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Derek J. Swarts

Edith Cowan University

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SOIL COMMUNITY STRUCTURE AND LITTER DECOMPOSITION UNDER IRRIGATED EUCALYPTUS GLOBULUS IN SOUTH WESTERN AUSTRALIA

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24 01 2006

Supervisor: Associate Professor Adrianne Kinnear
Supervisor: Dr Annette Koenders

This thesis is presented in fulfilment of the requirements for the award of Doctor of Philosophy in Biological Science
USE OF THESIS

The Use of Thesis statement is not included in this version of the thesis.
Credo ut intellegam

(I believe in order that I may understand)

Augustine
DEDICATION

This thesis is dedicated in loving memory to my great grand mother who passed away on the 16th of December 2003. Every week with out fail, Oma would ask if I had finished my studies and found work yet. I don’t think that Oma ever really understood what it was I was doing but I deeply regret that I will never be able to say to her “Yes, I have finished.”

Mrs Wijntje van den Berg
(27th May 1918 – 16th December 2003)
ACKNOWLEDGEMENT

In researching and writing this PhD, I, like most students have experienced great challenges, and enormous frustration. Tempering these ‘negative’ aspects of research have been the moments of scientific euphoria and the wonderful friendships I have been privileged to make. I do not really expect others to understand the pleasure that I have received from looking down a microscope for more hours than I care to count or the joy of working some horrendously difficult taxonomic key to identify a ‘bug’ that I only found once or twice, but I know that this mass of paper currently in your hands is the work of a collaborative rather than just mine. Although my name sits on the front cover, many others have assisted in various forms to bring this study to its conclusion. Here in this small section I will attempt to thank you all. (My sincere apologies to anyone inadvertently omitted).

My principal supervisor Associate Professor Adrianne Kinnear deserves an extra special mention, not only for her unfailing encouragement, support and guidance but also for her belief in me. To my associate supervisor Dr Annette Koenders, thank you for your support and guidance. I greatly appreciated your involvement and insight.

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Numerous volunteers (Kevin, Amanda, Steven, Joel, Llewellyn, Jordan and Marcus) gave of their time to help me collect samples, grind sand and leaves and many other time consuming but very valuable jobs. To them I say a very big “Thank You”.

To my family who are unanimously of the opinion that its about time I finished studying, thank you for your support. Finally to my wife, Amanda, thank you for your love and support and for your unshakeable belief that no matter what new hurdle appeared, that I would eventually finish.

Thank you All

D. J. Swarts
"If we knew what it was we were doing, it would not be called research, would it?"

Albert Einstein
ABSTRACT

Plantations provide a range of benefits, including the potential to ameliorate salinity and soil erosion, enhance biodiversity, and provide timber and wood chips. They are increasingly important because of their role in carbon sequestration (Adolphson, 2000; Anonymous, 2005; Jones et al., 2005; Kozlowski, 2002; Paul and Polglase, 2004). Recent research has highlighted the connection between plantation health and soil fertility (Johnston and Crossley Jr, 2002). Within an Australian context there is little published data on the composition of the soil and litter fauna and their contribution to litter decomposition under plantation systems (Adolphson, 2000). The Albany Effluent Irrigated Tree Farm provided an opportunity to research plantation (*Eucalyptus globulus*) soil flora and fauna communities, rates of litter decomposition and to describe the impact of irrigation (both mains-water and effluent) on these communities.

This study described soil communities and rates of litter decomposition under *E. globulus* irrigated with effluent and mains-water over the period 2000 and 2001. The replicated experimental design allowed for the use of parametric statistics and nested-ANOVA to determine the impact of season and treatment. Community level analysis was conducted using classification and ordination routines.

Soils at the Albany Effluent Irrigated Tree Farm have a duplex profile, comprising a grey white to grey yellow sandy topsoil overlying structured medium clay subsoil. A
lateritic duracrust (300 - 600mm) is present immediately above the clay. Soils are acidic (pH 4.2 – 4.7) with low levels of %TC (3.2 – 7.6) and %TN (0.33 – 0.35).

The soil microflora was described in terms of microbial biomass and microbial respiration. Levels of microbial biomass (307 – 1001 µg microbial C g⁻¹ soil) were calculated using the chloroform fumigation and extraction method and were comparable to published values. Rates of soil microbial respiration were very low and ranged from 0.24 – 0.49 g CO₂ g⁻¹ soil 24 hr⁻¹, comparable to rates of microbial respiration observed from recently glaciated soil in the northern hemisphere. Neither microbial biomass nor respiration was significantly affected by irrigation.

Soil nematodes were extracted using the whitehead tray method. Nematode densities (0.9 – 49.9 N m⁻² x 10⁴) and diversity (14 species) were always very low, regardless of treatment or season and were not significantly affected by irrigation. Irrigation affected nematode functional composition, increasing levels of bacterial feeding nematodes. This change in the functional composition of the nematode community is reflective of a shift in the microbial community towards a bacterial dominated microflora.

Soil acari were extracted using high gradient temperature extraction. Acarine densities were low, ranging from 3.4 to 29.8 N m⁻² x 10³ and were numerically dominated by species with r-selected characteristics (Tyrophagus sp and Tarsonemidae sp.). A total of 47 species were identified and species richness was consistently higher in non-irrigated treatments, but not significantly so. Prostigmatid
mites dominated acarine diversity, with 23 species followed by 12 mesostigmatid species (although mites of this order were rare and frequently immature), 11 oribatid and 1 astigmatid mite species. Irrigation did not affect acarine community composition. However, at a species level several oribatid mites with K-selected characteristics (Oppiids, *Brachychthonius* sp. 2., and *Tectocepheus velatus*) were negatively affected by irrigation, supporting the view that mites from this order may be useful as indicators of disturbance.

Rates of litter decomposition were slow ($k = 0.46 – 0.53$) but were not significantly affected by irrigation (effluent or mains-water). The eleven-month study may have been too short to detect any later irrigation-related responses. The chemical composition of mature (non-senesced) leaves (%TC and %TN) did not vary significantly between treatments. Acari communities were more numerous and species-rich in irrigated litter bags sampled 9 and 11 months after placement in the field. Classification and ordination revealed three distinct stages of faunal succession corresponding to months one and two after placement in the field, months three to seven, and months nine to eleven.

Soil communities at the Albany research site are dominated by bottom-up processes. The low soil organic carbon and soil acidity constrains the activity of the microbial resource that in turn limits the density of the micro and mesofaunal communities. Species diversity was constrained by two factors, the pasture history and the perturbation sequence associated with plantation establishment. Pasture sites are synonymous with depauperate mesofaunal communities and so provided
this research site with a very small micro and mesofaunal reservoir to begin with. The sequence of perturbation at the site has resulted in a soil dominated by micropores, with limited stratification and heterogeneity. Finally, the sustained perturbation associated with the previous land use and the plantation development has selected for a faunal community dominated by \( r \)-selected species.
DECLARATION

I certify that this thesis does not, to the best of my knowledge and belief

I. incorporate without acknowledgment any material previously submitted for a degree or diploma in any institution of higher education;

II. contain any material previously published or written by another person except where due reference is made in the text; or

III. contain any defamatory material.

_________________

Derek Juan Swarts

Dated: 24/01/2006
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“Revision plays a very large role in writing. Sometimes it seems to be all revision, and the longer I write the more I revise – and its never completely right”

Ellen Hunnicutt
1.0 Introduction and Review of Literature

1.1 INTRODUCTION

The Albany Effluent Irrigated Tree Farm represents a unique and very modern approach to the dilemma of traditional municipal wastewater disposal. The goal of the Albany Effluent Irrigated Tree Farm is that nutrients (including C, N & P) and moisture remaining in the effluent after wetland stripping are incorporated into the soil and/or plant (Eucalyptus globulus) biomass. Following maturity of the plantation, the operators of the Albany Effluent Irrigated Tree Farm (the Water Corporation of Western Australia) intend to harvest the timber for wood chips.

Development of the site occurred early in the 1990’s and particular attention during the planning stage was focused on the site’s hydrology to the exclusion of predicting and developing management strategies for the impact of effluent irrigation on both short and long-term nutrient cycles. For the Water Corporation, both the gate-keeping role of the soil microbial populations in mediating below-ground nutrient cycles and the impact of litter decomposition on the nutrient cycles are ‘black boxes’ of understanding. Given the pivotal role performed by soil microbes in the mobilisation/immobilisation phase of the nitrogen cycle (Aber et al., 1998; Aber et al., 1989; Aber, 1992), and the primary importance of litter nutrient quality, rates of litter fall and the decomposition process as a major source of nutrient inputs into the
below-ground nutrient cycles, this project was developed to ‘open up’ and shed some light on these ‘black boxes’ of misunderstanding.

Although the soil microbial fauna are gatekeepers of the below-ground nutrient cycles, their levels of activity and biomass are regulated by higher trophic levels of both micro and mesofauna (Anderson et al., 1983; Bardgett et al., 1993b; Kuikman and Van Veen, 1989). Given the critical ‘controlling’ role occupied by these fauna over the level and activity of the soil microbes, this project described the species and functional composition of the micro and mesofaunal soil and litter communities.

The Albany Effluent Irrigated Tree Farm also offered the opportunity to study the impact of developing *E. globulus* plantations on previously long-term pasture sites. In 1999, Aggangan et al., (1999) reported that *E. globulus* plantations occupied 100,000 ha in the south west of Western Australia and were being established at a rate of approximately 20,000 ha per year.

Clearly, the establishment of *E. globulus* plantations within Western Australia represents a significant change in patterns of land use. As such, it deserves both attention and research. This project did not set out to compare and contrast pasture with plantation (this research has already been conducted e.g. Adolphson, (2000) and Ananthakrishnan et al., (1993), but rather concentrated on *E. globulus* plantations and described the soil and litter fauna biodiversity resulting from the conversion of pasture to plantation.
Another rationale for this study was to advance the current level of knowledge regarding Australian soil and litter fauna. The project utilised a holistic, in-depth, cross-faunal approach to describe not only the community characteristics of individual soil and litter faunal groups, but also to describe their interactions. While necessarily limited by the paucity of relevant Australian invertebrate taxonomy and knowledge of functional morphology, this study aimed to advance the boundaries of our knowledge in the fields of soil and litter micro- and meso-flora and fauna, without being a specifically taxonomic work.

The final rationale for this research was to describe the impact of irrigation on the soil and litter fauna and rates of litter decomposition. Specifically, this project aimed to qualify the difference between the impact of irrigation with mains-water and irrigation with treated effluent. While extensive research (both local and international) has been conducted on the impact of effluent irrigation on soil chemistry and hydrology, the impact of irrigation (effluent or otherwise) on the soil and litter fauna and litter decomposition has never been studied within Australia, and only rarely around the world. This research seeks to redress this lack of information and examines the impact of both effluent and mains-water irrigation on soil and litter fauna and the rate of litter decomposition.

1.1.1 Summary of Research Aims

1. To describe the soil and litter microflora, micro-, and mesofauna under an *E. globulus* plantation recently developed on long-term pasture sites.
2. To describe the impact of irrigation (effluent and mains-water) on these soil and litter micro- and meso-flora and fauna communities.

3. To describe the impact of irrigation (effluent and mains-water) on rates of *E. globulus* leaf litter decomposition.

1.2 LITERATURE REVIEW

The literature review introduces the soil environment and considers the groups that are the focus of this research, their functions and contribution to ecosystem processes as well as to soil chemistry. It continues with the litter environment and an evaluation of plantation biology and includes a summary of the relevant research dealing with effluent and mains-water irrigation. The review concludes with a summary of the research hypotheses.

1.2.1 Soil and Litter Fauna and Ecosystem Function

Setala (2002) reports that more than three-quarters of the annual net primary production of a terrestrial ecosystem enters the soil directly. Soil food webs link the above- and below-ground environments and are responsible for the decomposition of organic matter, the cycling of nutrients and the maintenance of soil structure (Abbott, 1989; Bardgett, 2002; Behan-Pelletier and Newton, 1999; Bowman, 1998; Brussaard, 1998; Catovsky *et al.*, 2002; Jones and Bradford, 2001; Liiri *et al.*, 2002; Naeem, 1998; Rantalainen *et al.*, 2005; Setala, 2002). The linkages between fauna in the detritus food web are extremely complex, reflecting not only high levels of
species diversity but also a wide range of trophic positions (Jones and Bradford, 2001; Scheu et al., 2003; Setala, 2002).

Microorganisms breakdown the organic matter into its constituent elemental components in a process called decomposition (Bjornlund and Christensen, 2005; Lavelle, 2002). The micro-, meso-, and macrofauna typically lack the requisite cellulase enzymes to be able to breakdown soil organic material (leaves, faeces, dead organisms and roots). However, they are capable of indirectly affecting the rate of microbial decomposition by comminution (Seastedt, 1984), microbial inoculation of litter (Beare et al., 1992) and selective grazing of microbes (Hanlon, 1981). In addition, they carry 'hotspots' of high concentrations of microbial fauna that can exist in their gut and in their excrement (Brussaard, 1998). In the following sections, I consider the role of microflora, microfauna and mesofauna in detail.

1.2.1.1 Microflora

Estimates of the number of soil and litter microbial species are very high. Hawksworth and Mound (1991) estimated that there are 1,500,000 species of fungi, 60,000 species of algae and 30,000 species of soil bacteria. Recent advances in molecular methods have suggested that the true level of microbial diversity is probably far greater then these numbers suggest (Sly, 1998).

Functionally, microorganisms are the main decomposers, responsible for more then 90% of the mineralisation occurring in soils (Fitter, 2005; Lavelle, 2002; Lavelle and
Spain, 2001; Suzuki et al., 2005). Jenkinson and Ladd (1981) used mathematical models to predict generation intervals of 456 days for microbes in Australian soils. These estimates are between 1,000 to 10,000 times longer then those obtained for microbial colonies under optimal laboratory conditions (Lavelle and Spain, 2001). Jenkinson and Ladd (1981) characterise field microbial communities as ‘huge, largely dormant populations, with an enormous richness of species and an ability to survive hard times.’ This disparity between the potential for rapid microbial (bacterial) turnover and field observations of long periods of microorganism dormancy has been termed the ‘Sleeping Beauty Paradox’ (Lavelle et al., 1995).

Lavelle et al., (1995) postulated that the reason for these long periods of dormancy (Sleeping Beauty) is directly related to the inability of microorganisms to move through the soil profile. (It must be acknowledged that fungi are capable of ‘movement’ via the production of mycelia.) Consequently, they must wait for food to be brought to them rather than migrate from an area with low levels of food to an area with higher levels. Alternatively, bacteria and fungal propagules can be transported on or in meso- and macrofauna. In contrast, meso- and macroorganisms mix and transport soil in their search for food and modify the soil environment at a microorganism scale and so are capable of interrupting microbial dormancy, termed the ‘Prince Charming’ effect. They further suggest that macro-organisms are major regulators of microbial activity. This research project will describe the levels of soil microbial biomass and rates of respiration in both non-irrigated control and irrigated treatment sites.
1.2.1.2  Microfauna

The soil microfauna includes both the protozoa and nematodes (Pankhurst and Lynch, 1994). In this literature review, I will concentrate only on the soil nematodes. Nematodes are found in a wide range of habitats including the oceans, soil, fresh water and as parasites of plants, vertebrates and invertebrates (Freckman and Baldwin, 1990). Nematodes are aquatic organisms that can exist within the soil water film surrounding soil particles. Nematodes feed on a wide variety of fauna and flora and can be divided into functional groups including predators, omnivores, fungivores, microbivores and phytophages (Freckman and Baldwin, 1990; Gupta, 1994; Yeates et al., 1993). Nematodes are important in the decomposition process since they consume bacteria, fungi and other decomposers, thereby exerting an indirect effect on the rate of decomposition.

1.2.1.2.1  Nematoda

Yeates and King (1997b) described the impact of 'improving' native grassland with fertilized and introduced sward in New South Wales by comparing the nematode communities in these two grasslands. They noted that the development of improved pasture resulted in a four-fold increase in total nematode density and a significant reduction in species richness, with a shift from plant-feeding species to microbial-feeding species. They observed that the increase in soil fertility in improved pasture is closely related to the increase in microbial feeding species (especially bacterial-feeders), levels of nematode activity and nutrient cycling.
Bongers and Ferris (1999) suggested that nematode trophic structure could be used as an indicator of the level of disturbance in soil systems. They report that in general, nematodes respond rapidly to disturbance and particularly to nutrient enrichment, but do not quickly migrate from stressful conditions. Further, they point out the key trophic position occupied by nematodes, namely that they feed on most soil microorganisms and in turn are prey for other soil meso- and macro-organisms. Finally, they observe that there is a very clear link between nematode morphology and trophic function, that is, the feeding behavior (trophic function) of nematodes can be deduced from the structure of the mouth and pharynx.

The use of nematodes as indicators of disturbance in soil is not as simple as these authors have suggested. While feeding traits of nematodes can be inferred from the structure of the mouth and pharynx, this requires specialist equipment and a high level of training before an appropriate degree of confidence and consistency is achieved. Additionally the lack of taxonomic resolution, particularly in the poorly described Australian fauna, is a hindrance in confirming functional classifications. Conversely, the small volume of soil required for extractions and the standard methods available for extraction are an advantage particularly when compared with other soil fauna. This research project will examine the nematode fauna and classify them at a functional level. Particular attention will be paid to total density, species richness and possible changes in the relative density of functional groups.
1.2.1.3 *Mesofauna*

The soil mesofauna includes a wide variety of animal groups traditionally classified on the basis of length (minimum length of 0.2 mm and a maximum length of 10 mm (Wallwork, 1970), whose major direct contribution to the process of decomposition is via comminution. Comminution is defined as the act of reducing to a fine powder or to small particles (Lawrence, 1995). A wide variety of soil mesofauna and macrofauna have mouthparts specifically adapted for the process of comminution (Reddy, 1995; Siepel, 1990; Swift *et al.*, 1979). Comminution increases the surface area of the detritus that is available for microbial colonisation and so increases the rate at which fungi and bacteria can degrade the litter (Huhta *et al.*, 1991; Lensing *et al.*, 2005; Reddy, 1995).

There is substantial field and laboratory evidence that microbial-feeding soil mesofauna are able to influence both microbial activity and biomass (Bardgett *et al.*, 1998; Hedlund and Ohm, 2000; Klironomos and Kendrick, 1995). By so doing, they can indirectly control the rate of decomposition in two ways. Firstly, soil microbivores are able to mobilise nutrients bound within microbial biomass by direct grazing (Anderson *et al.*, 1983; Kuikman and Van Veen, 1989). Secondly by selectively grazing (Bardgett *et al.*, 1993b), microbivores are able to alter the level of competition between microbial species and so affect the microbial community structure (Newell, 1984; Parkinson *et al.*, 1979).
Irmler (2000) suggested that the fragmentation of litter by comminution and its subsequent transit through the gut of soil fauna increases the palatability of the litter for the soil microbes. Ramani and Haq (1991) report on a study of two oribatid mites, *Meristacarus degradatus* and *Xylobates rhomboides* feeding on leaf and woody litter, which the mites excrete as faecal pellets enriched with organic matter and minerals. They also proposed that the soil microarthropods play a role in the dissemination of microorganisms (particularly fungi) by ingesting spores and bacteria and excreting them in different areas.

Santos and Whitford (1981) described the impact of microarthropods on the rate of decomposition in the Chihuahuan desert. They utilised buried litter-bags and exposed them to varying regimes of insecticide, fungicide, and both fungicide and insecticide. After 90 days in the field they found litter exposed to both fungicide and insecticide had experienced less then 10% mass loss. Litter exposed to the insecticide only, lost approximately 15% of its initial mass, while litter exposed to the fungicide only, experienced approximately 25% mass loss yet the control litter-bags over the same period displayed more then 40% mass loss.

In their paper (Santos and Whitford, 1981) examined the possibility that the changes in the rates of decomposition (measured as percentage mass loss) were related to the chemicals used to prevent colonisation by insects, fungi or both. Using both experimental data and by referring to previously published work, the authors defend their experimental design and concluded that the soil microarthropods make a
significant indirect contribution to the decomposition process in these desert soil ecosystems.

Since then, further studies have supported this observation (Beare et al., 1992; Hasegawa and Takeda, 1996; Irmler, 2000; Setala, 2002; Setala et al., 1988; Setala et al., 1998; Setala et al., 1996; Vreeken-Buijs and Brussaard, 1996). However the degree to which top-down predatory pressure impacts on the rate of decomposition appears to vary depending on the system being studied. Vreeken-Buijs and Brussaard (1996) studied mesofaunal dynamics in relation to wheat residue decomposition in the Netherlands, and found that when mesofaunal predators were excluded from litter bags, the fungivorous mesofaunal populations were able to retard the rate of decomposition by over-grazing on the fungal population. Setala et al., (1988) observed the impact of soil fauna on birch litter decomposition in microcosm experiments and found that there was a clear trend of higher mass loss in the presence of soil fauna. Litter experiments produced 32.0% more CO₂ in the presence of fauna.

1.2.1.3.1 Acari

Franchini and Rockett, (1996) suggested that soil mites (in particular oribatids) are good indicators of the level of soil disturbance in agricultural systems. They examined the impact of conventional, reduced, and no-tillage regimes on soil oribatids. They observed that at a species level, oribatid mites displayed a variety of responses including insensitivity to the tillage regime, reduced density (negatively
sensitive) while others displayed increased density (positively sensitive). The authors postulated that the compacted nature of the soil in conventional tilled soil may have selected for small species such as *Oppiella nova*, that were more abundant in conventional tilled soils, and against larger oribatids that tended to be absent.

Behan-Pelletier, (1999) considered the biology, ecology and life history of oribatid mites and their role as possible bioindicator organisms. She noted that oribatids typically have high diversity, occur in high numbers, are easily sampled and extracted and are relatively easy to identify (at least in central Europe and the United States of America). She further noted that the use of oribatids as indicator species is most effective at the species level. Given the incomplete taxonomy of the oribatid fauna, particularly in the southern hemisphere, it is hardly surprising that their use is not yet widespread. Following the advice of both Franchini and Rockett (1996) and Behan-Pelletier (1999), this research will examine the soil acarine communities and, if appropriate, focus on the oribatid mite fauna.

### 1.3 Structuring of Soil and Litter Communities

At the centre of many soil and litter research projects (including this one) is the aspiration to explain how one or more factors work to structure soil and litter communities. While such projects tend to focus on density, species diversity and
other measures of community structure, they operate within an ecological framework (Crawford et al., 2005; de Ruiter et al., 1998). The application of ecological theory to the study of soil ecology is a relatively new development (Eijsackers, 2001; Fitter et al., 2005; Neher, 1999). In this next section, I review trophic dynamic theory and the concept of dual pathways for below-ground energy flow.

### 1.3.1 Trophic Dynamic Theory

R. E. Lindeman first proposed trophic dynamic theory in 1942 (Lindeman, 1942) as a result of a five-year study of ecological succession in a senescent lake (Cedar Creek Bog). Lindeman presented a theoretical model of nutrient cycling based on energy flow through aquatic plants, phytoplankton, grazing and predatory zooplankton, benthic worms, insect larvae, crustaceans and fish. The model quantified energy flow and emphasized that an ecosystem is composed of both biotic and abiotic components and includes the interactions within and between both these components.

Associated with trophic dynamic theory are two elements, namely ‘top-down’ predatory effects and ‘bottom-up’ resource effects (Pimm, 1982). These two forces work to structure the faunal density and diversity of a food web. Gutierrez et al., (1994) suggested that bottom-up processes determine maximum productivity while top-down processes determine the realized growth of an ecosystem. The clear delineation between top-down and bottom-up processes that exists in theory is very difficult to separate in practice.
Trophic cascades have become a central tenet of trophic dynamic theory. They can be described as the inverse patterns in density (or biomass) that can be seen across several trophic levels (Carpenter and Kitchell, 1993) when levels of predators or predation intensity vary. The concept of trophic cascades was developed in the 1960’s based on field experiments conducted in aquatic systems (Pace et al., 1999; Paine, 1980; Strong, 1992). He found that a food chain composed of three levels (top predators, mid-level consumers and basal producers) increased levels of top predators resulting in reduced numbers of the mid-level consumers, which in turn resulted in higher density of the basal producers (Pace et al., 1999).

While trophic cascades have been identified in aquatic systems (Carpenter and Kitchell, 1993; Strong, 1992), the case for trophic cascades structuring communities in terrestrial research is far from conclusive (Wardle et al., 2005). An examination of the literature reveals terrestrial systems that have found no evidence of cascades (Mikola and Setala, 1998b), limited or contradictory evidence (Dawes-Gromadzki, 2002; Strong, 1992) and those that show distinct cascades (Moran and Scheidler, 2002).

There are a number of significant differences between aquatic food webs and soil food webs. In general, aquatic food webs (particularly those in which strong trophic cascades have been identified) tend to be significantly simpler than their terrestrial equivalents. Research in New Zealand streams on the impact of the brown trout (Salmo trutta) has found that the brown trout lowers the density of the grazing
invertebrates (e.g. *Delatidium* spp.) which leads to an increase in the biomass of algae (Pace *et al*., 1999). In contrast, soil food webs rarely (if ever) display such strong interactions between trophic levels. This has been explained by the high diversity of soil food webs (Strong, 1992), their high level of functional redundancy (Setala, 2002), the high levels of omnivory (Abrams, 1993) and finally the heterogeneity that exists within trophic levels (Mikola and Setala, 1998b; Mikola and Setala, 1998a).

The high level of soil species richness is associated with a degree of functional redundancy. Consequently, the soil fauna may compensate for predation by a higher-level trophic organism on any one mid-level group. High levels of omnivory relate not only to the traditional meaning of feeding on both plant and animal material, but also to the fact that many soil organisms feed at multiple trophic levels (Moran and Scheidler, 2002). This means that simple cause and affect relationships are very difficult to predict and possibly do not even exist in the field.

Soil faunal heterogeneity contrasts with trophic dynamic theory which states that within a trophic level, the fauna acts in a similar manner (homogeneity). Mikola and Setala (1998b) utilized microcosms to examine the existence of trophic cascades in simplified food webs. They set up three different food chains; the first corresponding to one trophic level consisted of 10 species of bacteria and 10 species of fungi. The second (two trophic levels) consisted of 10 species of bacteria and fungi plus a bacterial and a fungal feeding nematode. The third (three trophic levels) consisted of 10 species of bacteria and fungi plus a bacterial and a fungal feeding nematode.
and a predatory nematode. Trophic dynamic theory predicts that in this simplified system, microbial biomass would increase when predators are added, since they regulate the microbial feeding nematodes. The authors found that soil microbial populations, for example, did not act in a homogenous manner and that there were differing responses by bacteria and fungi to increased rates of nematode grazing. Specifically, they noted that fungal biomass was most abundant in the presence of grazers, but did not vary significantly when the food chain was increased from two to three. They also reported that the density of bacteria was not affected by food chain length. Clearly, neither bacteria nor fungi responded to changes in the length of the food chain in the way predicted by trophic-dynamic theory.

Other researchers (Dawes-Gromadzki, 2002; Gutierrez et al., 1994; Moran and Scheidler, 2002; Scheu and Schaefer, 1998) have studied the impact of bottom-up controls on soil fauna. Scheu and Schaefer (1998) studied the impact of manipulating food resources on the soil macrofauna in a beachwood (Fagus sylvatica L.) forest in Germany. They manipulated levels of carbon, nitrogen and phosphorus in an attempt to manipulate the microbial biomass in litter and soil. They proposed that variations in the microbial component of the diet of saprophagous soil animals would allow them to study the impact of bottom-up forces on soil and litter faunal trophic levels. They observed that the application of glucose and other nutrients did result in an increase in the levels of soil and litter microbial biomass. However, none of the animal groups sampled (Lumbricidae, Isopoda, Diplopod, Chilopoda) responded to the increase in microbial biomass. The authors did note that certain saprophagous macrofauna taxa where controlled by the
availability of resources, thereby supporting the view that bottom-up forces do
structure decomposer communities.

Trophic dynamic theory has done much to further our understanding of the manner
in which food webs can be structured and how variations in resource availability and
predatory pressure permeate through the food web. The continued publication of
contradictory results, particularly concerning the presence or absence of trophic
cascades, shows that trophic dynamic theory as it relates to the below ground
ecosystem, needs to be both refined and progressed.

### 1.3.2 Below-ground Dual Energy Pathways

The idea that the soil microbial population can be divided into two components
(bacteria and fungi) and that these two components are responsible for fast cycling
(bacterial pathway) and slow cycling (fungal pathway) of nutrients is relatively new.
Strong field experimental evidence for this line of thinking can be found in a paper
by Beare *et al.*, (1992), although the formalization of the theory was more fully
presented by Bardgett *et al.*, (1996; 1998). Both studies focused on agricultural
settings. One compared conventional and no-tillage systems (Beare *et al.*, 1992),
while the other examined the impact of varying grazing pressure in grassland
(Bardgett *et al.*, 1996).

Beare *et al.*, (1992) conducted a very complex field exclusion study to examine the
food webs of agroecosystems. In particular, they tested the hypotheses that
conventional tillage agroecosystems contain bacterial-based food webs that increase litter decomposition and nutrient mineralisation (fast cycle), while no tillage agroecosystems contain fungal-based food webs that encourage slower litter decomposition and greater nutrient retention (slow cycle). Their research built on earlier field experimental work by Hendrix et al., (1986) and Holland and Coleman (1987), who developed the model which Beare et al., (1992) tested.

Beare et al., (1992) conducted a litter-bag study. They stratified litter-bags by placing them on the surface and burying others approximately 10 cm below-ground. Aqueous biocides or water was applied via a watering can. Sampling included both soil flora and fauna. Bacteria and fungi were measured using biovolumes, while Nematoda, Acari and Collembola were assigned to functional groups based on previous research at the same site.

They concluded that in general the rates of litter decomposition and nitrogen dynamics can be explained by differences in the composition of the decomposer communities and their trophic interactions (Beare et al., 1992). In conventional tillage agroecosystems, bacteria were more influential than fungi with regard to buried litter decomposition and N dynamics. Furthermore, the population structure of bacterial-feeding nematodes was linked to their bacterial food source. In non-tilled agroecosystems, fungi exerted a greater impact than bacteria on the rate of litter decomposition and the retention of N by immobilizing it within their hyphae. Additionally the populations of fungal feeding microarthropods were linked to the growth and activity of the saprophytic fungi. They concluded that the placement and
availability of litter (as the primary resource for bacteria and fungi) was responsible for the structuring of the decomposer community and the resulting trophic interactions. This can be viewed as a form of ‘bottom-up’ resource control of community structure.

Bardgett et al., (1996) studied the impact of withholding fertilizer, lime and sheep-grazing on the ratio of soil fungi to bacteria biomass in reseeded grasslands. Respiration was measured using the substrate-induced respiration (SIR) method, while biomass of both bacteria and fungi was calculated using phospholipid fatty acid analysis (PFLA). The cessation of fertilization resulted in a decline in soil pH from 5.4 to 5.1. The cessation of fertilizer and liming saw a further drop to 4.7 while the cessation of fertilizer, liming and grazing reduced soil pH to 4.5 (Bardgett et al., 1996). There was a strong correlation between the bacterial fatty acid (18:2 omega 6) and the ratio of fungal to bacterial substrate-induced respiration. They concluded that the fungal biomass was greater in non-grazed soils relative to grazed soils.

They postulated that heavy grazing favours a ‘fast cycle’ dominated by high levels of mineralization mediated by bacteria, while lightly grazed to non-grazed soils support a predominantly ‘slow cycle’ of decomposition and nutrient flow mediated by fungi and characterized by high levels of immobilization.

Yeates et al., (1997a) proposed that a measure of the success of conversion from intensive grassland to organic grassland farming could be the degree of shift towards a soil biotic community dominated by fungi and fungal feeding fauna. This
statement based on the results of grassland field experimental from Wales is supported by the conclusion that ‘a key feature of natural ecosystems is a soil community that is dominated by fungal pathways of decomposition’ (Bardgett and McAlister, 1999; Bardgett et al., 1998; Smith et al., 2003).

Field based research suggests that fungi and fungal feeding organisms dominate natural below-ground systems. Fungal pathways of decomposition are associated with slow, highly conserved cycling of nutrients. Bacteria and bacterial-feeding organisms tend to dominate in disturbed soil systems. Bacterial pathways of decomposition are associated with rapid cycling of nutrients and have an increased potential for nutrient leaching. This research project will attempt to infer bacterial/fungal pathways based on the relative density of the soil mesofaunal functional groups.

1.4 IMPACT OF E. GLOBULUS PLANTATIONS ON SOIL COMMUNITIES

We can view natural bushland and pasture as two extremes on a gradient of disturbance. Natural bushland with complex vegetation existing in multiple stories and consisting of numerous species represents the pristine end of the scale, while pasture with its simple vegetation structure of a few and frequently only one species, with no vertical stratification, representing the degraded end of the scale. The development of an E. globulus plantation on pastureland (as was the case in the Albany Effluent Irrigated Tree Farm) can be viewed as a small movement back towards the natural bushland end of the scale.
The revegetation of pasture with *E. globulus* results in a more complex system than the previous land use system and one that only begins to approach the complexity and stability of the natural bushland system. Some of the changes include the development of a litter layer, and with time the formation of an over-storey canopy that limits the amount of direct light and rain reaching the soil.

The development of a litter layer results in an insulating cover above the soil that limits fluctuations in soil moisture and temperature. Following litter fall, decomposition results in the formation of humus and the release of nutrients into the soil. The existence of the canopy results in a protective ‘interception’ layer that limits the amount of direct sunlight reaching the soil surface (and so reduces fluctuations in soil temperature (Swift et al., 1979)) and also limits the amount of direct rainfall (and so reduces the erosive forces of rain (Luce, 1997)).

Adams and Attiwill, (1986a) noted that the slow rate of decomposition under eucalypts was a feature of the genus in studies based in south-eastern Australia. They point out that this results in the storage of significant amounts of nutrients in the litter. Research conducted in the United Kingdom (Briones and Ineson, 1996) examined the rate of decomposition and nutrient loss from leaf litter of *E. globulus*, *Q. petraea* (oak), *F. excelsior* (ash) and *B. pendula* (birch). They report that *E. globulus* decomposed (measured as mass loss) as rapidly as *B. pendula* (birch), a species considered to exhibit fast rates of decomposition. They also concluded that mixed litter, while difficult to predict, tends to decompose faster than pure litter
samples (Smith and Bradford, 2003), an observation supported by Hansen (1999) and Hansen and Coleman (1998) in a similar study. This observation was disputed by Wardle et al., (2002), who conducted litter mixing experiments in the laboratory and found that there was a negative correlation between the decomposition rate of a litter and the decomposition rate of the same litter when mixed with other litters.

Adolphson (2000) described the soil and litter mite communities in pasture, *E. globulus* plantation and native forest sites in Collie, in the South West of Western Australia. While the study is pseudoreplicative in design and therefore necessarily limited in statistical analysis, the author makes a number of useful observations. She observed that the oribatid mite fauna were absent from pasture samples, present in *E. globulus* samples and most abundant in native bushland. Acarine density in the *E. globulus* plantation sites were consistently higher than in the native forest sites, while pasture sites exhibited extreme variation, depending on season. In spring, density values in pasture sites were significantly less than those in the plantation and native forest sites while in autumn, density in pasture sites was significantly higher than the other two sites. The variation in density in pasture sites was directly related to the density of the astigmatid mite *Tyrophagus* sp.

An analysis of the species composition at each site for both soil and litter fauna reveals that, irrespective of season, species richness was highest in the native forest, reduced (approximately 50%) in *E. globulus* plantations, and further reduced (approximately 50%) in pasture. Clearly, the soil acarine community under *E.*
*globulus* plantation in this study represents a stage on the disturbance gradient between pasture and native bushland and exhibited characteristics of both.

### 1.5 Wastewater Sources, Treatment and Disposal

Wastewater is defined as “superfluous water, or water that has served its purpose, allowed to run away” (Hawkins, 1982). Wastewater is produced by a variety of sources including municipal (sewage treatment plants), agriculture (dairy and piggery), and industry (fruit processing, winery and mining) (Cameron *et al.*, 1997; Tillman and Surapaneni, 2002). Wastewater is typically treated in some form prior to disposal. For example, treatment of municipal effluent aims to remove solids, pathogens and other contaminants and so produces a wastewater that is relatively clean and suitable for recycling back into the environment (Cameron *et al.*, 1997).

Disposal of municipal wastewater has traditionally involved pumping treated effluent into a nearby waterway. Cameron *et al.*, (1997) reported that 60% of all sewage in New Zealand was discharged into coastal waters following secondary treatment. Hope (1996), quoted in Sarooshi *et al.*, (2002), notes that historically most sewage from towns along the New South Wales coast including Sydney, has been primary treated and disposed of in the ocean. The discharge of treated effluent into local waterways has typically resulted in a reduction of dissolved oxygen levels (Tillman and Surapaneni, 2002), an increase in salinity and nutrient enrichment leading to eutrophication (Balks *et al.*, 1998; Bond, 1998; Cameron *et al.*, 1997; Falkiner and
Polglase, 1999; Myers et al., 1998; Myers et al., 1996; Smith and Bond, 1999; Snow et al., 1999).

Over the past 20 years, there has been an increase in the level of awareness of the detrimental impacts of traditional aquatic wastewater disposal. Studies of the Murray-Darling River system in New South Wales and nutrient loads within the river clearly suggest that the nutrient load in wastewater additions to the river has been a significant contributor to the problem of algal blooms (Tillman and Surapaneni, 2002).

Studies of the Albany harbours in the South West of Western Australia, including Princess Royal Harbour, have linked elevated nutrient levels to the decline in sea grass meadows, eutrophication and an increase in marine algae (EPA, 1990). Disposal of primary treated effluent by the Water Corporation into the adjacent King George Sound was identified as a significant contributor to the elevated levels of marine nutrients (Silifant, 1997).

As an awareness of the negative effects of traditional methods of wastewater disposal has grown, so too has the pressure to develop long-term sustainable alternatives (Tillman and Surapaneni, 2002). The most common alternative to traditional aquatic disposal is a land-based system. The principal aim behind land-based treatment systems is to assimilate the waste component using the biological, physical and chemical properties of the land system (Cameron et al., 1997). To be sustainable, such systems must not adversely affect the structure of the soil or
cause contaminants to be passed into the ground water or atmosphere (Cameron et al., 1997).

Land based treatment systems for municipal effluent offer a number of advantages over aquatic based systems. In many environments, particularly within Australia, water is a limiting resource. As such, the water component of effluent is a potentially valuable commodity (Tillman and Surapaneni, 2002). It can be recycled and reused for a variety of uses including as an irrigation source for crops or timber. Additionally, land based effluent disposal systems can utilise the nutrient composition of the effluent to enhance the biomass of the irrigated plantation.

While effluent quality varies not only seasonally but also according to its source, municipal effluent typically has elevated levels of N, P and K (Bond, 1998). While these nutrients contribute to eutrophication in aquatic systems (Cameron et al., 1997; Heckrath et al., 1995; Tillman and Surapaneni, 2002), the nutrient deficient quality of Australian soils and the ‘fertilizer’ like qualities of effluent are seemingly well matched.

Land based disposal of effluent presents its own unique set of complications. The significant levels of Na+ and other dissolved salts can adversely affect both salinity and soil sodicity (Balks et al., 1998; Tillman and Surapaneni, 2002). The typically basic pH of effluent (Cameron et al., 1997) can impact negatively on levels of soil organic C (Degens et al., 2000; Tillman and Surapaneni, 2002). The application of soluble N in a variety of forms (NH₄⁺ and NO₃⁻) creates the potential for N leaching
and subsequent contamination of below-ground aquifers (Bond, 1998; Cameron et al., 1997; Smith and Bond, 1999; Snow et al., 1999; Tillman and Surapaneni, 2002; Zaman et al., 1999).

An increasingly popular solution to the disposal of effluent is its utilization for irrigation of tree plantations. Such systems offer a solution to the environmental problem of traditional wastewater disposal and provide an opportunity to generate additional wood resources for an economic return. One such system is the Albany Effluent Irrigated Tree Farm, which is the experimental site for this research. An obvious trend amongst published research on land-based treatment systems for effluent is that few (if any) distinguish between the application of additional nutrients and the application of additional water. Consequently it is impossible to attribute variation between irrigated and control sites to either the increased availability of moisture or the increase availability of nutrients of to the interaction between the two.

1.5.1 Mains-Water Irrigation Effects on Soil and Litter Fauna

di Castri et al., (1981) reported that soil moisture levels were possibly the most significant determinant of soil and litter faunal community structure. Studies that have examined the impact of irrigation have focused on simulating long-term climate change in order to describe the impact on soil and litter faunal communities. Typically, such studies have tended to focus on the impact of drought in preference to increased irrigation, reflecting the long-term prediction for less rainfall in most of
the world’s temperate regions (Frampton et al., 2000; Hulme, 2005; Lindberg et al., 2002; Taylor et al., 2004).

The two major published studies of the impact of experimental irrigation and drought on soil fauna have both been conducted in Europe, in Sweden (Lindberg et al., 2002) and in Britain (Frampton et al., 2000). Despite significant variation in the methods used to collect and extract fauna and the degree of taxonomic resolution between the two papers, the results are remarkably similar. Both studies found that both drought and irrigation (water only) caused significant variation in the soil faunal community structure. Functional group analysis (Frampton et al., 2000) revealed that predators, herbivores, omnivores and mycophages were consistently more abundant under irrigation.

An in-depth Swedish taxonomic study observed that drought decreased and irrigation increased the density of Collembola, Oribatida, enchytraeids, mesostigmatid mites and macroarthropod predators (Lindberg et al., 2002). This study also found that oribatid species diversity increased under irrigation and decreased following drought. An analysis of the species diversity (Shannon-Wiener) of the oribatid fauna resulted in Irrigation (2.35) > Control (1.99) > Drought (1.33). These differences were all statistically significant ($F=51.76$, $P<0.001$). Species richness, however, was not significantly different between Irrigation and the Control but was significantly different between Drought and the Control and Drought and Irrigation (Lindberg et al., 2002). Each of the three treatments developed different Oribatid assemblages. Of the forty-five oribatids identified, seven existed only in the
irrigated plots. These were *Hypochthonius rufulus*, *Phthiracarus cf. piger*,
*Euphthiracarus cribarius*, *Microtritia minima*, *Platynothrus peltifer*, *Trimalaconothrus vietsi*, and *Nanhermannia coronata* (Lindberg *et al.*, 2002). Collembolan diversity displayed no significant treatment effects.

An English study examined the impact of artificially manipulated drought and irrigation on above-ground farmland arthropods (Collembola, Araneae, Coleoptera, Diptera, Hemiptera and Hymenoptera) over a short-term period (50-100 days) (Frampton *et al.*, 2000). This study reported that the effects of drought on the density of herbivores, mycophages, omnivores and predators were negative, while those of irrigation were positive. Species richness of all these functional groups was greatest in the irrigated plots, and least in the drought plots (Frampton *et al.*, 2000). There were substantial limitations in this research project because of its lack of replication over time, the short-term nature of the study and because the authors excluded the Acari from their samples. It must, however, be noted that the ability to artificially simulate enhance or reduced rainfall by shielding or irrigating large areas of soil at will requires significant engineering.

It is thought that changes in soil moisture levels may have resulted in an altered fungal species composition that in turn affected the density and diversity of the fungivores (Lindberg *et al.*, 2002). It was hypothesised that irrigated plots may have lower levels of microhabitat diversity due to the constant conditions provided by the irrigation treatment. The stable and predictable environment produced by irrigation may have allowed species to specialize and so result in increased diversity.
(Lindberg *et al.*, 2002). Clearly much more research is required to determine if increases in diversity are linked more to microhabitat heterogeneity or to environmental stability and species specialisation.

### 1.5.2 Effluent Irrigation

The nutrient quality of wastewater varies not only with time but also according to its source (Cameron *et al.*, 1997; Tillman and Surapaneni, 2002). Published research to date (November, 2003) has focused on the impact of N, C, P and Na, with a particular emphasis on soil chemistry. I have found only one recent study that has examined the impact of wastewater nutrients on soil and litter fauna, that of Yeates (1995). In the following pages, I briefly examine the impact of wastewater irrigation on soil structure and chemistry and then review the research dealing with wastewater irrigation and soil and litter fauna.

