-legume mix (Curry and Boyle 1987). Initial colonisation of the peat bogs by earthworms can be accelerated by deliberate introductions (Curry and Cotton, 1983). However with time this effect is obscured by natural colonisation (Curry and Boyle, 1995). Herbage yields were 25% greater in the 2nd year and 49% greater in the third year following inoculation of *L. terrestris* and *A. caliginosa* into reclaimed peat bogs (Curry and Boyle, 1987).

The reclaimed polders in the Netherlands are areas of land reclaimed from marine lakes. The soils are initially devoid of earthworms. Under these conditions, decaying organic matter accumulates on the surface forming a dense mat. The thickness of this mat varies from 0.5 to 2.5 cm (Hoogerkamp *et al.*, 1983). When earthworms invaded an area, the mat was incorporated into the soil in about three years and an A horizon began to develop. The soil characteristics changed from being a weak plate-like or moderate block-like structure in the upper 10 cm to granular. Water infiltration capacity increased considerably. The pasture yield increased on average by 10% (Hoogerkamp *et al.*, 1983).

In New Zealand, when land was cleared of native vegetation for pasture production, the native megascolecoid earthworms died out. Exotic lumbricids, inadvertently brought in by European colonists, invaded these pasture areas (Lee, 1961) although the process was enhanced by deliberate introductions (Stockdill, 1966). The introduction of lumbricids

into soils lacking them (Stockdill, 1982) gave rise initially to 72% increase in pasture production as the root mat was mineralised, releasing bound up nutrients and increasing water infiltration. This yield increase stabilised at 25% over a ten year period.

In these three examples, earthworms were introduced into pasture soils to increase the primary productivity of pastures by changing the soil structure and altering nutrient and energy flows. The benefits to the landholder are increased plant production with no extra inputs required, and improved soil structure which increases water infiltration and reduces runoff. A program in progress in Australia aims to carry out similar introductions into high-rainfall pastures in an effort to obtain similar benefits. This effort is described in the following section.

1.4.2 Australian high rainfall pastures

In Australian agricultural systems, native earthworm species are rare and the fauna is dominated by European lumbricid species which were accidentally introduced (Baker *et al.* 1992a,b). The dominant exotic species in these areas are similar to those in other countries with similar climates where Europeans have settled. They include *A. trapezoides*, *A. caliginosa*, *A. rosea*, *L. rubellus* and *Microscolex dubius* (Fletcher) (Kingston and Temple-Smith, 1989; Mele, 1991; McCredie and Parker, 1992; Baker *et al.*, 1992a,b; Baker *et al.*, 1993a,b,c; Garnsey, 1994). The distribution of these exotic species is patchy and there is scope to redistribute some species to areas where they have not already colonised (Baker, 1989, 1991).

In Australia, there have been some isolated attempts to redistribute earthworms species into agricultural areas. The exotic lumbricids *A. caliginosa* and *A. longa* were introduced to pastures in northern Tasmania, increasing pasture production by up to 75% within three years (Temple-Smith, 1991; Temple-Smith *et al.*, 1993; Garnsey, 1994). Noble *et al.* (1970) introduced *A. caliginosa* to irrigated pastures in New South Wales. This resulted in the decomposition of a thick litter mat and reduced bulk density. Earthworms

(*Aporrectodea* spp. and *Eukerria saltensis*) were introduced into an irrigated wheat field in New South Wales which resulted in increased air permeability (Blackwell and Blackwell, 1989).

There are two exotic species, *A. longa* and *L. terrestris*, which have successfully colonised areas like North America and New Zealand, but are conspicuously missing from mainland Australia, although *A. longa* is found in Tasmania. These are anecic species, an ecological group which is largely missing from the fauna in agricultural areas of Australia (Baker, 1991). The activity of anecic species would increase water infiltration, root penetration and soil mixing to greater depths than from the activity of the endogeic species that are already present in Australian soils (Baker, 1991). Syers and Springett (1983) found that when *A. longa* was introduced into a pasture containing only epigeic and endogeic species, there was a 20% increase in pasture production. The potential of introducing *A. longa* to increase primary productivity in permanent pastures in temperate, southern Australia is currently under investigation (Baker *et al.*, 1994b).

One species of any organism may have many ecotypes which are adapted to different environments and habitats (Briones, 1993; O'Connor, 1994). The exotic earthworm species that were accidentally introduced in Australia probably came from climatic areas which are very different to conditions found in Australia, such as England (Baker *et al.*, 1994b). However these species have broad distributions in their native habitats in Europe and northern Africa, covering a wide range of climatic and edaphic conditions (Baker, 1989, 1991). It has been suggested that ecotypes be introduced into Australia from areas with similar climates to Australia (Baker, 1989, 1991; Baker *et al.*, 1994b).

To obtain the maximum benefit from an earthworm redistribution program, an understanding of the limitations and problems of establishing particular earthworm species into new environments is required. The following section describes some of the

potential impediments to introducing earthworms into high rainfall pastures in southern Australia.

1.5. Potential impediments to introducing earthworms into Australian high rainfall pastures

1.5.1 Abiotic factors

Earthworm activity and distribution are dependent on climatic conditions (Waters, 1951; Daniel, 1991; James, 1992; Springett *et al.*, 1992 and see 1.1 Basic biology of earthworms). Temperature and moisture requirements vary between different species, but most species require temperatures between 0 and 25°C and soil water potentials ranging between -10 to -100 kPa (Lee, 1985). In southern Australia, the activity of exotic species is limited to the cool, moist months of the year (Baker *et al.*, 1993a; Garnsey, 1994), except where the areas are under irrigation and earthworm activity is extended over a longer season (Lobry de Brun, 1993). Earthworms can evade climatic extremes by burrowing deeper into the soil where they aestivate until conditions improve or by producing cocoons which have greater resistance to climatic extremes.

Soil chemical factors can have a strong influence on earthworms. Earthworms require adequate organic carbon and nitrogen in forms which are easily assimilated. Nitrogen is often a limiting nutrient for many organisms and in the soil environment organic matter with a low C:N ratio is of higher food value for most organisms (White, 1993). Evans and Guild (1948) showed that earthworms grew faster and produced more cocoons when fed on diets with high concentrations of nitrogen. In Hawaii, populations of earthworms are higher under stands of *Albizia falcataria* compared to *Eucalyptus saligna*, in part due to the higher nitrogen content of the albizia litter (Zou, 1993).

Earthworms are tolerant to quite wide extremes of pH, although each species has a narrower pH range over which they are most active (Edwards and Lofty, 1977). In soils of high acidity, liming may increase earthworm abundance (Hyvonen *et al.*, 1994).

Because earthworms consume large quantities of soil, they can accumulate high concentrations of heavy metals in their body tissues in contaminated areas (Lee, 1985). They are notoriously sensitive to copper (Nielsen, 1951; Ma, 1982 in Christensen, 1991; Abdul Rida and Bouché, 1995), and are also sensitive to lead (Abdul Rida and Bouché, 1995) although Malecki *et al.* (1982) suggest that some earthworm species are tolerant to high concentrations of lead. Earthworms are unaffected by the normal application of many pesticides especially herbicides (Edwards and Bohlen, 1990), although they are intolerant of many fungicides (Lee, 1985).

Although earthworms are able to modify the structure of a soil, their activity may be limited in very compact soils. Burrowing by *A. caliginosa* has been shown to be unaffected by soil strengths up to penetrometer resistances of 3 MPa (Dexter, 1978) but above this their burrowing activity is reduced. Earthworms can move through the soil by pushing their way through or by ingesting soil. Pressures exerted by earthworms pushing their way through soil are probably at the lower end of the scale of those exerted by plant roots (<1 MPa), and to burrow through compact soil they must ingest soil particles (Lee and Smettem, 1995).

Earthworm abundance has been found to be significantly positively correlated with high clay contents of the soil in two separate surveys (Nordström and Rundgren, 1974; Baker *et al.*, 1992b), although clay content may be interrelated with other soil factors, such as soil water retention. High clay content may be detrimental to earthworms in some areas of very high rainfall, where the soil becomes waterlogged and earthworm activity is limited by gas exchange between the soil and the atmosphere (Lee, 1985).

1.5.2 Biotic factors

1.5.2.1 Plant communities

Different plant guilds support different communities of earthworms (Dash and Senapati, 1991; Westernacher and Graff, 1987; Aplet, 1990; Babel *et al.*, 1992; Muys *et al.*, 1992;

Zou, 1993). In New Zealand, when native vegetation was cleared for agriculture the native earthworm fauna became locally extinct in cleared areas, possibly because they could not survive on the litter of the pasture plant species. However introduced lumbricids were able to establish themselves successfully in the pastures and agricultural land when planted with species with which they are usually found to be associated (Lee, 1961). When these pastures were planted with an exotic species, *Pinus radiata*, the earthworm community changed again in response to the changing quality of the litter input (Yeates, 1988).

1.5.2.2 Predators, pathogens and parasites

Earthworms are high in protein and make an excellent food source for predators if they can be caught. They are preyed upon by a large variety of animals including birds, snakes, mammals, beetles and flatworms (Table 1.2). Most of these studies were carried out in the northern hemisphere and there is scant information on predation of earthworms in the southern hemisphere.

There are a number of different types of parasites that can infest earthworms. Earthworm cocoons are subject to parasitism by various mite (Gjelstrup and Hendriksen, 1991) and nematode species (Poinar, 1978; Gunnarsson and Rundgren, 1986). Earthworms can be parasitised by nematodes (Poinar, 1978) and dipteran larvae (Fuller, 1933; Morris and Pivnick, 1991). In southern Australia, earthworms are generally active in the cool, wetter months of winter while the dipterans are most active in the warmer months. The larvae only become significant parasites of earthworms during wet springs or on irrigated agricultural land in spring and summer when earthworm and dipteran activity coincide (G. Kilpin pers. comm.; Kingston, 1988; Baker *et al.*, 1992a).

1.5.2.3 Food

An understanding of the effect of food type on survival, growth and reproduction of earthworms is required; to improve the ability to culture vermicomposting species

Table 1.2 Predators of earthworms

Predator	Location	Habitat	Reference
Birds			
Woodcock	Maine, USA	woodland	Vander-Haegen *et al* 1993
Woodcock	North Carolina	farmland	Stribling and Doerr (1985)
Woodcock	Derbyshire, England	woodland/pasture	Hirons and Johnson 1987
Great Snipe	central Norway	sub-alpine/low-alpine	Loefaldli *et al* 1992
Red-Billed Coughs	Cornwall, England	farmland	Meyer 1990
Stone Curlew			Green and Tyler 1989
Golden Plovers			Thompson and Barnard 1984
Rooks			Waite 1981
Brown Kiwis	North Island, NZ		Reid *et al* 1982
Ring-Billed Gull	Ontario, USA	farmland	Tomlin and Miller 1988
invertebrate feeding birds	Vale of Aylesbury, England	farmland	Tucker 1992

Table 1.2 continued. Predators of earthworms

Predator	Location	Habitat	Reference
other vertebrates			
Badger	north-east Scotland		Brown 1983
Badger	Swiss Midlands		Stocker and Lueps 1984
Badger	northern Scotland		Kruuk and Parish 1981, 1985
Badger	Monte Baldo, Italy		Kruuk and de Kock 1981
Garter Snake	Hidalgo, Mexico	aquatic	Macias-Garcia and Drummond 1988
invertebrates			
beetles (*Carabus* and *Cychrus*)			Gruntal and Segeyeva 1989
Chilopod		beechwood	Judas 1989
New Zealand flatworm	Britain		Boag *et al* 1993
New Zealand flatworm	Northern Ireland		Blackshaw and Stewart 1992
New Zealand flatworm	Northern Ireland	grassland	Blackshaw 1995

(Neuhauser *et al.*, 1980), to investigate what food types are most suitable for culturing soil dwelling species (Hartenstein and Amico, 1983; Butt *et al.*, 1992; Butt, 1993; Butt *et al.*, 1994), to investigate where earthworms are most likely to obtain food resources in a field situation (Shipitalo *et al.*, 1988) and to model the effects of earthworms on nutrient turnover (Marinissen and Ruiter, 1993; Zwart *et al.*, 1994). Finally, it has been of interest to compare the diets of coexisting earthworms (Piearce, 1978) to determine if they are competing for the same food resources (see section 6.2).

Identifying what earthworms consume is difficult due to the poor understanding and definition of the different types of soil organic material which makes up their diet. Unfortunately in studies of earthworm diets, many of the prominent food groups that are described are poorly identified in terms of their source, chemical composition and likely digestibility (Judas, 1992; Piearce, 1978; Gunn and Cherret, 1993). For example, food materials labelled "undetermined" and "non-*Fagus* particles" made up between 40 to 70% of the particulate material in the gut in a study by Judas (1992).

Other studies select identifiable materials and try to determine which food types earthworms consume or prefer. There is abundant evidence that earthworms consume and thrive on herbivore dung (Barley, 1959; Hendriksen, 1991; Holter, 1991; Butt *et al.*, 1992). Litter from certain plant species are consumed and preferred over others (Dickschen and Topp, 1987; Hendriksen, 1990; Phillipson *et al.*, 1976; Lawson, 1993). Preference may depend on nitrogen concentration (Evans and Guild, 1948; Abbott and Parker, 1981; Shipitalo *et al.*, 1988; Boström, 1987, 1988) or the presence of chemical deterrents (Boström, 1987; Westernacher and Graff, 1987). Slightly decomposed material seems to be preferred over fresh material (Waters, 1951; Barley, 1959; Piearce, 1978; Heine and Larink, 1993) due to the lower C:N ratio or disappearance of plant toxins (Boström, 1987). Endogeic species spend most time in the soil layers where roots may be a principle source of organic material. This can be as fresh material such as rootlets, root hairs and mycorrhizal fungi (Gunn and Cherrett, 1993) or older, partially

decomposed roots. Cortez and Bouche (1992) used the short-pulse labelling technique (McDougall and Rovira, 1965) to label living fine roots of plants with ^{14}C. After 24 hours, radioactive material was detected in earthworms which were active in the soil. It was argued that after 24 hours, the ^{14}C has not had enough time to reach the rhizosphere and so earthworms must be consuming roots. However, there is a possibility that ^{14}C could have been consumed from root hairs, mycorrhizal fungi or fine rootlets which are all food sources for earthworms.

Even when it is possible to determine what earthworms consume, it is often difficult to measure which particular components of organic matter earthworms assimilate and digest. Martin *et al.* (1992) used ^{13}C labelled material to determine the pool of organic carbon from which earthworms obtained the majority of their energy and showed that earthworms feed mainly on recent soil organic pools (< 3 years). Enzyme studies show that earthworms are able to digest a wide range of organic compounds particularly carbohydrates (including cellulose) and proteins (Table 1.3) although some studies contradict each other (see Table 1.4). For example, Tracey (1951), Urbasek (1990) and Urbasek and Pizl (1991) all found cellulase enzymes in the intestines of *A. caliginosa*, whereas Nielsen (1962) did not.

Earthworms can digest food directly in their intestines or may form mutualistic associations with microorganisms either in their intestine (Barois and Lavelle, 1986; Barois, 1992; Trigo and Lavelle, 1993) or externally, by lining their burrows with organic materials and mucus which encourages microbial activity (Lee, 1985). Gilot-Villenave (1994) found that the population of microorganisms in the intestines of the tropical earthworm *Millsonia anomala* (Omodeo and Vaillaud) determined what organic materials could be digested by the earthworm and that the community of microorganisms remained in the gut during the life of the earthworm. In enzyme studies, enzymes such as cellulase have been found to have both earthworm and microbial

Table 1.3. Enzymes present in various earthworm species and their origin (W - enzymes were found active in intestinal wall, F - enzymes were found active in gut flora, WF - enzymes were found active from non-sterile intestinal wall and possibly are of microbial origin).

Species	Enzymes found	Origin	Authors
Aporrectodea	chitinase, cellulase.	WF	Tracey 1951
caliginosa	di- and tri-saccharides, amylase.	WF	Nielsen 1962
	cellulases.	W	Urbasek 1990
	amylase, xylanase, lichinase, gycoamylase, laminarinase, cellulase, protease.	W	Urbasek and Pizl 1991
A. chlorotica	chitinase, cellulase.	WF	Tracey 1951
A. icturna	chitinase, cellulase.	WF	Tracey 1951
A. longa	chitinase, cellulase.	WF	Tracey 1951
A. nocturna	chitinase, cellulase.	WF	Tracey 1951
A. rosea	cellulases	W	Urbasek 1990
Bimastus eiseni	cellulase	WF	Tracey 1951
Dendrobaena mammalis	cellulase	WF	Tracey 1951
D. octaedra	di- and tri-saccharides, amylase.	WF	Nielsen 1962
	cellulases.	W	Urbasek 1990
	amylase, glycoamylase, xylanase, laminarinase, lichenase, cellulases, protease	W	Urbasek and Pizl 1991
D. rubidus	cellulases	W	Urbasek 1990
D. rubida	cellulase	WF	Tracey 1951
D. subrubicundra	chitinase, cellulase.	WF	Tracey 1951
D. vejdovskyi	cellulases	W	Urbasek 1990
Eisenia lecens	urease, xylase, arabinase, lactase, glucase, mannitolase, sucrase.	F	Marialigeti 1979
E. rosea	chitinase, cellulase.	WF	Tracey 1951

Table 1.3 continued. Enzymes present in various earthworm species and their origin (W - enzymes were found active in intestinal wall, F - enzymes were found active in gut flora, WF - enzymes were found active from non-sterile intestinal wall and possibly are of microbial origin).

Species	Enzymes found	Origin	Authors
Eiseniella tetraedra	cellulase.	WF	Tracey 1951
Lumbricus castaneus	cellulase.	WF	Tracey 1951
	cellulases.	W	Urbasek 1990
	amylase, glycoamylase, xylanase, laminarinase, lichenase, cellulases, protease	W	Urbasek and Pizl 1991
L. rubellus	chitinase, cellulase.	WF	Tracey 1951
	laminarinases.	WF	Nielsen 1963
	cellulase.	W	Urbasek 1990
L. terrestris	chitinase, cellulase.	WF	Tracey 1951
	laminarinases.	WF	Nielsen 1963
	cellulases.	W	Urbasek 1990
	cellulase, chitinase.	W	Parle 1963b
	chitinase.	F	Parle 1963b
Octolasium cyaneum	chitinase, cellulase.	WF	Tracey 1951
O. lacteum	chitinase, cellulase.	WF	Tracey 1951
	cellulases.	W	Urbasek 1990
	amylase, glycoamylase, xylanase, laminarinase, lichenase, cellulases, protease	W	Urbasek and Pizl 1991
Pontoscolex corethrurus	laminarinase, amylase, maltase, N-acetylglucosaminidase.	W	Zang *et al* 1993
	galactomannase, mannanase, glucomannanase, pullulanase, glycosidases, amylase, cellulase, licheninase, xylanase	WF	Zang *et al* 1993

Table 1.4. Enzymes not found in various earthworm species (W - tested on intestinal wall only, F - tested on gut flora only, WF - tested on non-sterile intestinal wall)

Species	Enzymes absent	Origin	Authors
Aporrectodea caliginosa	sucrase, melezitase, pectinase, xylanase, galactase, chitinase, trehalase, cellulase	WF	Nielsen 1962
Dendrobaena octaedra	pectinase, xylanase, galactase, chitinase, trehalase, cellulase	WF	Nielsen 1962
Eisenia lecens	cellulase, amylase.	F	Marialigeti 1979
Lumbricus terrestris	N-acetylglucosaminidase	?	Li and Shetlar 1965
Pontoscolex corethrurus	cellulase, mannanase	W	Zang *et al* 1993

origins, although their origins remain difficult to distinguish (Parle, 1963a,b; Marialigeti, 1979; Urbasek and Pizl, 1991; Zhang *et al.*, 1993)

Earthworm feeding rates have been estimated by measuring the rate of removal of organic material placed on the soil surface (Van Rhee, 1963), or by measuring the surface casting rate (Tomlin *et al.*, 1995). Both methods may underestimate earthworm feeding, the first because earthworms may also ingest organic material associated with the soil and the second because many species do not always cast on the soil surface and sub-surface casting is often ignored. Estimates of cast production in the field range from 1.5 to 2600 Mg ha^{-1} yr^{-1}, although the higher values are only found in tropical soils and values in temperate soils never exceed 100 Mg ha^{-1} yr^{-1} (Tomlin *et al.*, 1995). Cast production per earthworm have been calculated for various species and range up to 6700 mg g live $worm^{-1}$ day^{-1} (Table 1.5). Food ingestion and casting rates are influenced by food source (Van Rhee, 1963), food supply (Raw, 1962), age and chemical composition of the food (Edwards and Heath, 1975), pH (Springett and Syers, 1984; Nielsen, 1951), temperature (Knollenburg *et al.*, 1985), moisture (Satchell, 1967), soil texture (Thomson and Davies, 1974) ecological grouping (Van Rhee, 1963) and earthworm species, body size and maturity (Barley, 1959). Food ingestion rates are often highest when moderate levels of food concentration are supplied (Martin 1982b). At higher concentrations of food, earthworms do not need to consume as much soil to extract sufficient energy and nutrients and at low food concentrations, earthworms cannot extract enough energy to fuel rapid feeding rates.

The evidence gathered to date strongly suggests that earthworms are generalist feeders, moving through the soil and ingesting a large volume of substrate, but assimilating only a small proportion of the contents. The majority of the material assimilated comes from relatively recent organic matter. The digestive process can be the result of direct enzyme secretion from the earthworm intestine, of mutualistic associations with flora in the gut,

Table 1.5 Casting rates of earthworms (mg g live worm^{-1} day^{-1})

Species	**Casting rate**	**Reference**
A. caliginosa	362 - 2353	Curry *et al.*, 1995
	3750 - 4730	Martin, 1982b
	280 - 420	Barley, 1959
A. trapezoides	2630 - 4190	Martin, 1982b
A. rosea	1130 - 1270	Bolton and Phillipson, 1976
L .rubellus	1920 - 3010	Martin, 1982b
	80 - 460	Shipitalo *et al.*, 1988
	0.43 - 2.55	Dickschen and Topp, 1987
L. terrestris	242 - 713	Curry *et al.*, 1995
	70 - 180	Shipitalo *et al.*, 1988
	6.5 - 16.9	Heine and Larink, 1993
tropical species	6700	Lavelle, 1974

or of increased microbial activity in organic materials mixed with earthworm mucus and deposited on burrow walls.

An understanding of the food requirements of earthworms is important to increase the understanding of how earthworms affect nutrient dynamics in the soil, to be able to predict which environments various species would be able to colonise and to predict which species might compete with each other. Research into the food requirements of earthworms has been hampered, firstly because of the difficulty in categorising organic matter groups in the soil, and secondly because of the difficulty in determining what earthworms actually assimilate. NMR-analysis may be a useful tool to help categorise soil organic matter. A comparison of the NMR spectrum of soil which had passed through an earthworm intestine to the NMR spectrum soil which had not could be made to determine which of the major carbon compounds were lost in the process of digestion (carbohydrates, alkyls, aromatics etc.). The physiology of earthworm intestines may be able to be more fully understood using cell culture techniques to grow intestinal cells in-vitro (C. Lattaud pers. comm.). These cell cultures could be used to measure what compounds earthworms can digest and what compounds can be assimilated.

1.6. Competition as a potential impediment to introducing earthworms

The potential impact of competition on the successful (or otherwise) introduction of lumbricid earthworm species in southern Australia is unknown at present. This section briefly reviews whether the current ecological theory will predict that competition will have a strong impact on introduced earthworms and discusses the problems involved in the predictions of the theory and how they could be overcome. Following this is a discussion on how experiments should be designed to test competitive effects. The final part of this section reviews the current knowledge of competition in earthworm communities and discusses where further research needs to be done.

1.6.1 Competition theory

The importance of competition as a driving force in determining population and community structure became accepted after the publication of Darwin's "Origin of Species" in 1859. Schoener (1983) and Gurevitch *et al.* (1992) surveyed and reviewed many field experiments on competition between species. Schoener (1983) found that in 90% of the studies in the survey, competition was suggested to be the factor most likely to explain observations such as decreases in population size or decreases in survival and reproduction. Competition had a large overall negative effect on the biomass of individuals and populations in the survey by Gurevitch *et al.* (1992). The idea that competition occurs across a wide range of organisms and ecosystems was supported by both surveys. Competition occurs when the growth or reproductive output of one or more organisms is reduced, due to other organism(s) either consuming a shared resource (scramble competition) or physically or chemically blocking access to a resource (interference competition) (Nicholson, 1954). These two different types of competition will be discussed separately below and then the difficulties involved in attempting to detect and measure competition will be discussed.

1.6.1.1 Scramble competition

The theory that relates to scramble competition is the competitive exclusion principle (Hardin, 1960) which implies that when two species occupy the same niche, one will outcompete the other when resources are limited (Gause, 1934). Arthur (1987) calls this principle "the simplest general model on competition between two species, and thus continues to occupy a special place in ecological theory". There are situations where earthworm species with similar ecological requirements coexist in the one area (see section 1.6.3 *Do earthworms compete?*), and the competitive exclusion principle would predict that competition would be occurring between these species. However the principle has been widely criticised because of problems associated with testing the theory such as difficulties in measuring niche overlap between coexisting organisms.

Furthermore, extenuating circumstances have been found which allow two organisms to coexist while using the same resources. These criticisms are discussed below.

1.6.1.1.1 Measuring niche overlap

A practical problem involved in testing the theory of competitive exclusion is measuring the niche space of two coexisting organisms. There has been some debate over the definition of 'niche' (Vandermeer, 1972; Kroes, 1977; Austin, 1985) but for the purposes of this review, I will define a niche as all the resources required for a population to survive and reproduce at a maximum rate. It comprises food, water, shelter, and encompasses the idea of habitat, which includes temperature, light, oxygen, and altitude. Therefore a niche is multidimensional by nature and includes the set of constraints within which a species can survive (Hutchinson, 1957).

Because a niche is multidimensional, it is difficult to describe completely. In field studies, it is never really known whether all the characteristics of a niche have been included within the measurements taken, or whether some important parameters have been overlooked. For example, small differences in resource requirements between two sympatric grassland sparrows only became apparent after careful measurements of where each species spent most time gathering food (Wiens, 1973). These small differences were enough to circumvent direct competition and explain coexistence. Nevertheless, it is possible to measure some of the more obvious resource overlaps between two species. In practice this is usually done by quantifying how two or more species divide up food resources and/or space resources such as nesting sites.

Measuring niche overlap between organisms in the decomposer community is exceedingly difficult. Soils are opaque and it is much more difficult to observe the behaviour of soil organisms than organisms in other environments, although there has been some attempt to circumvent this problem using rhizotrons (Gunn and Cherrett, 1993). There are numerous potential food resources in the soil that are protected and

bound in time and space in many different ways (Golchin *et al.*, 1994a,b). Attempts to categorise food resources in the soil have been totally inadequate for determining whether coexisting species use the same or similar resources (section 1.5.2.3 *Food*). In soil, it is probable that many species affect the supply of a resource to a given species (Hendrix *et al.*, 1986; Verhoef and Brussard, 1990; Schaefer, 1995). Consequently one species may be in competition with a large number of other species (*diffuse competition* - see MacArthur, 1972; Diamond, 1975). In this situation, resource overlap becomes even more difficult to measure because it becomes necessary to measure the resource use of a large number of species.

1.6.1.1.2 Coexistence of species using the same resources

Individuals from two separate species may compete at local scales, but on larger scales species may coexist in an environment that is spatially and temporally variable because of differing life-history characteristics and responses to environmental conditions (Shmida and Ellner, 1984; Warner and Chesson, 1985; Siepel, 1994). Two species may also coexist if other factors (predation, abiotic) keep population densities down so that resources are rarely limiting (Janzen, 1970; Huston, 1979).

The competitive exclusion principle is based on the equations of Lotka (1925) and Volterra (1926) which assume unlimited mobility of individuals. This assumption may have some validity for mobile vertebrates such as birds, reptiles and mammals but may be inappropriate for less mobile organisms such as invertebrates, microorganisms and plants. Siepel (1994) developed a spatial simulation model of species interactions based on measured life history and feeding characteristics of soil microarthropods. In the model, competitive exclusion occurred rarely, or not at all, when mobility was restricted because of differences between organisms in terms of assimilation efficiency, mobility and environmental tolerance. However when mobility was unlimited, competitive exclusion occurred regularly and quickly in the model. Siepel went on to suggest that there is a high diversity of microarthropods in the soil because many species can have the

same niche due to their limitations in mobility and differences between species in terms of their behavioural and life-history characteristics.

The competitive exclusion principle is based on the assumption that the environment is stable, whereas in reality the environment is in constant flux. Species can coexist if the poorer competitor can "hang on" until conditions change and it becomes competitively superior to the other species (Chesson and Warner, 1979). The competitively superior species may not drive the other to extinction before a change in environment reversed it's competitive superiority. This was termed the *storage effect* (Warner and Chesson, 1985) because a species stores enough resources (in terms of offspring or body mass) in periods of resource abundance to overcome competition for resources in periods of resource limitation. Warner and Chesson suggest that this type of strategy would work best for long-lived organisms which have high fecundity, such as trees and many marine species.

Shmida and Ellner (1984) put forward a model whereby plants which used the same basic resources (nutrients, light, water etc.) can coexist by responding differently to resource availability. Competitively inferior species may be able to survive in favourable microsites or refuges which they can exploit because of distinct life-history characteristics and response to resource availability.

These models all have the underlying premise that organisms which use the same resources can coexist by having limited mobility and different responses to the resources and environment. In a heterogenous environment that fluctuates temporally, more than one species may be able to exploit the same resource and coexist by using the resource in a different way, or by being much more mobile and able to exploit new resource patches quickly before being pushed out by other species (pioneer species). Therefore it may be that the competitive exclusion principle is not a good general model for competition between two species, especially for species which are poorly mobile.

1.6.1.2 Interference competition

Interference competition occurs when one organism interferes with the ability of another organism to gain access to a required resource (Birch, 1957), whether that be food, shelter or mates. Examples of interference competition include interrupting mating behaviours (Kuno, 1992; McWilliams, 1992; Kuester and Paul, 1992), disrupting feeding behaviour (Desgranges and Gagnon, 1994; Trandem and Lampe, 1993; Griffith and Poulson, 1993; Shealer and Burger, 1993; Englund *et al.*, 1992; Ziv *et al.*, 1993), direct attack (Thurber *et al.*, 1992; Anderson and Patel, 1994; Griffiths, 1993) and production of chemical deterrents (Nilsson, 1994). In the soil and litter habitat, microorganisms have been shown to chemically inhibit other competitors (Safar and Cooke, 1988), ants have been shown to attack competing species and drive them away from food resources (Fox *et al.*, 1985; Anderson and Patel, 1994) and ant-lions have been shown to be aggressively territorial, with larger ant-lions taking the territories with highest resource value (Griffiths, 1993). Interspecific interference competition usually results in one species gaining access to a superior resource while other species are forced to use resources of lower quality.

Interference competition is often expressed as territoriality between individuals of the same species, especially in mammals and birds (Chitty, 1960). Intraspecific territoriality keeps population growth at a level below that imposed by food exhaustion and ensures that food supply will not run out (Wynne-Edwards, 1962). When the food supply changes, the size of the territory adjusts accordingly (Village, 1982). For example, Matczak and Mackay (1990) found that when food concentrations were high, filter-feeding caddisflies (Hydropsychidae) were close together (no more than 3 mm apart), but when food concentrations were low, the distance between larvae was greater (> 11 mm).

There is no general theory of interference competition, although any species with a set territory is likely to aggressively defend the boundaries from other members of the same species. As such, there is no reported cases of earthworms defending a territory, but this does not rule out interference behaviour by earthworms against other members of the same species or against different species.

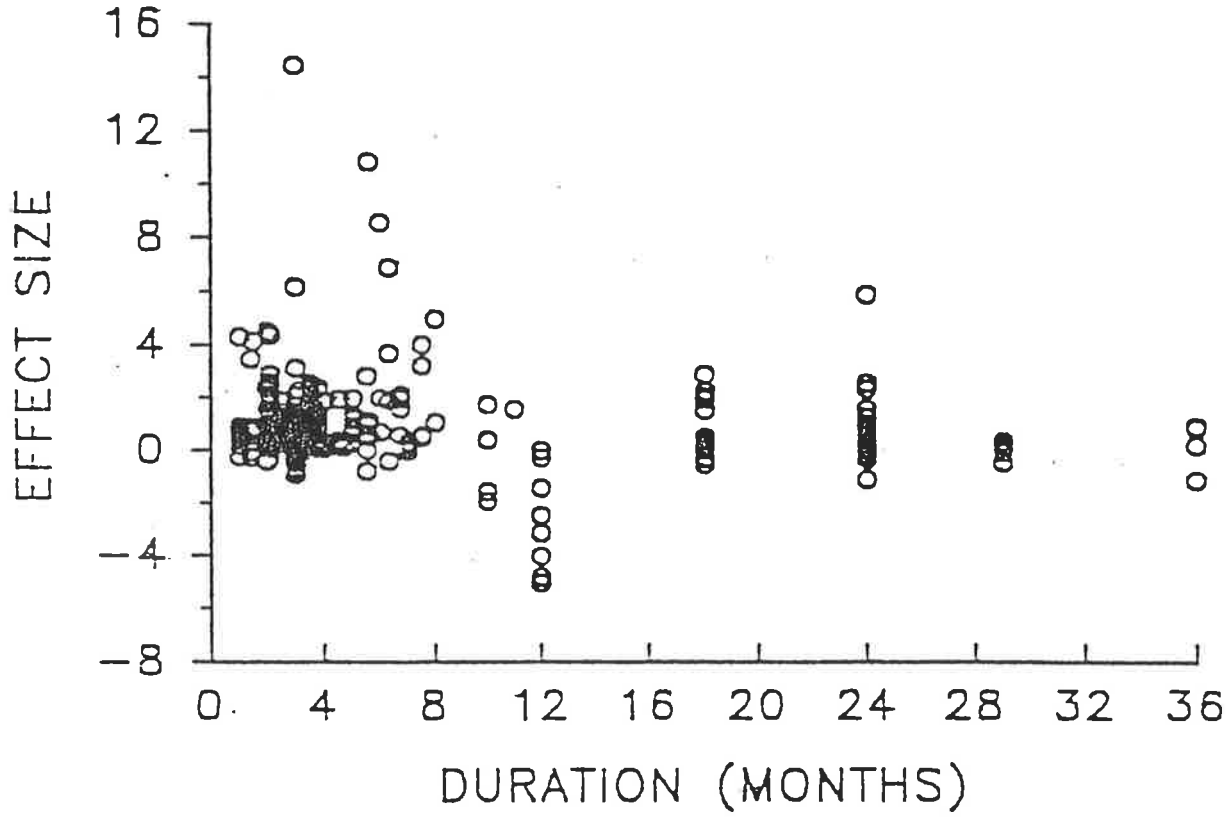
1.6.2. Detection and measurement of competition

There has been some criticism of the experimental designs of some field experiments used to detect or measure competition. Wiens (1977) suggested that some field studies have been too short, spending only a few weeks or a single season assaying populations in any given location. The median length of experiments was four months in the analysis of field studies by Gurevitch *et al.* (1992). However, competition was not found to occur any more or less often in the longer studies. What was found was that shorter experiments gave a greater scatter in magnitude of competitive effect (Fig. 1.3). Therefore, if it is only possible to run short experiments to detect competition, a number of separate experiments should be carried out to account for the variance in effect.

It is important to measure resource use by species in competition studies carefully (Wiens, 1973; and see above section). Resources may come in pulses rather than being present continuously (Grover, 1990), and these periods need to be taken into account when designing experiments. For example, in Rye Lake near New York, the cyanobacterium *Synechococcus* was the superior competitor in the plankton community only under low light and low P, but surface nanoplankton (*Cyclotella stelligera* and *Chlamydomonas gloecapsa*) were stimulated so that they were of equal biomass to *Synechococcus* following rapid and ephemeral increases in P concentration (Wehr, 1993).

Connor and Simberloff (1979) argue that some field experiments have no controls and so do not test the null hypothesis that competition is not occurring. An example of a

Fig. 1.3 The magnitude of the competitive effect plotted against the duration of the experiments. Most experiments were not extended beyond one year and the median length of all the experiments was four months. Effect size was the standarized difference between the means of the experimental and control groups. From Gurevitch *et al.* (1992).



competition experiment with no control is a study in which densities of two rodents, *Mus musculus* and *Peromyscus maniculatus*, were monitored in a ranch building. The maximum abundance of one species coincided with the least abundance of the other. This was seen as evidence for the existence of competition (Scheppe, 1967). However, with no control, there are many other hypotheses that could be developed to explain this phenomenon. For example, the distribution of rodents may be determined by environmental factors rather than direct competition. To determine whether competition has occurred, each species must be monitored in the presence of other competing species and on its own.

Competition is undoubtably important in natural ecosystems and will play a critical role in determining the survival, growth, reproduction and dispersal of some populations. But in many cases it is likely to be an intermittent process, occurring in patches across an ecosystem. Care must be taken when designing experiments to include proper controls, to carry out experiments for a sufficient length of time, and to measure resource use by the interacting species carefully.

1.6.3 Do earthworms compete?

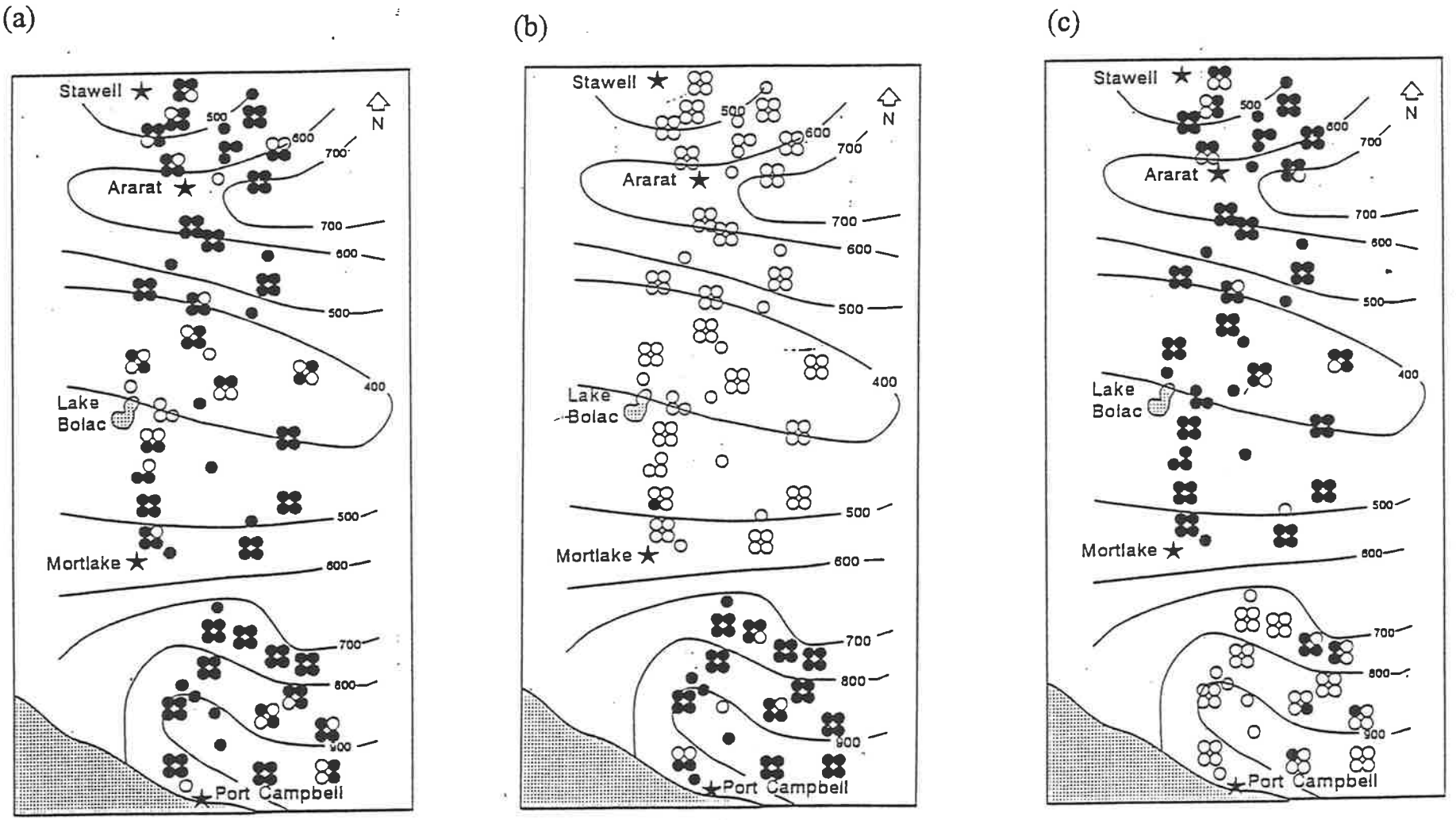
Studies of competition between different earthworm species are surprisingly few, especially since there have been many cases where earthworm species have been introduced into areas where they previously did not exist. The most commonly reported examples of introductions are where lumbricids from Europe have been introduced with agriculture into colonised lands such as North America, New Zealand and Australia (Lee, 1961; Baker, 1992 a,b; Fragosa, 1993; Alban and Berry, 1994; Fender, 1995). Miller *et al.* (1955 in Barley, 1961) and Lee (1961) concluded that in New Zealand, exotic lumbricids were not competing with the native megascolecids. The natives were first being reduced in abundance by the removal of the native vegetation and then the lumbricids (mostly from Europe) were accidentally introduced and prospered under the European agricultural practices and plants. In studies based largely on surveys in

Mexico, Fragosa (1993) suggested that competition best explained morphological and ecological patterns in earthworm communities in only a few situations.

There are cases in Australia where native and exotic earthworm species coexist within the same paddock (Jamieson, 1981; Baker *et al.*, 1992a; Baker *et al.*, 1992b). In a survey carried out by Lawson (1993) exotic lumbricids were found to have invaded native habitat in a site in southern Australia, particularly where there was some human-induced disturbance. The native species was not found to be present in the adjacent pasture, although this species (*G. lateralis*) has been found in agricultural pastures in this area (Baker *et al.*, 1993c). A survey in southern Australia found the native earthworm *Spenceriella* sp. coexisting with *A. trapezoides*, but not with *L. rubellus* even though *L. rubellus* was found over a large proportion of the area surveyed (see Fig. 1.4, Baker, 1996). It is not known whether this distribution pattern is due to soil or climatic preferences, or due to competitive exclusion of one species by the other. There is obviously a potential to examine whether competition occurs between introduced and native earthworm species in southern Australia.

Abbott (1980) compared fresh weight gains of three different earthworm species in single- and multi-species cultures in the laboratory. Competition effects in the form of weight loss were found between *Eisenia fetida* (Savigny) and *M. dubius* but not between *E. fetida* and *A. trapezoides*. Both *M. dubius* and *E. fetida* are epigeic species that consume organic material on the soil surface whereas *A. trapezoides* has characteristics of both endogeic and anecic species and consumes organic material mixed with organic matter as well as organic matter from the soil surface (Martin, 1982b; Lee, 1985; Edwards and Fletcher, 1988). This supports the theory that competition occurs principally between species with similar resource requirements. The paper by Abbott is the only published study where competition between earthworm species was determined using the appropriate controls of a single species by itself as well as in groups with other species.

Fig. 1.4 Distribution of the introduced (a) *Aporrectodea trapezoides*, (b) *Lumbricus rubellus* and the native (c) *Spenceriella* sp. in 163 pasture stires near Mortlake, Victoria. Presence at site indicated by (•), absence at site sampled indicated by (°). Rainfall isohyets are included in the maps (From G.H. Baker, in press).



Earthworms are regarded as being generalist detritivores. Their feeding action is similar to that of a filter feeder because they ingest a large volume of substrate, most of which is indigestible, and extract only a small amount of nutrients and energy per volume of substrate (section 1.5.2.3 *Food*). Therefore like other filter feeders, the concentration and physical placement of food within the soil matrix determines the ability of an earthworm to extract food resources, rather than the total amount of food present. It is possible that earthworms only compete with other individuals if they alter the distribution of food so that the availability of that food is reduced or by promoting microbial decomposition of soil organic matter which reduces the concentration of food in the soil.

Endogeic and anecic earthworms can alter the distribution of organic matter within the soil by their burrowing and feeding behaviour. The action of mixing the soil or burying surface litter may dilute patches of high food value, or remove a food resource from the spatial habitat occupied by another species. For example, if an anecic species buries surface litter or ephemeral patches of food such as dung pats, the density and biomass of epigeic species that could be supported would be reduced as the food and shelter was removed.

Earthworms may also compete with other earthworms of the same or different species within the same spatial habitat. Constant burrowing and mixing may reduce patches of high food value. Soil that has passed through the intestine of an earthworm often has a higher microbial activity than the surrounding soil (section 1.3.3 *Altering energy and nutrient flows*). Therefore in the short term, casts may be microsites of high food availability due to the high microbial biomass, but over time the overall concentration of easily assimilated organic matter in the soil may be reduced by the higher microbial activity and so reduce survival, growth and reproduction of earthworms. It is unlikely that the storage effect (section 1.6.1.1.2 Coexistence of species using the same resources) will be able to maintain populations of competitively inferior species of

earthworms for any great length of time. Earthworms are not particularly long-lived animals, (although some species may live up to ten years, Lee, 1985), nor are they prolific producers of cocoons (see section 1.2.2 *Population biology*). However, as the various models discussed above suggest, if competing earthworm species had slightly different life-history strategies and environmental constraints and were poorly mobile, then they may still be able to coexist in a heterogenous soil. Such information for earthworms species is fragmentary and mostly limited to a few lumbricid species (section 1.2.2 *Population biology*). Mobility of earthworms is known to be quite restricted (section 1.2.4 *Dispersal*) and soil has been shown to be remarkably heterogenous at both micro- (Foster, 1988) and macro-scales (Robertson, 1992).

Competition for food resources has been demonstrated between earthworms species, however the mechanism of competitive effects and how widespread competition can be found to be occurring in earthworm communities is unknown. Research into relationships between earthworms is important in order to gain an understanding of ecological interactions in the decomposer community. An understanding of whether earthworms will compete with each other is required before mass redistributions take place across southern Australia. Of particular interest are interactions between introduced species and species already established in agricultural areas, and the possibility that introduced earthworms may pose a threat to native species and native habitats.

1.7. Summary

Earthworms are keystone species in decomposing communities, altering nutrient and energy flows and changing the soil structure which in turn affects microorganisms and plants. Because of this, they have been introduced into agricultural areas where they have increased the Net Primary Productivity of those systems. There are numerous abiotic and biotic constraints to earthworm activity which limits their geographic range. Competition may be an important constraint on earthworm activity, although there is

little data to support or refute this hypothesis. There are documented cases where two species of earthworms share similar food and space resources. However our understanding of what earthworms actually assimilate makes it difficult to measure total niche overlap. There may be cases where one species of earthworms modifies the soil environment in such a way as to advantage or disadvantage other species. Further research into competition between earthworm species is required to understand fully the constraints on introducing and manipulating earthworms in agricultural soils.

Chapter 2 SITE DESCRIPTIONS, COMMON METHODS AND VALIDATION OF METHODS

2.1. Introduction

The field sites, materials and methods which are commonly used and testing of some methods for their suitability and validity are described in this chapter.

The testing of three methods is described; placing earthworms on moist filter paper to remove their intestinal contents and standardise the water content of their tissue, the effect of three biocides on earthworm growth and survival and removal of earthworms from soil without significantly altering the soil structure.

2.2. Description of field sites

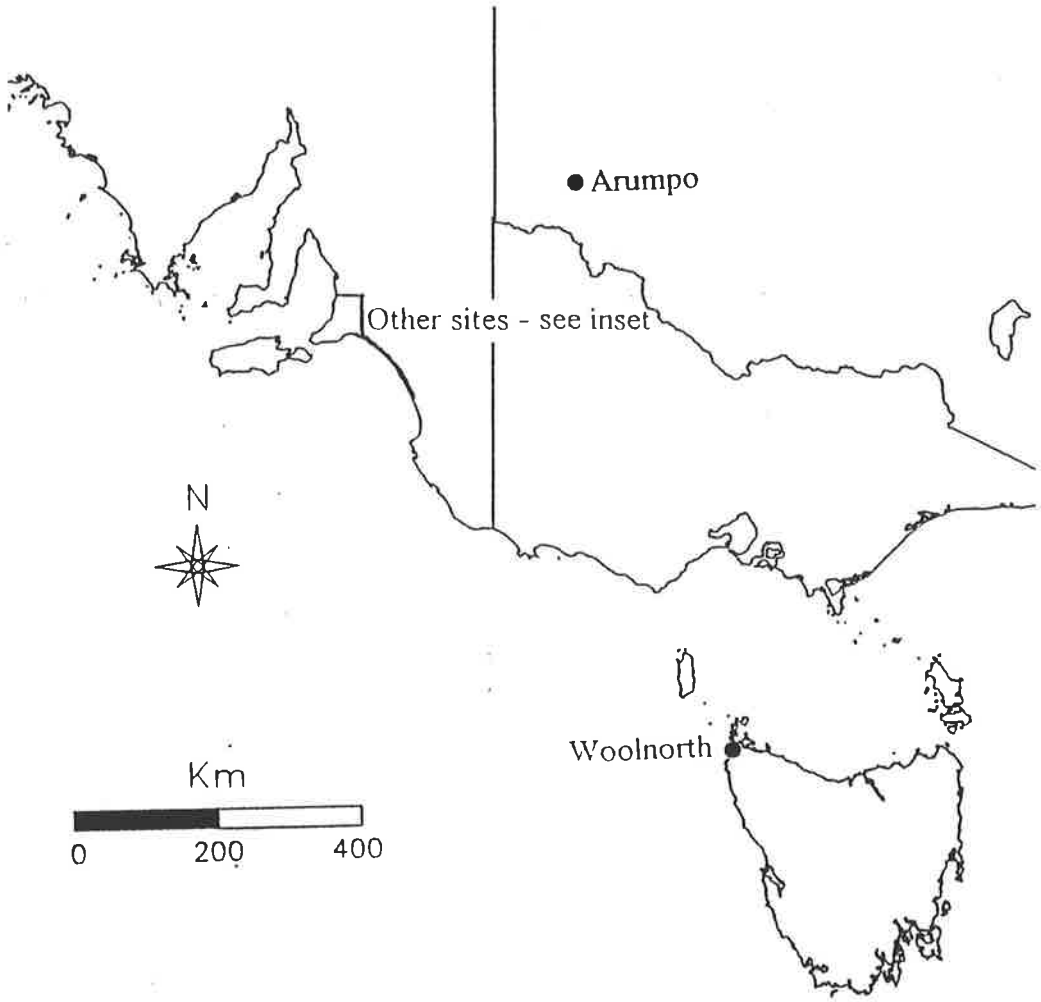
This section describes the location of the earthworm collection sites and the location, topography, rainfall patterns, soil type and chemical composition, vegetation, resident earthworm populations and land use patterns of the field experimental sites.

The collection sites were at the Waite campus of the University of Adelaide [*Aporrectodea trapezoides* (Dugès), *Aporrectodea rosea* (Savigny), *Microscolex dubius* (Fletcher)], Willow Creek (*A. trapezoides*), Woolnorth (*Aporrectodea longa* (Ude), *A. caliginosa* (Savigny)], and Deep Creek Conservation Park [*Gemascolex lateralis* (Spencer)]. The experimental sites were Springmount, Deep Creek, Macclesfield, Myponga and Woodside. The location of each site is shown on Fig 2.1. A description of the experimental sites is given below and is summarised in Table 2.1.

2.2.1 Deep Creek Conservation Park

The location of the experimental and collection site in the Deep Creek Conservation Park is shown in Fig 2.2. The study site is in a relatively undisturbed area. The collection

Fig. 2.1 Location of the experimental and collection sites in the south east of Australia



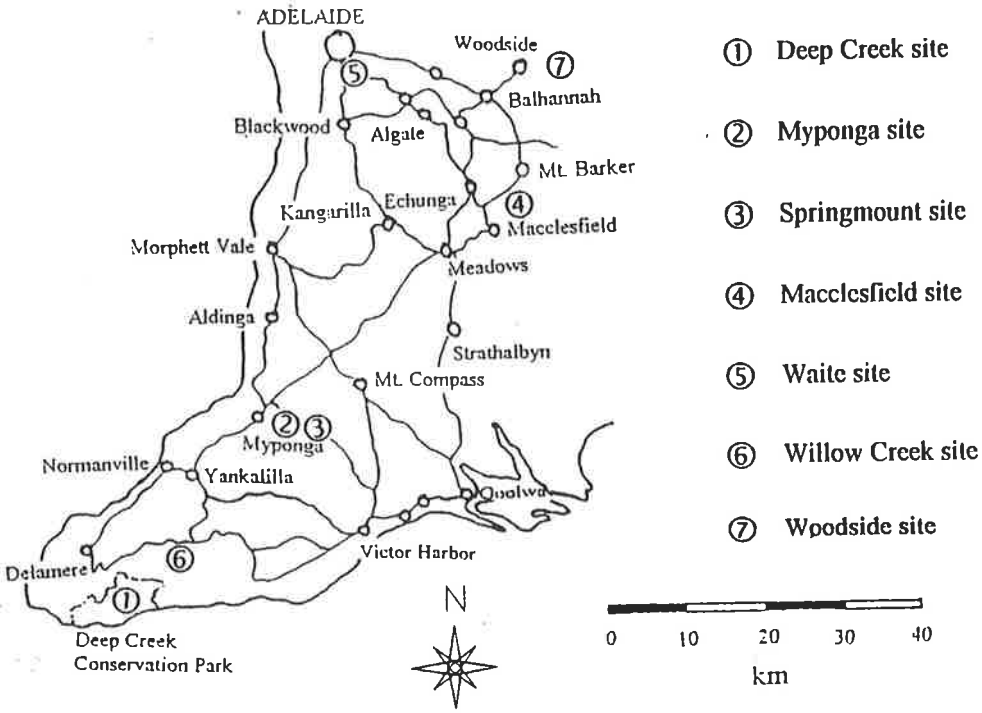


Table 2.1 Environmental variables of the field sites, % carbon determined by combusting at 1200^{o}C in a LECO high-temperature carbon analyser (Merry and Spouncer, 1988). Clay content determined by P0 method (Beech, unpublished).

Site	Annual rainfall (mm)	Soil classification	pH (CaCl2)	Clay (%)	Carbon (%)	Nitrogen (%)
Deep Creek	800†	Bleached, Mesotrophic, Brown Kurosol; sandy loamy	4.8†	17†	6.7†	0.26†
Macclesfield	770^{+}	Bleached-Mottled, Eutrophic, Brown Chromosol; loamy	5.2^{+}	18	3.1	0.19^{+}
Myponga	800^{+}	Melacic, Eutrophic, Grey Kurosol; clay loamy	4.4^{+}	7	5.2	0.53^{+}
Springmount	800*	Bleached, Eutrophic, Brown Chromosol; clay loamy	4.9*	20*	5.1*	0.53*
Woodside	800‡	Malanic-Mottled, Eutrophic, Brown Chromosol; loamy	4.59**	14**	3.1**	na

Soil types are classified using the Australian Soil Classification system (Isbell 1995). Physical and chemical data are based on samples taken from the top 10 cm of soil

* data taken from Baker *et al.*, 1992.

** data from R. Merry (unpublished)

na - data not collected

† data taken from Lawson, 1993

$^{+}$ data from G.H. Baker (unpublished)

‡ Bureau of Meteorology, South Australia

and experimental site were on an area of land that had a slope of between 0 and 12° facing south. Deep Creek receives an annual average rainfall of 800 mm, with the majority of the rain falling between June and October. The soil type is sandy loamy. The vegetation was dominated by stringybark eucalypt [*Eucalyptus obliqua* (L'Hér) - see Fig. 2.3]. Other plants found commonly at the site were thatch grass (*Lepidosperma* sp.) and bracken fern [*Pteridium esculentum* (Forst.f.)] (Lawson, 1993). This vegetation is typical of the Mount Lofty Ranges (Specht, 1972). The experimental and collection site was an area of 10 x 20 m that was 30 m from the fence line that abutted a pasture. The vegetation was similar right up to the fence line.

The distribution and abundance of native and introduced earthworm species in native scrub and in pastures adjacent to the scrub was investigated by Lawson (1993). The dominant earthworm species at Deep Creek was *G. lateralis* which is native to the region. Three introduced species (*A. trapezoides*, *A. caliginosa* and *O. cyaneum*) were also found in the scrub area but were less common and less abundant than *G. lateralis*. The mean abundance and season of peak population abundance are shown in Table 2.2. The native species, *G. lateralis*, is an epigeic species that is active in the litter layer for most of the year. *A. caliginosa* and *A. trapezoides* are exotic, endogeic species and are active only when the soil is moist. During the warmer, drier times of the year they burrow into the soil and become quiescent. The land use for this particular site is for conservation purposes. The site is not used for grazing and there is minimal human disturbance.

2.2.2 Myponga

The Myponga site was on flat land (Fig. 2.4) with an average annual rainfall of 800 mm that falls mostly between the months of June and October. The soil is loam in texture. Myponga has acid soils, which are relatively high in organic carbon and nitrogen (Table 2.1). The vegetation is a mix of grasses and clover and the paddock is used for cattle grazing.

Fig. 2.2. Location of Deep Creek Conservation Park and location of field site

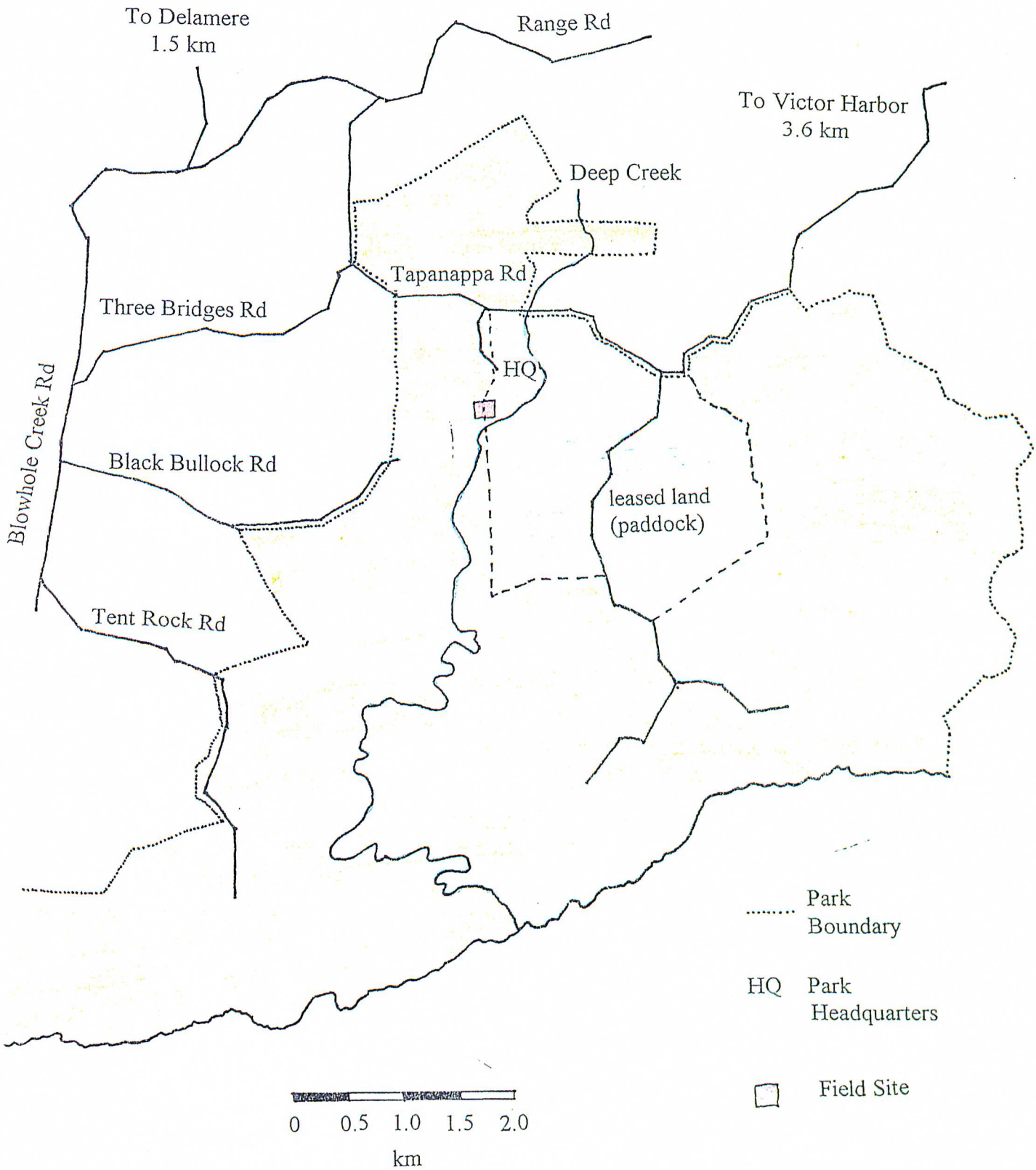


Fig. 2.3 Stringybark eucalypt (*E. obliqua*) forest at Deep Creek Conservation Park

Forest floor of stringybark eucalypt forest at Deep Creek

Table 2.2. Mean abundance and period of activity of earthworms at Deep Creek Conservation Park in the scrub area (Lawson, 1993)

	G. lateralis	*A. caliginosa*	*A. trapezoides*
Mean abundance (ind. m^{-2})	7.8	1.2	0.2
Time of activity	all year	April-Nov.	July-Sept.

