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# **Effects of Vermicomposted Spent Mushroom Compost on Growing Medium Characteristics, Plant Growth, Yield and Abiotic Stress Tolerance**

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A thesis submitted to the National University of Ireland, Cork in  
fulfilment of the requirements for the degree of Doctor of  
Philosophy.

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Sciences

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## **Declaration**

This work has not been previously accepted in substance for any degree and is not being concurrently submitted in candidature for any degree. This thesis is the result of my own independent work/investigation, except where otherwise stated.



Tara Duggan

## **Abstract**

Treatment of agricultural biodegradable wastes and by-products can be carried out using composting or vermicomposting, or a combination of both treatment methods, to create a growing medium amendment suitable for horticultural use. When compared to traditional compost-maturation, vermicompost-maturation resulted in a more mature growing medium amendment i.e. lower C/N and pH, with increased nutrient content and improved plant growth response, increasing lettuce shoot fresh and dry weight by an average of 15% and 14%, respectively. Vermicomposted horse manure compost was used as a growing medium amendment for lettuce and was found to significantly increase lettuce shoot and root growth, and chlorophyll content. When used as a growing medium amendment for tomato fruit production, vermicomposted spent mushroom compost increased shoot growth and marketable yield, and reduced blossom end rot in two independent studies. Vermicompost addition to peat-based growing media increased marketable yield by an average of 21%. Vermicompost also improved tomato fruit quality parameters such as acidity and sweetness. Fruit sweetness, as measured using Brix value, was significantly increased in fruits grown with 10% or 20% vermicompost addition by 0.2 in truss one and 0.3 in truss two. Fruit acidity (% citric acid) was significantly increased in plants grown with vermicompost by an average of 0.65% in truss one and 0.68% in truss two. These changes in fruit chemical parameters resulted in a higher tomato fruit overall acceptability rating as determined by a consumer acceptance panel. When incorporated into soil, vermicomposted spent mushroom compost increased plant growth and reduced plant stress under conditions of cold stress, but not salinity or heat stress. The addition of 20% vermicompost to cold-stressed plants increased plant growth by an average of 30% and increased chlorophyll fluorescence by an average of 21%. Compared to peat-based growing medium, vermicompost had consistently higher nutrient content, pH, electrical conductivity and bulk density, and when added to a peat-based growing medium, vermicomposted spent mushroom compost altered the microbial community. Vermicompost amendment increased the microbial activity of the growing medium when incorporated initially, and this increased microbial activity was observed for up to four months after incorporation when plants were grown in it. Vermicomposting was shown to be a suitable treatment method for agricultural biodegradable wastes and by-products, with the

resulting vermicompost having suitable physical, chemical and biological properties, and resulting in increased plant growth, marketable yield and yield quality, when used as an amendment in peat-based growing medium.

# Chapter 1

General Introduction and Literature  
Review

## **1.1 Introduction**

Biodegradable waste has been defined as ‘any waste that is capable of undergoing anaerobic or aerobic decomposition’ according to the EU Landfill Directive (Council Directive 1999/31/EC). Biodegradable waste is generated by a variety of sources including municipal, industrial and agricultural. Biodegradable waste from municipal and industrial sources were traditionally landfilled in the EU, with no further re-use or recycling potential. Since the introduction of the EU Landfill Directive in 1999, diversion of biodegradable waste from landfill is encouraged, with every member state obliged to reduce the amount of biodegradable municipal waste going to landfill in 2016 by 65% (by weight) of the biodegradable municipal waste produced in 1995. This has resulted in an increase in municipal waste recycling in the EU by composting and anaerobic digestion to 71 kg per capita in 2013, compared to only 30 kg per capita in 1995, and an increase in municipal waste incineration from 67 kg per capita in 1995 to 123 kg per capita in 2013 (Eurostat, 2015).

Composting and vermicomposting are both aerobic treatment processes for biodegradable wastes. Composting is a thermophilic process, with breakdown of the organic matter being carried out by bacteria and fungi, while vermicomposting occurs at ambient temperature and organic matter breakdown is carried out by worms, bacteria and fungi. Both processes produce a dark, crumbly, soil-like material that can be used in agriculture and horticulture as a plant fertiliser, soil enhancer or growing medium component. A more in-depth description of both processes will be given later in this chapter (1.3).

Anaerobic digestion is the controlled mesophilic breakdown of organic matter by bacteria in the absence of oxygen to produce a methane-rich biogas and a nutrient-rich digestate. The biogas can be burnt for heat and electricity generation, or

processed further for entry into the gas grid or for biofuel generation. The digestate is a sludge-like residue which can be spread onto agricultural land, or dewatered to create a separate liquor and semi-solid fraction (Saveyn & Eder, 2014). The liquor can then be re-used in the digestion process, while excess liquor can be spread onto agricultural land, much like animal slurry. The solid fraction can also be spread on land or stabilised by aerobic maturation for use in agriculture and horticulture.

Another method for municipal biodegradable waste diversion from landfill is energy recovery by incineration in the presence of oxygen which involves thermal oxidation of the waste at high temperatures ( $>900^{\circ}\text{C}$ ) (Oppelt, 1987). The waste, usually as mixed municipal waste with some segregation, is incinerated for heat generation and energy recovery, with the resulting ashes ordinarily disposed of at landfill (Ferreira *et al.*, 2003).

The end-of-waste criteria for compost and digestate have been recently set-out as required in the Waste Framework Directive (Council Directive 2008/98/EC, as amended) in the 'End-of-waste criteria for biodegradable waste subjected to biological treatment (compost & digestate): Technical proposals final report' (Saveyn & Eder, 2014). This report has set out the allowable input materials that are applicable to these end-of-waste criteria, and the treatment process requirements. It also sets out the allowable limits for human pathogens, heavy metals, organic pollutants, weed seeds and physical contaminants, established minimum organic matter content, stability levels and product testing requirements of compost and digestate material. These criteria determine whether a compost or digestate is to be considered a product or a waste. By meeting these criteria, further use of the compost or digestate material can be authorised safely. There is, as yet, no specific quality standard for vermicompost, although, due to the similar nature of the process and the

same end-use for both compost and vermicompost, compost quality standards can be obtained for sanitised vermicompost products e.g. I.S. 441:2011 (Irish standard) and BSI PAS 100 (UK standard).

## **1.2 Biodegradable wastes from agricultural sources**

Animal manure is not technically regarded as a waste product once it is applied to agricultural land or treated e.g. composted, on the farm it was produced. Although, once it is removed from the farm for treatment e.g. composting or anaerobic digestion in an external facility, it is regarded as a waste product and any facility treating this waste requires authorisation according to the EU Waste Framework Directive (Council Directive 2008/98/EC) sections 1(f) and 2(b), as amended. Animal manure and bedding is regarded as a Category 2 animal by-product according to Regulation (EC) No 1069/2009. According to the regulations it can be land-spread and transported without authorisation, but only once it can be demonstrated to the competent authority that these activities do not pose a significant risk of disease transfer.

Animal manure in the form of slurry is spread on land for the purpose of fertilisation, whereas animal manure in the form of manure and bedding is spread for fertilisation and soil enhancement purposes. As slurry is a more convenient way of collecting and spreading manure, this is the more popular practice amongst modern farmers (Burton & Turner, 2003). Managing excess animal manure and slurry can become an issue, particularly where there is an excess of this material produced in a particular area or geographical region where arable land, which can make use of this manure, is located elsewhere (Burton & Turner, 2003). For instance, on a small scale, pig farmers have a very high density of livestock (as high as 10.8 Livestock Units (LU) ha<sup>-1</sup> (Burton & Turner, 2003)) on a small area of land, and due to the lack of

requirement for grassland on pig farms, farmers must find alternative land where they can spread their excess manure, or find alternative treatment methods for this manure. This can lead to a high nutrient loading in a particular area, especially if there is a paucity of arable farmers in the locality able or willing to use this manure.

There are also temporal restrictions regarding the spreading of animal manure on agricultural land. For example, in Ireland, the European Communities (Good Agricultural Practice for the Protection of Waters) Regulations 2010 (S.I. 610 of 2010) restricts the spreading of animal manure from October to January (exact dates depending on region), and it also restricts the excessive use of fertiliser, capping the nitrogen application (in the form of livestock manure) at  $170 \text{ kg N ha}^{-1}$ .

When animal manure and slurries are spread excessively on land, e.g. in large quantities at one time, or during times of heavy precipitation, nutrient losses occur through leaching and denitrification. This results in the eutrophication of waterbodies, leading to unsafe drinking and bathing water quality and greenhouse gas emissions (Ogden, 2001; Flessa *et al.*, 2002).

It is important that excess animal manure be regarded as a resource rather than a waste product. When sanitised and stabilised efficiently, value is added to the material and it can provide many agricultural and horticultural benefits over raw manure and slurries. During treatment its bulk is reduced, leading to easier and cheaper transportation (DeLuca & DeLuca, 1997). Compost can be spread more frequently throughout the year i.e. it can be spread throughout the allowable spreading period, while animal manure is commonly spread only once during the year during the autumn or spring period, as it requires time to breakdown in the soil. The treatment and sustainable use of these animal manures in agriculture or horticulture can reduce greenhouse gas emissions to the atmosphere (Pattey *et al.*,

2005), and nutrient (DeLuca & DeLuca, 1997) and pathogen movement into waterways on the farm level. When used in horticulture as a soil-enhancer and growing medium amendment, aerobically and anaerobically treated animal manures can reduce the need for chemical fertilisers, replace some peat use in horticultural growing media, and, as they have been demonstrated to be potentially suppressive against plant pests and diseases (Cotxarrera *et al.*, 2002; McKellar & Nelson, 2003; Vallad *et al.*, 2003), can reduce pesticides and fungicide use.

As food production is set to rise with the forecasted increase in human population to 9 bn by 2050, the amount of animal manure produced is also due to increase and sustainable ways of managing this manure must be implemented. In Ireland, for example, the value of primary agricultural production is set to increase by 65% in 2025 compared to 2012-2014 baseline to meet Food Wise 2025 targets (Department of Agriculture Food and the Marine, 2015). These increases are estimated to come largely from the livestock sector due to increasing demand for meat in developing world markets and changes in EU policies such as the abolition of milk quotas in 2015.

Treated animal manure can be used for high-quality horticultural purposes i.e. as a fertiliser or a growing medium additive. Composting and vermicomposting are both potential strategies that can transform this type of waste into value-added products for horticultural use.

### **1.3 Composting and vermicomposting of agricultural biodegradable wastes**

The process of composting and vermicomposting produces compost and vermicompost, respectively. Both products are dark, crumbly, soil-like materials with a high organic matter content that can be used as a plant fertiliser, soil enhancer

or growing medium component, and can also be used for more niche purposes such as water filtration (Jordão *et al.*, 2007), as a biofilter (Ergas *et al.*, 1995) or for soil remediation (van Herwijnen *et al.*, 2007).

Composting is the thermophilic breakdown of organic matter by bacteria and fungi in the presence of oxygen while vermicomposting relies on worms to carry out most of the organic matter fragmentation. During vermicomposting the waste is fragmented by the action of the worms as it passes through their gizzard and digestive tract; it then remains in the worm bed for an extended period of time where additional microbial decomposition results in waste stabilisation and maturation (Fornes *et al.*, 2012). The worms derive their nutrition not from the waste itself, but from the microorganisms that have colonised the waste. The worm species most commonly used in the vermicomposting process are the epigeic earthworm species *Eisenia fetida* (tiger worm) (Figure 1.1) and *Eisenia andrei* (red worm), though other earthworm species can also be used e.g. *Eudrilus eugeniae* (African nightcrawler) and *Dendrobaena veneta* (European nightcrawler) (Dominguez & Edwards, 2010a). *Eisenia fetida* is a very suitable worm for commercial vermicomposting as it can survive in wide range of temperatures (0-35°C), can eat up to its own bodyweight daily and can live and reproduce in dense colonies (Munroe, 2004).

The composting process should have three distinct phases; sanitisation, stabilisation and maturation (Benton & Foster, 2008). The process can take anywhere from 6 weeks to 12 months depending on the system of composting employed, feedstock, management of the process etc. The three stages of composting are summarised in Table 1.1. The vermicomposting process has no distinct phases but usually consists of an initial phase where the worms ingest the waste and mechanically break it

down, followed by a period of time where the waste is mineralised by the bacteria, fungi and other decomposer organisms present in the vermicomposting bed.



Figure 1.1 Tiger worms (*Eisenia fetida*), commonly used in the vermicomposting process (photograph credit: Stephen Bean)

Temperature is one of the best measures to monitor whether the composting process is proceeding correctly. For example if the compost mix is right, there will be lots of microorganisms generating heat. Low temperatures in the first week are a signal that something is wrong. Temperature is also a good measure of compost stability i.e. during the maturation phase, a compost pile which remains 5-10°C above ambient temperature indicates stable, mature compost (once the moisture content is greater than 40%) (Benton & Foster, 2008).

Table 1.1 Features, characteristics and duration of the three stages of composting (amended from Benton and Foster (2008))

Composting Stage	Key Features	Stage Characteristics	Approximate Duration
Sanitisation	Microorganisms consume forms of carbon they can easily break down e.g. sugars and starches	High rate of biological activity characterised by high oxygen demand and heat generation. Tendency for the pH to drop below neutral and then rise above neutral as composting proceeds.	4-40 days depending on composting technology and feedstock materials
Stabilisation	Microorganisms consume forms of carbon they can break down moderately readily e.g. cellulose and lignin	Biological activity starts to decline. Oxygen demand gradually decreases. Heat generation declines. Tendency for the pH to remain above 8.	20-60 days depending on composting technology and feedstock materials
Maturation	The amount of available carbon is much reduced and microbial consumption slows down, re-colonisation by soil microorganisms occurs	Reduced biological activity. Medium to low oxygen demand and little heat generation. Temperatures should fall below 50°C. Oxidation of ammonium to nitrate ions. Tendency for the pH to fall towards neutral.	Variable duration depending on the curing methodology used and intended end use

Composting is a thermophilic process while vermicomposting takes place under ambient temperatures. The high temperatures generated by the microorganisms during composting are essential for killing off certain harmful pathogenic bacteria e.g. *Salmonella spp.* and *Escherichia coli*, and weed seeds. These biological

contaminants are controlled by maintaining adequate temperatures for a long enough period of time e.g.  $>55^{\circ}\text{C}$  for fourteen days (Brinton, 2000),  $65^{\circ}\text{C}$  for seven days (British Standards Institution, 2011), or  $70^{\circ}\text{C}$  for one hour (EC No. 1774/2002). Too-high a temperature ( $>70^{\circ}\text{C}$ ) will inhibit composting and potentially cause odour, as well as reducing nitrogen content through ammonification. When the compost is to be used in horticulture, waste materials should only be accepted if the contaminants can be controlled and minimised.

Worms cannot survive in temperatures exceeding  $35^{\circ}\text{C}$  (Tognetti *et al.*, 2005) and therefore vermicomposting must take place under ambient temperatures, with optimum temperatures of between  $15^{\circ}\text{C}$  and  $25^{\circ}\text{C}$  (Dominguez & Edwards, 2010b). Due to the lack of heat during the process, pathogenic bacteria and weed seeds are not destroyed and therefore the vermicomposting process is usually preceded by a short thermophilic composting step in order to meet safety criteria (Ndegwa & Thompson, 2001; Fornes *et al.*, 2012).

Maintaining oxygen levels during the composting and vermicomposting processes is essential to ensure worms and the desired aerobic microorganisms stay alive, and it also stops the compost and vermicompost from becoming anaerobic. During the composting and vermicomposting process, the oxygen content within the pile should remain above 10% but should never fall below 5% (Benton & Foster, 2008). Aeration is controlled in the composting process through the frequent turning of the compost piles or by forced aeration and by the action of worm burrowing in the vermicomposting process.

As well as vermicomposting and composting process controls, optimum composting and vermicomposting of agricultural biodegradable wastes requires that the

feedstock be prepared correctly. To prepare wastes for either composting or vermicomposting, the right mix and consistency must be achieved. The most important characteristics to be met when preparing wastes for composting are C/N ratio, pH, moisture content, and particle size.

The optimum carbon to nitrogen ratio (C/N) is 25-35:1 for composting (Bernal *et al.*, 2009) and 25:1-30:1 for vermicomposting (Ndegwa & Thompson, 2000; Dominguez & Edwards, 2010b). If the appropriate C/N ratio is not achieved, neither process will operate efficiently. For example, if there is too much nitrogen, all the carbon is taken up before the nitrogen is fully stabilised. This excess nitrogen can then be lost to the atmosphere as ammonia or nitrous oxide where it can cause odour and, in vermicomposting, worm mortality (Suthar, 2009), or it can be lost by leaching (Bernal *et al.*, 1998). This loss of nitrogen will also reduce role of the finished compost/vermicompost as a plant nutrient source (Velasco-Velasco *et al.*, 2011). If there is too much carbon, all the nitrogen is absorbed quicker than the carbon and the bacteria have to source their nitrogen requirement from the air. This will delay the composting and vermicomposting processes.

Initial feedstock pH should, if possible, be between 5.5 and 8 for composting (deBertoldi *et al.* (1982), cited by Bernal *et al.* (2009)) and close to neutral for vermicomposting (Ndegwa & Thompson, 2000; Fornes *et al.*, 2012). Highly alkaline or highly acidic feedstock materials can inhibit the colonisation of composting micro-organisms and worms, while a high pH promotes volatilisation and loss of ammonia (Elvira *et al.*, 1998).

Particle size and porosity are very important for composting, but less so for vermicomposting. With composting it is important to have larger-sized particles as there must be gaps within the compost pile to allow air to circulate through the

material, though the particle size must not be too large as to have a low surface area for microbial colonisation (Bernal *et al.*, 2009). The microorganisms need a lot of oxygen to break down the material, and therefore air must be able to circulate through the compost. There should be between 45% and 60% air space within the compost pile (Benton & Foster, 2008).

In the vermicomposting process, aeration is managed by the action of the worms and therefore large particles are not required. In fact, large particles, due to their size, are difficult for the worms to ingest and therefore must be broken down by bacteria and fungi into smaller particles first before the worms can ingest them. This microbial action in fresh feedstock can cause it to heat up in the vermicomposting bed as it is being composted instead of vermicomposted. This is to be avoided in vermicomposting as worms can only survive in temperatures of below 35°C (Munroe, 2004).

The moisture content is very important for both composting and vermicomposting as the microorganisms require water to live. For composting, if there is too much water in the compost pile, the amount of air space is reduced, and the pile can become anaerobic. A moisture content of 55-65% is optimum for the initial feedstock and the moisture content should not be allowed to drop below 40% throughout the composting process (Benton & Foster, 2008). Moisture contents can be controlled by watering with water or with leachate generated during the composting process. A moisture content of <40% would not be enough for the microorganisms to thrive in and would inhibit the composting process. It would also increase the generation of dust and bioaerosols (Kummer & Thiel, 2008), which would pose a risk to human health. For vermicomposting, the worms perform best in higher moisture contents of 80-90% (Elvira *et al.*, 1998).

Bulking materials are often used to increase the carbon content, porosity, reduce the water content and amend the pH of the initial feedstock. Commonly used bulking materials for composting include wood chippings (Doublet *et al.*, 2011; Nolan *et al.*, 2011), straw (Barrington, 2002; Nolan *et al.*, 2011), maize husks (Doublet *et al.*, 2011) and shredded green waste (Doublet *et al.*, 2011; Storey *et al.*, 2014), while in vermicomposting bulking materials commonly used include chopped straw or a straw-based waste product (Contreras-Ramos *et al.*, 2005; Das *et al.*, 2014), shredded paper (Ndegwa & Thompson, 2001; Nair *et al.*, 2006) and shredded cardboard (Arancon *et al.*, 2008).

Agricultural biodegradable wastes are very suitable wastes for composting and vermicomposting as they usually contain adequate nitrogen (from the manure) and a high carbon content (from the manure and bedding). The pH of these waste types is generally within the suitable range for composting and vermicomposting. The particle size of animal manure and dewatered slurry is suitable for vermicomposting and bulking agents are readily available on farms i.e. straw can be used to increase porosity for composting and increase carbon content. These wastes can also have suitable water content, depending on farm type and manure collection method. A further benefit of these wastes is the control over contaminants such as plastics, glass, stones and heavy metals. As the manure is often managed by one or a few people on each farm, contamination is easier to eliminate than for other waste streams such as food waste or particularly municipal biodegradable waste. A more detailed description of agricultural biodegradable wastes and their suitability for composting and vermicomposting can be found in Table 1.2.

Table 1.2 Widely available biodegradable agricultural wastes and their suitability for composting and vermicomposting

Manure Type	Positive characteristics	Negative characteristics	Suitable without amendment	Suitable with amendment	References
Cattle manure (straw bedding)	suitable N and C content	low porosity	<input checked="" type="checkbox"/> Composting† <input checked="" type="checkbox"/> Vermicomposting	<input checked="" type="checkbox"/> Composting <input checked="" type="checkbox"/> Vermicomposting	Parkinson, 2004; Lazcano <i>et al.</i> , 2008
Cattle slurry	good N source	low C content, low porosity, requires dewatering	<input checked="" type="checkbox"/> Composting†§ <input checked="" type="checkbox"/> Vermicomposting§	<input checked="" type="checkbox"/> Composting <input checked="" type="checkbox"/> Vermicomposting	Brito <i>et al.</i> , 2008
Pig slurry	good N source	low C content, low porosity, requires dewatering‡	<input checked="" type="checkbox"/> Composting† <input checked="" type="checkbox"/> Vermicomposting§	<input checked="" type="checkbox"/> Composting <input checked="" type="checkbox"/> Vermicomposting	Nolan <i>et al.</i> , 2011
Poultry manure (sawdust bedding)	good N source	low C and moisture content, low porosity, odorous	<input checked="" type="checkbox"/> Composting <input checked="" type="checkbox"/> Vermicomposting	<input checked="" type="checkbox"/> Composting <input checked="" type="checkbox"/> Vermicomposting	Tiquia & Tam, 2000; Pramanik <i>et al.</i> , 2011
Sheep manure (straw bedding)	good N source	low C content, low porosity, odorous	<input checked="" type="checkbox"/> Composting <input checked="" type="checkbox"/> Vermicomposting	<input checked="" type="checkbox"/> Composting <input checked="" type="checkbox"/> Vermicomposting	Solano <i>et al.</i> , 2001; Velasco-Velasco <i>et al.</i> , 2011
Horse manure (straw bedding)	good C source	can require additional N, low moisture content when fresh	<input checked="" type="checkbox"/> Composting <input checked="" type="checkbox"/> Vermicomposting	<input checked="" type="checkbox"/> Composting <input checked="" type="checkbox"/> Vermicomposting	Airaksinen <i>et al.</i> , 2001
Spent mushroom compost	good C/N ratio, moisture content and porosity, partially decomposed	none	<input checked="" type="checkbox"/> Composting <input checked="" type="checkbox"/> Vermicomposting	<input checked="" type="checkbox"/> Composting <input checked="" type="checkbox"/> Vermicomposting	Szmidt, 1994; Tajbakhsh <i>et al.</i> , 2008a
Anaerobic digestate fibre	good N source	low C content, low porosity, high moisture content	<input checked="" type="checkbox"/> Composting† <input checked="" type="checkbox"/> Vermicomposting	<input checked="" type="checkbox"/> Composting <input checked="" type="checkbox"/> Vermicomposting	Abbasi & Abbasi, 2010

†Suitable for a mechanically turned composting processes, but not forced aeration ‡Pig slurry has a smaller solid particle fraction than cattle slurry, making dewatering more difficult. §Suitable for composting/vermicomposting after dewatering to ≤85% moisture.



a



b

Figure 1.2 Windrow composting (a) and turning equipment (b) used to compost horse manure

Composting and vermicomposting systems include outdoor and in-vessel technologies, the suitability of which depends on the feedstock to be treated, climate, labour and electricity costs etc. Aeration can be managed by mechanical turning or by forced aeration and there are a number of different processing options.

Examples of composting operations using mechanical turning systems include open air windrows (Figure 1.2 a and b) and rotating drums. Forced aeration can come from positive pressure, negative pressure or a combination of both (Dominguez & Edwards, 2010b). Forced aeration compost piles are usually static piles with a small number of turning events to ensure homogenisation of the material. Forced aeration can occur outdoors, in small containers and in large buildings. A recent report on Irish compost production and use (McGovern, 2012) demonstrated that in October 2012 there were c. 45 composting facilities in operation in the Republic of Ireland with a combined treatment capacity of 386,100 tonnes. Of these 45 facilities, there was one vermicomposting facility, 17 windrow facilities and the remainder used in-vessel composting technology. These facilities treated a variety of waste streams, mainly green waste, source segregated brown-bin wastes, and sludges.

Vermicomposting systems, like composting systems, vary depending on the nature of the feedstock, climate, labour costs etc. They usually take place indoors or under cover to regulate temperature and precipitation, and can be batch or flow-through systems. Batch systems are where the worms are added to a single batch of feedstock, either containerised or piled loosely. The worms are left to vermicompost this feedstock completely, and upon completion of the vermicomposting process, the worms are mechanically (e.g. sieving) or behaviourally (i.e. by getting them to migrate to a fresh food source) separated from the finished vermicompost, and moved to a new batch of feedstock (Munroe, 2004). Flow-through vermicomposting systems (Figure 1.3) are more expensive and more technical, though they reduce labour considerably (Dominguez & Edwards, 2010b).

In a flow-through system the waste is fed frequently (usually every day) to the worms on the top of the bed in shallow (5-10 cm) layers. The worms eat this waste

and, at the same time, a layer (2-5 cm) of finished vermicompost is harvested from the bottom of the bed using a breaker bar which runs along the bottom of the bed and pushes the vermicompost through a wire screen at the bottom. Because the fresh feedstock is always fed onto the top of the bed, the worms remain in the top 20-30 cm and therefore do not need to be separated from the finished vermicompost. Due to the nature of this system, the volume within the vermicomposting bed also remains constant and can be manipulated to influence temperature. For example, increased volume in the winter months keeps the vermicomposting beds warmer, while reducing the volume in the summer cools the beds (T Herlihy 2013, pers. comm.).



Figure 1.3 Flow-through vermicomposting beds (sonomavalleywormfarm.wordpress.com)

Vermicomposting and composting processes produce similar products with the same end-uses but due to the increased perceived value of vermicompost (Tognetti *et al.*, 2005), it has a higher market value than compost. A number of authors have compared the efficacy of composting and vermicomposting, or some combination of

both treatments, using the same initial feedstock. These studies have varied, but, in general, have found that vermicompost has a higher nutrient quantity, especially total N (Short *et al.*, 1999; Ngo *et al.*, 2011), with some studies showing no change in nutrient content (Frederickson *et al.*, 2007; Fornes *et al.*, 2012). They found that vermicompost had an unchanged (Tognetti *et al.*, 2005; Frederickson *et al.*, 2007; Ali, 2011; Fornes *et al.*, 2012), or lower (Vincelas-Akpa & Loquet, 1997; Ngo *et al.*, 2011) carbon to nitrogen ratio when compared to compost and a lower pH and electrical conductivity (Tognetti *et al.*, 2005; Frederickson *et al.*, 2007; Fornes *et al.*, 2012).

As well as physicochemical characteristics, biological comparison of compost and vermicompost from the same feedstock has also been made by some authors. Vivas *et al.* (2009) found that microbial functional diversity and bacterial population size and diversity were increased in the finished vermicompost compared to finished compost using a mixed feedstock of vermicomposted olive mill waste and sheep manure. Bacterial community structure was also distinctly different in the vermicompost, compared to the community structure in the corresponding compost and original waste material. In comparison to this, Tognetti *et al.* (2005) found that microbial biomass C and dehydrogenase activity was reduced in vermicomposted municipal organic waste, compared to composted municipal organic waste. When comparing fungal content, Lazcano *et al.* (2008) found, when using a cattle manure feedstock, that vermicomposting alone and a combined composting-vermicomposting treatment increased the fungal content in the finished vermicomposts, compared to composting alone. Earthworms have been shown to modify the microbial diversity and abundance of soil by selective grazing,

inoculating with specific gut bacteria, increasing the surface area for colonisation and production of additional food sources (Moody *et al.*, 1995; Bernard *et al.*, 2012).

The aforementioned studies focused on the effect of vermicomposting on the end product, with few authors, namely Tognetti *et al.* (2005), Frederickson *et al.* (2007), Hernandes *et al.* (2010) and Ali (2011), looking at the effect of compost and vermicompost made from the same feedstock on plant growth. When comparing compost and vermicompost from the same feedstock (biodegradable municipal waste), Tognetti *et al.* (2005) found that vermicompost resulted in increased ryegrass growth, when compared to compost. Ali (2011) found that vermicomposted cotton residues had reduced phytotoxicity when compared to compost made from the same feedstock, but resulted in slower ryegrass growth, while Frederickson *et al.* (2007) and Hernandes *et al.* (2010) found that growing tomato, marigold and radish, and lettuce, respectively, in composted or vermicomposted biodegradable municipal waste and cattle manure, respectively, had no effect on plant growth.

Vermicomposting as a compost maturation treatment has been shown to increase nutrient content and organic matter degradation, to reduce C/N content, to increase microbial activity, and to increase plant growth effects (Vinceslas-Akpa & Loquet, 1997; Tognetti *et al.*, 2005, 2007; Lazcano *et al.*, 2008; Vivas *et al.*, 2009), when compared to composting alone. Its improved quality and increased market value over compost makes it an attractive treatment method for agricultural biodegradable wastes.

#### **1.4 Vermicompost for horticultural use**

When developing growing medium in horticulture, especially for use in pots, the medium needs to be light, friable, water retaining but resistant to water logging, have

a low conductivity, a moderate nutrient content and be free from physical, chemical and biological contamination. Peat is commonly used as the main component of such growing media, usually mixed with amendments such as lime, fertiliser, coconut coir, perlite and vermiculite to create a multi-purpose growing medium or one for specific purposes, e.g. as for seed germination or potting-on.

In 2007, 29 million m<sup>3</sup> of peat was used to create horticultural growing media in Europe, of which 59% was used in the professional market, including the production of mushrooms, and the remainder in the domestic or 'hobby' market (Altmann, 2008). Peat harvesting mainly occurs in central and north America, Asia, and Europe (World Energy Council, 2006). In Ireland, 50,000 ha of blanket bog has been impacted upon by industrial harvesting (Bullock *et al.*, 2012). Peat is a non-renewable resource, and where peatland remains intact, it provides a range of ecosystem services, including hydrological services, CO<sub>2</sub> sequestration, wildlife habitat and cultural aspects (Bullock *et al.*, 2012). Any strategy which can reduce the extraction of peat without impacting on the end product quality, or even improve it, should be welcomed. The United Kingdom, for example, now aims to reduce and possibly exclude the use of peat in growing media by 2030 in the hobby and professional market under the UK Government peat reduction targets (Department of Environment Food and Rural Affairs, 2011). To meet these targets, alternative materials need to be found to reduce the use of peat as a growing medium constituent, or to recycle peat in horticulture.

Vermicompost can be characterised as dense, with a high water holding-capacity, nutrient content (compared to peat, coconut coir and other growing media components) and conductivity. It can be phytotoxic at high concentration and therefore it is usually used in low to moderate amounts of 5-50% in growing media.

As with compost, vermicompost characteristics can vary widely and are dependent on the initial feedstock material used (Table 1.3). For example, Bachman & Metzger (2007) found that when vermicompost made from two different manure sources, cattle and pig, were mixed with a peat based-growing medium, the different manure sources affected physical and chemical characteristics of the growing medium to different extents. Warman & Anglopez (2010) also found that vermicomposts made from different municipal and agricultural waste sources had different effects in plant germination assays. The nutrient availability of vermicompost is largely dependant on the original nutrient status of the feedstock material as well as other parameters such as the C/N ratio, processing time and vermicompost maturity. When comparing composts and vermicomposts made from the same feedstock some authors have found that vermicompost had a higher nutrient content, especially total N than the corresponding compost (Short *et al.*, 1999; Ngo *et al.*, 2011). This may be due to the stimulation of bacteria in the worm gut (Drake & Horn, 2007) which could contribute to enhanced nutrient availability.

Table 1.3 Nutrient analyses of vermicomposts derived from a number of different feedstocks

Vermicompost Feedstock:	-----Total Nutrients-----			-----Plant-Available Nutrients-----			
	N	P	K	NH <sub>4</sub> -N	NO <sub>3</sub> -N	PO <sub>4</sub> -P	K
	-----%-----			-----mg L <sup>-1</sup> -----			
Food waste with cardboard (50:50)	1.8	0.5	2.0	<0.01	172	8	2050
Horse manure	1.7	0.7	1.8	<0.01	110	269	1370
Sewage waste with horse manure (33:66)	2.8	1.3	0.9	3	587	347	818
Sewage waste with horse manure (50:50)	3.5	2.0	0.6	17	611	335	425
Horse manure with seaweed (94:6)	1.7	0.6	2.3	<0.01	20	232	1740
Horse manure with spent brewers grain (50:50)	2.7	1.2	1.5	2	453	477	756

The stability and maturity of vermicompost are two important parameters to consider when determining whether it is suitable for horticultural use. Stability is a measure of microbial activity and is commonly determined by measuring the oxygen uptake rate, dehydrogenase activity, carbon dioxide production rate, or heat generated as a result of microbial activity. Maturity is associated with the plant-growth potential of compost and its phytotoxicity, and can be assessed using a number of parameters such as C/N and  $\text{NH}_4/\text{NO}_3$  ratios, microbial stability and seed germination and root length assays (Bernal *et al.*, 1998).

The additional nutrient content of vermicomposts is beneficial for use as a growing medium additive though the physical properties of vermicompost may limit its suitability. As previously mentioned, growing medium needs to be light, friable, water retaining but resistant to water logging. Vermicompost is a dense material when compared to peat, and therefore its addition can increase the weight and therefore the transport cost of the growing medium (Schmilewski, 2008). The addition of vermicompost to a peat-based growing medium was also found to reduce the air space and porosity of the growing medium (Atiyeh *et al.*, 2001), which may have negative effects on plant growth.

Previous vermicompost growth trials have shown significant increases in fruit yield in tomato (Atiyeh *et al.*, 2000; Arancon *et al.*, 2003), pepper (Arancon *et al.*, 2003, 2004), and strawberry (Arancon *et al.*, 2003, 2006). As well as increased fruit yield, vermicompost has also been shown to increase shoot and root biomass in lettuce (Papathanasiou *et al.*, 2012), tomato (Bachman & Metzger, 2008; Lazcano *et al.*, 2009) and ornamental flowers (Atiyeh *et al.*, 2002; Bachman & Metzger, 2008). Vermicompost can increase plant growth, both as a fertiliser, and as a biostimulant (biostimulant; effects over and above nutritional effects). In a trial evaluating the

fertiliser and biostimulant properties of pig manure vermicompost, Atiyeh *et al.*, (2001) showed that shoot length and shoot dry length of tomato plants were significantly increased when vermicompost was used as a growing medium amendment with or without fertigation. Papathanasiou *et al.* (2012) also found that lettuce biomass increased with vermicompost application when compared to a fertilised and unfertilised control. Root dry weight was also increased compared to a fertilised control (Atiyeh *et al.*, 2002), but not consistently (Atiyeh *et al.*, 2001).

