Vermicomposting manure: ecology and horticultural use

Bob Jacques

Thesis submitted in partial fulfilment of the requirements of Aberystwyth University for the degree of Master of Philosophy

2019

Institute of Biological, Environmental and Rural Sciences Aberystwyth University

Mandatory Layout of Declaration/Statements:

Word Count of thesis: DECLARATION	30,644
This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.	
Candidate name	Robert Jacques
Signature:	
Date	14/08/2019

STATEMENT 1

This thesis is the result of my own investigations, except where otherwise stated. Where *correction services have been used, the extent and nature of the correction is clearly marked in a footnote(s).

Other sources are acknowledged by footnotes giving explicit references. A bibliography is appended.

Signature:	
Date	14/08/2019

[*this refers to the extent to which the text has been corrected by others]

STATEMENT 2

I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loan, and for the title and summary to be made available to outside organisations.

Signature:	
Date	14/08/2019

NB: *Candidates on whose behalf a bar on access (hard copy) has been approved by the University should use the following version of Statement 2:*

I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loans after expiry of a bar on access approved by Aberystwyth University.

Signature:	
Date	14/08/2019

KESS Funding Statement:

Funding for this project was received from Knowledge Economy Skills Scholarships and the company sponsor, Martins TLC.

Ysgoloriaethau Sgiliau Economi Gwybodaeth
Knowledge Economy Skills Scholarships

UNDEB EWROPEAIDD
EUROPEAN UNION

Llywodraeth Cymru
Welsh Government

Cronfa Gymdeithasol Ewrop
European Social Fund

Knowledge Economy Skills Scholarships (KESS 2) is a pan-Wales higher level skills initiative led by Bangor University on behalf of the HE sector in Wales. It is part funded by the Welsh Government's European Social Fund (ESF) convergence programme for West Wales and the Valleys.

MARTINS
TLC
ENVIRONMENT FRIENDLY

Summary:

The project researched the ecology of vermicomposting manures and the use of vermicompost in horticulture. It was carried out in collaboration with Martins TLC Ltd., a vermicomposting company based in Ceredigion, Wales and the Institute of Biological, Environmental & Rural Sciences, Aberystwyth University. The project was part-funded by the European Social Fund through KESS2.

Vermicomposting is a form of composting that uses high densities of particular earthworm species to rapidly decompose, stabilise and release plant nutrients from organic matter. The earthworms promote the action of decomposing bacteria and fungi. The vermicompost researched in this project is used as a constituent of peat-free multipurpose compost as a horticultural growing medium.

The horticultural use of vermicompost was investigated by comparing its plant growth performance and key plant nutrient levels against those of other peat-free and peat-based growing media in a greenhouse pot trial. The results showed that all composts performed similarly well and all had high levels of most nutrients. The main conclusion was that this vermicompost would be a suitable alternative to peat-based growing media.

The ecology of vermicomposting is key to the efficacy of the vermicompost, and was investigated in a laboratory experiment that explored the effects of the presence or absence of earthworms on the biological, chemical and physical properties of the compost. Additionally, the presence or absence of springtails with and without earthworms was researched in the same experiment. Springtails, which are similar to tiny insects (usually less than 2 mm long) are known to be very important in soil organic matter decomposition and the recycling of plant nutrients. However, their role in vermicomposting has had little attention. This research found that in vermicomposting, springtails can enhance the effects of earthworms in increasing the availability of some key plant nutrients in the vermicompost.

Acknowledgements:

My academic supervisors Dr John Scullion and Dr Gareth Griffith who encouraged and supported me throughout, and my Company supervisor, Charlie Martin who made it all possible.

The KESS team in Aberystwyth and Bangor Universities - administration support and encouragement.

My family - my wife - for unerring practical and emotional support and maintaining the household finances, and my children - for encouraging me to embark on this venture.

My fellow post-graduate researchers for - practical help, advice and good humour.

Administrative and technical support of IBERS and other University departments and services.

Guidance, tuition, access and patience with experimental techniques, identification:

Dr Andrew Detheridge - indispensible help with many analytical techniques, statistical methods, and general support.

Dr Gordon Allison - infrared spectrometry, access interpretation and multivariate statistics.

Rhyn Fuchan, Dr Sue Lister - access to, tuition in and support with key laboratory equipment and techniques.

Tom Thomas - access to growing areas and laboratories at Botany Garden, as well as indispensible advice and support.

Dr Peter Shaw, UK Recorder for Collembola (Roehampton University) - expertise in springtail taxonomy and definitive morphological identification of specimens

Paul Brown, Senior Curator Natural History Museum London - access to and guidance with the museum's Collembola collection.

Caio Ambrosio Leal Dutra and Lina Avila Clasen - support, guidance and assistance with key experimental techniques.

Tom Moore - assistance in lab work.

Contents

Abbreviations

df	Degrees of freedom
LSD	Least significant difference (post hoc test of significance)
n	Number of replicates, sample size
NS	Not significant (result of a statistical test)
SE	Standard error
IRGA	Infrared gas analyser

1 Introduction

The project researched the ecology of vermicomposting manures and the use of vermicompost in horticulture. It was carried out in collaboration with Martins TLC Ltd., a vermicomposting company based in Ceredigion, Wales and the Institute of Biological, Environmental & Rural Sciences, Aberystwyth University.

Composting is a biological process involving the decomposition of organic material by microorganisms. In vermicomposting, earthworms also play a vital role which enhances the action of the microorganisms, as well as reducing the time to achieve a stable product suitable for horticultural use (Stiles 2017). Other fauna are inevitably present during vermicomposting, including nematodes, enchytraeids, mites and springtails (Monroy *et al.* 2011). Hence understanding the roles of different groups of fauna and their interactions with other organisms, particularly earthworms, is important for understanding the vermicomposting process and product. Research into these aspects may also contribute to the body of knowledge about fauna involved in the degradation of organic matter and the release of plant nutrients.

Two broader environmental and societal concerns make research into vermicompost important. There is increasing interest in developing growing media that reduce or eliminate the use of peat; vermicompost can be a constituent of peat-free composts (Barrett *et al.* 2016, Guerra *et al.* 2018). In addition vermicomposting can be an appropriate means of processing and stabilising waste organic matter from agriculture, industry and households (Stiles 2017).

This study sought to demonstrate that a peat-free growing medium containing vermicompost can perform as well as or better than peat-based and other peat-free, composts, in terms of plant growth. It also aimed to increase understanding of the role of earthworms and associated springtails in the vermicomposting process and their effects on the nutrient status and other characteristics of the vermicompost.

2 Literature Review

2.1 What is vermicompost and vermicomposting?

Vermicomposting is the deliberate decomposition of organic matter in association with high densities of certain earthworm species to produce a stable vermicompost for use as fertilisers, soil conditioners or growing media. Domínguez and Gómez-Brandón (2013) define vermicomposting as "a bio-oxidative process in which detritivorous earthworms interact with microorganisms and other fauna within the decomposer community, accelerating the stabilisation of organic matter, and greatly modifying its physical and biochemical properties". Vermicompost is generally higher in macro and micro nutrients than compost produced from the same raw material, has a lower C:N ratio, higher porosity and water holding capacity and is relatively safe in terms of risks to human and environmental health (Gómez-Brandón and Domínguez, 2014, Lim *et al.* 2015, Stiles, 2017). Vermicomposting does not necessarily include a "hot" or thermophilic phase (Tognetti *et al.* 2005).

2.2 Vermicompost and plant growth

Interest in vermicomposting stems from its potential as a safe and environmentally acceptable means of processing and utilising a range of animal wastes, sewage sludge, plant residues from agricultural production and other organic materials from industrial processes (Lim *et al.* 2015). Vermicompost can be used as a soil amendment in field crops and as a part of a medium for soilless plant production (Gómez-Brandón and Domínguez 2014). Additional interest relates to it potential as a constituent of peat-free or peat-reduced growing media (Guerra, 2018).

2.2.1 Vermicompost as a soil amendment

There are a great many studies into the effects of vermicompost on plant growth; in searching the literature for a meta-analysis Blouin *et al.* (2019) found 949 references relevant to aspects of plant growth and vermicompost published between 1997 and 2016. Of these, 625 referred to yields of plants grown in the presence of vermicompost compared to a control without vermicompost. The resulting extensive meta-analysis found that the

presence of vermicompost produced on average increases of 26% in commercial yield and 78% in shoot biomass. However, the analysis only included studies comparing soil amended with vermicompost to soil without any added fertilizer or organic matter as a control, so is of limited use for comparing the influence of vermicompost compared to other composts or growing media. Lim *et al.* (2015) cites 18 studies comparing vermicompost with various different controls and plant varieties; results of these studies were variable, sometimes with the presence of vermicompost apparently reducing yields, though unfortunately these authors give no indication of the statistical significance of the yield data from these trials.

2.2.2 Vermicompost in growing media

While studies of the plant growth effects of vermicompost as a soil amendment provide evidence for its benefits to plant growth, the current project relates more to the use of vermicompost in growing media. Studies comparing vermicompost with other composts or growth media give rather mixed results depending partly on the species and cultivar of plants grown and the proportion of vermicompost included in the experimental mix. Others are inconclusive because they do not compare the effects of vermicompost with non-vermicompost growing media.

Variable results were found by Bachman *et al.* (2008), who used vermicompost from pig manure to compare the yields of varieties of French marigold, tomato, pepper and cornflower in 10% and 20% vermicompost mixes (by volume) in MM360 commercial potting compost with an unamended MM360 control. After 12 days, compared to the control, shoot dry weight was significantly greater for French marigold in 10% vermicompost, significantly greater for tomato plants in 20% vermicompost, significantly lower for pepper in 20%, and not significantly different for cornflower at either amendment rate, or for pepper in 10% vermicompost.

Using pig manure vermicomposted by *Eisenia* spp, Atiyeh *et al.* (2000) found significant increases in yields of greenhouse tomatoes with a range of concentrations of vermicompost compared to peat-based MM360 commercial growing medium. Significantly improved germination and seedling growth occurred at 20%, 30% and 40% concentrations of vermicompost mixed with MM360. The 20% mix yielded 12.4% by weight more of fruit than the MM360 only control. These researchers claim that yield increases could not be solely as

a result of the nutrient status of the vermicompost because all plants were supplied with adequate nutrients by fertiliser application, and that the improvements were due to biological properties. However, they do not cite any direct evidence for such claims.

Roberts *et al.* (2007) did not find significant marketable yield increases when they trialled vermicompost made by *Dendrobaena veneta* Rosa 1886 from four different UK suppliers mixed with peat-based compost in varying proportions as growing media for tomatoes. Nor did they detect higher fruit vitamin C content. However, they found improved germination as well as improved proportions of unblemished fruit in vermicompost mixes, compared to using peat-based compost alone. This was especially the case at a mixing ratio of 20% vermicompost to 80% peat-based compost. However, no significant effects on fruit yield, weight of individual fruits or vitamin C content were demonstrated. Percentages of vermicompost by volume in peat-based compost used were 0% (control), 10%, 20% 40% and 100%. Germination rates were significantly higher than in the control in all the vermicompost mixes except 100%. These effects were irrespective of the source of the vermicompost. Total fruit yield per plant was slightly, but not significantly, greater in 20% and 10% vermicompost. The percentage marketable fruit per plant (i.e. fruits that were unblemished by blossom end rot or cracking) was significantly greater than in the control in the 40% and 100% vermicompost mixes. However, the lack of significantly increased total yield in the vermicompost mixtures compared to the control, resulted in the unblemished fruit benefits not being translated into increased marketable yield. These authors concluded that although vermicompost was a potential substitute for peat, there was little evidence of it being of horticultural advantage, and that many of the claims made by vermicompost producers were not substantiated by research.

Vermicompost has been compared to conventional compost as a peat substitute at rates of 0% (i.e. pure peat) to 100% (i.e. pure compost or vermicompost), presumably by volume, though the authors do not state this Lazcano *et al.* (2009). These researchers compared the effects on container-grown tomatoes of substituting a peat-based growing medium with either vermicompost produced from pig manure by *E. fetida,* or commercial compost produced from cow manure. They found that in both vermicompost and compost similar increases in root and top-growth biomass were obtained compared to the control at rates of up to 50% substitution. At rates of substitution higher than 50%, vermicompost continued

to show greater biomass than the peat-only based control, while the conventional compost at the same concentrations caused plant mortality. A difficulty with this study is that the vermicompost and compost were produced from different feedstocks (pig and cow manure), and there is evidence that vermicompost made from these two materials differ significantly in their properties (Domínguez and Gómez-Brandón 2013).

In another study using tomatoes Tringovska *et al.* (2012) compared vermicomposts from five different sources mixed at 10% by volume with a 1:1 mix of peat and perlite, against a control with no additives. The authors reported up to 2.2 fold increases in shoot biomass in the vermicompost mixes compared to the control, which they attributed largely to increased nutrient levels due to the presence of vermicompost. While this study achieved its aim of trialling the suitability of different vermicomposts as growing media constituents, the control had no added nutrients or alternative compost, so not surprisingly yielded less growth than the vermicompost-amended peat.

Compost quality comparisons of *E. fetida* vermicomposts from different wastes, including cow manure, using sandy loam as a control and base for vermicompost mixes at 5% and 10%, demonstrated a significant decrease in germination of radish, marigold and upland cress, but an increase in biomass (Warman and AngLopez, 2008). However, this study did not compare vermicompost with any other compost or inorganic source of nutrients, so the vermicompost effects could have been due to improved plant nutrition alone. Further, the duration of vermicomposting was up to 90 days, which may not be long enough to remove phytotoxins. Compared to the control, the cow manure-derived vermicompost showed no significant reduction in germination at 90 days for radish and cress, whereas at 45 days the reduction in all plants was significant.

Other potential problems with the use of vermicompost in potting mixtures were demonstrated by Lazcano and Domínguez (2010) in a greenhouse trial of ornamentals (pansies and primulas). This study used experimental pig slurry vermicompost and commercial vermicompost mixed at rates of 0% (control) 5%, 15% and 25% by volume with a peat-based commercial growing medium. They found a reduction in the biomass of both plants as the vermicompost content increased, this effect became significant at 5% vermicompost for primulas and 15% for pansies.

2.2.3 Vermicompost in peat-reduced and peat-free growing media

Sphagnum peat is not sustainable as growing medium, due to its effective non-renewability, as well as the carbon emissions and biodiversity loss resulting from its extraction (e.g. Guerra *et al.* 2018, Barrett *et al.* 2016). Hence there is increasing interest amongst growers and the public for sustainable alternatives, including vermicompost and coconut fibre (coir).

The successful substitution of peat with vermicompost by Lazano *et al.* (2009) has already been described. In a more recent study, Guerra *et al.* (2018) investigated mixtures of vermicompost and coconut fibre (coir) substrates in a soilless cultivation system. They used 20% vermicompost (by volume) with 80% coconut fibre as control, citing many studies that considered this proportion to be a suitable growing medium without adversely affecting plant performance, and compared this mix with 40% and 60% vermicompost mixed with coconut fibre. The aim of this research was to explore the potential for substrates capable of sustaining four crop-cycles. The plants used were melon (two cultivars), tomato and lettuce. These researchers concluded that the 40% vermicompost mixture met the physicochemical and biological characteristics required for the production system.

2.3 The role of earthworms in vermicomposting

Gómez-Brandón and Domínguez (2014) cite evidence for "manifold benefits" of vermicompost as a substitute for mineral fertiliser in potting media, including increasing nutrient availability, suppression of plant diseases and increasing soil microbial abundance, community structure and activity. The rate of decomposition and quality of the final product are largely determined by the effects of earthworms in their ingestion of the substrate, their influence on the microbial and fungal communities in the substrate and the subsequent microbial population and activity in the earthworm casts. Passage through the earthworm gut modifies the physical and biochemical properties of organic material, and total size of microbial population is reduced, while the composition of the microbial community is altered and its activity is increased (Aira and Domínguez 2009).

2.3.1 The earthworms used in vermicomposting

Eisenia fetida Savigny 1826 and *Eisenia andrei* Bouché 1972 are the two commonest species of earthworm used in temperate vermicomposting (Domínguez and Edwards 2011). Römbke

et al. (2014) have shown through DNA barcoding that these are likely to be cryptic species and that though *E. fetida* can be distinguished morphologically from *E. andrei*, the reverse is not always true. Another species used in vermicomposting in the UK is *Dendrobaena veneta* Rosa 1886 (Sherlock 2018). Domínguez and Edwards (2011) give some aspects of the biology and optimal abiotic conditions for vermicomposting earthworms. *E. fetida* and *E. andrei* are very similar in these respects, reaching maturity in 28-30 and 21-28 days respectively, both with a life cycle lasting 45-51 days, with the same optimal temperatures (25°C) and temperature limits (0-35°C), and the same optimal substrate moisture levels (80%-85%) and moisture limits (70% - 90%). *D. veneta* has a longer time to maturity (65 days) and life cycle (100-150 days), prefers lower temperatures (15 - 25°C), but has broader limits. *D. veneta* has lower optimal moisture level (75%) but wider limits than *Eisenia spp.*. (Note that there are some contradictions in the data for temperature and moisture for *D. veneta* between table 3.1a and the text in (Domínguez and Edwards 2011); the figures given here are taken from the text).

2.3.2 Interactions between earthworms and microorganisms

Interaction between the earthworms and microorganisms is a key factor in determining the rate of decomposition and quality of the final product. The earthworms modify the structure, activity and functional diversity of microbial and fungal communities during vermicomposting (Aira and Domínguez, 2009). Earthworms prefer to feed on substrates colonised by particular fungi, and fungi may be an important part of their diet. Survival of fungal propagules is differentially affected by passage through the gut according to earthworm species (Gómez-Brandón and Domínguez, 2014).

In a 72-hour microcosm experiment with *E. fetida* in pig slurry at varying population densities, Aira *et al.* (2008) found that earthworm density significantly affected carbon and nitrogen mineralisation, microbial activity and fungal biomass in different ways depending on the dose of manure. Earthworm densities were 0 (control), 25, 50 and 100 per 500 ml microcosm (mean individual weight was 0.3g +/- 0.007g). Over this period, fungal biomass, as measured by ergosterol content, correlated positively with earthworm densities, with ergosterol content at high earthworm density being twice that of the control. Higher densities of earthworms decreased microbial respiration, but increased it at low densities.

The nature of earthworm interactions with bacteria and fungi have been shown to depend on the source material, the species of earthworm, the duration vermicomposting and the length of the maturation period after the earthworms have left the substrate. Aira and Domínguez (2009) showed that the production of casts (the initial stage in vermicomposting) from pig and cow manure by *E. fetida*, resulted in differential effects on bacterial biomass and activity, and fungal biomass between the two manures. Bacterial biomass was higher in castsfollowing digestion of pig manure by earthworms, while it did not change in the case of cow manure. Fungal biomass increased in casts from cow manure but did not change significantly in pig manure. Basal respiration did not change in casts from pig manure, but was reduced in cow manure. These researchers interpreted the reduction in basal respiration as a reduction in microbial activity resulting in stabilisation of the vermicompost. They also suggested that the increased enzyme potential found in the casts might lead to a further thorough degradation of the manures during maturation after the earthworms have left through cast-associated processes.

Gómez-Brandón et al. (2011) compared phospholipid fatty acid (PLFA) profiles and basal respiration measurements in the presence and absence of earthworms at different stages of the vermicomposting process (from 2 to 36 weeks). They found that in vermicomposting pig slurry with *E. fetida*, the younger layers of material had reduced microbial biomass and diversity (according to the abundance of bacterial and fungal phospholipid fatty acids), but increased total microbial activity (measured by basal respiration), compared to the control without earthworms. However, in older layers microbial activity was lower. Gómez-Brandón et al. (2012) found that levels of bacterial and fungal activity in casts of *E. fetida* from rabbit manure decreased after 200 days of vermicomposting. Conversely, Haynes and Zhou (2016) found that after 18 weeks of composting, respiration rates in vermicomposts from a range of feedstocks, including cow manure, were higher than thermophilic compost from the same substrates.

2.3.3 Plant nutrients in vermicompost

In the experiment by Aira and Domínguez (2009) with pig and cow manure *E. fetida* casts were sampled daily for a range of nutrients. Ammonium concentration in the casts in both pig and cow manure was lower than in the undigested manure. However nitrate content

was significantly reduced (by 4.3-fold) in earthworm casts in the cow manure, but significantly increased (by 3.5-fold) in casts from pig manure. These researchers concluded that after casting, other factors influencing bacterial and fungal communities come in to play as the cast material ages, resulting in further degradation and increased nitrogen mineralisation.

In a 16 week experiment Domínguez and Gómez-Brandón (2013) compared the composting of fresh cattle manure and sewage sludge in the presence and absence of *E. andrei*. Microcosms with 500 g of substrate were sampled periodically and analysed for nutrients and microbiological parameters. The experimental microcosms contained 25 juvenile earthworms (approximately 65 g fresh weight); earthworms were absent from the control microcosms. Cattle manure showed a significant reduction in ammonium over time, but the opposite was the case in sewage sludge. The researchers suggest that the increase in ammonium in sewage sludge with earthworms present may have been due to earthworm mortality. The decrease in ammonium in cattle manure was significant in the first week in both the presence and absence of *E. andrei*. After nine weeks the concentration of nitrate increased significantly in the presence of earthworms until the end of the experiment, but the increase was significantly much weaker in the control. In the sewage sludge treatments, ammonium concentration increased rapidly both with and without earthworms, but to a significantly higher level in the presence of earthworms. After nine weeks, ammonium levels started to fall. Nitrate levels in the sewage sludge increased throughout the experiment, but significantly more so in the presence of earthworms. Domínguez and Gómez-Brandón (2013) also report a significant increase in available potassium in the presence of earthworms, though differences between the K concentrations with and without earthworms were small.

Lim *et al.* (2015) provide meta-data that show increased levels of nitrogen and phosphorous in eight *E. fetida* vermicomposted substrates compared to composted controls from the same feedstocks. The substrates include animal manures, sewage sludge, kitchen waste, and agricultural waste vermicomposted. Hence, in general research into nutrient levels in vermicompost show increases in both total plant nutrients and greater mineralisation attributable to the vermicomposting process.

2.4 Collembola and vermicomposting

In addition to earthworms and microorganisms, an array of other organisms are present and active during vermicomposting (Sampedro and Domínguez 2008, Monroy *et al.* 2011, Steel and Bert 2012), including microfauna (e.g. nematodes and protozoa), mesofauna, such as Collembola, mites, pseudoscorpions, macrofauna (e.g. woodlice, millipedes) as well as the larval stages of insects such as true flies and beetles (Steel and Bert 2012).

It is well established that mesofauna play an important role in regulating microbial activity, decomposition and nutrient cycling in soil food webs (Moore *et al.* 1988), and that Collembola one of the most important soil invertebrate groups in terms of their diversity, abundance and functions (Potapov *et al.* 2016). However the role of mesofauna in composting, and particularly vermicomposting, has received relatively little attention, one of the few is Sampedro (2008).

Gómez-Brandón and Domínguez (2014) allude to the interaction of earthworms and other decomposer fauna during vermicomposting, through both facilitative and antagonistic relationships, including direct ingestion of protozoa and nematodes. However they say little about the possible effects of these interactions on the vermicomposting process or the properties of the vermicompost, and do not mention Collembola or other mesofauna at all.

2.4.1 Presence of Collembola in compost

The food web in composts includes a rapidly changing complex community of fauna, including Collembola, which are found in every kind of compost at nearly all stages of composting, except the thermophilic stages (Steel and Bert 2012). Koleva *et al.* (2017) examined four composts, two from forestry waste and two from agricultural residues, each of which was composted in two different (unspecified) particle sizes for 145 days. This group found variations in diversity and abundance of different species in the four composts, though many of the species occurred in all four (e.g. *Protaphorura* sp.), and most in at least one of the agricultural and forestry composts (e.g. *Proisotoma minima*). It is suggested in this study that the species composition of Collembola could be used as an indicator of compost maturity, however Steel and Bert (2012) considered that Collembola would not be a useful indicator of maturity compared to nematodes.

Table 2-1. Collembola species recorded in composts

Order/family	Species	Citing literature
Entomobryomorpha/Tomoceridae		
	Tomocerus minor Lubbock 1862	Koleva *et al.* (2017)
Entomobryomorpha/ Entomobryidae		
	Lepidocyrtus curvicollis Bourlet, 1839	Koleva *et al.* (2017)
	Lepidocyrtus cyaneus Tullberg, 1871	Koleva *et al.* (2017)
	Heteromurus nitidus Templeton, 1835	Koleva *et al.* (2017)
	Orchesella flavescens Bourlet, 1839	Koleva *et al.* (2017)
	Orchesella villosa Geoffroy 1762	Koleva *et al.* (2017)
Entomobryomorpha/Isotomidae		
	Folsomia candida Willem, 1902	Koleva *et al.* (2017), Sampedro and Domínguez (2008)
	Folsomia fimetaria Linnaeus, 1758	Koleva *et al.* (2017)
	Folsomia quadrioculata Tullberg, 1871	Koleva *et al.* (2017)
	Isotoma viridis Bourlet, 1839	Koleva *et al.* (2017)
	Proisotoma minima Absolon, 1901	Koleva *et al.* (2017)
	Proisotoma minuta Tullberg, 1871	Sampedro and Domínguez (2008)
Poduromorpha/Hypogastruridae		
	Hypogastrura manubrialis (Hopkin (2007) mentions misidentification of *H. assimilis* as *H. manubrialis*)	Fjelberg (1998) cited in Steel and Bert (2012)
	Hypogastrura purpurascens	Gisin (1960) cited in Steel and Bert (2012)
Poduromorpha/Onychiuridae		
	Onychiurus spp. armatus -group Gisin, 1952 (= *Protaphorura* spp. (Sun *et al.* 2017)	Koleva *et al.* (2017)
Symphypleona/Katiannidae		
	Sminthurinus aureus Lubbock, 1862	Koleva *et al.* (2017)
Symphypleona/Sminthuridae	*Sminthurus viridis* Linnaeus, 1758	Koleva *et al.* (2017)

Koleva *et al.* (2017) attempted to assign the Collembola species found in their composts to their corresponding life forms and feeding specialisations. However, it is necessary to consult the broader literature relating to Collembola in soil to understand the potential function of Collembola in compost.

2.4.2 Collembola in vermicompost

In an experiment involving mesocosms of pig slurry, Monroy and Domínguez (2011) found that the abundance of Collembola in the presence of *E. fetida* increased by 67 fold compared to those in the absence of earthworms, with populations reaching over 300 individuals g^{-1} of dry weight of compost. Collembola formed over 50% of the arthropods present. Mesostigmatid and Astigmatid, and to a lesser extent Prostigmatid and Oribatid mites, also increased in the presence of earthworms. Collembola numbers were at their highest after vermicomposting of 4-11 weeks duration, after which the population declined rapidly. Two different doses of pig manure were given, with no significant effect on springtail populations. These researchers concluded that the increase in Collembola was not only due to increases in microbial biomass, which only increased by 1.3 times. They suggested that burrowing and casting by earthworms resulted in greater accessibility of food resources. The work of other researchers (eg Potapov *et al.* 2016) suggests that, depending on the trophic guild of Collembola present, direct consumption of earthworm-worked organic material may also have been a factor. This research did not identify lower taxa, so the diversity of Collembola, and how the relative abundance of lower tax or guilds may have changed with time are unknown.

The increase in Collembola found by Monroy and Domínguez (2011), suggests that earthworms and springtails in vermicompost are not in direct competition. Earlier stable isotope studies (Sampedro and Domínguez, 2008) of earthworms, Collembola, enchyraeids Diptera and Coleoptera in vermicomposting pig and cow manure concluded that these taxa occupied different trophic levels. Enchytraeids occupied the lowest tropic positions in both manures, together with by Diptera and Coleoptera larvae in the cattle manure, being primary decomposers. Adult *E. fetida* were in an intermediate position, and in the pig slurry, younger *E. fetida* were in a higher trophic position than that of the adults. Collembola were in the highest in trophic position of the taxa studied, being secondary decomposers, feeding

on organic matter as well as microorganisms and microfauna. Sampedro and Domínguez (2008) did not differentiate between the two species of Collembola named (*Proisotoma minuta* Tullberg 1871 and *Folsomia candida* Willem 1902), a shortcoming recognised by the authors.

2.4.3 Interactions between earthworms and Collembola in soil

The research cited in section 2.4.2 gave some indication of interactions between earthworms and Collembola in vermicompost; a much larger number of studies of such relationships in soil are available. Grubert *et al.* (2015) identified three types of interspecific interactions; facilitative, antagonistic and neutral. Using the incorporation rate of ^{15}N into beech saplings, this group of researchers examined relationships between two earthworms, one anecic (*Lumbricus terrestris* Linnaeus, 1758) and one endogeic (*Aporrectodea caliginosa* Savigny, 1826) and two Collembola species, *Heteromurus nitidus* Templeton, 1835 ("litter associated") and *Protaphorura armata* Tullberg, 1869 ("soil-associated") in different combinations. It was expected that the fauna with the same ecological traits (i.e. litter associated and soil associated) would be antagonistic. Antagonism, shown by reduced incorporation of ^{15}N into saplings compared to the control, was apparent when two species were present, but facilitation was suggested when three species (*L. terrestris* and both Collembola) were present. When only one species (either *L. terrestris* or *H. nitidus*) was present, incorporation increased significantly. This illustrates the complexity of the potential interactions between earthworms and Collembola, suggesting that interactions are species- rather than trait-specific.

Earlier work by Gutiérrez-López *et al.* (2010) and Eisenhauer (2010) had found both positive and negative relationships between earthworms and microarthropods. In the former study, positive relationships were observed between the endogeic earthworm *Hormogaster elisae* Álvarez, 1977 and Isotomidae, and between *Aporrectodea trapezoides* Dugès, 1828 (also endogeic) and Entomobryidae, but a negative relationship was found between *H. elisae* and Poduromorpha. In considering non-trophic interactions between different earthworm ecological groups and microarthropods, Eisenhauer (2010) found that each group had a different effect, but that the effect did not vary between microarthropod groups. The moderate densities of earthworms were beneficial but high densities detrimental. This is in

contrast to the vermicompost study by Monroy and Domínguez (2011), where the high densities of earthworms in vermicompost had a facilitative effect on Collembola. The reason for this could relate to different effects between different species of earthworms and Collembola (Grubert *et al.* 2016). Monroy and Domínguez (2011) did not specify which species of Collembola were present in the vermicompost, but the earthworms were *E. fetida* and being epigeic from a different ecological group than the earthworms in Eisenhauer (2010); the substrates were also very different (vermicompost versus soil) in composition, organic matter content and nutrient levels.

2.4.4 Life forms, feeding guilds and trophic niches of Collembola in soil

Stable isotope research using the differences in $^{15}N/^{14}N$ and $^{13}C/^{12}C$ found in different parts of the soil food web has enabled more understanding of relationships between Collembolan taxonomy, life forms and trophic niches to be elucidated (meta-analyses and reviews by Potapov *et al.* 2016 and 2019a and 2019b).

Chahartaghi *et al.* (2005) identified three trophic levels for Collembola, based on $^{15}N/^{14}N$ ratios of specimens of 20 different taxa from three forest stands compared to those for a range of potential food sources. The trophic niches were:

- Phycophages/herbivores, feeding mostly on lichens, algae and plant tissues - particularly species of the Symphypleona order (Sminthuridae and Dicyrtomidae) - hemiedaphic or epigiec springtails.
- Primary decomposers, feeding on detritus and associated fungi and bacteria - some members of the Entomobryomorpha order, particularly larger species from the Entomobryidae - epigeic springtails.
- Secondary decomposers, mostly feeding on fungi and other microorganisms - the largest group, made up of euedaphic species from the Poduromorpha, as well as Entomobryomorpha (some Entomobryidae and several Isotomidae). A subgroup of the secondary decomposers in the Hypogastruridae and Neanuridae (Poduromorpha) had diets that probably included microfauna (nematodes, Protozoa and rotifers), animal body parts and eggs in addition to fungi.