Fig. 2.4 Myponga field site

2.2.3 Macclesfield

The Macclesfield site was on sloping ground of 10° (Fig. 2.5) with an average annual rainfall of 770 mm that falls mostly between the months of June and October. The soil is loam in texture. Macclesfield has soils which are relatively high in organic carbon and nitrogen (see Table 2.1). The vegetation is a mix of grasses, clover and broadleaf weeds and the paddock is used for cattle grazing.

2.2.4 Springmount

The site at Springmount (Fig. 2.6) is described in Baker *et al.*, (1992a) where the following information was obtained. It is on a north facing slope (13°), receives an annual average rainfall of 800 mm, with the majority of the rain falling between June and October. The soil is loam in texture. Springmount has acid soils, which are relatively high in organic carbon and nitrogen (Table 2.1). The vegetation is a mix of grasses, clover and broadleaf weeds and the paddock is used primarily for sheep grazing but is occasionally used for cattle grazing.

Springmount has been used as a survey site for earthworm populations by G.H. Baker since 1988. The earthworm population is made up of *A. trapezoides* which is present at a density of between 200 and 400m^{-2} and *A. caliginosa* that is present at a density of about 150 to 350m^{-2} (Baker unpublished data). Both species are active and are at their greatest abundance between June and October in the top ten centimetres of the soil and for the rest of the year are found quiescent further down the soil profile (Baker *et al.*, 1992a).

2.2.5 Woodside

The experimental site at Woodside is on gently sloping land (4°), facing east (Fig. 2.7); it has an average annual rainfall of 800 mm yr-1 with the majority of the rainfall falling between April and October. The soil is a grey loam which has a high carbon and nitrogen content (Table 2.1). The vegetation is dominated by grasses and the land is

Fig. 2.5 Macclesfield field site showing field cages within a bird exclosure

Fig. 2.6 Springmount field site during summer showing bird exclosure

used for cattle grazing. The resident population of earthworms include *A. rosea*, *A. trapezoides*, *A. caliginosa* and *M. dubius* (G.H Baker, *unpublished data*).

2.3. Description of commonly used methods

2.3.1 Field cages

Cages were constructed from 300 mm diameter PVC plastic pipe, cut into 20 cm lengths. The details of the method are outlined in Baker *et al.*, (1996). Fine, plastic mesh was secured over the base and top of the cages to keep earthworms contained within the unit (Fig. 2.8). Soil in the cages could either be mixed or intact.

To collect intact soil cores, lengths of pipe were inserted into the soil in early spring (September to October) while the soil was moist. The cages were removed with the soil column intact in the dry summer period (December to February), when the majority of earthworms had moved down the soil profile to aestivate. Fine, nylon mesh was secured over the base before the cages were placed back in the soil. These cages could then be used in the following growing season (autumn-winter).

For mixed soil, a motorised post-hole digger was used to create a hole large enough for a cage to be placed inside. A fine, nylon mesh base was secured over the base of the cage and soil was handsorted back into the cage to remove any earthworms. These cages could be used immediately.

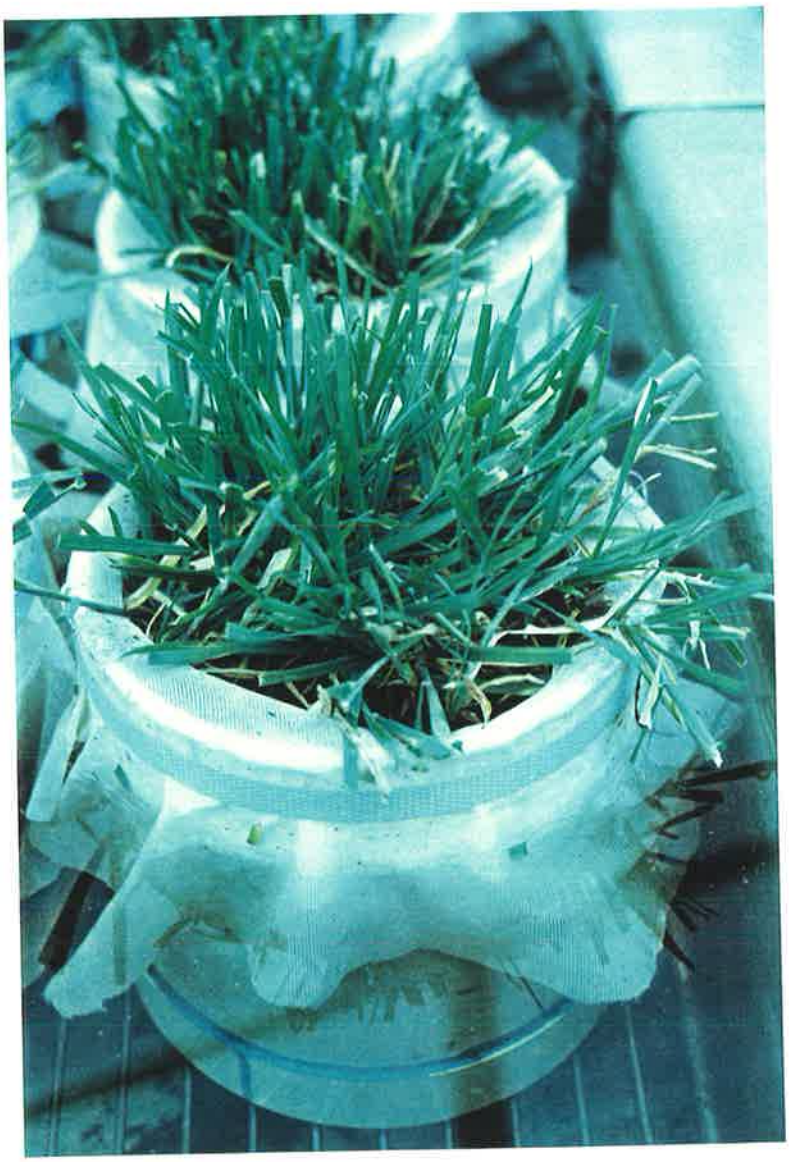
2.3.2 Unmixed soil cores for laboratory experiments

Soil cores were taken for laboratory studies by inserting 15cm diameter, 20cm long PVC pipe into soil and then removing the pipe, keeping the soil column intact. A solid plastic base was glued to the base of the core and fine, nylon mesh was secured over the top of the core to stop earthworm escapes (Fig. 2.9). In some cases, a 10cm diameter hole was cut into the mesh to allow plants to grow through.

Fig. 2.7 Woodside field site

Fig. 2.8 Field cages with fine, nylon mesh supported above the pipe with a wire frame. The field cages are placed within a bird exclosure.

Fig. 2.9 Intact soil cores showing the fine, nylon mesh with a hole cut to allow grass to grow through. These pots were placed in a water bath which is set at 15°C.



2.3.3 Relating soil moisture to water potential

Gravimetric soil moisture is a common and simple measurement of the water content of soils. However it is the water potential (osmotic + matric potential) created by a given gravimetric water content which is biologically important and the relationship between moisture content and water potential varies between soils.

For earthworms, there is a critical limit of water potential, above which they no longer remain active. Some species enter a stage of diapause, where they cease feeding, empty their alimentary canal and construct spherical, mucus lined chambers (Olive and Clark, 1978). Once in this state, they experience almost no dehydration. Other species become quiescent where they cease feeding and enter a state of torpor. They do not excavate chambers lined with mucus and so often suffer quite extreme dehydration (Olive and Clark, 1978). Adults of *A. longa* and *A. caliginosa* were both found to induce diapause whereas juveniles of the same species and both juveniles and adults of *A. rosea* and *A. chlorotica* only became quiescent (Satchell, 1967). The optimum range of water potentials under which different earthworm species are able to be most active varies between species (Lavelle, 1974; Nordström, 1975; Nordström and Rundgren, 1972; Bouché, 1983) depending on the permeability of their cuticle and the osmotic potential of their body fluids (Lee, 1985). For the species *A. longa*, individuals were able to maintain a maximum water content at water potentials less than -60 kPa. Water potentials greater than -620 kPa induced aestivation or quiescence (section 1.3.4.1). When values were intermediate between these two values, earthworm moisture contents were closely governed by the variation in water potential (Kretschmar and Bruchou, 1991).

The water potential of soils in earthworm experiments should be kept between -10 kPa and -60 kPa so that earthworm activity is not limited by water stress. Therefore the relationship between water potential and gravimetric soil moisture was assessed for all soils which were used in laboratory experiments in this and subsequent chapters.

2.3.3.1 Determining water potential using the filter paper method

The method to determine the water potential of various soils was based on the filter-paper method (Greacan *et al.*, 1989). This method, initially developed by Gardner (1937) and Fawcett and Collis-George (1967) was refined further by McQueen and Miller (1968), Hamblin (1981) and Greacan *et al.*, (1989).

A single, labelled filter-paper (Whatman ashless #42, 5.5 cm diameter) that had been heated at 105°C for 30 minutes to kill microorganisms was placed in the soil, ensuring that the filter paper did not touch the sides of the container. The soil was sealed from outside air to stop loss of moisture from the soil and allowed to equilibrate with the papers at 22°C for between 24 hours to six days depending on the moisture level. The filter papers were removed and any soil adhering to them was quickly brushed off and they were immediately weighed to ±1mg. They were then dried at 105°C for at least four hours before being weighed again. The water potential was determined using the formulae in Greacan *et al.*, (1989) :

W: 0.278 to 0.453 $S = \exp(12.27 - 17.93W)$

0.453 to 1.784 $S = \exp(5.55 - 3.11W)$

where S (kPa) is the water potential and W (g/g) is the gravimetric water content of the filter paper

To construct water potential curves to show how the water potential of a soil changes with increasing soil moisture, each soil was wetted to different gravimetric soil moistures and the water potential determined for each moisture. The range of soil moistures tested was kept within that which is optimal for earthworm activity. The soils which were used to determine water potential curves were used in pot experiments where it was important to set the soil moisture content so that earthworms would be fully hydrated active. The soils that were used to construct water potential curves are described in section 2 (Deep

Creek - 2.2.1, Springmount - 2.2.4, Woodside - 2.2.6). For Springmount soil, curves were constructed for soil which was collected in intact soil columns and for soil which had been collected, sieved and repacked into cores. A loam ('artificial soil') was used in some experiments and a water potential curve was also constructed for this soil type with various clay and dung amendments.

2.3.3.2 Springmount - mixed soil

Air-dried soil was sieved through a 5 mm sieve to removed large root clumps, stones and large soil clods. The soil was wetted to a number of moisture levels; 14, 16, 20, 23.1, 25.6, 28.1, 33.3% (g/g). 100g of wet soil was placed into a 300 ml plastic pot and a filter paper placed on top. Another 100g of soil of the same gravimetric moisture was placed on top of the filter paper and compacted to a set volume. A plastic lid was secured to the top of the pot and the seal covered with plastic adhesive tape to stop air flow across the seal. There were two replicate pots for each moisture level.

The relationship between gravimetric soil moisture and water potential of mixed Springmount soil is shown in Fig. 2.10. To maintain a water potential between -10 and -60 kPa, the soil must be kept between 14 and 20% moisture (g/g).

2.3.3.3 Springmount, Woodside and Deep Creek - unmixed soil

Intact soil cores were collected by inserting 7.5 cm dia., 10 cm length, PVC plastic to a depth of 7 to 8 cm in soil at Springmount, Deep Creek and Woodside. The soil cores were placed in a 60°C oven for seven days to kill earthworms (section 2.6) and dry the soil. The cores were placed in a plastic cup (80mm diameter, 65mm length) then weighed and watered to various gravimetric moisture contents (Table 2.3). There were five replicate cores for each moisture level. The core and cup were then placed inside a plastic bag that was secured at the top with an elastic band (Fig. 2.11). The soil was left to equilibrate for seven days at 22°C. After equilibration, a single, labelled filter-paper

Fig. 2.10 The relationship between gravimetric soil moisture and water potential of mixed Springmount soil

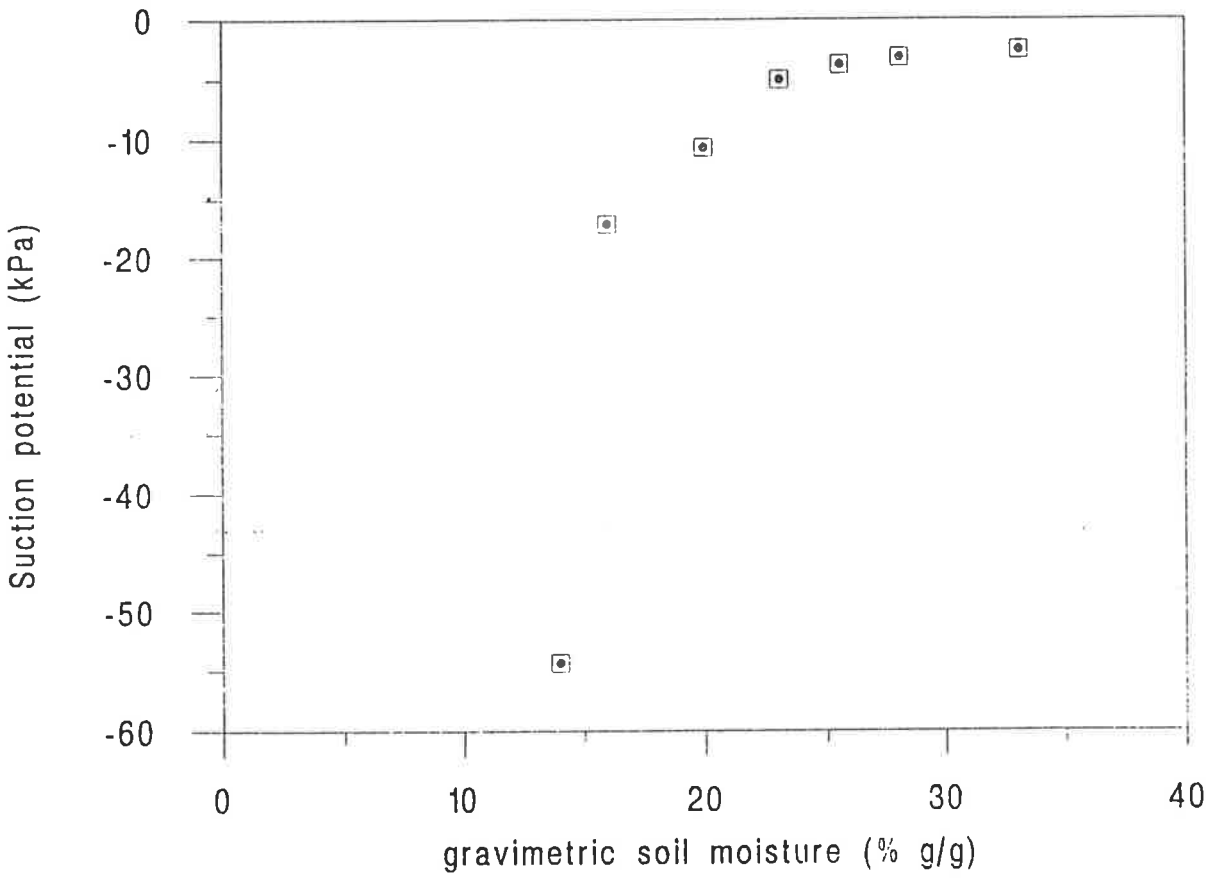


Table 2.3 Gravimetric and volumetric soil moistures and bulk density of soil cores used to determine the relationship between water potential and soil moisture in Springmount, Deep Creek and Woodside soils.

Site	Gravimetric soil moisture (%)	Volumetric soil moisture (%)	Bulk density (g cm-3)
Deep Creek	10	10.5	1.05
	20	20.6	1.03
	25	25.5	1.02
	30	30.3	1.01
	35	35.4	1.01
Springmount	10	11.2	1.12
	15	16.9	1.13
	20	22.3	1.12
	25	27.5	1.10
	30	33.5	1.12
Woodside	15	17.2	1.15
	20	23.7	1.13
	25	28.1	1.13
	30	33.0	1.10
	35	38.6	1.10

Fig. 2.11 Unmixed soil cores placed in a cup, enclosed in a plastic bag

(Whatman ashless #42, 5.5 cm diameter) that had been pre-treated at 105°C for 30 minutes was placed in a slit made in the soil with a spatula and the plastic bag was resecured. The filter papers were then removed after six days and processed as described above.

The relationships between soil moisture and water potential for these three soils are shown in Figs. 2.12, 2.13 and 2.14. To maintain a water potential in intact soil between -10 and -60 kPa, the soil must be kept between 10 and 18% moisture (g/g) for Springmount soil, 10 and 15% (g/g) for Deep Creek soil and 15 to 25% for Woodside soil.

2.3.3.4 "Artificial" soil

A soil was required for a number of pot experiments (Chapter 6), which had no food available for earthworms. Commercially-available, garden-loam which contained low levels of organic carbon (0.18%) was mixed with a clay for this purpose. Clay was added because pure loam has a poor water holding capacity and the range of soil moistures between field capacity and wilting point is very low. The loam was sieved through a 5 mm sieve to removed stones and different types and quantities of food were then added to the soil. The clay was a bentonite taken from a deposit on "Arumpo" station in south-western New South Wales (Fig. 2.1) which also had low levels of organic carbon (0.12%).

An experiment was carried out to determine the concentration of clay required to expand the range of moisture contents suitable for earthworm activity. Four mixes were used; 0% clay, 1% clay, 5% clay and 10% clay. The same filter paper method was used as for mixed Springmount soil (section 2.3.3.2), with the exception that the filter-paper was sandwiched between two blocks of 50g of soil in a 100 ml container. The moisture levels used are shown in Table 2.4.

Fig. 2.12 The relationship between gravimetric soil moisture and water potential of intact cores soil from Springmount

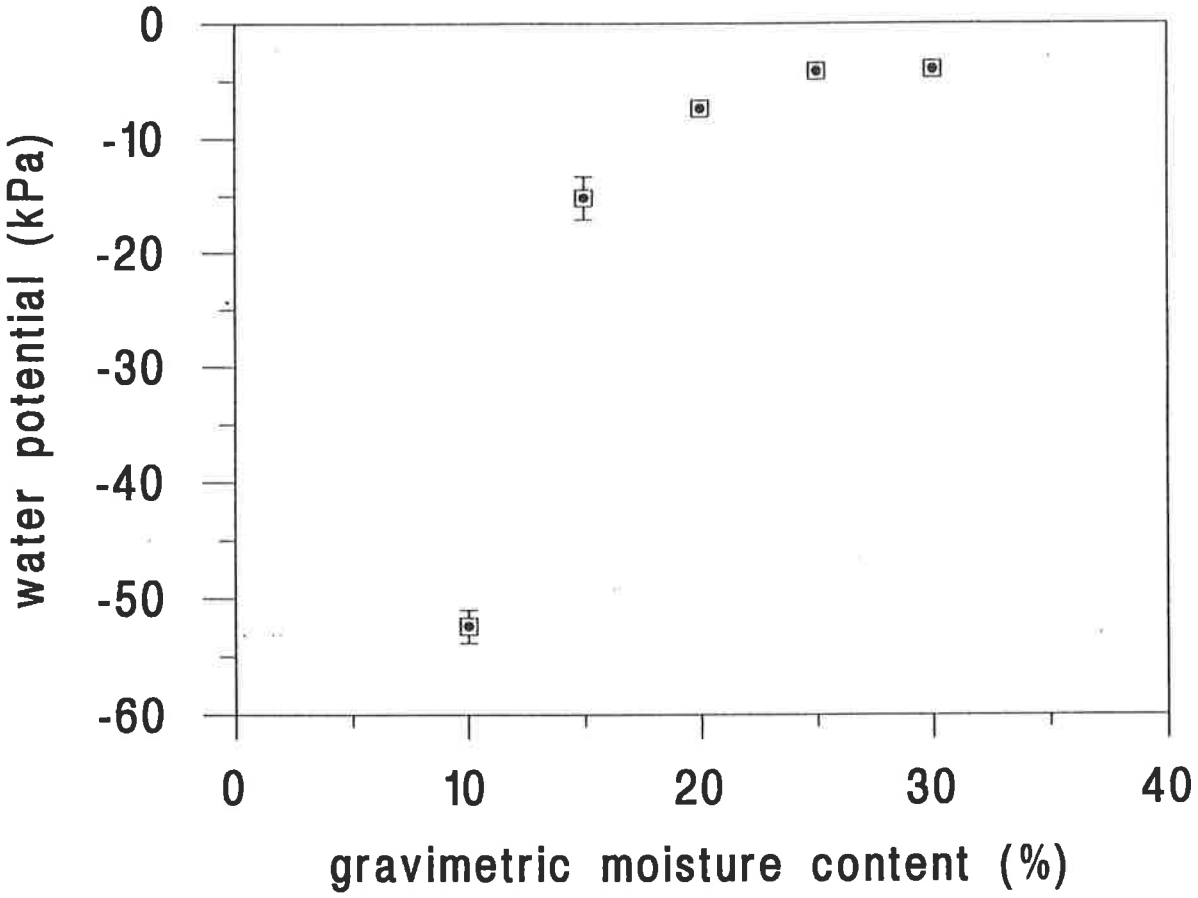


Fig. 2.13 The relationship between gravimetric soil moisture and water potential of intact cores soil from Deep Creek

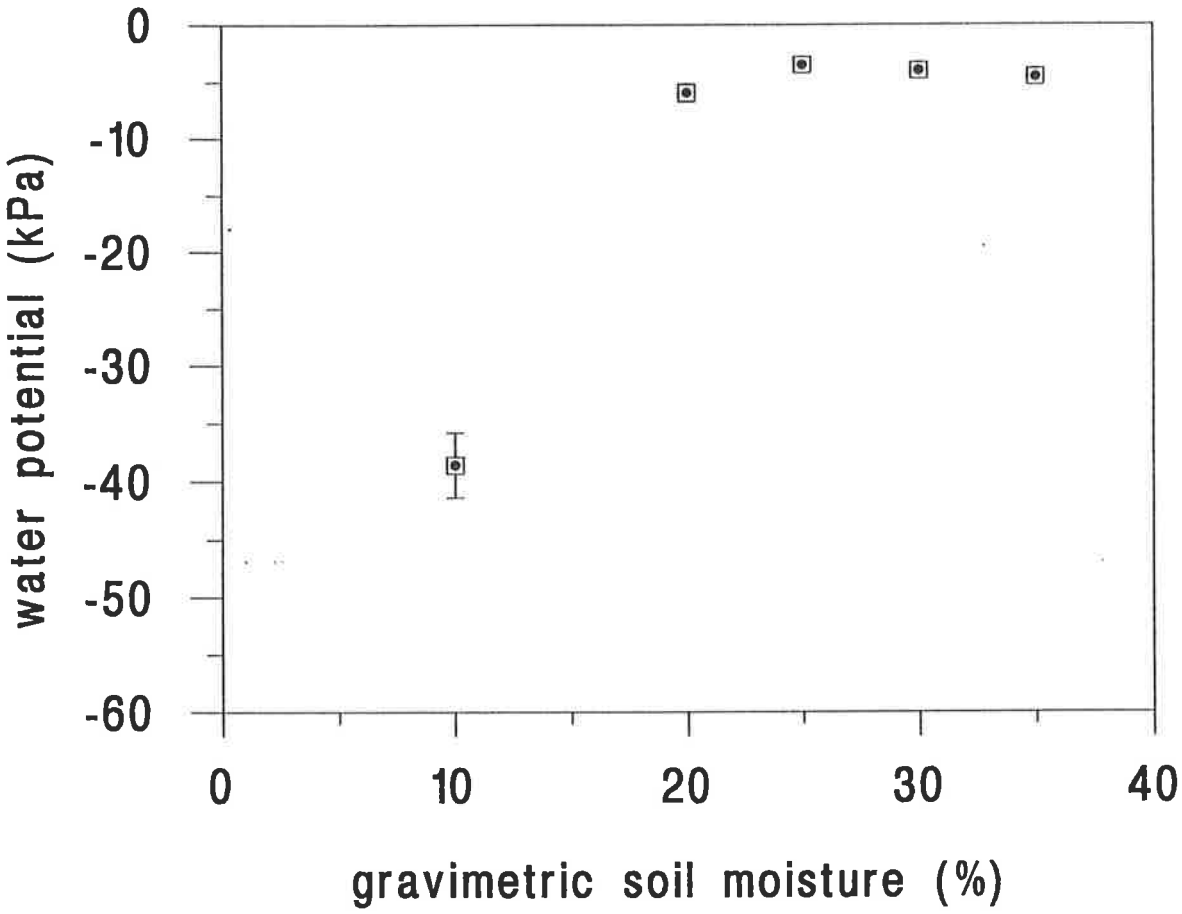


Fig. 2.14 The relationship between gravimetric soil moisture and water potential of intact cores soil from Woodside

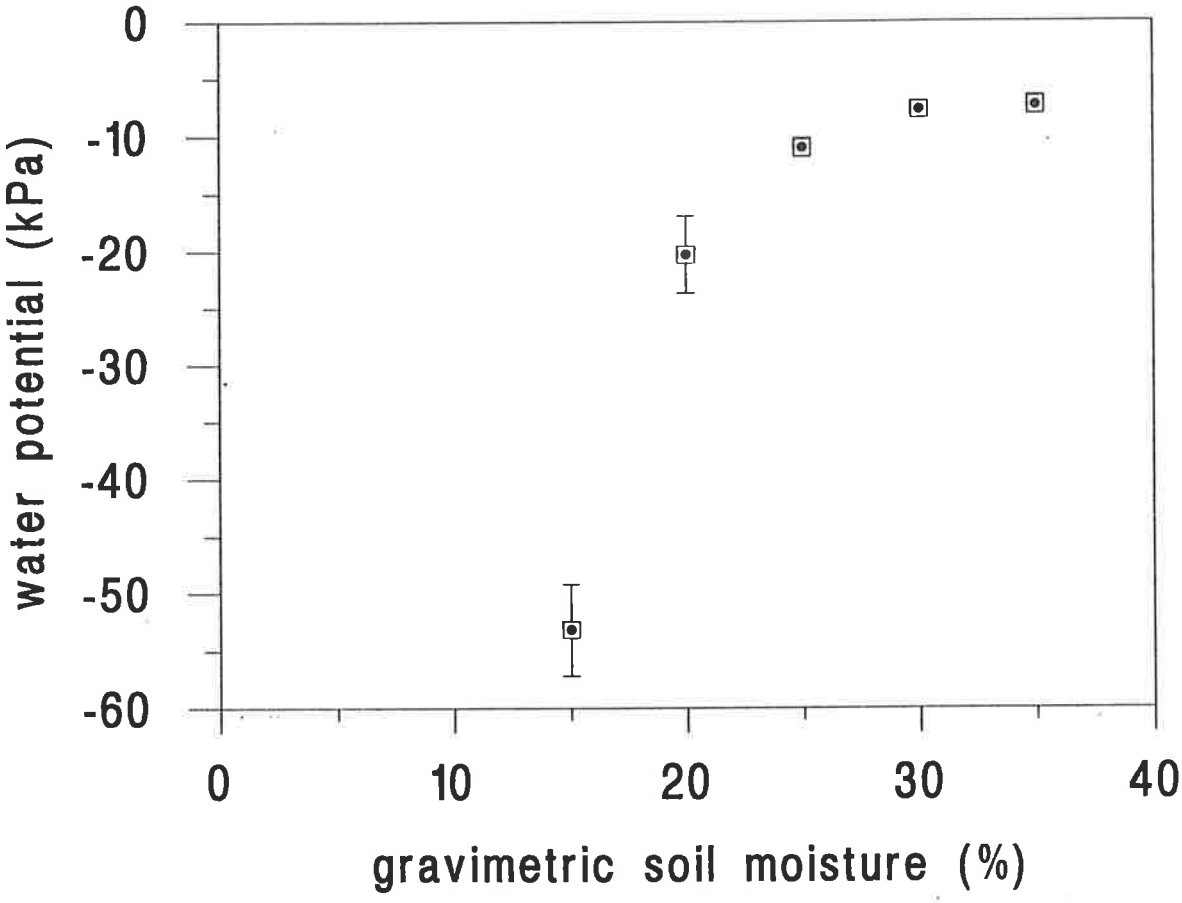


Table 2.4 Adjusted gravimetric moisture levels of garden loam mixed with different concentrations of 'Arumpo' clay and sheep dung to determine the relationship of gravimetric moisture to water potential in the various soil mixes.

clay (%)	dung (%)	moisture levels (%)
0	0	3 4 5 6 7 8
1	0	4 4.3 4.6 5 7 10
5	0	6 6.5 7 10 12 20
10	0	9 9.6 10 10.5 14 20
10	0.7	10 12 14 16 18
10	2.8	13 14 16 18 20 22
10	7.0	13 15 17 19 20 22 24

A second experiment was carried out using a mix of 10% clay : 90% loam to investigate the effect of adding sheep dung on the relationship between gravimetric soil moisture and the water potential of the soil. Three concentrations of dried (60°C), ground (1mm) sheep-dung were added; 0.7, 2.8, 7.0% (g/g) and a control. The moisture levels used are shown in Table 2.4.

Adding clay increased the range of moisture levels at which the water potential was suitable for earthworm activity (Fig. 2.15). The range of soil moisture that kept the water potential between -10 and -60 kPa is 3 to 5% for no added clay, 4 to 8% for 1% clay, 6 to 12% for 5% clay and 7 to 15% for 10% clay added. A 1:10 clay:loam mix was therefore used in further experimentation because it has the greatest water holding capacity and furthermore, high clay contents have been shown to favour earthworm survival and activity (see section 1.5.1). Adding sheep dung also changed the water holding capacity of the soil (Fig. 2.16) so that to keep the water potential between -60 and -10 kPa, the soil moisture must be in the range 12 to 20% moisture (g/g/) when 0.7 or 2.8% (g/g) dung is added and between 13 and 22% moisture (g/g) when 7.0% (g/g) dung is added to a 1:10 clay:loam mix.

2.4. Assessing a "filter paper method" for pre-treatment of earthworms to remove soil from their intestines and to standardise the water content of their tissue

2.4.1 Introduction

Change in live earthworm weight was used as a measure of earthworm performance. The live weight of an earthworm is made up of its body weight (biomass) which is between 65 and 90% water depending on the state of hydration (Lee, 1985) and the amount of soil and organic material in the intestines which varies between individuals (Martin, 1986). It would be desirable if the water content of earthworm tissue could be standardised and the material in the intestine removed, so that live weight bears a direct

Fig. 2.15 The relationship between gravimetric soil moisture and water potential of garden loam mixed with different concentrations of 'Arumpo' clay

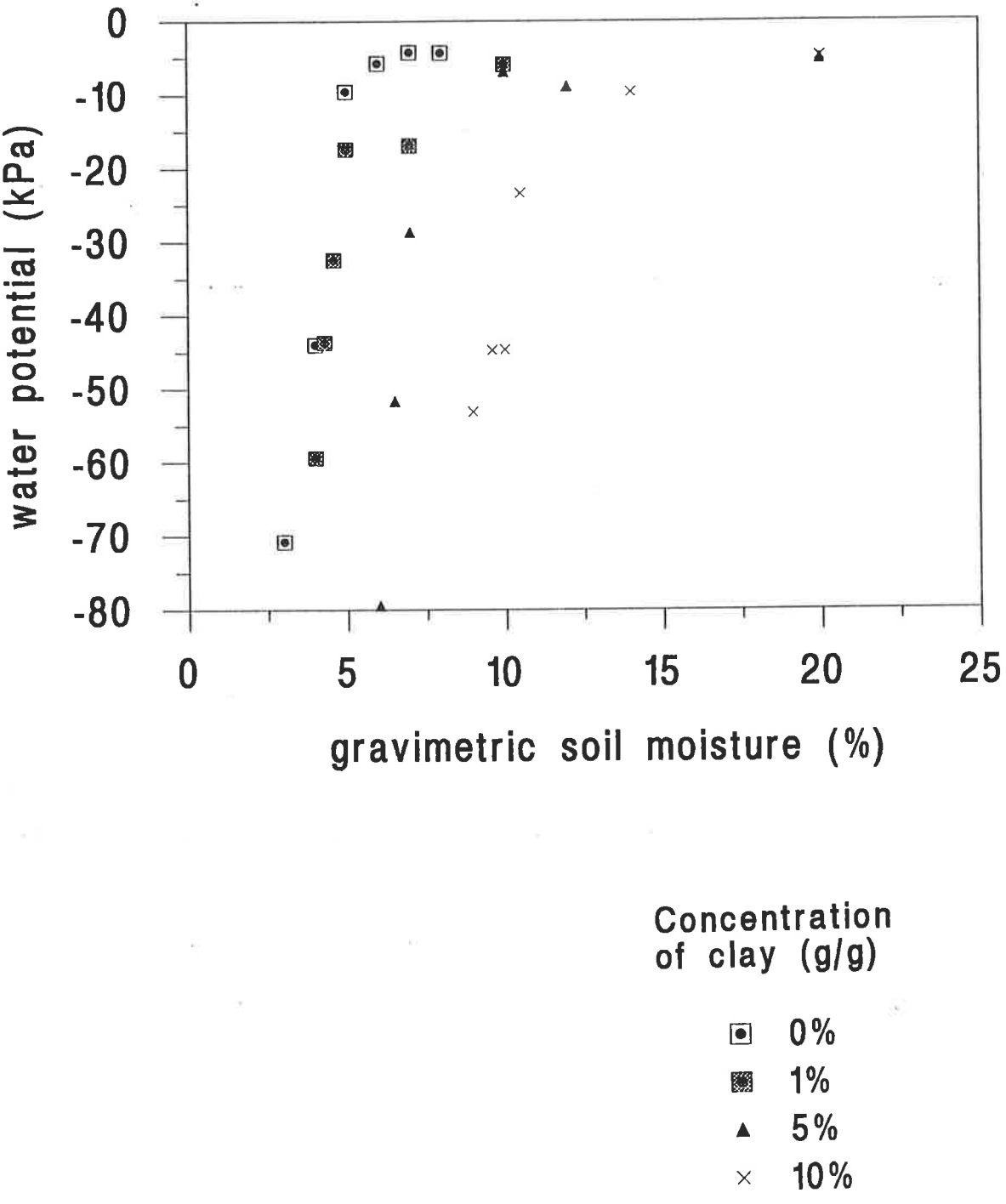
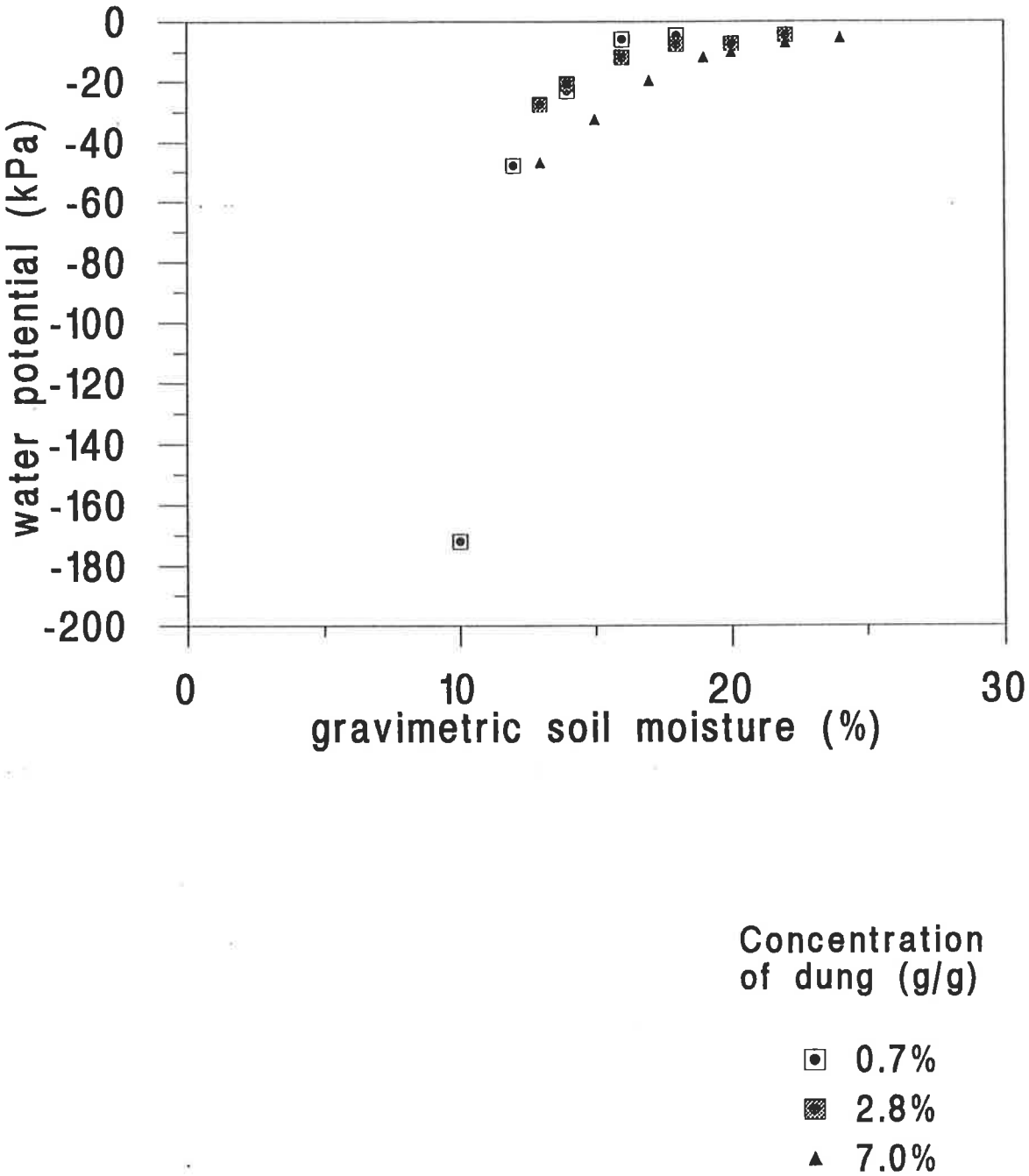


Fig. 2.16 The relationship between gravimetric soil moisture and water potential of garden loam and 10% 'Arumpo' clay mixed with different concentrations of sheep dung (gravimetric).



relationship to the dry weight of the earthworm, and the performance of earthworms in terms of growth can be assessed.

A number of methods have been used to void the intestinal loads of earthworms. Springett and Gray (1992) gently squeezed the gut contents out of the intestines by hand. Martin (1986) kept earthworms in jars with a few millimetres of water for 24 hours, encouraging the avacuation of the intestines by occassionally pressing the anal end of the worm. Bouché (1966) inserted a catheter into earthworm intestines and flushed them with water using a syringe. These methods either risk physically damaging earthworms, are time consuming or are unlikely to have any effect on standardising the water content of earthworm tissue. Placing earthworms on moist filter paper (Joest, 1897; Knight *et al.,* 1992; Thompson *et al.,* 1993) or moist cellulose powder (Doeksen and Couperus, 1962) are methods that have been used to void intestinal contents, but their efficiencies and their influence on voiding intestinal contents or standardising tissue water content have not been evaluated.

In this experiment, four earthworm species were kept in soil at two different water potentials (-5kPa or -60kPa). The earthworms were subsequently placed on saturated filter paper to measure how much intestinal material was eliminated and to determine the water content of earthworm tissue.

2.4.2 Material and methods

The experimental unit was a 300 ml plastic pot containing 300 g of soil and one earthworm. The soil was collected from the top 15 cm of the profile at Springmount and sieved (5 mm). Soil moisture was adjusted to a water potential of either -5kPa or -60kPa (see section 2.3.3.2). Thirty pots at each moisture content were set up for each of the four earthworm species; *A. trapezoides*, *A. caliginosa*, *A. rosea* and *A. longa*, which were collected from mass rearing boxes. The live weight (including intestinal contents) of individual earthworms at the start of the experiment ranged from 150 - 550

mg (mean = 360 mg) for *A. trapezoides*, 170 - 580 mg (mean = 310 mg) for *A. caliginosa*, 120 - 850 mg (mean = 250 mg) for *A. rosea*, and 200 - 670 mg (mean = 370 mg) for *A. longa*. The pots were sealed with plastic lids and tape and kept at 15 ± 1°C for 2 weeks. The weights of the pots were checked every 3 or 4 days. No water loss was detected.

At the end of the conditioning period, all earthworms were handsorted from the soil. Ten earthworms of each species from each moisture content were kept aside (referred to in the text as 0 h). The remaining twenty earthworms from each treatment were transferred individually to screw-top, plastic containers, each containing a disk of Whatman No. 24 filter paper (5.5 cm dia.), saturated with reverse osmosis water by adding water to the filter paper until free moisture just began to appear at the bottom of the containers. Ten earthworms from each treatment were removed from these containers after 24h and the remaining ten after 72 h.

Cast material voided from the earthworms at 24 h (from earthworms kept in containers for both 24 and 72 h) or 72 h (from earthworms kept in containers for 72 h) was collected by filtering any soil material found in the containers through a tared Whatman No. 24 filter paper. This was then dried at 60°C and weighed. The weight of fresh casts was calculated from dry cast weight by assuming that the cast moisture content was equivalent to that of the surrounding soil.

Weight of residual intestinal contents was determined on the 72 h sample by combusting the dried earthworms at 1200°C in a LECO high-temperature carbon analyser (Merry and Spouncer, 1988) and weighing the remaining mineral material. This particular soil loses 7.0% mass on combustion. The mineral content of earthworm tissue was 1.82%, determined by dissecting out the intestines, washing the remaining earthworm tissue of any soil particles, drying at 40°C and ashing at 1200°C. To calculate the weight of soil material that was left remaining in the intestines the following equation was used:

$$IC_{72} = (M_{72} - (0.0182 \times DW_{72})) / 0.9118$$

Where IC_{72} is the calculated intestinal contents after 72 h, M_{72} is the mineral material remaining after earthworms collected at 72 h were incinerated and DW_{72} is the dry weight of earthworms (including any gut contents) after 72 h (see Appendix A).

Total intestinal loads were estimated from the earthworms left in containers for 72 h by adding together the cast material voided at 24 and 72 h and the intestinal contents remaining after 72 h.

At 0, 24 and 72 h, measurements of earthworm live weight and dry weights were made and the water content of earthworms was calculated (all include intestinal contents). The fresh weight of earthworm tissue (excluding intestinal contents) was calculated from these values and the values for intestinal contents (see below).

Live weight was measured by washing earthworms, drying with tissue paper, then weighing. Dry weight was measured after drying these earthworms for 24 h at 60°C. Percentage water content of earthworm tissue after 0, 24 and 72 h was determined using the formula:

$$WC = ((LW - DW) / LW) \times 100\%$$

WC is the water content of earthworms, LW is the live weight of earthworms and DW is the weight of earthworms after drying at 60°C.

To estimate the fresh weight of earthworm tissue at 0 h, live weight was adjusted by subtracting the total intestinal contents of earthworms that were kept on filter paper for 72 h (see below) from the live weight to obtain an estimate of their tissue weight. This assumes that the intestinal loads of these 10 earthworms, isolated at 0 h, were not

significantly different from those of the earthworms that were kept on filter paper for 72 h. The fresh weight of tissue from earthworms removed from containers after 24 h was estimated by subtracting from the live weight the amount of fresh cast that had been voided between 24 and 72 h, and the intestinal contents of earthworms after 72 h. The fresh weight of earthworm tissue after 72 h was estimated by subtracting the amount of intestinal contents found after incineration at 1200°C.

Comparisons of total intestinal loads between incubation treatments were done using a one-way analysis of variance for all means within one species ($P<0.05$). Non-parametric tests were used for analysing the difference in water contents between treatments within a species because variances were significantly different between individual means (Bartlett's test). A Mann-Whitney U test was used to test for significant differences between two means (2-tailed, $P<0.05$).

2.4.3 Results and Discussion

When first removed from the pots, the water content of earthworm tissue was significantly higher in the dry soil compared to the wet soil for *A. longa, A. trapezoides* and *A. caliginosa*, but was not significantly different between soil types for *A. rosea* (Table 2.5).

Placing earthworms on saturated filter paper for 24 or 72 h eliminated any differences in moisture content between the earthworms from the two soil moisture treatments. The method is therefore suitable for standardising earthworm moisture contents.

After 72 h, the amount of soil remaining in the intestines as measured by the combustion method ranged from 0.2 to 1.5 mg (Table 2.6). It appears that there is a certain minimum amount of soil which cannot be eliminated during starvation. Although further starvation may remove this material, it is likely that small pieces of soil material get trapped in the invaginations of the intestines and so longer starvation may still not remove all intestinal

Table 2.5. Water content of the live earthworm, including tissue and intestinal contents (n=10, ± standard error), after the earthworms were removed from either the wet or dry soil and placed in containers with moist filter paper for 0, 24 and 72 hours (see text). Values followed by the same letter (a,b,c,d) are not significantly different at the $P<0.05$ level, within the same species.

	water content (% live weight)	
A. trapezoides	*wet soil*	*dry soil*
0 h	73 ± 1 a	80 ± 2 b
24 h	83 ± 1 bc	84 ± 0 c
72 h	84 ± 0 c	85 ± 0 c
A. caliginosa	*wet soil*	*dry soil*
0 h	73 ± 1 a	83 ± 0 b
24 h	85 ± 1 c	85 ± 0 c
72 h	84 ± 1 bc	85 ± 0 c
A. rosea	*wet soil*	*dry soil*
0 h	72 ± 1 a	73 ± 1 a
24 h	80 ± 1 b	80 ± 0 bc
72 h	85 ± 3 c	83 ± 1 bc
A. longa	*wet soil*	*dry soil*
0 h	73 ± 1 a	81 ± 2 b
24 h	83 ± 1 bc	85 ± 1 cd
72 h	85 ± 0 d	85 ± 0 d

Table 2.6. Dry weight (mg) of earthworm casts produced on moist filter paper after 24 or 72 hours, and soil remaining in the intestines after 72 hours. Figures in brackets are the percentage of casts produced or soil remaining in the intestine as a proportion of the total amount of soil in the intestines before the earthworms were placed in the containers. Values followed by the same letter (a,b) are not significantly different at the $P<0.05$ level, within the same species.

	dry-weight of casts (mg)	
A. trapezoides	*wet soil (-5kPa)*	*dry soil (-60kPa)*
24 h	24.8 (73.8)	6.0 (62.5)
24-72 h	7.3 (21.7)	2.5 (26.0)
remaining	1.5 (4.5)	1.1 (11.5)
total	**33.6 a**	**9.6 b**
A. caliginosa	*wet soil*	*dry soil*
24 h	11.8 (85.5)	1.9 (79.2)
24-72 h	1.3 (9.4)	0.3 (12.5)
remaining	0.7 (5.1)	0.2 (8.3)
total	**13.8 a**	**2.4 b**
A. rosea	*wet soil*	*dry soil*
24 h	11.7 (80.1)	14.0 (72.9)
24-72 h	2.2 (15.1)	4.6 (24.0)
remaining	0.7 (4.8)	0.6 (3.1)
total	**14.6 a**	**19.2 a**
A. longa	*wet soil*	*dry soil*
24 h	25.5 (90.1)	14.3 (93.5)
24-72 h	2.3 (8.1)	0.4 (2.6)
remaining	0.5 (1.8)	0.6 (3.9)
total	**28.3 a**	**15.3 b**

material. However the amount remaining in the intestines after 72 hours was small compared to the final average live weight of the earthworms which was 308 (±32) mg for *A. trapezoides*, 256 (±30) mg for *A. caliginosa*, 191 (±20) mg for *A. rosea* and 323 (±33) mg for *A. longa*.

The intestinal contents of *A. trapezoides, A. caliginosa* and *A. longa* were significantly lower in individuals from drier soil suggesting that their feeding rates are lower. *A. rosea* had similar intestinal contents from wet and dry soil. This suggests that it is a species that may be better adapted to dry soil than the other three species. This is consistent with *A. rosea*'s distribution in southern Australia where it is dominant in the drier, wheat-growing areas while *A. trapezoides* and *A. caliginosa* are dominant in the wetter, permanent-pasture areas (Baker *et al.*, 1995). However Bouché (1972) found little difference in preferences for soil moisture between the four species.

The ash content of earthworms measured by removing the intestines first (1.82%) is lower than that measured in other studies which ranged between 2.7% (Edwards and Niederer, 1988), 4.6 to 7.9% (Fisher, 1988), 15.06 to 23.07% (French *et al.*, 1957) and 4.2 to 35.2% (Stafford and Tacon, 1988). It is likely that the ash contents in these other studies were measured while there was still some material remaining in the intestines.

The method appears to standardise tissue water content and eliminate most of the intestinal contents and was used to prepare earthworms for weighing in this study.

This work forms the basis of a paper (Appendix B); P.R. Dalby, G.H. Baker and S.E. Smith (1996) "Filter paper method" to remove soil from earthworm intestines and to standardise the water content of earthworm tissue. *Soil Biology and Biochemistry*. In Press.

2.5. Assessing the safety of three biocides for use in pot and field experiments

2.5.1 Introduction

In some field and pot experiments, it was desirable to apply various biocides to kill weeds or insect pests. Glyphosate (broad spectrum herbicide) and 2,4-DB (broadleaf herbicide) were used in the field to remove weeds from field cores (Chapter 3) and dimethoate (insecticide and acaricide) was used in glasshouse trials to kill aphids attacking ryegrass plants (Chapter 6). The half-lives of the three chemicals in the soil have been measured at 60 days for glyphosate (Rueppel *et al.*, 1977), less than 7 days for 2,4-DB (Smith, 1978) and 30 days for dimethoate (Patil *et al.*, 1987). To ensure the application of these biocides did not affect the outcome of experiments, the effect of glyphosate, 2,4-DB and dimethoate on the growth and survival of four earthworm species; *A. trapezoides, A. rosea, A. caliginosa* and *A. longa*, was tested in soil from Springmount (The only soil on which the chemicals were applied).

Biocides can enter the soil either by direct application to the soil surface or by being translocated through plant roots (Greaves *et al.*, 1976). The three that were used here can be absorbed through plant foliage and translocated through the plant (Kidd and James, 1992). When they were used to control weeds or insects, they were always applied in situations where plants were growing. Their influence on earthworm growth and survival were therefore tested by applying them separately to both the soil surface and to plants growing in the soil media.

Glyphosate has been reported to reduce the growth of *A. caliginosa* when repeatedly applied to laboratory cultures at two week intervals, at a rate lower than that commercially recommended (Springett and Gray, 1992). However, in another pot experiment (Martin, 1982), it had no effect on *A. caliginosa* when the chemical was mixed with the soil. The herbicide, 2,4-DB, has not been tested on earthworms but a similar chemical, 2,4-D, had no measurable effect on earthworm numbers in the field (Potter *et al.*, 1990), nor on earthworm growth in a microcosm even though residues of

2,4-D were found in the earthworm tissue (Giles, 1983). In contrast, other studies have found that 2,4-D can significantly decrease earthworm growth when mixed into the soil (Martin, 1982), or when earthworms were immersed in a solution of 2,4-D (Ghabbour and Iman, 1967). Dimethoate has been shown to have a small, negative effect on earthworm numbers in pot experiments (Fayolle, 1979 and Atlavinyte, 1981 in Edwards and Bohlen, 1990).

The first experiment described below tested the effects of the biocides on earthworms when the biocides were applied directly to the soil with no plants present. Biocides could only come into contact with earthworms by leaching through the soil, or if earthworms fed or were active on the soil surface. The second experiment tested the effects of the biocides on earthworms when the biocides were applied to the shoots of ryegrass plants growing in the soil. In this situation, earthworms may come into contact with biocides that have been translocated through the plants to the roots and perhaps to the rhizosphere, as well as directly from the soil.

2.5.2 Material and methods

The two experiments were carried out at different times. The experimental design for both experiments was a 4 x 4 factorial. The treatments were a combination of three biocide treatments (glyphosate, 2,4-DB, dimethoate) and a water control and four earthworm species (see above) with six replicates per treatment. The experimental unit was a 1 L pot (dia. 112mm, height 125mm) containing approximately 800 g of air-dried, sieved (5 mm) soil collected from the top 15 cm at Springmount. The pots were kept in a water bath set at 15^{o}C (±1^{o}C) and to maintain a gravimetric soil moisture between 25 and 30% (water potential between -5 and -10 kPa). In Experiment 1 (see below) watering was not required and in Experiment 2, 20 - 30 g water was added per pot each week following biocide application to maintain a constant water potential of between -5 and -10kPa.

Aporrectodea trapezoides and *A. rosea* were collected from Waite (section 2.5) and *A. caliginosa* and *A. longa* were collected from Woolnorth (section 2.7). Earthworm live-weight was determined at the beginning and end of the experiment for each pot. Earthworms were treated before weighing by placing them in a container that contained water-saturated filter paper for 72 hours (section 2.4). The average starting weights of individual earthworms in experiments 1 and 2 are shown in Table 2.7. The starting weight of earthworms was different in Experiment 1 compared to Experiment 2 due to the differences in availability of earthworms.

In Experiment 1, four earthworms were added per pot which was an effective density of 406 m^{-2}. After three days, the biocides were applied to the soil surface using a small hand sprayer that had been calibrated to apply a set volume of spray to a pot (see Table 2.8). The experiment was terminated 10 days after the biocides were applied.

In Experiment 2, perennial ryegrass (*Lolium perenne* L. var. Victoria) was sown at a density of 9 seeds per pot. These were allowed to grow for 12 weeks before the earthworms were added. In Experiment 1, earthworm growth was very poor, and so the density of earthworms was reduced to 2 per pot (203 m^{-2}) in Experiment 2 for *A. caliginosa*, *A. trapezoides* and *A. rosea*, and 1 per pot (102 m^{-2}) for *A. longa* to reduce any effects of intra-specific competition. Biocides were applied in the same manner and rate as in Experiment 1, three days after the earthworms were added to the pots. The experiment was terminated three weeks after the biocides were applied, to allow time for them to be translocated through the plants to the roots.

The effects of biocides were analysed separately for each species, using Kruskal-Wallis analysis because the data could not be normalised.

Table 2.7. Average starting weight of total earthworms (mg pot^{-1}). There were four individuals pot^{-1} in experiment 1. There were two individuals pot^{-1} for all species except for *A. longa* (which had a single individual pot^{-1}) in experiment 2. Figures in brackets are standard error of the mean.

Biocide	*A.trapezoides*	*A.caliginosa*	*A.longa*	*A.rosea*
experiment 1				
control	1884 (124)	1177 (57)	1810 (161)	1171 (42)
dimethoate	1959 (104)	1282 (51)	1810 (109)	1174 (60)
glyphosate	1723 (117)	1175 (40)	1777 (211)	1204 (59)
2,4-DB	1669 (104)	1268 (79)	1835 (126)	1123 (27)
experiment 2				
control	635 (278)	655 (149)	1951 (591)	223 (35)
dimethoate	702 (161)	618 (154)	1891 (324)	210 (38)
glyphosate	662 (178)	671 (162)	1959 (281)	212 (51)
2,4-DB	643 (183)	637 (131)	1854 (393)	206 (47)

Table 2.8. Dilutions and rates of application of glyphosate, 2,4-DB and dimethoate to pots in Experiments 1 and 2.

Biocide	recommended dilution	application rate (ml pot^{-1})
glyphosate	1.0%	3.3
2,4-DB	0.5%	5.2
dimethoate	1.0%	5.8

2.5.3 Results and Discussion

The average survival of earthworms (all species combined) was 96% (range = 80 to 100%) in Experiment 1 and 90% (range = 70% to 100%) in Experiment 2. There were no significant differences in survival between treatments for any of the species in either experiment ($H<3.60$ (exp 1), $H<3.96$ (exp 2), $P>0.05$). Earthworms generally gained weight in Experiment 1 and always lost weight in Experiment 2 (Table 2.9), even though earthworm densities were reduced in the second experiment in an attempt to improve earthworm performance (see below). The biocide treatments in both experiments had no significant effect on weight change of any earthworm species.

Little can be deduced from the difference in performance of earthworms between the "soil only" and "soil + plants" experiments because the experiments were carried out at different times of the year, and for different lengths of time.

A single application at recommended field rates of either glyphosate, dimethoate or 2,4-DB had no effect on the growth or survival of *Aporrectodea trapezoides, A. rosea, A. caliginosa* or *A. longa* under the conditions we used, regardless of whether the biocides were applied directly to the soil surface, or onto living plants. It is concluded one application of either glyphosate, 2,4-DB or dimethoate at recommended rates does not harm *A. trapezoides, A. rosea, A. caliginosa* or *A. longa* in pot or field experiments.

This work forms the basis of a paper (Appendix B); P.R. Dalby, G.H. Baker and S.E. Smith (1995) Glyphosate, 2,4-DB and dimethoate: effects on earthworm survival and growth. *Soil Biology and Biochemistry*. **27**: 1661-1662.

Table 2.9. Effect of glyphosate, 2,4-DB and dimethoate on average weight change of total earthworms (mg pot^{-1}) over the period of the experiments. Values in brackets are standard errors of the mean. There were no significant differences between biocide treatments for each species in either experiment.

Biocide	*A.trapezoides*	*A.caliginosa*	*A.longa*	*A.rosea*
Experiment 1				
control	23 (19)	38 (15)	8 (14)	-12 (4)
dimethoate	2 (14)	- 7 (17)	- 2 (10)	13 (4)
glyphosate	27 (7)	37 (15)	8 (10)	9 (7)
2,4-DB	44 (14)	33 (6)	3 (14)	30 (7)
Experiment 2				
control	-109 (49)	- 14 (30)	-308 (229)	-64 (16)
dimethoate	- 48 (44)	-112 (38)	-201 (65)	-65 (15)
glyphosate	- 78 (76)	- 44 (48)	-114 (57)	-80 (18)
2,4-DB	-105 (85)	- 9 (21)	-271 (193)	-40 (14)

2.6. Obtaining earthworm free, unmixed soil that is safe to use in pot experiments

2.6.1 Introduction

In Chapter 6, a method was required to produce intact, earthworm-free soil cores. The methods that have been developed for removing earthworms without disturbing the soil have mostly been designed for sampling field populations of earthworms. However they may not necessarily be useful for producing earthworm free soil for laboratory experiments, because they do not remove all earthworms, they may adversely affect earthworms that are subsequently reinoculated and they may affect other soil organisms or plants. The criteria I used to select a method were that it must remove all earthworms from the soil and have minimal effect on the physical structure of the soil, or on earthworms that are experimentally reintroduced at a later date.

A number of methods have been used which reduce earthworm populations. Earthworms can be excluded from intact soil cores by removing the cores when the soil has dried out (during the warm, dry summer) and securing the base with plastic mesh (Baker *et al.*, 1996). The basis of this method is that earthworms burrow down the soil profile past the bottom edge of the core (15 cm) to aestivate as the soil dries, leaving the soil core relatively earthworm-free. This method excludes most earthworms from the soil column but does not remove cocoons that hatch once the cores are remoistened, contaminating the cores. This is particularly important for epigeic species, such as *M. dubius,* which do not burrow into the soil to aestivate, but survive as cocoons near the surface in dry conditions. In some years, the soil remains moist over summer and all earthworms stay near the surface for nearly the whole year. A further problem with this method is that it does not exclude some juvenile earthworms which remain quiescent in the top 15 cm and become active once the soil is remoistened.