#### 1.5.2.1 Effluent Irrigation and Soil Chemistry

Applications of sewage effluent to soil can result in significant increases in soil N levels (Bernal *et al.*, 1998; Falkiner and Smith, 1997; Smith and Bond, 1999). Polglase *et al.*, (1995) reported that the amount of N cycled in pine plantations irrigated with sewage effluent may be unusually large due to the regular additions of water and soluble nutrients in wastewater. They note that the water component of effluent accelerates the growth of trees and the uptake of N, turnover of soil N (Zaman *et al.*, 1999), denitrification and volatilisation. They caution that if effluent is
applied at a rate in excess of a plantation’s capacity to assimilate the water, nutrient leaching may occur.

Degens et al., (2000) examined the impact of long-term (22 years) dairy effluent irrigation on levels of soil organic C in grass pasture systems. They point out that a loss of organic C can detrimentally impact on soil structure, nutrient retention, water-holding capacity, and nutrient cycling. They examined organic C levels in the top 75 cm of soil and found that C storage had not changed after 22 years of dairy effluent application in a grass pasture system. They did find that C distribution had been affected, with C being redistributed from higher parts of the soil profile to lower parts (Degens et al., 2000).

Phosphorus applied to soil in effluent irrigated plantations can move through the soil profile by leaching vertically through the soil profile and horizontally via sub-surface and surface lateral movement (Falkiner and Polglase, 1999; Heckrath et al., 1995; Redding et al., 2002). Falkiner et al., (1999) report that P application rates in effluent irrigated plantations are typically between 50-100 kg P/ha/yr, while accumulation of P into plant biomass is between 5-15 kg P/ha/yr. In general, the ability of most Australian and New Zealand soils to retain P within their profile is sufficient to consider P leaching as a low risk (Cameron et al., 1997).

Falkiner et al., (1999) examined the fate of P applied to *Pinus radiata* and *Eucalyptus grandis* plantations in eastern Australia (Wagga Wagga). They reported that one half of the inorganic P in the top 0.5 m of the soil profile did not engage in P
cycling and was resistant to leaching. They also reported that P was unlikely to constrain the longevity of effluent irrigated plantations relative to the leaching of salt, N and the impacts of sodicity (Falkiner and Polglase, 1999).

The salts contained in wastewater can have an important detrimental effect on the soil (Tillman and Surapaneni, 2002). Salinity is a measure of the total salt concentration in either water or soil. Commonly occurring salts in wastewater include chlorides, carbonates, bicarbonates and sulfates of Na, Ca, Mg and K (Jayawardane et al., 2001; Tillman and Surapaneni, 2002). Salts will tend to accumulate near the top of the soil profile when insufficient water is applied to facilitate the leaching of salt through the soil profile (Myers et al., 1999; Tillman and Surapaneni, 2002). Elevated salt levels impact on soil osmotic water potential and so decrease the amount of water available to plants (Cameron et al., 1997). With the exception of halophytes, most plants are relatively intolerant of elevated levels of salt (Tillman and Surapaneni, 2002).

Sodicity differs from salinity in that it is a measure of the Na concentration relative to the concentration of others ions (e.g. Ca and Mg). When sodicity increases it results in a reduction in the stability of the soil aggregates (Cameron et al., 1997). Elevated levels of Na also negatively impact on the dispersion rate of soil (Balks et al., 1998; Halliwell et al., 2001).
1.5.2.2   Effluent Irrigation and Soil and Litter Fauna

Possibly the only published study within the last decade to examine the effect of effluent irrigation on soil and litter fauna was published by Yeates (1995). He studied the soil and litter fauna under a pine plantation (Pinus radiata) that had been established on sand dunes in Waitarere, North Island, New Zealand. Spray irrigation of domestic sewage was initiated 10 years after the establishment of the plantation. Sampling occurred 7 years after the commencement of effluent irrigation, in May 1992 and March 1993.

Yeates (1995) found that the increased moisture content was responsible for increases in the populations of soil earthworms and soil nematodes in effluent treated areas. He also found that there was a significant increase in predatory and bacterial feeding nematodes and a significant decrease in fungal feeding nematodes. None of the litter faunal groups was positively affected by effluent irrigation, while the density of three groups of litter fauna, including aphids, spiders, and adult diptera, were negatively affected. Significant limitations of this study include the low degree of sample replication (n = 5, per sample date), the lack of taxonomic resolution (typically to order) and the simplicity of the extraction technique.

Dindal et al., (1975) published a brief overview of their findings of the impact of municipal sewage effluent on soil invertebrates. They studied the impact in four different field trials, including reed canary grass monoculture (Phalaris arundinacea)
L.), a 70 year old oak hardwood (dominated by *Quercus alba*), a ten year old stand of red pine (*Pinus resinosa* Ait.) and in fields of newly planted white spruce (*Picea glauca*). Microbial respiration was measured by the evolution of CO₂. Earthworms were dug up and hand sorted, while microarthropods were extracted using Tullgren funnels over a period of 7 days (Dindal, 1977; Dindal *et al.*, 1975). Overall they found no significant variation in soil respiration values with treatment. They found that wastewater irrigation generally increased the population of annelids, whereas acarine density and species diversity (Shannon Wiener - H') were suppressed by wastewater irrigation. Collembolan density displayed both positive and negative responses to irrigation while species diversity decreased (Dindal *et al.*, 1975).

Dindal (1977) published more results from this study two years later. In this paper, he concentrated on the species level responses of oribatid mites to wastewater irrigation. He observed that in spring the number of oribatid species was reduced from 21 to 8 because of irrigation with wastewater. In spring only two mites, *Quadroppia* sp., and *Nothrus silvestris*, exhibited a positive response to wastewater irrigation. Interestingly, Dindal notes that neither of these two species was found in the control sites. He comments, that in general, the species present in the control sites were selected against, with most disappearing in samples irrigated with wastewater. In autumn oribatid, species richness declined from 22 to 10 species. Overall, the impact of wastewater irrigation was to cause a significant reduction in species richness.
We know very little about the impact of effluent irrigation on the soil and litter fauna. Considering the critical links between biota and ecosystem processes, if we are to accurately model the sustainability of alternative wastewater disposal systems, we must develop a greater understanding of the impacts of wastewater irrigation on soil and litter fauna.

1.5.2.3  *Effluent Irrigation and Litter Decomposition*

Baker *et al.*, (1990) examined the impact of spray irrigation with secondary treated domestic sewage effluent on the decomposition of litter in a *Pinus radiata* forest in Waitangi Forest in the North Island of New Zealand. They observed that effluent irrigation resulted in reduced organic matter within the litter, increased mass loss and increased levels of N, P, Ca and Mg in litter. They also observed that there was no significant impact on the levels of soil organic matter. Effluent irrigation increased the rate of decomposition measured as the rate of organic matter loss from litter-bags.

They hypothesized that effluent irrigation increased the rate of litter decomposition because of the elevated levels of moisture and nutrient availability. Increased levels of moisture and nutrients leads to enhanced microbial activity that is supported by increased levels of effluent derived carbon, that in turn affects the decomposer population which results in an increase in the rate of litter decomposition.
Guo and Sims (2001; 2002) studied the impact of meatworks effluent on litter decomposition and nutrient release under stands of one, two and three year old *E. globulus* plantations in Dannevirke, New Zealand. They found that effluent irrigation increased the rate of litter decomposition in 1-year-old stands, made no significant difference in 2-year-old stands and retarded growth in 3-year-old stands. The authors hypothesized that nutrient and moisture additions from effluent may increase soil microbial activity but that this activity may slow soon after canopy closure occurs (usually observed in two – three year old stands) and hence explains the variation in rates of litter decomposition. They also hypothesized that canopy closure may impact on soil temperature.

In an earlier study, Guo and Sims (2000) reported that light, temperature and effluent irrigation all influenced litter decomposition in controlled environments. They found that litter irrigated with effluent and exposed to light decomposed significantly faster than litter irrigated with water, however there was no significant difference between water and effluent irrigation treatments when the litter was shaded.

Based on these studies it is reasonable to hypothesise that irrigation with mains water and treated effluent is likely to cause changes to the soil and litter biotic composition and to alter the soil and litter physicochemical properties. Specific hypotheses now follow.
1.6 RESEARCH HYPOTHESES

1. Application of effluent and mains-water irrigation will result in increased levels of soil moisture relative to the non-irrigated control sites. This will be particularly evident during summer when irrigated levels are expected to be maximal and rainfall levels minimal.

2. Due to the alkaline pH of the effluent, soils irrigated with effluent will be more alkaline than non-irrigated control, and mains-water irrigated soils.

3. Soil microbial biomass will increase in effluent and mains-water irrigated soil supporting the trend reported by Barkle et al., (2000).

4. Based on the observation of Yeates, (1995) soil nematode populations will be dominated by bactivorous and predatory species in effluent irrigated soils.

5. Soil acarine species richness will be negatively affected by irrigation following the trend reported by Dindal et al., (1975).

6. Soil oribatid mites will be particularly sensitive to irrigation, supporting previous reports that Oribatid mites are particularly sensitive to perturbations (Behan-Pelletier, 1999; Dindal et al., 1975; Franchini and Rockett, 1996).

7. Effluent and mains-water irrigation is expected to enhance the rate of litter decomposition supporting the trend reported by Baker et al., (1990).
"If enough data is collected, anything may be proven by statistical methods."

Unknown
2.0 Site Description and Experimental design

2.1 LOCATION

The city of Albany is located approximately 400 km southeast of Perth on the southern coast of Western Australia (Fig 2.1). It has a population of approximately 25,000 and is a major center for the surrounding agricultural, tourist and marine industries. In 1992, because of increasing eutrophication of its harbors, the Albany Effluent Irrigated Tree Farm was developed as a land based system for the disposal of nutrient loads in municipal effluent wastewater. The experimental sites for this project are located within the Albany Effluent Irrigated Tree Farm (34.57°S, 117.48°E). The Albany Effluent Irrigated Tree Farm is managed by the Water Corporation of Western Australia, and is situated on 550 ha of land approximately 10 km north of Albany (Figure 2.1) and 68 m above sea level.

2.2 CLIMATE

The Bureau of Meteorology operates a weather reporting station at the Albany Airport that is directly opposite the Albany Effluent Irrigated Tree Farm and less then five km from the experimental sites. The Water Corporation also records weather information at the Albany Effluent Irrigated Tree Farm. All long-term climatic data has been provided by the Bureau of Meteorology, whereas the Water Corporation of Western Australia has provided all specific week-to-week climatic data relating to the study period.
Figure 2.1. Map of Australia and the Albany district (Western Australia) detailing the position of the Albany Effluent Irrigated Tree Farm.
Albany experiences a Mediterranean type climate with dry, warm summers and wet, cool winters. Annual rainfall varies between 600 and 950 mm with the bulk of it falling between May and October (Figure 2.2). Temperatures range from $15^\circ$ - $23^\circ$C in January and from $8^\circ$ - $16^\circ$C in July, and temperatures never went below $2^\circ$C for the duration of the study period. The mean annual number of rain days is 178.2, and mean annual rainfall is 934.3 mm (Bureau of Meteorology, 2002).

Soil sampling at the research site occurred 3 times in March 2000, October 2000, and February 2001. Rainfall in January 2000 was significantly higher than average, while rainfall in February 2000 was slightly higher than average. Rainfall in March 2000 was more than double the monthly average. Rainfall in October 2000 was less than half the monthly average, and followed reduced rainfall in both August 2000 and September 2000. Rainfall in February 2001 was less than the average monthly rainfall and followed a slightly less than average December 2000 and January 2001 (Fig. 2.2).

2.3 SITE HISTORY

Prior to its development as the Albany Effluent Irrigated Tree Farm, the land had been used for a variety of agricultural activities. Annual pastures of clover (Trifolium sp.) and ryegrass (Lolium sp.) were grazed by cattle for beef and by sheep for both wool and meat production (Kinhill Engineers, 1992a). Meadow hay was cut from most of the upland regions, despite being waterlogged for most of winter each year.
Figure 2.2. Long-term rainfall trend, actual rainfall and mean maximum temperature at the Albany Effluent Irrigated Tree Farm for the period January 2000 to June 2002. Data sourced from the Australian Bureau of Meteorology and the Water Corporation of Western Australia.
There was also a large piggery (2,500 pigs) on site. Effluent from the piggery was disposed of in two large evaporation ponds (Kinhill Engineers, 1992a).

The holding ponds that form part of the treatment system for effluent at the Albany Effluent Irrigated Tree Farm have been developed from these two evaporation ponds. The Public Environmental Review (Kinhill Engineers, 1992a) noted that past disposal of effluent from the piggery was probably contributing nutrients into Seven Mile Creek. The Albany Effluent Irrigated Tree Farm is situated at the head of the Seven Mile Creek catchment (Eade, 2001).

Purchasing of the land by the Water Corporation was conducted during 1992 and 1993. The development of the site began in 1993, with 222 ha of *Eucalyptus globulus* planted by 1994. Irrigation with effluent began in March of 1995 (Silifant, 1997). The Water Corporation planted 52 ha of *E. globulus* trees in 1993, 170 ha in 1994 and 35 ha in 1996 (Silifant, 1997). Approximately one hundred ha of *E. globulus* trees were planted in 1995. In 1996, two further areas, one of 30 ha and another of 16 ha, were planted. By mid-2000, additional irrigation was installed and commissioned which increased the available irrigation area to 300 ha (Eade, 2001). The remaining 150 ha of *E. globulus* act as a buffer zone, to intercept effluent runoff.

### 2.4 Site Design

Effluent from the Albany municipality is collected and pumped to the Timewell Road Treatment Plant. Following traditional primary and secondary treating, the effluent is
then pumped to the Land Treatment Site and stored in two holding ponds (Silifant, 1997). From here the treated effluent is pumped to the overland flow area, referred to as an ‘intermittently loaded specialized wetland’ (Silifant, 1997). A schematic representation of the site can be found in Figure 2.3.

The overland flow area consists of 14 ha of pasture that is divided into two groups of 17 bays. The individual bays are approximately 40 m long with slopes ranging from $1^\circ$ to $4^\circ$. Effluent pumped from the holding ponds to the top of the overland flow bays, runs down the gradient into the main dam. The purpose of the overland flow bays is to reduce the level of nitrogen in the effluent. The nitrogen component of the effluent is reduced by more than 50% of its initial level by a combination of microbial activity, volatilization and the sequestration of nitrogen into plant biomass (Silifant, 1997).

The main irrigation dam, which has a capacity of 365,000 m$^3$ (Silifant, 1997), is the principal storage facility on site, and represents 90 days of storage at the average daily flow rate (Eade, 2001). The dam is used as a storage facility during the winter months when it is too wet to irrigate.

Effluent from the dam is pumped through a bank of sand filters that remove all particulate matter down to 120 μm (Silifant, 1997). After filtration the effluent is pumped about the Land Treatment Site to some 300 hectares of *E. globulus*. The effluent is distributed via black flexible piping with built in drip emitters.
Figure 2.3 Schematic representation of the Albany Effluent Treatment system including the Albany Effluent Tree Farm (from Sillifant, 1997).
The design of the plantation required that non-irrigated *E. globulus* trees surround effluent irrigated *E. globulus* trees. This ensured that non-irrigated trees were strategically placed to intercept any effluent run-off and prevent it leaving the Albany Effluent Irrigated Tree Farm. The goal of the Albany Effluent Irrigated Tree Farm is to ensure that nutrients remaining in the effluent after wetland stripping are incorporated into the soil and/or plant biomass. Irrigation flow rates are controlled to maintain plantation soil at or near field capacity. This means that effluent flow rates vary inversely with rainfall, being highest in summer months and minimal or zero in winter months. Harvesting of the plantation for woodchips once the trees have reached maturity is hoped to make the Albany Effluent Irrigated Tree Farm economically viable in the long term.

### 2.5 Effluent Description

Effluent variables are measured in the main dam prior to distribution across the site. The connection of industrial effluent, and the conversion of septic tanks to the main sewer system, along with growth in the Albany City, has resulted in increased N levels in the main dam over time (Eade, 2001). Selected chemical characteristics of the effluent and a comparison with drinking water from the Albany region can be found in the next chapter.
2.6 **Experimental Design**

In 1996, six replicate experimental sites were set up within the Albany Effluent Irrigated Tree Farm. Three sites were set up within *E. globulus* planted in 1993 and 3 within *E. globulus* planted in 1994. These sites were maintained from 1996 until early 1999 by the Ecosystem Research Group (ERG) headed by Associate Professor Mark Adams of the University of Western Australia (Silifant, 1997). Three of the 6 sites were re-commissioned late in 1999 for this research project (Figure 2.4). The 3 replicate experimental sites used for this research project are approximately 1.5 km apart within trees planted in 1994. Within each experimental site, rows of 6 year-old *E. globulus* trees have been subjected to one of three treatments (Table 2.2).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment A</td>
<td>Non-Irrigated Control</td>
</tr>
<tr>
<td>Treatment B</td>
<td>Effluent irrigated to field capacity</td>
</tr>
<tr>
<td>Treatment C</td>
<td>Mains-Water irrigated at equivalent rates to treatment B</td>
</tr>
</tbody>
</table>

Trees subject to treatments B (effluent irrigated) and C (mains-water irrigated) were planted in double rows or “tramlines” (Plate 2.1) with 1 m between trees within a tramline and 5 m between each set of tramlines. Trees were planted 2 m apart along the length of each row. Row length is variable across the site. The non-
Figure 2.4 Position of replicate transects at each sampling site. Transects 1, 2 & 3 = Non-Irrigated Control Treatment, 4, 5 & 6 = Effluent Irrigated Treatment and 7, 8 & 9 = Mains-Water Irrigated.
irrigated trees (treatment A) were planted in single rows, 2 m apart down the rows and with 5 m spacings between rows. No irrigation system was installed below these trees (Plate 2.2). Treatments B and C were applied to 3 sets of double rows of trees. Trees subject to treatment B and C were separated by 9 double rows of buffer trees to minimize any possible edge effects and cross contamination from one treatment regime to another.

At the time of this study, trees subject to treatment B and C were approximately 12 meters in height with closed canopies with filtered sunlight only reaching the soil surface. Trees from control sites (treatment A) were of a similar height but the site had open canopies. (This is due to the difference in planting design Plate 2.3.)

Plate 2.1. ‘Tramline’ planting design of trees irrigated with effluent or mains water.
Plate 2.2. Trees from the non-irrigated control sites, displaying single row planting design.

Plate 2.3. *E. globulus* planted in 1994 in a non-irrigated control site, approximately 12 m in height.
Weed infestations at the sites were mainly composed of a variety of grasses (*Avena barbarta, Avena sativa*, and *Dichelachne* sp.)) During winter, there is an increase in the presence of the noxious inkweed, *Phytolacca octandra*. However, this quickly dies off towards the end of spring / beginning of summer.

### 2.7 Soils at the Site

Soils at the Albany Effluent Irrigated Tree Farm have a duplex profile, with a grey white to grey yellow sandy topsoil overlying a structured medium clay subsoil. A lateritic duracrust of 300 – 600 mm lies above the clay. Topsoils are acidic (pH 4.0 – 5.0), with moderate levels of organic carbon (3-6%) (Kinhill Engineers, 1992a). A ferruginous pan occurs over much of the site at a varying depth of between 0.1 – 0.7m (Kinhill Engineers, 1992a). Prior to planting of *E. globulus*, the surface was ripped to a depth of 1 m and the resulting overburden mounded for seedlings (Silifant, 1997).

### 2.8 Sampling Design and Common Methods

This section details sampling design and all the methods common to the soil faunal groups. Specific information regarding variations from the normal sampling regime is detailed in the appropriate chapter. The statistical design is a nested or hierarchical ANOVA (Figure 2.5). Samples (*n = 10*) were nested within unique transects (*n = 9*) that were nested within each treatment (*n = 3*, Non-Irrigated
Control, Effluent Irrigated and Mains-Water Irrigated). Sampling was conducted in three seasons (Autumn, Spring and Summer) over 2000 and 2001.

![Figure 2.5 Schematic representations of both the experimental site design and the experimental statistical design. The statistical design clearly shows the nested / hierarchical design of this project and highlights the unique independence of each transect](image)

### 2.8.1 Soil Sampling

Soil samples were collected over a 12-month period in 2000 and 2001. Autumn samples were collected in March 2000, spring samples in October 2000, and summer samples in February 2001. Winter samples were not collected because
sampling was designed to coincide with expected peaks in faunal density and because of the rationalisation between sampling effort and the associated ‘bench time’ required to process the resulting samples (limited resources and time).

Samples were collected from between randomly chosen trees, and equidistant between trees to standardise sampling and to ensure that a soil core could be inserted and extracted. Soil was taken from the top 10cm and placed in zip lock plastic bags. Since collecting took more than one day to carry out, samples were stored on ice overnight and transported back to the laboratory the following day.

On each of the 3 sampling occasions (March 2000, October 2000, and February 2001) each of the replicate experimental sites (n=3) was sampled. Ten soil samples were taken from each transect (n=9). There were replicate transects per treatment (n=3) and multiple treatments per season (n=3). Ninety soil cores were taken per sampling occasion/season (10 samples x 3 replicate transects x 3 treatments).

2.8.2 Carbon and Nitrogen Analysis

Soil from the Albany Effluent Irrigated Tree Farm was analysed for total N, total C, and pH. These specific physicochemical properties were chosen to specifically compliment the more detailed physicochemical data that was being collected concurrently by the Water Corporation and the University of Western Australia. Total N and C were analysed simultaneously on an Automated Nitrogen and Carbon Auto-Analyzer - Gas Solid Liquid (ANCA-GSL) at Edith Cowan University. Crushed,
homogeneous soil samples (2 mg) were placed into tin preparation units. Following combustion that converted the sample to gas, the sample was passed through a mass spectrophotometer and the percentage N and C of the sample was calculated. Using the initial dry weight of the sample, the total N and C per sample was calculated.

2.8.3 Percentage Soil Moisture Content

Soil moisture levels were calculated by drying 20g of field moist soil at 105°C for 8 hours. Soil dry weights were compared to original field weights and converted to a percentage (Forster, 1995b).

2.8.4 Soil Particle Analysis

Soil particle analysis was conducted on soil from the dried soil cores following extraction of soil fauna. Oven dried soil was passed through a standard set of soil sieves in order to determine the percentage contribution of each particle size (McDonald et al., 1990).

2.8.5 Soil pH

Soil pH was measured using the method of Foster (1995a). Ten grams of air dried soil was mixed with 25 mL of distilled water ($n = 90$ per sampling occasion). After stirring for one minute the solution was allowed to stand for 1 hour, than following a brief second stirring soil pH was measured using a CyberScan 500.
2.8.6 Faunal Identification

Soil and litter fauna was sorted and identified to the lowest possible taxon using an Olympus SZH10 dissecting microscope (10-70x) and a compound Olympus BX-50 (40-1,000x) microscope with Nomarksi attachment. Specimens were cleared on cavity slides in 50% lactic acid on slide-warming trays (Wright, 1988). Permanent mounts of Acari and Collembola were developed using Hoyers solution (Krantz, 1978). Once dried at 45°C, slides were ringed with Glyceel to prevent desiccation (Krantz, 1978).

All acarine fauna were separated and identified to species. Individuals that could not be identified to species were identified as morphospecies and assigned a unique identification number. The oribatid mite fauna was identified to Family and Genera where possible using the keys of Balogh et al., (1988; 1990), Balogh et al., (1983) and Norton (1990) and specialised taxonomic literature where available. The Prostigmata were identified using Keithly (1990), the Astigmata using Philips (1990) while Mesostigmata were identified using Krantz et al., (1990) and Evans and Till (1979).

2.8.7 Statistical Analysis

The sampling design is a replicated nested or hierarchical ANOVA with samples nested within transects nested within treatments. SPSS (v11) was used to
determine the significance of differences in mean population densities and diversity between treatments and sampling occasions. Densities were log transformed to ensure normality and homogeneity of variance prior to analysis. Post Hoc multiple comparison tests were conducted using the Bonferroni’s test in SPSS (v11).

The software package PRIMER (Plymouth Routines In Multivariate Ecological Research) v5 (Clarke and Gorley, 2001) was used for the classification and ordination of the species-by-samples data set and for the calculation of diversity estimates (Shannon Wiener - H') and evenness values (Peilou’s evenness - J').

Classification (hierarchical agglomerative clustering) and ordination (non-metric multi-dimensional scaling) of the data were based on transformed total densities of faunal species. Specific information regarding transformation procedures and other relevant information pertaining to classification and ordination analysis is provided in each appropriate chapter.

The PRIMER (v 5.0) statistical package was used to conduct an analysis of similarity (termed ANOSIM). ANOSIM computes a test statistic that reflects the observed differences in similarities between sites and contrasts them with differences in similarities among the replicates within sites (Clarke and Warwick, 1994). The analysis of similarity (ANOSIM) is based on the corresponding rank similarities between samples in the data set calculated in a similarity matrix (Clarke and Warwick, 1994). This similarity matrix is the same matrix used to develop the hierarchical agglomerative cluster analysis and MDS ordination analysis presented.
earlier. ANOSIM generates an R statistics that is scaled to lie between –1 and +1. A value of zero represents no difference between a set of samples. In ANOSIM differences between groups (pair-wise R) are scaled between 0 (indistinguishable) and 1 (all similarities within groups are less than any similarity between groups). Following the recommendation of the PRIMER manual I interpreted pair-wise R values >0.75 as well separated; R>0.5 as overlapping, but clearly different and R<0.25 as barely separable at all (Clarke and Warwick, 1994).
“We know more about the movement of celestial bodies
then about the soil underfoot”

Leonardo da Vinci
3.0 Site History and Soil Physicochemical Properties

3.1 INTRODUCTION

This chapter describes the site history and soil physicochemical properties of the Albany Effluent Irrigated Tree Farm. It begins with a summary of the site history and the potential ecological consequences of the site’s perturbation regime, followed by the soil physicochemical analyses conducted as part of this research study and concludes with a synthesis of the research carried out by the University of Western Australia in parallel with this study that focused on nutrient cycling. While it is unusual to begin a results section with a discussion chapter that draws on three sources of information (historical data, my own research and simultaneous complementary research), it provides an informative backdrop for the soil biology that follows it.

Relevant hypotheses for this section are:

1. Application of effluent and mains-water irrigation will result in increased levels of soil moisture relative to the non-irrigated control sites. This will be particularly evident during summer when irrigated levels are expected to be maximal and rainfall levels minimal.

2. Due to the alkaline pH of the effluent, soils irrigated with effluent will be more alkaline than non-irrigated control, and mains-water irrigated soils.
3.2 SITE HISTORY

The site of the Albany Effluent Irrigated Tree Farm was cleared of native bushland more than fifty years ago. After the removal of the native bushland, grasses suitable for pasture (Trifolium sp., Lolium sp. and meadow hay) were cultivated and non-native fauna including cattle, sheep and pigs were farmed (Kinhill Engineers, 1992b; Kinhill Engineers, 1992a). Prior to the planting of *E. globulus* in 1993 and 1994, the soil was prone to regular seasonal (winter) flooding. In preparation for planting with seedlings, the soil was ripped to a depth of one meter and mounded (Silifant, 1997). Following the establishment of the monospecific *E. globulus* plantation, irrigation with effluent and mains-water commenced in 1995 (Silifant, 1997).

This sequence of events from native bushland, to pasture, to deep ripped soil and mono-specific plantation irrigated with treated effluent or mains-water represents a progression of perturbations that has structured the microbial and faunal communities described in the following chapters. It is useful to consider the ecological impacts of each of these perturbations because they have the potential to be strong structuring forces of the biological communities described in the following chapters.

The conversion of native bushland into pasture results in the loss of a dense and diverse vegetation structure, that in turn results in extreme fluctuations in levels of soil moisture and temperature (Tian *et al.*, 1997). Fluctuations in soil humidity and temperature are considered to be the most important climatological factors affecting
the density and distribution of soil Acari and so would be expected to have a
negative impact on the diversity of the soil acarine population (Adolphson, 2000; di

In addition, the conversion of bushland to pasture results in a loss in the diversity
and volume of the litter layer (Tian et al., 1997) that results in significant changes in
soil chemistry, which are most frequently observed as a decrease in both soil C and
N (Murty et al., 2002). Other researchers have observed that a reduction in the
quality and quantity of the leaf litter can result in a depauperate soil and litter
decomposer community (Blair et al., 1990; Paoletti and Bressan, 1996; Paoletti et
al., 1991; Paoletti et al., 1992; Wall and Moore, 1999; Watt et al., 2002).
Immediately prior to site preparation and planting with *E. globulus*, the soil
mesofaunal communities were likely to have been depauperate.

To prepare the soil for planting with *E. globulus* seedlings, ripping was carried out to
a depth of one meter. Ripping was conducted to facilitate enhanced water
drainage and to promote deeper root growth of the *E. globulus* seedlings (Silifant,
1997). Soil ripping breaks down the compacted layers of soil and so enhances the
rate of water infiltration, thereby reducing levels of water runoff and flow
concentrations (Luce, 1997; Rokich et al., 2000). Increased water infiltration and
enhanced vegetation growth (as a result of ripping) combine to form a more stable
soil profile that protects against topsoil erosion (Luce, 1997). In the first instance,
ripping results in the loss of the vertical stratification that exists in soil and, secondly
surface organic matter is incorporated into the below-ground soil profile (Mueller et al., 1990; Perdue and Crossley, 1990; Schrader and Lingnau, 1997).

The structure of the soil profile is an important determinant of soil micro- and mesofaunal distribution (Perdue and Crossley, 1989; Perdue and Crossley, 1990; Roper and Gupta, 1995). In undisturbed ecosystems, soil mite distributions are vertically stratified, with highest densities in the top five centimetres. Up to 75% of the Acari and Collembola are found in the top four centimetres of the soil (Holt, 1985). It is thought that the availability of small soil pores in this region affects the distribution of the mesofauna and particularly the small oribatid mite (<125 μm) (Holt, 1985). It is also thought that the micro soil pores provide protection from predators (Holt, 1985). These suggestions, coupled with the greater availability of these soil pores in the uppermost region of the soil profile, have been suggested as an explanation for the disproportionate density of soil mesofauna in this part of the soil profile (Holt, 1985). In tilled soil, soil mites display a very even vertical distribution over the top 25 centimetres, which is unlike the distinction observed in undisturbed soil (Perdue and Crossley, 1989). Tillage, like deep ripping, appears to result in a loss of the soil’s vertical stratification by the destruction of small soil pores and the incorporation organic matter directly into the soil profile (Perdue and Crossley, 1990). Microbial biomass concentration displays a similar distribution response, with microbial biomass concentrated in the top 5 centimetres of the soil profile in undisturbed soils and more evenly distributed throughout the soil profile in tilled systems (Roper and Gupta, 1995). Following the establishment of the *E. globulus* plantation, the soil micro and mesofauna would be expected to display a very even
vertical distribution, reflecting the destruction of the vertical profile of the soil as a result of deep ripping.

In summary, at the time of *E. globulus* planting, soil at the Albany Effluent Irrigated Tree Farm would be characterised as highly disturbed, with the following four characteristics;

1. Reduced levels of C and N (due to deforestation).
2. Fluctuating levels of soil temperature and moisture (due to limited litter and canopy layer) that would be expected to have negatively impacted on the diversity of the soil mesofaunal populations.
3. A highly disturbed soil profile with no vertical stratification (due to deep ripping). This activity would have reduced an already depauperate soil faunal community and selected for species adapted to perturbation and against species adapted to a stable, well-developed soil profile.
4. Organic matter incorporated deep into the soil profile (due to deep ripping) that is expected to result in a more even distribution of the soil fauna throughout the soil profile.

This research project took place 6 years after the establishment of the *E. globulus* plantation. During those 6 years, the growth and maturation of the plantation would be expected to have a significant impact on the soil profile and fauna. As the trees grew and developed a canopy, this would have resulted in a moderation of the soil temperature and moisture. In addition, the development of the tree canopy would
have resulted in an initially small but increasing litter layer. The development of the litter layer would have further ameliorated the impact of sunlight and rain on soil temperature and moisture and resulted in a more stable environment, thereby creating a resource and a habitat in which litter fauna could exist. This would also have facilitated the development of the litter decomposition process. The growth and maturation of the *E. globulus* root system would have stabilised the soil profile and slowly developed the stratification of the soil. The extent to which the growing *E. globulus* plantation has improved the soil profile, litter layer and habitat for the soil and litter micro and mesofauna is as yet unknown.

3.2.1 Soil Moisture and Irrigation (Effluent and Mains-Water)

Irrigation at the Albany Effluent Irrigated Tree Farm is the primary mechanism for effluent disposal for the city of Albany. The hydrological design of the Land Treatment Site aims to keep soil at or near the soils water-holding capacity (Kinhill Engineers, 1992b). Irrigation rates are negatively correlated with rainfall and so periods of low rainfall are associated with high rates of irrigation and vice versa. Levels of soil moisture were calculated using the method outlined in section 2.8.3. Soil samples were collected as outlined in section 2.8.1 and collected at the same time as samples for faunal extraction. Forty-five soil samples were analysed for percentage soil moisture each sampling occasion.
The Mediterranean climate of Albany results in an annual wet (May – July) and dry (December – February) cycle (Fig 2.2) with highest mean temperatures observed in summer (dry) and lowest mean temperatures in winter (wet). Irrigation uncouples soil moisture from climate resulting in relatively consistent levels of soil moisture in irrigated sites. It is reasonable to expect that the impact of irrigation on levels of soil moisture and faunal community structure will be most pronounced in summer when the long term association of highest temperatures and lowest levels of soil moisture exhibited by the non-irrigated control site are replaced with high levels of soil moisture in irrigated (effluent and mains-water) sites. Evidence of this impact can be seen in Table 3.1. The two irrigated sites (effluent and mains-watered) always displayed higher levels of soil moisture than the non-irrigated control sites. Further, the level of soil moisture in both irrigated plots varied little over the three sampling periods in contrast to the cyclical nature of soil moisture levels in the non-irrigated control sites.

Table 3.1. Soil moisture content at the Albany Effluent Irrigated Tree Farm under three treatment regimes: non-irrigated control, effluent irrigated, and mains-water irrigated.

<table>
<thead>
<tr>
<th>Season</th>
<th>Site</th>
<th>% Soil H₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autumn 2000</td>
<td>Non-Irrigated Control</td>
<td>20.1 ± 2.80</td>
</tr>
<tr>
<td></td>
<td>Effluent Irrigated</td>
<td>33.5 ± 5.15</td>
</tr>
<tr>
<td></td>
<td>Mains-Water Irrigated</td>
<td>28.9 ± 6.42</td>
</tr>
<tr>
<td>Spring 2000</td>
<td>Non-Irrigated Control</td>
<td>12.3 ± 3.21</td>
</tr>
<tr>
<td></td>
<td>Effluent Irrigated</td>
<td>30.2 ± 4.89</td>
</tr>
<tr>
<td></td>
<td>Mains-Water Irrigated</td>
<td>34.2 ± 5.67</td>
</tr>
<tr>
<td>Summer 2001</td>
<td>Non-Irrigated Control</td>
<td>5.5 ± 1.37</td>
</tr>
<tr>
<td></td>
<td>Effluent Irrigated</td>
<td>38.6 ± 6.41</td>
</tr>
<tr>
<td></td>
<td>Mains-Water Irrigated</td>
<td>32.1 ± 5.84</td>
</tr>
</tbody>
</table>

(Values are means ± 1 SE, n = 45 per sampling occasion)
Soil moisture varied significantly due to treatment within each season. The non-irrigated control site was significantly drier (as expected) than the two irrigated sites in each season ($F(2,6)=7.285$, $p<0.05$). There was no significant difference between irrigated (effluent and mains-watered) sites in any season ($F(2,6)=0.495$, $p=0.632$). These findings confirm the research conducted by the University of Western Australia that reported monthly gravimetric analysis of soil moisture 10 centimetres below the surface displayed no seasonal variation in effluent irrigated soils with moisture content ranging from $>30\%\;v/v$ for each month, except February which dropped to $20\%\;v/v$ (Adams et al., 2001). Soil moisture in the non-irrigated control sites displayed strong seasonal variation with less than $10\%\;v/v$ content in summer months and approximately $20\%\;v/v$ water content in winter months (Adams et al., 2001).

Levels of soil moisture observed in the autumn non-irrigated control samples were higher than expected, given the average long-term rainfall for this month (38.7mm). Sampling occurred over two days on March 8th and 9th (2000). In the week of March 4th to 10th (2000) a total of 65.8 mm of rain fell. Clearly, this significant rainstorm event has resulted in the high levels of soil moisture observed in this sampling period.

In summary, the soil moisture levels of the non-irrigated control sites at the Albany Effluent Irrigated Tree Farm displayed distinct seasonal variation, consistent with a Mediterranean pattern of warm dry summers and a cool wet winters, while the two
irrigated treatment sites consistently display elevated levels of soil moisture (relative to the non-irrigated control sites) independent of rainfall.

Another factor for consideration is the quality of the irrigation water. Table 3.2 details the major chemical properties of effluent sampled from the main storage dam.

Previous studies by the Water Corporation have shown that there is no significant difference in effluent quality measured at the point of irrigation and in the main storage dam (Silifant, 1997) so these values can be considered indicative of the effluent quality exiting the irrigation system at the base of the *E. globulus* trees. The table also includes chemical properties for drinking water sampled in Albany and Australian National Health guidelines for safe levels of water contaminants. This

Table 3.2. Chemical characteristics of irrigation water at the Albany Effluent Irrigated Tree Farm.

<table>
<thead>
<tr>
<th></th>
<th>Effluent Irrigation</th>
<th>Mains-water³</th>
<th>Drinking Water ²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1997¹</td>
<td>1999¹</td>
<td>2000¹</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total nitrogen</td>
<td>19</td>
<td>36</td>
<td>29</td>
</tr>
<tr>
<td>Total Kjeldahl nitrogen</td>
<td>13</td>
<td>32</td>
<td>27</td>
</tr>
<tr>
<td>Ammonia as N</td>
<td>13</td>
<td>28</td>
<td>23</td>
</tr>
<tr>
<td>Nitrate + nitrite as N</td>
<td>6.4</td>
<td>4.2</td>
<td>8.1</td>
</tr>
<tr>
<td>Total phosphorus</td>
<td>2.9</td>
<td>7.9</td>
<td>1.4</td>
</tr>
<tr>
<td>Calcium Carbonate</td>
<td>-</td>
<td>227</td>
<td>224</td>
</tr>
<tr>
<td>Magnesium</td>
<td>12</td>
<td>13</td>
<td>13</td>
</tr>
</tbody>
</table>

data relating to the chemical composition of Albany’s drinking water is particularly important, as it is indicative of the water quality used in the mains-water irrigated treatment. Because the two water types (effluent and drinking water) are used for differing purposes, slightly different chemical properties are measured for each.

Overall, pH was very similar between mains-water and effluent-water, while almost all other chemical characteristics vary substantially between the two water types. Total nitrogen, ammonia, nitrate and nitrite in the effluent were substantially higher than mains-water or the maximum safe levels set out in the Australian Health Guidelines. Levels of phosphorus in the effluent were highly variable from year to year but were low relative to the levels of total nitrogen. Little is known about safe levels of phosphorus in effluent or drinking water, as there are no health guidelines at a state or national level (Anonymous, 2004). The World Health Organisation does not publish guidelines of acceptable levels of phosphorus in drinking water (Anonymous, 1993). The high level of calcium (measured as CaCO$_3$) in mains-water at Albany is characteristic of the source supplying this locality. The majority of Albany’s drinking water is drawn from underground aquifers surrounded by significant limestone formations.

In summary, irrigation has two effects on soil at the Albany Effluent Irrigated Tree Farm. In the first instance effluent and mains-water irrigation were responsible for sustained elevated levels of soil moisture while, in the second instance, effluent irrigation results in the application of high levels of soluble nitrogen in a form readily available for biological uptake and variable levels of additional phosphorus.
3.3 Soil Physicochemical Properties at the Albany Effluent Irrigated Tree Farm

3.3.1 Soil Chemistry

Soil physicochemical properties were calculated using the method outlined in section 2.8.2 and 2.8.5. Soil samples were collected as outlined in section 2.8.1 and collected at the same time as samples for faunal extraction. Forty-five soil samples were analysed for %TC and %TN each sampling occasion while ninety soil samples were analysed for soil pH each sampling occasion. Table 3.3 presents mean values for soil pH, and % TC and TN. Treatment had no statistically significant impact on soil pH ($F_{(2,6)} = 3.549, p=0.096$), which varied little across all sampled soils.

Percentage TC did not vary significantly due to treatment ($F_{(2,6)} = 2.126, p=0.200$) and levels of % TC are high reflecting the significant quantities of CaCO$_3$ in the area. Previously published values for levels of organic C in the Albany area are very low and range from 0.17 to 2.5% (McArthur, 1991). Percentage TN varied significantly by season ($F_{(2,6)} = 38.203, p<0.001$).

Post hoc analysis revealed a significant decline in percentage total N from autumn to spring ($p<0.05$) to summer ($p<0.001$). However it is doubtful whether a 0.1% variation in percentage total N represents a biologically meaningful result.
Table 3.3. Soil physicochemical characteristics at the Albany Effluent Irrigated Tree Farm under three treatment regimes: non-irrigated control, effluent irrigated, and mains-water irrigated.

<table>
<thead>
<tr>
<th>Season</th>
<th>Site</th>
<th>pH</th>
<th>Total C (%)</th>
<th>Total N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autumn 2000</td>
<td>Non-Irrigated Control</td>
<td>4.5 ± 0.09</td>
<td>5.2 ± 0.83</td>
<td>0.35 ± 0.005</td>
</tr>
<tr>
<td></td>
<td>Effluent Irrigated</td>
<td>4.4 ± 0.15</td>
<td>4.4 ± 0.73</td>
<td>0.35 ± 0.007</td>
</tr>
<tr>
<td></td>
<td>Mains-Water Irrigated</td>
<td>4.4 ± 0.15</td>
<td>3.6 ± 0.61</td>
<td>0.35 ± 0.014</td>
</tr>
<tr>
<td>Spring 2000</td>
<td>Non-Irrigated Control</td>
<td>4.2 ± 0.10</td>
<td>3.2 ± 0.67</td>
<td>0.34 ± 0.040</td>
</tr>
<tr>
<td></td>
<td>Effluent Irrigated</td>
<td>4.2 ± 0.19</td>
<td>4.7 ± 0.12</td>
<td>0.33 ± 0.036</td>
</tr>
<tr>
<td></td>
<td>Mains-Water Irrigated</td>
<td>4.4 ± 0.11</td>
<td>5.9 ± 1.95</td>
<td>0.34 ± 0.051</td>
</tr>
<tr>
<td>Summer 2001</td>
<td>Non-Irrigated Control</td>
<td>4.4 ± 0.06</td>
<td>5.5 ± 0.45</td>
<td>0.33 ± 0.005</td>
</tr>
<tr>
<td></td>
<td>Effluent Irrigated</td>
<td>4.4 ± 0.15</td>
<td>7.6 ± 3.10</td>
<td>0.33 ± 0.009</td>
</tr>
<tr>
<td></td>
<td>Mains-Water Irrigated</td>
<td>4.7 ± 0.10</td>
<td>5.5 ± 0.58</td>
<td>0.33 ± 0.059</td>
</tr>
</tbody>
</table>

(Values are means ± 1 SE, n = 90 for pH and n = 45 for % C and N per sampling occasion)

3.3.2 Soil Particle Size Analysis

Soil from the replicate sites at the Albany Effluent Irrigated Tree Farm were similar in particle structure and displayed no statistically significant difference ($F_{(2,6)} = 2.407$, $p=0.171$; Table 3.4). Soils were dominated by particles greater than 2 mm (gravel) and particles between 1 mm and 0.05 mm (fine sand). This data confirms previous work carried out for the Water Corporation of Western Australia, that observed topsoil at the Albany Effluent Irrigated Tree Farm was predominately sandy in composition (Kinhill Engineers, 1992b).
Table 3.4. Soil particle size analysis at each experimental site subjected to three treatment regimes: non-irrigated control, effluent irrigated, and mains-water irrigated.

