The effects of waste derived fertilisers and composts on crop production

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The candidate confirms that the work submitted is her own. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others.

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Abstract

The waste from olive oil production is a valuable source of nutrients that has the potential, when used well, to be a useful organic amendment to aid crop growth. It does have some drawbacks with potential phytotoxicity due to being likely to have a high amount of phenols present and a high conductivity. The waste from olive oil production used in these trials was composted with different animal manures to produce a number of products with different characteristics. In some of the trials a replacement form of organic amendment in the form of chicken manure was used instead. In addition to these amendments, some of the trials included biochar as an additional organic amendment to see if the properties of the biochar had any effect on plant development and yield.

The trials discussed in this thesis take place over a 3 year period on 3 different crops: winter wheat, oilseed rape and strawberries. The wheat was grown at both field and pot scale in the trials.

The field scale trials with oilseed rape and winter wheat showed no significant differences between yields when comparing a mineral fertiliser to a substitute chicken manure fertiliser. In the first harvest year the oilseed rape showed significantly higher yields on the plots that received biochar as an amendment, however this result was not replicated by the winter wheat in that or the subsequent harvest year.

When the olive mill waste compost was used as a substrate replacement for strawberries, the strawberry crop showed no significant patterns or changes in development due to the amendment applied in the first year. In the subsequent when used again as a growth media and more graduated inclusion amounts (% v/v) the OMW caused plant mortality in plants receiving more than 20% inclusion of compost. The compost used in this year had a high conductivity to which the high plant mortality was attributed. The plants that received more than15% OMW compost also produced significantly less marketable fruit, and the fruits produced were more poorly pollinated. In the same year, the OMW

was applied to wheat at pot trial scale, but used as a fertiliser and applied at the same nitrogen loading rate as the mineral nitrogen applied. In this trial there was no plant mortality of the wheat, and no discernible difference in the plant development.

These trials have shown that compost of olive mill waste and animal manures can be used as an alternative to traditional mineral fertilisers on both arable and horticultural crops in the UK and as a soilless media replacement, however it is important to consider the characteristics of the OMW before applying it.

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Chapter 1 Introduction

This chapter sets out the layout of this thesis and the broad topics covered within it.

1.1 Population growth and environmental pressures

The human population in Europe is declining, with the current population at 738 million, and predicted to be 646 million by the year 2100. Global population growth has also slowed down to an increase of 1.18% in 2015, dropping from 1.24% growth in 2005 (Nations, 2015) although the global population is still predicted to increase from 7.3 billion to 11.2 billion by the end of this century. This is despite a drop in fertility on a global scale. In order to allow for this population increase, there has to be a corresponding 70% increase in global food production by 2050 (Lal, 2013), not only of the correct produce but given rising fuel and transportation costs the food need to be sustainably produced and from the same local area.

The use of waste as a way to improve crops is certainly not a new concept, but the margins of error in the use of waste as a resource and the risk of contamination from them are growing smaller as the management of our wastes improves. The use of wastes in their raw or composted forms can both have benefits for crop growth and sustainability depending on the amount added to the substrate and the timing of the application. This work looks at adding wastes in their composted and raw processed forms on both arable and horticultural crops. The need for sustainability and reuse of wastes and residues in both forms of food production is needed alongside improved technology to plan and use the limited available space for food production. Innovations in crop production are developing all the time with research into increasing daylight hours for plants by using low cost LED technology, similar to that used to growth plants in the International Space Station.

Alongside reuse of waste is the importance of the sustainability of food production with the availability of both arable and horticultural substrates for

plant growth under stress. Soil is a limited resource that takes time to develop nutrients and structure and can be destroyed in a much smaller time period than it takes to be created. The year 2015 was designated as the International Year of Soil by the United Nations Food and Agriculture Organisation (FAO) to recognise the importance of preserving this resource. In balance, there are moves within the horticulture sector to increase sustainability by moving away from using peat as a growing media due to the fragility of peat ecosystems and the slow regeneration time of peat meaning it is not easily replaced.

1.2 Aim of this research

The aim of this research is to use agricultural waste as a soil and substrate amendment in order to study the effects of this on plant development and crop yield. The importance of creating and maintaining a circular economy with organic waste going back into the base substrate, providing nutrients for food production is paramount. This research uses organic based agricultural wastes on arable and horticultural crops in both a field and pot based setting to give a broad spectrum view on the potential effects. This aim will be achieved through specific objectives within each chapter. The objectives centre around comparing the effects of the different amendments on each crop grown, as well as the soil and substrate characteristics. In total there are nine objectives from the four experimental chapters.

From Chapter 2 the work on large scale arable trials:

- **Objective 1**: to assess the impact of a high nitrogen organic amendment, in this case chicken manure addition, on arable crops compared to mineral fertiliser in terms of crop yield.
- **Objective 2**: To assess the effects of biochar as an additional amendment to the chicken manure in comparison to a mineral fertiliser used by itself by measuring crop yield from the experimental plots.
- **Objective 3**: To assess the effects of the organic amendments on soil quality by carrying out chemical analysis of the soil.

From Chapter 3 the work on small scale arable trials:

- **Objective:** To study the effects of different organic fertiliser treatments against a mineral fertiliser used as a control both with and without the addition of a biochar application.

Objectives from Chapter 4 the first horticultural trials:

- **Objective 1:** To compare the effects of liquid fertiliser with chicken manure as a replacement fertiliser on strawberry growth with and without the addition of biochar.
- **Objective 2:** To compare ratios of OMW compost varieties and their effects on strawberry growth with and without the interaction with biochar.
- **Objective 3:** To compare the addition of a significant amount of biochar at a 50% rate and its effect on strawberry growth.

Finally the objectives from Chapter 5 the second set of horticultural trials:

- **Objective 1:** Determine the impacts of increasing the fraction (v/v) of OMW compost present in the growing media for strawberry plants and alongside this compare the effects of two common base substrates, coir and peat against each other. The different treatments set up in order to achieve this objective are given in Table 5.1.
- **Objective 2:** Compare the effects of a substrate with OMW compost added in the presence and absence of liquid fertilisation.

1.3 Layout of this thesis

This thesis is broken down into seven chapters. The first chapter being this introduction within which is contained a literature review section covering the broad topics discussed in the thesis.

Following the literature review are four experimental chapters detailing different trials completed. Each of these experimental chapters is focused on one type of trial only. As such, each experimental chapter has its own introduction and literature review for that tranche of work, followed by the methodology, results and discussions sections. The first data chapter, Chapter 2 is for the large scale arable trials completed over 2 seasons in 2013 and 2014. Chapter 3 focuses on a pot scale arable trial carried out in 2015 to build on the work in the previous chapter. After this are two chapters using strawberries grown indoors as the experiment plant, one chapter covering the work carried out in 2014, and the last data chapter for the work in 2015 on strawberries. Following the final experimental chapter is a concise overall conclusions chapter, highlighting further some of the most important aspects from the preceding chapters, which is then followed by a chapter highlighting potential further work. It is hoped that by presenting the data and research in this way that the appropriate detail and focus can be concentrated on each trial, with the final conclusions chapter being in place to bring the work together in one combined piece of work.

1.4 Literature review

1.4.1 Modern food production

The modern world of food production is one driven by technology and innovation and heading further into the 21st century it is these tools backed up by a solid knowledge and understanding of soil and substrate function that will feed the next generations.

1.4.1.1 Food security and climate change

This aspect of food security and food production is one which has received increasing attention over the past 50 years or so. More recently climate change and the resulting food poverty has been linked to civil war. It has been postulated that 21% of civil conflicts since 1950 can be connected with the El Nino Southern Oscillation (ENSO) which dominates our modern climate and effects crop production (Hsiang et al., 2011). The effects of climate change on

conflict are thought to be due to two factors, the first is increased global temperatures as discussed by Burke in 2009 when considering conflict in Africa (Burke et al., 2009). The other factor affecting conflict is thought to be drought, although the mechanisms between both of these factors and a violent response are not clear cut (Linke et al., 2015). The need to provide water, nutrients and resources for food production will only increase with the global population increase. The inclusion of organic waste management into this situation promotes the idea of a circular economy whereby waste is considered a resource rather than something that needs to be discarded.

1.4.2 Olive mill waste: composting and uses

The growing of olives and the extraction of olive oil is a worldwide industry, predominantly based in Mediterranean countries. The olive tree, *Olea europea,* is one of the main Mediterranean crops with a cultivated area of approximately 8.2Mha (López-Piñeiro et al., 2008a). This crop produces an annual volume of 10 Mm^3 of olive mill wastewater and 6 Mm^3 of solid olive mill by products; consisting of olive stones, leaves, and pomace (Nektarios et al., 2011). The combination of having this amount of organic waste, and the need for a good substrate for crop production should be a positive association. These oil mill wastes as well as being a source of organic matter, can be rich in nutrients such as potassium, nitrogen and phosphorus. There are various ways of recycling these wastes into crop production and one of these is to deliver them as some form of compost.

A drawback of using the wastewater from olive oil production as an amendment to produce compost is often the high phenol content which can prove extremely phytotoxic to crops (Cucci et al., 2008). This is due to the monometric phenolics known to be contained in OMW. These give OMW its dark colour and are the reason for the phytotoxic and antibacterial effects that OMW can have (Piotrowska et al., 2006). Piotrowska found that at application rates of 80m^3/ha that olive mill wastewater had a strong phytotoxic effect which led to a decline in the germination activity within the soil (Piotrowska et al., 2006). The process of composting can go some way to ameliorating this effect

by destroying the phenols present during the composting process. Other phytotoxic aspects of olive mill wastewater are its potential high conductivity. High conductivity in soil can prove inhibiting and at the extreme, fatal to plants due to the osmotic potential of the soil being far higher than the plant can tolerate. The result of this is that the plant cannot take in enough moisture to survive.

There have been many studies using the waste from olive production as compost on a range of crops. Alburquerque et al (2006) used an olive mill waste product called alperujo compost (ALC). This was the compost material produced using the solid residue from a two-phase centrifugation system for olive oil extraction. During greenhouse trials there was no evidence of adverse effects on the plant growth of peppers or phytotoxicity symptoms in ALC plots. The alperujo produced similar yields to the other organic amendments of cattle manure and sewage sludge with yields of 99.6, 98.3 and 97.7 t/ha respectively. The ALC amended plots showed little change in soil organic matter (SOM) content post-harvest, but SOM was significantly reduced in both cattle manure and sewage sludge compost amended plots (Alburquerque et al., 2006).

There have been successful outcomes using olive mill waste as a compost in other horticultural trials, one of these was in the work of Altieri and Esposito (2010). They used tomato and lettuce crops and two varieties of olive mill waste; one was untreated and stored in a stacked pile in net sacks (15kg each) and the other was composted by turning mechanically twice a week. Analysis of the compost and stacked pile showed optimum levels of nutrients, mainly K and N, which indicated the potential of OMW produced in these ways as a potentially good organic fertiliser. Phytotoxicity tests showed high values of germination index (GI) for both materials (Altieri and Esposito, 2010). Using these two forms of OMW their performance was found to compare favourably to standard treatments. In their trials they used tomato and lettuce crops and found the yield of the crops was comparable to the control. This suggests that for even short term crops it is possible to produce OMW that contains sufficient levels of nutrients to support plant growth (Altieri and Esposito, 2010).

Cucci et al (2008) applied olive oil pomace to sunflower and wheat crops over 3 seasons. They recorded a positive difference in the total accumulated organic matter in the soil and total N content. P and potassium (K) also increased with higher amounts of applied pomace. The build-up of organic matter made a contribution to the formation and stabilisation of the soil aggregates against the dispersing action of water. The application of organic matter to soil improves its water holding ability both at field capacity and wilting point (Cucci et al., 2008).

Other studies have combined the use of olive mill cake and poultry manure as a compost to improved soil fertility. In one of these studies the combination of these that gave the best crop yield on semi-early spunta potatoes was a mixture of 75% poultry manure and 25% olive mill cake, (Hachicha et al., 2006). Research has also been carried out into the impact of OMW application on seedling emergence. One particular study showed that OMW can be used as a safe agronomic amendment on maize seedlings, provided the application is reasonable and incremental and plant development stages are avoided, notably the preliminary development stages when applying the OMW as a liquid (Hanifi and Hadrami, 2008).

1.4.3 Biochar: functionality and uses

The use of the term biochar was first noted around 1998 for the solid residual from biomass pyrolysis (Bapat and Manahan 1998 in (Spokas et al., 2012a)). There was a shift in the 1980s for the purpose of biochar production as a way of sequestering carbon from its previous purpose as an energy and chemical resource (Spokas et al., 2012a). The first people to utilise biochar as a soil modification were the Amazonians using 'slash and char' agricultural practices to create the Terra Preta soils or Amazonian Dark Earths which have higher soil fertility relative to standard tropical soils (Spokas et al., 2012a, Atkinson et al., 2010, Warnock et al., 2007, Van Zwieten et al., 2010). Biochars are often referred to as a black carbon (BC) resource and this is classed as the material remaining following incomplete biomass combustion (Harvey et al., 2012).

Pyrolysis is the process of thermochemical decomposition of organic materials in the absence of oxygen at elevated temperatures (Spokas et al., 2012a,

Lehmann et al., 2011). There are three product streams from the creation of biochar: non-condensable gases, combustible bio-oil, and biochar (Spokas et al., 2012a). Pyrolysis can be endothermic or exothermic depending on the temperature of the reactants. It becomes increasingly exothermic as the reaction temperature decreases. Biochar formation commences at low temperatures where autogenous pyrolysis begins.

Soil fertility is influenced by a balance of abiotic and biotic reactions that are influenced themselves by the season and their location in relation to other resources. Previous studies have shown that adding biochar to soils may have immediate effects on properties such as soil nutrition, water retention, and microbial activity (Atkinson et al., 2010, Lehmann et al., 2011). Because of the mineral elements within biochars their incorporation into the soil influences soil structure, texture, porosity, particle size distribution and density. Biochar is known for its capacity for nutrient adsorption and due to its potential for surface oxidation it also has the ability to improve the cation exchange capacity (CEC) of a soil (Liang et al., 2006).

An influential physical attribute of biochar is its porous structure and large surface area. The structure can provide refugia for beneficial soil microorganisms such as mycorrhizae and bacteria and it also influences the binding of nutritive cations and anions (Atkinson et al., 2010, Warnock et al., 2007). Because of its general recalcitrant nature biochar may have long term impacts on soil formation. If it is to serve a beneficial role there should be a noted increase in the quantity of plant available nutrients within the biochar and its nutrition retention capacity. Organic compounds that get sorbed onto this black carbon have been postulated to interfere with soil microbial nitrification and denitrification reactions (Clough et al., 2010, Spokas et al., 2011, Spokas et al., 2012b).

Spokas et al 2012 noted that of the biochar studies they compiled approximately 50% of the studies observed short-term positive yield or growth impacts, 30% reported no differences and 20% noted negative yield or growth impacts (Spokas et al., 2012a). This indicates that the manner in which biochar interacts with soil and plants is some way from being fully understood.

1.5 Statistical analyses

The statistical analyses carried out in this research have been completed using SPSS version 19. For continuous data such as the yield data which is found in all chapters, it was at first confirmed to be normally distributed using a Shapiro-Wilk test for normality (Dytham, 2011). Continuous data that was not normally distributed was subject to mathematical transformations such a square root, Ln or a cubic function to normalise it prior to statistical analysis. It was then analysed using an ANOVA with a significance level at 0.05 with a Tukey's test as a post-hoc analysis to compare the means (Fowler and Cohen, 1990, Sokal RR, 1987).

For non-parametric data, such as the count data for the plant characteristics in Chapter 5, this was analysed in the first instance using a Kruskal-Wallis (Dytham, 2011) test with a Chi-Square analysis. In this analysis the data is converted into ranks so it can be ordered in terms of value, with the lowest median being assigned the lowest rank. If this showed significant differences between the treatments, the treatments were then further analysed using a Mann-Whitney U test to determine the treatments which were different from each other. Non-parametric data is shown as a median value is graphs and tables as this is the best representation of non-parametric data (Dytham, 2011).

In some instances data displayed in tables is supported by notation to identify which treatments are significantly different from one another. In this case, treatments with the same letter following their identification are not statistically different from one another, and treatments that are different are followed by different notation. This is quite detailed for some sets of data with up to 4 letters following a number to identify where any significances lie.

Chapter 2 Large scale arable trials

2.1 Introduction

This chapter will discuss in detail the large scale field trials which were carried out over a two year period using two different crops. This will consist of a review of the previous work carried out in this area and background information relating to the work, in addition to a detailed account of the methodology used in this study, and a full description and discussion of the results from trials carried out in 2013 and 2014. The work described in this chapter is a precursor to the small scale arable trials work discussed in Chapter 3.

In this chapter the organic amendments used in the trials are pelletised chicken manure and a commercially produced biochar. It might be expected that the chicken manure as a nitrogen replacer will compare similarly to the inorganic mineral fertiliser applied as a control, although the mineralisation of the organic nitrogen source could slow down the uptake of nitrogen by the plants. The biochar applied in these trials is added as an organic amendment and not as a fertiliser. The addition of biochar in the trial plots could improve the concentrations of plant available nutrients and reduce leaching of nutrients.