Electroshocking has been used to sample earthworms, or reduce their numbers in the field (Walton, 1933; Satchell, 1969; Thielemann, 1986; Blair *et al.*, 1995). Electroshocking removes about 67% of earthworms and it is unlikely to affect soil structure, plant growth, other soil fauna (Blair *et al.*, 1995) or have any effects on earthworms reintroduced at a later stage.

Freezing the soil to -20°C has been used to kill soil fauna and leave the soil essentially intact (M. Judas, pers. comm.). However unless the soil is quite dry, freezing the soil may affect soil structure as ice particles form between soil aggregates, forcing them apart. The method may be acceptable in work on soils where freezing regularly occurs during winter, but would not be appropriate in southern Australia.

A number of chemicals have been tested for field sampling of earthworms, including formalin (Raw, 1959; Baker, 1985), potassium permanganate (Dawson *et al.*, 1938 in Bouché & Gardner, 1984) and mustard (Gunn, 1992). Mustard extracted more earthworms than the other vermifuges (Gunn, 1992) and is likely to break down quickly in the soil, cause minimal damage to plants and is cheap, safe and simple to use. However, mustard is likely to act as an irritant to other meso- and macro-fauna and it may act as a food source for microorganisms.

Formalin has been shown to be both more effective than potassium permanganate in extracting earthworms (Nordström & Rundgren, 1972; Bouché & Gardner, 1984) and less effective (Raw, 1959; Gunn, 1992). Although formalin does not extract 100% of earthworms, it is likely that those remaining in the soil die from exposure to the chemical (Gunn, 1992). Formalin also kills some plants (eg. clover plants; Gunn, 1992), microorganisms and other soil fauna and so may not always be suitable. Furthermore, formalin may retain activity for some time after it has been applied, and may therefore kill or alter the behaviour of earthworms that are reintroduced to the soil.

Heating soil to 60°C for 7 days was used by Blakemore (1994) to ensure intact soil cores were free of resident earthworms, leaving the soil structure undisturbed. This method is likely to kill many soil organisms other than earthworms (Wiseman *et al.*, 1996) and result in a microbial community dominated by thermophilic species and those which can survive the heating process in heat-resistant, life stages. Heating to 60°C for 7 days also kills all plants growing in the soil. It may have a small effect on soil structure, but this is unlikely to affect subsequent earthworm activity significantly.

This experiment tested three methods for removing or killing earthworms in intact soil cores in the laboratory. These were: heat treatment, mustard extraction and formalin extraction, chosen because they were likely to be effective, simple to set up and use and unlikely to affect soil structure significantly. Heat treatment and mustard were unlikely to adversely affect reintroduced earthworms, and the effect of formalin on reintroduced earthworms was unknown.

2.6.2 Materials and Methods

Intact soil cores were collected from Springmount by inserting 15 cm dia., 20 cm length PVC plastic pipe to a depth of 15 cm. The cores were then capped on the bottom with a solid base.

There were three treatments (heat, mustard, formalin) with ten cores for each treatment. The heat treatment was placing cores in an oven at 60°C for seven days (Blakemore, 1994), the mustard treatment involved applying 177 mls (≡10 L m^{-2}) of a 15 g L^{-1} solution of Keens® English Mustard powder evenly to the soil surface of each core using a small watering can (Gunn, 1992)and the formalin treated cores had 283 mls (≡16 L m^{-2}) of 1.1% formalin added to the surface (Baker, 1985). Following the chemical treatment, any earthworms that came to the surface in the first hour were removed and counted.

One hour after treatment (or after the cores had cooled in the case of the heat treated cores), five of the soil cores were handsorted to determine how many earthworms survivedand the remaining five had four *A. trapezoides* added to each. *A. trapezoides* was added 24 hours after the cores were removed from the oven, whereas the mustard and formalin treated cores were left for seven days after treatment before *A. trapezoides* was added to allow the active components to dissipate. The cores had fine, nylon mesh secured over the top to stop earthworm escapes and then were kept in the laboratory at a constant temperature of 15°C for seven days. After this period, they were handsorted for earthworms.

A. trapezoides were kept in containers with moist filter paper for 72 hours to standardise the water content of their tissue and eliminate most of the intestinal contents (section 2.4) before they were carefully dried with tissue paper, weighed and added to the cores. Dead earthworms were identified by the fact that they were inactive and not fully turgid when placed in water.

The data for numbers of earthworms recovered were analysed using the Kruskal-Wallis non-parametric test. The percentage change in weight of earthworms was analysed using a 1-way ANOVA, after the data had been normalised using the arcsin-square root transformation.

2.6.3 Results

No earthworms were found in the cores 24 hr. after heat treatment (Table 2.10). Formalin extracted 54% of the *A. caliginosa* and 47% of the *A. trapezoides* in the hour after the chemical was added. All the earthworms remaining in the soil after this time were dead (Table 2.10). Mustard extracted 41% of *A. caliginosa* and 44% of *A. trapezoides* (Table 2.10) in the hour after chemical application, and those remaining in the soil were alive.

Table 2.10. Number of *A. caliginosa* (A. cal.) and *A. trapezoides* (A. trap.) extracted or killed after the three various treatments

Treatment	Extracted		Killed		Remaining alive	
	A. cal.	A. trap.	A. cal.	A. trap.	A. cal.	A. trap.
Heat	0 (0)	0 (0)	all*	all*	0 (0)	0 (0)
Formalin	4.0 (1.9)	1.4 (0.5)	3.4 (0.6)	1.6 (0.6)	0 (0)	0 (0)
Mustard	4.2 (0.7)	1.4 (0.4)	0 (0)	0 (0)	6.0 (1.5)	1.8 (0.2)

Standard errors of means of data from 5 replicate cores are shown in parentheses

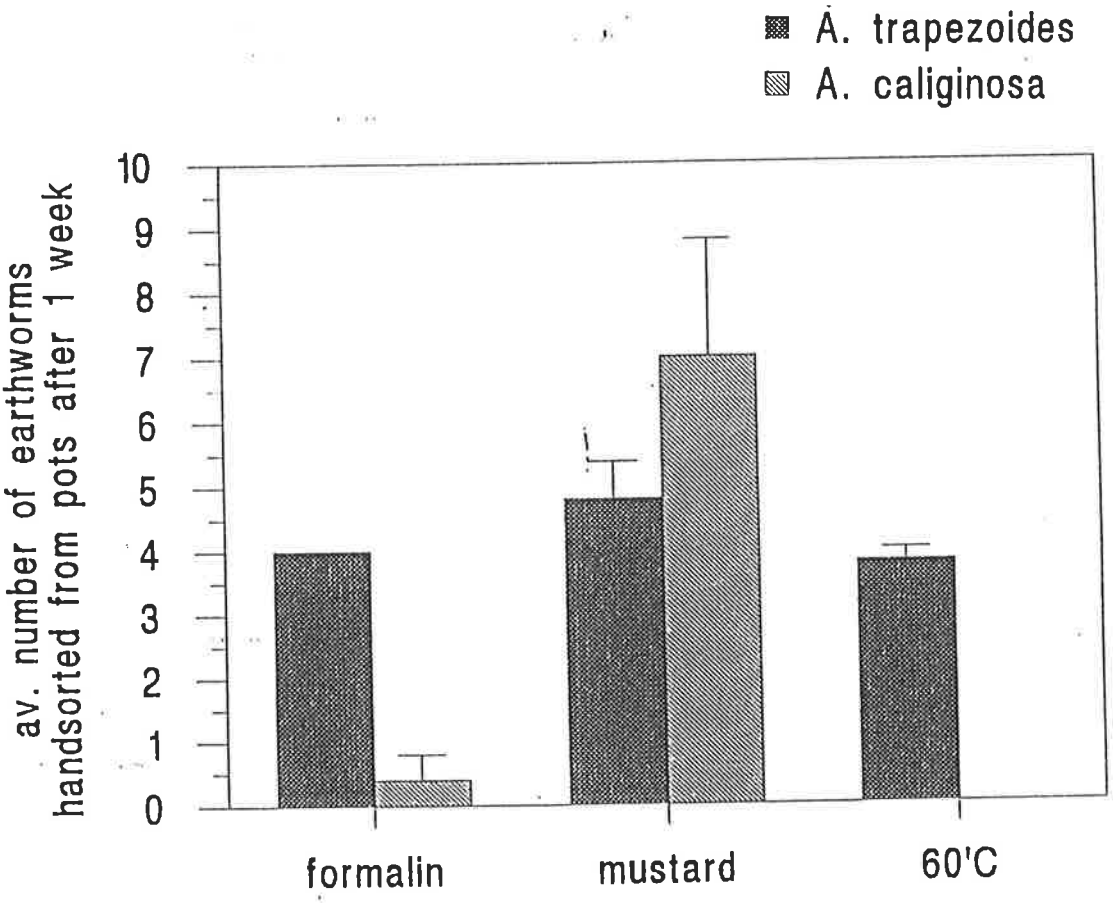
*No earthworms were found in the soil after 1 week in a 60°C oven and it was assumed that all were killed.

The mean numbers of reintroduced *A. trapezoides* that were recovered after 7 days were 4.0 for the formalin treatment, 4.8 for the mustard treatment and 3.8 for the heat treatment (Fig. 2.17). There was no significant difference in the recovery of *A. trapezoides* between treatments (Kruskal-Wallis, $H=2.84$, $P>0.05$). However, the mustard treatment was the only treatment in which the number of *A. trapezoides* remaining in the cores was greater than the 4.0 that were added. On average, *A. trapezoides* gained weight in the cores over the seven day period (between 1 and 11%) and there was no significant difference between the percentage change in weight between treatments (1-way ANOVA, $F=1.12$, $P>0.05$). There were no significant differences in the number of adult *A. trapezoides* between treatments when they were added to the cores (Kruskal-Wallis, $H=0.154$, $P>0.05$) or when they were handsorted from the cores after 7 days (Kruskal-Wallis, $H=0.688$, $P>0.05$). The average number of adults in the population was 1.5 out of 4.0 when they were added and 1.7 when they were handsorted.

The average numbers of residual *A. caliginosa* that were recovered after seven days were 0.4 for the formalin treatment, 7.0 for the mustard treatment and 0.0 for the heat treatment (Fig. 2.17). Significantly more *A. caliginosa* remained in the cores that had been treated with mustard than the other cores (Kruskal-Wallis, $H=11.68$, $P<0.05$).

A flush of fungal growth occurred in all treatments on the exposed soil surfaces after 7 days. The mustard treated soil cores had white fungal hyphae and the other cores had small colonies of fungi that were blue and white in colour.

Fig. 2.17. The average number of *A. caliginosa* and *A. trapezoides* collected from soil cores after the cores had been treated with mustard, formalin or heat (60°C) and four *A. trapezoides* had been reintroduced into each core.



2.6.4 Discussion

All treatments are likely to have had some effect on soil biota other than earthworms. To overcome this problem, soils should be left for a few weeks to allow the soil community to readapt to normal temperature and moisture conditions.

The flush in hyphal growth that followed treatment was probably a result of soil organisms (including earthworms) being killed by the treatments and providing a large food source for the microorganisms that survived the process. The addition of mustard would also have provided a food source for organisms that could utilise it. This flush of fungal growth may have had a short-term effect on subsequent earthworm growth and nutrient cycling in the soil, although this was not measurable after 7 days. It may be desirable to leave cores for a couple of weeks to allow this flush to dissipate.

Some earthworms were torn in two in their haste to reach the surface after formalin and mustard were applied to the soil. The violent reaction of earthworms to mustard or formalin may reduce the value of these methods for estimating earthworm numbers and biomass in the field. More dilute solutions may be nearly as effective at bringing earthworms to the surface, without earthworms tearing themselves.

This experiment aimed to compare the suitability of heat treatment with formalin and mustard application for producing intact, earthworm-free soil cores. The criteria that were set out in section 2.6.1 for a method to be useful for producing intact, earthworm-free soil were that the method must remove all of the earthworms from the soil and have no effect on reintroduced earthworms. The mustard and formalin treatments did not meet these criteria because they did not kill or extract all earthworms. The heat treatment did meet these criteria and also dries the soil so that all soil cores can be watered to a known gravimetric moisture content. It was the method chosen to create earthworm-free, intact soil cores in Chapter 6. These cores were left for 15 weeks after

heat treatment to give time for sown ryegrass to grow and allow the soil community to adapt to the temperature and moisture conditions imposed (see Chapter 6).

2.7 Conclusions

1. The filter paper method appears to standardise tissue water content and eliminate most of the intestinal contents.

2. One application of either glyphosate, 2,4-DB or dimethoate has no measurable effect on *A. trapezoides*, *A. caliginosa*, *A. rosea* or *A. longa*.

3. To remove earthworms from soil, the soil should be heated to 60°C for 7 days

Chapter 3 THE EFFECT OF SPECIES INTERACTIONS ON SURVIVAL, GROWTH AND REPRODUCTION OF THREE SPECIES OF EARTHWORMS

3.1 Introduction

Aporrectodea trapezoides (Dugés), *A. caliginosa* (Savigny), and *A. rosea* (Savigny) are the three most commonly found earthworm species found in pasture soils of southern Australia (Baker *et al.*, 1992 a,b). They have a similar ecology and so there is a potential for strong competitive interactions between them. They are all active in the top ten centimetres of the soil profile, are quiescent during the dry summer months and are most active during the cooler, wetter months (Baker *et al.*, 1992b). *A. rosea* and *A. caliginosa* are regarded as being endogeic and *A. trapezoides* has characteristics of both anecic and endogeic species (section 1.2.1). *A. trapezoides* and *A. caliginosa* are so closely related that they are considered by some to be morphs of the same species (Sims and Gerard, 1985), although they have different environmental tolerances (Briones, 1993).

In 1993 when this work was started, it was the belief of the author that of the three species mentioned above, *A. trapezoides* had the greatest potential for introduction into areas where it is not already present to improve soil properties and increase pasture productivity. If *A. trapezoides* is to be used for such a purpose, it needs to be understood whether intra- or inter-specific competition will limit population densities, or whether there are other, density-independent factors which limit population size. It should be noted that subsequent work has shown that *A. caliginosa* has the same potential as *A. trapezoides* for increasing pasture production and that it's interactions with other species also need to be understood. However the study described here concentrates on investigating interactions between *A. trapezoides* and the other two species.

This chapter reports on a series of experiments that aimed to determine whether there is competition for food resources between these three earthworm species in a pasture soil. The null hypothesis in all experiments was that adding a second species to or increasing the density of the first species would have no effect on the survival or growth of the first species (ie. there was no competitive effect). Investigations of interactions between or within a species were repeated over a number of years to maximise the opportunity to detect competition (section 1.6.2). In all experiments, there was a control of single species (section 1.6.2) for each species.

3.2. Laboratory experiments

3.2.1 Materials and methods

The presence or absence of food was a factor included in the experimental design of laboratory experiments. If adding food eliminated a measured competitive effect, then it would be assumed that reduced growth or survival was due to competition for food resources. The food source was oven-dried pea straw (60°C) that had been ground through a 1 mm sieve. The particle size distribution of the ground straw was 3.6% (>1.0 mm), 49.5% (0.5 - 1.0 mm) and 6.9% (<0.5 mm).

Pea straw was ground through a 1 mm sieve because there is a size limit to organic fragments that can be ingested by earthworms (Bolton and Phillipson, 1976; Piearce, 1978). Furthermore, earthworms have a higher assimilation efficiency when fed finely ground organic matter than when fed organic matter which contains large fragments (Neuhauser *et al.*, 1980; Boström and Lofs Holmin, 1986). Most of the pea straw (94.6%) passed through a 1 mm sieve and 46.9% passed through a 0.5 mm sieve. Piearce (1978) found that *A. caliginosa* could consume particles as large as 3 mm, although the earthworms he used were about twice the size [calculated from Piearce (1972) to average 500 to 800 mg] as the earthworms used here.

3.2.1.1 Interactions between A. caliginosa, A. trapezoides and A. rosea - 1993

This experiment aimed to determine whether there were interactions between *A. caliginosa, A. trapezoides* and *A. rosea* in soil from Springmount in the laboratory. There were eighteen treatments (see Table 3.1) and five replicates for each treatment. Soil was collected from Springmount down to a depth of 15 cm, air-dried, sieved (5 mm) and 3.5 kg added to each pot. The pots were 190 mm in diameter and 185 mm in depth.

A. trapezoides and *A. rosea* were collected from the first Waite collection site and *A. caliginosa* was collected from Myponga. Earthworms were pre-treated (section 2.4) before being added to the pots. The average fresh weight of *A. rosea* in each pot ranged from 241 - 662 mg (mean = 417 mg), *A. trapezoides* ranged from 340 - 810 mg (mean= 528 mg) and *A. caliginosa* ranged from 221 - 486g (mean = 389 mg).

The pots were covered with a nylon mesh to ensure that no earthworms escaped. 2.8 kg of soil was added to each pot. The pots were sealed at the base and the water potential was maintained at -4 kPa (gravimetric moisture of 40%, see section 2.3.3.1) by watering to weight once every week. However the pots dried out to -15 kPa (18% gravimetric moisture) for four days, six weeks into the experiment. The pots were kept in a constant temperature room at 19^{o}C (±1^{o}C).

After 12 weeks, any pea straw remaining on the soil surface was removed with a portable vacuum cleaner (Black and Decker "Dustbuster") and any soil particles remaining in the pea straw were removed by flotation in a saturated sodium chloride solution (360g L^{-1}). The pea straw was then rinsed and oven-dried (60^{o}C for 24 hr) before weighing. Earthworms were hand-sorted out of the soil, pre-treated with moist filter paper (see section 2.4) and dried carefully with tissue paper before weighing.

Table 3.1. List of treatments for the pot experiments. Six earthworms pot^{-1} is equivalent to 200 m^{-2}. In 1993, all treatments were included in the experimental design and in 1995, the treatments with *A. rosea* were omitted. The symbols are used to identify the treatments in the text. R = *A. rosea*; C = *A. caliginosa*; T = *A. trapezoides*; 6 and 12 refer to the number of earthworms pot^{-1} in the single species treatments; *f* = pea straw (food) was added at 14.5 g pot^{-1}; *n* = no pea straw was added.

Single species, low density (6 worms pot^{-1})	**R6*f***	*A. rosea* + pea straw
	R6*n*	*A. rosea*
	C6*f*	*A. caliginosa* + pea straw
	C6*n*	*A. caliginosa*
	T6*f*	*A. trapezoides* + pea straw
	T6*n*	*A. trapezoides*
Single species, high density (12 worms pot^{-1})	**R12*f***	*A. rosea* + pea straw
	R12*n*	*A. rosea*
	C12*f*	*A. caliginosa* + pea straw
	C12*n*	*A. caliginosa*
	T12*f*	*A. trapezoides* + pea straw
	T12*n*	*A. trapezoides*
Two species (6 + 6 worms pot^{-1})	**RC*f***	*A. rosea* + *A. caliginosa* + pea straw
	RC*n*	*A. rosea* + *A. caliginosa*
	RT*f*	*A. rosea* + *A. trapezoides* + pea straw
	RT*n*	*A. rosea* + *A. trapezoides*
	CT*f*	*A. caliginosa* + *A. trapezoides* + pea straw
	CT*n*	*A. caliginosa* + *A. trapezoides*

3.2.1.2 Interactions between A. caliginosa, and A. trapezoides - 1995

This experiment aimed to determine whether there were interactions between *A. caliginosa* and *A. trapezoides* in unmixed soil from Springmount in the laboratory using smaller earthworms than those used in the previous experiment. The list of treatments is shown in Table 3.1 and there were five replicates per treatment. The pots used were the same as for the previous experiment (section 3.2.1.1). *A. trapezoides* was collected from Willow Creek and *A. caliginosa* was collected from Woolnorth. Earthworms were pretreated before being weighed and added to the pots (section 2.4). The average weight of *A. trapezoides* in each pot ranged from 320 - 322 mg (mean = 321 mg) and of *A. caliginosa* ranged from 246 - 248 mg (mean = 247 mg). The percentages of earthworms which were clitellate (not measured in 1993) were 27% (±3%) for *A. trapezoides* and 33% (± 0%) for *A. caliginosa*. No significant differences were found between treatments in terms of average starting weights or percentage of clitellate individuals in the population for each species (1-way ANOVA, d.f.=5, F=0.00, P>0.05). The experiment was then carried out as described in section 3.2.1.1 except that the pots were watered once every week so that the gravimetric moisture was always between 20 and 30% (-10 to -5 kPa, see section 2.3.3.1).

3.2.1.3 Data analysis

A 2-way ANOVA was used to determine whether there were significant differences between earthworm treatments (ie. T6, T12, CT, RT) and food treatments (ie. *f*, *c*) separately. A 1-way ANOVA was used to determine whether there were significant differences between all treatments (ie. T6*f*, T6*c*, T12*f*, T12*c*). A Tukey's test used to determine whether differences between individual means were significant. Data expressed as percentages were transformed to arcsin(square-root(x+0.5)). When a Bartlett's test showed that the variances between treatments were not equal (P<0.05), a Kruskal-Wallis analysis was used instead of an ANOVA. This is identified in the text.

3.2.2 Results

3.2.2.1 Interactions between A. trapezoides, A. caliginosa and A. rosea - 1993

No significant differences between treatments were found in the amount of straw buried (Kruskal-Wallis, H=0.66, P>0.05), with 8.2 to 10.2g (57% to 70%) of the ground pea straw that had been added to the pots still present on the soil surface after 10 weeks (Fig. 3.1). There were no significant effects of adding pea straw on weight change or survival of *A. trapezoides* (2-way ANOVA, d.f.=1, F=1.50 or 0.50, P>0.05) or *A. caliginosa* (2-way ANOVA, d.f.=1, F=1.50 or 3.17, P>0.05). There was no significant effect of adding pea straw on weight loss of *A. rosea* (2-way ANOVA, d.f.=1, F=0.02, P>0.05), but survival of *A. rosea* was significantly lower (2-way ANOVA, d.f.=1, F=9.07, P<0.05) in the treatment with pea straw (89.5%) compared to the treatment without pea straw added (99.2%).

Although the exact numbers of individuals which were quiescent were not measured in this experiment, it was noted that after 10 weeks most *A. caliginosa* and *A. trapezoides* were quiescent whilst most *A. rosea* were still active.

Earthworm survival in each treatment was high in this experiment, ranging from 89.5 to 100%. There was no significant difference in survival between earthworm treatments of all three species (2-way ANOVA, d.f.=3, F<1.98, P>0.05) when the data from straw added and control treatments were pooled.

Earthworms lost weight in all treatments (Fig. 3.2, 3.3, 3.4). There was no significant difference between treatments in terms of weight loss for either *A. trapezoides* (2-way ANOVA, d.f.=3, F=2.14, P>0.05) or *A. caliginosa* (2-way ANOVA, d.f.=3, F=3.18, P>0.05). Because there were no effects of pea straw on weight change, the data from the treatment with added pea straw and the control were pooled for the following analyses. Weight loss by *A. rosea* was significantly greater (2-way ANOVA, d.f.=3, F=5.95, P<0.05) in the single species, low density treatment (R6) than the single species, high

Fig. 3.1 Pot experiment, 1993. Pea straw buried from and remaining on the surface of Springmount soil in pots after 10 weeks at 19 (±1)°C.

. There were no significant differences between treatments in the amount of straw buried at the 5% level. R = *A. rosea*, C = *A. caliginosa*, T = *A. trapezoides*, 6 = six earthworms added pot^{-1}., 12 = twelve earthworms added pot^{-1}. RC, RT and CT are mixed species treatments where six individuals of both species are added pot^{-1}.

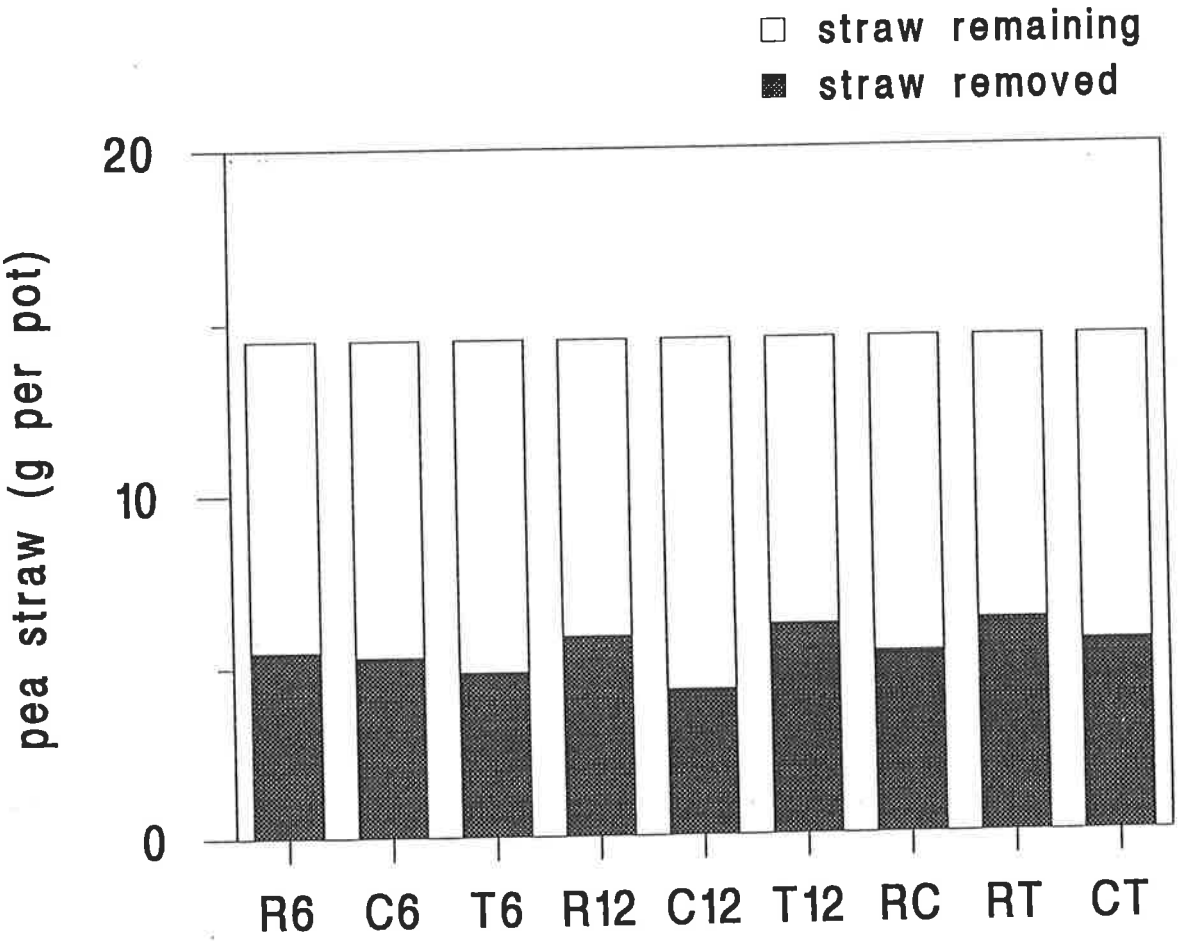


Fig. 3.2 Pot experiment, 1993. Change in weight of *A. trapezoides* kept in pots containing Springmount soil (pea straw added and control combined) for 10 wks at 19 (± 1)°C. Vertical bars represent standard errors of the means, n=10. There were no significant differences between treatments. R = *A. rosea*, C = *A. caliginosa*, T = *A. trapezoides*, 6 = six earthworms added pot^{-1}, 12 = twelve earthworms added pot^{-1}. RT and CT are mixed species treatments where six individuals of both species are added pot^{-1}.

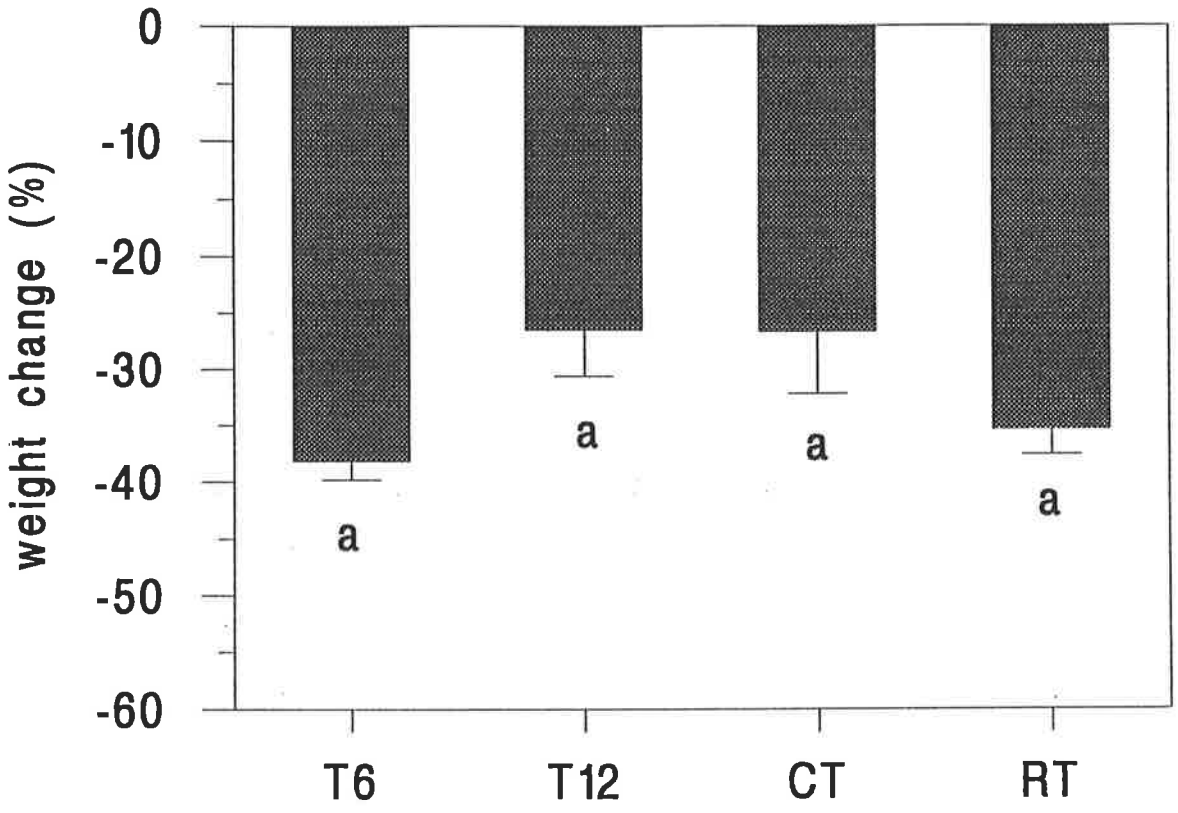


Fig. 3.3 Pot experiment, 1993. Change in weight of *A. caliginosa* kept in pots containing Springmount soil (pea straw added and control combined) for 10 wks at 19 (±1)°C. Vertical bars represent standard errors of the means, n=10. There were no significant differences between treatments. R = *A. rosea*, C = *A. caliginosa*, T = *A. trapezoides*, 6 = six earthworms added pot^{-1}., 12 = twelve earthworms added pot^{-1}. RC and CT are mixed species treatments where six individuals of both species are added pot^{-1}.

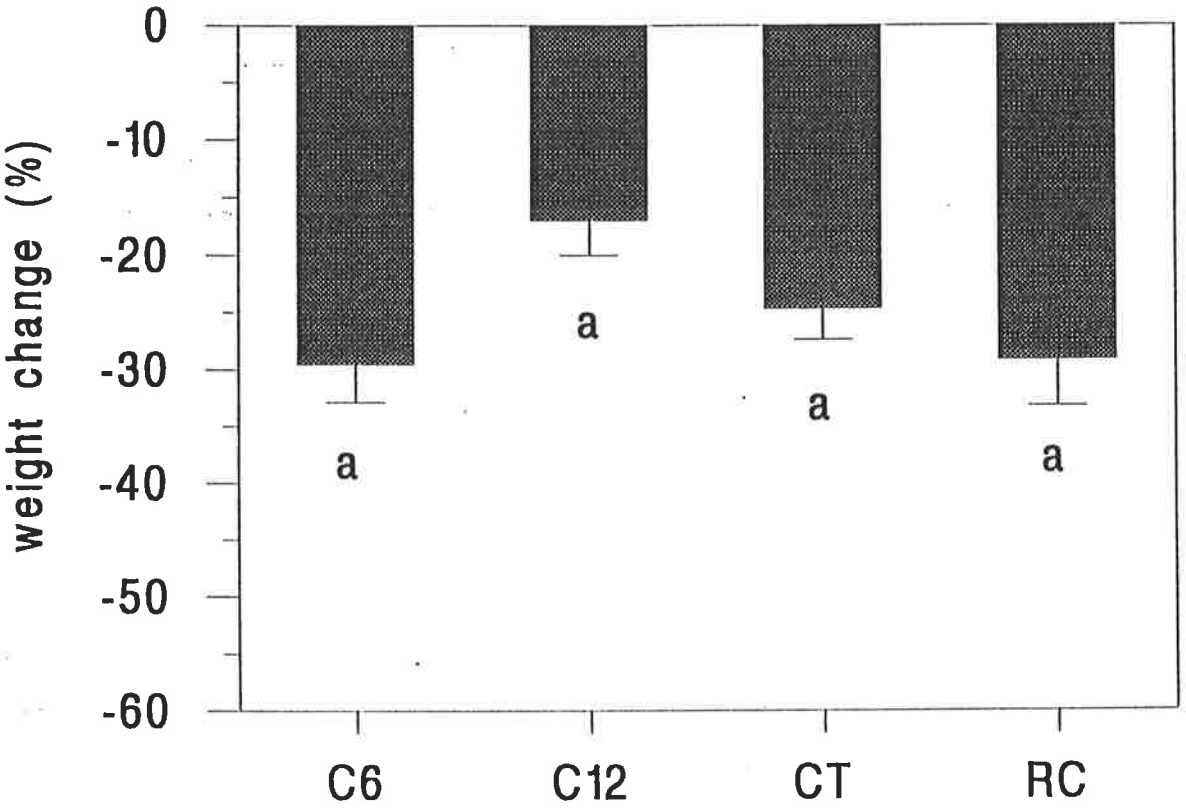
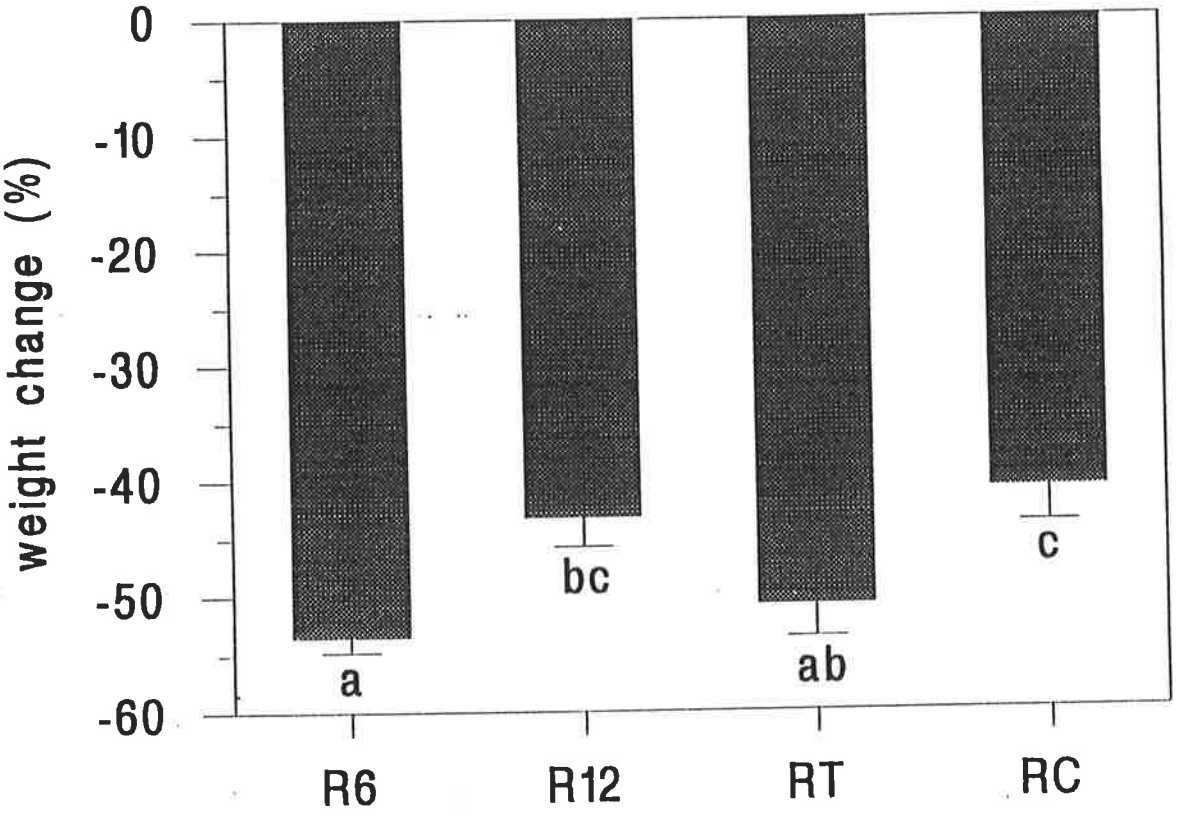


Fig. 3.4 Pot experiment, 1993. Change in weight of *A. rosea* kept in pots containing Springmount soil (pea straw added and control combined) for 10 wks at 19 (±1)°CC. Vertical bars represent standard errors of the means, n=10. Bars with the same letters are not significantly different at the 5% level. R = *A. rosea*, C = *A. caliginosa*, T = *A. trapezoides*, 6 = six earthworms added pot^{-1}., 12 = twelve earthworms added pot^{-1}. RC and RT are mixed species treatments where six individuals of both species are added pot^{-1}.



density treatment (R12) or the treatment with *A. caliginosa* (CR). Weight loss was also significantly higher in the treatment with *A. trapezoides* compared to the treatment with *A. caliginosa* (Fig. 3.4).

3.2.2.2 Interactions between A. caliginosa, and A. trapezoides - 1995

Between 0.28 and 2.27 g (2 and 16%) of pea straw remained on the soil surface after 10 weeks (Fig. 3.5) and the differences between treatments in the amount of straw remaining were not significant (Kruskal-Wallis, $H=4.71$, $P>0.05$). However, the disappearance of pea straw expressed per gram of earthworm tissue at the end of the experiment was significantly lower (Kruskal-Wallis, $H=11.23$, $P<0.05$) in the C12 and CT treatments compared to the other three treatments (Fig. 3.6).

The proportions of individuals of *A. trapezoides* and *A. caliginosa* that were quiescent were significantly higher (2-way ANOVA, d.f.=1, $F>20.39$, $P<0.05$) in the treatments with no added pea straw (Fig. 3.7, 3.8). The proportions of *A. trapezoides* that were quiescent at the end of the experiment averaged 0.12 in the treatments with added food and 0.32 in the treatments with no pea straw added. For *A. caliginosa*, the proportions that were quiescent averaged 0.30 for the treatments with straw and 0.72 for the treatments without. When the treatments of food and no food were pooled, there were no significant differences between earthworm treatments for either *A. trapezoides* or *A. caliginosa* (2-way ANOVA, d.f.=2, $F=2.79$ or 0.02, $P>0.05$).

There was some contamination by *A. caliginosa* in the soil of this experiment. Contaminants that were significantly smaller than the experimentally added earthworms were easily identified, however there was a certain cut-off point where it was no longer obvious which were resident and which were experimentally added. In the absence of straw, the selected size cut-off

Fig. 3.5 Pot experiment, 1995. Pea straw removed from and remaining on the surface of Springmount soil kept at 19 (±1)°C in pots after 10 wks. C = *A. caliginosa*, T = *A. trapezoides*, 6 = six earthworms added pot^{-1}., 12 = twelve earthworms added pot^{-1}. CT has both *A. trapezoides* and *A. caliginosa* (six individuals of both species are added pot^{-1}).

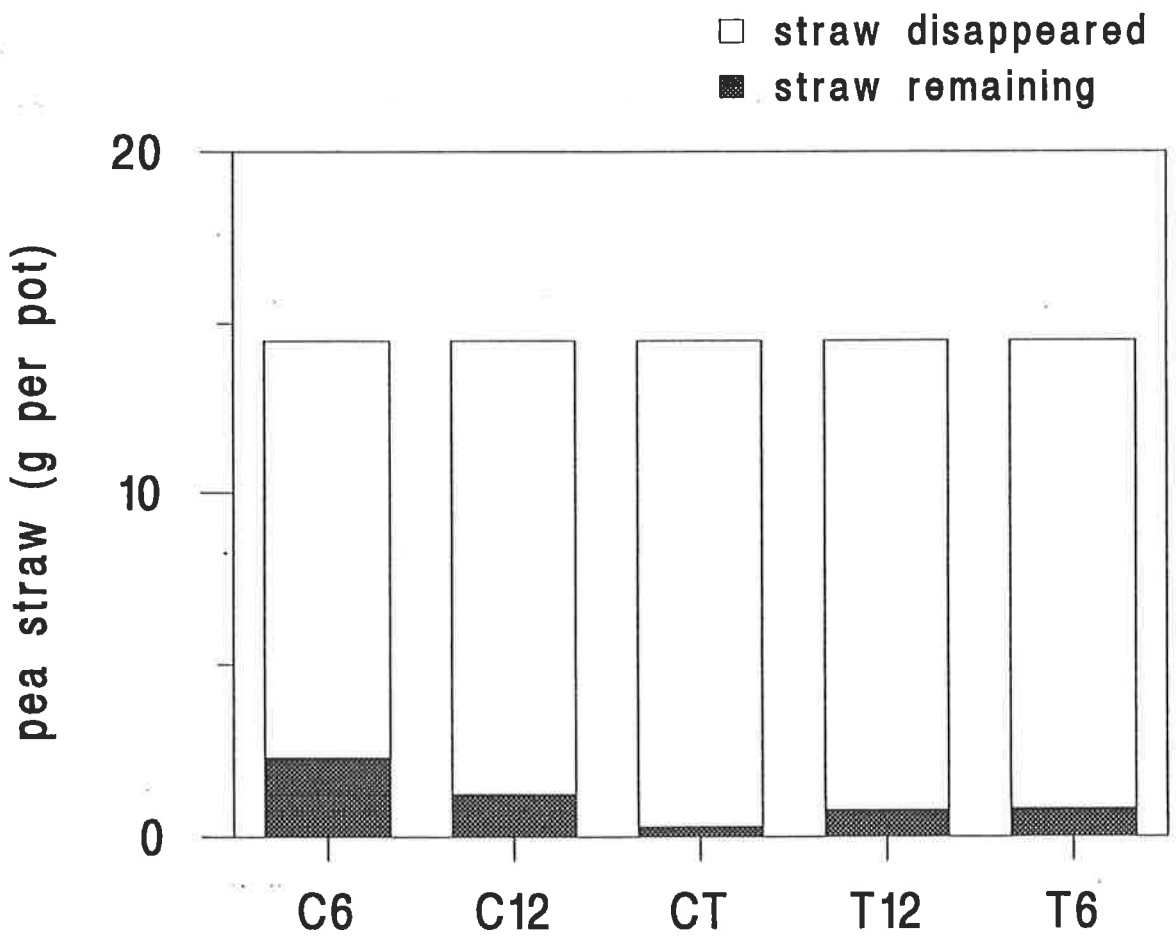


Fig. 3.6 Pot experiment, 1995. Pea straw removed from the surface of Springmount soil kept at 19 (±1)°C in pots after 10 wks expressed per gram of earthworm tissue at the end of the experiment. Vertical bars represent standard errors of the means, n=5. Bars with the same letters are not significantly different at the 5% level. C = *A. caliginosa*, T = *A. trapezoides*, 6 = six earthworms added pot^{-1}., 12 = twelve earthworms added pot^{-1}. CT has both *A. trapezoides* and *A. caliginosa* (six individuals of both species are added pot^{-1}).

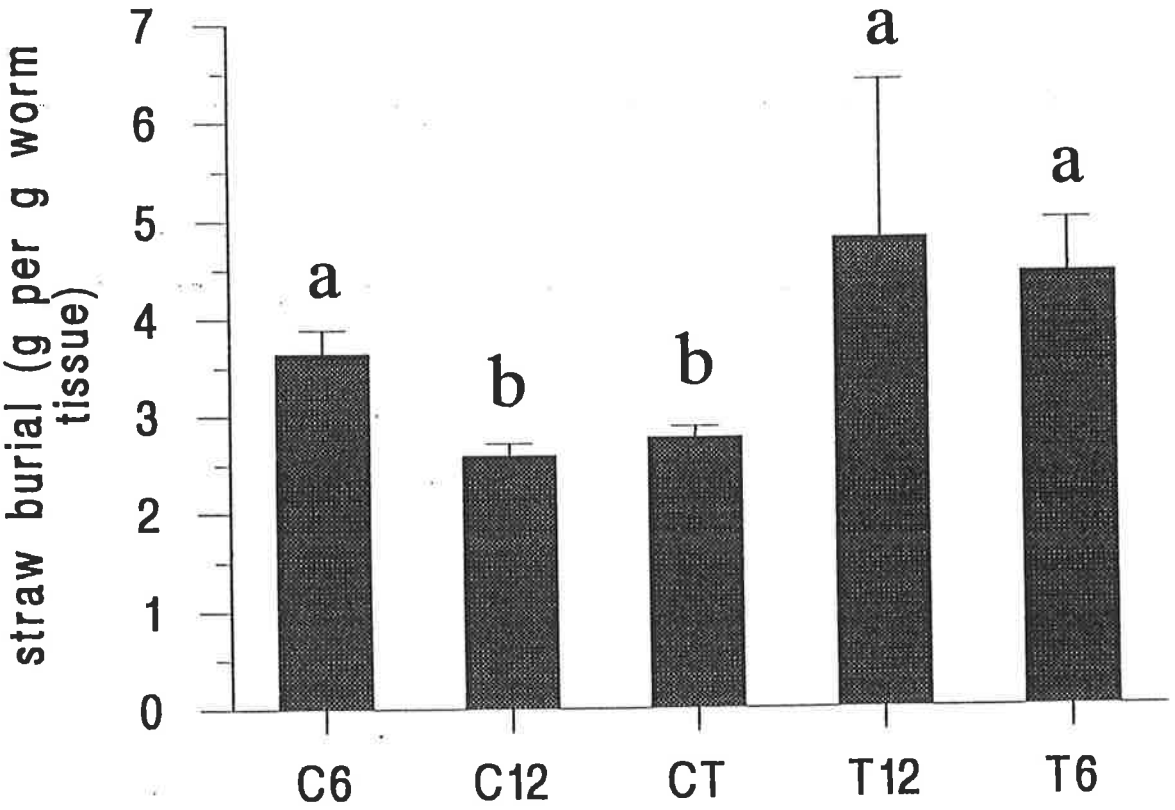


Fig. 3.7 Pot experiment, 1995. Proportion of *A. trapezoides* which were quiescent in pots containing Springmount soil kept at 19 (±1)°C measured after 10 wks (earthworm treatments combined). Vertical bars represent standard errors of the means, n=10. Bars with the same letters are not significantly different at the 5% level. pea straw = 14.5 g of pea straw added to the surface of the pots, control = no pea straw added.

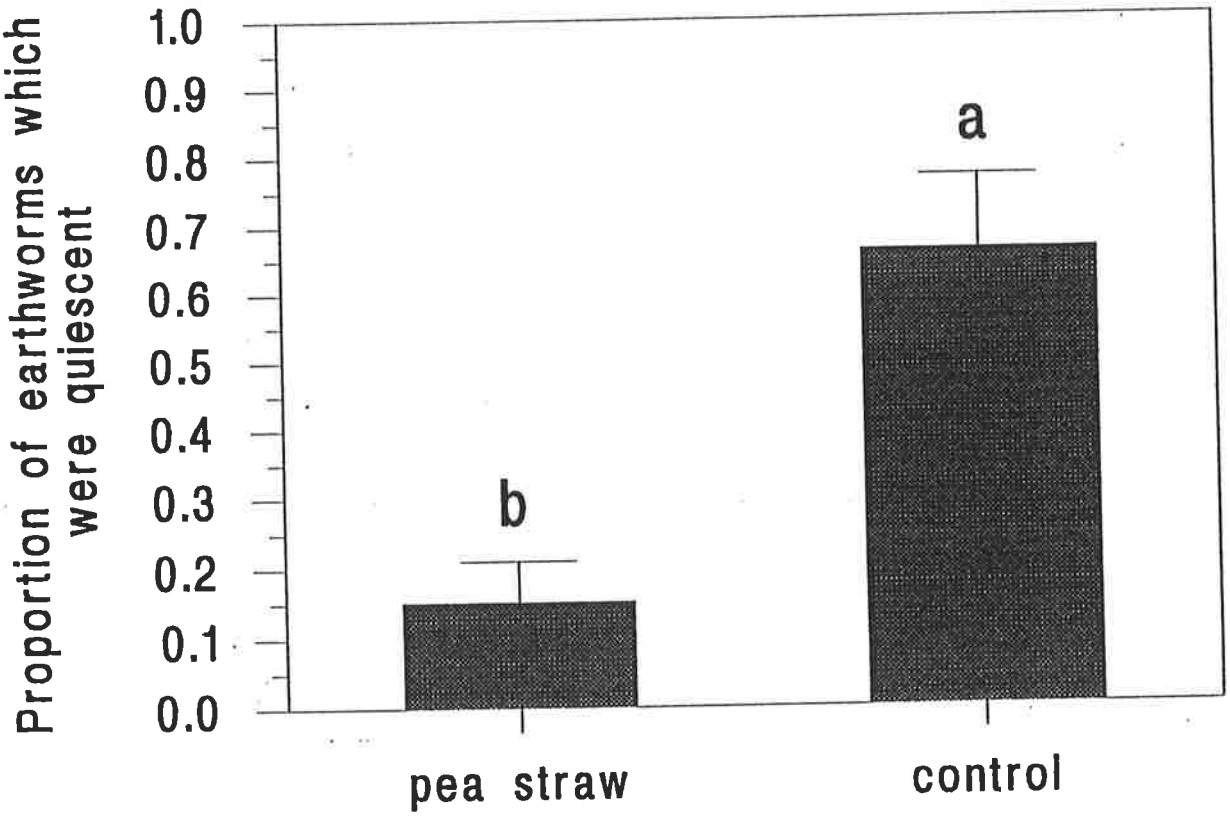
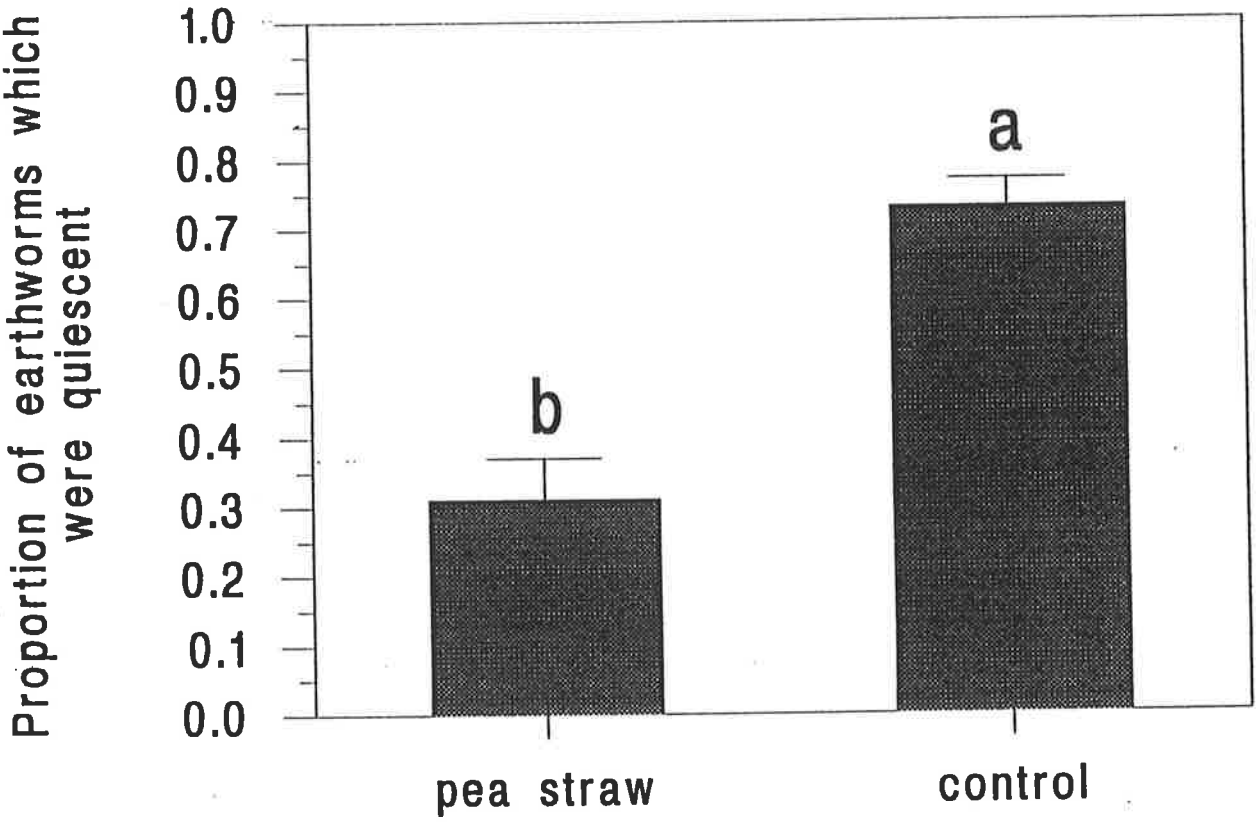


Fig. 3.8 Pot experiment, 1995. Proportion of *A. caliginosa* which were quiescent in pots containing Springmount soil kept at 19 (±1)°C measured after 10 wks (earthworm treatments combined). Vertical bars represent standard errors of the means, n=5. Bars with the same letters are not significantly different at the 5% level. pea straw = 14.5 g of pea straw added to the surface of the pots, control = no pea straw added.



was < 100 mg and with pea straw it was < 200 mg (see Table 3.2). In the pots without *A. caliginosa* (control), there were an average of 2.2 *A. caliginosa* subadults which weighed 712 mg total with pea straw and 1.0 subadults which weighed 201 mg with no straw (see Table 3.2). This was taken to be the average contamination after the small resident earthworms had been removed (see above). The average numbers and weights of residents (subadults + juveniles) were removed from the total numbers and weights for most calculations, except in the calculations for total number of individuals which were quiescent and for the removal of pea straw per gram of earthworm tissue at the end of the experiment.

No significant effects of adding pea straw on survival of *A. trapezoides* were found (2-way ANOVA, d.f.=1, F=0.25, P>0.05) or *A. caliginosa* (2-way ANOVA, d.f.=1, F=2.08, P>0.05). There was no significant effect of earthworm treatments on the survival of *A. trapezoides* (2-way ANOVA, d.f.=2, F=0.31, P>0.05), which averaged 80% across all treatments or for *A. caliginosa* (2-way ANOVA, d.f.=2, F=1.25, P<0.05) which averaged 90%.

The final average weights of both *A. trapezoides* and *A. caliginosa* were significantly higher in the pots which had pea straw added compared with those that did not (2-way ANOVA, d.f.=1, F=136 and 33.92, P<0.05) (Fig. 3.9 and 3.10). There was no significant difference between earthworm treatments in the final average weight of *A. caliginosa* (2-way ANOVA, d.f.=2, F=1.15, P<0.05). There was no significant difference in the final average weight of *A. trapezoides* between treatments which had no pea straw added, but weight was significantly lower(1-way ANOVA, d.f.=5, F=29.0, P<0.05) in the T12*f* treatment (12 *A. trapezoides*, with straw) compared to the T6*f* treatment (6 *A. trapezoides*, with straw).