<table>
<thead>
<tr>
<th>Replicate Site</th>
<th>Treatment</th>
<th>Gravel (&gt; 2 mm)</th>
<th>Coarse Sand (&lt; 2 mm &amp; &gt; 1 mm)</th>
<th>Fine Sand (&lt; 1 mm &amp; &gt; 0.05 mm)</th>
<th>Silt (&lt; 0.05 mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 1</td>
<td>Non-Irrigated Control</td>
<td>33.6 ± 1.60</td>
<td>14.7 ± 1.31</td>
<td>51.1 ± 2.94</td>
<td>0.6 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>Effluent Irrigated</td>
<td>50.1 ± 2.45</td>
<td>13.2 ± 1.37</td>
<td>36.3 ± 2.20</td>
<td>0.4 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>Mains-Water Irrigated</td>
<td>50.7 ± 2.71</td>
<td>12.9 ± 1.37</td>
<td>35.5 ± 8.70</td>
<td>0.9 ± 0.05</td>
</tr>
<tr>
<td>Site 2</td>
<td>Non-Irrigated Control</td>
<td>15.2 ± 3.81</td>
<td>19.4 ± 3.12</td>
<td>64.5 ± 6.24</td>
<td>0.9 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>Effluent Irrigated</td>
<td>9.7 ± 4.65</td>
<td>17.5 ± 0.83</td>
<td>72.3 ± 3.71</td>
<td>0.6 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>Mains-Water Irrigated</td>
<td>17.9 ± 2.47</td>
<td>14.7 ± 3.38</td>
<td>67.1 ± 16.9</td>
<td>0.2 ± 0.19</td>
</tr>
<tr>
<td>Site 3</td>
<td>Non-Irrigated Control</td>
<td>9.4 ± 5.75</td>
<td>6.75 ± 1.27</td>
<td>83.7 ± 0.79</td>
<td>0.1 ± 0.31</td>
</tr>
<tr>
<td></td>
<td>Effluent Irrigated</td>
<td>48.7 ± 10.65</td>
<td>10.7 ± 2.81</td>
<td>40.0 ± 15.4</td>
<td>0.5 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>Mains-Water Irrigated</td>
<td>17.2 ± 6.49</td>
<td>11.3 ± 0.31</td>
<td>71.0 ± 5.35</td>
<td>0.6 ± 0.08</td>
</tr>
</tbody>
</table>

(Values are means ± 1 SE, n = 5 per treatment per replicate site)

3.4 NITROGEN CYCLING AT THE ALBANY EFFLUENT IRRIGATED TREE FARM

Central to the success of the Albany Effluent Irrigated Tree Farm is the complete immobilisation or assimilation of nitrogen inputs from both effluent and litter into the site plant and microbial biomass. Nitrogen is the primary nutrient of interest to the Water Corporation because it is the major chemical component of the effluent (c.f. previous discussion on effluent quality) and because of the reporting requirements set down by the Environmental Protection Authority.

Figure 3.1 is a synthesis of data extracted from a report titled “Improved Management of the Albany Effluent Irrigation Tree Farm – 2001” (Adams et al., 2001). This report focused on water, N and P budgets and covered C:N ratios and
Nitrogen uptake by plants (23,600 kg) is almost equal to the amount of nitrogen applied to the site via effluent irrigation (24,000 kg). Litter fall adds a total of 8,500 kg of N into the system. A small component of the excess N applied to the soil at this site is fixed within the soil matrix, while 1,300 kg leaches through the soil profile.
presumably into the below-ground water system. Denitrification results in the loss of 2,200 kg of N into the atmosphere. The impact of volatilisation was not quantified (although it is presumed to be small) and a total of 5,400 kg of N could not be accounted for. The amount of nitrogen mineralised by the soil fauna is equal to the amount of nitrogen inputs into the system via both effluent irrigation and litter fall.

Based on these data, nitrogen inputs into the Albany Effluent Irrigated Tree Farm from effluent irrigation approximated the amount of nitrogen being sequestered by the *E. globulus* plantation. Adams *et al.*, (2001) concluded that, of the 8500 kg of nitrogen being added to the soil system in 1998, only a small amount of this was being bound within the soil profile (367 kg). A further 1,300 kg was leaching through the soil profile into the ground water while 2,200 kg was being denitrified. In addition, a total of 5,400 kg of nitrogen could not be accounted for. On a percentage basis, this unaccounted nitrogen represents more than 16% of the total nitrogen additions into the nitrogen cycle at the Albany Effluent Irrigated Tree Farm.

In summary, the bulk of the nitrogen in irrigated effluent approximately equates to the amount incorporated into *E. globulus* biomass. However, the model cannot account for a sizeable portion of the nitrogen. As expected from research hypothesis one irrigated soils did display elevated levels of soil moisture but contrary to expectation effluent irrigation did not have any affect on levels of soil pH (research hypothesis 2.)
In the following biological chapters, I will describe the response of the microbes and mesofauna to the addition of irrigated effluent. Of particular interest initially will be the response of the microbial populations to the application of soluble nitrogen, since previous research has shown that microbes are able to both mineralise and immobilise available nitrogen, depending on the availability of other nutrients such as carbon and phosphorus (Barkle et al., 2001; Hogberg et al., 2003; Shen et al., 1984).

The following biological chapters must be read in the context of this discussion of the soil environment at the Albany Effluent Irrigated Tree Farm. Key points to remember throughout the following chapters include:

1. The soil profile has been highly perturbed over a significant period and displayed little vertical stratification at the time of *E. globulus* plantation.
2. Soils at the Albany Effluent Irrigated Tree Farm are acidic with relatively low levels of organic C and TN.
3. Irrigation results in two major physicochemical affects
   a. Soil moisture in irrigated soils is independent of rainfall
   b. Effluent irrigation results in the application of readily available soluble nitrogen and phosphorus.
4. *E. globulus* trees are sequestering large quantities of nitrogen within their biomass, yet significant amounts of nitrogen are unaccounted for in the nitrogen model.
“It is sometimes as dangerous to be run into by a microbe as by a trolley car”

J.J Walsh
4.0 Soil Microbes

4.1 INTRODUCTION

In this chapter, I describe selected soil microbial characteristics under *E. globulus* plantations at the Albany Effluent Irrigated Tree Farm and their response to effluent and mains water irrigation. The baseline data from non-irrigated control sites provides a reference point against which the results from irrigated soil are compared. Soil microbial populations are characterised by biomass (a measure of total microbial population size including both active and inactive microbes) and respiration (a measure of microbial activity). I explore statistically the impact of both season and treatment on these microbial parameters. The aims of this part of the chapter are to describe:

a) baseline soil microbial biomass data;

b) baseline soil microbial respiration data;

c) soil microbial biomass and respiration data in irrigated (effluent and mains-water) sites; and

d) the impact of season and treatment on soil microbial biomass and respiration.

Research hypothesis 3. Soil microbial biomass will increase in effluent and mains-water irrigated soil supporting the trend reported by Barkle *et al.*, (2000).
4.2 Methods

Soil samples were collected using the method detailed in section 2.8.1 (Sampling Design and Common Methods – Soil Sampling). Once collected soil, samples were mixed and then used for calculating microbial biomass or respiration.

4.2.1 Microbial Biomass

Soil microbes are defined as the part of organic matter in soil that constitutes living microorganisms smaller than 5-10 μm³ (Alef and Nannipieri, 1995; Lawrence, 1995). Microbial biomass was calculated from the release of ninhydrin-positive compounds following fumigation of soil with chloroform. The method used was based on that of Amato and Ladd (1988) with modifications by Joergensen and Brookes (1990) and Sparling et al., (1993).

Following fumigation of soil with chloroform, ninhydrin-positive compounds collect in the soil, mainly as alpha-amino acids, as soil enzymes react with N-compounds released from lysed cells (Amato and Ladd, 1988; Amato and Ladd, 1994). Ninhydrin forms a purple complex with molecules containing alpha-amino nitrogen, ammonium and other compounds with free alpha-amino groups (Alef and Nannipieri, 1995).
The ninhydrin-positive compounds (NPC) were extracted into K$_2$SO$_4$. The liquid solution was then analysed on a Shimadzu UV-Vis Spectrophotometer at 570 nm ($n = 90$, per sampling occasion). Microbial biomass was then estimated using the multiplier calculation developed by Amato and Ladd (1988) (Biomass C (μg g$^{-1}$) = NPC flush x 21).

### 4.2.2 Microbial Respiration

Microbial respiration is an indication of microbial activity and was measured in the laboratory using the method of Anderson (1982). Fifty grams of soil was placed in a 100 mL beaker inside a 1L screw top jar ($n = 90$ per sampling occasion). Ten mL of KOH was added to a second beaker inside the 1L jar. As CO$_2$ was respired by the soil microbes it was absorbed into the KOH solution and reacted to form HCO$_3^-$ and CO$_3^{2-}$ (Anderson, 1982).

The amount of CO$_2$ absorbed by the KOH was calculated by titrating the remaining alkali against a standard acid (0.1 M HCl). Titrations were carried out using a 716 DMS Titrino Metrohm autotitrator. The rate of respiration was then calculated by dividing the respired CO$_2$ by the incubation period (Anderson, 1982).

### 4.2.3 Statistical Analysis

Statistical analysis was carried out using the method detailed in section 2.8.6. (Sampling Design and Common Methods – Statistical Analysis). Two-way nested
ANOVA was used to determine if there were seasonal or treatment effects on soil microbial biomass or respiration. Where appropriate, Bonferroni’s post hoc analysis was used to determine significant differences between treatments.

4.3 RESULTS

4.3.1 Microbial Biomass

4.3.1.1 Seasonal Responses

A statistically significant seasonal effect (Figure 4.1 and Table 4.1) was the approximately double levels of microbial biomass in the non-irrigated control sites in autumn samples ($F_{(2,6)}=3.989$, $p=0.079$). Microbial biomass in autumn was significantly higher than levels of microbial biomass observed in both spring ($p<<0.05$) and summer ($p<0.001$), however spring and summer were not significantly different ($p=0.061$).

<table>
<thead>
<tr>
<th></th>
<th>Autumn</th>
<th>Spring</th>
<th>Summer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Irrigated Control</td>
<td>1002 ± 68.5</td>
<td>657 ± 63.8</td>
<td>489 ± 96.6</td>
</tr>
<tr>
<td>Effluent Irrigated</td>
<td>589 ± 47.8</td>
<td>519 ± 60.7</td>
<td>558 ± 50.9</td>
</tr>
<tr>
<td>Mains-Water Irrigated</td>
<td>556 ± 66.6</td>
<td>571 ± 48.3</td>
<td>306 ± 26.8</td>
</tr>
</tbody>
</table>

(Values are means ± 1 SE, $n = 90$ per sampling occasion)

Microbial biomass in soil irrigated with effluent ($F_{(2,6)} = 0.480$, $p=0.640$) and mains-water ($F_{(2,6)} = 2.218$, $p=0.190$) were insensitive to season.
Figure 4.1. Mean microbial biomass (μg microbial C g⁻¹ soil) under three treatments sampled in autumn, spring and summer (values are means ± 1 SE, n = 90 per sampling occasion).

In this thesis, I have chosen to present relevant data both in graphical and table form. While it is convention to present data in one or other I have presented it in both forms because frequently the trend in the data (most easily demonstrated with a figure) is as important and the actual values (most easily read from a table). This thesis presents important baseline data from a poorly studied region of the world. To that end, both the values and the trends are of importance. While the data sets could have been placed within an Appendix, this proved cumbersome and resulted in the reader frequently turning large numbers of pages to verify statements made with the text.
4.3.1.2 Treatment Responses

A statistically significant treatment effect was the suppressive effect of irrigation on microbial biomass in the autumn irrigated soil \((F(2,6) = 6.042, \ p<0.05)\). Microbial biomass in autumn control samples was significantly higher than levels of microbial biomass observed in both effluent irrigated samples \((p<0.01)\) and mains-water irrigated samples \((p<0.01)\) however effluent and main-water irrigated samples were not significantly different \((p=0.352)\).

4.3.2 Microbial Respiration

4.3.2.1 Seasonal Responses

All three treatments displayed similar seasonal trends with highest levels of microbial respiration in autumn and summer and lowest levels in spring (Figure 4.2 and Table 4.2). Mean soil microbial respiration \((\mu g \ CO_2 \ g^{-1} \ 24hr^{-1})\) varied season \((F(2,6) = 4.023, \ p=0.078)\) with spring significantly lower than autumn \((p<<0.01)\) and summer \((p<<0.01)\). The same pattern of seasonal variation was observed in the effluent irrigated plots with spring significantly lower than both autumn \((p<<0.01)\) and summer \((p<<0.01)\).
Figure 4.2. Mean soil microbial respiration (μg CO₂ g⁻¹ soil 24 hr⁻¹) under three treatments sampled in autumn, spring and summer (values are means ± SE, n = 90 per sampling occasion).

Table 4.2. Mean soil microbial respiration (μg CO₂ g⁻¹ soil 24 hr⁻¹) under three treatments samples in autumn, spring and summer (values are means ± SE, n = 90 per sampling occasion).

<table>
<thead>
<tr>
<th></th>
<th>Autumn</th>
<th>Spring</th>
<th>Summer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Irrigated Control</td>
<td>10.7 ± 0.86</td>
<td>4.8 ± 0.64</td>
<td>6.5 ± 0.77</td>
</tr>
<tr>
<td>Effluent Irrigated</td>
<td>10.3 ± 0.67</td>
<td>5.9 ± 0.64</td>
<td>9.8 ± 1.67</td>
</tr>
<tr>
<td>Mains-Water Irrigated</td>
<td>11.1 ± 0.72</td>
<td>6.8 ± 0.91</td>
<td>9.8 ± 0.81</td>
</tr>
</tbody>
</table>

A similar trend was observed in the mains-water irrigated plots with spring significantly lower than autumn (p<<0.01), however spring was not significantly different from autumn (p=0.376).
4.3.2.2 Treatment Responses

There were no statistically significant treatment effects on soil microbial respiration.
Autumn ($F_{(2, 6)} = 0.710, \ p = 0.527$), spring ($F_{(2, 6)} = 0.007, \ p = 0.993$), and summer
($F_{(2, 6)} = 0.360, \ p = 0.712$).

4.4 Summary of Results

The significant outcomes from this section are:

1. Irrigation (both effluent and mains-water) resulted in relatively stable levels of
   microbial biomass that were lower than levels of microbial biomass observed
   in the non-irrigated control samples.

2. Levels of microbial respiration were insensitive to irrigation but displayed a
   consistent pattern of seasonal variation irrespective of treatment, with
   maxima in autumn, summer, and minima in spring.

In summary, while levels of microbial biomass were sensitive to irrigation (i.e. stable
levels of microbial biomass irrespective of season), they were insensitive to the
quality of the irrigation type leading to the rejection of research hypothesis three.

The high nitrogen load of the effluent did not result in any statistically significant
differences in levels of microbial biomass compared to irrigation with mains-water.
Levels of microbial respiration displayed no sensitivity to irrigation quality or
irrigation itself and microbial respiration varied consistently with season.
4.5 DISCUSSION

4.5.1 Microbial Biomass

4.5.1.1 Levels of Microbial Biomass

Traditionally, assessments of soil microbial biomass have been made using direct microscopy or a variation on the culture media technique. Both of these methods are tedious and time consuming and are routinely described as unreliable (Dalal, 1998). In the last 25 years microbial biomass has been estimated using biochemical and physiological techniques including chloroform fumigation incubation (Jenkinson and Powlson, 1976), chloroform fumigation extraction (Brookes et al., 1985; Vance et al., 1987) and substrate-induced respiration (Anderson and Domsch, 1978).

Sparling and Zhu (1993) have published some preliminary information on microbial biomass and respiration for 24 different sites (including Albany) within south-west Australia. I have utilised the same methods as these authors for calculating both microbial biomass and respiration, though with one difference. Microbial biomass fumigation for this study was conducted for 10 days while Sparling and Zhu (1993) completed theirs in 24 hours. Microbial biomass-C was calculated using a revised calibration conversion factor of $C = 21 \times NPC$ (Amato and Ladd, 1988; Sparling and Zhu, 1993).

Levels of soil microbial biomass at the Albany Effluent Irrigated Tree Farm ranged from $307 \pm 26.8$ to $1001 \pm 68.5 \mu g$ biomass-C g$^{-1}$ (Table 4.3). These values are between two and five times higher than the estimates of microbial biomass
Table 4.3 Selected physicochemical data from four studies and levels of soil microbial biomass (¹ Sparling and Zhu, 1993, ² This Study, ³ Aggangan et al., 1999, ⁴ Mendham et al., 2002a, ⁵ Organic C)

<table>
<thead>
<tr>
<th>Land Use</th>
<th>Sparling¹</th>
<th>Sparling¹</th>
<th>Sparling¹</th>
<th>Albany Effluent Irrigated Tree Farm²</th>
<th>Aggangan³</th>
<th>Mendham⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Native Bushland</td>
<td>Grass Pasture</td>
<td>Clover Pasture</td>
<td>E. globulus plantation on long term pasture</td>
<td>E. globulus plantation on long term pasture</td>
<td>E. globulus plantation on long term pasture</td>
</tr>
<tr>
<td>pH</td>
<td>5.78</td>
<td>4.43 – 4.71</td>
<td>4.10</td>
<td>4.2 – 4.7</td>
<td>4.94 – 5.40</td>
<td>5.1 – 6.3</td>
</tr>
<tr>
<td>%TC</td>
<td>2.22</td>
<td>2.22 – 3.04</td>
<td>2.74</td>
<td>3.2 – 7.6</td>
<td>3.36 - 7.45⁵</td>
<td>1.9 – 8.3</td>
</tr>
<tr>
<td>%TN</td>
<td>0.088</td>
<td>0.133 – 0.191</td>
<td>0.150</td>
<td>0.33 – 0.35</td>
<td>0.163 – 0.477</td>
<td>0.12 – 0.40</td>
</tr>
<tr>
<td>C:N</td>
<td>25.2</td>
<td>20.9 – 24.4</td>
<td>18.3</td>
<td>9.41 – 23.0</td>
<td>15.6 – 20.6</td>
<td>15.8 – 20.75</td>
</tr>
<tr>
<td>Microbial Biomass (µg microbial C g⁻¹ soil)</td>
<td>213</td>
<td>120 - 202</td>
<td>160</td>
<td>307 - 1001</td>
<td>449 - 1125</td>
<td>140 - 450</td>
</tr>
</tbody>
</table>
published by Sparling and Zhu (1993). Five of their sites were within the shire of Albany. Regrettably there is no specific information regarding exact locations, as each site receives only a general description of ‘native bush’, ‘grass pasture’ or ‘clover pasture’. Soil for their study was sourced from the top 10 cm of the soil profile and at least 20 cores were bulked to obtain representative samples. Microbial biomass in the ‘Albany native bush’ site was 213 $\mu$g biomass-C g$^{-1}$, 160 $\mu$g biomass-C g$^{-1}$ in the ‘clover pasture’, while in the ‘grass pasture’ it ranged from 120 to 202 $\mu$g biomass-C g$^{-1}$. These authors provide no information regarding the season or time of year in which they sampled and provide only a very general description of the location in which they sampled thereby limiting any discussion of the impact of season or site specific difference on these very low microbial biomass values. The similarity between the levels of microbial biomass of all the samples reported by Sparling and Zhu suggests that land use had no impact on the level of microbial biomass.

Levels of soil microbial biomass reported from the Albany Effluent Irrigated Tree Farm compare favourably with estimates of total microbial biomass reported from soil with similar physicochemical characteristics and land use histories in the south-west Australia (Aggangan et al., 1999). Although measured in the laboratory, soil for their microlysimeter study was sourced from the southwest Australia (Scott River, Augusta). The site history is very similar to the sites from this Albany based study, in that long-term pasture sites (21 years) were deep ripped and mounded prior to planting with *E. globulus* seedlings. Soils at their sample sites were described as sandy (Arenosols), frequently overlying laterite, humus and iron podozols. Levels of
soil pH, organic C and %TN were comparable between this study by Aggangan et al., (1999) and the values reported in this Albany based study (Table 4.3). These authors reported microbial biomass values of between 449 – 1125 μg biomass g⁻¹ (Table 4.3).

Mendham et al., (2002a; 2002b) reported on levels of microbial biomass and cumulative respiration rates from ten E. globulus plantations in the south west of Western Australian (Mendham et al., 2002a). Soil texture was quite variable between the ten sample sites with five of the sites described as sandy loam (similar to this study). The remainder of the sites were described as sandy, loamy sands or fine sandy loams. Average rainfall varied between 661 and 1358 mm per year. All plantations were between 7 and 10 years old, and clearing had occurred between 20 and 71 years before sampling. Soil physicochemical properties were directly comparable between this study by Mendham et al., (Mendham et al., 2002a) and those reported from the Albany Effluent Irrigated Tree Farm (Table 4.4). Microbial biomass was estimated using a chloroform fumigation extraction method and ranged from 140 – 450 μg biomass-C g⁻¹.

In summary the range of soil microbial biomass reported in this study from the Albany Effluent Irrigated Tree Farm (307 ± 26.8 to 1001 ± 68.5 μg biomass-C g⁻¹) is directly comparable to published studies of levels of soil microbial biomass measured under E. globulus plantations in the same geographical regions subjected to similar land use histories (Table 4.4).
4.5.1.2 Trends in Microbial Biomass

The seasonal variation in soil microbial biomass in the non-irrigated control plots across the three sampling events correlated with variation in levels of soil moisture (Table 4.4). There was a positive association between soil moisture and biomass maxima in the non-irrigated control sites (Fig. 4.3). The level of soil microbial biomass in autumn 2000, in the non-irrigated control site was nearly double that recorded from the two irrigated sites or any other value (Table 4.4). This peak in microbial biomass may be related to rainfall. Analysis of the rainfall data for autumn 2000 reveals that in the seven days prior to sampling, a total of 65.8 mm of rain fell on the experimental site. This is approximately 25 mm more than the monthly average (Fig 2.2) and followed a particularly dry winter the year before (1999).

Microbial communities in Western Australian soils are able to respond very rapidly (within 24 hours) to simulated rainfall events (Murphy et al., 1998) and such spikes in microbial biomass have been recorded following rainfall particularly during warm, dry periods (Kieft et al., 1987; Ross, 1987; van Gestel et al., 1992; West et al., 1989; West et al., 1988a; West et al., 1988b).

As already noted, seasonal fluctuation in microbial biomass occurred only in the non-irrigated control plots. If (as suggested) rainfall was responsible for this peak in microbial biomass observed in autumn 2000 in the non-irrigated control plots it had no effect on levels of microbial biomass in the two irrigated soils in any season. Irrigation artificially maintains high levels of soil moisture, resulting in very stable levels of soil microbial biomass that are unaffected (or unable) to respond to rainfall.
Figure 4.3. Plot of mean microbial biomass (μg microbial C g⁻¹ soil) versus mean % soil moisture. (Au = autumn, Sp = spring, Su = summer).

Given the Water Corporation's policy of maintaining the irrigated soil at or near its water holding capacity, rainfall results in a reduction or cessation in irrigation and in real terms results in very small fluctuations in soil moisture. The impact of irrigation (per se) on levels of soil microbial biomass appears to have been to replace the natural seasonal variation dependant on rainfall, with a very stable, rainfall and seasonally independent biomass (Fig 4.3)
Table 4.4. Selected physicochemical properties, microbial biomass, microbial respiration, metabolic quotient and carbon quotient for three seasons (autumn, spring and summer) and three treatments (NIC = non irrigated control, EI = effluent irrigated, MWI = mains-water irrigated).

<table>
<thead>
<tr>
<th>Season</th>
<th>Site</th>
<th>pH</th>
<th>Soil H₂O (%)</th>
<th>Total C (%)</th>
<th>Total N (%)</th>
<th>C:N</th>
<th>Microbial Biomass (µg microbial C g⁻¹ soil)</th>
<th>Microbial Respiration (g CO₂ g⁻¹ soil 24 hr⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autumn 2000</td>
<td>NIC</td>
<td>4.5 ± 0.09</td>
<td>20.1 ± 2.80</td>
<td>5.2 ± 0.83</td>
<td>0.35 ± 0.005</td>
<td>14.8</td>
<td>1001 ± 68.5</td>
<td>0.49 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>EI</td>
<td>4.4 ± 0.15</td>
<td>33.5 ± 5.15</td>
<td>4.4 ± 0.73</td>
<td>0.35 ± 0.007</td>
<td>12.6</td>
<td>589 ± 47.8</td>
<td>0.39 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>MWI</td>
<td>4.4 ± 0.15</td>
<td>28.9 ± 6.42</td>
<td>3.6 ± 0.61</td>
<td>0.35 ± 0.014</td>
<td>10.3</td>
<td>556 ± 66.6</td>
<td>0.46 ± 0.04</td>
</tr>
<tr>
<td>Spring 2000</td>
<td>NIC</td>
<td>4.2 ± 0.10</td>
<td>12.3 ± 3.21</td>
<td>3.2 ± 0.67</td>
<td>0.34 ± 0.040</td>
<td>9.41</td>
<td>657 ± 63.8</td>
<td>0.25 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>EI</td>
<td>4.2 ± 0.19</td>
<td>30.2 ± 4.89</td>
<td>4.7 ± 0.12</td>
<td>0.33 ± 0.036</td>
<td>14.2</td>
<td>519 ± 60.7</td>
<td>0.24 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>MWI</td>
<td>4.4 ± 0.11</td>
<td>34.2 ± 5.67</td>
<td>5.9 ± 1.95</td>
<td>0.34 ± 0.051</td>
<td>17.4</td>
<td>571 ± 48.3</td>
<td>0.24 ± 0.03</td>
</tr>
<tr>
<td>Summer 2001</td>
<td>NIC</td>
<td>4.4 ± 0.06</td>
<td>5.5 ± 1.37</td>
<td>5.5 ± 0.45</td>
<td>0.33 ± 0.005</td>
<td>16.6</td>
<td>490 ± 96.6</td>
<td>0.41 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>EI</td>
<td>4.4 ± 0.15</td>
<td>38.6 ± 6.41</td>
<td>7.6 ± 3.10</td>
<td>0.33 ± 0.009</td>
<td>23.0</td>
<td>558 ± 50.9</td>
<td>0.37 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>MWI</td>
<td>4.7 ± 0.10</td>
<td>32.1 ± 5.84</td>
<td>5.5 ± 0.58</td>
<td>0.33 ± 0.059</td>
<td>16.6</td>
<td>307 ± 26.8</td>
<td>0.31 ± 0.04</td>
</tr>
</tbody>
</table>

(Values are means ± 1 SE, SE could not be calculated for C:N)
A question to be considered, is why does irrigation result in stable levels of microbial biomass at the lower end of levels of microbial biomass reported from the non-irrigated control soils? Wardle (1992), in his review of the factors that affect levels of soil microbial biomass noted that organic matter (Alvarez et al., 1998; Beyer, 1995; Bhattacharyya et al., 2001), temperature and levels of soil moisture are the three most important determinants of levels of soil microbial biomass. Soils from the South West Land Division of Western Australia have low organic matter content (Spain et al., 1983), with a high proportion of sand, low levels of fertility and medium to low levels of pH (Frost, 1991). The physicochemical composition of all major soil types within the south-west of Australia have been sampled (McArthur, 1991). Seven ‘reference’ soils exist within the Albany region. Owing to the inherent heterogeneity of soil, none of these reference sites corresponds exactly to the soil composition at the Albany Effluent Irrigated Tree Farm. Mean topsoil pH for McArthur’s (1991) seven Albany sites was 5.67 and ranged from 4.8 to 7.0, mean organic carbon was 3.16% and ranged from 0.38 to 9.5% while mean total nitrogen was 0.117% and ranged from 0.01 to 0.36% (McArthur, 1991). Undisturbed soils under native bushland in this geographical region are characterised by moderate to low pH, and low levels of both organic C and TN.

Of the three variables suggested by Wardle (1992) to be determinants of microbial biomass, the level of soil moisture is the factor most obviously affected by irrigation. Given that there is a positive relationship between levels of soil moisture and levels of soil microbial biomass (Insam and Haselwandter, 1989; Insam et al., 1991; Maraun and Scheu, 1996a; Maraun and Scheu, 1996b; Wardle, 1992) it may be that
the level of soil microbial biomass at the Albany Effluent Irrigated Tree Farm is constrained by low levels of soil organic matter. The peak in microbial biomass observed in the autumn control samples was linked to the significant rainfall event in the days immediately prior to sampling after a fifteen-month drought. During this period of drought levels of soil moisture would have been a significant limiting factor on levels of microbial biomass in the non-irrigated control sits. As a result, rates of litter decomposition would have slowed resulting in a gradual build up of litter organic matter. With the breaking of the drought, the microbial biomass peaked possibly due to the availability of soil moisture and the temporarily 'high' levels of available soil carbon due to the leaching of soluble carbons from the leaf litter into the soil (Leaching of carbon associated with rainfall was observed in the litter decomposition experiment described in Chapter 7). It follows, that this peak in microbial biomass would be associated with a peak in microbial respiration. Clearly, this is not the case (Figure 4.2.). However, rates of microbial respiration can peak and plateau very rapidly (in the order of hours) in response to changes in soil moisture (Murphy et al., 1998). It appears, therefore, that the autumn sampling was conducted after a peak and subsequent return to typical rates of microbial respiration but during the associated peak in levels of microbial biomass.

The lack of a seasonal response in levels of microbial biomass may suggest that temperature is not a significant limiting factor at this site when levels of soil moisture are non-limiting. Levels of microbial biomass in the non-irrigated control sites peaked when both air temperature and levels of soil moisture were high and declined when either temperature (i.e. in spring) or moisture levels (i.e. summer)
were at a minima. This interaction between both temperature and soil moisture suggests that a determinant of levels of soil microbial biomass in the non-irrigated soil may be soil humidity as determined by the interaction of both temperature and soil moisture.

4.5.2 Microbial Respiration

4.5.2.1 Rates of Microbial Respiration

The most important outcome of this part of the research is the consistent very low rates of microbial respiration, irrespective of treatment, site or season, when compared with rates elsewhere in the world. For example, rates of soil microbial respiration from sites representing a variety of climatic regimes in North America ranged from 52 to 487 $\mu g$ CO$_2$ g$^{-1}$ hr$^{-1}$ (Insam, 1990), up to three orders of magnitude greater than the 0.24 - 0.49 $\mu g$ CO$_2$ g$^{-1}$ hr$^{-1}$ range recorded in this study. Outside Australia, comparable rates of microbial respiration to those of my study are from recently glaciated soil! Soil from the Rotmoos Glacier (Austria) that become ice free 1-3 years before sampling but had no plant cover respired at a rate of 0.6 ± 0.2 $\mu g$ CO$_2$ g$^{-1}$ hr$^{-1}$ (Insam and Haselwandter, 1989). Five year old soil from the Athabasca Glacier (Canada) respired at a rate of 1.2 $\mu g$ CO$_2$ g$^{-1}$ hr$^{-1}$ while 225 year old soil from the same glacier respired at a rate of 4.5 $\mu g$ CO$_2$ g$^{-1}$ hr$^{-1}$ (Insam and Haselwandter, 1989).

The lack of suitable geographic data makes it difficult to place rates of microbial respiration from this study into an appropriate context. Rates of microbial respiration
from the same five sites in Albany (discussed above) ranged from 0.12 to 0.20 μg CO₂ g⁻¹ hr⁻¹ (albeit with lower rates of microbial biomass) (Sparling and Zhu, 1993). Soil from the Western Australian wheatbelt (Moora) sampled in the spring of 1999 respired at rates of ≈ 0.23 to 0.29 μg CO₂ g⁻¹ hr⁻¹ (similar to the rate of microbial respiration observed in this study), while soil microbial biomass varied between ≈ 390 and 710 μg biomass-C g⁻¹ (Luxhoi et al., In Preparation) (similar to the level of microbial biomass observed in this study). The soil from the western Australian wheat belt has a higher pH (5.5 - CaCl₂ method) but lower levels of %TN (0.06%) and %TC (0.66%) when compared to the soil at the Albany Effluent Irrigated Tree Farm. This finding suggests that soils in the southern half of West Australia have very low rates of microbial respiration that are lower even than rates of respiration from recently glaciated soils in the Northern Hemisphere.

A study of thirty soils sourced from eastern Australia (New South Wales, Victoria and Queensland) reported rates of microbial respiration that varied between 0.17 to 3.09 μg CO₂ g⁻¹ hr⁻¹ with a mean value of 1.12 μg CO₂ g⁻¹ hr⁻¹ (Wang et al., 2003). Rates of microbial respiration were measured using a method directly comparable to the method utilised in this study. Unfortunately, the authors provided no information regarding the land use management regime for any of the sites. Total organic carbon ranged from 0.7 to 6.3%, total nitrogen from 0.05 to 0.6% while pH varied between 5.0 and 8.6. Clay content ranged from 30 to 760 mg g⁻¹. The authors observed that rates of soil respiration were generally limited by the supply of biologically available substrate (organic carbon) under favourable temperature and moisture conditions and that there was no consistent relationship between rates of
microbial biomass and microbial respiration. They argue that caution should be used when interpreting the size of microbial biomass estimates independent of their respiration rate (Wang et al., 2003). The results from this Albany based study clearly support this warning, as levels of microbial respiration were very low despite the relatively high levels of microbial biomass. This finding also supports the view that the factors limiting microbial respiration can be different to the factors limiting microbial biomass.

A study of rates of soil respiration in five different sites in each of three different Australian forests concluded that rates of microbial respiration are limited by a lack of readily available carbon rather than nitrogen or phosphorus (Maheswaran and Attiwill, 1989). This study based in south-east Australia examined wet sclerophyll forest (Eucalyptus regnans F. Muell.) established on red-brown earths overlying granodiorite, intermediate forest between wet and dry sclerophyll (E. regnans and Eucalyptus oblique L’Herit.) on gradational brown earths overlying granodiorite and dry sclerophyll forests (Eucalyptus sideroxylon A. cunn. ex Woolls and Eucalyptus microcarpa) on shallow soil with a high proportion of gravel (upto 50%). Total N ranged from 0.00 to 0.80%, TC ranged from 7.0 to 11.6% while pH ranged from 4.6 to 5.7. Each of these characteristics is broadly similar to the soil physicochemical data reported from the Albany Effluent Irrigated Tree Farm

In this south-eastern Australian study of the factors limiting rates of soil microbial respiration, the authors examined the impact of N, P, glucose, N and P, and N and glucose additions to the rate of soil respiration (Maheswaran and Attiwill, 1989).
While additions of phosphorus had no significant effect on rates of respiration (either positive or negative), it is possible that added phosphorus was absorbed and bound within the soil matrix and so was unavailable to the soil microbes. Additions of N in the form of KNO₃ and NH₄NO₃ decreased the rate of microbial respiration in all soils (with the exception of one) by up to 28%. The negative response of respiration to the addition of inorganic N was interpreted to indicate that N was not a limiting resource in these soils. The rate of soil respiration increased rapidly from 0.19 to 1.15 nmol g⁻¹ s⁻¹ when increasing amounts of glucose (water soluble carbon) were added to the soil (0 – 2 cm). Rates of microbial respiration peaked when the soil concentration of glucose reached 1.25%, indicating that other factors were limiting the rate of microbial respiration at this point. Given the levels of total C observed in this study (Table 4.3) were less than the levels reported by Maheswaran and Attiwill (1989), rates of microbial respiration at the Albany Effluent Irrigated Tree Farm may be similarly limited by the very low levels of available C, rather than soil moisture, nitrogen or phosphorus.

Soil microbial activity (as measured by respiration) is known to be limited by strongly acidic conditions (pH <4.5) and by shortages of decomposable organic matter, rather than by shortages of inorganic nutrients (Salonius, 1972). It has been suggested that at these pH levels the soil organic material might be unavailable to the soil microflora (Salonius, 1972). Phosphorus has also been suggested as limiting resource for both microbial biomass and microbial respiration at low pH because it may bind to Fe⁺ and/or Al⁺ and so make it unavailable to the soil microbial population (Gallardo and Schlesinger, 1994). The soils from this study also
have a low pH (Table 4.4) and there is some preliminary evidence that phosphorus is limiting the growth of *E. globulus* trees on this experimental site (Adams *et al.*, 1995; Silifant, 1997). This suggests that the rate of microbial respiration at the Albany Effluent Irrigated Tree Farm may also be limited by the acidic pH and low level of available phosphorus.

The *E. globulus* plantation soils at the Albany Effluent Irrigated Tree Farm are characterised by high levels of sand, low levels of clay, low acidic pH, low levels of TC (half the levels of oxidisable C reported by Maheswaran and Attiwill (1989)) and low levels of TN. This indicates that the low levels of microbial respiration reported in this study may be due primarily to a lack of available carbon. Compounding the lack of available carbon may be the low soil pH, which may be binding the carbon (and other nutrients such as phosphorus) in an insoluble form that makes these nutrients unavailable for microbial utilisation.

Another commonly reported factor limiting rates of soil microbial respiration is soil moisture (Garcia *et al.*, 1994; Mathes and Schriefer, 1985; Orchard and Cook, 1983; Pohhacker and Zech, 1995; Sparling, 1997). However, it is unlikely that soil moisture is a major limiting factor of rates of microbial respiration at these sites because irrigation has had no significant effect on rates of microbial respiration.

In summary, rates of soil microbial respiration at the Albany Effluent Irrigated Tree Farm are well below commonly reported values from around the world, however they are consistent with the small volume of published respiration data available for
the south of Western Australia. It seems probable given the discussion above that rates of microbial respiration are limited by the availability of carbon with the potential for complex phosphorus related interactions. In addition, it appears that very low rates of microbial respiration may be a characteristic of soils within southwest Australia.

4.5.2.2 Trends in Microbial Respiration

Rates of microbial respiration were insensitive to both types of irrigation and displayed similar patterns of seasonal variation irrespective of treatment. Rates of microbial respiration peaked in autumn and summer, coinciding with peaks in temperature. This positive correlation between ambient temperature maxima and minima and rates of microbial respiration is commonly reported in the literature (Mathes and Schriefer, 1985; O'Connell, 1990; Pohhacker and Zech, 1995). This suggests that climate in the form of temperature, is a primary determinate of seasonal variation in microbial respiration.

4.5.2.3 Microbial Metabolic Quotient

In addition to microbial biomass and microbial respiration, the microbial community can be described in terms of the microbial metabolic quotient. The quotient has been suggested as a useful measure of change in the microbial populations due to disturbance (Anderson and Domsch, 1985). The microbial metabolic quotient is variously termed microbial biomass quotient, specific respiration of biomass or $q_{CO_2}$
and is based on Odum’s theory of ecosystem succession (Odum, 1969; Odum, 1985). Proposed in 1985, it is the ratio of soil microbial respiration to microbial biomass. It is suggested that this quotient declines after recovery from a disturbance and during succession since the microflora become more efficient at conserving carbon (Insam and Haselwandter, 1989; Pohhacker and Zech, 1995; Wardle and Ghani, 1995).

Published values for the metabolic quotient usually vary between 1 to 10 x 10^{-3} (\mu g CO_{2}-C g^{-1} h^{-1} \mu g biomass-C) (Wardle and Ghani, 1995). \( q_{CO_2} \) values from agricultural chronosequences built on reclaimed open pit mines in Germany ranged from 1.8 to 2.6 x 10^{-3} in recently reclaimed soils sites and declined to 1 to 1.5 x 10^{-3} in sites that were more than 50 years old (Insam and Domsch, 1988). \( q_{CO_2} \) values from recently reclaimed mine sites that have been developed as forest ranged from 0.7 to 2.8 x 10^{-3} while sites that were 40 years or older ranged from 1 to 2 x 10^{-3} (Insam and Domsch, 1988). In a review of the impact of disturbance on \( q_{CO_2} \), Wardle and Ghani (1995) present data from 32 sites covering forests (Beech, Scots pine, and Spruce) grassland and arable fields. The microbial metabolic quotient varied between 0.56 and 6.1 x 10^{-3} with a mean value of 2.0 x 10^{-3}. These values are greater than those reported in my study (Table 4.5) by an order of magnitude. This observation is explained by the very low rates of microbial respiration (Table 4.3) that are orders of magnitude below commonly reported values.
The use of $q_{CO_2}$ as a bioindicator or measure of ecosystem disturbance or development has been heavily criticized of late. Wardle and Ghani (1995) present a critique of the microbial metabolic quotient and state that its usefulness is limited because it confounds the effects of disturbance with those of stress. They suggest that evaluation of $q_{CO_2}$ is not always a reliable or consistent bioindicator of disturbance or ecosystem development. The microbial metabolic quotient has been shown to be particularly sensitive to variations in the quality and amount of respirable C-substrate (Sparling, 1997). Given the earlier suggestion that rates of microbial respiration are limited by a lack of available carbon it would seem that the low $q_{CO_2}$ values reported in Table 4.5 reflects a highly constrained microbial population as opposed to a highly efficient and stable microbial population. This finding supports the criticism of $q_{CO_2}$ values and suggests that it should be used with caution.

Table 4.5. Microbial Metabolic Quotient and the Microbial Carbon Quotient for three seasons and treatments at the Albany Effluent Irrigated Tree Farm

<table>
<thead>
<tr>
<th>Season</th>
<th>Site</th>
<th>Microbial Metabolic Quotient ($q_{CO_2}$)</th>
<th>Microbial Carbon Quotient (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autumn 2000</td>
<td>Non-Irrigated Control</td>
<td>0.00049</td>
<td>1.93</td>
</tr>
<tr>
<td></td>
<td>Effluent Irrigated</td>
<td>0.00066</td>
<td>1.34</td>
</tr>
<tr>
<td></td>
<td>Mains Water Irrigated</td>
<td>0.00082</td>
<td>1.54</td>
</tr>
<tr>
<td>Spring 2000</td>
<td>Non-Irrigated Control</td>
<td>0.00038</td>
<td>2.05</td>
</tr>
<tr>
<td></td>
<td>Effluent Irrigated</td>
<td>0.00046</td>
<td>1.10</td>
</tr>
<tr>
<td></td>
<td>Mains Water Irrigated</td>
<td>0.00042</td>
<td>0.97</td>
</tr>
<tr>
<td>Summer 2001</td>
<td>Non-Irrigated Control</td>
<td>0.00083</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>Effluent Irrigated</td>
<td>0.00066</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>Mains Water Irrigated</td>
<td>0.00101</td>
<td>0.56</td>
</tr>
</tbody>
</table>
4.5.2.4 *Microbial (Carbon) Quotient*

The microbial carbon quotient is the ratio between microbial biomass C and soil TC. It has been suggested as a more useful measure of soil processes or soil health than microbial C or total C (Insam and Domsch, 1988; Sparling, 1997). It is thought that microbial C pools will decline at a faster rate than total organic matter pools when C is being exploited and so lead to a decrease in the microbial carbon quotient (Sparling, 1997). When a soil or ecosystem undergoes succession, the proportion of total organic matter as microbial biomass tends to be greater than in established soil, producing an increase in the microbial carbon quotient (Insam and Domsch, 1988; Sparling, 1997). The values in Table 4.5 are at the low end of published global values that typically range from 1% to 6%. Given the direct connection between this derived measure of the microbial population and levels of soil carbon and the previous discussion regarding the potentially limiting nature of carbon it is not surprising that the microbial quotient values are so low. Reanalysing the data presented by Sparling and Zhu (1993) we find microbial carbon quotient values that range from 0.54 to 0.95%. These values are directly comparable to the values observed in summer 2001 (Table 4.5) and slightly lower than values recorded for both autumn and spring 2000.

Both these derived measures of the microbial community clearly highlight the unique nature of the microbial communities at the Albany Effluent Irrigated Tree Farm. The levels of microbial biomass were affected by irrigation, however they appear to be
constrained by the lack of organic matter and the low soil pH. Rates of microbial respiration are insensitive to irrigation yet they are so low that comparable rates are reported from recently glaciated soils and so may be unable to respond to irrigation. Overall, rates of microbial respiration appear to be constrained by the low levels of organic C. Given that these soils support a very small active microbial biomass that is the primary resource for the remaining soil faunal community, it is expected that this will result in a bottom-up resource structuring affect on the remaining soil fauna.
"Nematode worms, account for four of every five animals living on Earth – and are so abundant that if the planet's surface vanished, its "ghostly outline" could still be made out in the biomass of nematodes, almost all of species unknown."

E.O. Wilson
5.0 Soil Nematoda

5.1 INTRODUCTION

Soil nematodes are ubiquitous microfauna in the soil. Along with protozoa and acari, they are important microbivores and can exert important top down pressure on microbial populations (Griffiths, 1994; Ingham et al., 1985). Nematode community structure has been suggested as a potential bioindicator in environmental monitoring (Bongers and Ferris, 1999; Bongers and Yeates, 1988). Nematodes occupy key positions in soil food webs in that they feed on many soil organisms and are an important food source for many other soil organisms. Previous research has reported that nematodes respond rapidly to disturbance and enrichment, and has highlighted the connection between microbial activity and nematode community structure (Bongers and Ferris, 1999; Griffiths, 1994; Yeates et al., 1997b).