2.2 Literature Review

2.2.1 The Importance of soil preservation and the use of organic amendments

During the 20th century there was recognition that soil was an exhaustible resource, and the importance of the soil as an environmental component. There was also an acknowledgment of the need to maintain or improve the soil's ability to perform the host of functions that it is taken for granted that soil provides (Nortcliff, 2002). The importance of soil has been known for thousands of years with records in Roman literature of soil tests being undertaken in order to assess a soil's fertility (McNeill and Winiwarter, 2004).

2015 was the international year of soil as designated by the United Nations Food and Agriculture Organisation (FAO). This was intended to increase understanding of the importance of soil for food security, which along with a healthy soil has intrinsic benefits for ecosystem function and biodiversity. Until recently soil was left behind air and water when being considered in legislative decisions and has historically received less statutory protection than either air or water (England, 2015). We now recognise the importance of soil as a resource for food production, biodiversity and as a buffer against extreme weather events such as flooding, drought and other pollution events. It is thought that around 95% of our global food supplies are provided by the soil, either directly or indirectly (FAO, 2015) and that without a healthy soil the global population would be unable to sustain itself. It is estimated that by 2050 world food production must increase by 60% compared to production in 2015, and in developing nations this figure is almost 100%. Intensification of agriculture has led to increased productivity and efficiency in recent decades, but this goes hand in hand with negative impacts on soil fertility and biodiversity (D'Hose et al., 2012).

One of the major obstacles to achieving increased food production globally is the degradation of soil which can occur in several ways, the primary causes are:

- Loss of organic matter through crop cultivation,
- Erosion by wind and water,
- Contamination through the build-up of toxic elements
- Compaction from farm machinery and livestock,
- Salinisation through irrigation water or coastal flooding; and
- Acidification from atmospheric pollutants. (POST, 2015)

Soil erosion is a large problem for agriculture and for the environment, it is estimated that some 12Mha of land are seriously affected and then abandoned on an annual basis globally (Pimentel et al., 1995). The use of chemical pesticides and mineral fertilisers can compensate in terms of crop yield for the loss of soil through erosion. However they bring with them their own problems such as increased resistance to pesticides which has been well documented

(Whalon et al., 2008) and to the creation of environmental pollution and degradation of the natural environment.

The primary benefit of applying organic matter to soil is the provision of nutrients for crop growth and for sustaining the microbial population within the soil which in turn improves the functionality of the soil and should translate into the need for less or no application of mineral fertiliser (England, 2015, Roig et al., 2012). The addition of organic matter to the soil has several additional benefits; it can increase the water retention capacity of the soil due to the improvement in the soil structure. The improved soil structure also leads to benefits of greater porosity and a decrease in the risk of erosion and greater protection against flooding (Powlson et al., 2012).

As well as the environmental and social impacts of soil degradation there is also a real economic cost. In the UK alone, in 2012 DEFRA estimated the costs at between £0.9 and £1.4bn per year with 45% of the cost attributed to loss of organic content, 39% to compaction and 13% to erosion (DEFRA, 2012). The costs incurred as a result of the changing soil are related to loss of provisioning costs, greenhouse gas emissions, flood related costs and water quality related costs.

2.2.2 The importance of soil functionality and nutrient cycling for arable crops

2.2.2.1 The Nitrogen Cycle and the use of nitrogen as a resource by plants

Nitrogen is often considered to be the foremost limiting factor in plant growth (Franche et al., 2009, Daughtry et al., 2000) and is the most essential nutrient given that it is a building block of all proteins and nucleic acids present in the plant (Russell, 1973). The mineralisation of nitrogen is the process that converts the N in organic compounds into assimilable nitrogen in the form of ammonia and nitrates (Russell, 1973). The process behind this conversion is shown in Figure 2.1 and is completed through soil microorganisms in the form of symbiotic bacteria (Russell, 1973, Cassada and Russell, 1975, Xu et al., 2012).The form in which the plant is able to take up the nitrogen is dependent

on a plant's adaptability to the conditions and the pH of the soil, with plants in acid soils able to more readily assimilate ammonium whereas plants more adapted to higher pH will likely prefer nitrate (Masclaux-Daubresse et al., 2010). It is the process of oxidation that converts inorganic nitrogen into nitrates, and a reduction process which converts them into ammonia (Connell, 2005).

The majority of nitrogen uptake by plants is through transportation of the nitrogen into the shoots where it is used in the leaves through its inclusion in the chloroplasts (Xu et al., 2012). Most of the nitrogen present in the leaves is therefore contained within chlorophyll molecules so there is a closely linked relationship between chlorophyll levels in the leaves and the nitrogen content (Daughtry et al., 2000). In the case of wheat the leaf nitrogen is then transferred into the grain as the plant enters the senescence phase, and the speed and onset of flag leaf senescence in wheat are linked to nitrogen remobilisation efficiency and also crop yield through grain filling (Masclaux-Daubresse et al., 2010). A sign of nitrogen deficiency in wheat is yellowing or red tinged leaves.

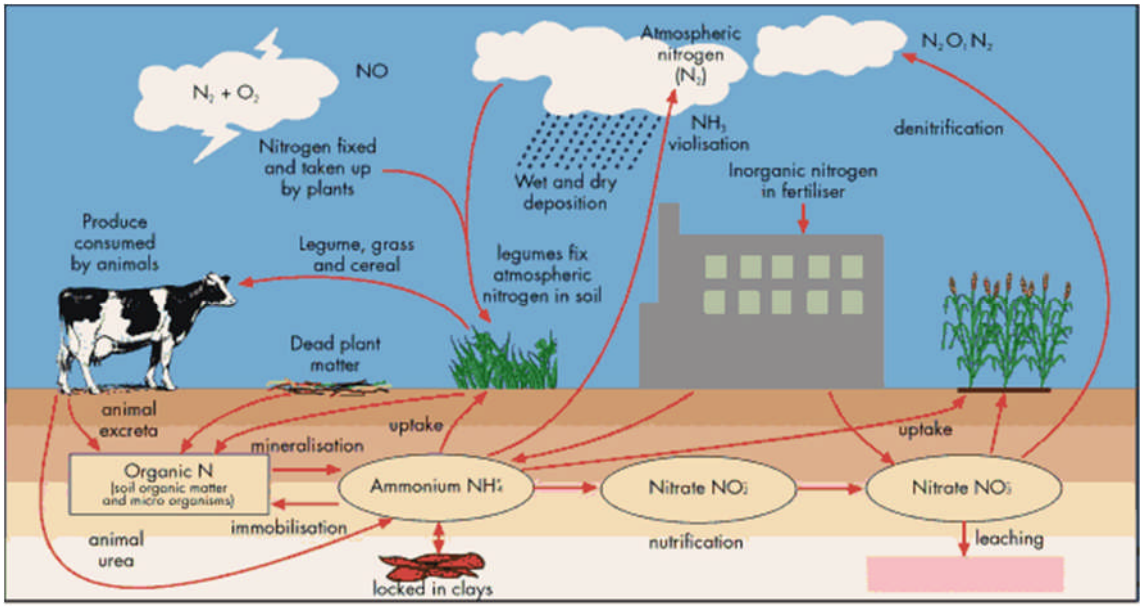


Figure 2.1 The aspects of the nitrogen cycle important in the soil and for plant growth. Source: Agricultural Bureau of South Australia (undated) http://bettersoils.soilwater.com.au/module2/images/27.gif

2.2.2.2 The role of phosphorus in plant growth

In its role as a phosphate (P_2O_5) in the soil phosphorus plays a vital role in enzyme reactions in the plant that require phosphorylation and is necessary for cell division and the growth of meristem tissue (Russell, 1973). The nature of the phosphorus cycle can be termed open or sedimentary due to there being no interchange with the atmosphere (Rodríguez and Fraga, 1999). The biggest reserves of phosphorus are in rock form, and in that sense phosphorus is a limited resource, however farmyard manures are a rich natural source of phosphorus that can be applied to land with pig manure having the highest amounts of total and available phosphorus (DEFRA, 2010).

Excess phosphorus applications and runoff can cause damaging effects in the environment, especially in water where it can cause eutrophication, the result of which can be algal blooms, a decrease in water oxygen levels, and a reduction in biodiversity (Jarvie et al., 2006, DEFRA, 2010, Sharpley et al., 2000). The control of applications of phosphorus to soil is regulated through the Water Framework Directive (WFD) Directive 2000/60/EC, 2000; which requires control of phosphorus inputs to river systems to maintain and improve the ecology of the riparian systems (Jarvie et al., 2006, EC, 2000).

2.2.2.3 The role of potassium in plant growth

Potassium is not a building block of a plants make up, rather it is important in the creation of amino acids and proteins from ammonium ions. Plants deficient in potassium will show stunted growth (Russell, 1973). Potassium is the most abundant inorganic component of plants, contributing to the osmotic potential of the cells and the electrolytic properties of the cytoplasm and is key is maintaining the turgidity of plant tissues (Johnston, 2012). Potassium in agricultural terms is referred to as potash (K_2O). Potassium allows plants to fully utilise the beneficial effects of nitrogen and increase yield as a response.

Potassium is found in farmyard manures and is especially high in poultry manures in its available form (DEFRA, 2010). Layer poultry manures have 9.5 kg potash per ton with 90% of this in a plant available form compared to 3.2 kg/t

at 90% availability in cattle manure and 2.4kg./ton potash with 90% availability in pig manures (DEFRA, 2010).

2.2.3 Organic amendments applied to soil

2.2.3.1 Manure application

The application of manure to crops has occurred since the hunter gatherers started farming following the last ice age 10 000 years ago (Pringle, 1998, McNeill and Winiwarter, 2004) and the animals they kept gave back into the land they fed from. As societies were primarily small communities in one place the nutrient cycle was small and local and what came out of the soil often went back in (McNeill and Winiwarter, 2004).

Extensive work has been done to assess the effects of using manures as a substitute for chemical fertilisers to improve the sustainability of the soil whilst at the same time utilising what would otherwise be considered a waste product. The long term application of manures instead of mineral fertilisers can have a positive effect on soil quality including lower bulk density, higher porosity, higher content of organic matter and increased amounts of P, K, Ca, Mg and nitrate in the topsoil (Edmeades, 2003). The increase in soil organic matter following application of a manure improves the biological activity within the soil and the infrastructure value of it, reducing erosion, as well as improving the soil's physical properties (Gong et al., 2009). The review paper by Edmeades in 2003 summarised the results of fourteen studies comparing the use of inorganic fertilisers and manures on crops. This identified that 13 of these studies showed no significant difference between the yield of crops for plots that had mineral fertiliser applied and those where manure was used. In four of the trials bulk density was measured and in three of those the bulk density decreased with manure application (Edmeades, 2003).The Askov long term field experiments began in 1894 in Denmark and were set up to compare mineral fertilisers to animal manures. The crop yields were measured every year from 1894 and the soil carbon, nitrogen, potassium and phosphorus content were analysed every four years from 1923 onwards (Christensen, 1997). The results for the effects on yield and soil properties have been discussed in several papers; the yield

results for the crops in these trials showed 2.5 and 3 times greater yield for manure and inorganic fertilisers respectively when compared to untreated plots (Christensen, 1997).

Chicken manure has long been used as a rich organic amendment to improve crop growth. The addition of poultry manure alongside an NPK fertiliser was shown in Ayoola (2006) to be the most effective method of fertilisation for cassava, melon and maize crops when compared with NPK alone, and poultry manure alone. This was attributed to the additional benefits provided by adding an organic amendment in terms of the increased water retention ability of the soil (Ayoola and Adeniyan, 2006). Chicken manure applied by itself has also been shown to have beneficial effects on the yield of timothy grass when compared with an inorganic fertiliser and a dairy manure fertiliser replacement (Warman, 1986). The rate of nitrogen mineralisation has to be taken into account when applying manures to crops as this will determine the amount of plant available nitrogen from manures. Not all the nitrogen contained within a manure amendment will become available in a form capable of being assimilated by plants .Temperature is a key factor in the rate of mineralisation (Slaton et al., 2008), when temperatures fall the rate of mineralisation falls to less than 3% of total nitrogen recovered as nitrate when incubated at 5^{o}C, compared to 31% at 25^{o}C (Gordon et al., 2014). Chicken manure when applied as an amendment has been shown to increase levels of phosphorus and potassium in the soil as well as nitrogen (Limon-Ortega et al., 2009).

2.2.3.2 Biochar

Biochar can be differentiated from charcoal through its use as a soil amendment rather than a fuel (Lehmann et al., 2011). Soil fertility is influenced by a balance of abiotic and biotic reactions that are influenced themselves by the season and their location in relation to other resources (Spokas et al., 2012a). Previous studies have shown that adding biochar to soils may have immediate effects on properties such as soil nutrition, water retention, or microbial activity (Atkinson et al., 2010, Lehmann et al., 2011) as well as increase carbon sequestration and improved agronomic performance (Spokas et al., 2012a). The mitigating

effects for climate change aren't solely related to sequestering carbon but extend to impacting on the emission of greenhouse gases from soil. Biochar has been shown in laboratory experiments to decrease the methane and nitrous oxide emissions from soil (Karhu et al., 2011). Because of the mineral elements within biochars their incorporation into the soil influences soil structure, texture, porosity, particle size distribution and density. Biochar as a soil amendment can improve soil structure by reducing bulk density (Singh et al., 2010a) and reducing nutrient leaching through absorption of ammonia preventing volatilisation of nutrients from the soil which has the benefit of increasing fertiliser efficiency (Stavi and Lal, 2013). The biochar is able to sorb ions such as ammonia from the soil through a combination of electrostatic and capillary forces on its surface (Singh et al., 2010a) and its high surface area and internal porosity aid this function (Laird et al., 2010). The effectiveness of the reduction in nutrient leaching depends on nutrient, soil and biochar type (Yao et al., 2012). The high cation exchange capacity (CEC) demonstrated by many biochars are also contributed to by the high specific surface area of biochar (Liang et al., 2006). Biochar is thought to be of most use with soils that are intrinsically poor and thought to have little benefit to rich high quality soils (Quilliam et al., 2012). There is a direct link between the quality of the feedstock for the biochar and the quality of the biochar. A low quality feedstock is likely to produce a low quality biochar, and there is the potential for the introduction of contaminants into the soil when using some biochars (Jeffery et al., 2015).

Studies with biochar produced from papermill waste trialled on two soil varieties showed an increase in N uptake in plants when biochar was applied and also in soil fertility due to the increase in pH when applied to acid soils (Van Zwieten et al., 2010). There was recognition by Van Zwieten as well as Yao that there needs to be clarification of which instances biochar can be beneficial to crops, dependent on the soil and crop type. On applying biochar produced from grinding charcoal made from oak *Quercus* spp and hickory *Carya* spp to soil columns Laird et al (2010) described that they reduced total N and total dissolved P leaching by 11% and 69%, respectively when measured in soil columns receiving swine manure along with a 20 g biochar/kg soil treatment (Laird et al., 2010). In 371 studies Biederman and Harpole (2013) found that the

crop yield along with soil microbial biomass, total nitrogen, phosphorus and potassium on average increased with the addition of biochar when compared to a control which received no biochar. In the 2013 paper it was suggested that the addition of biochar to crops has a positive impact on 'energy, carbon storage and ecosystem function' (Biederman and Harpole, 2013). One aspect of biochar for which there is little data is the effects of this recalcitrant material on downstream non-target environments.

2.3 Aims and objectives of the large scale arable crop trials

The aim of the work described in this chapter was to evaluate the effects of the application of a high nitrogen organic amendment in the form of chicken manure with and without biochar on the growth of arable crops (winter wheat and oilseed rape) and the quality of the soil after harvest. Chicken manure applied in a pelletised form was selected for use as the organic amendment given the ubiquitous use of chickens as a protein source, in eggs and meat and the high volume of litter available as a result. The university farm has a chicken unit on site conducting research with both broiler and laying breeds so there is the potential for this amendment to be available as a continued resource for crop production.

This aim will be met through the following objectives:

Objective 1: to assess the impact of a high nitrogen organic amendment, in this case chicken manure addition, on arable crops compared to mineral fertiliser in terms of crop yield.

Objective 2: To assess the effects of biochar as an additional amendment to the chicken manure in comparison to a mineral fertiliser used by itself by measuring crop yield from the experimental plots.

Objective 3: To assess the effects of the organic amendments on soil quality by carrying out chemical analysis of the soil.

2.4 Methodology

2.4.1 Location of the trials

The area selected for the large scale arable trials is part of a large research farm located in Tadcaster, West Yorkshire (lat. 53^{o} 86' long. -1^{o} 33' alt.55m), owned and operated by the University of Leeds. The farm in Tadcaster is used as a resource for Yorkshire wide projects from a variety of disciplines. The farm is host to projects in partnership with York and Sheffield universities for research into soil quality. The farm covers approximately eighty hectares and grows wheat, barley, potato, legume and maize crops. The farm also hosts animal science research on monogastric species such as chicken and pigs which generates a large amount of manure as a potential resource.

The primary soil type in the chosen fields is the Wothersome series. This soil type was selected in order to allow the planting of one or two different crops onto the same substrate to ensure that there was minimal variability in the results due to soil type. The soil maps of the farm show a great variation in soil type from field to field so choosing fields with the right crops and the same soil took some consideration. Details of the Wothersome series are given in Table 2.1; this gives an indication of the drainage and composition of the soil in the chosen fields, both important aspects when considering crop growth and success.

Table 2.1. Description of the Wothersome series soil type.