Feeding strategies are clearly linked to microhabitat and morphological adaptations in Collembola as in other taxa, particularly of mouthparts, with collembolan plant and fungal feeders having chewing mouthparts whereas scavengers and predators (and some fungal hyphae feeders) have scratching or piercing mouthparts (Malcicka *et al.* 2017). Since traditional taxonomy is largely based on morphology, it follows that taxonomic rankings higher than species are likely to give some indication of habitat and trophic group. Potapov *et al.* (2016) linked collembolan taxonomy, trophic niches and life forms in a meta-analysis of stable ^{15}N and ^{13}C isotope composition of 82 species. This revealed clear taxonomic separation into families that related to both life forms and trophic niche, and led to delimitation of four functional guilds:

- “Epigeic plant and microorganisms consumers” - most atmobiotic and epedaphic Entomobryomorpha and Symphypleona - feeding on higher plants and microscopic algae, and to a lesser extent fungi. Functional role - regulation of fungal communities, and therefore influencing the first stages of litter decomposition.
- “Epigeic animal and microorganisms consumers” - Hypogastruridae and Neanuridae, representing the epedaphic and hemiedaphic Poduromorpha. Functional role - regulation of population density of microorganisms as well as their animal predators (microbivores). Neanuridae may affect the rate of decomposition of wood by feeding on fungivores. These families are often in similar habitats, however they have different mouthparts, the Hypogastruridae with chewing, the Neanuridae with sucking mouthparts.
- "Hemiedaphic microorganism consumers” - many hemiedaphic and euedaphic Isotomidae, likely to be less selective in feeding in nature than suggested by laboratory experiments, which suggest preferences for fungi, consuming plant material as well as the bacteria and fungi decomposing it. Functional role - comminution of organic matter to form the physical structure of litter and regulation of the microorganisms decomposing litter, affecting net mineralisation.
- “Euedaphic microorganisms consumers” - the Onychiuridae (Poduromorpha) in the lower litter layers and mineral soil, feeding on fungal hyphae and amorphous organic matter. Functional roles - regulation of communities of microorganisms and fungal

hyphae, influencing nutrient uptake by plant roots and decomposition of organic matter.

Further meta-analysis of stable isotope ratio data has suggests partial confirmation of trophic niche conservatism. In 92% of 415 species from 21 orders of soil fauna species analysed by Potapov *et al.* (2019a), stable isotope values did not differ significantly within genus, and hence could be a guide to their ecosystem functions. However, this was not the case in all taxa, so for the present vermicompost study it was considered preferable to identify to species level where possible.

2.4.5 Effects of Collembola on soil nutrient cycling

Studies of Collembola in soil suggest that different species assemblages affect nitrogen turnover in different ways. A field study of several species of collembolans showed that the presence of a single species tended to immobilise nitrogen, whereas a mixture of species increased nitrogen mineralisation (Mebes and Filser, 1998). These authors give two possible explanations for their findings; differing environmental conditions between the study areas, resulting in different influences on mineralisation or immobilisation or the different effects of different species combinations. In the light of Potapov's (2019a) findings, the influence of combinations of Collembolans from different functional guilds, with one or more guilds having facilitating the promotion of mineralisation by other guilds, rather than species differences *per se*. Partsch *et al.* (2006) found evidence for differential effects on plant root growth of associations between earthworms and Collembola. Plant root biomass decreased when only either one of these groups was present, but increased when both were present. These effects were related to enhanced rates of nitrogen mineralisation and uptake in the presence of both groups of fauna, compared to when only one group was present. This was likely to be another example of facultative interactions between different functional groups.

Filser (2002) cites a study by Mebes in which a strong positive correlation ($R_S = 0.94$) between nitrate levels and *Mesophorura* sp. (Poduromorpha), but points out that such correlations from the field are generally weak.

2.5 Development of hypotheses for experimental work

This review of the literature enables some broad observations to be made in order to develop hypotheses for further investigation.

The results of trials of vermicompost as part of a growing medium are mixed, but the general thrust, as well as the claims of vermicompost producers (Roberts *et al.* 2007 and Martin Pers. Comm. 2017), suggest that vermicompost is superior to conventional composts as a growing medium, due to improved plant nutrients and microbial populations. While many of the studies cited here compare different levels of substitution of commercial growing media with vermicompost with the commercial media as control, the company sponsor's priorities were related more to comparing the Martins TLC vermicompost with other premium composts. In addition, a key aspect of the company's vermicompost is that it is peat-free, and the reduction of peat use is becoming an important consideration in commercial growing media (Guerra *et al.* 2018, Barrett *et al.* 2016). Hence it was considered important to trial the company's multipurpose compost (i.e. the mix of 25% vermicompost and 75% coir by volume) against a proven peat-based growing medium to demonstrate that the company's medium could achieve improved plant growth as suggested by some of the literature. In addition, it was important to show that vermicompost could compete favourably with other premium peat-free composts widely used by commercial growers. With these requirements in mind the following hypotheses were developed:

Hypothesis 1a, plants grown in vermicompost would exhibit significantly increased plant growth and above ground biomass on harvesting, compared to those grown in other peat-based and peat-free premium, non-vermicompost growing media.

Hypothesis 1b, any improved parameters of plants grown in vermicompost would be reflected in levels of key available nutrients.

The key to vermicomposting is in the action of earthworms, both directly in ingestion and comminution of organic matter, the passage of organic matter through the gut, and the effects on microorganisms, leading to increased nutrient mineralisation. In addition, cast-associated processes continue after the ingested organic matter is eliminated, continuing the process of mineralisation and stabilisation of the vermicompost. Collembola are ubiquitous in composts and clearly play a variety of key ecological functions in soil, often in

association with earthworms and other fauna, in facilitative, neutral or antagonistic relationships, depending on the trophic niches occupied. The presence of earthworms in the vermicompost has been shown to increase the abundance of Collembola and other mesofauna. Therefore it would be expected that the presence of Collembola would have an effect on nutrient mineralisation and other processes in vermicomposting. These aspects led to another main objective of the study; to gain an understanding of the roles of earthworms and Collembola, and their interactions, in vermicomposting and the quality of the vermicompost. Hence another experiment was required for which the following hypotheses were developed:

Hypothesis 2a, the presence *E. fetida*/*E. andrei* in vermicomposting manures would increase the concentrations of water-soluble nutrients and affect other physical, chemical and biological properties of the substrate, and interactions between earthworms and Collembola would enhance these effects.

Hypothesis 2b, the presence of earthworms in vermicomposting manures would increase the abundance of Collembola.

3 Materials and Methods

The project had three main objectives; (1) to characterise the key biological, chemical and physical aspects of vermicomposting through fieldwork at the vermicomposting site, (2) to compare the vermicompost with other premium growing media through a greenhouse pot trial, (3) to explore the influence of earthworms and Collembola on the properties of vermicompost through a microcosm fermentation experiment.

Practical or logistical constraints on the work constrained certain aspects of the experimental work. (1) The research was undertaken part time, therefore timings of experimental set up, measurements and sampling could not always be carried out at ideal times and frequencies. (2) Work at the vermicomposting site was further constrained because the site is a commercial enterprise, so it was not possible to design an experiment based on site and the fieldwork had to be co-ordinated with vermicomposting operations.

3.1 Common procedures and measurements

This section describes the aspects of the methodology that were used in more than one of the experiments. Any deviations from or additions to the procedures and measurements described here are detailed in the appropriate section.

3.1.1 Substrate respiration

This was carried out in November and December 2017 at the same time as and immediately adjacent to the locations of mesofauna samplings, the positions of which were determined using a random numbers grid. Ambient temperature was checked at the start and end of each series of measurements to ensure that as far as possible fluctuations of more than two degrees C in each series were avoided.

Substrate respiration was sampled by placing a soil respiration chamber (SRC-1, PP Systems, www.ppsystems.com) connected to an infrared gas analyser (IRGA) (EGM-4, PP Systems). This IRGA measures the concentration of CO_2 from which the proprietary software calculates the substrate respiration rate (PP Systems, 2005 and 2010).

3.1.2 Physical properties

All samples collected for laboratory analyses were placed immediately in ziplock plastic bags and placed in a cool box or opaque bag. Short term storage was in a cold room maintained at 2-5 °C. Longer term storage was in freezers maintained at -20 °C.

As far as possible the methods for compost and growing media laboratory analysis used by NRM Laboratories were followed; these conform to the relevant British Standards (NRM, pers. com. 2017).

A method for fresh bulk density determination suitable for small samples devised, based on the methods used for compost by NRM laboratories. A calibrated 100 ml beaker was loose-filled with approximately 80 ml of the sample. Standard pressure was applied using a 100 g weight. The contents were then weighed and the fresh bulk density calculated.

Moisture content was determined by drying a known weight of fresh sample to constant weight in a 105°C oven and re-weighing.

Water-holding capacity was estimated for composts used in the pot trial (section 3.3) by filling a 1-litre plant pot with drainage holes with the substrate, immersing in water for 30 minutes, followed by draining for three hours. The substrates were then sub-sampled and moisture contents determined by drying to constant weight at 105 °C.

The dry bulk density was calculated from the moisture content and fresh bulk density.

The percentages by weight of particles less than 2 mm were estimated using freeze dried samples. A known weight was placed into a 2 mm sieve and shaken by hand for one minute. The ratio of the weight of substrate falling thorough the sieve to the original weight allowed the calculation of the <2 mm particle size percentages.

3.1.3 Chemical properties

Key cations and anions concentrations in water extracts of the substrates were determined by ion chromatography (Metrohm 700 series, Metrohm UK, Runcorn, https://www.metrohm.com/en-gb/company/metrohm-uk/). The water extract was prepared for each sample by shaking a weight equivalent to 60 ml of fresh substrate in 300

ml distilled water for 1 hour at approximately 22 °C and 300 rpm followed by filtering through grade 1 filter paper (NRM, pers.com. 2017). Initial attempts at ion chromatography with undiluted extracts were unsuccessful due to the high levels of nutrients present in the composts that were beyond the calibration range of the equipment, as well as high levels of phenols that were suspected of clogging ion exchange columns. To overcome these problems the extracts were further diluted to 20% of the initial extract. The ion concentrations in the compost could then be calculated from the concentrations in the diluted water extract.

Electrical conductivity and pH were carried out on subsamples of the extracts used for ion chromatography before further dilution (NRM, pers.com., 2017). Electrical conductivity was measured using an Omega CDH-SD1 meter (Maranta-Madrid S. L., Algete, Spain). pH was measured using a Mettler Toledo FE20 FiveEasy benchtop pH meter (Mettler-Toledo, Leicester, England).

The C:N ratios were determined by elemental analysis (Elementar Vario Max Q CN analyser). Oven-dried samples were milled to 50 µm using a Janke & Kunkel mill. Two sub samples of each milled sample were weighed into pre-dried and weighed Elementar reaction tubes. Each sub-sample was approximately 0.1g, weights of sub-samples and tubes were taken to 4 decimal places and recorded. The reaction tubes containing the sub-samples were run through the Elementar at 900°C, together with a 200mg aspartic acid standard for calibration. The “plant” method was used so as to introduce higher oxygen levels to achieve complete combustion, as recommended for organic material (Elementar manual).

3.1.4 Mesofauna sampling

Samples were taken using steel soil cores, 6 cm diameter, 6 cm height. Three samples were taken from each sampling location. Samples were transported in their cores in a cool box and placed in Tullgren funnels within 18 hours of completion of sampling. The Tullgren apparatus (Burken Scientific Ltd., www.burkardscientific.co.uk) had 24 funnels. The heat and light required to extract the mesofauna were provided by one 25 watt incandescent bulbs mounted above each funnels, and a 30 ml tube containing 10 ml of 70% alcohol (ethanol or IMS) was fixed via a rubber bung to the bottom of each funnel to collect the extracted

animals. Samples were removed from the steel cores as they were placed in the Tullgren apparatus. One funnel was used for each core.

After the samples had been in the Tullgren apparatus for 7 days, the collection tubes were removed. During the extraction period the apparatus was checked in case of bulb failure; no failures occurred during the extraction.

Identification of fauna was carried out alongside counting, using the relevant keys. Hopkin (2007) was used to identify Collembola to species as far as possible. Identification of Collembola species was confirmed by comparison with specimens held by the Natural History Museum (London) and by Dr. Peter Shaw of Roehampton University, the UK recorder for Collembola.

For Acari, Shepherd and Crotty (2017) was used to separate into three groups (1) Mesostigmata (order), (2) Oribatida (excluding Astigmatina) (sub-order) and (3) juvenile or other taxa. The rationale for the separation of mites in this way was a balance of practicality and scientific requirements; the level of expertise in mite identification was not sufficient to confidently identify lower tax efficiently, and the main focus of the study was on Collembola, hence it was considered adequate to identify the main competitors (Oribatida, excluding the Astigmatina) and predators (Mesostigmata) of springtails amongst the Acari. These very broad divisions inevitably led to inaccuracy in that juveniles could have been from any taxon, some Mesostigmata (such as some Uropodina) are omnivores or nematode feeders, and that some Prostigmata and Astigmatina may be predators or competitors of springtails (Shepherd and Crotty 2017). However, the method enabled rapid identification and counting while capturing the key information required for the study.

Sherlock (2012 and 2018) were used to identify any earthworms to species and to identify other invertebrates to order Tilling (2014) was used.

Mesofauna extracted by the Tullgren funnels were counted using a Bogorov counting chamber. This is a perspex block with a groove machined in it in which the sample is poured, facilitating systematic counting.

Mesofauna numbers were recorded with the aid of a virtual click-counter (Thing Counter, Karuma, Google Play Store) on an android tablet PC, enabling the recording of multiple categories.

3.1.5 Earthworm sampling

Earthworms were sampled using a 10 cm depth by 3 cm diameter made from plastic drainage pipe to estimate the numbers earthworms present in the vermicomposting beds. Numbers of individual, numbers cocoons and total fresh weight (earthworms plus cocoons) were recorded. Before recording weights, the earthworms were brushed lightly with a soft brush to remove adhering substrate.

3.2 Field work at the vermicomposting site

The purposed of the fieldwork were to characterise key biological, chemical and physical changes during vermicomposting at Martins TLC and to inform a laboratory-based microcosm fermentation experiment. The objectives of the fieldwork were to determine at different stages of the vermicomposting process (a) substrate respiration rates, (b) moisture content, density and particle size (c) determine key plant nutrient levels, electrical conductivity and pH, and (d) determine the diversity and abundance of invertebrate taxa present, particularly the earthworms and Collembola.

Sampling of vermicomposting beds was carried out on in November and December 2017 and December 2018. Sampling depth was used as a proxy for age of the vermicompost. Because the earthworm beds are surface fed regularly with layers of manure feedstock approximately 5 cm deep, the lowest layer is considerably older than the uppermost layer, with an age gradient between. The age of each layer was estimated by consulting the feeding records kept by the company, and assuming increasing rates of volume loss due to decomposition and compaction with depth.

The analytical procedures are described in section 3.1.

3.3 The greenhouse pot trials

Greenhouse pot trials were carried out in order to compare the Martins TLC vermicompost with other premium peat-free and peat-based composts.

The hypotheses were:

Hypothesis 1a, plants grown in vermicompost would exhibit significantly increased plant growth and above ground biomass on harvesting, compared to those grown in other peat-based and peat-free premium, non-vermicompost growing media.

Hypothesis 1b, any improved parameters of plants grown in vermicompost would be reflected in levels of key available nutrients.

The trial used four composts, each with different key constituents, three of which (including the vermicompost) were peat-free composts used by commercial organic growers, and one a premium peat-based compost (Table 3-1).

Table 3-1. Composts used in the plant trial.

Compost description	Peat status	Main constituents	Code for plant trial	Product name, supplier
Coir-based multipurpose compost	Peat-free	Coir, with small amounts of loam and vermiculite (unknown quantities)	CBC	Fertile Fibre, http://www.fertilefibre.com
Multipurpose vermicompost in coir	Peat-free	25% vermicomposted horse/cow manure with sawdust and straw bedding, 75% coir.	MTLC	Martins TLC multipurpose compost, http://martins-tlc.co.uk/
Peat-based premium compost	Peat-reduced	80% Irish Peat, 20% wood fibre	PBC	Incredicompost, http://www.thompson-morgan.com
Wood fibre-based growing medium	Peat-free	Fine bark blended with coir (unknown proportions, but bark is the main constituent)	WBC	SylvaGrow Sustainable Growing Medium, https://www.melcourt.co.uk/

Peat-free composts CBC and WBC were chosen because they are widely used by organic growers (Martin, pers. com., 2017), with different main constituents. PBC was chosen because it is a premium peat-based compost best compost overall for "germination and potting" (Which? Gardening magazine, 2016) and so a high standard against which to compare the performance of the peat-free composts.

The trial used tomato (Money Maker) (*Solanum lycopersicum* L.) and leek (Lyon) (*Allium ampeloprasum* L.) plants, seeds were purchased from Newmans Garden Centre, Aberystwyth. Tomato is commonly used for plant trials (eg Roberts *et al.* 2007, Lazcano *et. al.* 2009), leek is of interest because of its relatively poorly developed root system.

Seeds were sown in covered seed trays and placed on a heated bench in a greenhouse for germination and initial growth. The seed trays were filled with Levington F2 standard seed compost; the same compost was used for all seeds for uniformity (this compost was not one of the ones used in the pot trials).

After germination seedlings were pricked out into 19 cm 3 litre pots filled with the composts to be trialled, at a rate of one seedling per pot. Using this size of pot was intended to reduce the risk of potential effects on plant growth due to restricted space for root growth.

Nine replicates of each treatment for each plant were used, giving 36 pots for each plant, 72 pots in total. This allowed for three destructive samplings of three replicates during the course of the trial.

Pots were be placed on benches in the unheated greenhouse in a randomised block design in order to minimise the effects of variations in temperature, humidity and light levels. A saucer was placed under each pot to collect drainage water.

Watering was carried out with tap water as necessary, watering each pot at the same time with the same volume of water.

Plant height was measured each week. After each destructive harvesting, shoots and leaves were removed and roots carefully washed to remove compost. Top growth and root biomass were determined by drying to constant weight at 105 °C.

3.4 The microcosm fermentation experiment

The literature review had identified that as well as the key role played by earthworms in vermicomposting, other fauna, particularly Collembola were likely to influence, and be influenced by, the process. A microcosm fermentation experiment was designed to gain an understanding of the roles of earthworms and Collembola, and their interactions, in vermicomposting and the quality of the vermicompost.

The fermentation experiment was intended to test two main hypotheses:

Hypothesis 2a, the presence *E. fetida*/*E. andrei* in vermicomposting manures would increase the concentrations of water-soluble nutrients and affect other physical, chemical and biological properties of the substrate, and interactions between earthworms and Collembola would enhance these effects.

Hypothesis 2b, the presence of earthworms in vermicomposting manures would increase the abundance of Collembola.

3.4.1 Treatments

There were six different treatments all of which contained defaunated manure, with a control with no added fauna, two treatments with earthworms, one of which also had mesofauna present, and a treatment with mesofauna only. In addition two treatments had added organic nitrogen, one of which also had mesofauna present. There were four destructive samplings. Each treatment and each destructive sampling batch was replicated four times. This required a total of 96 microcosms, 16 of each treatment, four of each treatment were destructively sampled on four occasions. This is summarised in Table 3-2.

The added organic nitrogen was intended to mimic the potential effects of earthworm mortality and decomposition. To determine the total carbon and nitrogen, earthworm dry weights were recorded after killing in hot water (Scullion, pers. com., 2017) and drying to constant weight at 105°C. Samples were then ground to 50 µm and the total C and N were determined by elemental analysis (section 3.1.3). These values were used to calculate the weight if arginine equivalent to the total N in 11g fresh weight of earthworms. 10 ml of arginine solution in distilled water at appropriate concentrations was introduced on two occasions during the experiment. Four days before the start of the experiment (Table 3-3)

0.28g of arginine in 10 ml water was added to each of the N and MN treatments, equivalent to the nitrogen content of 5g fresh weight of earthworms. Immediately after the third destructive sampling (day 106) a further 0.34g of arginine per microcosm was added to these treatments, equivalent to the total nitrogen content of 6g fresh weight of earthworms. Hence by the fourth destructive sampling, 0.62g of organic nitrogen had been introduced to each N and MN treatments, equivalent to the full 11g fresh weight N content of earthworms added to the E and ME treatments.

Table 3-2. Summary of treatments, destructive samplings and replicates used in the fermentation experiment; 1 denotes presence, 0 denotes absence.

Treatment	Earth worms added	Mesofauna added	Organic N added	Destructive samplings	Replicates per destructive sampling	No. of microcosms
X	0	0	0	4	4	16
E	1	0	0	4	4	16
M	0	1	0	4	4	16
ME	1	1	0	4	4	16
N	0	0	1	4	4	16
MN	0	1	1	4	4	16
Totals					24	96

3.4.2 Manures and thermophilic composting

Cow manure (CM): less than 1 day old cow faeces were collected from Trawscoed farm (Aberystwyth University). Horse manure (HM): 1-3 day old horse droppings with small amounts of bedding straw were collected from Lluest equestrian centre (Aberystwyth University). Both manures were collected on the same day. Thermophilic composting took place inside an unheated polytunnel. The manures were mixed with straw and formed into a prism-shaped windrow approximately (80x180x35 cm width x length x height). Composting continued for 111 days.

3.4.3 Defaunation

The partially composted manure was homogenised by mixing in cement mixer followed by shredding. Moisture content was determined by drying to constant weight in a 105°C oven and found to be 70%. The partially composted manure was transferred to 100g ziplock bags with 400g (approximately 0.8l) in each. It was then defaunated by freezing at -80C for at least 24 hours (Cole *et al.* 2004). One bag was retained without defaunation for the first microorganism inoculation. Both defaunated and non-defaunated composts were stored in a cold room at 2-5°C.

The events and timings of the experiment are summarised in Table 3-3

3.4.4 Re-inoculation with microorganisms

Inocula of microorganisms were prepared and introduced at stages during the experiment (Cole *et al.* 2004). Each inoculum was prepared from a different non-defaunated substrate expected to contain microorganisms representative of the next stage of vermicomposting. Substrates were mixed with distilled water at a rate of 4-500g to 1500 ml respectively and stirred by hand. A stack of soil sieves, 2 mm, 1 mm, 750µm, 500µm, 250µm and 125µm, was used to filter the resultant mix and the filtrate was used as the inoculum. The limit of 125µm was intended to prevent contamination by enchytraeids, mesofauna and their eggs while allowing the majority of nematodes and Protozoa to pass through (Cole *et al.* 2004).

At each inoculation, 10 ml of inoculum was introduced evenly to the surface of each microcosm using a pipette.

The substrates used for the inocula were:

Inoculum 1 The same partially composted manure mixture as used in the microcosms; this was intended to reintroduce similar communities of microorganisms to that which would have been present before defaunation.

Inoculum 2 Partially vermicomposted manure collected from a recently fed earthworm bed at the vermicomposting site (Martins TLC Ltd.).

Inoculum 3 Further vermicomposted manure collected from the vermicomposting site.

Inoculum 4 Mature vermicomposted manure collected from the vermicomposting site.

Table 3-3. Main events and timings of the fermentation experiment.

	Event	Days since start
Week -1	inoculum 1 + organic N added	-4
Week 0	**start** (first earthworms added)	0
	2nd earthworms added	3
Week 1	1st two mesofauna introductions	7
Week 2	2nd two mesofauna introductions	15
Week 3	3rd two mesofauna introductions	21
Week 5	**1st destructive sampling**	36
	inoculum 2 added	36
Week 11	**2nd destructive sampling**	79
	inoculum 3 added	79
Week 15	**3rd destructive sampling**	106
	inoculum 4 + organic N added	106
Week 19	**4th destructive sampling**	136

3.4.5 Microcosm construction and preparation

The microcosms used were food-grade, clear PET straight cylindrical jars with 100 mm black screw lids with nominal volumes of 1000 ml, obtained from Ampulla (https://www.ampulla.co.uk), with external measurements of 14 cm height (with cap) and 10.2 cm diameter. In each lid a hole of approximately 5 cm diameter was made using a hole saw. A piece of 20µm polyester mesh (Plastok Ltd, https://www.plastok.co.uk) was hot-melt glued to the underside of the lid using a glue gun, covering the hole.

Prior to filling, each microcosm was labelled and its empty weight recorded. In each microcosm was placed 120 ml (equivalent to 1.5 cm depth) of hydrated coir at 80% moisture. On top of this was an inverted plant pot saucer drilled with 6 mm holes, as a screen to reduce mixing of coir and partially composted manure by worms and prevent sampling of the coir in subsequent measurements. Above the screen was placed 400g

(equivalent to 800 ml) of defaunated, partially composted manure at approximately 70% moisture.

The filled microcosms were weighed again to enable the desired moisture content to be achieved.

10 ml of microflora/microfauna suspension was pipetted on top of the compost, in the N and MN treatments the required concentration of arginine solution was also added.

The microcosms were then brought up to 80% moisture by adding the requisite amount of water and placed in the dark at a temperature range of approximately 17-25C. Microcosms were maintained at 80% moisture as the optimum for *E. fetida/andrei* (Domínguez and Edwards, 2010).

Maximum and minimum temperatures of the cupboards containing the microcosms were recorded at least weekly using a mercury max/min thermometer. Two digital temperature loggers were used to monitor differences in temperatures between shelves.

3.4.6 Introduction of Mesofauna

A lure method was devised to extract mesofauna from MTLC vermicompost beds. Litter bags of 500µm mesh containing defaunated manure were placed on compost beds and covered with a shallow layer of composting manure. After two to three days, the bags were removed and taken in zip-lock bags to the laboratory. The bag contents were placed in a large beaker of tap water to enable the mesofauna (chiefly Collembola) to float to the surface (Hopkin 1997 and Mitschunas 2008).

The mesofauna were then scooped off in small batches using mesh strips. One strip was added to each the microcosms requiring mesofauna to be introduced (i.e. the M, ME and MN treatments).

This process was repeated twice a day on three different days in varying random orders so as to reduce the variability of the initial diversity and abundance of mesofauna. At each introduction six sample tubes were placed at random amongst the microcosms to collect additional mesofauna samples for extraction in Tullgren funnels. This was in order to

estimate the mean abundance and diversity of taxa entering each of the microcosms Table 3-4 and to assess the variability between microcosms resulting from this method.

Table 3-4. Abundances of each taxon extracted from the test samples (individuals per sample).

	Test samples							
Taxa	1	2	3	4	5	6	Mean	SE
Protaphorura aurantiaca	110	135	61	90	85	68	92	11.20
Anurida granaria	0	0	0	0	2	0	0	0.33
Fiesea truncata	6	6	5	5	9	3	6	0.80
Hypogastrura assimilis	0	0	4	0	1	0	1	0.65
Coecobrya tenebricosa	67	49	75	89	67	59	68	5.58
Pseudosinella sexoculata	0	1	0	0	9	1	2	1.45
Proisotoma minima	22	14	15	16	29	16	19	2.36
Parisotoma notabilis	65	85	64	69	76	71	72	3.20
Magalothorax minimus	3	0	1	0	0	3	1	0.60
other Collembola	0	2	0	0	0	0	0	0.33
Total Collembola	273	292	225	269	278	221	260	12.03
Oribatida	0	1	0	3	0	0	1	0.49
Mesostigmata	1	0	2	3	2	1	2	0.43
Other Acari	0	2	1	1	1	1	1	0.26
Total Acari	1	3	3	7	3	2	3	0.83
Other invertebrate orders	1	2	2	2	1	3	2	0.31

The abundances shown in Table 3-4 suggest that variation in taxa between samples was in general low. If this was representative of the microcosms then similar abundance and

diversity would have been introduced into each. However, with 48 microcosms with mesofauna present, there would have been an unknown number with greater variation from the mean for each taxon than others. In addition, the higher taxa (mites and other invertebrate orders) had low mean abundances with relatively high standard errors, so would have been present in some microcosms, but absent in others.

3.4.7 Introduction of *Eisenia* spp.

A weight of 11 g of earthworms, equivalent to 45-55 individuals of mixed size and age, was introduced into the top of each microcosm. The earthworms were washed in tap water before introduction to reduce the risk of contamination of microcosms with unwanted mesofauna. They were partially dried with a muslin cloth.

3.4.8 Data collected during the experiment

Any additions to or deviations from the common procedures and measurements described in section 3.1 are given here.

Substrate respiration

The EGM-4 IRGA was used with an adapted Soil Respiration Chamber (SRC) into which the entire microcosm could be placed and measurements taken without removing the lid. The SRC was adapted as using a nominal 1.5 l capacity white jar with water-vapour-tight screw top (https://www.auer-packaging.com). The lid had a 104 mm diameter circular hole cut in it with a heated scalpel. Into the hole is fitted the standard SRC, and kitchen grade silicone sealant is used to seal the join. This enabled the microcosm to be measured to be placed inside and the lid with SRC attached screwed down to reduce CO_2 escape to a minimum. The volume and area settings are adjusted on the IRGA to take in to account the non-standard volume and area of the adapted SRC (PP Systems 2005). A custom-made calculator tool in MS Excel is used to calculate the volume for each microcosm at each measurement, by entering the height of the substrate from the base of the microcosm.

IRGA measurements were made more than once in first few weeks to capture any rapid changes early in the fermentation. Subsequently they were made at least once between each destructive sampling.

3.4.9 Data collected following destructive sampling

Tullgren extraction of mesofauna

Carried out at each destructive sampling, using cores constructed from 50 ml Sterilin tubes cut to 6 cm depth. Two cores taken from each of 24 microcosms at each sampling and placed together in Tullgren funnel. Cores remained in the funnels for 6 days. Collembola were identified as far as possible to species level, mites (Acari) were identified to relevant sub taxa where possible. Other invertebrates were identified to order. The number of individuals in each group was recorded.

Earthworm biomass

Earthworms were collected after each destructive sampling, brushed clean and weighed and counted. Any earthworm cocoons were also counted and included in the earthworm weights.

3.4.10 Physical and chemical analyses

The composted manure from microcosm was emptied on to a tray and subsampled into 4 batches:

(1) 60 ml used to determine bulk density and for ion chromatography, stored at -20 °C

(2) approximately 80 ml for further analyses, stored at -80 °C prior to freeze drying and milling.

(3) approximately 80 ml held in reserve, stored at -20 °C

(4) approximately 10g fresh weight for moisture determination.

The coir bases were not sampled. The analytical techniques used are described in the common procedures and measurements section (3.1).

3.4.11 Near Infrared spectroscopy (NIRS)

NIRS (and subsequent chemometric analysis) was used as a screening technique to detect differences in the overall chemical composition in samples of compost that related to treatment and time. The approach, whilst lacking in sensitivity compared to more exacting

analytical approaches, has the advantage of being fairly broad brush and provides information rapidly and at high rates of sample throughput. NIRS was selected over Fourier transform IR spectroscopy (FTIR) in this project as it required minimum preparation, so increasing throughput, and being a higher energy spectroscopy, it was possible to take spectral measurements from much larger amounts of sample, so helping ensure that spectra were more representative, which for a complex heterogeneous material like compost is of particular importance. Unlike FTIR, which reports on fundamental vibrations, NIRS reports on overtones and harmonics, and consequently is more difficult to interpret. However, such considerations are only of importance with spectra from pure substances, as with complex samples non-Beer Law interactions e.g. peak shift, broadening, and constructive and destructive superimposition, often make identification of specific absorbance bands, or likely classes of components, difficult if not impossible (Allison, pers. comm, 2019). With NIRS this is further compounded by the spectra not reporting the fundamental absorbencies. However, the usefulness of NIRS for prediction of component concentration by use of multivariate regression is well documented (e.g. Ben-Dor *et al.* 1997, Ehsani *et al.* 1999, McWhirt *et al.* 2002, Ilani *et al.* 2016), and the advantages of reduced sample preparation and higher energy made this an ideal analytical technique for the present study.

Samples were freeze-dried and then ground by forcing through a 2 mm soil sieve with a pestle. The dried and ground samples were stored in a -20C freezer and freeze-dried a second time the day before the analysis. NIR spectra were acquired from 4-5g sub-samples of the composts in ring cups of 36 mm diameter using a NIRSystems model 6500 nearinfrared scanning monochromator (FOSS NIRSystems Inc., Laurel, MD). The samples were scanned (average of 32 successive scans) over the wavelength range from 400 to2498 nm at 2 nm intervals using WinISI II 1.04a (Infrasoft International LLC, State College, PA) software.