Vermicompost and vermicompost extracts have also shown potential as a fungal suppressant of plant pathogens such as *Rhizoctonia* (Chaoui *et al.*, 2002; Simsek Ersahin *et al.*, 2009), *Pythium* (Chaoui *et al.*, 2002; Jack, 2012), *Verticillium* (Chaoui *et al.*, 2002), and *Fusarium* (Szczecz, 1999), amongst others. It is not yet fully understood how vermicompost suppresses pathogens. The majority of authors suggest that beneficial bacteria in the vermicompost out-compete these pathogens (Szczecz, 1999; Chaoui *et al.*, 2002; Simsek Ersahin *et al.*, 2009; Jack, 2012). Others conclude that vermicompost may induce plant resistance, or cause the plant to produce antifungal compounds (Meghvansi *et al.*, 2011).

Vermicompost and vermicompost extracts have also been shown to reduce the severity of pest damage such as cucumber beetles, tobacco and tomato hornworm, mealy bugs and aphids (Arancon *et al.*, 2005; Yardim *et al.*, 2006; Edwards *et al.*, 2010), as well as others. It is hypothesised that the amount of water-soluble compounds, such as phenols, are increased in plants grown with vermicompost and vermicompost extract, thus reducing the severity of pest damage to plants (Yardim *et al.*, 2006; Edwards *et al.*, 2010) and also possibly due to the slower mineralisation of nutrients in the vermicompost treatments (Arancon *et al.*, 2005; Yardim *et al.*, 2006), although the exact mechanism is still under discussion (Arancon *et al.*, 2005).

Little work has been carried out on the effect of vermicompost and vermicompost extracts on abiotic stress. Ahmad *et al.* (2009) has shown that vermicompost had positive plant growth effects on ginger grown in saline soil, by increasing the fresh and dry weight of the shoot and rhizome, as well as increasing the chlorophyll, carbohydrate and protein content of the plant. Similar increases in stress tolerance in saline soils were seen by Ahmad & Jabeen (2009) in the growth and yield of field-grown sunflowers treated with vermicompost. In contrast, Sallaku *et al.* (2009) found that vermicompost did not increase cucumber transplant growth following fertigation with saline irrigation water. Apart from saline stress, vermicompost extract and vermicompost-derived humic acids were also found to alleviate drought stress in tomatoes (Chinsamy *et al.*, 2013; García *et al.*, 2014).

### **1.5 Spent mushroom compost as a vermicompost feedstock**

One agricultural by-product which potentially lends itself very well to vermicomposting and horticultural use is spent mushroom compost (SMC). SMC is a widely-available by-product of the mushroom industry, with approximately 200,000 tonnes produced in Ireland every year (Teagasc Mushroom Stakeholder Consultative Group, 2013). Further to this, it is mainly produced in a small region around the border counties of Ireland and Northern Ireland (Williams *et al.*, 2001). The majority (72%) of the material is applied to land (Maher *et al.*, 2000). As transport of this material is difficult due to its low bulk density, the production of such large quantities in a small region leads to an excess of P and K in these border counties, when compared to the theoretical capacity for fertilisers in the region (Maher *et al.*, 2000). Currently, SMC has little or no value as a soil enhancer, due to expensive transport and land-spreading costs. This by-product could be further

treated and improved by vermicomposting, converting it into a high-value product for use in amateur and professional horticulture.

SMC originates from mushroom compost, which typically consists of partially-composted farmyard manure, commonly horse and chicken manure. These materials, along with straw, gypsum and water are composted at high temperatures, approximately 80°C, for short periods of time (up to 2 weeks) to achieve pathogen destruction and weed seed deactivation. After this thermophilic composting stage, the material is known as phase I mushroom compost (Williams *et al.*, 2001). To make phase II mushroom compost, the compost is placed in pasteurisation units, and maintained at lower temperatures (40-60°C) for approximately one week to reduce the ammonia content to below 10 ppm (Williams *et al.*, 2001), making the compost suitable for mushroom mycelium culture. The mushroom mycelium, as an inoculated monoculture on grain, is then added and incubated for a further two to three weeks until colonised (phase III mushroom compost). At this stage, the mushroom compost is sold to the mushroom grower, who lays the phase III mushroom compost on shallow mushroom culture beds and adds a layer of casing (Jordan *et al.*, 2008), usually consisting of moss peat and lime. The mushroom grower can then proceed to harvest mushrooms for approximately 8-10 weeks, after which, the compost can no longer be re-used for mushroom growing (Maher *et al.*, 2000) and is known as 'spent' mushroom compost.

SMC is a very suitable feedstock for vermicomposting as it;

- is partially composted
- has been through thermophilic pasteurisation
- is a very consistent product

- is not contaminated with plastic, glass or heavy metals
- contains the appropriate C/N ratio for vermicomposting
- does not require a waste facility permit to handle/process
- is a very cheap material to purchase, at times, free

Other authors have demonstrated that SMC is a very good feedstock for vermicomposting (Tajbakhsh *et al.*, 2008a, 2008b; Abu Bakar *et al.*, 2014), but little work has been carried out on the effect of vermicomposted SMC on plant growth.

## **1.6 Research objectives**

The objectives of this study are to evaluate the effect of vermicomposting as a post-stabilisation method for compost and specifically determine whether vermicomposting offers opportunity for conversion of spent mushroom compost into value-added products.

It aims to evaluate the effects of vermicompost addition on the physical, chemical and biological properties of growing medium and to assess its efficacy when used as an additive in a peat-based and a peat-reduced growing medium.

It will investigate the effect of vermicompost, when used as a growing medium additive, on growth, development and yield quality of crops with different economic sinks, and will determine whether vermicompost increases plant growth under conditions of abiotic stress.

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# Chapter 2

Effect of compost and vermicomposted  
compost on shoot and root growth of  
lettuce (*Lactuca sativa* cv. Webb's  
Wonderful)

## Abstract

Immature compost can have negative plant and soil effects such as phytotoxicity, soil N immobilisation, and reduced plant growth, especially when used in horticultural applications e.g. as a growing medium. Composting involves three main stages; sanitisation, stabilisation, and maturation. This study investigates the use of vermicomposting as a post-stabilisation method to increase the maturation rate of composted horse manure. It also compares the effects of three grades (ungraded, >3 mm and <3 mm particle size), and increasing concentrations (0, 10, 20, 50, and 75% (vol/vol) in a peat-based growing medium) of the compost and the corresponding vermicomposted compost on lettuce shoot and root growth. Compared to composting, vermicomposting reduced the pH, C/N ratio and increased the electrical conductivity and nutrient content. Mean shoot fresh and dry weights were significantly higher in plants grown with vermicompost, compared to those grown with compost. The addition of either amendment increased root fresh and dry weight and reduced root water content significantly. Mean shoot fresh and dry weights were significantly higher in plants grown with the 10 and 20% amendment concentrations than in all other concentrations, while plants grown with 50 and 75% concentrations had shoot fresh and dry weights significantly higher than those grown with 0% concentration. Shoot water content responded differently with increasing concentration of either vermicompost or compost amendments. Grading of the different amendments affected shoot dry weight only, increasing shoot dry weight in the ungraded and small grade amendments, compared to plants grown in the large grade amendments. Vermicomposting increased the rate of maturation, resulting in significantly larger plants, with reduced conductivity stress and root/shoot ratio, especially at higher amendment concentrations.

**Keywords:** composting, vermicomposting, growing medium, maturation, phytotoxicity

## 2.1 Introduction

The recovery of biodegradable wastes by composting is encouraged through EU waste reduction targets (Council Directive 1999/31/EC). A high-value use for compost is as a growing medium component in horticulture (Rosen *et al.*, 1993). Currently the use of compost for high-value purposes is restricted due to product quality issues. For example, as little as ca. 6% (250,000 m<sup>3</sup>) of compost produced in Germany is used for the professional and hobby growing medium market (Schmilewski, 2008). The benefits of compost use in horticulture include increased recycling of organic matter, reduced use of peat, presence of plant-accessible and slow-release nutrients (Nendel & Reuter, 2007; Tognetti *et al.*, 2008), as well as improved stress tolerance and disease resistance of plants grown in it (McKellar & Nelson, 2003; Borrero *et al.*, 2004; Tuitert *et al.*, 2007; Walker & Bernal, 2008).

When using compost in horticulture, the main considerations are soluble salts, contamination and maturity (Gouin, 1993; Herity, 2003). High-salinity composts can be phytotoxic (Ribas *et al.*, 2009) and, by increasing the water potential in the soil, such composts reduce water uptake by the plant. Physical, chemical and biological contamination can be controlled through careful waste acceptance procedures and by maintaining adequate composting temperatures for long enough periods of time. The plant-growth potential of the compost (compost maturity) is dependent on the composting procedure, and when the compost facility operators deem the compost to be ready. Often space is a restriction in composting facilities (Herity, 2003), and compost can be deemed to be ready too quickly, resulting in sale of immature compost that is phytotoxic, and can become anaerobic during storage risking the production of dangerous and noxious gases such as H<sub>2</sub>S (Velusami *et al.*, 2013). This immature compost, when used in horticulture, will reduce plant growth, especially in

young plants (Zuconni *et al.*, 1981; Cruz *et al.*, 1990 (cited by Cuartero & Fernández-Munoz, 1999)). Compost maturity, is distinct to compost stability. Compost stability is a measure of the compost's microbial activity and is commonly determined by measuring the oxygen uptake rate, carbon dioxide production rate, or heat generated as a result of microbial activity. Compost maturity can be assessed using a number of parameters such as C/N and  $\text{NH}_4/\text{NO}_3$  ratios, microbial stability and seed germination and root length assays (Bernal *et al.*, 1998).

Compost is traditionally matured passively i.e. it is left to stand in static piles/windrows, with little or no forced aeration or mechanical turning. Mesophilic (<40°C) microorganisms recolonise the compost after the thermophilic stage, further breaking down the material (Bernal *et al.*, 2009). During maturation, the C/N ratio falls (Bernal *et al.*, 1998), ammonium is transformed into nitrate (Vega-Sánchez, 1987; Sánchez-Monedero *et al.*, 2001), pH decreases (Sánchez-Monedero *et al.*, 2001), further organic matter humification occurs (Binner *et al.*, 2011) and phytotoxic chemicals in the compost such as phenolic acids and volatile fatty acids are broken down (Zuconni *et al.*, 1981).

Compost can also be matured by vermicomposting, i.e. feeding the sanitised and stabilised material to worms. It is desirable that vermicomposting be carried out on sanitised biodegradable material as this thermophilic process eliminates human pathogens and weed seeds (Frederickson *et al.*, 1997; Ndegwa & Thompson, 2001; Lazcano *et al.*, 2008).

A limited number of authors have compared the efficacy of composting and vermicomposting, or some combination of both treatments, using the same initial feedstock. The results of these studies have varied, but in general have found that

vermicompost has a higher nutrient quantity, especially total N than the corresponding compost (Short *et al.*, 1999; Ngo *et al.*, 2011), although some studies showing no change in nutrient content (Frederickson *et al.*, 2007; Fornes *et al.*, 2012). Most authors found that vermicompost had an unchanged carbon to nitrogen ratio when compared to compost (Tognetti *et al.*, 2005; Frederickson *et al.*, 2007; Ali, 2011; Fornes *et al.*, 2012), and a lower pH and electrical conductivity (Tognetti *et al.*, 2005; Frederickson *et al.*, 2007; Fornes *et al.*, 2012). Most of these studies focused on the effect of vermicomposting on the end product quality, with few authors looking at the effect of compost and vermicompost made from the same feedstock on plant growth (Tognetti *et al.*, 2007; Frederickson *et al.*, 2007; Hernandes *et al.*, 2010; Ali, 2011).

Another common industrial practice when processing compost and especially vermicompost for market is screening the material into different size fractions (Munroe, 2004). This removes bulky materials and lumps of compost, improving the visual appearance of the compost (Rynk *et al.*, 1992). The smaller grades are usually sold on their own as a fertiliser, growing medium additive or as a soil enhancement product, while the larger grades are sold as mulch or for field application in agriculture, re-composted or re-fed to the worms to break down further, or disposed of as a waste product (Rynk *et al.*, 1992; Waste & Resources Action Programme, 2008).

The current study looks at the effect of vermicomposting on the end-product quality and also the effect of compost and vermicompost made from the same feedstock on lettuce shoot and root growth. This study also aims to compare the effects of ungraded and graded size fractions of both compost and vermicompost on the physical, chemical and plant growth parameters of the end-products.

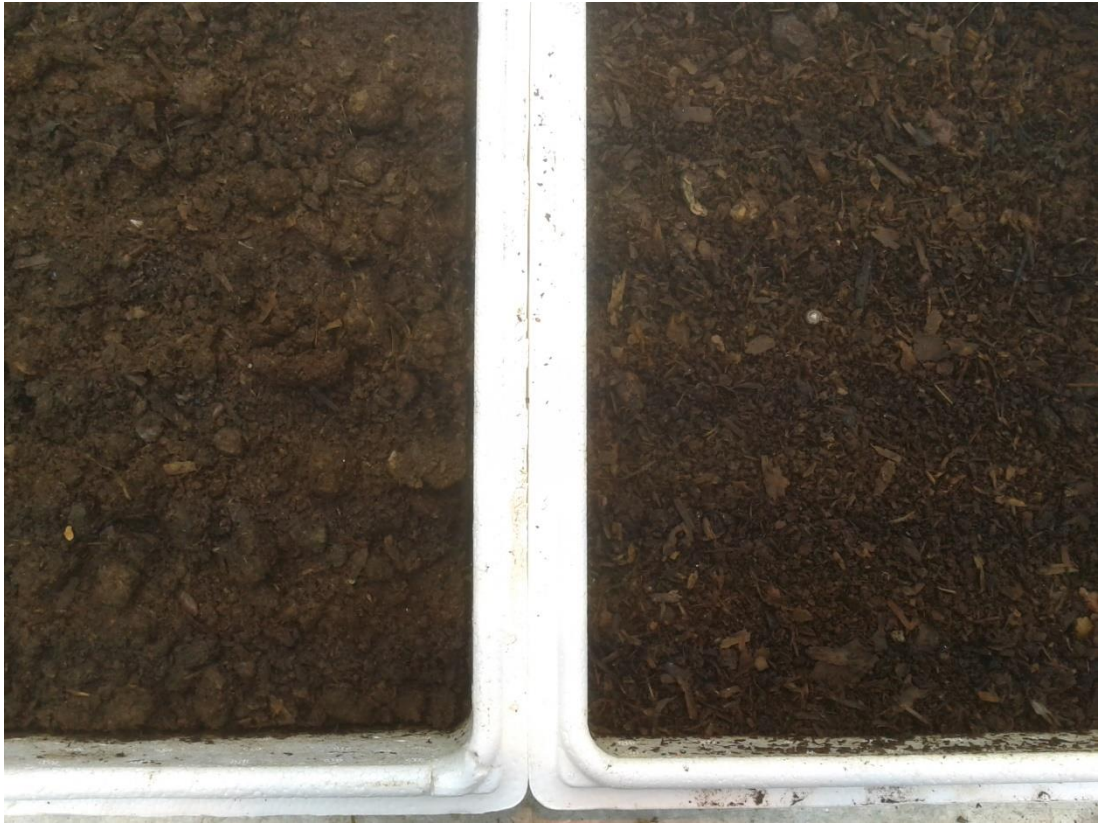


Figure 2.1. Composting microcosm (LHS) and vermicomposting microcosm (RHS) at the end of the vermicomposting process

## 2.2 Materials and methods

### 2.2.1 Compost maturation

Commercially available horse manure compost, Gee-Up<sup>®</sup> (Gee-Up, Blarney, Ireland), consisting of composted horse manure and bedding and composted using open-air turned windrows, was matured in two ways. It was conventionally matured by letting it compost for longer, allowing for further stabilisation, or by vermicomposting, feeding the material to worms. Maturation microcosms were set up using 10 L capacity, ventilated, sealable, polystyrene boxes (500 x 300 x 129 mm). Three replicates of each maturation treatment were set up and to each, 6 L of the horse manure compost was added. Mature vermicomposting worms (*Eisenia fetida*) were added to three of the microcosms (vermicomposting) (60 g per microcosm) but not the other three (composting) and the boxes were sealed. Neither

the compost nor vermicompost was manually turned during this time, as previous experience with this material had demonstrated that it would not go anaerobic. The boxes were kept in a glasshouse maintained at a minimum temperature of 18°C until the vermicomposting process was complete (assessed visually) (Figure 2.1). This took approximately 30 days, after which, the worms were manually separated from the vermicompost, and the compost and vermicomposted compost were stored in a cool dry place until use. For nutrient analysis and the plant growth study, the replicates of each treatment were pooled.

### 2.2.2 *Plant growth study*

The compost and vermicompost were each separated into three different grades based on size. The first grade (“ungraded”) was not sieved, the second grade consisted of the material which did not pass through a 3 mm sieve ( $>3$  mm) (“large grade”), and the third grade consisted of the material which passed through the 3 mm sieve ( $<3$  mm) (“small grade”). The different grades were diluted with a fertilised peat-based growing medium containing green waste compost, vermiculite, perlite and sand (hereafter referred to as the base growing medium). The different grades of compost and vermicompost were used in a factorial experiment as an amendment to this base growing medium at rates of 0, 10, 20, 50 or 75% vol/vol (25 treatments).

Seeds of crisphead lettuce (*Lactuca sativa* cv. Webb’s Wonderful) were sown on 16/08/2012 in the base growing medium without amendment. After seven days the seedlings were potted up into 0.3 L pots containing the different growing medium treatments. Ten replicate plants were used for each treatment. They were grown-on in a glasshouse in a replicated randomised block arrangement at a minimum temperature of 18°C.

The plants were harvested four weeks after transplanting. Prior to harvest, chlorophyll content of the youngest fully expanded leaf of each plant was measured in triplicate using a portable hand-held chlorophyll meter, (Minolta SPAD model 501, Konica Minolta Inc., Tokyo, Japan). The shoots of the lettuce plants were then cut, and fresh and dry weights obtained. Roots of replicates 4, 6 and 9 from each treatment were gently washed to remove adhering growing medium, patted dry, and fresh and dry weights were obtained. Plant biomass was dried at 60°C for seven days before weighing.

Pooled oven-dried samples (60°C for seven days) of each fraction of the vermicompost and compost amendments were subjected to physical and chemical analysis. Carbon and total nitrogen were measured using the Dumas method according to AOAC (1990). Total phosphorus and potassium was determined after digestion in aqua-regia (concentrated hydrochloric and nitric acid) and analysed using ICP-OES (Ministry of Agriculture Fisheries and Food, 1981; United States Environmental Protection Agency, 1996). Compacted dry bulk density was carried out in accordance with BS EN 13040:2000 (British Standards Institution, 2000).

Organic matter was determined by loss on ignition at 550°C for 24 h (Vincelas-Akpa & Loquet, 1997). Electrical conductivity (EC) and pH was measured in a 1:10 soil/distilled water suspension (Laos *et al.*, 2002). This suspension was placed on a shaking table for 1 hour, left to settle for 15 minutes, after which the pH of the solution was determined. The solution was then filtered through a Whatman Grade 1 filter paper, and EC was measured using a portable conductivity meter (WTW Cond 330i, WTW GmbH & Co., Weilheim, Germany). Organic matter, pH and EC analysis were carried out separately on the three replicates of vermicompost or

compost grades, while single measurements were carried out on pooled vermicompost or compost grades for the nutrient and bulk density analysis.

### *2.2.3 Statistical analysis*

Organic matter (OM), pH and EC results were analysed using parametric two-way ANOVAs, followed by Tukey's range test. Plant data were non-normal, but were normalised by square root transformation and analysed by parametric three-way ANOVAs. Data presented represents mean values of the untransformed data. Multiple comparison tests were conducted using Tukey's range test. Post-hoc tests were conducted on the main effect means where there were no significant interaction effects, or if the p-value of the interaction was an order of magnitude lower than the p-value of the main effect. Parametric linear and quadratic regressions were performed on leaf and root water contents with respect to increasing amendment concentrations, for the different grades and amendment types. Statistical analysis was carried out using IBM SPSS Statistics Package v.21.

## **2.3 Results**

### *2.3.1 Chemical and physical analysis*

Compared to composting, vermicomposting significantly reduced pH, increased EC ( $p < 0.001$ ), but had no effect on OM content in the end-product (Table 2.1). The pH was lowest in the base growing medium, followed by the vermicompost and the compost. EC was also lowest in the base growing medium, significantly lower than in the other growing medium components. Grade had a significant effect on the EC of both compost and vermicompost, with the small amendment grade having

significantly higher EC than the corresponding large amendment grade of compost and vermicompost (Table 2.1).

Nutrient analysis showed that vermicomposting the horse manure compost increased the N, P, and K concentrations in the ungraded and small grade vermicompost and reduced the N, P, and K concentration in the large grade vermicompost, compared to composting. Vermicomposting the compost reduced the C/N ratio in the ungraded and small grade samples, but made no difference to the large grade. Vermicompost bulk density was increased in the ungraded sample, reduced in the large grade, and was unchanged in the small grade sample, compared to the corresponding compost sample. The bulk density and nutrient content of compost and vermicompost were higher than those of the base growing medium.

Table 2.1. Values for pH, EC and % organic matter (OM) ( $\pm$ SD) of the peat growing medium, and compost and vermicompost grades

Amendment Type	Grade	pH	EC mS cm <sup>-1</sup>	OM %
Peat Growing Medium	ungraded	5.14(0.13)a	1.33(0.04)a	54(8)a
Compost	ungraded	7.11(0.03)c	2.83(0.03)b	51(3)a
	large	7.19(0.02)c	2.74(0.11)b	47(9)a
	small	7.11(0.03)c	2.94(0.05)b	56(2)a
Vermicompost	ungraded	6.88(0.03)b	3.36(0.16)cd	45(6)a
	large	6.87(0.03)b	3.23(0.06)c	47(4)a
	small	6.80(0.05)b	3.51(0.03)d	49(1)a
		df	ANOVA	
F value Amendment Type†		1	381.14	170.80
Sig.			***	***
F value Grade		2	8.26	11.49
Sig.			**	**
F value Amendment x Grade		2	4.85	0.28
Sig.			*	ns

Means in the same column followed by the same letter were not significantly different,  $p > 0.05$ , according to Tukey's range test. ns = not significant,  $* = p \leq 0.05$ ,  $** = p \leq 0.01$ ,  $*** = p \leq 0.001$  according to two-way ANOVAs. †For the two-way ANOVAs, only the compost and vermicompost amendment types were included as they were the only two amendments that were separated into grades.

Table 2.2. Nutrient analysis and bulk density of the peat growing medium, and compost and vermicompost grades

Amendment Type	Grade	N % w/w	P -----g/kg-----	K	C/N	Bulk Density g/l
Peat Growing Medium	ungraded	0.86	0.58	1.23	28	290
Compost	ungraded	1.42	5.13	10.49	19	620
	large	1.47	5.45	10.92	18	700
	small	1.57	5.88	11.15	17	670
Vermicompost	ungraded	1.53	5.64	11.61	16	760
	large	1.31	4.88	9.75	18	630
	small	1.65	6.22	11.96	15	670

### 2.3.2 Plant growth parameters

Both the compost and vermicompost amendments resulted in increased shoot weight compared to the unamended base medium (Table 2.3). Mean shoot fresh and dry weights were significantly higher in plants grown with vermicompost compared to plants grown with compost by an average of 15% and 12%, respectively. There was no effect of amendment type on root growth. Compost and vermicompost grades did not significantly affect shoot fresh weight nor root fresh and dry weight, but they did affect shoot dry weight. Mean shoot dry weight was significantly higher in plants grown with the small grade and ungraded amendments, followed by the large grade (Table 2.3).

Raising the amendment concentrations (averaged over compost and vermicompost) to 10 and 20% increased shoot fresh and dry weight significantly, compared to 0% amendment. For plants grown with 50% and 75% amendment, shoot weights was significantly increased compared to 0%, but significantly lower than for plants grown at 20% (Table 2.3). Increasing the amendment concentration resulted in significantly increased root fresh and dry weight. The highest root fresh weight was

in plants grown with 20% amendment concentration, and the highest root dry weight was in plants grown with 50% amendment concentration (Table 2.3).

Mean root/shoot fresh weight ratios were affected by amendment type and concentration, but not grade (Table 2.3). Compared to the compost amendment, plants grown with vermicompost had a significantly reduced (-15%) root/shoot fresh weight ratio. Plants grown with the highest amendment concentration, 75%, had significantly greater root/shoot ratio than plants grown at all the other amendment concentrations (Table 2.3). There was a highly significant interaction effect in shoot water content (Table 2.3) between amendment and concentration ( $p \leq 0.0001$ ), showing that the compost and vermicompost amendments did not cause a similar response in shoot water contents with increasing concentration. This relationship was further investigated using Tukey's range test and regression analysis (Figure 2.1).

Tukey's range test (data not shown) revealed that plants grown with no amendment to the base growing medium had the highest shoot water content, significantly higher than that for plants grown in ten of the twelve compost treatments, and for seven of the twelve vermicompost treatments. The only treatment where the shoot water content was significantly different in plants grown with compost and vermicompost amendments was the small grade vermicompost and compost amendments at 75% concentration, with vermicompost having higher shoot water content by 3.7%. The shoot water content of the small grade 75% compost amendment was also significantly lower than the other two grades of 75% compost (Table 2.3).

Regression analysis revealed different responses in shoot water content to increasing amendment concentrations of compost and vermicompost (Figure 2.1). Shoot water content from each grade followed more closely a negative linear relationship for the

Table 2.3 Effect of compost and vermicompost amendment on main effect means ( $\pm$ SD) of plant growth parameters recorded during harvest

Main Effect		Shoot Fresh Weight	Shoot Dry Weight	Root Fresh Weight	Root Dry Weight	Root/Shoot Fresh Weight	Shoot Water Content	Root Water Content	Chlorophyll
		g					%	%	SPAD
Amendment Type	Compost	10.27(4.28)a	0.67(0.28)a	3.07(1.27)a	0.17(0.10)a	0.31(0.07)b	93.43(1.64)	94.88(0.02)a	27.08(2.61)
	Vermicompost	11.82(4.48)b	0.75(0.30)b	3.05(1.08)a	0.16(0.10)a	0.27(0.04)a	93.83(1.03)	95.12(0.03)a	28.72(3.27)
Grade	ungraded	11.03(4.28)a	0.72(0.29)b	3.09(1.14)a	0.17(0.11)a	0.29(0.05)a	93.58(1.33)	94.74(0.03)a	27.75(2.84)a
	large	10.81(4.47)a	0.65(0.29)a	3.14(1.27)a	0.16(0.10)a	0.28(0.05)a	94.07(0.89)	95.43(0.02)a	27.93(3.08)a
	small	11.28(4.60)a	0.76(0.30)b	2.94(1.13)a	0.16(0.09)a	0.29(0.08)a	93.24(1.70)	94.82(0.02)a	28.01(3.29)a
Concentration	0%	5.88(2.33)a	0.29(0.12)a	1.50(0.33)a	0.04(0.03)a	0.27(0.04)ab	95.16(0.48)	97.28(0.02)a	24.12(1.38)
	10%	14.09(2.65)d	0.87(0.17)cd	3.52(0.56)c	0.19(0.06)b	0.25(0.05)a	93.77(1.03)	94.48(0.02)b	28.83(2.38)
	20%	14.58(3.35)d	0.95(0.21)d	3.93(0.97)c	0.21(0.07)b	0.28(0.04)ab	93.46(0.75)	94.68(0.01)b	28.67(2.50)
	50%	11.90(3.15)c	0.80(0.20)c	3.73(0.73)c	0.22(0.07)b	0.30(0.05)b	93.22(1.16)	94.16(0.02)b	29.07(2.73)
	75%	8.70(3.25)b	0.61(0.20)b	2.64(1.03)b	0.16(0.13)b	0.35(0.08)c	92.68(1.76)	94.11(0.03)b	28.41(3.06)
		df	ANOVA						
F value Amendment Type		1	22.32†	11.83	0.00	0.27	18.68	12.02	0.24
Sig.			***	***	ns	ns	***	***	ns
F value Grade		2	0.65	7.55	0.46	0.38	0.11	18.21	0.81
Sig.			ns	***	ns	ns	***	***	ns
F value Concentration		4	113.24†	145.16	34.90	22.33	12.21	53.60	5.63
Sig.			***	***	***	***	***	***	***
Amendment x Grade		2	ns	ns	ns	ns	ns	*	ns
Amendment x Concentration		4	***	ns	ns	ns	ns	***	ns
Grade x Concentration		8	ns	*	ns	ns	ns	***	ns
Amendment x Grade x Concentration		8	ns	ns	ns	ns	*	***	ns

ns = not significant, \*= $p \leq 0.05$ , \*\* =  $p \leq 0.01$ , \*\*\* =  $p \leq 0.001$ , according to three-way ANOVAs. Means in the same column, within the same main effect, followed by the same letter were not significantly different,  $p > 0.05$ , according to Tukey's Range Test. †  $p \leq 0.0001$

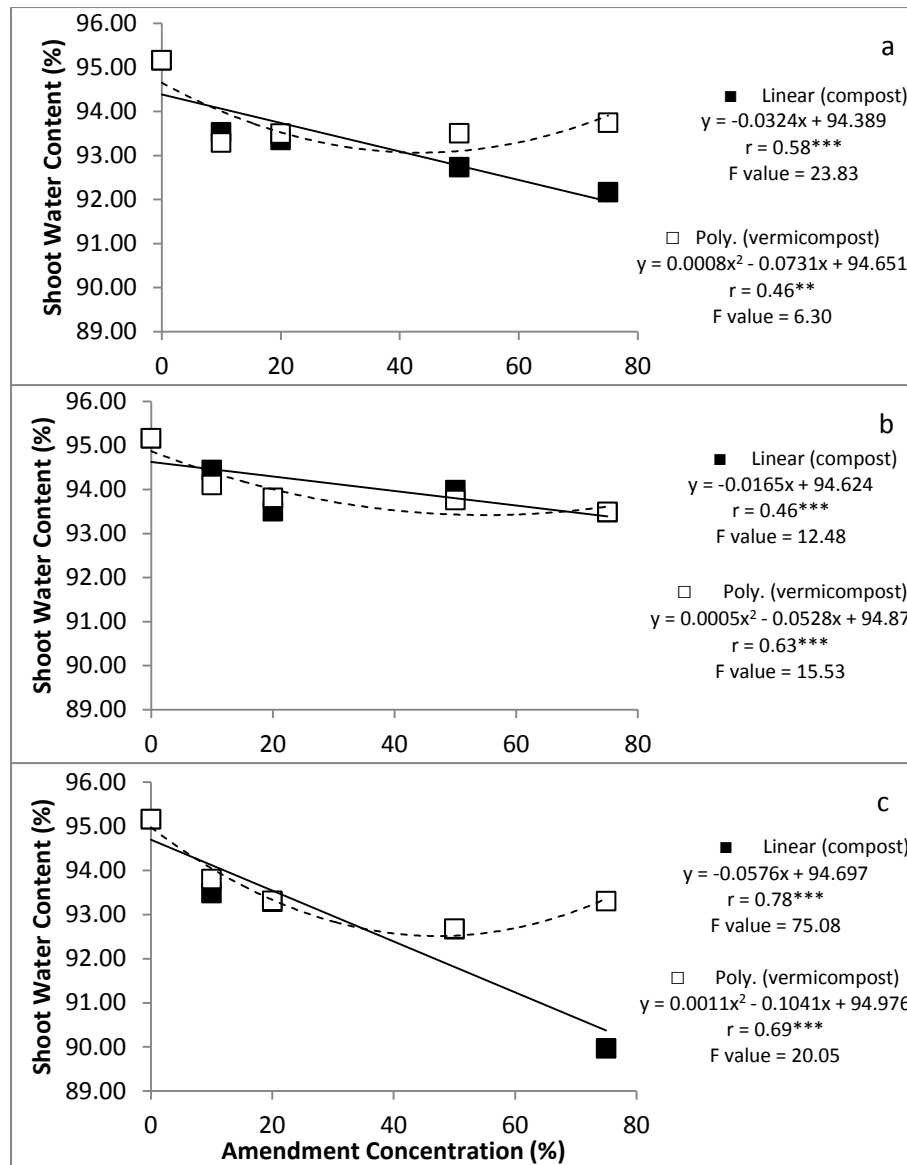


Figure 2.1 (a-c) Response of mean shoot water content of plants grown with ungraded (a), large grade (b), and small grade (c) compost and vermicompost amendments with increasing concentration. Significant  $r$  values ( $p \leq 0.05$ ) are denoted by  $*$  =  $p \leq 0.05$ ,  $**$  =  $p \leq 0.01$ ,  $***$  =  $p \leq 0.001$ .

compost amendments, and a quadratic relationship for the vermicompost amendments (Figure 2.1), with the 50% vermicompost amendment resulting in the lowest shoot water content compared to the other vermicompost amendment concentrations. The large grade amendments had the least effect on shoot water content (Figure 2.1).

Root water content was significantly affected by amendment concentration, but not by amendment type or grade (Table 2.3). The addition of 10-75% amendment significantly reduced root water content by an average of 3%, and on average, root water content was higher than shoot water content. There were no significant interaction effects, suggesting a similar dose response of root water content to the two amendments. This was also seen in regression analysis of root water content (data not shown) where there were no clear differences in root water response between compost and vermicompost, unlike what was seen with shoot water content (Figure 2.1). Only two amendments formed significant linear correlations with increasing concentration: ungraded compost,  $r = 0.68$  ( $F_{1,13} = 11.37$ ,  $p \leq 0.01$ ), and small grade compost,  $r = 0.66$  ( $F_{1,13} = 10.07$ ,  $p \leq 0.01$ ). Root water content was negatively affected by increasing the concentrations of these two compost grades, while there was no significant linear or quadratic relationship with any of the vermicompost grades, or the large grade compost.

Tukey's range test (data not shown) found that plants grown with no amendment had the lowest chlorophyll content, significantly lower than all the vermicompost treatments, and half of the compost treatments ( $F_{24,226} = 10.89$ ,  $p \leq 0.001$ ). The only treatments where plants grown in compost or vermicompost produced significantly different chlorophyll contents were the small grade vermicompost and compost at 75% concentration, with the plants grown with vermicompost having a higher mean chlorophyll content by 26%.

## **2.4 Discussion**

Vermicomposting had a significant effect on compost characteristics (Table 2.1 and Table 2.2). Although statistical analysis was not possible because replicates were

pooled for analysis, the trend of increased N, P and K in all grades of vermicompost, except for the large grade (Table 2.2), suggests that vermicomposting did increase the nutrient quantity in the end-product, as was also reported by Vincelas-Akpa & Loquet (1997) and Ngo *et al.* (2011). The increased EC in all of the vermicompost grades, compared to the compost grades, is thought to be due to the increased nutrient content also seen in the vermicompost (Table 2.2), and possibly an increased nutrient 'concentration effect' (Bernal *et al.*, 1996) in the vermicompost, compared to the compost, as also indicated by increased bulk density in ungraded vermicompost, compared to ungraded compost. The increased EC in the small grade compost and vermicompost compared to the corresponding large grade amendments is also thought to be due to these factors. Frederickson *et al.* (2007) reported significantly lower levels of N, P and K in screened vermicompost made from source-segregated household waste, when compared to screened compost of the same material. This was attributed either to leaching or to increased digestion of paper by the worms, lowering the nutrient content of the vermicompost.