In this chapter, I examine the soil nematode populations and trophic structure under non-irrigated *E. globulus* plantations as ‘baseline’ data. I then analyse the nematode trophic structure in soil irrigated with effluent or mains-water, and explore statistically the impact of both season and treatment on soil nematode populations and trophic structure. The aims of this chapter are to describe:

a) baseline soil nematode diversity and density;
b) baseline soil nematode trophic structure;
c) soil nematode density and diversity in irrigated (effluent and mains-watered) sites;
d) soil nematode trophic structure in irrigated (effluent and mains-water) sites; and
e) the impact of season and treatment on nematode density, diversity and trophic structure.

Research hypothesis 4. Based on the observation of Yeates, (1995) soil nematode populations will be dominated by bactivorous and predatory species in effluent irrigated soils.

5.2 METHODS

Soil samples were collected using the method detailed in section 2.8.1 (Sampling Design and Common Methods – Soil Sampling) with the following modifications. Soil was collected with a metal core (5 cm diameter x 10 cm depth) and placed into zip lock plastic bags. Approximately 400 g of soil was collected for each sample.

5.2.1 Extraction

Prior to extraction, soil samples were gently sieved through a 5 mm screen and carefully stirred. Nematodes were extracted into water using Whitehead trays (Whitehead and Hemming, 1965). Six soil samples were randomly chosen from each transect within each treatment within each replicate site. This resulted in 18 samples from each treatment for a total of 56 samples per sampling occasion. Stainless steel mesh trays (170 mm x 170 mm) were lined with Kimwipes™. One hundred grams of soil was placed into the tray and spread evenly over the tissues. The mesh trays were then lowered into plastic containers filled with distilled water so
that the top of the water just touched the base of the soil. The trays were covered with plastic (Gladwrap™) and left to stand for 36 hours. On completion of the extraction procedure, the soil and Kimwipes were removed while the water (with extracted nematodes) from each plastic container was washed through a 45 μm sieve with 80% ethanol. The resulting samples were stored in 40 mL specimen jars in 80% ethanol. It must be noted that this method does not allow for the distinction between active and anhydribiotic nematodes.

5.2.2 Identification

Samples were sorted under a dissecting microscope (Olympus SZH 10-70x). Temporary mounts of specimens for identification were made using cavity slides and small quantities of 25% lactic acid. To aid in clearing specimens, slides were gently warmed on a slide-warming tray. Trophic status was inferred based on stoma structure and stylet morphology (Freckman and Baldwin, 1990; Yeates et al., 1993). Nematodes were assigned to a morphospecies based upon stylet morphology, buccal characteristics and stoma shape and size. Nematodes were identified to Order using the key of Freckman et al., (1990) and a compound microscope (Olympus BX-50 40-1,000x).

5.2.3 Statistical Analysis

Statistical analysis was carried out using the methods detailed in section 2.8.6. (Sampling Design and Common Methods – Statistical Analysis). Two-way nested
ANOVA was used to determine if there were seasonal or treatment effects on mean soil nematode density. Where appropriate, Bonferroni’s post hoc analysis was used to determine significant differences between treatments. Seasonal effects on mean soil nematode density were analysed for each treatment.

5.3 RESULTS

5.3.1 Nematoda Density

5.3.1.1 Seasonal Responses

There were statistically significant seasonal effects on nematode densities in non-irrigated control and mains-water irrigated sites. Although nematode densities in the effluent irrigated sites displayed the same seasonal trend, this was not statistically significant ($F_{(2,6)}=2.807, p=0.134$). Nematode densities declined significantly between autumn and spring samples at the non-irrigated control and mains-water irrigated sites (non-irrigated control $F_{(2,6)}=38.623, p<0.001$; mains-water irrigated $F_{(2,6)}=7.020, p<0.05$), then increased in summer (non-irrigated control $p<0.001$; mains-water irrigated $p<0.05$).

5.3.1.2 Treatment Responses

There were statistically significant treatment effects on levels of soil nematode density in autumn ($F_{(2,6)}=10.502, p<0.05$), but not spring ($F_{(2,6)}=0.351, p=0.717$), or summer ($F_{(2,6)}=1.545, p=0.285$).
Nematode density in autumn in the irrigated sites (effluent irrigated $p<0.001$; mains-water irrigated $p<0.001$) was significantly lower than in the non-irrigated control sites. This trend was repeated in summer although the difference between the non-irrigated control and the two irrigated treatments was not as great.
5.3.2 Nematoda Species Diversity

Table 5.2 lists species richness (SR), species diversity (Shannon-Wiener diversity \( H' \)) and species equitability or evenness (Pielou’s evenness \( J' \)) for each treatment within each season. Species richness is simply the total number of different species sampled within each treatment within each season. The Shannon-Wiener diversity index is a measure of the ability to accurately predict the species type of the next individual collected. The Shannon-Wiener function combines two diversity components; (1) the number of species and (2) the evenness of individual allotments among the species (Krebs, 1994). The greater the value of \( H' \) the larger the diversity or the less likely that we can accurately predict the species of the next

<table>
<thead>
<tr>
<th>Season</th>
<th>Treatment</th>
<th>SR</th>
<th>( H' )</th>
<th>( J' )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autumn</td>
<td>Non-Irrigated Control</td>
<td>10</td>
<td>2.14</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>Effluent Irrigated</td>
<td>10</td>
<td>1.99</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>Mains-Water Irrigated</td>
<td>10</td>
<td>1.96</td>
<td>0.84</td>
</tr>
<tr>
<td>Spring</td>
<td>Non-Irrigated Control</td>
<td>10</td>
<td>1.99</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>Effluent Irrigated</td>
<td>8</td>
<td>1.59</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>Mains-Water Irrigated</td>
<td>9</td>
<td>1.45</td>
<td>0.68</td>
</tr>
<tr>
<td>Summer</td>
<td>Non-Irrigated Control</td>
<td>14</td>
<td>1.87</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>Effluent Irrigated</td>
<td>10</td>
<td>1.93</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>Mains-Water Irrigated</td>
<td>13</td>
<td>1.59</td>
<td>0.67</td>
</tr>
</tbody>
</table>
individual collected. The maximum $H'$ value is equal to $\log_e S$ where $S$ is the total number of species.

Pielou's evenness ($J'$) is a measure of how evenly each species is distributed within a sample. Measures of evenness range between 0 and 1. Zero indicates an absolutely uneven distribution while 1 indicates an absolutely even distribution of all species (Clarke and Gorley, 2001).

The primary result within Table 5.2 is the consistently low level of nematode species richness irrespective of treatment and season. Given the very low species richness in all treatments and seasons, further statistically analyses of either species richness or the diversity indices would be of questionable value. Table 10.2 (Appendix) lists each species and its density. High standard errors are a characteristic of the data and reflect the strongly aggregated distribution of soil nematodes.

5.3.3 Classification and Ordination Analysis of Nematoda Density

The impact of season and treatment on the faunal community structure (species composition and density) was analysed using multivariate analyses (Figs 5.2 and 5.3). Mean nematode species density per $m^2$ was square root transformed prior to the calculation of Bray-Curtis similarities for the construction of both classification (hierarchical agglomerative) and ordination (MDS) plots.
Cluster patterns were dominated by seasonal effects. The spring nematode communities characterized by significantly lower densities and lower species richness clustered separately from both autumn and summer communities. There was no indication of a consistent treatment effect on nematode communities in any season.

Figure 5.3 is a 2-dimensional MDS plot of $\sqrt{\text{transformed standardized Nematoda densities}}$. The stress level (0.01) is very low and suggests a very high degree of confidence that the two dimensional graphical representation (Fig 5.3) is a faithful representation of the multidimensional data set (Clarke and Warwick, 1994).
The ordination pattern of Figure 5.3 supports the cluster analysis of Figure 5.2 with nematode communities separating based on season (summer and autumn separate from spring) with no treatment effect. I will continue to examine the effect of irrigation on nematode density and species richness in the next section when I consider individual nematode species and nematode functional groups.

5.3.4 Nematoda Functional Groups

Nematodes were classified into functional groups based on information regarding feeding habits and stylet morphology extracted from the literature. Nematodes were grouped into one of four functional groups including plant feeding (Tylenchida sp. 1
Plate 5.1. Tylenchida sp. x 1,000 (Plant feeding)

Plate 5.2. Monochida sp. x 400 (Animal predator)

Plate 5.3. Rhabditida sp. x 1,000 (Bacterial feeding)

Plate 5.4. Rhabditida sp. x 400 (Bacterial feeding)
Table 5.3. Species richness and relative density (%) of nematode functional groups under three treatment regimes: non-irrigated control, effluent irrigated, and mains-water irrigated sampled in autumn, spring and summer.

<table>
<thead>
<tr>
<th>Functional Group</th>
<th>Autumn Control</th>
<th>Effluent</th>
<th>Watered</th>
<th>Spring Control</th>
<th>Effluent</th>
<th>Watered</th>
<th>Summer Control</th>
<th>Effluent</th>
<th>Watered</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant Feeding</td>
<td>2 (15.1)</td>
<td>3 (21.4)</td>
<td>3 (33.0)</td>
<td>3 (30.4)</td>
<td>3 (35.7)</td>
<td>4 (54.9)</td>
<td>4 (42.1)</td>
<td>3 (12.9)</td>
<td>4 (7.7)</td>
<td>4</td>
</tr>
<tr>
<td>Animal Predator</td>
<td>1 (20.4)</td>
<td>-</td>
<td>1 (0.3)</td>
<td>1 (2.2)</td>
<td>1 (0.1)</td>
<td>1 (1.0)</td>
<td>1 (0.01)</td>
<td>-</td>
<td>1 (0.4)</td>
<td>1</td>
</tr>
<tr>
<td>Bacterial Feeding</td>
<td>6 (55.7)</td>
<td>6 (66.7)</td>
<td>5 (61.2)</td>
<td>4 (47.2)</td>
<td>3 (32.1)</td>
<td>2 (37.9)</td>
<td>7 (55.4)</td>
<td>5 (76.3)</td>
<td>7 (87.1)</td>
<td>7</td>
</tr>
<tr>
<td>Hyphal Feeding</td>
<td>1 (8.7)</td>
<td>1 (11.9)</td>
<td>1 (5.5)</td>
<td>2 (20.2)</td>
<td>1 (32.1)</td>
<td>2 (6.3)</td>
<td>2 (2.4)</td>
<td>2 (10.9)</td>
<td>1 (4.8)</td>
<td>2</td>
</tr>
<tr>
<td>Total number of species</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>8</td>
<td>9</td>
<td>14</td>
<td>10</td>
<td>13</td>
<td>14</td>
</tr>
</tbody>
</table>

(\(n = 54\) per sampling occasion)

– 4, Plate 5.1), animal predator (Monochida sp. 1, Plate 5.2), bacterial feeding (Rhabditida sp. 1 – 5, Plate 5.3 and 5.4, and Araeolaimida sp. 1 and 2) and hyphal feeding (Dorylamida sp. 1 and 2). Classifications were based on feeding habits inferred from stoma and oesophageal structure as discussed by Yeates et al., (1993).

The species richness of soil nematodes, functional groups and the percentage contribution of each functional group to total density are presented in Table 5.3. While caution must be utilised when interpreting any patterns based on such low number of species a number of season and treatment effects can be identified. Most obvious is the large percentage contribution of the single animal predator species, Monochida sp. 1, to nematode density in the non-irrigated autumn control site where it contributed approximately 20%. In these autumn non-irrigated control
sites this nematode contributed nearly twenty percent of the total nematode population. This peak in nematode density in the autumn non-irrigated control sites and the one off large percentage contribution by the animal predator nematodes coincides with the high levels of microbial biomass described in the previous chapter.

In both autumn and summer samples irrigation was consistently associated with an increase in the percentage contribution of bacterial feeding nematodes. In the non-irrigated control sites, the percentage contribution of bacterial feeding nematodes to total density is relatively constant across each season.

5.4 SUMMARY OF RESULTS

The significant outcomes from this section are:

1. Nematode density was insensitive to irrigation but displayed a consistent pattern of seasonal variation irrespective of treatment, with maxima in autumn and summer and minima in spring.

2. Nematode densities (like microbial biomass) were lower in irrigated sites in autumn when compared with the non-irrigated control sites.

3. In the autumn non-irrigated control site maximum nematode densities coincided with maximum levels of microbial biomass.

4. Irrigation was associated with an increase in the percentage contribution of bacterial feeding nematodes when densities were maximal (autumn and summer).
In summary, levels of nematode density were dominated by season and were negatively affected by irrigation in both autumn and summer, although this result was only statistically significant in autumn. Community level analysis of the nematode populations suggested that nematode communities were dominated by seasonal effects. Analysis of the functional composition of the nematode community revealed that all communities were dominated by bacterial feeding nematodes, but irrigation increased the relative dominance of the bacterial feeding nematodes supporting research hypothesis four.

5.5  DISCUSSION

5.5.1  Nematode Densities and Species Richness

Soil nematode density in this study ranged from a spring low of 0.89 to an autumn high of 49.88 x 10^4 individuals m^{-2}. Very little is known about nematode populations in Australian temperate soils. A study of nematode populations at Moora, West Australia (30°36'S, 116°6'E) under canola-wheat-lupin crop rotation reported mean nematode density values that ranged from approximately 3 x 10^5 (winter) to 1.6 x 10^6 (spring) individuals m^{-2} (Osler et al., 2000). Nematode densities at the Albany Effluent Irrigated Tree Farm are an order of magnitude lower than nematode densities reported from Moora, West Australia.
Soil nematode density typically ranges from 1 to $10 \times 10^6$ individuals m$^{-2}$ (Yeates and Bongers, 1999). In their classic review of soil fauna populations, Peterson and Luxton (1982) report that nematode densities range widely from a recorded low of $8.1 \times 10^3$ individuals m$^{-2}$ from a tropical rain forest in Puerto Rico to a high of $30 \times 10^6$ individuals m$^{-2}$ from a meadow steppe in the former USSR. They note that the majority of studies report nematode densities ranging between $10^6$ and $10^7$ individuals m$^{-2}$ that are one to two orders of magnitude greater than levels of nematode density recorded at the Albany Effluent Irrigated Tree Farm. Comparisons between nematode studies are complicated by the use of different extraction procedures with variable extraction efficiencies (Peterson and Luxton, 1982).

There appears to be little correlation between nematode densities and vegetation type although particularly low nematode densities ($10^5$ individuals m$^{-2}$) have been reported from extreme environments such as dry sandy heathlands, permanently moist habitats such as moors and bogs (Peterson and Luxton, 1982) as well as desert and extreme tundra soils (Lavelle and Spain, 2001). Levels of nematode density at the Albany Effluent Irrigated Tree Farm are low by comparison to global estimate of nematode density.

Nematode density is influenced by levels of soil moisture and levels of soil organic matter (Anderson et al., 1997a; Anderson et al., 1997b; Bakonyi and Nagy, 2000; Freckman and Mankau, 1986; Gupta et al., 1998; Lavelle and Spain, 2001; Sohlenius, 1985). While there appears to be a positive correlation between levels of organic matter and nematode density the relationship between levels of soil
moisture and nematode density is more complex (Lavelle and Spain, 2001; Pen-Mouratov et al., 2004). Soil moisture appears unlikely to be a significant constraint on nematode density at the Albany Effluent Irrigated Tree Farm since there was no statistically significant difference between nematode density under the two irrigated treatments relative to the non-irrigated control sites as would be expected if levels of soil moisture were a significant limiting factor. It seems most likely that the consistent very low levels of nematode density observed at the Albany Effluent Irrigated Tree Farm are a function of the low levels of active microbial biomass that in turn are a function of the low levels of organic carbon and acidic soil pH.

Levels of species richness at the Albany Effluent Irrigated Tree Farm ranged from eight to fourteen and did not vary significantly due to treatment or season. Such low species richness is comparable to levels reported from studies conducted in extreme environments, such as the sixteen nematode species reported from a study of tundra soil on Signy Island, Antarctica (Collins et al., 1975; Peterson and Luxton, 1982) and the seventeen nematode taxa identified in a study of soil from the Negev Desert (Pen-Mouratov et al., 2004). Levels of species richness or nematode diversity are typically thought to be controlled by levels of soil temperature (Bakonyi and Nagy, 2000; Pen-Mouratov et al., 2004). While temperature may be a significant controlling variable of nematode species richness in extreme environments such as deserts and Antarctic tundra, it seems unlikely that temperature alone would explain the low species richness at the Albany Effluent Irrigated Tree Farm, given the prevailing Mediterranean type climate.
The site history and soil physicochemical properties at the Albany Effluent Irrigated Tree Farm may be a major constraint on the soil nematode population. As previously mentioned, the low levels of total carbon (a component of soil organic matter) are thought to be a significant limiting factor of nematode density (Bakonyi and Nagy, 2000; Freckman and Mankau, 1986; Gupta et al., 1998; Lavelle and Spain, 2001; Sohlenius, 1985). The low levels of soil organic matter and acidic pH was suggested as a significant potential constraint on the levels of soil microbial respiration and that there may be a connection between low levels of microbial respiration and nematode density and species richness.

Prior to the establishment of the *E. globulus* plantation at the Albany research site the vast majority of the land was used for pasture. During the winter months, this land was subject to regular flooding (Silifant, 1997). After planting of the *E. globulus* plantation, flooding has not been observed, suggesting that the increased uptake of water by the plantation has been successful in lowering the water table and preventing the development of water logged soils. As noted earlier, permanently moist (water logged) habitats display very low levels of nematode density (Peterson and Luxton, 1982). While pasture sites are typically associated with large numbers of nematodes (Yeates, 1996) the seasonal flooding of the soils at the Albany research site are likely to have resulted in anaerobic soil conditions that are known to limit nematode activity and density (Freckman and Baldwin, 1990). Therefore, at the time of plantation establishment the nematode population may have been very small and species poor. When sampling occurred some years later, the nematode
population might have been increasing in size and diversity from this very small initial population.

5.5.2 Effect of Irrigation on Soil Nematodes

Irrigation tended to dampen the seasonal pattern of nematode density in summer and autumn similar to the effect irrigation had on levels of microbial biomass. Nematode sensitivity to high levels of soil moisture (as discussed on the previous page) could explain this observation. Given the high levels of soil moisture observed in the irrigated sites, it is possible that this limits nematode density in the irrigated sites.

In the non-irrigated control sites, bacterial feeding nematodes represent approximately half of the nematode population in each season. Irrigation is associated with increases in the percentage contribution of bacterial feeding nematodes in both autumn and summer where they account for two thirds and three quarters of the population respectively (Table 5.4). The dominance of bacterial feeding nematodes under E. globulus plantations, as observed in this Albany based study, is consistent with previous research showing that bacterial feeding nematodes dominate in managed forest soils (Yeates and Bongers, 1999). In general, highly disturbed sites tend to be dominated by bacterial feeding nematodes, while undisturbed sites tend to display more even distributions of bacterial and fungal feeding nematodes (Bardgett et al., 1999; Bardgett et al., 1996; Bardgett and
McAlister, 1999; Bardgett et al., 1998; Beare et al., 1992; Moore and de Ruiter, 1997).

Table 5.4. Percentage contribution (%) and nematode total density under three treatment regimes: non-irrigated control, effluent irrigated and mains-water irrigated.

<table>
<thead>
<tr>
<th></th>
<th>Bacterial Feeding Nematodes (%)</th>
<th>Total Density (N m⁻² x 10⁴)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autumn</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Irrigated Control</td>
<td>55.7</td>
<td>49.9 ± 2.15</td>
</tr>
<tr>
<td>Effluent Irrigated</td>
<td>66.7</td>
<td>7.6 ± 0.19</td>
</tr>
<tr>
<td>Mains-Water Irrigated</td>
<td>61.2</td>
<td>9.3 ± 0.22</td>
</tr>
<tr>
<td>Spring</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Irrigated Control</td>
<td>47.2</td>
<td>1.3 ± 0.07</td>
</tr>
<tr>
<td>Effluent Irrigated</td>
<td>32.1</td>
<td>2.6 ± 0.49</td>
</tr>
<tr>
<td>Mains-Water Irrigated</td>
<td>37.9</td>
<td>0.6 ± 0.15</td>
</tr>
<tr>
<td>Summer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Irrigated Control</td>
<td>55.4</td>
<td>22.5 ± 1.66</td>
</tr>
<tr>
<td>Effluent Irrigated</td>
<td>76.3</td>
<td>9.1 ± 0.67</td>
</tr>
<tr>
<td>Mains-Water Irrigated</td>
<td>87.1</td>
<td>14.2 ± 1.12</td>
</tr>
</tbody>
</table>

(Values are means ± 1 SE, n = 54 per sampling occasion)

In autumn, bacterial feeding nematode densities were significantly higher ($F_{(2,6)}=8.918$, $p<0.05$) in the non-irrigated control sites than in both irrigated treatments (effluent irrigated $p<0.001$; mains-water irrigated $p<0.001$). As noted earlier this significant increase in nematode density is thought to be associated with the increased levels of microbial biomass also observed in these autumn non-irrigated sites. Bacterial feeding nematode densities did not vary significantly in summer between treatments ($F_{(2,6)}=0.640$, $p=0.558$).

There are a number of published studies on the impact of irrigation on nematode density. A significant proportion of these studies have been based in desert ecosystems in the Negev Highlands of Israel (i.e. Alon and Steinberger, 1999; Liang and Steinberger, 2001; Steinberger and Sarig, 1993; Steinberger and Wallwork,
1985) or in the Chihuahuan desert in New Mexico (i.e. Freckman et al., 1987; Whitford et al., 1981; Whitford et al., 1982). The nematode response to irrigation is inconsistent, with some authors reporting that irrigation has no significant effect on nematode density (Bakonyi and Nagy, 2000; Freckman et al., 1987) while others report a positive correlation between irrigation and nematode density (Steinberger and Sarig, 1993; Whitford et al., 1981). Mesocosm-based analysis of nematode responses to irrigation have shown that individual species respond very differently to variation in soil moisture content, with some species positively affected by irrigation and others negatively affected (Sohlenius, 1985). In general, fungal feeding nematodes tend to be negatively affected by irrigation while bacterial feeding nematodes tend to be positively affected by irrigation (Bakonyi and Nagy, 2000; Sohlenius, 1985) a trend supported by the results from this research study.

In a New Zealand study (Waitarere), nematode density and functional composition was analysed in soil under a *Pinus radiata* plantation established on dune sands that were spray irrigated with treated domestic sewage effluent (Yeates, 1995). After two years of effluent irrigation the total number of nematodes was not significantly affected, however nematode functional composition was significantly different between the control and irrigated sites. Nematode populations under effluent irrigation displayed significant increases in the density of predatory and bacterial feeding nematodes, with significant declines in the density of fungal feeders and omnivores. A similar trend was observed in this Albany based study.
5.5.3 Nematode Community Structure in the Autumn Non-Irrigated Control Site

The nematode community in the autumn non-irrigated control site deserves special attention for two reasons. First, a high percentage contribution by animal predator nematodes occurred only in this season and site and second, the high level of nematode density highlights a potential relationship with the high level of microbial biomass observed in the same season and site.

Animal predator nematodes feed on other nematodes and protozoa (Freckman and Baldwin, 1990; Yeates et al., 1993). Their low contribution to nematode populations in all other seasons and treatments may be a consequence of the overall low levels of nematode density. It seems reasonable that low prey densities will support low predator densities.

Other than a significant contribution to the nematode community in the autumn non-irrigated control site, this predatory nematode was rarely encountered. Of the eighteen soil cores analysed for the autumn non-irrigated control sites Monochida sp.1 was only found in two samples. Of these two samples, this predatory nematode was counted ten times in one sample, and more than a thousand times in the second sample. This reflects the aggregated pattern of soil nematode distribution. The second reason for focusing on the nematode population in the autumn non-irrigated control was the overall increase in the nematode density relative to the two irrigated treatments and the potential connection between this and the increased
level of microbial biomass observed in the same season and sites. Even if the animal predator nematode is removed from the data set, the overall nematode density in the autumn control site is still substantially higher than the nematode density observed in the two irrigated treatments. This increase in overall nematode density is largely attributable to the increase in bacterial and hyphal feeding nematodes. Bacterial feeding nematodes were extracted from all eighteen soil samples, while hyphal feeding nematodes were rarely encountered (three samples). Bacterial feeding nematodes were approximately five times as abundant in the autumn non-irrigated control site compared to both irrigated sites. Pearson's correlation coefficient was calculated using SPSS to determine if densities of bacterial feeding nematodes correlated with levels of microbial biomass in autumn. As expected there was no significant correlation in autumn effluent ($p=0.214$) and mains water irrigated sites ($p=0.051$). However, the non-irrigated control sites despite their elevated levels of microbial biomass and bacterial feeding nematodes also displayed no significant correlation ($p=0.256$). This lack of a significant correlation is probably a result of the aggregated pattern of distribution.

In summary, levels of nematode density and species richness at the Albany Effluent Irrigated Tree Farm are very low and comparable to extreme water stressed environments such as deserts, tundra or Antarctica. The low level of nematode density and species richness has made it difficult to identify statistically significant irrigation related effects. However there is evidence that irrigation has affected the nematode communities by dampening seasonal fluctuations in density and by selecting for bacterial feeding nematodes.
“the tiny mites sustain our planet earth”

Juan B. Morales-Malacara
6.0 Soil Acari

6.1 INTRODUCTION

Acari (mites) are ubiquitous, tiny (typically >1 mm), inhabitants of aquatic, terrestrial, arboreal and parasitic habitats (Walter and Proctor, 1999). They are one of the largest and most biologically diverse groups within the class Arachnida (Evans, 1992; Krantz, 1978). More than any other habitat, mites dominate the soil-litter stratum where they function as comminuting microbivore-detritivores (bacterial, fungal or detritus feeding), piercing-sucking microbivores (fluid feeders), filter feeders, direct plant parasites, indirect plant parasites, nematophages and arthropod predators (Walter and Proctor, 1999).

Experiments conducted in the Chihuahuan Desert examined the contribution of the acarine fauna to decomposition by actively excluding the Acari from litter samples and comparing their rates of decomposition with litter samples which were allowed to develop natural assemblages of acarine fauna (Santos et al., 1981; Whitford et al., 1980; Whitford et al., 1983). These authors found that when the acarine fauna were excluded (via fungicides and insecticides) from the decomposition process the rate of decomposition was reduced by as much as 30%. This reduction in litter decomposition was linked to the comminuting role of the acarine fauna. Comminution, the physical breakdown of organic matter, promotes the process of decomposition by increasing the surface area available for microbial colonization.
and is the major indirect impact of the soil Acari on ecosystem processes (Edwards et al., 1970; Lebrun, 1979; Moore et al., 1988; Visser, 1985).

The single major indirect effect of mites on decomposition is via selective grazing. By actively predating specific species, the acarine fauna can mediate microbial succession and so impact on the rate of decomposition and nutrient cycling (Bardgett et al., 1999; Klironomos and Kendrick, 1995; Parkinson et al., 1979). It has been suggested that populations of Acari may be regulated by the density of microbial flora (bottom-up resource control) and the density of predatory fauna (top-down predatory control). However, the experimental evidence to support both of these processes is inconclusive and contradictory. Studies of trophic cascades in soil systems have presented a confusing and ambiguous picture. Some authors have reported strong evidence for top-down and bottom-up control (Santos et al., 1981; Scheu, 2002), others have found no evidence for either process (Gutierrez et al., 1994; Mikola and Setala, 1998b; Ponsard et al., 2000) while still others have found intermittent evidence for these structuring forces (Dawes-Gromadzki, 2002; Moran and Scheidler, 2002).

Soil mites, in particular the oribatid mite fauna, have been suggested as potential indicators of disturbance, stress and rehabilitation (Aoki, 1979; Behan-Pelletier, 1999; Franchini and Rockett, 1996; Kay et al., 1999; Linden et al., 1994; Paoletti and Bressan, 1996; Paoletti et al., 1991; Wardle et al., 1995). The particular focus on oribatids is proposed because of their life history characteristics, sensitivity to perturbation and stress and because they typically display high diversity, occur in
large numbers, are relatively easy to sample and extract, and are relatively easy to identify (Behan-Pelletier, 1999). Oribatid mites in agricultural soil have been found to display a variety of responses to tillage including insensitivity to the tillage regime, reduced density - negative sensitivity while other oribatid mites displayed increased density - positive sensitivity (Franchini and Rockett, 1996). The use of oribatid mites as indicators of stress or rehabilitation success is still very much in its infancy. The lack of life history and similar biological data severely limits the analysis of direct causal links between perturbation and/or stress and the resulting impact on soil oribatid mite populations. This Albany based study hopes to add to this small but growing body of research that specifically examines the impact of perturbation on oribatid mite fauna.

In this chapter, I describe the soil acarine populations and trophic structure under non-irrigated E. globulus plantations, presented as the ‘baseline’ data. I then analyse the acarine trophic structure in soil irrigated with effluent and mains-water, and explore statistically the impact of both season and treatment on soil acarine populations and trophic structure. The aims of this chapter are to describe,

a) baseline soil acarine diversity and density;
b) baseline soil acarine trophic structure;
c) soil acarine density and diversity in irrigated (effluent and mains-water) sites;
d) soil acarine trophic structure in irrigated (effluent and mains-water) sites;
e) the impact of season and treatment on acarine density, diversity and trophic structure.
Research hypothesis 5. Soil acarine species richness will be negatively affected by irrigation following the trend reported by Dindal et al., (1975).

Research hypothesis 6. Soil Oribatid mites will be particularly sensitive to irrigation, supporting previous reports that Oribatid mites are particularly sensitive to perturbations (Behan-Pelletier, 1999; Dindal et al., 1975; Franchini and Rockett, 1996).

6.2 METHODS

Soil samples were collected using the method detailed in section 2.8.1 (Sampling Design and Common Methods – Soil Sampling) with the following modifications.

Soil samples were taken using metal cores (5 cm diameter x 10 cm). Once extracted from the ground each metal core was wrapped in medical gauze to reduce movement of the soil and placed in a zip lock plastic bag for transport. A total of ten samples were taken from each treatment regime (n = 3) within each replicate site (n = 3), for a total of 90 samples per sampling occasion.

6.2.1 Extraction

Soil fauna were extracted from inverted soil cores by dry heat extraction using infra-red extractors after the method of Kempson, et al., (1963). Extractions were carried out over 10 days, with the final surface temperatures exceeding 65°C. This resulted in a vertical temperature gradient of around 40°C across the soil core (65°C above
the soil core and 20°C immediately below the soil core). This ensured that in the final two days of extraction, all parts of the soil core were exposed to temperatures exceeding 40°C. Soil fauna was collected into picric acid (Kempson et al., 1963), and following filtration was stored in 70% alcohol.

6.2.2 Identification

Samples were sorted under a dissecting microscope (Olympus SZH 10-70x). Temporary mounts of specimens for preliminary identification were made using cavity slides and small quantities of 50% lactic acid. To aid in clearing specimens, slides were gently warmed on slide drying benches (Electrotermal®). Permanent mounts were made using Hoyer’s solution, and all specimens were stored in the Edith Cowan University Invertebrate Collection. Soil mites were identified to Order and where possible to family and morphospecies. In the remainder of the thesis ‘species’ refers to ‘morphospecies’.

6.2.3 Statistical Analysis

Statistical analysis was carried out using the method detailed in section 2.8.6. (Sampling Design and Common Methods – Statistical Analysis). All raw data was log (natural) transformed to ensure normal distributions and equal variances. Multivariate analysis (classification and ordination) was carried out on standardised
and 4th root transformed data (designated $\sqrt[4]{\sqrt{\hat{\cdot}}}$) following the recommendation of Clarke and Gorley (2001).

**6.3 RESULTS**

**6.3.1 Acarine Density**

Mean soil acarine densities for all treatments and season are presented in Figure 6.1 and Table 6.1. Maximum and minimum acarine densities were both recorded in the mains-water irrigated treatment. Maximum densities of $29.8 \pm 3.80 \text{ N m}^{-2} \times 10^3$ were observed in spring and minimum densities of $3.4 \pm 0.67 \text{ N m}^{-2} \times 10^3$ were observed in summer. Acarine densities in all sites were lowest in summer 2001 samples, when levels of soil moisture were lowest (Fig 6.1 and Table 6.1). Spring densities in all treatments were dominated by the astigmatid mite Acaridae Tyrophagus sp.

**6.3.1.1 Seasonal Responses**

Mean acarine densities in the non-irrigated control site did not vary significantly due to season despite the substantial reduction in density observed in the summer samples relative to the autumn and spring samples ($F(2,6)=2.211, p=0.188$). The acarine density in this site appears to be correlated with patterns of soil moisture and temperature. Mean acarine density was highest in spring associated with warm moist soil conditions and lowest during summer, associated with hot dry soil conditions.
Figure 6.1. Mean soil Acari density (N m^{-2}) under three treatment regimes: non-irrigated control, effluent irrigated, and mains-water irrigated (values are means ± 1 SE, n = 90 per sampling occasion).

Table 6.1. Mean soil Acari density (N m^{-2} x 10^3) under three treatment regimes: non-irrigated control, effluent irrigated, and mains-water irrigated.

<table>
<thead>
<tr>
<th></th>
<th>Autumn</th>
<th>Spring</th>
<th>Summer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Non-Irrigated</td>
<td>18.9 ± 5.08</td>
<td>21.2 ± 3.25</td>
<td>7.6 ± 2.76</td>
</tr>
<tr>
<td>Effluent Irrigated</td>
<td>9.5 ± 2.13</td>
<td>28.3 ± 4.02</td>
<td>4.5 ± 1.41</td>
</tr>
<tr>
<td>Mains-Water Irrigated</td>
<td>15.2 ± 7.65</td>
<td>29.8 ± 3.80</td>
<td>3.4 ± 0.67</td>
</tr>
</tbody>
</table>

(Values are means ± 1 SE, n = 90 per sampling occasion)
Acarine densities in both irrigated treatments varied significantly due to season (effluent irrigated $F_{(2,6)}=13.060$, $p=0.006$; mains-water irrigated $F_{(2,6)}=29.676$, $p=0.000$). In both treatments, spring densities were significantly higher than both autumn (effluent irrigated $p<0.001$; mains-water irrigated $p<0.001$) and summer densities (effluent irrigated $p<0.05$; mains-water irrigated $p<0.001$) but autumn densities were not significantly different to summer (effluent irrigated $p=0.059$; mains-water irrigated $p=0.122$). The high spring densities responsible for this statistically significant seasonal variation are almost exclusively due to the very high densities of *Tyrophagus* sp. This astigmatid mite routinely contributed more than 80% of the total acarine density within each soil core in the spring sample. In a subsequent section, (Acarine Ordinal Abundance) I will consider the impact of *Tyrophagus* sp., on acarine density and community composition more fully.

### 6.3.1.2 Treatment Responses

There were no statistically significant treatment effects on soil acarine density in autumn ($F_{(2,6)}=0.070$, $p=0.933$), spring ($F_{(2,6)}=0.827$, $p=0.480$), or summer ($F_{(2,6)}=0.324$, $p=0.734$).

### 6.3.2 Acarine Species Composition

Forty-seven species was identified (Table 6.3). Forty species were identified from the non-irrigated control soil, thirty-four species from effluent irrigated soils and thirty-three species from mains water irrigated soils. The Prostigmata was the most
species rich order with twenty-three species in fourteen families. Twelve species of Mesostigmata were also identified (the majority from one family, Ascidae) though mites of this order were rare and frequently immature. Three of the twelve mesostigmatid species were single occurrences. Oribatid mites were the next most species rich group with eleven species in eight families. The Astigmata were represented by the single species Acaridae Tyrophagus sp. Five species were ubiquitous, being found in all treatments in all seasons, Tyrophagus sp., two Tydeidae - Ididorryia sp., and Tydeidae sp 2., Tarsonemidae sp., and an Oppiidae.

### 6.3.2.1 Species Samples Curves

If both density and species richness are considered concurrently it becomes clear that a small subset of species is responsible for a large proportion of the overall density. The three species Tyrophagus, Tydeidae sp 2., and Tarsonemidae sp., were responsible for >80% of the total density. This pattern of distribution in which a small number of species are responsible for a disproportionately large percentage of the density is not uncommon, particularly in soil communities (Krebs, 1989; Krebs, 1994).

Species samples curves can be utilised to determine if sampling effort (number of soil cores) has been sufficient. The resulting curves are typically logarithmic in shape (Krebs, 1989). That is, initial small increases in sampling effort result in a large increase in the cumulative number of species. As sampling effort increases
<table>
<thead>
<tr>
<th></th>
<th>Autumn</th>
<th>Spring</th>
<th>Summer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Effluent</td>
<td>Water</td>
</tr>
<tr>
<td>ACARIDAE Tyrophagus sp</td>
<td>46.7 ± 16.42</td>
<td>45.1 ± 9.82</td>
<td>21.9 ± 8.54</td>
</tr>
<tr>
<td>Brachychthoniidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brachychthonius sp 1</td>
<td>-</td>
<td>-</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>-</td>
<td>1.9</td>
</tr>
<tr>
<td>Brachychthonius sp 3</td>
<td>0.3</td>
<td>0.3</td>
<td>1.0 ± 0.53</td>
</tr>
<tr>
<td>Brachychthonius sp 4</td>
<td>-</td>
<td>-</td>
<td>0.2</td>
</tr>
<tr>
<td>Ceratozetidae</td>
<td>0.2</td>
<td>-</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>-</td>
<td>0.2</td>
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<td></td>
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<td>-</td>
<td>0.2</td>
</tr>
<tr>
<td>Ophiidae sp 1</td>
<td>6.6 ± 0.51</td>
<td>3.6 ± 1.34</td>
<td>2.4 ± 0.33</td>
</tr>
<tr>
<td></td>
<td>1.6 ± 1.00</td>
<td>0.2</td>
<td>2.0 ± 0.34</td>
</tr>
<tr>
<td></td>
<td>1.6 ± 1.00</td>
<td>0.2</td>
<td>2.0 ± 0.34</td>
</tr>
<tr>
<td>Tectocephidae</td>
<td>0.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>-</td>
<td>0.2</td>
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<tr>
<td></td>
<td>0.2</td>
<td>-</td>
<td>0.2</td>
</tr>
<tr>
<td>Cryptognathidae</td>
<td>0.7 ± 0.23</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0.7 ± 0.23</td>
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<td></td>
<td>0.2</td>
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<td>0.2</td>
<td>-</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>-</td>
<td>0.2</td>
</tr>
<tr>
<td>Nanorchestidae</td>
<td>1.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Penthalodidae</td>
<td>0.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pygmephoroidea</td>
<td>1.5 ± 0.41</td>
<td>1.7</td>
<td>0.7 ± 0.52</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>Spring</td>
<td>Summer</td>
</tr>
<tr>
<td>----------------------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Effluent</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Water</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Effluent</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Water</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**PYGMEPHOROIDEA sp 2**
- - 1.0 ± 0.53 1.7 ± 0.31 0.3 0.2 0.2 - -

**RAPHIGNATHIDAE Raphignathus sp 1**
1.4 ± 0.25 0.3 0.2 0.2 0.5 ± 0.31 - 4.6 ± 2.81 2.9 ± 0.41 6.6 ± 0.27

**Raphignathus sp 2**
- - 0.2 - - 0.3 - 4.2 ± 2.51 5.1 ± 0.31 4.6 ± 0.22

**RHAGIDIIDAE Rhagidia sp**
0.9 - - - - 0.3 - -

**STIGMAEIDAE Eustigmaeus sp**
0.9 ± 0.25 0.3 1.9 - 0.2 0.2 1.0 ± 0.11 1.4 ± 0.32 2.2 ± 0.13

**STIGMAEIDAE Stigmaeus sp**
1.7 ± 0.67 - 0.2 0.3 0.2 - 0.2 - 2.6 ± 2.04

**TARSONEMIDAE sp**
47.6 ± 39.47 10.4 ± 4.58 7.8 ± 1.5 9.5 ± 0.32 25.9 ± 13.81 6.0 ± 0.31 4.9 ± 0.51 3.4 ± 0.31 2.2 ± 1.25

**TYDEIDAE Ididorryia sp**
5.4 ± 1.39 1.2 ± 0.51 3.8 ± 1.4 1.0 1.9 ± 0.11 0.5 ± 0.22 0.9 ± 0.86 0.5 0.7 ± 0.25

**TYDEIDAE Pretydeus sp**
0.7 - 0.2 - - - - -

**TYDEIDAE sp 1**
- 0.2 - 0.2 - - - -

**sp 2**
60.7 ± 22.93 16.3 ± 4.73 44.4 ± 26.71 4.4 ± 0.92 5.3 ± 0.31 4.4 ± 1.01 20.9 ± 13.81 8.7 ± 6.61 3.7 ± 0.61

**sp 3**
0.5 - - - - - - -

**ASCIDAE Asca sp**
0.2 0.3 0.3 - - - 0.5 0.2 0.5

**ASCIDAE Gamesellodes sp**
0.3 0.5 ± 0.21 0.2 0.7 ± 0.21 0.2 0.2 0.7 ± 0.51 0.3 -

**ASCIDAE Protogamacellus sp**
0.9 ± 0.12 - 0.9 - 0.3 0.7 ± 0.12 - - 0.3

**AMEROSEIIDAE sp**
0.2 - 17.5 ± 2.42 0.2 - 0.5 - - -

**ASCIDAE sp 1**
- - - - - - - -

**sp 2**
0.2 - - 0.2 - 0.2 - - 0.3

**sp 3**
- 0.2 - - - - - 0.2 -

**sp 4**
- - 0.3 - - - - -

**DIGIMASELLIDAE sp**
- 0.2 0.9 ± 0.22 - 0.3 1.3 ± 0.42 0.3 0.7 ± 0.17 0.7 ± 0.17

**PHYTOSEIDAE sp**
0.2 0.2 33.9 0.2 - 0.5 ± 0.31 - - -

**RHODACARIDAE sp**
- - 0.2 - 2.0 ± 0.41 - - -

**Mesostigmata sp 1**
0.5 2.0 ± 0.73 0.5 ± 0.31 - - 0.2 - - -

(Values are means ± 1 SE, n = 90 per sampling occasion. SE values could not be calculated for some individuals with low frequencies of occurrences)
the number of new species tends to decrease until it reaches an asymptote were no
new species are identified despite substantial increases in sampling effort. Most of
the ninge graphs in Figure 6.2 display an initial increase in species count with
sample effort followed by a plateau in the number of species identified at higher
levels of sampling effort. This suggests that sufficient soil cores were sampled.

6.3.2.2 Species Distribution

Organisms are distributed in their habitat in one of three spatial patterns: uniform,
random or aggregated (Krebs, 1989). The standardized Morisita index of dispersion
is considered to be one of the best measures of dispersion because it is
independent of population density and sample size and because it provides results
on an absolute scale of –1 (maximum uniformity), 0 (random), +1 (maximum
aggregation) (Krebs, 1989). The NEGBINOM program version 4.2 within Krebs
Ecological Software allows for the computation of the standardized Morisita index of
dispersion. This program was used to analyse acarine data. The acarine
distributions at the Albany Effluent Irrigated Tree Farm were always highly
aggregated with all values exceeding 0.5 which is the 95% confidence level for this
index (Krebs, 1989). Aggregated distribution is defined as ‘given the location of one
individual there is an increased probability that another individual is nearby’ (Krebs,
1989; Krebs, 1994). This pattern of distribution for soil organisms is particularly
common in Mediterranean climates and is thought to be linked to the varying organic
matter content found in soil (di Castri and Vitali-di Castri, 1981; Usher, 1976; Usher
et al., 1982). While the distribution of organic matter is thought to be primarily
Figure 6.2. Species samples curves for each season and treatment displaying sampling effort and cumulative numbers of species.
responsible for differences in species distribution, species diversity and community structure, variation in water availability due to climate is thought to be the chief determinant of population size in Mediterranean climates (di Castri and Vitali-di Castri, 1981; Huhta and Hanninen, 2001; Wallwork et al., 1986; Zak and Freckman, 1991).