3.5 Statistical analyses

3.5.1 Field work at the vermicomposting company

One or two-way ANOVA and MANOVA were the basic statistical test used order to identify any significant differences in biological, chemical or physical between ages of vermicompost. Where data were normally distributed and Levene's test showed homogeneity of variance, ANOVA was carried out in SPSS 25 (IBM) with LSD pairwise comparisons for post hoc tests. Where the data were normal, but unequal variance was shown, ANOVA with Welch's F test was used with Tukey's HSD post hoc test, carried out in PAST 3.25 (Hammer 2019). Where data were not normal one-way or two-way PERMANOVA (NPMANAOVA) was carried out in PAST 3.25, with 9999 permutations with pairwise post hoc multiple comparisons; where taxa data were involved the Bray-Curtis similarity index was used, otherwise the standard Euclidean index was used (Hammer 2019). PERMANOVA was used both for univariate and multivariate non-parametric ANOVA tests, as justified by Anderson (2017).

3.5.2 The greenhouse pot trials

Repeated measures ANOVA, carried out in SPSS 25, was used to identify any significant differences in plant growth parameters in different treatments over time. Other tests used were PERMANOVA and ANOVA with Welch's F test with Tukey's HSD as described in section 3.5.1.

3.5.3 The microcosm fermentation experiment

In most cases ANOVA and PERMANOVA were adequate, with their adaptations and post hoc tests as described section 3.5.1. were adequate for identifying differences between treatments.

However, for the NIRS a different approach was required (Allison per. comm 2019). NIR spectra were exported to .csv format using WinISI software and subsequently imported into Matlab R2019a software (Mathworks Inc) using a custom importation script, and subjected to principal component analysis (PCA) using the PLS Toolbox (Eigenvector. Inc) version 8.7 in MatLab (Mathworks) version 9.6. Spectra were processed prior to PCA to remove noise and

scatter (multiplicative scatter correction, MSC), and mean centred. Model fit was evaluated by Venetian blinds cross validation and component number optimised to reduce overfitting whilst explaining maximal variance. Sample outliers were identified and removed from the model to improve fit and reduce component number. In some cases, PCA models were decluttered by use of general least squares regression (GLS), which is an optional pre-processing algorithm on the PLS toolbox. The success of GLS decluttering was evaluated by consideration of model fit, cross validation, and variance explained, and GLS was only utilised when it was of demonstrable benefit to the model. PCA was achieved by singular value decomposition (SVD); error of fit and cross validation were determined as root mean squared error of correlation (RMSEC) and root mean squared error of cross-validation (RMSECV) respectively, and both were plotted against principle component number to follow model fit and robustness. To identify differences in the PCA scores between treatments and with compost age, MANOVA and CVA were carried out in Genstat 18th Edition.

4 Results

This chapter presents the results obtained from the field work, greenhouse plant trial and microcosm experiment using the methods described in Chapter 3.

4.1 Field work at the vermicomposting site

The aims of the fieldwork were to characterise key biological, chemical and physical changes during vermicomposting at Martins TLC and to inform a laboratory-based microcosm fermentation experiment. An initial study was made of substrate respiration rates and the diversity and abundance of earthworms and mesofauna, a follow up investigation was carried out to determine the physical and chemical properties of the vermicompost at different ages.

4.1.1 Vermicomposting at Martins TLC

Martins TLC peat-free multipurpose compost (MTLC) consists of vermicomposted manures mixed with coir at ratio of 25% worm casts to 75% coir. The feedstock for the vermicompost is horse manure (mixed with sawdust bedding) and cow manure (mixed with straw and bedding). The manures are stored in covered windrows, where partial thermophilic composting occurs. Fermentation occurs in 0.5 m deep vermicomposting beds constructed from concrete-blocks housed in polytunnels with concrete floors. The vermicomposting beds contain high densities of epigeic earthworms, *Eisenia fetida* Savigny, 1826 or *E. andrei* Bouché, 1972 dominate, with *Dendrobaena veneta* Rosa, 1886 observed occasionally. Feeding is carried out as necessary, usually at one to two week intervals, by adding a mixture of both manures in layers approximately 5 cm deep and watered with collected rainwater. The surfaces of the beds are covered by discarded carpet material and cardboard to exclude light, retain moisture and as thermal insulation.

4.1.2 Substrate respiration

Vermicompost respiration rates were obtained on two occasions directly from the surface of the earthworm beds. Respiration rates are shown in Figure 4-1. Mean substrate temperatures were 10.1 °C (SE 0.228) and 4.7C (SE 0.414). Three IRGA readings were taken at each sampling site.

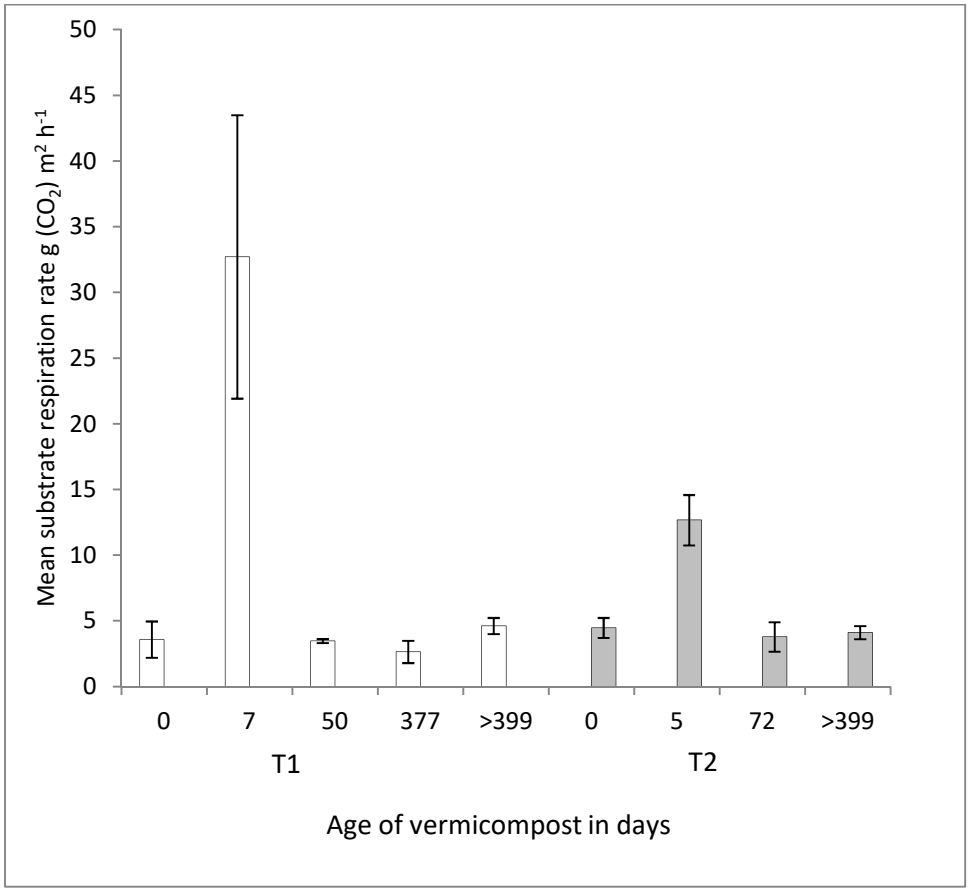


Figure 4-1. Mean substrate respiration rates ($g_{(CO_2)}$ m^{-2} h^{-1}) at different ages of vermicompost at two sampling temperatures T1 (10.1C) and T2 (4.7C). 0 days represents the manure feedstock before being applied to the compost beds, other figures are estimated days since manure was applied to the sampled area. All sampling was at the compost/manure surface (n = 3).

The 7 day-old substrate (T1) showed an elevated respiration rate compared to the manure feedstock, as did the 5-day old (T2). Respiration rates were much lower in the older substrates.

4.1.3 Physical properties during vermicomposting

In the follow-up investigation, the vermicompost was sampled at different depths as a proxy for age. Dry bulk density, moisture content and particle size determined.

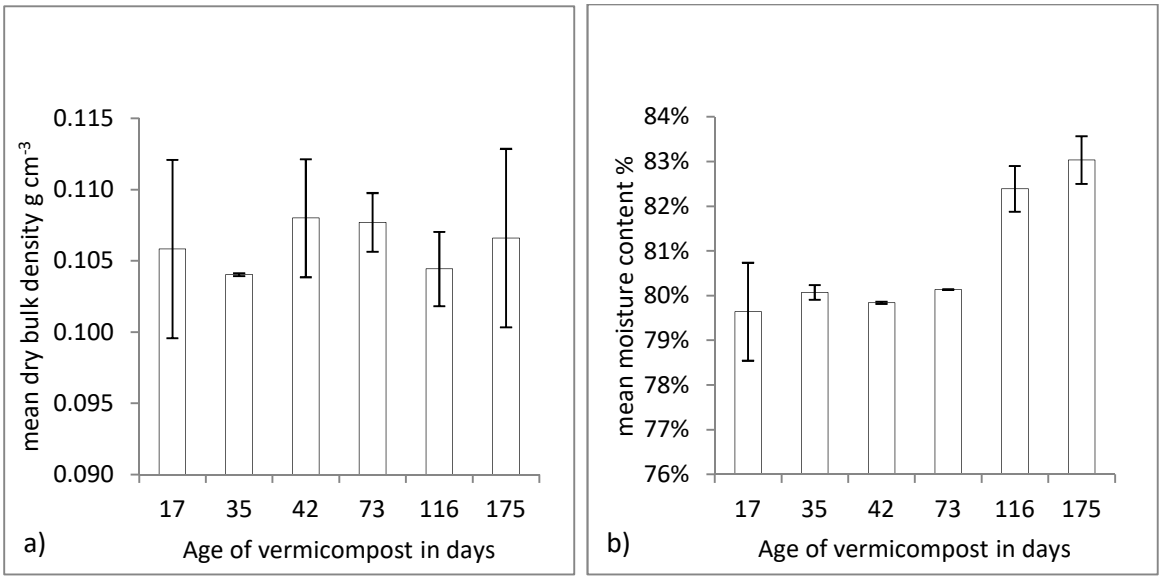


Figure 4-2. Dry bulk density in g ml^{-1} (a) and percentage moisture content (b) of varying ages of vermicompost (n = 2). Error bars show SE.

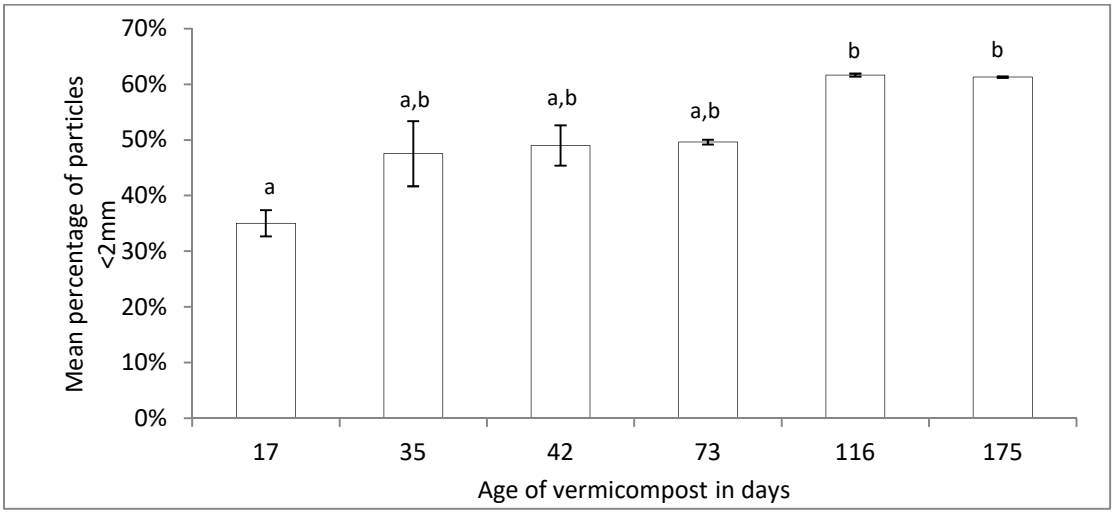


Figure 4-3. Percentage of particles (by weight) less than 2 mm in relation to age of vermicompost in days (n = 2). Values with the same letters (a, b) are not significantly different from each other (p > 0.05)Error bars show SE.

The percentage of particles less than 2 mm in size (i.e. passing through a 2 mm sieve) increased significantly with age (Welch F test in the case of unequal variances: F = 78.73, p = 0.005), as shown in Figure 4-3. Post hoc pairwise comparisons (Tukey's HSD) showed significantly higher percentages of particles less than 2 mm in the 116 and 175-day old vermicomposts compared to the 17 day old substrate.

4.1.4 pH and electrical conductivity (EC)

Mean pH and electrical conductivity (EC) readings from a range of ages are shown in Table 4-1. One-way ANOVA on the pH data returned a significant result ($F = 6.087$, $p = 0.024$), and an LSD post hoc test showed significant increases in pH with age. There were no significant differences in EC between vermicompost ages.

Table 4-1. Mean pH and electrical conductivity (EC) of samples from vermicomposting beds. Same letter suffixes (a, b, c) in the pH column denote no significant differences between ages.

Age of vermicompost days	pH			EC µS cm^{-1}		
	n	Mean	SE	n	Mean	SE
17	2	7.125^{a}	0.015	2	861.0	55.0
35	2	7.500^{a,b}	0.150	2	1074.0	71.0
42	2	7.655^{b}	0.165	2	1142.5	33.5
73	2	7.700^{b}	0.000	2	1152.5	91.5
116	2	7.595^{b}	0.175	2	1111.0	118.0
175	2	8.065^{c}	0.105	2	1091.0	144.0

4.1.5 Water-soluble nutrients

Concentrations of water-soluble nutrients detected by ion chromatography are shown in

Table 4-2, Table 4-3 and Table 4-4. A One-way PERMANOVA with age as the factor and all the nutrients detected as variables was not significant. One-way PERMANOVA tests with individual nutrients as single variables showed chloride and sulphate to increase significantly with age ($F = 11.58$, $p = 0.027$ and $F = 10.21$, $p = 0.008$ respectively), post hoc pairwise comparisons revealed no significant differences. No other significant differences in water-soluble nutrients were shown.

Table 4-2. Mean concentrations (mg l^{-1}) of water-soluble nutrients in samples from different depths of the vermicomposting beds (n = 2): ammonium, nitrite and nitrate

Age of vermicompost days	**Ammonium**		**Nitrite**		**Nitrate**	
	Mean	SE	Mean	SE	Mean	SE
17	1.480	0.055	0.180	0.180	75.635	16.080
35	1.193	0.038	0.165	0.165	150.713	2.213
42	0.658	0.658	0.515	0.105	138.545	0.075
73	0.703	0.703	0.195	0.195	139.885	26.430
116	nd	nd	nd	nd	109.648	50.243
175	nd	nd	nd	nd	79.390	63.770

Table 4-3. Mean concentrations (mg l^{-1}) of water-soluble nutrients in samples from different depths of the vermicomposting beds (n = 2): phosphate, potassium, sulphate.

Age of vermicompost days	**Phosphate**		**Potassium**		**Sulphate**	
	Mean	SE	Mean	SE	Mean	SE
17	38.0	2.520	110	5.540	4.97	0.443
35	48.8	1.010	154	1.260	8.28	0.138
42	40.6	0.220	160	6.668	5.05	0.240
73	44.3	0.078	168	13.492	5.17	0.977
116	50.2	5.650	162	13.665	9.58	0.295
175	46.4	2.955	162	18.630	11.0	1.680

Table 4-4. Mean concentrations (mg l^{-1}) of water-soluble nutrients in samples from different depths of the vermicomposting beds (n = 2): calcium, magnesium, sodium and chloride.

Age of vermicompost days	**Calcium**		**Magnesium**		**Sodium**		**Chloride**	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
17	17.40	0.222	4.43	0.013	37.5	0.275	68.1	2.428
35	24.0	2.527	10.6	1.630	49.9	0.862	93.7	1.360
42	19.3	1.102	6.21	1.333	53.1	0.430	102	6.407
73	20.8	3.225	8.64	1.545	50.7	2.955	104	3.050
116	17.4	2.973	5.54	0.325	48.6	4.488	109	2.947
175	16.1	2.118	5.12	0.698	49.7	5.210	109	7.625

4.1.6 Earthworm biomass

Data were collected on the number of earthworms and cocoons and the fresh weight of earthworms and cocoons combined, in each vermicompost age group. From these data the mean weight of individuals could be calculated (Table 4-5). Univariate, one-way PERMANOVA tests with age of vermicompost as the factor and the individual variables showed that the number of earthworms decreased significantly with age ($F = 30.86$, $p = 0.036$), as well as the total fresh weight of earthworms and cocoons decreased significantly with age ($F = 62.29$, $p = 0.039$), neither with any significant post hoc pairwise comparisons. The reduction in the number of cocoons with age and the reduction in the mean weight of individual earthworms with age were not significant.

Table 4-5. Mean numbers per litre of earthworms and cocoons, total fresh weight (earthworms and cocoons), and mean weight per individual at different ages of vermicompost.

Age of vermi-compost days	**Number of earthworms per litre**		**Number of cocoons per litre**		**Total fresh weight (earthworms + cocoons) g l^{-1}**		**Mean weight per individual g**	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
17	665	70.73	42	14.15	71.23	2.76	0.11	0.01
35	99	70.73	7	7.07	8.42	7.14	0.07	0.02
42	50	49.51	35	21.22	3.89	3.89	0.08	<0.01
73	42	14.15	21	7.07	2.76	1.20	0.06	0.01
116	7	7.07	0	-	0.21	0.21	0.03	<0.01
175	7	7.07	0	-	0.28	0.28	0.04	<0.01

4.1.7 Mesofauna abundance and diversity

The Tullgren funnel extraction from vermicompost samples collected from the earthworms beds yielded several species of Collembola, as well as Acari (including Oribatida and Mesostigmata), other arthropods (Chilopoda, Coleoptera, Diptera, Hemiptera, Isopoda, Pauropoda, Psocoptera, Psuedoscorpiones), Enchytraeidae and juvenile *Eisenia* spp. (Lumbricidae).

All sampling was at the compost/manure surface. The abundance of the higher invertebrate taxa is summarised in Table 4-6, that of the Collembola species and main groups of Acari in Figure 4-4 and Figure 4-5.

Table 4-6. Mean abundance (individuals per litre), number of taxa and diversity indices of invertebrate taxa extracted by Tullgren funnel. Diversity indices were calculated in PAST 3 and are based on total numbers of individuals of Collembola, Acari, other arthropods (Chilopoda, Coleoptera, Diptera, Hemiptera, Isopoda, Pauropoda, Psocoptera, Psuedoscorpiones), and annelids (Enchytraeidae and Lumricidae).

	Age of vermicompost - days									
	0		5		72		377		>399	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Collembola	3146	491.59	3425	1779.73	3775	251.54	143	12.89	367	86.19
Acari	377	260.18	460	94.32	863	206.87	138	45.96	69	11.95
Other arthropods	63	10.40	143	56.37	45	1.97	16	5.20	12	3.40
annelids	0	0	138	37.49	4	3.93	0	0	2	1.97
Total inverts	3586	590.48	4166	1883.38	4687	98.70	297	52.99	450	79.26
Number of taxa	8	1.15	10	0.58	12	1.2	8	0.58	9	0.88
Simpson_1-D	0.414	0.231	0.436	0.259	0.654	0.162	0.733	0.067	0.704	0.019
Shannon_H	0.889	0.469	0.931	0.527	1.435	0.453	1.597	0.254	1.501	0.089

Collembola is clearly the dominant taxon, with Acari of less importance. Other arthropods are present in low numbers in all ages of vermicompost, and annelids appear mostly in the 5 day age group.

One-way PERMANOVA tests (Bray-Curtis similarity index) showed that the distribution of total individuals was significantly different across vermicompost age groups ($F = 16.86$, $p = 0.001$, no significant post hoc pairwise comparisons), but there was no significant difference in the number of taxa. There were significant differences in both the Simpson 1-D ($F = 2.553$, $p = 0.004$) and Shannon_H diversity indices ($F = 2.97$, $p = 0.006$), neither diversity index showed significant post hoc pairwise comparisons.

Abundance of collembolan species was greatest, but highly variable, in the 0, 5 and 72 day old vermicompost and lower and less variable in the 377 and >399 age groups (Figure 4-4). Mite taxa followed a similar pattern (Figure 4-5).

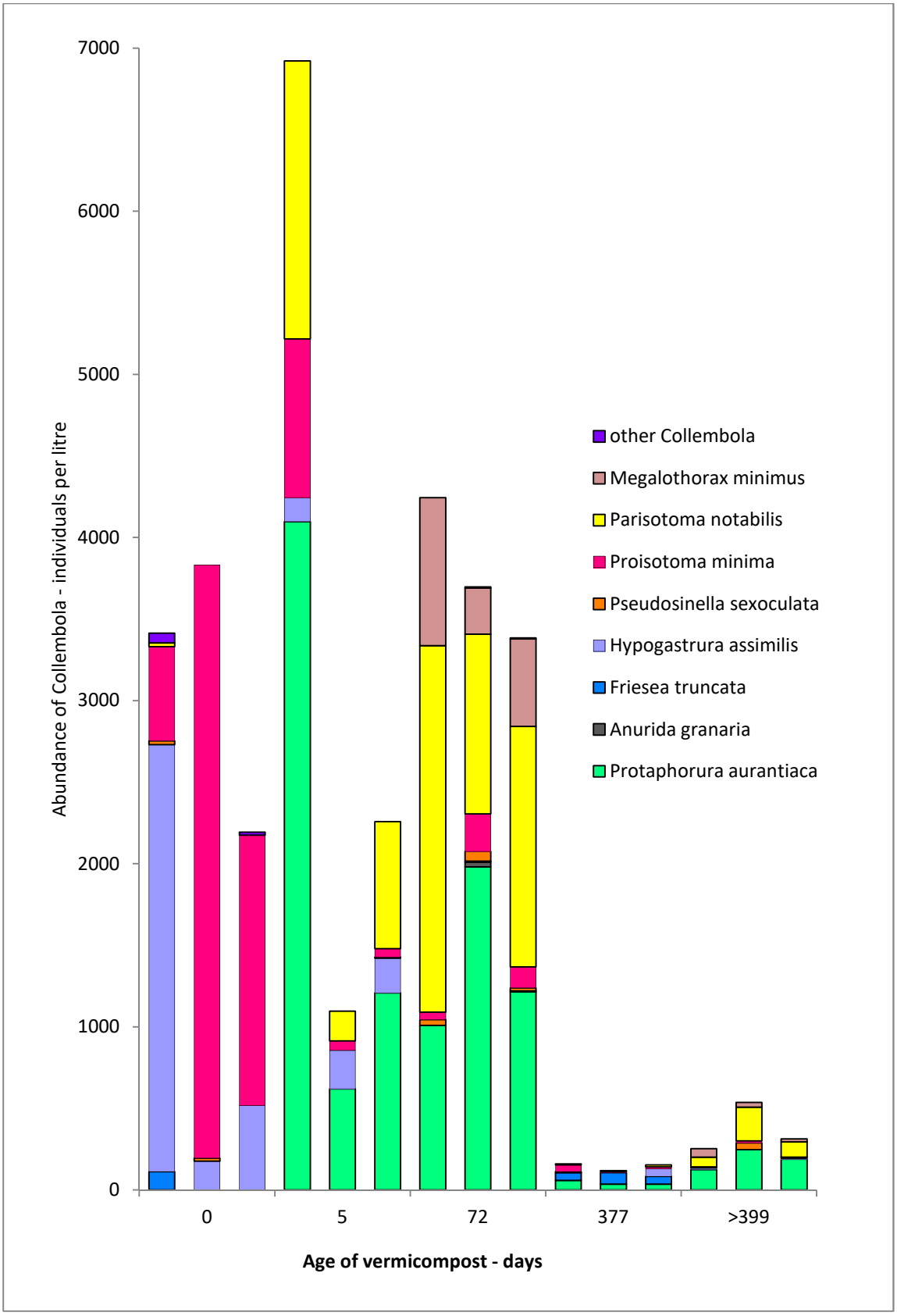


Figure 4-4. Abundance of Collembola species in the vermicompost samples (individuals per litre).

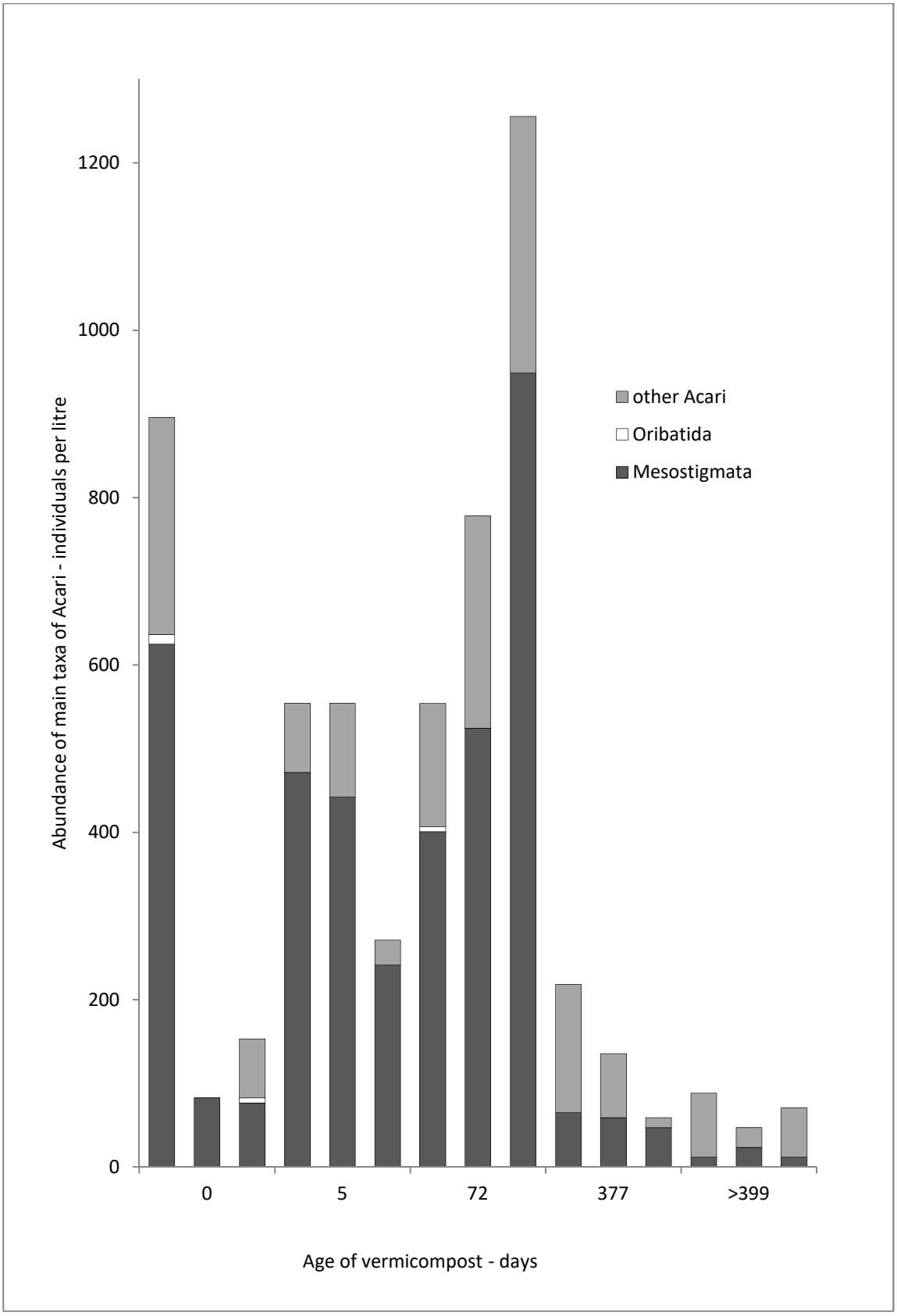


Figure 4-5. Abundance of main taxa of Acari in the vermicompost (individuals per litre).

A one-way multivariate PERMANOVA test (Bray-Curtis similarity index) showed that the distribution of total individuals of each species of Collembola was significantly different across vermicompost age groups ($F = 14.63$, $p < 0.001$, no significant post hoc pairwise comparisons), but there was no significant difference in the number of taxa. There were significant differences in both the Simpson 1-D ($F = 7.301$, $p = 0.005$) and Shannon_H diversity indices ($F = 4.074$, $p = 0.007$), neither diversity index showed significant post hoc pairwise comparisons.

4.2 The greenhouse pot trials

The purpose of the greenhouse trial was to test the hypotheses (a) that plants grown in vermicompost would exhibit significantly increased plant growth and above ground biomass on harvesting, compared to those grown in other peat-based and peat-free premium, non-vermicompost growing media, and (b) that any improved parameters of plants grown in vermicompost would be reflected in levels of key available nutrients.

Of the four growing media used, three were entirely peat-free: coir-based (CBC), vermicompost produced by the company sponsor (MTLC), a wood fibre based growing medium (WBC). The fourth was premium peat-based compost (PBC). Nine replicates of each plant species in each compost were established, 72 pots in total. This allowed for destructive samplings as the trial progressed.

4.2.1 Tomato growth in the trial composts

Tomato plants were destructively sampled days 25, 53 and 74. Three replicates of each of the four compost treatments were harvested at each sampling.

Plant height

All plants demonstrated significant growth in stem height at each measurement ($p < 0.001$, Figure 4-6 and Figure 4-7). Repeated measures ANOVA for days 4, 11, 19 and 25 (Table 7-2) showed a significant interaction for stem height between compost type and date of sampling ($p = 0.001$), as well as significant differences between composts ($p = 0.004$). Pairwise comparisons between composts over the four samplings showed that the vermicompost (MTLC) and the peat-based compost (PBC) exhibited significantly greater stem height than the coir-based compost (CBC). Figure 4-6 shows the interaction between compost type and date of sampling, with MTLC showing greatest height up to day 19, and PBC leading by day 25.

From day 35 to the final destructive sampling on day 74 there were no significant differences in mean tomato height between treatments.

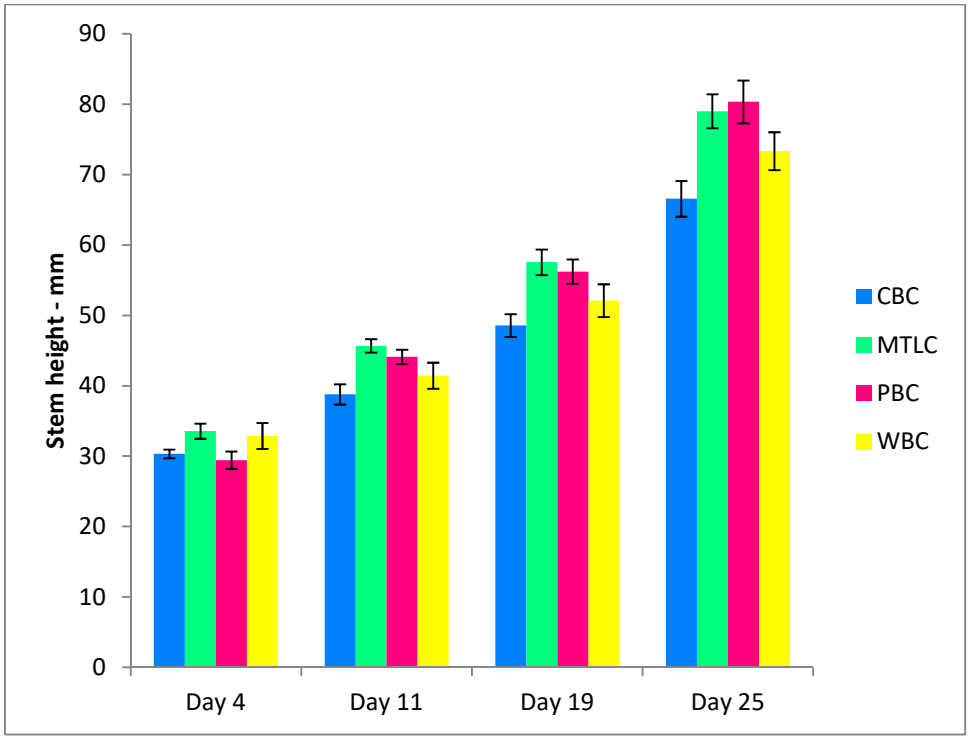


Figure 4-6. Mean stem height in all tomatoes to Day 25 (n = 9). Error bars show SE.