Soil Type	
Genetic Group	Brown Forest Soil – High Base Status
Parent Material	Boulder Clay derived from Magnesium Limestone and Red Marl
Drainage	Imperfect
Soil Horizons	
0 - 9”	Warm reddish brown heavy loam, cloddy, moderately compact few stones.
9 - 16”	Brownish-red sandy clay loam, weak prismatic structure, compact and tenacious, few stones.
16 - 30”	Red sandy clay to clay, prismatic structure, compact and tenacious very few stones

2.4.2 Field and plot set up, and treatments applied

The plots were located within fields that were being commercially grown and harvested with pesticides, herbicides and fertilisers applied when required. Pesticides and herbicides were applied to the experimental plots to mirror large scale food production as much as possible, the only difference in the treatments being the change of fertiliser. The dosing of the organic fertiliser was applied based on matching the N content of the inorganic fertiliser, with no additional nutrients taken into account. The plots were located in the centre of the tractor tramlines with a 6m buffer either side to neutralise any effect that may occur due to wind effects and spray from the standard fertiliser applied by the tractors to other crops in the area. Both fields were enclosed by hedges and the plots were located 18m from the nearest boundary hedge. A randomised complete block design (RCBD) was chosen; with the treatments randomised within each block. This reduces the amount of random variation by eliminating variation between blocks from any errors that might be incurred in comparing the

treatments (Mead et al., 2002). The stratification of the blocks allows the hedge boundary located 18m from the plots to be accounted for and any variations due to field slope.

Table 2.2 Details of the five crop treatments applied during the initial field trials

Treatment	*Description*
A	Standard ammonium nitrate fertiliser treatment
B	Replacement organic fertiliser in the form of pelletised organic chicken manure
C	Double dose of replacement organic fertiliser
D	Treatment B with the addition of biochar
E	Treatment C with the addition of biochar

There were six replicates of each of the treatments on each crop and within each RCBD block there was one of each of the treatments. Each plot measured 6 x 4m, this size was chosen to enable the research harvester with a 2m width to harvest through the centre of each plot. There was a 1m buffer between each set of plots to allow for effective harvesting and to prevent contamination from adjacent plots. The arrangement and management of the plots can be seen in Plate 2.1. The borders between the plots were maintained by cutting any wheat that grew in these areas using a petrol push along mower. This and the markers labelling each column of treatments enabled each plot to be managed effectively.

Plate 2.1 The set up and management of the plots in the field

The layout of the treatments in the field can be seen in Figure 2.2 with ten columns of plots and 3 rows. For reasons of simplicity the 1m borders between each column are not shown on Figure 2.2. The plots were marked out with canes and string in the first season of 2012. The exact locations of the outer post markers for the end of the plot area were identified using a theodolite working from two permanent markers before the posts were removed at harvest. This enabled the posts to be reinserted in the exact positions the following growth season using the two established permanent markers and the theodolite to accurately reposition the post markers.

Block 3	C_1	A_1	B_3	E_2	D_2	E_4	D_6	B_5	C_5	A_5
Block 2	C_2	B_2	E_1	A_3	D_3	D_4	E_5	C_4	B_6	A_6
Block 1	B_1	C_3	A_2	D_1	E_3	D_5	B_4	A_4	E_6	C_6

Figure 2.2 Plot layout of the treatments for the large scale arable trials arranged in 3 blocks. Letters indicate treatments, numbers indicate the replicates Treatments are as follows: A) Standard ammonium nitrate fertiliser treatment; B) Replacement organic fertiliser in the form of pelletised organic chicken manure; C) Double dose of replacement organic fertiliser; D) Treatment B with the addition of biochar; E) Treatment C with the addition of biochar.

In February 2013 the oil seed rape (OSR) plots showed extensive signs of damage due to rabbit grazing across the entire field in which the plots were located, a picture of this damage is shown in Plate 2.2. The subsequent recovery of the plants in April was rapid, with leaves and stems already regenerating with no visible signs of the previous damage. The implications of the rabbit grazing damage are thought to be minimal given that it occurred across the whole field crop and is a widespread and common problem within the UK agricultural industry.

Plate 2.2 Effect of rabbit grazing on the OSR crop over the 2012-2013 winter period

2.4.3 Crops, organic amendments and inorganic fertiliser used

The chosen crops for the 2012-2013 season were oilseed rape *Brassica napus* (OSR) and winter wheat *Triticum aestivum* (WW), the crop for the 2013-2014 season was WW. The crops chosen for the trials dominate the agricultural landscape in the UK; the latest statistics from DEFRA show 1.96 million hectares of wheat *Triticum spp* grown in the UK in 2011 and 705 thousand hectares of oilseed rape *B. napus* ((DEFRA, 2015a)). Wheat is the most abundant crop grown in the UK, with oilseed rape being third after barley. Wheat growth dropped to 1.93 million hectares in 2014 with OSR falling in consecutive years to 675 thousand hectares in 2014. Barley and wheat both

count as cereal crops so choosing OSR over barley gives more variation in the test crops. This makes OSR and wheat ideal crops to test as they are grown across a widespread area and this research could have an impact on how these common crops are grown in the UK.

The initial trials described were carried out using a locally available replacement enhanced organic N source in the form of dried chicken manure alone and in combination with commercially available biochar and were compared to the use of a traditional inorganic fertiliser.

The chemical attributes of the chicken manure used as an organic fertiliser were analysed prior to application in order that the correct nitrogen amount could be applied to the crop. The chemical analysis of the chicken manure used as a fertiliser is shown in Table 2.3. The additional nutrients present in the chicken manure such as Mg and Cu are not applied to the land in a mineral form. These nutrients are applied in traditional farming methods by combining manure into the soil prior to drilling in the seed.

Table 2.3 Table showing the chemical constituents of the raw organic chicken manure

Parameter	*Units*	*Value*
pH water (1:2.5)		8.74
EC	µS/cm	5830
Total Phosphorus	% w/w	1.53
Total Potassium	% w/w	2.38
Total Magnesium	% w/w	0.697
Nitrate Nitrogen (fresh)	mg/kg	13.3
Ammonium Nitrogen (fresh)	mg/kg	861
Total Nitrogen	% w/w	4.48
Total Sulphur	% w/w	0.51
Total Copper	mg/kg	73
Total Zinc	mg/kg	461
Total Sodium	% w/w	0.447
Total Calcium	mg/kg	80340
Total Phenols (index)	mg/kg	<1
Dry Matter (fresh)	%	89.9

2.4.4 Application of organic amendments and inorganic fertiliser

The treatments were all applied by hand. The manual application was completed by weighing out the correct amount of each treatment for each plot and then applying this by hand through walking transects up and down the 24m^2 plots. The spread of the treatments was controlled by walking the same speed and with the same width transect in each plot. The inorganic fertiliser was applied following consultation with the guideline documents produced by the Home Grown Cereals Authority (HGCA) (HGCA, 2008, HGCA, 2012) on both wheat and oilseed rape and using the Fertiliser Manual RB209 produced by the

Department for Environment, Food and Rural Affairs (DEFRA). The Fertiliser Manual (DEFRA, 2010) requires that the soil type be classified to estimate the soil nitrogen supply (SNS) prior to fertiliser being applied. The SNS is worked out using Equation 1.

$$Soil\ Nitrogen\ Supply\ (SNS)\ =\ SMN\ +\ estimate\ of\ nitrogen\ already\ in\ the\ crop\ +\ estimate\ of\ mineralisable\ soil\ nitrogen$$

Equation 1 Soil nitrogen supply SMN = soil mineral nitrogen.

The amount of fertiliser required based on the SNS can be worked out using the field assessment method whereby the nitrogen requirements are classified according to the type of soil, the previous crop grown on the land, and annual rainfall in the area. This information was supplemented by local information from the local land agent and farmer who manages the land where the trials took place. Details of applications and dates are given in Table 2.4

Table 2.4 Details of applications for the large scale arable trials from 2012 – 2014

Date	*Crop*	*Fertiliser application nitrogen rate (kg/ha)*	*Action description*
21.08.2012	OSR		OSR planted
19.09.2012	OSR	30	
05.10.2012	WW		Biochar at 8.3MKg/ha*
08.10.2012	WW		WW planted
30.10.2012	OSR		Biochar at 10MKg/ha
21.02.2013	OSR	40	
25.02.2013	WW	40	
17.10.2013	WW		WW planted
16.04.2014	WW	40	
17.04.2014	WW		Biochar at10Mkg/ha

*rate of biochar application was intended to be 10MKg/ha, however supply meant that this was the rate for the first application

The biochar used for these trials was a commercially available biochar produced by Carbon Gold, with a feedstock of predominantly green waste. The analysis for the biochar is given in Table 2.5. The table shows that the biochar has high levels of calcium and potassium. The biochar was applied as a top dressing on the soil for OSR in 2013 and WW in 2014. The biochar was applied prior to seed drilling on the WW in 2013 so was incorporated into the soil. The growing season for OSR starts earlier than that for WW so in 2012 the crop had already been drilled prior to the trials starting, only enabling the biochar to be applied as a top dressing. Given the positive results of top dressing on the OSR in the 2013 harvest (results given in 2.5.1.1) this was repeated on the in 2013 for the sowing of the WW

Table 2.5 Characterisation of the biochar from Carbon Gold

Parameter	*Concentration (mg/kg)*
P	1371.46
K	8084.33
Ca	40347.73
Mg	2034.79
Na	1842.98
S	1546.75
Fe	2836.24
Cu	26.73
Mn	10.61
Zn	242.92
Mo	3.87
B	24.32
Cd	0.00
Cr	8.62
Ni	10.59
Pb	23.09
Al	1706.52

2.4.5 Soil and plant sampling and analysis

Chlorophyll readings were taken on a weekly basis throughout the growing season up until harvest using a Minolta soil plant analysis development (SPAD) meter. Chlorophyll is a useful measure of N uptake in plants, as there is a close correlation between leaf N and chlorophyll since most leaf N is contained within chlorophyll molecules. The SPAD meter produces a value that is proportional to the amount of chlorophyll in the leaf. The leaves for chlorophyll measurements were selected and sampled in a ‘W’ pattern with five readings taken from different plants within each plot. Sampling in this ‘W’ pattern is a good way of sampling across a plot. Using this sampling method and without marking any leaves for weekly monitoring it is likely that no leaf was measured twice for chlorophyll due to the random selected process within the ‘W’ shaped sampling pattern.

Soil samples were taken towards the end of the season before harvest. These were pooled samples taken from 5 cores sampled to 150mm using a 200mm x 20mm soil corer and the soil stored in ziplock bags which were then kept refrigerated. These samples were taken from across each plot and sampled in a similar way to the chlorophyll measurements.

Samples of grain and seed were harvested using a 2m wide combined harvester with the weight of yield per 12m^2 harvested measured by the machine. Samples of the OSR seeds in 2013 were kept for further laboratory analysis for dry matter and oil and glucosinolate content. The dry weight of seed is used as an indicator of seed quality as the greater the storage of dry matter within the seed, the greater the success of germination and seedling emergence (Moshatati and Gharineh, 2012). The water content of seeds also has impacts on the length of time that seed can be stored for without degenerating (Rao et al., 2006). The oil value gives a quality indicator for the OSR crop, high oil content is desirable for producers and for quality the glucosinolates value should be low. Glucosinolates are compounds contained in pungent mustard type plants such as OSR and contain sulphur and nitrogen derived from amino acids and glucose. The rapeseed meal produced from crushing the seeds for oil is sold on for animal feed and this is the main reason why the levels of glucosinolate present need to be less than 18mmol, the higher the level of glucosinolates, the more bitter the taste and the more unsuitable it is for recycling into animal feed.

In addition to these crop measurements, in 2014 five plants were selected at random from the plots using the line intercept sampling method. In this method a 500mm cane was thrown at random into each plot and the closest five stems of winter wheat were selected for analysis. Each stem was carefully removed by hand and stored in a ziplock bag. These plants were assessed in greater detail, with the plant height, spike length, number of leaves, number of nodes, and number of grains counted individually for each plant. The fresh and dry weight of the plants and grains were also assessed. The grains were counted using a Numigral grain counter.

2.4.6 Analysis methods

2.4.6.1 Dry weight

The dry weight of the WW and OSR were calculated in the lab by weighing the samples in small crucibles then drying them in a drying oven for a minimum period of 24 hours before being weighed again to calculate the water lost. In

2014 the stems sampled using the line sampling method were also measured for height, number of leaves, number of nodes, spike length and number of grains.

2.4.6.2 pH and EC

The pH and EC of the soil samples were analysed by mixing 3g of soil in 30ml of water. This solution was then agitated on a mechanical shaker for a minimum of 30 minutes to ensure thorough mixing and full suspension of the sample before the pH and EC were measured using a HACH HQ with an EC probe and a pH probe attached.

2.4.6.3 Analysis of ions

The nutrient analysis of anions and cations present in the samples was completed using a Metronohm Ion Chromatograph or the IC. After taking measurements for pH and EC the suspended samples were placed in an Eppendorf 5810 centrifuge and spun at 4000rpm for 20 minutes to separate the solid fraction. The supernatant was removed and retained and the precipitate was discarded. The supernatant was then filtered through a 42µm syringe filter to enable the sample to be processed using the IC. For the compost samples which had not fully separated following centrifuging they were filtered using a standard filter paper and conical flask, and then filtered with a 42µm syringe filter.

The aqueous samples were then diluted down to 1:10000 and analysed using the IC to give a preliminary analysis of the ions present. Following that the samples were tested at 1:1000, 1:100 and for the samples whose values were still low enough at those dilutions there were diluted at 1:10 and put through the IC again. Samples whose predicted ion content would exceed a maximum of 30mg/l were not analysed at the next level of dilution.

The IC tested for sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg) cations and for fluoride (F), chloride (Cl), bromide (Br), nitrate (NO3), phosphate (PO4) and sulphate (SO4) anions.

2.4.6.4 TKN

The TKN analysis was completed using acid digestion. This was done with 1g of sample in 50ml of distilled water in a flask. To this a copper catalyst tablet was added along with some glass beads to prevent flash boiling and finally 10ml concentrated H_2SO_4 was added to each tube. This was placed in a Buchi digester block for approximately 2 hours until all the samples had been digested and they were a straw or greenish colour. The samples were allowed to cool prior to distillation. The samples were distilled in a Buchi K-360 distiller using NaOH with 50ml of indicating boric acid in a duran bottle for the sample to be precipitated into.

The distilled sample in the indicating boric acid solution was then placed at the bottom of a burette containing 10mM H_2SO_4. The sample was titrated until the colour of the boric acid solution returned indicating the endpoint.

2.4.6.5 Ammonia

The methodology for the ammonia analysis is similar as per the TKN analysis but without the acid digestion of the sample prior to distillation. For this 1g of sample was placed in 50ml of distilled water and placed in the Buchi K-360 distiller. The sample was processed and the precipitate dripped into a duran bottle of indicating boric acid solution. The duran bottle following distillation was placed under the burette with 10mM H_2SO_4 in and titrated to its endpoint.

2.4.6.6 Biological oxygen demand analysis

To calculate the biochemical oxygen demand (BOD) of the samples they were analysed using the OxiTop method. Using this equipment the BOD was measured over a 5 day period to give a reading for a BOD5. For this method 20g of sample was placed in the WTW OxiTop flask with 5ml of water to dampen the sample and a small beaker of 30ml NaOH in the top of the flask close to the seal and measuring head. This NaOH absorbs the CO2 produced through the decomposition of the organic matter and changes gradually into a weak acid solution. The BOD measurement is based on a pressure change in the flask which decreases over time due to the oxygen being used up by the decomposition taking place. Each flask was placed in an incubator at 20oC and left for 5 days. The measurement head on the flask was set to measure the

change in pressure every twenty minutes therefore during the five days 360 points were recorded for each sample.

The BOD of the samples was calculated using a manometric respirometric test using the OxiTop® (WTW) method. For this method 20g of sample was placed in the 2.5L OxiTop flask with 5ml of water to dampen the sample and a small beaker of NaOH in the top of the flask close to the seal and measuring head. This NaOH absorbs the CO_2 produced through the decomposition of the organic matter and changes gradually into a weak acid solution. The BOD measurement is based on a change in pressure within the flask which decreases over time due to the oxygen being used up by the decomposition of the organic matter taking place. Each flask was placed in an incubator at 20^{o}C and left for 24 hours. A reading from the measuring heads was taken after 24 hours, and then the heads were reset to run for another 24 hours giving 48 hours total decomposition time. The measurement head on the flask was set to measure the change every four minutes giving data 360 points were recorded for each sample over each 24 hour period. The amount of consumed oxygen is calculated by using the following equation

$$RA = \Delta p \times \frac{MO2}{R \times T} \times \frac{V_{ges} - Vabs - Vsample}{msample}$$

Where:

Δp is the difference in pressure [hPa];

M_{O2} the molecular mass

O_2 [=31,998 mg _ mol_{-1}];

R the ideal gas constant [=83,140 ml _ hPa _ (K _ mol)$_{-1}$];

T the temperature in Kelvin [=293.15 K];

V_{ges} the total volume [=2500 ml]; V

$_{abs}$ the volume of medium of absorbance [ml];

V_{sample} the volume sample [ml] and
$m_{sample,dry}$ is the dry mass of sample [g DM] (Binner et al., 2012).