The C/N ratio is commonly used as an indicator of compost maturity (Bernal *et al.*, 1998; Herrera *et al.*, 2008; Lazcano *et al.*, 2008), with a compost C/N ratio of 15-20:1 being suitable for nursery plant production (Rosen *et al.*, 1993). As the worms digest the material, they fragment it further through the grinding action in their gizzard. This increases the surface area of the material, allowing for further colonisation by bacteria and fungi, and increased breakdown rate. This action also further concentrates the material, as seen in the increase in bulk density and electrical conductivity of the ungraded vermicompost, compared to the ungraded compost, and release of organic acids such as humic and fulvic acids, reducing pH (Elvira *et al.*, 1998). The combined C/N change and reduced pH suggests that worms accelerated

maturation (Ali, 2011), when compared to traditional compost maturation techniques (Frederickson *et al.*, 1997; Ngo *et al.*, 2011).

Many of the chemical and physical parameters remained unchanged (i.e. C/N, bulk density) or lower (N, P and K contents) in the large grade vermicompost compared to the large grade compost. This may be explained by the worms' feeding habit. The fact that there are large particles in the large grade vermicompost amendment indicates that the worms did not ingest that particular material, although it is important to note that some of these particles could be aggregates of smaller particles which would have been digested by the worms. As much of this material may not have gone through the gut of the worm, it was not all concentrated or amended by vermicomposting, and hence, it had similar chemical characteristics to the same grade of compost.

The reduction of N, P, and K in this large grade vermicompost is interesting as it might suggest that the worms favour feeding on higher-nutrient materials, leaving lower-nutrient feedstock undigested, and leading to a higher nutrient concentration in the smaller particles present in the ungraded and small grade vermicompost, than in the large grade vermicompost. Frederickson *et al.* (2007) also suggested the action of the worms' feeding habit may influence the nutrient concentration of the finished vermicompost. It is hypothesised that the material in the large grade vermicompost (evidenced by its size) was not amended by the worms to the same extent as the ungraded and small grade vermicompost, and had similar chemical and physical characteristics to the compost grades. This suggests that it is the action of the material being eaten by the worms that improves its characteristics.

The pH, EC, nutrient content and bulk density of the compost and vermicompost were higher than those of the base growing medium (Table 2.1 and Table 2.2). This is due to the high nutrient content and salt content of animal manure, and the naturally acidic peat in the peat-based base growing medium. Arancon *et al.* (2004) also found that vermicomposted food waste had a higher pH, EC and nutrient content, and Atiyeh *et al.* (2001) found a higher EC, bulk density and NO<sub>3</sub>-N content in pig manure vermicompost, when compared to a commercially available, fertilised peat-based growing medium.

The root and shoot growth of plants grown with the compost and vermicompost amendments were higher than in the base growing medium with no amendment. Vermicompost increased shoot growth (fresh and dry weight) significantly (Table 2.3) when compared to compost, even with increased conductivity in the former. Tognetti *et al.* (2005) also reported that vermicompost significantly increased ryegrass growth by 15-17%, compared to compost made from the same feedstock. This may be due to increased nutrient content, reduced phytotoxic effects (Ali, 2011), or more suitable pH in the more mature vermicompost compared to the compost. Root/shoot ratio was reduced in plants grown with vermicompost amendment compared to those grown with compost. This was due to the increased shoot fresh weight biomass and the unchanged root fresh weight biomass with the vermicompost amendment, compared to the compost amendment.

Root growth was not significantly affected by amendment type. Root growth is less sensitive than shoot growth to salinity stress (Cuartero & Fernández-Muñoz, 1999; Shannon & Grieve, 1999), such as could be caused by high-EC vermicomposts and composts. This might explain the similar root growth compared to increased shoot growth with vermicompost, compared to compost. This trend was also seen in shoot

and root water content (Figure 2.1 and Table 2.3). The response of shoot water content to increasing concentrations of compost had a negative, linear trend, while plants grown with vermicompost followed a more complex response, indicating that, at high concentrations of vermicompost (>50%), plants use succulence as a mechanism for coping with salinity stress (Cuartero & Fernández-Muñoz, 1999; Ouhibi *et al.*, 2014). Plants grown with vermicompost and exposed to salinity stress had higher water contents and were more capable of escaping this stress than were those grown with the compost amendment. Again, as with root growth, the root water content was not affected by amendment type (Table 2.3), indicating that roots are less affected by salinity than shoots. It is difficult to ascribe a reason why the vermicompost amendments induce this stress escape response in lettuce, compared to the compost amendments. Some authors have suggested that beneficial bacteria (Pathma & Sakthivel, 2012) and humic acids (Arancon *et al.*, 2004) in the vermicompost are responsible for biostimulant plant growth, stress tolerance and disease resistance.

Amendment grade had an effect on shoot dry weight only. The EC of the large grade amendments were lower than the small grade amendments (Table 2.1). The lower EC reduced the osmotic potential in the root zone resulting in increased plant-water uptake and increased shoot water content in the large grade amendments. The increase in shoot dry weight in the small grade amendment is possibly due to increased N, P and K in this amendment compared to the large and ungraded amendments, resulting in higher biomass allocation. Fitzpatrick *et al.* (1994) found that there was no difference in growth of roots and shoots of West Indian mahogany (*Swietenia mahagoni* L.) when grown in screened (<19 mm) and unscreened sewage sludge and green waste compost. On the other hand, Frederickson *et al.* (2007) found

that screening (<10 mm) both compost and vermicompost made from the same feedstock (source-segregated household waste) reduced the nutrient content, pH and EC, and increased the C/N of the vermicompost compared to the compost.

The response of shoot and root growth to increasing concentrations of compost or vermicompost amendments resulted in an increase in plant fresh and dry weight (compared to 0%) at lower concentrations followed by a reduction in plant weight at higher concentrations. Perez-Murcia *et al.* (2006) also reported a decrease in broccoli growth when composted sewage sludge was incorporated into growing medium at a concentration of 50%. In the current study, plant growth reduced as amendment concentration increased from 20 to 50% or 50-75%. Reduced plant growth is likely due to increased conductivity and excessive nutrient concentrations (Arancon *et al.*, 2008), and also possibly due to phytotoxic compounds in the high-amendment treatments (Delgado *et al.*, 2010). As shoot biomass decreased with increasing conductivity, root biomass also decreased, but not to the same extent. This increased the ratio of root biomass to shoot biomass with increasing amendment concentration (Table 2.3). Root water content also fell with increasing amendment concentration, again, likely due to increasing conductivity.

The addition of compost at most concentrations, and vermicompost at all concentrations resulted in significantly increased chlorophyll content in lettuce plants compared to those plant grown in the unamended control. Leaf chlorophyll content is an indicator of plant productivity and is an indirect measure of leaf nitrogen (Xue & Yang, 2009). Reductions in chlorophyll content have occurred due to plant stress (Carter & Knapp, 2001). Plants grown in the small grade compost-amended base growing medium, at 75% concentration, had significantly lower chlorophyll content than those grown in the same concentration and grade of

vermicompost. Plants grown at this concentration of compost may have been affected to a greater extent from EC or phytotoxicity stress than plants grown at this concentration of vermicompost, and therefore the improved stress tolerance in the vermicompost-grown plants may also explain the increased chlorophyll levels. Wilson *et al.* (2001) found a negative linear relationship between chlorophyll content in the tropical herb *Orthosiphon stamineus* (popularly known as Java tea) and increasing concentration of compost used in peat and coir-based growing media. The authors proposed that nutrient mineralisation was slow, resulting in insufficient supply of nutrients, especially at higher concentrations.

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# Chapter 3

Comparison of composted and  
vermicomposted spent mushroom compost  
as components of horticultural growing  
media

## Abstract

Waste management policy supports the conversion of biodegradable wastes into value-added products. Spent mushroom compost (SMC) is a widely-available, low-value by-product of the mushroom industry. It has little or no value as a soil enhancer, mainly due to high transport and land-spreading costs. The main objectives of this study were to identify whether SMC could be used as a major component of peat-reduced horticultural growing media, and to investigate if it could be vermicomposted and used as a growing medium additive. Tomato seedlings were transplanted into an industry-standard peat-based growing medium, or into a peat-reduced (50/50 vol/vol vermiculite to SMC) growing medium, with both growing media being prepared with and without the amendment of 10% vermicomposted SMC. Plants were harvested on days 53 and 170. The peat-based growing medium had higher shoot and root growth, earlier flowering dates, and increased number and fresh weight of fruits compared to the peat-reduced growing medium. The addition of vermicomposted SMC had no effect on plant growth 53 days after sowing, but, 170 days after sowing, the addition of vermicompost significantly increased shoot fresh weight, shoot water content, fruit dry weight, and fruit quality in both growing media. The addition of vermicompost to the peat-based growing medium also significantly increased shoot dry weight and the percentage marketable yield, and reduced the incidence of blossom end rot. This study demonstrates that vermicomposted SMC can represent a suitable growing medium amendment, especially when added to a peat-based growing medium.

**Keywords:** growing media, spent mushroom compost, vermicompost, blossom end rot

### 3.1 Introduction

Spent mushroom compost (SMC) is a by-product of the mushroom industry. Ireland is one of Europe's largest mushroom producers, producing 63,600 tonnes of mushrooms in 2012 and approximately 200,000 tonnes of SMC every year (Teagasc Mushroom Stakeholder Consultative Group, 2013). SMC is primarily disposed of by land spreading, although it has little or no commercial value; contractors charge approximately €10 tonne<sup>-1</sup> to spread SMC ([www.carbolea.ul.ie](http://www.carbolea.ul.ie)).

Mushroom compost is a very consistent product used in the production of button mushrooms (*Agaricus bisporus*). It is made largely from wheaten straw, poultry litter, horse manure and gypsum. The mushroom compost is inoculated with mushroom mycelium, and delivered to the mushroom grower who adds a layer of peat casing, which is a source of moisture for the mushrooms. Two to three flushes of mushrooms are grown, taking approximately six to eight weeks, after which the mushroom compost and peat casing mix is removed from the farm and is known as 'spent' mushroom compost. SMC is approximately 20% peat by volume.

In Europe, there is a very strong demand for peat in horticultural growing media. A recent study calculated a market value for horticultural peat of €1.26bn in 2005 (Altmann, 2008). Peat, as a non-renewable resource, is becoming less desirable as a growing medium, especially for the hobby market. SMC could be a viable material for use in peat recycling and peat reduction in growing media.

SMC is an actively decomposing, immature compost. Szmidt (1994) found that the temperature of SMC increased rapidly once emptied and piled. Due to its immaturity, fresh SMC can be unsuitable for land spreading. As the material is still

decomposing, it can use up nitrogen and oxygen in the soil, and also be a source of phytotoxic compounds (Tiquia *et al.*, 1996).

SMC, both fresh and matured, is most commonly used in agricultural and horticultural applications. When applied to cereal (Courtney & Mullen, 2008) and vegetable (Maynard, 1994) crops, at high application rates (50-112 t/ha), SMC has been shown to result in yields similar to those achieved with chemical fertilisers. When used as an amendment to horticultural peat-based growing medium, SMC was found to be suitable at low concentrations (25% vol/vol dry weight), for the production of tomato, courgette and pepper seedlings (Medina *et al.*, 2009). Higher concentrations (>50%) of SMC was found to be unsuitable for vegetable seedling growth (Medina *et al.*, 2009), but high concentrations of SMC was found to be a suitable additive for the production of containerised shrubs (Chong *et al.*, 1994).

High electrical conductivity (EC) of SMC had been described in previous studies as the main phytotoxic component when used on containerised plants (Guo *et al.*, 2001; Ribas *et al.*, 2009). Jordan *et al.* (2008) suggested that the high K content, and, to a lesser extent, Na content in SMC is the principal contributor to the high EC, thus limiting the use of SMC as a potting substrate.

SMC can be actively or passively matured to improve its soil-enhancing properties (Brunetti *et al.*, 2009). Commonly, SMC is piled on farmland and matured passively for up to a year before it is land spread (Velusami *et al.*, 2013). Without aeration, and when piled in large enough quantities, SMC can start to decompose anaerobically (Guo *et al.*, 2001; Velusami *et al.*, 2013). Anaerobic decomposition results in the production of dangerous and noxious gasses (Velusami *et al.*, 2013), phytotoxic compounds, and the reduction of nitrate and ammonium to ammonia and molecular

nitrogen (denitrification), thus reducing the fertiliser value of the compost (Bueno *et al.*, 2008).

An alternative to passive maturation is active maturation, whereby the compost is actively managed to speed up the maturation process. Active maturation practices include mechanical turning, forced aeration, (to increase oxygenation) and vermicomposting. Vermicomposting physically breaks the SMC down further by the action of worms and other decomposers. Actively re-composting SMC increases its stability and maturity (Brunetti *et al.*, 2009). Compost maturity can be described as the plant-growth potential of the compost, while compost stability is a measure of the compost's microbial activity. Szmidt (1994) found that re-composting SMC for 4-5 weeks generated a growing medium suitable for tomato production at 100% concentration.

Vermicomposting the SMC is an alternative to composting. Tajbakhsh *et al.* (2008) found that vermicomposting SMC increased its plant-available nutrient content and maturity, while Abu Bakar *et al.* (2014) showed that during vermicomposting, worms grew and multiplied more successfully in SMC than in other organic wastes. More research is needed to assess the effects of vermicomposted SMC on plant growth.

The main negative considerations when using SMC as a plant growth substrate are its immaturity, and high conductivity. In this study, SMC was matured in two ways: composting or vermicomposting. The EC of the matured SMC products was then reduced by mixing them with other growing medium materials. Composting the SMC is a high-throughput solution for the treatment of SMC, while vermicomposting is a low-throughput solution. Therefore, composted SMC was used

as a growing medium additive at a higher rate (50% vol/vol) than vermicomposted SMC (10% vol/vol). The composted SMC was mixed with vermiculite, an expanded silicate material commonly used in horticulture, to create a peat-reduced growing medium. The vermicomposted SMC was mixed with this peat-reduced growing medium, and also with a peat-based growing medium. Tomatoes were then grown in each growing media, with and without the addition of vermicomposted SMC, to evaluate the effect of these SMC products on plant growth and yield.

## **3.2 Materials and methods**

### *3.2.1 Compost maturation*

The SMC was obtained from a commercial mushroom producer in Co. Westmeath (Reilly Mushrooms Ltd., Athlone, Ireland) and matured in one of two ways. To compost the SMC, it was stored indoors and mechanically turned using a front-end loader once every two weeks for approximately 90 days. For vermicomposting, the SMC was fed to worms in a medium-scale (1 m<sup>3</sup> capacity) vermicomposting bin, with no mechanical turning or forced aeration. In this system, the compost was fed to the worms at a daily rate of approximately 5-8 kg/day by spreading the SMC on the surface of the bin. The worms used to vermicompost the SMC were a combination of epigeal worm species, mainly consisting of *Eisenia fetida*, but also other species, such as potworms (*Enchytraeus spp.*). Other decomposer organisms were also present in the vermicomposting bins, including a range of fungi, bacteria and other commonly occurring, soil-dwelling arthropods. When the vermicomposting process was complete (approximately 90 days), and the bin was full, worms were separated from the vermicompost using separating equipment. The vermicompost and compost

were collected on 16<sup>th</sup> Dec 2012, and stored in breathable sacks in a cool dry place until use.

### 3.2.2 *Nutrient analysis*

Nutrient analysis of the composted SMC, vermicomposted SMC, and peat-based growing medium was carried out on air-dried samples that were ground and passed through a 2 mm sieve (Jordan *et al.*, 2008). The analysis was carried out at the Aquatic Services Unit, University College Cork. Available nitrogen was extracted in potassium chloride (International Organization for Standardization, 2003) and measured using flow injection analysis (Lachat Quik-Chem 8000 FIA). For total nitrogen and total phosphorus, the material was first digested using the Kjeldahl method (Persson *et al.*, 2008). Ammonia was measured using flow injection analysis, phosphorus by the manual colorimetric method (Murphy & Riley, 1962), and potassium by flame atomic absorption spectroscopy (Varian, 1989). Electrical conductivity and pH was measured in a 1:10 soil/distilled water suspension (Laos *et al.*, 2002). This suspension was placed on a shaking table for 15 min, after which the pH of the solution was determined (Thermo Scientific Orion 3 Star). The solution was then filtered through a Whatman Grade 1 filter paper, and EC was measured using a portable conductivity meter (WTW Cond 330i, WTW GmbH & Co., Weilheim, Germany). All analyses were carried out on three replicate samples.

### 3.2.3 *Trial set-up and harvesting*

Seeds of an indeterminate tomato F<sub>1</sub> hybrid (*Solanum lycopersicum* cv. ‘Grande’) were sown on 20<sup>th</sup> Dec 2012 in a commercially available peat-based growing medium. They were germinated and grown on in a heated glasshouse maintained at a

minimum temperature of 18°C. Daylight was supplemented with 400 W artificial sodium vapour lamps, creating a 16 h photoperiod.

After 15 days, the tomato seedlings were transplanted into 1 L pots containing one of four different growing media, two peat-based and two peat-reduced. The first was a commercially available peat-based growing medium (Shamrock Multipurpose Compost, Bord na Móna, Kildare, Ireland) containing approximately 60% (vol/vol) limed peat moss to 40% green waste compost. The second peat-based growing medium consisted of the commercially available medium mentioned above, but with the addition of 10% vermicomposted SMC. The first peat-reduced growing medium was non-commercial and consisted of 50% composted SMC and 50% vermiculite. The second peat-reduced growing medium contained 50:40:10 composted SMC:vermiculite:vermicomposted SMC.

Forty plants were used in total, ten replicates for each of the four growing media. The plants were arranged in the glasshouse in a replicated randomised block design. The plants were fed 22 days after sowing, and every subsequent two weeks, with a commercially available soluble plant food, Miracle-Gro<sup>®</sup> (The Scotts Miracle-Gro Company, Ohio, US), containing 24:8:16 N:P:K, and the trace elements B (0.02%), Cu (0.07%), Fe (0.15%), Mn (0.05%), Mo (0.0005%) and Zn (0.06%) (w/w), at a dilution of 30 ml powder per 9 L tap water.

On day 53, replicates 1, 3, 5, 7, and 9 of each treatment were harvested. Plant fresh and dry weight, separated into shoots (leaves and stem) and roots, were recorded. Harvested plant biomass was dried at 60°C for a minimum of 7 days before weighing. At 53 days, the remaining five replicate plants from each treatment were potted up into 6 L pots containing the respective growing medium, and grown on

until fruiting. The plants were supported with stakes, side shoots were pinched out weekly, and the main stem was pinched out above the second truss. Flowering date (when the first flower was fully opened) of each plant was recorded. Pollination was aided by striking the stake of each plant three times a week during the flowering period. On day 81, to add to the volume of growing media available, an additional 3.5 L seed tray filled with the appropriate growing medium mix was put underneath each pot for the plants to root into.

On day 170, when the majority of the tomatoes were ripe (visual assessment), the last five replicates was harvested. Plant height, truss one height (measured as the distance between the base of the stem and position on the stem of truss one), truss two height (distance between the base of the stem and position on the stem of truss two), and shoot fresh and dry weight were recorded. Fruit parameters measured were number, fresh and dry weight of ripe and unripe fruits, fruit class (according to EC No. 543/2011), number of fruits with blossom end rot (BER), and percentage marketable yield.

The percentage marketable yield was calculated as:

$$\frac{\text{total number of fruits} - \text{number of fruits with blossom end rot}}{\text{total number of fruits}}$$

EC No. 543/2011 lays down the marketing standards for fruits and vegetables, including tomatoes, sold in the EU. Tomatoes are graded, under this regulation, into three quality classes. ‘Extra’ class is the highest quality class, and contains ‘superior quality fruits, that are firm and characteristic of the variety, free from greenback and free from all but the very slightest of superficial defects’. Class I is the second highest quality class and contains ‘good quality fruits, that are reasonably firm, free of cracks and greenback, with some slight defect allowed’. Class II is the lowest

quality class and contains ‘lower quality fruits than ‘Extra’ class and Class I, reasonably firm, must not show unhealed cracks, and some defects are allowed’.

#### *3.2.4 Statistical analysis*

Normality tests were conducted on all variables and where data was skewed, they were transformed (square root). Nutrient, pH and EC results were analysed using parametric one-way ANOVAs. Plant and fruit data were analysed statistically with parametric two-way ANOVAs. Multiple comparison tests were conducted using Tukey’s range test. Data presented represents mean values of the untransformed data. Count data were analysed using non-parametric two-way ANOVAs, followed by Kruskal-Wallis multiple comparison test. Statistical analysis was carried out using IBM SPSS Statistics Package v.21.

### **3.3 Results**

#### *3.3.1 Chemical analysis*

The pH, EC and nutrient content of the peat-based growing medium, the composted SMC and the vermicomposted SMC are shown in Table 3.1. The compost and vermicompost made from SMC had a similar nutrient content and EC, and were not significantly different from one another for any measured parameter. When compared to the peat-based growing medium, both composted and vermicomposted SMC had similar available nitrogen contents, but significantly higher total nitrogen, phosphorus, potassium and calcium contents. The pH and EC were also significantly higher in the composted and vermicomposted SMC than in the peat-based growing medium. Composted and vermicomposted SMC was used at a rate of 50% and 10%, respectively, and therefore, while the phosphorus, potassium and calcium

concentrations of the peat-reduced growing medium were higher compared to the peat-based growing medium, the available nitrogen content was lower.

Table 3.1 Nutrient analysis ( $\pm$ SD) of the main growing medium components

	Available Nitrogen	Total Kjeldahl Nitrogen	Total Phosphorus	Total Potassium	Total Calcium	pH	EC
	-----% of dry weight-----						mS cm <sup>-1</sup>
Peat-Based Growing Medium	0.19(0.00)	1.32(0.03)	0.14(0.03)	0.26(0.08)	1.09(0.16)	5.20(0.09)	1.97(0.27)
Composted SMC	0.21(0.01)ns	1.82(0.08)**	0.44(0.01)***	1.70(0.27)***	6.92(0.44)**	7.43(0.03)***	5.63(0.35)***
Vermicomposted SMC	0.21(0.04)ns	2.06(0.27)**	0.53(0.09)***	1.43(0.09)***	6.41(0.93)**	7.27(0.20)***	5.39(0.19)***
F value	0.38	16.51	43.15	58.12	86.06	292.98	162.66
p value	0.701	0.004	<0.001	<0.001	<0.001	<0.001	<0.001

When compared to the peat-based growing medium, within a column, ns = not significant, \*\* =  $p \leq 0.01$ , \*\*\* =  $p \leq 0.001$  according to Tukey's range test.

### 3.3.2 Day 53 harvest and flowering

Fifty three days after sowing, shoot and root fresh (but not dry) weights were significantly higher in the peat-based than in the peat-reduced growing media; there was no vermicompost effect in either medium (Table 3.2). Shoot and root water content were also significantly higher in the peat-based growing media than in the peat-reduced media. Root/shoot dry weight ratio was significantly lower in the peat-based growing media, than in the peat-reduced, and tomato plants in the peat-based growing media flowered significantly earlier than the plants in the peat-reduced growing media by an average of five days.

Table 3.2 Effect of growing media on shoot and root fresh and dry weight, root/shoot ratio, and percentage water content on day 53, and flowering date ( $\pm$ SD)

Treatment		Shoot Fresh Weight	Shoot Dry Weight	Root Fresh Weight	Root Dry Weight	Root/ Shoot Dry Weight	Shoot Water Content %	Root Water Content %	Flowering Date  Days after sowing
		-----g-----							
Peat-Based – VC		22.80(9.74)b	1.70(0.77)a	6.78(3.11)a	0.44(0.24)a	0.25(0.03)a	93(0.22)b	94(0.78)bc	70(2)a
Peat-Based + VC		16.91(4.32)ab	1.30(0.33)a	5.37(1.25)a	0.31(0.07)a	0.24(0.02)a	92(0.18)b	94(0.50)c	73(4)ab
Peat-Reduced – VC		11.78(3.83)a	1.02(0.36)a	4.33(1.36)a	0.31(0.09)a	0.31(0.03)b	91(0.29)a	93(0.63)ab	77(3)b
Peat-Reduced + VC		10.42(2.54)a	0.87(0.22)a	3.83(0.86)a	0.29(0.07)a	0.34(0.05)b	92(0.22)a	92(0.48)a	76(3)ab
	df	ANOVA							
F value Peat-Based/Reduced (P)	1	13.02	7.15	6.13	1.51	25.95	74.10	25.51	11.18
Sig.		**	*	*	ns	***	***	***	**
F value $\pm$ Vermicompost (V)	1	1.77	1.73	1.11	1.43	0.65	0.02	0.01	0.18
Sig.		ns	ns	ns	ns	ns	ns	ns	ns
P x V	1	ns	ns	ns	ns	ns	*	ns	ns

ns = not significant, \*= $p \leq 0.05$ , \*\* =  $p \leq 0.01$ , \*\*\*= $p \leq 0.001$  according to two-way ANOVAs. Means in the same column followed by the same letter were not significantly different,  $p > 0.05$ , according to Tukey's Range Test. – VC = without vermicompost amendment, + VC = with 10% vermicompost amendment.

### 3.3.3 Day 170 harvest

#### *Vegetative parameters*

Similar to the harvest on day 53, plants grown in the peat-based growing media had significantly higher shoot fresh weight (+18%) than the plants grown in peat-reduced growing media (Table 3.3). Unlike the harvest on day 53 however, there was also a significant vermicompost effect on shoot fresh weight and shoot water content (Table 3.3). The addition of 10% vermicomposted SMC to either growing media increased shoot fresh weight by an average of 20%, and water content by an average of 1% (Table 3.3). Shoot dry weight was highest in the peat-based growing medium with vermicompost amendment, being significantly higher than that in the peat-based growing medium without vermicompost amendment (+24%), and the peat-reduced growing medium with vermicompost amendment (+13%).

The distance between the base of the stem to the first and second truss differed between plants grown in the various growth media. The first truss on plants grown in the peat-reduced growing media developed at a significantly higher point on the stem compared to plants grown in peat-based growing media. There was a significant difference in truss one and two heights between the peat-based growing medium (26 and 42 cm, respectively) with no vermicompost amendment, and the peat-reduced growing medium (36 and 54 cm, respectively) with no vermicompost amendment (Table 3.3). There was no significant vermicompost effect on height of either truss, though there was a significant  $P \times V$  interaction for truss two height, as a result of vermicompost reducing truss two height in the peat-reduced growing medium compared to vermicompost increasing truss two height in the peat-based growing medium (Table 3.3).

Table 3.3 Effect of growing media on shoot fresh and dry weight, and percentage water content on day 170, and distance between the base of the stem and truss one, and the base of the stem and truss two ( $\pm$ SD)

Treatment		Shoot Fresh Weight	Shoot Dry Weight	Shoot Water Content	Distance to Truss 1	Distance to Truss 2
		g		%	cm	
Peat-Based – VC		513.68(96.56)a	81.81(8.62)a	84(2)b	26(3)a	42(3)a
Peat-Based + VC		662.86(31.04)b	101.09(4.05)b	85(1)b	32(5)ab	48(2)ab
Peat-Reduced - VC		473.06(37.61)a	91.36(5.38)ab	81(1)a	36(5)b	54(3)b
Peat-Reduced + VC		521.62(18.91)a	89.47(5.53)a	83(1)b	34(5)ab	48(8)ab
	df	ANOVA				
F value Peat-Based/Reduced (P)	1	12.40	0.14	26.60	7.11	9.76
Sig.		**	ns	***	*	**
F value $\pm$ Vermicompost (V)	1	15.91	10.06	7.19	0.68	0.01
Sig.		***	**	*	ns	ns
P x V	1	ns	***	ns	ns	**

ns = not significant, \*= $p \leq 0.05$ , \*\* =  $p \leq 0.01$ , \*\*\* =  $p \leq 0.001$  according to two-way ANOVAs. Means in the same column followed by the same letter were not significantly different,  $p > 0.05$ , according to Tukey's Range test. – VC = without vermicompost amendment, + VC = with 10% vermicompost amendment.

### *Fruit parameters*

Mean total fruit fresh weight, and mean ripe fruit fresh weight were higher in the peat-based growing media than in the peat-reduced growing media ( $p \leq 0.001$ ), by 65% and 64%, respectively (Table 3.4). There was no vermicompost effect on fruit fresh weight. For total and ripe fruit dry weight however, there was no significant difference between the peat-based and peat-reduced growing media, but there was a significant vermicompost effect. The addition of 10% vermicompost to either growing media significantly increased fruit dry matter production by an average of 18% in ripe fruits ( $p=0.048$ ), and the total fruit dry matter content was also slightly higher (+14%) in total fruits (ripe and unripe fruits combined), though not significantly so ( $p = 0.052$ ).

The number of fruits per plant was significantly lower in plants grown in the peat-reduced growing media (Table 3.4), by a median of three fruits, though the amendment of the peat-reduced growing medium with vermicompost resulted in no significant difference in fruit number from the peat-based growing medium. BER affected all plants in the trial, but BER incidence in some treatments was significantly higher than in others. The addition of vermicompost to the peat-based growing medium significantly reduced the number of fruits with BER by a median number of seven fruits per plant. The addition of vermicompost to the peat-reduced growing medium, however, did not have a significant effect on BER incidence (Table 3.4). The percentage marketable yield was increased in both growing media when amended with vermicompost, significantly so when compared to the peat-based growing medium with no vermicompost amendment. The addition of

Table 3.4 Effect of growing media on fruit fresh and dry weight, median number of fruits, and fruit quality parameters recorded on day 170

Treatment		% Marketable Yield	Total Fruits	Ripe Fruits	Total Fruits	Ripe Fruits	Number of Fruits	Blossom End Rot	Fruit Quality Classes†		
			mean fresh weight		mean dry weight				‘Extra’ Class	Class I	Class II
			-----g-----		-----g-----						
			mean								
Peat-Based – VC		58(10)a	747.69(160.67)b	681.58(169.26)b	46.01(8.21)a	41.56(8.89)a	19(2)b	9(2)b	0(1)ab	11(2)a	2(2)a
Peat-Based + VC		82(13)b	716.16(84.82)b	683.85(99.10)b	50.74(8.05)a	48.24(9.74)a	18(1)ab	2(2)a	1(1)b	12(2)a	1(1)a
Peat-Reduced - VC		77(11)ab	401.16(53.16)a	375.97(49.39)a	45.57(4.94)a	39.43(3.95)a	15(1)a	4(2)ab	0(0)a	10(2)a	2(1)a
Peat-Reduced + VC		79(9)b	487.52(76.52)a	455.99(80.83)a	50.67(5.36)a	46.96(5.67)a	17(2)ab	3(1)ab	0(1)ab	10(3)a	3(1)a
		df	ANOVA								
F value Peat-Based/Reduced (P)	1	2.35	45.26	34.63	0.29	0.20	15.58	2.42	7.33	1.20	1.51
Sig.		ns	***	***	ns	ns	***	ns	*	ns	ns
F value ± Vermicompost (V)	1	7.15	0.75	1.15	4.42	4.61	0.04	5.56	10.48	1.12	1.21
Sig.		*	ns	ns	ns	*	ns	*	**	ns	ns
P x V	1	*	ns	ns	ns	ns	ns	*	ns	ns	ns

ns = not significant, \*= $p \leq 0.05$ , \*\* =  $p \leq 0.01$ , \*\*\* =  $p \leq 0.001$  according to two-way ANOVAs. Numbers in the same column followed by the same letter were not significantly different,  $p > 0.05$ , according to Kruskal-Wallis multiple comparison test (number of fruits, number of fruits with blossom end rot, fruit quality classes) or Tukey's range test (fruit fresh and dry weight, and % marketable yield). †Fruits were graded into different classes according to EC No. 543/2011. – VC = without vermicompost amendment, + VC = with 10% vermicompost amendment.

vermicompost to the peat-based growing medium significantly increased marketable yield by 41%.

Any ripe fruits not affected by BER were graded for quality. The number of Class I and Class II fruits did not differ between treatments, although the number of 'Extra' class fruits was significantly different. The median number of 'Extra' class fruits was higher in the peat-based growing media, compared to the peat-reduced growing medium ( $p < 0.05$ ), and also higher with vermicompost ( $p < 0.01$ ) than without, by a median of one fruit per plant (Table 3.4).

### 3.4 Discussion

High conductivity of SMC was shown in previous studies to be phytotoxic (Guo *et al.*, 2001; Ribas *et al.*, 2009). This study also found that SMC had high conductivity,  $K^+$  and  $Na^+$  contents (Table 3.1), significantly higher than that in the peat-based growing medium. During mushroom production, the mushroom mycelium produce a number of metabolites that contribute to the high conductivity of the spent mushroom compost, mainly  $Ca^{2+}$ , but also  $K^+$ ,  $Mg^{2+}$  and  $Na^+$  (Beyer, 1998). There was no significant effect of maturation type (composted vs. vermicomposted) on nutrient content. Similar results were reported by Frederickson *et al.* (2007), who found that there was no difference in macronutrient quantity between unscreened green waste that was thermophilically composted, followed by vermicomposting, and unscreened green waste that had only undergone thermophilic composting for the same period of time. The  $NH_4-N$  concentration of both the composted and vermicomposted SMC was 0.003% (data not shown), well below the 0.04% maximum proposed by Bernal *et al.* (1998) for mature compost. This indicates that the two processes were equally successful in maturing SMC.

Compared to a recent study of fresh SMC in Ireland (Walsh *et al.*, 2013), the treated SMC in this study had higher total N, P and K, especially P. Phosphorus loss is generally low during composting, and mainly due to leaching (Eghball *et al.*, 1997). In this study re-composting and vermicomposting were conducted indoors in order to control nutrient losses. Therefore as the SMC was broken down it became more concentrated (Bernal *et al.*, 1996), leading to a higher N, P and K value than the original feedstock material.

Walsh *et al.* (2013) found the mean N:P:K ratio of fresh SMC was 5.6:1:3.6. This study found a similar N:P:K ratio of 4.2:1:3.2 for both re-composted and vermicomposted SMC (**Error! Reference source not found.**). Nitrogen volatilisation is common during composting and reduces the fertiliser value of the compost, resulting in as much as 40% N loss (Eghball *et al.*, 1997). The high N concentration of the matured SMC in this study suggests that possibly the N content of the raw SMC used was high, or that both treatment methods were suitable for reducing nitrogen volatilisation during treatment, i.e. correct aeration, pH, and temperatures.

On day 53, there was a clear negative effect of the peat-reduced growing medium on plant growth and development (flowering date). The plants were well fertilised throughout the trial, thus the poorer plant growth in the peat-reduced growing medium is likely due to high conductivity. In this study, the peat-reduced growing medium would have had a much higher EC than present in a typical growing medium of 1.2 – 1.5 mS cm<sup>-1</sup> (Maher *et al.*, 2000). Tomato plants are salinity-tolerant plants (Medina *et al.*, 2009), although early shoot and root growth, and root and shoot water content were negatively affected by the large amount of composted SMC used (50%) in the peat-reduced growing media. Previous studies demonstrated

that tomato root and shoot biomass were negatively affected by high conductivity (Cuartero & Fernández-Muñoz, 1999), and shoot biomass was affected to a greater extent than root biomass (Maggio *et al.*, 2007), resulting in an increased root/shoot dry weight ratio with salinity stress. The current trial also found a significantly increased root/shoot dry weight ratio in the peat-reduced growing media (0.32), compared to the peat-based growing medium (0.25), reflecting the larger reduction in shoot biomass compared to root biomass caused by high conductivity (Table 3.2).