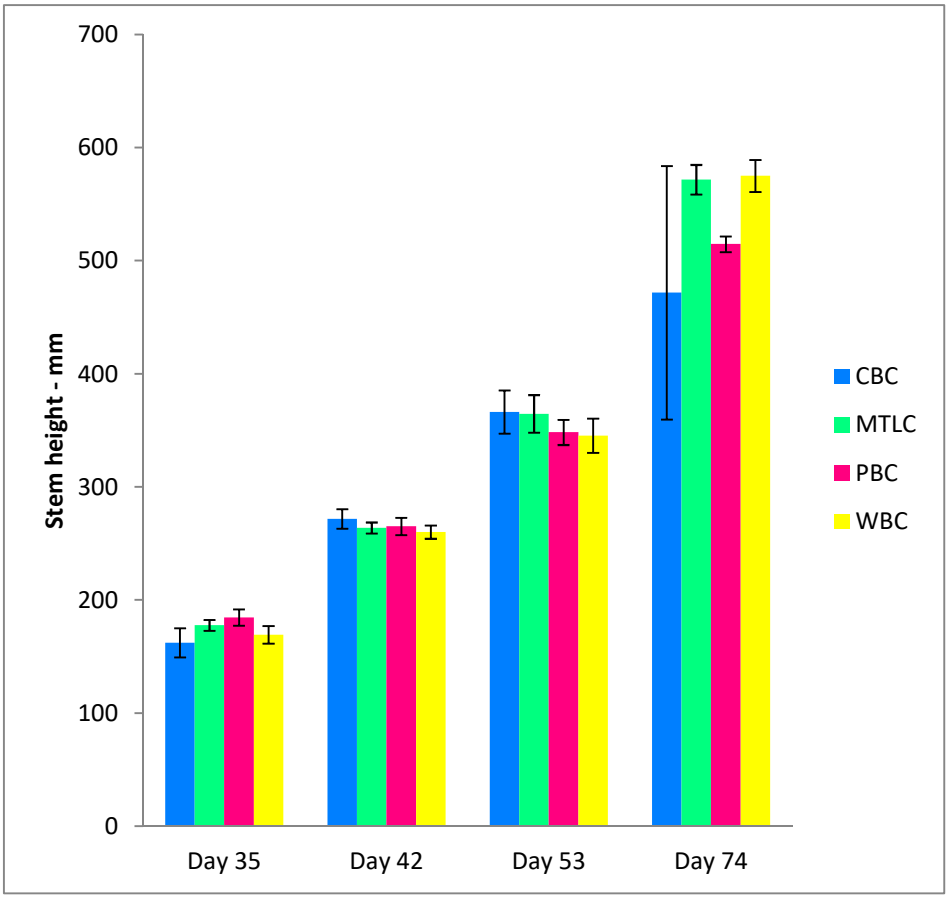


Figure 4-7. Mean stem height in tomatoes on Days 35, 42 (n = 6), 53 and 25 (n = 3, due to the destructive sampling on day 53). Error bars show SE.

Degree of wilting under water stress

Wilting in tomatoes was observed on days 53, 56, 61 and 67. This was a consequence of water stress due to high temperatures during periods when watering was not feasible. Though unintended, wilting gave the opportunity to observe any differences between treatments, as all treatments were given the same volume of water before at each watering. On these occasions a "wilting category" between 1 an 4 was recorded for each plant, with 1 denoting no wilting and 4 severe wilting. On day 53, 24 tomato plants remained after the first destructive sampling. A univariate PERMANOVA test for wilting category on day 53 between composts returned a significant result ($n = 6$, $F = 15.13$, $p < 0.001$); post hoc pairwise comparisons showed significantly less wilting in CBC than in PBC and WBC ($p = 0.002$ and 0.016 respectively), as well as in MTLC compared to PBC and WBC ($p = 0.002$ and 0.015 respectively). On days 56, 61 and 67, 12 plants remained ($n = 3$). A multivariate PERMANOVA test for all these days showed significant differences ($F = 4.083$, $p = 0.036$), but pairwise comparisons failed to show any significant differences between composts, though the data suggests a similar situation; plants in MTLC and CBC suffered significantly less wilting than in PBC and WBC.

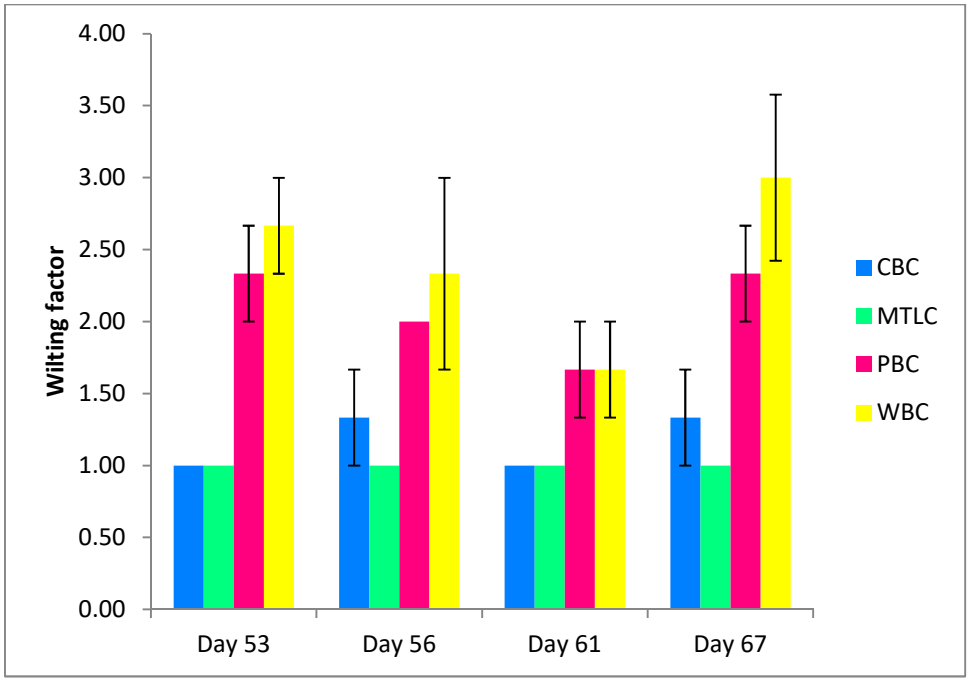


Figure 4-8. Mean wilting category in tomatoes (day 53, n = 6, days 56 -67, n = 3). 1: no wilting, 2: slight wilting, 3: moderate wilting, 4: severe wilting. Error bars show SE.

Dry weights

Mean total dry weights for each destructive sampling of tomatoes are shown in Figure 4-9. There were no significant differences between composts for top, root or total dry weights. However, PERMANOVA showed that there was a significant difference in root/shoot ratio between composts ($F = 6.27$, $p = 0.020$) at day 25. Though no significant result was returned in post hoc pairwise comparisons, mean root/shoot ratio appears to be significantly higher in CBC than the other media, notwithstanding high variability in the other three (Figure 4-10). There were no significant differences in mean root/shoot ratios at subsequent destructive samplings.

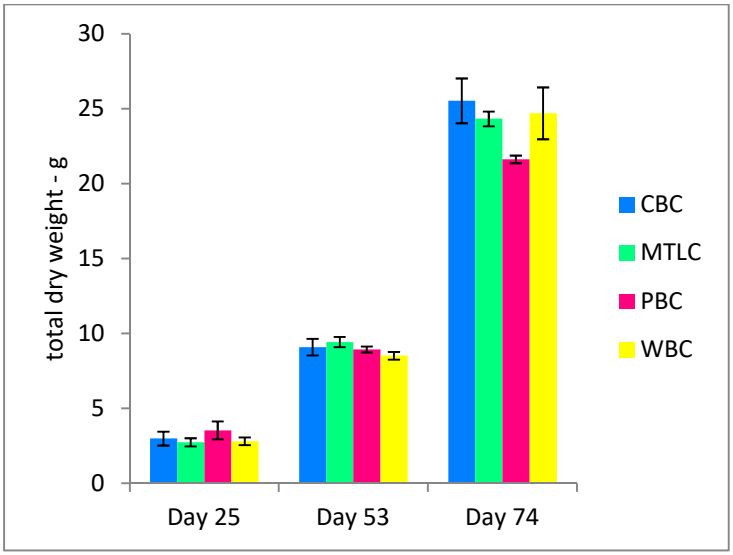


Figure 4-9. Mean total dry weights of tomato plants at destructive sampling (n = 3). Error bars show SE.

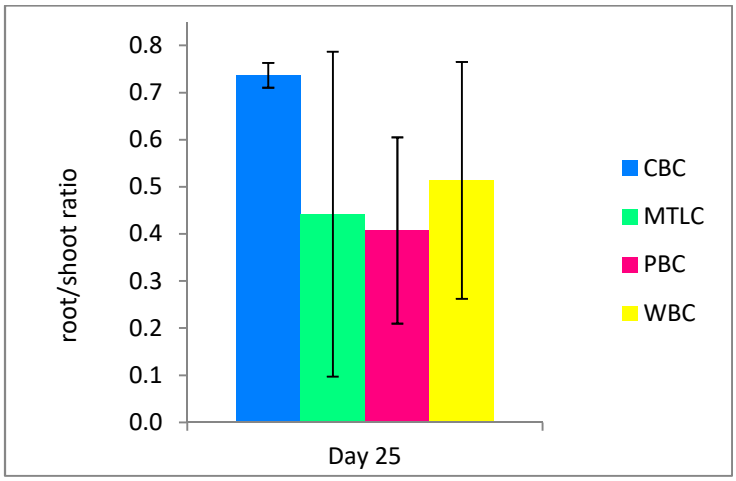


Figure 4-10. Tomato root/shoot ratio by dry weight at day 25 (n=3). Error bars show SE.

4.2.2 Leek growth in the trial composts

All the leeks were destructively sampled on day 103; there were 32 plants in total, eight replicates of each compost treatment.

Plant height

Figure 4-11 shows mean leek height over the course of the trial. Repeated measures ANOVA (Table 7-3) showed that all plants demonstrated significant growth over time ($p < 0.001$), with no significant interactions between date and compost (within subject effects). However a significant result was returned for compost type in the between subject effects ($p = 0.034$). Multiple comparisons using least significant difference (LSD) showed mean height in PBC to be significantly greater than in MTLC ($p = 0.027$) and WBC ($p = 0.007$).

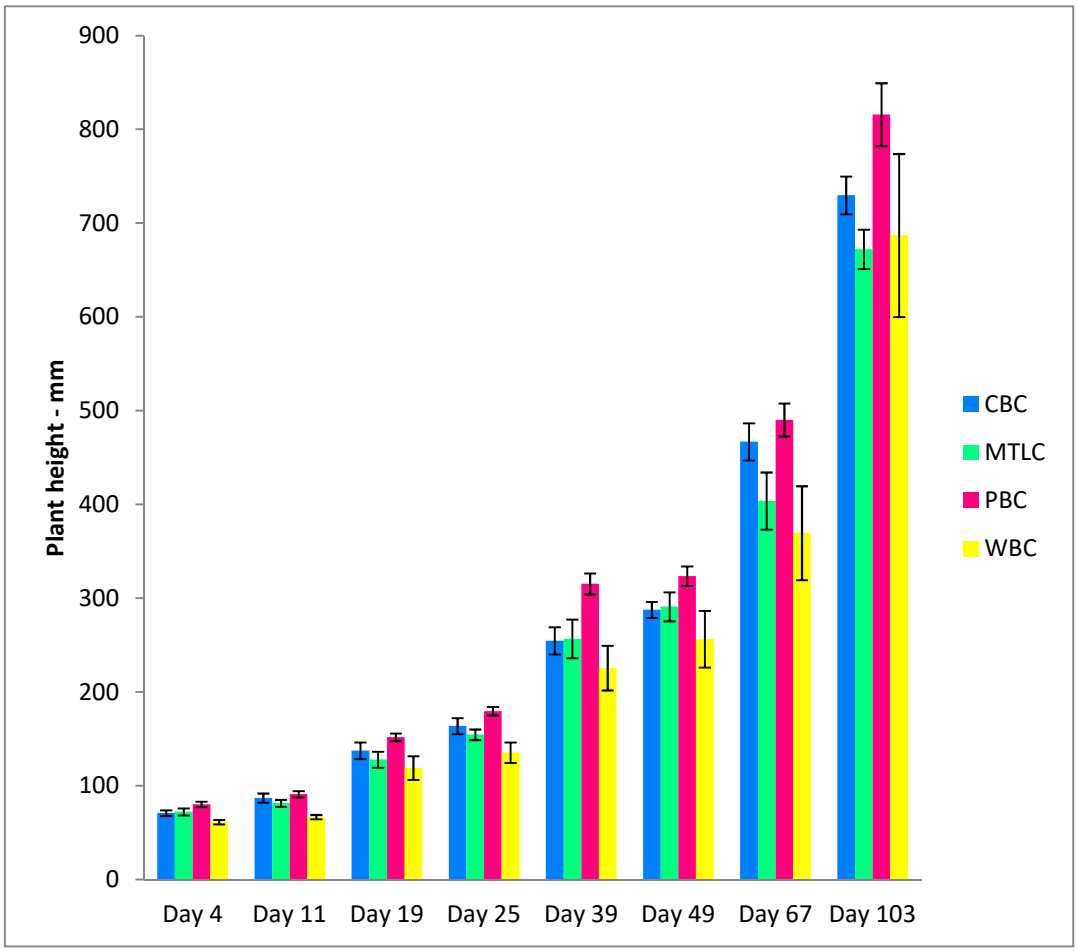


Figure 4-11. Mean plant heights of leeks over the course of the trial (n = 8).

Dry weights

Dry weights of top growth and roots were taken following destructive sampling on day 103 (Figure 4-12). There were no significant differences between top or root dry weights. However one-way ANOVA showed the total dry weights were significantly different (p = 0.028). LSD post hoc tests showed that PBC produced significantly greater total dry weight than both MTLC and WBC

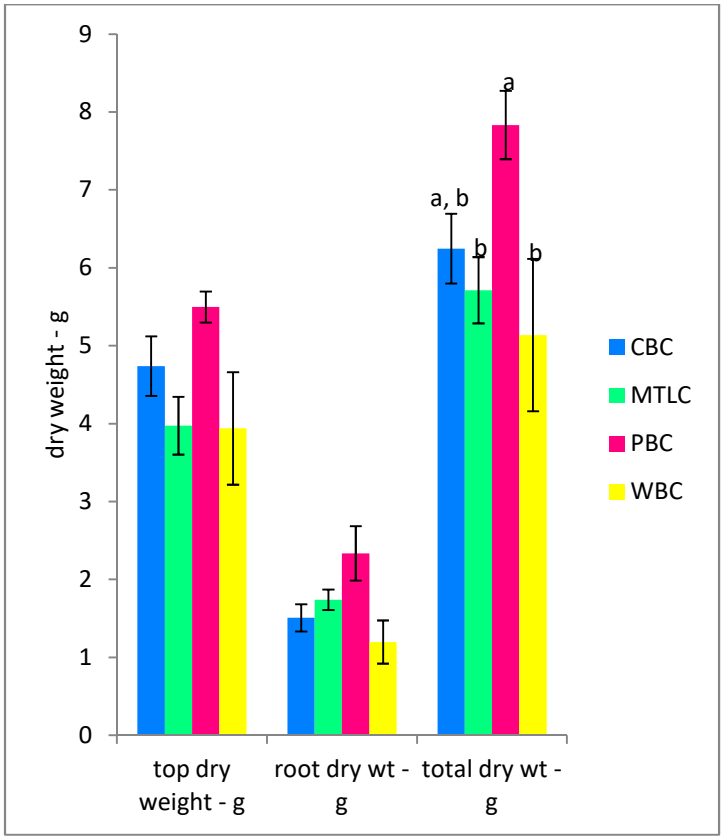


Figure 4-12. Mean dry weights of leek plants at destructive sampling (n = 8). Significantly different means are denoted by different annotations (a, b) ($p < 0.05$ in ANOVA). Error bars show SE.

4.2.3 Physical and chemical properties of the composts used in the pot trial

The composts used in the trial were sampled to determine their moisture content, water holding capacity, dry bulk density, pH and electrical conductivity, as well as concentrations of water-soluble nutrients.

The mean out-of-the-bag moisture contents of the composts were CBC 72.15% (SE 0.200), MTLC 74.94% (SE 0.080), PBC 64.61% (SE 0.510)and WBC 63.66% (SE 0.180) w/w. The water holding capacities were CBC 86.67%, MTLC 84.85%, PBC 85.39%, WBC 76.18% w/w. The mean dry bulk densities in g cm^{-3} of the composts were: CBC 0.109 (SE 0.0022), MTLC 0.118 (SE 0.0020), PBC 0.125 (SE 0.0016), WBC 0.169 (SE 0.0029).

The two coir based composts, CBC and MTLC had highest out-of-the bag moisture content (NS) and the lowest bulk densities. The bulk density of CBC was significantly lower than that of both PBC and WBC, while that of MTLC was significantly lower than that of WBC ($F = 60.31$, $p = 0.012$). The water holding capacities were determined without replication, however that of WBC was lower than those of the other composts.

pH and electrical conductivity (EC)

Data for pH and EC are shown in Figure 4-13. The pH of MTLC was significantly higher than that of the other composts ($F = 29.79$, $p = 0.018$). There were no significant differences in EC between composts, though that of WBC was higher than the rest.

Total nitrogen and carbon

The results of elemental analysis show significant differences between the composts in their percentages of nitrogen and carbon as well as their C/N ratios (Table 4-7). MTLC had significantly higher total nitrogen than the other three composts. Each compost had significantly different total carbon than each of the others; the order from highest to lowest was PBC, WBC, MTLC, CBC. The C/N ratio of MTLC was significantly lower than that of all the other composts.

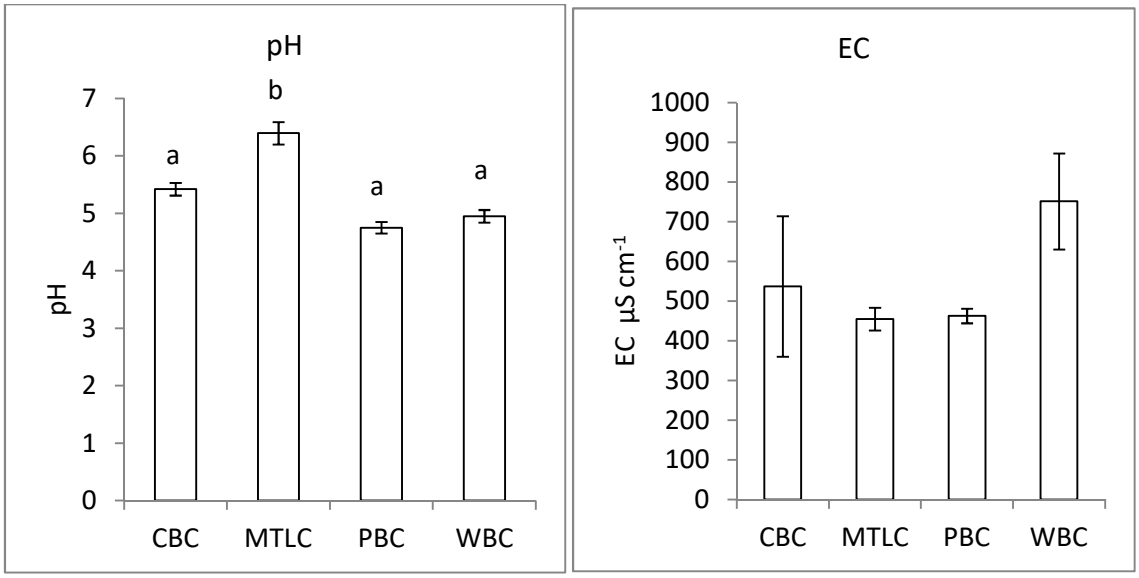


Figure 4-13. pH and electrical conductivity of the composts used in the trial. Significantly different means are denoted by different annotations (a, b) ($p < 0.05$). Error bars show SE.

Table 4-7. Total nitrogen and carbon percentages and carbon-nitrogen ratios of the composts. Groups with significantly different means are denoted by different annotations (a, b, c, d) ($p < 0.05$).

		n	Mean	SE	F	p
N%	CBC[(c)]	2	.672	.0325	96.2	0.010
	MTLC[(a)]	2	1.238	.0075		
	PBC[(b)]	2	1.039	.0100		
	WBC[(b)]	2	1.025	.0760		
C%	CBC[(d)]	2	31.850	1.3745	2613	0.001
	MTLC[(c)]	2	36.535	.0445		
	PBC[(a)]	2	50.432	.1110		
	WBC[(b, c)]	2	45.149	.7770		
C/N ratio	CBC[b]	2	47.440	.2289	46.59	0.009
	MTLC[a]	2	29.528	.2082		
	PBC[b]	2	48.584	.3563		
	WBC[b]	2	44.236	2.5371		

Water soluble nutrients

Figure 4-14 and Figure 4-15 show the concentrations of water soluble nutrients in the composts, as calculated from the concentrations in the extracts analysed by ion chromatography. The extracts had detectable Cl^-, NO_3^-, PO_4^{3-}, SO_3^{2-}, SO_4^{2-}, Na^+, NH_4^+, K^+, Ca^{2+}, Mg^{2+} , whereas NO_2^- ions had no detectable concentrations in any of the composts.

CBC had significantly lower phosphate concentrations than the other three composts ($F = 392.1$, $p = 0.006$). CBC also had the lowest concentration of calcium ($F = 34.55$, $p = 0.030$). CBC and MTLC had significantly higher concentrations of chloride than the other two composts ($F = 268.1$, $p = 0.005$). Sodium concentrations CBC and MTLC were also significantly higher ($F = 360.3$, $p = 0.006$) than PBC and WBC, however these were not identified by Tukey's post hoc test. MTLC had the highest concentration of sulphite (NS), ammonium concentrations were higher in CBC and MTLC, but not significantly so. CBC and PBC had significantly higher concentrations of sulphate than the other composts, and WBC significantly higher than MTLC ($F = 58.69$, $p = 0.0171$). PBC had the lowest concentration of potassium (ns), while its concentration of magnesium was the highest ($F = 4717$, $p < 0.001$). WBC had the highest (though variable) concentrations of nitrate (NS).

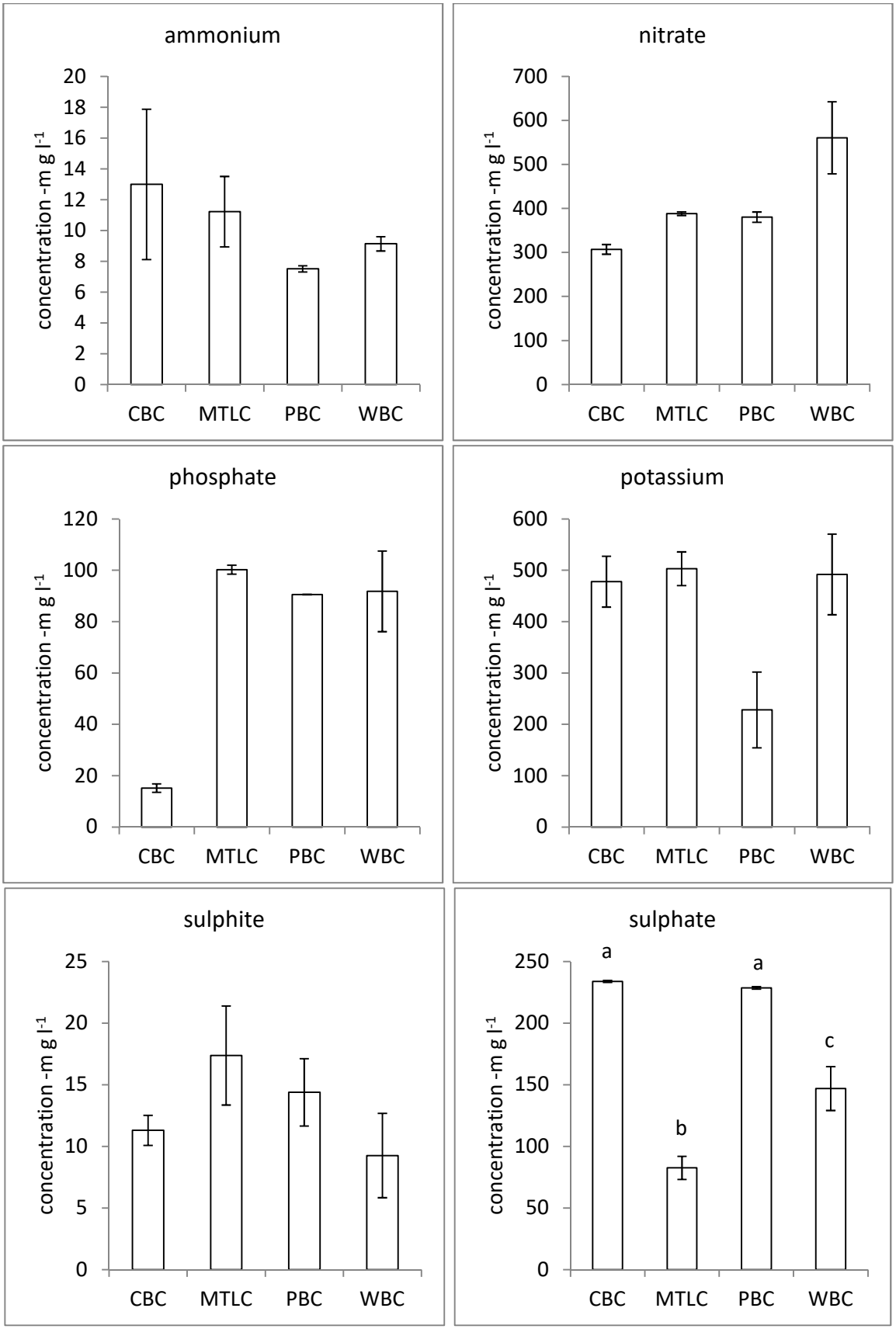


Figure 4-14. Concentrations of water-soluble nutrients in composts used in the pot trial (ammonium, nitrate, phosphate, potassium, sulphite, sulphate). Significantly different means are denoted by different annotations (a, b, c) ($p < 0.05$). Error bars show SE, n=2.

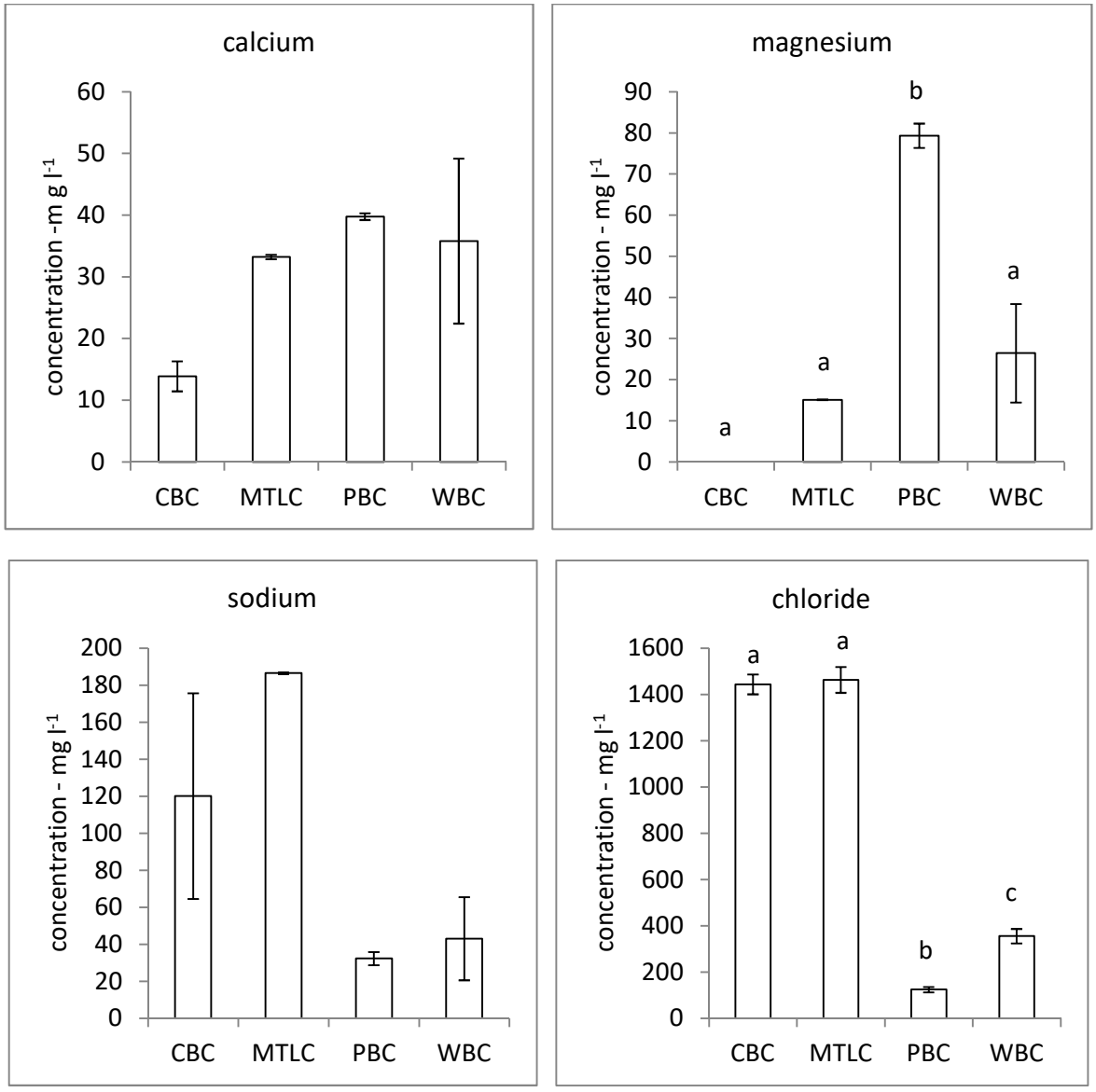


Figure 4-15. Concentrations of water-soluble nutrients in composts used in the pot trial (calcium, magnesium, sodium, chloride). Significantly different means are denoted by different annotations (a, b, c) ($p < 0.05$). Error bars show SE, n = 2.

4.3 The microcosm fermentation experiment

4.3.1 Purpose and overview of the experiment

The microcosm fermentation experiment was designed to test two main hypotheses that: (a) the presence *E. fetida*/*E. andrei* in vermicomposting manures would increase the concentrations of water-soluble nutrients and affect other physical, chemical and biological properties of the substrate, and interactions between earthworms and Collembola would enhance these effects, and (b) the presence of earthworms in vermicomposting manures would increase the abundance of Collembola.

The design was partially factorial with a total of six treatments of defaunated co-composting horse and cow manures, with added microorganism inocula:

X	control (no fauna added)
E	earthworms added
M	mesofauna added
ME	mesofauna and earthworms added
N	organic nitrogen added (no fauna)
MN	organic nitrogen and mesofauna added

Each treatment was replicated 16 times, making 96 microcosms in total. Successive destructive samplings of four replicates of each treatment were made at 36, 79, 106, 136 days from the start of the experiment.

4.3.2 Temperature of the experimental areas

Temperature fluctuations in the experimental area were avoided as par as possible and the temperature range was 14 to 23°C (Figure 4-16). The maximum temperature difference between experimental blocks at any time was 1°C.