2.4.6.7 Cation exchange capacity (CEC)

The cation exchange capacity (CEC) of the sample was analysed using an atomic absorption spectrophotometer. Methods were adapted from those given in Gaskin (2008) given the type of samples (Gaskin et al., 2008).

A 1g sample was added to 20ml of ultra-high quality (UHQ) water in a falcon tube and placed in a shaker for 15 minutes at 22oC at 130rpm. The samples were then centrifuged at 4000rpm for 8 minutes, following this the supernatant was discarded and the sample rehydrated with 20ml UHQ water. This was repeated five times. The next step, after the fifth time of the supernatant being discarded 10ml of sodium acetate was added. The pH of this sodium acetate had been adjusted to pH7 using concentrated acetic acid. This sample was placed in a shaker for 5 minutes at 22^{o}C at 130rpm, then centrifuged for 8 minutes at 4000rpm. The supernatant was discarded and the process repeated 3 times. The next step after the third time of discarding the supernatant 10ml of ethanol was added, placed in a shaker bath for 15 minutes at 22oC at 130rmpm then centrifuged at 4000rpm for 8 minutes. This was repeated 3 times. The final stage was to add 10ml of pH adjusted sodium acetate, place in a shaker for 5 minutes at 22oC at 130rpm, and then centrifuged for 8 minutes at 4000rpm. In this last step the supernatant was kept in a separate container to be kept. This process was repeated three times to end with 30ml of solution.

Before analysis in the AAS the samples needed to be diluted. This was completed using a potassium solution using 10ml of sample with 10ml 2000ppm K+ solution made up to 100ml with UHQ water.

2.5 Results of the large scale crop trials carried out in 2013

2.5.1 Oilseed rape

2.5.1.1 Oilseed rape yield from the experimental plots

The overall yield data can be seen in Figure 2.3 and this data was taken from the weight of seed harvested from a 12m^2 area. The table shows the mean yield data for each treatment and the standard deviation obtained from the six replicates for each treatment. The table also shows the values for the expected values of grain in MKg per hectare calculated from the yield recorded. These can be compared to the mean OSR yield from the University farm in the same

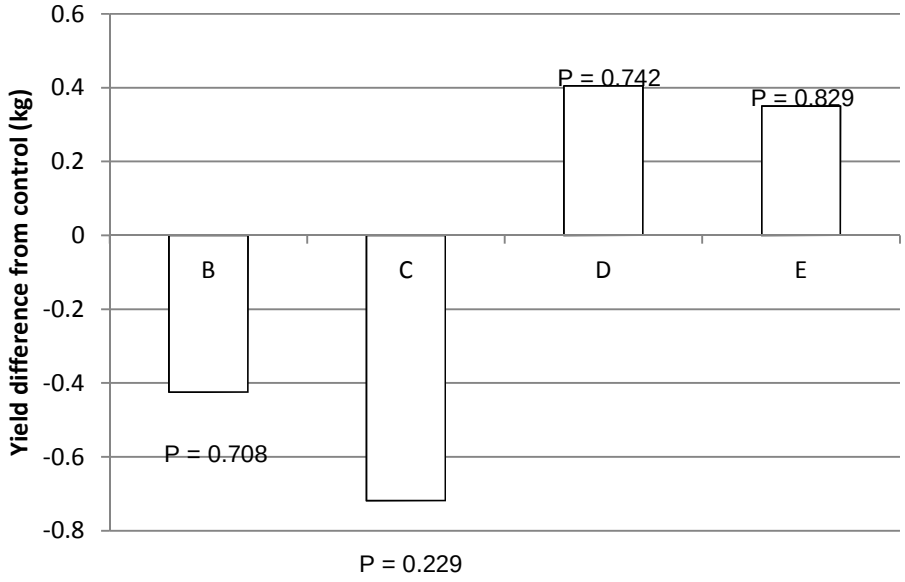
year which for 2013 was 4.9MKg/ha. This can be considered to be the optimal growth conditions for the OSR crop. The graph shows the difference of each of the treatments B-D and their variance from the control. Overall from the table it can be seen that yields varied from a low of 3.86 kg/12m^2 up to 4.98 kg/12m^2. The table shows that there was a difference in the treatments in terms of the yield compared to the control with some treatments yielding more and some treatments yielding less than the control treatment. Overall the yields per hectare obtained from the experimental plots were generally lower than the overall yield from the University farm the same year. This suggests that the conditions in this trials were poorer for the OSR crop than for farm yield. Only treatments D and E were comparable to the yields obtained on the farm. This could be due to an effect of scale as on a smaller plot size there may be less efficiency over a smaller area that can be minimised by a larger harvester.

Figure 2.3 shows that treatment C (double dose of chicken manure) showed the lowest yield of all the treatments for 2013 with only 3.86 kg/12m^2 or 3.22 MKg/ha. In comparison treatment E which is the same dose of chicken manure with biochar added produced a yield of 4.92 kg/12m^2 or 4.11 MKg/ha. In comparison to the control treatment (ammonium nitrate fertiliser) treatment C showed a yield of 0.71 kg less that the control treatment and when biochar was added (treatment E) the yield was 0.35kg higher than the control treatment.

The treatments using a comparable chicken manure dose without and with biochar (treatments B and D respectively) showed a similar trend with lower yields in comparison to the control when no biochar was added and yields higher than the control treatment when biochar was added.

The yield data was assessed as being normally distributed with a Shapiro-Wilk p-value of > 0.05 for all treatments. The yield data from the plots for 2013 showed that treatments D and E with the biochar addition had significantly higher yields than treatment C and B with values of 0.001 in both cases for treatment C and 0.012 and 0.020 respectively for treatment B in a Tukey's test where $p < 0.05$. This would suggest that the biochar addition to these plots improved the yield for oilseed rape in 2013.

By comparing the yields of the crops by block as well as by treatment it is possible to see if there is any effect of the hill slope on yield. The yield between block 1 and block 2 and 3 (both 0.001) are significantly different with the yield being significantly higher in row 1 in both cases. This could be due to a variety of factors. Row 1 in the OSR field was at the top of the slope in the experiment, and was also the closest to the field boundary hedge. Hedges act like sinks that can provide beneficial effects through storage of additional nutrients, harbouring natural enemies for agricultural pests (Bianchi et al., 2006) and can act as a windbreak against weather effects providing a warmer and more humid climate closer to the hedge (Cleugh, 1998). The fertiliser effects on the OSR crop of the chicken manure is the same as the inorganic fertiliser.



a

Treatment	Mean (kg/12m^2)	Standard deviation	Predicted mean (MKg/ha)
A	4.57	0.59	3.82
B	4.15	0.58	3.46
C	3.86	0.75	3.22
D	4.98	0.37	4.15
E	4.92	0.51	4.11

b

Figure 2.3 The mean average yield and standard deviation for the treatments on the OSR are shown and the bar graph demonstrates the difference between the control (Treatment A) and each of the trial treatments with the P value for this difference shown adjacent to each bar.

2.5.1.2 Dry weight of oilseed rape seed produced

Figure 2.4 shows the results for the percentage dry weight of oilseed rape seeds produced in 2013 for the different treatments used. The photo in Plate 2.3 shows the OSR seeds after being oven dried.

Plate 2.3 Photo showing the OSR seeds after oven drying

There is a significant difference in the dry weight of the seeds between treatments A (seed dry weight 95%) and C (seed dry weight 90%) with a value of 0.044 in a Tukey's test where $p < 0.05$. Given that there are no other patterns in the difference in seed dry weight and the larger standard deviation for treatment C it is likely that this difference is due to experimental error. Two of the replicates for treatment C have much lower values for seed dry matter at 82% and 86%, if these results are discarded there are no longer any significant differences in seed dry weight.

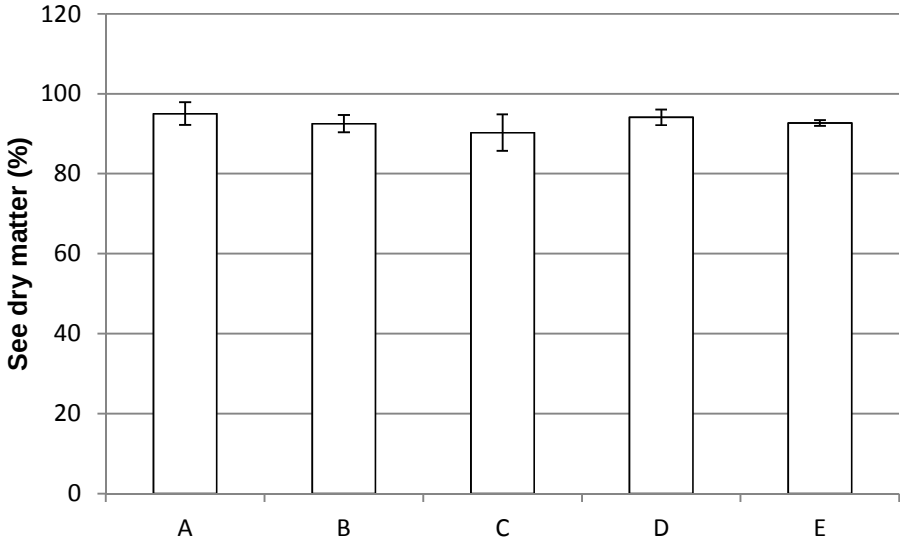


Figure 2.4 The OSR seed dry matter content for all treatments with the standard deviation for each indicated by an error bar.

2.5.1.3 Oil and Glucosinolates content of the oilseed rape seeds

Samples of the OSR seeds were sent to the National Institute for Agricultural Botany (NIAB) laboratories to be analysed for oil and glucosinolate content.

The results from the oil analysis are shown in Figure 2.5. The figure shows that there was very little variation in the oil content for the different treatments and overall it ranged from 51.08 to 51.75 g/100g. As expected for the oil content an ANOVA analysis using a Tukey's test showed no significant differences between treatments for the amount of oil produced from the seeds when $p < 0.05$.

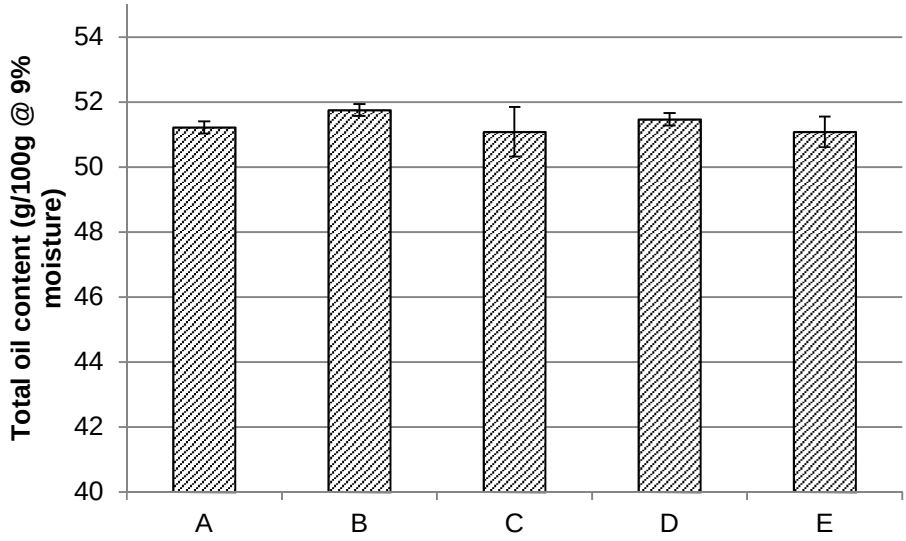


Figure 2.5 Average total oil content in the OSR seeds with error bars showing standard deviation

The glucosinolate content in the OSR seeds was measured and the results are shown in Figure 2.6. Once again there appears to be very little difference between the glucosinolate content of the seeds for the different treatments. The results using the Tukey's test show no significant difference between treatments for glucosinolate levels in the seeds. Levels of glucosinolates in all of the treatments were below 18mmol and the oil from seed pressing would therefore be suitable for recycling into animal feed.

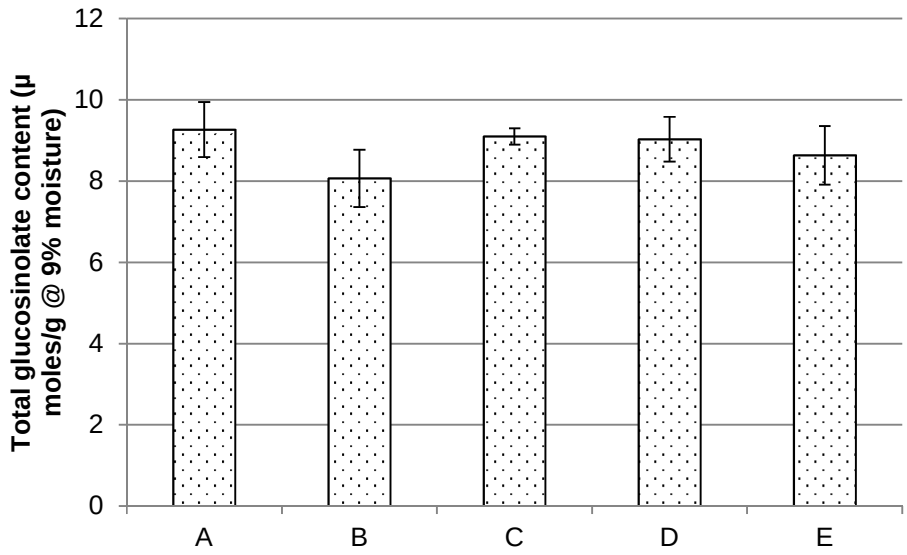


Figure 2.6 Average total glucosinolate content in the OSR crop (µ moles/g) from 2013

2.5.1.4 Leaf chlorophyll content

Figure 2.7 shows the concentration of chlorophyll in the leaves of the oilseed rape plants measured at intervals throughout the growing season. It can be seen that regardless of the treatment used the chlorophyll concentrations initially increase over the first 10 days from around 45-50 SPAD units and then drop steadily over the next 2 months to around 25-30 SPAD units before remaining stable over the last couple of weeks. The trend throughout the season for all of the treatments was a falling level of chlorophyll, representing a falling level of leaf nitrogen. This occurs through the growing season as accumulated nitrogen in the plant, mainly the leaf and stem, is redistributed into the pod walls, and eventually the seeds (Schjoerring et al., 1995, Ogunlela et al., 1989)

Comparison between treatments shows that there were no significant differences between treatments in terms of the leaf chlorophyll measurements from the dates surveyed throughout the season. The comparisons made between the treatments on the same date all had p values greater than 0.05.

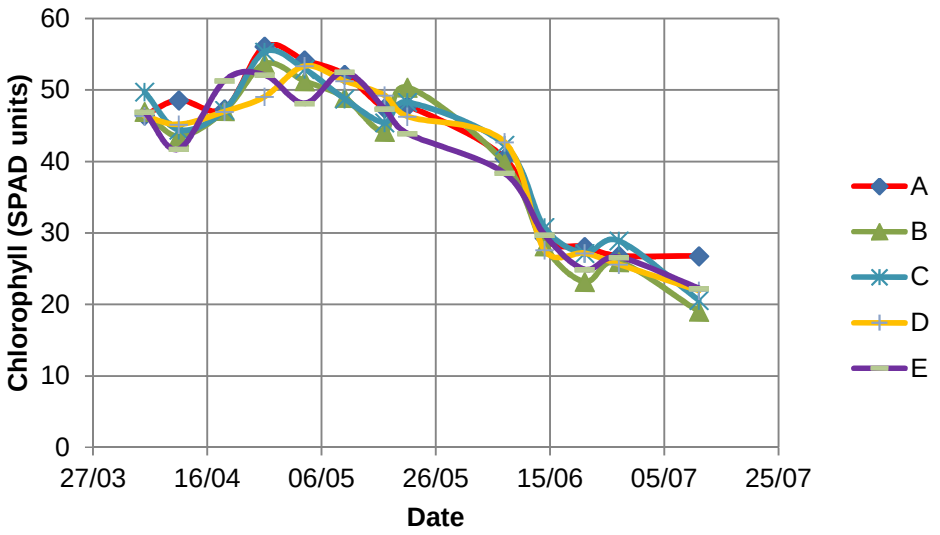


Figure 2.7 Chlorophyll levels in the leaves in the OSR throughout the 2013 growing season

2.5.1.5 Soil quality

The soil samples from these trials were sent to the laboratories at Natural Resource Management (NRM) for chemical analysis. The results are shown in Table 2.6 as averages of the samples sent for analysis. Two pooled samples, each consisting of three of the replicates were submitted for analysis.
Table 2.6 shows the results from the laboratory tests carried out on the soil samples before harvest had taken place. The range of values for the pH goes from a low of 7.25 up to 7.65 with no trends shown in data relating to treatment type. The pH values from the soil tested for the plots in 2013 are not significantly different from one another when compared using a Tukey's test where $p < 0.05$. This shows that the chicken manure and inorganic fertiliser had no different impacts from each other when applied to the soil and that the biochar similarly did not impact on soil pH.

The element showing the least amount of variation between treatments in this analysis was Mg. Treatments A, B and D all have the same values of 333.5 mg/l with treatments C and E not varying greatly from this median. The values for nitrogen show that the nitrates and ammonium nitrogen vary between treatments, however the value for total nitrogen shows that treatments C, D and

E have very similar levels of nitrogen present. Treatment A has the highest level of nitrogen shown in the soil sample.