Shoot fresh weight biomass on day 170 was significantly increased in the peat-based growing media (Table 3.3) when vermicompost was added. As the plants were fully fertilised throughout the trial, the significantly increased shoot growth in the peat-based growing medium with added vermicompost could be attributed to a biostimulant plant growth effect, relative to plants grown in the peat-based growing medium without vermicompost. Atiyeh *et al.* (2000) reported significantly increased tomato shoot growth in a peat-based growing medium supplemented with 10% pig manure vermicompost, compared to the same growing medium with no vermicompost addition, when all nutrients were supplied. On day 170 the average water content of the tomato plants grown with vermicompost was significantly higher (Table 3.3). In the peat-reduced growing media, there was a significant increase in water content in the plants grown with vermicompost (84%) than without vermicompost (83%). This suggests that vermicomposted SMC reduced osmotic stress as the plants matured through succulence, a stress escape mechanism which reduces salinity stress by mitigating against excessive ion concentration (Flowers *et al.*, 1991), another possible biostimulant effect observed in plants grown with vermicompost.

The distance from the base of the stem to truss one and truss two indicates truss initiation rates, and is therefore an indication of plant development (de Koning, 1994). Truss one and two developed at significantly lower points on the stem on the plants grown in peat-based growing media without vermicompost, compared to the peat-reduced growing media without vermicompost (Table 3.3). The lower the truss develops on the stem the faster the plant was developing, indicating the high-conductivity peat-reduced growing media affected plant development as well as plant growth.

Total and ripe fruit fresh weight of plants in the peat-reduced growing media were lower than the peat-based growing media by an average of 39%, while fruit dry weight was not significantly different (Table 3.4). Reina-Sánchez *et al.* (2005) also found that percentage fruit dry weight increased, while fruit fresh weight yield was reduced significantly with increased salinity in four tomato cultivars. This was due to reduced fruit water acclimation, an osmotic effect of high salinity in the root zone (Cuartero & Fernández-Muñoz, 1999).

Vermicompost amendment increased fruit dry matter production significantly (Table 3.4). Gutiérrez-Miceli *et al.* (2007) also found that the addition of sheep manure vermicompost to tomatoes grown in soil increased tomato soluble and insoluble solid content significantly, compared to tomatoes grown in soil alone. Tomato dry matter components e.g. sugars, organic acids and minerals, contribute to tomato quality (Garcia & Barrett, 2006). Reduced fruit water content is a desirable quality in processing tomatoes (e.g. for tomato paste), as less energy is required during processing (Zegbe-Domínguez *et al.*, 2003).

Reduced tomato fruit yield, caused by high conductivity in the root zone, is associated with a lower fruit number and lower average fruit weight (Cuartero & Fernández-Muñoz, 1999). Table 3.4 shows a reduced average fruit number in the high-conductivity, peat-reduced growing media, compared to the peat-based media. Long periods of salinization, as in this study, can reduce the number of flowers per truss, the number of pollen grains per flower, and percentage fruit set (Cuartero & Fernández-Muñoz, 1999), but vermicompost compensated for these effects.

BER was significantly reduced in the peat-based growing medium when vermicompost was added (Table 3.4). BER is caused by a local  $\text{Ca}^{2+}$  deficiency in the blossom-end of the fruit (Cuartero & Fernández-Muñoz, 1999). Addition of vermicomposted SMC increased the  $\text{Ca}^{2+}$  content of the peat-reduced growing medium (Table 3.1), therefore reducing the incidence of BER. Even though the concentration of  $\text{Ca}^{2+}$  was much higher in the peat-reduced growing medium, BER was not significantly reduced. Increased salinity, as was the case in the peat-reduced growing medium, can increase the incidence of BER (Cuartero & Fernández-Muñoz, 1999; Magán *et al.*, 2008), although different tomato varieties have shown different BER response patterns (Reina-Sánchez *et al.*, 2005). The estimated amount of  $\text{Ca}^{2+}$  in the peat-reduced growing medium was  $34.6 \text{ g kg}^{-1}$  (Ca content of SMC was 6.92%, diluted by 50% with vermiculite). According to Mayfield & Kelley (2012), this concentration exceeds the needs of tomatoes grown in soil. Despite this, it is possible that increased salinity reduced water (and hence  $\text{Ca}^{2+}$ ) uptake in the root zone (Adams & Ho, 1992), resulting in increased BER incidence.

A small, though significant difference in fruit class was found in the median number of 'Extra' class fruits only (Table 3.4). The median number of fruits in this class was higher in the peat-based growing media than in the peat-reduced media, and also in

plants grown with vermicomposted SMC than without. In this trial, 'Extra' class fruits were the largest, as they were most characteristic of the large, plum-sized tomato shape of this variety. A higher number of smaller fruits with increasing salinity, as also reported by Magán *et al.* (2008), explains the difference between the peat-based and peat-reduced growing media. It is hypothesised that a higher number of large fruits without BER, contributed to the increased number of 'Extra' class fruits in the peat-based growing medium with vermicompost added, than in the peat-reduced growing medium without vermicompost addition. The increase in percentage marketable yield in both growing media with vermicompost addition, compared to the peat-based growing medium without vermicompost, is likely due to reduced BER incidence in the growing media with added vermicompost. Increased tomato marketable yield when plants were grown with vermicompost was also reported by Gutierrez-Miceli *et al.* (2007).

This study shows that both composting and vermicomposting are suitable treatment methods for maturing SMC for use as growing media components. The overall effects of the growing medium types were that the peat-based growing medium outperformed the peat-reduced growing medium, while vermicompost addition affected plant dry-matter production, succulence, fruit quality and yield parameters. The high conductivity of the peat-reduced growing medium was the main limiting factor. This study found that during early plant growth, tomato shoot fresh weight was most affected by high conductivity, followed by roots, and during late plant growth, tomato fruit fresh weight was most affected by high conductivity, followed by shoot fresh weight. To reduce the conductivity further, the SMC would need to be used at a lower rate, 20-40% vol/vol with vermiculite, or other low-conductivity, peat-free growing media substrates such as coconut coir, wood fibre, perlite etc. Further

studies would be required to formulate such a peat-reduced growing media, containing composted SMC, with the right bulk density, air capacity, pH, EC, and nutrient quantities suitable for plant growth.

As the plants were fully fertilised with all the macro nutrients, except for  $\text{Ca}^{2+}$ , some of the plant-growth effects observed in the plants grown with vermicompost, can be attributed to biostimulant effects. The increased shoot weight of fully fertilised plants in the peat-based growing medium with vermicompost, and the increased succulence in the peat-reduced growing medium when vermicompost was added, demonstrates the positive biostimulant effects of vermicompost on unstressed and stressed tomato plants. Vermicompost addition also increased the percentage marketable yield, and reduced the number of fruits affected by BER due to the increased  $\text{Ca}^{2+}$  content. Although the addition of vermicomposted SMC to both growing media did not affect fruit fresh weight yield, the effect of the vermicompost on fruit quality and yield parameters, makes it a valuable addition to a peat-based growing medium for tomato fruit production.

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# Chapter 4

Effect of vermicomposted spent mushroom compost on tomato plant growth and biological properties of peat-based growing media, throughout a tomato growing cycle

## Abstract

Animal manures and agricultural by-products have been previously analysed for their effect on plant growth when used as growing medium additives, but less work has been carried out on the effects of these additives on yield quality and growing media properties. Spent mushroom compost, a by-product of the mushroom industry, was fed to worms (vermicomposted) and used as an additive to a peat-based growing medium at a rate of 0, 10 or 20% (vol/vol). Tomato plants were germinated and grown on in these three growing media, and the plants and growing medium were analysed at regular intervals to observe the effects of vermicompost on plant growth, yield and growing medium biological properties. Concurrently, a sample of the same vermicompost was stored for six months and analysed monthly to assess the effect of storage on vermicompost quality. When vermicompost was used at 10% and 20% amendment concentration it had no negative effects on shoot growth and tomato yield, and, at some harvests, it increased shoot fresh weight and percentage marketable fruit yield. There were indications of salinity stress, e.g. delayed flowering and ripening in plants grown with 20% vermicompost, but this was not enough to negatively affect plant growth nor fruit weight and yield parameters. Vermicompost also improved fruit quality parameters which are known to positively influence tomato flavour. Initially, after incorporation of the vermicompost into the growing medium, physicochemical parameters were more affected than were the biological parameters, although later on in the plant's development stage, vermicompost did significantly increase rhizosphere bacterial numbers and richness, and, in the bulk growing medium, microbial respiration. Vermicompost storage had some effect on vermicompost microbial properties but these changes were not detrimental to the quality of the vermicompost.

**Keywords:** tomato, vermicompost, spent mushroom compost, yield, fruit quality, bacterial diversity, microbial activity, vermicompost storage

#### 4.1 Introduction

Use of animal manure composts as components of plant growing media can reduce fertiliser and peat use with added benefits such as disease resistance (McKellar & Nelson, 2003) and increased plant growth under conditions of abiotic stress (Mamo *et al.*, 2000; Tartoura & Youssef, 2011). Use of spent mushroom compost (SMC) as a component of growing media is potentially a very effective and environmentally sustainable re-use of this organic matter.

SMC originates from mushroom compost, a mix of composted chicken and horse manure, wheaten straw and gypsum, to which mushroom mycelium is added and allowed to grow when it is referred to as phase III mushroom compost. Once it has reached this phase, the mushroom compost is delivered to the mushroom grower who adds a layer of peat casing, approximately 20% peat by volume. Two to three flushes of mushrooms are grown over an six to eight weeks period, after which the mushroom compost and peat casing mix is removed from the farm and is known as ‘spent’ mushroom compost.

SMC is an actively decomposing material (Szmidt, 1994; Bazermore *et al.*, 2000) and it requires further processing to make it a suitable growing medium component. This can be done by further composting or by vermicomposting. Vermicomposting has potential as worms readily eat this material (Abu Bakar *et al.*, 2014), and have been shown to increase its plant-available nutrient content and maturity (Tajbakhsh *et al.*, 2008). The main issues associated with SMC when used in a growing medium are its high electrical conductivity (Zhang *et al.*, 2012), and, if not matured correctly, a high concentration of phytotoxic compounds (Curtin & Mullen, 2007) such as organic acids, phenolic compounds and salts.

Vermicomposts made from a variety of other biodegradable wastes and by-products have been shown to be effective growing medium additives for tomato plant and fruit production. Tomato seedling growth was significantly increased compared to fertilised and unfertilised controls when grown in pig manure and cattle manure vermicompost (Atiyeh *et al.*, 2000b, 2001; Paul & Metzger, 2005), while mature tomato plant growth remained largely unchanged with addition of vermicomposted cattle manure, and vermicomposted food and cotton wastes (Paul & Metzger, 2005; Zaller, 2007a).

Vermicompost is a material which has been described as a plant growth promoting product due to its high microbial activity (Atiyeh *et al.*, 2000b). In an experiment with tomato transplants comparing the effect of different organic amendments on bacterial rhizosphere community structure of mature field-transplanted plants, the only organic amendment with a significantly different community structure from the control at the end of the trial was the vermicompost treatment (Jack *et al.*, 2011). This shows that vermicompost has the potential to affect bacterial community structure long after its initial use. In contrast, Albiach *et al.* (2000) found that yearly addition of a small amount of vermicompost to soil ( $2.5 \text{ t ha}^{-1} \text{ yr}^{-1}$ ) did not affect microbial biomass or enzyme activities.

The potential effects of increased microbial activity include enhanced nitrification, nutrient cycling and organic matter degradation (Ingham *et al.*, 1985), the production of humic substances, plant growth promoting compounds and plant hormones (Arancon *et al.*, 2006b), and protection against plant parasitic nematodes (Arancon *et al.*, 2003b) and plant pathogens (Szczec, 1999).

There have been a number of recently published studies investigating the effect of storage on mature vermicompost properties (Das *et al.*, 2014; Karthikeyan *et al.*, 2014; Gupta *et al.*, 2014; Tereshchenko *et al.*, 2014). Storage duration, storage conditions and the parameters measured varied between studies and these different conditions produced varying results. For example, in a three-month study of vermicompost storage under ambient, aerobic conditions ( $29 \pm 4^{\circ}\text{C}$ ), vermicompost pH and electrical conductivity remained constant,  $\text{NO}_3\text{-N}$  concentration increased for the first five weeks, followed by a gradual decline until week twelve, plant-available phosphorus, potassium, percentage moisture and urease activity decreased gradually over the 12 weeks, and dehydrogenase activity fell to almost zero after eight weeks of storage (Karthikeyan *et al.*, 2014). Similar trends in  $\text{NO}_3\text{-N}$  and available phosphorus and potassium were observed in a six-month study of vermicompost storage at ambient conditions ( $30 \pm 2^{\circ}\text{C}$ ) (Das *et al.*, 2014), although  $\text{NO}_3\text{-N}$  peaked at 90-105 days in this study, in comparison to 35 days in the aforementioned study. To maintain the quality of the vermicompost over a three-month period, Karthikeyan *et al.* (2014) suggested that the best storage conditions were in air-tight containers, after the vermicompost had been air-dried for 24 hours.

This study aims to evaluate the effect of vermicomposted SMC as a growing medium additive for tomato plant production and vine tomato culture. It examined the effect of vermicompost on plant and fruit growth from seedling to fruiting stage as plants were harvested regularly up until the ripening of the second fruit truss. As well as assessing plant growth during these different stages, this study also evaluated the effect of vermicompost addition on the physical, chemical and biological properties of the three growing media upon initial mixing, and also monitored the changes in microbial activity and diversity of the different growing media over the

lifetime of the plant. A further goal was to evaluate the effect of storage on chemical and biological parameters of the vermicompost, and to determine whether the quality of the material changed over the storage period.

## 4.2 Materials and methods

### 4.2.1 Trial set up

Tomato seeds, *Solanum lycopersicum* F<sub>1</sub> hybrid cv. Grande, an indeterminate vine cultivar, were sown on the 27<sup>th</sup> June 2014 (day 0) in 84-cell trays. The trays were surface sterilised by soaking for 15 minutes in a 1% NaOCl solution followed by a thorough rinsing with tap water. Each tray was filled with a different growing medium:

- 100/0 (vol/vol) Plagron™ Lightmix/vermicomposted SMC (0% VC)
- 90/10 Plagron Lightmix/vermicomposted SMC (10% VC), or
- 80/20 Plagron Lightmix/vermicomposted SMC (20% VC)

Plagron Lightmix (Plagron, Ospel, Netherlands) is a commercially available peat-based growing medium consisting of peat, perlite, and a small amount ( $1.5 \text{ kg m}^{-3}$ ) of added fertiliser (12-14-24 NPK) The vermicompost was made by feeding SMC (Reilly Mushrooms Ltd., Athlone, Ireland), consisting of straw, poultry manure, horse manure, peat, lime and gypsum, to worms in a 12 m x 2 m x 1 m (l x h x w) flow-through vermicomposting bed. In this system, the SMC was fed at a rate of approximately  $180 \text{ kg day}^{-1}$  to epigeal worm species, primarily *Eisenia fetida* (redworm). Other decomposer organisms were also present in the vermicomposting bins including a range of fungi, bacteria and other commonly occurring soil-dwelling arthropods and pot worms. The density of worms in the top layer of the

vermicomposting bed was 60 g kg<sup>-1</sup>. Concurrent to daily feeding, finished vermicompost was harvested from the bottom of the bed at a daily rate of approximately 90 kg day<sup>-1</sup>, and it took approximately 90 days for the SMC to go through the system. The vermicompost was harvested on 10<sup>th</sup> June 2014, and stored in breathable sacks in a cool dry place until it was used 17 days later.

The tomato seedlings were transplanted 16 days after sowing. Seventy eight tomato plants were transplanted into surface-sterilised pots (wiped with 70% ethanol) containing their respective growing medium mix as follows: five seedlings from each treatment into 2 L pots, five seedlings from each treatment into 6 L pots and sixteen seedlings from each treatment into 14 L pots.

To ensure adequate fertilisation in each of the three growing medium mixes, Osmocote<sup>TM</sup> (Everris Ltd., Ipswich, UK) 3-4 month slow-release fertiliser containing 16% N, 9% P<sub>2</sub>O<sub>5</sub>, 12% K<sub>2</sub>O, 2% MgO, 0.45% Fe, 0.06% Mn, 0.02% B, 0.055% Cu, 0.02% Mo, and 0.02% Zn (w/w), was added at the recommended rate of 2.5 g L<sup>-1</sup> to the Plagron Lightmix, before mixing with the other growing medium components. To reduce blossom end rot incidence, the calcium content of the three growing media was equalised by adding gypsum (19% Ca<sup>2+</sup>) at a rate of 1.03 g L<sup>-1</sup> to the 0% VC treatment, 0.51 g L<sup>-1</sup> to the 10% VC treatment, and 0 g L<sup>-1</sup> to the 20% VC treatment. This equated to a total of 444 mg L<sup>-1</sup> Ca<sup>2+</sup> in each of the three growing medium mixes.

The pots were kept spatially separated by placing each on an upturned surface-sterilised seed tray (wiped with 70% ethanol), and the plants were grown on in a heated glasshouse maintained at a minimum of 18°C (Figure 4.1). Daylight was supplemented by 400 W artificial sodium vapour lamps (16 h photoperiod) from 8<sup>th</sup>



Figure 4.1 Tomato trial set-up on week 4 (a), 6 (b), 9 (c) and 13 (d)

October 2014. The plants were arranged in a replicated, randomised block design. The plants were supported with stakes, side shoots were pinched out weekly, and the apical bud on the main stem was pinched out at the point below the third truss. The plants were treated with Bayer Organic Bug Free™ insecticide (Bayer, Leverkusen, Germany) for the control of thrips on the 6<sup>th</sup> August 2014 (active ingredient: 2% w/w fatty acids). The trusses were pruned to six fruits per truss on the 5<sup>th</sup> September 2014 to replicate vine tomato cultivation. Pollination was aided by striking the stake of each plant three times a week during the flowering period. The plants were spaced out and the blocks moved around the glasshouse every two weeks until the fruits started to ripen (17<sup>th</sup> October 2014), after which, the risk of damage to the fruits was considered to be too high.

#### 4.2.2 *Plant parameters*

Plants were harvested at regular intervals throughout the trial to assess the effect of vermicompost on plant growth throughout the tomato growing cycle. The day before transplanting (day 15), five seedlings from each plug tray were harvested and plant fresh and dry weight measured. Plant biomass was dried at 60°C until a constant weight was obtained. Each of the five replicate plants in the 2 L pots were harvested 1 month after transplanting, the five replicate plants from the 6 L pots were harvested 2 months after transplanting, five replicates from the 14 L pots were harvested 3 months after transplanting, a further five replicates were harvested 4 months after transplanting, and the remaining six replicates from the 14 L pots were harvested when all the fruits were fully ripe (one week after the previous harvest). During each harvest the following shoot parameters were collected: plant height, stem diameter at the base of the plant, number of true leaves, leaf fresh and dry weight, and stem fresh and dry weight. The average chlorophyll content (Minolta SPAD model 501, Minolta Corporation Ltd., Japan) was also measured, but measurements were taken on different leaves depending on the development stage of the plants. For month 1-3 harvests the chlorophyll content of the youngest fully expanded leaf was measured, while at month 4 harvest and the final harvest, the chlorophyll contents of leaves 4, 7 and 10 were measured.

Fruit data were collected from each plant from month 2 harvest onwards. The full inflorescence fresh and dry weight was measured during month 2 harvest only, after which the inflorescence was separated into fruit and truss stem (peduncle, pedicels, and sepals) for fresh and dry weight, with individual fruit fresh weight, and grouped truss dry weight being recorded.

The number of fruits with blossom end rot (BER) was recorded each month, and severity of BER, fruit marketability and ripening was recorded 3 and 4 months after transplanting and during the final harvest. Marketability was scored as 1 = not marketable or 2 = marketable. Ripening was scored as follows: 0 = green, 1 = breaker (when red coloration is first evident), 2 = light red (more than 60%, but less than 90%, of the fruit surface is pink or red), and 3 = red (more than 90% of the fruit surface is red in colour) (Cano *et al.*, 2003).

Fruit class for each fruit was determined 4 months after transplanting and during the final harvest using EC No. 543/2011, which lays down the marketing standards for fruits and vegetables, including tomatoes, sold in the EU. Tomatoes were graded under this regulation into three quality classes. 'Extra' class is the highest quality class and contains 'superior quality fruits, that are firm and characteristic of the variety, free from greenback and free from all but the very slightest of superficial defects'. Class I is the second highest quality class and contains 'good quality fruits, that are reasonably firm, free of cracks and greenback, with some slight defects allowed'. Class II is the lowest quality class and contains 'lower quality fruits than 'Extra' class and Class I, reasonably firm, must not show unhealed cracks, and some defects are allowed'.

Tomato soluble solids, pH and titratable acidity were measured during the final harvest. The first marketable fruit from each truss was pooled by truss and by treatment. The pooled samples were blended for two minutes in a Waring blender (MX-700G), and the pH of the diluted tomato mixture (15 g of the tomato homogenate in 100 ml distilled water) was measured using a Thermo Scientific Orion 3 Star benchtop pH meter. Titratable acidity was determined by titration with 0.1 M NaOH to pH 8.0 and expressed as % citric acid. A sample of the homogenised

fruit was centrifuged for 10 minutes at 2000 g and soluble solids of the supernatant was measured using an Atago hand-held refractometer (ATC-S/Mill-E). Soluble solids was reported as °Brix at 20°C.

A consumer acceptance test was carried out using a panel of 24 untrained persons aged 23 to 40 from the School of Biological, Earth and Environmental Sciences, University College Cork (Figure 4.2). The fruit used for the consumer acceptance panel was the second marketable tomato from truss two of each plant. Three samples of tomato (each sample consisting of one-quarter of a fruit) were served at random to each of the panellists on white plates marked with a three digit code. Using 15 cm unstructured line scales, the panellists were asked to rate the acceptability of each sample with respect to appearance, smell, sweetness, sourness, overall tomato flavour, juiciness, texture, and overall acceptability of the tomato.



Figure 4.2 Consumer acceptance panel test

Plant and fruit parameters collected between the harvest periods included plant height, flowering date, fruit ripening and BER incidence. Plant height was measured and flowering recorded as the days on which the first flower opened fully on truss one and truss two. Fruit ripening was scored (as described above) on a weekly basis starting on 11<sup>th</sup> October and finishing on 18<sup>th</sup> November 2014, by which time all the fruits had ripened. Concurrently, the number of fruits with blossom end rot was recorded.

#### 4.2.3 *Growing medium parameters*

A sample of the Lightmix peat-based growing medium and of the vermicomposted SMC was sampled on day 0. The Lightmix and vermicomposted SMC were analysed for water-soluble macro- and micro-nutrients, bulk density, dry density, pH, EC and air-filled porosity at NRM Laboratories, Berkshire, UK. EC, pH, dry matter, dry density and bulk density were measured according to BS EN 13040 2000 (British Standards Institution, 2000). The samples were extracted with deionised water 1:5 (vol/vol), pH was determined, and filtered samples were analysed for EC.  $\text{Cl}^-$ ,  $\text{SO}_4\text{-S}$ , and  $\text{NO}_3\text{-N}$  was determined by ion chromatography,  $\text{NH}_4\text{-N}$  was determined by colorimetric analysis, and P, K, Mg, Ca, Na, B, Cu, Fe, Mn, Mo, and Zn were analysed using inductively coupled plasma – optical emission spectroscopy (Ministry of Agriculture Fisheries and Food, 1981; United States Environmental Protection Agency, 1996).

On day 15 the three growing medium mixes and the vermicomposted SMC were sampled for water-holding capacity. Water-holding capacity was analysed by saturating 10 g oven-dried (60°C) samples of each mix, and the vermicomposted SMC, with water for thirty minutes, the excess water was then allowed to drain and

the volume of water that drained from the sample was measured to obtain water-holding capacity.

The three growing medium mixes, and the separated bulk growing medium and rhizosphere samples of five tomato seedlings from each treatment, were also analysed for bacterial numbers and diversity, and for microbial activity (bulk samples only). The bulk and rhizosphere samples were separated by taking each of the seedlings out of the cell tray and gently shaking the bulk growing medium away from the roots. The collected medium was regarded as the bulk growing medium sample. The remaining roots with the growing medium still adhering to them after shaking, was regarded as the rhizosphere sample. Sampling equipment was sterilised between samples.

Bacterial numbers and diversity were measured by community-level physiological profiling using Biolog EcoPlates™ (Biolog Inc., Hayward, USA). Samples (2 g) were diluted with quarter-strength Ringer's solution to make a 1:5000 dilution, inoculated onto EcoPlates, and incubated at 24°C. The EcoPlates consist of 31 carbon substrates replicated three times per plate. The bacteria in the solution attempt to metabolise these carbon sources; if successful, this results in a release of purple-coloured formazan in the wells and the development of a colour change (Preston-Mafham *et al.*, 2002). This colour change was measured as absorbance in a microplate reader (Bio Rad, Model 680) at 600 nm, 48, 60, 72, and 84 h after inoculation. The speed and pattern of breakdown of the carbon sources indicated bacterial numbers and diversity. Bacterial numbers are denoted by average well colour development (AWCD) and species richness. AWCD was calculated by dividing the total well colour development (minus control well absorbance) by the number of wells for each sample (31). Species richness was calculated by counting

the number of positive wells with an absorbance of greater than 0.122, after subtracting the control well. These were compared after an incubation period of 96 h for bulk growing medium samples and 75 h for rhizosphere samples. Shannon index was used to calculate bacterial diversity, and principal component analysis was used to visualise the profiles of the bacterial community of the different samples. When comparing Biolog data for the Shannon index and principal component analysis, Garland (1996) suggested that only samples with similar AWCD be compared, which, in the data presented, corresponded to an AWCD of approximately 0.150. Biolog results comparing vermicompost and Lightmix samples only were normalised by volume, and Biolog results comparing the three bulk growing medium mixes were normalised by dry weight. Control well subtraction for each incubation period before analysis in this study was difficult due to the respiration of certain bacteria in the absence of a carbon source, resulting in the frequent development of colour in the control wells, a phenomenon which others have reported (G Mozolowski 2014, pers. comm.). Therefore, the control well absorbance to be subtracted from all treatments at all incubation times was measured 48 h after inoculation, before colour formation occurred in the control wells.

Dehydrogenase activity was used as a measure of microbial activity using the method based on that of Thalmann (1968). Bulk growing medium samples (1 g) were incubated with 1.5 % (w/v) triphenyl tetrazolium chloride in Tris-HCL buffer (pH 7.6) for 24 h at 30°C, after which acetone was used to extract the triphenyl formazan (TPF) produced from the reduction of triphenyl tetrazolium chloride by the respiring microbes. A 2 ml aliquot of the sample was centrifuged at 3000 g for 10 minutes and the supernatant absorbance read at 550 nm on a microplate reader (Bio Rad, Model 680). Absorbance values were converted to µg TPF using a calibration

curve of TPF (>90% purity, Sigma-Aldrich), and dehydrogenase activity was then calculated as  $\mu\text{g TPF g}^{-1}$  growing medium dry weight or rhizosphere fresh weight.

Fungal biomass in the three growing medium mixes, Lightmix base growing medium and SMC vermicompost was determined by measuring ergosterol content. Ergosterol was extracted from 1.6 g bulk growing medium samples with 4 g acid-washed beads (2 g 250-500  $\mu\text{m}$  diameter and 2 g 1000  $\mu\text{m}$  diameter) and 10 ml of methanol using the physical disruption method described by Gong *et al.* (2001) with extended extraction times i.e. the vials were vortexed for 30 s twice, followed by intensive shaking for 1.5 h on a bench-top shaker (500 rpm), and then centrifuged at 14,000 g for 10 minutes. Ergosterol was measured using a UPLC H-Class Core System with an Acquity UPLC TUV Detector (dual wavelength) and Acquity Column Heater 30-A. The system was interfaced with Empower 3 software (Waters Corp., Milford, MA, USA). The core system included an Acquity UPLC H-Class quaternary solvent manager, and an H-Class Sample Manager-FTN. The column used was an Acquity UPLC BEH C18 1.7 $\mu\text{m}$ , 2.1 x 50 mm column maintained at 25°C. Elution was monitored at 282 nm with an isocratic run of HPLC grade methanol at a flow rate of 0.92 ml min<sup>-1</sup>. The total run time was 2 min per sample. Under these conditions, the retention time of ergosterol was 25 seconds. Pure ergosterol (>95% purity, Sigma-Aldrich) was used to generate a standard curve of  $A_{282}$  vs. ergosterol concentration.

Bulk growing medium and rhizosphere samples from each of the five replicate pots per treatment were also sampled when the plants were harvested at months 1, 2, 3, and 4 after transplanting. Samples were analysed immediately using Biolog plates and dehydrogenase activity, and bulk samples were oven-dried at 60°C to determine moisture content. Bulk sample results were normalised by dry weight. Biolog plates

were read more frequently for these samples (two times per day for 7 days), as colour development of these plates, especially for the rhizosphere samples, was very rapid.

#### *4.2.4 Effects of storage on vermicompost characteristics*

The vermicomposted SMC used in this trial was harvested on 10<sup>th</sup> June 2014, and stored for six months in a breathable sack in a cool dark environment. Using sterile gloves and sampling equipment, samples were taken on day 0 (the day the vermicompost was harvested from the worm bed), and every following month for six months. The samples were analysed in triplicate for pH, EC, OM, dehydrogenase activity and Biolog analysis, and a single sample taken for nutrient analysis at NRM Laboratories Ltd., Berkshire, UK, using the methods described in section 4.2.3, on day 0, month 3 and month 6 of storage. Monthly analysis of organic matter was determined by loss on ignition at 550°C for 24 h, while EC and pH were measured in a 1:10 soil/distilled water suspension (Laos *et al.*, 2002) which was placed on a shaking table for 15 minutes, left to settle for one hour, after which the pH of the solution was determined. The solution was then filtered through a Whatman Grade 1 filter paper, and EC was measured using a portable conductivity meter (WTW Cond 330i, WTW GmbH & Co., Weilheim, Germany). Dehydrogenase activity and Biolog analysis were carried out using the methods described in section 4.2.3.

#### *4.2.5 Statistical analysis*

Normality tests were conducted on all parametric variables and where data was skewed, they were transformed (square root or log<sub>10</sub> transformed (dehydrogenase activity only)). Plant and fruit data were analysed statistically by parametric one-way ANOVAs. Multiple comparison tests were conducted using Tukey's range test.

Data presented represents mean values of the untransformed data. Count data (number of true leaves and number of fruits with BER) were analysed using a Kruskal-Wallis test, followed by a Kruskal-Wallis multiple comparison test. Parametric linear regressions were performed between vermicompost concentration and consumer acceptance parameters, and vermicompost concentration and leaf chlorophyll content measured during the month 4 and final harvests.

Growing medium characteristics analysed by parametric one-way ANOVAs followed by Tukey's range test, or using a Kruskal-Wallis test, followed by a Kruskal-Wallis multiple comparison test (for the discontinuous non-normal variable richness). Bulk and rhizosphere dehydrogenase samples from each month were compared using two-way ANOVAs. Data presented represents mean values of the untransformed data. Principal component and cluster analyses were carried out on Biolog absorbance patterns at day 0 using the software Multiple-Variate Statistical Package v. 3.21, Kovach Computing Services, Anglesey, Wales.

Linear and quadratic regressions were performed on vermicompost storage parameters with respect to increasing time in storage. Principal component and hierarchical cluster analyses of the vermicompost over the storage period were carried out on Biolog absorbance patterns using the software Multiple-Variate Statistical Package v. 3.21, Kovach Computing Services. All statistical analyses described, except for principal component and cluster analysis, were carried out using IBM SPSS Statistics Package v.21.

### **4.3 Results**

The physicochemical and biochemical properties of the Lightmix growing medium and the vermicomposted SMC are shown in Table 4.1. Lightmix is a very good

quality commercial growing medium with plant-appropriate pH, EC and air fill porosity values. The nutrient content is purposefully low to allow the user to add the type and level of fertiliser desired. Vermicompost had a higher pH, EC, dry weight and fresh weight density compared to Lightmix. Its addition to Lightmix would make a heavier growing medium, with a lower air fill porosity and water-holding capacity. Vermicompost contained a much higher macro- and micro-nutrient content than Lightmix, particularly  $K^+$ ,  $Ca^{2+}$ ,  $Na^+$  and  $SO_4^{2-}$ , although the water-soluble P content was similar in both, while the Mn content was lower for vermicompost (Table 4.1).

Table 4.1 Mean ( $\pm$ SD) physicochemical properties of the Lightmix peat-based growing medium and the vermicomposted spent mushroom compost

Parameter	Unit	Lightmix	Vermicompost
pH		5.59 $\pm$ 0.01	6.88 $\pm$ 0.04
EC <sup>†</sup>	mS cm <sup>-1</sup>	1.05 $\pm$ 0.06	5.49 $\pm$ 0.14
Fresh Density	kg m <sup>-3</sup>	531	769
Dry Density	kg m <sup>-3</sup>	142.8	215.3
Dry Matter	%	26.9	28.0
Air Fill Porosity	%	9.1	5.7
Water Holding Capacity	ml L <sup>-1</sup>	305.9 $\pm$ 50.7	227.9 $\pm$ 64.6
NH <sub>4</sub> -N	mg L <sup>-1</sup>	5.2	11.1
NO <sub>3</sub> -N	mg L <sup>-1</sup>	170.3	545.4
P	mg L <sup>-1</sup>	70.7	80.1
K	mg L <sup>-1</sup>	230.0	2288.2
Mg	mg L <sup>-1</sup>	39.5	260.1
Ca	mg L <sup>-1</sup>	245.7	1237.0
Na	mg L <sup>-1</sup>	35.9	494.7
SO <sub>4</sub> <sup>2-</sup>	mg L <sup>-1</sup>	280.7	4254.0
B	mg L <sup>-1</sup>	0.17	0.43
Cu	mg L <sup>-1</sup>	<0.06	0.09
Mn	mg L <sup>-1</sup>	0.20	0.11
Zn	mg L <sup>-1</sup>	<0.06	0.12

<sup>†</sup> EC = electrical conductivity

The Biolog data showed similar bacterial levels in the Lightmix and vermicompost (Table 4.2), although fungal biomass (measured as ergosterol content) was approximately 50% higher in vermicompost than in Lightmix, and there was a 25-fold difference in dehydrogenase activity, with the vermicompost being the more microbially active.

Table 4.2 Mean or median (richness only) ( $\pm$ SD) biochemical properties of the Lightmix peat-based growing medium and the vermicomposted spent mushroom compost

Parameter	Unit	Lightmix	Vermicompost
Ergosterol	$\mu\text{g ml}^{-1}$	5.90 $\pm$ 1.16	9.99 $\pm$ 1.63
Dehydrogenase Activity	$\mu\text{g TPF ml}^{-1}$	3.09 $\pm$ 1.76	77.62 $\pm$ 9.75
AWCD <sup>†</sup>		0.12 $\pm$ 0.02	0.16 $\pm$ 0.01
Richness		7 $\pm$ 1	8 $\pm$ 2
Shannon		1.35 $\pm$ 0.11	1.74 $\pm$ 0.17

<sup>†</sup>AWCD = Average Well Colour Development

#### 4.3.1 Plant parameters

There was no significant difference between the size of plants grown in any of the three growing media at the seedling stage (15 days after sowing) (Table 4.3). At month 1 harvest, plants grown with 20% vermicompost had a significantly lower stem dry weight (-14%) than those in 0 and 10% vermicompost mixes, and a significantly higher shoot water content than those grown in 0% (Table 4.3). This was the only month where a significant difference in the number of leaves was detected ( $p = 0.038$ ), although this difference was not detected by the post-hoc Kruskal-Wallis multiple comparison test ( $H$  value = 6.77) with the closest rank mean difference being between plants grown with 0 and 20% vermicompost at 6.0.