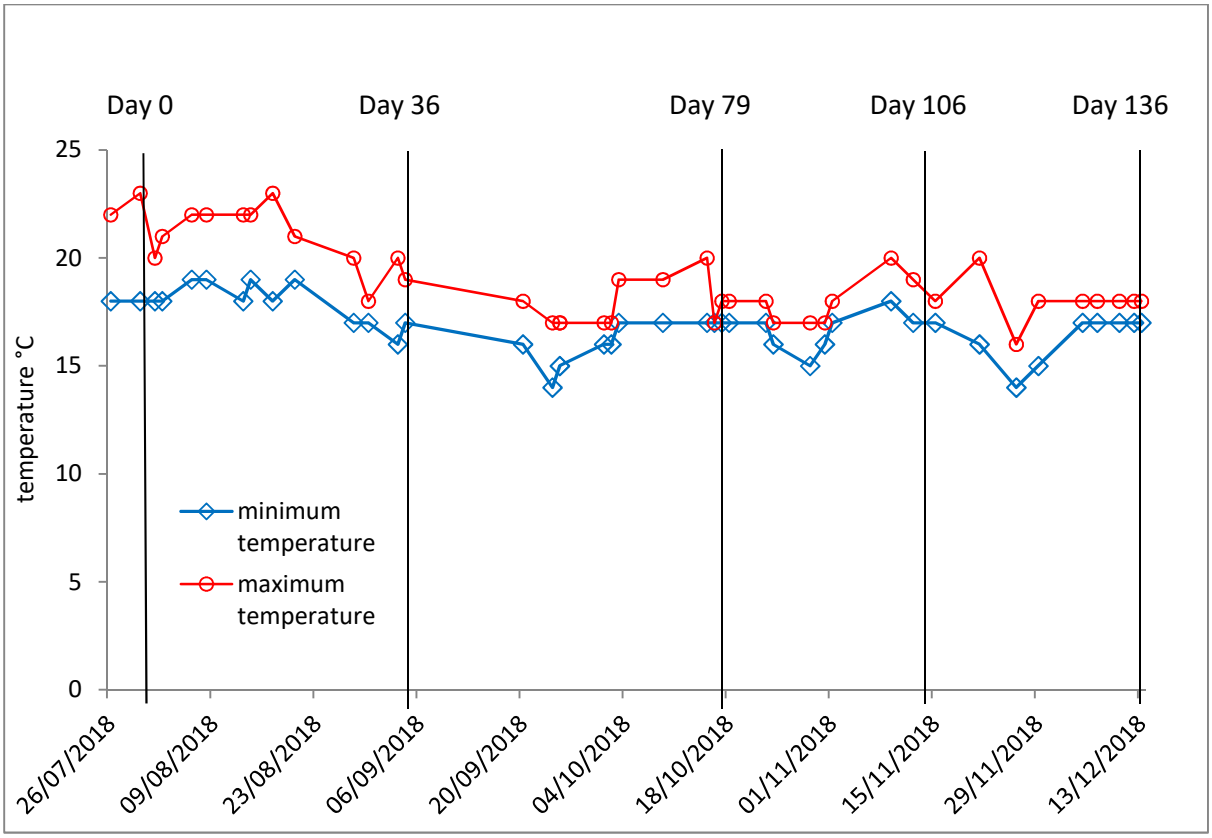


Figure 4-16. Maximum and minimum temperatures of the experimental area during the microcosm experiment. Timings of key events are shown (vertical lines): the start of the experiment (day 0) and the four destructive samplings on days 36, 79, 106 and 136 (the end of the experiment).

4.3.3 Substrate respiration

Measurements of CO_2 flux were taken periodically throughout the experiment using the IRGA, as detailed in Chapter 3. The first measurement was taken before the start (day -4), i.e. after the first inoculation with microorganisms but before earthworms, mesofauna and organic N were added. There were no significant differences between groups selected for different treatments, the mean respiration rate was 4.071 g (CO_2) m^{-2} h^{-1} (SE 0.141). Bar charts of subsequent substrate respiration rates are shown in Figure 4-17 and Figure 4-18.

On day 18, treatments with earthworms present (E and ME) had significantly higher respiration rates than the control (X) and N and MN ($F = 6.094$, $p = 0.001$), mean respiration rates across all treatments had fallen to 2.149 g (CO_2) m^{-2} h^{-1} (SE 0.118). By day 31, the mean

respiration rate for all treatments was lower again at 1.884 g (CO_2) m^{-2} h^{-1} (SE 0.051), and the treatments with added organic nitrogen had significantly lower respiration rates than all the other treatments (F= 20.83, p < 0.001). At the first destructive sampling on day 36, there were no significant differences between treatments) and mean respiration rates across all treatments had fallen to 2.080 g (CO_2) m^{-2} h^{-1} (SE 0.099).

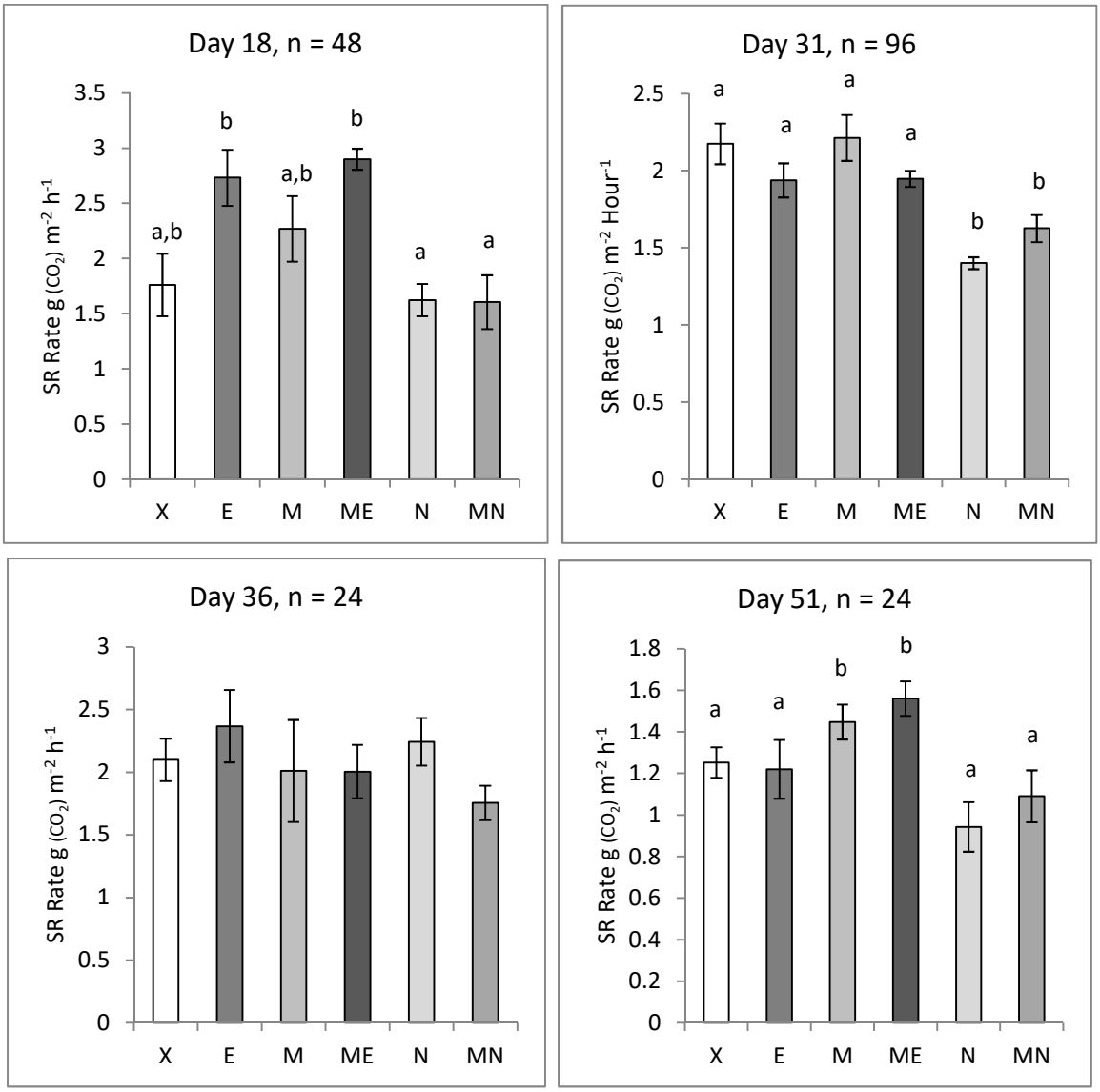


Figure 4-17. Substrate respiration on days 18, 31, 36 and 51. Means with the same lower case letter (a, b or c) indicates that there are no significant differences between them. Error bars show SE. (Treatment codes X, E, M, ME, N and MN are given in section 4.3.1).

On day 51, the mean respiration rate across all treatments was 1.252 g (CO_2) m^{-2} h^{-1} (SE 0.0580) and two of the treatments with mesofauna, M and ME, had significantly higher respiration rates than the other treatments (F = 4.448, p = 0.008). In subsequent samplings,

no significant differences between treatments were found, although the respiration rates of the control (X) and the mesofauna only (M) treatments tended to be higher than the others. By day 135 the mean respiration rate across all treatments was 0.709 g (CO2) m^{-2} h^{-1} (SE 0.0612), and the respiration rates of both treatments with earthworms present (E and ME) were lower than the others (not significant).

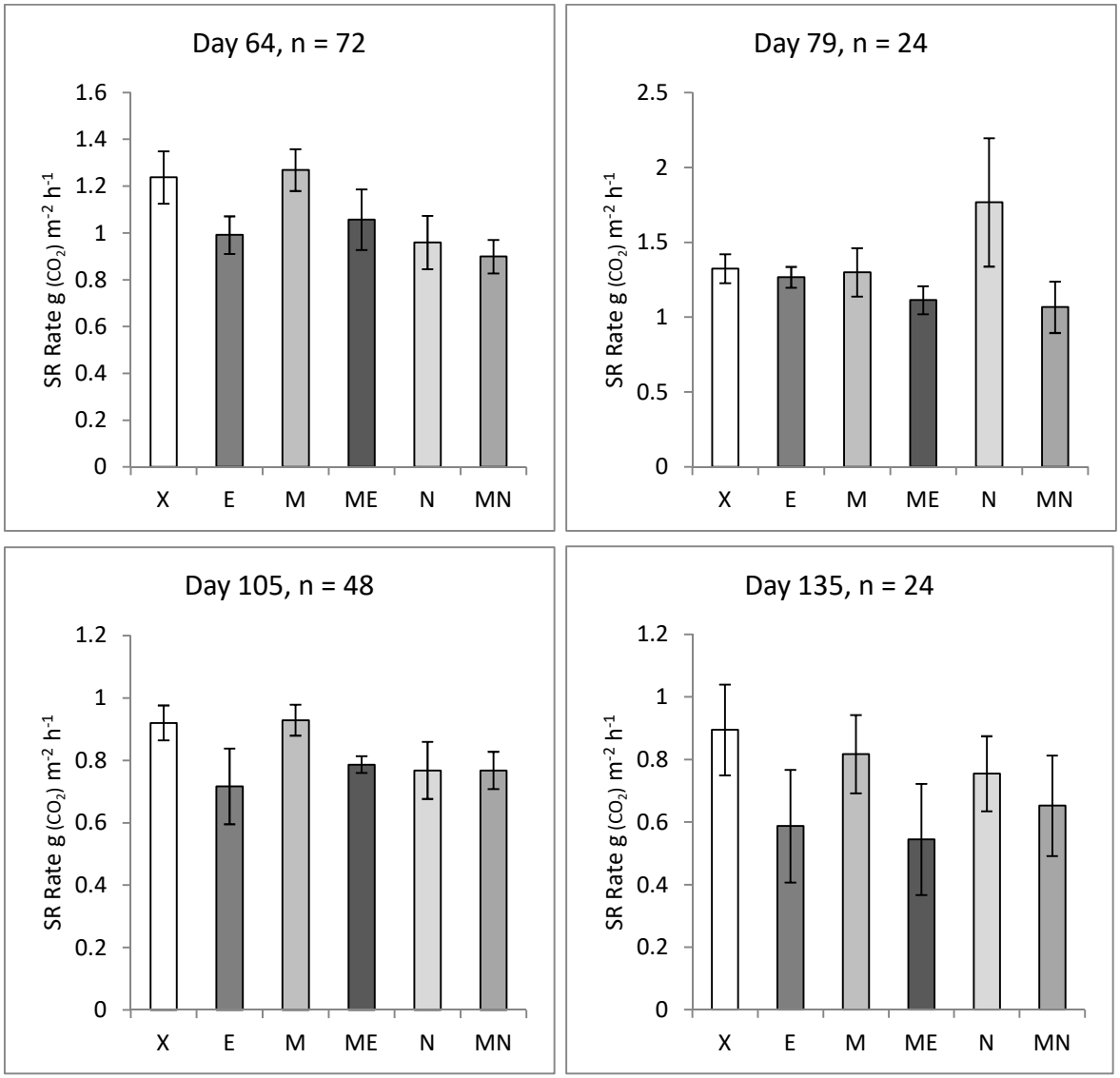


Figure 4-18. Substrate respiration on days 64, 79, 105 day 135. Error bars show SE. (Treatment codes X, E, M, ME, N and MN are given in section 4.3.1).

4.3.4 Physical properties

No significant differences were found in mean dry bulk densities between treatments or compost age groups (Table 4-8).

Table 4-8. Mean dry bulk density of treatments with compost age.

Compost age - days	Treatment	Mean dry bulk density g ml^{-1}
36	X	0.129
79	X	0.142
106	X	0.134
136	X	0.131
36	E	0.122
79	E	0.130
106	E	0.131
136	E	0.124
36	M	0.121
79	M	0.133
106	M	0.138
136	M	0.129
36	ME	0.121
79	ME	0.126
106	ME	0.127
136	ME	0.128
36	N	0.121
79	N	0.133
106	N	0.134
136	N	0.127
36	MN	0.121
79	MN	0.136
106	MN	0.136
136	MN	0.127

The percentage of particles passing through a 2 mm sieve showed significant differences in relation to treatment and compost age, as well as their interaction (Figure 4-19); the results of a two-way PERMANOVA on this basis are shown in Table 4-9.

Table 4-9. Two-way PERMANOVA of particle size in relation to date of destructive sampling (age) and treatment

	Sum of squares	df	Mean square	F	p
Treatment	1.4465	5	0.28929	187.70	<0.001
Age	0.032022	3	0.010674	6.93	0.001
Interaction (treatment x age)	0.097751	15	0.006517	4.23	<0.001
Residual	0.11097	72	0.001541		
Total	1.6872	95			

A one-way PERMANOVA followed by pairwise comparisons confirms that treatments with earthworms had significantly greater percentages of particles of less than 2 mm than those without earthworms ($p < 0.001$). The particle size effects increased significantly with time in the earthworm treatments, but not in the other treatments (treatment x age). There was no significant effect relating to mesofauna.

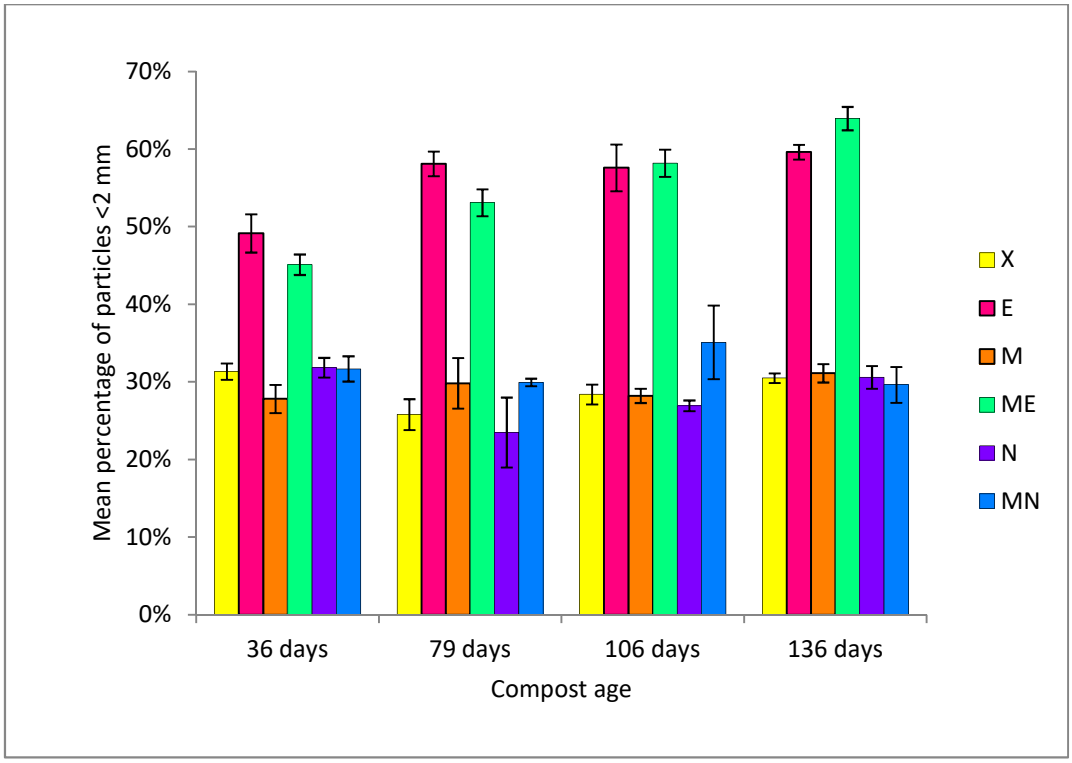


Figure 4-19. Mean percentage of particles <2 mm at successive destructive samplings (ages of compost). Error bars show SE.

4.3.5 Water-soluble nutrients

The mean concentrations of water-soluble nutrients in the composts at each destructive sampling are presented in Table 4-10.

Concentrations consistently greater than zero were obtained for chloride, nitrate, phosphate, sulphate, sodium, potassium, calcium and magnesium. Ammonium and nitrite are also included in the statistical analyses, although concentrations of these two ions were too low to detect in some of the extracts, and are therefore apparently absent from some treatments.

A two-way PERMANOVA with sampling day and treatment as factors and all ions as variables returned significant results for both age (destructive sampling day) ($F = 30.22$, $p < 0.001$) and treatment ($F = 22.08$, $p < 0.001$) as well as their interaction (age x treatment) ($F = 2.08$, $p = 0.011$) (Table 7-5). One-way ANOVAs of each nutrient in each age group are shown in Table 4-10.

Table 4-10. Mean concentrations and one-way ANOVA of water-soluble nutrients in composts (mg l^{-1}) by compost age and treatment (n = 4).

36 days	chloride		nitrite		nitrate		phosphate		sulphate	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
X	627.06 ^{a}	26.50	2.14	1.25	1248.74 ^{a}	50.39	515.19 ^{a,b}	18.65	87.89 ^{a}	4.80
E	572.40 ^{a}	14.70	nd	nd	1750.58 ^{b}	54.82	659.91 ^{c}	17.01	91.59 ^{a}	2.17
M	445.24 ^{b}	39.60	3.11	1.32	823.78 ^{c}	113.89	471.44 ^{a}	12.06	69.14 ^{b}	4.66
ME	564.83 ^{a,b}	35.52	nd	nd	1704.38 ^{b}	112.10	658.94 ^{c}	34.19	96.52 ^{a}	5.70
N	588.34 ^{a}	15.36	0.81	0.81	1415.07 ^{a,b}	113.04	536.03 ^{b}	16.67	81.41 ^{a,b}	3.59
MN	530.14 ^{a,b}	24.52	3.12	1.21	1247.85 ^{a}	79.49	572.01 ^{b}	17.14	73.41 ^{a,b}	3.91
ANOVA	F	5.069	F		F	14.03	F	14.20	F	6.172
	p	0.004	p	ns	p	<0.001	p	<0.001	p	0.002

36 days	sodium		ammonium		potassium		calcium		magnesium	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
X	425.94 ^{a}	15.63	10.07	2.45	1085.78 ^{a,b}	33.48	120.63 ^{a,b}	7.34	56.51 ^{a}	5.18
E	422.30 ^{a}	8.60	13.54	1.19	1082.56 ^{c}	22.02	147.14 ^{a,c}	6.54	77.56 ^{a,b}	3.28
M	323.81 ^{b}	20.71	7.83	2.32	816.20 ^{a}	57.46	107.49 ^{b}	5.13	61.50 ^{a}	9.95
ME	425.71 ^{a}	21.47	7.69	2.47	1088.45 ^{c}	62.99	151.67 ^{c}	5.82	90.34 ^{b}	4.31
N	412.64 ^{a}	7.16	10.48	3.57	1051.86 ^{a,b}	22.18	117.91 ^{b}	8.51	67.08 ^{a,b}	5.82
MN	389.02 ^{a}	15.88	8.93	2.13	994.11 ^{b,c}	41.35	125.66 ^{a,b,c}	2.39	68.48 ^{a,b}	0.76
ANOVA	F ^{P}	6.284	F ^{P}		F ^{P}	6.083	F	7.700	F	4.652
	p	0.001	p	ns	p	0.003	p	0.001	p	0.007

79 days	chloride		nitrite		nitrate		phosphate		sulphate	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
X	607.61 ^{a}	22.43	nd ^{a}	nd	1375.62 ^{a}	48.86	567.94 ^{a}	24.96	89.88 ^{a}	2.36
E	570.30 ^{a}	24.41	nd ^{a}	nd	2243.06 ^{b}	87.60	764.49 ^{b}	20.11	120.88 ^{b}	3.88
M	753.79 ^{b}	30.07	3.33 ^{b}	0.13	1771.93 ^{c}	38.17	727.49 ^{c}	10.63	117.26 ^{b}	2.78
ME	555.29 ^{a}	16.50	nd ^{a}	nd	2252.94 ^{b}	73.09	759.96 ^{b}	11.62	114.30 ^{b,c}	3.88
N	618.22 ^{a}	27.82	nd ^{a}	nd	1854.91 ^{c}	98.18	631.71 ^{a,c}	31.26	101.55 ^{a,c}	2.86
MN	670.43 ^{a,b}	76.84	0.79 ^{a,b}	0.79	2043.46 ^{b,c}	227.00	677.05 ^{b,c}	63.34	101.84 ^{a,c}	10.15
ANOVA	F ^{P}	3.584	F ^{P}	15.34	F	8.503	F	5.803	F	5.424
	p	0.019	p	0.001	p	<0.001	p	0.002	p	0.003

79 days	sodium		ammonium		potassium		calcium		magnesium	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
X	457.19	34.13	16.96	8.29	1113.93	88.03	128.31	7.54	70.79	4.11
E	472.41	32.40	5.81	0.79	1168.45	93.74	204.55	18.16	105.52	4.55
M	615.13	20.35	19.79	8.91	1520.41	25.51	181.39	11.22	94.89	1.74
ME	465.32	34.36	8.33	5.09	1147.32	75.05	222.59	29.89	127.86	16.74
N	521.40	37.09	21.62	6.32	1358.30	123.93	188.93	28.98	95.41	13.00
MN	547.43	88.62	7.83	4.88	1331.67	178.42	192.03	42.15	106.59	22.83
ANOVA	F		F		F		F		F	
	p	ns	p	ns	p	ns	p	ns	p	ns

106 days	chloride		nitrite		nitrate		phosphate		sulphate	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
X	530.28	91.75	nd	nd	1379.33 ^{a}	237.47	501.68 ^{a}	73.27	83.09 ^{a}	12.41
E	625.43	8.38	nd	nd	2747.58 ^{b}	21.77	781.07 ^{b}	8.01	116.71 ^{b}	1.44
M	658.58	19.77	nd	nd	1744.44 ^{a}	46.49	626.66 ^{a}	13.06	95.96 ^{a}	2.16
ME	607.77	9.87	nd	nd	2756.90 ^{b}	27.91	791.37 ^{b}	7.36	120.49 ^{b}	1.58
N	534.91	87.35	nd	nd	1963.60 ^{a,b}	417.96	589.21 ^{a,b}	98.04	93.54 ^{a,b}	14.78
MN	611.04	27.61	nd	nd	2112.30 ^{a}	191.40	636.31 ^{a}	42.93	97.76 ^{a}	4.14
ANOVA	F		F		F ^{P}	6.699	F ^{P}	4.451	F ^{P}	3.123
	p	ns	p	na	p	0.001	p	0.008	p	0.033

106 days	sodium		ammonium		potassium		calcium		magnesium	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
X	319.69	87.81	nd	nd	791.18	222.04	109.51 ^{a}	24.24	65.08 ^{a,b}	23.24
E	466.03	2.99	1.41	1.41	1167.12	6.93	231.26 ^{b}	4.91	127.21 ^{b,c}	5.36
M	449.34	11.76	nd	nd	1138.31	19.72	125.96 ^{a}	6.38	73.28 ^{a}	2.49
ME	453.15	3.95	nd	nd	1140.68	8.53	239.74 ^{b}	5.40	136.38 ^{c}	4.46
N	357.94	92.72	nd	nd	889.43	242.63	144.09 ^{a}	42.55	74.54 ^{a,b}	23.50
MN	362.94	63.15	nd	nd	900.26	169.37	153.49 ^{a,b}	15.85	83.38 ^{a}	14.81
ANOVA	F		F	na	F		F ^{P}	6.611	F ^{P}	4.086
	p	ns	p	na	p	ns	p	0.001	p	0.013

136 days	chloride		nitrite		nitrate		phosphate		sulphate	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
X	668.43	62.08	0.34	0.34	1469.42 ^{a}	113.05	541.18 ^{a}	32.48	94.00 ^{a}	5.08
E	618.56	13.63	nd	nd	2650.31 ^{b}	206.75	728.24 ^{b,c}	37.78	118.88 ^{a,b}	8.54
M	589.59	8.87	0.46	0.46	1698.71 ^{a,c}	59.04	583.54 ^{a,c}	20.96	94.87 ^{a}	5.61
ME	640.63	8.35	nd	nd	3062.36 ^{d}	47.12	824.88 ^{d}	10.15	135.30 ^{b}	1.25
N	584.62	20.05	nd	nd	2438.26 ^{b}	57.92	688.82 ^{b}	9.06	95.01 ^{a}	4.22
MN	569.96	15.03	nd	nd	2278.86 ^{b,c}	187.88	673.99 ^{a,b,c}	38.82	93.15 ^{a}	6.22
ANOVA	F		F	na	F ^{p}	21.24	F	13.46	F ^{P}	10.03
	p	ns	p	na	p	<0.001	p	<0.001	p	<0.001

136 days	sodium		ammonium		potassium		calcium		magnesium	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
X	427.54 ^{a,b,c}	18.41	nd	nd	1027.31 ^{a,b}	27.44	158.07 ^{a,c}	30.14	70.48 ^{a}	10.20
E	458.74 ^{a,b}	10.57	3.27	3.27	1114.88 ^{a,c}	16.53	223.91 ^{b}	24.39	124.26 ^{b,c}	13.85
M	313.16 ^{c}	55.68	nd	nd	775.73 ^{b}	140.38	138.90 ^{c}	9.67	67.39 ^{a}	8.43
ME	475.20 ^{b}	4.96	2.19	2.19	1163.07 ^{c}	13.19	249.93 ^{b}	5.71	147.03 ^{c}	2.44
N	430.95 ^{a,c}	14.22	nd	nd	1082.75 ^{a,b}	14.18	199.30 ^{a,b}	4.20	106.66 ^{b}	3.41
MN	369.34 ^{a,c}	48.42	nd	nd	935.11 ^{a,b}	128.48	209.48 ^{a,b}	23.54	82.80 ^{a,b}	17.97
ANOVA	F ^{P}	3.600	F	na	F ^{P}	3.199	F	4.675	F	8.607
	p	0.016	p	na	p	0.015	p	0.007	p	<0.001

Treatments with the same superscript letters (a, b, c, d) are not significantly different to each other ($p > 0.05$, LSD). Superscript *P* denotes non-parametric ANOVA (pairwise comparisons by PERMANOVA). nd = not detected, i.e. nutrient levels were too low to detect.

There were significant differences in mean nitrate, phosphate and sulphate concentrations between treatments within each age group. Nitrate concentrations (Figure 4-20) were significantly higher in treatments containing earthworms (E and ME) than in all others at each destructive sampling. At 136 days, nitrate levels in ME were also significantly higher than in E. Nitrate also shows significant increases in concentrations within some treatments with age. Treatments E and ME showed significantly higher levels of nitrate with age to 106 days in both E ($F = 15.31$, $p = 0.001$) and ME ($F = 67.84$, $p < 0.001$), while mean nitrate concentration ME (not E) increased significantly between 106 and 136 days as well. The control (X) showed no significant change in nitrate concentrations with age. Nitrate in the mesofauna only treatment (M) was significantly lower than all other treatments at 36 days, had increased significantly by 79 days ($F = 41.85$, $p = 0.001$) to a significantly higher concentration than X, and then fell to levels not significantly different to X at 106 and 136 days. The treatments containing organic nitrogen (N and MN) were not significantly different from each other in nitrate concentrations in all age groups.

Ammonium and nitrite ions were not detected in many of the compost samples after 36 days; nitrite was never detected in the E and ME treatments. At 36 days there were no significant differences in concentrations of ammonium and nitrite between treatments. At 79 days nitrite was detected in M and MN (only in one sample of the latter), giving a statistically significant result for M ($F = 15.34$, $p = 0.001$).

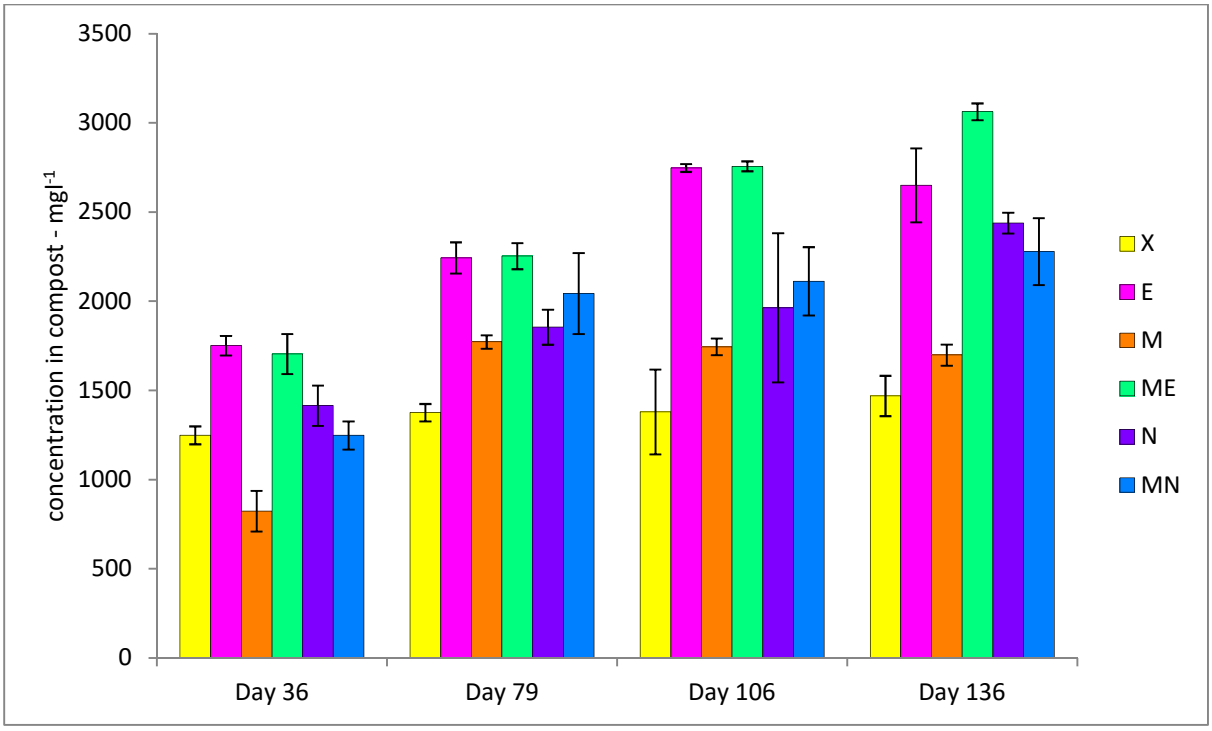


Figure 4-20. Concentrations of nitrate in the composts in mgl^{-1} at successive destructive samplings.

Mean concentrations of phosphate were significantly higher in E and ME than in X and M treatments in the first three age groups (36, 79 and 106 days). At 136 days, ME was significantly higher in phosphate than all the other treatments, including E, which was significantly higher to X. Overall, phosphate concentrations showed less significant change with time than did nitrate concentrations. As in the case with nitrate, X did not change significantly in phosphate concentrations throughout the experiment. Levels in E and ME increased significantly between 36 and 79 days in the former ($F = 5.288$, $p = 0.018$) and between 79 and 106 days in the latter ($F = 14.05$, $p = 0.002$). Mean phosphate concentrations in M showed a significant increase between 36 and 79 days followed by a significant decrease from 79 to 106 days ($F = 15.31$, $p < 0.001$).