A. trapezoides gained weight when pea straw was added (24.3%) but lost weight when no pea straw was added (-28.8%) and these were significantly different from each other

Table 3.2 Pot experiment, 1995. Average contamination pot^{-1} of *A. caliginosa*. Juveniles were any small earthworms which were significantly smaller than the rest of the population (see section 3.2.2.2). na = not measured because the contaminant subadults *A. caliginosa* could not be distinguished from subadult *A. caliginosa* which were experimentally added. C = *A. caliginosa*, T = *A. trapezoides*, 6 = six earthworms added pot^{-1}., 12 = twelve earthworms added pot^{-1}. CT has both *A. trapezoides* and *A. caliginosa* (six individuals of both species are added pot^{-1}). *f* = 14.5 g of pea straw was added to the surface of each pot, *n* = no pea straw was added

treatment	juveniles		subadults	
	numbers	average weight (mg)	numbers	average weight (mg)
C6*f*	3.4	426	na	na
C6*n*	2.4	204	na	na
C12*f*	2.2	199	na	na
C12*n*	2.0	165	na	na
CT*f*	2.0	138	na	na
CT*n*	0.4	54	na	na
T6*f*	1.2	135	2.6	896
T6*n*	0.4	22	1.0	222
T12*f*	0.6	39	1.8	527
T12*n*	1.0	80	1.0	180

Fig. 3.9 Pot experiment, 1995. Average weight of *A. trapezoides* in pots containing Springmount soil kept at 19 (±1)°C after 10 wks. Vertical bars represent standard errors of the means, n=5. Bars with the same letters are not significantly different at the 5% level. C = *A. caliginosa*, T = *A. trapezoides*, 6 = six earthworms added pot^{-1}., 12 = twelve earthworms added pot^{-1}. CT has both *A. trapezoides* and *A. caliginosa* (six individuals of both species are added pot^{-1}). *f* = 14.5 g of pea straw was added to the surface of each pot, *n* = no pea straw was added

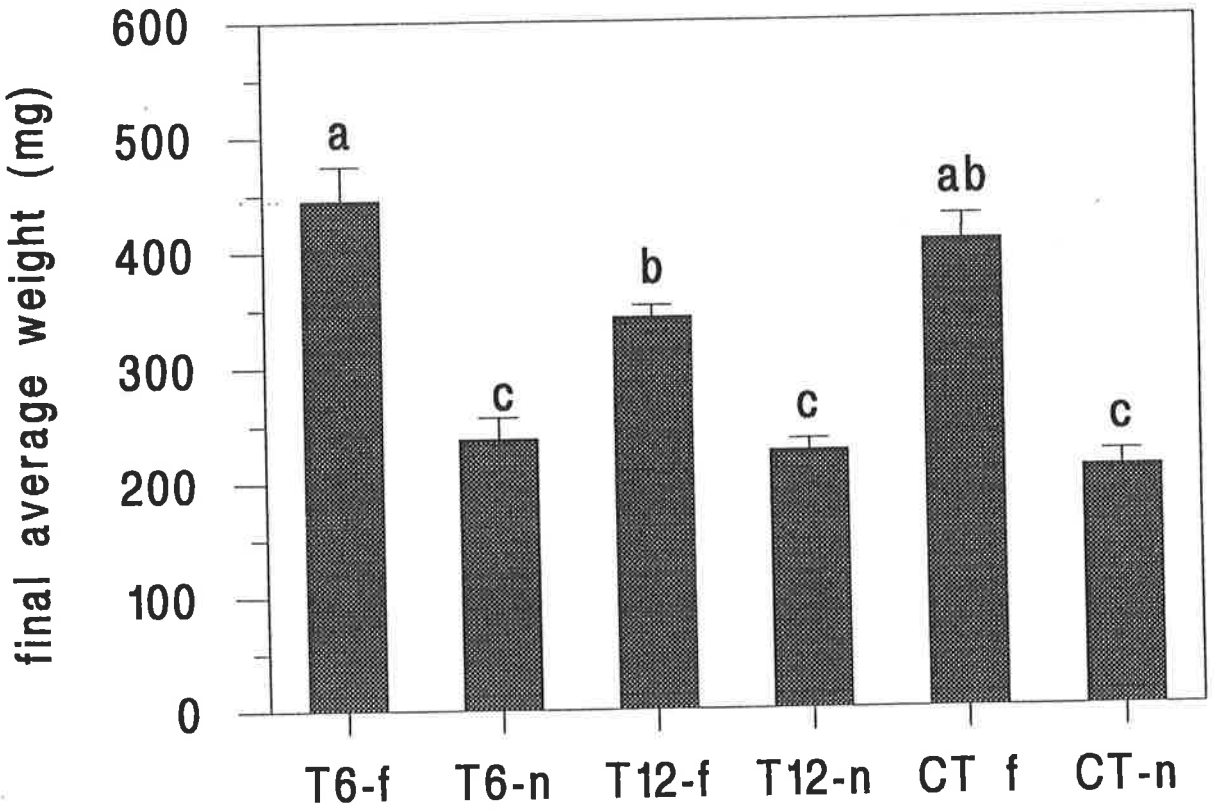
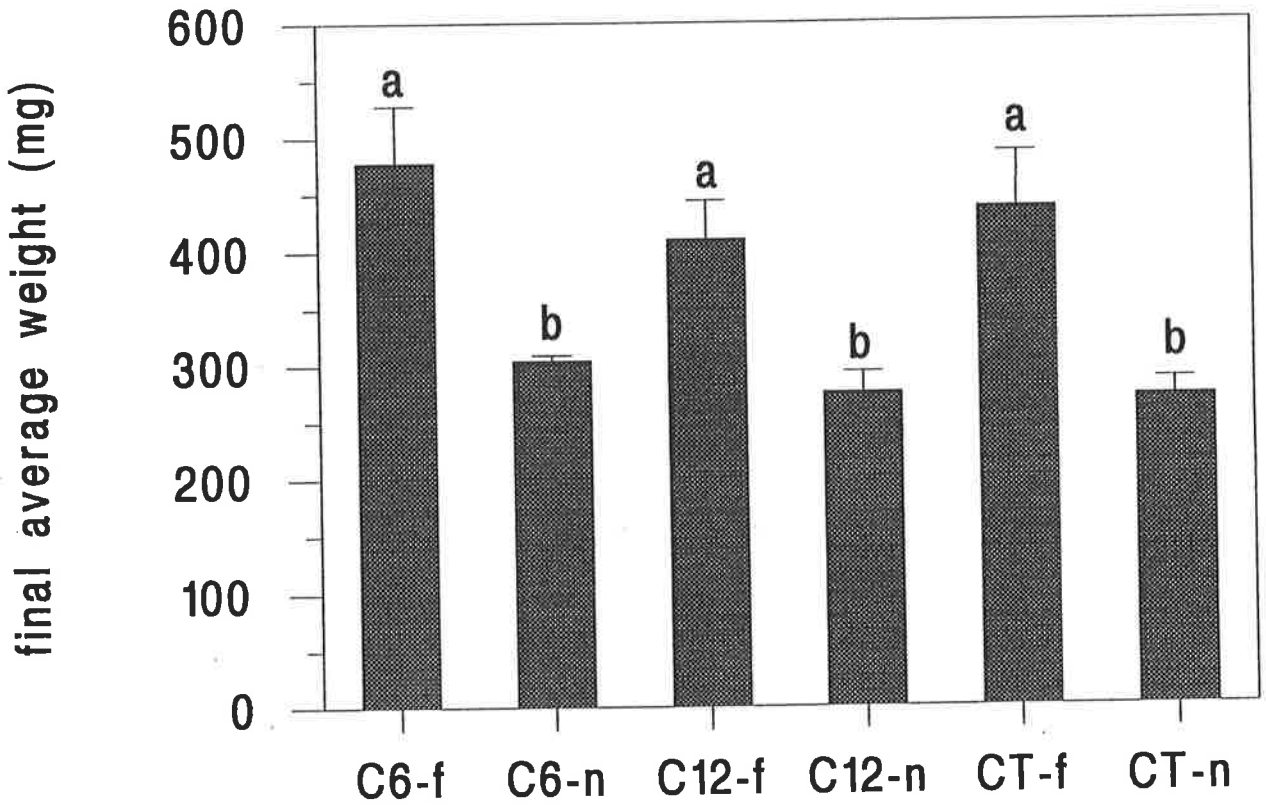


Fig. 3.10 Pot experiment, 1995. Average weight of *A. caliginosa* in pots containing Springmount soil kept at 19 (±1)°C after 10 wks. Vertical bars represent standard errors of the means, n=5. Values followed by different letters are significantly different at the 5% level. Bars with the same letters are not significantly different at the 5% level. C = *A. caliginosa*, T = *A. trapezoides*, 6 = six earthworms added pot^{-1}., 12 = twelve earthworms added pot^{-1}. CT has both *A. trapezoides* and *A. caliginosa* (six individuals of both species are added pot^{-1}). *f* = 14.5 g of pea straw was added to the surface of each pot, *n* = no pea straw was added



(2-way ANOVA, d.f.=1, F=106, P<0.05) (Fig. 3.11). When straw and control treatments are combined for each earthworm treatment, *A. trapezoides* gained weight in the T6 treatment (6.8%) and lost weight in the T12 (-10.5%) and CT treatments (-3.1%). There was no significant difference between treatments in terms of earthworm weight change when no pea straw was added but when pea straw was added to the treatments, earthworms in T6 gained significantly more weight (39.1%) than the T12 treatment (7.2%) (1-way ANOVA, d.f.=5, F=3.81, P<0.05).

A. caliginosa gained weight in all treatments (Fig. 3.12) particularly in the treatments with pea straw added (78.2%) compared to those with none (10.2%) (2-way ANOVA, d.f.=1, F=35.3, P<0.05). *A. caliginosa* gained most weight in the C6 treatment (54.4%) compared to the C12 (37.6%) and CT (40.6%) treatments but this was not significant (2-way ANOVA,

A. trapezoides gained 1.10 mg ind.$^{-1}$ day^{-1} when pea straw was added and lost 1.32 mg ind.$^{-1}$ day^{-1} when no pea straw was added (earthworm treatments combined). Growth of *A. caliginosa* was 2.78 mg ind.$^{-1}$ day^{-1} when pea straw was added and 0.52 mg ind.$^{-1}$ day^{-1} with no straw.

The proportion of clitellate individuals in the population was significantly higher in the treatments with added pea straw for both *A. trapezoides* (2-way ANOVA, d.f.=1, F=5.52, P<0.05) and *A. caliginosa* (2-way ANOVA, d.f.=2, F=12.67, P<0.05), however there were no significant differences between earthworm treatments for either species (2-way ANOVA, d.f.=2, F=0.01 or 1.32, P<0.05) (Fig. 3.13 and 3.14). The proportions of clitellate individuals in the treatments with pea straw were 0.36 for the *A. trapezoides* and 0.56 for *A. caliginosa,* and without straw they were 0.22 for *A. trapezoides* and 0.18 for *A. caliginosa* (compared to starting proportion of 0.27 for *A. trapezoides* and 0.33 for *A. caliginosa*). Most clitellate *A. trapezoides* had only retained a tuberculum pubertatis whereas clitellate *A. caliginosa* usually had a fully developed clitellum.

Fig. 3.11 Pot experiment, 1995. Change in weight of *A. trapezoides* in pots containing Springmount soil kept at 19 (±1)°C measured after 10 wks. Vertical bars represent standard errors of the means, n=5. Values followed by different letters are significantly different at the 5% level. Bars with the same letters are not significantly different at the 5% level. C = *A. caliginosa*, T = *A. trapezoides*, 6 = six earthworms added pot^{-1}., 12 = twelve earthworms added pot^{-1}. CT has both *A. trapezoides* and *A. caliginosa* (six individuals of both species are added pot^{-1}). *f* = 14.5 g of pea straw was added to the surface of each pot, *n* = no pea straw was added

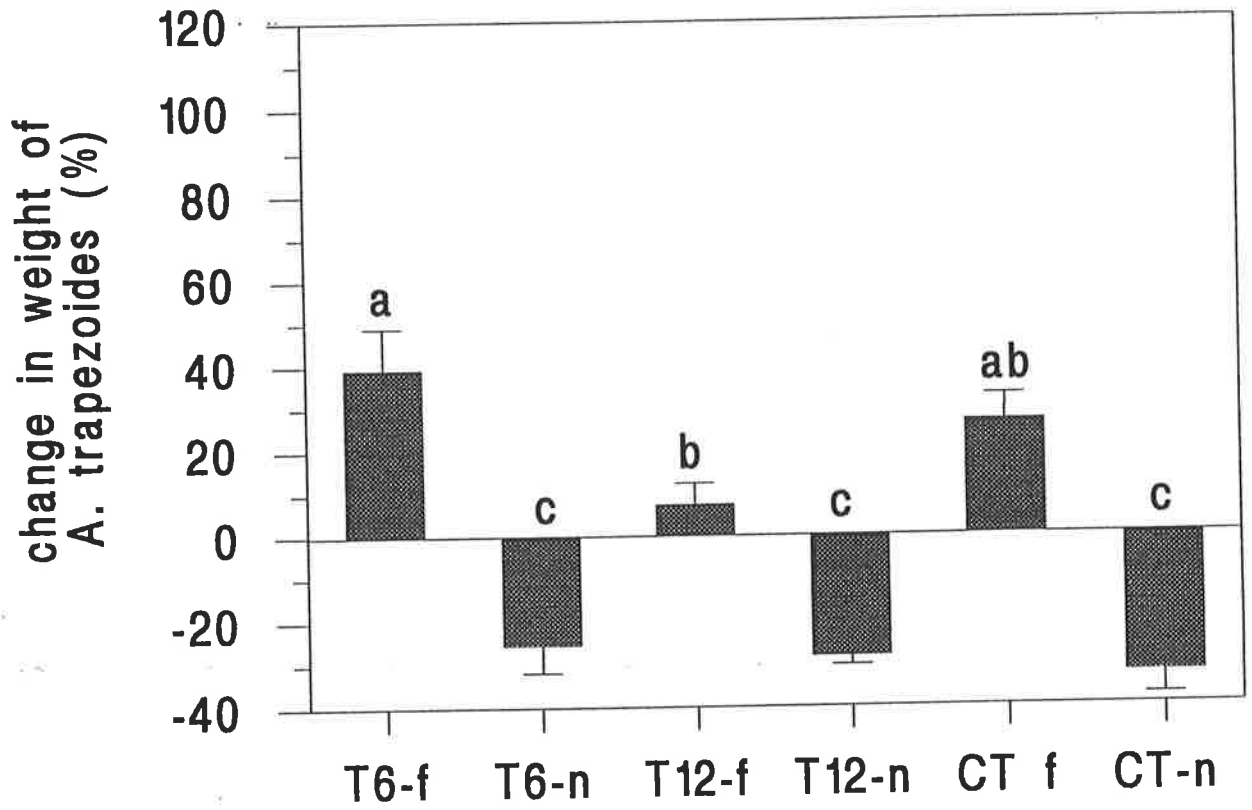


Fig. 3.12 Pot experiment, 1995. Change in weight of *A. caliginosa* in pots containing Springmount soil kept at 19 (±1)°C measured after 10 wks. Vertical bars represent standard errors of the means, n=5. Bars with the same letters are not significantly different at the 5% level. C = *A. caliginosa*, T = *A. trapezoides*, 6 = six earthworms added pot^{-1}., 12 = twelve earthworms added pot^{-1}. CT has both *A. trapezoides* and *A. caliginosa* (six individuals of both species are added pot^{-1}). *f* = 14.5 g of pea straw was added to the surface of each pot, *n* = no pea straw was added

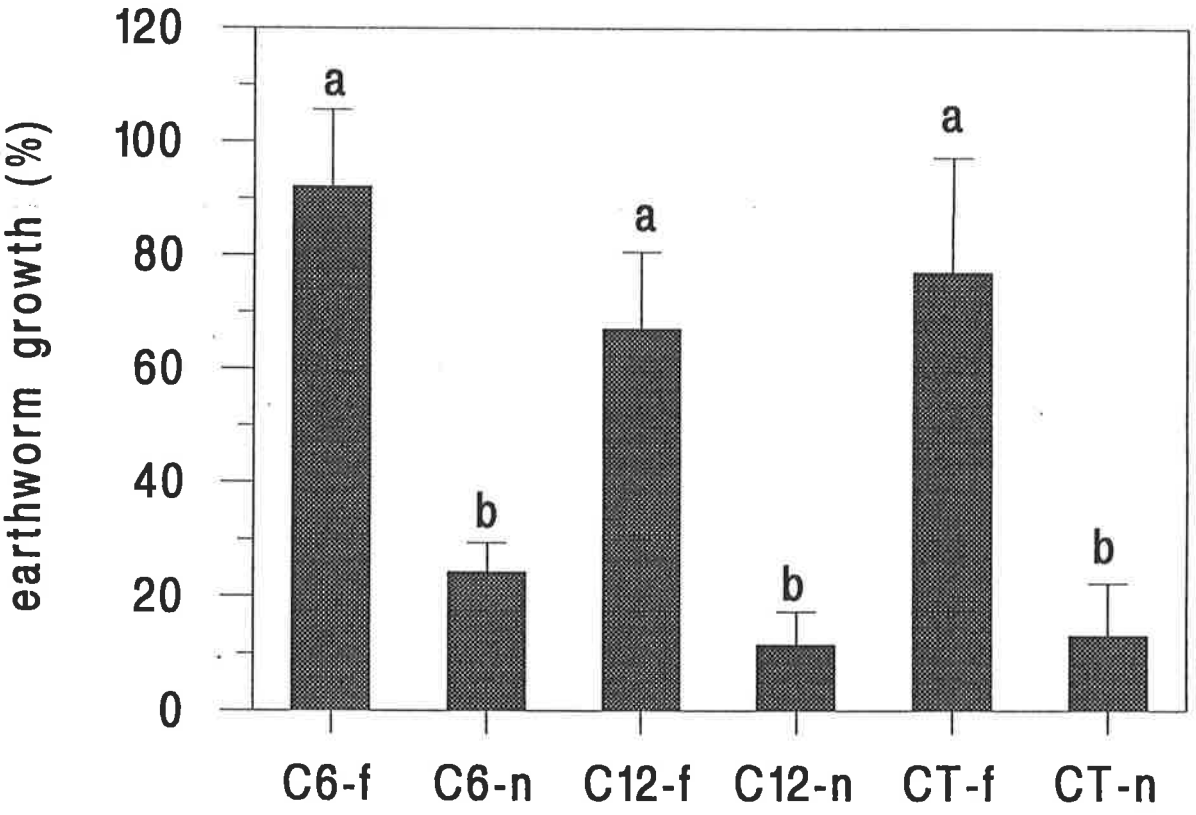


Fig. 3.13 Pot experiment, 1995. Proportion of *A. trapezoides* which were adults in pots containing Springmount soil kept at 19 (±1)°C measured after 10 wks (pea straw and control combined). Vertical bars represent standard errors of the means, n=5. Bars with the same letters are not significantly different at the 5% level. C = *A. caliginosa*, T = *A. trapezoides*, 6 = six earthworms added pot^{-1}., 12 = twelve earthworms added pot^{-1}. CT has both *A. trapezoides* and *A. caliginosa* (six individuals of both species are added pot^{-1}).

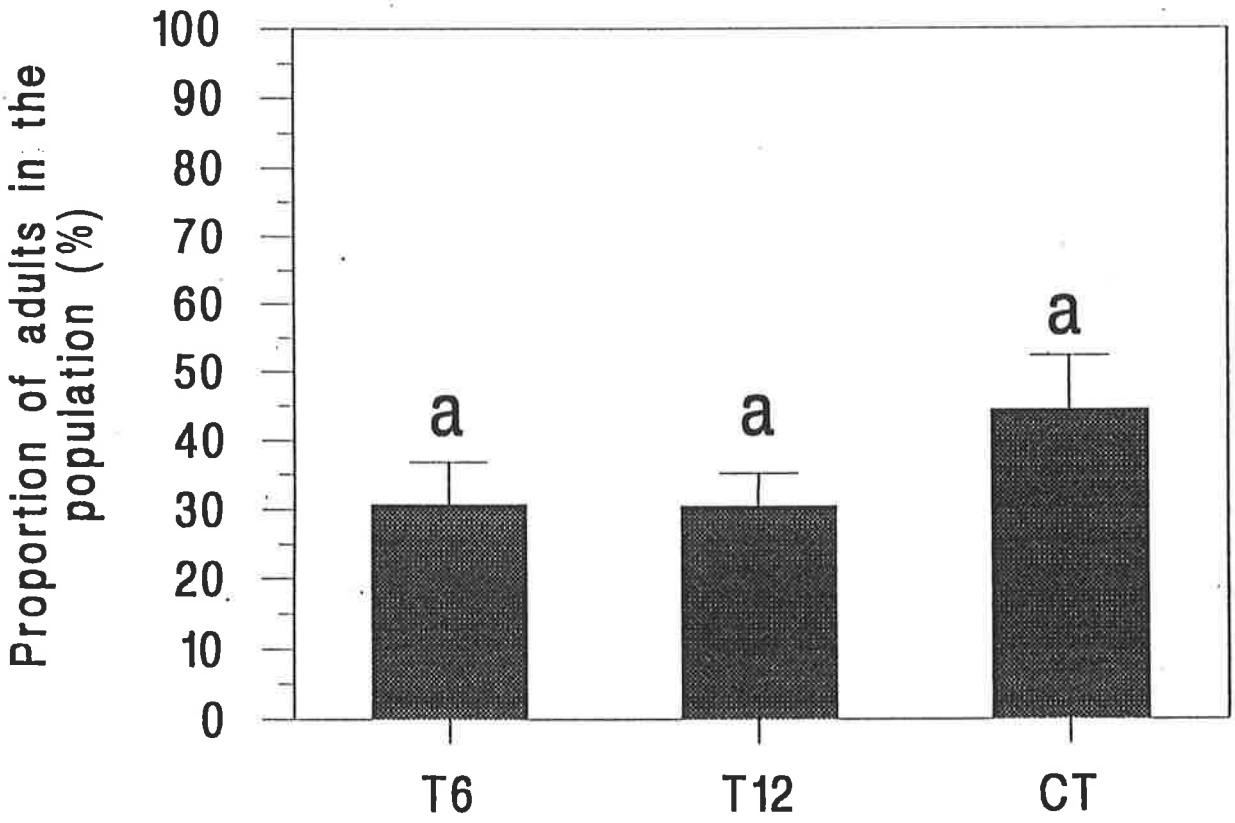
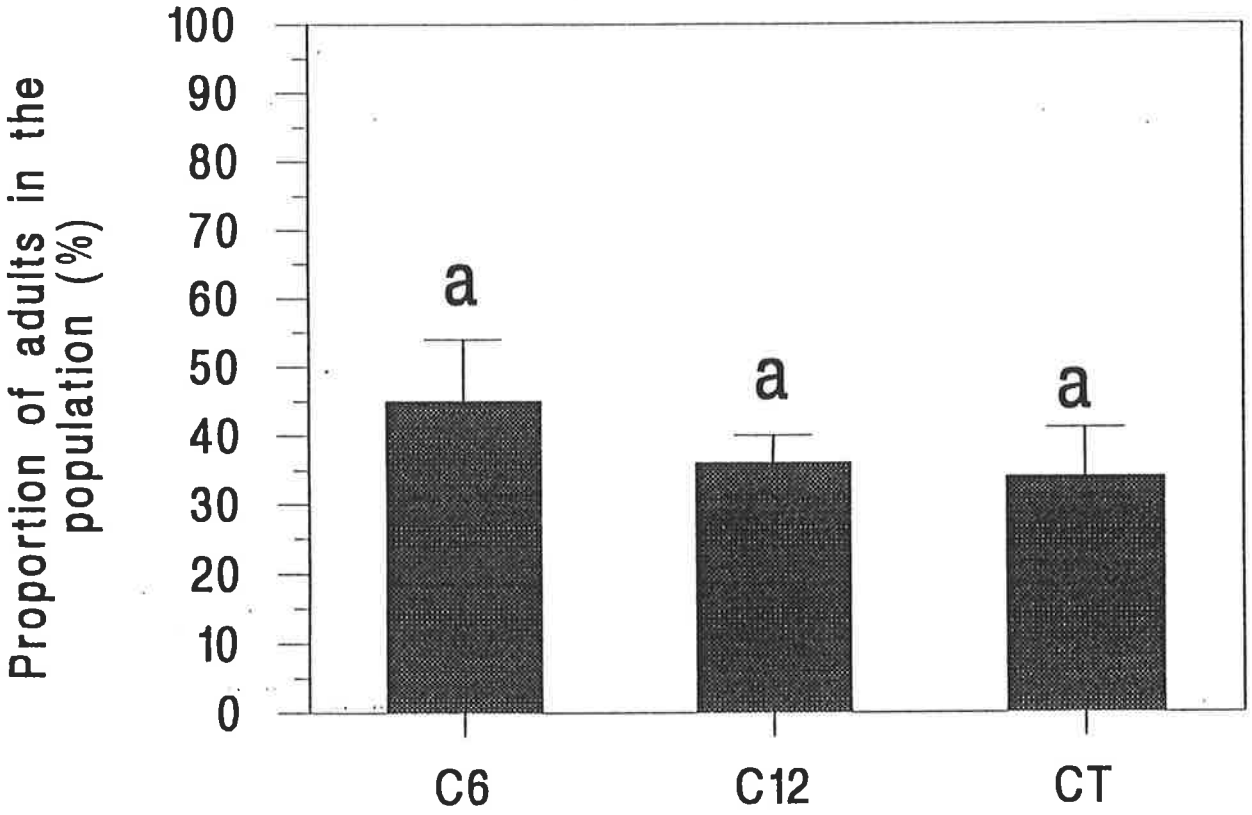


Fig. 3.14 Pot experiment, 1995. Proportion of *A. caliginosa* which were adults in pots containing Springmount soil kept at 19 (±1)°C measured after 10 wks (pea straw and control combined). Vertical bars represent standard errors of the means, n=5. Bars with the same letters are not significantly different at the 5% level. C = *A. caliginosa*, T = *A. trapezoides*, 6 = six earthworms added pot^{-1}., 12 = twelve earthworms added pot^{-1}. CT has both *A. trapezoides* and *A. caliginosa* (six individuals of both species are added pot^{-1}).



3.3. Interactions between *A. caliginosa*, *A. trapezoides* and *A. rosea* at the field site at Springmount

3.3.1 Materials and Methods

3.3.1.1 1993 - mixed soil

The aim of this experiment was to determine whether the results of the first pot experiment (1993) could be repeated under field conditions at Springmount in mixed soil. The treatments are listed in Table 3.3 and there were seven replicate cages for each treatment. Cages containing mixed soil were constructed as described in section 2.3.1. The soil was initially handsorted back into the cages, but because few earthworms were seen, the soil was then replaced without handsorting for most of the cages.

A. trapezoides was collected from Parawa, and ranged in size from 327 - 591 mg (average = 439 mg). *A. rosea* was collected from the first Waite collection site and ranged in size from 311 - 397 mg (average = 348 mg). *A. caliginosa* was collected from Woolnorth and their size ranged from 338 - 471 mg (average = 397 mg). The earthworms were pre-treated using the filter paper method (section 2.4) and weighed before being placed into the cages in early winter (June), when soils were sufficiently moist for it to be likely that the earthworms would survive.

The field experiment used approximately the same densities of earthworms per surface area of soil as the pot experiment (200 worms m^{-2} and 400 worms m^{-2}). Thirty earthworms $cage^{-1}$ in the field was equivalent to twelve earthworms pot^{-1} (400 worms m^{-2}). A set of control cages, to which no earthworms were added, was also included to estimate the potential contamination by resident earthworms in the other cages. Sources of contamination could include cocoons and small, quiescent juveniles that were not seen during the handsorting of the soil.

Any plants growing in the cages were killed with a 1.4% solution of Roundup (glyphosate, 360 g L^{-1} a/i - Monsanto) applied at a concentration of 30 ml $cage^{-1}$ in late

Table 3.3. List of treatments for the 1993 field experiment at Springmount in mixed soil. Fifteen earthworms cage^{-1} is equivalent to 200 m^{-2}. A similar design was used at Macclesfield and Myponga except that treatments with *A. caliginosa* were excluded. The symbols are used to identify the treatments in the text. R = *A. rosea*; C = *A. caliginosa*; T = *A. trapezoides*; 15 and 30 refer to the number of earthworms cage^{-1} in the single species treatments; Con = Control.

Single species, low density (15 worms cage^{-1})	**R15**	*A. rosea*
	C15	*A. caliginosa*
	T15	*A. trapezoides*
Single species, high density (30 worms cage^{-1})	**R30**	*A. rosea*
	C30	*A. caliginosa*
	T30	*A. trapezoides*
Two species (15+ 15 worms cage^{-1})	**RC**	*A. rosea* + *A. caliginosa*
	RT	*A. rosea* + *A. trapezoides*
	CT	*A. caliginosa* + *A. trapezoides*
Control	**Con**	no earthworms added

July. Round-up applied in this manner has no detrimental effect on any of the three species used (section 2.5). Perennial ryegrass (*Lolium perenne* var. Victoria) was sown in early August at 20 kg ha^{-1} (14.1 g $cage^{-1}$) as recommended by the seed supplier (Adelaide Seed Co., Ridleyton, South Australia). Germination in some cages was poor and seeds were re-sown at the same density late in August.

The experiment was terminated on the 27th October. The above-ground plant biomass was removed, dried at 60°C and weighed. Two soil cores, each 50 mm in diameter, were taken down the soil profile within each cage to determine total soil organic carbon and total soil nitrogen. Earthworms were handsorted out of the cages, pre-treated (section 2.4) and weighed.

3.3.1.2 1994 - mixed soil

The experimental design was similar to that of 1993, except that all densities were doubled (Table 3.4). Soil was collected from the Springmount site, to a depth of 15 cm. The soil was thoroughly mixed in a concrete mixer before being added back into the cages (section 2.3.1) in autumn 1994. *A. trapezoides* and *A. rosea* were collected from the Waite collection site. *A. caliginosa* was collected from the Woolnorth collection site in Tasmania. The average weights pot^{-1} ranged from 434 to 594 mg (average = 525 mg) for *A. trapezoides*, 110 to 154 mg (average = 130 mg) for *A. rosea* and 223 to 283 mg (average = 255 mg) for *A. caliginosa*. For each species, there was no significant difference in the average starting weight between treatments (1-way ANOVA, d.f.=3, $F<0.62$, $P>0.05$). The earthworms were pre-treated using the filter paper method (section 2.4) and weighed before being placed into the cages in early August.

The experiment was terminated in late October. Earthworms were handsorted out of the cages, pre-treated (section 2.4) and weighed.

Table 3.4. List of treatments for the 1994 field experiment at Springmount in mixed soil. Thirty earthworms cage^{-1} is equivalent to 400 m^{-2}. The symbols are used to identify the treatments in the text. R = *A. rosea*; C = *A. caliginosa*; T = *A. trapezoides*; 30 and 60 refer to the number of earthworms cage^{-1} in the single species treatments; (H) = high density (30+30 worms cage^{-1}) mixed species treatment; Con = Control.

Single species, low density (30 worms cage^{-1})	**R30**	*A. rosea*
	C30	*A. caliginosa*
	T30	*A. trapezoides*
Single species, high density (60 worms cage^{-1})	**R60**	*A. rosea*
	C60	*A. caliginosa*
	T60	*A. trapezoides*
Two species (30 + 30 worms cage^{-1})	**RC(H)**	*A. rosea* + *A. caliginosa*
	RT(H)	*A. rosea* + *A. trapezoides*
	CT(H)	*A. caliginosa* + *A. trapezoides*
Control	**Con**	no earthworms added

3.3.1.3 1995 - unmixed soil

The treatments are listed in Table 3.5. Unmixed soil was used in the cages (section 2.3.1) instead of mixed soil (section 3.7.7) and there were nine replicate cages for each treatment. A 20 cm hole was cut into the mesh on the top of the cage to allow the pasture plants to grow through. Smaller earthworms were added in comparison to the other field experiments because large earthworms often lost weight in Springmount soil (see section 3.7.2.2)

A. trapezoides was collected from Willow Creek, and the average weights in each treatment ranged from 285 to 289 mg (average = 288 mg). *A. caliginosa* was collected from Woolnorth and the average weights in each treatment ranged from 284 to 293 mg (average = 289 mg). The proportion of adults in the population ranged in each treatment from 0.011 to 0.015 (mean = 0.013) for *A. trapezoides* and 0.289 to 0.304 for *A. caliginosa* (mean = 0.293). For both *A. caliginosa* or *A. trapezoides* there were no significant differences between treatments in terms of average starting weight (1-way ANOVA, d.f.=2, $F<0.61$, $P>0.05$) and proportion of adults in the population (1-way ANOVA, d.f.=3, $F<0.70$, $P>0.05$). The earthworms were pre-treated (section 2.4) and weighed before being placed into the cages in early winter (June), when soils were sufficiently moist for survival.

The experiment was terminated on the 17th October. The above-ground plant biomass was removed, dried at 60^{o}C and weighed. Earthworms were handsorted out of the cages, pre-treated and weighed (section 2.4).

3.3.1.4 Data analysis

The mean weight and number of contaminants was subtracted from values of weights and numbers measured in cages of the respective experiments before the data were analysed, because they had a disproportionate effect on the values of treatments with lower numbers of earthworms and so biased the results.

Table 3.5. List of treatments for the 1995 field experiment at Springmount in unmixed soil. Thirty earthworms cage^{-1} is equivalent to 400 m^{-2}. The symbols are used to identify the treatments in the text. R = *A. rosea*; C = *A. caliginosa*; T = *A. trapezoides*; 30 and 60 refer to the number of earthworms cage^{-1} in the single species treatments; (H) = high density (30+30 worms cage^{-1}) mixed species treatment; Con = Control.

Single species, low density (30 worms cage^{-1})	**C30** **T30**	*A. caliginosa* *A. trapezoides*
Single species, high density (60 worms cage^{-1})	**C60** **T60**	*A. caliginosa* *A. trapezoides*
Two species (30 + 30 worms cage^{-1})	**CT(H)**	*A. caliginosa* + *A. trapezoides*
Control	**Con**	no earthworms added

Data analysis was done using a 1-way ANOVA and differences between individual means were determined using Tukey's test except where a Bartlett's test showed significant difference between variances in which case a Kruskal-Wallis analysis was used. Means of survival, percentage weight loss and proportion of adults in the population were transformed using the arcsin-squareroot transformation. Total weight and absolute weight loss was transformed using the log transformation.

3.3.2 Results

3.3.2.1 Contamination by resident earthworms

Contamination occurred in all field experiments. Sources of contamination would include cocoons and small juveniles that were not seen during the handsorting of the soil at the beginning of the experiment. The mean contamination for all five field experiments are shown in Table 3.6 and were calculated by determining the average density in the control cages.

Contamination by *A. caliginosa* was similar in all field experiments at Springmount in 1993 and 1994 (Table 3.6), but was significantly higher in 1995 (1-way ANOVA, d.f.=3, $F=9.92$, $P<0.05$). The differences between sites in the number of *A. trapezoides* in the control cages was not significant (1-way ANOVA, d.f.=3, $F=1.15$, $P>0.05$).

3.3.2.2 1993 - mixed soil

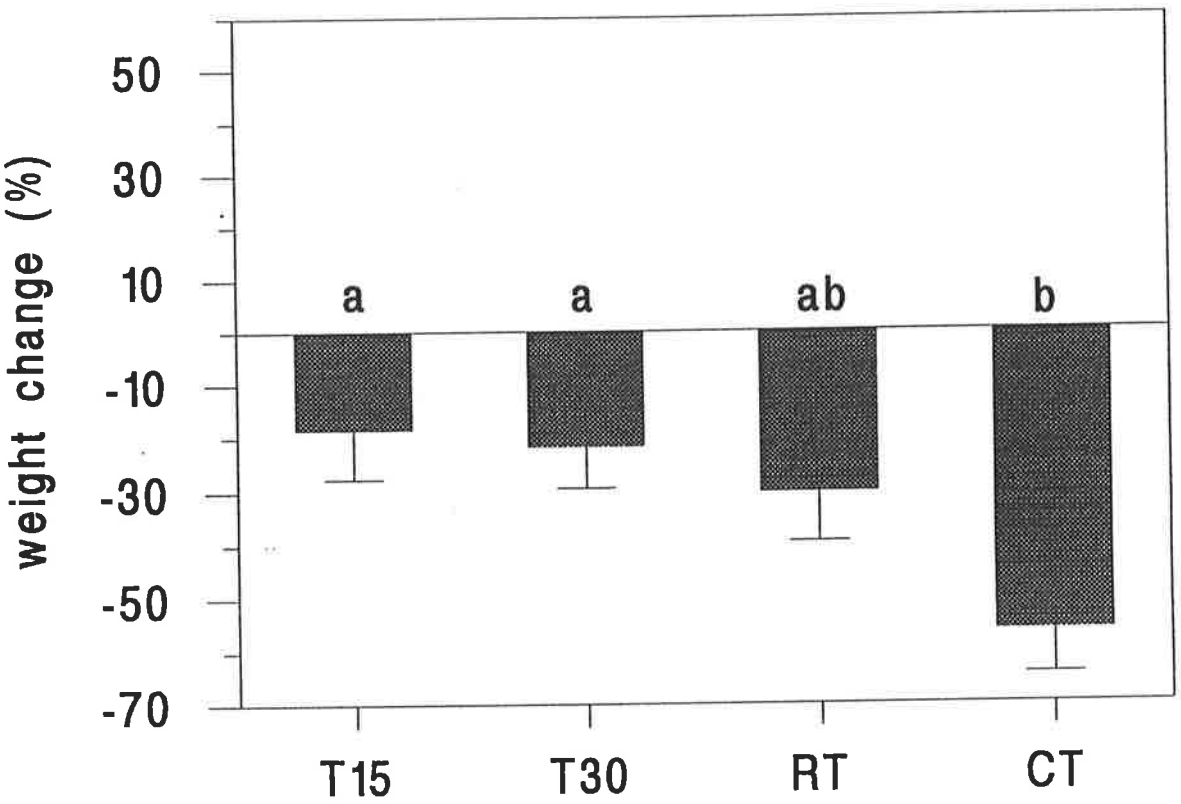
Survival was not significantly different between treatments for *A. caliginosa* (Kruskal-Wallis, $H=1.15$, $P>0.05$), *A. trapezoides* (1-way ANOVA, d.f.=3, $F=0.28$, $P>0.05$) or *A. rosea* (1-way ANOVA, d.f.=3, $F=0.50$, $P>0.05$). Survival was low, ranging from 51 - 66% for *A. caliginosa*, 34 - 47% for *A. trapezoides* and 19 - 33% for *A. rosea*.

Earthworms in most treatments lost weight over the period of the experiment. *A. trapezoides* lost between 18 and 56% of their mean weight individual^{-1} (Fig. 3.15), *A. caliginosa* gained weight in two treatments (27% in C15 and 10% in RC) and lost

Table 3.6 Contamination of cages at field sites at Springmount, Myponga and Macclesfield between 1993 and 1995 in soil that had been mixed or left unmixed.

Experiment/ date	*A. caliginosa*		*A. trapezoides*		*A. rosea*		*M. dubius*		*Gemascolex spp.*	
	number cage^{-1}	weight g cage^{-1}	number cage^{-1}	weight g cage^{-1}	number cage^{-1}	weight g cage^{-1}	number cage^{-1}	weight g cage^{-1}	number cage^{-1}	weight g cage^{-1}
Springmount 1993 unmixed	4.7	0.92	4.8	1.38	0	0	0	0	0	0
Springmount 1993 mixed	4.9	1.02	1.6	0.38	0	0	0	0	0	0
Myponga 1993 unmixed	3.4	1.10	3.9	2.03	0.4	.10	0.4	0.98	0.02	na
Macclesfield 1993 unmixed	0.6	0.10	0.9	0.24	1.4	.10	0.3	0.03	0	0
Springmount 1994 mixed	5.6	0.90	1.0	0.25	0	0	0	0	0	0
Springmount 1995 unmixed	24.3	6.12	9.6	2.20	0	0	0.13	0.02	0	0

Fig. 3.15 Springmount 1993, mixed soil. Change in weight of *A. trapezoides* in field cages measured after 19 wks (pea straw and control combined). Vertical bars represent standard errors of the means, n=7. Bars with the same letters are not significantly different at the 5% level. C = *A. caliginosa*, T = *A. trapezoides*, R = *A. rosea*, 15 = fifteen earthworms added pot^{-1}., 30 = thirty earthworms added pot^{-1}. RT and CT are mixed species treatments where fifteen individuals of both species are added pot^{-1}.



weight in two treatments (1% in C30 and 31% in CT) (Fig. 3.16) and *A. rosea* lost between 46 and 50% of it's mean weight individual^{-1} (Fig. 3.17). Weight loss was significantly greater in the CT treatment for both *A. caliginosa* (Kruskal-Wallis, H=6.21, P<0.05) and *A. trapezoides* (1-way ANOVA, d.f.=3, F=4.23, P<0.05) compared to their respective single-species, low density treatments (C15 and T15).

3.3.2.3 1994 - mixed soil

Survival ranged from 72% to 83% for *A. caliginosa*, 43 to 45% for *A. rosea* and 65 to 67% for *A. trapezoides*. There were no significant differences in survival between treatments for either *A. caliginosa* (1-way ANOVA, d.f.=3, F=0.48, P>0.05), *A. rosea* (1-way ANOVA, d.f.=3, F=0.39, P>0.05) or *A. trapezoides* (1-way ANOVA, d.f.=3, F=0.29, P>0.05).

A. caliginosa gained between 1 and 18% of starting weight in the four treatments. *A. rosea* lost between 16 and 17% of it's starting weight and *A. trapezoides* lost between 35 and 37%. There were no significant differences between treatments in terms of weight change for either *A. caliginosa* (1-way ANOVA, d.f.=3, F=0.57, P>0.05), *A. rosea* (1-way ANOVA, d.f.=3, F=0.27, P>0.05) or *A. trapezoides* (1-way ANOVA, d.f.=3, F=0.50, P>0.05).

The final proportion of adults in the population was less than 5% for *A. rosea* in all treatments. The proportion of adults was also low for *A. caliginosa* with all treatments having less than 20%. The differences between treatments in terms of the proportion of adults in the population at the end of the experiment were not significantly different for *A. caliginosa* (1-way ANOVA, d.f.=3, F=0.56, P>0.05) or *A. rosea* (1-way ANOVA, d.f.=3, F=1.22, P>0.05). There was a significantly higher proportion of adults of *A. trapezoides* in the final population (1-way ANOVA, d.f.=3, F=4.13, P>0.05) in the TR(H) treatment (57%) compared to the CT(H) (37%) or T30 treatments (34%) (Fig. 3.18).

Fig. 3.16 Springmount 1993, mixed soil. Change in weight of *A. caliginosa* in field cages measured after 19 wks. Vertical bars represent standard errors of the means, n=7. Bars with the same letters are not significantly different at the 5% level. C = *A. caliginosa*, T = *A. trapezoides*, R = *A. rosea*, 15 = fifteen earthworms added pot^{-1}., 30 = thirty earthworms added pot^{-1}. RC and CT are mixed species treatments where fifteen individuals of both species are added pot^{-1}.

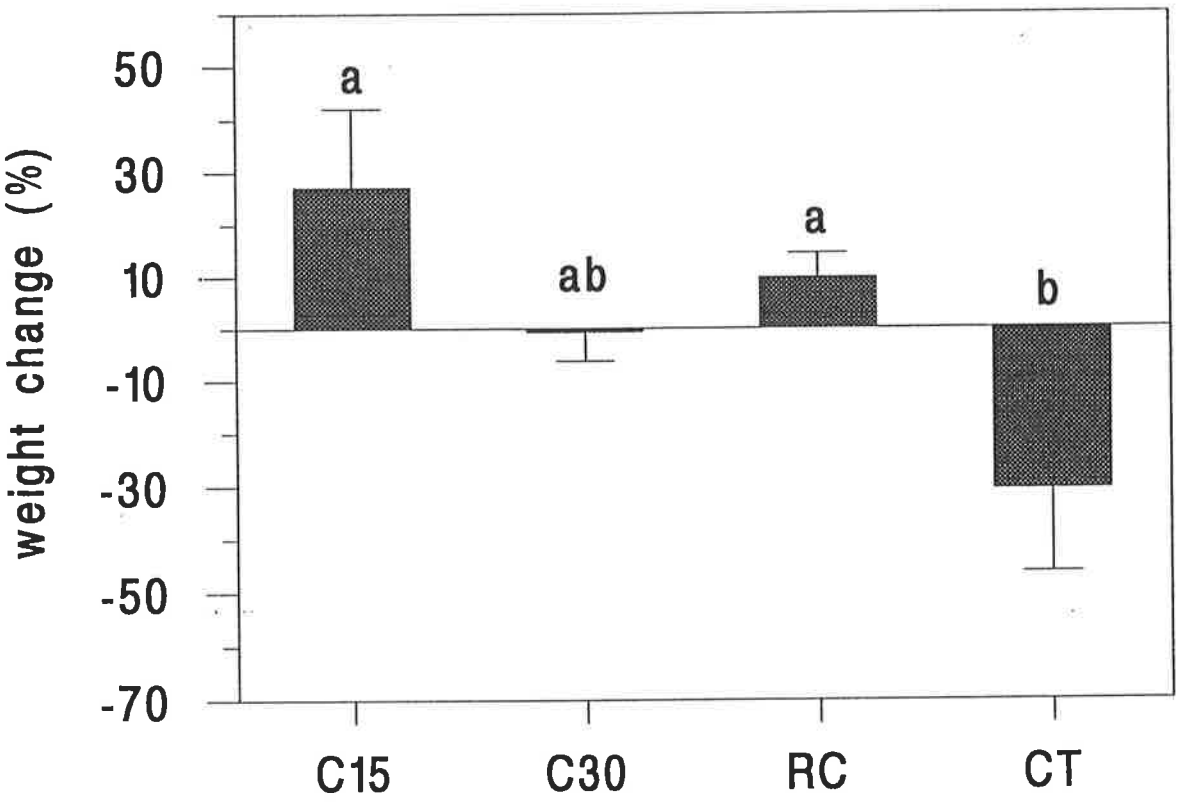


Fig. 3.17 Springmount 1993, mixed soil. Change in weight of *A. rosea* in field cages measured after 19 wks. Vertical bars represent standard errors of the means, n=7. Bars with the same letters are not significantly different at the 5% level. C = *A. caliginosa*, T = *A. trapezoides*, R = *A. rosea*, 15 = fifteen earthworms added pot^{-1}., 30 = thirty earthworms added pot^{-1}. RC and RT are mixed species treatments where fifteen individuals of both species are added pot^{-1}.

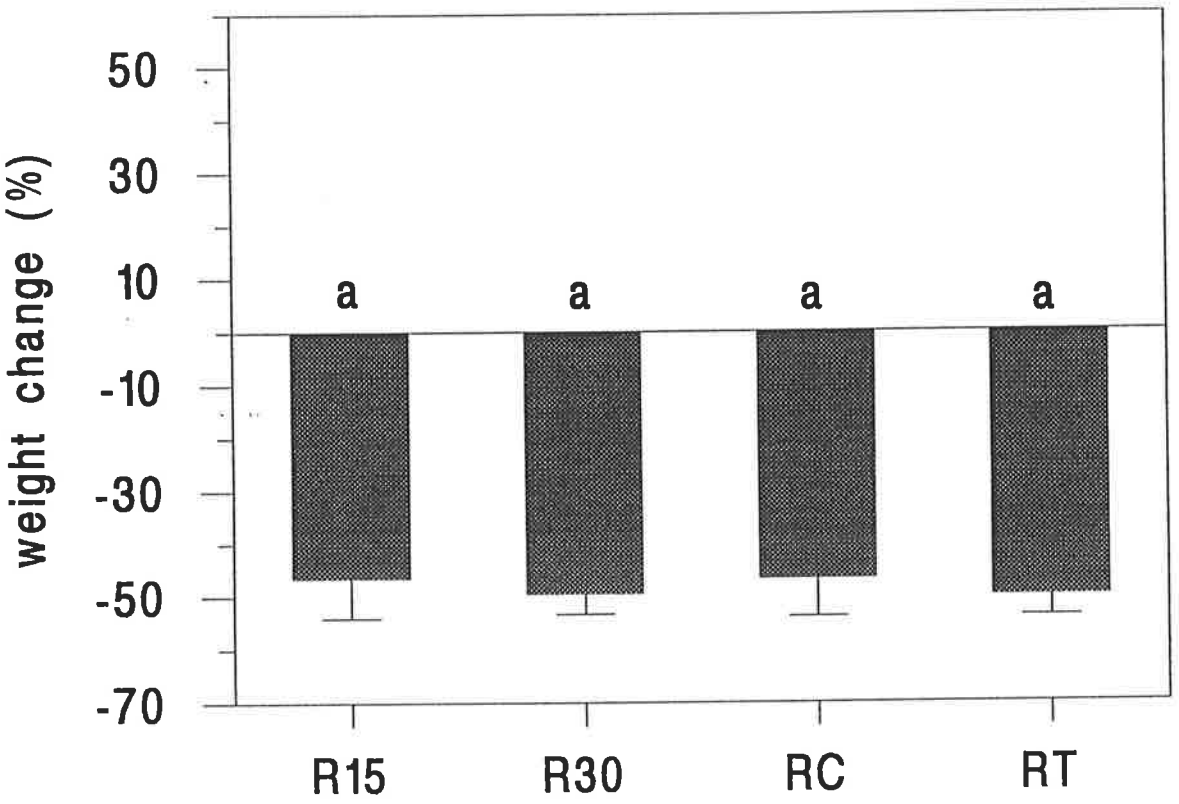
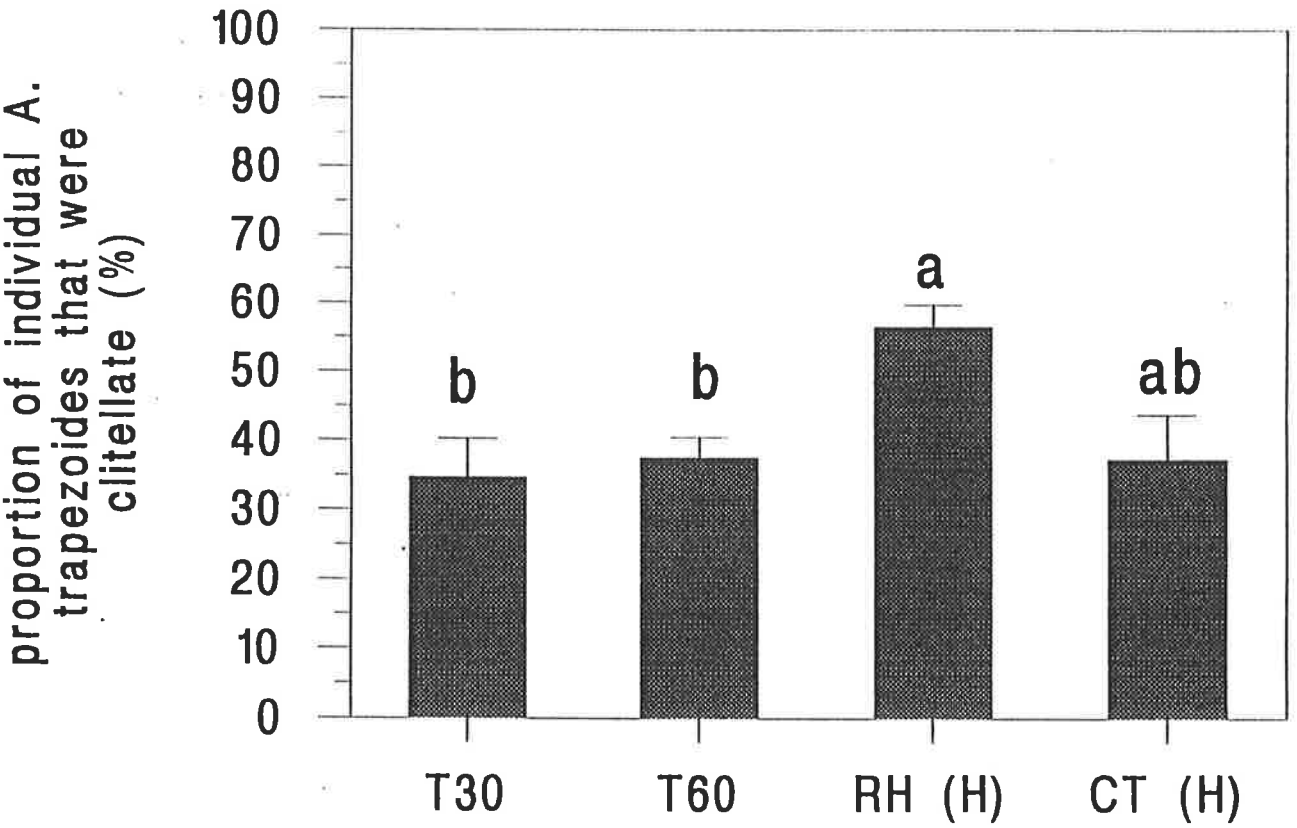


Fig. 3.18 (Springmount, 1994 - mixed) Proportion of adults in the population of *A. trapezoides* in field cages measured after 12 wks. Vertical bars represent standard errors of the means, n=7. Bars with the same letters are not significantly different at the 5% level. C = *A. caliginosa*, T = *A. trapezoides*, R = *A. rosea*, 30 = thirty earthworms added pot^{-1}., 60 = sixty earthworms added pot^{-1}. RT(H) and CT(H) are mixed species treatments where thirty individuals of both species are added pot^{-1}.



3.3.2.4 1995 - unmixed

Survival averaged 80.0% across all treatments for *A. caliginosa* and 37.7% for *A. trapezoides*. with no significant differences between treatments for each species (1-way ANOVA, d.f.=3, $F<2.22$, $P>0.05$).

A. caliginosa gained weight in the C30 treatment (6.7%) and lost 17.9 and 21.5% of their starting weight in the C60 and CT treatments respectively with no significant differences between treatments (Kruskal-Wallis, $H=1.82$, $P>0.05$). *A. trapezoides* gained 62.5% in the T30 treatment and lost 39.8% and 20.6% of their starting weights in the T60 and CT treatment respectively (Fig. 3.19). *A. trapezoides* lost significantly more weight in the T60 treatment compared to the T30 treatment (Kruskal-Wallis, $H=7.77$, $P<0.05$).

The proportion of adults in the population ranged from 0.11 to 0.72 for *A. caliginosa* and 0 to 0.36 for *A. trapezoides*. However, due to the high value of the variances, there were no significant differences between treatments (1-way ANOVA, d.f.=3, $F<1.59$, $P>0.05$).

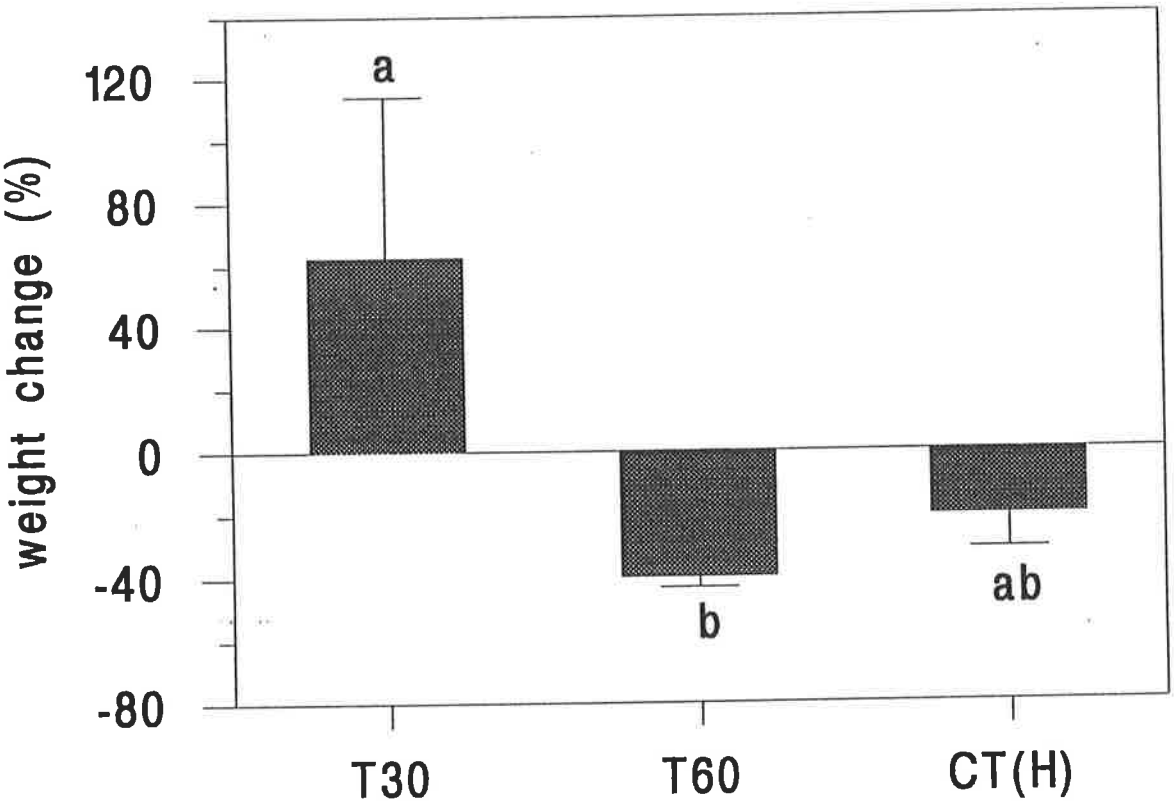
3.4 Intra-specific interactions between individuals of *A. trapezoides* at Springmount in unmixed soil (1993)

3.4.1 Materials and methods

In this experiment, one of the species (*A. trapezoides*) was used in an experiment to investigate intra-specific interactions using a wider range of earthworm densities than had been used in the previous experiments at Springmount. Unmixed soil cores were prepared in the manner described in section 2.3.1.

Four different densities of *A. trapezoides* were used; 8, 15, 30, 60 cage^{-1} (100, 200, 400, and 800 m^{-2}). There was also a control with no earthworms added to assess

Fig. 3.19 Springmount 1995, unmixed soil. Change in weight of *A. trapezoides* in field cages measured after 18 wks. Vertical bars represent standard errors of the means, n=9. Bars with the same letters are not significantly different at the 5% level. C = *A. caliginosa*, T = *A. trapezoides*, 30 = thirty earthworms added pot^{-1}., 60 = sixty earthworms added pot^{-1}. CT(H) are mixed species treatments where thirty individuals of both species are added pot^{-1}.



contamination in other cages. For each treatment there were seven replicates of each treatment.

A. trapezoides was collected from Parawa in the Mount Lofty Ranges. The average earthworm weight ranged from 229 - 327 mg (average = 267 mg). The earthworms were pre-treated using the filter paper method (section 2.4) before being weighed and placed into the cages in mid June. The cages were sprayed with a solution of 0.8% Buticide (24-DB, 400 g/L^{-1} a/i - Monsanto) at a concentration of 17 ml $cage^{-1}$ in late July to kill the capeweed which was smothering the ryegrass. 24-DB applied at this rate to this soil has no detrimental effect on the three species (section 2.5).

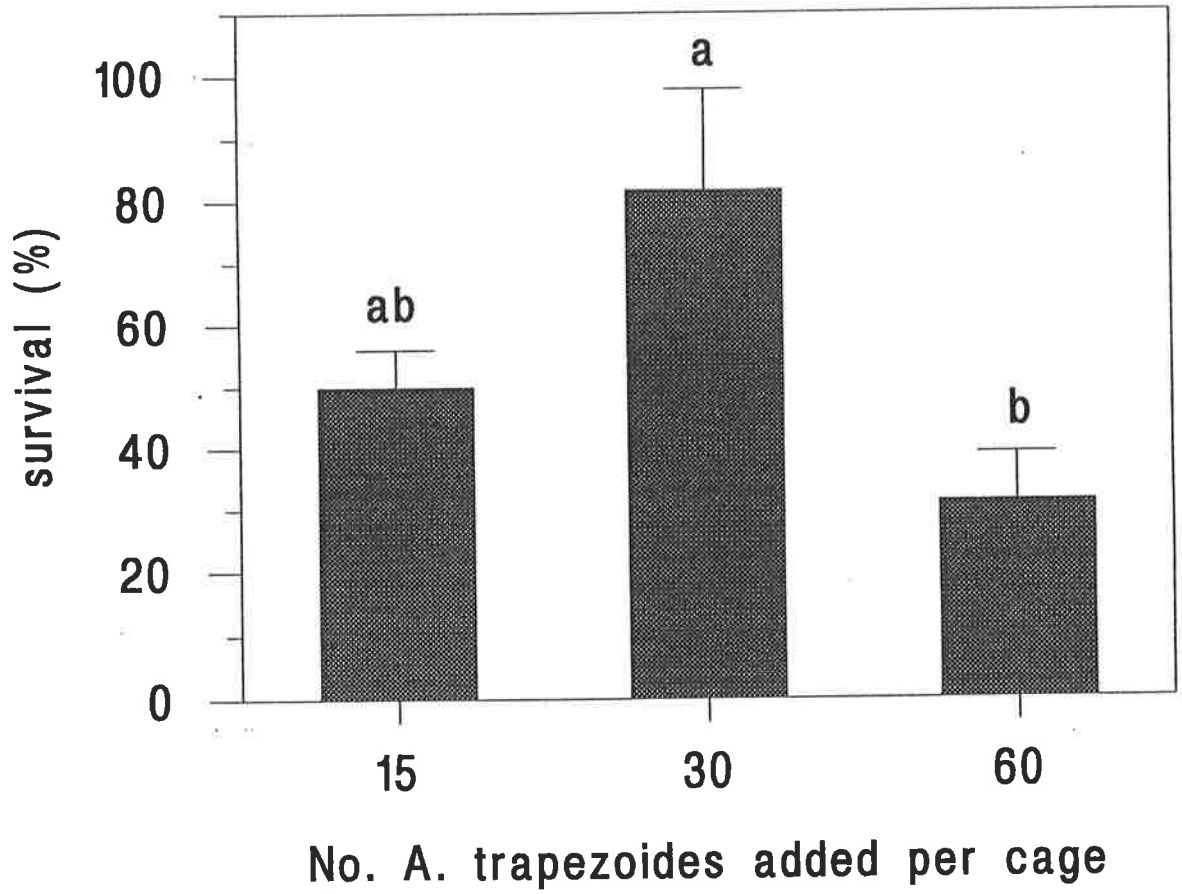
The experiment was terminated on the 15th November. Earthworms were handsorted out of the cages and pre-treated (section 2.4) before weighing. Data analysis was as described in section 3.3.1.4.

3.4.2 Results

When the average values of numbers and weight of resident earthworms was removed from the data, the values for survival and growth for the treatment with 8 worms $cage^{-1}$ were very variable and in one case negative. Therefore this treatment was removed from the analysis.

Survival ranged from 31 to 82% (Fig. 3.20). The treatment with 60 worms $cage^{-1}$ had significantly lower survival than 30 $cage^{-1}$ (1-way ANOVA, d.f.=4, F=, P>0.05), but was not significantly different from the treatment with 15 worms $cage^{-1}$. Earthworms in all treatments gained weight (14 to 30%), but there was no significant difference between treatments (1-way ANOVA, d.f.=2, F=0.76, P>0.05). The average proportion of adults in the population at the end of the experiment ranged from 3.1 to 4.1% with no significant differences between treatments (Kruskal-Wallis, H=0.99, P>0.05).

Fig. 3.20 Springmount 1993, Different densities of *A. trapezoides* in unmixed soil. Survival of *A. trapezoides* in field cages measured after 20 wks. Vertical bars represent standard errors of the means, n=7. Bars with the same letters are not significantly different at the 5% level. 15 , 30 and 60 = fifteen, thirty and sixty *A. trapezoides* added pot^{-1}.



3.5 Interactions between *A. trapezoides* and *A. rosea* at Macclesfield and Myponga in unmixed soil (1993)

3.5.1 Materials and methods

Interactions between *A. trapezoides* and *A. rosea* were investigated at Macclesfield and Myponga to determine if different sites altered the outcome of the experiments. At the Myponga site, *A. trapezoides* was the dominant species in the local community while at the Macclesfield site, *A. rosea* and *A. trapezoides* were the dominant species.