Table 4.3 Effects of vermicompost incorporation on shoot parameters ( $\pm$ SD) of tomato plants

Harvest	% VC†	Plant Height cm	Stem Diameter mm	Median No. True Leaves	Leaf Fresh Weight	Leaf Dry Weight	Stem Fresh Weight	Stem Dry Weight	Shoot Fresh Weight	Shoot Dry Weight	Shoot Water Content
					-----g-----						%
Day 15	0								0.25(0.04)	0.01536(0.0023)	93.83(0.26)
	10				-----n/a-----						93.87(0.40)
	20								0.21(0.03)	0.01324(0.0022)	93.72(0.27)
F value									1.54	1.19	0.32
Sig.									ns	ns	ns
Month 1	0	56(3)	8.71(0.62)	13(0)a	70.92(2.38)	7.09(0.30)	30.20(1.34)	2.22(0.12)b	101.12(2.50)	9.30(0.37)	90.80(0.25)a
	10	57(2)	9.25(1.03)	14(1)a	73.66(3.19)	7.20(0.72)	31.34(2.49)	2.23(0.19)b	105.00(5.06)	9.43(0.91)	91.03(0.56)ab
	20	55(2)	8.39(0.94)	14(0)a	70.52(5.84)	6.47(0.62)	30.06(2.41)	1.92(0.16)a	100.58(8.19)	8.39(0.78)	91.66(0.24)b
F value/Chi <sup>2</sup>		1.16	1.21	6.52	0.88	2.32	0.54	5.84	0.88	3.08	6.84
Sig.		ns	ns	*	ns	ns	ns	*	ns	ns	**
Month 2	0	86(8)	12.66(0.95)	13(1)	294.42(32.90)a	34.99(3.11)	95.5(11.11)	14.12(1.76)	389.92(37.04)a	49.11(4.01)	87.38(0.72)
	10	85(6)	12.21(1.01)	13(1)	342.84(19.11)ab	36.81(2.88)	102.9(11.03)	12.98(1.69)	445.74(28.21)ab	49.79(4.52)	88.82(0.85)
	20	91(6)	13.44(0.38)	13(1)	370.58(36.09)b	40.49(6.71)	110.72(4.12)	16.95(4.61)	481.30(36.52)b	57.44(8.56)	88.10(1.11)
F value/Chi <sup>2</sup>		1.27	2.82	1.17	8.08	1.88	3.38	2.39	9.06	2.92	3.19
Sig.		ns	ns	ns	**	ns	ns	ns	**	ns	ns
Month 3	0	84(7)	14.13(0.29)	14(1)	607.86(42.57)	71.48(5.45)b	126.68(5.79)	18.80(1.60)b	755.19(44.68)	93.70(6.63)b	87.60(0.37)a
	10	83(6)	13.77(1.36)	12(1)	515.58(75.17)	56.74(6.36)a	116.32(14.57)	15.09(2.28)ab	652.65(86.84)	75.07(8.50)a	88.47(0.26)b
	20	83(8)	13.55(1.18)	13(1)	558.42(59.57)	61.76(6.05)ab	119.04(15.75)	15.03(1.97)a	697.22(74.52)	79.77(8.06)a	88.55(0.26)b
F value/Chi <sup>2</sup>		0.03	0.39	2.87	2.90	7.89	0.89	6.01	2.62	7.78	15.81
Sig.		ns	ns	ns	ns	**	ns	*	ns	**	***
Month 4	0	85(7)	13.98(1.04)	13(1)	550.53(46.01)a	69.34(5.80)	128.14(13.18)	21.80(2.45)	701.59(44.18)a	95.07(5.69)	86.44(0.40)
	10	87(4)	15.03(2.14)	13(1)	564.64(118.47)a	68.64(14.76)	139.73(24.99)	23.19(4.86)	726.93(143.27)ab	95.55(19.70)	86.88(0.23)
	20	85(4)	15.87(1.61)	13(1)	706.61(46.03)b	84.52(8.96)	143.29(14.14)	24.64(4.40)	872.68(58.22)b	113.02(13.32)	87.07(0.70)
F value/Chi <sup>2</sup>		0.38	1.64	0.38	5.73	3.45	0.95	0.58	4.73	2.52	2.27
Sig.		ns	ns	ns	*	ns	ns	ns	*	ns	ns
Final Harvest	0	86(8)	15.19(1.51)	13(1)	574.17(48.51)a	78.53(8.39)	145.84(17.50)	28.19(4.48)	744.32(55.15)a	110.89(12.20)	85.12(1.12)
	10	85(2)	15.63(1.97)	13(1)	625.52(39.91)ab	77.25(8.63)	149.63(8.39)	27.28(2.83)	798.22(44.53)ab	108.68(9.21)	86.36(1.25)
	20	86(6)	14.87(1.85)	13(0)	671.40(62.88)b	92.13(16.04)	148.07(15.17)	27.68(3.40)	844.05(70.75)b	124.43(17.77)	85.29(1.39)
F value/Chi <sup>2</sup>		0.11	0.27	1.18	5.01	2.71	0.12	0.09	4.13	2.21	1.63
Sig.		ns	ns	ns	*	ns	ns	ns	*	ns	ns

ns = not significant, \* $p \leq 0.05$ , \*\* $p \leq 0.01$  according to one-way ANOVAs (F value) or Kruskal-Wallis test (Chi<sup>2</sup>) (no. true leaves only). Means harvested in the same month and column followed by the same letter were not significantly different,  $p > 0.05$ , according to Tukey's Range Test or Kruskal-Wallis Multiple Comparison test (no. true leaves only). Variables where no significant differences were found were not assigned letters. †VC = vermicompost, n/a = not available.

At month 2 harvest, leaf fresh weight and shoot fresh weight were significantly higher in plants grown with 20% vermicompost than those in 0% vermicompost (by 26% and 23%, respectively). At month 3 harvest, leaf, stem and shoot dry weights were all significantly lower in one or both of the vermicompost treatments than the control while there was a significant increase in shoot water content with vermicompost addition. As a consequence, there was no reduction in leaf, stem, or shoot fresh weight with either 10 or 20% vermicompost addition to the growing medium.

Month 4 harvest and the final harvest were within seven days of one another. At both harvests, plants grown with 20% vermicompost showed significant increases (compared to 0% vermicompost) in leaf fresh weight (by 28% (month 4) and 17% (final harvest), respectively) and shoot fresh weight (by 24% and 13%, respectively). There was no significant vermicompost effect on leaf, stem or shoot dry weight during these harvests, and there was no significant difference between 0, 10 and 20% vermicompost at any harvests for plant height or stem diameter.

Chlorophyll content of the youngest fully expanded leaf was significantly lower in plants grown with 20% vermicompost, compared to those grown with 0%, at month 1 harvest (Table 4.4). Other significant chlorophyll effects were not seen until month 4 harvest and final harvest, where the chlorophyll content of leaf 10 was significantly higher in one (final harvest) or both of the vermicompost treatments (month 4 harvest) than the control. At the final two harvests, there were also significant positive regressions between percentage vermicompost concentration and chlorophyll content of the youngest fully expanded leaf measured (leaf 10) ( $F_{1,14} = 11.70$ ,  $p \leq 0.01$  and  $F_{1,14} = 10.89$ ,  $p \leq 0.01$ ), respectively (data not shown).

Significant positive regressions between chlorophyll content and vermicompost concentration were not found with either leaf 4 or leaf 7 during month 4 and final harvests.

Table 4.4 Effect of vermicompost incorporation on plant chlorophyll content ( $\pm$ SD)

Harvest	% VC <sup>‡</sup>	Chlorophyll YFEL <sup>†</sup>	Chlorophyll Leaf 4	Chlorophyll Leaf 7	Chlorophyll Leaf 10
		-----SPAD-----			
Month 1	0	47.8(2.2)b			
	10	46.1(1.2)ab	-----n/a-----		
	20	43.7(1.2)a			
F value		8.06			
Sig.		**			
Month 2	0	47.6(1.2)			
	10	44.4(3.2)	-----n/a-----		
	20	44.4(2.7)			
F value		2.71			
Sig.		ns			
Month 3	0	47.4(4.9)			
	10	46.7(1.9)	-----n/a-----		
	20	47.6(2.2)			
F value		0.12			
Sig.		ns			
Month 4	0		19.5(7.9)	29.7(9.9)	28.0(5.9)a
	10	n/a	26.2(11.7)	33.0(6.1)	37.5(6.5)b
	20		30.5(6.2)	36.7(3.5)	40.0(3.5)b
F value			1.51	1.31	6.95
Sig.			ns	ns	**
Final Harvest	0		17.7(9.1)	23.6(8.3)	28.2(7.4)a
	10	n/a	19.7(8.4)	30.2(4.7)	35.9(3.4)ab
	20		23.3(10.4)	29.5(12.2)	40.7(7.4)b
F value			0.38	0.75	5.20
Sig.			ns	ns	*

ns = not significant, \*= $p \leq 0.05$ , \*\* =  $p \leq 0.01$  according to one-way ANOVAs. Means harvested in the same month and column followed by the same letter were not significantly different,  $p > 0.05$ , according to Tukey's Range Test. Variables where no significant differences were found were not assigned letters. <sup>†</sup>YFEL = youngest fully expanded leaf. <sup>‡</sup>VC = vermicompost, n/a = not available.

Average flowering dates for plants grown in 0, 10, and 20% vermicompost were days 52, 52, and 53, respectively for truss one and 55, 56 and 57, respectively for truss two (data not shown). The differences in flowering dates of truss one were not significant, while for truss two, plants grown in 0% vermicompost flowered

significantly earlier than plants grown in 20% vermicompost ( $F_{2,42} = 4.08$ ,  $p < 0.05$ ). There were no significant differences in plant height or truss height between the treatments at the time of flowering of truss one and two (data not shown).

There was no significant difference in ripening of truss one fruits between the different treatments (Table 4.5). There was a small but significant delay in ripening of truss two fruits of plants grown in 20% vermicompost compared to those grown in 0%. At week 17, plants grown in 20% vermicompost had a significantly higher percentage of green fruits than 0%, a significantly lower percentage of red fruits at week 18, and a significantly higher percentage of breakers, and lower percentage of red fruits at week 19. Fruits from all treatments were 100% red (truss 1 and 2) by week 20 (Table 4.5). There was one week (week 19) where there was a significant difference in the number of fruits with BER. During this week truss 2 fruits had a significantly higher % BER in the 0% vermicompost treatment than in the 20% vermicompost treatment (Table 4.5).

There was no significant difference in fruit fresh or dry weight in the plants grown in the different growing medium mixes at the different harvest dates (Table 4.6). Fruit water content (data not shown) was unchanged between treatments except for the final harvest where the water content (95.59%) of the fruits from plants grown in the 0% vermicompost treatment was significantly higher ( $F_{2,14} = 4.7$ ,  $p = 0.03$ ) than the water content (94.93%) of the fruits in the 20% treatment. The percentage marketable fruit yield was significantly higher during month 2 and month 3 harvest in plants grown with 10% or 20% vermicompost compared to those grown with 0%, and the number of fruits with BER during these harvests was significantly lower in plants grown with 20% vermicompost than those grown with 0%. There was no effect of vermicompost addition on fruit quality classes, although the number of

Table 4.5 Effect of vermicompost incorporation on fruit ripening and blossom end rot (BER) incidence ( $\pm$ SD) recorded during weeks 15 - 20

Harvest	% VC†	Ripening-----								-----BER-----	
		% Green	% Breaker	% Light Red	% Red	% Green	% Breaker	% Light Red	% Red	% Fruits with BER	
		-----Truss 1-----				-----Truss 2-----				Truss 1	Truss 2
Week 15	0	97(7)	2(6)	1(4)	0(0)	100(0)	0(0)	0(0)	0(0)	2(6)	0(0)
	10	98(6)	2(6)	0(0)	0(0)	100(0)	0(0)	0(0)	0(0)	0(0)	0(0)
	20	100(0)	0(0)	0(0)	0(0)	100(0)	0(0)	0(0)	0(0)	0(0)	0(0)
F value		1.57	1.07	1.00						2.14	
Sig.		ns	ns	ns						ns	
Week 16	0	85(12)	7(9)	8(12)	0(0)	100(0)	0(0)	0(0)	0(0)	3(11)	0(0)
	10	85(12)	9(11)	6(8)	0(0)	100(0)	0(0)	0(0)	0(0)	0(0)	0(0)
	20	86(13)	11(11)	3(7)	0(0)	100(0)	0(0)	0(0)	0(0)	0(0)	0(0)
F value		0.05	0.37	0.89						1.11	
Sig.		ns	ns	ns						ns	
Week 17	0	22(16)	42(23)	18(17)	18(15)	53(19)a	38(18)	7(9)	2(5)	7(16)	0(0)
	10	29(20)	42(20)	9(11)	20(13)	61(20)ab	30(23)	8(17)	1(5)	0(0)	3(7)
	20	36(28)	35(23)	14(10)	15(12)	79(21)b	18(19)	2(5)	1(5)	5(15)	0(0)
F value		1.19	0.40	1.35	0.36	4.51	2.66	0.87	0.00	0.78	2.12
Sig.		ns	ns	ns	ns	*	ns	ns	ns	ns	ns
Week 18	0	5(11)	7(12)	13(15)	75(20)	11(8)	22(16)	22(16)	45(19)b	6(16)	8(18)
	10	1(5)	9(16)	20(19)	70(18)	21(22)	24(17)	9(14)	46(20)b	0(0)	3(10)
	20	8(11)	9(9)	30(26)	53(31)	29(20)	30(28)	23(27)	18(17)a	6(15)	0(0)
F value		1.09	0.13	1.81	2.54	2.33	0.47	1.58	7.42	0.90	1.37
Sig.		ns	ns	ns	ns	ns	ns	ns	**	ns	ns
Week 19	0	0(0)	2(5)	5(8)	93(12)	0(0)	0(0)a	12(11)	88(11)b	10(18)	15(27)b
	10	0(0)	0(0)	9(11)	91(11)	0(0)	6(8)ab	20(19)	74(22)ab	0(0)	3(7)ab
	20	0(0)	2(5)	6(8)	92(11)	6(20)	15(16)b	14(21)	65(19)a	8(16)	0(0)a
F value			0.53	0.54	0.12	0.95	5.60	0.59	4.40	1.59	3.37
Sig.			ns	ns	ns	ns	**	ns	*	ns	*
Week 20	0	0(0)	0(0)	0(0)	100(0)	0(0)	0(0)	0(0)	100(0)	13(22)	7(9)
	10	0(0)	0(0)	0(0)	100(0)	0(0)	0(0)	0(0)	100(0)	0(0)	3(7)
	20	0(0)	0(0)	0(0)	100(0)	0(0)	0(0)	0(0)	100(0)	8(20)	0(0)
F value										0.89	1.51
Sig.										ns	ns

ns = not significant,  $*=p\leq 0.05$ ,  $**=p\leq 0.01$ ,  $***=p\leq 0.001$  according to one-way ANOVAs. Means harvested in the same month and column followed by the same letter were not significantly different,  $p>0.05$ , according to Tukey's Range Test. Variables where no significant differences were found were not assigned letters. †VC = vermicompost.

Table 4.6 Effect of vermicompost incorporation on tomato fruit traits ( $\pm$ SD)

Harvest	% VC <sup>‡</sup>	Fruit Fresh Weight	Fruit Dry Weight	% Marketable Yield	Fruit Quality Classes			Fruits with BER <sup>†</sup>
		-----g-----			Extra Class	Class I	Class II	
					-----median no.-----			
Month 2	0	167.30(28.48)	13.72(1.91)	70(16)a				3(2)b
	10	163.18(40.09)	12.80(2.82)	98(4)b		n/a		0(0)ab
	20	131.56(31.96)	11.13(2.55)	100(0)b				0(0)a
F value/Chi <sup>2</sup>		1.73	1.43	15.35				11.71
Sig.		ns	ns	***				**
Month 3	0	933.51(222.94)	53.70(11.26)	70(17)a				4(2)b
	10	1126.60(56.36)	60.40(3.36)	98(4)b		n/a		0(0)ab
	20	1017.64(32.59)	57.76(0.80)	100(0)b				0(0)a
F value/Chi <sup>2</sup>		2.52	1.31	13.64				11.68
Sig.		ns	ns	***				**
Month 4	0	1445.44(129.07)	70.43(7.85)	85(17)	6(2)	3(1)	1(2)	2(2)
	10	1245.58(199.76)	63.80(8.93)	98(4)	6(2)	4(2)	1(2)	0(0)
	20	1311.91(123.37)	68.35(10.45)	97(5)	9(1)	3(1)	1(1)	0(1)
F value/Chi <sup>2</sup>		2.10	0.69	2.43	3.59	2.24	1.67	3.01
Sig.		ns	ns	ns	ns	ns	ns	ns
Final Harvest	0	1245.51(185.26)	55.02(10.33)	90(14)	5(3)	2(2)	2(1)	1(2)
	10	1336.24(54.67)	63.53(6.52)	99(3)	8(2)	3(2)	1(1)	0(0)
	20	1177.52(239.88)	61.32(13.58)	96(10)	9(2)	2(1)	1(2)	0(1)
F value/Chi <sup>2</sup>		1.22	0.90	1.09	3.53	0.52	2.65	2.93
Sig.		ns	ns	ns	ns	ns	ns	ns

ns = not significant, \*= $p \leq 0.05$ , \*\* =  $p \leq 0.01$ , \*\*\*= $p \leq 0.001$  according to one-way ANOVAs (F value) (fruit fresh and dry weight and marketable yield) or Kruskal Wallis Test (Chi<sup>2</sup>) (fruit quality classes and number of fruits with BER). Means harvested in the same month and column followed by the same letter were not significantly different,  $p > 0.05$ , according to Tukey's Range Test or Kruskal-Wallis multiple comparison test. Variables where no significant differences were found were not assigned letters. Fruit quality classes were assigned according to EC No. 543/2011. <sup>†</sup>BER = Blossom End Rot. <sup>‡</sup>VC = vermicompost. n/a = not applicable.

‘Extra’ class fruits of plants grown with 20% vermicompost was 50% higher than those from plants grown with 0% during month 4 ( $p=0.17$ ) and final harvests ( $p=0.17$ ) (Table 4.6).

There was a significant difference in fruit quality parameters in the plants grown in the vermicompost mixes compared to those in the 0% vermicompost control (Table 4.7). Soluble solid content was significantly increased in truss one fruits in the 10% vermicompost, and increased in the truss two fruits in the 20% vermicompost treatment compared to the control (Table 4.7). Fruit pH was unchanged in truss one, while, in truss two, fruits from plants grown in 20% vermicompost had significantly lower pH than those from plants grown in 0 and 10% vermicompost (Table 4.7). Fruit titratable acidity was significantly increased in fruits from plants grown in 10% and 20% vermicompost in both trusses compared to 0% vermicompost, and fruits from 20% vermicompost also had significantly higher titratable acidity in truss two than those from 10% (Table 4.7).

Although these fruit chemical tests showed a number of significant improvements in tomato flavour determinants with vermicompost addition (Table 4.7), these differences were largely not detected by the consumer acceptance panel (Table 4.8). ANOVA analysis showed no significant difference in treatment means, although regression analysis detected a significant positive trend in fruit overall acceptability score with increasing vermicompost concentration ( $y = 0.675x + 6.644$ ,  $r = 0.48$ ,  $F_{1,16} = 4.89$ ,  $p = 0.04$ ), but not for any other consumer acceptance parameters (data not shown).

Table 4.7 Effect of vermicompost incorporation on fruit quality parameters ( $\pm$ SD)

		Truss	Soluble Solids Brix at 20°C		pH		Titratable Acidity % Citric Acid	
			1	2	1	2	1	2
	% VC†							
Final Harvest	0		3.8(0.1)a	3.3(0.2)a	4.38(0.04)	4.50(0.02)b	7.26(0.15)a	6.48(0.07)a
	10		4.0(0.1)b	3.4(0.0)a	4.41(0.04)	4.48(0.01)b	7.92(0.12)b	6.98(0.12)b
	20		3.8(0.0)a	3.6(0.0)b	4.40(0.02)	4.44(0.02)a	7.90(0.06)b	7.34(0.10)c
F value			22.75	9.72	1.07	18.23	51.42	90.15
Sig.			***	**	ns	***	***	***

ns = not significant, \*= $p \leq 0.05$ , \*\* =  $p \leq 0.01$ , \*\*\*= $p \leq 0.001$  according to one-way ANOVAs. Means in the same column followed by the same letter were not significantly different,  $p > 0.05$ , according to Tukey's Range Test. Variables where no significant differences were found were not assigned letters. †VC = vermicompost.

Table 4.8 Effect of vermicompost incorporation on fruit consumer acceptance parameters

	Appearance	Smell	Sweetness	Sourness	Overall Flavour	Juiciness	Texture	Overall Acceptability
	-----mean acceptability score-----							
0% VC†	10.4(1.3)	10.0(1.5)	7.2(2.0)	7.1(2.0)	7.6(1.5)	7.5(2.6)	6.9(1.1)	7.4(1.0)
10% VC	10.9(2.0)	9.9(0.7)	6.9(1.4)	7.6(2.0)	7.6(1.5)	8.1(1.5)	7.3(1.3)	7.9(1.0)
20% VC	11.0(1.2)	10.4(1.3)	7.0(1.3)	8.4(1.4)	8.0(1.4)	8.3(2.4)	8.0(2.4)	8.7(1.2)
ANOVA								
F value	0.23	0.30	0.04	0.79	0.13	0.19	0.95	2.34
Sig.	ns	ns	ns	ns	ns	ns	ns	ns

ns = not significant according to one-way ANOVAs. Variables where no significant differences were found according to Tukey's range test were not assigned letters. †VC = vermicompost.

#### 4.3.2 *Growing medium parameters*

Table 4.9 shows the different characteristics of the growing media on day 0 of the trial. Of the different biological characteristics, only AWCD and dehydrogenase activity were statistically significant differences observed between treatments (Table 4.9). There was no difference in fungal biomass between the media. Growing medium with 10% added vermicompost had significantly lower AWCD than that containing 20% vermicompost, while with dehydrogenase activity, both 10% and 20% vermicompost had significantly higher activity than 0% (Table 4.9). The chemical characteristics measured, pH and EC, showed significant increases in both parameters with each increase in vermicompost concentration.

PCA and hierarchical cluster analysis of the bacterial profiles of the three growing medium treatments on day 0 can be seen in Figure 4.1 a and b. One replicate from the 20% vermicompost treatment was removed from this analysis as it was clearly an outlier. PCA analysis shows that there is a shifting trend to the right of axis two with increasing vermicompost concentration. Increasing the vermicompost concentration to 20% also resulted in a wider distribution around axis 1. Cluster analysis showed some grouping of the 20% vermicompost samples together, then the 0% vermicompost samples, and then the 10% vermicompost samples (Figure 4.1 b).

The development of the microbial parameters throughout the trial harvests can be seen in Table 4.10. There were some small, one-off significances found in the early months of the trial, while there were some clearer trends observed in month 3 and month 4 harvests. During these latter two harvests, rhizosphere AWCD and richness was significantly higher in plants grown with 10% vermicompost compared to 0%.

Table 4.9 Effect of vermicompost incorporation on growing medium biological, physical and chemical characteristics ( $\pm$ SD), day 0

	AWCD†	Richness median no.	Shannon	DA¶ $\mu\text{g g}^{-1}$ TPF	Ergosterol $\mu\text{g g}^{-1}$	WHC‡ $\text{ml L}^{-1}$	pH	EC§ $\text{mS cm}^{-1}$
0% VC†	0.147(0.021)ab	9(2)	1.75(0.25)	17.70(10.09)a	11.10(2.19)	305.88(50.66)	5.59(0.01)a	1.05(0.06)a
10% VC	0.140(0.017)a	7(2)	1.67(0.18)	51.07(15.72)b	10.02(1.07)	306.68(74.38)	5.64(0.01)b	1.65(0.12)b
20% VC	0.184(0.034)b	9(2)	1.69(0.23)	77.87(31.25)b	11.36(0.04)	246.31(79.56)	5.73(0.01)c	2.21(0.07)c
F value/Chi <sup>2</sup>	4.52	3.02	0.20	12.65	0.76	0.83	196.00	133.19
Sig.	*	ns	ns	***	ns	ns	***	***

ns = not significant, \*= $p \leq 0.05$ , \*\* =  $p \leq 0.01$ , \*\*\*= $p \leq 0.001$  according to one-way ANOVAs (F value) or Kruskal Wallis Test (Chi<sup>2</sup>) (richness only). Means in the same column followed by the same letter were not significantly different,  $p > 0.05$ , according to Tukey's Range Test. Variables where no significant differences were found were not assigned letters. †AWCD = Average Well Colour Development. ‡WHC = water holding capacity. §EC = electrical conductivity. ¶DA = Dehydrogenase Activity. †VC = vermicompost.

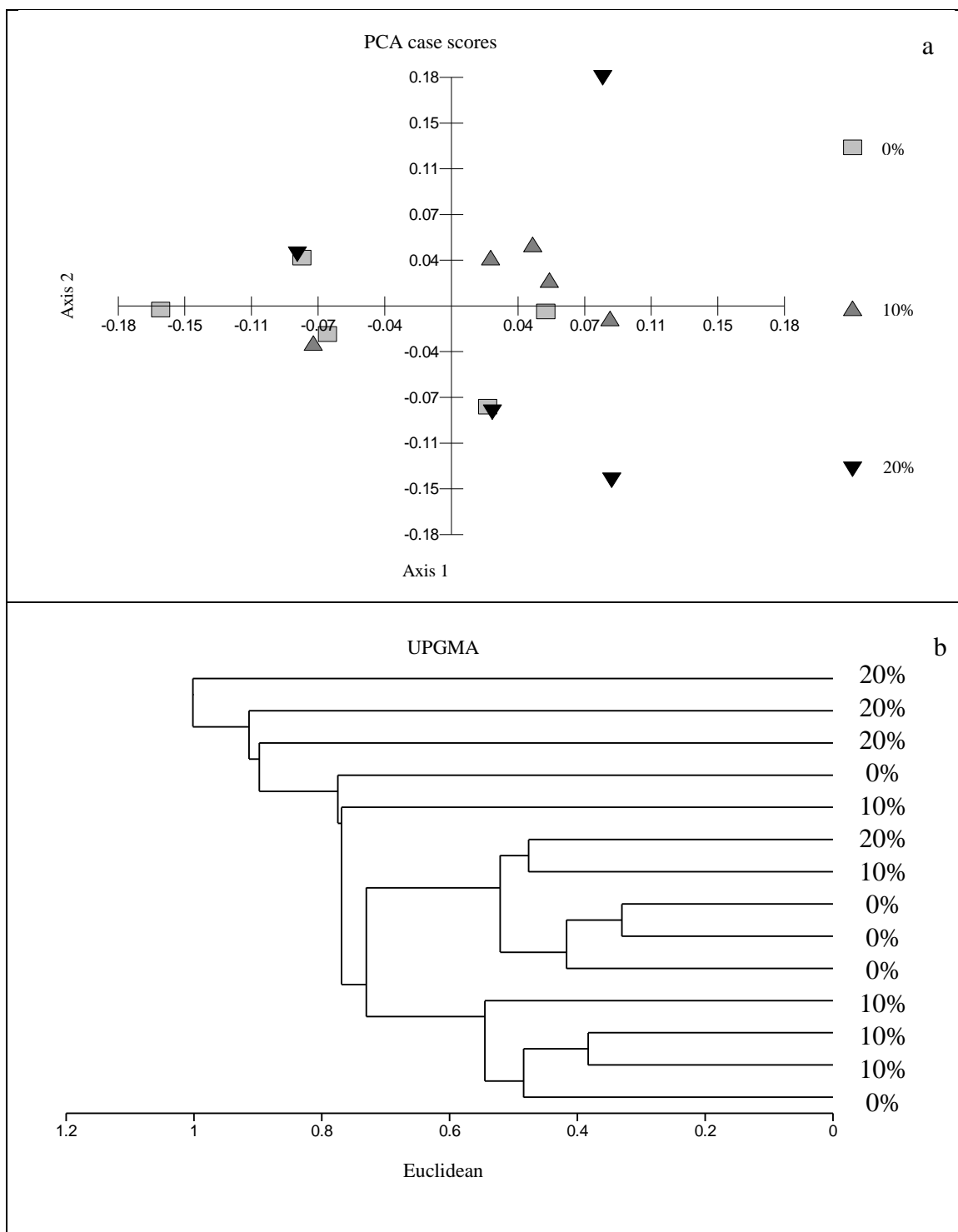


Figure 4.1 a and b. Effect of vermicompost incorporation on PCA and Hierarchical Cluster Analysis, respectively, of microbial profile at Day 0 (cumulative % of axes 1 and 2 = 49.20%)

Table 4.10 Growing medium biological characteristics ( $\pm$ SD) day 15 to month 4

Medium Analysed		AWCD <sup>†</sup>		Median Richness		Shannon		Dehydrogenase Activity	
Harvest	% VC <sup>¶</sup>	B <sup>‡</sup>	R <sup>§</sup>	B	R	B	R	B	R
$\mu\text{g g}^{-1}$									
Day 15	0	0.246(0.100)	0.219(0.085)	13(3)	13(5)	1.95(0.30)	2.19(0.37)	60.83 (38.37)	35.31(13.04)ab
	10	0.238(0.064)	0.287(0.065)	15(4)	15(2)	1.85(0.16)	2.29(0.21)	56.31(41.61)	32.20(10.05)a
	20	0.219(0.022)	0.305(0.074)	15(1)	16(2)	1.84(0.21)	2.09(0.31)	72.07(31.07)	81.33(36.19)b
F value/Chi <sup>2</sup>		0.19	1.84	0.34	2.01	0.36	0.54	0.80	4.39
Sig.		ns	ns	ns	ns	ns	ns	ns	*
Month 1	0	0.175(0.027)	0.282(0.090)	13(2)	15(1)a	2.06(0.28)	2.24(0.23)	58.44(17.97)	51.29(25.99)
	10	0.240(0.116)	0.291(0.079)	13(5)	16(4)ab	1.75(0.36)	2.17(0.11)	64.89(29.93)	70.09(59.24)
	20	0.154(0.027)	0.320(0.065)	11(1)	24(2)b	1.93(0.11)	1.96(0.60)	93.57(21.22)	55.16(37.18)
F value/Chi <sup>2</sup>		2.05	0.32	2.83	8.63	1.71	0.76	2.62	0.08
Sig.		ns	ns	ns	*	ns	ns	ns	ns
Month 2	0	0.246(0.092)	0.272(0.101)	14(6)	15(3)	2.05(0.14)	2.10(0.50)	298.35(98.62)	778.30(377.80)
	10	0.196(0.060)	0.265(0.089)	11(5)	18(5)	1.95(0.12)	2.20(0.28)	402.82(206.97)	785.90(286.40)
	20	0.206(0.102)	0.307(0.066)	11(6)	22(3)	2.05(0.31)	2.25(0.26)	447.33(261.54)	878.72(286.23)
F value/Chi <sup>2</sup>		0.47	0.34	1.51	5.78	0.42	0.21	0.16	0.19
Sig.		ns	ns	ns	ns	ns	ns	ns	ns
Month 3	0	0.163(0.051)ab	0.120(0.050)a	12(5)	10(4)a	1.84(0.35)	1.95(0.18)	297.81(119.59)	1399.78(844.27)b
	10	0.249(0.105)b	0.318(0.082)b	17(8)	23(7)b	1.69(0.53)	1.88(0.34)	243.35(58.53)	326.17(139.16)a
	20	0.107(0.042)a	0.209(0.052)ab	8(3)	14(4)ab	1.77(0.35)	2.01(0.36)	306.46(73.72)	363.77(239.34)a
F value/Chi <sup>2</sup>		4.96	12.46	4.80	9.78	0.15	0.20	0.70	5.81
Sig.		*	***	ns	**	ns	ns	ns	*
Month 4	0	0.183(0.074)	0.179(0.056)a	15(4)	15(3)a	2.33(0.14)	2.21(0.20)	434.17(219.67)a	839.18(338.62)
	10	0.221(0.086)	0.356(0.092)b	18(6)	22(4)b	2.16(0.20)	2.34(0.31)	540.53(149.67)ab	814.03(226.18)
	20	0.289(0.096)	0.250(0.016)ab	23(4)	21(3)ab	2.18(0.32)	2.11(0.50)	894.44(172.69)b	663.73(132.36)
F value/Chi <sup>2</sup>		1.96	10.96	5.84	7.80	0.77	0.54	5.56	0.74
Sig.		ns	**	ns	*	ns	ns	*	ns

ns = not significant, \*= $p \leq 0.05$ , \*\* =  $p \leq 0.01$ , \*\*\*= $p \leq 0.001$  according to one-way ANOVAs (F value) or Kruskal-Wallis test (Chi<sup>2</sup>) (median richness only). Means harvested in the same month and column followed by the same letter were not significantly different,  $p > 0.05$ , according to Tukey's Range Test or Kruskal-Wallis Multiple Comparison test (median richness only). Variables where no significant differences were found were not assigned letters.

<sup>†</sup>AWCD = Average Well Colour Development. <sup>‡</sup>B = bulk. <sup>§</sup>R = rhizosphere. <sup>¶</sup>VC = vermicompost.

The addition of 20% vermicompost to Lightmix significantly increased rhizosphere dehydrogenase activity compared to 0% during day 15 harvest, while during month 3 harvest the 0% vermicompost rhizosphere samples had significantly higher dehydrogenase activity than the 10% and 20% vermicompost samples. When comparing the dehydrogenase activity of bulk and rhizosphere samples, rhizosphere samples had significantly higher mean dehydrogenase activities in month 2, 3 and 4 harvests ( $F_{1,2} = 17.75$ ,  $p = <0.001$ ,  $F_{1,2} = 7.34$ ,  $p = 0.01$  and  $F_{1,2} = 4.91$ ,  $p = 0.04$ , respectively) than bulk samples (data not shown).

#### 4.3.3 *Vermicompost storage results*

During storage, some vermicompost parameters varied over time while others remained constant (Table 4.11). The pH range during the storage period was narrow for a composted organic material, between 6.61 and 7.00. Months 1 and 5 had lower pH values than the other months sampled, while month 3 and month 4 had the highest pH of the dates sampled but there was no trend with pH over time found with regression analysis (Table 4.12).

EC showed a positive quadratic trend with increasing storage time (Table 4.12), even though the mean moisture content ( $\pm$ SD) of the VC did not change over time ( $70.83\% \pm 0.57\%$ ) (data not shown). At months 5 and 6, vermicompost had the highest EC of the seven sampling dates. Dehydrogenase activity over time had a significant positive linear regression,  $r = 0.516$  with increasing storage time. AWCD, richness and Shannon index all showed similar quadratic relationships with storage time, with values in all three parameters dipping during the middle of the storage period, months 2 to 4, and rising again at the end of the storage period, month 5 and 6 (Table 4.11).