6.3.2.3 *Species Richness, Diversity and Evenness*

Table 6.3 details species richness, species diversity and evenness measures for each treatment within each season. Species richness was highest in the autumn (25 to 30) and lowest in summer (23 to 17) and, within each season, was always higher in the control sites than in the irrigated sites. Despite the reduced summer densities, species richness remained relatively high. Statistically however, there was no significant impact of season (non-irrigated control $F_{2,6}=0.201$, $p=0.824$; effluent irrigated $F_{2,6}=1.775$, $p=0.248$; mains-water irrigated $F_{2,6}=0.311$, $p=0.744$) or treatment (autumn $F_{2,6}=0.163$, $p=0.853$; spring $F_{2,6}=1.674$, $p=0.265$; summer $F_{2,6}=1.178$, $p=0.370$) on species richness.

Species richness, evenness and diversity were similar across treatments and seasons with the exception of spring levels of evenness and diversity. In this season the relative dominance of *Tyrophagus* sp. (>80%) at each treatment site, resulted in substantially lower diversity ($H'$) and evenness ($J'$) values in spring relative to autumn and summer, although levels of species richness are comparable to autumn and summer levels.
Table 6.3. Acari species richness (SR), diversity ($H'$) and evenness ($J'$) under three treatment regimes: non-irrigated control, effluent irrigated, and mains-water irrigated for autumn, spring and summer.

<table>
<thead>
<tr>
<th>Season</th>
<th>Treatment</th>
<th>SR</th>
<th>$H'$</th>
<th>$J'$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autumn</td>
<td>Non-Irrigated Control</td>
<td>30</td>
<td>1.75</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>Effluent Irrigated</td>
<td>25</td>
<td>1.73</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>Mains-Water Irrigated</td>
<td>28</td>
<td>2.06</td>
<td>0.62</td>
</tr>
<tr>
<td>Spring</td>
<td>Non-Irrigated Control</td>
<td>25</td>
<td>0.84</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>Effluent Irrigated</td>
<td>19</td>
<td>0.72</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>Mains-Water Irrigated</td>
<td>21</td>
<td>0.38</td>
<td>0.13</td>
</tr>
<tr>
<td>Summer</td>
<td>Non-Irrigated Control</td>
<td>23</td>
<td>2.22</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>Effluent Irrigated</td>
<td>17</td>
<td>2.14</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>Mains-Water Irrigated</td>
<td>17</td>
<td>2.39</td>
<td>0.85</td>
</tr>
</tbody>
</table>

6.3.3 Classification and Ordination Analysis of Acarine Density

Classification (Fig 6.3) and ordination (Fig 6.4) was conducted on standardized 4th root transformed mean acarine species densities to identify similarities or differences in acarine community composition at each of the treatment sites within each season. Fourth root transformations were applied following the advice of Clarke and Gorley (2001) who observed that the 4th root transformation has a ‘fairly’ severe effect in down-weighting the importance of the very abundant species so that less dominant, and even rare species play some role in determining the similarity between samples. Given the dominance in the spring samples by *Tyrophagus* sp., particularly in spring, it is appropriate to utilise this transformation.
Figure 6.3. Dendogram for hierarchical clustering of nine acarine communities using Bray-Curtis similarities calculated on standardized $\sqrt{V}$-transformed densities.

Figure 6.4. MDS ordination of nine acarine communities based on standardized $\sqrt{V}$-transformed densities.
Figure 6.3 shows all sites clustering by season rather than by treatment with all communities grouped at a Bray-Curtis level of similarity just above 50%. The widely spread and variable distance between ordinated communities (Fig. 6.4) suggest a less interpretable pattern than the cluster analysis (Fig. 6.3), with no consistent treatment or season response. ANOSIM revealed no significant seasonal or treatment related effects (Global R=0.053, p=39.3%)

### 6.3.4 Acarine Ordinal Analysis

Analysis of the soil acarine communities at the level of order has been conducted as a precursor to the more complex species level analysis of the next section. Within the ecological literature, studies of soil invertebrates frequently identify soil acari to the level of order and compare ordinal ratios. It has been suggested that the ratio is sensitive to levels of organic matter (Edwards and Lofty, 1969). In both autumn and summer, prostigmatid mites dominated the acarine communities, with small contributions from mesostigmatid and oribatid mites while astigmatid mites dominated the spring samples.

#### 6.3.4.1 Astigmata

Astigmatid mite (*Tyrophagus* sp.) densities (Table 6.4) varied significantly between seasons in all treatments (p<0.05) with spring densities > autumn > summer densities (non-irrigated control $F_{(2,6)}=10.952$, p=0.010), effluent irrigated ($F_{(2,6)}=11.331$, p=0.009), and mains-water irrigated ($F_{(2,6)}=51.565$, p<0.001). However
within each season astigmatid densities did not vary significantly due to treatment (autumn $F_{(2,6)}=0.497$, $p=0.631$, spring $F_{(2,6)}=0.482$, $p=0.640$, summer $F_{(2,6)}=0.253$, $p=0.123$).

6.3.4.2 Prostigmata

There were no statistically significant effects of season (autumn $F_{(2,6)}=0.269$, $p=0.772$, spring $F_{(2,6)}=1.555$, $p=0.281$, summer $F_{(2,6)}=0.275$, $p=0.768$) or treatment (non-irrigated control $F_{(2,6)}=0.733$, $p=0.517$), effluent irrigated ($F_{(2,6)}=2.238$, $p=0.182$), and mains-water irrigated ($F_{(2,6)}=0.282$, $p=0.762$) on the density of prostigmatid mites. In both autumn and summer, prostigmatid mites (Table 6.4) were the dominant acarine order, comprising between 36.57% and 62.33% of the populations.

In autumn, in the non-irrigated control sites, prostigmatid mite densities were two to four times greater than densities in the irrigated treatments. There may be a bottom-up resource link between this high level of prostigmatid mite density and the higher levels of nematode density and microbial biomass observed simultaneously at this site.

6.3.4.3 Mesostigmata

There were no statistically significant effects of season (autumn $F_{(2,6)}=0.977$, $p=0.427$, spring $F_{(2,6)}=0.217$, $p=0.810$, summer $F_{(2,6)}=0.706$, $p=0.549$) or treatment (non-irrigated control $F_{(2,6)}=0.384$, $p=0.701$), effluent irrigated ($F_{(2,6)}=0.612$, $p=0.565$),
Table 6.4. Mean soil acarine ordinal density (N m$^{-2}$) and relative density (%) under three treatment regimes: non-irrigated control, effluent irrigated, and mains-water irrigated.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Astigmata (Tyrophagus sp.)</th>
<th>Prostigmata</th>
<th>Mesostigmata</th>
<th>Oribatida</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N m$^{-2}$)</td>
<td>(relative density)</td>
<td>(relative density)</td>
<td>(relative density)</td>
<td></td>
</tr>
<tr>
<td><strong>Autumn</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Irrigated</td>
<td>4675 ± 1311 (23.7)</td>
<td>12784 ± 3484 (62.3)</td>
<td>544 ± 105 (4.4)</td>
<td>935 ± 181 (9.5)</td>
<td>18938 ± 5082</td>
</tr>
<tr>
<td>Effluent Irrigated</td>
<td>4522 ± 922 (38.9)</td>
<td>3791 ± 753 (36.5)</td>
<td>680 ± 229 (11.7)</td>
<td>476 ± 228 (12.7)</td>
<td>9469 ± 2134</td>
</tr>
<tr>
<td>Mains-Water</td>
<td>2210 ± 662 (10.9)</td>
<td>6766 ± 1715 (38.3)</td>
<td>5576 ± 5050 (44.1)</td>
<td>663 ± 220 (6.5)</td>
<td>15215 ± 7649</td>
</tr>
<tr>
<td><strong>Spring</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Irrigated</td>
<td>17425 ± 2028 (71.6)</td>
<td>2482 ± 806 (13.3)</td>
<td>629 ± 187 (6.4)</td>
<td>636 ± 230 (8.5)</td>
<td>21172 ± 3252</td>
</tr>
<tr>
<td>Effluent Irrigated</td>
<td>23443 ± 2860 (77.8)</td>
<td>3723 ± 900 (14.2)</td>
<td>510 ± 91 (2.9)</td>
<td>595 ± 173 (4.9)</td>
<td>28271 ± 4024</td>
</tr>
<tr>
<td>Mains-Water</td>
<td>27608 ± 3182 (85.7)</td>
<td>1190 ± 360 (6.5)</td>
<td>544 ± 173 (5.0)</td>
<td>459 ± 81 (2.6)</td>
<td>29801 ± 3796</td>
</tr>
<tr>
<td><strong>Summer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Irrigated</td>
<td>697 ± 143 (10.5)</td>
<td>5355 ± 1942 (56.0)</td>
<td>255 ± 40 (8.6)</td>
<td>1275 ± 635 (24.7)</td>
<td>7582 ± 2761</td>
</tr>
<tr>
<td>Effluent Irrigated</td>
<td>748 ± 88 (16.6)</td>
<td>3383 ± 1253 (60.7)</td>
<td>136 ± 17 (9.0)</td>
<td>204 ± 50 (13.6)</td>
<td>4471 ± 1408</td>
</tr>
<tr>
<td>Mains-Water</td>
<td>442 ± 130 (20.1)</td>
<td>2635 ± 487 (51.5)</td>
<td>221 ± 37 (13.4)</td>
<td>136 ± 21 (14.8)</td>
<td>3434 ± 675</td>
</tr>
</tbody>
</table>

(Values are means ± 1 SE, n = 90 per sampling occasion)
and mains-water irrigated ($F_{(2,6)}=0.789, p=0.481$) on the density of mesostigmatid mites.

Mesostigmatid mite densities were similar across all treatments and seasons (Table 6.4), with the exception of the autumn mains-water irrigated sites. Mesostigmatid densities in this particular season and treatment were approximately ten times higher than in any other treatment or season. This peak in mesostigmatid density was due to singular large increases in the density of two mites, Ameroseiidae sp. and Phytoseiidae sp.

6.3.4.4 Oribatida

There were no statistically significant effects of season (autumn $F_{(2,6)}=0.804, p=0.487$, spring $F_{(2,6)}=2.957, p=0.144$, summer $F_{(2,6)}=0.856, p=0.479$) or treatment (non-irrigated control $F_{(2,6)}=0.421, p=0.674$), effluent irrigated ($F_{(2,6)}=0.453, p=0.659$), and mains-water irrigated ($F_{(2,6)}=1.374, p=0.306$) on the density of oribatid mites.

Although not statistically significant, oribatid mite densities (Table 6.4) were always lower in the irrigated treatments relative to the non-irrigated control. In summer, when temperatures and irrigation were maximal, oribatid mite densities were substantially lower in both irrigated sites relative to the non-irrigated control site.
6.3.5 Classification and Ordination Analysis of Acarine Ordinal Density

Acarine ordinal composition was analysed using hierarchical agglomerative classification (Fig. 6.5) and MDS ordination (Fig. 6.6). The most obvious outcome is the high similarity between all sites. Consistent with the previous results, spring sites are clustered separately from autumn and summer sites reflecting the impact of *Tyrophagus* sp. on community structure. The MDS pattern in Figure 6.6 supports the pattern seen in the cluster analysis, with spring samples separating from autumn and summer samples.

![Dendogram for hierarchical clustering of nine acarine communities using Bray-Curtis similarities calculated on standardized √n-transformed acarine ordinal densities.](image)

Figure 6.5. Dendogram for hierarchical clustering of nine acarine communities using Bray-Curtis similarities calculated on standardized √n-transformed acarine ordinal densities.
ANOSIM results revealed that there was a significant effect of season on community structure (Global $R=0.794, p=0.7\%$) with autumn ($R=0.963, p=10\%$) and summer ($R=1.000, p=10\%$) significantly different to spring but not significantly different from each other ($R=0.222, p=20\%$).

6.3.6 Oribatid Mites

6.3.6.1 Classification and Ordinal Analysis of Oribatid Mite Densities

Since the effect of irrigation type on oribatid mite densities was not statistically significant, data from both irrigated treatments was pooled in order to determine if
irrigated communities were different to non-irrigated communities. Figure 6.7 shows the control and irrigated treatments clustering separately before joining at a Bray-Curtis similarity level of >55%. Despite the high degree of similarity, non-irrigated communities clustered separately from irrigated communities, suggesting that irrigation was associated with different oribatid mite communities.

The MDS pattern of Figure 6.8 supports the pattern of the cluster analysis (Fig 6.7) with irrigated and non-irrigated communities clearly separating from each other. ANOSIM results revealed that similarities within communities were less than any similarities between communities (Global R=0.704, p=10%).

![Dendogram](image)

**Figure 6.7.** Dendogram for hierarchical clustering of six acarine communities using Bray-Curtis similarities calculated on standardized √-transformed oribatid mite density data.
6.3.6.2 Oribatid Mite Species

Eleven species of oribatid mites from eight families were identified (Table 6.5). Of these eleven species, five species (Oppiidae sp., Brachychnthionus sp 2., & sp 3., Tectocepheus velatus, and Zygoribatula sp.) were chosen for further analysis due to their high frequency of occurrence and/or treatment/season response.
Table 6.5. Mean soil oribatid mite species density (N m$^{-2}$) under three treatment regimes: non-irrigated control, effluent irrigated, and mains-water irrigated.

<table>
<thead>
<tr>
<th></th>
<th>Autumn</th>
<th>Spring</th>
<th>Summer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Effluent</td>
<td>Water</td>
</tr>
<tr>
<td>BRACHYCHTHONIIDAE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brachychthonius sp 1</td>
<td>-</td>
<td>-</td>
<td>17</td>
</tr>
<tr>
<td>Brachychthonius sp 2</td>
<td>17</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Brachychthonius sp 3</td>
<td>34</td>
<td>34</td>
<td>102 ± 510</td>
</tr>
<tr>
<td>Brachychthonius sp 4</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CERATOZETIDAE sp</td>
<td>17</td>
<td>-</td>
<td>85</td>
</tr>
<tr>
<td>COSMOCHTHONIIDAE</td>
<td>-</td>
<td>17</td>
<td>-</td>
</tr>
<tr>
<td>Cosmochthonius sp</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAPLOCHTHONIIDAE</td>
<td>17</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Haplochthonius sp</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOTHRIDAE sp</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Oppiid species 1</td>
<td>668 ± 477</td>
<td>357 ± 1382</td>
<td>238 ± 272</td>
</tr>
<tr>
<td>ORIBATULIDAE</td>
<td>153 ± 1020</td>
<td>17</td>
<td>204 ± 346</td>
</tr>
<tr>
<td>Zygoribatula sp</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TECTOCEPHEIDAE</td>
<td>34</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tectocephalus velatus</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Values are means ± 1 SE, n = 90 per sampling occasion. SE values could not be calculated for some individuals with low numbers of occurrences)
Three of the oribatid species (oppiid mites, *Brachychthonius* sp 2., and *Tectocepheus velatus*) displayed a consistent negative response to irrigation. The oppiid mites were present in every treatment in all seasons. Although oppiid densities were lower in summer, when rates of irrigation were highest and rainfall was lowest, this result was not statistically significant ($F_{(2,6)}=0.311, p=0.750$).

*Brachychthonius* sp 2., and *Tectocepheus velatus*, were present in each season but only in the non-irrigated control sites. *Brachychthonius* sp 3., displayed no treatment response being present in all treatment in both autumn and spring but was absent from all treatments in summer. Although *Zygoribatula* sp., was present in nearly all treatments and seasons it displayed no consistent treatment or seasonal variation.

Variation in oribatid mite communities associated with irrigation (Figures 6.7 and 6.8) was largely due to the Oppiidae, *Brachychthonius* sp 2., and *Tectocepheus velatus*. If these three species are removed from the oribatid mite data, classification and ordination reveals no season or treatment related pattern and ANOSIM returns a negative result (Global $R = -0.704, p=100\%$).

The oppiid mites comprise two species Oppiiinae *Vietoppia* sp? and Oppiellinae sp. Unfortunately, these two species were not separated in the early samples. Therefore, "Oppiidae" is a 2-species group. It was therefore not possible to separate the species’ responses.
6.3.7 Acarine Functional Groups

All adult acarine fauna were allocated to one of seven functional groups (phytophagous, predacious, fungivorous, saprophagous, algivorous, parasitoid, and nematophagous) as defined by Smith *et al.*, (1999) using information sources listed in Table 6.6. The percentage contribution of each functional group to the overall acarine density and the number of species within each functional group is presented in Table 6.7.

Classification of acarine fauna into functional groups allowed for an analysis of season and treatment on those fauna thought occupying similar trophic levels. Due to their low relative density and low levels of species richness, saprophagous, algivorous and parasitoid mites have been excluded from further statistical analysis. Although there were no statistically significant treatment or seasonal affects on acarine functional groups there were interesting faunal group responses. The phytophagous group was dominated exclusively by Acaridae *Tyrophagus* sp., and as noted previously this species’ densities peaked in spring at all sites. The density of nematophagous mites was consistently lowest in spring irrespective of treatment. In both autumn and summer, the relative density of nematophagous mite was consistently lower in the irrigated treatments relative to the non-irrigated control. The substantial decline in the relative density of nematophagous mites in spring coincided with a significant reduction in nematode densities in spring (Table 5.1 and Fig. 5.1).
Table 6.6. Possible functional groupings of soil mite fauna found under *E. globulus* plantations in Albany, South West Australia. (Soil fauna are listed alphabetically. Where a soil mite could be ascribed to multiple functional groups I choose the first functional group listed).

<table>
<thead>
<tr>
<th>Taxonomic Classification</th>
<th>Functional Group</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACARIDAE <em>Tyrophagus</em> sp</td>
<td>Phytophagous</td>
<td>(Kethley, 1990; Krantz, 1978; Mueller et al., 1990; Walter and Proctor, 1999)</td>
</tr>
<tr>
<td>AMEROSEIIDAE sp</td>
<td>Predacious</td>
<td>(Koehler, 1999; Smith et al., 1999)</td>
</tr>
<tr>
<td>ASCIDAE <em>Asca</em> sp</td>
<td>Predacious, Fungivorous</td>
<td>(Koehler, 1999; Smith et al., 1999)</td>
</tr>
<tr>
<td>ASCIDAE <em>Gamesellodes</em> sp</td>
<td>Predacious, Fungivorous</td>
<td>(Koehler, 1999; Smith et al., 1999)</td>
</tr>
<tr>
<td>ASCIDAE <em>Protogamacellus</em>sp</td>
<td>Predacious, Fungivorous</td>
<td>(Koehler, 1999; Smith et al., 1999)</td>
</tr>
<tr>
<td>ASCIDAE sp 1</td>
<td>Predacious</td>
<td>(Koehler, 1999; Smith et al., 1999)</td>
</tr>
<tr>
<td>ASCIDAE sp 2</td>
<td>Predacious</td>
<td>(Koehler, 1999; Smith et al., 1999)</td>
</tr>
<tr>
<td>ASCIDAE sp 3</td>
<td>Predacious</td>
<td>(Koehler, 1999; Smith et al., 1999)</td>
</tr>
<tr>
<td>ASCIDAE sp 4</td>
<td>Predacious</td>
<td>(Koehler, 1999; Smith et al., 1999)</td>
</tr>
<tr>
<td>BDELLIDAE <em>Spinibdella</em> sp 1</td>
<td>Predacious, Nematophagous</td>
<td>(Smith et al., 1999; Walter and Proctor, 1999)</td>
</tr>
<tr>
<td>BRACHYCHTHONIIDAE <em>Brachychnthionius</em> sp 1</td>
<td>Fungivorous, Algivorus</td>
<td>(Smith et al., 1999)</td>
</tr>
<tr>
<td>BRACHYCHTHONIIDAE <em>Brachychnthionius</em> sp 2</td>
<td>Fungivorous, Algivorus</td>
<td>(Smith et al., 1999)</td>
</tr>
<tr>
<td>BRACHYCHTHONIIDAE <em>Brachychnthionius</em> sp 3</td>
<td>Fungivorous, Algivorus</td>
<td>(Smith et al., 1999)</td>
</tr>
<tr>
<td>BRACHYCHTHONIIDAE <em>Brachychnthionius</em> sp 4</td>
<td>Fungivorous, Algivorus</td>
<td>(Smith et al., 1999)</td>
</tr>
<tr>
<td>CERATOZETIDAE sp</td>
<td>Saprophagous, Fungivorous, Predacious</td>
<td>(Smith et al., 1999)</td>
</tr>
<tr>
<td>CHEYLETIDAE sp 1</td>
<td>Predacious</td>
<td>(Smith et al., 1999; Walter and Proctor, 1999)</td>
</tr>
<tr>
<td>CHEYLETIDAE sp 2</td>
<td>Predacious</td>
<td>(Smith et al., 1999; Walter and Proctor, 1999)</td>
</tr>
<tr>
<td>COSMOCHTHONIIDAE <em>Cosmochnthionius</em> sp</td>
<td>Algivorus</td>
<td>(Smith et al., 1999)</td>
</tr>
<tr>
<td>CRYPTOGNATHIDAE <em>Cryptognathus</em> sp</td>
<td>Predacious</td>
<td>(Smith et al., 1999)</td>
</tr>
<tr>
<td>CUNAXIDAE <em>Cunaxa</em> sp</td>
<td>Predacious</td>
<td>(Smith et al., 1999; Walter and Proctor, 1999)</td>
</tr>
<tr>
<td>DIGAMASELLIDAE sp</td>
<td>Predacious</td>
<td>(Koehler, 1999; Smith et al., 1999)</td>
</tr>
<tr>
<td>EUPODIDAE <em>Eupodes</em> sp 1</td>
<td>Fungivorous</td>
<td>(Krantz, 1978; Smith et al., 1998a)</td>
</tr>
<tr>
<td>EUPODIDAE <em>Eupodes</em> sp 2</td>
<td>Fungivorous</td>
<td>(Krantz, 1978; Smith et al., 1998a)</td>
</tr>
<tr>
<td>HAPLOCHTHONIIDAE <em>Haplochnthionius</em> sp</td>
<td>Fungivorous</td>
<td>(Koehler, 1999; Smith et al., 1999)</td>
</tr>
<tr>
<td>Taxon</td>
<td>Diet</td>
<td>Reference 1</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>---------------------------</td>
<td>------------------------------</td>
</tr>
<tr>
<td><strong>Mesostigmata sp 1</strong></td>
<td>Predacious</td>
<td>(Koehler, 1999; Smith et al., 1999)</td>
</tr>
<tr>
<td><strong>NANORCHESTIDAE Speleorchestes sp</strong></td>
<td>Fungivorous, Phytophagous</td>
<td>(Smith et al., 1999; Walter and Proctor, 1999)</td>
</tr>
<tr>
<td><strong>NOTHRIDAE sp</strong></td>
<td>Saprophagous</td>
<td>(Smith et al., 1999)</td>
</tr>
<tr>
<td><strong>OPPIIDAE sp 1</strong></td>
<td>Fungivorous</td>
<td>(Norton, 1990; Smith et al., 1999)</td>
</tr>
<tr>
<td><strong>ORIZATULIDAE Zygoribatula sp</strong></td>
<td>Fungivorous, Phytophagous</td>
<td>(Norton, 1990; Smith et al., 1999)</td>
</tr>
<tr>
<td><strong>PENTHALODIDAE Stereotydeus sp</strong></td>
<td>Phytophagous</td>
<td>(Walter and Proctor, 1999)</td>
</tr>
<tr>
<td><strong>PYEMOTOIDEA sp</strong></td>
<td>Parasitoid</td>
<td>(Smith et al., 1999; Walter and Proctor, 1999)</td>
</tr>
<tr>
<td><strong>PYGMEOBOIDEA sp 1</strong></td>
<td>Fungivorous</td>
<td>(Smith et al., 1999; Walter and Proctor, 1999)</td>
</tr>
<tr>
<td><strong>PYGMEOBOIDEA sp 2</strong></td>
<td>Fungivorous</td>
<td>(Smith et al., 1999; Walter and Proctor, 1999)</td>
</tr>
<tr>
<td><strong>PYTOSEIDAE sp</strong></td>
<td>Predacious</td>
<td>(Smith et al., 1999; Walter and Proctor, 1999)</td>
</tr>
<tr>
<td><strong>RAPHIGNATHIDAE Raphignathus sp 1</strong></td>
<td>Predacious</td>
<td>(Krantz, 1978; Smith et al., 1999)</td>
</tr>
<tr>
<td><strong>RAPHIGNATHIDAE Raphignathus sp 2</strong></td>
<td>Predacious</td>
<td>(Krantz, 1978; Smith et al., 1999)</td>
</tr>
<tr>
<td><strong>RHAGIDIIDAE Rhagidia sp</strong></td>
<td>Predacious</td>
<td>(Krantz, 1978; Smith et al., 1999)</td>
</tr>
<tr>
<td><strong>RHODACARIIDAE sp</strong></td>
<td>Predacious</td>
<td>(Koehler, 1999; Smith et al., 1999)</td>
</tr>
<tr>
<td><strong>STIGMAEIDAE Eustigmus sp</strong></td>
<td>Predacious, Phytophagous</td>
<td>(Krantz, 1978; Smith et al., 1999; Walter and Proctor, 1999)</td>
</tr>
<tr>
<td><strong>STIGMAEIDAE Stigmaeus sp</strong></td>
<td>Predacious, Phytophagous</td>
<td>(Krantz, 1978; Smith et al., 1999; Walter and Proctor, 1999)</td>
</tr>
<tr>
<td><strong>TARSONEMIDAE sp</strong></td>
<td>Fungivorous, Phytophagous, Parasitic, Parasitoid, Predacious</td>
<td>(Koehler, 1999; Smith et al., 1999)</td>
</tr>
<tr>
<td><strong>TECTOCEPHEIDAE Tectocephus velatus</strong></td>
<td>Fungivorous</td>
<td>(Krantz, 1978; Smith et al., 1999; Walter and Proctor, 1999)</td>
</tr>
<tr>
<td><strong>TYDEIDAE Ididorryia sp</strong></td>
<td>Nematophagous, Fungivorous, Phytophagous</td>
<td>(Smith et al., 1999; Walter and Proctor, 1999)</td>
</tr>
<tr>
<td><strong>TYDEIDAE Pretydeus sp</strong></td>
<td>Nematophagous, Fungivorous, Phytophagous</td>
<td>(Smith et al., 1999; Walter and Proctor, 1999)</td>
</tr>
<tr>
<td><strong>TYDEIDAE sp 1</strong></td>
<td>Nematophagous, Fungivorous, Phytophagous</td>
<td>(Smith et al., 1999; Walter and Proctor, 1999)</td>
</tr>
<tr>
<td><strong>TYDEIDAE sp 2</strong></td>
<td>Nematophagous, Fungivorous, Phytophagous</td>
<td>(Smith et al., 1999; Walter and Proctor, 1999)</td>
</tr>
<tr>
<td><strong>TYDEIDAE sp 3</strong></td>
<td>Nematophagous, Fungivorous, Phytophagous</td>
<td>(Smith et al., 1999; Walter and Proctor, 1999)</td>
</tr>
</tbody>
</table>
Table 6.7. Species richness and relative density (%) of acarine functional groups under three treatment regimes: non-irrigated control, effluent irrigated, and mains-water irrigated.

<table>
<thead>
<tr>
<th>Functional Group</th>
<th>Autumn Control</th>
<th>Autumn Effluent</th>
<th>Autumn Watered</th>
<th>Spring Control</th>
<th>Spring Effluent</th>
<th>Spring Watered</th>
<th>Summer Control</th>
<th>Summer Effluent</th>
<th>Summer Watered</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytophagous</td>
<td>1 (25.5)</td>
<td>2 (49.7)</td>
<td>1 (15.0)</td>
<td>1 (83.7)</td>
<td>1 (83.7)</td>
<td>1 (93.6)</td>
<td>1 (9.6)</td>
<td>1 (17.3)</td>
<td>1 (13.5)</td>
<td>2</td>
</tr>
<tr>
<td>Predacious</td>
<td>14 (4.6)</td>
<td>11 (5.4)</td>
<td>7 (39.4)</td>
<td>7 (0.9)</td>
<td>8 (1.5)</td>
<td>9 (1.3)</td>
<td>11 (11.7)</td>
<td>8 (25.5)</td>
<td>9 (61.5)</td>
<td>22</td>
</tr>
<tr>
<td>Fungivorous</td>
<td>10 (33.0)</td>
<td>7 (18.9)</td>
<td>9 (12.0)</td>
<td>12 (12.1)</td>
<td>8 (12.3)</td>
<td>7 (3.2)</td>
<td>7 (4.2)</td>
<td>5 (34.9)</td>
<td>4 (9.9)</td>
<td>14</td>
</tr>
<tr>
<td>Nematophagous</td>
<td>4 (36.9)</td>
<td>3 (19.4)</td>
<td>3 (33.1)</td>
<td>3 (2.7)</td>
<td>2 (2.6)</td>
<td>2 (1.7)</td>
<td>3 (2.7)</td>
<td>2 (1.2)</td>
<td>5 (1.6)</td>
<td>5</td>
</tr>
<tr>
<td>Saprophagous</td>
<td>1 (0.1)</td>
<td>1 (0.6)</td>
<td>2 (6.6)</td>
<td>1 (0.6)</td>
<td>1 (0.2)</td>
<td>1 (0.7)</td>
<td>1 (0.2)</td>
<td>1 (0.2)</td>
<td>1 (0.1)</td>
<td>2</td>
</tr>
<tr>
<td>Algivorous</td>
<td>- (0)</td>
<td>1 (0)</td>
<td>- (0)</td>
<td>1 (0)</td>
<td>- (0)</td>
<td>- (0)</td>
<td>1 (0)</td>
<td>1 (0)</td>
<td>1 (0)</td>
<td>1</td>
</tr>
<tr>
<td>Parasitoid</td>
<td>- (0)</td>
<td>1 (0.2)</td>
<td>- (0)</td>
<td>1 (0)</td>
<td>- (0.1)</td>
<td>- (0)</td>
<td>1 (0)</td>
<td>1 (0.2)</td>
<td>1 (0)</td>
<td>1</td>
</tr>
</tbody>
</table>

(n = 90 per sampling occasion)

In both autumn and summer the relative density of fungivorous mites was negatively affected by irrigation relative to the non-irrigated control sites, although the relative density of fungivorous mites was high in the effluent irrigated treatment in summer (Table 6.7). All of the summer data must be interpreted in the light of the low acarine density. The relative density of predatory mites in the mains water irrigated treatment in both autumn and summer was consistently higher than densities recorded in the relative control and effluent irrigated sites. In the autumn samples, this is due to a large number of Phytoseidae sp., and Ameroseiidae sp. In summer, the overall low acarine density exaggerates the difference in the relative densities between the three treatments, particularly in the mains-water irrigated sites that had the lowest densities.
6.3.7.1 Classification and Ordination of Acarine Functional Groups

Acarine community composition based on functional groups was analysed using classification and ordination routines (Fig 6.9 & 6.10). Classification results consistently show treatments clustering within their respective seasons before all treatments cluster at >50% Bray-Curtis similarity. The ordination pattern in Figure 6.10 supports the separation of the spring communities from the autumn and summer communities reflecting the dominance of the phytophagous mites in this season.

Figure 6.9. Dendogram for hierarchical clustering of nine acarine communities using Bray-Curtis similarities calculated on standardized $\sqrt{N}$-transformed acarine functional group density data.
6.3.8 Acarine Species Composition

The advantage of identifying soil acarine fauna to the level of morphospecies is the ability to analyse the affect of treatment and season on individual species. This specific analysis allows for the identification of trends in species responses otherwise obscured by higher-level taxonomic analysis.

Acarine species that were present in any season (autumn, spring and summer) or in any one of the three sites (control, effluent or mains water irrigated) with density values in excess of 1,000 individuals per m² were analysed for their site distribution patterns. Twenty-three species were excluded from further analysis due to their low
frequency of occurrence or uninterruptible distribution pattern. These twenty-three species contributed <2% of the total soil mite density.

Six mite species were present at greater than 1,000 individuals per m². They were *Tyrophagus* sp. (Plate 6.1), *Tydeidae* sp. 2, *Tarsonemidae* sp. (Plate 6.2), *Pygmephoroidea* sp. 1 (Plate 6.4), *Ameroseiidae* sp., and *Phytoseidae* sp. 1. These six species contributed >85% of the total soil mite density (Table 6.5). The affect of season and treatment on *Tyrophagus* sp., has been discussed previously. The prostigmatid mites *Tydeidae* sp. 2 and *Trasonemidae* sp., displayed no statistically significant variation in densities due to treatment or season although both mites displayed a negative response to irrigation in autumn and summer. *Pygmephoroidea* sp. 1., and the two-mesostigmatid mites could not be analysed statistically for their seasonal and treatment responses owing to their very low frequency of occurrence.

Table 6.8. Density (N m⁻²) of the six acarine species that contributed more than 1,000 individuals m⁻² in any treatment regardless of season under three treatment regimes: Non-irrigated control, effluent irrigated and mains-water irrigated.

<table>
<thead>
<tr>
<th>Species</th>
<th>Autumn</th>
<th>Spring</th>
<th>Summer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Effluent</td>
<td>Watered</td>
</tr>
<tr>
<td>Acaridae <em>Tyrophagus</em></td>
<td>4658</td>
<td>4505</td>
<td>2193</td>
</tr>
<tr>
<td><em>Tydeidae</em> sp. 2</td>
<td>6069</td>
<td>1632</td>
<td>4437</td>
</tr>
<tr>
<td><em>Tarsonemidae</em> sp.</td>
<td>4760</td>
<td>1037</td>
<td>782</td>
</tr>
<tr>
<td><em>Pygmephoroidea</em> sp. 1</td>
<td>153</td>
<td>170</td>
<td>68</td>
</tr>
<tr>
<td><em>Ameroseiidae</em> sp.</td>
<td>17</td>
<td>-</td>
<td>1751</td>
</tr>
<tr>
<td><em>Phytoseidae</em> sp. 1</td>
<td>17</td>
<td>3383</td>
<td>-</td>
</tr>
</tbody>
</table>
Although a number of species were found to be unique to a specific treatment, statistical analysis of these patterns of occurrence was precluded due to the low levels of density and their infrequency of occurrence (frequently single or duo events). Six species were found exclusively in the control non-irrigated sites. These were *Speleorchestes* sp., *Tydeidae* sp. 3, *Mesostigmata* sp. 5, *Brachychthonius* sp. 2 (Plate 6.6), *Tectocepheus velatus* (Plate 6.5), and *Nothridae* sp. In contrast, three species were unique to the effluent irrigated sites, *Steretydeus* sp., *Mesostigmata* sp. 7, and *Mesostigmata* sp. 8. Only one species was unique to the mains water irrigated sites, *Cheyletidae* sp. 1 (Plate 6.4). An additional two species (*Eupodes* sp. 2., and *Cosmochthonius* sp.) were only encountered in the irrigated (effluent and mains watered) sites. Table 10.2 in the appendix lists the presence/absence data for each species by treatment and by season.

In summary, of the forty-seven mite species identified at the Albany Effluent Irrigated Tree Farm, seven displayed a consistent negative response to irrigation in both autumn and summer samples. Summer samples are particularly important because during this season, rates of irrigation (effluent and mains-water) are maximal and levels of rainfall are minimal. The seven species were, *Brachychthonius* sp. 2., *Oppiidae* sp., *Tectocepheus velatus*, *Pygmephoroidea* sp. 1., *Tarsonemidae* sp., *Ididorryia* sp., and *Tydeidae* sp. 2. In contrast, in summer, the mite *Eustigmaeus* sp., displayed a positive response to irrigation.
Plate 6.1. Acaridae *Tyrophagus* sp. ventral view x 100

Plate 6.2. Tarsonemidae sp. ventral view x 100

Plate 6.3. Tydeidae *Ididorryia* sp. ventral view x 100

Plate 6.4. Pygmephoroidea sp. 1 dorsal view x 100
Plate 6.5. Tectocepheidae *Tectocepheus velatus* sp. ventral view x 200

Plate 6.6. Brachychthoniidae *Brachychthonius* sp. 2. dorsal view x 100

Plate 6.7. Oribatulidae *Zygoribatula* sp. ventral view x 100

Plate 6.8. Stigmaeidae *Eustigmaeus* sp. dorsal view x 100
6.3.9 Collembola

Twenty-four collembola were extracted from all 270-soil cores. Seventeen collembola were extracted in the autumn and the remaining seven were extracted in the spring samples. Two species were identified, Isotomidae sp. 1 and Brachystomellidae sp. In autumn, there were five Brachystomellidae sp. and twelve Isotomidae sp. 1. In spring, there were three Brachystomellidae sp. and four Isotomidae sp. 1. The collembola were found in both irrigated treatments and in the non-irrigated controls sites. Due to the low collembolan densities, no statistical analysis was conducted. (The two-collembolan species extracted from soil samples were also found in litter samples.)

6.3.10 Summary of Results

The significant outcomes from this chapter are:

1. Large acarine densities in spring were due to very high densities of the Acaridae Tyrophagus sp.

2. Species richness varied with season, and was reduced by irrigation supporting research hypothesis five.

3. Oribatid mite fauna were less dense in irrigated soils in both autumn and summer, when irrigation is likely to be maximal and rainfall minimal supporting research hypothesis six.

4. Soil mites including Tydeidae Ididorryia sp., Tarsonemidae sp., Tydeidae sp. 2., Brachychthonius sp 2 and members of the family Oppiidae displayed a consistent negative response to irrigation.
5. The mite Stigmaeidae *Eustigmaeus* sp. (Plate 6.8), was typically most abundant in the irrigated sites.

6. Nematophagous fauna displayed reduced density in irrigated sites relative to non-irrigated sites.

7. Collembolan density was always extremely low.

6.4 DISCUSSION

6.4.1 Acarine Densities

Studies of soil mite populations under *E. globulus* plantations are very rare. I know of three published studies, one conducted in Portugal (Serralheiro and Madeira, 1990), another in Chile (di Castri and Vitali-di Castri, 1981) and another in Collie, in south western Australia (Adolphson, 2000) making it difficult to place acarine densities from this Albany study into an appropriate context. Acarine densities at the Albany Effluent Irrigated Tree Farm ranged from $3.4 \pm 0.67 \times 10^3$ m$^{-2}$ in summer to $29.8 \pm 3.80 \times 10^3$ m$^{-2}$ in spring. These values are substantially lower than the $75 \pm 19.5 \times 10^3$ m$^{-2}$ (spring) – $181 \pm 27.1 \times 10^3$ m$^{-2}$ (autumn) densities reported for *E. globulus* plantations in Collie (Table 6.9), south western Australia (Adolphson, 2000). Adolphson’s study was conducted under an eight year old *E. globulus* plantation developed on long term pasture sites approximately 200 km northwest of the Albany research site and is particularly useful for comparison because of the similar geographical location (south western Australia), similar site histories (long
Table 6.9. Comparison between four studies of soil acarine densities conducted under *E. globulus* plantations including minimum and maximum densities, plantation age, and selected soil characteristics.

<table>
<thead>
<tr>
<th>Location</th>
<th>Plantation Age</th>
<th>Soil pH</th>
<th>Soil H₂O</th>
<th>Minimum density (m⁻²)</th>
<th>Maximum density (m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albany, Western Australia</td>
<td>6 – 7 years</td>
<td>4.2 – 4.7</td>
<td>5.5 – 38.6%</td>
<td>3.4 ± 0.67 x 10³</td>
<td>29.8 ± 3.80 x 10³</td>
</tr>
<tr>
<td>Collie, Western Australia</td>
<td>8 years</td>
<td>5.6 – 6.3</td>
<td>11.3 – 20.6%</td>
<td>75 ± 19.5 x 10³</td>
<td>181 ± 27.1 x 10³</td>
</tr>
<tr>
<td>Portugal</td>
<td>&gt; 11 years</td>
<td>-</td>
<td>-</td>
<td>≈ 1 x 10³</td>
<td>≈ 18 x 10³</td>
</tr>
<tr>
<td>Chile</td>
<td>-</td>
<td>-</td>
<td>1070 dm³</td>
<td>1070 dm³</td>
<td>1070 dm³</td>
</tr>
</tbody>
</table>

*a* Study conducted at the Albany Effluent Irrigated Tree Farm, extraction process as described in materials and methods; *b* Study conducted in Collie, south western Australia by Adolphson (2000), using identical extraction method to this study; *c* Study conducted in Portugal by Serralheiro and Madeira (1990), soil was sampled to a depth of 25cm using soils cores of 80 x 65mm dia. Extractions were conducted using the Berlese-Tullgren funnel method with temperatures reaching 40±2°C; *d* Study conducted in Chile, reported by di Castri and Vitali-di Castri (1981); no information is provided regarding the size or number of soil cores, age of plantation, or season in which sampling occurred. Extractions were conducted using the Berlese-Tullgren funnel method.
term pasture), similar site preparation histories (ripping of the soil and subsequent mounding prior to *E. globulus* planting), similar plantation age (eight years old) and identical methods of soil sampling and invertebrate extraction. The Collie samples were dominated by extremely high levels of *Tyrophagus* sp. a known opportunistic mite that can demonstrate extreme fluctuations in density. Levels of soil pH were higher in the Collie based study compared to this Albany study (Table 6.9) while levels of soil moisture although comparable did not vary as much in the Collie study compared to this Albany based study (Table 6.9).

Levels of acarine density reported from this Albany based study are within the same order of magnitude (Table 6.9) as those reported in a similar study conducted in Portugal (Serralheiro and Madeira, 1990). This study published arthropod (including *Acari*) densities under *E. globulus* plantations sampled once per season (Serralheiro and Madeira, 1990). In the Portuguese study, the low acarine densities recorded in autumn coincided with annual low levels of soil moisture and the annual high levels of mean temperature. Oribatids were the dominant mite fauna, although *Acari* were identified as Oribatid or ‘Other Mite Fauna’ (Serralheiro and Madeira, 1990). The plantation is older (specific age is unknown) than the plantation of the Albany based study as it was coppiced at the end of the second rotation, eleven years after its establishment.

A study conducted in the Mediterranean-climate zone of Chile recorded an acarine density of 1070 individuals per dm$^3$ of soil (di Castri and Vitali-di Castri, 1981). This lack of information on sampling and plantation age makes it impossible to convert
the acarine density into units suitable for comparison with this study. The lack of
detail provided in this study and the overall lack of studies of acarine populations
under *E. globulus* plantations in geographically appropriate sites make it difficult to
place the acarine densities observed in this Albany based study in an appropriate
context.

Comparable total acarine densities to those in this present study have been reported
from sites situated in Australian deserts (Wood, 1971), Australian semi-arid zones
(Kinnear, 1991; Kinnear and Tongway, 2004; Noble *et al.*, 1996), Arctic/Antarctica
(Hodkinson *et al.*, 2004; Peterson and Luxton, 1982) and tundra (Peterson and
Luxton, 1982). All of these sites are characterized by some form of water stress and
limited levels of organic mater. Deserts and semi-arid regions receive limited rainfall
while Arctic, Antarctica and tundra regions typically have relatively high
concentrations of water but because of the low temperatures the water is in a solid
(ice) form and so unavailable to the soil micro-arthropods. Given that the Albany
Effluent Irrigated Tree Farm experiences a Mediterranean type climate and that the
two experimental sites were irrigated, it seems unlikely that the availability of water
per se is responsible for the low acarine density. Low levels of total carbon and the
acidic soil pH were suggested earlier as a potential limiting factor for rates of
microbial respiration, and may also be limiting rates of acarine density. This may
represent a form of bottom up control in that the level of organic carbon and acidic
soil pH regulates the microbial populations and so indirectly limits the density of the
microarthropod community (Maraun and Scheu, 2000).
In summary the acarine density at the Albany Effluent Irrigated Tree Farm compares favourably with densities reported from *E. globulus* plantations in Portugal but was lower than total densities reported from a nearby *E. globulus* plantation in Collie. Comparable densities are reported from studies of water stressed (desert, tundra, and Artic/Antarctica) and nutrient poor soils.