The treatments were as shown in Table 3.3. Cages containing unmixed soil (see section 2.3.1) were established. Earthworms were placed in the cages in early June.

A. trapezoides and *A. rosea* were collected from the Waite campus. At Myponga, the average weight of earthworms $cage^{-1}$ ranged in size from 439 to 573 mg (average = 483 mg) for *A. trapezoides* and from 305 to 423 mg (average = 362 mg) for *A. rosea* while at Macclesfield, *A. trapezoides* ranged from 465 to 631 mg (average = 532 mg) and *A. rosea* ranged from 329 to 491 mg (average=365 mg).

Pasture cuts were taken three times during the season. Cuts were made on the 24th August, 21st September, and 2nd November at Macclesfield and on 25th August, 22nd September and 9th November at Myponga. Plants were dried at 60°C and weighed. On the November 9th , earthworms were handsorted out of the cages and pre-treated before weighing. Data analysis was as for section 3.3.1.4.

3.5.2 Results

3.5.2.1 Macclesfield

Contamination in the control cages was low at the Macclesfield site, with *A. rosea* and *A. trapezoides* being the dominant species (Table 3.6). Survival of introduced *A. rosea* ranged from 67 to 71% with no significant differences between the three treatments

(Kruskal-Wallis, H=0.16, P>0.05). Survival of *A. trapezoides* ranged from 56 to 68% with no significant differences between treatments (Kruskal-Wallis, H=0.50, P>0.05). The average final weight of *A. rosea* individuals was 23 (R30) to 37% (R15) lower than the starting average weights. *A. trapezoides* either lost a small amount of weight or increased in weight by up to 14%. Weight change was not significantly different between treatments for *A. trapezoides* (Kruskal-Wallis, H=1.42, P>0.05) and *A. rosea* (Kruskal-Wallis, H=2.91, P>0.05).

The average plant growth (dry weight) ranged between 27.3 to 33.8g $cage^{-1}$ (3.9 to 4.8 t ha^{-1}). Plant growth was significantly higher (1-way ANOVA, d.f.=5, F=2.91, P<0.05) in the treatment with 30 *A. trapezoides* than the treatment with 15 *A. rosea*, but neither of these treatments were significantly different from the control.

3.5.2.2 Myponga

Contamination in the control cages at Myponga was higher than at Macclesfield and *A. trapezoides* and *A. caliginosa* were the dominant species (Table 3.6). In the experiment run at this site, survival ranged from 45 to 64% for *A. rosea* and 31 to 69% for *A. trapezoides* after due allowance for contaminants with no significant differences between treatments for *A. trapezoides* (1-way ANOVA, d.f.=2, F=3.36, P>0.05) or *A. rosea* (1-way ANOVA, d.f.=2, F=0.23, P>0.05).

A. rosea lost between 6 (R15) and 19% (TR) of their average starting weight with no significant differences between treatments (1-way ANOVA, d.f.=2, F=0.73, P>0.05). *A. trapezoides* increased in weight by between 51% (TR) and 67% (T15)) with no significant differences between treatments (1-way ANOVA, d.f.=2, F=0.20, P>0.05).

The proportion of adults in the final population ranged from 68 to 87% for *A. rosea* and 36 to 88% for *A. trapezoides* with no significant differences between treatments for

either *A. trapezoides* (1-way ANOVA, d.f.=2, F=3.14, P>0.05) or *A. rosea* (1-way ANOVA, d.f.=2, F=0.49, P>0.05).

Plant growth ranged from 15.6 to 20.3 g cage^{-1} (2.2 to 2.9 t ha^{-1}) with no statistical differences between treatments (1-way ANOVA, d.f.=5, F=0.81, P>0.05).

3.6 Comparison of growth and survival between field experiments

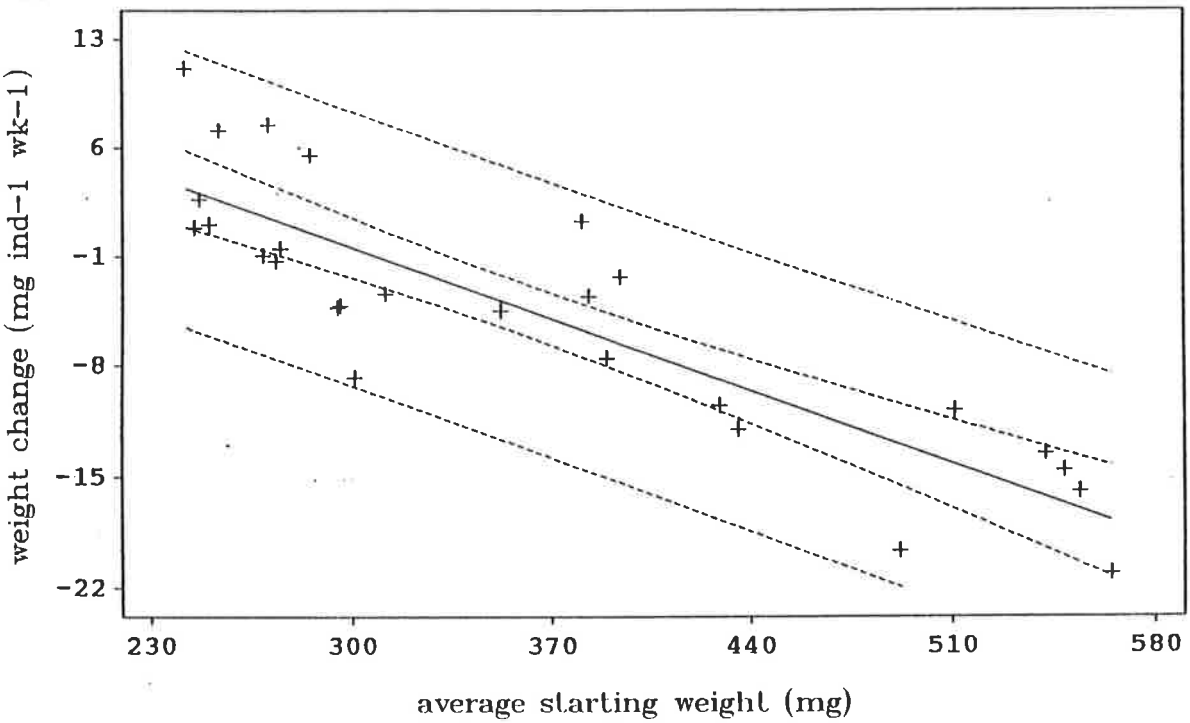
The treatment of *A. trapezoides* @ 30 cage^{-1} was common to all field experiments. This treatment was used to compare the performance of earthworms across experiments.

Weight change (mg individual^{-1} week^{-1}) and survival (%) were calculated for the cages with *A. trapezoides* @ 30 cage^{-1} from all experiments. The experiments from Springmount were grouped for analysis into those where the soil was mixed and those in which soil was left unmixed. The relationship between the average starting weight of earthworms in a cage and the final survival or weight change was determined using correlation and regression analyses.

Weight change individual^{-1} week^{-1} was positive for *A. trapezoides* @ 30 individuals cage^{-1} in the experiments at Macclesfield, Myponga and in unmixed soil at Springmount in 1993 and negative in experiments at Springmount in 1993 (mixed soil), 1994 (mixed) and 1995 (unmixed).

If the data from the Springmount site are considered separately, weight gain was significantly higher in the experiments in which the soil was unmixed compared to the experiments where it was mixed (1-way ANOVA, d.f.=1, F=24.94, P<0.05). There was also a strong, significant correlation (-0.87) between weight change and starting weight (Fig. 3.21) when the data were pooled from the Springmount experiments (Linear regression, d.f.=1, F=77.84, P<0.05, r^2=0.757).

Fig. 3.21 Correlation between weight change and starting weights of *A. trapezoides* at a starting density of 30 cage^{-1} in the four field experiments at Springmount. y = 19.178 - 0.0654x. 95% confidence (inner, curved pair of dotted lines) and predicted (outer, straight pair of dotted lines) interval lines are shown.



Average survival of *A. trapezoides* @ 30 cage^{-1} ranged from 42.2% Springmount 1993, mixed soil. to 97.6% (Springmount 1993 - unmixed). There were no significant correlations between average starting weight and survival and there was no significant difference between mixed and unmixed cages in terms of survival of *A. trapezoides* when the Springmount experiments were analysed in isolation (1-way ANOVA, d.f.=1, F=32, P>0.05).

3.7 Discussion

This discussion deals separately with the results of the pot and field experiments. It goes on to discuss inter- and intra-specific competition between species and tries to summarise and draw general conclusions about the nature, extent and importance of competition between the three species of earthworms used. A series of tables which summarise the results of all eight experiments (Table 3.7, 3.8) are presented. The growth response of earthworms is discussed in some detail and compared between experiments and other studies. Finally, the effect that mixed and unmixed soil has on earthworm performance is discussed.

3.7.1 Pot experiments

All *A. trapezoides* and *A. caliginosa* were quiescent at the end of the first pot experiment (1993) and therefore were not active throughout its term. Less were quiescent in the second experiment especially in the pots where pea straw was added. In the second experiment, earthworms performed better in terms of growth in the treatments with pea straw compared to the control, but this was not the case in the first experiment. Nearly all pea straw was buried in the second experiment, but less than half was buried in the first. The likelihood that earthworms were inactive for a significant period in the first experiment suggests that the results should be interpreted with caution. Any measured effects on survival or growth may not be due to interactions between earthworms in this experiment.

All earthworms lost weight in the first experiment. This may have been because the soil was too dry or earthworms were too large to be able to maintain their weight in Springmount soil (section 3.7.2.2). In the second experiment, the soil was watered to weight more often and was not allowed to become drier than -10 kPa. The size of earthworms added was also smaller; 321 mg (compared to 528 mg in the first experiment) for *A. trapezoides* and 247 mg (compared to 389 mg in the first experiment) for *A. caliginosa*.

A. rosea lost significantly less weight in the higher density treatment (R12) and in the treatment with *A. caliginosa* added (RC) compared to the low density, single species treatment (R6). It has been suggested that *A. caliginosa* can increase the numbers of *A. longa* in the field (Temple-Smith *et al.*, 1993). It is therefore possible that *A. caliginosa* could have enhanced the performance of *A. rosea*. The mechanism of such a relationship may be that *A. caliginosa* is able to make food resources more available to *A. rosea* (and *A. longa*) through it's digestive process, or by bringing food from the surface into the spatial habitat of *A. rosea* which rarely feeds from the soil surface. However, as mentioned earlier, the results of this experiment may not be due to species interactions because the period of activity of *A. caliginosa* could have been too brief.

The reasons why the survival of *A. rosea* was lower when pea straw was added in the first experiment are unclear, especially as earthworm activity was probably quite low. It may be that pea straw encourages the growth of some microorganisms such as nematodes or mites which were parasitic on earthworm tissue (see section 1.5.2.2). Further investigation is required to answer this question.

A. trapezoides gained less weight in T12 (high density) compared to T6 (low density) in the second experiment, but only when pea straw was added. This seems to be counter-intuitive because if earthworms are competing for food, then it would be expected that adding food would reduce any negative intraspecific interactions. However, this negative

effect when food was added may have been because there was less opportunity for interactions in the control (no pea straw) treatment, because more individuals were quiescent, and activity in general would have been much lower. This density effect was also observed in field cages containing unmixed soil in 1993 and 1995 and is discussed in more detail in section 3.7.4.

Quiescence of earthworms in response to low food concentrations has not been recorded before. Earthworms become inactive in response to drought (Olive and Clark, 1978), and construct mucus-lined chambers to protect themselves from long periods of water stress. Inactivity can be obligatory (akin to diapause in insects) in some species such as sexually mature *A. caliginosa* and *A. longa*. However the response of most species (and the juveniles of *A. caliginosa* and *A. longa*) to stress (drought, temperature, chemical toxicity) is facultative quiescence, with the worms entering a state of torpor without excavating chambers or lining them with mucus. They can quickly return to an active state once the stress has been alleviated (Satchell, 1967; Bouché, 1972). Whether quiescence in the face of food shortage is a hormonal response to stress, or whether the earthworms literally run out of energy is not known and further investigation is required to elucidate the mechanism.

Straw burial was significantly lower per gram of earthworm tissue in the CT and C12 treatments compared to the C6, T12 and T6 treatments. This may be the result of a competitive effect whereby the performance of *A. caliginosa* is reduced at higher densities, or of different feeding behaviours. However; neither growth, survival nor number of adults of *A. caliginosa* was significantly different between CT and C12 and the other treatments. Furthermore, the total amount of pea straw buried was not significantly different between treatments. The total weight of earthworms was greater in the CT and C12 treatments (4.48 and 4.27g) compared to the other treatments (2.20 to 3.00g) and this accounts for the lower amount of pea straw buried on a per weight basis.

3.7.2 Interaction experiments at Springmount

A. caliginosa was the species that performed best in terms of growth and survival in the field experiments at Springmount. *A. trapezoides* fared slightly worse, and *A. rosea* did not seem to be well adapted to the conditions because it's survival was always less than 50% and on average it always lost weight. For this reason, *A. rosea* was not used in the pot or field experiments in 1995.

The numbers of earthworms added to the cages at Springmount were doubled in 1994 and 1995, because no significant interactions were found in the 1993 experiment, and I wanted to be sure that if competition did play a role in these communities, it could be detected. The densities of earthworms have been measured at Springmount in survey work carried out by G.H. Baker between 1989 and 1995 (unpublished). Numbers varied from 150 to 350 m^{-2} for *A. caliginosa* and 200 to 400 m^{-2} for *A. trapezoides*. The total density of *A. caliginosa* and *A. trapezoides* combined has never exceeded 700m^{-2}. The total biomass of earthworms at Springmount has ranged between 120 and 260 g m^{-2} depending on the seasonal conditions. The total biomass of earthworms added experimentally in 1994 and 1995 was 105 to 110 g m^{-2} in the low density treatments and 210 to 220 g m^{-2} in the high density treatments. Therefore the densities and biomass of earthworms used in these experiments were similar to the maximum densities found in the field.

Soil was left unmixed in the 1995 experiment because analysis of the data from earlier experiments indicated that earthworms performed better in unmixed soil (section 3.7.7). This relationship is investigated further in Chapter 7.

3.7.2.1 Contamination

The reasons for higher contamination by *A. caliginosa* in 1995 compared to similar cages in 1993 are unclear. Perhaps this was due to a higher number of cocoons and juveniles being produced in the previous year, earthworms not moving out of the soil cores over

summer, earthworms moving into the cores before the bases were added due to early autumn rains, or the mesh at the base of the cages was not secured correctly.

3.7.2.2 Smaller earthworms gain more weight

The trend for earthworms to gain more weight in cages with smaller worms was noticed after analysing data from 1993 and 1994 and so in 1995, I used subadult *A. trapezoides* in experiments which weighed between 100 to 300 mg (ie. smaller than before). Earthworms which were smaller than this were not used because of difficulties involved in handling them, in particular they are easily damaged and are more difficult to find when handsorting through soil. There is a trade-off that must be made between using small enough worms so that they can grow in the soil, and using large enough earthworms so that experimental error in sampling does not become too high.

3.7.3 Interaction experiments at Macclesfield and Myponga

There were no significant interactions between *A. trapezoides* or *A. rosea* in the experiments carried out at Macclesfield and Myponga which is consistent with all other experiments (Table 3.7, 3.8). This is discussed further in section 3.7.5. Contamination was low at Macclesfield and Myponga (Table 3.6) and had no affect on the outcome of the analysis.

3.7.4 Intra-specific interactions

Increasing the density of *A. trapezoides* decreased earthworm growth in the 1995 pot experiment (when pea straw was added) and in cages containing unmixed soil in 1993 and 1995. There was a strong trend for survival to be lower in the high density *A. trapezoides* treatment in the Macclesfield experiment, but this was not significant at the 5% level. The mechanism for this competition is not known. Possibly the worms compete directly for food or space, or physically interfere with each other, reducing their activity or feeding rates. Further work needs to be done to elucidate the mechanisms.

Table 3.7 Effect of earthworm interactions on growth of *A. trapezoides*, *A. caliginosa* and *A. rosea* in pot and field experiments

Species	*A. trapezoides*	*A. caliginosa*	*A. rosea*
A. trapezoides			
- Pot 93	0	0	0
- Pot 95	-	0	na
- Spr 93M	0	-	0
- Spr 94	0	0	0
- Spr 95	-	0	na
- Spr 93U	-	na	na
- Mac	0	na	0
- Myp	0	na	0
A. caliginosa			
- Pot 93	0	0	0
- Pot 95	0	0	na
- Spr 93M	-	0	0
- Spr 94	0	0	0
- Spr 95	0	0	na
A. rosea			
- Pot 93	0	+	+
- Spr 93M	0	0	0
- Spr 94	0	0	0
- Mac	0	na	0
- Myp	0	na	0

0 = no effect
+ = positive effect
- = negative effect
na = not available because treatments not included in experimental design
Pot 93 = first pot experiment
Pot 95 = second pot experiment
Spr 93M = field experiment in mixed soil at Springmount in 1993
Spr 94 = field experiment at Springmount in 1994
Spr 95 = field experiment at Springmount in 1995
Spr 93U = field experiment in unmixed soil at Springmount in 1994
Myp = field experiment at Myponga in 1993
Mac = field experiment at Macclesfield in 1993

Table 3.8 Effect of inter- and intra-specific interactions on the proportion of individuals which were adults at the end of the experiments for *A. trapezoides*, *A. caliginosa* and *A. rosea* in pot and field experiments

	A. trapezoides	A. caliginosa	A. rosea
A. trapezoides			
- Pot 95	0	0	na
- Spr 94	0	0	+
- Spr 95	0	0	0
- Spr 93U	0	na	
- Myp	0	0	0
A. caliginosa			
- Pot 95	0	0	na
- Spr 94	0	0	0
- Spr 95	0	0	0
A. rosea			
- Spr 94	0	0	0
- Myp	0	0	0

0 = no effect
+ = positive effect
- = negative effect
na = not available because treatments not included in experimental design
Pot 93 = first pot experiment
Pot 95 = second pot experiment
Spr 93M = field experiment in mixed soil at Springmount in 1993
Spr 94 = field experiment at Springmount in 1994
Spr 95 = field experiment at Springmount in 1995
Spr 93U = field experiment in unmixed soil at Springmount in 1994
Myp = field experiment at Myponga in 1993
Mac = field experiment at Macclesfield in 1993

Intraspecific competition between individuals of *A. trapezoides* was not detected in the 1993 pot experiment or the cages containing mixed soil at Springmount in 1993 and 1994. Earthworm activity may have been reduced due to food stress in 1994, and so earthworms may have not had an opportunity to compete because their activity was either low or they were quiescent. It is relevant to note that intra-specific competition was only demonstrated in the 1995 pot experiment when food was added and worms were growing and was not shown in the control pots where earthworms lost weight. Another explanation may be that competition is intermittent and depends on what limits it's performance in that particular year (see section 3.7.5.1).

Increasing the density of *A. caliginosa* or *A. rosea* had no affect on the survival or growth of either species. This may have been because the earthworms were not active enough for interactions to occur or because the densities were not high enough for intraspecific competition to be a factor for these species. However, the experimental densities of *A. caliginosa* were higher than the natural densities found at Springmount and *A. caliginosa* gained weight in the 1995 pot experiment, and at Springmount in 1993 (unmixed soil), 1994 and 1995 and so it seems unlikely that it was inactive. Therefore at Springmount, intraspecific competition would seem to be of relatively small importance as a regulatory force in maintaining the population of *A. caliginosa* below a certain density. Other factors which may be more important are discussed in section 3.4.8. *A. rosea* lost weight in all experiments except the Myponga experiment and so may have been inactive in most experiments. Further investigation into intraspecific interactions of *A. rosea* is warranted at sites where it can be found to grow and survive well.

3.7.5 Inter-specific interactions

It is difficult to interpret the results of investigations involving *A. rosea* and the other two species because *A. rosea* performed poorly in most experiments and may not have been fully active. At the Myponga site however, both *A. trapezoides* and *A. rosea* coexisted and gained weight when they were added to the cores at densities which were higher

than that measured at the site (82 *A. trapezoides* m^{-2}; 3 *A. rosea* m^{-2}; G.H. Baker, unpublished data). No interactions were detected in this experiment, indicating that competition does not occur between these species at this site. *A. trapezoides* and *A. rosea* have been shown to have some differences in their ecology which might be explanations for coexistence. For example, *A. trapezoides* is active at the surface whereas *A. rosea* rarely comes to the surface (Baker *et al.*, 1994a) and in southern Australia, *A. trapezoides* is dominant in high rainfall pastures whereas *A. rosea* is the dominant species in drier, cropping systems (Baker *et al.*, 1994b).

In Springmount soil, where *A. caliginosa* and *A. trapezoides* are found in high densities, there was a significant negative effect on the growth rates of both species in two experiments out of five. This suggests that although competition could not be demonstrated between these species at all times, it would be a factor in determining the performance of earthworm populations under some conditions. It is not known whether competition under these circumstances is enough to be a major factor in limiting the densities of these two species at Springmount. Long-term sampling of earthworm densities at these sites which is being carried out by G.H. Baker may provide some clues. Otherwise long term experiments with mixed and single species cultures will be required to answer this question.

It would be interesting to know whether *A. trapezoides* and *A. caliginosa* use similar resources. If they do, then it suggests that for much of the time they avoid competition for resources. To answer the question of whether they use the same resources, some measure of what food resources each uses needs to be obtained first. Food resource-use would be difficult to determine because of the complex nature of soil organic matter, although the novel use of some recent technologies may overcome this problem (see section 1.5.2.3).

An analysis of spatial distribution and movement in the soil may resolve whether earthworms are likely to compete for space. In an unpublished experiment, *A. caliginosa* and *A. trapezoides* were shown to avoid each other (Clare L. Griffin, unpublished data). Ground up cow dung was placed on top of a mesh cylinder containing sieved soil. This cylinder of soil and dung was placed in a larger container and more sieved soil was placed around the cylinder. Earthworms were then added to the containers. The three experimental treatments were *A. caliginosa*, *A. trapezoides* and *A. caliginosa* + *A. trapezoides*. In the mixed species treatment, *A. trapezoides* was significantly more abundant within the soil cylinder while *A. caliginosa* was significantly more abundant around the perimeter of the soil cylinder compared to when they were in the single species treatments. The mechanism for this displacement is not known. Earthworm species may dislike being in the presence of a high density of another species and move away, creating an aggregation effect. This aggregation effect was also demonstrated in a separate experiment in Chapter 6, where *A. longa* aggregated in pasture soil, which resulted in *A. trapezoides* becoming more aggregated in the adjacent forest soil.

3.7.6 Earthworm growth

Earthworm growth, or loss of weight in the experiments is summarised in Table 3.9. *A. rosea* only gained weight in one treatment in one experiment. No intra-specific competition was detected for *A. rosea* and so it seems unlikely that the densities of earthworms added were too high. The size of the earthworms may have been too large to be supported in the soils, handling the earthworms may have stressed them so that they entered a state of quiescence and lost weight, or the soils may have been unsuitable for *A. rosea* (although it was found low densities at Macclesfield and Myponga).

Table 3.9 Minimum, maximum and average growth rates of *A. trapezoides*, *A. caliginosa* and *A. rosea* per treatment in all pot and field experiments.

Species	Experiment	Growth rate (mg ind.$^{-1}$ wk.$^{-1}$)		
		Minimum	Average	Maximum
A. trapezoides	Pot 93	-26.8	-16.6	-9.9
	Pot 95	-3.4	-0.8	2.1
	Spr 93 M	-11.6	-6.9	-3.9
	Spr 93 U	1.4	5.8	19.7
	Spr 94 M	-15.7	-15.1	-13.7
	Spr 95 U	-6.0	-0.1	9.2
	Mac U	-1.2	0.9	3.6
	Myp U	3.6	4.6	6.9
A. caliginosa	Pot 93	-13.3	-9.9	-5.7
	Pot 95	-4.8	11.6	34.6
	Spr 93 M	-5.7	0.3	5.0
	Spr 94 M	0.2	2.1	3.8
	Spr 95 U	-3.8	-2.1	1.1
A. rosea	Pot 93	-28.8	-20.9	-14.5
	Spr 93M	-53.9	-11.2	-9.4
	Spr 94 M	-10.1	-1.4	2.1
	Mac U	-6.9	-5.5	-4.3
	Myp U	-4.7	-3.3	-2.0

Pot 93 = first pot experiment
Pot 95 = second pot experiment
Spr 93 M = field experiment in mixed soil at Springmount in 1993
Spr 94 M = field experiment in mixed soil at Springmount in 1994
Spr 95 U = field experiment in unmixed soil at Springmount in 1995
Spr 93 U = field experiment in unmixed soil at Springmount in 1994
Myp U = field experiment in unmixed soil at Myponga in 1993
Mac U = field experiment in unmixed soil at Macclesfield in 1993

A. trapezoides gained weight at all sites but lost weight in Springmount soil when it was mixed. The soils at all three sites were obviously suitable for *A. trapezoides*. The average growth rate ranged between a loss of 16.6 and a gain of 5.8 mg ind^{-1} wk^{-1} for *A. trapezoides* and a loss of 9.9 and a gain of 11.6 mg ind^{-1} wk^{-1} for *A. caliginosa*. The maximum growth rate of any treatment was 19.7 for *A. trapezoides* and 34.6 mg ind^{-1} wk^{-1} for *A. caliginosa*. This compares with measurements of 1.5 to 2.1 mg ind^{-1} wk^{-1} for *A. caliginosa* in field situations and up to 21 mg ind^{-1} wk^{-1} in the laboratory (Fig. 1.1). Therefore the growth rates measured in these experiments are similar to other growth rates recorded in the literature, and in some circumstances, the growth rates are equivalent to those measured by *A. caliginosa* under optimum conditions (Boström 1988).

3.7.7 Differences in growth and survival between mixed and unmixed soil

There is a trend for the growth and survival to be higher in unmixed soil compared to mixed soil, although comparing data sets from different experiments in different years should be done with caution. Nevertheless, unmixed soil was used in pot and field experiments in 1995 in case there was a real effect of mixing soil on earthworm performance. This section discusses some of the reasons why there might be a difference in earthworm performance between mixed and unmixed soil.

Two factors may have caused the difference in growth and survival between *A. trapezoides* from mixed and unmixed soil. Firstly, mixing the soil dilutes the carbon originally concentrated in the top layers through a larger volume of soil (see Table 3.10). The mixing action also results in a mineralisation of organic matter, as previously unavailable carbon supplies are brought into contact with soil microorganisms. This would result in a lower concentration of food sources in the soil for earthworms. They

Table 3.10. Soil organic carbon distribution down soil profile in cages with mixed or unmixed soil at Springmount collected in 1993. Values followed by the same letter are not significantly different at the 5% level.

Depth (cm)	% carbon in soil	
	Mixed	Unmixed
0-5	2.24 (0.11) a	4.27 (0.27) c
5-10	2.30 (0.08) a	3.37 (0.30) b
10-15	2.22 (0.15) a	2.18 (0.09) a

may suffer from a "relative" resource shortage (Andrewartha and Browning, 1961), whereby earthworms have to expend more energy tunnelling through the soil to consume the same quantity of food, and subsequently their assimilation efficiency would be lower. A lower assimilation efficiency will obviously result in lower growth rates and survival.

Secondly, in the unmixed soil, there was a high production of plant material on the surface. This would have been mirrored by root production underground which can add large amounts of carbon into the soil ecosystem (Fogel, 1985). Roots may be an important resource for earthworms. Earthworms feed on microorganisms (Edwards and Fletcher, 1988) which are particularly abundant in the rhizosphere (Rovira, 1965). Earthworms have also been reported to feed on dead roots (Edwards and Lofty, 1982a; Bouché and Kretzschmar, 1974), live roots (Cortez and Bouché, 1992; Gunn and Cherrett, 1993) and possibly mycorrhizal hyphae (Pattinson, G. unpubl.). Martin *et al.* (1992) used C3 and C4 plant material to show that earthworms obtain the majority of their carbon resources from plant material recently produced by measuring $^{13}C/^{12}C$ ratios. Therefore, a reduction of organic material in the soil cages, especially a reduction in new organic material from plant roots, could be expected to have an adverse effect on earthworm growth and survival that was recorded in these experiments. This is investigated further in Chapter 7.

The effect of soil disturbance on earthworm growth found in these experiments may be one explanation as to why direct-drilling or minimum-tillage increases earthworm numbers in comparison to conventionally cultivated soils (Edwards and Lofty, 1982b; Rovira *et al.*, 1987). Cultivating soil dilutes the food resource in upper soil layers throughout the relatively poorer lower soil layers in much the same way as handsorting the soil does. Fallow periods are also likely to reduce earthworm numbers, because fallowing greatly reduces the input of new plant material into the soil ecosystem.

There may be other factors that caused the lower growth of earthworms in the mixed soil apart from a food shortage. The structure of mixed soil has been changed which may have affected earthworms in a number of ways. With the structure destroyed, any remnant burrows will have also been destroyed. Therefore the earthworms added to the pots would have had to construct new burrows. It is unknown whether a soil mixed in this manner is any worse or better for burrowing. Finally, the relationship of water with the soil would be different in mixed compared to unmixed soil. The same amount of rain may have produced very different soil water potential in mixed and unmixed soils because of the differences in pore size distributions. Therefore further investigation into the effect of food distribution and type as well as the effect of changing the soil structure on earthworm activity is required to determine the mechanism of poorer earthworm growth and survival found in mixed soils. This is investigated in Chapter 7.

3.8 Conclusions

1. Earthworm performance (growth, survival etc.) was disappointingly low in some experiments. This may have been due to; the unsuitability of the species for the particular soil types, individuals were too large to be able to be supported in the soil, or the soil structure and food distribution had been changed as a result of mixing the soil (investigated further in Chapter 7). *A. caliginosa* was the species that performed best in terms of growth and survival in the field experiments at Springmount, *A. trapezoides* gained weight in at least some treatments where the soil was left undisturbed at all three sites and *A. rosea* lost weight at all sites and only gained weight in one treatment at one site.

2. *A. trapezoides* compete intraspecifically for food or space (intraspecific competition) or may physically interfere with each other (interference competition) under a range of conditions, although the interaction was not measured in all cases. Further work needs to be done to elucidate the mechanisms.

3. No intraspecific interactions were measured for *A. caliginosa* or *A. rosea*, although conditions may not have been sutiable for A. rosea and so the tests may not have been valid.

4. *A. caliginosa* and *A. trapezoides* had a measured negative impact on each other one experiment out of five, but not all. This suggests that although competition does not occur between these species all of the time, it will be a factor intermittently. It would be interesting to know whether the mechanism for competition is for food resources (scramble competition), or whether they interfere with or stress each other in some way (interference competition).

5. There was no evidence that *A. rosea* had any interspecific associations or interactions with either *A. caliginosa* or *A. trapezoides* suggesting that these species either do not have similar resource requirements to *A. rosea* or there is some mechanism that avoids competition for resources. There are some ecological differences between *A. rosea* and the two other species which suggest that there are differences in their resource requirements.

6. *A. trapezoides* and *A. caliginosa* respond to food stress by entering a state of quiescence.

7. Small earthworms (100 - 300 mg) should be used in further experimentation because there is a significant negative correlation between earthworms weight and earthworm growth.

8. Mixing the soil may reduce subsequent growth of earthworms placed in the soil.

9. Further work is required to determine what components of the soil organic matter earthworms digest and assimilate.

Chapter 4 INTERACTIONS BETWEEN *APORRECTODEA LONGA* (UDE) AND OTHER SPECIES IN PASTURES

4.1 Introduction

Aporrectodea longa (Ude) is being considered for introduction into the high rainfall agricultural areas of southern Australia (section 1.4.2). If a species of earthworm is to be introduced into an area where it currently does not exist, it needs to be understood whether it will compete with species which are already established in that area. There is very little known about species interactions in earthworm communities in general (section 1.6), and also little pertaining specifically to *A. longa*.

The most widespread species of earthworms found in pastures in southern Australia are *Aporrectodea trapezoides* (Dugés), *Aporrectodea caliginosa* (Savigny) and *Aporrectodea rosea* (Savigny) (Baker *et al.*, 1995). In the Mount Lofty Ranges, South Australia, the area in which this study was based, *Microscloex dubius* (Fletcher) is also widespread (Baker *et al.*, 1992a,b).

In Tasmania, both *A. caliginosa* and *A. longa* had higher population densities in areas where both species coexisted compared to areas where only one species was present (Temple-Smith *et al.*, 1993). Furthermore, these two species are often found together in permanent pastures in Tasmania (R. Garnsey, *pers. comm.*) and New Zealand (J. Springett, *pers. comm.*). Whether this is because of positive interactions between the two species, or a sharing of similar habitat preferences, is not known.

This chapter reports on a series of experiments that aimed to determine whether there is competition between *A. longa* and four other species; *A. caliginosa*, *A. trapezoides*, *A. rosea* and *M. dubius*. The experimental designs used were similar to those set out in Chapter 3. The null hypothesis in most experiments was that adding a second species to the first species had no effect on the survival or growth of the first species (ie. there was

no competitive effect). Two experiments investigated the ability of *A. longa* to consume earthworm cocoons or artificial spheres that had been mixed in soil. Consumption of cocoons and spheres was monitored either by determining the proportion remaining in soil at the end of the experiment or by presence in casts.

4.2 Pot experiment - Interaction between *A.longa* and *A. caliginosa*

4.2.1 Materials and methods

The aim of this experiment was to determine if there were significant competitive or mutualistic interactions between *A. longa* and *A. caliginosa* as measured by changes in growth or survival of each species. There were five treatments in the experiment; *A. caliginosa* added at 6 pot^{-1} or 12 pot^{-1}, *A. longa* at 6 pot^{-1} or 12 pot^{-1} and *A. longa* and *A. caliginosa* together, with each being added at 6 pot^{-1}. The earthworms were added to 4 litre pots (20 cm diameter, 20 cm depth) containing Springmount soil. There were ten replicates per treatment.

Soil was collected from Springmount to a depth of 15 cm, air-dried then sieved through a 5 mm sieve. Each pot contained 2.85 kg soil (on a dry weight basis).

Both *A. longa* and *A. caliginosa* were collected from the Woolnorth collection site in early June 1993 and stored in a mix of soil and sphagnum moss for four weeks prior to the experiment. Earthworms were pre-treated (section 2.4), then weighed. The average starting weights of *A. longa* and *A. caliginosa* were 1282 mg and 361 mg respectively. There was no significant difference in starting weights between treatments for each species (1-way ANOVA, $d.f.=2$, $F<1.30$, $P>0.05$).

The earthworms were added to the pots in early July. The pots were kept in a constant temperature water bath at 15°C (±1°C). The water potential was kept above -10 kPa (section 2.3.3.1) by weekly watering to weight.

Ten weeks after the earthworms were added to the soil, the experiment was terminated. Earthworms were removed from the soil and pre-treated before weighing (section 2.4).

Statistical analysis was carried out using a 1-way ANOVA, using Tukey's Pair-wise comparison to determine differences between individual means. The percent weight loss and survival was transformed to arcsin (square root((x/100)+1)). In some cases, the assumptions of the ANOVA test could not be met, and in these cases a Kruskal-Wallis test was used. A linear regression was used to analyse the relationship between the average starting weight of *A. longa* pot^{-1} and the average growth rate pot^{-1}.

4.2.2 Results

Overall survival of *A. caliginosa* and *A. longa* was greater than 95%, with no significant differences in survival between treatments for either *A. caliginosa* (Kruskal-Wallis, H=2.00, P>0.05) or *A. longa* (1-way ANOVA, d.f.=2, F=0.00, P>0.05).

Both species of earthworms lost weight over the period of the experiment, with *A. caliginosa* losing between 11 and 30% of the average starting weight pot^{-1}, or 0.6 to 1.6 mg $ind.^{-1}$ day^{-1}, and *A. longa* losing between 47 and 55% of the average starting weight pot^{-1}, or 8.4 to 10.5 mg $ind.^{-1}$ day^{-1} (Fig. 4.1 and 4.2). *A. caliginosa* lost significantly more weight (Kruskal-Wallis, H=13.72, P<0.05) in the treatment with *A. longa* than they did in the low density, single species treatment. *A. longa* lost significantly more weight (1-way ANOVA, d.f.=2, F=6.63, P<0.05) in the treatment with *A. caliginosa* than in the low density, single species treatment. There was a significant negative relationship (Linear regression, Student's T=-3.76, r^2=0.336, P<0.05) between starting weight of *A. longa* and it's growth rate (Fig. 4.3).

Fig. 4.1 Pot experiment - interactions between *A. longa* and *A. caliginosa*. Weight change of *A. caliginosa* in pots containing Springmount soil kept at 15°C (±1°C) for 10 wks. Vertical bars represent standard errors of the means, n=10. Bars with same letters are not significantly different at the 5% level. C6 = *A. caliginosa* at a density of 6 worms pot^{-1}. C12 = *A. caliginosa* at a density of 12 worms pot^{-1}. CL = *A. caliginosa* and *A. longa* at a density of 6 worms pot^{-1} each.

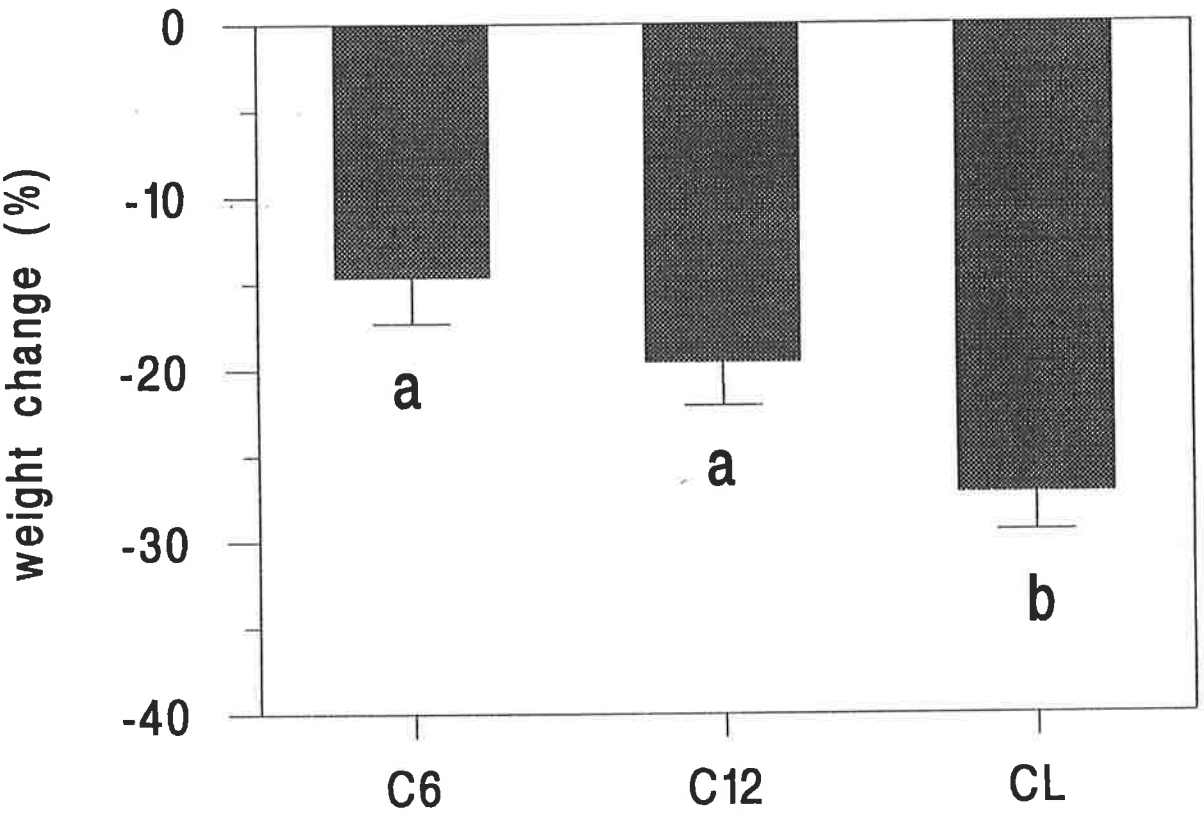


Fig. 4.2 Pot experiment - interactions between *A. longa* and *A. caliginosa*. Weight change of *A. longa* in pots containing Springmount soil kept at 15°C (±1°C) for 10 wks. Vertical bars represent standard errors of the means, n=10. Bars with same letters are not significantly different at the 5% level. L6 = *A. longa* at a density of 6 worms pot^{-1}. L12 = *A. longa* at a density of 12 worms pot^{-1}. CL = *A. caliginosa* and *A. longa* at a density of 6 worms pot^{-1} each.

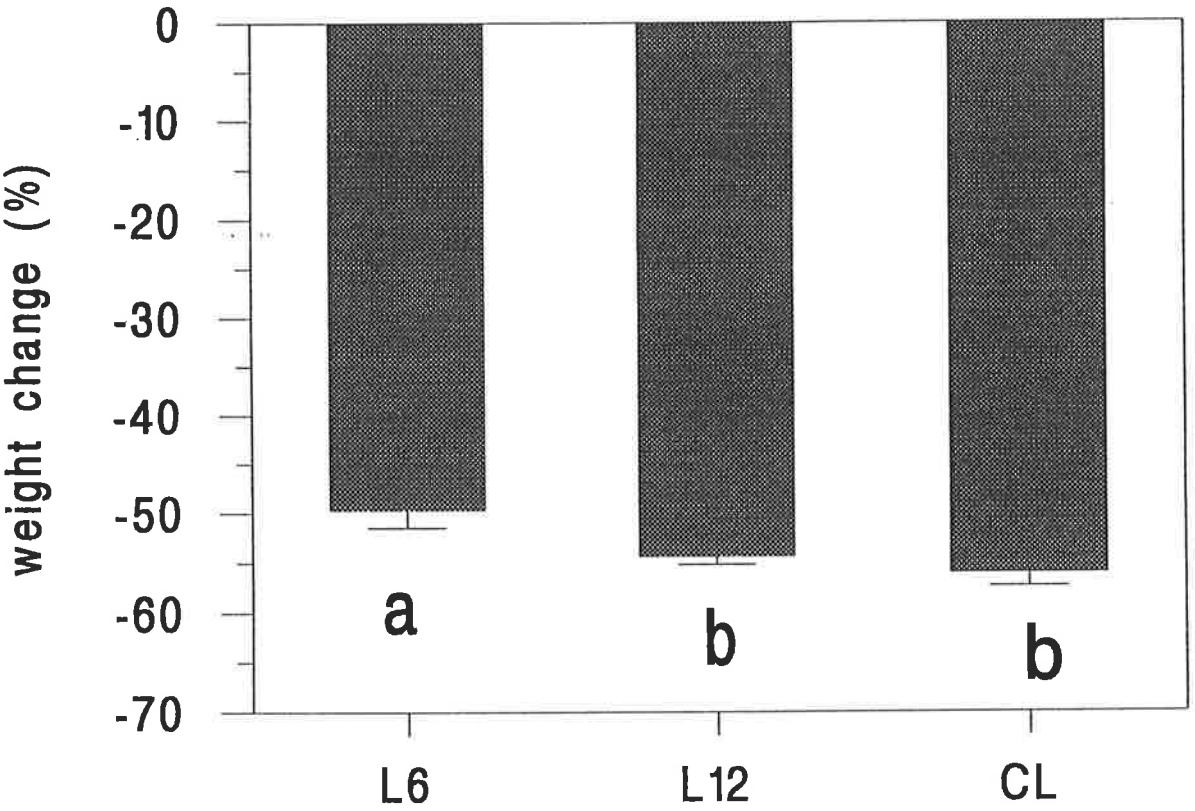
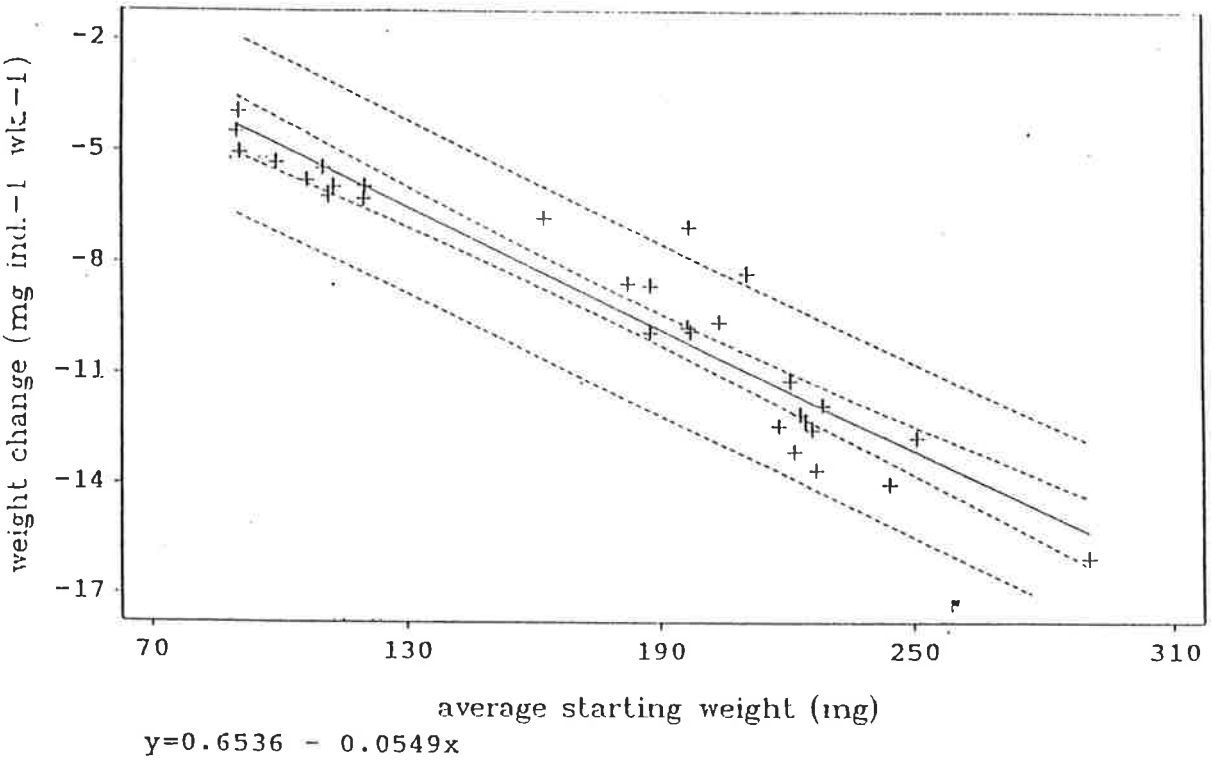


Fig. 4.3 Pot experiment - interactions between *A. longa* and *A. caliginosa*. Relationship between weight change and average starting weight for *A. longa* in pots containing Springmount soil kept at 15°C (±1°C) for 10 wks. 95% confidence (inner, curved pair of dotted lines) and predicted (outer, straight pair of dotted lines) intervals are shown. y = 0.654 - 0.0549x



4.3 Field experiment at Springmount - effect of *A. caliginosa*, *A. trapezoides* and *A. rosea* on *A. longa*

4.3.1 Materials and methods

4.3.1.1 1994 - mixed soil

This experiment aimed to determine if growth, survival or reproduction of *A. longa* was significantly reduced by the presence of *A. caliginosa*, *A. trapezoides* or *A. rosea* at Springmount in 1994. There were five treatments: *A. longa* added at 8 and 15 worms cage^{-1} and 8 *A. longa* cage^{-1} plus either another 30 *A. trapezoides*, 30 *A. caliginosa* or 30 *A. rosea*. For each treatment there were seven replicates.

Cages were constructed as described in section 2.3.1. Individuals of *A. longa* and *A. caliginosa* were collected from the Woolnorth site in mid-June 1994 and stored in a mix of soil and sphagnum moss. Individuals of the species *A. trapezoides* and *A. rosea* were collected from the Waite collection site in late-June 1994.

Earthworms were pre-treated as described in section 2.4 and added to the cores in late-June 1994. The average starting weight for individual *A. trapezoides* was 469 mg, 352 mg for *A. caliginosa*, 237 mg for *A. rosea*, and 1465 mg for *A. longa*. There were no significant differences between treatments in terms of the starting weight of *A. longa* (1-way ANOVA, d.f.=4, $F=0.36$, $P>0.05$).

The experiment was terminated in late-September 1994. The soil in the cages was handsorted for earthworms. These were pre-treated (section 2.4) before weighing. The handsorted soil was then wet-sieved through a 2 mm sieve to remove cocoons.

Data was analysed in the same manner as for section 4.2. Cocoon density was transformed to log (x+1).

4.3.1.2 1995 - unmixed soil

This experiment aimed to determine whether there were any significant interactions between *A. longa* and either *A. caliginosa* or *A. trapezoides*. There were six treatments; *A. longa* added at 7 or 14 cage^{-1}, *A. longa* (7 cage^{-1}) plus either *A. trapezoides* (30 cage^{-1}) or *A. caliginosa* (30 cage^{-1}), *A. trapezoides* at 30 cage^{-1} and *A. caliginosa* at 30 cage^{-1}. For each treatment there were nine replicates.

Cages containing undisturbed soil were set up at Springmount in 1995 as described in section 2.3.1. Individuals of *A. longa* and *A. caliginosa* were collected from Tasmania from the Woolnorth site in mid-June 1995. These were stored in a mix of soil and sphagnum moss. Individuals of the species *A. trapezoides* were collected from the Willow Creek collection site in late-June 1995.

Earthworms were pre-treated (section 2.4) and added to the cores in late-June 1995. The average starting weight for individual *A. trapezoides* was 288 mg, for *A. caliginosa* 289 mg, and for *A. longa* 961 mg. There were no significant differences between treatments in terms of the starting weights of *A. longa* (1-way ANOVA, d.f.=4, F=1.05, P>0.05), *A. caliginosa* (1-way ANOVA, d.f.=3, F=0.61, P>0.05) or *A. trapezoides* (1-way ANOVA, d.f.=3, F=0.11, P>0.05). The percentages of the starting population that had a clitellum (ie. were mature adults) were 15% for *A. longa*, 29% for *A. caliginosa* and 1.3% for *A. trapezoides*. Again there were no significant differences between the number of clitellate earthworms in each treatment for *A. longa* (1-way ANOVA, F=0.13, d.f.=3, P>0.05) or *A. caliginosa* and *A. trapezoides* (1-way ANOVA, F<0.57, d.f.=2, P>0.05).

The experiment was terminated in mid-October 1995 after 15 weeks. The soil in the cages was handsorted for earthworms which were then pre-treated (section 2.4) and weighed.

A multiple regression between the different numbers of earthworms of each species at the end of the experiment in each treatment was used to analyse effect of species interactions on survival. Otherwise the data was analysed in the same manner as described in section 4.2.

4.3.2 Results

4.3.2.1 1994 - mixed soil

Survival of *A. longa* in the different treatments ranged from 71.6 to 91.4%, with an average across treatments of 87.0%. There were no significant differences between treatments in terms of survival (1-way ANOVA, d.f.=4, F=0.54, P>0.05).

A. longa lost 23.6 mg ind.$^{-1}$ wk.$^{-1}$, which over the period of the experiment represented a weight loss of 18% of their starting weight. Weight loss was greatest in the treatment with *A. trapezoides* and this was significantly higher (1-way ANOVA, d.f.=4, F=2.98, P<0.05) than the treatment with *A. rosea* (Fig. 4.4) but not significantly different from the single species treatment of *A. longa* (8 cage^{-1}).

The proportion of individuals which were clitellate ranged from 6 to 34.5%, with an average of 19% (Fig. 4.5). There was a significantly lower proportion of adults (1-way ANOVA, d.f.=4, F=2.84, P<0.05) in the population of *A. longa* when *A. rosea* was added to the cages compared to the control population with only 8 *A. longa* cage^{-1}.

The average density of cocoons of each species at the end of the experiment was 0.37 cage^{-1} for *A. longa*, 1.6 cage^{-1} for *A. trapezoides*, 0.14 cage^{-1} for *A. rosea*, and 9.1 cage^{-1} for *A. caliginosa*. Significantly more *A. caliginosa* cocoons were produced than for any other species (1-way ANOVA, d.f.=3, F=24.3, P<0.05). *A. longa* produced

Fig. 4.4 Springmount 1994 - interactions between *A. longa* and either *A. caliginosa, A. trapezoides* or *A. rosea.* Weight change of *A. longa* in cages containing mixed soil after 11 weeks. Vertical bars represent standard errors of the means, n=7. Bars with same letters are not significantly different at the 5% level. L8 = *A. longa* (8 cage^{-1}), L15 = *A. longa* (15 cage^{-1}), CL = *A. caliginosa* (30 cage^{-1}) and *A. longa* (8 cage^{-1}), LR = *A. rosea* (30 cage^{-1}) and *A. longa* (8 cage^{-1}), LT = *A. trapezoides* (30 cage^{-1}) and *A. longa* (8 cage^{-1}).

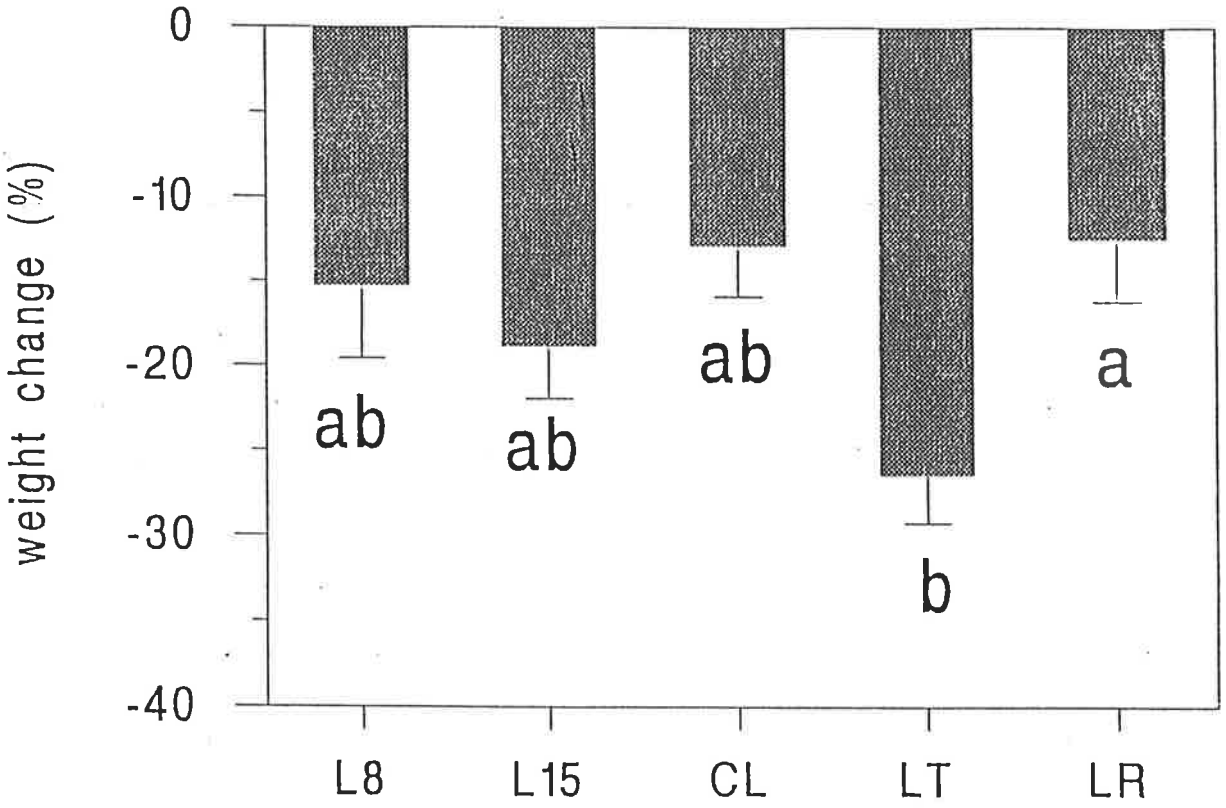
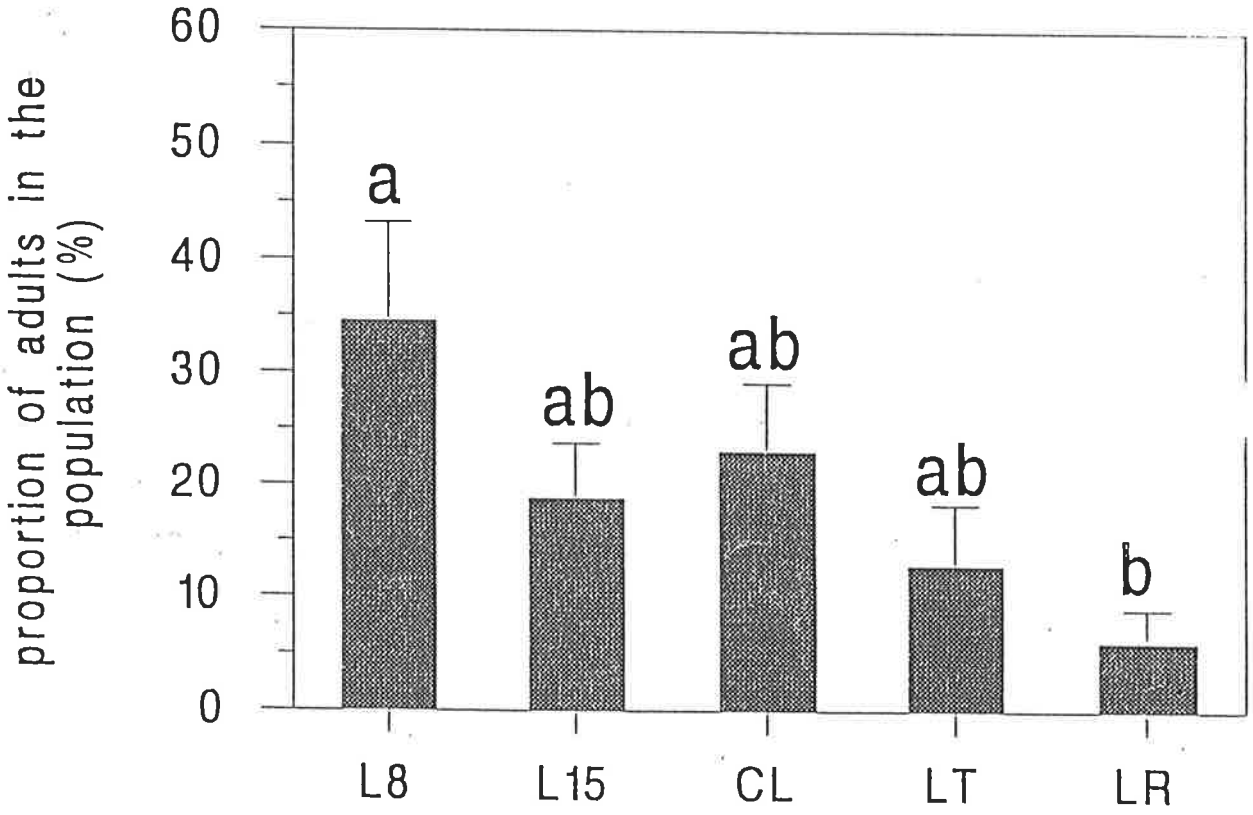


Fig. 4.5 Springmount 1994 - interactions between *A. longa* and either *A. caliginosa*, *A. trapezoides* or *A. rosea*. Proportion of adult *A. longa* in cages containing mixed soil after 11 weeks. Vertical bars represent standard errors of the means, n=7. Bars with same letters are not significantly different at the 5% level. L8 = *A. longa* (8 cage^{-1}), L15 = *A. longa* (15 cage^{-1}), CL = *A. caliginosa* (30 cage^{-1}) and *A. longa* (8 cage^{-1}), LR = *A. rosea* (30 cage^{-1}) and *A. longa* (8 cage^{-1}), LT = *A. trapezoides* (30 cage^{-1}) and *A. longa* (8 cage^{-1}).



significantly more cocoons (Kruskal-Wallis, d.f.=4, H=11.13, P<0.05) in the treatment with *A. trapezoides* than the other treatments (Fig. 4.6). There was a significant relationship (Linear regression, Student's T=-3.51, r^2=0.272, P<0.05) between the average starting weight of *A. longa* and it's weight change over the period of the experiment (Fig. 4.7).