Changes in sulphate concentrations followed similar trends over time to those of phosphate, with significant increases in E and ME after 36 days ($F = 7.944$, $p = 0.007$, and $F = 19.99$, $p < 0.001$ respectively). Unlike phosphate and nitrate, at 36 days there was no significant difference in sulphate between treatments with earthworms and the other treatments except M, which was significantly lower. Concentrations of sulphate in M were

significantly higher at day 79, and were not significantly different to those of E and ME, followed by a significant decrease from 79 to 106 days ($F = 23.66$, $p < 0.001$).

Relatively higher concentrations of sodium, potassium, calcium and magnesium in E and ME compared to other treatments were also apparent in some age groups. However, these were only significant in calcium (E: $F = 5.851$, $p = 0.017$, ME: $F = 7.949$, $p = 0.005$) and magnesium (E: $F = 8.277$, $p = 0.004$, ME: $F = 7.485$, $p = 0.005$). In all these cations, concentrations did not change significantly in X over time.

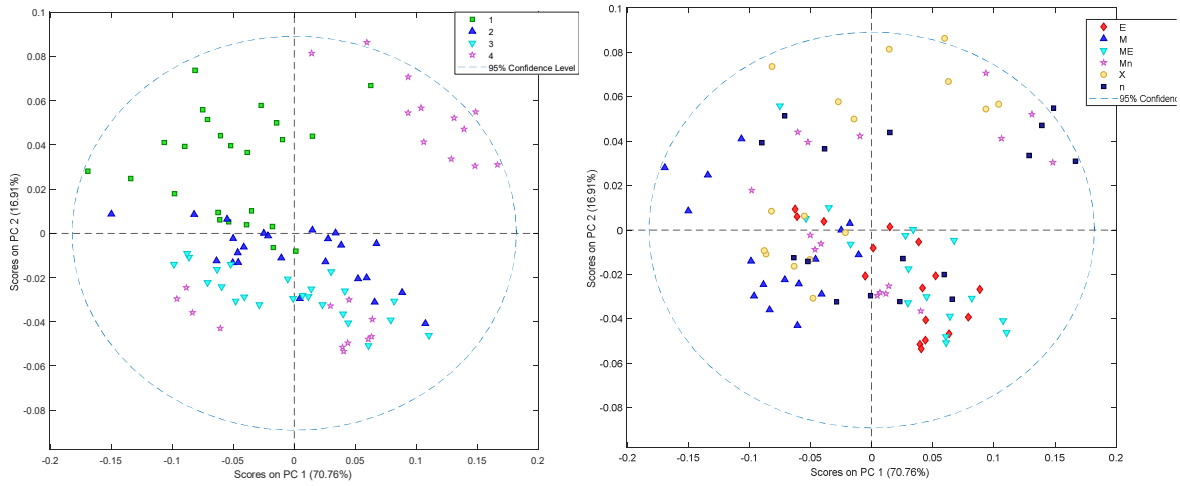
Mean chloride concentrations in M were significantly lower than in the treatments without mesofauna (X, E and N) at 36 days, but significantly higher than in X, E, ME and N at 79 days. Thereafter there were no significant differences between treatments.

There were few significant differences in mean nutrient concentrations between the treatments with added nitrogen (N and MN) and the control (X). However, nitrate levels at 136 days were significantly higher in both N and MN compared to X. This followed a significant increase in NO_3^- concentrations between 36 and 79 days and 106 and 136 days in N ($F = 3.523$, $p = 0.053$, and from 36 to 79 days in MN ($F = 6.501$, $p = 0.009$). Nitrate did not increase significantly in MN after the second addition of organic nitrogen after 106 days, while it did in the case of N.

4.3.6 Near infrared spectroscopy (NIRS)

A total of 96 samples were scanned, four replicates of each treatment at each of four destructive sampling days. An initial PCA was carried out on the spectra and cross-validation showed that a 5 PC model accounted for 99.35% of the variation (RMSEC = 0.000236289, RMSECV = 0.000352435).

This basic PCA model showed strong separation for compost age (i.e. destructive sampling day), and a visible but weak grouping for treatment (Figure 4-21). PC1 explained 70.76% of the variation, PC2 16.91%; loadings for PC1 and PC2 are shown in



(a) (b)

Figure 4-21. First (basic) PCA model plots of PC1 against PC2 showing (a) compost age and (b) treatment. (a) ages are shown as 1: 36 days (green squares), 2: 79 days (darker blue upward triangles), 3: 106 days (lighter blue downward triangles), 4: 136 days (pink stars). (b) treatments are shown as X: yellow circles, E: red diamonds, M, darker blue upward triangles, ME: lighter blue downward triangles, N: dark blue squares, MN: pink stars.

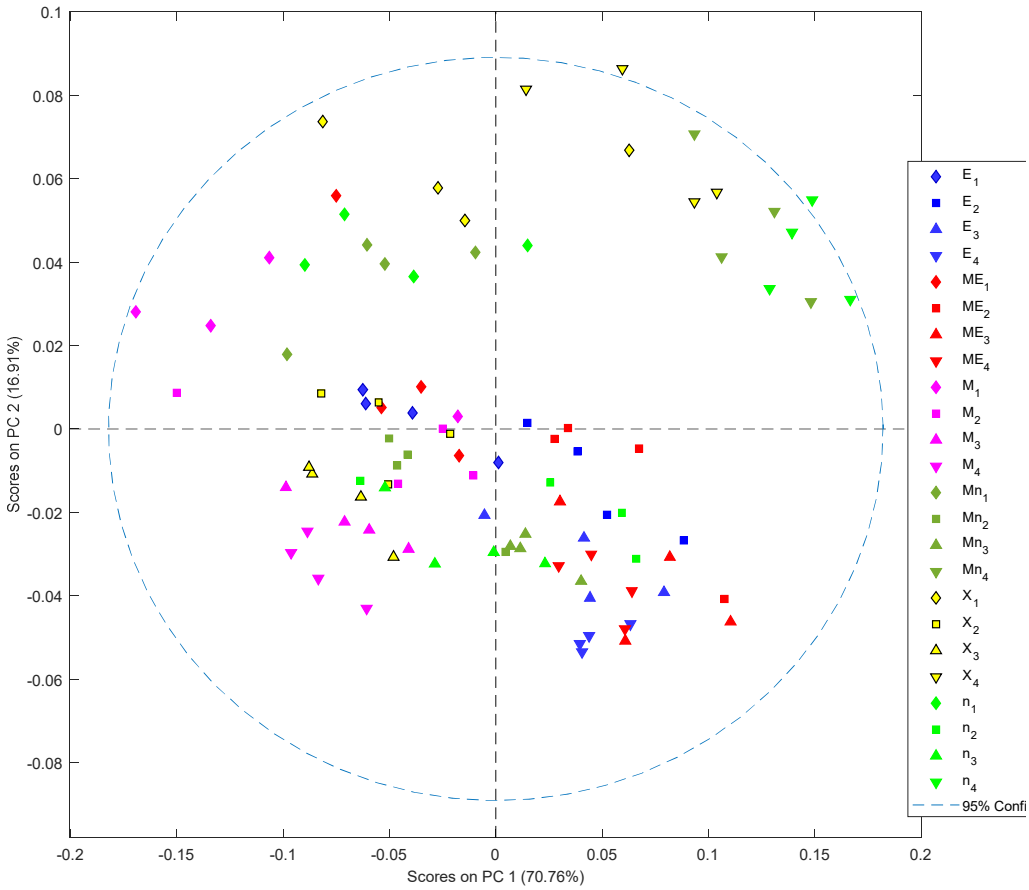


Figure 4-22. First (basic) PCA scores combined to show treatment scores with age. In the legend the subscript numerals 1-4 denote ages 36, 79, 106, and 136 days respectively.

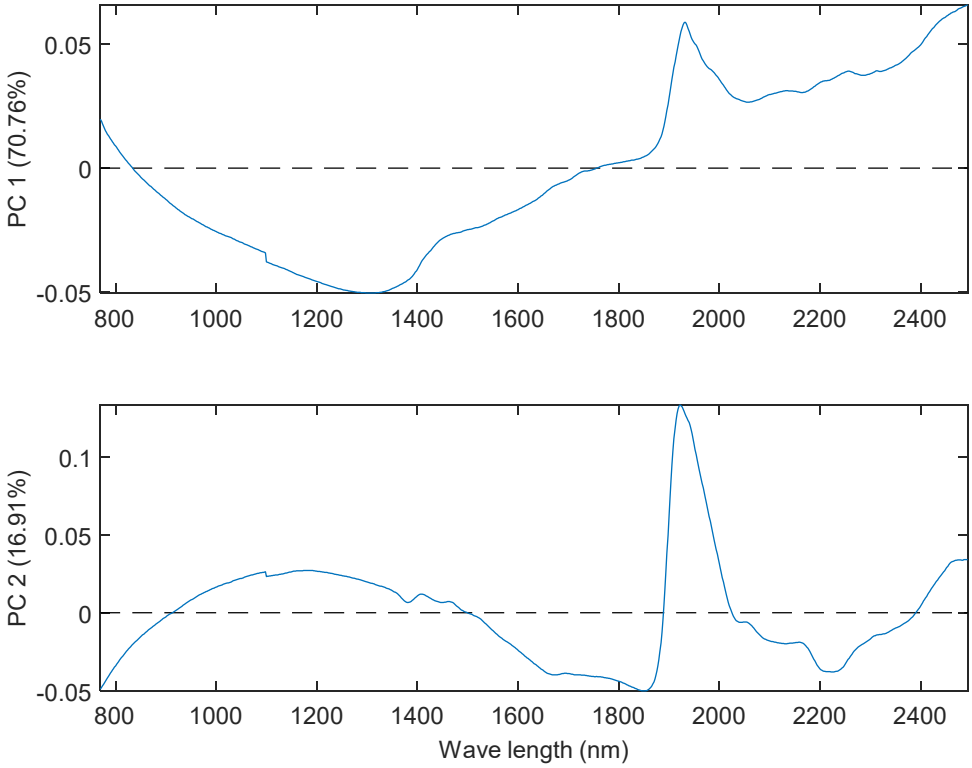


Figure 4-23. First PCA model loadings for PC1 and PC2.

Further development of the PCA model was carried out using GLS weighting in the pre-processing. Cross-validation showed that a 5 PC model accounted for 88.67% of the variation (RMSEC = 0.000086468, RMSECV = 0.000126423); with a particularly good fit for PCs 1-3 (84.75% of the variation). This second PCA model showed clear separation of the samples by destructive sampling day and treatment (Figure X-3). PC1 explained 52.73% of the variation, and suggested separation largely along a time dimension. PC2 explained 26.98% of the variation, apparently related to treatment. Loadings for PC1 and PC2 are shown in Figure 4-24. Loadings for this model are shown in Figure 4-25.

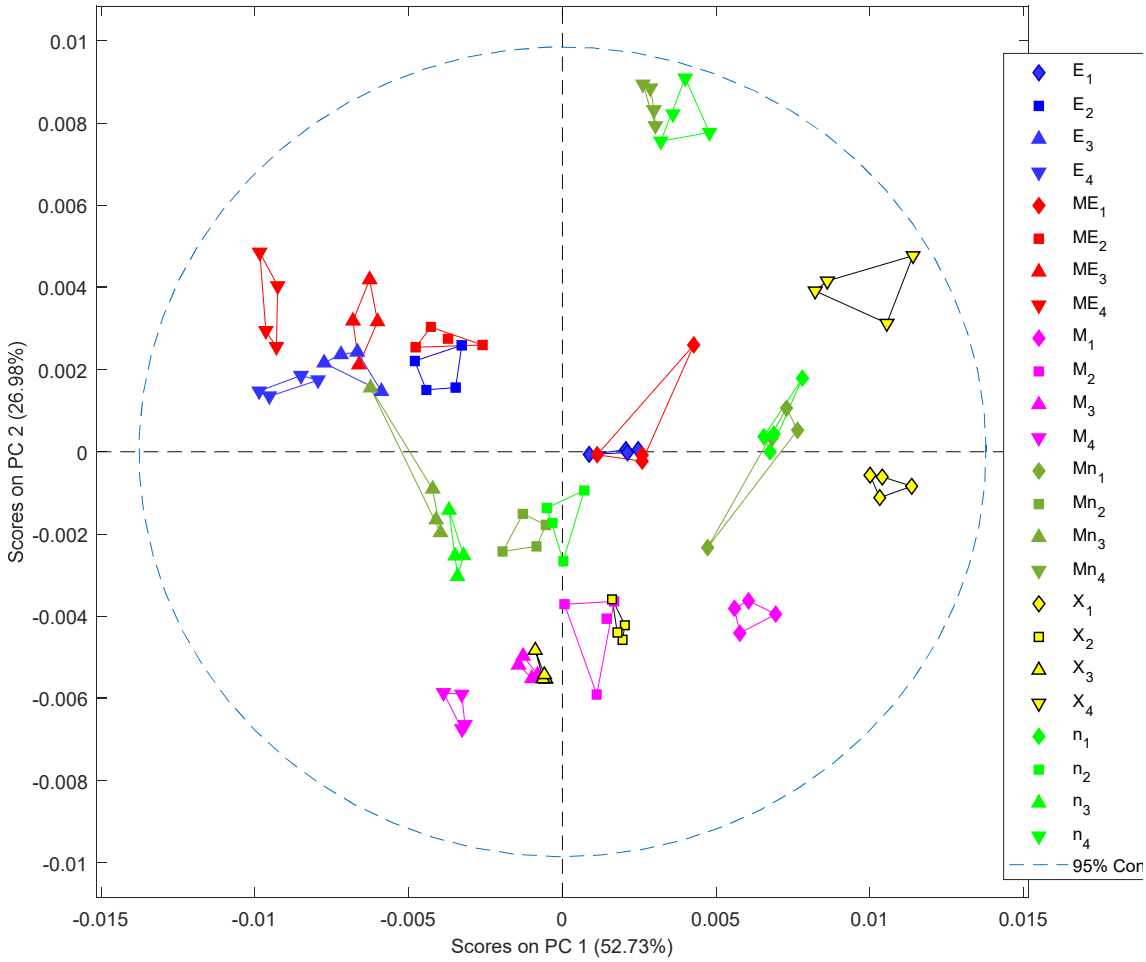


Figure 4-24. Second (GLS) PCA model plot of PC1 against PC2. In the legend the subscript numerals 1-4 denote ages 36 days (diamonds), 79 days (squares), 106 days (upward triangles), and 136 days (downward triangles) respectively. Treatments are shown as X: yellow with black boundary, E: blue, M: pink-purple, ME: red, N: bright green, MN: dark green.

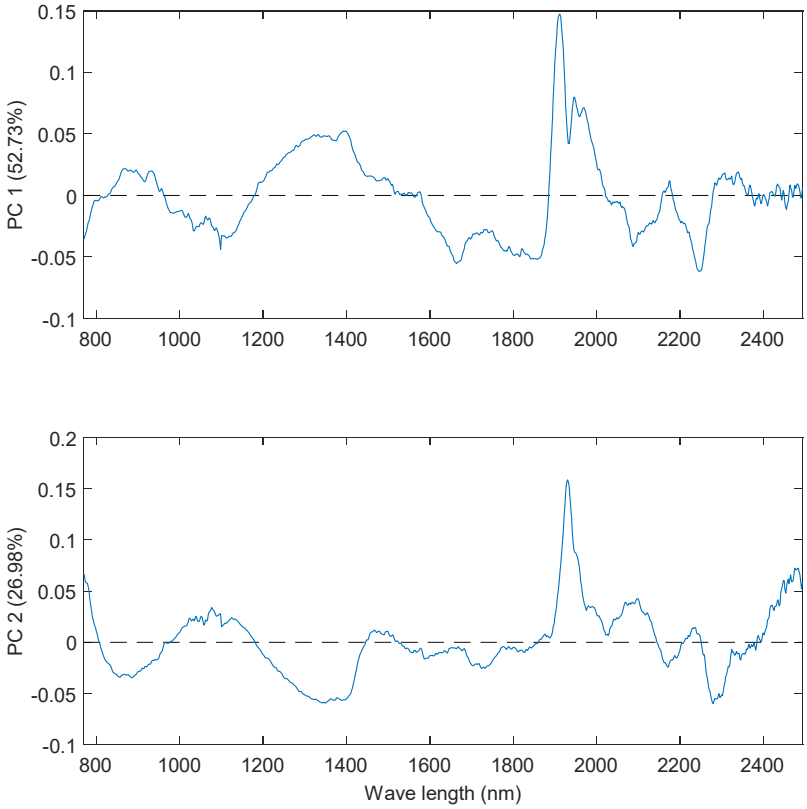


Figure 4-25. Second (GLS) PCA model loadings for PC1 and PC2.

One-way ANOVA of the second (GLS) model PC 1-3 scores by treatments (Table 7-6) showed all 3 PCs to be highly significant (PC1: $F = 11.93$, PC2: $F = 13.11$, PC3: $F = 9.03$, all PCs: $p < 0.001$). One-way MANOVA of treatment against PC1, PC2 and PC3 was also significant ($p < 0.001$). Canonical variant analysis (CVA) (Figure 4-26) showed significant differences between the control (X) and M as well as between each of these and all the other treatments. There was considerable overlap between E and ME 95% confidence intervals, with only weak (non-significant) separation, mainly on CV2. The N and MN treatments showed least separation. Two-way ANOVA of treatment x compost age showed significant differences for PC1, PC2, PC3 ($F = 222.71$, 107.29, 18.88 respectively, $p < 0.001$ for all). However two-way MANOVA of treatment x compost age against PC1, PC2 and PC3 was not significant; this may have been due to the GLS weighting (Allison, pers. comm.).

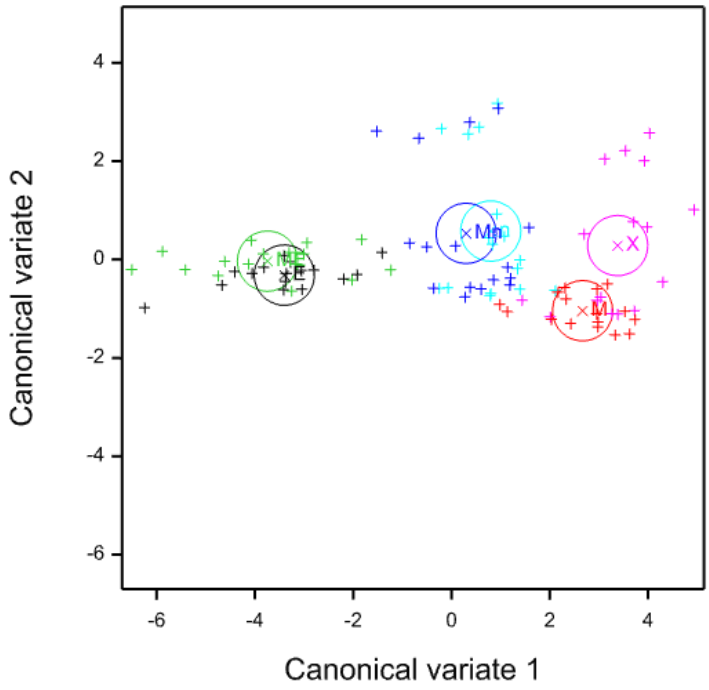


Figure 4-26. CVA following MANOVA of the second (GLS) PCA model for treatment with 95% confidence ellipses.

To avoid this possible distortion, similar analyses were applied to the first (basic) PCA model. One-way ANOVA on the basic model for treatment gave significant results for PC1, PC2 and PC4 ($F = 7.26, 6.26, 5.93$ respectively, $p < 0.001$ for all). The PC3 result was not significant. One-way MANOVA of treatment against PC1, PC2 and PC3 and PC4 was also significant ($p < 0.001$). CVA (Figure 4-27) demonstrated significant differences between X and M and between each of these and all the other treatments. The 95% confidence intervals of the N and MN treatments partially overlapped but showed a degree of separation, mainly on CV1. There was more overlap between E and ME, with very weak separation.

PC1, 2 and 3 of the basic model showed significance to compost age in one-way ANOVA ($F = 12.35, 17.5, 7.86$ respectively, $p < 0.001$ for all). PC4 was not significant. One-way MANOVA of PC1, 2, 3 and 4 against date gave a significant result ($F = 11.63$, $p < 0.001$). CVA (Figure 4-28) showed significant differences between the 36 day and the 136 age groups, and between each of these and the 79 and 106 day groups. However no significant differences were demonstrated between the 79 and 106 age groups.

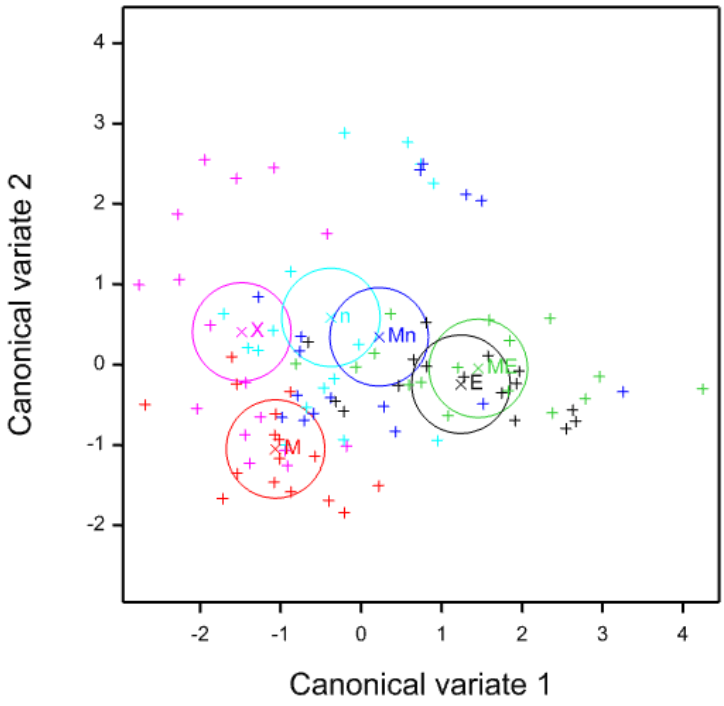


Figure 4-27. CVA following MANOVA of the first (basic) PCA model for treatment with 95% confidence ellipses.

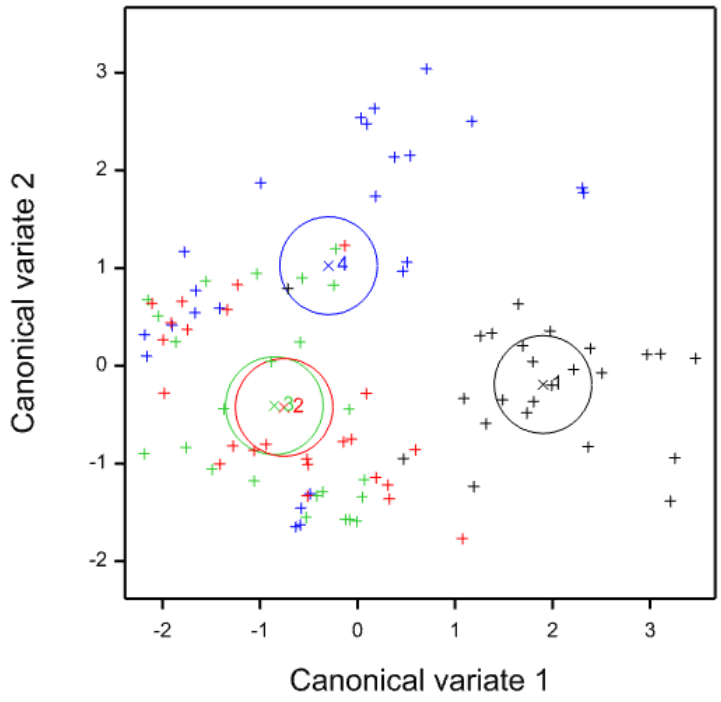


Figure 4-28. CVA following MANOVA of the first (basic) PCA model for compost age with 95% confidence ellipses. Ages shown as 1: 36 days, 2: 79 days, 3: 106 days, 4: 136 days.

Two-way ANOVA for age x treatment on PC1, 2, 3 and 4 showed all these to be significant (F = 13.60, 30.76, 1.99, 2.28, $p < 0.001$, < 0.001, $= 0.014$, $= 0.004$ respectively). Two-way MANOVA on these returned a significant result ($F = 14.33$, $p < 0.001$).

4.3.7 Fauna - earthworms

The total fresh weight of earthworms was lower than the initial mean of 11g (SE 0.01) per microcosm at each destructive sampling. There was also a marked decrease in biomass between each sampling.

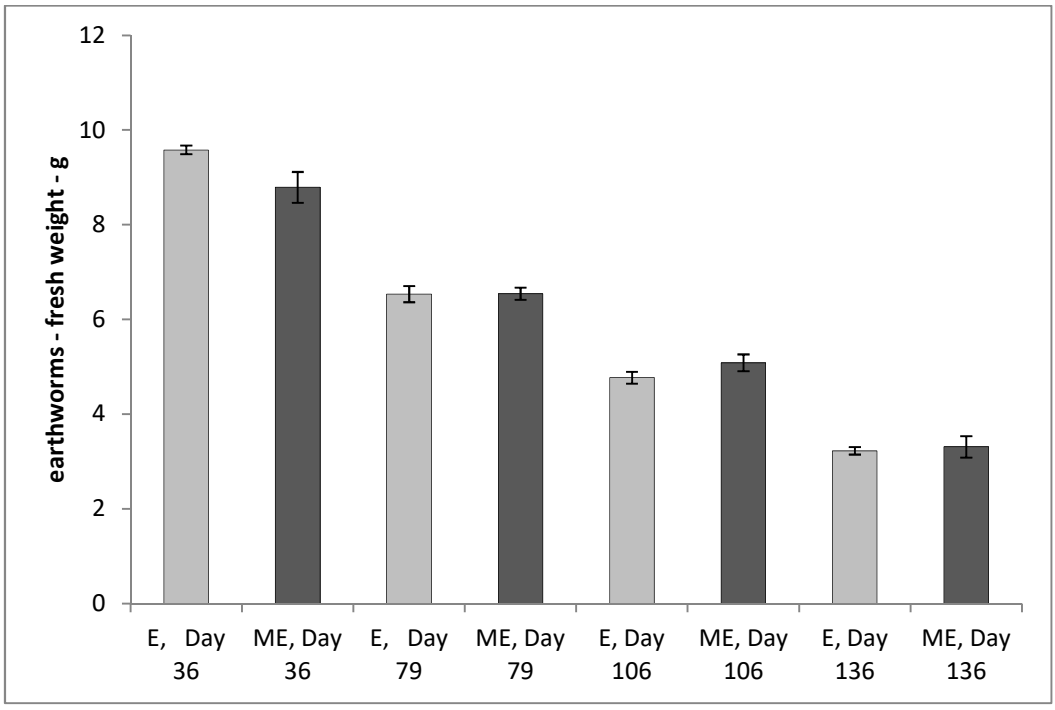


Figure 4-29. Mean total earthworm biomass at successive destructive samplings (n = 4).

A two-way ANOVA on treatments with earthworms (E and ME) of earthworm biomass with treatment and age of compost as the two factors returned a significant result for age ($F = 384.52$, $p < 0.001$) and the interaction between treatment and age ($F = 3.50$, $p = 0.031$, but no significant difference for treatment. The decline in biomass between each destructive sampling was significant ($p < 0.001$ in LSD post hoc tests). The numbers of earthworms also declined significantly with age ($F = 22.16$, $p < 0.001$). Mean numbers were 49 (2.02) and 45 (2.21) for E and ME respectively) on day 79, to 33 (1.08) and 30 (3.6) on day 136 (figures in brackets are SE). Mean individual weight also fell significantly ($F = 12.49$, $p < 0.001$) with age.

4.3.8 Fauna - mesofauna

Abundance of major taxa

As intended, mesofauna were found only in very small numbers in treatments X, E and N until the final destructive sampling on day 136, when some mites were extracted from treatments X and N, and very high numbers of juvenile mites (deuteronymphs) from two replicates of treatment E (an estimated 1,549 individuals in one and 3,098 in the other). Collembola were generally totally absent from treatments X, E and N; the only exception was one replicate of treatment E on day 106 (one individual of *Proisotoma minima*, equivalent to approximately 8 individuals per litre). Data for all treatments at each destructive sampling are shown in Table 4-11.

4.3.9 Fauna - Collembola

Five main species of Collembola were identified as present in the microcosms; occasional other unidentified species were found and recorded as "other Collembola" (Table 4-12). Since Collembola were almost absent from treatments X, E and N, the statistical analyses considered only treatments M, ME and MN.

The apparent greater abundance of Collembola in association with earthworms (treatment ME) throughout the experiment was significant. The increase in abundance after 36 days was also significant, but changes in abundance after 79 days were not significant. A two-way multivariate PERMANOVA on abundance of all species of Collembola as variables with treatment and age of compost as factors, showed significant differences for both treatment ($F = 20.02$, $p < 0.001$) and age ($F = 7.417$, $p < 0.001$). The interaction of treatment and age was also significant ($F = 2.167$, $p = 0.032$).

Table 4-11. Mean abundance of major taxa (individuals per litre) of mesofauna at successive destructive samplings (n = 4).

Compost age/ Treatment		Collembola		Acari		Other taxa		Total mesofauna	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
36 days	X	0	0	0	0	4	2.54	4	2.54
	E	0	0	0	0	2	2.25	2	2.25
	M	138	35.40	0	0	5	2.89	143	37.67
	ME	708	181.88	12	6.06	5	2.75	725	182.55
	N	0	0	0	0	20	12.18	20	12.18
	MN	200	38.21	29	23.18	2	2.25	231	52.24
79 days	X	0	0	19	9.60	7	4.31	26	12.85
	E	0	0	8	7.75	0	0	8	7.75
	M	756	123.42	13	12.75	0	0	768	133.24
	ME	1766	347.74	13	9.71	9	3.49	1788	356.15
	N	0	0	44	26.27	3	2.50	47	28.54
	MN	547	56.74	16	6.61	2	2.25	564	51.53
106 days	X	0	0	9	6.63	7	4.09	15	5.76
	E	2	2.00	0	0	0	0	2	2.00
	M	779	130.02	39	27.43	0	0	818	128.93
	ME	1485	117.65	49	29.34	21	10.14	1556	140.94
	N	0	0	9	5.25	0	0	9	5.25
	MN	822	85.02	10	6.00	2	2.00	834	82.60
136 days	X	0	0	67	15.01	2	1.75	69	15.65
	E	0	0	1223	709.85	0	0	1223	709.85
	M	466	82.26	11	6.54	0	0	477	78.54
	ME	1406	244.68	21	20.75	0	0	1427	265.27
	N	0	0	129	34.98	0	0	129	34.98
	MN	590	98.85	26	9.64	2	1.75	617	88.88

Table 4-12. Abundance of Collembola species (individuals per litre) in treatments with added mesofauna. Means bearing the same superscript letters (a, b, c) are not significantly different from each other ($p > 0.05$), n =4.