### 6.4.2 Acarine Seasonal Variation

Acarine densities ranged from a minimum of $3.4 \pm 0.67 \times 10^3$ m$^{-2}$ in summer to a maximum of $29.8 \pm 3.80 \times 10^3$ m$^{-2}$ in spring. This seasonal variation with minimum densities during the hottest and driest part of the year and maximum densities during the cooler, more moist period of the year is consistent with the seasonal trend in acarine populations reported from other Mediterranean-type ecosystems (di Castri and Vitali-di Castri, 1981; Majer and Greenslade, 1988). In systems where soil carbon is low and Prostigmata dominate (as in these soils), the abiotic controls of temperature and moisture are considered to be particularly important (Whitford, 1989). It is thought that the combination of high temperatures and low rainfall results in low levels of soil moisture that in turn limit soil acarine densities. The causal link between the edaphic constraint of low soil moisture and decreased soil faunal density is not fully understood (Maraun and Scheu, 2000). For aquatic soil fauna such as nematodes, the link between decreased soil moisture and decreased nematode density is easier to explain. The decreased levels of soil moisture clearly limit the habitat availability for these aquatic organisms. However, the connection is not quite so clear for the non-aquatic soil fauna such as soil mites. As temperature
increases and rainfall decreases, the soil water availability ($pF$) falls below a critical threshold and fauna that do not employ an aestivation or anhydrobiosis strategy to 'over-summer' either migrate lower into the soil profile (Mackay et al., 1987; Metz, 1971; Perdue and Crossley, 1990; Steinberger and Wallwork, 1985) or die (di Castri and Vitali-di Castri, 1981).

Seasonal fluctuations in density were not restricted to the non-irrigated control sites but were also observed in the two irrigated treatments. This suggests that despite the local scale structuring force of irrigation (be it effluent or mains-water) the densities of the acarine communities were regulated by the broadest structuring force of climate (di Castri and Vitali-di Castri, 1981; Ferguson and Joly, 2002). Indeed the impact of seasonal variation on the acarine density in the two irrigated treatments exceeds that observed in the non-irrigated control sites (Fig. 6.1). In contrast, soil moisture (as expected) was most variable in the non-irrigated control sites and most stable in the two irrigated treatments (Table 3.3). Of the 47 species recorded half (24) were never found in the summer non-irrigated samples and most were not found in the late spring samples. Thus, the maintenance of soil moisture levels throughout the normally drier summer period did little to dampen the seasonal fluctuations in the biota. This suggests that the availability of moisture is not the prime determinant of soil acarine density. It may be due to complex interactions between moisture and temperature and their effect on relative soil humidity.
6.4.3 Acarine Species Richness

The total number of species reported in this study (47) is slightly higher than the 33 species recorded from soil under an *E. globulus* plantation in Collie, Western Australia (Adolphson, 2000). While there is substantial difference in the species composition of the two studies (Table 6.10), the difference in the species richness at the two sites is most obvious with the Prostigmata; 23 species at Albany compared with 17 species at Collie, and Mesostigmata; 12 species at Albany compared with 4 species at Collie. Owing to a lack of published research of soil acarine fauna under *E. globulus* plantations, it is difficult to know if the level of species richness at the Albany Effluent Irrigated Tree Farm is low, moderate or high. The Portuguese study of soil acarine fauna under *E. globulus* unfortunately provides no information at the species level, either of total species richness or individual species identifications (Serralheiro and Madeira, 1990).

In considering the experimental design of the Collie based project, it is important to recognize that the three experimental sites of pasture, plantation (*E. globulus*) and native bushland (*E. marginata* with *E. calophylla* as the minor overstorey) were immediately adjacent to each other (Adolphson, 2000). It seems reasonable to suggest that the endemic fauna within the native bushland may have acted as a faunal reservoir that inoculated the adjacent pasture and plantation sites and so contributed to the levels of species richness observed in that study. The *E. globulus* plantations at the Albany Effluent Irrigated Tree Farm are surrounded by large
Table 6.10. Acarine species richness and ordinal composition from six Australian studies

<table>
<thead>
<tr>
<th>Environment</th>
<th>Species Richness</th>
<th>Prostigmata</th>
<th>Oribatida</th>
<th>Mesostigmata</th>
<th>Astigmata</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albany Effluent Irrigated Tree Farm (^a)</td>
<td>47</td>
<td>23</td>
<td>11</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>(E. globulus plantation 6-7 years old)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collie, south western Australia (^b)</td>
<td>33</td>
<td>17</td>
<td>11</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>(E. globulus plantation 8 years old)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jarrahdale, south western Australia (^c)</td>
<td>14</td>
<td>3</td>
<td>9</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>(E. marginata rehabilitation site 5 years old)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eastern Goldfields, Western Australia (^d)</td>
<td>75</td>
<td>38</td>
<td>26</td>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>(semi-arid soils)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Semi-arid systems, Eastern Australia (^e)</td>
<td>44</td>
<td>34</td>
<td>7</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>(chenopod shrubland)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Semi-arid systems, Eastern Australia (^e)</td>
<td>35</td>
<td>28</td>
<td>5</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>(semi-arid soils)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Albany Effluent Irrigated Tree Farm—this study; \(^b\) Adolphson, 2000; \(^c\) Cuccovia, 1997; Cuccovia and Kinnear, 1999; \(^d\) Kinnear and Tongway, 2004; \(^e\) Noble et al., 1996.
expanses of pasture, with a small central heavily degraded remnant bushland too far away from the research sites to be a source of fauna for the plantation sites. Nevertheless, species richness at the Albany Effluent Irrigated Tree Farm is higher than in the Collie study.

The level of species richness and ordinal composition at the Albany Effluent Irrigated Tree Farm is comparable to the other Australian studies cited in Table 6.10 with the exception of the *E. marginata* study. The rehabilitation study conducted in the jarrah forest, contrasts with the other studies for two reasons. First, the low level of species richness and second the dominance of the oribatid mite fauna relative to the other acarine orders. Perhaps the high levels of oribatid mite fauna reflect higher levels of organic matter, vegetation type, total organic carbon, and soil pH.

A consistent trend through the remaining Australian studies (Table 6.10) is the dominance of the acarine species richness by prostigmatid mites. This trend has been previously identified in Australian semi-arid and arid soils (Kinnear, 1991; Kinnear and Tongway, 2004; Wood, 1971). Some authors have pointed out that the low levels of organic matter (<8.6%) typically encountered in desert (and most Australian) soil systems (including the Albany Efluent Irrigated Tree Farm) favour prostigmatid mites and select against oribatid and mesostigmatid mites (Steinberger and Wallwork, 1985). Wood (1971) heavily emphasized the positive correlation between levels of soil organic matter and oribatid mites because of their known preference for highly organic soils. This supports the results from a South African study (Loots and Ryke, 1967) where researchers found an inverse relationship
between prostigmatid mites and organic matter content and a positive correlation between oribatid mites and organic matter content.

In summary, levels of acarine density at the Albany Effluent Irrigated Tree Farm are lower than those reported from a nearby *E. globulus* plantation in Collie, south western Australia, but comparable to densities reported from soils subject to water stress or low levels of soil organic material. Levels of species richness were higher than all but one study cited in Table 6.10. As noted in the results forty-four of the forty-seven species make an almost negligible contribution to overall density (<20%), while the remaining three species were responsible for more than 80% of the total density. Prostigmatid mites dominated the overall species richness accounting for half of all species identified, a trend reported by several authors sampling acarine communities from organic matter poor soils.

6.4.4 Non-Oribatid Acarine Species Response to Irrigation

The community characteristics of the soil acarine populations from all *E. globulus* plots are strongly suggestive of early-succession or disturbed communities. With the exception of the large populations in spring of the opportunistic *Tyrophagus* sp., the soil acarine densities are low and comparable to those found in extreme natural environments such as arid or semi-arid soils (Kinnear, 1991; Kinnear and Tongway, 2004; Wood, 1971), reclaimed quarry sites (Davis, 1963), coal pit heap sites (Hutson, 1980a; Hutson, 1980b), and coal-shale tips undergoing reclamation (Luxton, 1982). All of these extreme or rehabilitated environments were dominated
by a small number of opportunistic species such as *Tyrophagus* sp., or Tarsonemidae, which were also two of the three most abundant species, sampled in these Albany soils.

*r*-strategist mites are uniquely suited to exploiting recently disturbed soil and dominating the available niche spaces (Behan-Pelletier, 1999). A study of eight undisturbed and five disturbed environments from the Mediterranean-climate zone of Chile reported that members of the astigmatid mite family Acaridae rarely made a substantial contribution to total acarine density in the undisturbed environments, but in disturbed environments acarid mites contributed more than 75% of the total acarine density (di Castri and Vitali-di Castri, 1981). Both disturbed environments involved irrigation, one of crops and the other of pasture. That acarid mites are able to respond positively to an increase in the levels of soil moisture may explain the increase in *Tyrophagus* sp. density observed in spring in this Albany based study.

Acarine densities under *E. globulus* in the Collie, south western Australia study were dominated by Acaridae *Tyrophagus* sp in both spring (98%) and autumn (70%) (Adolphson, 2000). (Comparisons between the faunal specimens deposited by Adolphson at Edith Cowan University and my samples confirm that this is the same species as I observed). Astigmatid mites are known to be specialists in exploiting spatially and/or temporally restricted microhabitats (Norton *et al.*, 1993). Astigmatid populations are often significantly increased following disturbance, including agriculture (Behan-Pelletier, 1999) and irrigation (Philips, 1990). It is thought that this increase in astigmatid density is related to the decline in predator populations.
(see earlier discussion on the relationship between perturbation and its disproportionate negative affect on higher order trophic organisms), the short generation time of astigmatid mites and the presence of an effective dispersal stage – the hypopus (Behan-Pelletier, 1999).

In agricultural systems, astigmatid mite populations have also been found to increase rapidly following tillage and chemical application (Behan-Pelletier, 1999; Franchini and Rockett, 1996; Philips, 1990). Recently disturbed agricultural soils display similarly high relative astigmatid mite densities (Behan-Pelletier, 1999). This supports the view that the soil acarine community at the Albany Effluent Irrigated Tree Farm was in an early or intermediate stage of succession (Odum, 1984).

Small prostigmatid mites belonging to the genus Tarsonemidae are commonly reported in studies of Australian soils. For example, these mites were reported by Noble et al., (1996) from soil under annual grasses in the semi-arid zones of eastern Australia. A study conducted in the chenopod shrublands of the semi-arid zone in Western Australia found that Tarsonemus sp. dominated (>90%) the acarine populations in heavily grazed sites (Kinnear and Tongway, 2004). A tarsonemid mite was regularly found in conventional and non-conventional tilled agricultural soil and remnant bushland in York, Western Australia in densities ranging between 15 - 30 x 10⁴ individuals m⁻² (Swarts, 1998). Tarsonemid mites were the second most abundant mite family in eleven disturbed environments in the Chihuahuan Desert of New Mexico (Kay et al., 1999). Tarsonemid mites made substantial contributions to total acarine density in four reclaimed coal pit heap sites in Northumberland,
England (Hutson, 1980a). In the first year the relative contribution of tarsonemid mites to overall density in the Northumberland study ranged from 3.1 to 17.2% while in the second year it ranged from 8.7 to 45.8%. Luxton, (1982) reported that relative density of tarsonemid mites ranged from 0.2 to 9.3% on two coal-shale tips in Lancashire, England. Tarsonemid density was reported from eight natural ecosystems within the Mediterranean-climate zone of Chile and ranged from 1.5 to 150.9 individuals dm$^{-3}$ (di Castri and Vitali-di Castri, 1981). The same authors reported tarsonemid densities from five human-modified ecosystems within the Mediterranean-climate zone of Chile that varied from 7.9 to 79.3 individuals dm$^{-3}$.

Clearly, species of the family Tarsonemidae are cosmopolitan inhabitants of soils around the world. Members of this family are variously considered to be microphytophages, predators, plant feeders and parasites of both vertebrates and invertebrates (Evans, 1992; Kethley, 1990; Krantz, 1978). Tarsonemid mites, much like *Tyrophagus* sp., are known to be able to respond positively to disturbance, colonize organic matter in large numbers and display characteristics of $r$-strategists (Crossley *et al.*, 1992; Kinnear and Tongway, 2004; Santos and Whitford, 1981; Whitford *et al.*, 1988).

In summary, the presence and numerical dominance of the astigmatid mite *Tyrophagus* sp. and the prostigmatid mite from the genus Tarsonemidae must be interpreted in the light of the perturbation and stress history of the Albany Effluent Irrigated Site. Comparisons with the literature have clearly indicated that recently perturbed and early succession sites are typically dominated by $r$-strategist mites.
such as *Tyrophagus* sp., and Tarsonemidae (Behan-Pelletier, 1999; di Castri and Vitali-di Castri, 1981; Franchini and Rockett, 1996; Hutson, 1980a; Hutson, 1980b; Kinnear and Tongway, 2004; Luxton, 1982; Philips, 1990; Santos *et al.*, 1981; Santos and Whitford, 1981). The dominance of these mites and their frequency of occurrence support the view that the Albany Effluent Irrigated Tree Farm has the faunal characteristics of a highly perturbed early succession community.

Given the perturbation history of the Albany Effluent Irrigated Tree Farm it is likely that the soil structure is dominated by micropores. The biological processes required to develop meso and macropores occur over centuries (Lavelle and Spain, 2001) and require the services of “ecosystem engineers” including earthworms, termites and ants (Young *et al.*, 1998) that were notable absent from this site. The large numbers of tydeid mites is possibly a reflection of the niche sizes available in the early succession soil at the Albany Effluent Irrigated Tree Farm. The ecological literature dealing with tydeid mites almost exclusively considers them in the context of critical controlling fauna of nematode density and colonization rates in desert systems (Santos *et al.*, 1981). In a subsequent discussion (6.4.6), I will consider the correlation between tydeid mite density, microbial biomass, and nematode density. I will focus primarily on the possibility of evidence for bottom up resource-driven control of the density of the nematode and tydeid mite fauna.
6.4.5 Oribatid Mite Responses to Irrigation

One of the specific research aims of this study was to consider the potential use of oribatid mites as indicator species of disturbance and stress (research hypothesis six). In natural ecosystems with high levels of soil organic matter, oribatid mites typically constitute the main component of the acarine population (Peterson and Luxton, 1982). A small contribution by oribatid mites to overall acarine density (as observed in this study) is typically associated with communities dominated by large numbers of Prostigmata and has been reported from tundra and desert sites characterized by some form of water stress and low levels of soil organic matter (Peterson and Luxton, 1982).

Oribatid mites may display intolerance to altered levels of soil moisture by either reduced densities at, or absence from irrigated sites (Dindal, 1977; Dindal et al., 1975). A study examining acarine responses to irrigation in Pennsylvania, United States of America, recorded substantial reductions in the number of oribatid and prostigmatid species in mixed oak hardwood and old field herbaceous communities that had been irrigated for several years (Table 6.11). In this Albany based study, oribatid mite species richness also displayed a slight negative response to irrigation in both autumn and spring (Table 6.11). Oribatid species richness in the non-irrigated control sites was low (5-10) in the Albany study compared to Pennsylvania study (17-18, Table 6.11).
Soil acari may display intolerance to altered soil moisture regimes by reduced densities, or reduced frequency of occurrence rather than absence from the irrigated soil. In the Albany based study, oribatid mite densities were lower in all irrigated sites in all seasons although not statistically so. These differences were due largely to the two-oppiid species, whose densities at the non-irrigated control sites were 2 – 6 times higher than those at the other treatment sites supporting hypothesis six. That some oppiiid species are negatively effected by experimentally-increased soil moisture is also suggested by a Swedish study that utilized irrigation to simulate increased “seasonal rainfall” to monitor the responses of soil mite communities (Lindberg et al., 2002). Dindal et al., (1975) also reported lower mite densities (including oribatids) and shifts in the dominant families and species following irrigation.

Table 6.11. The impact of irrigation (effluent and mains-water) on soil acarine species richness at the Albany Effluent Irrigated Tree Farm and Pennsylvania, United States of America.

<table>
<thead>
<tr>
<th>Season</th>
<th>Oribatida</th>
<th>Prostigmata</th>
<th>Mesostigmata</th>
<th>Total SR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autumn</td>
<td>Non-Irrigated</td>
<td>7</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Irrigated</td>
<td>6</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td>Spring</td>
<td>Non-Irrigated</td>
<td>10</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Irrigated</td>
<td>7</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>Summer</td>
<td>Non-Irrigated</td>
<td>5</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Irrigated</td>
<td>5</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>(Dindal et al., 1975)</td>
<td>Control *</td>
<td>17</td>
<td>21</td>
<td>9</td>
</tr>
<tr>
<td>(Dindal et al., 1975)</td>
<td>Treated *</td>
<td>6</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>(Dindal et al., 1975)</td>
<td>Control +</td>
<td>18</td>
<td>36</td>
<td>7</td>
</tr>
<tr>
<td>(Dindal et al., 1975)</td>
<td>Treated +</td>
<td>8</td>
<td>12</td>
<td>4</td>
</tr>
</tbody>
</table>

(*’Irrigated’ sites are a combination of both effluent and mains-water irrigated sites, n = 30 samples for the non-irrigated control site and n = 60 samples for the combined irrigated sites; *Mixed Oak Forest Community, Spring 1972; +Old Field Herbaceous Community, Spring 1972)
The distribution pattern of *Brachychthonius* sp. 2 is of particular interest. This soil mite was only found in the non-irrigated control sites in each of the three seasonal sampling events, however the density and frequency of occurrence of this species is very low. While this does not necessarily preclude the possibility of this being a genuine irrigation response, (indeed rare species can be negatively affected by perturbation or stress just as much as commonly encountered species), the fact that this species was only found once in autumn and spring samples and three times in summer samples makes it difficult to suggest one outcome (irrigation response) over another (difficulty of sampling rare species with aggregated patterns of distribution).

There is some evidence in the literature to suggest that mite species of the family Brachychthoniidae are negatively affected by irrigation. Dindal (1977) reported that spring samples extracted from untreated soil under old field – white spruce communities were dominated by Brachychthoniidae spp. while soil treated with municipal wastewater irrigation did not support any Brachychthoniidae spp. Autumn sampling in the mixed-oak hardwood site found that Brachychthoniidae spp. were a subdominant species in the control sites but was rarely encountered in sites irrigated with municipal effluent. A Swedish study reported that the density of *Brachychthonius* sp. in irrigated soil was $50 \pm 25 \times 10^3 \text{ m}^{-2}$ a substantial (but not statistically significant) reduction from the $74 \pm 23.8 \times 10^3 \text{ m}^{-2}$ individuals recorded in the control soil (Lindberg *et al.*, 2002).
Tectocepheus velatus sp was only ever encountered in the non-irrigated control sites. However like Brachychthonius sp. 2 this species was only ever extracted from one soil core in each of the three seasonal samplings so it is difficult to suggest one outcome (treatment effects) over the other (sampling effects). The ecological literature presents a confusing picture with regard to the family Tectocepheidae and its response to irrigation. Dindal (1977) reports that Tectocepheus velatus was a rare species in both untreated and treated (irrigated) old field – white spruce communities when sampled in spring. In the same study Tectocepheus velatus was a dominant species in the untreated spring samples in a mixed-oak hardwood but was absent in the effluent irrigated soil. In autumn at the same site, Tectocepheus velatus was a dominant species in the untreated soil and a rare species in the effluent irrigated soil. The Swedish study by Lindberg et al., (2002) reported that the density of Tectocepheus velatus increased (albeit non-significantly) from $20.0 \pm 5.64 \times 10^3 \text{ m}^{-2}$ in the non-irrigated control soil to $25.0 \pm 3.19 \times 10^3 \text{ m}^{-2}$ in the mains water irrigated soil. This variable response to irrigation by Tectocepheus velatus suggests that there is no simple relationship between its density and irrigation. Clearly more research is required to assess the usefulness of Tectocepheus velatus as an indicator of stress or perturbation.

6.4.6 Nematophagous Mites

In autumn 2000, in the non-irrigated control sites, high levels of microbial biomass corresponded with high levels of nematode fauna and high levels of nematophagous acarine fauna relative to the irrigated treatments (Table 6.12). Elevated level of
microbial biomass observed in the autumn non-irrigated control sites was possibly a response to above average rainfall observed in mid to late summer of 2000 following a prolonged period of drought.

The data suggests that the increase in microbial biomass (approximately two times the levels observed in the experimental sites) supported a substantial increase in the nematode density in the non-irrigated control sites relative to the two irrigated treatments (Table 6.12). An analysis of the functional composition of the nematode community reveals that ≈80% of the population was composed of bacterial feeding and animal predatory nematodes. Although this increase in the nematode density (approximately 6 times the levels observed in the two irrigated treatments) did not result in an increase in acarine density it did result in a variation in the functional

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Table 6.12. Selected measurements of the soil floral and faunal community from all three treatments in autumn 2000.

<table>
<thead>
<tr>
<th></th>
<th>Non-Irrigated Control</th>
<th>Effluent Irrigated</th>
<th>Mains-Water Irrigated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Microbial Biomass</strong> (µg C g⁻¹ soil)</td>
<td>1002 ± 68.5</td>
<td>589 ± 47.8</td>
<td>556 ± 66.6</td>
</tr>
<tr>
<td><strong>Microbial Respiration</strong> (µg CO₂ g⁻¹ soil 24 hr⁻¹)</td>
<td>10.7 ± 0.86</td>
<td>10.3 ± 0.67</td>
<td>11.1 ± 0.72</td>
</tr>
<tr>
<td><strong>Nematode Density</strong> (number 10³ m⁻²)</td>
<td>498 ± 21.5</td>
<td>76 ± 1.9</td>
<td>93 ± 2.2</td>
</tr>
<tr>
<td>% Bacterial Feeding Nematodes</td>
<td>55.7</td>
<td>66.7</td>
<td>61.2</td>
</tr>
<tr>
<td>% Animal Predator Nematodes</td>
<td>20.4</td>
<td>0.0</td>
<td>0.3</td>
</tr>
<tr>
<td><strong>Acarine Density</strong> (number 10² m⁻²)</td>
<td>18.9 ± 5.08</td>
<td>21.2 ± 3.25</td>
<td>7.6 ± 2.76</td>
</tr>
<tr>
<td>% Nematode Feeding Acari</td>
<td>36.9</td>
<td>19.4</td>
<td>33.1</td>
</tr>
</tbody>
</table>
composition as evidenced by the increase in the percentage contribution of the nematode feeding acari (Table 6.12). The most abundant nematophagous acarine fauna in the autumn non-irrigated control site were two species of Tydeidae, Ididorryia sp. and Tydeidae sp. 2.

These data reflects the ability of the nematode population to respond to an increase in the microbial community (specifically microbial biomass) and suggest that the nematode populations were constrained in part by the microbial population (bottom-up resource control). Secondly, the data reflects the importance of bottom-up resource control on both density and functional composition of the fauna occupying higher trophic positions within the food web. Previous authors have commented that bottom-up resource controls dominate in disturbed environments, including mine site rehabilitation, and reforestation, because higher trophic levels that would be responsible for top-down predatory forces, are disproportionately affected by disturbance (Chesson and Case, 1986; Pimm, 1982; Scheu and Schaefer, 1998).

While the increase in microbial biomass was linked to changes in both nematode density and functional composition, and a change in acarine functional composition it did not affect acarine density. Perhaps this can be explained as; the net affect to a functional web caused by a change in a resource is directly proportional to the size of the change and the trophic proximity of the affected fauna. Using the current food web as an example, the net affect of the increase in microbial biomass to the functional web was clearly identified in both the nematode and acarine fauna. Since nematodes feed directly on microbial biomass both density and functional
composition were affected. Since soil acari feed on microbial biomass, either as fungal feeders or indirectly via soil nematodes, they displayed only a change in functional composition but not overall density.

6.4.7 Summary

Continuous irrigation had the effect of smoothing out the annual wet-dry soil moisture cycle that is typical of the Albany environment. Although not statistically significant, acarine densities and species richness were lower in irrigated soils in both autumn and summer when rates of irrigation were maximal and rainfall was minimal. Reductions in acarine density and species richness with irrigation are thought to occur because irrigation reduces below-ground habitat heterogeneity (di Castri, 1973; Huhta and Hanninen, 2001; Lindberg et al., 2002; Lindberg and Persson, 2004). Variations in temperature and moisture produce temporal and spatial heterogeneity that may promote diversity, a hypothesis that was tested by Huhta and Hanninen (2001) in a microcosm study. They concluded that their results supported this hypothesis, but the differences they obtained were not marked.

Despite the importance of the Albany sites perturbation history in structuring the soil acarine community, climate was found to result in significant seasonal variation in levels of acarine density similar to trends in acarine density reported from other Mediterranean climates (di Castri and Vitali-di Castri, 1981; Majer and Greenslade, 1988; Postle et al., 1986; Postle et al., 1991). The acidic nature of the soil and its very low levels of organic matter content were cited as an explantation for the low
density and species richness of the Oribatida and the high species richness and
density of the Prostigmata (23 of 47 species).

Although not statistically significant, Oribatid mite densities were substantially lower
in irrigated soils relative to the non-irrigated control soils. At an individual species
level, mites of the family Oppiidae, Brachychthonius sp. 2, and Tectocepheus
velatus, all displayed a consistent negative response to irrigation. However, the
oribatid mites were poorly represented in this study, even in the non-irrigated sites,
possibly reflecting the perturbation history, acidic soils and low levels of soil organic
matter. The strongest evidence for the negative impact of irrigation on oribatid soil
fauna comes from studies conducted in mature, well established vegetation sites in
which the oribatid mite fauna are typically found to dominate both density and
species richness prior to the application of irrigation (Dindal, 1977; Dindal et al.,
1975; Lindberg et al., 2002; Lindberg and Persson, 2004).

Analysis of the soil acarine species composition at the Albany Effluent Irrigated Tree
Farm demonstrated that these populations were dominated by r-strategist,
pioneering mite species, reflecting the importance of the perturbation history on the
acarine community. The continuing effect of the perturbation history is proposed as
a possible explanation for the lack of clear and statistically significant differences in
acarine communities between treatments. It is suggested that the characteristics of
the dominant mite fauna that allow them to be such effective r-strategist colonising
species may be the same characteristics that allow them to tolerate the impact of
irrigation.
“Don't be afraid to go out on a limb … that's where the fruit is.”

Anonymous
7.0 Litter Decomposition

7.1 INTRODUCTION

Litter mesofauna are important indirect contributors to the process of litter decomposition and to the maintenance of soil fertility (Hansen, 1999; Santos and Whitford, 1981; Whitford et al., 1983). Several papers have reported that litter decomposition rates are enhanced by the addition of effluent (Baker et al., 1990; Guo and Sims, 2001; Guo and Sims, 2002). Guo et al., (2002) also reported that litter subjected to effluent irrigation experienced more rapid losses of N, K, Ca, Mg and Mn than litter that was not irrigated with effluent.

In this chapter, I describe the rate of litter decomposition and the litter-bag mesofaunal density and diversity and trophic structure under the *E. globulus* plantation. I analysed the rate of decomposition and the litter-bag mesofaunal density and diversity and trophic structure under plantations irrigated with effluent and mains-water. I also analysed the rate of Total C and Total N loss from litter over time.

The aims of this chapter are to describe:

a) baseline rates of litter decomposition;
b) baseline litter-bag mesofaunal density and diversity;
c) rates of litter decomposition in irrigated (effluent and mains-water) sites; and
d) litter-bag mesofaunal density and diversity in irrigated (effluent and mains-water) sites.

Research hypothesis 7. Effluent and mains-water irrigation is expected to enhance the rate of litter decomposition supporting the trend reported by Baker et al., (1990)

7.2 METHODS

7.2.1 Litter Decomposition

Litter decomposition, measured as the rate of mass loss, was recorded over 11 months using litter bags of 10 cm by 15 cm with eight mm mesh (Arunachalam et al., 1998; Baker et al., 1990; Gallardo and Merino, 1999; Irmler, 2000; Kuperman, 1999). Litter was collected in the field during autumn of 2001. Eight grams of litter (± 0.05 g) was placed inside each of 480 mesh bags. The mesh bags were returned to the field and sampled sequentially on eight occasions over 11 months.

7.2.1.1 Litter Collection

Freshly fallen E. globulus leaves were collected from the Albany Effluent Irrigated Tree Farm in autumn 2001 (March - April). Anti bird netting (black nylon plastic mesh size 51 x 51mm) of size 10 x 5m was set up 1.5m above the ground between double rows of trees. Leaves were collected twice from the netting, once at 4 weeks and again at the conclusion of the collection period (8 weeks). Freshly harvested leaves were transported back to the laboratory and air dried at 25°C.
Litter-bags (10 cm x 15 cm) were constructed from nylon mesh (8 mm). Eight grams (± 0.05 g) of air-dried litter was placed inside each bag. Litter-bags were closed at either end with thin strips of heat sealed plastic. Each litter-bag was labelled with an aluminium tag stamped with its initial dry weight and a unique identifying number.

Litter-bags were placed in the field in the first week of June 2001 and placed randomly but equidistant between trees. Field observations suggested that the rate of litter decomposition might be higher in the immediate vicinity of the drip line. To control for this observation, litter-bags placed under trees subject to treatments B (Effluent Irrigated) and C (Mains Water Irrigated) were stratified by distance from the drip line. One half of the litter-bags were placed on the drip line and the other half were placed off the drip lines, on the mounded soil between the irrigated trees.

Litter-bags were collected eight times over 11 months, at 0, 1, 2, 3, 5, 7, 9, and 11 months. On each sampling occasion, eight litter-bags were collected from both irrigated sites, while four litter-bags were collected from each control site. There were three replicate sites. Four hundred and eighty litter-bags were placed in the field and subsequently harvested. All litter-bags were analysed for mass loss. On each sampling occasion, litter from two bags was bulked for mesofaunal extractions. Litter from remaining bags (n = 2) was used for chemical analysis.
7.2.1.3  Litter Mass Loss

Samples were examined for contamination by soil and other plant material (mainly grasses). These were removed prior to calculating mass loss. Samples were air dried to a constant weight in the laboratory at 55°C. Leaf material was then removed from the litter-bags and weighed.

Observed rates of litter decay were correlated with predicted rates of decay to test the appropriateness of the single negative exponential decay model (Blair et al., 1990). Annual decay rate constants were calculated from the percentage mass remaining using a single negative exponential decay model $X/X_0 = e^{-kt}$. Where $X/X_0$ is the final amount of litter divided by the initial amount of litter in grams, $t$ is the amount of time elapsed in years, and $k$ is the annual decay rate constant (Olsen, 1963).

7.2.2  Litter Chemical Analysis

Soil carbon and nitrogen were analysed using the method detailed in section 2.8.2 (Sampling Design and Common Methods – Carbon and Nitrogen Analysis). Litter was oven dried to a constant weight at 60°C and then ground to a fine powder using a mortar and pestle prior to placement in aluminium sampling containers.
7.2.3 Litter-bag Fauna

7.2.3.1 Extraction Procedure

Individual litter-bags were carefully removed from the ground and placed into large paper bags for transport back to the laboratory. Litter fauna were extracted into picric acid using modified infra-red heat extractors (Kempson et al., 1963). Extractions were conducted over ten days with final surface temperatures reaching 45°C. Samples were filtered and stored in 80% ethanol.

7.2.3.2 Identification

Identification of litter fauna was carried out using the method described in section 2.8.5 (Sampling Design and Common Methods – Faunal Identification). Litter macrofauna were identified to Order using Dindal (1977). Litter mites were identified to Order and where possible to species or morphospecies (2.8.5 Faunal Identification). Litter Collembola were identified to Order and where possible to species or morphospecies using Greenslade (1991).

7.2.4 Statistical Analysis

Statistical analysis was carried out using the method detailed in section 2.8.4. (Sampling Design and Common Methods – Statistical Analysis). Raw density values per litter-bag were converted to numbers of individuals per kg⁻¹ litter dry weight so that comparisons between sampling events could be made. The
experimental design allowed for an analysis of the impact of proximity to the dripper line on the rate of decomposition, total C and total N loss and mesofaunal composition. Statistical analysis of the impact of litter-bag placement in relation to the dripper line revealed no significant differences in litter mass loss, total N, total C or mesofaunal composition. Therefore, the results from each position (on dripper line and off dripper line) were combined.

7.3 RESULTS

7.3.1 Rate of Litter Decomposition

Litter-bags for month 0 (Plate 7.1) were placed in the field and immediately harvested to calculate the percentage mass loss due to handling. All subsequently harvested samples were adjusted to reflect mass loss due to handling (<1%).

The rate of litter decomposition of recently abscised *E. globulus* was rapid initially during the winter-spring months. Approximately 30% of the initial litter mass (8.00 ± 0.05 g) was lost in the first 3 months (Fig 7.1) Over the next 8 months the rate of litter mass loss was reduced by only approximately 10% (Fig 7.1).
Plate 7.1. Samples of litter-bag contents from each sampling period to show pattern of leaf disappearance.
Figure 7.1. The percentage mass remaining of *E. globulus* litter under three treatment regimes: non-irrigate control, effluent irrigated, and mains-water irrigated over an 11-month period. (Values are means ± 1 SE, initial weight for all litter bags was 8.00 ± 0.05 g).

There were no treatment effects on litter mass loss in any month (Month 0 $F_{(2,6)}=1.272$, $p=0.342$; Month 1 ($F_{(2,6)}=0.271$, $p=0.771$); Month 2 ($F_{(2,6)}=0.290$, $p=0.758$); Month 3 ($F_{(2,6)}=0.383$, $p=0.694$); Month 5 ($F_{(2,6)}=0.029$, $p=0.971$); Month 7 ($F_{(2,6)}=3.038$, $p=0.119$); Month 9 ($F_{(2,6)}=0.136$, $p=0.875$); Month 11 ($F_{(2,6)}=0.96$, $p=0.910$).

Annual decay rates ($k$) based on 11 months of decomposition data were 0.53 in the control non-irrigated sites, 0.52 in the effluent irrigated sites, and 0.46 in the mains water irrigated sites. The lack of a significant difference between the annual decay rates ($k$) supports the ANOVA result above that found no significant
treatment effects across the 11 months. Correlations between actual decay rates and predicted decay rates over the eleven months revealed a highly significant correlation for each treatment (Control $r^2 = 0.757; p<0.01$, Effluent $r^2 = 0.631; p<0.01$, Mains Water $r^2 = 0.774; p<0.01$). The highly significant correlation between the actual rate of decay and the predicted rates of decay suggests that the single negative exponential decay model is a good predictor of the rate at which this litter decays.

### 7.3.2 Litter Carbon and Nitrogen

#### 7.3.2.1 Total Carbon (TC)

Percentage TC in mature leaves harvested directly from mature *E. globulus* trees displayed no significant difference due to treatment ($F_{(2,6)}=0.565, p=0.595$). Despite some fluctuations in %TC (Figure 7.2) levels over the first seven months there were no significant differences between treatments ($F_{(2,6)}=0.349, p=0.718$) or between sampling date within a treatment (non-irrigated control $F_{(5,12)}=0.945, p=0.487$; effluent irrigated $F_{(5,12)}=1.761, p=0.196$; mains-water irrigated $F_{(5,12)}=1.740, p=0.200$). Leaf litter sampled nine months after placement in the field displayed a statistically significant treatment response, with lower levels of %TC in mains-water irrigated plots compared with non-irrigated and effluent irrigated plots ($F_{(2,6)}=11.719, p=0.005$). Leaf litter sampled in the twelfth month also displayed a significant treatment response with both irrigated plots significantly lower than the non-irrigated control plots ($F_{(2,6)}=14.556, p=0.001$).
Figure 7.2. Percentage TC plotted against time from placement in the field under three treatment regimes: non-irrigated control, effluent irrigated, and mains-water irrigated. Data also includes values for mature leaves harvested directly from *E. globulus* trees (On Tree).

### 7.3.2.2 Total Nitrogen (TN)

Percentage TN (Figure 7.3) for leaves harvested directly from mature *E. globulus* trees did not vary significantly with treatment ($F_{(2.6)}=1.206, p=0.363$) but were significantly higher than levels of %TN in litter packs (non-irrigated control $F_{(2.6)}=2.815, p=0.032$; effluent irrigated $F_{(2.6)}=3.830, p=0.009$; mains water irrigated $F_{(2.6)}=5.030, p=0.002$). Although %TN tended to increase with sampling date, this trend was not significant for any treatment (non-irrigated control $F_{(6,14)}=1.606, p=0.204$; effluent irrigated $F_{(6,14)}=1.886, p=0.139$; mains water irrigated $F_{(6,14)}=4.451, p=0.006$).
Figure 7.3. Percentage TN plotted against time from placement in the field under three treatment regimes: non-irrigated control, effluent irrigated, and mains-water irrigated. Data also includes values for mature leaves harvested directly from *E. globulus* trees (On Tree).

### 7.3.3 Faunal Density

Since there were no fauna in month 0, these samples are not included in this analysis. Faunal densities in litter packs ranged from a low of 457 ± 142 in month one to a high of 3.2 x 10³ ± 605 in month 7 (Figure 7.4). Figure 7.4 clearly shows that faunal densities increased significantly in litter packs in months nine and eleven (non-irrigated control $F_{(6,14)}=9.127$, $p<0.001$; effluent irrigated $F_{(6,14)}=32.306$, $p<0.001$; mains water irrigated $F_{(6,14)}=32.480$, $p<0.001$). Litter bags sampled nine
months after placement in the field displayed a significant treatment response with mean faunal density significantly higher in effluent irrigated litter relative to the non-irrigated control litter \( F(6, 14) = 4.379, p = 0.059 \). Faunal densities in the eleventh month did not vary significantly due to treatment \( F(6, 14) = 1.509, p = 0.279 \). It is interesting to note that in the eleventh month, faunal densities declined in both irrigated treatments and continued to rise in the non-irrigated control litter. Despite this reduction in faunal densities in the irrigated leaf litter, densities remained twice those of the non-irrigated control.

Figure 7.4. The effect of treatment on mean faunal density (number kg\(^{-1}\) litter dry weight) under three treatment regimes: non-irrigated control, effluent irrigated, and mains-water irrigated.
Community Structure of Litter bag Fauna

Classification analysis of species density (Fig 7.5) displayed clear separation of treatments and samples into three distinct groups, forming a temporal sequence at a bray-curtis similarity of 40%. Samples taken during months one and two grouped together with two exceptions (2-W and 3-C), samples taken in the third, fifth and seventh month grouped together, and samples from the ninth and eleventh month formed a third group.

Figure 7.5. Hierarchical agglomerative classification of samples based on \( \sqrt{ } \) transformed mean faunal density (number kg\(^{-1}\) litter dry weight). Identification tags equal time in months since placement in the field while letter indicates treatment. C = Non-Irrigated Control, E = Effluent Irrigated, and W = Mains-Water Irrigated.
The ordination analysis (Fig 7.6) supports the classification pattern of Figure 7.5 with three distinct temporal sequences (ANOSIM Global R=0.719, p=0.1%). The distinction between stage one (months 1 and 2) and stage two (months 3, 4 and 5) is not entirely consistent compared with the distinction between all stage two and stage three samples (Table 7.1). The existence of these ‘aberrant samples’ (2-W and 3-C) is explained by their density. The 2-W sample recorded a density of 1133 individuals kg\(^{-1}\) that is more similar to samples collected in the third month, while the 3-C sample recorded a density of 361 individuals kg\(^{-1}\) that is more similar to samples collected in the second month (Table 7.2). This response was most likely to be observed in the first three months due to the more intense sampling effort during this period (monthly instead of every two months). This means that there was less time between sampling events compared to later samples and therefore less likely that
litter-bag communities were significantly different from one sampling event to the next.

Table 7.1. Analysis of Similarity, Pairwise tests between litter-bag communities

<table>
<thead>
<tr>
<th>Pairwise Tests (Groups)</th>
<th>R Statistic</th>
<th>Significance Level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>One, Two</td>
<td>-0.074</td>
<td>70</td>
</tr>
<tr>
<td>One, Three</td>
<td>0.778</td>
<td>10</td>
</tr>
<tr>
<td>One, Five</td>
<td>0.963</td>
<td>10</td>
</tr>
<tr>
<td>One, Seven</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>One, Nine</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>One, Eleven</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Two, Three</td>
<td>0.333</td>
<td>20</td>
</tr>
<tr>
<td>Two, Five</td>
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<td>Two, Seven</td>
<td>0.337</td>
<td>10</td>
</tr>
<tr>
<td>Two, Nine</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Two, Eleven</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Three, Five</td>
<td>0.185</td>
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<td>Three, Nine</td>
<td>0.963</td>
<td>10</td>
</tr>
<tr>
<td>Three, Eleven</td>
<td>1</td>
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</tr>
<tr>
<td>Five, Seven</td>
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<td>10</td>
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<td>Five, Nine</td>
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<td>10</td>
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<td>10</td>
</tr>
<tr>
<td>Seven, Eleven</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Nine, Eleven</td>
<td>-0.074</td>
<td>50</td>
</tr>
</tbody>
</table>
Table 7.2. The effect of treatment and time since placement in the field on litter mesofaunal density (N kg\(^{-1}\) litter dry weight) under three treatment regimes: non-irrigated control, effluent irrigated, and mains-water irrigated.

<table>
<thead>
<tr>
<th>Month</th>
<th>Treatment</th>
<th>Acari</th>
<th>Collembola</th>
<th>Arachnida</th>
<th>Other Insecta</th>
<th>Other Arthropoda</th>
<th>All Other Fauna</th>
<th>Total Density per kg(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Non-Irrigated Control</td>
<td>45</td>
<td>-</td>
<td>-</td>
<td>115</td>
<td>-</td>
<td>-</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>Effluent Irrigated</td>
<td>255</td>
<td>-</td>
<td>46</td>
<td>23</td>
<td>-</td>
<td>-</td>
<td>324</td>
</tr>
<tr>
<td></td>
<td>Mains Water Irrigated</td>
<td>168</td>
<td>-</td>
<td>23</td>
<td>71</td>
<td>-</td>
<td>-</td>
<td>262</td>
</tr>
<tr>
<td>2</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>199</td>
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<td>Effluent Irrigated</td>
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<td>-</td>
<td>52</td>
<td>74</td>
<td>-</td>
<td>-</td>
<td>499</td>
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<td></td>
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<td>177</td>
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<td>115</td>
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<td>218</td>
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<td>25</td>
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<tr>
<td></td>
<td>Effluent Irrigated</td>
<td>482</td>
<td>-</td>
<td>401</td>
<td>762</td>
<td>55</td>
<td>-</td>
<td>1700</td>
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<td></td>
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<td>1159</td>
<td>-</td>
<td>164</td>
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<td>7</td>
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<td>859</td>
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<td>47</td>
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<td>3809</td>
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<td>89</td>
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<td>119</td>
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<td></td>
<td>Effluent Irrigated</td>
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<td>704</td>
<td>3048</td>
<td>2640</td>
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<td>557</td>
<td>1646</td>
<td>24</td>
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<td>1439</td>
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<td>2246</td>
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</table>
7.3.5 Mesofaunal Composition

All litter mesofauna were identified into one of six taxa; Acari, Collembola, Arachnida, Other Insecta, Other Arthropoda and All Other Fauna. Both the acarine and collembolan fauna were identified to lowest possible taxon or morphospecies. Table 7.2 lists density of each taxon for each sampling occasion and for each treatment. List 10.1 lists all the acarine and collembolan species that were identified from *E. globulus* leaf litter.

Figure 7.7 displays the percentage contribution of these six faunal categories for each treatment within each sampling event. Overall, the Acari were the dominant litter fauna followed by the Other Insecta. Typically these two groups accounted for >80% of the litter fauna in each treatment within each sampling event. The dominant litter insects excluding the Collembola were members of the orders Coleoptera, Diptera and occasional numbers of Hymenoptera.

7.3.5.1 Acari

The litter-bag acarine fauna were represented by 23 species. The Prostigmata (9 species) and Oribatida (8 species) were the most species rich acarine orders. There were five mesostigmatid mite species and one astigmatid species (Acaridae *Tyrophagus* sp.).
Figure 7.7. Relative density (%) of the six faunal categories to total faunal density (number kg$^{-1}$ litter dry weight).
Acarine species richness (Table 7.3) tended to increase with time from placement of litter-bags in the field and peaked at 9 months. Species richness in the 11th month was similar to that in the 7th month. In every sampling period, the species richness was lowest in the non-irrigated control. Despite low species richness, the consistency of this trend is obvious, particularly in the seventh, ninth and eleventh months.

Table 7.3. The effect of time since placement in the field on litter acarine species richness under three treatment regimes: non-irrigated control, effluent irrigated, and mains-water irrigated.