4.3.2.2 1995 - unmixed soil

The average level of survival was 58.5% for *A. longa*, 80.7% for *A. caliginosa* and 31.6% for *A. trapezoides*. There was a significant negative relationship (Fig. 4.8) between the number of *A. caliginosa* and the number of *A. longa* handsorted from the cages at the end of the experiment (Multiple regression, d.f.=2, F=3.44, P<0.05). For every extra 7.0 *A. longa* found in the treatment with *A. caliginosa* at the end of the experiment there was an average of 1.4 less *A. caliginosa* compared to cages without *A. longa.*

A. longa lost weight in all treatments, with those in the high density treatment losing significantly more weight (40%) compared to the other three treatments which showed losses between 13 and 24% (Kruskal-Wallis, d.f.=3, H=10.73, P<0.05). *A. caliginosa* gained 6.7% in the treatment with 30 $cage^{-1}$, but lost 18% in the treatment with 60 $cage^{-1}$ and 24% in the treatment with *A. longa* present. There were no significant differences between treatments (Kruskal-Wallis, d.f.=2, H=1.54, P>0.05). *A. trapezoides* gained 62.5% at a density of 30 $cage^{-1}$ and 1.2% with *A. longa* present and lost 40% at a density of 60 $cage^{-1}$ (Fig. 4.9). The weight loss at 30 $cage^{-1}$ was significantly different than at 60 $cage^{-1}$ (Kruskal-Wallis, d.f.=2, H=6.82, P<0.05).

The proportion of *A. longa* which were adults was 12% with no significant differences between treatments (1-way ANOVA, d.f.=3, F=0.19, P>0.05). The proportion of *A. caliginosa* which were adults was 29% and 14% for *A. trapezoides*, again with no

Fig. 4.6 Springmount 1994 - interactions between *A. longa* and either *A. caliginosa, A. trapezoides* or *A. rosea*. Average density of *A. longa* cocoons in cages containing mixed soil after 11 weeks. Vertical bars represent standard errors of the means, n=7. Bars with same letters are not significantly different at the 5% level. L8 = *A. longa* (8 cage^{-1}), L15 = *A. longa* (15 cage^{-1}), CL = *A. caliginosa* (30 cage^{-1}) and *A. longa* (8 cage^{-1}), LR = *A. rosea* (30 cage^{-1}) and *A. longa* (8 cage^{-1}), LT = *A. trapezoides* (30 cage^{-1}) and *A. longa* (8 cage^{-1}).

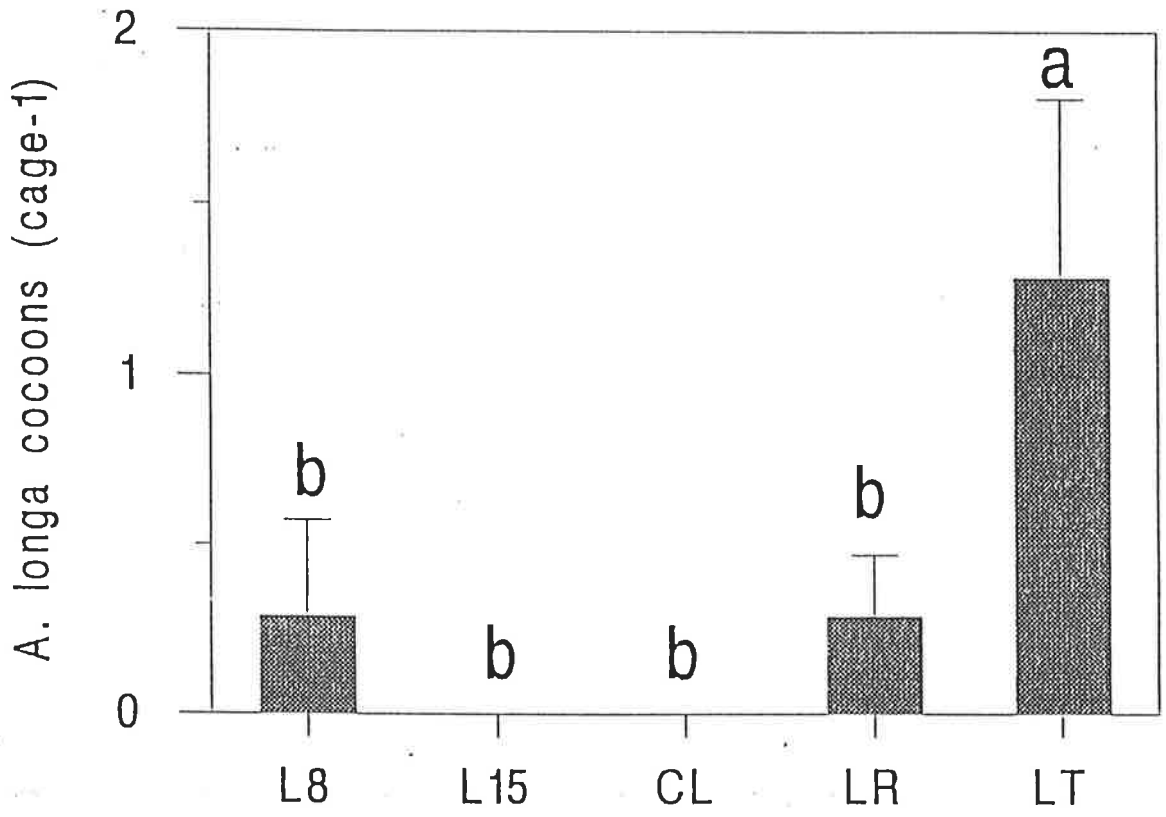


Fig. 4.7 Springmount 1994 - interactions between *A. longa* and either *A. caliginosa, A. trapezoides* or *A. rosea*. Relationship between weight change and average starting weight for *A. longa*. 95% confidence (inner, curved pair of dotted lines) and predicted (outer, straight pair of dotted lines) intervals are shown. y = 34.0 - 0.0393x

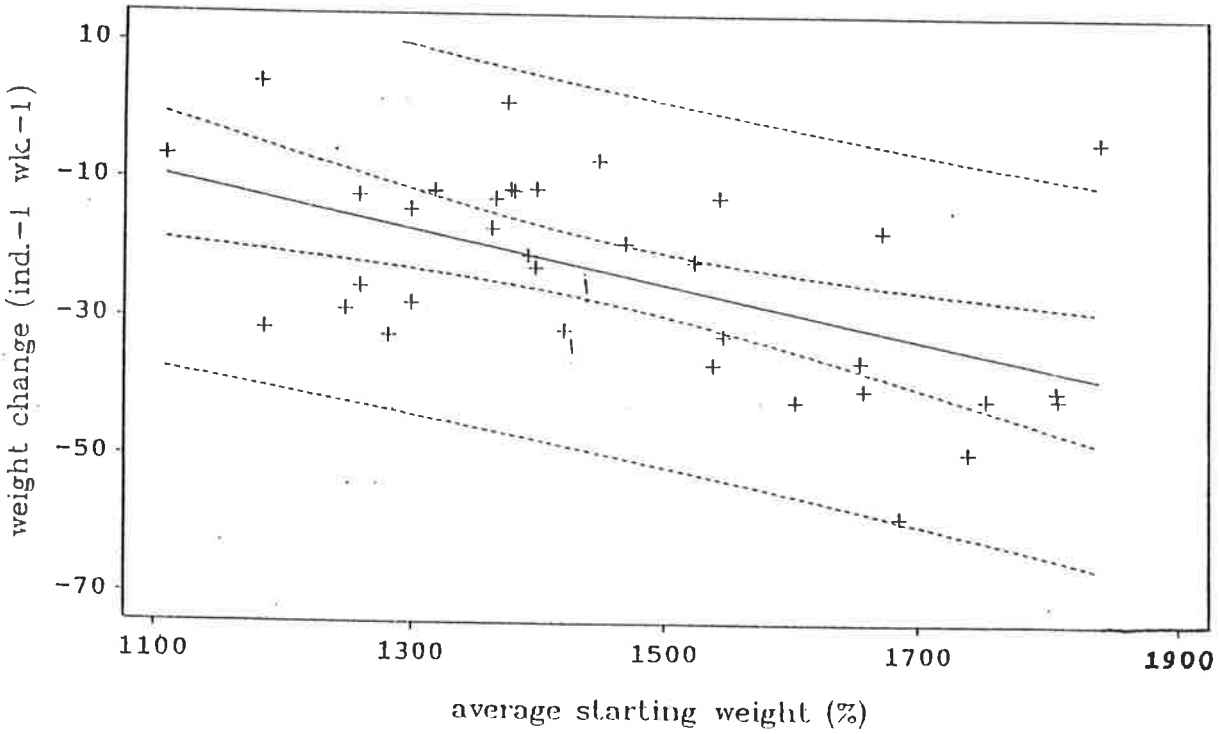


Fig. 4.8 Springmount 1995 - interactions between *A. longa* and either *A. caliginosa* or *A. trapezoides*. Relationship between the density of *A. caliginosa* and *A. longa* in undisturbed soil. 95% confidence (inner, curved pair of dotted lines) and predicted (outer, straight pair of dotted lines) intervals are shown.

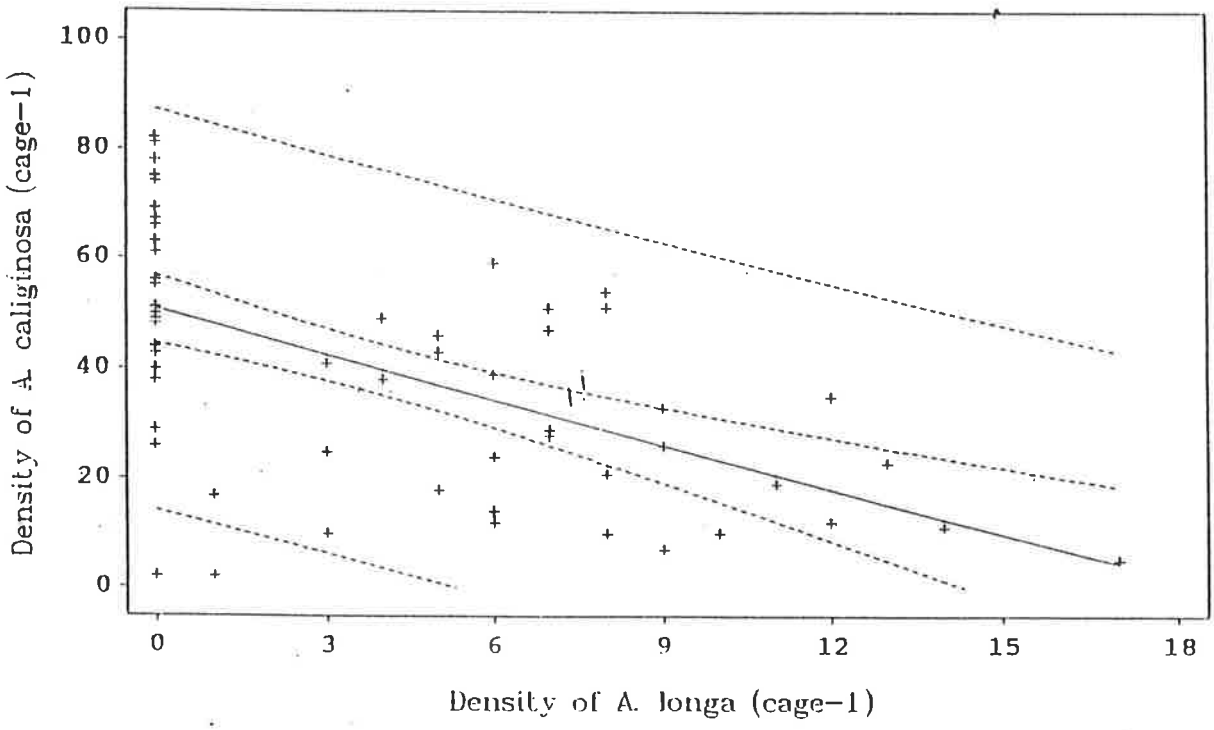
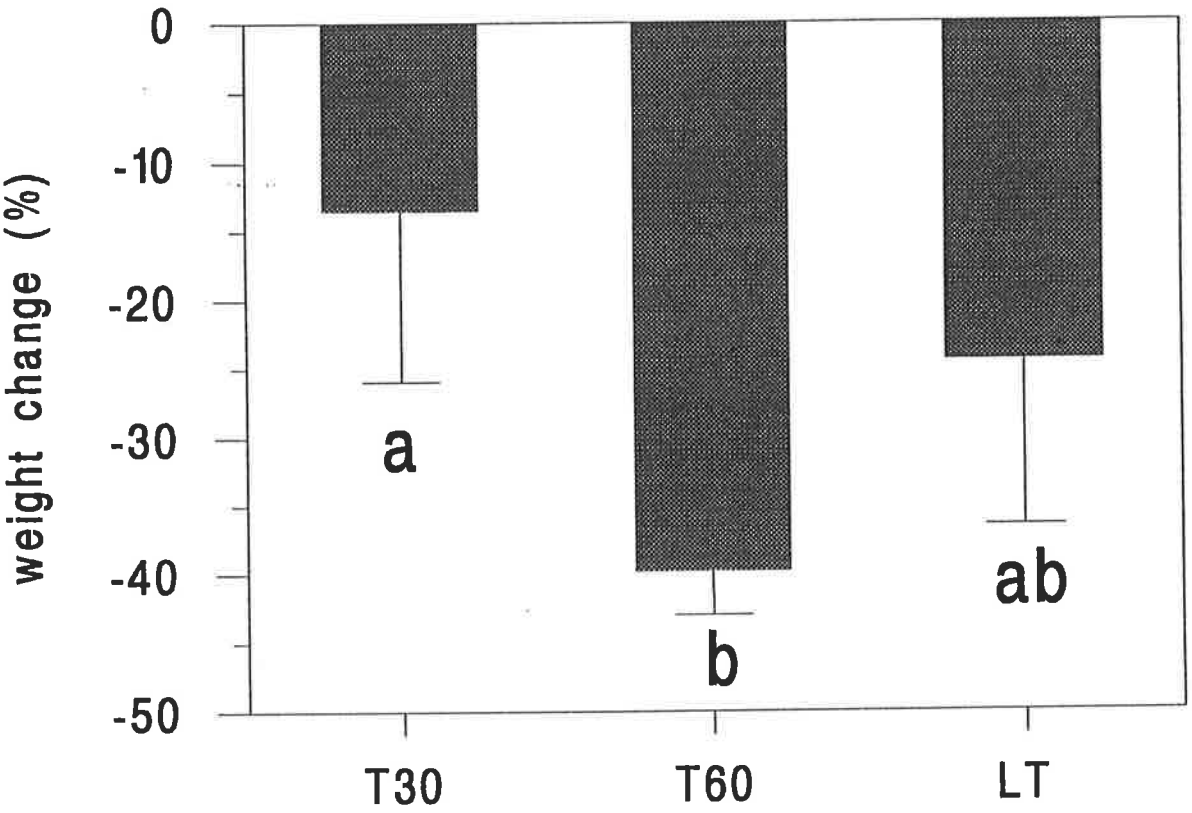


Fig. 4.9 Springmount 1995 - interactions with *A. caliginosa* and *A. trapezoides*. Weight change of *A. trapezoides* in unmixed soil over 15 weeks. Vertical bars represent standard errors of the means, n=9. Bars with same letters are not significantly different at the 5% level. T30 = *A. trapezoides* at a density of 30 worms cage^{-1}. T60 = *A. trapezoides* at a density of 60 worms cage^{-1}, LT = *A. trapezoides* (30 cage) and *A. longa* (8 cage^{-1}).



significant differences between treatments for either of these species (1-way ANOVA, d.f.=2, $F<0.97$, $P>0.05$). There was no significant interaction between average starting weight and weight change for *A. longa* (Linear regression, Student's T = 23.8, $r^2=0.0108$, $P>0.05$).

4.4 Interactions between *A. longa* and *M. dubius*

4.4.1 Materials and methods

4.4.1.1 Woodside 1994 - Effect on resident M. dubius

The experiment was set up at Woodside by Geoff H. Baker. Baker was interested in determining the impact of different population densities of *A. longa* on lime incorporation and pasture growth. The experiment consisted of four different densities of *A. longa*; 8, 15, 30 and 45 cage^{-1} and a control with no worms added. There were seven replicate cages containing intact soil (section 2.3.1) for each density. On analysis of the results, there was a significant interaction between the density of *A. longa* and the density of resident *M. dubius*. The difference in population densities of *M. dubius* was analysed using a 1-way ANOVA after the data had been transformed to square root(x+0.5).

4.4.1.2 Pot experiment - Effect on M. dubius

The aim of this experiment was to investigate whether there is a negative impact of *A. longa* on the survival, growth or reproduction of *M. dubius*. There were four treatments (2x2 factorial); resident *M. dubius* with and without *A. longa* (4 core^{-1}) and resident *M. dubius* plus two extra *M. dubius* core^{-1}, with and without *A. longa* (4 core^{-1}). The density of *A. longa* was set at 4 core^{-1} because this is equivalent to the density of *A. longa* in the previous field experiment (~200m^{-2}) which had shown that *A. longa* reduced the resident populations of *M. dubius*. The density of added *M. dubius* was set at 2 core^{-1} because this is equivalent to the average density of *M. dubius* found at Woodside (~100m^{-2}). The resident treatments were included in case the added *M. dubius* all died. *M. dubius* had not survived experimental handling in previous experiments (data not shown) . For each treatment there were 8 replicates.

Soil cores were collected as described in section 2.3.1. The soil was watered to a water potential of -23 kPa (section 2.3.3.3). The cores were kept in a waterbath at 15°C in a glasshouse with air temperatures ranging from 9°C to 32°C. A square of fine nylon mesh was secured over the top of the core and a 10 mm dia. hole cut out to allow the pasture plants (mostly ryegrass) to grow through.

Sheep dung was collected from holding pens, dried and ground through a 1mm grill. 8.85g (dry weight) of this sheep dung was mixed with 18g of reverse osmosis water and added to each pot as a food source for *M. dubius*.

Clitellate *M. dubius* were collected from the Waite collection site. *M. dubius* was not pre-treated because of their very small size and their sensitivity to handling. The average weight of *M. dubius* individuals was 191 mg in the treatment with *A. longa* and 189 mg in the treatment without *A. longa* and these weightswere not significantly different (1-way ANOVA, d.f.=1, F=0.00, P>0.05).

A. longa were collected from the Woolnorth site. They were pre-treated (section 2.4), weighed and added to the pots. The average weight *A. longa* was 1074 mg in the treatment with added *M. dubius* and 1080 mg in the treatment with resident *M. dubius*, with no significant difference between treatments (1-way ANOVA, d.f.=1, F=0.02, P>0.05). Earthworms of this size were chosen because larger earthworms (1550 mg) lost weight under these conditions (data not shown). Out of the four *A. longa* in each core, an average of 1.13 (28%) had a clitellum.

Earthworms were added to the cores in August 1995. The shoots of the ryegrass plants were cut after two weeks and every week thereafter to simulate grazing.

The experiment was terminated after 10 weeks. Litter was removed from the surface by hand, separated from soil by flotation in a saturated sodium chloride solution (360 g L^{-1}), rinsed with RO water, dried (60°C for 24 hr.) and weighed. Earthworms were handsorted from the soil. *A. longa* was pre-treated before weighing (section 2.4) but *M. dubius* was weighed without pretreatment (see above). Cocoons were wet sieved from the soil through a 1.6 mm sieve which was fine enough to catch the smallest cocoons which belonged to *M. dubius* (2.6 mm long and 1.8 mm wide).

Data were analysed in the same manner as described in section 4.2.1. The difference in the numbers of *M. dubius* cocoons found at the end of the experiment was analysed using a 2-way ANOVA. The number of *M. dubius* and *A. longa* cocoons were transformed to log (x+1).

4.4.2 Results

4.4.2.1 Woodside 1994 - Effect on resident M. dubius

The number of resident *M. dubius* that were found at the end of the experiment in the control cages was 7.29 $cage^{-1}$. This was significantly higher (1-way ANOVA, d.f.=4, $F=8.04$, $P<0.05$) than for all of the treatments which had *A. longa* added (Fig. 4.10).

4.4.2.2 Pot experiment - Effect on M. dubius

Few *M. dubius* (<0.25 pot^{-1}) were recovered at the end of the experiment in either the pots with *M. dubius* added or the pots with no *M. dubius* added (but with a background resident population). Other residents were recovered from the soil (Table 4.1).

The average number of *M. dubius* cocoons sieved from the soil in each of the treatments ranged from 1.0 to 1.75 pot^{-1} (Fig. 4.11). Significantly more

Fig. 4.10 Woodside 1994 - Effect of *A. longa* on resident *M. dubius*. The density of *M. dubius* residents handsorted from cages with different starting densities (0 to 45 cage^{-1}) of *A. longa* after 18 weeks. Vertical bars represent standard errors of the means, n= 7. Bars with same letters are not significantly different at the 5% level.

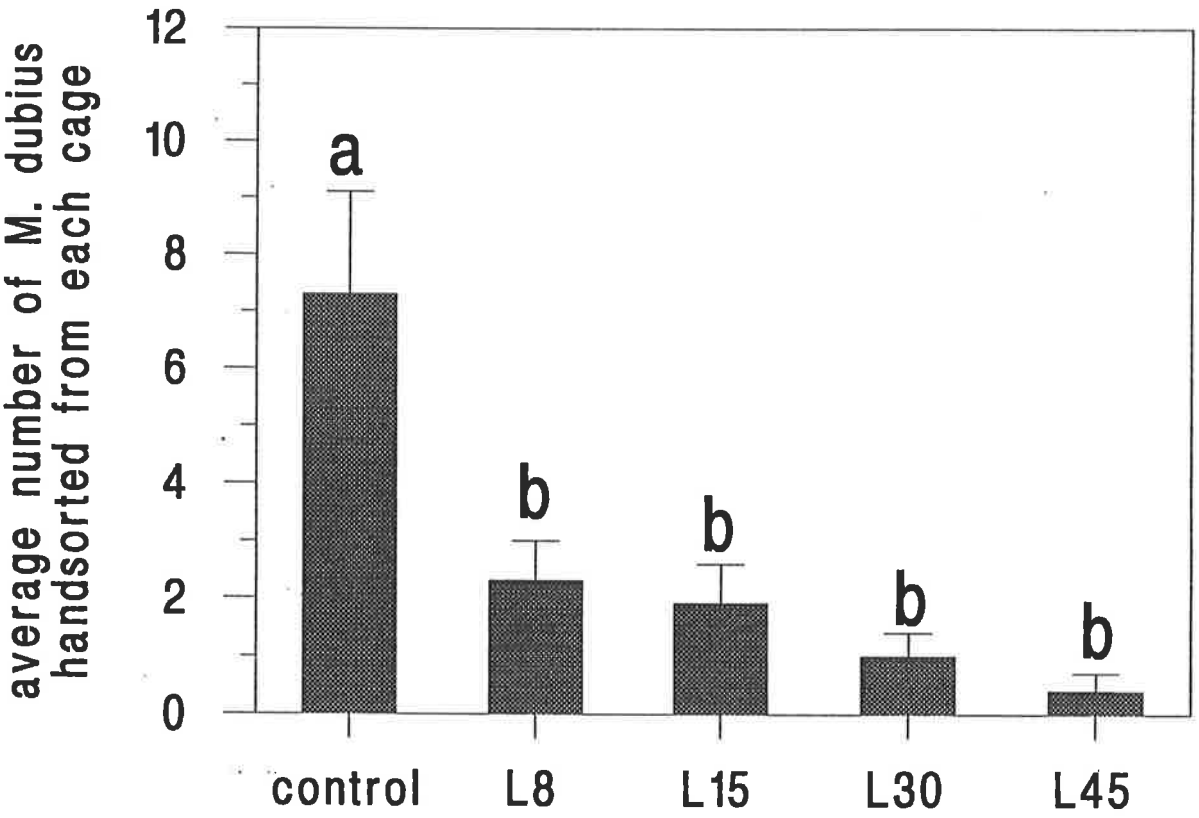
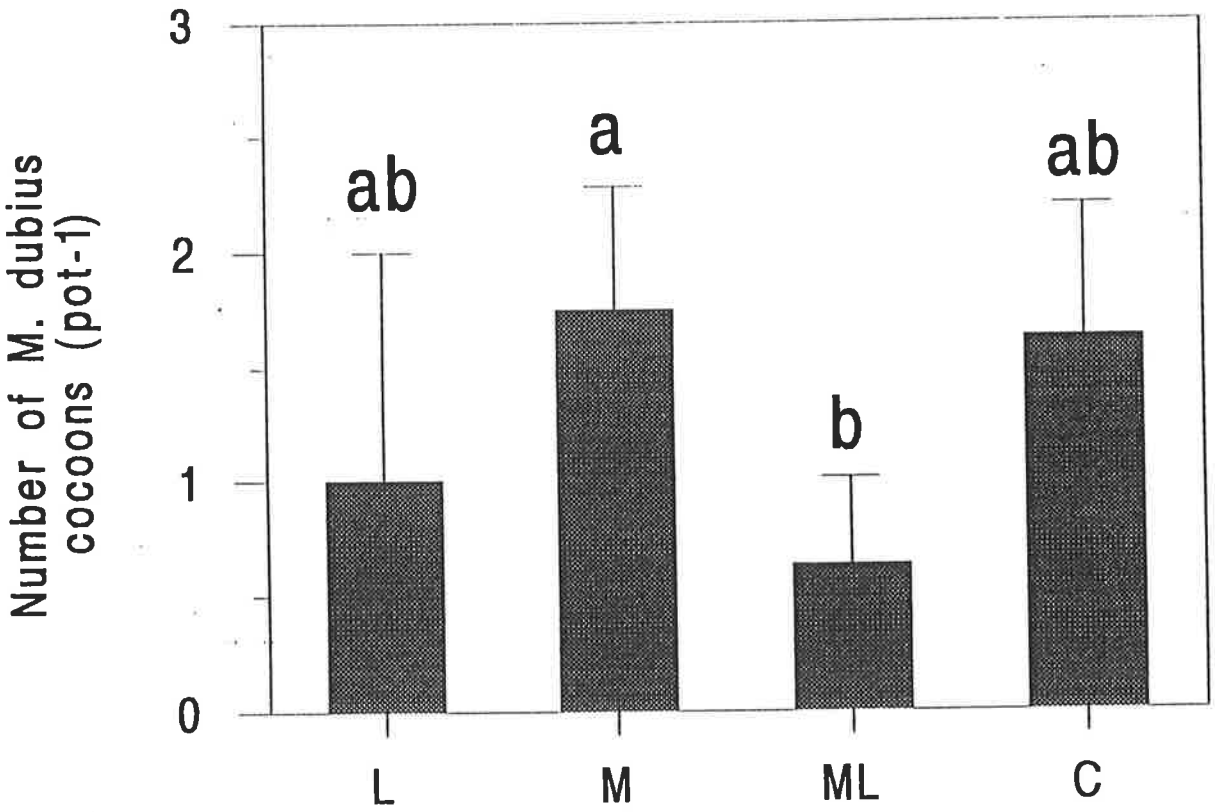


Table 4.1 Pot experiment - Effect of *A. longa* on *M. dubius*. Average number of resident earthworms (apart from *M. dubius*) recovered at the end of the experiment in unmixed soil cores from Woodside kept at 15°C for 10 weeks. Numbers in brackets are standard errors of the mean, n=7.

Treatment	*A. trapezoides*	*A. rosea*	*A. caliginosa*	unidentified juveniles
1. *M. dubius*	0.63 (0.26)	2.75 (0.82)	0.50 (0.50)	1.38 (0.65)
2. *M. dubius* + *A. longa*	1.38 (0.60)	2.25 (0.62)	0	1.63 (0.78)
3. *A. longa*	0.38 (0.26)	2.13 (0.79)	0	0.38 (0.26)
4. Control	0.50 (0.27)	2.38 (1.31)	0	0.50 (0.27)

Fig. 4.11 Pot experiment - Effect of *A. longa* on *M. dubius*. Average number of *M. dubius* cocoons wet sieved from the soil (1.6 mm sieve) from pots kept at 15°C containing intact Woodside soil after 10 weeks. Vertical bars represent standard errors of the means, n= 10. Bars with same letters are not significantly different at the 5% level. L = *A. longa*, M = *M. dubius*, ML = *A. longa* + *M. dubius*, C = Control (no earthworms added).



M. dubius cocoons were sieved from the treatments with no *A. longa* added compared to the treatments with *A. longa* added (2-way ANOVA, d.f.=1, F=4.47, P<0.05). There was no significant difference in the number of *M. dubius* cocoons sieved from the soil between the treatments with added *M. dubius* and those with no added *M. dubius* (2-way ANOVA, d.f.=1, F=0.14, P>0.05).

All *A. longa* were recovered from the treatments with *M. dubius* and 94% were recovered from the treatment with *A. longa* on it's own. *A. longa* increased in weight by 23.2% over the period of the experiment, representing an average growth rate of 6.63 mg. ind^{-1} $wk.^{-1}$. There were no significant differences between treatments in terms of weight change (1-way ANOVA, d.f.=1, F=1.52, P>0.05). There was no significant relationship between starting weight and weight change (Linear regression, Student's T=0.11, r^2=0.0009, P>0.05).

The number of *A. longa* cocoons wet sieved from the soil was 1.1 pot^{-1} on average for the pots which had *A. longa* added, with no significant differences between these treatments (1-way ANOVA. d.f.=1, F=0.59, P>0.05).

Pots with *A. longa* present had significantly less (1-way ANOVA, d.f.=3, F=5.06, P<0.05) litter remaining on the surface of the soil compared to pots without *A. longa* after ten weeks (Fig. 4.12).

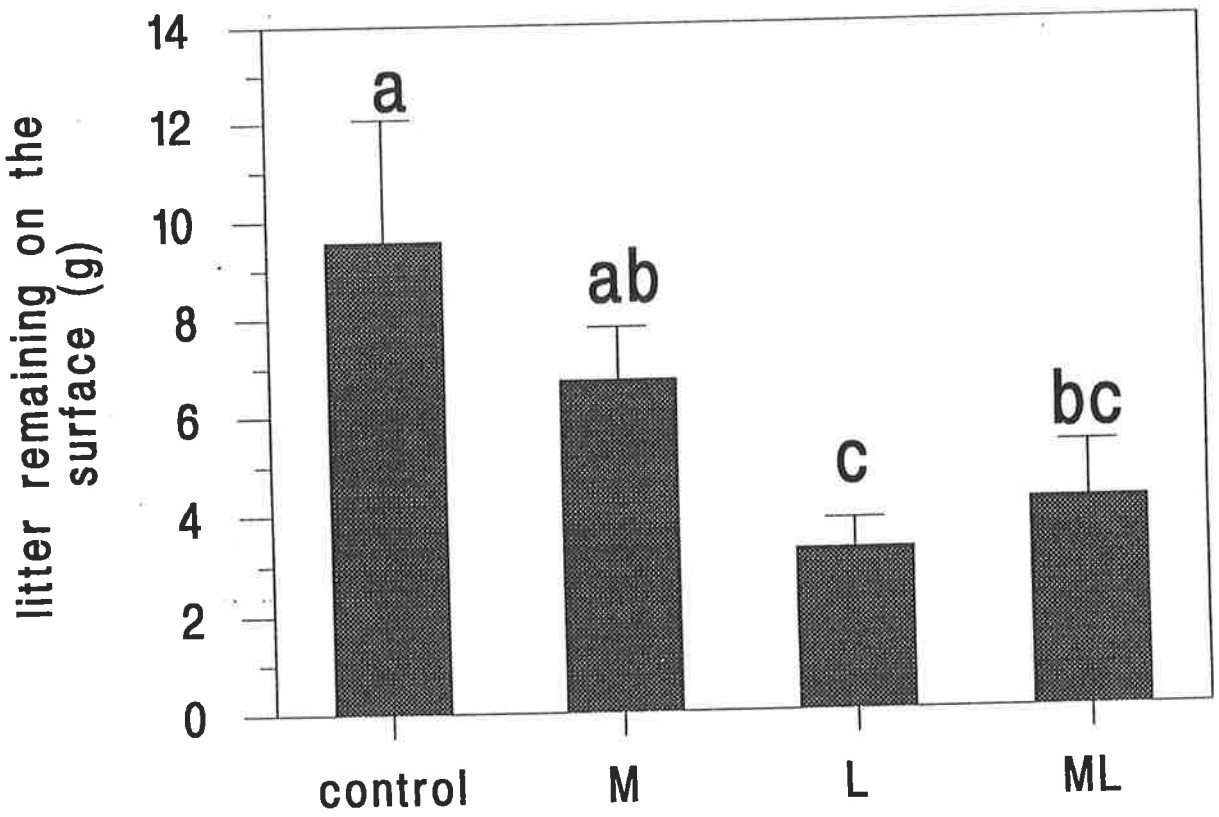
4.5 Does *A. longa* consume cocoons?

4.5.1 Materials and methods

4.5.1.1 Does A. longa consume M. dubius cocoons?

This experiment aimed to determine if *A. longa* consume cocoons of *M. dubius* and whether larger worms are more able to do this than small worms. Cocoons of *M. dubius* were collected from a site on the Waite campus by wet sieving the soil through a sieve with a 1.6 mm mesh and removing the cocoons with forceps. *A.*

Fig. 4.12 Pot experiment - Effect of *A. longa* on *M. dubius*. Plant litter and sheep dung remaining on the surface of pots kept at 15°C containing intact Woodside soil after 10 weeks. Vertical bars represent standard errors of the means, n= 10. Bars with same letters are not significantly different at the 5% level. L = *A. longa*, M = *M. dubius*, ML = *A. longa* + *M. dubius*, C = Control (no earthworms added).



longa were collected from Woolnorth and kept in a mixture of soil and sheep dung in the laboratory at 15°C (±1°C) before being used in the experiment. *A. longa* were separated into two size classes, large (av. wt. 3981 mg) and small (av. wt. 719 mg). A control (no earthworms) was included to ensure that cocoons were not disappearing for reasons other than the presence of *A. longa*. There were six replicates for each treatment.

The experiments were carried out in 50 ml plastic specimen containers with screw top lids. Twenty five grams (dry weight) of clay:loam mix (1:10 see Chapter 2) was added to containers and adjusted to a moisture content of 15% (g g^{-1}) which corresponded to a water potential of -10 kPa. Another layer was added on top which consisted of 10g of clay:loam (1:10) which contained 7% sheep dung and moistened to -10 kPa (23%). Five grams of the sheep dung:soil mix was added first, followed by five cocoons which were placed evenly onto the surface and then the remaining 5g of dung:soil mix was added on top.

Earthworm casts were collected from the soil every day for 48 days, and sieved through a 0.5 mm sieve to extract cocoons. After 48 days, earthworms were handsorted from the soil, pre-treated (section 2.4) and weighed. The soil remaining in the containers after 48 days was wet-sieved through a 1.6 mm mesh to extract cocoons of *M. dubius*.

A 1-way ANOVA was used to analyse the percentage weight loss after the data was transformed to arcsin(square root((x/100)+0.5)). The difference between treatments in the number of cocoons remaining in the soil at the end of the experiment was analysed using a Chi-square analysis.

4.5.1.2 Can A. longa consume artificial spheres of similar size to M. dubius cocoons?

Cocoons of *M. dubius* are difficult to collect because of their small size (1.8mm dia.) and low density in the soil (4.5 cocoons kg^{-1}). This makes it difficult to collect enough

cocoons to carry out experiments. This experiment aimed to test the hypothesis that *A. longa* can consume artificial spheres that are of similar size to *M. dubius* cocoons.

The three treatments were large earthworms (fresh weight = 3000 to 3200 mg), medium-sized earthworms (fresh weight = 1500 to 1800 mg) and small earthworms (fresh weight = 800 to 900 mg), with three replicates for each treatment. Treatments with different earthworm sizes were included to determine whether the size of an earthworm restricted it's ability to consume beads.

The experiments were carried out in 50 ml plastic specimen containers with screw top lids. The containers were filled with 40g (dry weight) of clay:loam mix (1:10 see Chapter 2) which contained 7% (g g^{-1}) sheep dung and was moistened to a moisture content of 23% (g g^{-1}) which corresponded to a water potential of -10 kPa. One earthworm was added to each container.

Small plastic beads of diameter 1.5 mm and 2.5 mm were added to the soil at 2.5 beads g^{-1} soil for each size class (2500 kg^{-1} soil). *Aporrectodea longa* were collected from Woolnorth and kept in a mixture of soil and sheep dung in the laboratory at 15°C (±1°C) before being used in the experiment. The containers were kept at a constant temperature of 15°C (±1°C).

Earthworm casts were collected twice during 7 days, by sorting through the soil and picking them out with forceps. Any beads adhering to the surface of the casts were removed and discarded because they might not have passed through the intestines of the earthworms. The casts were then sieved through a 710 μm sieve to separate the beads. Differences in the density of beads in the casts between earthworm size classes and between small and large beads were analysed using a 2-way ANOVA.

4.5.2 Results

4.5.2.1 Does A. longa consume M. dubius cocoons?

All earthworms survived over the 48 day period and all lost weight. The weight loss averaged 8.9% for large *A.longa* and 16.0% for small *A. longa*. There were no significant differences between large and small *A. longa* in terms of the percentage weight-loss (1-way ANOVA, d.f.=1, F=2.85, P>0.05).

No cocoons were found in surface-collected casts. All cocoons could be accounted for in the control treatment, although one cocoon had hatched. Not all cocoons could be accounted for in the treatments with *A. longa* added, with both earthworm treatments losing approximately one cocoon out of five (Fig. 4.13).

4.5.2.2. Can A. longa consume artificial spheres of similar size to M. dubius cocoons?

The average quantity of casts collected for each individual earthworm was 2.5g for the small earthworms (3.0 g g^{-1} starting weight of earthworm), 4.8g for the medium-sized earthworms (2.8 g g^{-1} starting weight of earthworm) and 7.8g for the large earthworms (2.5 g g^{-1} starting weight of earthworm). Both small and large beads were collected from the casts of every individual earthworm. The density of the beads in the casts was lower than the density found in the soil (2.5 beads g^{-1}), especially for the larger beads (Table 4.2). The density of beads g^{-1} cast material was significantly higher for smaller beads than for larger beads (2-way ANOVA, d.f.=1, F=6.59, P<0.05) but there were no significant differences in bead density between the different size classes of earthworms (2-way ANOVA, d.f.=2, F=1.21, P>0.05).

Fig. 4.13 Does *A. longa* consume *M. dubius* cocoons? Number of cocoons remaining in soil after 48 hours with and without *A. longa* in artificial soil kept at 15°C (±1°C). Vertical bars represent standard errors of the means, n= 6. Bars with same letters are not significantly different at the 5% level. C = control (no earthworm added), B = large *A. longa* added, S = small *A. longa* added.

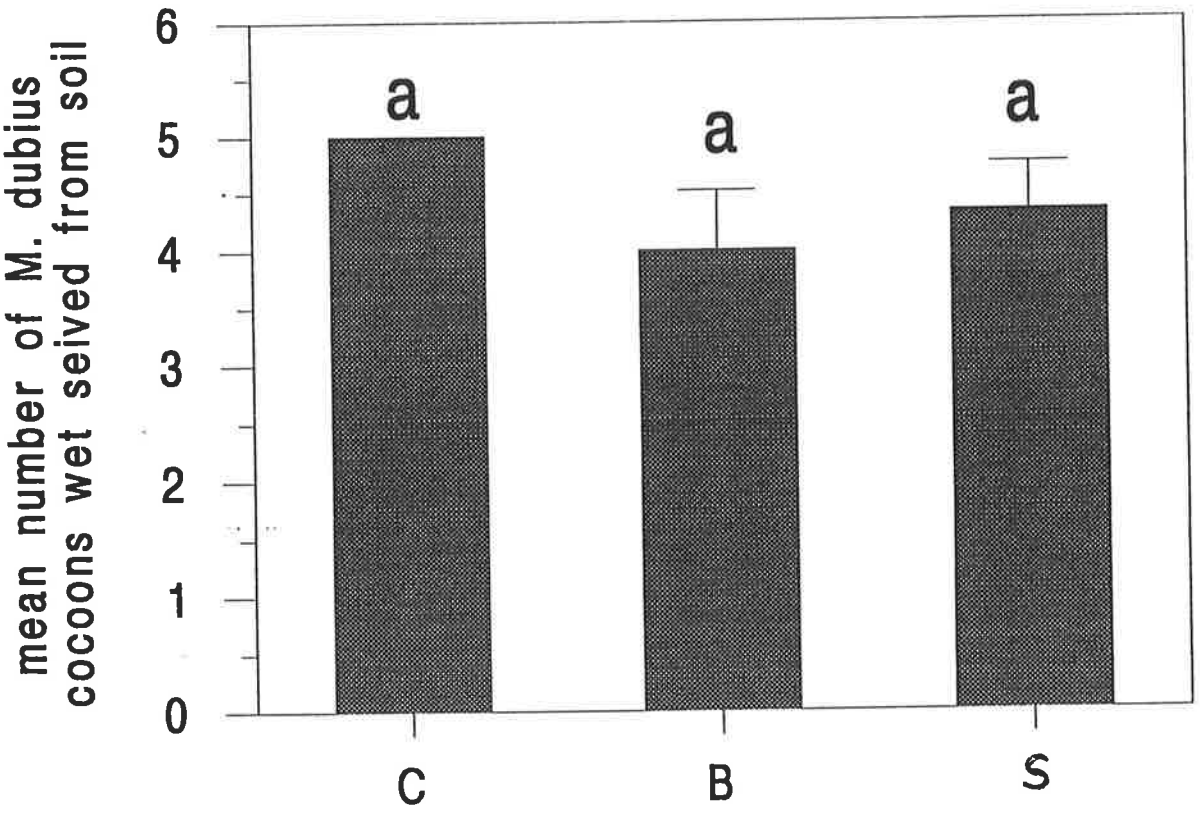


Table 4.2. Density of beads found in the casts of *A. longa* of three different size classes which had been placed in soil mixed with sheep dung for 7 days at 15°C (±1°C). The soil had small (1.5 mm diameter) and large (2.5 mm diameter) beads mixed throughout at 2.5 beads g^{-1} for each bead size. Numbers in brackets are standard errors of the mean, n=3.

Size Class of *A. longa* (g)	Density of Beads (beads g^{-1} cast material)	
	1.5 mm	2.5 mm
0.75 - 1.0	1.96 (0.30)	0.76 (0.28)
1.5 - 2.5	1.88 (0.76)	1.06 (0.12)
3.0 - 4.0	1.12 (0.19)	0.66 (0.06)

4.6 Discussion

4.6.1 Interactions between A. longa *and other species*

The presence of *A. rosea* significantly reduced the proportion of *A. longa* which were clitellate, although the effect was not a strong one. Further work is required to determine whether *A. rosea* poses a significant threat to the establishment of *A. longa* in pastures by reducing reproductive activity. An improved experimental design for investigating competition between earthworms species is discussed in Chapter 7.

There was no evidence in the experiments described above that *A. longa* had any adverse effect on the survival, growth or reproduction of *A. trapezoides*. In the 1994 field experiment at Springmount, *A. longa* produced more cocoons in the presence of *A. trapezoides* than it did in the single-species treatment (low density). There is no evidence for a mechanism for such a positive interrelation and further investigation is required to confirm the interaction and elucidate the mechanism (Chapter 7).

The competition between *A. longa* and *A. caliginosa,* demonstrated in the pot and field experiments, suggests that the positive association between *A. longa* and *A. caliginosa* found in Tasmania (Temple-Smith *et al.,* 1993) is due to similar habitat preferences rather than a positive interaction between the two species. The mechanism for competition between the species is unknown. It may be interference competition, whereby *A. caliginosa* is stressed by the presence of *A. longa* as was shown for *A. trapezoides* (Chapter 5). However, this is unlikely to be the case. Although the results in Chapter 5 show that *A. trapezoides* moves away from *A. longa*, this does not translate into a significant negative effect of *A. longa* on the growth and survival of *A. trapezoides* in the experiments described above. Furthermore, there was no evidence of *A. longa* forcing *A. caliginosa* from pasture soil to forest soil. Therefore interference competition is unlikely to be the cause of competition shown here. Scramble competition for food is the most likely cause of competition between *A. longa* and *A. caliginosa*. Both have similar habitat preferences, so there is a high chance that they use and compete for

similar resources. This suggests that earthworms act less like filter feeders (section 1.5.2.3) which slowly reduce the concentration of food resources in a habitat, but rather consume discrete packages of resources which reduces the fitness of other individuals using the same resource. This is discussed further in Chapter 7.

The mechanism for reduced cocoon production by *M. dubius* in the presence of *A. longa* could be direct competition for food resources, removal of the habitat of *M. dubius* through the burial of the litter layer by *A. longa*, or direct consumption of *M. dubius* cocoons by *A. longa*. These possibilities are discussed further below.

The first two possibilities are linked. *M. dubius* is epigeic in habit and the litter layer acts as the spatial habitat and food resource for it. *A. longa* was shown in section 4.4.2.2 to remove significant amounts of litter from the soil surface. This would result in lower amounts of food available for *M. dubius*, and would force *M. dubius* to seek shelter in the upper soil layers where the food quality is lower, resulting in a reduction in its reproductive output. To test this further, a similar experiment to that described in section 4.4.1.2 should be set up, but food in the form of organic matter such as animal dung should be added to the surface at regular time intervals so that food is never in short supply for *M. dubius* even in the presence of *A. longa*. If the competitive effect disappears under these conditions, then it could be concluded that competition for food reduced the reproductive output of *M. dubius* in the presence of *A. longa*. If the competitive effect is still apparent, then mechanisms other than competition for food are likely to be operating.

The results presented in section 4.4.2.3 suggest that *A. longa* can cause the destruction of *M. dubius* cocoons. It is not known whether this is the cause of reduced numbers of cocoons in experiments described in sections 4.4.2.1 and 4.4.2.2. In the field experiment, there were 7.29 adult *M. dubius* cage^{-1} in the control treatment, but only 2.29 in the treatment with 8 *A. longa* cage^{-1} a drop in survival of 69%. If it is assumed that adults do

not survive from one field season to the next, but survive as cocoons which hatch between July and August (Baker *et al.*, 1993c), the reduction in cocoons would explain the reduction in number of adults. If this reduction in cocoon numbers was due solely to *A. longa* consuming the cocoons, then it would be expected that the earthworms would have to consume approximately 69% of the soil which contained the cocoons. If it is assumed that all the *M. dubius* cocoons in these experiments were in the top five centimetres of the cage in the field experiment, which is reasonable considering that *M. dubius* is epigeic in habit, and the cage is 0.0706 m^2 then the cocoons would be present in 3.53 litres of soil. The soil has a bulk density of 1.13 Mg m^{-3}, so the cocoons are in a mass of soil of 3.99 kg. Cocoons would probably have hatched between the middle of July and early August, a period of six to eight weeks after *A. longa* was added. To account for the drop in cocoon numbers, *A. longa* would have to consume 69% of 3.99 kg soil in at least 42 days. As there were 8 *A. longa* $cage^{-1}$, this would require a consumption rate of 8.19 g soil $individual^{-1}$ day^{-1}. Each *A. longa* was approximately 1.3g and so the consumption rate would have to be 6.30 g soil g^{-1} day^{-1}. In the pot experiment, a similar calculation results in a consumption rate required to account for the drop in cocoon numbers of 2.86 g soil g^{-1} day^{-1}. This laboratory estimate is within the range of consumption rates measured for other lumbricid species of earthworms (Table 4.3), so it is theoretically possible that the reduction in *M. dubius* cocoons in the pot experiment can be accounted for by direct consumption by *A. longa*. However in the field experiment, the consumption rate of soil required to account for the population decline of *M. dubius* is higher than has been measured for similar species and so cocoon consumption may only explain part of the reduction in the population of *M. dubius*. The other likely mechanism for the lower population density of *M. dubius* when *A. longa* is added is that *A. longa* buries dung and litter from the surface of the soil, reducing the amount of food and shelter available to *M. dubius*.

Table 4.3 Consumption rates of soil by different species of lumbricid earthworms

Species	Consumption rate (mg day^{-1} g^{-1} earthworm)	Reference
A. caliginosa	362 - 2353	Curry *et al.*, 1995
	3750 - 4730	Martin, 1982b
	280 - 420	Barley, 1959
A. trapezoides	2630 - 4190	Martin, 1982b
A. rosea	1130 - 1270	Bolton and Phillipson, 1976
L .rubellus	1920 - 3010	Martin, 1982b
	80 - 460	Shipitalo *et al.*, 1988
	0.43 - 2.55	Dickschen and Topp, 1987
L. terrestris	242 - 713	Curry *et al.*, 1995
	70 - 180	Shipitalo *et al.*, 1988
	6.5 - 16.9	Heine and Larink, 1993

Individuals of *A. longa* which are larger than 800 mg can consume spheres as large as 2.5 mm in diameter. It is therefore conceivable that *A. longa* of this size would be able to consume the cocoons of other species if those cocoons were smaller than 2.5 mm. Such species include *Dendrobaena octaedra* (Savigny), *Allolobophora chlorotica* (Savigny), *Dendodrilus rubidus* (Savigny), *Eisenia fetida* (Savigny), *Eiseniella tetraedra* (Savigny) and small cocoons of the species *A. rosea* (Sims and Gerard, 1985). It is also possible that *A. longa*, as well as other large species of earthworms such as *Lumbricus terrestris* (L.), could consume cocoons larger than 2.5 mm diameter. *L. terrestris* is known to be able to consume seeds up to 3 mm in diameter and 6 mm in length (Piearce *et al.*, 1994). However, although they may physically be able to consume objects of this size, the data presented in section 4.5.2.2 shows that earthworms prefer not to consume larger beads and Shumway and Koide (1994) have similarly found that earthworms prefer to consume smaller objects (0.5 to 1.0 mm). Therefore these earthworm species are unlikely to have a significant impact on the numbers of larger cocoons in soil.

4.6.2 Relationship between weight change and starting weight of A. longa

There was a significant negative relationship between the average starting weight and the average weight change of *A. longa* at Springmount in the pot experiment and 1994 field experiment. There was no such relationship in the 1995 field experiment at Springmount or the pot experiment using Woodside soil. The negative relationship is similar to that found for other species of earthworm in Chapters 3 and 5, and is discussed further in Chapter 7.

4.6.3 Implications for introducing A. longa

Although *A. longa* and *A. caliginosa* compete against each other, the competition is weak so that the two species could be introduced into the same area. The analysis in section 4.3.2.2 suggests that on average, ten *A. longa* are required to reduce the numbers of *A. caliginosa* by two individuals in a population. This means that addition of ten A. longa to an area that supports a population of ten *A. caliginosa*, would reduce the

population of *A. caliginosa* to eight but the total earthworm population would increase to eighteen. Assuming individual earthworm activity does not change under these more crowded conditions, the total earthworm activity would be increased.

The effect that *M. dubius* can have on nutrient cycling and pasture production is unknown and therefore the impact of *A. longa* reducing *M. dubius* populations in pastures cannot be predicted accurately.

4.7 Conclusions

1. *A. longa* and *A. caliginosa* compete with each other

2. *A. longa* and *A. trapezoides* do not compete with each other

3. *A. longa* could be introduced into an area with *A. caliginosa* and *A. trapezoides* with no major impact in the short-term on the numbers of either species.

4. *A. longa* reduces the number of *M. dubius* in the soil. This may be as a result of burying surface litter thus depriving *M. dubius* of habitat and/or food and *A. longa* consuming cocoons of *M. dubius*.

5. *A. longa* are able to consume earthworm cocoons as large as 2.5 mm, but prefer to consume smaller objects (<1.5mm).

6. Smaller *A. longa* gain more weight per unit time than large *A. longa*.

Chapter 5 WILL *APPORECTODEA LONGA* POSE A THREAT TO NATIVE STRINGYBARK FORESTS IN THE MOUNT LOFTY RANGES?

5.1 Introduction

Aporrectodea longa (Ude) is a lumbricid species of earthworm which is exotic to Australia but is currently being considered for introduction into agricultural areas of high rainfall in southern Australia (Baker *et al.*, 1994). *A. longa* [along with *Aporrectodea caliginosa* (Savigny)] has been deliberately introduced into Tasmanian pastures, resulting in increased pasture production (Temple-Smith *et al.*, 1993), but it is not present in high numbers on mainland Australia (Baker *et al.*, 1994). The anecic habit of *A. longa* is an ecological type (Section 1.2.1) missing from the earthworm communities in agricultural areas of southern Australia (Baker *et al.*, 1994). Although *A. longa* can bring benefits to farmers, there is a risk that it may move from agricultural areas and colonise native areas. This would possibly have a dramatic effect on the soil processes in native areas.

A. longa casts prodigiously on the soil surface, buries surface litter (Parle, 1963b; Syers *et al.*, 1979; Piearce, 1978; Edwards and Lofty, 1982) and creates permanent burrows. The effect of burrowing, casting and burying leaf litter will alter the soil structure and the habitat for soil dwelling organisms, resulting in changes to the structure of the decomposer community (section 1.3.2). The effect of large numbers of macropores in native soils would be to change runoff patterns and the rate of water infiltration. The effect that this might have on the structure and productivity of the native ecosystem is unknown.

A. longa preferentially buries the seeds of some plant species compared with others (section 1.3.4), which would alter the plant community structure in the long term. Leaf litter has been shown to significantly affect seedling establishment of native plant species in eucalypt communities (Facelli and Kerigan, *in press*). The burial of this litter may

significantly affect the survival of some plant species. The production of surface casts and the burial of litter may allow exotic plants to germinate and become established.

The presence of earthworms is often associated with faster nutrient cycling (section 1.3.3). Increased nutrient cycling may result in a flush of nutrients which would alter the composition of the plant community and allow exotic plants to establish. Eucalypt forests often suffer under conditions of high nitrogen availability because conditions are more favourable for sap-sucking insects such as psyllids, which can defoliate trees over large areas of land (White, 1969; 1970a,b; 1993).

To date, no evidence has been produced suggesting that *A. longa* would successfully invade native habitats. *A. longa* was not found in native areas in Tasmania, except where the areas have been disturbed by human activities (T.J. Kingston, pers. comm.). A survey of the eastern slopes of Mt. Kosciusko (New South Wales, Australia) failed to find any invasion of native habitats by *A. longa*, even though the earthworm had invaded cleared areas where exotic plant species were established (Wood, 1974). Similarly, although *A. caliginosa*, another European lumbricid species, has invaded an area in Deep Creek Conservation Park, the site where it was found had been subjected to considerable human disturbance (Lawson, 1993). Studies in other regions have found that exotic species rarely become established in native areas (Fragosa, 1993), except in small, isolated and partially disturbed reserves (Dotson and Kalisz, 1989).

Although there is no evidence that the introduction of *A. longa* poses a threat to native habitats, it is not absolutely certain that it would not. The experiments described in this chapter aimed to test whether *A. longa* can survive in a soil from a native forest at Deep Creek under laboratory and field conditions, whether the presence of *A. longa* in this soil would effect the local fauna, in particular *Gemascolex lateralis* (Spencer), and whether *A. longa* is likely to move from adjacent pasture soils to forest soils.

5.2 Survey of resident populations in forest and pasture soils

The characteristics of the Deep Creek site are described in section 2.1. Ten intact, rectangular soil samples, 20cm. x 40cm. x 10cm. deep, were collected both from the forest and from pasture at Deep Creek in September 1995. The soil samples were collected 10 m apart in a grid pattern, between 30 and 60 m from either side of the fenceline dividing the forest and the adjacent pasture (Fig. 5.1). The samples were stored at 15°C for no longer than 10 days before they were handsorted for earthworms.

The number of resident earthworms collected in the survey is shown in Table 5.1. *G. lateralis* was found only in the forest soil and *Aporrectodea trapezoides* (Dugés) and *A. caliginosa* were found only in the pasture soil.

5.3 Survival, growth and reproduction of *A. longa* and it's effect on protozoa and mesofauna

5.3.1 Materials and methods

5.3.1.1 Pot experiment - Performance of A. longa and effects on soil fauna

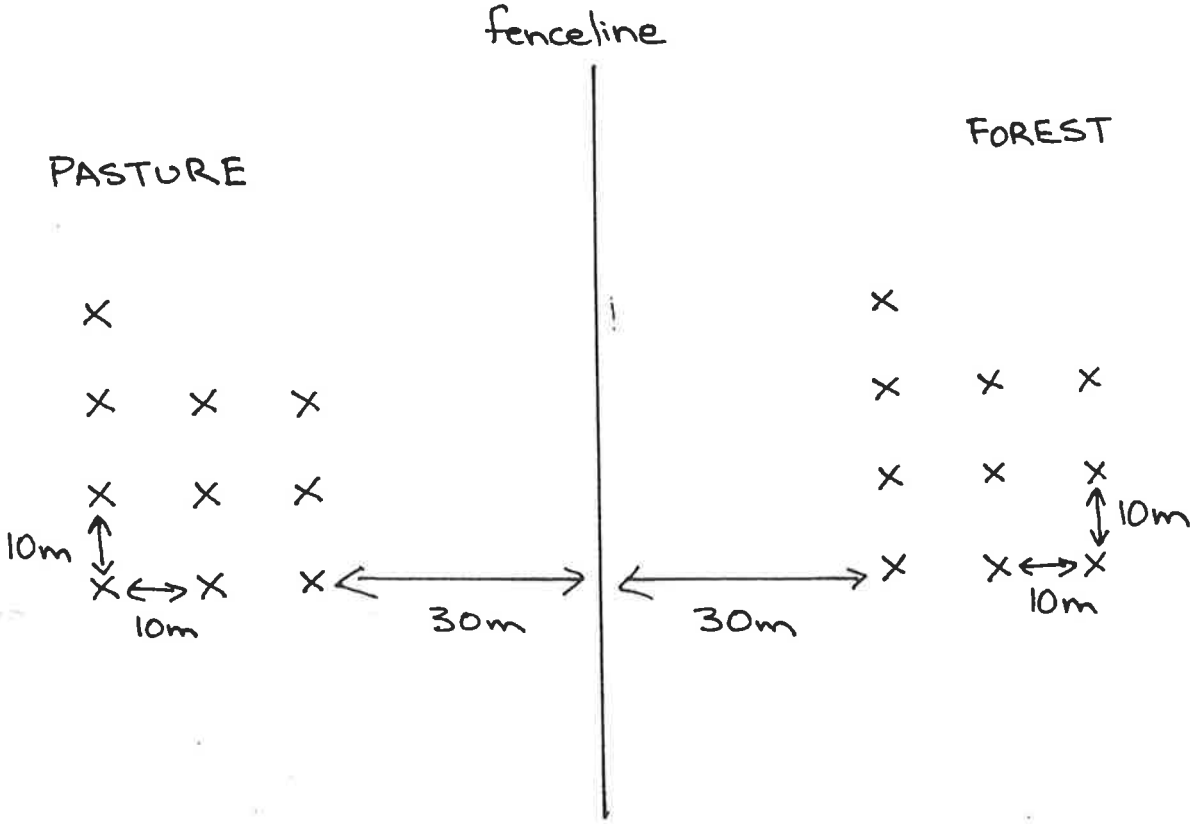
This experiment aimed to determine whether *A. longa* could survive, grow and reproduce in soil from the forest at Deep Creek and whether its presence affects the numbers of protozoa and mesofauna. Four densities of earthworms were used to determine what density of *A. longa* could be supported in the soil, if any. They were 1, 2, 3 and 5 *A. longa* core^{-1} with eight replicates per treatment. A density of 1 core^{-1} is equivalent to 57 m^{-2}.

Soil cores were collected from the Deep Creek site during summer, as described in section 2.3.2, after a rainfall event to make it easier to insert them into the soil. A solid base was secured to the base of the soil cores and sealed with a silicon sealant (section 2.3.2). The soil was watered to a water potential of -10 kPa (15% g/g; section 2.3.3.2), placed in a 15°C (±1°C) water bath and fine nylon mesh was secured over the top of the

Table 5.1 Resident earthworm densities (numbers m^{-2}) in pasture and forest soil at Deep Creek. Figures shown in brackets are standard errors of the mean from ten samples.