Nutrient analysis of the vermicompost during storage (Table 4.13) was quite variable, especially during month 3 where the macro-nutrient content was between 50 and 241% higher than day 0 and month 6 values. This increase in month 3 was not as distinctive in the Na and Cl contents during the sampling periods as it was for the plant macro-nutrients (Table 4.13). PCA and hierarchical cluster analysis of the Biolog data during the storage period (Figure 4.2 a and b) showed that the vermicompost had a similar bacterial community structure at each sampling date, with no clustering of the sampling dates or trends over storage time.

Table 4.11 Vermicompost chemical and biological parameters measured ( $\pm$ SD) during 6 months of storage

	pH	EC <sup>†</sup> mS cm <sup>-1</sup>	OM <sup>‡</sup> %	Dehydrogenase Activity µg TPF/g	AWCD <sup>§</sup>	Median Richness	Shannon
Day 0	6.88 $\pm$ 0.04	5.49 $\pm$ 0.14	59.26 $\pm$ 2.35	429.07 $\pm$ 88.55	0.203 $\pm$ 0.01	12 $\pm$ 3	2.036 $\pm$ 0.11
Month 1	6.65 $\pm$ 0.03	5.30 $\pm$ 0.12	62.11 $\pm$ 1.07	341.46 $\pm$ 14.24	0.156 $\pm$ 0.05	10 $\pm$ 3	1.715 $\pm$ 0.16
Month 2	6.92 $\pm$ 0.01	5.65 $\pm$ 0.19	59.78 $\pm$ 2.02	594.79 $\pm$ 22.55	0.070 $\pm$ 0.07	4 $\pm$ 3	1.675 $\pm$ 0.47
Month 3	6.99 $\pm$ 0.01	5.83 $\pm$ 0.05	59.39 $\pm$ 1.62	446.60 $\pm$ 65.55	0.114 $\pm$ 0.03	5 $\pm$ 1	1.499 $\pm$ 0.23
Month 4	7.00 $\pm$ 0.02	5.35 $\pm$ 0.11	58.86 $\pm$ 3.07	664.11 $\pm$ 126.64	0.100 $\pm$ 0.03	4 $\pm$ 1	1.44 $\pm$ 0.12
Month 5	6.61 $\pm$ 0.03	6.26 $\pm$ 0.04	58.83 $\pm$ 1.23	547.79 $\pm$ 24.28	0.179 $\pm$ 0.01	9 $\pm$ 2	1.808 $\pm$ 0.26
Month 6	6.93 $\pm$ 0.04	6.55 $\pm$ 0.22	59.75 $\pm$ 0.68	563.95 $\pm$ 132.00	0.159 $\pm$ 0.05	10 $\pm$ 1	1.923 $\pm$ 0.21

<sup>†</sup>EC = electrical conductivity. <sup>‡</sup>OM = organic matter. <sup>§</sup>AWCD = Average Well Colour Development.

Table 4.12 Regression analysis of the vermicompost physicochemical and biological parameters with time during the six month storage period

Parameter	Regression Equation	r value	Adjusted R <sup>2</sup> value	F value	P value
pH	$y = 0.006x + 6.838$	0.077	-0.046	0.113	ns
EC <sup>†</sup> (mS cm <sup>-1</sup> )	$y = 0.046x^2 - 0.105x + 5.495$	0.837	0.666	20.98	<0.001
OM <sup>‡</sup> (%)	$y = 60.354 - 0.214x$	0.231	0.003	1.069	ns
Dehydrogenase Activity (μg TPF g <sup>-1</sup> )	$y = 31.67x + 417.54$	0.516	0.228	6.892	<0.05
AWCD <sup>§</sup>	$y = 0.010x^2 - 0.062x + 0.196$	0.641	0.345	6.277	<0.01
Richness	$y = 0.825x^2 - 5.262x + 12.865$	0.791	0.584	15.046	<0.001
Shannon	$y = 0.053x^2 - 0.333x + 2.036$	0.655	0.366	6.772	<0.01

<sup>†</sup>EC = electrical conductivity. <sup>‡</sup>OM = organic matter. <sup>§</sup>AWCD = Average Well Colour Development. ns = not significant.

Table 4.13 Vermicompost pH, electrical conductivity (EC) and nutrient analysis during storage

	NO <sub>3</sub> -N	NH <sub>4</sub> -N	P	K	Ca	Na	Cl
	-----mg l <sup>-1</sup> -----						
Day 0	545.4	11.1	80.1	2288.2	1237.0	494.7	1120.6
Month 3	895.3	29.5	120.1	3334.7	4044.6	549.7	1490.2
Month 6	529.6	26.8	47.2	2322.7	1187.2	502.6	1042.5

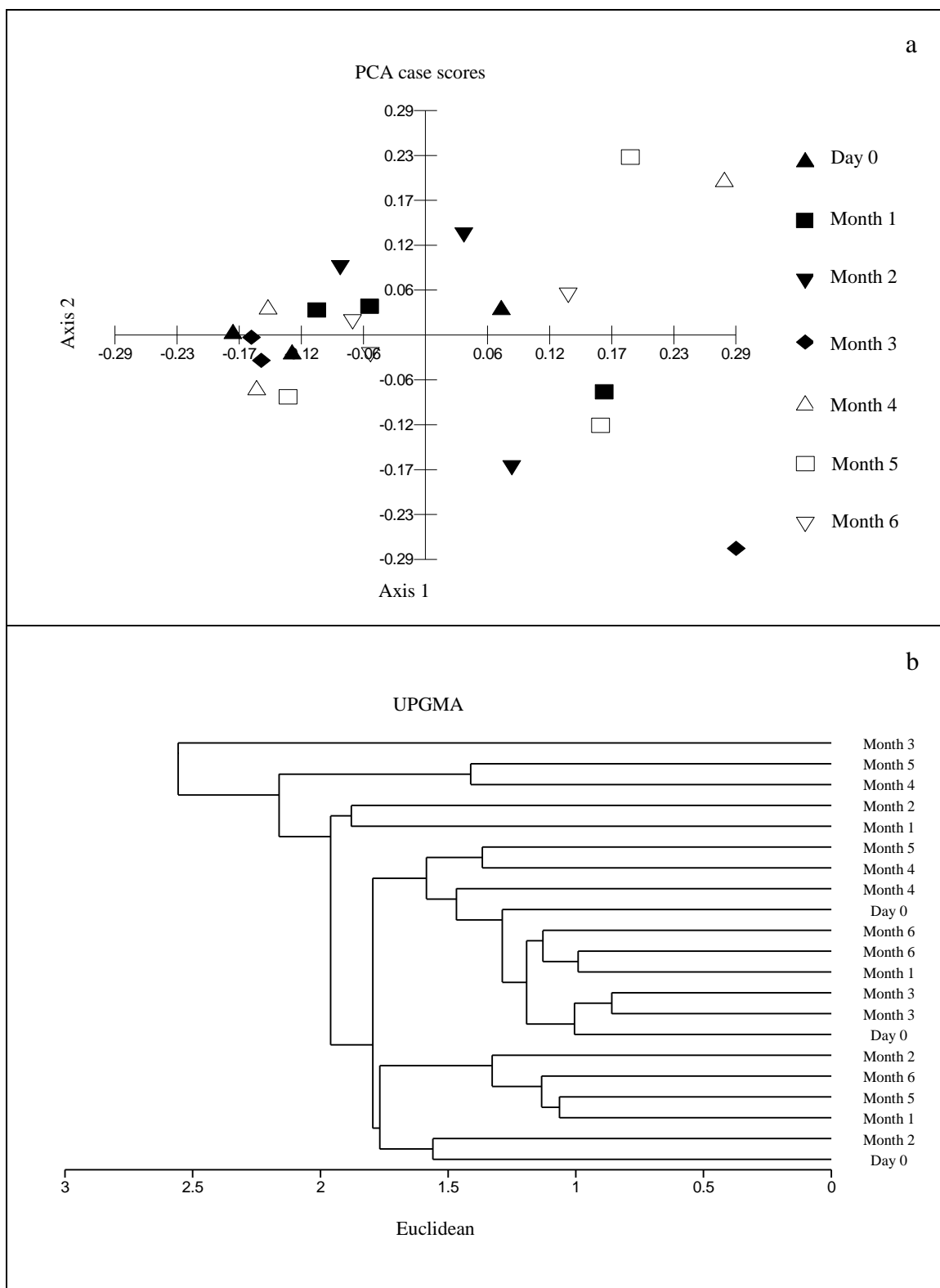


Figure 4.2 a and b PCA and Hierarchical Cluster Analysis, respectively, of 100% vermicompost from harvest to 6 months storage (cumulative % of axes 1 and 2 = 40.75%)

#### 4.4 Discussion

Compared to Lightmix, vermicompost had better chemical and biological growing medium properties i.e. richness and diversity of microbes, but some of the physical growing medium properties (Table 4.1) were better in Lightmix than in vermicompost e.g. air fill porosity density and water-holding capacity. Compared to growing medium and soil, vermicompost has been shown to have increased density of microbes (Masciandaro *et al.*, 2000; Atiyeh *et al.*, 2001; Chaoui *et al.*, 2003). This study also showed higher species diversity and richness of the bacterial community in the vermicompost, compared to the peat-based growing medium. Although vermicompost had increased macro- and micro-nutrient content, the addition of Osmocote and gypsum to the Lightmix would have provided enough nutrients to sustain the tomato plants throughout the growing cycle.

Increased bulk density and reduced air fill porosity in vermicompost, compared to peat-based growing medium, was also reported by Atiyeh *et al.* (2001) and Hidalgo & Harkess (2002). The effect of these parameters could increase the cost of transport of the growing media (Schmilewski, 2008), and reduce respiration and water availability in the root zone (Miller & Jones, 1995).

Vermicompost has been reported to increase water-holding capacity in a peat/perlite growing medium (Hidalgo & Harkess, 2002), while, as in this study, vermicomposted SMC was shown to reduce the water-holding capacity of peat-based compost, with increasing concentration of SMC (Medina *et al.*, 2009). The effect of vermicompost on the water-holding capacity of growing medium may be dependent on the initial feedstock used to create the vermicompost, or alternatively, it may depend on the initial water-holding capacity of the growing medium components that

the vermicompost is added to, i.e. peat has an higher water-holding capacity compared to perlite (Evans, 2011), and Evans & Stamps (1996) found that growing media containing coconut coir dust had higher water-holding capacities than those containing peat.

If the physical properties of the growing media were negatively impacted upon, this effect was not seen in plant growth, as only one of the six harvests (month 3) produced plants from either vermicompost treatment with significantly lower shoot dry weight than those grown in 0% vermicompost; in month 1 stem dry weight was significantly higher in plants grown in the 0% vermicompost treatment than those with 20% vermicompost.

Even though the quantity of chemical fertiliser was reduced, the 20% vermicompost treatment had significantly higher plant shoot fresh weight during three of the six harvest periods than did the 0% treatment (Table 4.3). This was driven by an increase in leaf fresh weight, rather than stem fresh weight. This increase in leaf fresh weight in the presence of adequate nutrition could be attributed to a biostimulant effect, as was also found by Atiyeh *et al.* (2000b) in tomato plants grown with vermicompost with added fertigation in young plants, but not in mature tomato plants (Atiyeh *et al.*, 2000a). The significant decrease in shoot and/or stem dry weight in plants in the 20% vermicompost treatment, compared to those in the 0% vermicompost treatment, at month 1 and month 3 harvests, was mitigated by the significantly increased shoot water content in one or both of the vermicompost treatments, resulting in no significant fresh weight differences. There were some significant effects of vermicompost on plant and fruit moisture content as was seen in other trials (see section 2.3.2 and 3.3.3), but these effects were not consistent throughout the trial. This will be discussed further in chapter 6.

Overall, the effect of vermicompost on plant growth was not uniform across each harvest. Importantly, despite the high EC of the vermicompost, early shoot fresh weight (day 15 and month 1 harvest) was not negatively affected by vermicompost addition to the growing medium, indicating that the addition of 10, and 20% vermicompost was suitable for tomato seedling germination. The use of 20% vermicomposted food waste, and 10, and 20% vermicomposted pig manure was also found to be suitable for tomato germination by Zaller (2007b) and Atiyeh *et al.* (2000a), respectively. It is important to note that plant chlorophyll content, an indirect measure of plant productivity and leaf nitrogen content (Xue & Yang, 2009), was reduced in plants grown with 20% vermicompost compared to those grown with 0% vermicompost at month 1 harvest (Table 4.4). In contrast to this, the chlorophyll content of the upper leaf canopy (leaf 10) was higher in plants grown with vermicompost addition compared to those with no vermicompost addition. This indicated delayed leaf senescence in the plants grown with vermicompost addition compared to those grown without.

Delayed leaf senescence could also be explained by delayed plant development, as was also signified by delayed flowering and ripening in truss two of plants grown with 20% vermicompost, compared to plants grown with 0% vermicompost. De Koning (1994) found that moderate increases ( $0.3 \text{ mS cm}^{-1}$  to  $0.9 \text{ mS cm}^{-1}$ ) in salinity significantly reduced flowering rate (trusses  $\text{d}^{-1}$ ) by 4%, resulting in delayed flowering. Despite the reduction in flowering and ripening, all three treatments were fully ripe by week 20, and had statistically similar truss positions on the stem (an indicator of plant development (de Koning, 1994)), signifying that, although there was some delay in plant development of plants grown with 20% vermicompost, this did not reduce the time to harvest, which was analysed every seven days.

Unlike shoot growth, the added vermicompost had no significant effect on fruit fresh or dry weight during the different harvest periods (Table 4.6). Fruit water content was reduced only in the final harvest, in fruits from plants grown with 20% vermicompost compared to those grown with 0%, which could indicate salinity stress (Cuartero & Fernández-Muñoz, 1999), although this did not affect overall fruit fresh or dry weight. The addition of vermicompost to Lightmix significantly increased fruit percentage marketable yield during month 2 and 3 harvests (Table 4.6). Increased marketable yield was also observed in studies carried out by (Atiyeh *et al.*, 2000a; Arancon *et al.*, 2003a; Gutiérrez-Miceli *et al.*, 2007).

This increase in marketable yield was driven by the reduction in fruit BER in the vermicompost treatments compared to the control, despite the similar levels of  $\text{Ca}^{2+}$  in each treatment. SMC is naturally high in  $\text{Ca}^{2+}$  as it is used in the manufacture of mushroom compost.  $\text{Ca}^{2+}$  is added in the form of gypsum and lime, and it is incorporated to soak up ammonia, prevent colloid materials forming a ‘greasy’ compost and to provide structure during the composting process (Waste & Resources Action Programme, 2007). BER is a physiological disorder resulting from an inadequate supply of calcium to the tomato fruits leading to lesions and necrosis at the blossom end of the fruit, rendering it unmarketable. BER occurrence can be brought on by lack of calcium within the growing medium (Saure, 2001), high salinity in the root zone (Cuartero & Fernández-Muñoz, 1999), inadequate watering (Adams & Ho, 1992) and competition for  $\text{Ca}^{2+}$  within the vegetative and reproductive plant biomass (Saure, 2001), amongst others. Premuzic *et al.* (1998) also found that fruits of tomato plants grown with vermicompost had a higher calcium content than those grown in sand or peat-perlite growing medium mixtures with added calcium fertigation. In contrast to this, Zaller (2007a) did not find

significantly increased fruit calcium content with vermicompost addition to a peat-based growing medium, and found, in one of the three cultivars tested, that 20% vermicompost significantly reduced calcium content compared to 0% vermicompost. Additional calcium content in the fruits may be due to reduced ion competition in the root zone (Adams & Ho, 1992). As the chemical fertiliser content was reduced in the vermicompost treatments, enhanced plant nutrient uptake with vermicompost addition may have occurred (Premuzic *et al.*, 1998; Atiyeh *et al.*, 2000a), thereby enhancing  $\text{Ca}^{2+}$  uptake by the roots (Adams & Ho, 1993).

Fruit quality parameters for fresh and processing tomatoes include total soluble solids, pH and titratable acidity (Cuartero & Fernández-Muñoz, 1999). Soluble solids are a measure of fruit sugar content, while pH and titratable acidity are a measure of acidity. A high sugar and acid content is required for best tomato flavour (Kader, 1986). Tomatoes are regarded as an acid food with a desired fruit pH of 4.25 and a maximum pH limit of 4.4 to reduce spoilage in processing tomatoes (Garcia & Barrett, 2006). In this experiment, the lowest pH was found in truss one fruits from each treatment, while pH of truss two fruits decreased with increasing vermicompost concentration, closely approaching the maximum desired pH of 4.4 (Table 4.7). In this study the addition of vermicompost significantly increased fruit soluble solids, titratable acidity and reduced pH compared to fruits of plants grown in 0% vermicompost, although the effectiveness of both vermicompost concentrations were not consistent amongst trusses. Fruits with higher soluble solids and titratable acidity would have better flavour characteristics and therefore demand increased price and result in reduced spoilage during storage (Cuartero & Fernández-Muñoz, 1999). The addition of vermicompost to soil or growing medium was also found to increase fruit

quality by increasing soluble solids (Gutiérrez-Miceli *et al.*, 2007), and increasing peel firmness and glucose and fructose contents (Zaller, 2007a).

Generally, a soluble solids level lower than 4.2 and a pH of above 4.4 does not meet industry standard. The fruits in this trial did not meet these requirements but this is most likely due to a cultivar unsuited to ripening in an Irish climate and harvesting the fruits late in the year (November) when daylight intensity, and day length period (even with supplemental light) was insufficient to ripen fruits with the correct pH and soluble solids content.

Increased soluble solids and titratable acidity with vermicompost addition is possibly explained by the increased electrical conductivity in the growing medium which is known to increase these parameters (Cuartero & Fernández-Muñoz, 1999). Increased soluble solids and titratable acidity with vermicompost addition was also found by Gutiérrez-Miceli *et al.* (2007) and Singh *et al.* (2010), while Olivares *et al.* (2015) found no difference in pH and Brix levels with the addition of 50% vermicompost to soil, compared to 100% soil. Although these results suggest fruits grown with vermicompost would have improved flavour than fruits grown without vermicompost, there was no significant difference found in sweetness, sourness, and overall flavour found by the consumer acceptance panel (Table 4.8), although overall tomato acceptability was found to be positively related to vermicompost addition.

Despite the fact that vermicomposted SMC had increased ergosterol content, dehydrogenase activity, bacterial richness and diversity than Lightmix peat-based compost (Table 4.2), the addition of 10 and 20% vermicompost only increased dehydrogenase activity significantly compared to the Lightmix control when mixed initially (Table 4.9). Vermicompost addition increased microbial activity, as

indicated by the increase in dehydrogenase activity, but it also shifted the bacterial community structure of the 10% and 20% vermicompost growing medium mixes compared to the mix containing 0% vermicompost (Figure 4.1 a). Atiyeh *et al.* (2000b), in contrast, found that the sum of biological activity of Biolog GN plates (a similar measurement to average well colour development) was significantly higher in 10% and 20% vermicompost-amended peat-based growing medium than in unamended peat-based growing medium, while Atiyeh *et al.* (2001) found that vermicompost significantly increased dehydrogenase activity after >50% vermicompost addition to a peat-based growing medium.

Vermicompost addition to soil has been commonly found to increase microbial activity (Masciandaro *et al.*, 2000; Chaoui *et al.*, 2003; Arancon *et al.*, 2006a), but, as peat is largely microbially inactive, this increase is not as distinct as it may be in soil. There was a significant effect of 10, and 20% vermicompost addition to growing medium pH and EC upon initial mixing, suggesting that the chemical effects of moderate additions of vermicompost to growing media were stronger than the biological effects.

After planting, the effect of vermicompost on the biological properties of the bulk growing medium and the rhizosphere samples varied between harvests, and significant differences were found mainly in the rhizosphere samples rather than in bulk samples. Month 3 and 4 harvests showed similar results with rhizosphere AWCD and richness with samples from 10% vermicompost having significantly higher values than those from 0% vermicompost. This indicated that even small amounts of vermicompost can have a lasting effect on the rhizosphere bacterial numbers and richness after a long period of time. This was also found by Jack *et al.* (2011), in a study looking at the effect of vermicomposted animal manure on

bacterial communities over the lifecycle of tomato plants, who found that vermicompost had a significant effect of rhizosphere communities towards the end of the plants' life cycle, at anthesis and maturity, more so than at the early stages, at pre-planting and transplanting. It is possible that this is due to the production of root exudates, which have a significant effect of the rhizosphere microbial community (Bais *et al.*, 2006).

It has been shown that tomato plants produce root exudates in different amounts and compositions at different times in their lifecycle with root exudate yield peaking at 54 days after germination, and falling to 43% and 20% of the peak value at flowering and fruiting, respectively (Davey & van Staden, 1976). Therefore it is possible that these root exudates are having a stronger effect on growing medium microbial community during the earlier, more vigorous vegetative and reproductive stages of tomato growth than at the latter stages, when 10% vermicompost was found to influence biological properties.

Storage had little effect on vermicompost chemical and biological parameters. There was a narrow pH range (0.4 units) during the six-month storage period. A narrow pH range (0.2 units) was also found by Karthikeyan *et al.* (2014) over a 12-week storage period. EC did increase towards the final months of storage; this could possibly be due to increased mineralisation of the organic matter as the microbial content increased. The increase in microbial activity with storage was found by Kleawklaharn & Iwai (2014) after vermicompost made from cassava waste, soil and cow dung was stored for three months in breathable sacks. Reduced microbial activity was found when the vermicompost was stored in air-tight bags, or when there was large losses in the moisture content of the vermicompost (Gupta *et al.*, 2014; Karthikeyan *et al.*, 2014).

The three Biolog variables all dipped in value during the middle of the storage period and recovered towards the end of the storage period. As there were no chemical or physical variables following the same relationship, it is unlikely that these are driving this process and is possibly due to cyclical processes within the activity of the bacterial community as proposed by Tereshchenko *et al.* (2014).

The addition of 10% and 20% vermicompost to a fertilised peat-based growing medium increased tomato plant growth, tomato fruit yield and yield quality parameters, all while reducing the peat-content of the growing medium and by reducing the concentration of chemical fertiliser used by 10% and 20%, respectively. This study highlights the potential benefits of vermicompost use in both amateur and professional horticulture for fruit production.

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# Chapter 5

Effect of vermicomposted spent mushroom compost on tolerance to abiotic stress in garden cress (*Lepidium sativum*)

## Abstract

Abiotic stresses, such as cold, heat and salinity, are some of the most important factors limiting crop production worldwide, resulting in large losses in yield and food production. This study investigated the effect of moderate vermicompost applications (10% or 20% vol/vol) to soil on plant growth under salinity, heat or cold stress using the model plant *Lepidium sativum* (garden cress). When exposed to moderate levels of salt stress, there was no effect on plant photochemical efficiency, though average plant fresh and dry weight fell significantly when exposed to salt stress. The addition of vermicompost did not alleviate the effects of salinity stress. When exposed to acute heat stress, plant photochemical efficiency was significantly reduced compared to unstressed plants, and it took 21 hours for the stressed plants to return to similar levels of photochemical efficiency as the unstressed plants. After a three-day recovery period, plant growth was still reduced in the heat-stressed plants compared to the unstressed plants, but there was no vermicompost effect on plant growth. The addition of vermicompost to soil, particularly at 20% vol/vol, increased plant fresh and dry weight in plants exposed to chronic cold stress, and increased photochemical efficiency significantly in two independent outdoor trials.

**Keywords:** heat stress, cold stress, salinity stress, chlorophyll fluorescence, vermicompost, *Lepidium sativum*

## 5.1 Introduction

Abiotic stress is currently one of the most important issues facing global crop production. Depending on the type and severity of the stress, it can damage or even eliminate a crop (Warrick, 1984; Mahajan & Tuteja, 2005), potentially with a complete loss of food value. Drought, temperature and salinity stress are the most damaging abiotic stressors, especially taking into account the potential cumulative effects of climate change bringing more frequent extremes of weather (Coumou & Rahmstorf, 2012). Combined with this, the world's increasing population will result in a greater demand for food. This demand will have to be met with increased yields and cropping intensity rather than land expansion, as arable land is set to increase in developing countries by 12% but reduce in developed countries by 8% (Food and Agriculture Organisation of the United Nations (FAO), 2009). Salinization of irrigated soils is becoming increasingly problematic due to the long-term use of irrigation, and the use of poor quality irrigation sources with salt concentrations high enough to cause damage to plants ( $>2 \text{ g L}^{-1}$ ) (Brouwer *et al.*, 1985). Between 1992 and 2010, 60% of the total water extracted worldwide was used for agriculture (FAO, 2015), and this is expected to grow by 11% by 2050 (FAO, 2009) as effects of climate change become more obvious. Heat stress is an ever-increasing issue due to climate change (Ahuja *et al.*, 2010). High temperatures cause plant stress either directly, by damaging enzymes or membranes, or indirectly via greater evapotranspiration. Cold stress can hinder early crop development by causing chilling and freezing injury (Yadav, 2010). The ability of plants to grow through cold periods is important as this would allow for extended growing periods and increased food production in cooler climates.

Plants develop morphological and physiological responses to stress via three mechanisms: tolerance, avoidance and escape. Stress tolerance involves developing mechanisms which allow the plants to adjust to, or acclimate to the stress, expanding the range of conditions under which a plant can operate. Stress tolerance mechanisms are diverse and can be stress-specific or stress non-specific. For example stress-specific tolerances include heat tolerance by the production of heat shock proteins (Vierling, 1991) and the exclusion of  $\text{Na}^+$  from the leaves as part of salinity tolerance (James *et al.*, 2011). Stress non-specific tolerances include the production of reactive oxygen species (ROS) scavengers i.e. antioxidants and ROS quenching enzymes, which are produced in reaction to a number of stressors such as heat, chilling and drought stress (Apel & Hirt, 2004) and the production of osmolytes, which reduce cellular dehydration and improve membrane functionality during cold (Yadav, 2010), heat and drought stress (Prasad *et al.*, 2008).

Stress avoidance involves the development of plant responses which allow the plant to operate under the stressful conditions without suffering from the stress itself. For instance, deep rooting plants can tap into moisture deep within the soil profile during periods of drought, therefore not suffering from drought stress (Chaves *et al.*, 2002).

Stress escape involves changes in plant phenology which allow the plant to grow without being exposed to the stressful conditions. For instance in hot climates, winter/spring annuals avoid the hottest period of the year, thereby escaping the heat and drought stress that is associated with the summer period (Chaves *et al.*, 2002).

Under field conditions, crops often experience multiple abiotic stressors consecutively or concurrently (Mittler, 2006). For example in the developing world, heat stress is commonly experienced alongside drought stress (Prasad *et al.*, 2008),

and salinity stress if the crop is irrigated, while in Europe cold stress (during spring) can be followed by heat stress (during summer). During these periods of multiple stressors, non-specific stress tolerance and stress avoidance mechanisms are required.

The addition of organic matter to poor-quality soil has been shown to alleviate abiotic stress in crops. Organic matter addition can reduce the effects a variety of abiotic stressors as it increases soil water-holding capacity (Khaleel *et al.*, 1981), and reduces soil compaction (Soane, 1990), leading to a deeper, more branched root system capable of gaining access to additional soil water and nutrient stores. It increases soil aggregation, allowing for a quicker infiltration of water and quicker drainage (Bot & Benites, 2005) which reduces water logging. The additional nutrients supplied by the organic matter (Jenkinson & Rayner, 1977), and the ability of organic matter to conserve nutrients (Khaleel *et al.*, 1981), can result in better soil nutrient status and added capacity for plants to tolerate or avoid abiotic plant stress (Papadopoulos & Rendig, 1983; Heckathorn *et al.*, 1996; Cakmak, 2005; Hu & Schmidhalter, 2005). Organic matter also supports a wide range of micro-biological activity, from mycorrhizal fungi to plant growth-promoting bacteria, both of which have been shown to increase plant growth under conditions of abiotic stress (Ruiz-Lozano, 2003; Yang *et al.*, 2009).

The use of vermicompost, vermicompost extracts and vermicompost-derived humic acids as a means of alleviating plant stress have previously been investigated. Vermicompost has been found to alleviate salinity stress in ginger shoots and rhizomes (Ahmad *et al.*, 2009), but not in cucumber seedlings (Sallaku *et al.*, 2009). Vermicompost extract has been found to alleviate salinity, heat and drought stress in tomato plants (Chinsamy *et al.*, 2013, 2014), while vermicompost-derived humic

acids were found to increase plant and root growth in rice plants exposed to drought stress (García *et al.*, 2014).

Garden cress (*Lepidium sativum*) is widely used as an model plant species in phytotoxicity and abiotic stress assays (Iglesias Jiménez & Perez Garcia, 1989; Gill *et al.*, 2012), and even for more specific bioassays such as the detection of the toxin microcystin-LR (produced by cyanobacteria in drinking water) (Gehring *et al.*, 2003). With regards to abiotic stress, cress has been shown to be a good indicator of soil heavy metal phytotoxicity (Plaza *et al.*, 2005) and salinity stress (El-Darier & Youssef, 2000). There has also been some investigation into the use of biostimulants to alleviate salinity stress in garden cress (Habibi & Abdoli, 2013), where it was found that a seed pre-treatment with salicylic acid increased percentage germination under salinity stress, but did not increase subsequent plant growth under salinity stress.

The aim of this study was to investigate whether the use of vermicomposted spent mushroom compost (SMC), incorporated into soil *ex situ*, alleviated heat, cold or salinity stress in garden cress, using either *in vivo* or *in vitro* trials.

## **5.2 Materials and methods**

### *5.2.1 Vermicompost manufacturing and soil/vermicompost analysis*

Vermicomposted SMC was made by feeding SMC (Reilly Mushrooms Ltd., Athlone, Ireland), consisting of straw, poultry manure, horse manure, peat, gypsum and lime to worms in a 12 m x 2 m x 1 m (l x h x w) flow-through vermicomposting bed. In this system, the SMC was fed at a rate of approximately 180 kg day<sup>-1</sup> to a combination of epigeal worm species, mainly consisting of *Eisenia fetida*. Other

decomposer organisms were also present in the vermicomposting bins including a range of fungi, bacteria and other commonly occurring soil-dwelling arthropods and pot worms. The density of worms in the top layer of the vermicomposting bed was  $60 \text{ g kg}^{-1}$ . Concurrent to daily feeding, finished vermicompost was harvested from the bottom of the bed at a daily rate of approximately  $90 \text{ kg day}^{-1}$ , and it took approximately 90 days for the SMC to go through the system. The vermicompost was harvested and stored in breathable sacks in a cool dry place until use.

Vermicompost and soil nutrient analysis was carried out by NRM Laboratories, Berkshire, UK. Electrical conductivity (EC), pH, dry matter, dry density and bulk density were measured according to BS EN 13040 2000 (British Standards Institution, 2000). The samples were extracted (1:5 vol/vol) with deionised water, pH was read, and filtered samples were analysed for EC.  $\text{Cl}^-$ ,  $\text{SO}_4\text{-S}$ , and  $\text{NO}_3\text{-N}$  was determined by ion chromatography,  $\text{NH}_4\text{-N}$  was determined by colorimetric analysis, and P, K, Mg, Ca, Na, B, Cu, Fe, Mn, Mo, and Zn were analysed using inductively coupled plasma–optical emission spectroscopy (Ministry of Agriculture Fisheries and Food, 1981; United States Environmental Protection Agency, 1996).

EC and pH of the soil/vermicompost mixtures collected at the beginning (heat and cold trials) or end (salinity trial) of the trial and were measured in a 1:5 soil/distilled water suspension. This suspension was placed on a shaking table for 1 hour, left to settle for 15 minutes, after which the pH of the solution was read (Thermo Scientific Orion 3 Star). The solution was then filtered through a Whatman Grade 1 filter paper, and EC was measured using a portable conductivity meter (WTW Cond 330i, WTW GmbH & Co., Weilheim, Germany).

### 5.2.2 Salinity stress trial

To test the effect of vermicompost addition to soil on cress response to saline irrigation, cress seeds, *Lepidium sativum* cv. Extra Curled, were sown in a commercially available Westland loamy sand topsoil (heat sterilised to eliminate weed seeds) (Westland Horticulture Ltd., Dungannon, United Kingdom), amended with 0%, 10% or 20% (vol/vol) vermicomposted SMC. Cress seeds were sown in each of the three soil treatments in 0.5 L pots (ten seeds per pot). The cress plants were grown on for sixteen days arranged in a replicated, randomised block design in a growth room (PAR  $110 \mu\text{mol m}^{-2} \text{s}^{-1}$ , 16 hour photoperiod,  $20^\circ\text{C} \pm 2$ , 60% relative humidity). Germination was recorded after seven days. The blocks were moved around once a week during the trial duration to minimise the effects of within-site variability. The day the seeds were sown the pots were watered with tap water, after this, the pots were watered every second day with 30 ml of one of three different distilled water/NaCl solutions:  $0 \text{ g L}^{-1}$  NaCl,  $5.4 \text{ g L}^{-1}$  NaCl and  $6.8 \text{ g L}^{-1}$  NaCl. There were ten replicate pots per treatment. These NaCl concentrations were chosen based on a previous dose-response trial (Figure 5.1) to achieve a 0%, 30%, and 50% reduction in cress dry weight, respectively. Ten pots from each soil treatment were watered with one of the three NaCl solutions for the sixteen-day duration, after which plant chlorophyll fluorescence (Imaging-PAM Chlorophyll Fluorimeter, Heinz Walz GmbH, Effeltrich, Germany), number of plants per pot, pot and plant fresh and dry weights (after oven drying at  $60^\circ\text{C}$  for seven days) were recorded.

Statistical analysis was carried out using IBM SPSS Statistics Package v.21. Normality tests were conducted on all parametric variables and where data was skewed, they were transformed (square root). Pot and plant fresh and dry weight, plant moisture content and chlorophyll fluorescence were analysed using parametric

two-way ANOVAs. Multiple comparison tests were carried out using Tukey's range test. Data presented represents mean values of the untransformed data. Non-normal variables, such as number of seeds germinated and number of plants per pot, were analysed using non-parametric two-way ANOVAs, and multiple comparison tests were carried out on the treatment means using Kruskal-Wallis multiple comparisons tests.

### 5.2.3 Heat stress trial

Cress seeds, *Lepidium sativum* cv. Extra Curled, were sown in Westland loamy sand topsoil amended with one of two levels of vermicompost or two levels of chemical fertiliser. Vermicompost was added at a rate of 10% or 20% (vol/vol) to the topsoil. Chemical fertiliser was added to horticultural grade sand at a rate of 924.8 mg L<sup>-1</sup> NH<sub>4</sub>, 120.1 mg L<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> and 3.33 g L<sup>-1</sup> K<sub>2</sub>O, to match the available nutrient content of vermicomposted SMC. Sand was used as a base with which to add the fertiliser, as this was an unfertilised base material which could be used to match the fertilised level of the vermicompost. This fertilised sand was added at a rate of 10% or 20% (vol/vol) to the topsoil to match the fertiliser value of the two vermicompost treatments. Cress seeds were sown in each of the four soil treatments in 0.5 L pots (ten seeds per pot) with six replicate pots sown per treatment.