Table 2.6 Soil analysis for the soil tested at the end of the growing season in OSR in 2013

Analysis	*Units*	*Treatment*				
		A	B	C	D	E
pH water (1:2.5)	-	7.55	7.25	7.5	7.65	7.58
Available Phosphorus (index)	mg/l	39.7	41.5	39.7	45.5	42.6
Available Potassium (index)	mg/l	172	182	194	205.5	199.75
Available Magnesium (index)	mg/l	333.5	333.5	328	335.5	331.75
Nitrate Nitrogen (fresh)	mg/kg	13.25	12.15	10.75	9.9	10.32
Ammonium Nitrogen (fresh)	mg/kg	2.13	1.51	1.46	2.41	1.93
Total Nitrogen	mg/kg	15.3	13.66	12.2	12.31	12.25
Dry Matter (fresh)	%	89.2	88.8	89.25	88.45	88.85

The results for the phosphorus content of the soil show a pattern of higher phosphorus in treatments D and E (45.5 and 42.6 mg/l respectively) which were the treatments with the 10 Mkg/ha addition of biochar. This correlates with a higher yield in both of these treatments. Treatments B and D and treatments C and E have the same application rate of manure as each other respectively. Table 2.6 shows that both treatments with a double dose of manure applied have a decreased amount of P in the soil. This result is misleading as when the raw data from the pooled samples sent for analysis are looked at it shows that the variation in the data for phosphorus for both treatments B and E is greater

than the standard deviation for C and D respectively. This can be interpreted as a misleading result due to too few samples being analysed. The values for B and C range from 39.8-43.2 mg/l and 39 – 40.4 mg/l and the values for D and E range from 44.6 – 46.4 mg/l and 42.4 – 46.6 mg/l respectively.

The potassium levels in the soil show the greatest trend from the soil data, there is an increasing pattern of K levels in the treatments from A-D, and reducing again to treatment E. Chicken manure is rich in K and this is shown in treatments B-E having higher levels of K than treatment A which only received mineral fertiliser. Treatment C received a double dose loading of chicken manure which gives a reason for the higher soils concentrations observed in C compared to B. Treatments D and E both have higher levels of K than treatments B and C despite having the same levels of manure applied respectively. This could be related to the biochar addition and its capabilities for retaining nutrients in the soil and making them more available for plant uptake.

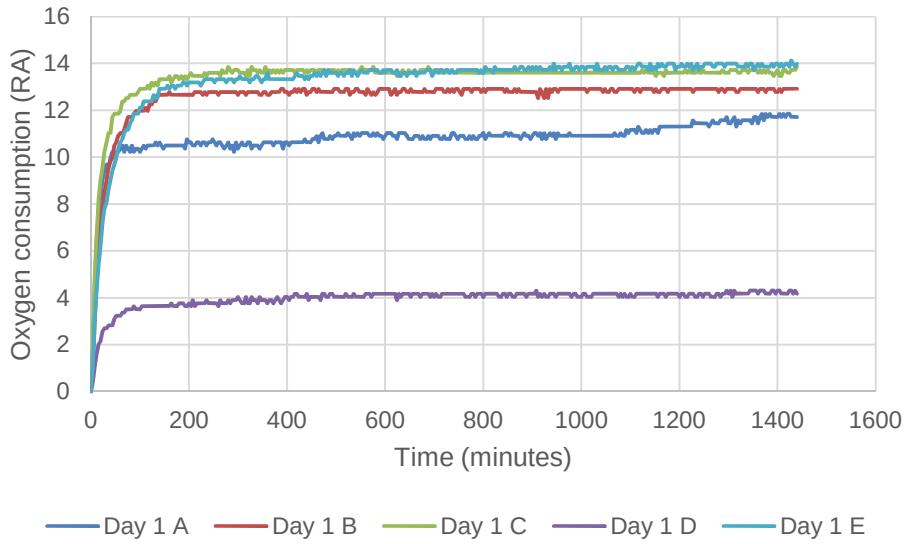
As not enough data was collected for the soil analysis, it is not possible to carry out any statistical analyses on the results from the 2013 fieldwork.

2.5.1.6 Biochemical oxygen demand (BOD)

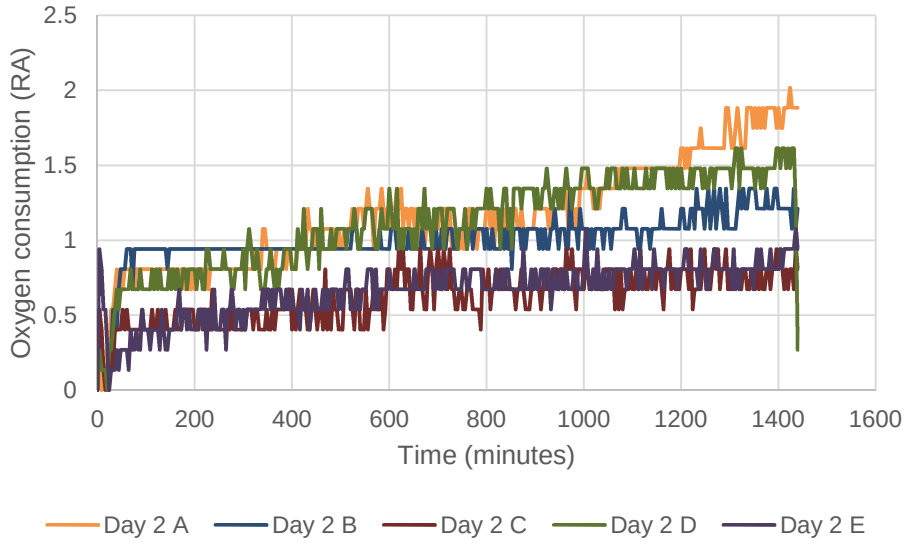
The BOD of a sample is an indication of the activity in the sample based on the microbial activity to break down nutrients such as carbohydrates and is described as the rate of biochemical oxidation of organic matter (Connell, 2005). The results for the soil BOD analysis are shown in Figure 2.8a and b. Figure 2.8a shows the oxygen uptake over the first 24 hours of the test and Figure 2.8b shows the oxygen uptake over the second 24 hour period.

The BOD for the first 24 hour period in the glass flasks shows an initial stage of rapid oxygen consumption followed by levelling off for all of the treatments. Treatments C and E both with a double dose application of chicken manure showed the highest BOD of all of the flasks with treatment D being the lowest. The second 24 hour period (Figure 2.8b) shows greatly reduced oxygen consumption measured within the flasks with not a great difference in the value between any of the treatments. For this period treatments C and E showed the lowest oxygen consumption of all the flasks. Nutrient metabolism and microbial

activity is greatest on the first day due to availability of both nutrients and oxygen within the sealed flask. By the second day the nutrients have been used up so the oxygen consumption of the sample is lower.



a



b

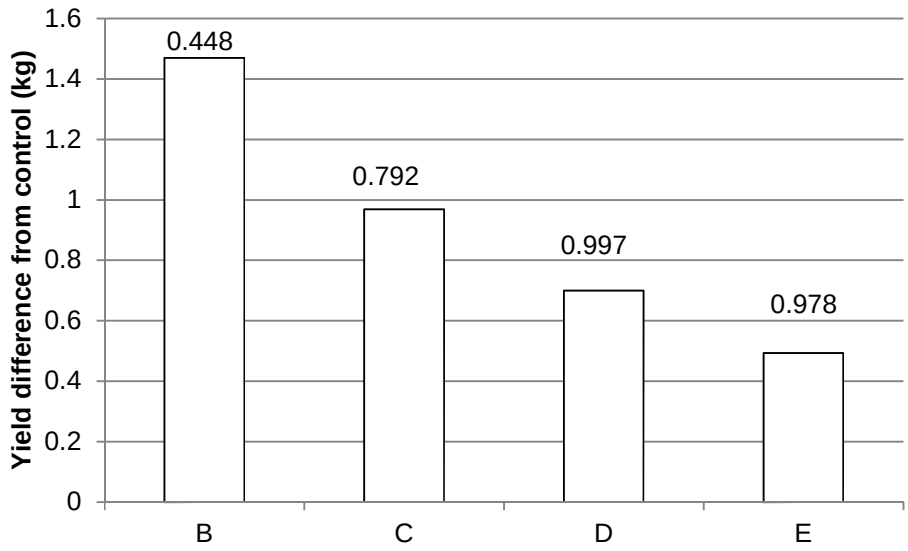
Figure 2.8 Graphs showing the oxygen uptake of the soil samples with the OxiTop flask over two 24 hour periods, a) shows the first 24 hours, and b) shows the second 24 hour period

2.5.2 Winter wheat

2.5.2.1 Winter wheat yield

The graph in Figure 2.9a and b shows the average mean yield for winter wheat for each treatment in 2013. This is for the $12m^2$ that was harvested for each of the six replicates that make up the average value for each treatment. The table shows the mean yield data for each treatment and the standard deviation obtained from the six replicates for each treatment. The table also shows the values for the expected values of grain in MKg per hectare calculated from the yield recorded. For a comparison between the trial plots and the wider farm yield from the soil using modern farming methods the average yield from the plots can be transformed from $Kg/12m^2$ to MKg/hectare. The average yield of winter wheat across the farm acreage in 2013 was 9.5MKg per hectare. This can be considered to be the optimal yield for the WW crop for the farm. In comparison to this value all of the treatments applied as part of these trials gave a higher value. The graph shows the difference of each of the treatments B-E and their variance from the control. Overall from the table it can be seen that yields varied from a low of $14.23kg/12m^2$ up to $15.7kg/12m^2$. The graph shows that there was a difference in the treatments in terms of the yield compared to the control with some treatments yielding more and some treatments yielding less than the control treatment. The data was shown to be normally distributed when analysed using a Shapiro-Wilk test. When compared using a Tukey's test there were no significant differences in yield between treatments when compared treatment to treatment. There is no significant difference for the WW yield between blocks as they were laid out in rows in the field. This trend is likely to be due to the WW plots being on level ground. The WW in the trials performed better than the optimal yield for WW on the university farm.

This is a contrast to the results for the same treatments on the OSR when both treatments D and E with the biochar addition showed significantly higher yields than the other treatments. The fertiliser effect of the chicken manure in this case works as well as the inorganic fertiliser.



a

Treatment	Mean (kg/12m^2)	Standard deviation	Predicted mean (MKg/ha)
A	14.23	1.17	11.86
B	15.70	2.03	13.08
C	15.20	1.72	12.67
D	14.93	1.39	12.45
E	14.73	1.29	12.27

b

Figure 2.9 The mean average yield and standard deviation for the treatments on the WW are shown in b) and a) the bar graph demonstrates the difference between the control (Treatment A) and each of the trial treatments with the P value for this difference shown adjacent to each bar.

2.5.2.2 Dry weight of the winter wheat grain produced

The grain dry weight was calculated for the WW grain and they are shown in Figure 2.10. A photo of the dried grain is shown in Plate 2.4.

Plate 2.4 The WW grain after being oven dried

Treatments A, B, C and D showed no significant differences between their dry weights. Treatment E showed to be significantly different from all other treatments when compared in a univariate analysis in a Tukey's test where $p < 0.05$. The reasons for treatment E being significantly different from all the other treatments, and consequently higher water content in the grain are not known although could be due to experimental error.

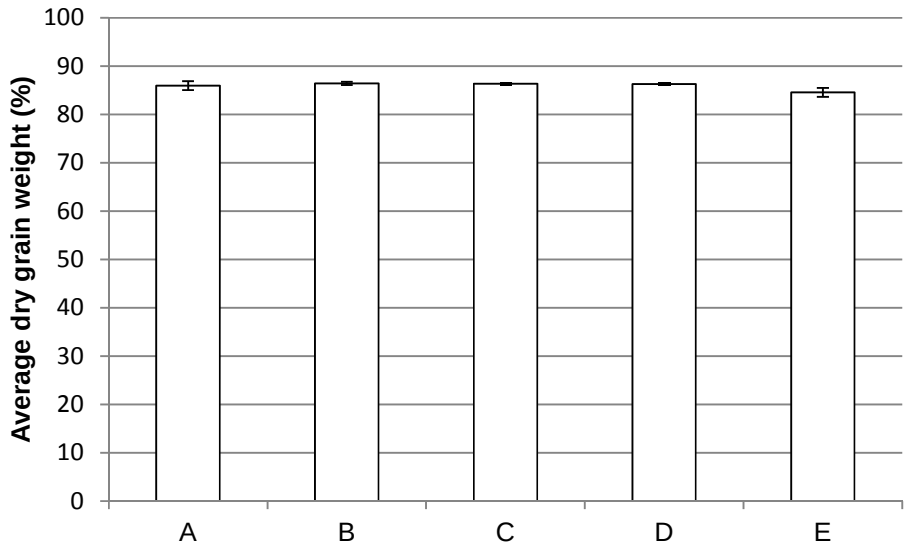


Figure 2.10 Boxplot showing the differences in dry weight of grain following harvest

2.5.2.3 Leaf chlorophyll content

The line graph in Figure 2.11 shows the chlorophyll levels in the leaves of the WW throughout the growing season in 2013. The trend in the chlorophyll in the leaves for the WW throughout the growing seasons was for steady growth through most of the growing period and then a sharp decline towards the end of the season (Figure 2.11). This is when the grain filling is taking place in the final growth stages of the plant when the grains gain the most protein and the leaves start to die back (HGCA, 2008). The range at the start of the measuring period was from 35 – 43 SPAD units and the range at the end was between 17 – 25 SPAD units. There was no significant difference in the chlorophyll levels in the leaves between the treatments.

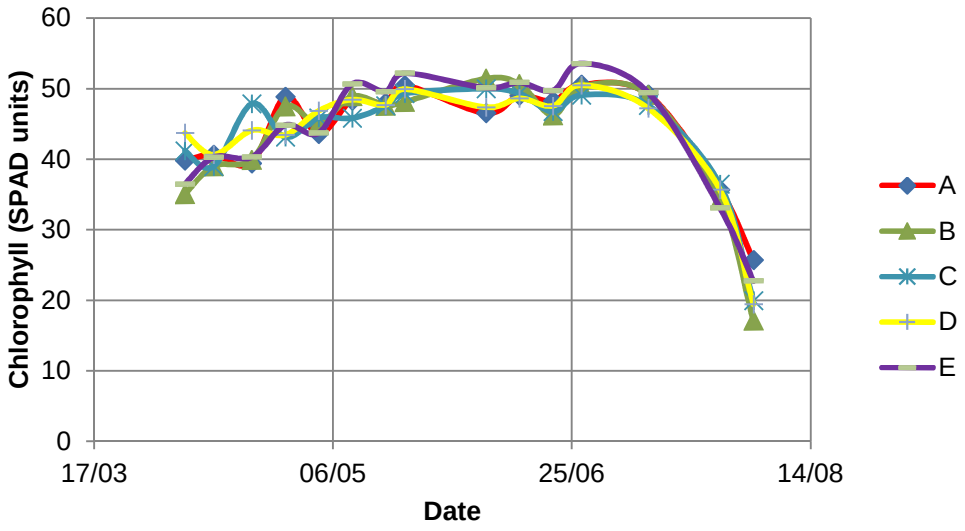


Figure 2.11 Mean chlorophyll levels in the leaves in the WW throughout the 2013 growing season

2.5.2.4 Soil quality

The pH results from the plots reveal that there is no significant difference in the pH between treatments when using a Tukey's test with a p value of 0.05.This shows that the treatments had no significant impact on soil pH when compared with a mineral fertiliser and each other.

Two pooled samples for each treatment were sent off the NRM laboratories for further chemical analysis. It was not possible to send a representative sample from treatment E in the winter wheat trial to the laboratory for further analysis due to sample loss. The results for this analysis are shown in Table 2.7.