<table>
<thead>
<tr>
<th>Month</th>
<th>Treatment</th>
<th>SR</th>
</tr>
</thead>
<tbody>
<tr>
<td>One</td>
<td>Control (Non-Irrigated)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Effluent Irrigated</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Mains Water Irrigated</td>
<td>3</td>
</tr>
<tr>
<td>Two</td>
<td>Control (Non-Irrigated)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Effluent Irrigated</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Mains Water Irrigated</td>
<td>7</td>
</tr>
<tr>
<td>Three</td>
<td>Control (Non-Irrigated)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Effluent Irrigated</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Mains Water Irrigated</td>
<td>6</td>
</tr>
<tr>
<td>Five</td>
<td>Control (Non-Irrigated)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Effluent Irrigated</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Mains Water Irrigated</td>
<td>9</td>
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<tr>
<td>Seven</td>
<td>Control (Non-Irrigated)</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Effluent Irrigated</td>
<td>9</td>
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<tr>
<td></td>
<td>Mains Water Irrigated</td>
<td>9</td>
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<tr>
<td>Nine</td>
<td>Control (Non-Irrigated)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Effluent Irrigated</td>
<td>10</td>
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<td></td>
<td>Mains Water Irrigated</td>
<td>12</td>
</tr>
<tr>
<td>Eleven</td>
<td>Control (Non-Irrigated)</td>
<td>6</td>
</tr>
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<td></td>
<td>Effluent Irrigated</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Mains Water Irrigated</td>
<td>9</td>
</tr>
</tbody>
</table>
Due to the overall low numbers of species neither species diversity ($H'$) nor evenness ($J'$) were calculated. The distribution patterns of the litter acarine fauna at a species level are more completely dealt with in section 7.3.6 Patterns of Acarine Succession.

### 7.3.5.2 Collembola

Five collembolan species were extracted from the leaf litter. Two Isotomidae species, one Brachystomellidae, Sminthuridae (Plate 6.2) and an Entomobryidae (Plate 6.3). Collembola were rarely extracted in the first seven months. However, they were found in all treatments during the last two sampling occasions (nine and eleven months). In the ninth month they represented $<5\%$ of the overall faunal density in each treatment, but in the eleventh month collembola represented $>30\%$ of the total faunal density in both the irrigated treatments while only accounting for $5\%$ of the fauna in the non-irrigated control sites. The increase in Collembola in the eleventh month in the two irrigated sites coincided with a reduction in the percentage contribution of the acarine species and an increase in the contribution of Other Insecta.

Analysis of the total density of collembolan fauna by month and by treatment revealed only one significant result. In the 9th month following placement of the litter-bags in the field collembolan density in the mains water irrigated sites was significantly higher ($F_{2,6}=21.338, p<0.05$) than in the effluent irrigated ($p<0.05$) and non-irrigated control sites ($p<0.05$). In the 11th month, there were no significant differences between the total densities of collembola due to treatments ($F_{2,6}=1.509, p>0.05$).
There was no significant difference in the total collembolan density from the 9th to the 11th month.

Although not significant ($F_{2,6}=4.628, p=0.053$), collembolan densities were highest in the two irrigated treatments and lowest in the non-irrigated control sites during stage three (months 9 and 11).

Plate 7.2. Sminthuridae sp. lateral view x 40

Plate 7.3. Entomobryidae sp. ventral view x 30
7.3.5.3 *Arachnida*

Spiders made a consistent contribution to the faunal density in the irrigated sites in all months. Percentage contribution to total faunal density in both irrigated sites between months 1-7 was typically in the range of 10-20%, and then decreased to <10% in months 9 and 11. In the non-irrigated control sites spiders were absent until the 5th month, and typically represented approximately 1% of the total fauna in the following months.

7.3.5.4 *Other Insecta, Other Arthropoda and All Other Fauna*

These broader taxa included the larger mesofauna and some macrofauna. Other Insecta included beetles, ants, and flies, while Other Arthropoda were predominantly Isopoda (slaters) and Chilopoda (centipedes). All Other Fauna included earthworms (Oligochaeta).

The Other Insecta were, together with the acari, the dominant litter fauna. From month 1 to 7 they typically contributed between 10 and 45% of the total faunal density. Analysis of the relationship between the percentage contribution of the Acari and Other Insecta fauna to total density revealed a significant negative correlation ($r^2 = 0.663$, $p << 0.01$). That is, high levels of Acarine fauna were associated with low levels of Other Insecta and vice versa.
The Other Arthropoda and All Other Fauna rarely made substantial contributions to the total density with the exception of the All Other Fauna (earthworms) in the 11th month and only in the effluent irrigated sites. Isopods (Other Arthropoda) were found in significant numbers in the third and fifth month but only in the mains water irrigated sites.

### 7.3.6 Patterns of Acarine Succession

Acarine densities and species richness peaked in stage three – months nine and eleven (Fig 7.8). *Tyrophagus* sp., (the same species as was encountered in the soil samples) was ubiquitous, and typically accounted for between 10 and 50% of the total acarine density. *Tyrophagus* sp., densities peaked in the third stage (autumn) but were substantially higher in the effluent ($17.9 \times 10^2 \text{ N kg}^{-1}$) and mains-water irrigated sites ($17.1 \times 10^2 \text{ N kg}^{-1}$) relative to the non-irrigated control ($5.5 \times 10^2 \text{ N kg}^{-1}$).

Litter comminuiting fauna (astigmatid and oribatid mite fauna) were present in all stages but densities peaked in stage 3. Stage 1 – months one and two (spring) was dominated by three litter comminuters (*Tyrophagus* sp., *Brachychthonius* sp. 3., and *Ceratozedidae* sp.) and one predatory mite (*Ascidae* sp. 1) in all three treatments.

Stage 2 – months three, five and seven (spring and summer) was characterised by the development of a more diverse community. In stage 2 prostigmatid mites were
present for the first time in significant numbers. Oribatid mites were absent from the non-irrigated control litter packs, but were present in both irrigated treatments. Stage 2 was also characterised by the development of a more species rich predatory mite functional group.

Stage 3 (autumn) litter communities were characterised by large increases in species richness and acarine densities. There were large increases in the densities of comminuting (Tyrophagus sp., Brachychthonius sp. 3., and Zygoribatula sp.) and predatory fauna (Protogamacellus sp., Ascidae sp. 1., and Ameroseiidae sp.). There were few prostigmatid litter mites recorded from non-irrigated control sites in stage 3 although prostigmatid mites including Spinibdella sp., Cheyletidae sp. 1., and Eustigmaeus sp. were regularly recorded in both irrigated sites.
Figure 7.8. The effect of treatment and stage on the density kg\(^{-1}\) dry litter of selected litter acarine fauna to overall acarine density. (Only species contributing >100 individuals (number kg\(^{-1}\) litter dry weight) in at least one treatment regardless of stage were included).
7.4  **Summary of Results**

The significant outcomes from this chapter are:

1. Irrigation did not affect levels of %TC or %TN in attached leaves.

2. Litter-bag mesofaunal densities were very low and increased from the seventh month.

3. Classification and ordination revealed three distinct stages of faunal succession.

4. Overall, the litter Acari tended to dominate the mesofaunal populations in *E. globulus* litter-bags.

5. Irrigation did not affect rates of litter mass loss rejecting research hypothesis seven.

6. Collembola and Acari shared an inverse relationship, with high numbers of Collembola coinciding with low numbers of Acari and vice versa.

7.5  **Discussion**

The rate at which litter decomposes (used in this discussion to describe both mineralization and physical breakdown) has a direct impact on the productivity of forest and plantation ecosystems. The rate at which litter is broken down and incorporated into soil affects the content of soil organic matter, the soil structure, soil
water relations and the rooting environment, as well as determining the rate at which nutrients within the litter are recycled and converted into a form available for plant uptake (Aggangan et al., 1998; Aggangan et al., 1999; Woods and Raison, 1982). Rates of decomposition are regulated by environmental conditions such as humidity (Santo et al., 1993), temperature (Pereira et al., 1998; Smith, 1990) and edaphic factors (Berg, 2000; Mentenmeyer and Berg, 1986). Within the same environment, rates of litter decomposition are affected by the physicochemical properties of the litter (Gallardo and Merino, 1993; Irmler, 2000; Pereira et al., 1998). Low rates of decomposition have been associated with high C:N ratios, high lignin content and/or tannin content and the ratio of toughness to N (Gallardo and Merino, 1999; Irmler, 2000; Pereira et al., 1998; Woods and Raison, 1982).

If, as is suggested, the rate of decomposition is in part a function of the nutrient and structural characteristics of the fallen litter, then changes to floral composition are likely to affect ecosystem functions by changing the litter characteristics (Pereira et al., 1998). Similarly, the application of irrigation, either water or an effluent with high levels of N and other soluble elements, is expected to impact on the litter subsystem’s ability to provide services such as decomposition, maintenance of soil structure, nutrient cycling and energy flow (Cepeda-Pizarro et al., 1996; Taylor et al., 2004). The rate of decomposition under Australian eucalypts is of particular interest since they regularly occupy sites of low nutrient status, receive low natural inputs of nutrients (Woods and Raison, 1982), yet display levels of productivity that are comparable to high productivity forests from around the world (Adams and Attiwill, 1986b). It has been suggested that the rate of litter decomposition may control the
long-term productivity of the forest community (Woods and Raison, 1982), so any change in the available nutrient load due to a variation in the floral composition may have substantial long term consequences.

Soil food webs are donor-controlled (Laakso et al., 2000; Pimm, 1982; Strong, 1992), and so limited by the rate at which organic matter falls to the ground and becomes available to the below-ground faunal system. In natural systems, the major input of energy and nutrients is via leaf litter. The critical ‘gate-keeping’ role of the soil and litter mesofaunal in mediating the process of litter decomposition means they can control the rate at which nutrients and energy are released into the below-ground system from detritus. At the Albany Effluent Irrigated Tree Farm, irrigation was expected to impact on the rate of decomposition by providing increased levels of soil moisture and in the case of effluent irrigation, soluble nutrients (N and P). It was hypothesised that this would impact on the structure of the soil and litter faunal communities.

Previous research at the Albany Effluent Irrigated Tree Farm reported that *E. globulus* stands irrigated with effluent produced between 40 and 60% more leaf litter than non-irrigated stands (Adams et al., 2001). This results in a substantial difference in the biomass of standing crop under irrigated (both types) and non-irrigated trees, which is immediately obvious to the casual observer when walking through the plantation. Rates of litterfall in the years 1997-98 and 1998-99 were found to vary significantly between each of the treatments with effluent irrigated (∼7500 kg ha⁻¹ yr⁻¹) > mains-water irrigated (∼7300 kg ha⁻¹ yr⁻¹) > non-irrigated
control sites (≈4500 kg ha\(^{-1}\) yr\(^{-1}\)) (Adams et al., 2001). It is difficult to place these figures in an appropriate context as a recent publication noted, due to the lack of studies on rates of litterfall in *Eucalyptus* plantations of any species in Australia (Moroni and Smethurst, 2003). *Eucalyptus diversicolor* regrowth forests in Western Australia produced 3700 – 4500 kg ha\(^{-1}\) yr\(^{-1}\) of litter (O'Connell and Grove, 1993). Rates of litterfall under three year old *Eucalyptus nitens* plantations in Tasmania ranged from 4763 kg ha\(^{-1}\) yr\(^{-1}\) under fertilized trees to 5368 kg ha\(^{-1}\) yr\(^{-1}\) under unfertilized trees (Moroni and Smethurst, 2003). Rates of litterfall under *Eucalyptus pilularis* forests in New South Wales and Queensland ranged from 4000 kg ha\(^{-1}\) yr\(^{-1}\) for trees under the age of twenty to 12000 kg ha\(^{-1}\) yr\(^{-1}\) for trees over the age of one hundred (Turner and Lambert, 2002). Mean annual litterfall from two dry woodland sites in central Queensland ranged from 1129 kg ha\(^{-1}\) yr\(^{-1}\) in an open *E. populina* woodland to 2318 kg ha\(^{-1}\) yr\(^{-1}\) in an open *E. cambagena* woodland (Grigg and Mulligan, 1999). Two year old *E. globulus* growing in a Mediterranean climate in Portugal produced between 1000 and 2500 kg ha\(^{-1}\) yr\(^{-1}\) (Madeira and Pereira, 1990/1991). Variation in the rates of litterfall has been related to geographical location, age of the plantation or forest and the specific species of eucalypt being studied. Rates of annual *E. globulus* litterfall at the Albany Effluent Irrigated Tree Farm for the control sites compare with rates of litterfall in other Australian studies, while rates of litterfall in the irrigated sites are higher.
7.5.1 Patterns of *E. globulus* Litter Decomposition at the Albany Effluent Irrigated Tree Farm

Following the example of previous decomposition studies (i.e. Baker and Attiwill, 1985; Guo and Sims, 1999; Guo and Sims, 2001; Guo and Sims, 2002; Singh *et al.*, 1993; Woods and Raison, 1982; Woods and Raison, 1983) I determined rates of litter decomposition using the decay rate constant ‘*k*’ (Olsen, 1963). Rates of litter decomposition ranged between *k* = 0.46 – 0.53 for both irrigated and non-irrigated *E. globulus* trees at the Albany Effluent Irrigated Tree Farm. There was no significant difference in rates of *E. globulus* litter decomposition between irrigated and non-irrigated trees over the eleven months of this study. These rates of litter decomposition compare favourably with previously published results for Australian, *k* = 0.24 to 0.94 (Guo and Sims, 1999), *k* = 0.47 to 0.68 (Woods and Raison, 1983), New Zealand, *k* = 0.39 to 0.59 (Baker and Attiwill, 1985) and Indian, *k* = 0.69 (Singh *et al.*, 1993) based studies of eucalypt litter decomposition.

The use of *k* as a measure of the rate of decomposition has been the subject of criticism in the literature (i.e. Woods and Raison, 1982). An underlying assumption of the equation is that the mass of accumulated litter is constant over time because the levels of standing crop are at equilibrium (Olsen, 1963). Research of standing crops under Australian eucalypts has found that litter loads may not approach an equilibrium point for many decades (30 - 60 years) after a disturbance (Birk and Simpson, 1980). Another assumption of the model is that rates of litter decomposition are constant throughout the life of the abscised litter (Olsen, 1963). A
complete review of the limitations of the decomposition constant $k$ is provided by Woods and Raison (1982). While these limitations can not be denied, the fact remains that scientists need a mechanism by which rates of decomposition can be calculated to facilitate intra and inter site comparisons. The main reason for the continued use of $k$, despite these limitations, is the high level of correlation between actual and predicated rates of decomposition based on the decay rate constant $k$.

Eucalypt leaves decompose more slowly than many European broad-leaved tree species (Briones and Ineson, 1996) and this can result in the storage of substantial volumes of nutrients within the standing crop (Adams and Attiwill, 1986b; Adams and Attiwill, 1986a). The rates of *E. globulus* litter decomposition observed in this study support the long held view that eucalypt litter decomposes slowly (Adams and Attiwill, 1986a). It has been suggested that the relatively slow decomposition of Australian eucalypts may be due to their high initial polyphenolic content (Wood, 1974). Recent studies have challenged this view and suggest that eucalypt litter can decay very rapidly given appropriate conditions (Briones and Ineson, 1996; Guo and Sims, 2001). Given that there are over 500 species within the genus *Eucalyptus*, it seems reasonable to assume that leaf litter from different species will display different rates of decomposition. Additionally, external factors including moisture (Guo and Sims, 2002), temperature (Bargali et al., 1993), levels of incident light (Guo and Sims, 2002), and the number of different litter types (Briones and Ineson, 1996) all impact on the rate of litter decomposition. Given the dependence of litter decomposition on so many factors, generalizations about rates of decomposition at a genus level may not be particularly useful (or accurate).
In this study, rates of litter decomposition did not vary significantly with treatment. Neither the application of water (mains-water irrigated) nor the addition of water and nutrients (effluent irrigated) had any significant impact on the rate of litter decomposition. A Portuguese study of rates of *E. globulus* litter decomposition and nutrient release reported that leaf litter collected from 26 year old *E. globulus* plantation did not display significantly different rates of decomposition despite being collected from trees subjected to a range of treatments including solid fertilization, irrigation, irrigation and solid fertilization, irrigation and liquid fertilization and irrigation, liquid fertilization and solid fertilization (Ribeiro *et al.*, 2002). The authors reported that the only difference between the leaf litters was in initial N concentrations. While variation in N concentration would normally be expected to result in different rates of decomposition these authors reported that leaves with high N concentrations leached N at a faster rate during the first 133 days while leaf litters with lower N concentrations showed no change or even increased in N concentration. After 133 days, all leaf litters increased in N concentration and although there were slight variations in k (0.42 – 0.47 at 317 days and 0.37 – 0.42 at 643 days) these were not statistically significant.

This is the opposite finding to that reported in a similar study conducted in New Zealand (Baker *et al.*, 1990). These authors described the impact of effluent irrigation on the rate of pine needle decomposition in a *Pinus radiata* plantation. They reported increased rates of decomposition following effluent irrigation, which they explained by hypothesizing that effluent irrigation supplied both moisture and
nutrients; two factors commonly reported as limiting overall rates of litter decomposition (Guo and Sims, 1999; Guo and Sims, 2001; Guo and Sims, 2002). They suggested that the increased availability of moisture, organic carbon and other nutrients led to an increase in microbial activity, with suggested bottom-up resource effects on the litter mesofaunal population that were responsible for the increased the rate of litter fragmentation and mass loss. In the New Zealand study by Baker et al., (1990) effluent was distributed by spray irrigation while in this Albany based study effluent was distributed via trickle drip irrigation. In the New Zealand study, effluent was spread over a very large surface area while in this Albany study effluent was specifically targeted at the base of the tree trunk. If, as is suggested, the level of available moisture is a significant limiting factor of rates of litter decomposition then it seems probable that the targeted method of effluent irrigation (drip irrigation) employed at the Albany Effluent Irrigated Tree Farm does not result in the widespread application of soluble nutrients over the litter and so does little to remove the limitations of available moisture and soluble nutrients except at positions immediately adjacent to the drippers.

A number of researchers have reported that rates of litter decomposition when measured by mass loss tend to follow a two stage pattern (Briones and Ineson, 1996; Hasegawa and Takeda, 1996; Pereira et al., 1998). The initial phase (stage one) is characterized by the rapid loss of leaf litter mass and is typically very short (in the order of two to four months). The second phase (stage two) is characterized by the gradual loss of leaf litter mass and can take many months. Even in the short time frame of this study (11 months), the pattern of mass loss observed in this
project displays two distinct stages of decomposition (not to be confused with the three stages of faunal succession). In the first three months *E. globulus* litter under all three treatments lost approximately 25% of its initial mass (Figure 7.1). Between the 3rd month and the 11th month, all three treatments lost a further 5 to 10% of their initial mass. It is thought that the initial rapid weight loss may be due to the leaching of water soluble compounds, tannins and other readily metabolisable materials from the leaf litter into the soil system (Briones and Ineson, 1996; Pereira et al., 1998; Prescott, 1995). Mass loss in the second stage of decomposition equates to the physical breakdown of the leaf litter mediated by the comminuting activities of the litter fauna (Briones and Ineson, 1996; Reddy, 1995). This trend was clearly visible in the third stage of litter decomposition at the Albany Effluent Irrigated Tree Farm when obvious fragmentation of the litter correlated with the significant increases in litter faunal density (c.f. Plate 7.1 months 9 and 11). Other studies of *Eucalyptus* litter decomposition have observed that the initial phase tends to be in the order of two to four months (Briones and Ineson, 1996; Gallardo and Merino, 1993; Gallardo and Merino, 1998; Gallardo and Merino, 1999). It is thought that the thicker cuticle and greater toughness of eucalypt litter retards the movement of soluble compounds during the initial phase of decomposition (Gallardo and Merino, 1993). It is worth noting that the litter decomposition experiment at the Albany Effluent Irrigated Tree Farm commenced in June (winter in the Southern Hemisphere). Rainfall during the months of June, July, August and September 2001 exceeded 50mm per month. It seems probable that the high rates of rainfall may have facilitated the rapid leaching of soluble materials from the leaf litter as evidenced by the initial rapid mass loss (Fig 7.1).
In summary, rates of litter decomposition were unaffected by irrigation treatments (effluent and mains-water). It was proposed that this lack of a response to the irrigation was due to the method of distributing irrigation. The use of drip irrigation at the Albany Effluent Irrigated Tree Farm results in highly targeted distribution of effluent and did not affect rates of decomposition at the site level. Given that rates of decomposition ($\lambda$) between the non-irrigated and two irrigation treatments are the same the greater rate of litter fall ($I$) observed in the two irrigated treatments explains the greater levels of litter standing crop ($X$) observed in the irrigated treatment plots.

**7.5.2 Litter Chemistry of Decomposing *E. globulus* Leaf Litter at the Albany Effluent Irrigated Tree Farm**

The relationship between litter quality and decomposition rates has been the focus of a large number of research projects over the last twenty years (Corbeels, 2001). A comprehensive review of the literature can be found in Heal *et al.*, (1997). Within the literature are two key themes. The first is that the C:N ratio is widely accepted as a general index of litter quality, while the second is that the levels of lignin and polyphenols are responsible for the recalcitrance of plant litter to decomposition (Corbeels, 2001). C:N ratios >25 are commonly described as low quality residues that are likely to promote immobilization while litter with C:N ratios of <25 tend to be classified as high quality residues and are expected to promote the release of N and other organic compounds via mineralization (Myers *et al.*, 1994). Other researchers
have reported that N mineralization occurs only when the N concentrations of the residue are above 2% (Palm and Sanchez, 1991).

Initial ratios of fallen *E. globulus* leaf litter were quite high (>55) for all three treatments, but slowly declined with time in the field to range from 40 to 50 after eleven months in the field. This high C:N ratio is expected to result in slower rates of decomposition and immobilization of nitrogen within the standing crop, a conclusion supported by the slow rates of decomposition discussed in the previous section. This level of C:N ratio is comparable to other levels reported in the literature for *E. globulus*. C:N ratios of recently fallen litter from non-irrigated eucalypts grown in New Zealand ranged from 37.3 (*E. botryoides*) to 53.46 (*E. globulus*) to 56.9 (*E. ovata*) while eucalypts *spray irrigated* with effluent ranged from 34.49 (*E. botryoides*) to 35.6 (*E. globulus*) to 26.86 under *E. ovata* (Guo and Sims, 2002).

Observed values for %TC and %TN for both mature and fallen *E. globulus* leaf litter are similar to other published results for Australian eucalypts (i.e. Hooda and Weston, 1999; Moroni and Smethurst, 2003). Neither effluent nor mains-water irrigation have significantly impacted on the nutrient composition of the leaf litter relative to the non-irrigated trees. Litter TC/TN dynamics in the three treatments are remarkably similar and occasional variations are more likely to be reflective of the sampling regime (i.e. small numbers of replicates \( n = 6 \) per sampling event for non-irrigated control and \( n = 12 \) for irrigated treatments per sampling event) and the inherent variability of individual leaves rather than representing any biologically significant result.
Percentage total carbon within the *E. globulus* litter tended to decrease with time since placement in the field. Both mature leaves (still attached to the tree) and abscised leaf litter displayed similar %TC. There was no effect of treatment on litter %TC over the first six months. Percentage TC declined rapidly in the ninth and eleventh months in the two irrigated treatments but were relatively stable in the non-irrigated control sites. This suggests that in the irrigated treatments, carbon and litter mass were initially lost at a similar rate, and as decomposition progressed, carbon was lost at a faster rate than litter mass. Despite eleven months in the field, litter from the non-irrigated control sites did not display any variation in percentage total carbon. This suggests that in the non-irrigated control sites, the rates of carbon loss and litter decomposition are similar.

It is unfortunate that this experiment did not continue for longer to observe when %TC would begin to decline in the non-irrigated control sites. The time frame is just a little too short to state that there is a biologically significant difference in the patterns of %TC in the irrigated sites relative to the non-irrigated control sites, although it appears as if this may be the case. The ninth and eleventh months correspond to March and May 2002 and fall within the southern hemisphere season of autumn. The importance of this observation is that autumn and summer are the two seasons of the year during which rates of irrigation are maximal and rainfall is minimal. It seems reasonable to suggest that if irrigation were to result in a different pattern of TC loss it would be most easily observed in summer/autumn when the
difference in soil moisture and hence litter humidity between the irrigated and the control sites would be greatest.

Percentage total nitrogen within the *E. globulus* litter tended to increase with time since placement in the field. Mature leaves (still attached to the tree) display levels of total N approximately double those of abscised litter. A Portuguese study of N resorption from mature leaves prior to abscission reported that between 32 and 65% was resorbed depending on treatment and season (Madeira *et al.*, 1995). The resorption of N from leaves prior to abscission is a commonly observed trend and allows for plants to redistribute nutrients within their biomass without losing potentially limiting nutrients to the below-ground ‘ecosystem’ (Raven *et al.*, 1999). Percentage TN remained relatively constant during the first three months of the experiment, after which they began to increase in all treatments. This suggests that, initially, nitrogen (like carbon) was lost from the litter at a similar rate to mass loss. Most likely, this similarity between the rates of N, C and mass loss is due to the leaching of highly mobile C and N compounds from the litter during these initial months. However, from the third month following placement in the field, %TN begins to increase. Previous studies have reported that C:N ratios decline as decomposition progresses (Bradford *et al.*, 2002). This result is due to a decline in the %TC concentration and an increase in the %TN concentration. Research has shown that the increase in %TN correlates with the presence and increasing dominance of fungal biomass (Hasegawa and Takeda, 1996). It is thought that the rise in %TN is due to the immobilization of N by fungi growing on the surface of the decomposing leaf litter (Baker and Attiwill, 1985; Baker *et al.*, 1990; Bradford *et al.*, 2002).
2002; Hasegawa and Takeda, 1996). Physical examination of the litter supports this, as obvious hyphal growth was first observed in litter collected three months after placement in the field. As time since placement in the field increased, the amount of fungi observed growing on the surface of the leaf litter also increased.

7.5.3 Litter Fauna and Patterns of Faunal Colonization in Decomposing E. globulus Leaf Litter at the Albany Effluent Irrigated Tree Farm.

Litter microarthropods are regulators of litter decomposition. When excluded from litter via small mesh sizes, or selected against by the application of chemicals, rates of decomposition slow, and primary productivity decreases (Carcamo et al., 2001; Heneghan et al., 1999; Seastedt, 1984; Setala and Huhta, 1991; Whitford et al., 1983). Since few of these microarthropods are able to directly consume decomposing leaf litter it is thought that much of the biological regulation which they impose on the decomposition process is exerted through the their trophic interactions with the microbial flora (Lussenhop, 1992; Moore et al., 2004; Moore et al., 1988).

Analysis of the faunal assemblages identified from decomposing E. globulus litter at the Albany Effluent Irrigated Tree Farm using MDS (Fig 7.6) and Classification (Fig 7.5) routines identified a three-stage pattern of faunal colonization. Stage one comprised the first two months and was characterized by low faunal densities and low species richness. Stage two included months three, five and seven and was distinguished by increased mesofaunal densities and levels of species richness.
Stage three included months nine and eleven and was typified by a significant increase in mesofaunal density and stable to slightly increased levels of species richness. In the following discussion I consider each of the stages in turn, highlighting the characteristics unique to each stage and where possible consider potential relationships between the patterns of faunal colonization and patterns of litter mass loss.

### 7.5.3.1 Stage One

All three treatments (Non-Irrigated Control, Effluent and Mains Water Irrigated) in stage one were characterized by low levels of mesofaunal density and species richness. The microarthropod composition within this stage was dominated by Acari, other Insecta (specifically Formicidae and Coleoptera) and small numbers of Arachnida. Contributions to acarine density in all three treatments were dominated by *Tyrophagus* sp., *Brachycthonius* *sp.* 3, *Ceratozetidae* sp., and *Asciidae* sp. 1. Both *Tyrophagus* sp. and *Brachycthonius* *sp.* 3., have been discussed in previous sections. Both of these mites are known r-strategists, and posses a range of adaptive strategies for invading new organic material which include the ability to reproduce parthenogenically, a short reproductive cycle (Santos and Whitford, 1981), resistance to desiccation (Behan-Pelletier, 1999), and tolerance of disturbance (Franchini and Rockett, 1996). Mites of the family *Ceratozetidae* are described as cosmopolitan in their distribution and have been found in forest litter, humus, soil and moss (Krantz, 1978). A study of acarine populations in both litter and soil under *E. globulus* plantations in Collie (South Western Australia) reported no ceratozetid mites, despite finding them in both soil and litter samples under
native *E. marginata* bushland (Adolphson, 2000). The predatory mite Ascidae sp. 1, was found in higher numbers in the second month suggesting that these mites were migrating into the fresh litter to prey on increasing densities of *Tyrophagus* sp., *Brachychthonius* sp. 3, and Ceratozetidae sp and other mesofauna.

A study of microarthropod succession in canola litter placed on the soil surface of a West Australian wheat field reported that Collembola were the most dominant microarthropod order during the first month and the second most dominant order (after prostigmatid mites) in the second month (Osler *et al.*, 2004). At the Albany Effluent Irrigated Tree Farm, collembola were not found in *E. globulus* litter until the third month (very low density) and then not again until the ninth and eleventh month when they were extracted in substantial numbers (255-2900 kg\(^{-1}\) litter dry weight). Collembola were rarely recorded from soil under *E. globulus* at the Albany Effluent Irrigated Tree Farm. The differences in the patterns of faunal succession may be related to the differences in litter type. Other authors have observed lower collembolan densities in soil under *E. globulus* than under native vegetation and have suggested that this may be related to the chemical composition of the leaf litter (Pinto *et al.*, 1997; Serralheiro and Madeira, 1990).

### 7.5.3.2 Stage Two

Stage two was characterized by increased species richness and faunal density dominated by litter Acari, Other Insecta (specifically Formicidae and Coleoptera) and Arachnida. The density of *Tyrophagus* sp. did not increase substantially from the first stage to the second. Across all three treatments there was a large increase in
the number of predatory mites, including Protogamacellus sp., Podocinella sp?, Ascidae sp 1., and the nematode feeding mite Ididorryia sp.

Thirteen mite species were extracted in stage two. Seven of these were found in the non-irrigated control, ten in the effluent irrigated, and twelve in the mains-water irrigated sites. Five mite species were common to all three treatments, Tyrophagus sp., Ididorryia sp., Protogamacellus sp., Ascidae sp 1., and Podocinella sp? As noted previously densities of Tyrophagus sp., did not increase significantly from the first stage to the second.

The prostigmatid mite Tydeidae Ididorryia sp. was ubiquitous across all treatments in this stage, which may suggest that there were substantial populations of nematodes on the leaf litter. A study of microarthropod succession on buried litter in the Chihuahuan desert reported that tydeid mites were the first mites in a consistent pattern of colonization to invade buried leaf litter (Santos et al., 1981; Santos and Whitford, 1981). These authors reported that tydeid mites were significant predators of bacteriophagic nematodes and so hypothesized that tydeid mites regulated rates of decomposition by regulating the population size of microbial grazing nematodes (Santos et al., 1981; Santos and Whitford, 1981).

The increase in acarine predatory mite density (Mesostigmata and Prostigmata) was consistent across all three treatments and appears to relate to the increase in overall acarine density (bottom-up resource control). Studies of microarthropod succession have noted that predatory mites are usually found in insignificant
numbers in the first phase of decomposition and increase in density with time, parallel with an increase in overall acarine density (Vreeken-Buijs and Brussaard, 1996). A study of wheat residue decomposition and the importance of mesofauna to the rate of decomposition reported that rates of decomposition were predator controlled (Vreeken-Buijs and Brussaard, 1996). When microarthropod predators (mesostigmatid mites) were excluded from litter-bags, rates of decomposition decreased. The authors suggested that this was due to over grazing of the microbial fauna by the microbivorous mesofauna (Vreeken-Buijs and Brussaard, 1996). They concluded that microarthropod predators regulate the density of the microbivorous mesofauna and so indirectly affect the grazing intensity of these microbivorous mesofauna (top-down predator control). A study of the microarthropod succession on canola litter material (*Brassica napus*) placed on the soil surface in a wheat field in West Australia, reported that mesostigmatid mites were never a dominant microarthropod order (Osler *et al*., 2004). This observation is almost certainly linked to the short period of time over which the four succession studies were conducted, since all studies were completed in less than 103 days (three and a half months).

Of the three mesostigmatid mite species that were found in all treatments within the second stage of faunal succession, the mesostigmatid mite Podocinidae *Podocinella* sp? deserves a little more attention. While members of the genus *Podocinum* are widespread and well known from Australian soils, species within the genus *Podocinella* are not. This particular unidentified species displayed characteristics of both genera, although it is more like the description of *Podocinella* than *Podocinum*. 
While it is not unusual to be faced with this kind of taxonomic dilemma, (particularly when dealing with the poorly described Australian fauna) this specific mite also displayed an unusual pattern of density, being only found in samples collected within the fifth month but from all samples irrespective of treatment. Further research into this particular mesostigmatid mite and clarification of its taxonomic position will be a particularly interesting follow up to this project.

Of the thirteen litter mite species extracted during stage two, six mite species were never recorded in litter samples from the non-irrigated control site. Of these six species, four species were recorded in both irrigated treatments suggesting a potential irrigation response. Three of these mite species are oribatids including; *Brachythionius* sp. 3., *Ceratozetidae* sp., and *Zygoribatula* sp., and one mesostigmatid mite, *Ameroseiidae* sp. The presence of these three fungal feeding oribatid mites coincides with the presence of significant quantities of fungal hyphae on the surface of *E. globulus* litter. Additionally, the presence of these fungal feeding mites supports the suggestion that the increase in %TN observed in this stage is potentially related to the immobilization of nitrogen within fungal hyphae, as observed in other research studies (Bradford *et al.*, 2002; Hasegawa and Takeda, 1996).

Rates of decomposition (mass loss) in stage two plateaued, suggesting that by September, three months after placement in the field, the high levels of rainfall recorded in the three previous months were sufficient to leach the mobile and soluble nutrients from the litter. This suggests that the mass loss observed from the
third to the eleventh month corresponded with the physical fragmentation of litter, rather than to mass loss due to leaching. This view is supported by the samples in Plate 7.1. When comparing the leaf litter from each month, there is a clear variation between the leaf litter collected at two months and three months after the commencement of the project, with litter in the third month showing obvious signs of initial comminution. There is another obvious increase in the level of fragmentation between litter harvested in the seventh and ninth months following placement in the field. These two months corresponded with the conclusion of the second stage of faunal colonization and the commencement of the third stage – months nine and eleven.

7.5.3.3 Stage Three

The third stage of mesofaunal colonization was characterized by significant increases in litter mesofaunal total density and species richness in the two irrigated sites and by incremental increases in both density and species richness at the non-irrigated control site (Fig. 7.3). Mesofaunal densities observed in the ninth month were up to thirty times higher than those observed in the seventh month. Proportionally, the litter acarine fauna accounted for >75% of the total mesofaunal density in each of the three treatments in both months (nine and eleven). Approximately half of the litter acarine density in both irrigated sites and a third in the non-irrigated site was due to the astigmatid mite Tyrophagus sp. The remaining acarine fauna were dominated by prostigmatid and mesostigmatid mites. The increase in species richness between stage two and stage three is almost entirely explained by the increase in the number of prostigmatid mite species in stage three.
Prostigmatid mites including *Spinibdella* sp., Cheyletidae sp 1., *Raphignathus* sp 1., and *Eustigmaeus* sp., were all regularly extracted from leaf litter in the irrigated sites but were never encountered in the non-irrigated control sites. These predatory mites may have been migrating into the litter-bags to prey on increased densities of mites and collembola.

The rapid increase in the total mesofaunal density observed in the ninth month for the two irrigated sites is possibly due to relatively high levels of soil/litter moisture. Given that this period of time coincides with the end of summer 2002, and consequently low levels of rainfall, higher temperatures, and maximum rates of irrigation, this may explain why this peak in mesofaunal density was observed in the two irrigated sites and not in the non-irrigated control sites. As noted earlier, this peak in mesofaunal density coincided with an increase in the obvious particulation and fragmentation of the litter, as displayed in Figure 6.1. While it appears that the increase in mesofaunal density may be related to the application of irrigation it is noteworthy that the absence of a more complex faunal community in the non-irrigated control litter had no discernable impact on rates of mass loss, levels of %TC and %TN and the C:N ratios. It is possible that the relationship between species richness and litter mass loss (decomposition) or changes in litter chemistry are not immediately obvious and may take some time (months?) to propagate through the litter subsystem. Studies of the relationship between birch litter decomposition in Germany and levels of litter species richness have observed a positive correlation between the two (Setala *et al.*, 1988; Setala *et al.*, 1998).
The other major contributor to overall mesofaunal density in this stage was the collembola. Five collembolan species were identified and densities ranged from 255 to 3547 individuals kg\(^{-1}\) litter dry weight. As noted earlier, the presence of collembola at the end of this study is the opposite trend to that reported from a canola decomposition study conducted in wheat fields in central Western Australia (Osler et al., 2004). In the study of canola decomposition, Collembola were found to be the most dominant microarthropods during the initial month of faunal colonization but became the least dominant fauna as decomposition entered the second and third month. A study of beech litter (\textit{Fagus sylvatica}) decomposition conducted in northern Germany reported that Collembola were early colonizing fauna (Irmler, 2000). This three year study reported that collembolan populations dominated at the beginning of the decomposition process while oribatid mites dominated towards the end (Irmler, 2000). At the Albany Effluent Irrigated Tree Farm, oribatid mites tended to be most abundant in the early stages of decomposition and were less abundant during the later stages when prostigmatid mite and collembolan density increased. A potential explanation of this ‘contradictory’ order of colonization may well be related to the chemical composition of the \textit{E. globulus} litter. Collembola have been reported as extremely sensitive to variation in levels of allelochemicals, being able to detect variations at the level of a picogram (Ananthakrishnan et al., 1993). Eucalypts are known to produce significant quantities of allelopathic chemicals (Briones and Ineson, 1996) which may potentially be excluding collembola during the early stages of litter decomposition. A Portuguese study of microarthropod densities under native vegetation dominated by \textit{Q. suber} and contrasted with populations under monoculture plantations of \textit{E. globulus} reported significantly lower levels of
collembola under *E. globulus* in each of the four sampling periods (Serralheiro and Madeira, 1990). Based on the sequence of mesofaunal colonization at the Albany Effluent Irrigated Tree Farm, it appears as if collembola may be excluded from the decomposition process by the presence of allelopathic chemicals during the early months and only occur in substantial numbers following the commencement of litter fragmentation in month nine associated with the significant increase in litter Acari when the allelopathic chemicals may have been leached from the litter.

With the exception of the mite *Tyrophagus* sp., no mesofaunal species was ever present in all treatments in all stages. This highly variable pattern of occurrence suggests a very transient mesofaunal community and supports the concept of mesofaunal succession on litter (Hasegawa and Takeda, 1996). These authors suggest that changes in mesofaunal succession are a result of variation in the structure, chemical and biological properties of the decomposing substrate. Strong patterns of mesofaunal succession have been observed in the Chihuahuan desert, where *buried* litter was found to be colonized first by tydeid mites, next by tarsonemids and pyemotids followed by predatory Gamasina and predatory prostigmatid mites. Once numbers of these predatory fauna increased, there was a marked drop in the numbers of tarsonemid and pyemotid mites. The final group to colonize the litter were Collembola and Psocoptera (Santos and Whitford, 1981). This clear pattern of short term mesofaunal succession in the Chihuahuan desert, irrespective of the time of placement, has not been reported for any other environment since (Osler *et al.*, 2004). Other authors have reported that mesofaunal community composition is strongly influenced by seasonality rather than
resource availability or quality (Vossbrinck et al., 1979). Still other authors have rejected the notion of seasonal control and have related changes in microarthropod community structure to resource quality (Lagerlof and Andren, 1985). The reality of these contradictory conclusions is that the structuring force of microarthropod communities on decomposing litter is probably a variety of forces as opposed to just one or two. That is, the factors responsible for structuring a specific decomposer community at a given point in time (i.e. at the time of sampling) may well be the result of complex interactions between season, temperature, moisture, resource quality, perturbation history and various site specific stressors. In addition, each of these regulating forces operates at varying scales and so result in complex cross scale dynamics that may be extremely difficult to identify and separate from each other.

In summary, rates of litter decomposition at the Albany Effluent Irrigated Tree Farm are comparable to rates of decomposition of eucalypts reported from similar geographical and Mediterranean climate regions. Rates of decomposition at the Albany Effluent Irrigated Tree Farm measured over eleven months support the view that *E. globulus* litter (at least for the first year) decomposes very slowly, particularly under monoculture plantations and are insensitive to irrigation or irrigation type. This finding leads to the rejection of the seventh research hypothesis (7. Effluent and mains-water irrigation is expected to enhance the rate of litter decomposition supporting the trend reported by Baker et al., (1990)). The two-phase pattern of mass loss reported from other studies of eucalypt decomposition was also observed at this experimental site and allowed the use of the exponential model (*k*). Initial
rapid mass loss in phase one was suggested to be associated with the leaching of soluble nutrients while the slower rate of decomposition in phase two was associated with fragmentation of the litter. Levels of total carbon and nitrogen were comparable to the literature and the high C:N ratio was suggested as a potential explanation for the low rate of litter decomposition.

Faunal colonization of abscised litter occurred in three distinct stages. Stage one was characterized by low levels of density and species richness. The majority of the litter acarine fauna were typically r-strategists and included *Tyrophagus* sp., *Brachythonius* sp 3., *Ceratozetidae* sp., and *Ascidae* sp 1. Stage two was characterized by increasing mesofaunal density and species richness with substantial increases in the number of predatory mesostigmatid mites, and the nematode feeding tydeid, *Ididorryia* sp. Stage three was characterized by a significant increase in mesofaunal density (up to thirty times higher than stage two) and species richness.

The increase in species density was largely attributable to the astigmatid mite *Tyrophagus* sp., while the increase in species richness was almost entirely due to the increase in predatory prostigmatid mite species. The increase in *Tyrophagus* sp., is thought to be related to the increased humidity resulting from increased summer temperatures and elevated levels of moisture due to irrigation. The significant increase in the mesofaunal density coincided with a substantial increase in the fragmentation and particulation of the leaf litter, although this did not correspond with a significant change in the rate of litter mass loss.
Despite the significant difference in mesofaunal density in irrigated sites relative to the non-irrigated control site in stage three, this did not translate into a significant difference in rates of litter decomposition, or litter chemistry. It may be that this litter decomposition experiment concluded before the impact of increased mesofaunal density and species richness could be detected in rates of decomposition or levels of litter chemistry. Alternatively, as observed by Riberio et al., (2002), irrigation and fertilization may have no effect on rates of litter decomposition except during the initial phase. These authors observed that during the initial phase of decomposition N and P were released in higher proportions from *E. globulus* leaf litter, with higher initial concentrations of these elements. Consequently, this resulted in a narrowing of the range of these elements in litter from trees subjected to irrigation, and the various combinations of irrigation and fertilization (see earlier discussion for complete list). This narrowing of the range of N and P in leaf litter following the first phase of decomposition was suggested as a potential explanation for the observation that over a one and two year period there was no significant difference in the rates of litter decomposition regardless of treatment.

Six years after planting, *E. globulus* trees at the Albany Effluent Irrigated Tree Farm displayed no significant difference in initial levels of C and N relative to their treatment. There is the potential for a substantial pulse of soluble nutrients from freshly abscised litter to enter the soil under effluent irrigated *E. globulus* during late summer and early autumn. This is because mature effluent irrigated trees are predicted to produce leaf litter with significantly higher levels of N (Adams *et al.*, 2003).
Effluent irrigated *E. globulus* trees produce more leaf litter relative to non-irrigated trees. The bulk of this litter falls during early summer, which coincides with the peak effluent irrigation period. These factors combine to result in a standing crop under effluent irrigated trees that is of greater volume and containing higher levels of soluble nutrients at the same time as the peak in irrigation, therefore providing the potential for rapid leaching of these soluble nutrients into the soil profile. It would be interesting to observe if this pulse in N flow actually occurs, since N additions from decomposing leaf litter have been poorly incorporated (if at all) into models of nutrient flow at the Albany Effluent Irrigated Tree Farm.
"There are two possible outcomes: If the result confirms the hypothesis, than you've made a measurement. If the result is contrary to the hypothesis, than you've made a discovery."