The cress plants were grown-on for seven days arranged in a replicated, randomised block design in a growth room (5.2.2). The blocks were moved around the growth room half way-through this seven-day period. After the seven days, half of the plants were heat stressed. This was carried out on the plants during the dark hours of the photoperiod to reduce heat stress avoidance by transpiration. Half of the pots remained in the growth room in dark conditions (unstressed plants), and the

remaining pots were placed in the dark in replicated randomised block design in an incubator maintained at initially 25°C which, once the pots were placed in it, was brought slowly up to 40°C over a period of 1.5 hours, and then the plants were kept at 40°C for 2 hours (stressed plants). After 2 hours at 40°C the plants were removed from the incubator and kept in the dark in the growth room. Chlorophyll fluorescence of the stressed plants was measured (Imaging-PAM Chlorophyll Fluorimeter, Heinz Walz GmbH, Effeltrich, Germany) on the stressed plants 3 minutes, 30 minutes, 1 hour, and 2 hours after removal from the incubator. The stressed and unstressed plants were then put back under lights in the growth room in time for the usual light phase of the photoperiod. Chlorophyll fluorescence of the stressed and unstressed plants (after dark adaption for a minimum of 20 minutes) was also measured 4 hours and 21 hours after the stressed plants were removed from the incubator. The images were processed using the imaging software Imaging Win v2.0 (Heinz Walz GmbH, Effeltrich, Germany).

The stressed and unstressed plants were allowed to grow on under non-stress conditions for a further three days after the stress period before they were harvested. During harvesting the number of plants per pot, pot and plant fresh weight and dry weight (after oven drying at 60°C for seven days) were recorded. Due to the low number of replicate pots that would fit in the incubator at one time (three pots per treatment), the experiment was repeated four times under the same conditions to obtain four replicates in a replicated randomised block design.

Normality tests were conducted on all parametric variables and where data was skewed, they were transformed (square root). Pot and plant fresh and dry weight, and plant moisture content were analysed statistically with parametric three-way ANOVAs. Data presented represents mean values of the untransformed data. The

non-normal variable, number of plants per pot, was analysed using a non-parametric three-way ANOVA.

Plant chlorophyll fluorescence data of plants in the heat stress trial over time was analysed using a repeated measures three-way ANOVA followed by Tukey's range test. Analysis of chlorophyll fluorescence of the stressed and unstressed plants 4, and 21 hours after the stressed plants were removed from the incubator was analysed using parametric three-way ANOVAs. All statistical analysis described was carried out using IBM SPSS Statistics Package v.21.

#### 5.2.4 Cold stress trials

Two cold stress trials were conducted outdoors between December 2013 and March 2014. Both trials were set-up in the same way. The first trial was set-up on 6<sup>th</sup> December 2013 and the second on 27<sup>th</sup> January 2014. Cress seeds, *Lepidium sativum* cv. Extra Curled, were sown in Westland loamy sand topsoil, amended with 0%, 10% or 20% (vol/vol) vermicomposted SMC. Cress seeds were sown in each of the three soil treatments in 0.5 L pots (ten seeds per pot) with ten replicate pots sown per treatment. The pots were placed in a replicated randomised block design on wire shelving on gravel to allow for clearance and drainage, and to keep the pots spatially separated.

One parameter, percentage germination (trial two only), was recorded during the trial. Sixty days after sowing the plants were harvested. Harvests were carried out on a morning after a ground frost and the plant were processed at 08:00 the following morning. The number of live plants per pot, chlorophyll fluorescence (Imaging-PAM Chlorophyll Fluorimeter, Heinz Walz GmbH, Effeltrich, Germany), and pot and plant fresh and dry weight (after oven drying at 60°C for seven days) were recorded.

During these trials temperature was recorded every three minutes using a Testo 175 T2 data logger (Testo Ltd., Hampshire, UK). This data was used to obtain the daily maximum and minimum temperatures. Minimum grass temperature and daily rainfall data were obtained from the Met Éireann weather station at Cork Airport, 10 km from the trial site.

Statistical analysis was carried out using IBM SPSS Statistics Package v.21. Normality tests were conducted on all parametric variables and where data was skewed, they were transformed (square root) and analysed using parametric one-way ANOVAs followed by Tukey's range test. Data presented represents mean values of the untransformed data. The non-normal variable, number of plants per pot, was analysed using a non-parametric Kruskal-Wallis test followed by a Kruskal-Wallis multiple comparison test.

## **5.3 Results**

### *5.3.1 Topsoil and vermicompost nutrient analysis*

Compared to the topsoil, vermicompost had similar pH values, lower dry weight bulk density, and higher EC and macro- and micro-nutrient content (except for Mn and Fe) (Table 5.1). There was a difference in the nutrient content of the topsoil used in the salinity and cold trials, and in the topsoil used in the heat trials, with the former having a higher N, P, and K value than the latter (Table 5.1).

### *5.3.2 Salinity stress trial*

Figure 5.1 shows the results of the preliminary salinity dose response trial on cress plant dry weight. Increasing salinity had a negative linear effect on cress plant

Table 5.1 Physicochemical properties of the topsoil and the vermicomposted spent mushroom compost

Parameter	Unit	-----Trials used in-----		
		Topsoil Salinity and Cold	Topsoil Heat†	Vermicompost All
pH		6.1	6.9	6.6
EC‡	mS cm <sup>-1</sup>	0.49	0.33	5.38
Fresh Density	kg m <sup>-3</sup>	890	947	788
Dry Density	kg m <sup>-3</sup>	851	910	223
Dry Matter	%	95.6	96.1	71.7
NH <sub>4</sub> -N	mg L <sup>-1</sup>	22.3	31.2	29.5
NO <sub>3</sub> -N	mg L <sup>-1</sup>	142.6	49.5	895.3
P	mg L <sup>-1</sup>	16.6	2.7	120.1
K	mg L <sup>-1</sup>	100.7	25.7	3334.7
Mg	mg L <sup>-1</sup>	89	32.2	506.0
Ca	mg L <sup>-1</sup>	226.5	193.4	4044.6
Na	mg L <sup>-1</sup>	42.6	40.1	549.7
SO <sub>4</sub> <sup>2-</sup>	mg L <sup>-1</sup>	405.2	88.3	12323.3
B	mg L <sup>-1</sup>	0.18	0.15	0.30
Cu	mg L <sup>-1</sup>	0.10	0.11	0.07
Mn	mg L <sup>-1</sup>	1.04	0.94	0.16
Zn	mg L <sup>-1</sup>	0.05	0.03	0.59
Fe	mg L <sup>-1</sup>	5.08	8.92	0.17

† = sample analysed before the addition of chemical fertiliser, ‡EC = electrical conductivity.

growth. Table 5.2 shows the soil pH and EC of the various treatments at the end of the trial. The pH of the different treatments increased slightly with increasing NaCl concentration. The electrical conductivity increased with NaCl treatment and with vermicompost concentration. The addition of 5.4 g L<sup>-1</sup> NaCl and 6.8 g L<sup>-1</sup> NaCl increased soil conductivity by an average of 209% and 200%, respectively while the addition of vermicompost and NaCl resulted in a soil conductivity increase of between 162% and 666%, compared to 0% vermicompost with no NaCl irrigation.

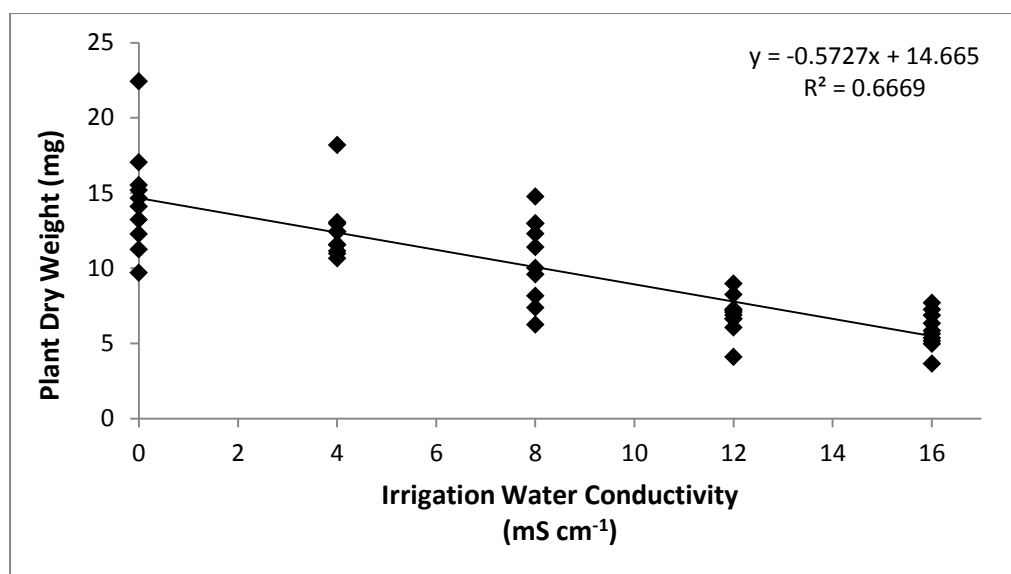


Figure 5.1 Effect of increasing concentration of saline irrigation water on cress plant dry weight

Table 5.2 Effect of vermicompost and salinity treatments on soil pH and electrical conductivity (EC) measured at the end of the trial

% Vermicompost	NaCl	pH	EC <sup>†</sup> mS cm <sup>-1</sup>
0	0 g L <sup>-1</sup>	5.75	0.644
10	0 g L <sup>-1</sup>	5.77	1.186
20	0 g L <sup>-1</sup>	5.90	1.697
0	5.4 g L <sup>-1</sup>	6.25	1.988
10	5.4 g L <sup>-1</sup>	5.85	2.330
20	5.4 g L <sup>-1</sup>	5.93	3.830
0	6.8 g L <sup>-1</sup>	5.81	1.932
10	6.8 g L <sup>-1</sup>	5.76	3.400
20	6.8 g L <sup>-1</sup>	6.16	4.930

<sup>†</sup> = electrical conductivity

Table 5.3 summarises the results of the salinity stress trial. There is a strong, clear, negative effect of increasing NaCl concentration on plant growth and plant moisture content, with increasing salinity. The addition of 5.6 g L<sup>-1</sup> and 6.8 g L<sup>-1</sup> to the irrigation water reduced mean plant fresh weight by 28% and 34%, respectively, compared to 0 g L<sup>-1</sup>. Plant moisture content was significantly reduced with increasing NaCl concentration with 0 g L<sup>-1</sup> NaCl having the highest mean plant

Table 5.3 Effect of NaCl and vermicompost addition on plant growth parameters ( $\pm$ SD) in the salinity stress trial

		Pot Fresh Weight	Plant Fresh Weight	Pot Dry Weight	Plant Dry Weight	Plant Moisture Content	Chlorophyll Fluorescence	Seeds Germinated	Plants per pot	
% Vermicompost	NaCl	-----mg-----				%	Fv/Fm	-----median no.-----		
0	0 g L <sup>-1</sup>	1899.4(205.8)b	196.7(25.0)cd	112.4(11.2)ab	11.6(1.1)abc	94.05(0.57)c	0.824(0.043)a	10(1)bc	10(1)bc	
10	0 g L <sup>-1</sup>	2250.3(405.5)b	231.6(35.3)d	133.4(21.7)b	13.7(1.9)c	94.04(0.41)c	0.829(0.031)a	10(1)bc	10(1)bc	
20	0 g L <sup>-1</sup>	2298.7(290.5)b	229.9(29.1)d	135.3(18.2)b	13.6(1.8)bc	94.11(0.33)c	0.828(0.042)a	10(0)c	10(0)c	
0	5.4 g L <sup>-1</sup>	1503.6(202.4)a	155.7(24.7)ab	102.4(13.9)a	10.6(1.7)a	93.18(0.32)b	0.825(0.038)a	10(0)b	10(0)bc	
10	5.4 g L <sup>-1</sup>	1468.4(166.5)a	163.9(18.6)bc	100.0(14.3)a	11.2(1.7)ab	93.20(0.35)b	0.833(0.046)a	9(1)a	9(1)a	
20	5.4 g L <sup>-1</sup>	1325.2(197.6)a	152.7(21.3)ab	92.98(16.5)a	10.7(1.7)a	93.00(0.26)ab	0.824(0.035)a	9(1)a	9(1)a	
0	6.8 g L <sup>-1</sup>	1489.1(332.2)a	157.5(25.5)ab	103.4(24.3)a	11.0(2.1)a	93.01(0.34)ab	0.839(0.037)a	10(0)b	10(1)b	
10	6.8 g L <sup>-1</sup>	1430.0(268.2)a	149.9(20.4)ab	104.9(22.8)a	11.0(1.8)a	92.69(0.35)ab	0.836(0.029)a	10(0)b	10(1)b	
20	6.8 g L <sup>-1</sup>	1223.8(224.2)a	127.4(20.8)a	90.6(19.7)a	9.5(1.9)a	92.62(0.43)a	0.838(0.022)a	10(0)bc	10(1)b	
		df	ANOVA							
F value/Chi <sup>2</sup>	Vermicompost	2	1.37	2.33	1.22	2.33	1.79	0.06	1.02	0.81
	Sig.		ns	ns	ns	ns	ns	ns	ns	ns
F value /Chi <sup>2</sup>	NaCl	2	73.25	74.39	21.71	17.94	89.67	0.83	9.35	7.39
	Sig.		***	***	***	***	***	ns	***	***
Vermicompost x NaCl		4	**	**	*	*	ns	ns	ns	ns

ns = not significant, \*= $p \leq 0.05$ , \*\* =  $p \leq 0.01$ , \*\*\* =  $p \leq 0.001$ , according to two-way ANOVAs. Means in the same column followed by the same letter were not significantly different,  $p > 0.05$ , according to Tukey's range tests or Kruskal-Wallis multiple comparison test (median number of seeds germinated and median number of plants per pot only).

water content (94.07%), followed by 5.4 g L<sup>-1</sup> NaCl (93.13%) and 6.8 g L<sup>-1</sup> NaCl (92.77%). There was no NaCl main effect on chlorophyll fluorescence, while median number of seeds germinated and number of plants per pot were significantly lower with 5.4 g L<sup>-1</sup> than with 6.8 g L<sup>-1</sup> NaCl. The addition of vermicompost did not have any significant effects on any parameters according to the two-way ANOVAs. There were significant vermicompost x NaCl interaction effects for all plant weight parameters, with the treatment means showing a trend of further reduced plant weight when plants were grown with vermicompost and NaCl, compared to those grown with vermicompost and no NaCl, or plants grown with NaCl and no vermicompost (Table 5.3). The addition of vermicompost and 5.4 g L<sup>-1</sup> NaCl reduced seed germination and number of plants per pot by a median average of one plant per pot, compared to 5.4 g L<sup>-1</sup> NaCl only. This effect was not seen at 6.8 g L<sup>-1</sup>.

### 5.3.3 Heat stress trial

Vermicompost incorporation into the soil resulted in slightly lower soil pH than chemical fertiliser addition but it did increase soil conductivity (Table 5.4).

Table 5.4 Effect of vermicompost and chemical fertiliser addition on soil pH and EC

	pH	EC <sup>†</sup> mS cm <sup>-1</sup>
0% Vermicompost + Chemical Fertiliser Level 1	5.98	0.623
0% Vermicompost + Chemical Fertiliser Level 2	5.99	1.268
10% Vermicompost	5.68	1.053
20% Vermicompost	5.79	1.787

<sup>†</sup> = electrical conductivity.

The acute heat stress applied in this trial was sufficient to significantly reduce overall plant fresh weight by an average of 19% (Table 5.5). It also significantly reduced plant dry weight, but it did not have a lasting effect on plant water content (Table 5.5) compared to the unstressed plants. There was no significant main effect on the

number of plants per pot at the end of the trial (data not shown). The addition of the higher level of fertiliser (the addition of 20% vermicompost, or the chemical fertiliser equivalent) had a negative effect on overall plant fresh weight and water content, but not dry weight. There was no significant vermicompost effect found in this trial, although plants grown with vermicompost had a higher average fresh weight and dry weight than plants grown without, but this difference was not significant.

Table 5.5 Effect of heat stress, vermicompost and fertiliser level on plant growth parameter main effect means ( $\pm$ SD)

Main Effects		Pot Fresh Weight	Plant Fresh Weight	Pot Dry Weight	Plant Dry Weight	Plant Moisture Content
		-----mg-----				%
Vermicompost	With	831.5(146.6)a	91.6(15.3)a	47.6(7.2)a	5.3(0.8)a	94.22(0.62)a
	Without	781.2(134.7)a	85.8(15.4)a	45.4(7.3)a	5.0(0.8)a	94.14(0.55)a
Fertiliser Level	Level 1	870.7(142.3)b	95.2(15.0)b	48.4(7.9)a	5.3(0.8)a	94.41(0.51)b
	Level 2	742.0(110.0)a	82.1(13.1)a	44.6(6.1)a	4.9(0.7)a	93.95(0.55)a
Stressed	Unstressed	889.1(135.2)b	98.0(14.5)b	50.2(7.6)b	5.5(0.8)b	94.32(0.60)a
	Stressed	723.6(90.0)a	79.4(9.7)a	42.8(4.5)a	4.7(0.5)a	94.04(0.53)a
		df	ANOVA			
F value Vermicompost (V)		1	2.10	2.39	0.96	1.16
Sig.			ns	ns	ns	ns
F value Fertiliser Level (F)		1	13.92	12.23	2.82	2.38
Sig.			***	**	ns	ns
F value Stressed (S)		1	22.93	24.32	10.73	11.84
Sig.			***	***	**	**
V x F		1	ns	ns	ns	ns
V x S		1	ns	ns	ns	ns
F x S		1	ns	ns	ns	ns
V x F x S		1	ns	ns	ns	ns

ns = not significant, \*= $p \leq 0.05$ , \*\* =  $p \leq 0.01$ , \*\*\* =  $p \leq 0.001$ , according to three-way ANOVAs. Means in the same column and same main effect, followed by the same letter were not significantly different,  $p > 0.05$ .

There was no significant main effect of vermicompost ( $F_{5,15} = 3.12$ ,  $p > 0.05$ ) or fertiliser level ( $F_{5,15} = 0.41$ ,  $p > 0.05$ ) on the chlorophyll fluorescence of stressed plants but there was a significant effect of time ( $F_{5,15} = 89.11$ ,  $p < 0.001$ ), with no interaction effects. Average chlorophyll fluorescence measurements during the

different time periods were significantly different (Figure 5.2), with the lowest measurement recorded three minutes after the stress. With each consecutive reading, the chlorophyll fluorescence rose until twenty one hours after the stress, when measurements ceased.

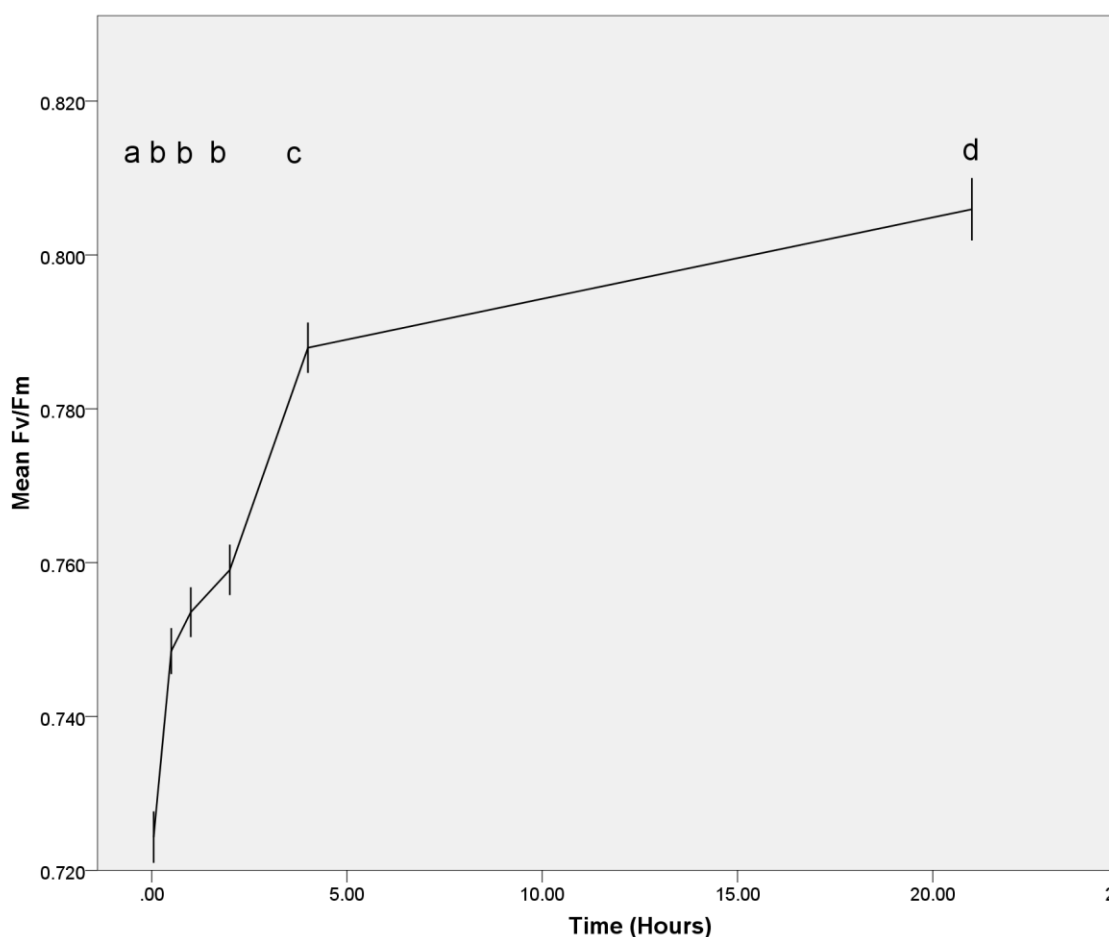


Figure 5.2 Time course of chlorophyll fluorescence ( $F_v/F_m$ ) ( $\pm$ SE) of heat-stressed plants after removal from the heat stress. Means with the same letter were not significantly different,  $p > 0.05$ , according to Tukey's Range Test

The chlorophyll fluorescence of the stressed and unstressed plants in this trial was compared four and twenty one hours after the stress conditions (Table 5.6). Four hours after the stress conditions, the stressed plants had significantly reduced chlorophyll fluorescence compared to the unstressed plants, but this was not found

after twenty one hours, when they were both statistically similar. There was a significant vermicompost effect found twenty one hours after the stress conditions, with plants grown with vermicompost having significantly lower chlorophyll fluorescence than the plants grown without vermicompost.

Table 5.6 Effect on chlorophyll fluorescence (Fv/Fm) ( $\pm$ SD) of heat stress, vermicompost and fertiliser level main effects, four and twenty one hours after stress conditions

Main Effects		Time elapsed after stress	
		4 h Fv/Fm	21 h Fv/Fm
Vermicompost	With	0.805(0.020)a	0.804(0.013)a
	Without	0.807(0.023)a	0.814(0.009)b
Fertiliser Level	Level 1	0.803(0.024)a	0.807(0.015)a
	Level 2	0.809(0.019)a	0.812(0.009)a
Stressed	Unstressed	0.824(0.007)b	0.812(0.006)a
	Stressed	0.788(0.013)a	0.806(0.016)a
		df	ANOVA
F value Vermicompost		1	0.69
Sig.			ns
F value Fertiliser Level		1	2.34
Sig.			ns
F value Stressed		1	93.45
Sig.			***
Vermicompost x Fertiliser Level		1	ns
Vermicompost x Stressed		1	ns
Fertiliser Level x Stressed		1	ns
Vermicompost x Fertiliser Level x Stressed		1	ns

ns = not significant, \*= $p \leq 0.05$ , \*\* =  $p \leq 0.01$ , \*\*\* =  $p \leq 0.001$ , according to three-way ANOVAs. Means in the same column and same main effect, followed by the same letter were not significantly different,  $p > 0.05$ .

#### 5.3.4 Cold stress trials

Vermicompost slightly increased soil pH and increased soil conductivity considerably with increasing vermicompost concentration (Table 5.7).

Table 5.7 Effect of vermicompost addition on soil pH and EC

	pH	EC† mS cm <sup>-1</sup>
0% Vermicompost	5.85	0.520
10% Vermicompost	5.98	0.960
20% Vermicompost	6.09	1.855

† = electrical conductivity.

Plant growth parameters from cold trial one (Table 5.8) shows that the addition of 20% vermicompost to the soil increased pot and plant fresh and dry weight compared to plants grown without vermicompost, and with each additional increase of vermicompost there was a significant increase in plant chlorophyll fluorescence (Figure 5.3). There was no significant effect of treatment on plant moisture content. The number of live plants per pot at the end of the trial was significantly higher in soil with the addition of 20% vermicompost, than 0% vermicompost (Table 5.8).

Table 5.8 Effect of vermicompost addition on plant growth parameters ( $\pm$ SD), cold stress trial one

	Pot Fresh Weight	Plant Fresh Weight	Pot Dry Weight	Plant Dry Weight	Plant Moisture Content	Chlorophyll Fluorescence	Plants per pot
% vermicompost	-----mg-----				%	Fv/Fm	Median no.
0	184.6(44.6)a	31.0(4.1)a	17.4(6.6)a	2.9(0.8)a	90.80(2.00)a	0.635(0.049)a	6(1)a
10	236.4(54.8)a	34.0(4.0)a	24.0(7.8)a	3.6(1.7)ab	89.62(3.69)a	0.706(0.032)b	8(2)ab
20	352.5(57.6)b	44.9(4.5)b	34.4(10.2)b	4.3(0.9)b	90.35(1.94)a	0.754(0.037)c	8(1)b
df	ANOVA						
F value/Chi <sup>2</sup>	25.05	30.04	10.32	3.73	0.50	22.51	6.72
Sig.	***	***	***	*	ns	***	*

ns = not significant, \* =  $p \leq 0.05$ , \*\* =  $p \leq 0.01$ , \*\*\* =  $p \leq 0.001$ , according to one-way ANOVAs or Kruskal-Wallis multiple comparison tests (median no. plants per pot only). Means in the same column, followed by the same letter were not significantly different,  $p > 0.05$ , according to Tukey's range tests or Kruskal-Wallis multiple comparison tests (median no. plants per pot only).

Plants grown as part of cold trial two (Table 5.9) had results similar to those from plants grown as part of cold trial one. Plants in cold trial two grown with 20% vermicompost, according to one-way ANOVAs, had significantly higher plant and pot fresh weight, and close to significant ( $p = 0.057$ ) higher plant dry weight,

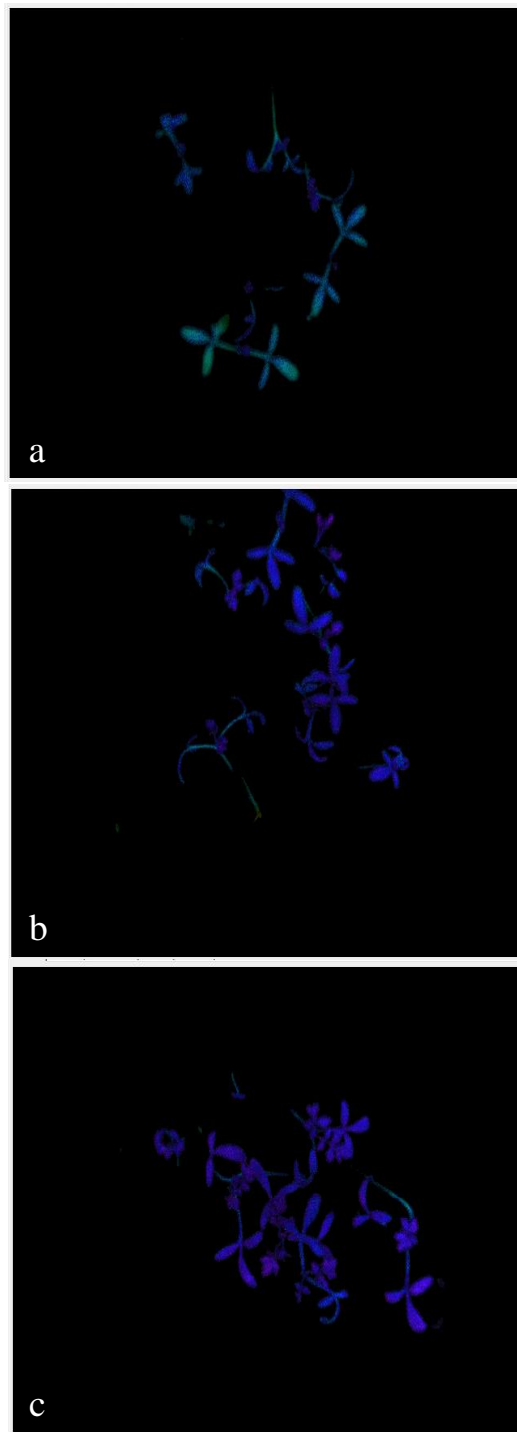


Figure 5.3 Chlorophyll fluorescence image of cress plants from cold stress trial one grown without vermicompost (a), with 10% vermicompost (b) or with 20% vermicompost (c)

compared to plants grown with 0% vermicompost. Tukey's range tests did detect significant differences between treatment means for plant dry weight, but not for pot fresh weight. Chlorophyll fluorescence was significantly higher in plants grown with vermicompost than without. There was no significant difference of vermicompost on plant moisture content (Table 5.9), the number of live plants per pot at the end of the trial, and the number of seedlings germinated ( $\chi^2_{2,28} = 3.24$ ,  $p = 0.20$ ) (data not shown).

Table 5.9 Effect of vermicompost addition on plant growth parameters ( $\pm$ SD), cold stress trial two

% vermicompost	Pot Fresh Weight	Plant Fresh Weight	Pot Dry Weight	Plant Dry Weight	Plant Moisture Content	Chlorophyll Fluorescence	Plants per pot
	-----mg-----				%	Fv/Fm	Median no.
0	195.4(34.8)a	21.9(1.9)a	30.6(8.1)a	3.3(0.5)a	84.57(2.57)a	0.488(0.063)a	10(2)a
10	226.2(31.2)a	22.8(2.8)ab	34.8(6.3)a	3.5(0.6)ab	84.65(1.16)a	0.585(0.064)b	10(0)a
20	228.6(25.4)a	25.2(2.0)b	35.3(3.6)a	3.9(0.3)b	84.51(1.21)a	0.596(0.030)b	9(1)a
	df	ANOVA					
F value/Chi <sup>2</sup>	2	3.68	5.58	1.72	3.21	0.16	11.75
Sig.		*	**	ns	ns	ns	***

ns = not significant,  $*=p \leq 0.05$ ,  $** = p \leq 0.01$ ,  $*** = p \leq 0.001$ , according to one-way ANOVAs or Kruskal-Wallis multiple comparison tests (median no. plants per pot only). Means in the same column, followed by the same letter were not significantly different,  $p > 0.05$ , according to Tukey's range tests or Kruskal-Wallis multiple comparison tests (median no. plants per pot only).

Temperature data showed that both trials had similar temperature profiles (Figure 5.4). Trial one was slightly colder with an average maximum temperature of 8.7°C and average minimum temperature of 2.9°C, while trial two had an average maximum temperature of 10.3°C and average minimum temperature of 3.0°C. The number of grass frosts that occurred during the two trials was similar, 33 and 37 for trial one and trial two, respectively. The rainfall data showed slightly different rainfall patterns during trials one and two (Table 5.10). Trial one had a higher cumulative rainfall amount by 18%, compared to trial two. There was a higher

number of wet days ( $>1$  mm precipitation day<sup>-1</sup> (McElwain & Sweeney, 2007)) in trial one than trial two, but a similar number of extreme rainfall events ( $>10$  mm precipitation day<sup>-1</sup> (McElwain & Sweeney, 2007)) in trial one and trial two (Table 5.10).

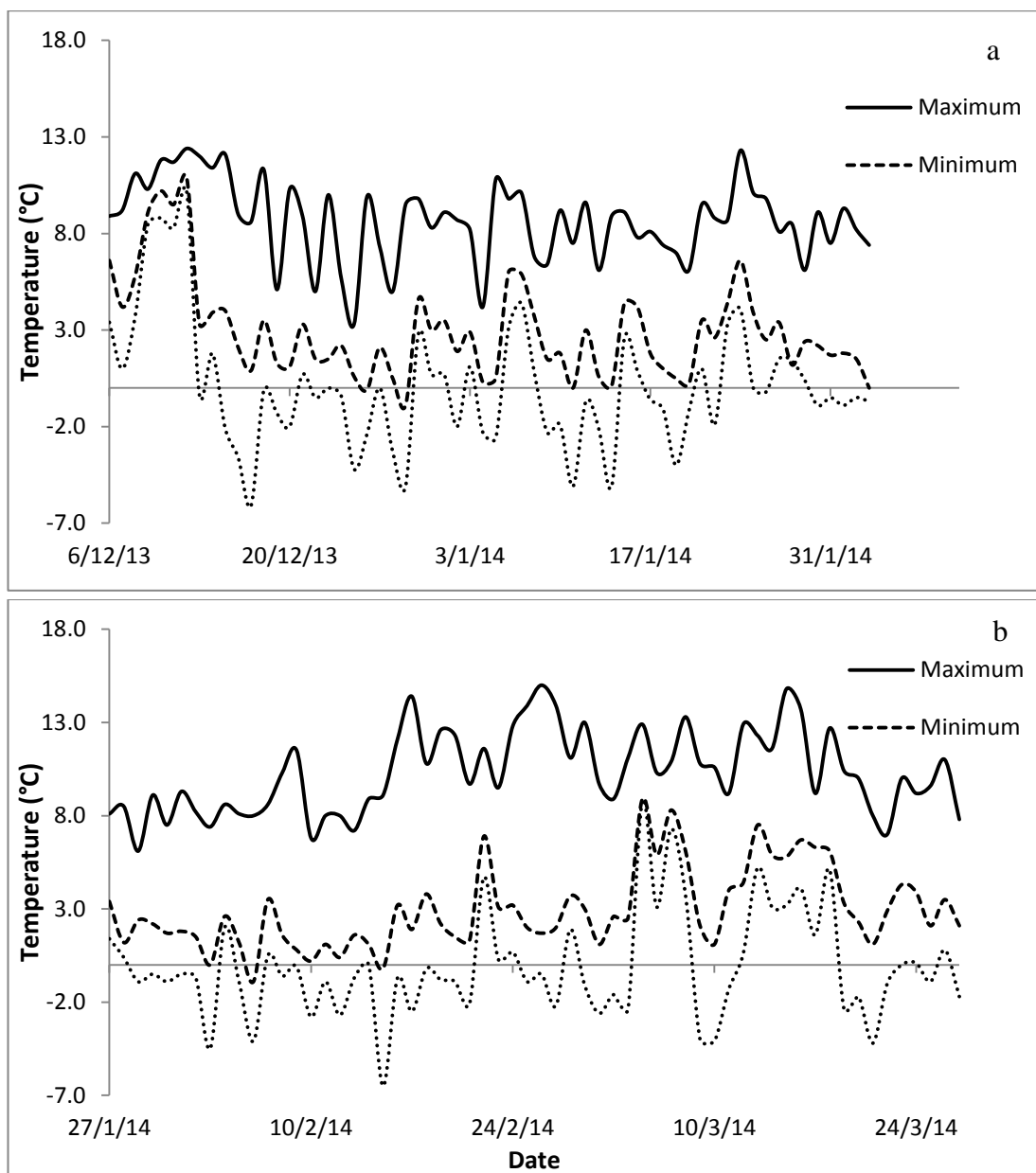


Figure 5.4 Temperature data over the duration of cold trial one (a) and two (b)

Table 5.10 Cold trial one and two rainfall data

	Cumulative rainfall (mm)	No. wet days <sup>1</sup>	No. extreme rainfall events <sup>1</sup>
Trial One	438.2	46	18
Trial Two	372.1	41	17

<sup>1</sup>As defined by McElwain & Sweeney (2007).