	A. trapezoides	*A.caliginosa*	*G. lateralis*
pasture	114.8 (15.9)	187.2 (38.9)	0 (0)
forest	0 (0)	0 (0)	21.3 (4.6)

Fig. 5.1 Sampling design for estimating resident populations of earthworms in forest and adjacent pasture soil at Deep Creek in 1995.



cores to stop earthworms escaping. Shade cloth (70%) was placed over all the cores to simulate the shade under a eucalypt forest.

Adult *A. longa* were collected from mass rearing boxes and pre-treated before being weighed (section 2.4) and added to the cores in February 1995. The average weight of each individual of *A. longa* was 2110mg.

Cores were watered to weight every two weeks, and each core lost 1.5g water $week^{-1}$ on average over this time. Any plants that germinated were removed. After 10 weeks earthworms were handsorted from the soil and pre-treated using the filter paper method (section 2.4) before being weighed and scored for the number of juvenile and adult earthworms.

Two separate 50g soil samples were taken from the mixed soil of each core to extract nematodes (Yeates and Bird, 1994). The soil was placed on two layers of "Kleenex" tissue paper supported on a 2 mm mesh inside a 7.5cm dia. funnel connected by a tube to within 1 cm from the bottom of a 20 x 2.5 cm pyrex test tube. Water was applied to the soil as a fine mist for 10 s, once every 10 min for five days. The water that had filtered through the soil over this period was trapped at the base of the funnel in the test tube. This water was allowed to settle before the majority of the water was removed by suction to within 2.5 cm of the bottom of the tube. The remaining water was plunged into hot water 60°C for five min. to kill the nematodes, and then a fixative was added (F.A. 4:1, Birds and Yeates, 1994). The samples were stored in 50 ml specimen jars. Nematodes were counted using a Wild Heerburg dissecting microscope.

Mesofauna were extracted from the soil using Tullgren funnels. Two separate samples of 200g of soil were placed on mesh (1mm cloth) in a funnel over 70% ethanol for five days at 20°C with no light. Mesofauna were separated into mites and collembolans and counted using the dissecting microscope described above.

Protozoan numbers were estimated using an MPN method (V.V.S.R. Gupta - pers. comm.). Saline Phosphate buffer solution was prepared by mixing together 0.34g KH_2PO_4, 1.21 g K_2HPO_4, 8 g NaCl in 750 ml of distilled water, adjusting the pH to 6.8-7.0 and making volume to one litre. The solution was autoclaved (20 min) before use. Straw infusion was prepared by adding 10 g finely chopped wheat straw to one litre of non-sterile phosphate buffer, shaking ~200 rpm for 24 g (at 5-15°C) and filtering (Whatman n0. 24) to remove coarse organic material. The filtrate was then filtered through 0.45 μm millipore filter into a bottle. This was autoclaved and stored at 4°C before use. Two 20g samples of soil were mixed separately with 95ml of Saline Phosphate buffer solution (pH 6.8) and shaken for 15 minutes. A series of 5 fold dilutions was made by mixing 0.2 μl of supernatant with 0.8 μl of phosphate buffer pH 6.8 and vortexing. Dilutions were made 5 times from the original dilution. Five samples of 0.1 μl were placed into 25-well plates with 1 μl of phosphate buffer, a suspension of *Enterobacter* (E.64 - prepared by V.V.S.R. Gupta) and straw extract. The plates were stored at 25°C and then scored for flagellates, ciliates and amoeba after 3 and 13 days. MPN values were calculated using standard MPN tables.

The remaining soil from the pot experiment was wet-sieved (0.5 mm) to extract cocoons and small juvenile earthworms.

The data were analysed by using a 1-way ANOVA. Proportions (proportional weight change, survival, proportion of adults in population) were transformed using arcsin-squareroot transformation before analysis. Where variances were significantly different between treatments, a Kruskal-Wallis analysis was used.

5.31.2 Field experiment - Performance of A. longa in comparison to A. caliginosa

The aim of this experiment was to determine whether *A. longa* could survive, grow and reproduce in soil from Deep Creek under field conditions over one season (1995). No

attempt was made to determine whether *A. longa* could aestivate over a summer period at the site due to time constraints. However, the species has been shown to be able to survive over summer in pastures in the Mount Lofty Ranges (G.H. Baker, pers. comm.). *A. caliginosa* was also included because it is known to be able to survive in the forest (Lawson, 1993) and if it could not survive, it would be as a result of being placed in cages rather than the habitat and so testing the performance of *A. longa* in field cages would be invalid.

There were two treatments; *A. longa* (2 cage^{-1}) and *A. caliginosa* (2 cage^{-1}). Two earthworms cage^{-1} is similar to the density of *A. caliginosa* (27m^{-2}) found at Deep Creek by Lawson (1993) and is half of the lowest density used in the previous pot experiment (section 5.2.2.1). For each treatment there were ten replicates.

Cages were constructed by inserting 20cm long, 30cm dia. PVC pipe into the soil in winter of 1995 when the soil was moist. The lengths of pipe were removed from the soil, keeping the soil column inside intact, and fine, nylon mesh was secured over the base of the pipe. The details of the method are described in section 2.3.1. The mesh was secured over the base and top of the cores with an epoxy-resin glue (Araldite®) and a tight band of packing tape to stop any earthworms escaping from the cages.

A. longa and *A. caliginosa* were collected from the Woolnorth site in Tasmania and were pre-treated and weighed before adding to the cages. The average weights were 1338mg and 331mg for *A. longa* and *A. caliginosa* respectively.

Earthworms were handsorted from the cages 12 weeks after they were added. Cocoons were wet-sieved from the soil through a 0.5 mm sieve.

A comparison of the number of resident earthworms in cages with *A. longa* and *A. caliginosa* was analysed using a one-way ANOVA after the data had been normalised using a square root transformation.

5.3.2 Results

5.3.2.1 Pot experiment - Performance of A. longa and effects on soil fauna

Survival of *A. longa* was not significantly different between treatments (Kruskal-Wallis, $P>0.05$). It was high in all treatments, with 86 to 100% of individual earthworms surviving the ten week period.

The average weight of *A. longa* at the end of the experiment was not significantly different between treatments. Earthworms only increased in weight (+3%) at the lowest density of one worm core^{-1} (56 worms m^{-2}), and lost between 5 and 19% of their starting weight in the other treatments. In the lowest density treatment, average earthworm growth was 1.91 mg individual^{-1} day^{-1}.

The proportion of individuals which were clitellate stayed at 100% in the lowest density treatment, but declined as the population density in the cores increased (Fig. 5.2). The proportion of individuals which were clitellate was significantly higher in the treatment with one earthworm core^{-1} than in the treatment with five earthworms core^{-1} (Kruskal-Wallis, $P<0.05$).

There was no significant difference between treatments in terms of cocoon production (Kruskal-Wallis, $P<0.05$), although there was an indication that the two treatments with the intermediate earthworm densities had higher cocoon production (Fig. 5.3). The rate

Fig. 5.2 The proportion of *A. longa* which were clitellate after 10 wks incubation in pots containing Deep Creek soil kept at 15°C. Vertical bars represent standard errors of the means, n=8. Values followed by different letters are significantly different at the 5% level.

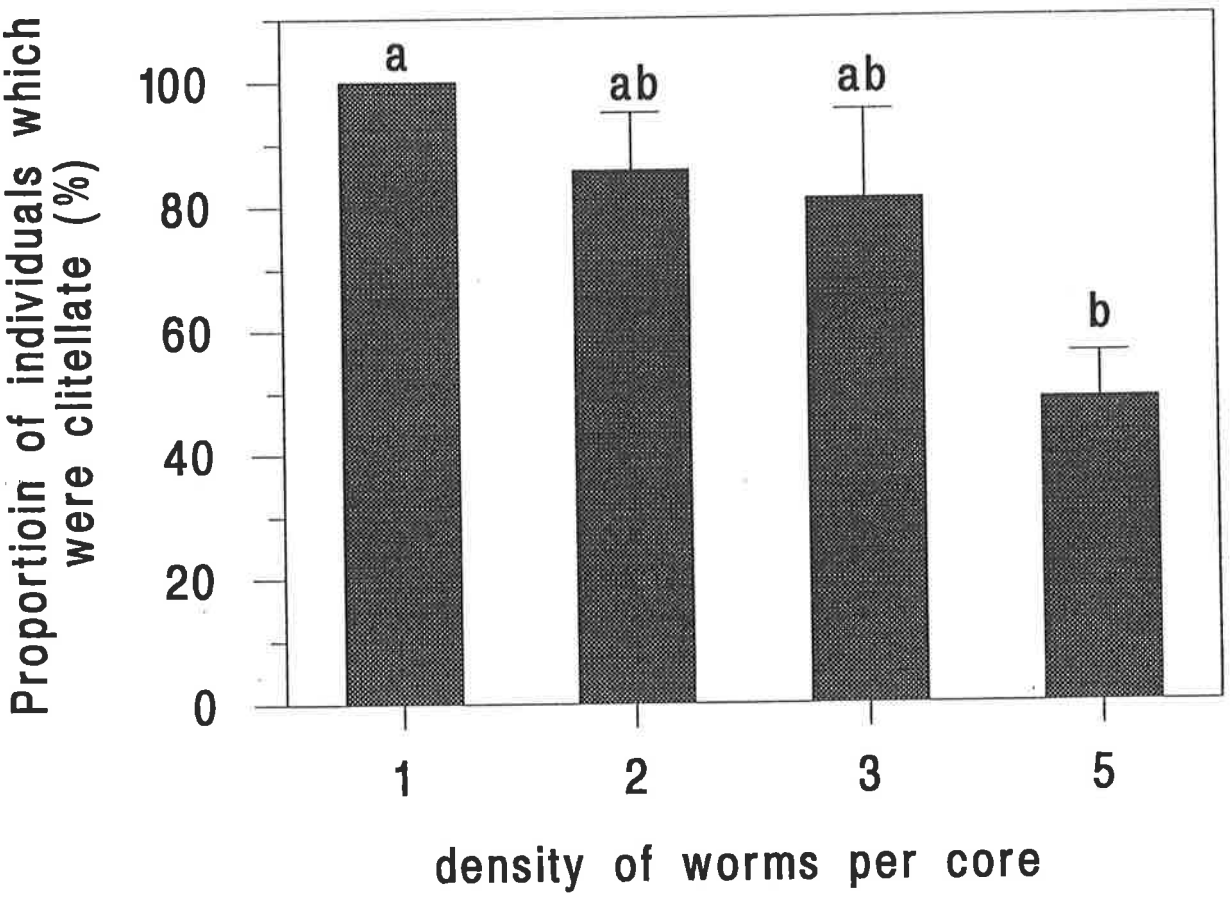
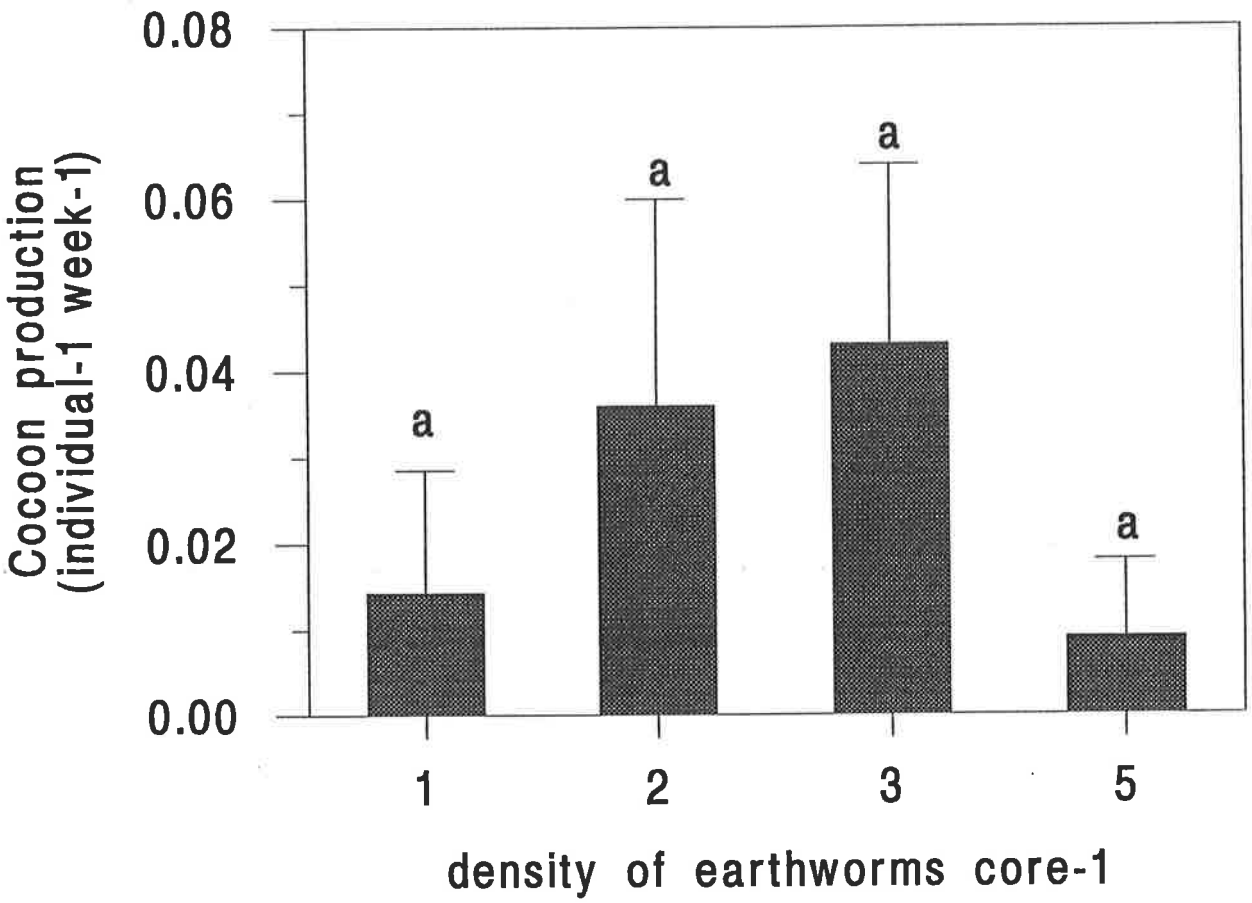


Fig. 5.3 Cocoon production by *A. longa* in pots after 10 wks incubation containing Deep Creek soil kept at 15°C. Vertical bars represent standard errors of the means, n=8. There were no significant differences between treatments at the 5% level.



Comparing the soil samples from the control and the treatment with five *A. longa* core^{-1}, there were no significant differences in the densities of mites, collembolans, flagellates, amoebae, or ciliates (1-way ANOVA; d.f.=1; F<2.23, P>0.05) (Table 5.2). However for flagellates, there was a significant difference between the variances of means (Bartlett's test, χ^2=6.17, P<0.05) with the control having a significantly higher variance than the *A. longa* treatment.

5.3.2.2 Field experiment - Performance of A. longa in comparison to A. caliginosa

All *A. longa* survived and 90% of *A. caliginosa* survived over the 12 weeks (Table 5.3). *A. longa* increased in weight by an average of 24% of the starting weight at a growth rate of 4.1 mg individual^{-1} day^{-1} (Table 5.3). *A. caliginosa* gained 106% of it's starting weight over 12 weeks at a growth rate of 3.9 mg individual^{-1} day^{-1} (Table 5.3).

On average, 39% of the individuals of *A. longa* in each core were adults (Table 5.3), having developed a tuberculum pubertatis; none were found to be fully clitellate. On average, 94% of the *A. caliginosa* were adult (Table 5.3) and all were fully clitellate. There were no *A. longa* cocoons found in any of the cages. Two of the cages containing *A. caliginosa* had cocoons (2 and 3 cocoons respectively).

Significantly (1-way ANOVA, d.f.=1, F=6.65, P<0.05) more *G. lateralis* were collected from the cages which contained *A. caliginosa* (5.3) than from which contained *A. longa* (2.0) (Table 5.4).

5.4. Does *A. longa* affect the native earthworm *G. lateralis*?

5.4.1 Materials and methods

5.4.1.1 At what density do individuals of G. lateralis have the highest survival, growth and reproduction?

The aim of this experiment was to determine at what density *G. lateralis* would have the highest survival, growth and reproduction. This density would then be used in the

Table 5.2 Numbers of mesofauna and protozoa (ind. g^{-1} soil) in soil subsamples from control and 5 *A. longa* treatments containing Deep Creek soil kept at 15°C and measured after 10 wks. There were no significant differences between treatments at the 5% level. There was a significant difference between variances for flagellates at the 5% level. Values in brackets are standard errors of the mean.

	mesofauna (n=6)		protozoa (n=8)		
	mites	collembolans	flagellates	ciliates	amoeba
control	0.197 (0.081)	0.037 (0.019)	1255 (513)	30.5 (5.4)	711 (101)
A.longa	0.142 (0.050)	0.010 (0.004)	503 (74)	24.3 (4.0)	508 (57)

Table 5.3 Survival, growth, numbers of adults and cocoon production of *A. longa* and *A. caliginosa* in field cages at Deep Creek over 12 weeks. Values in brackets are standard errors of the means (n=10).

	A. longa	*A. caliginosa*
Survival ($cage^{-1}$)	2.00 (0)	1.80 (0.13)
(%)	100 (0)	90 (7)
Growth (mg $ind.^{-1}$ day^{-1})	+4.1 (0.3)	+3.9 (1.0)
(%)	+24 (6)	+106 (9)
Adults ($cage^{-1}$)	0.78 (0.28)	1.70 (0.15)
Cocoons (total)	0	5
($ind.^{-1}$ $week^{-1}$)	0	0.023 (0.016)

Table 5.4 Number of resident native earthworms in field cages after 12 weeks with *A. longa* and *A. caliginosa* at Deep Creek. Figures in brackets are standard errors of the means (n=10). Values followed by different letters are significantly different at the 5% level within a row.

residents (cage^{-1})	*A. longa*	*A. caliginosa*
G. lateralis	2.00 (0.73) a	6.30 (1.64) b
G. spp.	3.00 (1.18) a	1.40 (0.58) a
Native spp.	0.11 (0.11) a	0.90 (0.41) a

5.4. Does *A. longa* affect the native earthworm *G. lateralis*?

5.4.1 Materials and methods

5.4.1.1 At what density do individuals of G. lateralis have the highest survival, growth and reproduction?

The aim of this experiment was to determine at what density *G. lateralis* would have the highest survival, growth and reproduction. This density would then be used in the experiment to test the effect of *A. longa* on *G. lateralis* in Deep Creek soil (section 5.2.3.2). The four densities used in this experiment were 1, 2, 3 and 5 individuals of *G. lateralis* pot^{-1} with eight replicates per treatment. The pots used were the same as those described in section 5.3.2.1. In order to determine whether the density of *G. lateralis* which resulted in the highest survival, growth and reproduction was affected by the starting size of the earthworms in the population (section 3.7.2.2), the whole experiment was divided into two blocks. One block (Block 1) had large earthworms (mostly adults) and the other block (Block 2) had small earthworms (mostly juveniles).

Soil was collected in the same manner as described above. Specimens of *G. lateralis* collected from the Deep Creek site immediately prior to setting up the experiment were pre-treated with the filter-paper method before being weighed and added to the pots in February 1995. The average weight of worms was 795 mg and 373 mg in Block 1 and Block 2 respectively.

Cores were kept in a 15°C (±1°C) waterbath and watered to weight every two weeks. Shade cloth (70%) was placed over all of the cores to simulate conditions under a eucalypt forest. Any plants germinating during this time period were removed. The cores were left to incubate for ten weeks before the earthworms were removed, pre-treated (section 2.4), weighed and populations scored for the number of juvenile and adult earthworms. The soil was subsequently wet-sieved (710μm) for cocoons and small juveniles.

Analysis was done using a 2-way ANOVA, with the densities as one factor and the Blocks (earthworm size groups) as the other. Any values expressed as a percent were transformed to arcsin(square root(x+0.5)). The number of cocoons and juveniles were transformed to log(x+1).

5.4.1.2 Interactions between A. longa and G. lateralis in pots

This experiment aimed to test the short-term impact of *A. longa* on *G. lateralis* in intact soil cores from Deep Creek under laboratory conditions. There were three treatments; *G. lateralis*, *G. lateralis* + *A. longa* (1 pot^{-1}), and *G. lateralis* + *A. longa* (3 pot^{-1}). A density of two *G. lateralis* pot^{-1} was used in all treatments because in the preliminary experiment (section 5.3.2.1), this density resulted in the highest reproduction, growth and proportion of adults in the population. It is also the density that is most similar to the average densities of *G. lateralis* found at Deep Creek by Lawson (1993). Two different densities of *A. longa* were chosen, 1 pot^{-1} and 3 pot^{-1}. The low density (1 pot^{-1}) was the only density in the preliminary experiment where *A. longa* gained weight. To test the effect of *A. longa* at densities that it might achieve in native soil under optimal conditions, a treatment with *A. longa* at a density of 3 pot^{-1} was also included. There were twenty replicates of each treatment.

Soil cores were collected as described above (section 5.3.2.1). The cores were placed in a 15°C (±1°C) water bath and fine nylon mesh was secured over the top of the cores to stop earthworms escaping. Shade cloth (70%) was placed over all of the cores.

A. longa was collected from Woolnorth and *G. lateralis* was collected from Deep Creek. The earthworms were pre-treated using the filter paper method before being weighed and added to the cores. The average weight of *A. longa* was 1047 mg and 30% of the individuals had partially developed clitella. The average weight of *G. lateralis* was 143 mg and all individuals were juveniles. The size of earthworms was chosen to maximise

the chance that they would be able to gain weight over the period of the experiment, based on the results of the previous experiments (see section 5.4).

Cores were watered to weight every two weeks. Any plants that germinated during this time period were removed. The experimental cores were left to incubate for 12 weeks before the earthworms were removed.

Earthworm casts were collected from the surface of the soil cores and could be identified as to whether they came from *A. longa* or *G. lateralis*. 5 g casts of each species were placed into separate plates (X cm dia) containing 95 ml phosphate buffer, a nutrient solution and Enterobacter (E64) for six days. The plates were scored for the number and type of different species of protozoa. Protozoa identifications were made by Robin Coles (CRC Soil and Land Management). Earthworms were handsorted from the soil and pre-treated using the filter paper method (section 2.4), before being weighed and scored for the total number of juvenile and adult earthworms. The remaining soil was subsequently wet-sieved (0.5 mm) for cocoons and small juveniles.

The data was analysed using a 1-way ANOVA. Proportional weight change and proportions of adults in population were transformed to arcsin(square root(x+0.5)) before analysis. Survival was transformed to log(x+1). Where variances were significantly different between treatments, a Kruskal-Wallis analysis was used.

5.4.2 Results

5.4.2.1 Optimal density of G. lateralis

Casts of the earthworms species *G. lateralis* contained five species of ciliates, two species of flagellates and one amoeba species (Table 5.5). *A. longa* casts contained four ciliate species, two flagellate species and one amoeba species (Table 5.5). Only one protozoa species was common between the casts of the two species (*Colpoda* sp. A).

Table 5.5 Protozoan species found in casts collected from the surface of soil cores taken from Deep Creek, belonging to the earthworm species *G. lateralis* and *A. longa*. The casts were incubated for six days in a nutrient solution at 25°C.

Protozoa	*A. longa* casts	*G. lateralis* casts
Ciliates	*Colpoda* sp. A	*Colpoda* sp. A
	Urostyla sp.	*Colpoda* sp. B,
	Stylonychia sp.	Sp. A1
		Carnivore sp.
Flagellates	*Bodo* sp. A,	Sp. F2
	Sp. F1	Sp. F3
Amoeba	*Leptomyxid* sp.	Sp. A1

Survival of *G. lateralis* ranged between 50 to 100% across treatments and there was no significant difference in survival between treatments or blocks (2-way ANOVA, d.f.=3 or 1, F=1.19 or 0.00, P>0.05).

In Block 2 where the small *G. lateralis* were added, the change in weight over the 10 week period varied from an increase of 108 mg (+12%) in the treatment with one worm core^{-1} to a loss of 71 mg (-15%) for the treatment with three worms core^{-1}. In Block 1, earthworms lost between 106 mg (-17%) in the treatment with three worms core^{-1} and 272 mg (-34%) in the treatment with five worms core^{-1} over the same period. There was no significant effect of density on the average change in weight between treatments (2-way ANOVA, d.f=3, F=0.82, P>0.05), although there was a trend for weight loss to increase with increasing density (Fig 5.4). In Block 2 there was an overall increase in earthworm weight and in Block 1 there was an overall decrease in weight (Fig. 5.5). The change in weight was significantly different between the two blocks (2-way ANOVA, d.f=1, F=14.34, P<0.05).

There was a significant negative relationship (R^2=0.497) between the average weight of individuals at the start of the experiment and the change in weight per individual (Fig. 5.6). From the regression line, 527mg is the critical size for earthworms above which they would lose weight.

A significantly higher proportion of individuals in the population were clitellate in Block 1 (85.4±5.4%) than in Block 2 (14.8±4.7%) at the start of the experiment (2-way ANOVA, d.f.=1, F=132.51, P<0.05). Although the proportion of clitellate individuals was still higher in Block 1 (65.4±8.8%) than Block 2 (48.2±8.1%) after the ten weeks of the experiment, it was no longer significantly different (2-way ANOVA, d.f.=1, F=1.73, P>0.05). There was no significant difference between treatments at the end of the experiment in terms of the proportion of clitellate individuals in the population (2-way ANOVA, d.f.=3, F=0.47, P>0.05).

Fig. 5.4 Effect of earthworm density on the weight loss of *G. lateralis* in pots containing Deep Creek soil kept at 15°C measured after 10 wks. Vertical bars represent standard errors of the means, n=8. Values followed by the same letter are not significantly different at the 5% level.

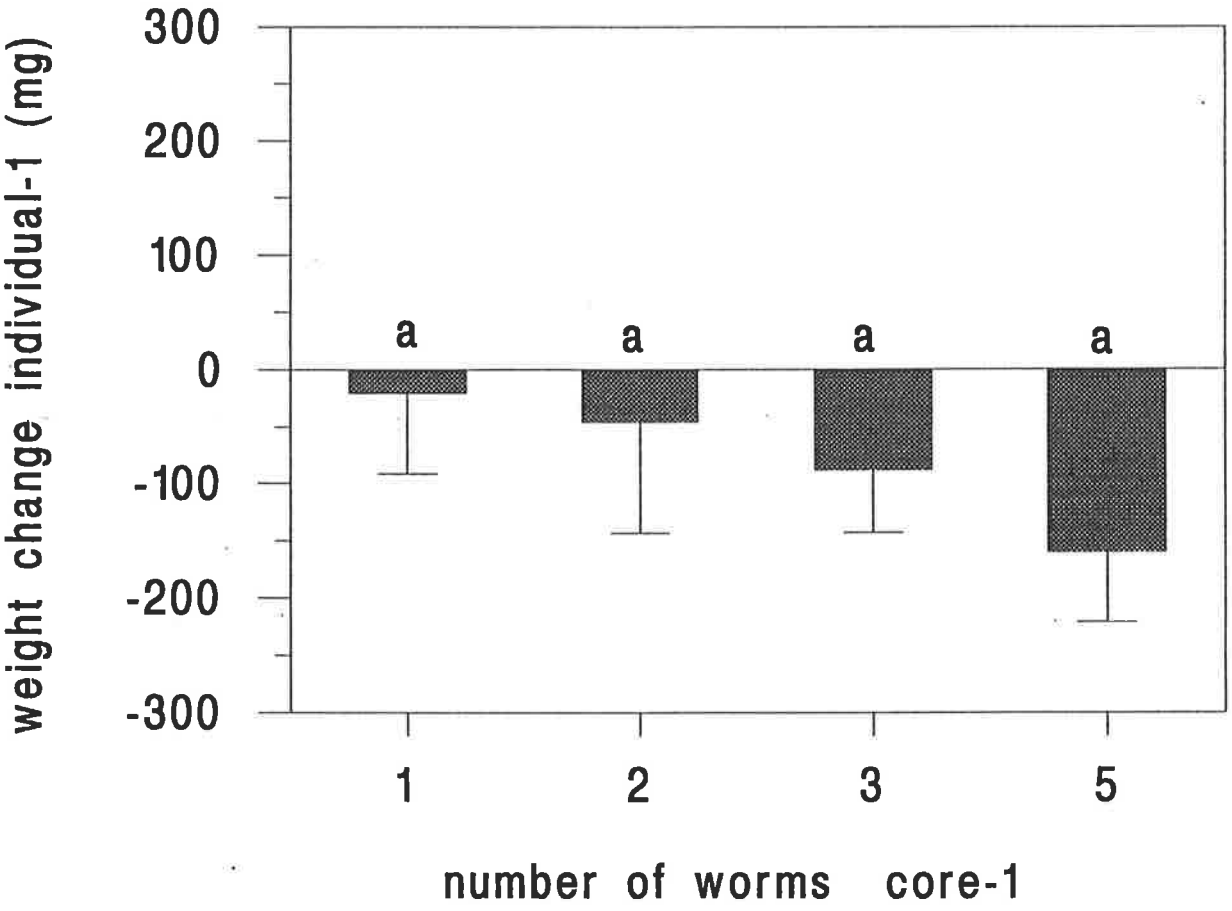


Fig. 5.5 The effect of earthworm size on final earthworm weight (average) in pots containing Deep Creek soil kept at 15°C measured after 10 wks. Vertical bars represent standard errors of the means, n=32. Values followed by the same letter are not significantly different at the 5% level. The average starting weight of earthworms in Block 1 was 373mg and in Block 2 was 795mg.

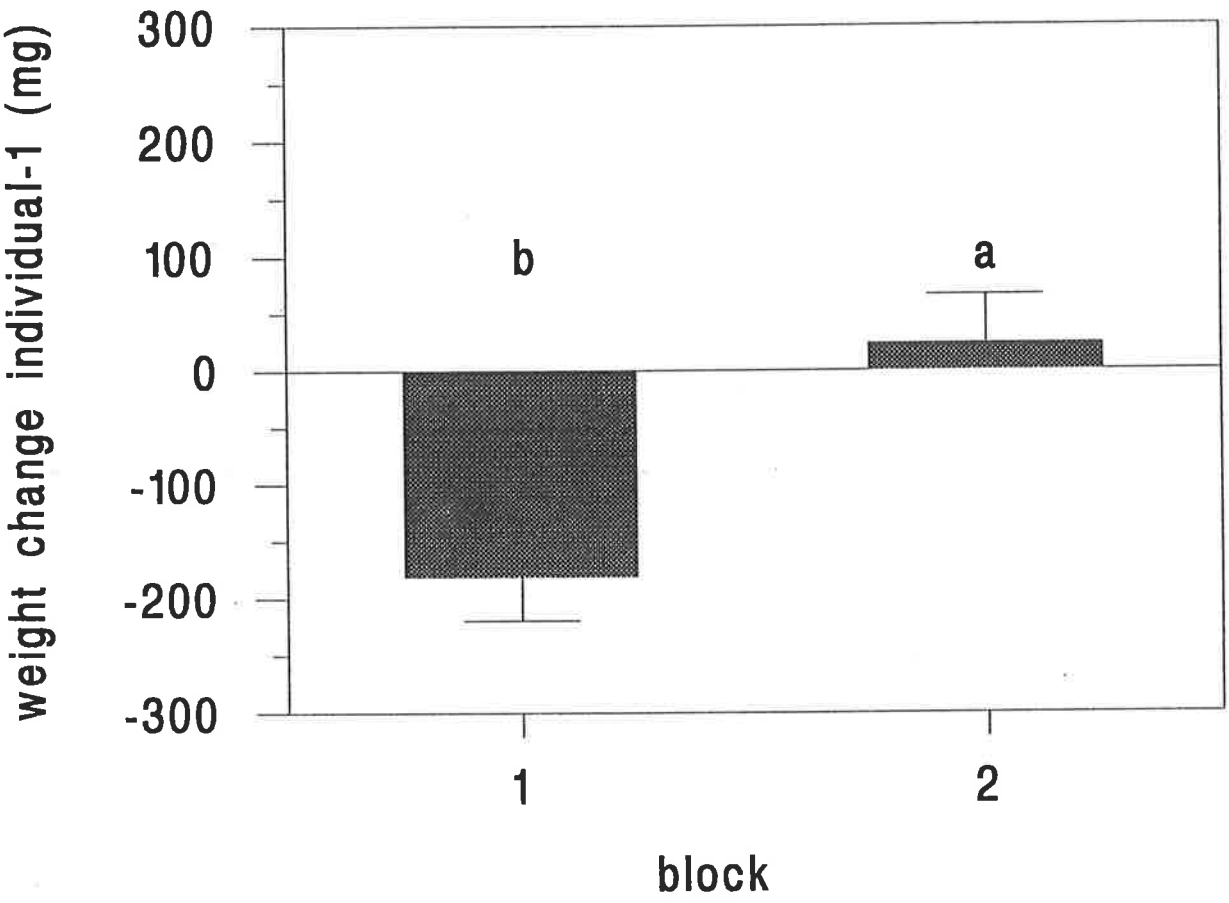
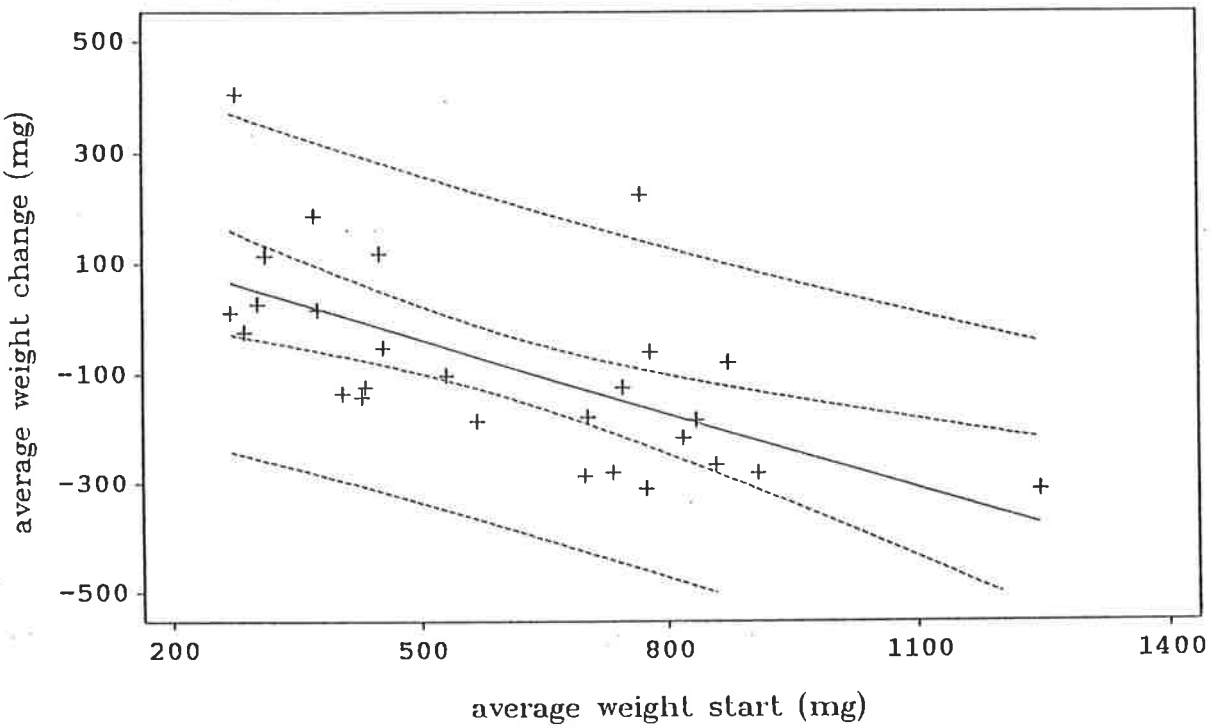


Fig. 5.6 Relationship between average starting weight and average change in weight of *G. lateralis* in pots containing Deep Creek soil kept at 15°C measured after 10 wks from all treatments (n=32). 95% confidence (inner, curved pair of dotted lines) and predicted (outer, straight pair of dotted lines) intervals are shown. y = 187 - 0.45x



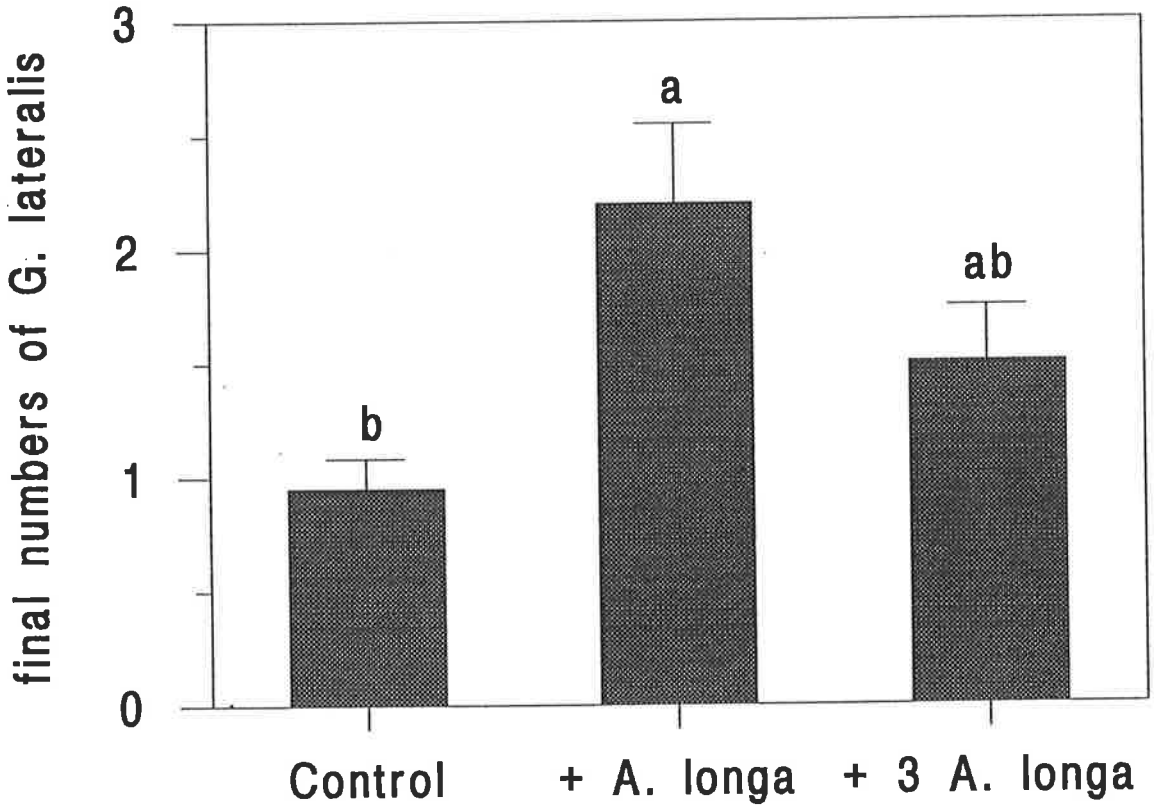
Cocoon production ranged from 0.025 to 1.4 cocoons individual^{-1} week^{-1} with an average across all treatments of 0.295 cocoons individual^{-1} week^{-1}. Total reproductive output ranged from 0.15 to 1.6 neonates (juveniles + cocoons) individual^{-1} week^{-1} with an average of 0.385 neonates individual^{-1} week^{-1} . There was no significant difference between treatments or blocks in terms of the rate of cocoon production (2-way ANOVA, d.f.=1 or 3, F=3.31 or 0.23, P>0.05), juvenile hatching (2-way ANOVA, d.f.=1 or 3, F=0.91 or 0.91, P>0.05) or total reproduction (2-way ANOVA, d.f.=1 or 3, F=1.28 or 0.54, P>0.05).

5.4.2.2 Interactions between A. longa and G. lateralis in pots

There was some contamination by *G. lateralis* in this experiment because it is a species which is active all year round at Deep Creek (Lawson, 1993). This made assessing survival of experimentally added *G. lateralis* difficult because it was not always clear which individuals had been added experimentally, and which were residents. The average number of individuals of *G. lateralis* handsorted from the pots was 0.95 for the control, 2.20 for the 1 x *A. longa* treatment and 1.50 for the 3 x *A. longa* treatment (Fig. 5.7). Significantly more *G. lateralis* were handsorted from the treatment with 1 *A. longa* compared to the control (1-way ANOVA, F=4.92, d.f.=2, P<0.05). There was no significant difference between treatments in terms of survival of *A. longa* (1-way ANOVA, F=0.22, d.f.=1, P>0.05) which was 90% in the treatment with 1 *A. longa* pot^{-1} and 83% in the treatment with 3 *A. longa* pot^{-1}.

The final growth rate of *G. lateralis* across all treatments was 2.9 mg ind^{-1} day^{-1} , with no significant difference between treatments. There was no significant difference (1-way ANOVA, d.f.=2, F=0.97, P>0.05) between growth rates of *A. longa,* which on average was 16.8 (±4.3) mg ind^{-1} day^{-1} in the treatment with 1 *A. longa* and 8.6 (±2.4) mg ind^{-1} day^{-1} in the treatment with 3 *A. longa.*

Fig. 5.7 Numbers of *G. lateralis* found in pots containing Deep Creek soil kept at 15°C measured after 12 wks. Vertical bars represent standard errors of the means, n=20. Values followed by different letters are significantly different at the 5% level.



The final proportion of *G. lateralis* that were fully clitellate ranged between 52% to 63%, with no significant differences between treatments (1-way ANOVA, F=0.23, df=2, P>0.05). For *A. longa*, the proportion of individuals that were clitellate was 0.389 for 1 *A. longa* and 0.278 for 3 *A. longa* and there were no significant differences between treatments (Kruskal-Wallis, H=0.001, df=1, P>0.05).

A. longa did not produce any cocoons over the period of the experiment. *G. lateralis* produced 0.10 (±0.07) cocoons pot^{-1} in the control, 0.45 (±0.33) cocoons pot^{-1} in the treatment with 1 *A. longa* and 0.83 (±0.78) cocoons pot^{-1} in the treatment with 3 *A. longa*. There were no significant differences between treatments (Kruskal-Wallis, H=0.033, df=2, P>0.05).

5.5. Does *A. longa* prefer to be in soil from the native area or from the adjacent pasture?

5.5.1 Materials and methods

Even if *A. longa* can be shown to survive, grow and reproduce in Deep Creek soil, if it were given a choice between forest and pasture soil, it might prefer to stay in a pasture soil and not move into the forest soil. The aim of this experiment was to determine whether *A. longa* preferred pasture soil over forest soil.

In this experiment, *A. longa* was added to boxes which contained blocks of soil from the Deep Creek site from both the native scrub and the adjacent pasture. There were ten replicate boxes.

The boxes were 28.4cm x 39.5cm and 11.5cm deep. Blocks of soil were cut with a spade from the native scrub and the pasture and placed side by side in the boxes so that each block filled up half of the space in the box (Fig. 5.8). Two *A. longa* were added to each block of soil. This density was equivalent to 0.6 pot^{-1}, 2.6 field $cage^{-1}$ and 36 m^{-2}.

Fig. 5.8 Boxes containing blocks of soil which were cut with a spade from the native scrub and the pasture and placed side by side, as described in section 5.5. (Does *A. longa* prefer to be in soil from the native area..) and section 5.6.1.1 (Does *A. longa* force *A. trapezoides* or *A. caliginosa* out of pasture soil into native soil?)

Fine nylon mesh was secured over the top of each box with an epoxy-resin glue (Araldite ®) to stop earthworms escaping. The pots were maintained at 15°C (±1°C) in the laboratory.

The experiment was harvested after three weeks which was considered long enough for the earthworms to move the length of the box and make a choice on the preferred soil. The two blocks were handsorted separately for earthworms.To determine whether *A. longa* preferred pasture soil over native soil, a one tailed Binomial Test was used.

5.5.2 Results

There were significantly more *A. longa* in the pasture soil than the forest soil (Fig. 5.9) after three weeks (Binomial test; n=40, T=30, P<0.05). Comparing the initial distribution of *A. trapezoides*, *A. caliginosa* and *G. lateralis* (Table 5.1) with the distribution after three weeks in the block experiments, *A. trapezoides* apparently move from the pasture soil to the forest soil in the presence of *A. longa* whereas *G. lateralis* moves from the forest to the pasture (Fig. 5.10).

5.6 Effect of *A. longa* on movement of *A. trapezoides* and *A. caliginosa* from pasture soil to native soil

5.6.1 Materials and methods

5.6.1.1 Does A. longa *force* A. trapezoides *or* A. caliginosa *out of pasture soil into native soil?*

In the experiment described in the previous section (5.5), individuals of *A. trapezoides* were found the forest soil when *A. longa* was added to the boxes. In the survey, *A. trapezoides* was only found in pasture soil (section 5.2). This experiment aimed to determine whether *A. longa* forces *A. trapezoides* into a soil that it would otherwise not move to. The experiment used the same method as in section 5.5.1 except that there were 18 boxes, 9 with *A. longa* and 9 without.

Fig. 5.9 The number of *A. longa* found in adjacent pasture or forest soil blocks after three weeks in the first (5.5 Does *A. longa* prefer to be in the forest) and second (5.6 Effect of *A. longa* on movement of *A. trapezoides* and *A. caliginosa*) soil choice experiments. Vertical bars represent standard errors of the means, n=10. Values followed by different letters are significantly different at the 5% level.

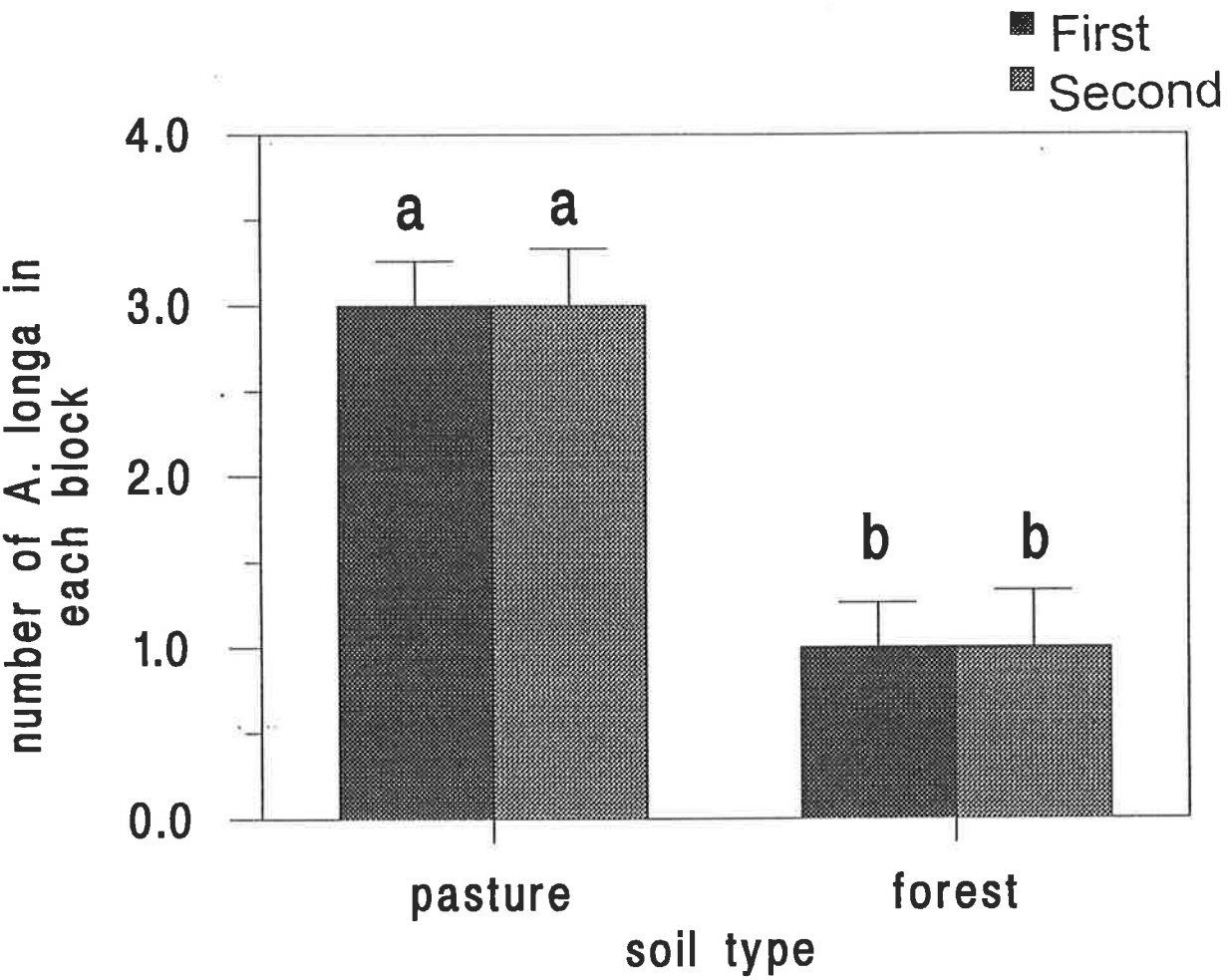
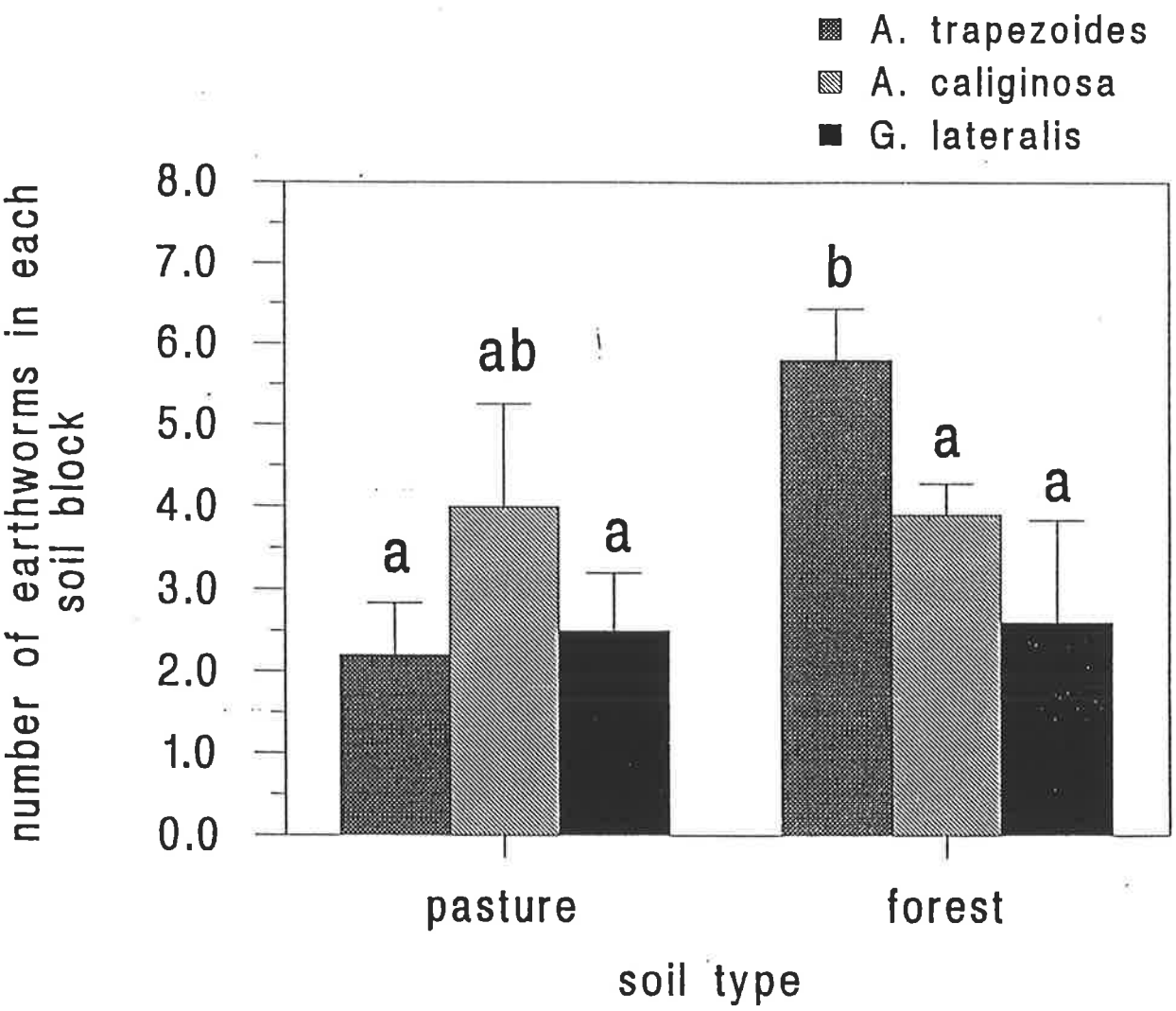


Fig. 5.10 The number of *A. trapezoides*, *A. caliginosa* and *G. lateralis* found in adjacent pasture or forest soil blocks after three weeks. Vertical bars represent standard errors of the means, n=10. Values followed by different letters are significantly different at the 5% level.



The experiment was harvested after three weeks which was considered long enough for the earthworms to move the length of the box and make a choice on the preferred soil. The two blocks were handsorted separately for earthworms.

To determine whether *A. longa* preferred pasture soil over native soil, a one tailed Binomial Test was used. To determine whether the numbers of *A. trapezoides* were higher in the forest soil when *A. longa* was present, a generalised linear model with binomial errors was used, after the proportion of *A. trapezoides* in the pasture compared to the forest soil was transformed to lambda (lambda = log ((r+0.5) / n-r+0.5)), where n = the total number of species in each box and r is the proportion of *A. trapezoides* in the pasture soil.

5.6.1.2 Does A. trapezoides *avoid mucus from* A. longa*?*

This experiment aimed to determine whether *A. trapezoides* avoided mucus obtained from *A. longa*. Mucus was obtained by placing 20g of *A. longa* (26 individuals) in 50 ml of RO water for 1 hour, after which, the mucus could be seen to be floating on the surface of the water. The earthworms were removed and the mucus solution was filtered through two "Kleenex" tissues to remove any soil particles that had been deposited by the earthworms. The mucus solution was thoroughly mixed and 2ml was pipetted onto one half of a 7cm glass, split petri-dish. 2ml of RO water was pipetted into the other half (control). In eight petri-dishes, one individual of *A. trapezoides* was added to the control side and in another eight petri-dishes, one individual of *A. trapezoides* was added to the mucus side.

The position of *A. trapezoides* in the petri dish was recorded after 24 hours. Individual *A. trapezoides* that straddled both compartments were allocated to the compartment in which the worms head was located. Data was analysed using Chi-squared analysis.

Table 5.6 The number of resident earthworms in adjacent pasture and forest soil after four weeks with and without *A. longa*. Standard errors of the mean are shown in brackets. The ratio of earthworms in the pasture soil compared to the forest soil is shown in the row labelled 'proportion'.

Species		*A. trapezoides*	*A.caliginosa*	*G. lateralis*	*G. spp*
Control -	pasture	5.00 (0.82)	4.56 (1.22)	2.56 (0.47)	0 (0)
	forest	2.67 (0.58)	3.33 (0.47)	1.44 (0.63)	0.44 (0.24)
	proportion	1.873	1.369	1.778	0.000
A. longa -	pasture	3.89 (0.79)	4.67 (0.55)	2.89 (1.06)	0 (0)
	forest	4.67 (0.82)	5.11 (0.59)	1.44 (0.18)	0.22 (0.22)
	proportion	0.833	0.914	2.007	0.000

5.6.2 Results

5.6.2.1 Does A. longa force A. trapezoides or A. caliginosa out of pasture soil into native soil?

There were significantly more individuals of *A. longa* in the pasture soil than the forest soil (see Fig. 5.9) after three weeks (Binomial test; n=36, T=27, $P<0.05$). The proportion of *A. trapezoides* in the pasture compared to the forest soil (Table 5.6) was significantly higher in the control than in the *A. longa* treatment (generalised linear model with binomial errors, $\chi^2=5.79$, $P<0.05$). There was no such effect of *A. longa* on the movement of *A. caliginosa*, *G. lateralis* or *Gemascolex* spp (Table 5.6).

5.6.2.2 Does A. trapezoides *avoid mucus from* A. longa*?*

After 24 hours, eight individuals were in the control sections and eight individuals in the mucus sections out of the 16 experimental dishes. Earthworms had been observed to move from one compartment to the other over the 24 h. period. 63% of earthworms were found in the same compartment as that in which they were originally placed.

5.7 Discussion

5.7.1 Growth, survival and reproduction of A. longa

Under laboratory conditions, *A. longa* can survive, grow and reproduce in soil from Deep Creek, which means that it can digest and assimilate leaf litter produced by the sclerophyllous flora or microorganisms growing on it. However under field conditions, *A. longa* did not reproduce, which means it will be unable to maintain a population in the field in the long term.

The growth rate of *A. longa* was dependent on the starting weight. In the first pot experiment, the weight of *A. longa* added was 2110 mg and the growth rate was 1.91mg individual^{-1} day^{-1} in the treatment with one individual core^{-1} (57 m^{-2}). In the field experiment, the starting weight was 1338mg and the growth rate was 4.1 mg individual^{-1} day^{-1} where the density was 2 cage^{-1} (27 m^{-2}). In the pot experiment with *G. lateralis*,

the average starting weight was 1047mg and the growth rate was 16.8 mg individual^{-1} day^{-1}, with one individual core^{-1} (57m^{-2}). Although the densities were not equal across this comparison, there was negative relationship between starting weight and growth that was found for other species (section 3.7.2.2). This is discussed further in Chapter 8. The growth rate of *A. longa* of 4.1 mg ind.$^{-1}$ day^{-1} in the field experiment at Deep Creek can be compared with values of -21.5 mg ind.$^{-1}$ day^{-1} at Springmount (8 cage^{-1}, mixed soil), 58.8 mg ind.$^{-1}$ day^{-1} at Woodside (8 cage^{-1}, unmixed soil, data not shown) and 32.6 mg ind.$^{-1}$ day^{-1} at Kuipto (8 cage^{-1}, unmixed soil, data not shown). Other studies carried out in pots have shown that *A. longa* can gain approximately 20 mg (Butt, 1993) or 28 mg (Lofs-Holmin, 1982) individual^{-1} day^{-1}. It is difficult to make comparisons across experiments because they were done in different years, in different soils and used different starting weights of earthworms which can have a significant impact on the growth rate of *A. longa*. It is also difficult to compare growth rates from mixed and unmixed soil because earthworms grow less in soil that has been mixed (Chapters 3 and 6). Nevertheless, the comparison shows that the growth rates of *A. longa* in Deep Creek soil are up to an order of magnitude lower than those measured at other sites such as Kuipto and Woodside. This suggests that conditions at Deep Creek are far from optimal for *A. longa*. Further investigation should compare the growth of *A. longa* in forest and adjacent pasture soil to determine whether it is the soil and climatic conditions which result in low growth rates, or the floral community and composition of the litter layer.

The reproductive rate of *A. longa* was 0.009 to 0.043 cocoons individual^{-1} week^{-1} in the first pot experiment, with no cocoons being produced in the other experiments. If the rate of production of cocoon in the first pot experiment is extrapolated over 20 weeks of the year (a reasonable estimate of the period of activity of adult earthworms in southern Australia - Baker *et al.*, 1992a), the cocoon output would be 0.18 to 0.86 individual^{-1} yr^{-1}. This is low compared to rates of cocoon production of 8.0 individual^{-1} yr^{-1} observed by Evans and Guild (1948). The rate of cocoon production required to maintain a population of *A. longa* in the field is not known and consequently no predictions are

possible with respect to the effectiveness of this rate of cocoon production to the long term viability of a population. *A. longa* may not have produced cocoons in the other experiments because the earthworms used were too small to be reproductive. The average weight in the first pot experiment was 2110 mg, while it was less than 1338 mg in the other two experiments. Moreover, in the first pot experiment, all earthworms added were clitellate whereas in the field experiment, none were fully clitellate and in the experiment with *G. lateralis*, 37% were adults in the pots with 3 A. *longa* (treatments with one *A. longa* were unlikely to contain cocoons because *A. longa* is obligatory biparental). This meant that there was greater opportunity for earthworms to mate and reproduce in the first pot experiment.

In the field experiment using cages, *A. caliginosa* grew to double their original size, became adults and reproduced. It is assumed therefore that the cages themselves did not limit growth and reproduction of *A. longa*.