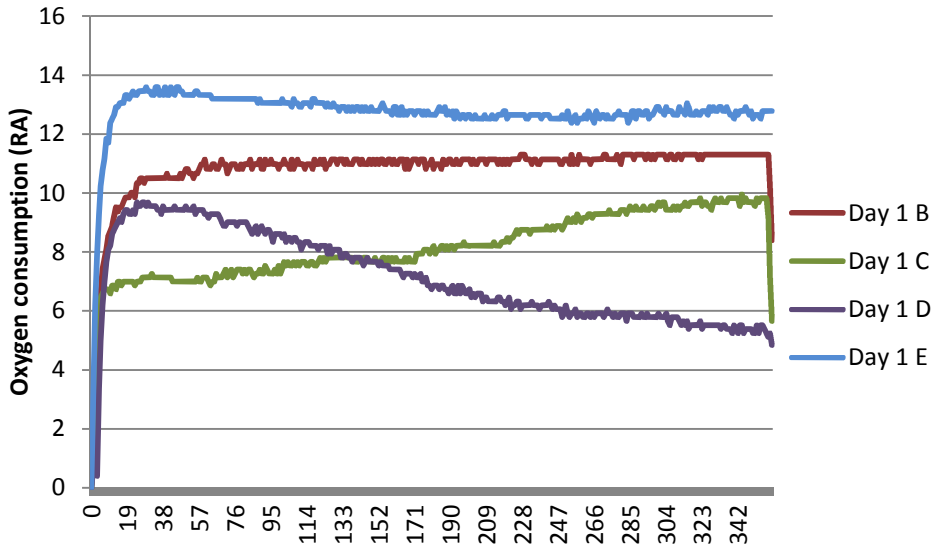
Table 2.7 Soil chemical analysis for the WW plots at the end of the 2013 season

	Treatment				
	Units	A	B	C	D
pH water (1:2.5)		6.95	7.2	6.9	7.5
Available Phosphorus (index)	mg/l	37.9	46.2	48	47.9
Available Potassium (index)	mg/l	183.5	207	214	206.5
Available Magnesium (index)	mg/l	326.5	336	333.5	342
Nitrate Nitrogen (fresh)	mg/kg	9.48	14.65	15.5	9.955
Ammonium Nitrogen (fresh)	mg/kg	1.465	1.83	2.11	1.83
Total Nitrogen	mg/kg	10.945	16.48	17.61	11.785
Dry Matter (fresh)	%	87.05	87.25	87.1	85.95

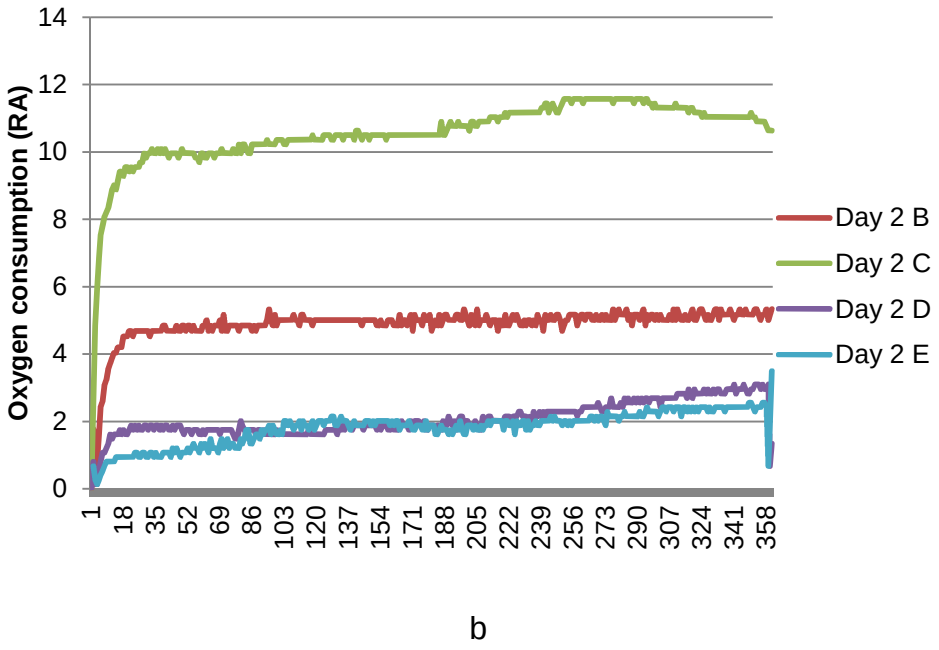
The levels of P in the samples are higher in the three treatments that received chicken manure as a fertiliser. This is due to the relatively high levels of P in the chicken manure, 1.53% P on a dry weight basis. The levels of K show a similar pattern with the chicken manure fertiliser containing 2.38% K. The pattern is the same for all the nutrients included in the analysis of the soil post-harvest with greater values in the treatments that received chicken manure as a replacement fertiliser.

2.5.2.5 Biochemical oxygen demand (BOD)

The methodology for the BOD was as in section 2.5.1.6 for treatments B-E. Treatment A was subject to a longer incubation period and a BOD_5 (test carried out over a 5 day period – this is standard practice with wastewater samples) was determined for this sample. The results for the OxiTop are shown in Figure 2.12a) and b) show similar varying patterns on both days with treatment D having low oxygen consumption on both days. There is a reasonable amount of variation within the OxiTop data between the treatments. It would be expected that treatments B-E would have a higher BOD than treatment A given the increased organic matter applied to the soil. However the consumption of oxygen by soil from treatment A is consistently higher than that of all other treatments, only reducing to similar values of consumption towards the end of the five days.



a



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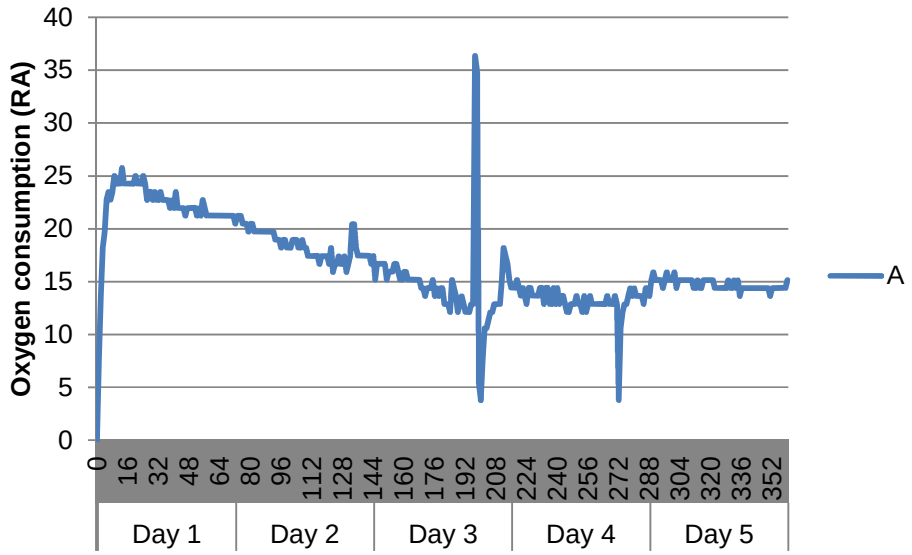


c

Figure 2.12 Graphs showing the oxygen uptake of the samples with the OxiTop flask a) shows the data over the first 24 hour period for treatments B-E and b) shows the data for B-E for the second 24 hour period, c) shows the BOD5 for treatment A

The reason for the sharp spike followed by a drop in the oxygen consumption for treatment A (Figure 2.12c) over five days is possibly due to acclimatisation of the sample. Looking at the raw data the change in oxygen consumption

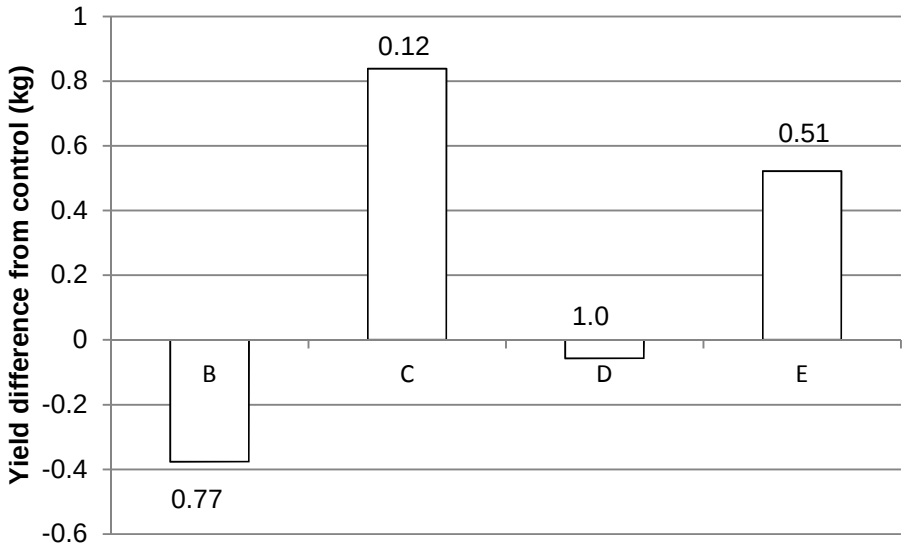
appears in all 6 replicates of the soil which would suggest the change is due to an external pressure such as extreme movement or a sudden change in temperature causing the oxygen consumption rate to change erratically. Excluding these points as outliers the curve levels off normally.

2.6 Results of the large scale crop trials carried out in 2014

2.6.1 Winter wheat

2.6.1.1 Winter wheat yield

The bar graphs in Figure 2.13a shows the variation in the treatments from the control value given by treatment A with the data for all treatments shown in Figure 2.13b. The treatment with the highest yield was treatment C with an average mean weight from 6 plots at 14.18kg per $12m^2$. The treatment with the lowest yield was treatment B with an average mean weight of 12.97kg per $12m^2$. The values for treatment B had a higher standard deviation than all other treatments with replicates B1 and B6 being the lowest of all. The data collected for the yield from the plots in 2014 was normally distributed with Shapiro-Wilk p values greater than 0.05. The yield data from the WW in 2014 shows no significant difference when analysed using a Tukey's test in SPSS with a p value of less than 0.05. This is a comparable result to those recorded in 2013 for wheat, with the change in the method of biochar application having no effect. The treatment closest to being significantly different from the control is treatment C with a p value of 0.12 and an average yield difference of 0.84 kg per $12m^2$. The fertiliser effects of the chicken manure and mineral fertiliser in this instance are no different between the treatments.



a

Treatment	Mean (kg/12m^2)	Standard deviation	Predicted mean (MKg/ha)
A	13.35	0.71	11.12
B	12.97	1.17	10.81
C	14.18	0.52	11.82
D	13.29	0.67	11.07
E	13.87	0.39	11.55

b

Figure 2.13 The mean average yield and standard deviation for the treatments on the WW are shown in b) and a) shows the bar graph demonstrating the difference between the control (Treatment A) and each of the trial treatments with the P value for this difference shown adjacent to each bar.

The position of the row had no effect on the yield of the plots, with none being significantly different from each other.

2.6.1.2 Analysis of grains and plant parameters

2.6.1.2.1 Fresh and dry weight

The fresh and dry weight of the grain and the plant were measured in 2014, the results of this are shown as a mean value in Table 2.8. From this data it is possible to see there is little variation in plant dry weight between treatments A, B and E with all values ranging between 62.4 and 62.7. Treatments C and D differ from this with mean dry weights of 65.1 and 61.6 respectively. There are no significant differences between any of the treatments for plant dry matter content. The results for grain dry matter show that treatments A-C have very similar values ranging from 51.1 to 52.0, with treatments D and E having lower means at 47.3 and 47.0 respectively. This shows that grain treatments D and E have significantly lower dry matter content than treatments A, B and C.

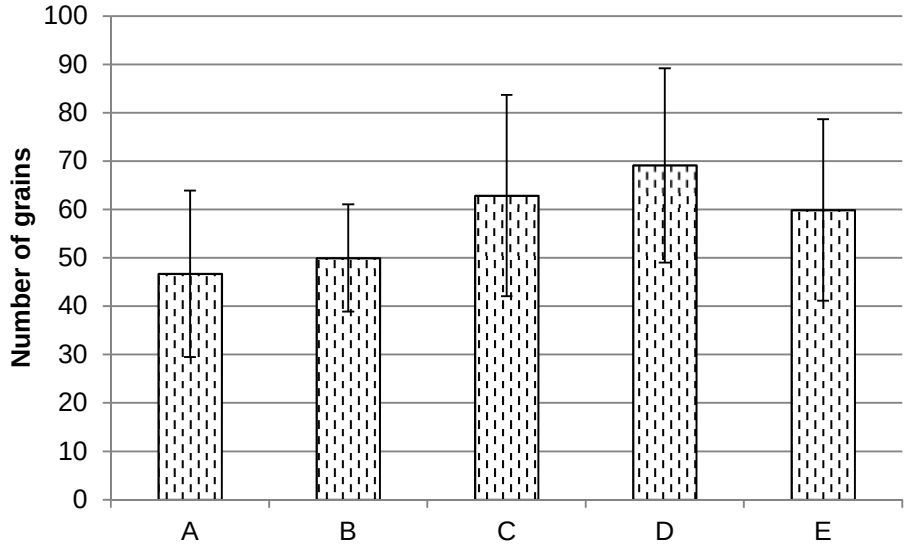
Table 2.8 The plant and grain dry weight expressed as a percentage of the as harvested weight

Treatment	*Plant dry weight (%)*	*Grain dry weight (%)*
A	62.4	51.1
B	62.7	51.8
C	65.1	52.0
D	61.6	47.3
E	62.7	47.0

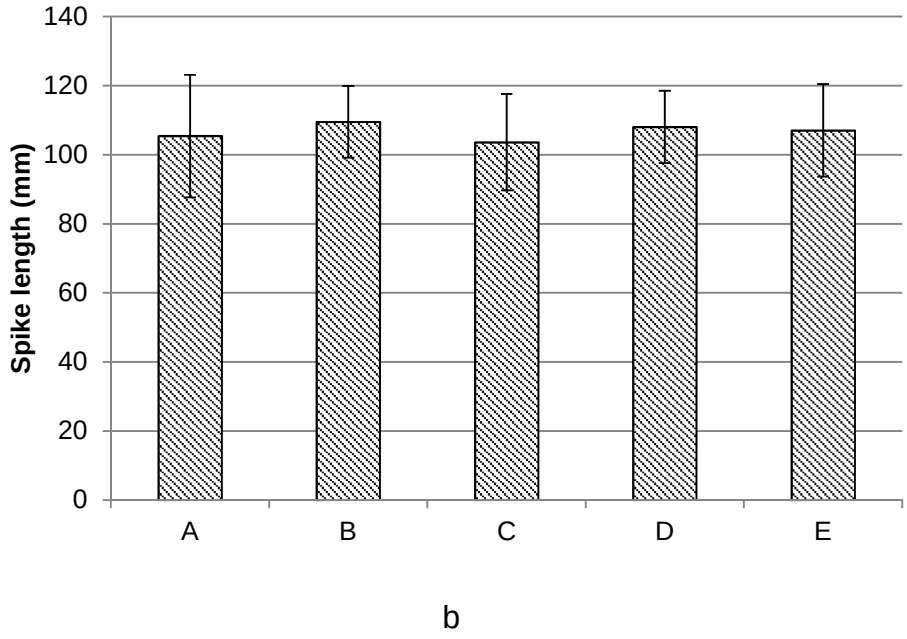
2.6.1.2.2 Plant characteristics

The detailed measurements of the five plants from each plot are shown in Figure 2.14a-c. The data is displayed with total height shown by the total height of the plant in graph c) and spike measurements in graph b). The number of grains on average per stem is shown in a). The largest plants were those from treatment D with the greatest overall height an average height of 809mm. The smallest plants were those from treatment B with a height of 792mm, although

plants in treatment B had the largest spike length with a mean value of 109mm. The smallest spike length was from plants in treatment C with a mean length of 103mm. The data collected from the plants for the height, spike length and number of grains was normally distributed with Shapiro-Wilk values above 0.05. This allowed the data to be analysed using a multivariate ANOVA. There were no significant differences in a Tukey's test with a p value of <0.05 for plant height or spike length between any of the treatments. The number of grains on each spike did show some significant differences between treatments. Plants grown as part of treatment A had significantly fewer grains than treatments C, D and E with p values of 0.03, 0.00 and 0.028 respectively. Treatments B had plants with significantly fewer grains that those on plants in treatments C and D with p values of 0.033 and 0.00 respectively.



a



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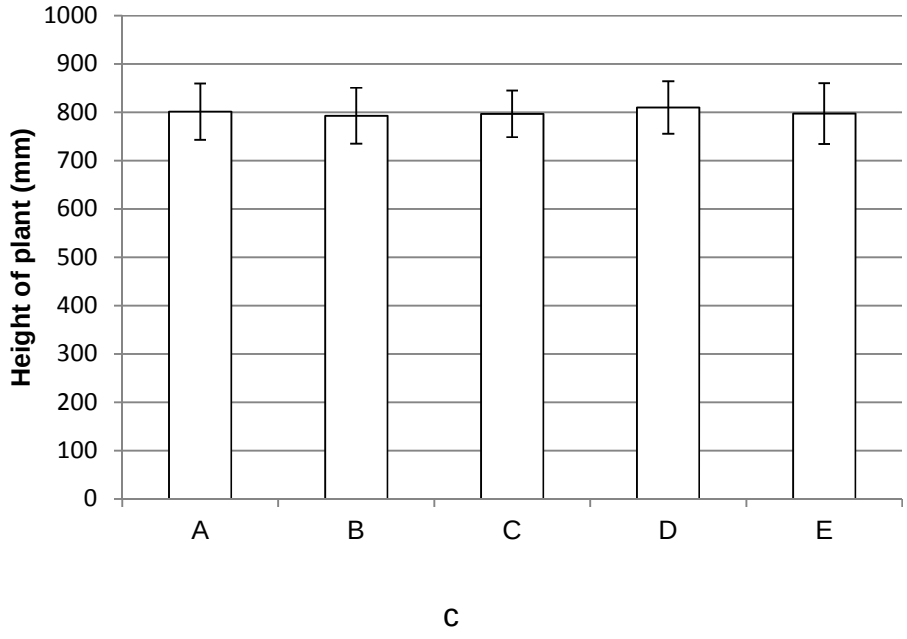


c

Figure 2.14 Bar graphs showing the wheat characteristics with a) showing the mean number of grains, b) showing the mean spike length and c) showing the mean plant height

The mean number of nodes per stem was the same for each treatment (mean = 4) and the number of leaves were the same for treatments A-D with a value of 5 with only treatment E having a mean number of 6 leaves. The data for the number of nodes and leaves was non-parametric and given the consistency of

the values between treatments further statistical analysis would not be more valuable.