## 5.4 Discussion

Increased nutrient content in the vermicompost, compared to the topsoil (Table 5.1), is largely due to the naturally high  $K^+$  content of poultry and horse manure. The high EC value of vermicompost compared to topsoil is due to a number of metabolites, e.g.  $Ca^{2+}$ ,  $K^+$ ,  $Mg^{2+}$  and  $Na^+$ , produced by the mushroom mycelium during mushroom production, (Beyer, 1998). Vermicompost had a lower dry weight bulk density than topsoil as vermicompost contains a higher organic matter content compared to mineral soil (Azarmi *et al.*, 2008).

A limitation of these abiotic stress trials were the different controls used for the salinity and cold stress trial (i.e. 0% vermicompost) and for the heat trial (i.e. 0% vermicompost + fertiliser equivalent). This was due to the changed nutrient concentrations of the Westland topsoil between batches purchased i.e. the batch used for the cold and salinity stress trial (batch one) had a higher fertiliser level than the batch used for the heat stress trial (batch two) (Table 5.1). Under normal plant growth conditions (i.e. 0%, 10% or 20% vermicompost without NaCl irrigation), plant growth in batch one topsoil with 0% vermicompost was statistically similar to 10 and 20% vermicompost (Table 5.3). As the plant growth response was the same, it was decided that no additional fertiliser was required. When batch two was used for the heat trials, it quickly became apparent that plant growth was much reduced compared to plants grown with 10%, and 20% vermicompost (data not shown). This

was due to a lower nutrient content of the topsoil (Table 5.1). As previous studies have demonstrated, nutrient content plays an important role in plant stress response (Papadopoulos & Rendig, 1983; Heckathorn *et al.*, 1996; Hu & Schmidhalter, 2005) and therefore to distinguish between a biostimulant rather than a nutrient effect, the control soils used for the heat trial were set up with additional fertiliser, as described in section 5.2.3.

Looking at the combined results of the trials (Table 5.11), vermicompost has different effects on plant growth when combined with each of the three abiotic stressors. The negative plant growth effect of vermicompost seen in the salinity trial, as was indicated by the significant vermicompost x NaCl interaction effect (Table 5.3), is due to the additional salinity resulting from the vermicompost itself (Table 5.2). This was also indicated by reduced germination in the plants grown with vermicompost and  $5.4 \text{ g L}^{-1}$  NaCl (Table 5.2). This osmotic effect of the vermicompost was also seen in the heat stress trial (due to the reduced chlorophyll fluorescence (Table 5.6)), although plants grown with vermicompost were still deemed to have a healthy photochemical efficiency of between 0.75 and 0.85. This slight reduction in chlorophyll fluorescence (-1%) may be due to the additional osmotic stress in the root zone, possibly leading to slower recovery of plants grown in vermicompost due to increased soil conductivity. It is harder for plants with increased conductivity in the root zone to take up water (Romero-Aranda *et al.*, 2001), and therefore this effect may have reduced the ability of the plants grown in vermicompost to recover as quickly as those grown without vermicompost. Despite this, there were no lasting negative effects of vermicompost on plant growth in the heat stress trial.

Table 5.11 Summary of abiotic stress trial results

		Plant Growth	Plant Moisture Content	Chlorophyll Fluorescence	No. plants per pot
Stress:	Effect of:				
Salinity	Stress	–	–	=	–
	Vermicompost	= / –	=	=	–
Heat	Stress	–	=	–	=
	Vermicompost	=	=	= / –	=
Cold	Stress	n/a	n/a	n/a	n/a
	Vermicompost	+	=	+	= / +

– (negative effect), = (no effect), + (positive effect), n/a (not applicable).

Vermicompost had a positive effect on plant growth, survival (trial 1 only), and chlorophyll fluorescence in the cold stress trials (Table 5.11), and it was this stress where vermicompost was found to be most effective. The reasons for these positive growth effects will be discussed later in this section. It is important to note that if one was to study the effect of these stressors on chlorophyll fluorescence, the stress that resulted in the lowest chlorophyll fluorescence (cold stress) had the best vermicompost effect, the next lowest chlorophyll fluorescence (heat stress) had no vermicompost effect, and the stress with the highest chlorophyll fluorescence (salinity stress) had no, or slightly negative, vermicompost effects. This possibly indicates that the more intensive the stress, the better effect on plant growth vermicompost may have on plant growth parameters and stress tolerance, and that when water uptake is required to recover from the stress, or the stress itself is due to an osmotic effect, vermicompost might not be the most suitable additive to induce stress tolerance as it can intensify this stress.

There was a clear negative effect of salinity on plant growth and moisture content (Table 5.3) as would be expected from salinity stress. The NaCl concentrations (5.6 g L<sup>-1</sup> and 6.8 g L<sup>-1</sup>) were chosen in this trial to affect a 30%, and 50% reduction in

plant growth, respectively. A 28% reduction in plant growth was achieved by  $5.6 \text{ g L}^{-1}$ , but only a 34% reduction in plant growth was observed with  $6.8 \text{ g L}^{-1}$ . This may be explained by the similar EC values of soils with no vermicompost addition and with increasing salinity in the irrigation water from  $5.4 \text{ g L}^{-1}$  to  $6.8 \text{ g L}^{-1}$  (Table 5.2). Similar reductions in cress seed plumule growth was observed after four days growth with  $10 \text{ mS cm}^{-1}$  ( $5.6 \text{ g L}^{-1} = 9.3 \text{ mS cm}^{-1}$ ) and  $12.5 \text{ mS cm}^{-1}$  ( $6.8 \text{ g L}^{-1} = 11.4 \text{ mS cm}^{-1}$ ) NaCl solution, by 29% and 36% respectively (Muhammed & Hussain, 2010). This suggests that a more concentrated NaCl solution than  $6.8 \text{ g L}^{-1}$  would be necessary to affect a 50% reduction in cress plant growth.

Chlorophyll fluorescence was not affected by increased salinity in the irrigation water (Table 5.3). Other authors have also found that chlorophyll fluorescence is not sensitive to salinity stress (Larcher *et al.*, 1990; Jimenez *et al.*, 1997). This could be due to the photosynthetic efficiency of the plants acclimating to the stress over the lifetime of the trial. This is commonly found during chronic stress trials (Ben *et al.*, 1987), as chlorophyll content is affected rather than chlorophyll fluorescence (Jimenez *et al.*, 1997; Lichtenthaler & Miehe, 1997) in chronic salinity stress trials such as this one.

When studying plant response to salinity stress, vermicompost did not significantly increase plant growth under stressful or non-stressful conditions. Sallaku *et al.* (2009) reported no significant differences in plant dry weight and relative growth rate of cucumber seedlings irrigated with saline nutrient solutions that were grown in peat, compared to 50/50 peat/vermicompost mixtures, and 100% vermicompost mixtures. Ahmad *et al.* (2009) found the addition of vermicompost to a sandy-loam soil/cow dung mix (9:1) increased ginger rhizome and ginger shoot growth under salinity stress. This was attributed to reduced  $\text{Na}^+$  accumulation by the plants grown

with vermicompost compared to the control and the increased availability of  $K^+$  by the vermicompost fertiliser. There was some indication that there was not enough fertiliser in the control mix of sandy-loam soil/cow dung in Ahmad *et al.* (2009), as plant growth was increased, chlorophyll content of the leaves was increased (a proxy measurement of leaf nitrogen content (Xue & Yang, 2009)), and the nitrogen content of the rhizomes increased in plants grown with vermicompost under non-saline conditions, compared to plants grown under non-saline conditions in the control soil mix. The additional fertiliser provided by the vermicompost may have driven the increased plant growth under the conditions of salinity stress, i.e.  $K^+$  and  $Na^+$  competition in the root zone. Vermicompost-derived products such as vermicompost leachate (Chinsamy *et al.*, 2013) was found to increase tomato seedling growth under salinity stress. This was due to increased stress tolerance mechanisms such as the accumulation of compatible solutes rather than to a nutrient effect of the vermicompost leachate.

There was no significant effect of vermicompost on plant moisture content under salinity stress as had been seen in other trials (see section 2.3.2 and 3.3.3). This will be discussed further in Chapter 6.

Vermicompost commonly has a high conductivity, depending on the feedstock and processing techniques used e.g. leaching. SMC also has a high conductivity (Ribas *et al.*, 2009). While the additional vermicompost amendment concentrations further increased soil conductivity (Table 5.2), with the possible risk of further inhibiting plant water uptake, this did not further reduce plant growth significantly (Table 5.3). A further avenue of study may be to control for soil salinity before the stress is applied, to test whether the addition of vermicompost would increase plant growth under the same level of salinity stress, although, in the field, application of

vermicompost would increase soil conductivity and it would not be possible for the grower to reduce the conductivity of the irrigation water. Therefore, although the trial design carried out resulted in increased conductivity in the vermicompost treatments, which may have increased the salinity stress experienced by the plants, this design replicated field conditions more closely than the aforementioned design.

Heat-stressed plants had lower fresh and dry weight than unstressed plants by 19% and 15%, respectively (Table 5.5). Saleh & Plieth (2009) found that the exposure of cress seedling to 6 hours at 42°C mobilised a variety of antioxidative activities in cress plants, and Camejo *et al.* (2005) found that the exposure of tomato plants (at the fourth true leaf stage) for 2 hours at 45°C reduced chlorophyll fluorescence and increased leaf electrolyte leakage in stressed plants compared to unstressed plants.

The significantly reduced pot and plant fresh weight and plant moisture content in the higher fertiliser level indicates that this level of fertiliser was high enough to induce salinity stress, as plants grown in saline conditions have been shown to have reduced plant growth and reduced plant moisture content (Romero-Aranda *et al.*, 2006). Overall, there was no significant effect of vermicompost on plant growth and moisture content, indicating that vermicomposted SMC did not increase a plant's ability to cope with heat stress when nutrient content is controlled for. Although this trial did not find that vermicompost induced heat stress tolerance in plants, vermicompost leachate has been found to increase plant growth, chlorophyll content, total sugars, and proline content in one-month old, heat stressed (30°C) tomato seedlings (Chinsamy *et al.*, 2014).

As would be expected, the chlorophyll fluorescence of heat-stressed plants recovered to the status of a typical healthy plant ( $F_v/F_m$  of 0.75 - 0.85) with time (Figure 5.2).

In this study it took 21 hours for the stressed plants to recover to the same chlorophyll fluorescence as unstressed plants (Table 5.6), results similar to those of Shi *et al.* (2006) who found that cucumber seedling chlorophyll fluorescence had recovered by 24 hours after heat stress.

Biostimulants have been shown to induce heat stress tolerance in plants. For example, Singh & Shono (2005) found that the foliar application of the brassinosteroid 24-epibrassinolide increased plant growth, fruit weight and survival of tomato plants exposed to heat stress, and also increased the production of heat shock proteins in plants exposed to high temperatures (30°C). A combination of a seaweed extract, humic acid and the fungicide propiconazole was found to increase the photochemical efficiency, root strength, and reduced visual damage of heat-stressed turfgrass (Zhang *et al.*, 2003), and plant growth promoting bacteria and fungi have been found to increase plant growth and germination under heat stress (Mastouri *et al.*, 2010; Ali *et al.*, 2011; Khan *et al.*, 2011; Aremu *et al.*, 2015). Vermicompost has been reported to contain plant hormones, humic acids and beneficial bacteria and fungi (Pathma & Sakthivel, 2012; García *et al.*, 2014), but possibly not in the same concentrations or combinations required to affect heat stress tolerance in the same manner observed in previous biostimulant trials.

In the cold stress trials, the addition of vermicomposted SMC to soil resulted in larger plants with a higher fresh and dry weight, higher chlorophyll fluorescence and a higher number of live plants (trial one only) at the end of the trial (Table 5.8 and Table 5.9), despite no significant differences in germination in trial two. The increased plant growth was likely due to additional release of nutrients during the continued mineralisation of the vermicompost throughout the 30-day period. Chaoui *et al.* (2003), in an incubation study, found that soil extractable nitrogen was at a

level 75 days after vermicompost incorporation into soil, similar to that present when it was first incorporated, and Stewart *et al.* (1998) found that in a laboratory incubation study that SMC application to soil resulted in at least 16 weeks of nitrogen leaching from the soil, while a field incubation study found that SMC released nitrogen for at least 30 weeks after application.

As the plants were kept outside and uncovered, the high number of extreme rainfall events (Table 5.10) would have quickly leached the water-soluble nutrient fraction from the pots. This would have likely limited the nutrient availability in the 0% vermicompost treatments. As previously explained, vermicompost and SMC mineralises in soil over time, and, due to the high organic matter content vermicompost also conserves nutrients (Khaleel *et al.*, 1981). Therefore, the two vermicompost treatments may have been slowly releasing nutrients throughout the trial. Nutrient availability plays a vital role in plant stress response (Grattan & Grieve, 1999; Cakmak, 2005; Hu & Schmidhalter, 2005). The additional nutrients released by the vermicompost resulted in more consistent nutrient availability and increased plant growth and stress tolerance (increased chlorophyll fluorescence) under cold stress conditions, especially with 20% vermicompost addition to soil.

Despite this result being attributed to a nutrient effect of the vermicompost rather than a biostimulant effect, it is important to note that it is unlikely that traditional chemical fertiliser inputs would have the same outcome as the 10% or 20% vermicompost application in cold wet conditions, due again to leaching of the soil and the quick loss of water-soluble chemical fertiliser. It is likely that only organic amendments such as vermicompost, compost and animal manures, or specially prepared slow-release chemical fertilisers would have similar increased effects on

plant growth and stress response under the type of cold stress conditions experienced in this trial.

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# Chapter 6

## General Discussion and Recommendations

## **6.1 Introduction**

With the world population estimated to reach 9.6 bn in 2050 (United Nations, 2013), food production will become ever more critical. World chemical fertiliser use is rising, and in the medium term, it is estimated to increase at a rate of 1.8% per annum from 2014 to 2018, and reach a global nutrient consumption of 200,500,000 tonnes in 2018 (Food and Agriculture Organisation of the United Nations, 2015). More sustainable alternatives are required to reduce the environmental impact of these fertilisers. Agricultural biodegradable wastes and by-products are currently under-utilised, and are often perceived by farmers as a nuisance. By using the right treatment methods, these wastes can be transformed into value-added products which can be used to reduce the use of chemical fertilisers and peat in horticulture and possibly provide an additional source of income for the farmer. When used in the right quantities and in the right circumstances, these products can have the same effect as chemical fertilisers, while also improving soil and growing media quality, reducing the negative effects of spreading excess amounts of agricultural biodegradable wastes, and increase plant growth and yield.

The objectives of this study were to take commonly available agricultural biodegradable manures and by-products (primarily spent mushroom compost) and mature them using a vermicomposting process to produce value-added by-products for use in horticulture. This study evaluated the effects of the vermicompost on growing medium characteristics, plant growth, yield, yield quality and abiotic stress response. These objectives were achieved using a number of studies which looked at the effect of the vermicomposting process on compost quality (Chapter 2 and Chapter 3), the use of vermicompost in peat-based and peat-reduced growing media (Chapter 3), and the effect of vermicompost on plant growth, development, yield and

yield quality parameters (Chapter 2, 3 and 4), on plant abiotic stress response (Chapter 5) and on growing medium physical, chemical and biological characteristics (Chapter 4). The following sections discuss the main conclusions of this study, including some limitations of the work, future research considerations and recommendations for use of the products studied.

## **6.2 Comparison of compost and the corresponding vermicompost**

When tested for specifically on a microcosm level, vermicompost-matured horse manure compost outperformed compost-matured horse manure compost with regards to maturation and plant growth effects (section 2.3.1 and 2.3.2). When spent mushroom compost was vermicompost-matured or compost-matured on an industrial scale, with less control and precision, compost characteristics were similar with regards to nutrients, pH and conductivity (Table 3.1). Though compost characteristics were similar, the addition of vermicompost to the peat-reduced growing medium still improved shoot water content under conditions of osmotic stress, even with increased salinity (Table 3.3). One limitation of Chapter 3 is that the compost-matured spent mushroom compost should also have been added at a rate of 10% to the peat-based growing medium, to compare the effects of compost and vermicomposted compost directly. The addition of vermicompost to the peat-reduced growing medium also brought many of the parameters closer (with regards to significance) to the peat-based growing medium e.g. truss development and flowering date, fruit number and % marketable yield. With regards to nutrients, the similarities between the chemical characteristics of vermicompost-matured and compost-matured spent mushroom compost may be due to the nutrient test methods i.e. total nutrients were measured in Chapter 3 (no significant differences), whereas plant-available nutrients (significant differences) were reported in Chapter 2.

Vermicompost has been shown to increase plant-available nutrient content in other studies (Short *et al.*, 1999; Tognetti *et al.*, 2005; Ngo *et al.*, 2011). These differences may also be due to the different feedstock used in Chapter 3 compared to Chapter 2.

### **6.3 Vermicompost effect on growing media and soil physicochemical and biological properties**

In each chapter, compared to the control compost/soil mix, vermicompost had increased pH, electrical conductivity (EC) and nutrient content (Table 2.1, Table 3.1, Table 4.1 and Table 5.1). Even when the equivalent level of fertiliser was added, as in the heat stress trial (Chapter 5), the EC was higher with vermicompost than with fertiliser (Table 5.4). Much of this increased conductivity in the vermicompost came from beneficial plant macro- and micro-nutrients, although, due to excessive amounts of sodium and chloride (Table 4.13), vermicompost addition resulted in plant osmotic stress in some of the trials (see section 6.4 for examples). Optimum growing medium conductivity is  $<1 \text{ mS cm}^{-1}$  for sensitive plants and  $<2.5 \text{ mS cm}^{-1}$  for moderately tolerant plants (Handreck & Black, 2002). The average EC of vermicompost measured in this study is 4.91, meaning that the EC value of vermicompost is a limiting factor for its use as a growing medium additive, especially for salt-sensitive plants such as onions, garlic, carrots, parsnip, radish etc. (Shannon & Grieve, 1999).

Compared to the peat-based growing medium controls, some of the physical properties of vermicompost were less suitable for pot-plant growth such as bulk density (Table 2.2 and Table 4.1), air fill porosity and water-holding capacity (Table 4.1). This was also found by other authors (Atiyeh *et al.*, 2001; Hidalgo & Harkess, 2002) when comparing vermicomposted animal manure to peat-based growing

media. Although the physical properties of the growing medium may have been slightly reduced with vermicompost addition, there was no indication that this negatively affected plant growth during these trials.

When compared to soil, vermicompost had a lower bulk density (Table 5.1), and therefore could improve soil physical properties e.g. reduced compaction and better drainage (Soane, 1990; Bot & Benites, 2005). Although the water-holding capacity of vermicompost has not been directly compared to that of soil in these studies, it is generally accepted that the addition of organic matter to soil will increase its water-holding capacity (Khaleel *et al.*, 1981). Therefore, the addition of vermicomposted spent mushroom compost should also increase soil water-holding capacity, which can increase plant growth and reduce plant stress during heat, drought and salinity stress (Sinha *et al.*, 1986; Rockstrom *et al.*, 2003; Xu & Zhou, 2006).

Effects of vermicompost on growing medium biological properties were generally positive, with initial vermicompost addition resulting in increased dehydrogenase activity and altered microbial community composition according to principal component analysis (Table 4.9 and Figure 4.1). During the plant's vigorous growth period, vermicompost did not affect growing medium biological properties although as plant growth was less vigorous, over a period of two months (which is quite considerable), vermicompost did increase root and rhizosphere bacterial numbers and richness (Table 4.10).

#### **6.4 Effect of vermicompost on plant growth, development and yield under normal and stressful conditions**

Vermicompost increased plant growth under non-stress conditions in chapters 2, 3 and 4, and either had no effect, or increased plant growth under abiotic stress

conditions (Table 6.1). In some instances, even with the recommended fertigation level (Table 3.3), or with reduced chemical fertiliser input (Table 4.3) plant growth was increased in the presence of vermicompost. Vermicompost performed best when compared to a control which was not adequately fertilised (Table 2.3). When comparing the effect of vermicompost addition to peat-based growing medium on tomato seedlings, Atiyeh *et al.* (2001) also found that there was significantly increased plant growth at more vermicompost concentrations when the plants were unfertilised, compared to fertigated plants.

Table 6.1 Summary of vermicompost effects on plant growth and yield quality

Chapter:	Plant Growth	Root Growth	Yield	Yield Quality	Stress Tolerance
2	+	+	+	+	+
3	+	=	+	+	+
4	+	n/a	+	+	=
5 (salinity stress)	=	n/a	n/a	n/a	=
5 (heat stress)	=	n/a	n/a	n/a	=
5 (cold stress)	+	n/a	n/a	n/a	+

– (negative effect), = (no effect), + (positive effect), n/a (not applicable)

Vermicompost was found to increase root growth in five-week old lettuce plants (Table 2.3) but not in 53 day old tomato plants (Table 3.2). This might be explained by the conductivity of vermicompost which was lower in Chapter 2, 3.36 mS cm<sup>-1</sup>, than in Chapter 3, 5.39 mS cm<sup>-1</sup>, or possibly that additional fertilisation was

provided to the plants in Chapter 3, but not Chapter 2. Higher conductivity in the root zone negatively affects root biomass (Cuartero & Fernández-Muñoz, 1999).

Bachman & Metzger (2007) found that seedling root dry weight responded differently to vermicompost addition depending on the plant, i.e. there was no effect of vermicompost (10% or 20% vol/vol) on tomato or cauliflower seedlings, but vermicompost increased the root dry weight of French marigold seedlings and reduced the root dry weight of pepper seedlings. Increased root growth in mature French marigold plants when grown with vermicompost amendment was also reported by Atiyeh *et al.* (2002), while reduced root growth in pepper transplants grown with vermicompost amendment was also recorded by Paul & Metzger (2005), indicating that root growth in some plant species responds better to vermicompost addition than in others, which is possibly a salinity tolerance response.

Vermicompost performed very well with regards to yield and yield quality (Table 6.1). Vermicompost increased marketable yield in Chapters 2, 3 and 4, and yield quality parameters in Chapter 2 (due to increased chlorophyll content and therefore more intense green colour), Chapter 3 (increased fruit dry matter and reduced blossom end rot) and Chapter 4 (reduced blossom end rot, improved fruit chemical properties and increased fruit acceptability according to the consumer acceptance panel). In the presence of vermicompost, yield quality parameters improved under adequate fertilisation (Table 3.4), reduced fertilisation (Table 4.5, 4.6 and 4.7), and limited fertilisation (Table 2.3). Vermicompost was also found to increase marketable yields in pepper (Arancon *et al.*, 2003, 2004a), tomato (Atiyeh *et al.*, 2000; Arancon *et al.*, 2003; Gutiérrez-Miceli *et al.*, 2007) and strawberry plants (Arancon *et al.*, 2003, 2004b), and to increase tomato yield quality parameters (Premuzic *et al.*, 1998; Gutiérrez-Miceli *et al.*, 2007; Zaller, 2007) in other studies.

Vermicompost addition was found to induce salinity stress in some trials, resulting in reduced plant growth at high concentrations (>50% vermicompost) (Table 2.3), but when used at lower concentrations (e.g. a minimum of 20% in Chapter 4) it significantly reduced plant development parameters i.e. increased the number of days to flowering, and delayed fruit ripening. When used at 10% concentration in Chapter 3 and Chapter 4, vermicompost did not reduce tomato plant development rate significantly. The effects of vermicompost further reduced plant growth under salinity stress (Table 5.3), especially at higher NaCl concentrations.

## **6.5 Are vermicompost effects nutritional or biostimulant effects?**

When it became clear that vermicompost increased plant growth under nutrient-limited conditions (Chapter 2), the remainder of the trials were designed with adequate (Chapter 3 and Chapter 5) or slightly reduced (Chapter 4) chemical fertilisation, as this more closely represents commercial horticultural conditions.

Combinations of nutritional and biostimulant plant-growth effects were observed throughout the trials (Table 6.2). Plant biomass response in Chapter 3 and Chapter 4 could be attributed to a biostimulant effect as adequate nutrition was provided, while the effects on marketable yield are more difficult to ascribe.

In Chapter 3,  $\text{Ca}^{2+}$  levels were not standardised. This was deliberate so that any additional effects of vermicomposted spent mushroom compost were identifiable. Therefore the increase in marketable yield observed in plants grown with vermicompost in this chapter can be attributed to a reduction in blossom end rot due to an increase in  $\text{Ca}^{2+}$  in the growing medium.  $\text{Ca}^{2+}$  was standardised in Chapter 4, and yet blossom end rot incidence was still reduced by the addition of vermicompost, and marketable yield increased in two of the four harvests, which

suggests a biostimulant response. This could also be due to increased plant nutrient uptake in plants grown with vermicompost, which was also reported by Premuzic *et al.* (1998) and Singh *et al.* (2010).

Table 6.2 Summary of vermicompost nutritional and biostimulant effects on plant growth

Chapter:	Nutritional Effects	Biostimulant Effects
2	shoot growth root growth leaf chlorophyll content	shoot water content
3	blossom end rot marketable yield no. 'extra' class fruits	shoot growth ripe fruit dry weight shoot water content
4	leaf chlorophyll content fruit quality	shoot growth blossom end rot marketable yield
5 (salinity stress)	none	none
5 (heat stress)	none	none
5 (cold stress)	shoot growth stress tolerance plant survival	none

Increased yield quality parameters could be attributed to a nutrient effect, although it is important to note that this would need confirmation with trials where the macro- and micro-nutrient quantities of the growing media with and without vermicompost was equalised. Increases in leaf chlorophyll when plants were grown with vermicompost (Table 2.3), which improves the visual quality of the plant, can be attributed to a nutrient effect as in this instance the increased nitrogen in the vermicompost was thought to have resulted in increased chlorophyll content.

Increased tomato fruit dry matter production (Table 3.4) is a desired quality in processing tomatoes (Zegbe-Domínguez *et al.*, 2003) and as all plants were given the recommended fertiliser level, this can be attributed to a biostimulant effect. The improved fruit quality parameters recorded in Chapter 4 (Table 4.5, 4.6 and 4.7) are more difficult to ascribe as it was only hypothesised that the additional conductivity in the root zone, a nutrient effect, increased plant soluble solids and titratable acidity compared to fruits grown without vermicompost. Other authors who have found increased fruit soluble solid content in plants (tomato and strawberry) grown with vermicompost or vermicompost leachate have attributed the effect to increased nutrient content of the vermicompost (Joshi & Pal Vig, 2010; Singh *et al.*, 2010).

As previously discussed (section 6.4), there is evidence that vermicompost induced salinity stress in some of the trials. As a response to that stress, vermicompost addition to growing media was also found to increase plant succulence under this salinity stress in Chapters 2 and 3 and during some of the harvests in Chapter 4 (Table 4.3). Mature tomato plant growth had increased succulence in Chapter 3 (Table 3.3), while earlier tomato plant growth (month 1 and 3 harvests) (Table 4.3) and relatively early (five-week old) lettuce plants (Table 2.3) had increased succulence in Chapters 4 and 2, respectively. Succulence has been described as a stress escape mechanism for salt-stressed plants (Cuartero & Fernández-Muñoz, 1999; Muhammed & Hussain, 2010), which results from increased leaf solute concentration and therefore increased leaf water uptake and leaf turgor pressure (Jennings (1976) (cited by Ouhibi *et al.*, 2014)).

Despite this response of increased plant succulence under salinity stress in tomatoes and lettuce, under controlled salinity stress vermicompost did not increase plant succulence in cress plants (Table 5.3). There may be a number of factors

contributing to this, such as the difference in cropping length, with cress plants being grown for a very short period of time compared to lettuce and tomato plants, while the increased salinity stress in Chapter 5 i.e. vermicompost and NaCl, compared to vermicompost only in Chapters 2, 3 and 4 could have contributed to the differences in plant succulence observed in the different chapters. In a study investigating the effect of increasing salinity on cress seedling growth, Muhammed & Hussain (2010) found increased cress succulence at low salinities ( $5.0 \text{ mS cm}^{-1}$ ) but reduced succulence at higher salinities. Therefore, the salinity experience by the combined vermicompost and NaCl treatments in Chapter 5 might have been too strong for the plants to escape using succulence.

## **6.6 Future Research**

The present research has identified a number of areas where future research could be undertaken. In particular, it is important that a more detailed study be carried out on an industrial scale on the effects of vermicomposting as a post-stabilisation method for compost maturation, compared to traditional compost maturation. This research should include the effect of these two maturation methods on the biological properties of the compost and vermicomposted compost, as well as the physical and chemical properties. Once this research has been undertaken, it would also be beneficial to evaluate the commercial viability of vermicomposting as a post-stabilisation method for compost maturation taking into account the additional costs and maintenance of a vermicomposting system, the increased market value of vermicompost compared to compost, and the potential for increased plant growth and yield quality as a result of vermicompost use.

This study investigated the effect of vermicomposted agricultural biodegradable wastes and by-products, primarily spent mushroom compost, on plant growth, yield and abiotic stress tolerance. The effect of vermicomposts made from other waste sources should also be investigated, to observe the effect of feedstock on vermicompost quality and subsequent plant growth response. Unpublished work from this research study also found beneficial responses when lettuce was grown in vermicompost from other feedstocks such as pelleted sewage sludge and food waste.

It was demonstrated in this study that vermicompost had a positive impact on yield and yield quality (Table 6.1) when used in containerised vegetable production. Large-scale grower trials should be used to confirm that these yield responses are also observed in horticultural production systems.

The effects of vermicompost application on peat-based growing medium properties have been investigated as part of this study, but more work is needed on the effects of vermicompost addition on soil physicochemical and biological properties. Vermicompost has more scope to be beneficial with regards to these parameters, especially the physical properties, when used as a soil amendment than when used as a growing medium additive as the high organic matter of growing media means it shares similar physical properties to vermicompost.

It would be beneficial to further examine the effect of vermicompost on the biological properties of growing media on other plants, and to test whether the proposed hypothesis that bacterial results were driven by root exudate production in Chapter 4 is in fact true.

The effect of vermicompost on root growth was not fully examined in these studies. The effects of vermicompost on plant root growth and development should be

investigated using mini-rhizotrons (Huck & Taylor, 1983). It would be beneficial to compare root growth in soil, with and without vermicompost, to observe the effect of vermicompost on soil physical properties and whether this affects root growth. It would also be beneficial to compare root growth of plants grown in peat-based growing media with and without vermicompost amendment, to observe the effect of vermicompost specifically on root growth.

This research looked at the effect of vermicompost on salinity, heat and cold stress but not drought stress, which is also an economically important abiotic stress. Abiotic stress usually occurs in combinations in the field e.g. drought and heat are usually experienced together. It would therefore be interesting to investigate the effect of vermicompost on drought stress; more importantly the effect of vermicompost on combined stressors e.g. on heat and drought together should be assessed.

To determine whether vermicompost addition definitively results in biostimulant plant growth responses, plants should be grown in different levels of vermicompost and compared to plants grown using the equivalent chemical fertiliser level (as was done in section 5.2.3), and the level of chemical fertiliser that has been shown to achieve maximum plant growth.

## **6.7 Recommendations**

Spent mushroom compost is a suitable feedstock for vermicomposting and vermicomposted spent mushroom compost has been shown in this research to make a good quality growing medium additive which can provide additional plant growth, yield, and yield quality effects, even under reduced fertilisation. The limitation of vermicomposted spent mushroom compost is still its high conductivity, although as

vermicomposting requires an additional step and additional facilities, it is more expensive to produce, and therefore it is not commercially viable to use vermicomposted spent mushroom compost in large quantities. The additional costs of production when a vermicomposting step is included in a composting facility consists of the purchase of additional machinery (i.e. vermicomposting vessel and equipment for; separating the worms from the finished product, loading and unloading the vermicomposting vessel and for screening the finished vermicompost), facilities (vermicomposting usually takes place indoors or under-cover), electricity (particularly for heat generation in cooler climates during winter), labour and the purchase of the worms themselves. Hence, it is recommended that vermicomposted spent mushroom compost should be used in small to moderate amounts (<50%) when used for containerised vegetable production.

Despite the negative environmental impacts, for commercial purposes peat is a very good quality growing medium. It has suitable physical properties and chemical properties that can be easily amended to make it suitable for plant growth (Schmilewski, 2008) e.g. addition of fertiliser and lime. There are currently no materials available in large enough quantities, with the same consistency, that can replace peat use in commercial horticulture. For example, coconut coir is available in a large enough quantity to replace peat, though currently it is more expensive. Coconut coir has suitable physical properties as a growing medium but it lacks consistency, can have high electrical conductivity value and as it must be shipped large-distances from developing countries such as India and Sri Lanka (Schmilewski, 2008). Because of this it comes with its own environmental sustainability issues, for example the environmental cost of long-distance shipping and the potential to introduce foreign pests and diseases.

Composted bark and wood fibres (thermally or mechanically extracted fibres from wood and wood waste) have also been investigated as alternatives for peat in growing media, but have worked best when used to dilute the volume of peat used in growing medium (usually up to 50% by volume). The physical properties of these materials are suitable as a growing medium additive but not for use as a peat-alternative. Due to the high carbon content of composted bark and wood fibre, additional nitrogen is required in the production process to limit N-fixation by these materials during plant growth (Maher *et al.*, 2008).

While for hobby gardeners, peat-free composts in the form of wood and coir-based composts can be suitable, commercial scale horticulturalists require a more consistent, professional and inexpensive product, and, for this reason, peat dominates the professional market. Vermicomposted spent mushroom compost could be used in the hobby gardener market for the dilution of peat-free growing medium. It is important to note, though, that vermicomposted spent mushroom compost does contain some peat and therefore if used as part of a peat-free growing medium the resulting mix would contain some recycled peat and may no longer be referred to as completely peat-free; as the peat is recycled, it would likely satisfy customer demands for an environmentally sustainable alternative to peat-based growing media. For use in the professional horticulture market, vermicomposted spent mushroom compost should be used for peat-dilution, not peat-replacement, to achieve the same or better plant growth responses that the peat-based growing media most commonly used in professional horticulture.

Vermicompost should be marketed as a plant growth promoting product and not as a replacement for chemical fertiliser. Its main attributes with regards to horticultural

use are that it can be used to reduce peat and chemical fertiliser inputs without negatively impacting on, but even enhancing yield, and importantly yield quality.

One potential new market for vermicompost is for ornamental transplant production. The trend now is that garden centre customers are looking to buy plants that are already in flower to provide instant results for their garden. One consequence of this purchasing trend is that plants are now being grown on to flowering stage in very small containers and bedding plug packs. The continued mineralisation of vermicompost could replace the need for slow-release fertilisers for when the plants are delivered to the garden centre, as vermicompost was found to increase shoot and root growth in plants grown with limited nutrients and in small containers (Table 2.3).

## **6.8 Reference List**

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