Enrico Fermi
8.0 General Discussion

8.1 INTRODUCTION

8.1.1 Context

Plantation estates in Western Australia cover nearly 370,000 hectares, the majority of which is located within the southwest corner (Parsons and Gavran, 2005). Most of these plantations have been established to revegetate pasture sites (Aggangan et al., 1998; Aggangan et al., 1999) and they have the potential to ameliorate environmental degradation such as increased salinity, soil erosion, and to enhance biodiversity (Adolphson, 2000; Anonymous, 2005; Kozlowski, 2002). Since tree health and soil fertility are interdependent, the development of management strategies that maintain or promote soil fertility are essential for the long-term sustainability of any plantation (Johnston and Crossley Jr, 2002). One such management strategy at a global level, is the growing focus on the carbon sequestration potential of soil and increasingly on the importance of the soil and litter fauna as significant rate modifiers of the decomposition process (Jones et al., 2005; Paul and Polglase, 2004). Data on the composition of the soil and litter fauna and their contribution to litter decomposition is sparse for Australian plantation systems (Adolphson, 2000). The Albany Effluent Irrigated Tree Farm provided an opportunity to study the soil and litter faunal communities under an *E. globulus*
plantation and in addition, to describe the impact of irrigation (both effluent and mains-water) on these communities.

This research is unique in that it is the first known Australian study to adopt a ‘whole of fauna’ approach to describing the soil and litter communities under plantations. The research focused on the microfauna (Nematoda) mesofauna (Acari and Collembola) with simultaneous monitoring of the microbial resource base but did not include the Protozoa. The paucity of West Australian and Australian research regarding soil and litter fauna and in particularly plantation communities provided constraints on the taxonomic resolution and setting of the results within an appropriate geographical context.

### 8.1.2 Major outcomes of the Research

The plantation soils established on ex-pasture sites were consistently characterised by very low levels of active microbial biomass, reduced faunal populations (both low densities and diversity) and slow rates of litter decomposition, all characteristic of low-productivity environments. When there were effects of irrigation, it was independent of irrigation type. At the species level, oribatid mites from the family Oppiidae, Brachychthoniidae and Tectocephidae displayed negative responses to irrigation that suggest they may be potential indicator species for this type of soil disturbance.
8.2 **WHAT FACTORS STRUCTURE THESE SOIL AND LITTER COMMUNITIES?**

The soil and litter faunal communities at the Albany research site characterised by low density and diversity are significantly different to the soil and litter faunal communities sampled from *E. marginata* forests within the same geographical location that were characterised by significantly higher density and diversity (c.f. Postle *et al.*, 1986; Postle *et al.*, 1991). This raises the question, what factors are responsible for structuring the soil and litter communities at the Albany research site? To answer this question I have used three interconnected models in soil biology. Central to the discussion is a hierarchical model of the factors determining soil processes in terrestrial ecosystems presented by Lavelle *et al.*, (1993). Aspects of this model can be applied to the Albany research site to explain why these soils support such low faunal densities. To explain the low species richness it is useful to focus on the factors hypothesised to influence biodiversity, specifically, climate, spatial heterogeneity, disturbance, and productivity and integrate these within the hierarchical model. The factors responsible for structuring density and species richness are not necessarily discrete or mutually exclusive. A factor may be responsible for limiting both density and species richness. Finally, disturbance and the concept of dual energy pathways will be employed to explain the trophic types and dominance of *r*-selected species as observed at this Albany research site.
8.2.1 Factors Limiting Population Density

Soil and litter organisms, particularly invertebrates, are exposed to a unique set of complicated limiting factors not seen within other ecosystems (Lavelle and Spain, 2001). Detritus serves as a habitat and habitat modifier of soil organisms (Moore et al., 2004). As a habitat, detritus provides shelter, refugia and breeding sites while as a habitat modifier detritus regulates levels of soil moisture, light and temperature (Moore et al., 2004). In addition, detritus is the primary source of energy and nutrients for soil organisms and so there is a complex interrelationship between soil and litter organism habitat and resource (Lavelle and Spain, 2001; Moore et al., 2004; Moore et al., 2003). The quality and composition of detritus is an important regulator of soil populations. The three principal constraints on soil invertebrate populations are moisture availability, temperature, and food resources (Lavelle and Spain, 2001). These principal constraints have been organised into a hierarchical model of the factors determining soil processes (Brussaard, 1998; Lavelle et al., 1993; Lavelle and Spain, 2001). In this model, there are four hierarchical levels: (i) climatic factors (moisture and temperature regimes), (ii) edaphic properties (soil composition and nutrient levels), (iii) the physical and chemical composition of the decomposing resource (litter type and nutrient composition), (iv) biological regulation via mutualistic or antagonistic relationships between species (Lavelle and Spain, 2001). Within the model, all levels interact and higher-level factors dominate over subordinate factors (di Castri, 1988; Lavelle and Spain, 2001). While this model has been confirmed at a global and continental scale (Aerts, 1997; Heal et al., 1981), at
the regional and site-specific scale the hierarchical sequence may be altered and other factors may become increasingly important (Coleman et al., 1990).

The prevailing Mediterranean type climate at Albany, resulted in seasonal fluctuations in soil mesofaunal density. Levels of soil and litter faunal density and diversity were very low and were comparable to levels of soil and litter faunal density and diversity reported in studies of water stressed environments (semi-arid, tundra and Antarctica). It appears that factors other than climate (temperature and moisture) possibly soil edaphic properties and litter quality were responsible for the low level of mesofaunal density.

Two key edaphic characteristics of the soil at the Albany research site were the low levels of soil organic carbon and soil pH. These two edaphic factors appear to be the limiting constraints on microbial activity. The active microbial biomass is the primary resource base for the remainder of the soil food web (de Ruiter and Moore, 1997; de Ruiter et al., 1998; Hendrix et al., 1986; Hunt et al., 1987). The small soil micro and mesofauna populations at the Albany research site appear to be severely constrained by the low rates of microbial activity, evidence of bottom-up resource control.

Soil communities are donor-controlled, dependent on rates of litter decomposition to input nutrients including carbon and nitrogen into the soil (Laakso et al., 2000; Pimm, 1982; Strong, 1992). Rates of decomposition are limited in part by the
nutrient composition of the leaf litter (Berg, 2000; Bradford et al., 2002; Carcamo et al., 2001; Gallardo and Merino, 1999; Guo and Sims, 2001; Koopmans et al., 1998; Lavelle and Spain, 2001; Pereira et al., 1998; Ponsard et al., 2000; Wardle et al., 2002; Wardle et al., 1998). As noted in the previous paragraph, microbial activity appears to be limited by the low levels of organic carbon and acidic soil pH. Organic carbon is input into the soil subsystem via decomposition. Rates of litter decomposition at the research site were, like those of eucalypts generally, very low reflecting the poor nutrient quality of the litter (C:N >55) and its single species origin. Low rates of litter decomposition limit the rate at which carbon moves from the above-ground system to the below-ground system.

In summary, climate, soil acidity, low soil organic carbon and the poor quality of the litter resource have all combined to structure the soil micro and mesofauna densities at the Albany research site. Bottom-up resource control is the dominant process operating in these soils. The small microbial resource is reflected throughout the food chain by low micro and mesofaunal densities.

8.2.2 Factors Limiting Species Richness

Tropical to polar gradients in species richness of above-ground fauna have been well documented and eight factors have been hypothesised to explain this (Krebs, 2001). Although below-ground taxa do not display this gradient (Freckman and Mankau, 1986; Hunt et al., 1987; Lavelle and Spain, 2001), the hypothesised factors
are still likely to be relevant. At a global scale, mesofauna are numerically dominant in soil communities from temperate soils with high levels of accumulated organic matter (Lavelle and Spain, 2001). Soil mesofaunal species richness is highly dependent on environmental factors and the scale of observation. At the local scale, mesofaunal species richness is determined by the diversity and abundance of resources. At a regional scale, soil mesofaunal species richness is more likely to reflect vegetation type and management practices, while at a continental scale, climate and latitude are likely to be most important (Lavelle and Spain, 2001; Peterson and Luxton, 1982). Of the eight hypotheses, three are relevant to this study, climate, spatial heterogeneity and disturbance. Of these, climate can be dismissed as a factor. The Mediterranean climate experienced at the Albany research site is unlikely by itself to be a limiting factor of diversity as other studies of Mediterranean soils have reported high levels of species richness (c.f. Postle et al., 1986; Postle et al., 1991). At this research site, climate is subordinate to disturbance as a structuring force of these communities.

The two remaining factors are spatial heterogeneity and disturbance. The outcomes of this research suggest these two factors are the major determinants of species richness in these communities. Mature, stable soils are characterised by vertical stratification and highly developed soil pores of varying size (Perdue and Crossley, 1989; Perdue and Crossley, 1990; Roper and Gupta, 1995). The habitat variability (spatial heterogeneity) that exists in stable mature soils may explain the observed high levels of mesofaunal species richness. Conversely, highly disturbed soils display a lack of vertical stratification and low levels of niche variability (Holt, 1985;
Pankhurst and Lynch, 1994; Perdue and Crossley, 1990), one of the reasons used to explain why tilled agricultural soils support low levels of mesofaunal species richness (Blumberg and Crossley, 1983; Loring et al., 1981; Stinner et al., 1988; Wardle, 1995; Winter et al., 1990). Owing to the disturbance history of the Albany Effluent Irrigated Tree Farm the soil is expected to display little vertical stratification and be dominated by micropores (Pankhurst and Lynch, 1994). Meso and macropores are developed by the activity of tree roots and soil macrofauna or ecosystem engineers sensu Jones et al., (1994) and develop over decades (Lavelle and Spain, 2001) and so would only just be developing at the time sampling took place because the plantation is only six years old.

Disturbance reduces the relative importance of the other factors regulating soil faunal populations (Lavelle and Spain, 2001). The experimental site has been converted from bushland to pasture for approximately 40 years, deep ripped, planted with a non-native monoculture plantation (E. globulus) and irrigated with effluent or mains-water for five years prior to sampling. When the site was utilised for pasture, soil temperature and moisture would have fluctuated widely since there was no canopy or litter layer to ameliorate the direct effect of sunlight and rainfall. This land use practise is associated with wind and water erosion leading to decreased levels of soil organic matter and disruption of soil aggregates (Post et al., 2004). Pasture systems are synonymous with low mesofaunal density and diversity (Adolphson, 2000; Andre et al., 2002; Peterson and Luxton, 1982; Swift et al., 1998). The current mesofaunal population has built up from this very low base
because of the establishment of the plantation (six years old at time of sampling) and the development of a litter layer with subsequent positive effects on moderating soil temperature and moisture.

While moderate levels of disturbance may be associated with increased species richness as explained by the intermediate disturbance hypothesis (Connell, 1978), the disturbance history at the Albany Effluent Irrigated Tree Farm represents a substantial and sustained disturbance regime conducted over a significant period. Disturbance of this nature is expected to destroy the soil profile and pore structure, and eradicate the original soil invertebrate population (Coleman et al., 1990; Pankhurst and Lynch, 1994; Young et al., 1998).

Disturbance selects for $r$-selected species and against $K$-selected species (Pianka, 1970). It does this by disrupting the hierarchy of factors structuring soil communities (climate, edaphic properties, vegetation and resource quality, biological interaction) and replacing them with a completely modified hierarchy of constraints (Lavelle and Spain, 2001). These disturbed environments are most suited to ecological generalists, that are short lived, and produce many small young (MacArthur and Wilson, 1967; Pianka, 1970). These populations typically fluctuate rapidly in size as these $r$-selected species exploit their environment (MacArthur and Wilson, 1967; Pianka, 1970). This selection pressure is reflected in the dominance of the soil mesofaunal populations at this Albany site by *Tyrophagus* sp., and Tarsonemidae.
sp., two \( r \)-selected acarine species and the significant increase in \textit{Tyrophagus} sp., density in the spring samples.

Three factors have been identified as structuring species diversity. First, pasture sites (the previous land use prior to plantation development) are associated with depauperate mesofaunal communities and so provided the Albany Effluent Irrigated Tree Farm with a very small mesofaunal reservoir to start with. Second, the highly disturbed, early succession soils of the Albany Effluent Irrigated Tree Farm are thought to display low levels of spatial heterogeneity, little vertical stratification and are dominated by micropores, thereby limiting species richness. Finally, the extreme perturbation history of the site has selected for \( r \)-selected type mesofauna and against \( K \)-selected types. Six years of \textit{E. globulus} plantation development on long-term ex-pasture sites has been insufficient time for the development of a heterogeneous soil structure at this research site.

Conventional tillage literature demonstrates clearly the impacts of repeated disturbance on soil communities. These communities are characterised by a microbial resource dominated by bacteria, microfauna that display significantly increased densities of bacterial feeding nematodes and acarine communities dominated by \( r \)-selected species such as \textit{Tyrophagus} and Tarsonemidae sp. The parallels with this research are striking.

It is not surprising that this disturbed soil profile and the resident early succession community is associated with nitrogen leaching. Studies of conventional tillage in
agricultural systems report that highly disturbed environments are associated with a microbial resource dominated by bacteria (Bardgett *et al.*, 1996; Bardgett and Leemans, 1995; Bardgett and McAlister, 1999; Bardgett *et al.*, 1998; Frey *et al.*, 1999; Yeates *et al.*, 1997a). Studies of grazing pressure reported that the abundance of both active and total fungal mycelium was higher in lightly grazed soils than in adjacent heavily grazed soils (Bardgett *et al.*, 1993a; Bardgett *et al.*, 1998). A study of soil microbial community structure across a prairie restoration chronosequence (replanted in 1979 and 1993) reported that bacterial communities recovered from disturbance faster than fungal communities (Bailey, 2003). If bacteria did dominate the microbial biomass at the Albany research site, the higher trophic levels (specifically the nematodes) should reflect this bacterial dominance of the microbial resource. Researchers have observed a close correlation between bacterial biomass and bacterial feeding nematode populations (Beare *et al.*, 1992). In the non-irrigated control sites bacterial feeding nematodes accounted for approximately 50% of the nematode populations, while fungal feeding nematodes contributed less than 20%, suggesting that the microbial populations were indeed dominated by bacteria.

Agricultural systems dominated by bacterial driven decomposer pathways are associated with rapid cycling of labile substrates and increased opportunities for nutrient leaching (Bardgett and McAlister, 1999; Bardgett *et al.*, 1998). The nitrogen leaching from the Albany Effluent Irrigated Tree Farm (Fig. 3.1) may be a reflection of a bacterial dominated microflora, reduced faunal elements and associated rapid but 'leaky' nutrient cycling.
8.2.3 Irrigation as a Structuring Force

The hypotheses relating to irrigation effects on soil and litter biota are rejected because irrigation did not significantly affect faunal densities or diversities nor did it significantly impact on microbial biomass or respiration, or rates of litter decomposition. Irrigation did not significantly affect the microbial resource because the microbial resource was carbon limited and the effluent was not high in carbon and because the faunal community was dominated by $R$-selected species pre-adapted to exploiting disturbed environments.

Mains-water irrigation was expected to result in higher densities and/or diversity of the soil communities by removing the potentially limiting factor of soil moisture. Effluent irrigation was expected to have an impact over and above that of mains-water irrigation on the soil communities. Similar to mains-water irrigation, effluent irrigation was expected to remove the hypothesised limiting factor of soil moisture. In addition, because of its nutrient load (nitrogen and phosphorus both nutrients known to be limiting in south west Australian soils) effluent irrigation was hypothesised to result in increased levels of microbial biomass and higher rates of respiration and so result in a bottom-up restructuring of the soil communities.

Soil communities can be affected by the application of irrigation (Dindal, 1977; Dindal et al., 1975; Yeates, 1995). This research points to subtle (but important) irrigation affects. Mains-water irrigation resulted in seasonally stable levels of
microbial biomass but had no effect on rates of microbial respiration. Rather, rates of microbial respiration appear to be limited by levels of soil organic carbon and acidic soil pH, not soil moisture. Irrigation did result in a substantial increase in the relative density of the bacterial feeding nematodes relative to the non-irrigated sites but this result was not statistically significant. Irrigation had no statistically significant effect on acarine density or species richness.

This lack of a community response to irrigation was unexpected particularly in the light of previous research (c.f. Dindal, 1977; Dindal et al., 1975; Yeates, 1995). There are two potential biological explanations for this result. In the first instance, increased levels of soil moisture did not impact on the level of microbial activity. Consequently, there was no increase in the microbial resource and no opportunity for a bottom-up restructure of the soil faunal community. Second, the acarine community as whole did not respond to irrigation because it was dominated by $r$-selected species and therefore pre-adapted to exploiting the disturbance the irrigation stressor caused. Given that the acarine communities were dominated by $r$-selected species, there was little opportunity for variation in community structure towards a community more dominated by $r$-selected species. If this line of thinking is correct, then the potential positive affect of irrigation on $r$-selected species should be matched by a potential negative affect of irrigation on the few $K$-selected species present.

Some oribatid mite species demonstrate $K$-selected life histories (Norton, 1994), some of which are strongly attuned to long-term rainfall patterns (Wallwork et al.,
Such species may be particularly vulnerable to perturbations in patterns of soil moisture (Lindberg et al., 2002; Lindberg and Persson, 2004). The oribatid species’ responses to irrigation can therefore be complex and individual, reflecting a particular species’ life history. Although oribatid mites were poorly represented in this study, three oribatid mite species did display a consistent negative response to irrigation (oppiid mites, Brachychthonius sp. 2., and Tectocepheus velatus). This negative response highlights the potential of these mites as indicator species of disturbance. Mites from these families have previously been suggested as potential indicator species of disturbance (c.f. Cuccovia and Kinnear, 1999; Hunt, 1994). This Albany based research study supports the view that some oribatid mites appear to be indicator species of disturbance, however more research needs to be conducted under a wide range of perturbation and rehabilitation regimes to confirm this trend.

Effluent irrigation resulted in no statistically significant differences in the soil community that were distinct from mains-water irrigation. This lack of a response to the nutrient load of the effluent irrigation further suggests that soil communities at the Albany Effluent Irrigated Tree Farm are constrained by factors other than nitrogen and phosphorus and supports the view that the microbial community was restricted by low levels of soil organic carbon. If organic carbon is the most limiting factor structuring a community, then the application of water, nitrogen and phosphorus would be expected to have little impact on this community.
8.3 **SUMMARY OF FINDINGS**

In summary, the soil communities at the Albany research site were characterised by

i. a microbial resource with very low rates of activity

ii. low mesofaunal densities and diversity, that are numerically dominated by
   \textit{r}-selected species (\textit{Tyrophagus} sp., and Tarsonemid mites)

iii. low rates of litter decomposition (typical of eucalypt communities) reflecting
    the high C:N and monospecific litter type

iv. Low levels of soil organic carbon and the acidic pH appear to be the main
    constraint on the activity of the microbial resource.

v. The microbial biomass appears to be dominated by bacteria

vi. The soil and litter micro and mesofaunal populations are dominated by
    bottom-up processes

vii. Other factors limiting density and diversity appear to include the previous
    long-term land use of pasture, the disturbance history of the site, and the
    low nutrient quality of the monospecific litter

8.4 **IMPLICATIONS FOR MANAGEMENT**

Plantation management in Western Australia is moving towards a broad base,

sustainable approach, reversing decades of degradation resulting from traditional

agricultural practises (Strauss, 2001). In this context, the Albany Effluent Irrigated

Tree Farm represents a novel approach to the issue of wastewater disposal. Rather

than view wastewater as a potential liability, it has been viewed as a valuable

commodity combining recycled water with a nutrient load. Any project that recycles

water is particularly relevant to the dry, water-limited climate of Western Australia.
In addition, the use of a land based method for wastewater disposal replaces marine
discharge, with its negative side effects of pollution, eutrophication and loss of
seagrass meadows (Silifant, 1997).

Within the limitations of this study, initial research shows that irrigation results in no
statistically significant difference in mesofaunal community composition and rates of
litter decomposition. This lack of a community level response must be viewed in the
light of the factors constraining the soil community and in the context that the faunal
community was dominated by species specifically adapted to exploiting the
disturbance created by the irrigation stressor.

Clearly, we know little about the long-term affects of effluent irrigation on soil
physicochemistry, flora and fauna. This initial study provides a baseline to which
future studies can be compared. The maintenance of a functional soil community is
critical to the long-term success of the Albany Effluent Irrigated Tree Farm.
Particularly, as the plantation ages, and the nitrogen contribution to the soil via litter
decomposition is predicted to exceed that via effluent irrigation (Adams et al., 2001),
it is essential that the nitrogen be maintained on site to prevent contamination of the
below-ground aquifer. In this context, the ‘sink’ capacity of the fungal component of
the microbial biomass may be important. Currently the microbial resource appears
to be dominated by bacteria, and as such is associated with a rapid nutrient cycle
coupled with the potential for nutrient leaching.
Previously, management practises at the Albany research site depleted organic carbon and acidified the soil. Management strategies to promote fungal biomass could be associated with increased rates of carbon sequestration and a slower but conservative nutrient cycle coupled with the potential for nutrient immobilisation (Bardgett and Cook, 1998; Bardgett et al., 1998). Since the soil subsystem at the Albany research site is constrained by the low levels of soil organic carbon, increasing the available carbon should be a priority.

Carbon pools within the soil could be increased by direct amendment with a carbon source. Additionally, the effluent treatment regime could be reviewed with a view to retaining carbon within the effluent rather than removing it prior to irrigation. The C:N ratio of the leaf litter was highlighted as a significant constraint on litter decomposition and the main natural source of carbon input into the soil. If a non-sclerophyllous understorey was planted under the current *E. globulus*, this could have a positive impact on rates of litter decomposition by reducing the C:N ratio and increasing the litter diversity. In addition, it would result in a more even spread of litter rather than its collection predominately in the tram lines immediately at the base of the trees.

In future plantations, the benefits of growing a mixed stand should be considered, because of the benefits to litter quality and diversity. Finally, alternative irrigation methods could be reviewed; particularly spray irrigation that would result in the spread of irrigation over a larger area as opposed to the current highly targeted approach of drip irrigation. Spray irrigation could result in increased litter moisture
levels and so potentially increase rates of decomposition due to leaching of soluble nutrients. In addition to these suggestions, time and the absence of further perturbation are key to the development of the soil profile, soil pore structure, more complex faunal assemblages and the transition from a communities dominated by $r$-selected to $K$-selected species.

The capacity of plantations to sequester carbon is an important contemporary issue with global implications (Batjes and Sombroek, 1997; Hulme, 2005; Karnosky et al., 2005). Australia state government laws already allow for the trading of carbon credits as separate property rights from the plantation wood fibre (Anonymous, 2005). To date, all attention had been on above-ground sequestration. The rate of C sequestration into below-ground pools is regulated by the rate of organic matter decomposition. Traditionally, the role of soil and litter fauna in this process has largely been ignored based on the assumption that they are always present in sufficient density and diversity to mediate the decomposition process (Paustian, 1994). Recently, this view has been challenged and the importance of climate change on soil communities and the litter resource and the potential subsequent effects on ecosystem function have been highlighted (Fontaine et al., 2004; Smith et al., 1998b; Staddon, 2004). In order to accurately model the carbon sequestration of plantations and the impact of climate change, it is important that we possess detailed information regarding the soil and litter fauna that act as important rate modifiers (decomposition fauna) of this process (Jones et al., 2005; Paul and Polglase, 2004). Given the ecological and financial implications of the future carbon-credit trading system, it essential that we develop a complex understanding
of the soil and litter communities in plantations and how they might respond to a range of perturbations including climate change.

8.5 Research Limitation and Future Directions

This research, like all similar research was constrained by time and funding. An additional constraint was the paucity of previous West Australia research in this field. Further constraints on this research included the following,

i. Limited temporal scale

Although there was replication in this study at the level of treatment (three transects for each treatment; non-irrigated control, effluent irrigation and mains-water irrigation) the study ran from March, 2000 to February 2001. As such, the seasonal results presented throughout this thesis are reflective of the results obtained in a season within the years 2000 and 2001. Whether or not the data is reflective of seasonal variation at the Albany Effluent Irrigated Tree Farm would require sampling across multiple seasons.

The eleven-month litter-bag study provided useful preliminary information regarding rates of litter decomposition and faunal colonisation of *E. globulus* litter. An extended study of litter decomposition and litter fauna would add to our understanding of litter decomposition in Australian monoculture plantations and
increase our knowledge regarding the contribution of mesofauna to rates of litter decomposition.

ii. Constraints on taxonomic groups

When designing this project, it was clear that it would not be possible to describe all the soil flora and fauna due to time, financial and logistic constraints. Attention was therefore directed at those faunal groups deemed to be most important. Research effort was focused on describing the soil micro and mesofaunal communities (nematode and acari) in detail. The soil microflora is time consuming and technically challenging to describe, consequently they were described in terms of total microbial biomass and microbial respiration.

It would particularly useful to do a follow-up experiment to determine if an ageing and increasingly stratified soil profile at the Albany Effluent Irrigated Tree farm would result in the development of a more species rich and $K$-selected species community, or to confirm if irrigation would continue the selection pressure for mesofauna with characteristics of $r$-selected species and the effect of either species suite on rates of litter decomposition.

This study highlighted a small number of oribatid mites that were sensitive to irrigation. It would valuable to concentrate on the oribatid mite response to irrigation by employing mesocosm techniques. Oribatid mites have been consistently
identified as potential indicator species of disturbance and this research could add significantly to our understanding of their response to perturbation.

iii. Taxonomic resolution

Finally, the potential for taxonomic research is significant. One of the most time consuming aspects was the identification of the fauna using taxonomic keys primarily based on European or North American species. The number of both general and species specific taxonomic keys to the Australian fauna is limited. I regularly found that the Australian soil and litter fauna could not be identified past family due to the incomplete taxonomy of the local fauna.
To err is human, but to really foul things up you need a computer.

- Paul Ehrlich
9.0 References


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“I don’t see the logic of rejecting data just because they seem incredible.”

Fred Hoyle
### 10.1 Nematoda Data


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<th>Nematode Species</th>
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<th>Effluent Irrigated</th>
<th>Mains Water Irrigated</th>
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Table 10.2. Mean nematode species density (N m\(^{-2}\) x 10\(^3\)) under three treatment regimes: non-irrigated control, effluent irrigated, and mains-water irrigated

![Table](attachment:table.png)

(Values are means ± 1 SE, \(n = 54\) per sampling occasion)
### 10.2  **Acarine Presence Absence Data**

Table 10.3. Soil acarine presence absence data by season and treatment.

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<td>Mesostigmata</td>
<td></td>
<td></td>
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</tbody>
</table>

(* indicates were incomplete species separation resulted in two species within the one group)
Table 10.4. List of Acari and Collembola identified from *E. globulus* litter-bags deposited in the field and harvested 8 times over 11 months.

**Astigmata**

ACARIDAE *Tyrophagus* sp

**Prostigmata**

CHEYLETIDAE sp 2
TYDEIDAE *Ididorryia* sp
EUPODIDAE *Eupodes* sp 1
EUPODIDAE *Eupodes* sp 2
RAPHIGNATHIDAE *Raphignathus* sp 1
BDELLIDAE *Spinibdella* sp
STIGMAEIDAE *Stigmaeus* sp
STIGMAEIDAE *Eustigmaeus* sp
PYEMOTOIDEA sp

**Mesostigmata**

AMEROSEIIDAE sp
ASCIDAE *Protogamacellus* sp
ASCIDAE sp 1
PHYTOSEIDAE sp
PODOCINIDAE *Podocinella*?

**Oribatida**

OPPIIDAE sp 1
ORIBATULIDAE *Zygoribatula* sp
BRACHYCHTHONIIDAE *Brachychthonius* sp 2
BRACHYCHTHONIIDAE *Brachychthonius* sp 3
BRACHYCHTHONIIDAE *Brachychthonius* sp 4
TECTOCEPHEIDAE *Tectocepeus velatus*
CERATOZETIDAE sp
NOTHRIDAE sp

**Collembola**

ISOTOMIDAE sp 1
ISOTOMIDAE sp 2
BRACHYSTOMELLIDAE sp
SMINTHURIDAE sp
ENTOMOBRYIDAE sp
10.3 **PUBLICATIONS RELATING TO THIS RESEARCH PROJECT**

The following paper was delivered as an oral presentation at the XI Congress of Acarology held in Merida, Yucatan Mexico in 2002. This paper was accepted for publication in the proceedings of the conference on the 13th of March, 2004.
Oral Presentation
Mites in Soil Habitats

THE IMPACT OF IRRIGATION ON THE SOIL MITE COMMUNITIES OF EUCALYPTUS GLOBULUS PLANTATIONS IN SOUTH WESTERN AUSTRALIA.

Derek Juan Swarts (d.swarts@ecu.edu.au) and Adrianne Kinnear (a.kinnear@ecu.edu.au)
Abstract

This study investigated the effects of effluent irrigation on the diversity and density of soil acarine communities under blue gum, *Eucalyptus globulus* plantations in south western Australia. The mite communities of these soils exhibited characteristics typical of disturbed communities and were dominated by prostigmatid mites with frequent, high densities of opportunistic species from Acaridae and Tarsonemidae. The communities exhibited strong seasonal fluctuations in density and species richness. Irrigation (whether effluent or mains-derived) reduced soil mite species richness and oribatid densities. The decrease in oribatid densities was due largely to a reduction in the densities of mites from the Family Oppiidae. The addition of a nitrogen-rich effluent had no additional effects on either mite densities or species richness. Possible reasons for the irrigation-induced changes in soil mite communities are discussed.
Introduction

Soil mite communities are sensitive to changes in their environment and are known to respond to disturbance with changes in density and species assemblages. Oribatid mites, in particular, have been suggested as possible disturbance indicators because of consistent responses of some species to perturbation. Irrigation is a soil disturbance commonly associated with agriculture and when it is constant and in high volumes, it can induce substantial changes in the soil mite communities (di Castri, 1973). Less drastic soil moisture perturbations also produce changes in mite assemblages, though the degree of impact varies and depends on the experimental approach taken, the pattern of moisture addition and the climatic characteristics governing the soil community itself (see for example, MacKay et al., 1986; Huhta and Hanninen, 2001; Lindberg et al., 2002).

This study describes the soil mite communities of an effluent-irrigated tree farm of Tasmanian bluegums, *Eucalyptus globulus* in south western Australia. The aim of the research was to determine if the density and species composition of the soil mite communities under these plantations is altered by the addition of nitrogen-rich effluent irrigation, and if so, can the observed changes be attributed to the nature of the effluent, or are they a result of the irrigation itself and the consequent changes to soil moisture.

Methods

The study sites

The sampling sites are situated within a bluegum plantation located near the city of Albany in south western Australia (43.57°S, 117.48°E). The climate is Mediterranean with an annual rainfall of between 600 and 900 mm, the bulk of which falls between May and October. Temperatures range from 15 - 23°C in January to 8 – 16°C in July.

In 1992, because of increasing eutrophication of local harbors, a land-based system for the treatment and disposal of nutrient loads in wastewater was developed. After traditional secondary treatment, wastewater effluent is passed through an artificial ‘intermittently loaded specialised’ wetland for nutrient stripping prior to dam storage. The water is then used to irrigate 283ha of *Eucalyptus globulus* (Tasmanian bluegum) plantations. The goal is that nutrients remaining in the effluent after wetland stripping are incorporated into the soil and/or plant biomass. Irrigation flow rates are controlled to maintain plantation soil at or near field capacity. This means that effluent flow rates vary inversely with rainfall, being highest in summer months and minimal or zero in winter months.

The site has a duplex soil profile, with grey white to grey yellow sandy topsoil overlying structured medium clay subsoil. A lateritic duracrust of 300 – 600mm lies above the clay. Topsoils are acidic (pH 4.0 – 5.0) with carbon levels of 3 – 6%. Prior to the development of the tree farm, the area was poorly drained with annual flooding during the winter months. No flooding occurred after establishment of the trees. Prior to planting the bluegums, the duracrust was ripped to one meter depth and the resulting overburden mounded for seedlings.

This study utilised a nested ANOVA design. Three replicate plantation sites, approximately 1.5km apart and 1500 m² in area were chosen. Nested within each of
these sites were the treatments – 3 rows of 6 year-old *E. globulus* trees subject to one of three treatments from the time of planting in 1994:

Treatment A:  Control site that was not irrigated.
Treatment B:  Effluent-irrigated. Effluent was filtered prior to delivery. The nutrient characteristics of the effluent are presented in Table 1.
Treatment C:  Mains water-irrigated at the same rate as in Treatment B.

Replicate soil samples for mesofaunal extractions were nested within each of the treatments that were, in turn, nested in each replicate site.

At the time of this study (2000 – 2001), the trees were approximately 12m high with closed canopies. There was no irrigation system installed at treatment sites A. At treatment sites B and C, effluent and mains water was delivered through a trickle drip irrigation system and the frequency of irrigation was maintained at a rate that approached, but did not exceed the topsoil’s water holding capacity.

**Faunal sampling**

Soil mites were sampled 3 times over a 12-month period beginning in 2000, in autumn (March), spring (October) and summer (February). On each sampling occasion, 5 soil cores (5cm dia. x 10cm deep) from each of the 3 treatments in each of the 3 replicate sites were taken (a total of 45 soil samples per season). Soil cores were taken equidistant between randomly selected trees along a row, transported back to the laboratory in insulated containers and the mesofauna extracted within 48 hours of sampling.

Soil fauna were extracted from inverted soil cores by dry heat extraction using infra-red extractors modified after Kempson *et al.*, (1963). Extractions were carried out over 10 days, with the final surface temperatures exceeding 50°C. This ensured that in the two final days of extraction, all parts of the soil core were exposed to temperatures exceeding 40°C. Mites were collected in picric acid, stored in 70% alcohol and sorted to species level where possible.

**Data analysis**

SPSS (v11) Nested ANOVA routine was used to determine the significance of differences in population densities and diversity between treatments and sampling occasions. Densities were log transformed to ensure normality and homogeneity of variance prior to analysis. Post Hoc multiple comparison tests were conducted using the Scheffe test in SPSS (v11).

**Results**

**Densities**

Mean mite densities were relatively low across all sites (Table 2). There were significant seasonal effects on mean densities (F=17.867; P<0.001; df=2). Post Hoc tests revealed that densities were different for all seasons with spring values > autumn > summer values. There were no significant treatment effects. The high spring densities
were due largely to very high numbers of *Tyrophagus* sp. in all treatments. Prostigmata and Oribatida were most abundant in autumn samples. The Mesostigmata and Oribatida populations rarely accounted for more than 20% of total density.

At the ordinal level we found significant seasonal effects for Astigmata and significant treatment effects for Oribatida. Astigmatid densities (*Tyrophagus* sp.) varied significantly between each of the seasons ($F=51.645; P<0.001; df=2$) with spring values > autumn > summer values. Both irrigated sites had consistently reduced densities of oribatids regardless of season, although this result was only statistically significant between the non-irrigated and mains-irrigated treatments ($P=0.05$).

*Insert Table 2 about here.*

**Species Richness and Diversity**

A total of 45 species was identified (Table 3). The Prostigmata was the most species rich order with 22 species in 14 families. Oribatid mites were the next most species rich group with 11 species in 8 families. Eleven species of Mesostigmata were also identified (the majority from one family, Ascidae) though mites of this order were rare and five of the 11 species were single occurrences. Astigmata were represented by the single species *Tyrophagus* sp.

*Insert Table 3 about here.*

There were significant treatment effects on species richness ($F=2.486; P=0.026; df=2$). The non-irrigated treatments always had higher numbers of species (35 in total) than either mains-irrigated (29) or effluent sites (30) regardless of season ($P=0.005$). Seven species were found only in non-irrigated sites, 3 oribatids (Nothridae sp., *Tectocepheus* sp., *Brachychthonius* sp. 2) 3 prostigmatids (*Tydeidae* sp. 3, *Speleorchestes* sp., *Cryptognathus* sp.) and 1 mesostigmatid species. Even though not statistically significant there was a consistent seasonal trend with highest species numbers in autumn and lowest in summer (Table 2). Both species diversity ($H'$) and evenness ($J'$) were lowest in the spring samples. This result was due to the dominant *Tyrophagus* sp. whose densities were an order of magnitude greater than those of other species. There were no apparent treatment effects on diversity and evenness.

Of the 45 species identified, 10 were ubiquitous, being found in all seasons and across all treatments (Table 3). The majority of species found were only present in autumn and spring samples, and rarely, if ever, appeared in summer samples. Three species were community dominants, *Tyrophagus* sp., and two unidentified species, a tydeid (species 2) and a tarsonemid species. These mites were found in relatively large numbers (*Tyrophagus* sp. reaching densities of 10 000 per m$^{-2}$ and the other two species 2 – 3 000 per m$^{-2}$) and they occurred in more than 50% of the samples. The families Tydeidae and Brachychthoniidae were the best represented with 4 and 3 species respectively.

**Discussion**

The experimental design allowed us to identify if there were effects due to irrigation with effluent, as distinct from effects due to irrigation alone (mains water). Overall, we found very few statistically significant treatment effects on the soil mite communities, and there were no significant effects of effluent irrigation, as distinct from irrigation *per se*. Recent research in these irrigated systems (Adams, 2001) suggests that the excess
nitrogen entering with the effluent is taken up directly and almost completely by the root systems of *E. globulus*, and very little remains available for the soil subsystem itself. (Less is known of the fate of any excess phosphorus). This, together with the low carbon availability in these soils (a limiting factor of microbial activity) may explain the lack of an observed response by the mite communities to effluent irrigation.

There were however, community responses to irrigation, whether effluent or mains-derived, though they were limited in degree. One was a reduced species richness in irrigated sites. Six species were never recorded from irrigated sites. Four of the six (Nothridae ap., Tydeidae sp., *Speleorchestes* sp., and *Cryptognathus* sp.) were only sampled from the most abundant autumn communities and, more importantly, only in low numbers and with low frequencies of occurrence. It is therefore not possible to conclude that their absence from irrigated sites (and from the other non-irrigated sites) reflects a true irrigation response. It may reflect the difficulty in sampling rare species, particularly from these small soil populations. *Tectocepheus* sp. was an exception, in that it was sampled from all rain-fed sites and no irrigated sites, but again, this species’ low numbers and frequencies of occurrence mean that any conclusions regarding its intolerance to constant soil moisture regimes need to be made cautiously.

That mite species’ may display intolerance to altered soil moisture levels by their absence from irrigated sites is suggested by the study of Dindal *et al.*, (1975) who recorded substantial reductions in numbers of oribatid and prostigmatid species in mixed oak hardwood and red pine which had been irrigated for several years. In our study, the reduction in species richness was only demonstrable at the total acarine level rather than the ordinal level, a difference that was seen mainly in the Prostigmata. Oribatid species numbers were very low (6 – 8 species) in our study across all treatments.

Species may display intolerance to altered soil moisture regimes by reduced densities rather than absence from irrigated sites. For example, in addition to reduced acarine species richness at irrigated sites, Dindal *et al.*, (1975) also recorded lower mite densitys (including oribatids) and shifts in the dominant families and species. In our study, oribatid mite densities were lower in irrigated sites, and even though only statistically significantly lower in the mains-irrigated sites, the densities in both irrigated treatments were similar, being 2 - 3 times lower than non-irrigated soils, regardless of season. These differences were due largely to the two oppiid species, whose densities at the non-irrigated sites were 3 – 6 times higher than those at the other treatment sites. That some oppiid species are affected negatively by experimentally-increased soil moisture is also suggested by the data of Lindberg *et al.*, (2002) who provided extra “seasonal rainfall” to sites in Sweden and monitored the responses of the soil mite communities.

Continuous irrigation at sites would have the effect of smoothing out the rainfall cycle which is typical of the Albany environment and which normally produces an annual wet-dry cycle. A consequent reduction in below-ground habitat heterogeneity has been suggested as one reason for the reductions in mite density and species richness associated with irrigation (di Castri, 1973, Huhta and Hanninen, 2001; Lindberg *et al.*, 2002). Fluctuations in, for example, temperature and moisture produce temporal and spatial heterogeneity that may promote diversity. Huhta and Hanninen (2001) tested the hypotheses that varying conditions of temperature and moisture in soil microcosms will permit more species to co-exist than uniform ones, and that higher species richness will be found in the more heterogeneous and varying environments. They concluded that the results supported the hypotheses, but the differences they obtained were not marked.
It is also reasonable to expect that the life history traits of resident soil fauna may be dependent on the natural wet-dry cycles and that perturbations to these cycles may impact negatively on these species. This reasoning has been used to explain the reductions in invertebrate density and species richness that occurs when the wet-dry cycles of freshwater lakes are artificially dampened by manipulating the water regime (Neckles et al., 1990). Some Oribatid species in particular demonstrate k-selected life history traits (Norton, 1994), some of which are strongly attuned to long-term rainfall patterns (Wallwork et al., 1986). Such species may be particularly vulnerable to perturbations in soil moisture patterns. The oribatid species’ responses to these kinds of manipulations may therefore be individual and complex, depending on a particular species’ life history characteristics. For example, soil oribatid communities adapted to high seasonal rainfall, when exposed to simulated drought conditions, responded negatively with reduced density and species richness but the individual species responses were complex and together exhibited increases, decreases or no change in density compared with controls (Lindberg et al., 2002).

Soil mites are known to exhibit seasonal density patterns which are correlated with annual rainfall cycles (Holt, 1985; Wallwork et al., 1986; Whitford, 1989), and the strong seasonal responses in mite densities and species richness we obtained, with the maxima and minima corresponding with peaks and troughs of rainfall, are typical. In dryland systems, the peaks are often caused by juvenile recruitment from seasonal breeding that is synchronized with long-term predictable precipitation (Zak & Freckman, 1991; Wallwork et al., 1984). In systems where soil carbon is low and Prostigmata dominate (as in the Albany soils), the abiotic controls of temperature and moisture on mesofaunal populations may be particularly important (Whitford, 1989). Of the 45 species we recorded, half (23) were never found in summer samples at non-irrigated sites and most were not found in the late spring samples. Thus, maintenance of soil moisture levels throughout the normally drier summer period did little to dampen these seasonal fluctuations in the biota. This is not a surprising outcome, given that it is likely to be interactions between temperature and moisture (e.g. in the determination of relative humidity) which are important, rather than either of these two variables alone.

The community characteristics of the mite communities from all the E. globulus plots are strongly suggestive of early-succession or disturbed communities. With the exception of the large populations in spring of the opportunistic Tyrophagus sp., the mite densities are low and comparable to those found in extreme natural environments such as arid or semi-arid soils (Kinnear, 1991; Wood, 1971), poorly-vegetated and reclaimed quarry sites (Davis, 1963), coal pit heap sites (Hutson, 1980) and coal-shale tips undergoing reclamation (Luxton, 1982). The species composition of the Albany communities is also suggestive of pioneer communities and disturbed environments. A small number of species known to be opportunistic, such as Tyrophagus sp. and Tarsonemidae (Behan Pelletier, 1999; Phillips, 1990; di Castri, 1973; Santos and Whitford, 1981) were found frequently and in relatively large numbers. This is not surprising given that the soils are strongly acidic and the site preparation prior to tree planting included deep ripping and mounding which would have degraded soil structure and destroyed soil horizons. If, as Luxton (1982) suggests, microarthropods colonise ‘new’ sites though the agency of wind and phoresy, the surrounding of our sampling sites by additional plantation plots may also slow the rate of diversity increase with time, even as soil conditions improve. Finally, the monospecific litter typical of these plantations may also be a factor in reducing diversity and density, at least for the oribatid fauna (Hansen and Coleman, 1998).
In conclusion, 6 years of mains water irrigation appears to have had the effect of reducing soil mite species richness and oribatid density. The use of a nutrient-rich effluent as an irrigate had no significant additional effect on either mite density or species richness. The mite communities of these plantation soils reflect both the disturbances and natural histories of the sites in that they exhibit characteristics of mite communities found in highly-disturbed environments, with low densities and dominants that are known colonisers of these kinds of soil systems.

Acknowledgements

This study was funded by the School of Natural Sciences and the Centre for Ecosystem Management, Edith Cowan University. Access to the study sites was provided by the Water Corporation of Western Australia who also provided funding to one of us (DS) to attend the Congress. We are grateful for the comments provided by the anonymous reviewer that substantially improved this paper.

References


Legends for Tables.

Table 1. Nutrient content (mgL\(^{-1}\)) and pH of stored effluent prior to irrigation of Eucalyptus globulus plots in 1997. Source: Water Corporation of Western Australia, 1997.

Table 2. Mean densities and diversity of soil mites in irrigated and non-irrigated Eucalyptus globulus plots. Density values are Nos.m\(^{-2}\) x 10\(^2\). Numbers in parentheses are percentages of total densities.

Table 3. Seasonal distributions (presence/absence) of soil mite species in irrigated and non-irrigated Eucalyptus globulus plots. (* Two oppiid species are included here and were not separated in the analysis).

Table 1.

<table>
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<tr>
<td>Non-Irrigated</td>
<td>63.18 ± 26.34</td>
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<td>Family</td>
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