5.7.2 Effect of A. longa *on native soil fauna*

5.7.2.1 Effect of A. longa *of mesofauna and protozoa*

There was no significant effect of the presence of *A. longa* on the total numbers of mesofauna and protozoa, but there was a reduction in the variance between samples for flagellates. The long term effect of making the soil a more homogenous environment is not well understood. A more complex environment is usually associated with greater diversity (Soberon and Llorente 1993, Van den Brink *et al.* 1994, Robertson and Hackwell 1995) and so reducing the heterogeneity would have long term implications for species diversity.

Marinissen and Bok (1988) and Yeates (1987) found that the presence of earthworms altered the species composition of meso- and micro-fauna (section 1.3.2). Out of a total of twelve protozoan species that were found in the casts of the two species, there was only one species which was common to casts of both species. Although this is likely to

reflect a difference in diet, it would also be due to differences in the ability of different protozoan species to survive passage through an earthworms intestines and could have long term implications for the community structure of the decomposer community if populations of *A. longa* were able to establish in native forest soils in high densities.

5.7.2.2 Survival, growth and reproduction of G. lateralis

Percent survival of *G. lateralis* was similar to that of a range of different species used in this study. Small *G. lateralis* grew proportionately more and showed greater absolute increases in weight than large individuals. This was also noted for other species of earthworms and is discussed further in Chapter 7. Cocoon production by *G. lateralis* averaged 0.295 cocoons $ind.^{-1}$ $wk.^{-1}$ in the pot experiment, but there was a large variance with some pots yielding up to 35 neonates (cocoons and juveniles) whilst others had neither cocoons nor juveniles. This variance would have been due partly to the developmental stage of the earthworms added experimentally, and partly due to the conditions in the soil. Some pots would have been, by chance, better environments for *G. lateralis* in terms of food resources which are patchy across the forest floor and this patchiness in resource availability would have been reflected in the patchiness of reproductive output.

5.7.2.3 Effect of A. longa *on* G. lateralis

There was no significant negative effect of *A. longa* on experimentally added *G. lateralis*. In the field experiment, there was some contamination by native species, and there were significantly fewer individuals of *G. lateralis* in cages with *A. longa* compared to cages with *A. caliginosa*. However, the density in the cages with *A. longa* was equivalent to a density of 28.3 m^{-2} which is similar to the density of earthworms found in the forest in the initial survey (Table 5.1). Furthermore, there was a significant positive effect of the presence of *A. longa* on the number of *G. lateralis* in the experiments on

interactions in pots. Therefore the negative result in the field experiment may have been due to chance presence of greater numbers of *G. lateralis* in the cages with *A. caliginosa*. To resolve this, the experiment should be repeated with higher replication, and control cages should be included with no earthworms added. There was no significant effect of *A. longa* on the other native species, but the numbers found were too low for a meaningful comparison.

5.7.3 Preference of A. longa *for native soil*

A. longa prefers to stay in pasture soil over forest soil. This is consistent with it's distribution in Tasmania and Mt. Kosciusko (see section 5.1) and suggests that *A. longa* will not pose a threat to native habitats, not because it cannot survive and grow in the soil, but because it prefers to stay in the pasture soil. It is possible that *A. longa* would enter native areas where there has been some degree of disturbance and exotic weeds have established, because it is better able to digest the plant litter of exotic species compared to native species.

Sclerophyll woodland, as found at Deep Creek, is typical of much of the Mount Lofty Ranges. However, the predicted potential distribution of *A. longa* using Climex (Fig. 5.11) covers an area in Australia which includes many different natural ecosystems. The potential threat to these habitats should be understood before a wide ranging campaign of redistribution of *A. longa* is started. Studies should be carried out to determine whether *A. longa* can survive, grow and reproduce in native soils and whether it would move into native soils from pasture soils.

5.7.4 Effect of A. longa *on movement of* A. trapezoides *and* A. caliginosa

The presence of *A. longa* forced *A. trapezoides* from the pasture soil to the forest soil. Although this is only likely to have an edge effect around the perimeter of native areas adjacent to pastures containing *A. longa*, it is interesting to speculate on what the mechanism might be. There was no effect of mucus produced by *A. longa* on the

Fig. 5.11 "Climex" matching of the climate of Mt Barker in the Mount Lofty Ranges, South Australia with other regions of Australia. (from Baker *et al.*, 1996 with permission).

movement of *A. trapezoides* and so it does not appear to act as a chemical deterrent. *A. longa* may change the soil characteristics to such a degree that the forest soil becomes more suitable for *A. trapezoides* than the pasture soil. However, this is unlikely to occur during the time frame of the experiments described above. Constant physical contact with other earthworm species may be distressing to *A. trapezoides* and result in movement away from *A. longa*. In laboratory studies, earthworms become active and move away when they are touched with various objects (pers. obs.). The effect of *A. longa* on the behaviour of *A. trapezoides* could be investigated further to determine what causes *A. trapezoides* to move away from soil containing *A. longa*. It would be interesting to investigate whether adding other species of earthworms, or other invertebrates would invoke similar movements due to the intolerance of earthworms to contact with other species.

5.8 Conclusions

1. *A. longa* is unlikely to have any significant effect on the native stringybark forests of the Mt. Lofty Ranges if it is introduced into pastures in the area.

2. Further investigation is required to test the impact of *A. longa* on other native ecosystems in southern Australia which are in areas where the species is predicted to become successfully established.

3. *A. longa* can affect the behaviour of *A. trapezoides*, forcing it to move from pasture soil into forest soil. The mechanism for this interaction is unknown and requires further investigation.

Chapter 6 WHY DID *APORRECTODEA TRAPEZOIDES* PERFORM BETTER IN UNMIXED SOIL COMPARED TO MIXED SOIL?

6.1 Introduction

In this chapter, experiments are described which aim to determine why *Aporrectodea trapezoides* (Dugés) performed better in terms of growth and survival in unmixed soil compared to mixed soil at the Springmount site (Chapter 3). Possible explanations include; *A. trapezoides* preferred food to be banded at the surface, living root-mass was higher in unmixed soil, there was an effect of mixing the soil which affects earthworm performance which is independent of food (ie. soil structure), smaller earthworms were added to the cages which contained mixed soil.

Geophagous earthworms such as *A. trapezoides* actively select soil which is rich in organic matter (Lee, 1985) and may therefore prefer organic material to be concentrated in patches, rather than spread homogenously through the soil profile. When soil remains unmixed, an organic layer consisting of plant material, animal faeces, urine and carcasses in various stages of decomposition, as well as living and dead microorganisms and soil fauna, builds up near the soil surface and becomes a "patch" with a high food value that can be utilised by various soil organisms, including earthworms. When soil is cultivated or mixed, as in the experiments outlined in chapter 3, this layer is diluted through a greater volume of soil. Although approximately the same amount of food would be available for earthworms in a pot or cage of mixed soil compared to an unmixed soil , the earthworms may suffer a "relative shortage" of food (Andrewartha and Browning, 1961). This would presumably reduce their assimilation efficiency, because they would have to increase the amount of energy spent on burrowing to ingest the same quantity of food. Martin (1982b) showed that *A. caliginosa* grow faster at higher concentrations of food. Edwards and Lofty (1982b) found that *A. longa* and *A. caliginosa* were rarer in plots under cultivation compared with direct drilled plots in a short term experiment and at a

number of long term trial sites. They did not determine whether this difference in abundance was due to mechanical damage, or dilution of the food source.

The potential importance of roots as a source of food for earthworms is discussed in section 1.5.2.3. Roots could be a source of food either directly in the form of living or dead organic material or indirectly by supporting a large community of soil microorganisms. Although plant roots probably are an important component of an earthworm's diet in the long term, the experiment described below tested whether living roots could be a source of food for earthworms.

There may be other effects of mixing the soil which affect earthworms and are not related to food supply. For example, earthworms may have to spend more energy constructing tunnels in mixed soil whereas they can use existing tunnels in unmixed soil. The relationship between water content and suction potential is slightly different in mixed and unmixed soil (section 2.3) and this may result in differences in earthworm performance over the length of a field season.

Cages containing unmixed soil had smaller earthworms on average than cages which contained mixed soil and smaller earthworms gain weight faster than larger worms (section 3.6.7) which might explain the differences in performance between mixed and unmixed soil.

6.2 Experiment 1 - Food (sheep dung): banded at the surface or mixed evenly through the soil

6.2.1 Materials and methods

This experiment aimed to determine whether *A. trapezoides* performed better when sheep dung was concentrated in a single band at the top of the soil or when it was mixed throughout the soil profile. The three treatments were: sheep dung banded at the surface (banded); sheep dung spread throughout the soil at a low weight of dung pot^{-1} (low

concentration); sheep dung spread evenly throughout the soil at a high weight of dung pot^{-1} (high concentration) with ten replicates for each treatment. The concentration of dung in the soil ($g\ g^{-1}$) was the same in the top band of soil in the banded treatment as in all the soil in the high concentration treatment. The total mass of sheep dung pot^{-1} was the same in the banded and low concentration treatments.

The soil used was a garden loam mixed with clay (section 2.3.3.3). The sheep dung was collected from sheep holding-pens, dried (60°C for 24 hr) and ground through a plant-grinding mill with a 2 mm screen. In the banded treatment, 225g of clay:loam was placed in a 300 ml plastic container. Another 72.9g of clay:loam with 2.1g (2.8% w/w) of dried, ground sheep dung mixed throughout was placed on top. This formed a band in the top 1/4 of the pot. In the low concentration, 2.1g (0.7% w/w) of sheep dung was mixed with 297.9g of clay:loam and placed in a 300 ml plastic container. In the high concentration treatment, 8.4g (2.8% w/w) sheep dung was mixed with 291.6g of clay:loam and placed in a 300 ml plastic container. The soil with no sheep dung was wetted to 20% gravimetric moisture and the soil with sheep dung added was wetted to 23% gravimetric moisture to ensure that the water potential was similar in all soil types (section 2.3.3).

A. trapezoides was collected from Willow Creek, pre-treated (section 2.4), weighed and one individual was added to each pot. The average starting weight of *A. trapezoides* was 230 mg, with no significant differences in weight between treatments (1-way ANOVA, d.f.=2, F=0.01, P>0.05). After four weeks, earthworms were handsorted from the soil, pre-treated (section 2.4) and weighed. It was recorded whether earthworms were found in the top 1/4 or bottom 3/4 of the pot.

Differences in growth were analysed using a 1-way ANOVA. A Tukey's test was used to determine differences between individual means. Proportions and percentages were transformed to arcsin(square-root(x+0.5)). The differences between treatments in the

number of earthworms aestivating and the numbers in the top layer of soil were analysed using Chi-square analysis.

6.2.2 Results

All earthworms were recovered at the end of the experiment. Earthworms gained weight in the high concentration treatment and this was significantly different (1-way ANOVA, d.f.=2, F=7.8, P<0.05) from the banded and low concentration treatments which lost weight (Table 6.1). There was no significant difference between treatments in the number of earthworms aestivating (Chi-square, d.f.=2, χ^2=3.33, P>0.05) (Table 6.1). Significantly more earthworms were found in the top 1/4 in banded (Chi-square, d.f.=1, χ^2=10.77, P<0.05) and high concentration (Chi-square, d.f.=1, χ^2=5.00, P<0.05) treatments compared to the low concentration treatment (Table 6.1).

6.3 Experiment 2 - Field experiment at Springmount 1994: banded versus mixed soil profile

6.3.1 Materials and methods

The aim of this experiment was to determine whether *A. trapezoides*, *A. caliginosa* and *A. longa* performed better when soil with high organic matter was concentrated in a single band at the top of the soil profile. The experiment was set up as a 4x2 factorial design, with four earthworm treatments, two soil treatments and seven replicates per treatment. The four earthworm treatments were: *A. trapezoides* (30 individuals cage^{-1}), *A. caliginosa* (30 individuals cage^{-1}), *A. longa* (8 individuals cage^{-1}) and a control where no earthworms were added. A density of 30 earthworms cage^{-1} is equivalent to 400 earthworms m^{-2}, and is slightly higher than the average of these two species found at this site (section 2.2.4). A density of eight *A. longa* core^{-1} (100 m^{-2}) represents a value slightly higher than the average densities found in soils in permanent pastures in Tasmania (Temple-Smith *et al.*, 1993). The two soil treatments were: 3.2 kilograms of the organic soil placed above 7.1 kilograms of the mineral soil (banded treatment), and 3.2 kilograms of the organic soil mixed thoroughly with 7.1 kilograms of the mineral soil

Table 6.1 Experiment 1. Change in weight of *A. trapezoides*, number aestivating and number found in the top 1/4 of the soil in pots of loam with sheep dung banded at the surface, or mixed at either a low or high concentration, kept at 15°C and measured after 4 wks. Values in brackets are standard errors of the means, n=10. Values followed by same letters within the same column are not significantly different at the 5% level.

Treatment	number recovered (total)	weight gain (%)	number aestivating	number found in top 1/4
Low concentration	10	-5.8 (2.0) a	6 a	0 b
High concentration	10	12.6 (6.7) b	2 a	4 a
Banded	10	-9.2 (2.5) a	4 a	7 a

(unbanded treatment). In the banded treatment, the organic soil formed a five centimetre band over the mineral soil which was ten centimetres in depth. In the unbanded treatment, the soil filled the core to a depth of fifteen centimetres.

The experiment was set up at Springmount in 1994. Cages containing mixed soil were constructed (section 2.3.1). Two types of soil were collected. The top five centimetres of soil was removed and put aside in June. This layer will be called the organic layer. The next ten centimetres of soil was then removed. This layer will be called the mineral layer. These two soil types were kept separate. Each was air-dried, mixed using a large cement mixer and then sieved through a five millimetre sieve.

A. trapezoides were collected from Waite in late-June 1994. *A. longa* and *A. caliginosa* were collected from Woolnorth in mid-June 1994 and stored in a mix of soil and sphagnum moss. Earthworms were pre-treated (section 2.4) before being weighed and added to the cores in late-June 1994. The average starting weight for individual *A. trapezoides* was 644 mg., for *A. caliginosa* 332 mg., and for *A. longa* 1349 mg. For each species, there were no significant differences between treatments in terms of starting weight (1-way ANOVA, d.f.=2, $F<0.21$, $P>0.05$).

The experiment was terminated in mid-October 1994. One soil core (2.5 cm dia.) was taken from each cage. This core was divided into two sections, a top section (5 cm) and a bottom section (10 cm). Soil was dried at 105°C and analysed for organic carbon using a Leco carbon analyser. The remaining soil in the cages was handsorted for earthworms which were then pre-treated (section 2.4) before weighing. The handsorted soil was then sieved to remove cocoons.

1-way ANOVA was used to analyse the data and a Tukey's test was used to determine differences between individual means. Proportions and percentages were transformed to arcsin(square-root(x+0.5)). The final weight of earthworms was transformed to

log(x+1). Where data could not be normalised or there was a significant difference between variances of the means (Barlett's test) a Kruskal-Wallis analysis was used. This is identified in the text. Small numbers of resident *A. caliginosa* and *A. trapezoides* were present in the cages. The average density and weight of these contaminants of each species was determined from the control cages and values were subtracted from values obtained from each cage containing the respective species before analysis.

6.3.2 Results

The concentration of organic carbon in the soils was significantly higher in the 0-5 compared with the 5-10 cm section and the concentration of organic carbon was higher in the top 5 cm of the banded cages compared with the unbanded cages (1-way ANOVA, d.f.=1, F=116.3, P>0.05) (Table 6.2). The average weight of organic carbon in the total soil volume in the cages was 273 g in the banded treatment and 276 g in the unbanded treatment and these means were not significantly different from each other (1-way ANOVA, d.f.=1, F=0.64, P>0.05).

There was no significant difference between treatments in terms of survival for either species which ranged from 76 to 78% for *A. caliginosa* (1-way ANOVA, d.f.=1, F=0.02, P>0.05), 50 to 56% for *A. trapezoides* (1-way ANOVA, d.f.=1, F=0.29, P>0.05) and 66 to 72% for *A. longa* (Kruskal-Wallis, H=0.01, P>0.05).

The average number of adults in the population at the end of the experiment was 10.9 cage^{-1} in the banded and 10.4 cage^{-1} in the unbanded treatment for *A. caliginosa*, 4.8 and 5.7 cage^{-1} for *A. trapezoides* and 0.28 and 0.75 cage^{-1} for *A. longa* in the banded and unbanded treatments respectively. There was no significant difference in proportion of adults in the population between treatments for *A. caliginosa* (1-way ANOVA, d.f.=1, F=0.00, P>0.05), *A. trapezoides* (1-way ANOVA, d.f.=1, F=2.24, P>0.05) or *A. longa* (1-way ANOVA, d.f.=1, F=1.51, P>0.05).

Table 6.2 Soil organic carbon (± standard error of the mean, n=21) in the banded and unbanded cages measured at the end of the field experiment in 1994. Bars followed by the same letter are not significantly different from each other at the 5% level.

treatment	soil depth	
	0-5 cm	5-15 cm
banded soil	3.66 ± 0.05 % a	2.22 ± 0.03 % d
unbanded soil	2.80 ± 0.06 % b	2.63 ± 0.03 % c

Individuals of *A. caliginosa* gained between 3.1 and 4.0 mg individual^{-1} wk^{-1} or 16-20% of their starting weight over the period of the experiment. Both *A. trapezoides* and *A. longa* lost weight over the period of the experiment. On average, *A. trapezoides* lost between 14.4 and 15.8 mg individual^{-1} wk^{-1} (-38 to -41.7% over 17 wks) and *A. longa* lost 12.9 to 14.8 mg individual^{-1} wk^{-1} (16 to 19% over 17 wks). There was no significant difference in percentage weight change between treatments for *A. caliginosa* (1-way ANOVA, d.f.=1, F=0.45, P>0.05), *A. trapezoides* (1-way ANOVA, d.f.=1, F=0.93, P>0.05), or *A. longa* (1-way ANOVA, d.f.=1, F=0.24, P>0.05).

6.4 Experiment 3 - Living plant roots as a food source for earthworms?

6.4.1 Materials and methods

The aim of this experiment was to determine firstly whether *A. trapezoides* performed better in soils with the greater plant root production and secondly whether it performed better in unmixed compared to mixed soil. Six treatments (2x3 factorial) were involved: two soil treatments (mixed or unmixed) and three plant treatments (no plants, heavily-clipped plants and lightly-clipped plants) with ten replicates per treatment. Plant shoots were lightly or heavily clipped to vary the amount of root mass produced by the plants. Eight *A. trapezoides* were added to each core, equivalent to a density of 400 m^{-2}.

Soil cores were collected from Springmount using the method described in section 2.3.2. The soil was heated to 60°C for seven days to remove earthworms and cocoons (section 2.6). In half of the cores, the soil was removed, thoroughly mixed and replaced into the cores. The soil was watered to a water potential of -10 kPa (20% gravimetric in mixed soil, section 2.3.3.1; 18% gravimetric in unmixed soil, section 2.3.3.2). The cores were left for 5 weeks to allow any seeds to germinate; seedlings were then killed with a 1.0% solution of Roundup (360g L^{-1} glyphosate - Monsanto) sprayed on the plants with a handsprayer at 5.9 ml pot^{-1}. This is the same application rate and same amount of herbicide added on an area basis as described in section 2.5.

Ryegrass (*Lolium perenne* L.) was planted at 11 mg seed core^{-1} into 40 of the pots. A nutrient solution was added in place of water once every second week for 10 weeks at 1/10th of the rate described by Hoagland and Arnon (1939). Water use was determined every three or four days by measuring water loss for each treatment and subtracting the water loss from the control pots from those with plants present to correct for evaporation. Water use was used as a measure of plant growth. Plants were clipped to maintain a water use of between 10 and 15 mg core^{-1} day^{-1} in the lightly clipped treatment and between 3 and 7 mg core^{-1} day^{-1} in the heavily clipped treatment. A square of fine nylon mesh was secured over the top of the core and a 10 mm dia. hole cut to allow ryegrass to grow through. The cores were kept in a waterbath (15°C±1°C) in a glasshouse with air temperatures ranging from 9°C to 32°C. Before earthworms were added, ryegrass plants were allowed to grow for 12 weeks. This allowed time for the community of soil microorganisms to return to structure not dominated by thermophilic species (after the heat treatment - section 2.6) and for plants to grow to a size where they were using up to 15 mg water core^{-1} day^{-1}.

A. trapezoides were collected from the Willow Creek collection site. They were pre-treated with filter paper, weighed and added to the pots in late August. The average weight of *A. trapezoides* was 221mg and there was no significant difference between treatments in terms of starting weight (1-way ANOVA, d.f.=5, F=0.36, P>0.05). Earthworms of this size were chosen because large adults (>350 mg) tend to lose weight in this soil and earthworms which are too small (<100 mg) are difficult to find and are easily damaged as a result of handling (section 3.6.7). Of the eight *A. trapezoides* that were added to each core, an average of 0.9 (range 0 to 2) had a clitellum.

The experiment was terminated on 15 January 1996. Earthworms were handsorted from the soil, and pre-treated (section 2.4) before being weighed. Plant roots were wet sieved from the soil through a 1 mm sieve and dried at 60°C before weighing.

Water use and root weight were analysed using a 2-way ANOVA. The data for root weight was transformed to log(x+1). Survival, earthworm weight and proportion of adults was analysed using a 2-way ANOVA adjusted for covariates (replicates, soil mass).

6.4.2 Results

Water use was significantly higher (2-way ANOVA, d.f.=1, $F=55.74$, $P<0.05$) in the lightly clipped treatments compared to heavily clipped treatments (Fig. 6.1) and significantly higher (2-way ANOVA, d.f.=1, $F=61.89$, $P<0.05$) in the mixed soil compared to the unmixed soil (Fig. 6.2). Root weight at the end of the experiment was significantly higher (2-way ANOVA, d.f.=1, $F=16.31$, $P<0.05$) in the lightly clipped pots compared to the heavily clipped pots and was significantly higher (2-way ANOVA, d.f.=1, $F=83.1$, $P<0.05$) in the mixed soil compared to the unmixed soil (Fig. 6.3).

Survival ranged from 65 to 79% and there was no significant difference between treatments (Kruskal-Wallis, d.f.=5, $H=4.86$, $P<0.05$). Weight change ranged from a gain of 2.24 mg ind.$^{-1}$ wk.$^{-1}$ to a loss of 2.75 mg ind.$^{-1}$ wk.$^{-1}$. The final weight of earthworms was significantly higher (Fig. 6.4) in the unmixed soil compared to the mixed soil (2-way ANCOVA, d.f.=1, $F=4.58$, $P<0.05$). There was no significant overall effect of plants on final earthworm weight (2-way ANCOVA, d.f.=2, $F=2.46$, $P<0.05$). However, if only the treatments with unmixed soil were analysed, earthworm weight was significantly higher (Fig. 6.5) in treatments with plants than the treatments without plants (2-way ANCOVA, d.f.=2, $F=6.00$, $P<0.05$). The number of adults in the population ranged from 30 to 80% with no significant differences between plant (2-way ANCOVA, d.f.=2, $F=0.02$, $P>0.05$) or soil treatments (2-way ANCOVA, d.f.=1, $F=0.01$, $P>0.05$).

Fig. 6.1. Plant roots experiment, Pot. Water use of ryegrass plants either heavily or lightly clipped kept in Springmount soil (mixed and unmixed soil treatments combined) at 15°C and measured over 10 wks. Vertical bars represent standard errors of the means, n=20. Bars followed by same letters are not significantly different at the 5% level.

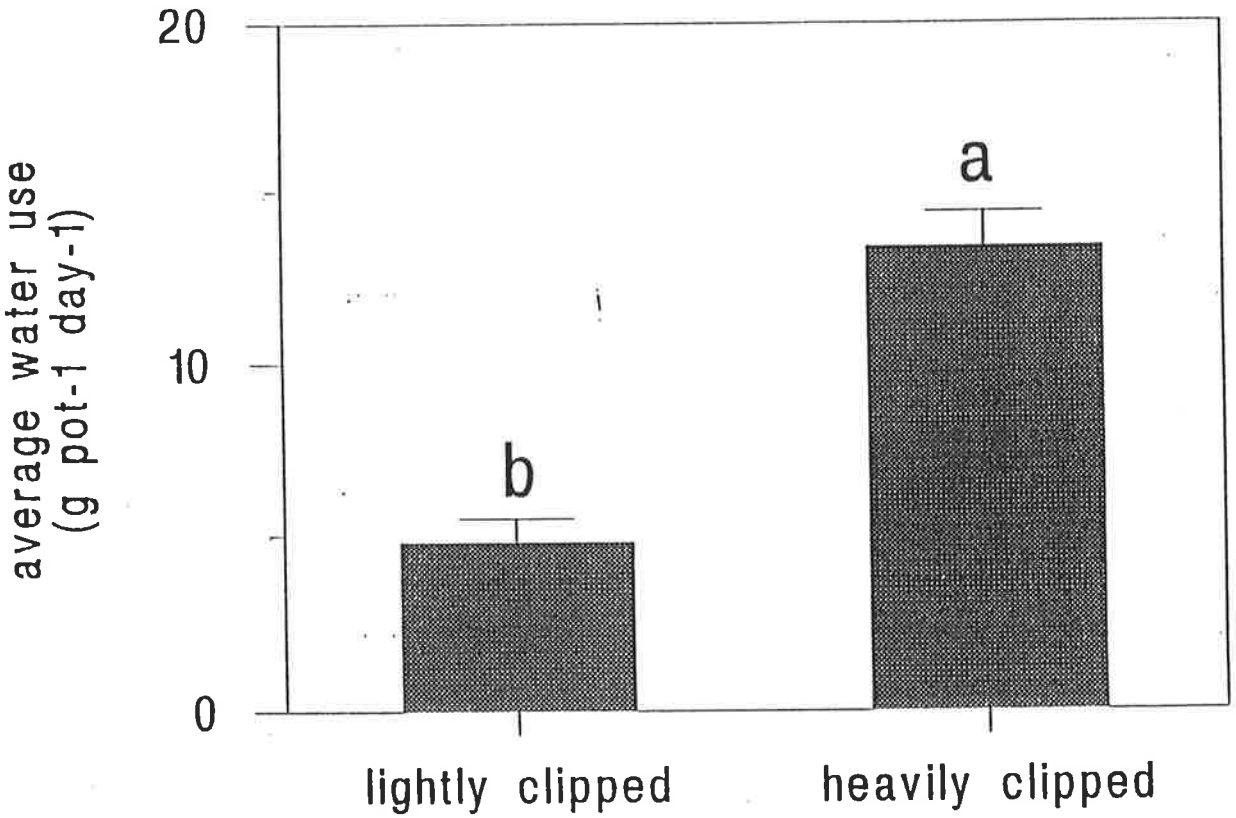


Fig. 6.2 Experiment 3. Water use of plants (light and heavy clipped plants combined) in pots of either mixed or unmixed soil from Springmount kept at 15°C and measured over 10 wks. Vertical bars represent standard errors of the means, n=30. Bars followed by same letters are not significantly different at the 5% level.

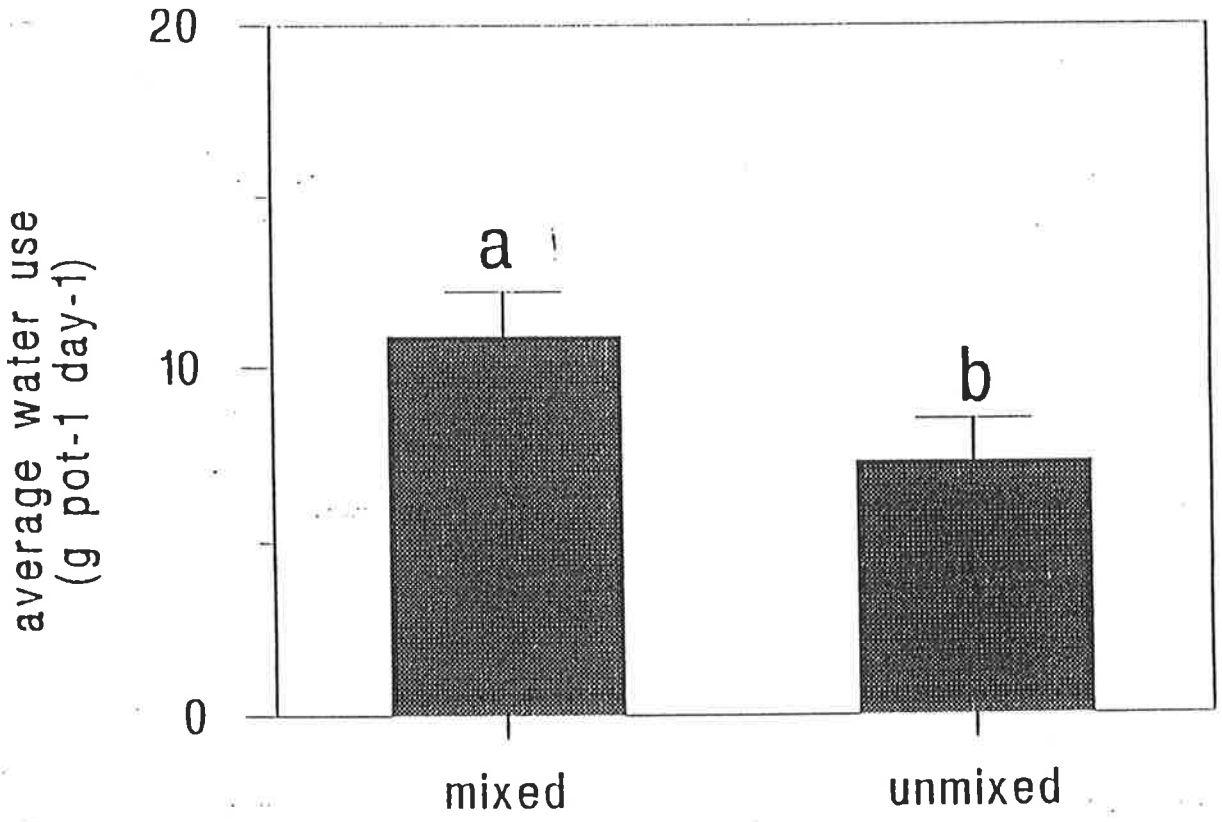


Fig. 6.3 Plant roots experiment, Pot. Root mass of ryegrass plants either heavily or lightly clipped kept in mixed or unmixed Springmount soil at 15°C after 25 wks. Vertical bars represent standard errors of the means, n=10. Bars followed by same letters are not significantly different at the 5% level. h = heavily clipped, l = lightly clipped, m = mixed soil, u = unmixed soil.

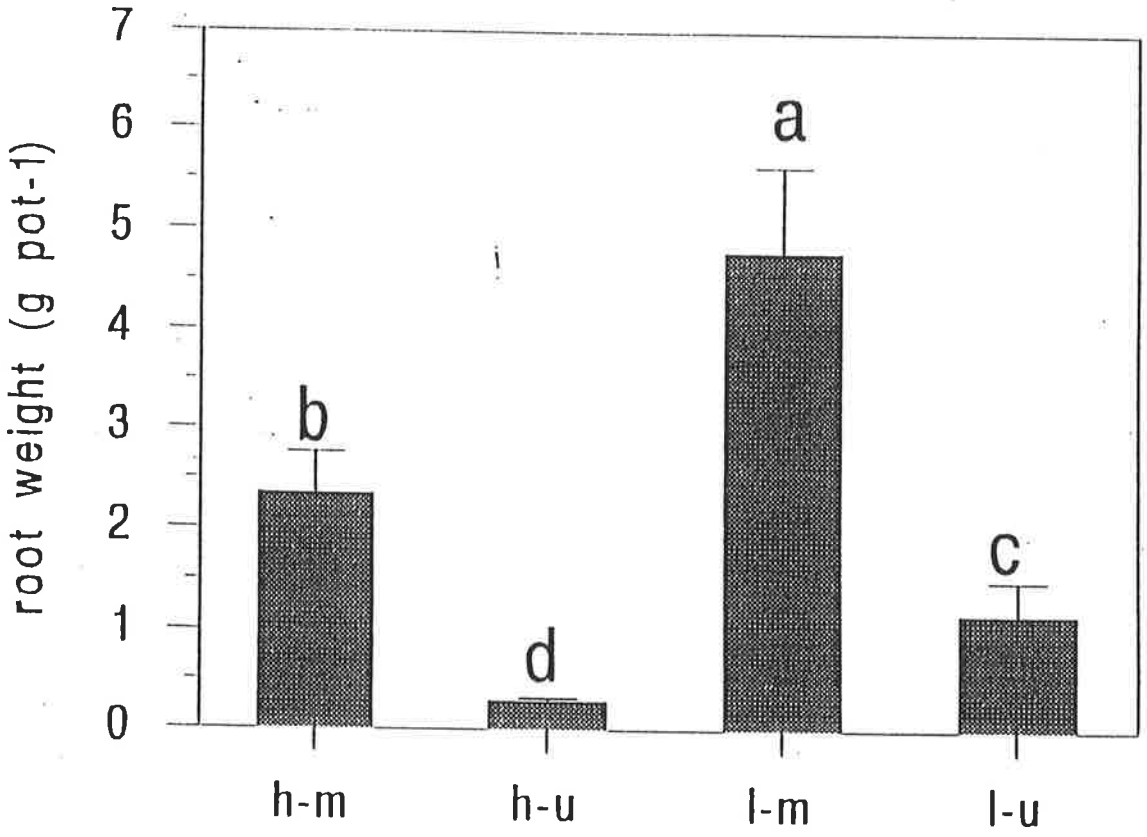


Fig. 6.4 Experiment 3. Final weight of earthworms in pots of either mixed or unmixed soil (plant treatments combined) from Springmount kept at 15°C and measured over 10 wks. Vertical bars represent standard errors of the means, n=30. Bars followed by same letters are not significantly different at the 5% level.

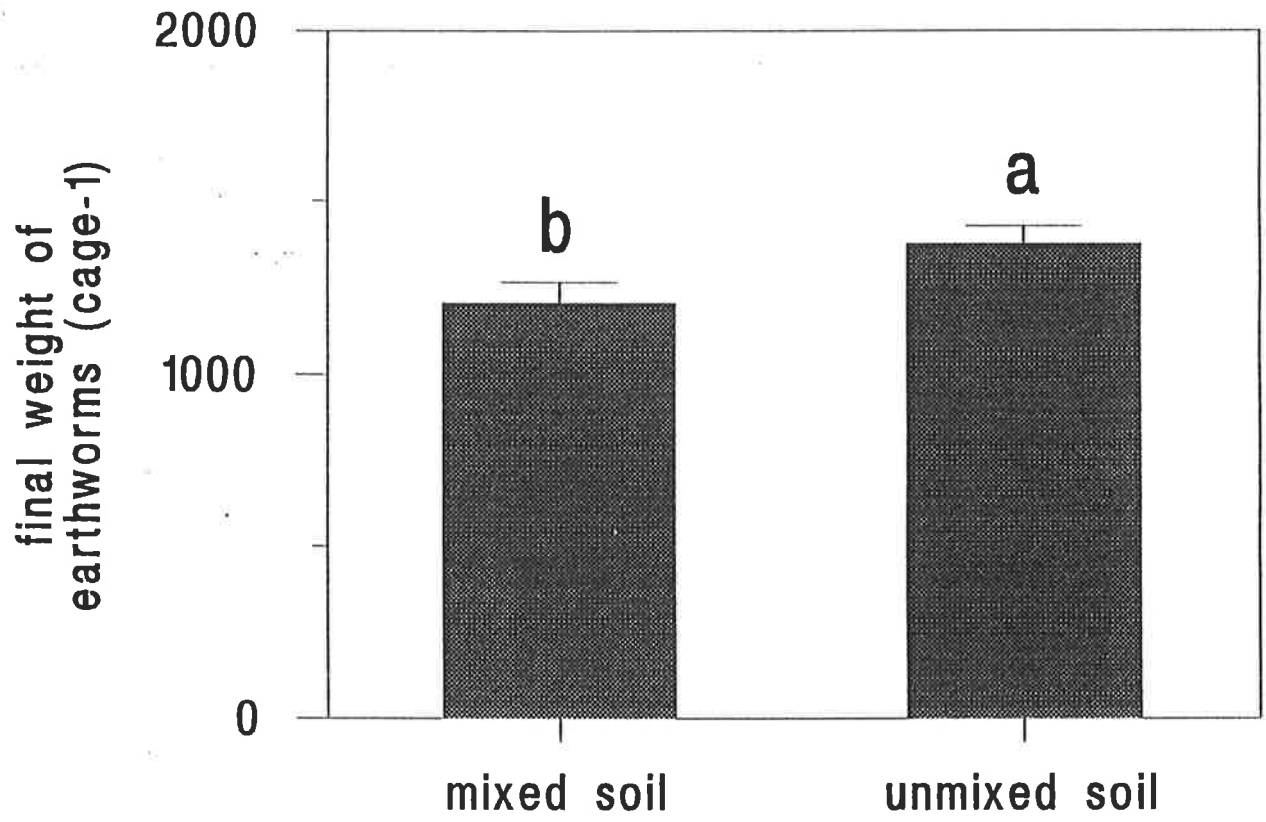
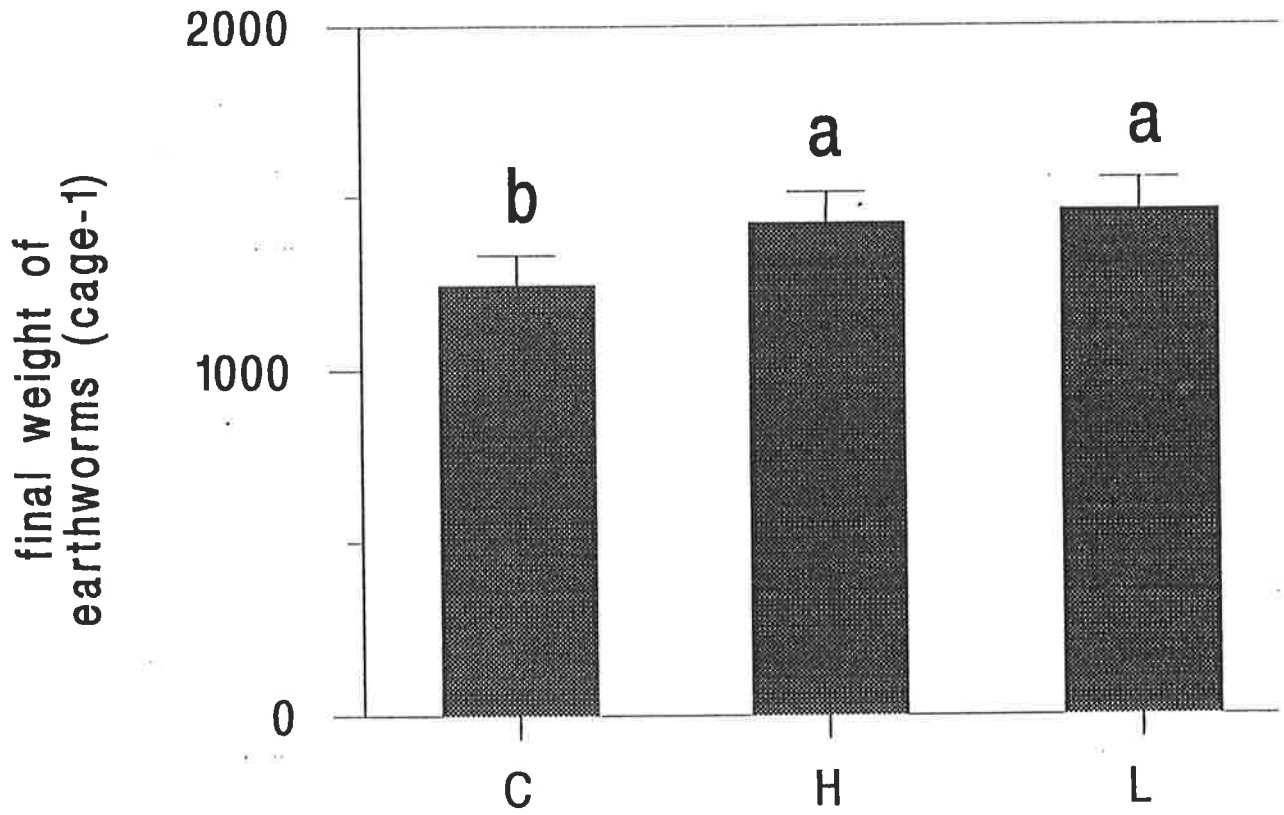


Fig. 6.5 Experiment 3. Final weight of earthworms in pots with unmixed soil from Springmount kept at 15°C and measured over 10 wks. Vertical bars represent standard errors of the means, n=30. Bars followed by same letters are not significantly different at the 5% level ($P<0.05$). C = control (no plants), H = heavily clipped plants and L = lightly clipped plants.



6.5 Discussion

6.5.1 Banded resources

There was no difference in earthworm performance between the banded and low concentration treatments in the pot or field experiments. These treatments had the same quantity of food which was allocated into either a high concentration band at the surface or mixed throughout the soil. Therefore there was no evidence that banding the food at the surface resulted in increased performance of earthworms compared to mixing food throughout the soil.

Although there was no effect on performance, there was an effect on the spatial distribution of earthworms in the pot experiment (not measured in the field experiment). Earthworms were significantly aggregated in the banded layer which is not surprising considering that this is where the resources were.

6.5.2 Plant roots as a food source

Earthworms performed better when plant roots were present in unmixed soil, but not in mixed soil. This means that the difference in earthworm performance between mixed and unmixed soil in Chapter 3 was at least in part because there were plants growing in the unmixed soil and almost no plant growth in the mixed soil.

Under some conditions at least, living roots or a microbial community associated with them are a significant source of food for earthworms. It is not known why earthworm growth was similar when plants were present in the mixed soil. It may be due to roots growing down established earthworm tunnels in unmixed soil making them a more available source of food, whereas in mixed soil earthworms activity and root activity may be much less related and roots would be less available for earthworms as a food source.

Root growth and water use was higher in mixed soil compared to unmixed soil. This was because plants in the unmixed soil suffered severe root disease infection soon after

earthworms were added. The reason root disease symptoms were less in the mixed soil is likely to be because soil disturbance breaks up the hyphal network of fungi which cause the disease (McGonigle and Miller, 1996). If earthworms can consume dead roots, but not living roots, then the increase in earthworm growth in the unmixed soil may have been as a result of root diseases killing off the roots and making their tissue available to the earthworms.

Clipping the plants was successful in reducing root growth in the experiment and is a useful method for comparing the effects of different root inputs into the soil on various soil processes.

6.5.3 Mixed vs. unmixed soil

Earthworm performance was significantly reduced in the mixed soil compared to the unmixed soil, independent of any plant effects. Any effects of changed soil structure on soil moisture would have been removed by watering the soil to a set water potential rather than a set moisture content. Breaking up the soil structure may result in earthworms having to spend more energy forming tunnels which were destroyed in the mixing process, reducing the energy they could expend on growth. The negative effect of tillage on earthworms is likely to be at least partly due to similar changes in soil structure.

6.5.4 Reasons earthworms gained more weight in unmixed soil compared to mixed soil in field experiments at Springmount

In Chapter 3 there was a suggestion that earthworms performed better in unmixed soil than in mixed soil when different experiments were compared. In this comparison, it was not known whether the differences were due to the differences in the size of earthworms added, the disturbance of the soil, the dilution of food resources through a larger volume of soil or because root growth was higher in the unmixed soil. The results from the experiments presented here show that the differences in growth are not affected by

diluting the food resources. There was a weak, but significant effect of mixing the soil, with higher earthworm growth recorded from the unmixed soil compared to the mixed soil treatment. In the unmixed soil, there was a strong relationship between plant biomass and earthworm growth. It is highly likely that the higher earthworm growth in the unmixed soil was due to the presence of plants.

6.6 Conclusions

1. Earthworms did not perform better in terms of growth or survival in soil where the resources were concentrated in a band at the surface compared to soil where the resources were spread evenly throughout the soil.

2. Earthworms had greater growth rate in soil which contained a higher quantity of food.

3. Earthworms performed better in pots of undisturbed soil when plants were present.

4. Earthworm growth was higher in unmixed compared to mixed soil.

5. The reason that earthworms performed better in unmixed soil in field experiments in Chapter 3 was probably due to the presence of plants in unmixed soil but not in mixed soil.

Chapter 7 GENERAL DISCUSSION

7.1 Introduction

The original aims set out at the start of this thesis were to determine if there are competitive interactions between earthworms in pasture soils in the high-rainfall areas of southern Australia and to assess the potential impact of introducing an exotic lumbricid, *Aporrectodea longa* (Ude), on a native habitat (Deep Creek) adjacent to a pasture. Detailed discussion on the experimental work carried out to meet these aims is set out in preceding chapters. This chapter discusses some of the issues raised in the preceding chapters in a more general context. The first two sections discuss how well the study had addressed the original aims. The next two sections discuss how the experimental methods could be improved and what the implications of this study are for the deliberate introduction of *A. longa* to high-rainfall agricultural areas. The final section is a summary of the main conclusions from the study.

7.2 Competition between introduced species

Competitive interactions were found between *A. caliginosa* and *A. trapezoides*, *A. longa* and *A. caliginosa* and *A. longa* and *M. dubius*. This is the first reported evidence that competition occurs between earthworm species under field conditions, and along with Abbott (1980) is the only experimental evidence that earthworms compete with each other. There are three possible mechanisms for competition between these species; interference competition, consumption of cocoons and scramble competition for food resources.

Interference competition was demonstrated between *A. longa* and *A. trapezoides* in section 5.5 and between *A. caliginosa* and *A. trapezoides* in section 3.7.5.1. The reasons for the migration of *A. trapezoides* away from *A. longa* and *A. caliginosa* away from *A. trapezoides* are unknown. No evidence was found that suggested *A. longa* produced a

chemical deterrent in their mucus (section 5.5). Constant physical contact with other earthworm species may be distressing to *A. trapezoides* with the result that individuals move away from *A. longa*. In laboratory studies, earthworms become active and move away when they are touched with various objects (*pers. obs.*). The effect of *A. longa* on the behaviour of *A. trapezoides* could be investigated further to determine what causes *A. trapezoides* to move away from soil containing *A. longa*. It would be interesting to investigate whether adding other species of earthworms, or other invertebrates would invoke similar movements due to the intolerance of earthworms to contact with other species.

The movement of *A. trapezoides* towards the fresh dung, and the movement of *A. caliginosa* away from the dung might be due to differences in food preferences as well as distress from physical contact. Both species have been found coexisting at high densities within a field at Springmount (Baker *et al.*, 1992), so interference obviously does not result in exclusion. Coexistence may be partly as a result of the difference in food preference between the two species. Further investigation would lead to a better understanding of the mechanism of this interference effect and may also shed light on food preferences and dietary requirements of the two species.

This is the first evidence of earthworms consuming cocoons of other species. *A. longa* consumed *M. dubius* cocoons (section 4.5) and it is possible that the reduction in the population and cocoon density of *M. dubius* in other experiments was due in part to cocoon consumption by *A. longa.* Removal of organic matter from the surface by *A. longa* is the other likely explanation for the observed decreases in population density of *M. dubius* in the presence *of A. longa*. Given that *A. longa* can consume cocoons of *M. dubius* and artificial spheres as large as 2.5 mm, it is probable that *A. longa* and other large earthworm species can consume cocoons of other species of earthworms (section 4.6.1) and possibly cocoons and eggs of other soil fauna such as snails and slugs.

Whether this would result in a significant impact on populations of these organisms requires further investigation.

Scramble competition could explain the observed competitive interactions between earthworm species. For scramble competition to occur, earthworms must feed on discrete resources which are scarce in the soil. A reduction in these resources by another species would reduce the performance of the first species. As competition was not demonstrated between all earthworm species, their diets could well be different. Other studies have also shown different dietary preferences between earthworm species (Piearce, 1978; Shipitalo *et al.*, 1988; Hendriksen, 1991; Rozen *et al.*, 1995). However, the concept that earthworms feed on limited, discrete food resources (ie. particulate organic-matter fragments of a particular size and chemical composition) is not consistent with the model of earthworms as filter feeders, moving through the soil and indiscriminately scavenging on a wide range of tiny organic-matter units (ie. bacteria, simple chemical compounds) (section 1.5.2.3). The filter-feeder model is also not supported by the result from section 6.2, where earthworms responded to the total quantity of food rather than the physical placement of food in the soil. If earthworms are filter feeders, they should grow better where food is concentrated in patches, where they can spend the majority of their feeding activity and maximise their assimilation efficiency. As the evidence presented here suggests they do not feed in this manner, then the total amount of food in the soil must be important rather than the placement.

If the species used in this study are competing for discrete packages of food, the obvious question is, what are these discrete packages? There is a poor understanding of the dietary requirements of earthworms (section 1.5.2.3) and increased knowledge would be useful for studies of community ecology such as presented here and similar studies in which the link between the spatial distribution of food and individual consumers can be used to predict competitive outcomes (Ives 1995). An increased understanding would also be applicable for developing models of nutrient turnover in the decomposer

community (Marinissen and Ruiter, 1993) and developing mass rearing techniques for earthworms.

The data obtained from these experiments cannot be used in the conventional models used to simulate competitive interactions between species (Roughgarden, 1979). Such models require data on population growth over a number of generations. The experiments in this study measured average individual growth, or cocoon production, over a set time period which did not span more than one generation. To estimate population growth over a number of generations would require larger experimental cages, which allow earthworms to burrow down the soil profile to aestivate over the dry summer period.

Inter-specific competition within the spatial scale of a cage may not translate into competitive exclusion at larger, field scales. There is greater opportunity for competitive avoidance at a larger scale because the level of heterogeneity increases with increasing area. Numerous theoretical demonstrations have shown that habitat subdivision can allow two species, a fugitive species and a superior competitor, to coexist as a stable metapopulation (Armstrong, 1976; Hastings, 1980; Shmida and Ellner, 1984). Metapopulation models are difficult to apply to earthworm populations in agricultural fields, because it is difficult to measure the boundaries of the one population, let alone numerous, neighbouring populations. Inoculation programs offer a unique opportunity to study the dispersal and colonising behaviour of earthworms and to study whether some species of earthworms outcompete other species, or reduce their population densities when they invade an area. As such, Introduction programs should be designed and implemented with this in mind.

7.3 Potential risks of introducing *A. longa* to native habitats

Native earthworm species are rare in agricultural areas across southern Australia which are dominated by exotic earthworm species (Baker *et al.*, 1992a,b; 1993a,b,c; 1994b). It

could be speculated that exotic species outcompeted the native species in these areas, however if native populations have decreased in size, it was probably a result of clearing native vegetation rather than competition between native species and exotic species (section 1.6.3). The results presented in Chapter 5 confirm those suggestions. *A. longa* did not perform well in soil from the forest area and showed a strong and consistent preference for pasture soil over soil collected from the native forest. It seems highly unlikely that *A. longa* would invade native areas similar to Deep Creek. Further work is required to test the likely impact of *A. longa* on other native ecosystem types in the high-rainfall areas of southern Australia (section 5.7.3).

There is a potential for competition between native and introduced earthworm species in pastures, where native species have been able to survive the change in habitat (section 1.6.3). Competition between *A. longa* and native earthworms in pasture soil was not tested in this study. It remains an area where further investigation could be done.

There is a small risk that *A. longa* would adapt to the native soil and litter over time, invade native areas and become a serious pest, if it were deliberately introduced across southern Australia. This is an unlikely event because complex and large shifts in the genetic makeup of *A. longa* would have to take place for it's digestive system to adapt from digesting plant species of European origin (grasslands, deciduous forests, agricultural areas), to being able to digest sclerophyllous organic material that dominates native forests. These changes are unlikely to take place in a time frame less than that which would see *A. longa* colonise agricultural areas naturally, from the few small colonies already found on the mainland (Wood, 1974) and so deliberately introducing *A. longa* is unlikely to damage to native areas over and above that which would happen naturally.

7.4 Improving the design of experiments to investigate the community ecology of earthworms

The basie of the experimental designs used in this study was that the densities used should be similar to those found in the field where possible, that there be a treatment with only one species which could be compared to a treatment with two species and that the length of time was similar to a field season in the case of field experiments, or 10 weeks in the case of pot experiments. This type of experimental design should be able to demonstrate competition between some species. However there are some limitations with this approach and improvements to these are discussed below.

Although it was possible to make educated guesses as to what density and biomass of earthworms could be supported in some soils such as at Springmount where long-term survey work had been carried out, it would be useful in cases where this information is unavailable to carry out preliminary experiments to determine the performance of earthworms across a range of earthworm densities (eg. sections 3.4, 5.4.2 and 5.4.1.1). Once the maximum density of earthworms which can be supported in the soil is known, this density can be used in a later experiments in which more than one species is added at a range of densities. Using a range of densities gives much more information than adding a second species at a single density. Of course, if the densities of earthworms are pushed high enough, competition should be demonstrable between any two species. What is important however, is whether competition occurs between species at densities that can be realistically attained under field conditions. Using a range of densities also allows a linear or multiple regression to be used to analyse the data so that the impact on the average survival of one species in the presence of another species can be determined. An example of this is shown in section 4.3, where the variability in survival and contamination meant that rather than having discrete treatments, there was a wide range of densities of *A. caliginosa* whose numbers could be related to the number of *A. longa* in the cores and vice versa. This analysis not only showed significant interaction between the two species, it also showed how the density of one species related to the density of

the other. In this case, it meant that 7 *A. longa* would result in a loss of 1.4 *A. caliginosa*.

The durations of field experiments described in this study were slightly less than the length of one field season (15 to 20 weeks), which is similar to the average length of field experiments surveyed by Gurevitch *et al.* (1992; see section 1.6.2). If it is only possible to run short experiments to detect competition, a number of separate experiments should be carried out, as was done in this study (see discussion in section 1.6.2). Longer term investigations of earthworm interactions would require a larger field cage which penetrated deeper into the soil to allow earthworms to burrow down the soil profile to survive over the dry, summer period. Although longer term experiments did not generally demonstrate competition any more or less than shorter term studies (Gurevitch *et al.* 1992), it would be interesting to determine whether the short term effects demonstrated here translate into long term competitive exclusion or greatly reduced population densities. This would also provide information on the population dynamics which could be used in the conventional population interaction models.

The size of the experimental units was never larger than 0.07 m^{-2}. It is quite possible that individuals are forced to compete at small scales, but at larger scales are able to avoid competition by utilising different spatial niches. A number of models which allow coexistence to occur between species using similar resources was discussed in section 1.6.1.1.2. These models all have the underlying premise that organisms which use the same resources can coexist by having limited mobility and different responses to the resources and environment. In a heterogenous environment that fluctuates temporally, more than one species may be able to exploit the same resource and coexist by using the resource in a different way, or by being much more mobile and able to exploit new resource patches quickly (pioneer species) before being pushed out by other species. Earthworms live in an environment which is under constant flux of different grazing pressures, prevailing weather, soil management, fertiliser inputs and invasions by new

species (including earthworms). Therefore some species like *A. trapezoides* and *A. caliginosa* may be able to coexist due to their differences in environmental tolerances (Chapter 3; Briones, 1993) even though they have been shown to compete under some conditions (Chapter 3) and are limited in mobility (section 1.2.4). Other species, such as *M. dubius* may be able to avoid competition with species such as *A. longa* (Chapter 4) by quickly exploiting high value resource patches such as dung before *A. longa* are able to locate it.

There was a consistent trend for smaller earthworms to gain more weight per unit time than larger earthworms (section 3.7.2.2, 4.6.2 and 5.7.1). Consequently small earthworms should be used in experiments where earthworm growth is used as a measure of performance, because it is the stage where the greatest impacts on growth of various treatments will occur (White, 1993). However, there is a lower limit to the size of earthworms which can be used because of difficulties involved in handling them. Small earthworms are easily damaged and more difficult to find by handsorting through soil. There is a trade-off between using worms small enough for them to have a large growth potential, and using large enough earthworms so that experimental error in sampling does not become too high.

Earthworms performed poorly in mixed soil in the experiments described in this study. Further work should use intact soil cores rather than sieved soil. Earthworms performed better in unmixed soil probably because of greater inputs of carbon from roots and less energy required for excavating tunnels.

A summary of the optimal experimental approach to test for earthworm interactions is shown in Table 7.1.

Table 7.1 Experimental conditions to test for competitive interactions between earthworms

Soil	intact soil cores
Earthworms	as small as possible without being too difficult to find in the soil, too easily damaged
Duration	at least 1 field season
Scale	studies at different scales will give different answers
Replicates	at least 7
Treatments	one species at a density set by preliminary investigation, other treatments with a second species at various densities.
Analysis	multiple regression

7.5 Relevance of study to the introduction of earthworms into agricultural soils

If earthworms are not greatly affected by inter-specific competition, then increasing the diversity of earthworm communities in agricultural soils may result in greater increases in plant production and improvements in soil properties. This is because more individual earthworms could be supported in a soil if the community was made up of a number of species, each of which used different components of the soil resource. Such a case has already been reported. Adding *A. caliginosa* and *A. longa* to pastures in Tasmania increased plant production in the pastures to a greater degree than adding either species separately (Temple-Smith *et al* 1993), even though there is strong evidence that they compete against each other on a small scale. There are of course a number of qualifications that need to be made. Not all earthworm species are equal in their effects on nutrient cycling and soil structure, and so only those species which have been identified as being able to improve agricultural production should be considered for introduction. Further work needs to be done to determine whether there is any advantage in mixing species of earthworms in terms of their effects on soil structure and fertility and pasture production, regardless of any adverse effects due to competition.

This research points out that the species composition of a particular area might be a poor predictor of what species could survive in that area, although comparing treatments between sites and between years should be interpreted with caution. It would be expected that where there is a large amount of traffic and movement of stock between individual fields, most species would have had enough opportunity to colonise most of the areas. However, if earthworms that are common within these areas could be successfully introduced into fields where they are currently not present, it suggests that we do not fully understand the restrictions of dispersal on earthworm communities at a regional scale. It would be interesting to see whether earthworms can be successfully introduced into areas of intensive agricultural production where they are not currently found such as the Mount Lofty Ranges.

7.6 Summary of Conclusions

1. Competitive interactions were found between *A. caliginosa* and *A. trapezoides* (Chapter 3)

2. Competitive interactions were found between *A. longa* and *A. caliginosa* and *A. longa* and *M. dubius* (Chapter 4)

2. There are three possible mechanisms for competition between earthworms; scramble competition for food resources (Chapter 3, 4, 5 and 6), interference competition (Chapter 3 and 5) and consumption of cocoons (Chapter 4).

3. *A. longa* probably does not pose a serious threat to native stringybark forests in the Mount Lofty Ranges, South Australia because it prefers pasture to forest soil and performs poorly in forest soil (Chapter 5).

4. Experiments investigating the community ecology of earthworms should be done using small earthworms, in unmixed soil, at a range of different scales, over a number of generations if possible and at a number of different sites. Competition studies should include a control treatment with only one species. Other species should be added at a range of densities (Table 7.1) (all Chapters).

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Appendix A. **Calculations for final intestinal contents after 72 h.**

Let DW_{72} = dry weight @ 72 h,

IC_{72} = intestinal contents @ 72 h,

TW_{72} = tissue weight @ 72 h,

Ft = ash content of TW_{72} (see text),

Fi = ash contents of IC_{72} (see text),

M_{72} = mineral material remaining after incineration of DW_{72}.

The dry weight of earthworms can be expressed by;

$$DW_{72} = IC_{72} + TW_{72} \quad (1)$$

$$\Rightarrow \quad TW_{72} = DW_{72} - IC_{72} \quad (1a)$$

The mineral material remaining after the incineration of dried earthworms can be calculated by the following equation:

$$M_{72} = (IC_{72} * Fi)) + (TW_{72} * Ft) \quad (2)$$

Substitute (1a) into (2),

$$M_{72} = (IC_{72} * Fi) + ((DW_{72}-IC_{72})*Ft)$$

$$\Rightarrow \quad IC_{72} = (M_{72} - (DW_{72}* Ft)) / (Fi - Ft)$$

Appendix B **Published and accepted papers arising from this study**

Dalby, P. R., Baker, G. H. & Smith, S. E. (1995). Glyphosate, 2,4-DB and dimethoate: effects on earthworm survival and growth. *Soil Biology and Biochemistry, 27*(12), 1661-1662.