2.6.1.3 Leaf chlorophyll content

The chlorophyll in the leaves for the WW was measured on six occasions throughout the growing season in 2014. The results of this are shown in Figure 2.15. The data as shown by the graph as highly correlated with each other, all sharing a similar distribution throughout the growing season. Treatment A shows the greatest mean values on the first (51.21) and last (18.65) measurements. Treatment B has the lowest SPAD values at both the start and end of the measuring period with a value of 45.3 and 10.0 respectively. The data was not normally distributed, and several transformations were unable to normalise it. In this case a non-parametric test was used, with a Chi-Square and Kruskal-Wallis test used to identify any significant differences on each date, then a Mann-Whitney-U test used to identify which treatments were different from each other.

The Kruskal-Wallis test identified differences in the data on the 21 May, 12 June and the 17 and 23 of July. The results of the Mann-Whitney U test showed that on the 21 May treatment A was significantly different higher than treatment B. On the 12 June treatment E had significantly higher chlorophyll values than treatment D. On the 17 July treatment A and E had significantly higher chlorophyll than treatment B. On the 23 July treatments A and E were both significantly higher than treatment B and D.

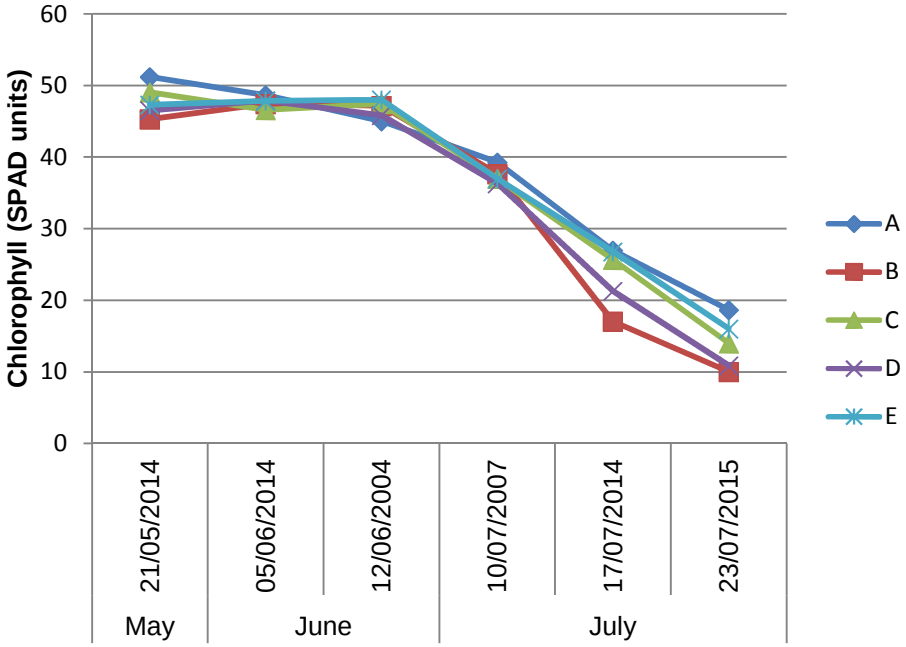


Figure 2.15 Line graph showing the chlorophyll data for the WW in 2014

2.6.1.4 Soil analysis

The soil analysis for the 2014 trials was completed at the University of Leeds and the values for pH, EC, TKN, ammonia, nitrate, Mg, Ca, Na, P, K, chloride and sulphate were determined. The data analysis showed that the spread of data was normally distributed using the Shapiro-Wilk test for normality, this allowed the data to be analysed using an ANOVA. The results for this analysis are shown graphically in Figure 2.16 and Figure 2.17. The results in Figure 2.16 have no error bars displayed as the standard deviation for these values was too great to display on the graph. The information in Figure 2.17 is shown with error bars displaying the standard deviation.

The pH values for the treatments can be seen from the graph in Figure 2.16 to be very similar with a range from 6.71 – 6.94 which were the values for treatments C and E respectively. The TKN values were also quite similar with a lowest value of 1.26g/kg for treatment D and a high value of 2.18g/kg for treatment E, with treatment A having the second highest value of 1.59g/kg. There were no significant differences between the treatments for either pH or TKN.

There was a large variation with the sodium measurements not only between treatments, but also between the replicates for each treatment. The lowest value was from treatment C at 2.54mg/l and the highest from treatment E with 11.93mg/l. There were no significant differences between any of the treatments for sodium. The magnesium values ranged from 8.42 to 12.26mg/l which were the values for treatments B and A. The levels of magnesium between treatments A and B were trending towards being significantly different, with a p value of 0.06.The levels of nitrate in the samples are the lowest in treatment B (4.35mg/l) and highest in treatment D (7.49mg/l) with none of the samples significantly different from one another. The levels of phosphate had the highest mean amount in D with 3.19mg/l and lowest in treatment C with 1.14mg/l. There were no significant differences between treatments for phosphates, however when the results were analysed by block they showed that block 3 had significantly higher levels of phosphate than the other two blocks. The raw data for phosphates showed many zero results in the data. The levels of sulphate in the samples show great variation with a range from 4.12mg/l (treatment C) to 13.43mg/l (treatment E). There was also a lot of variation within each replicate and there were no significant differences between any of the treatments.

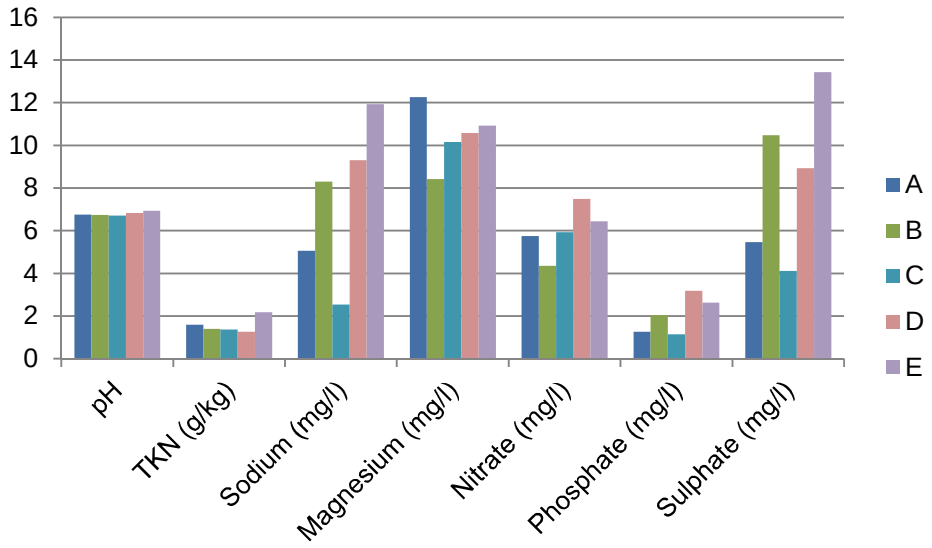


Figure 2.16 Mean values for the soil chemical qualities for each treatment the units for each measurement vary so are given under each cluster instead of on the vertical axis

In Figure 2.17 the results for conductivity showed a range from 160µS/cm (treatment B) to 217 µS/cm (treatment A), with the greatest variation in results shown by treatment C. There were no significant differences between any of the treatments, and in all cases the values were well below those which would have been inhibiting for plant growth. The results for the ammonia had a range from 137 to 179g/kg for treatments B and A respectively. There was a significant difference in the levels of ammonia in treatments A and B with A being significantly higher than B with a p value of 0.03. The levels of calcium show the lowest value for treatment B (19.13mg/l) with the highest value for treatment A (27.89mg/l). Treatments A and B were also significantly different with A again having a significantly higher level than treatment B with a p value of 0.03. There were no significant differences between any of the treatments for potassium and chloride, with treatment B having the lowest value in both cases with 20.21mg/l and 38.88mg/l respectively. The greatest value for potassium was treatment E at 33.06 and the greatest value for chloride was treatment D at 59.29mg/l. The values for total phosphorus were obtained from 1 replicate only so cannot be compared statistically. The greatest value for total phosphorus was recorded in treatment C with a value of 50mg/l and the lowest value from treatment A with 36.2 mg/l.

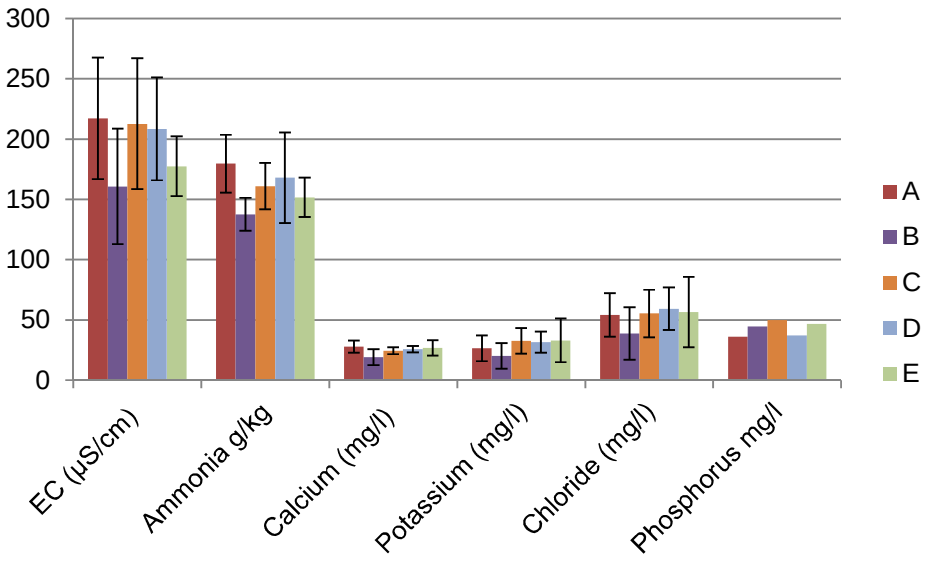


Figure 2.17 Bar graph showing the mean values for the soil chemical qualities for each treatment the units for each measurement vary so are given under each cluster instead of on the vertical axis

2.6.1.5 Biochemical oxygen demand (BOD)

The methodology for the BOD follows that described in section 2.5.1.6 except for the length of time the measuring heads were left to measure the oxygen consumption. In this instance instead of recording two consecutive 24 hour periods, the flasks were left in the incubator at 20^{o}C for five days to enable a BOD_5 to be calculated. BOD_5 is a common measurement for calculating the BOD of a soil or substrate sample. The measurement head on the flask was set to measure the change every twenty minutes therefore during the five days 360 points were recorded for each sample.

The results for the BOD in 2014 are shown in Figure 2.18 which shows three graphs stacked on top of each other. The reason for the jump in the oxygen consumption in treatment E is unexplained (Figure 2.18a), although examination of the raw data shows that two of the replicates (E2 and E5) had similar consumption values to replicates in other treatments with the other four being higher. E6 displayed consumption values similar to those in E2 and E5 up until day four when this replicate suddenly increased to four times its

previous consistent value. Removing this data from the analysis (Figure 2.18b) does eliminate the sharp increase in the data; however treatment E still has consistently higher oxygen consumption than the other treatments.

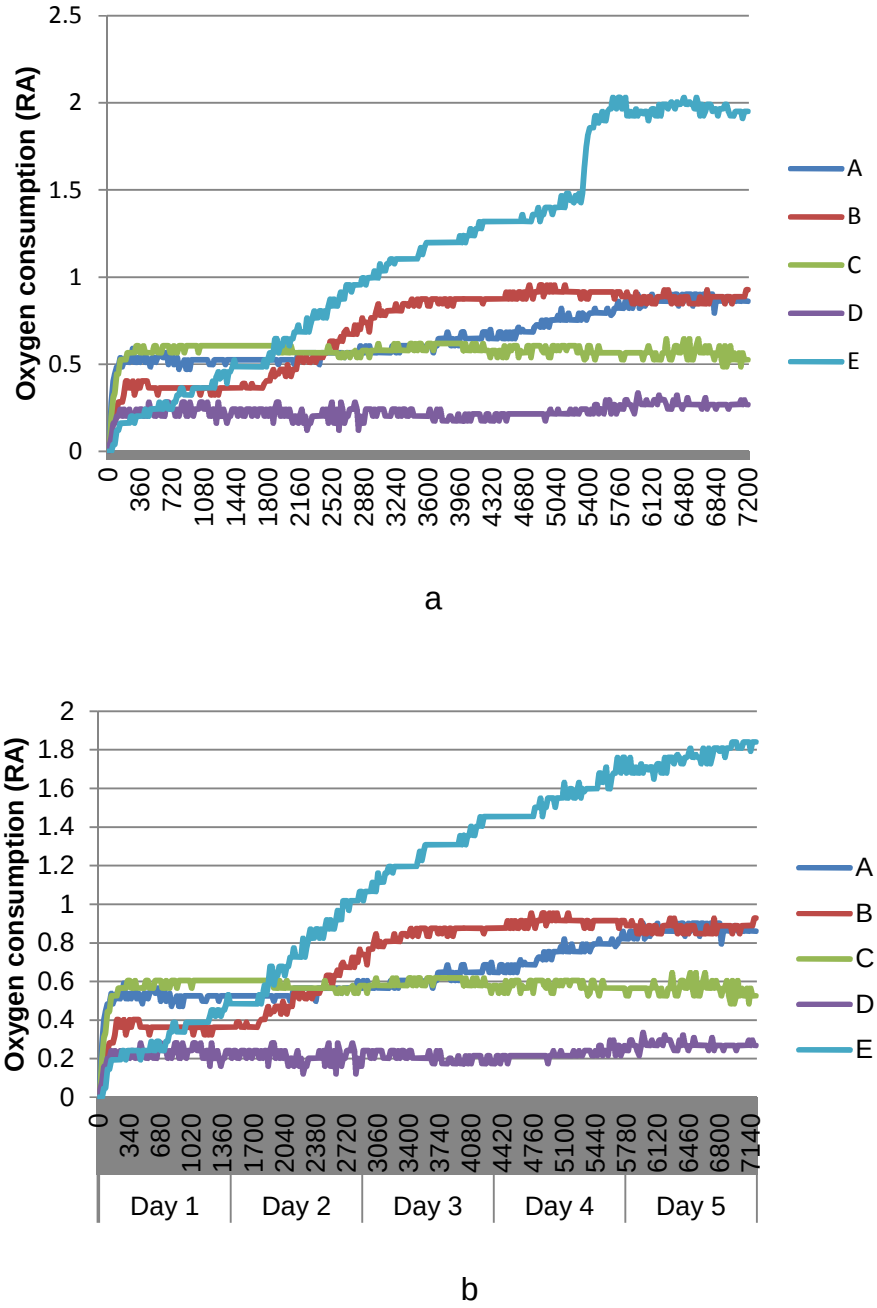


Figure 2.18 Line graphs illustrating the OxiTop results from the arable soils with a) showing the lines with the inclusion of all data and b) showing the lines with the exclusion of the outlier data in treatment E

2.6.1.6 CEC

The CEC analysis was completed on the soil samples from 2014. This analysis was carried out solely on treatments A, B and D to assess the effects of different treatments and the combining effect of biochar on the soil CEC. The results for the CEC analysis have a normal distribution so were analysed using an ANOVA. The mean value for the CEC showed that treatment D had the highest value at 10.89 The results of the ANOVA show that the results for the treatments are not significantly different from one another when $p<0.05$. The results for this analysis are shown in Figure 2.19 with error bars to show the standard deviation from the mean for the values. The standard deviation for treatment B is larger than for treatments A and D but not out of the ordinary expectation.

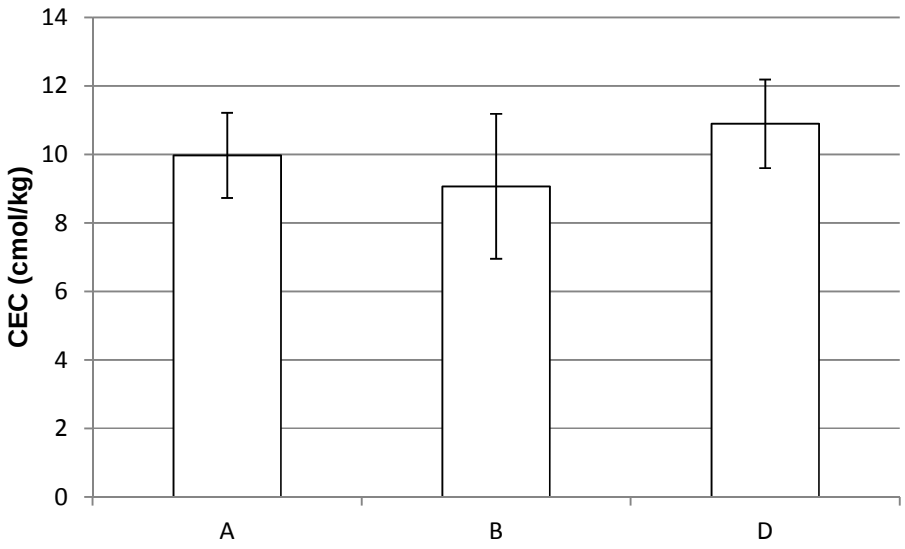


Figure 2.19 The results of the CEC analysis for the three treatments with error bars showing the standard deviation

2.7 Discussion

Given the amount of data available for the two years of large scale field trials a clearer discussion of the results should be achieved by separating them into each objective before discussion.

In relation to the aims and objectives of this work set out in 2.3 these are addressed in the following sections.