Species	Compost age (days)	M		ME		MN			
		mean	SE	mean	SE	mean	SE	*F*	*p*
Protaphorura aurantiaca	36	47	13.83	133	48.79	42	12.51		ns
	79	54[a]	20.32	418[b]	85.29	17[a]	9.26	18.96	.001
	106	30[a]	4.21	208[b]	40.59	29[a]	17.15	16.19	.006
	136	47[a]	12.24	503[b]	138.35	11[a]	6.30	11.65	<0.001
Fiesea truncata	36	4	2.46	32	32.25	2	2.25		ns
	79	104	36.72	168	47.73	89	33.60		ns
	106	112	39.55	87	47.91	105	4.77		ns
	136	111	10.92	195	56.46	79	18.34		ns
Coecobrya tenebricosa	36	32	10.61	128	40.55	39	23.12		ns
	79	255	33.32	120	43.01	270	72.18		ns
	106	251[a]	26.27	46[b]	15.33	252[a]	53.49	11.13	0.009
	136	137[a,b]	36.18	35[b]	14.02	281[a]	43.78	13.37	0.001
Proisotoma minima	36	32[a]	11.21	201[b]	50.84	107[a,b]	28.06	6.168	0.008
	79	184[a]	58.53	866[b]	243.01	154[a,b]	82.17	7.037	0.012
	106	293[a]	159.19	1040[b]	60.36	421[a]	114.84	11.34	0.057
	136	168	40.93	537	251.14	200	84.16		ns
Parisotoma notabilis	36	21[a,b]	13.05	198[b]	107.94	11[a]	8.04	2.786	0.009
	79	160[a]	49.99	194[a]	44.91	18[b]	3.48	5.786	0.033
	106	93	55.64	105	18.22	16	5.45		ns
	136	4[a]	3.75	134[b]	34.83	20[a]	6.37	11.85	<0.001
Other Collembola	36	2	2.00	17	10.13	0	0		ns
	79	0	0	0	0	0	0		ns
	106	0	0	0	0	0	0		ns
	136	0	0	4	4.25	0	0		ns
Total Collembola	36	138[a]	35.40	708[b]	181.88	200[a]	38.21	8.197	0.002
	79	756[a]	123.42	1766[b]	347.74	547[a]	56.74	9.159	0.001
	106	779[a]	130.02	1485[b]	117.65	822[a]	85.02	12.37	0.005
	136	466[a]	82.26	1406[b]	244.68	590[a]	98.85	10.24	0.002

A one-way multivariate PERMANOVA test on abundance of all species of Collembola as variables with age of compost as factor gave $F = 3.923$, $p < 0.001$; and pairwise comparisons showed significant increases in abundances from 36 days to 79 days but no significant differences between thereafter (Table 4-13).

Table 4-13. Pairwise comparison of the influence of day of sampling on total Collembola abundance in treatments with added mesofauna.

	36 days	79 days	106 days
79 days	$F = 7.009$, $p < 0.001$		
106 days	$F = 8.87$, $p < 0.001$	ns	
136 days	$F = 4.192$, $p = 0.002$	ns	ns

The increasing abundance of Collembola in the presence of earthworms and with duration of composting appears to be species specific.. The results of one-way univariate PERMANOVA tests on each species, as well as total Collembola, by treatment in each age group are summarised in Table 4-12. Overall, the total numbers of Collembola were significantly greater in the presence of earthworms in each age group. Mean abundance of *Protaphorura aurantiaca* was not significantly different between treatments at 36 days, but was present in the ME treatment in much higher numbers than in the other two in subsequent age groups. *Proisotoma minima* showed a similar trend to *P. aurantiaca*, particularly in ME compared with M, and at 106 days abundance of this species was significantly much higher in ME than in both M and MN. Conversely *Coecobrya tenebricosa* was significantly less numerous in ME at 106 days than in both M and MN, and significantly much lower than in MN at 136 days (it was also much less abundant than in M, but this was not significant due to relatively high SEs). Trends in the abundances of *Parisotoma notabilis* were less clear; at 36 days this species was significantly less abundant in MN than in ME, at 79 days it was less numerous in MN than in both the others (the situation was similar, but not significant, at 106 days. At 136 days *P. notabilis* seemed to have declined in M, remained high in ME and low in MN; the abundance in ME was significantly greater than in both M and MN. *Fiesea truncata* and "other Collembola" showed no significant differences between treatments in any of the age groups. The abundance of species as a percentage of total Collembola illustrate these trends (Figure 4-30).

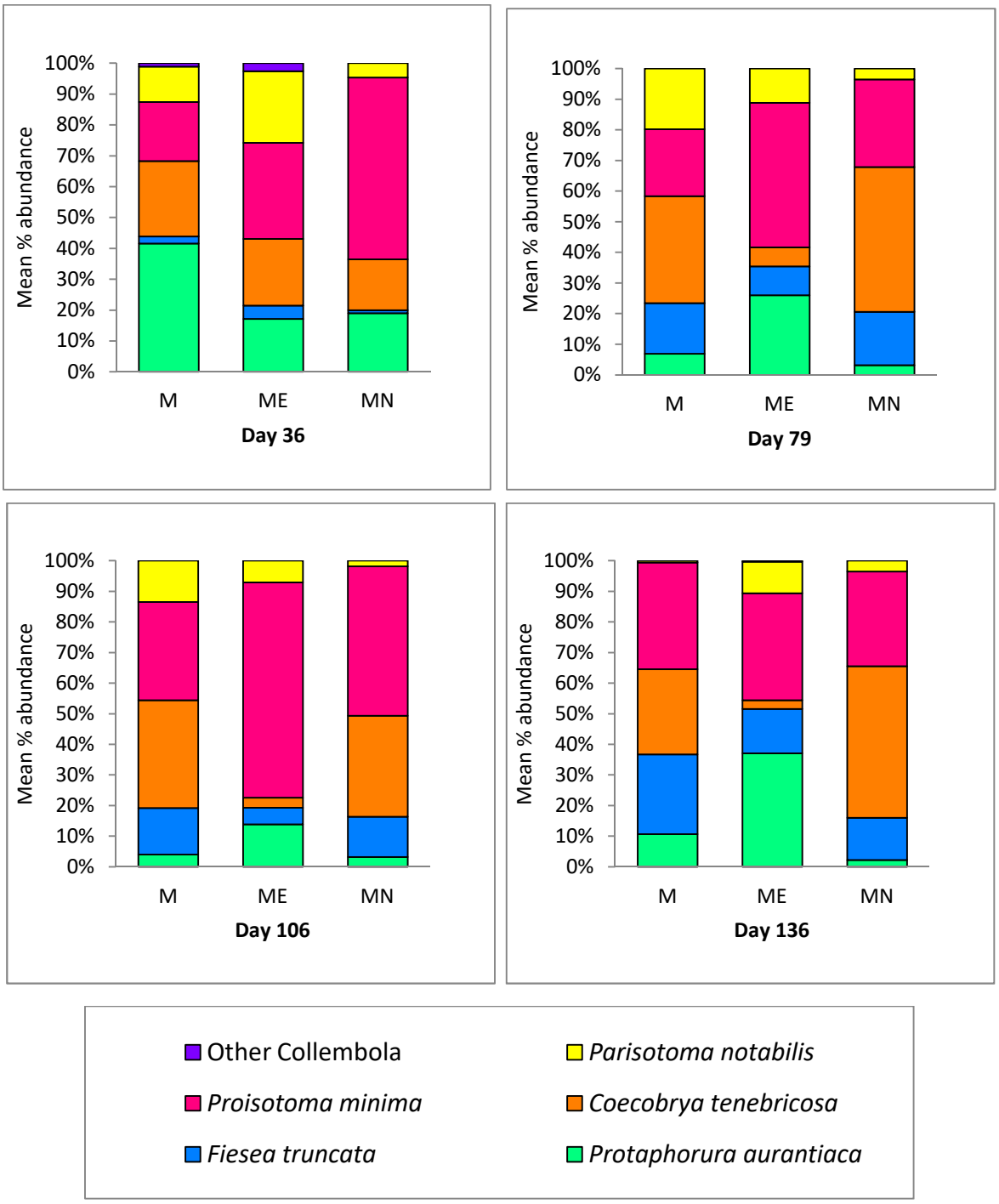


Figure 4-30. Mean abundance of Collembola species as percentages of mean total numbers of Collembola per litre.

5 Discussion

This chapter discusses the results from different aspect of the work, and relates these aspects together and with the literature.

5.1 Vermicomposting in the microcosm experiment and on site

The fieldwork carried out at the vermicomposting site was designed to characterise some ot the biological, chemical and physical properties of the vermicomposting process, as well as to inform the fermentation experiment. The fermentation experiment was designed to explore the effects of earthworms and Collembola on vermicomposting. In this section the two are dealt with together and some of the similarities and contrasts drawn out.

The hypotheses of the fermentation experiment were:

(a) the presence *E. fetida*/*E. andrei* in vermicomposting manures would increase the concentrations of water-soluble nutrients and affect other physical, chemical and biological properties of the substrate, and interactions between earthworms and Collembola would enhance these effects.

(b) the presence of earthworms in vermicomposting manures would increase the abundance of Collembola.

The experiment had six treatments and used 96 microcosms of 1 litre capacity filled with defaunated, partially composted manure (Table 5-1). The tops of the microcosms were finely meshed to prevent the passage of mesofauna. Destructive samplings were carried out 36, 79, 106 and 136 days from the start of the experiment.

Table 5-1. Fermentation experiment design: treatments, destructive samplings and replicates used in the fermentation experiment; 1 denotes presence, 0 denotes absence

Treatment	Earth worms added	Mesofauna added	Organic N added	Destructive samplings	Replicates per destructive sampling	No. of microcosms
X	0	0	0	4	4	16
E	1	0	0	4	4	16
M	0	1	0	4	4	16
ME	1	1	0	4	4	16
N	0	0	1	4	4	16
MN	0	1	1	4	4	16
Totals					24	96

5.1.1 Substrate respiration and water-soluble nutrients

The substrate respiration rates throughout the fermentation experiment were lower than those recorded in the vermicompost beds (section 4.1.2), where mean respiration rates were never less than 2.6 $g_{(CO_2)}\ m^{-2}\ h^{-1}$, compared to less than 1 $g_{(CO_2)}\ m^{-2}\ h^{-1}$ for all treatments by the end of the experiment at 135 days. The highest mean rate recorded after the earthworms had been introduced into the experiment was 2.9 $g_{(CO_2)}\ m^{-2}\ h^{-1}$ (ME at 18 days), compared to over 32 $g_{(CO_2)}\ m^{-2}\ h^{-1}$ in the earthworm bed (7-day old vermicompost). These differences are likely to be related to several factors, including: (1) the age of the manure feedstock, which in the experiment had been composting for several months before introduction to the microcosms, (2) earthworm density, a mean of 665 individuals per litre (71g fresh weight) in 17-day old vermicompost sampled in the field (i.e. at the vermicomposting site), compared to approximately 50 individuals per litre (11g fresh weight) in the E and ME treatments in the experiment, (3) differences in feedstock, both were horse and cow manure, but from different sources with different bedding material, and may have been mixed in very different proportions. It is also possible that at such high densities of earthworms, there may be a significant contribution to CO_2 evolution by the earthworms themselves (Uvarov 2016) so not all the elevation in respiration can be attributed to microbial activity.

Observations in the field showed that in the first 7 days of vermicomposting at least substrate respiration increased dramatically compared to that of the feedstock. By day 18 of the fermentation experiment the respiration rate had fallen in all the treatments from a mean of 4.1 $g_{(CO_2)}\ m^{-2}\ h^{-1}$, four days before introducing the earthworms, to 2.1 $g_{(CO_2)}\ m^{-2}\ h^{-1}$; in the treatments with earthworms, it had fallen less to 2.7 (E) and 2.9 $g_{(CO_2)}\ m^{-2}\ h^{-1}$ (ME). It is possible that an increase in E and ME would have been observed if measurements earlier in the experiment. Aira *et al.* (2008) demonstrated a linear increase in CO_2 evolution with increasing densities of earthworms in a 72-hour experiment, though in their experiment the highest earthworm density was 100 earthworms in 500 ml (30g fresh weight) and the substrate used was fresh pig manure.

Although in the fermentation experiment substrate respiration was considerably lower than in the vermicomposting beds, there was a significantly elevated respiration rate due to the presence of earthworms at day 18 compared to the non-earthworm treatments. Thereafter substrate respiration declined at varying rates in different treatments. Most of the differences in respiration between treatments in this later phase were not significant, but in general respiration rates in the treatments with earthworms present (E and ME) fell more quickly and to a lower level by the end of the experiment than in the treatments where earthworms were absent. This suggests that the presence of earthworms increased the rate of stabilisation of the compost (Domínguez and Gómez-Brandón 2013).

Mesofauna effects on respiration were unclear. Mean respiration rates in the mesofauna only (M) treatment were often higher than in the control (X) after day 18, but only at day 51 were both M and ME showing significantly higher respiration rates than the control, and in fact all the other treatments. This may relate to the potentially contrasting effects of Collembola on organic matter mineralisation; depending the abundance and combination of species present and trophic interactions, springtails may increase carbon mineralisation or promote immobilisation (Filser 2002). For example, at relatively high biomass, Collembola have been shown to lead to a reduction in soil respiration, presumably because of microbial grazing, while at lower levels grazing can stimulate fungal growth, increasing soil respiration (Chauvat and Wolters 2014).

Hence, in the presence of earthworms there was an initial elevation in microbial respiration as the earthworms stimulated microbial decomposition of the substrate, followed by a reduction in the respiration rate as microbial activity was reduced in the earthworm casts (Gómez-Brandón and Domínguez 2014). Similar, much less pronounced effects of increased substrate respiration due to short-term stimulation of microbial activity in faeces, followed by a reduction in activity over time (Frouz 2018), may also have been occurring in the presence of mesofauna.

The initial elevated respiration in the presence of earthworms may be linked to the significantly higher nitrate concentration in E and ME compared to the non-earthworm treatments at 36 days. This is presumably due to enhanced microbial mineralisation of nitrogen. Nitrate levels continued to increase in E and ME after day 36, while respiration rates in these treatments were generally lower relative to the other treatments, but not significantly so. This may be because of a time-lag in the microbial activity in the non-earthworm treatments such that the respiration rate declined more slowly in these than in the earthworm treatments.

At 136 days, nitrate levels in the presence of earthworm remained significantly higher than in the other treatments and a significant mesofauna effect was also seen, with nitrate in ME significantly greater than that in E. This effect was also apparent in phosphate and sulphate concentrations, which throughout the experiment followed a similar pattern to that of the nitrate concentrations. Domínguez and Gómez-Brandón (2013) reported increases in phosphate concentrations over 112 days in cattle manure vermicomposted in the presence of *E. andrei,* compared to the same manure composted without earthworms. Increased nitrate concentrations due to decomposer diversity (earthworms together with Collembola) have been reported in soils by Eisenhauer *et al.* (2018); the current study suggests this combined effect due to the presence of both groups may occur in vermicompost.

Nitrate did not increase significantly in the MN treatment (mesofauna present with added organic nitrogen) after the second addition of organic nitrogen after 106 days, while it did in the case of treatment N. This could be an example of reduced nitrogen mineralisation due to the specific combination of collembolan trophic groups and their interactions with microorganisms (Filser 2002, Briones 2018). However, there was a significant increase in

nitrate from 36 to 79 days in M (824 to 1772 g ml^{-1}, significantly higher than the control), suggesting increased nitrogen mineralisation due to Collembola, which could relate to the significantly higher respiration rate of M compared to X at 51 days. The increased nitrogen mineralisation and higher respiration rate in M could be related to the increase in mean collembolan abundance from 36 to 79 days, from 138 to 756 Table 4-11. The mean relative abundance of different species also changed in this time period; *P. aurantiaca* abundance changed very little while abundance of all the other speceis increased by an order of magnitude. This could have resulted in increased functional dissimilarity which, according to Heemsbergen *et al.* (2004), who found a positive correlation between functional dissimilarity both soil respiration and mass loss of leaf litter. After day 51 there were no significant differences in respiration rates between any of the treatments and after day 79 no significant differences in nitrate levels between the M treatment and the control (X). A possible explanation for this is longer term effect of increased abundance of Collembola resulting in increased grazing pressure and a reduction of microbial activity, and hence reduced nitrogen mineralisation Table 4-10.

Hence the mineralisation of nitrogen appears to be enhanced in the ME treatment, but may be enhanced or inhibited at different times in the M and MN treatments, due to different species mixes of Collembola that relate to their differing trophic niches or functional dissimilarity. This effect in epigeic earthworms complements research by Grubert *et al.* (2016) who found facilitative and antagonistic interactions between different collembolan species in association with an endogeic and an anecic earthworm species.

Although trends in mean nitrate were observed in the samples of different ages in the vermicomposting beds, no significant differences were demonstrated, though sulphate did increase significantly with age. The trend in nitrate shows an initial doubling from 17 days to 35 days, followed by gradual decline to 116 days, then a sharp decrease at 175 days. However, the standard errors at are high in the older layers, suggesting high variability. It is possible that compaction and water-logging in the bottom of the vermicomposting beds could have led to anaerobic pockets where denitrification occurred (Killham, 1994), this may be related to the increase in pH in the 175 day vermicompost. Nitrate concentrations in the microcosm experiment were an order of magnitude higher than those in the vermicomposting beds, irrespective of treatment. This is probably due to a number of

factors (1) the different sources of the manures, and the carefully controlled conditions of initial composting in the absence of earthworms prior to the microcosm experiment, which reduced leaching in the feedstock, compared to the external, though covered, storage of feedstock at the vermicomposting site, (2) in the microcosms moisture levels were kept constant, their closed construction prevented any leaching of nutrients and their small size reduced the likelihood of aerobic conditions prevailing.

5.1.2 Earthworm biomass

In the vermicomposting beds there was a significant decrease in earthworm numbers with age, particularly from 17-day to 35 days, followed by a more gradual decline; mean individual earthworm weight declined from 0.11 g to 0.04 g as the vermicompost age increased, however this was not significant. It would be expected that earthworms would tend to migrate from the lower, older layers to the upper younger layers where there would be fresh feedstock (Aira *et al.* 2011). In the microcosm experiment earthworm biomass declined significantly throughout the experiment, however there was also a significant reduction in mean individual weight, suggesting that mortality was not the main cause of biomass loss but lack of available nutrition. In the closed microcosms the earthworms were unable to migrate to find fresh food, so a reduction in individual weight was not surprising.

5.1.3 Associations between earthworms and Collembola

The significant differences in total collembolan abundance between the ME treatment and the mesofauna treatments in the absence of earthworms (M and MN) give a clear indication that the presence of earthworms in vermicomposting promotes increases in springtail populations. This supports the findings of Monroy *et al.* (2011), who showed that Collembola populations were significantly increased in the presence of *E. fetida*, particularly from 4-11 weeks of composting in pig manure. The positive effect of high density of epigeic earthworms contradicts other studies that suggest that high densities tend to have negative effects on Collembola (Eisenhauer 2010), however these studies were on soil epigeics, where densities are much lower than in vermicompost, and none involved *Eisenia* species.

Monroy *et al.* (2011) did not differentiate between collembolan taxa, so any variation in the influence of earthworms between different species of Collembola in vermicompost was not

apparent. However, in the current study, associations between earthworms and Collembola appear to have had varying and sometimes opposing effects depending on species. The presence of earthworms (ME) had positive effects on *P. aurantiaca* and negative effects on *C. tenebricosa*. In contrast, the absence of earthworms (M and MN) appears to have had negative effects on *P. aurantiaca* and positive effects on *C. tenebricosa.* Numbers of *P. aurantiaca* increased in the presence of earthworms at successive destructive samplings (except at day 106), but remained low in the absence of earthworms. Conversely, *C. tenebricosa* abundance tended to decrease in the presence of earthworms and increase in their absence. Hence *Eisenia fetida/andrei* appears to be facilitative to *P. aurantiaca* but antagonistic to *C. tenebricosa*. This is not only interesting in terms of species dynamics in vermicomposting, but also because according to Hopkin (2007), *C. tenebricosa* is an alien synanthrophic species. It was not recorded in the initial mesofauna sampling field work at Martins TLC, but was present in the vermicomposting beds in large numbers at the time of collecting mesofauna for introduction to the microcosms in the fermentation experiment. The presence of *C. tenebricosa* in vermicomposting beds appears contradictory to the supposition that earthworms are antagonistic to this springtail, but it was observed only in the unusually hot and dry summer of 2018, and only in the surface feedstock from recently fed earthworm beds. Hence it is possible that the unusual conditions, coupled with the fact that the earthworms tend to retreat to lower substrate layers in hot dry conditions, encouraged the increase in the *C. tenebricosa* population.

5.1.4 Mesofauna abundance and diversity in the vermicomposting beds

There are clear trends relating to the age of the vermicompost, which can be tentatively related to feeding habits and trophic niches (Table 5-2).

With this variability in mind, some tentative conclusions can be made about invertebrate abundance and diversity. The changes in the Simpson 1-D and Shannon diversity indices suggest the evenness and diversity of the vermicompost community was greatest in the older compost from 72 days. Mean total abundance of invertebrates was greatest in the younger age groups from 0 to 72 days. The mid-range of 72 day old material had the highest abundance as well as being one of the most diverse age groups.

Collembola were overwhelmingly dominant in all but the 377 day-old compost, where Acari were present in similar numbers (Figure 4-4, Figure 4-5). It could be conjectured that the drop in mean springtail numbers per litre between 72 days (3775 individuals) and 377 days (143 individuals) was due in part to predation by mesostigmatid mites, which were at their height (401 to 949 individuals) at 72 days. In general, Acari abundance followed that of Collembola throughout the age profile. This could be evidence of the trophic cascade effect of predation resulting in top-down regulation of prey populations (Briones 2014).

Oribatid mites were scarce in the vermicompost, which was unexpected since these mites are considered detritivores (Shepherd and Crotty, 2017). This may relate to competition with Collembola, however Oribatida and Collembola have been identified as having different functional guilds and trophic niches (Potapov 2016, 2019), with oribatids being involved particularly in litter fragmentation (Briones 2014).

5.1.5 Trophic interactions between Collembola taxa

Some of the trends and variations in Collembola populations in both the vermicomposting beds and the fermentation experiment are likely to relate to differences in the food resources they exploit, which as discussed previously may relate to variations in substrate properties. The feeding preferences of Collembolan species are still little understood (Briones 2014). Assumptions that the majority are fungal feeders have been shown to be over-simplifications. Springtails as a taxon occupy several trophic levels and may be predators or scavengers, bacteriovores, fungivores, herbivores or detritivores (Rusek 1998, Potapov 2018). Possible feeding preferences of some of the taxa found in the vermicompost beds and microcosm experiment are shown in Table 5-2, compiled from the work of Addison *et al.* (2003) (based on gut contents), Malcicka *et al.* (2017) (type of mandibula, stable isotope studies, lipids, digestive enzymes, gut contents and field observations), and a major meta-analysis of stable isotope data by Potapov (2019b).

Table 5-2. Collembolan feeding habits and trophic niches of taxa relevant to species found in the vermicomposting beds and fermentation experiment (Addison *et al.* 2003), Malcicka *et al.* 2017) and Potapov 2019b).

		Potapov *et al.* 2019b							Addison *et al.* 2003			Malcicka *et al.* 2017		
			$\Delta^{13}C$, ‰			$\Delta^{15}N$, ‰			percentages of gut contents					
Genus/ (family)	Species	Reliability	mean	variation	species effect	mean	variation	species effect	>40 %	>20-40%	>10-20%	vertical stratification	Mandibula	Diet
(Entomobry-idae)		h	2.4	h	n	0.7	h	y						
Proisotoma		l	4.4	l	-	0.8	l	-						
Parisotoma	*P. notabilis*	h	4.3	h	-	1.7	m	-		Fh	Fd			
Isotomidae		h	4	h	y	1.9	h	y						
(Neelidae)		l	2.6	l	-	2	l	-						
Megalothorax		l	2.3	-	-	2	-	-						
(Hypogastrur-idae)		m	4.5	h	n	4.2	h	n						
Hypogastrura		l	3.2	h	-	4.8	h	-	Fd		Fh			
(Onychiur-idae)		h	4	m	n	5	h	n						
Friesea	*F. mirabilis*	l	4.2	-	-	5.1	-	-						
Friesea		l	4.2	-	-	5.1	-	-						
(Neanuridae)		h	5.5	h	y	5.1	h	y						
Protaphorura		m	4	m	n	6.1	h	n	Fh		Fd			
Protaphorura	*P. armata*	m	3.7	h	-	7.6	h	-				E	C	P, F
Megalothorax	*M. minimus*									POMvf AOM	POM			
Proisotoma	*P. minuta*													
Friesea	*F. truncata*											H	S/ P	N
Hypogastrura	*H. viatica*											H	C	P

In the stable isotope columns (Potapov *et al.* 2019b) : reliability (of the data) relates to the number of populations/species studied, variation/trophic flexibility is based on standard deviations (l = low, m = medium, h = high), species effect is based on significant results in ANOVA and indicates whether differences between species belonging to a taxon have been detected (y = effect present, n = no effect), cells with no data are due to only one population/speceis being studied. In the gut contents columns (Addison *et al.* 2003) AOM = amorphous organic matter, POMvf = very fine particulate organic matter, POM = particulate organic matter, Fh = Fungal hyphae - hyaline, Fd = Fungal hyphae - dark, Fs = Fungal spores. In the Malcicka et al. (2017) columns: vertical habitat stratification - E = Euedaphic, H = Hemiedaphic, Mandibula type - C =chewing, S/P = scratching/piercing, Diet - F = fungivores/bacterivores, P = herbivores, N = scavengers/predators.

Hypogastrura assimilis occurred in great abundance in the manure feedstock (0 days old), but was reduced in the 5 day old vermicompost and absent in many of the older samples. *H. assimilis* is considered scarce in the British Isles by Hopkin (2007), with the only published records being from vermicompost beds and a manure heap, so its occurrence in the partner company's vermicompost is not surprising. Another species of this genus, *H. viatica* is hemiedaphic in its vertical habitat stratification and tends to be herbivorous with chewing mouthparts (Malcicka *et al.* 2017). Such traits would fit with the abundance of *H. assimilis* in the feedstock, but scarcity in older vermicompost. *P. minima* followed a similar pattern to *H. assimilis* but persisted to a greater extent in the older layers.

The distribution of *Megalothorax minimus* is quite distinct, being totally absent in all samples of the 0, 5 and 377 day vermicomposts, but the third most abundant collembolan species at 72 days and also present at >377 days. This springtail may exploit different resources to the other species found in the vermicompost, according to both gut content studies (Addison *et al.* 2003) and stable isotope studies (Potapov *et al.* 2019b). However the latter authors advise caution as only one population was available for the stable isotope study and was at genus rather than species level.

P. notabilis and *P. aurantiaca* similar temporal patterns, though the former was generally less abundant. Both were almost absent from the manure but were abundant and dominant in the 5 and 72-day old vermicompost, as well as relatively abundant and dominant in the older layers (>399 days) where Collembola and other mesofauna number were reduced. They appear to coexist in both the vermicomposting beds and the ME treatment of the microcosm experiment, possibly relating to the exploitation of different resources; the mean Δ^{15}N for the *P. notabilis* is 1.7‰ and for the *Protaphorura* genus 6.1‰ (Potapov *et al.* 2019b).

The lowest abundance of Collembola as well as other mesofauna was in the 377 day old vermicompost. *Friesea truncata* was present in this age group (as well as in one of the manure samples). It is possible that *F. truncata,* together with the Mesostigmata, was preying on other Collembola (Rusek 1998, Malcicka *et al.* 2017), contributing in particular to the reduction in abundance of *P. notabilis* and *P. aurantiaca.*

5.1.6 The effects of earthworms on physical properties

No significant differences in dry bulk density were found in fermentation experiment or in the field. However in the vermicomposting beds, although no significant differences were found in moisture content or bulk density at different depths (depth was used as a proxy for age), it was observed that in the lowest (oldest) layers, a certain amount of compaction and water-logging had occurred. Aira *et al.* (2011) found accumulation of vermicomposted cow manure led to compaction in the lower layers of a continuous feeding-industrial vermicompost system, reducing earthworm activity and therefore minimising aeration. Epigeic earthworms tend to exert lower penetrating forces than anecic and endogeic groups (Briones 2014), so may not have the ability to access compacted organic matter. This finding is noteworthy for the vermicomposting process; it may be worth taking steps to reduce to increase aeration or improve earthworm access to the lowest layers.

In the microcosm experiment, the influence of earthworms on mean particle size was cumulative as the experiment progressed, with the percentage of particle less than 2 mm reaching 60% (E) and 64% (ME) by the end of the experiment. In the absence of earthworms the percentages of particles <2 mm ranged between 23% and 30%, and did not change significantly throughout the experiment. The patterns of treatment responses in terms of particle size were very similar to the higher rates of microbial activity found initially in the presence of earthworms, as well as to the increased concentrations of water-soluble nitrate, phosphate and sulphate in the E and ME treatments. These findings support those of Hanc and Dreslova (2016), who found improved nutrient availability in vermicompost compared to compost, with finer substrates having higher availability than coarser substrates. No significant mesofauna effect occurred in the present experiment, but the ME treatment by 136 days had a slightly higher percentage of <2 mm particles than E, and at 79 and 106 days MN had a higher percentage of the smaller particles than the N treatment.

5.1.7 Near Infrared spectroscopy (NIRS)

In the fermentation experiment clear differences between treatments and trends over time were shown in NIR spectra (section 4.3.6). In the (M)ANOVA and CVA of both the Basic and GLS PCA models clear earthworms effects were seen, with E and ME separated from the no-

earthworm treatments. A mesofauna effect was also apparent in the separation between X and M. Separation was also seen in compost age (Figure 4-28), with 36 days and 136 days significantly different from all others, though with no significant difference between 79 and 106 days. In the PCA plot of the GLS model, there is tight grouping on treatment and age in most cases. Broadly, in this model, PC1 appears to relate to age, with scores becoming more negative with increasing age. PC2 generally fits to treatment, with the earthworm treatments tending to be more positive, mesofauna only and control more negative, and the added-nitrogen treatments nearer zero. The outliers of N and MN at 136 days are likely to represent a significant biochemical change due to the inoculation with additional organic nitrogen after the 106 day destructive sampling. The control at 136 days was also an outlier, though it is likely to be a real effect, since replicates are closely grouped. There is no obvious reason for this separation in the control at 136 days, but it could relate to an anomaly in sample preparation, such as insufficient drying (as discussed below, positive scores on PC1 and PC2 in the GLS model could be related to spectral peaks due to moisture).

Attempts to tentatively assign specific peaks and troughs in the PCA loadings to particular chemical constituents met with variable success. A broad trough in PC1 and a broad peak in PC2 were observed in the basic PCA model loadings (Figure 4-23) between 1100 and 1400 nm. This area of the NIR spectrum has been assigned to C-H and N-H stretches of aliphatic groups (Ilani *et al.* 2016). However, in the basic model the most negative scores on PC1 and the most positive scores in PC2 relate to treatments without earthworms early in the experiment, e.g. X, M, N, MN at day 36. Conversely, E (earthworms only) and ME (earthworms and mesofauna present) at 79, 106 and 136 days, score positively on PC1 and negatively on PC2. In the GLS model the situation is similar, but reversed, with respect to 1400 nm, but different at around 1100 nm. Here there is a minor negative peak in PC1 and a minor positive peak in PC2; these could relate to the later stages of the treatments with earthworms. In which case, this could represent increased levels of aliphatic compounds, which would be associated with more advanced progression of microbial breakdown (Ilani *et al.* 2016) and increased levels of key nutrients.

A major differentiating factor in both treatment and time is nitrate concentration, so it might be expected that this would be represented in the NIRS analysis. A number of authors have ascribed the wavelengths around the 1900 nm region to nitrate (e.g. Ilani *et al.* 2016,

Ehsani *et al.* 1999). However, the latter authors point out that this region is also influenced by moisture, soil organic matter content and texture, so careful substrate-specific calibration is required before assignment of wavelengths to specific compounds can be carried out with confidence. McWhirt *et al.* (2002) also ascribe this region to water and Ben-Dor *et al.* (1997) to water, cellulose, lignin and other complex organic molecules. In the loadings for PC1 and PC2 in both models, the region at approximately 1900 nm gave the strongest positive peak. Since the highest scores for PC1 and PC2 were for X, N and MN at the 136 days, and nitrate concentrations in X were significantly different to those in N and MN, NO_3^- seems to be an unlikely candidate for this wavelength region in this experiment.

Although it was not possible to assign specific wavelengths to specific chemical constituents, the NIRS technique was successful in its intended purpose of detecting differences between treatments due to their overall chemical composition. The results also suggest that differentiation according to compost maturity, shown by increasing separation between the earthworm and non-earthworm treatments with compost age, is possible by NIRS. This is supported by the findings of other researchers (Moral *et al.* 2009) who have reported the potential for NIRS to quickly evaluate compost quality process monitoring. This is an area where further research would be valuable to establish practical methods for assessing vermicompost maturity using NIRS.

5.2 The greenhouse pot trials

The trial found little evidence to support the hypothesis that tomato and leek plants grown in vermicompost would exhibit significantly increased growth and above ground biomass on harvesting, compared to those grown in a range of non-vermicompost growing media. However, the trial did demonstrate that the sponsoring company's vermicompost (MTLC: 25% vermicompost, 75% coir by volume) gave similar results in terms of plant growth to two other premium peat-free growing media (CBC and WBC) and a premium peat-based compost (PBC). In one respect, resistance of plants to wilting, the vermicompost performed better than the other media.

The present study did not confirm the findings of some researchers such as Atiyeh *et al.* (2000) who demonstrated improved tomato seedling growth in a vermicompost mix compared to a standard commercial growing medium. By contrast at the end of the 74 day

tomato trial in the current study, there were no significant differences in mean stem height or for mean top, root or total dry weights between composts. However, in the first 25 days the vermicompost and the peat-based compost showed significantly greater stem height than the coir-based and wood-fibre based media. This might make MTLC the more suitable choice of peat-free compost, compared to CBC and WBC, for growers who raise plants in pots or modules for transplanting.

The observations on wilting under water stress enabled further useful comparison of the growing media. Martins TLC compost (MTLC) suffered the least wilting, significantly less than PBC and WBC. This could be more a consequence of the coir content than the vermicompost content in MTLC. In an under-watering regime, Alexander *et al.* (2013) found wood fibre based compost to produce inferior fuchsia plants compared to those produced in coir and peat-based composts. It is possible that the reduced water stress shown by plants in MTLC was due to the combined effects of coir and vermicompost, and the loam content of the CBC may have provided similar benefit to the coir-based compost, resulting in both composts performing better in reducing wilting than the peat-based compost. In a commercial context, the frequency and of irrigation and the volume of water used can be important factors in the choice of growing media.

The vermicompost performed less well in the leek trial, where the peat-based compost demonstrated significantly higher stem heights than MTLC and WBC in repeated measures ANOVA. Similarly, PBC produced the greatest mean total dry weight, significantly greater than MTLC and WBC.

Variability in growth parameters with different plant varieties has been shown by others, e.g. Bachman *et al.* (2008) who found increased shoot dry weight in tomatoes, but decreased dry weight in pepper shoots in vermicompost.

While the vermicompost in the present study did not perform better than the other three media in terms of tomato and leek growth, it showed itself to be comparable in quality. This confirms the conclusions of researchers such as Lazcano *et al.* (2009) and Guerra *et al.* (2018) who showed that growing media containing vermicompost could be viable alternatives to peat- based compost. Roberts *et al.* (2007) also fond this to be the case,

though the inclusion of vermicompost brought no significant benefits in terms of tomato fruit yield.

5.2.1 Plant responses to the physical and chemical properties of the composts

Some of the chemical properties can be interpreted using the ADAS index system for loamless compost (ADAS, undated) (Table 5-3), with additional reference to WRAP (2014).

The main conclusion from using the ADAS index is that all the composts exhibit high concentrations of nutrients except in a few cases.

On the ADAS index, all composts would be classed at 7 (very high) in nitrate levels and at 0 (very low) for ammonium. These conform well with the WRAP (2014) guidelines, which specify ammonium as low as possible and lower than nitrate.

Although mean phosphate levels were much lower in CBC than the others, on the ADAS system CBC would still be designated 3 (high) (compared to the others at 8, very high). Magnesium was the main nutrient where there was high variability on the ADAS index, with 0 (very low) for CBC, 2 (medium) for MTLC, 4 (very high) for WBC and 6 (very high) for PBC.

All the composts were high or very high for electrical conductivity, however they are close to, or exceed in the case of WBC, the WRAP (2014) guidelines which recommend less than 600 $\mu S\ cm^{-1}$. These high levels could be a concern in terms of plant growth, especially as the WRAP guidelines are intended primarily for composts before being mixed with carrier materials with lower EC and nutrient levels, at rates not usually exceeding 40%.

The observed differences in the degrees of wilting of tomatoes in the different composts can only partly be explained by the latters' differing water retention properties. Tomatoes in WBC suffered the most wilting and had lower water retention than the rest at 76%. However PBC was also prone to wilting but had 85% water retention, very close to CBC (87%) and MTLC (85%). It is possible that the high EC of WBC contributed to increased wilting in this medium, through higher osmotic pressure. However CBC has a similarly high EC, but plants growing in it exhibited significantly less wilting.

Table 5-3. Interpretation of some key chemical parameters of the composts used in the pot trials using the ADAS index for loamless compost (ADAS, undated). Treatment codes are: CBC - coir-based, MTLC - vermicompost, PBC - peat-based, WBC - wood fibre-based).

Nutrient/ parameter		ADAS Index	mg l^{-1}	ADAS Interpretation
Nitrate	CBC	7	> 300	very high
	MTLC	7	> 301	very high
	PBC	7	> 302	very high
	WBC	7	> 303	very high
Phosphate	CBC	3	12 - 18	high
	MTLC	8	76 - 100	very high
	PBC	8	76 - 100	very high
	WBC	8	76 - 100	very high
Potassium	CBC	6	401 - 650	very high
	MTLC	6	401 - 650	very high
	PBC	4	176 - 250	very high
	WBC	6	401 - 650	very high
Ammonium	CBC	0	0 - 20	very low
	MTLC	0	0 - 20	very low
	PBC	0	0 - 20	very low
	WBC	0	0 - 20	very low
Magnesium	CBC	0	0 - 5	very low
	MTLC	2	11 - 15	medium
	PBC	6	51 - 85	very high
	WBC	4	26 - 35	very high
Electrical Conductivity			µS cm^{-1}	
	CBC	4	501 - 600	very high
	MTLC	3	401 - 500	high
	PBC	3	401 - 500	high
	WBC	6	701 - 900	very high

The other water-soluble nutrients in the analysis are not featured in the ADAS index system. Chloride was significantly higher in MTLC and CBC, and sodium was also relatively high in these two. These factors might possibly relate to the salt content of the coir base, however they appear not to have been reflected in the electrical conductivity values. Mean concentrations of calcium were not significantly different between composts.

The composts used in the plant trial differ from each other in various physical and chemical properties, but that there is little evidence for these influencing plant growth during the trial, since both tomatoes and leeks performed similarly in each compost. One possible explanation for this is that none of the parameters were growth-limiting.

5.3 Conclusions

The project had three main objectives; (1) to characterise the key biological, chemical and physical aspects of vermicomposting through fieldwork at the vermicomposting site, (2) to compare the vermicompost with other premium growing media through a greenhouse pot trial, (3) to explore the influence of earthworms and Collembola on the properties of vermicompost through a microcosm fermentation experiment.

The fieldwork was a useful means of exploring some key properties of the vermicompost and the vermicomposting process, such as the species and densities of the earthworms and Collembola involved, and water-soluble nutrient levels and substrate respiration. Such information was important for developing and interpreting the fermentation experiment. Any future on-site investigations would benefit from a structured fieldwork programme repeated several times in a year to monitor substrate respiration, nutrient levels and fauna as the vermicomposting beds developed from first feeding to final harvesting. Such a programme would also explore seasonal variations, particularly changes in earthworm and Collembola populations.

The plant trial did not support the hypothesised increased growth and biomass in the vermicompost compared to the other three composts. However it did demonstrate that vermicompost could be a suitable alternative to the other media in horticulture. Water-soluble nutrient levels were essentially very similar between the four composts trialled, with most being high or very high on the ADAS index. One possible advantage of the vermicompost over two of the others was the reduced wilting experienced by plants growing in it, which could be the subject of further study.

The microcosm fermentation experiment successfully demonstrated the hypothesis that the presence of earthworms would increase concentrations of some water-soluble nutrients, particularly nitrate, in the compost. It also showed clearly that there were facilitative effects of earthworms on the abundance of some species of Collembola, and provided evidence for possible antagonistic effects on one species (*C. tenebricosa*). The influence of Collembola was less clearly demonstrated, but there was evidence of significantly increased nitrate and phosphate levels in the presence of springtails by 136 days of vermicomposting.

6 References

ADAS, undated. ADAS index system for loamless compost (obtained from NRM laboratories, Pers. Comm., 2017).

Addison, J.A., Trofymow, J.A. and Marshall, V.G., 2003. Functional role of Collembola in successional coastal temperate forests on Vancouver Island, Canada. Applied Soil Ecology, 24(3), pp.247-261.

Aira, M. and Domínguez, J. (2009). Microbial and nutrient stabilization of two animal manures after the transit through the gut of the earthworm *Eisenia fetida* (Savigny, 1826). Journal of Hazardous Materials 161 1234–1238.

Aira, M., Gómez-Brandón, M., González-Porto, P. and Domínguez, J., 2011. Selective reduction of the pathogenic load of cow manure in an industrial-scale continuous-feeding vermireactor. Bioresource technology, 102(20), pp.9633-9637.

Aira, M., Sampedro, L., Monroy, F. and Domínguez, J., 2008. Detritivorous earthworms directly modify the structure, thus altering the functioning of a microdecomposer food web. Soil Biology and Biochemistry, 40(10), pp.2511-2516.

Alexander, P.D., Williams, R.H. and Nevison, I.M., 2011, October. Improving gardeners' understanding of water management in peat and peat-free multi-purpose growing media: an assessment with fuchsia. In International Symposium on Growing Media, Composting and Substrate Analysis 1013 (pp. 257-263).

Anderson, M. J., 2017. Permutational Multivariate Analysis of Variance (PERMANOVA). Wiley StatsRef: Statistics Reference Online. https://doi.org/10.1002/9781118445112.stat07841.

Atiyeh, R.M., Arancon, N., Edwards, C.A. and Metzger, J.D., 2000. Influence of earthworm-processed pig manure on the growth and yield of greenhouse tomatoes. Bioresource Technology, 75(3), pp.175-180.

Atiyeh, R.M., Domínguez, J., Subler, S. and Edwards, C.A., 2000. Changes in biochemical properties of cow manure during processing by earthworms (Eisenia andrei, Bouché) and the effects on seedling growth. Pedobiologia, 44(6), pp.709-724.

Atiyeh, R.M., Edwards, C.A., Subler, S. and Metzger, J.D., 2001. Pig manure vermicompost as a component of a horticultural bedding plant medium: effects on physicochemical properties and plant growth. Bioresource technology, 78(1), pp.11-20.

Bachman, G.R. and Metzger, J.D., 2008. Growth of bedding plants in commercial potting substrate amended with vermicompost. Bioresource technology, 99(8), pp.3155-3161.

Ben-Dor, E., Inbar, Y. and Chen, Y., 1997. The reflectance spectra of organic matter in the visible near-infrared and short wave infrared region (400–2500 nm) during a controlled decomposition process. Remote Sensing of Environment, 61(1), pp.1-15.

Blouin, M., Barrere, J., Meyer, N., Lartigue, S., Barot, S. and Mathieu, J., 2019. Vermicompost significantly affects plant growth. A meta-analysis. Agronomy for Sustainable Development 39:34.

Briones, M.J.I., 2014. Soil fauna and soil functions: a jigsaw puzzle. Frontiers in Environmental Science, 2, p.7.

Briones, M.J., 2018. The serendipitous value of soil fauna in ecosystem functioning: the unexplained explained. Frontiers in Environmental Science, 6, p.149.

Chamberlain, P.M., Bull, I.D., Black, H.I.J., Ineson, P. and Evershed, R.P., 2006. Collembolan trophic preferences determined using fatty acid distributions and compound-specific stable carbon isotope values. Soil Biology and Biochemistry, 38(6), pp.1275-1281.

Cole, L., Dromph, K.M., Boaglio, V. and Bardgett, R.D., 2004. Effect of density and species richness of soil mesofauna on nutrient mineralisation and plant growth. Biology and Fertility of Soils, 39(5), pp.337-343.

Chahartaghi, M., Langel, R., Scheu, S. and Ruess, L., 2005. Feeding guilds in Collembola based on nitrogen stable isotope ratios. Soil Biology and Biochemistry, 37(9), pp.1718-1725.

de Vries, F.T., Thébault, E., Liiri, M., Birkhofer, K., Tsiafouli, M.A., Bjørnlund, L., Jørgensen, H.B., Brady, M.V., Christensen, S., de Ruiter, P.C. and d'Hertefeldt, T., 2013. Soil food web properties explain ecosystem services across European land use systems. Proceedings of the National Academy of Sciences, 110(35), pp.14296-14301.

Chauvat, M. and Wolters, V., 2014. Response of soil biota to manipulation of collembolan biomass. European journal of soil biology, 60, pp.53-57.

Domínguez, J. and Edwards, C.A., 2011. Biology and ecology of earthworm species used for vermicomposting. Vermiculture technology: earthworms, organic wastes, and environmental management. CRC Press USA. Elvira, C., Sampedro.

Domínguez, J. and Gómez-Brandón, M., 2013. The influence of earthworms on nutrient dynamics during the process of vermicomposting. Waste Management & Research, 31(8), pp.859-868.

Ehsani, M.R., Upadhyaya, S.K., Slaughter, D., Shafii, S. and Pelletier, M., 1999. A NIR technique for rapid determination of soil mineral nitrogen. Precision agriculture, 1(2), pp.219-236.

Eisenhauer, N., 2010. The action of an animal ecosystem engineer: identification of the main mechanisms of earthworm impacts on soil microarthropods. Pedobiologia, 53(6), pp.343-352.

Eisenhauer, N., Vogel, A., Jensen, B. and Scheu, S., 2018. Decomposer diversity increases biomass production and shifts aboveground-belowground biomass allocation of common wheat. Scientific reports, 8(1), p.17894.

Filser, J., 2002. The role of Collembola in carbon and nitrogen cycling in soil: Proceedings of the Xth international Colloquium on Apterygota, České Budějovice 2000: Apterygota at the Beginning of the Third Millennium. Pedobiologia, 46(3-4), pp.234-245.

Filser, J., Faber, J.H., Tiunov, A.V., Brussaard, L., Frouz, J., Deyn, G.D., Uvarov, A.V., Berg, M.P., Lavelle, P., Loreau, M. and Wall, D.H., 2016. Soil fauna: key to new carbon models. Soil, 2(4), pp.565-582.

Frouz, J., 2018. Effects of soil macro-and mesofauna on litter decomposition and soil organic matter stabilization. Geoderma, 332, pp.161-172.

Guerra, P.A.M., Salas Sanjúan, M.D.C. and López, M.J., 2018. Evaluation of physicochemical properties and enzymatic activity of organic substrates during four crop cycles in soilless containers. Food Science & Nutrition, 6(8), pp.2066-2078.

Gómez-Brandón, M., Aira, M., Lores, M. and Domínguez, J., 2011. Changes in microbial community structure and function during vermicomposting of pig slurry. Bioresource Technology, 102(5), pp.4171-4178.

Gómez-Brandón, M. and Domínguez, J., 2014. Recycling of solid organic wastes through vermicomposting: microbial community changes throughout the process and use of vermicompost as a soil amendment. Critical Reviews in Environmental Science and Technology, 44(12), pp.1289-1312.

Grubert, D., Butenschoen, O., Maraun, M. and Scheu, S., 2016. Understanding earthworm–Collembola interactions and their importance for ecosystem processes needs consideration of species identity. European journal of soil biology, 77, pp.60-67.

Gunadi, B., Blount, C. and Edwards, C.A., 2002. The growth and fecundity of Eisenia fetida (Savigny) in cattle solids pre-composted for different periods. Pedobiologia, 46(1), pp.15-23.

Gutiérrez-López, M., Jesús, J.B., Trigo, D., Fernández, R., Novo, M. and Díaz-Cosín, D.J., 2010. Relationships among spatial distribution of soil microarthropods, earthworm species and soil properties. Pedobiologia, 53(6), pp.381-389.

Hammer, Ø., 2019. PAST (PAleontological Statistics) Version 3.25 Reference manual, Natural History Museum, University of Oslo. https://folk.uio.no/ohammer/past/

Hanc, A. and Dreslova, M., 2016. Effect of composting and vermicomposting on properties of particle size fractions. Bioresource technology, 217, pp.186-189.

Haynes, R.J. and Zhou, Y.F., 2016. Comparison of the chemical, physical and microbial properties of composts produced by conventional composting or vermicomposting using the same feedstocks. Environmental Science and Pollution Research, 23(11), pp.10763-10772.

Heemsbergen, D.A., Berg, M.P., Loreau, M., Van Hal, J.R., Faber, J.H. and Verhoef, H.A., 2004. Biodiversity effects on soil processes explained by interspecific functional dissimilarity. Science, 306(5698), pp.1019-1020.

Hopkin, S.P., 1997. *Biology of the springtails: (Insecta: Collembola)*. OUP Oxford.

Hopkin, S.P., 2007. A key to the Collembola (springtails) of Britain and Ireland. FSC publications.

Huhta, V., 2007. The role of soil fauna in ecosystems: A historical review. Pedobiologia, 50(6), pp.489-495.

Ilani, T., Herrmann, I., Karnieli, A. and Arye, G., 2016. Characterization of the biosolids composting process by hyperspectral analysis. Waste management, 48, pp.106-114.

Killham, K., 1994. Soil ecology. Cambridge University Press.

Koleva, L., Yordanova, M. and Dimitrov, G., 2017. Collembola Communities in Different Compost Types as Bioindicator of Substrate Quality. Journal of Tekirdag Agricultural Faculty, Special Issue of 2nd International Balkan Agriculture Congress, May 16-18, 2017.

Lazcano, C., Arnold, J., Zaller, J.G., Martín, J.D. and Salgado, A.T., 2009. Compost and vermicompost as nursery pot components: effects on tomato plant growth and morphology. Spanish Journal of Agricultural Research, (4), pp.944-951.

Lazcano, C. and Domínguez, J., 2010. Effects of vermicompost as a potting amendment of two commercially-grown ornamental plant species. Spanish Journal of Agricultural Research, 8(4), pp.1260-1270.

Lim, S.L., Wu, T.Y., Lim, P.N. and Shak, K.P.Y., 2015. The use of vermicompost in organic farming: overview, effects on soil and economics. Journal of the Science of Food and Agriculture, 95(6), pp.1143-1156.

Malcicka, M., Berg, M.P. and Ellers, J., 2017. Ecomorphological adaptations in Collembola in relation to feeding strategies and microhabitat. European journal of soil biology, 78, pp.82-91.

Martin, C., Pers. Comm. 2017. Discussions with the company sponsor Martins TLC Ltd.

McWhirt, A.L., Weindorf, D.C., Chakraborty, S. and Li, B., 2012. Visible near infrared diffuse reflectance spectroscopy (VisNIR DRS) for rapid measurement of organic matter in compost. Waste Management & Research, 30(10), pp.1049-1058.

Mebes, K.H. and Filser, J., 1998. Does the species composition of Collembola affect nitrogen turnover?. Applied Soil Ecology, 9(1-3), pp.241-247.

Mitschunas, N., Wagner, M. and Filser, J., 2008. Increased field emergence of seedlings at high densities of fungivorous soil mesofauna. The Journal of the Torrey Botanical Society, 135(2), pp.272-281.

Monroy, F., Aira, M. and Domínguez, J., 2011. Epigeic earthworms increase soil arthropod populations during first steps of decomposition of organic matter. Pedobiologia, 54(2), pp.93-99.

Moody, S.A., Briones, M.J.I., Piearce, T.G. and Dighton, J., 1995. Selective consumption of decomposing wheat straw by earthworms. Soil Biology and Biochemistry, 27(9), pp.1209-1213.

Moore, J.C., Walter, D.E. and Hunt, H.W., 1988. Arthropod regulation of micro-and mesobiota in below-ground detrital food webs. Annual review of Entomology, 33(1), pp.419-435.

Moral, R., Paredes, C., Bustamante, M.A., Marhuenda-Egea, F. and Bernal, M.P., 2009. Utilisation of manure composts by high-value crops: Safety and environmental challenges. Bioresource Technology, 100(22), pp.5454-5460.

NRM, pers. com. 2017. Method principles for compost and growing media analysis: 4.5 water soluble nutrients 4.6 electrical conductivity, 4.8 compacted bulk density and dry bulk density, 4.9 pH. Contact: www.cawoodscientific.uk.com/nrm.

Partsch, S., Milcu, A. and Scheu, S., 2006. Decomposers (Lumbricidae, Collembola) affect plant performance in model grasslands of different diversity. Ecology, 87(10), pp.2548-2558.

Potapov, A.A., Semenina, E.E., Korotkevich, A.Y., Kuznetsova, N.A. and Tiunov, A.V., 2016. Connecting taxonomy and ecology: Trophic niches of collembolans as related to taxonomic identity and life forms. Soil Biology and Biochemistry, 101, pp.20-31.

Potapov, A.M., Scheu, S. and Tiunov, A.V., 2019a. Trophic consistency of supraspecific taxa in below-ground invertebrate communities: Comparison across lineages and taxonomic ranks. Functional Ecology, 33(6), pp.1172-1183.

Potapov, A.M., Tiunov, A.V. and Scheu, S., 2019b. Uncovering trophic positions and food resources of soil animals using bulk natural stable isotope composition. Biological Reviews, 94(1), pp.37-59.

PP Systems, 2005. SRC-1 / CPY-2 Closed System Chambers for use with all EGM's (1/2/3/4) and CIRAS-1. Operator's Manual Version 3.31. http://www.ppsystems.com.

PP Systems, 2010. EGM-4 Environmental Gas Monitor For CO_2. Operator's Manual, Version 4.18. http://www.ppsystems.com.

Roberts, P., Jones, D.L. and Edwards-Jones, G., 2007. Yield and vitamin C content of tomatoes grown in vermicomposted wastes. Journal of the Science of Food and Agriculture, 87(10), pp.1957-1963.

Römbke, J., Aira, M., Backeljau, T., Breugelmans, K., Domínguez, J., Funke, E., Graf, N., Hajibabaei, M., Pérez-Losada, M., Porto, P.G. and Schmelz, R.M., 2016. DNA barcoding of earthworms (*Eisenia fetida/andrei* complex) from 28 ecotoxicological test laboratories. Applied Soil Ecology, 104, pp.3-11.

Rusek, J., 1998. Biodiversity of Collembola and their functional role in the ecosystem. Biodiversity & Conservation, 7(9), pp.1207-1219.

Sampedro, L. and Domínguez, J., 2008. Stable isotope natural abundances (δ13C and δ15N) of the earthworm *Eisenia fetida* and other soil fauna living in two different vermicomposting environments. applied soil ecology, 38(2), pp.91-99.

Shepherd, M. and Crotty, F., 2017. A key to the soil mites of Britain and Ireland (Test version 6: April 2017). Course material for "Soil Mesofauna: ecology and identification", FSC Preston Montford, April-May 2017.

Sherlock, E., 2012. Key to the Earthworms of the UK and Ireland. 1st edition. FSC and Natural History Museum.

Sherlock, E., 2018. Key to the Earthworms of the UK and Ireland. 2nd edition. FSC and Natural History Museum.

Steel, H. and Bert, W., 2012. Biodiversity of compost mesofauna and its potential as an indicator of the composting process status. Dynamic Soil Dynamic Plant, 5(spec. iss. 2), pp.45-50.

Stiles, W., 2017. Vermicomposting: an alternative method for organic waste management. Farming Connect https://businesswales.gov.wales/farmingconnect/posts/vermicomposting-alternative-method-organic-waste-management.

Sun, X., Zhang, F., Ding, Y., Davies, T.W., Li, Y. and Wu, D., 2017. Delimiting species of Protaphorura (Collembola: Onychiuridae): integrative evidence based on morphology, DNA sequences and geography. Scientific reports, 7(1), p.8261.

Tilling, S.M., 1987. A key to the major groups of British terrestrial invertebrates. Field Studies, 6(4), pp.695-766.

Tognetti, C., Laos, F., Mazzarino, M.J. and Hernandez, M.T., 2005. Composting vs. vermicomposting: a comparison of end product quality. Compost Science & Utilization, 13(1), pp.6-13.

Tringovska, I. and Dintcheva, T., 2012. Vermicompost as substrate amendment for tomato transplant production. Sustainable Agriculture Research, 1(526-2016-37844).

Uvarov, A.V., 2016. Density-mediated earthworm effects on soil respiration. Polish Journal of Ecology, 64(4), pp.534-547.

Warman, P.R. and AngLopez, M.J., 2010. Vermicompost derived from different feedstocks as a plant growth medium. Bioresource Technology, 101(12), pp.4479-4483.

Which? Gardening magazine. 2016. Compost trial report, Jan/Feb 2016 issue. https://www.which.co.uk.

WRAP, 2014. Guidelines for the Specification of Quality Compost for use in Growing Media. http://www.wrap.org.uk/sites/files/wrap/Growing_Media_Specification.pdf

Xie, H.T., Yang, X.M., Drury, C.F., Yang, J.Y. and Zhang, X.D., 2011. Predicting soil organic carbon and total nitrogen using mid-and near-infrared spectra for Brookston clay loam soil in Southwestern Ontario, Canada. Canadian Journal of Soil Science, 91(1), pp.53-63.

7 Appendix

The detailed results of statistical tests are shown here.

7.1 Field work at the vermicomposting site

Table 7-1. One-way PERMANOVA of the distribution of mesofauna with age of compost.

Age - individuals	
One-way PERMANOVA	Bray-Curtis
Permutation N:	9999
Total sum of squares:	2.66E+00
Within-group sum of squares:	3.43E-01
F:	16.86
p (same):	0.0009
pairwise comparisons	ns
Age - Simpson_1-D	
One-way PERMANOVA	Bray-Curtis
Permutation N:	9999
Total sum of squares:	0.4412
Within-group sum of squares:	0.2183
F:	2.553
p (same):	0.0037
pairwise comparisons	ns
Age - Shannon_H	
One-way PERMANOVA	Bray-Curtis
Permutation N:	9999
Total sum of squares:	0.4218
Within-group sum of squares:	0.1928
F:	2.97
p (same):	0.0061
pairwise comparisons	ns

7.2 The greenhouse pot trials

Table 7-2. Repeated measures ANOVA of stem heights of all tomato plants (n = 9) for days 4, 11, 19, 25. Significant results are shown in bold.

Tests of within subject effects

Source	Correction for sphericity	Type III Sum of Squares	df	Mean Square	*F*	*p*
Date of measure	Greenhouse-Geisser	36837.910	2.130	17298.102	767.096	**<0.001**
Date x compost	Greenhouse-Geisser	575.618	6.389	90.098	3.995	**0.003**
Error (date)	Greenhouse-Geisser	1536.722	68.147	22.550		

Note: the Greenhouse-Geisser correction was used because the data do not show sphericity (Mauchly's test: $p < 0.05$, $\varepsilon < 0.75$).

Tests of between-subjects effects

Source	Type III Sum of Squares	df	Mean Square	*F*	*p*
Intercept	368955.007	1	368955.007	4604.044	**<0.001**
Compost	1295.354	3	431.785	5.388	**0.004**
Error	2564.389	32	80.137		

Multiple comparisons (LSD)

Compost	Compost	Mean difference	*p*	95% Confidence Interval for Difference	
				Lower bound	Upper bound
CBC	MTLC	-7.889	**0.001**	-12.187	-3.591
	PBC	-6.472	**0.004**	-10.770	-2.174
	WBC	-3.889	0.075	-8.187	0.409
MTLC	CBC	7.889	**0.001**	3.591	12.187
	PBC	1.417	0.507	-2.881	5.715
	WBC	4.000	0.067	-0.298	8.298
PBC	CBC	6.472	**0.004**	2.174	10.770
	MTLC	-1.417	0.507	-5.715	2.881
	WBC	2.583	0.230	-1.715	6.881
WBC	CBC	3.889	0.075	-0.409	8.187
	MTLC	-4.000	0.067	-8.298	0.298
	PBC	-2.583	0.230	-6.881	1.715

Table 7-3. Repeated measures ANOVA of stem heights of leek plants (n = 8) for days 4, 11, 19, 25, 67 and 103. Significant results are shown in bold.

Tests of within subject effects

Source	Correction for sphericity	Type III Sum of Squares	df	Mean Square	*F*	*p*
Date of measure	Greenhouse-Geisser	10896797.71	1.390	7838451.802	607.370	**<0.001**
Date x compost	Greenhouse-Geisser	84396.307	4.171	20236.416	1.568	0.200
Error (date)	Greenhouse-Geisser	502346.813	38.925	12905.565		

Note: the Greenhouse-Geisser correction was used because the data do not show sphericity (Mauchly's test: $p < 0.05$, $\varepsilon < 0.75$).

Tests of between-subjects effects

Source	Type III Sum of Squares	df	Mean Square	*F*	*p*
Intercept	13719012.13	1	13719012.13	1276.678	**<0.001**
Compost	107107.724	3	35702.575	3.322	**0.034**
Error	300884.313	28	10745.868		

Multiple comparisons (LSD)				95% Confidence Interval for Difference	
Compost	Compost	Mean difference	*p*	Lower bound	Upper bound
CBC	MTLC	23.9583	0.267	-19.3859	67.3026
	PBC	-25.5000	0.238	-68.8442	17.8442
	WBC	36.1458	0.099	-7.1984	79.4901
MTLC	CBC	-23.9583	0.267	-67.3026	19.3859
	PBC	-49.4583	**0.027**	-92.8026	-6.1141
	WBC	12.1875	0.569	-31.1567	55.5317
PBC	CBC	25.5000	0.238	-17.8442	68.8442
	MTLC	49.4583	**0.027**	6.1141	92.8026
	WBC	61.6458	**0.007**	18.3016	104.9901
WBC	CBC	-36.1458	0.099	-79.4901	7.1984
	MTLC	-12.1875	0.569	-55.5317	31.1567
	PBC	-61.6458	**0.007**	-104.9901	-18.3016

Table 7-4. One-way ANOVA and LSD post hoc tests of total leek dry weight at destructive sampling (n = 8). Significant results are shown in bold.

	Sum of Squares	df	Mean Square	*F*	*p*
Between Groups	32.259	3	10.753	3.512	**0.028**
Within Groups	85.722	28	3.062		
Total	117.981	31			

Multiple comparisons (LSD)				95% Confidence Interval for Difference	
Compost	Compost	Mean difference	*p*	Lower bound	Upper bound
CBC	MTLC	0.53375	0.547	-1.2583	2.3258
	PBC	-1.58625	0.081	-3.3783	0.2058
	WBC	1.11000	0.215	-0.6821	2.9021
MTLC	CBC	-0.53375	0.547	-2.3258	1.2583
	PBC	-2.12000	**0.022**	-3.9121	-0.3279
	WBC	0.57625	0.515	-1.2158	2.3683
PBC	CBC	1.58625	0.081	-0.2058	3.3783
	MTLC	2.12000	**0.022**	0.3279	3.9121
	WBC	2.69625	**0.005**	0.9042	4.4883
WBC	CBC	-1.11000	0.215	-2.9021	0.6821
	MTLC	-0.57625	0.515	-2.3683	1.2158
	PBC	-2.69625	**0.005**	-4.4883	-0.9042

7.3 The microcosm experiment

Table 7-5. Two-way PERMANOVA with sampling day and treatment as factors for chloride, nitrite, nitrate, phosphate, sulphate, sodium, ammonium, potassium, calcium and magnesium as variables.

	Sum of squares	df	Mean square	*F*	*p*
Age	1.61×10^7	3	5.38×10^6	30.22	<0.001
Treatment	1.96×10^7	5	3.93×10^6	22.08	<0.001
Interaction (treatment x age)	5.55×10^6	15	3.70×10^5	2.08	0.011
Residual	1.28×10^7	72	1.78×10^5		
Total	5.41×10^7	95			

Table 7-6. One-way ANOVA of the second (GLS) model PC 1-3 scores by treatments.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
PC1 GLS (NIRS)	Between Groups	0.00114919	5	0.00022984	11.932	0.000
	Within Groups	0.00173357	90	0.00001926		
	Total	0.003	95			
PC2 GLS (NIRS)	Between Groups	0.001	5	0.000	13.110	0.000
	Within Groups	0.001	90	0.000		
	Total	0.001	95			
PC3 GLS (NIRS)	Between Groups	0.000	5	0.000	9.035	0.000
	Within Groups	0.000	90	0.000		
	Total	0.000	95			
PC4 GLS (NIRS)	Between Groups	0.000	5	0.000	3.648	0.005
	Within Groups	0.000	90	0.000		
	Total	0.000	95			
PC5 GLS (NIRS)	Between Groups	0.000	5	0.000	8.764	0.000
	Within Groups	0.000	90	0.000		
	Total	0.000	95			