2.7.1 Objective 1

To trial chicken manure on arable crops with a mineral fertiliser application used as a control treatment for comparison by measuring crop yield from the experimental plots

For objective 1 there were no significant differences in the yield for either of the crops over the two year period. This would suggest that using a chicken manure amendment applied at the same nitrogen loading rate as the mineral fertiliser is able to produce the similar yield. The OSR was the only crop to have a lower expected rate of yield relative to the expected yield per hectare on the farm as a whole, in MKg/ha for all of the treatments applied.

In the 2014 trials the grain count by spike on the wheat stem was not significantly different to the control for a matched nitrogen loading rate in a chicken manure application, however a double dose of manure significantly increased the number of grains when compared to the control and the matched nitrogen loading rate chicken manure treatment. The amount of nitrogen available to the plant at certain developmental stages, such as double ridge which occurs between growth stage 1 and 2, can influence the number of grains produced (Langer and Liew, 1973, Acevedo, 2002, Novoa and Loomis, 1981). This would suggest that more nitrogen was available at this stage in the double dose chicken manure treatment.

The rate of mineralisation of nitrogen from manures is slow so that uptake by the plants is incremental and not all the nitrogen in the manure will become plant available. The double dose of manure was used as a treatment to counteract the mineralisation speed of manures and make more nitrogen available to the plants. The grain results indicate that this may have occurred in the double dose rate by producing more grains per spike, but this has had no effect on yield between treatments. It is not clear why the higher number of grains did not transfer into a greater yield, however it could be a result of only 5 plants being collected per plot. Or this result would suggest that although grains

on the treatments with a double dose are more numerous than other treatments, their overall weight was lower giving no difference in yield result. A bigger sample size could have ameliorated this difference shown in the grain number if by chance the stems selected at random had more grains that the average across the plot.

The chlorophyll levels in the leaves for the crops in 2013 showed no significant differences between treatments. The wheat trials in 2014 showed a significant difference between treatments A and B, with treatment A on three occasions having a significantly higher chlorophyll content in the leaves. This would suggest that in 2014 the nitrogen uptake in the leaves for the mineral fertiliser was better than in 2013 when compared to uptake with a chicken manure fertiliser. Ghosh in 2004 reported similar results in their trials with a soybean crop, with the highest chlorophyll levels in the treatments that received the 100% mineral fertiliser treatment (Ghosh et al., 2004). The measure of chlorophyll gives an idea of the amount of matter assimilated by the plant (Ghosh et al., 2004) and an indication of its dry matter content. The chlorophyll levels in treatment A in this case did not transfer to any differences in yield, dry weight of the grains or plant, or the plant height.

2.7.2 Objective 2

To assess the effects of biochar as an additional amendment to the chicken manure in comparison to a mineral fertiliser used by itself by measuring crop yield from the experimental plots

The plots with the biochar added on the OSR crop in 2013 showed a significantly higher yield than the plots with just chicken manure added. This would suggest that the addition of biochar improved the yield; however the WW in 2013 and 2014 showed no significant differences between these treatments. The difference in the biochar between crops in 2013 was that the biochar application was applied as a top dressing on the OSR and was incorporated into the soil for the WW. The month of March in 2013 was one of the coldest in recorded history with average temperatures in the UK dropping 2.5^{o}C below the monthly average from historical records (Office, 2015). In this instance the

biochar application may have acted as a thermal protective layer either adding extra bulk on top of the roots as frost protection or due to its black colour conveying a thermal effect of increased heat absorption. The use of plastic mulches in agriculture, referred to as plasticulture is a well-used method of improving crop productivity by increasing soil temperatures, as well as providing protection from weeds and soil moisture conservation (Touchaleaume et al., 2016, Kasirajan and Ngouajio, 2012). Higher soil temperatures improve nutrient uptake by the crop, increases the nutrient mineralisation and availability within the soil and also increases microbial activity. Crops typically grown under plastic are peppers, musk melon, cucumbers, tomato, watermelon and aubergines, with an estimated 1 million tonnes of plastic mulch film used worldwide on an annual basis (Kasirajan and Ngouajio, 2012). In experiments comparing different types of plastic film and mulch all plastic covers raised the mean daily soil temperature by at least 1.5^{o}C when compared with bare soil (Touchaleaume et al., 2016). Although the addition of biochar on top of the soil is not the same as growing crops under plastic, in many cases the plastic used is black in colour to enhance the thermal effects of the treatment.

The lack of improvement that was otherwise shown in the plots where biochar was added onto winter wheat could be due to the low loading rate of 10Mkg/ha that was applied in both years. The short length of this study meant that any cumulative effects of the biochar being applied on the same locations would have limited impact on the trial. Biochar is often applied at rates greater than 10Mkg/ha, with both Quillam (2012) and Jones (2012) applying biochar at 25 and 50Mkg/ha for three seasons with Quillam reporting that the biochar application rates did not affect plant growth and Jones finding the biomass production in the third year in grass production was improved significantly at both rates when compared to a control (Quilliam et al., 2012, Jones et al., 2012). This would suggest that the effects of the build-up of biochar in the case of Jones 2012 had a positive effect on the plant production and yield. The 10Mkg/ha of biochar applied in these trials had no cumulative effects on plant development and yield over two seasons when compared to plots that received no biochar amendment.

The SPAD values for leaf chlorophyll showed no significant differences between treatments with and without biochar for the 2013 trials. This supports the result of Quillam (2012) who found the same results. In 2014 there was a difference in the chlorophyll levels with treatments A and E being significantly higher than treatments B and D. This is a result that does not follow any links between biochar and chlorophyll as the two receiving biochar were significantly different from one another. This could be related to the nitrogen in the double dose of chicken manure amendment being more effectively taken up by the plant in the presence of biochar. In work completed by Asai (2009) and Lehmann (2003) they reported that in some instances of N deficiency in the soil the addition of a biochar amendment can reduce leaf uptake of nitrogen (Asai et al., 2009, Lehmann et al., 2003, Quilliam et al., 2012). This matches the result of treatment D having a lower chlorophyll measurement, whilst in treatment E where there has been more manure applied giving a higher nitrogen application rate, the plant can assimilate more nitrogen into the leaves leading to a significantly higher chlorophyll measurement.

2.7.3 Objective 3

To assess the effects of the organic amendments on soil quality by carrying out chemical analysis of the soil

It is unfortunate that the methods of soil analysis and preparation were not consistent across the two year trial period, which means they cannot be compared against one another effectively.

2.7.3.1 2013

The treatments that received the biochar as an amendment, treatments D and E, on the OSR crop showed higher phosphorus and potassium levels than all other treatments. The chicken manure used for the trials had rates of 1.5% and 2.4% (of dry solids) of phosphorus and potassium respectively, as shown in Table 2.3. This pattern is not followed for phosphorus for treatments B and C which received the same amounts of chicken manure as D and E. The

functionality of the biochar may have reduced the leaching of phosphorus through the soil, making it more available for uptake in the plants. It is possible in some cases for biochar to absorb phosphates on its surface (Yao et al., 2012). The levels of potassium in the samples are the lowest where only mineral fertiliser was applied, which correlates with the chicken manure having 2.4% of dry solids potassium concentration. Treatment A, with the mineral fertiliser only, had the highest total nitrogen of all treatments.

In the winter wheat crop in 2013 the levels of phosphorus and potassium for treatment D were not higher than the other treatments. The total nitrogen in treatment A for the winter wheat was also lower than the other treatments showing a different pattern to the results compared to the OSR trial. The results for 2013 are hard to compare further as only two samples were sent for analysis for each treatment.

2.7.3.2 2014

The effects of the applications on soil quality did show a significance in 2014 on the WW when compared between the blocks the plots were arranged in. Block 3 had significantly higher levels of phosphate than both of the other blocks. The data is patchy, however the results with a value of 0 from the other blocks need to be accounted for and treated as accurate due to the samples being treated and analysed in the same way. This would suggest that this is a field effect rather than an effect of the treatments due to the randomised block design of the experiment. Block 3 was located downhill from both the other blocks which could suggest that the higher amounts of phosphorus present are a result of leaching through the soil. Excess nutrients leaching through the soil can be transported into the groundwater or surface waters on an area of land (Laird et al., 2010, Vanden Nest et al., 2014). In this case as we are only looking at soil samples the effect of transfer from groundwater can be ignored as the groundwater level was below the level of the base of the soil. Nutrient leaching can cause soil acidification, loss of nutrients, reduced crop yields and have impacts on the downstream environments (Laird et al., 2010, Yao et al., 2012, Lehmann et al., 2005). Application of the chicken manure in its raw pelletised

form could have increased the P losses through the soil due to its low C/P and N/P ratios. In work by Vandacasteele (2013) composting chicken manure along with green waste increased the C/P ratio and transformed the raw chicken manure into a more suitable soil amendment with P losses made less likely (Vandecasteele et al., 2013). In the same trial it was found that combining the chicken manure compost with a biochar made from oak as a feedstock further reduced the readily available P in the amendment. In this instance the effects of biochar on the chicken manure amendment have not reduced the nutrient leaching of phosphate. The levels of total phosphorus in the samples cannot be compared statistically as only one sample was submitted for analysis.

The soil analysis in 2014 showed that treatment A had significantly higher levels of ammonia and calcium in the soil than treatment B. The higher levels of ammonia were thought to be due to the amount that was readily available in the mineral fertiliser that was applied. The raised levels of calcium are not understood, but it could have been due to chance patchy distribution of calcium deposits in the field. These in turn also help increase the amounts of ammonia that remain in the soil by reducing the amount of ammonia that is subject to volatilisation (Fenn et al., 1981). Otherwise the analysis of the soils after plant growth showed variable results and were not correlated in relation to the chemical components of the materials that were applied.

2.8 Conclusions

These trials completed at field scale did not show any consistent differences in plant development and yield between the treatments applied in both years on both crops. The only difference shown was in the yield between the plots receiving biochar and those that didn't in the OSR crop in 2013. Given these results, in can be concluded that under these conditions a chicken manure replacement fertiliser can in the short term be used as an effective fertiliser on cereal crops. Long term use of chicken manure on field crops, given its high amounts of phosphorus and potassium could lead to excessive phosphorus

leaching from the soil and affecting groundwater and downstream environments.

A smaller more controlled trial growing arable crops in a pot experiment with comparable treatments to the large scale trial would allow a more in depth analysis of the effects on plant growth. A trial to assess this has been completed and is discussed in Chapter 3 Small scale arable trials.

Chapter 3 Small scale arable trials 2015

3.1 Introduction

The work described in this chapter further develops the trials and methods used in Chapter 2 and builds on this to create a broader trial under stricter control and management. This is done using some of the same treatments as given in Chapter 2 alongside more novel amendments to compare the crop development.

The organic amendments used in this trial are two forms of an OMW compost produced using chicken and pig manure, pelletised chicken manure and a commercially produced compost. The OMW compost applied at nitrogen rates to match that of the inorganic fertiliser used as a control should compare similarly as the potential phytotoxic elements within the OMW will be applied at a low rate. The biochar used in this trial was applied as an organic soil amendment and not as a fertiliser.

3.2 Literature review

3.2.1 Wheat's economic and political importance

Wheat is a wild grass from the Gramineae family and its use as a food goes back to the Stone-Age. It is native to dry countries in western Asia (Cornell and Hoveling, 1998, Perkins, 1997), the fertile crescent as it is known. Wheat breeding came to the fore from 1875 – 1925, involving a global community of plant scientists (Perkins, 1997). There are different varieties of wheat grown in different climates that have undergone significant genetic modification, both historical and modern, to achieve the optimum growth in that climate. This has led to the wheat plant being the most widely grown crop on a global scale (Satorre and Slafer, 1999) with only rice rivalling it for abundance (Perkins, 1997). The latest statistics for wheat production state that in 2013 the

production in the UK was over 1.6Mha of wheat, for Europe this was over 57.5Mha and globally wheat production stood at 218.5Mha (FAOSTAT, 2013). Approximately 95% of the wheat produced globally is a bread wheat, with the remaining 5% of the yield from durum, or pasta wheats.

Despite its relatively recent domestication, bread wheat has over 25 000 varieties developed from around 600 genera of grasses (Cornell and Hoveling, 1998) displaying a wide genetic variation in the family. Production of wheat increased dramatically during the 20th century, in the first half due to a greater area being cultivated for this crop, but in the second half of the century this increase in production can be attributed to an improvement in yields (Satorre and Slafer, 1999). The increase in the second half of the 20th century can be attributed to better farming practices using better plant varieties and the use of more fertiliser, referred to as the Green Revolution (Perkins, 1997, McClung, 2014, Godfray and Garnett, 2014). The instigation of these changes can be credited to the rise in agricultural science, more specifically those with expertise in plant breeding and those with an understanding of soil science and fertility (Perkins, 1997).

The rise in agricultural science was also a result of political leaders attempting to maintain stability within their populations. Resource exhaustion leading onto hunger is commonly associated with political instability and the collapse of society (Hochberg and Brown, 2015). The rise in agricultural research into crop varieties and improving yields has also been affected by times of conflict, most notably World War II (Perkins, 1997). The idea that famines would be an intrinsic part of development was first raised by Malthus in the 1700s when he suggested that because population growth is geometric, and agricultural growth would only ever be arithmetic that at some point, people would go hungry (Perkins, 1997, Godfray and Robinson, 2015). Food production and population growth are linked in an arms race with agricultural production under pressure to keep up with population growth, there remains around 1 billion people that are still chronically underfed (McClung, 2014).

By 2050 the global population is expected to rise to close to 10 billion people (Nations, 2015). A projected 100% increase in the demand for food was

predicted by Tilman in 2011. To meet this demand we will be reliant on clearing more land for production and dramatically increasing yields particularly in developing countries where access to technology to improve crop yields is currently poor (Tilman et al., 2011). It isn't enough for production in agriculture to be high either, it also has to be sustainable in the long term (McClung, 2014) with regards to the use of water and unsustainable soil management (Tscharntke et al., 2012). Agricultural production also has to counter the effects of climate change such as rising temperatures (McClung, 2014), changes in regional rainfall and extreme weather events (Godfray and Garnett, 2014).

3.2.2 Using wheat as a trial crop

Brunetti et al. (2007) conducted experiments using durum wheat as a test crop and applying organic amendments made from OMW. These amendments consisted of raw OMW left for 60 days in an OMW lagoon and a catalytically digested OMW that was left for 60 days after an application of MnO_2. These OMW were then both applied at 300 and 600m^3/ha. They found that the amendments positively impacted on the yield by increasing kernel weight, spike density and the number of kernels per unit area in comparison to a control that received no amendment or fertiliser (Brunetti et al., 2007). The use of a raw semisolid OMW amendment in trials by Lopez-Pineiro (2006) showed an increase in the yield of wheat grown in pot trials using two types of soil as a base media. This material was trialled at dosage rates from 0-40kgN/ha, the 40kgN/ha showed in the 2nd year an increase in the yield of 29% and 198% in the two different soils (López-Piñeiro et al., 2006). This OMW amendment was also shown to be a valuable source of N, K and organic matter. In 2008 Lopez-Pineiro achieved similar results in terms of wheat yield in the second year using different soils and a solid OMW product, although on this occasion one of the soils showed a depressed yield in the first year of trials (López-Piñeiro et al., 2008b).

Santonoceto (2010) demonstrated that durum wheat grown in Italy had the best yield with the Italian recommended dose rate of OMW of 8 l/m^2 when compared to a control that received only water and treatments receiving16 l/m^2 and 32

l/m^2. The number of fertile spikelets and the number of kernels per ear was significantly higher for the recommended dose rate of 8 l/m^2 compared to all other treatments (Santonoceto et al., 2010).

Rinaldi also conducted experiments on durum wheat in Italy, over a 3 year period. Plots were either treated with 50 Mkg/ha of OMW (equivalent to approx. 5l/m^2) (the maximum recommended amount in Italy) or left untreated. No significant differences were observed between yields from treated and untreated plots throughout the 3 years, however yields in both plots showed a decline year on year (Rinaldi et al., 2003). This highlights that an OMW amendment that has had no pre-treatment would not compare favourably to a more traditional mineral fertiliser addition.

Mekki in 2006 found that using an OMW treated with a fungal pre-treatment under aerobic conditions, followed by anaerobic digestion could produce a viable crop amendment. This designed crop amendment created from OMW showed increased yields in durum wheat when compared to plots treated with water as a control. Crops that received untreated raw OMW performed the worst (Mekki et al., 2006a). This study by Mekki did not compare the use of OMW amendments and the use of widely used mineral fertilisers.

The effects of using biochar and manure on arable crops has been discussed in Chapter 2.

3.2.2.1 Development stages

The growth of wheat in the UK is commonly categorised into decimal developmental stages known as growth stages (GS). The growth of wheat can be categorised into two broad headings, firstly from sowing up to the production of fertile grains, and secondly the filling of the grains with carbohydrate (AHDB, 2015b, Development et al., 1980). This decimal code system was formalised and further described by Tottmann in 1987 (Tottman, 1987). This system of charting the growth of wheat starts as GS10 with the emergence of the first leaf through the coleoptile. The coleoptile is the first leaf to emerge from the seed following germination. This scale is broken down in to 9 main sections made up of different stages ending with the ripening stages at GS93. Along with this are

descriptive benchmarks of wheat development that correspond to a particular growth stage. The key phases of wheat growth and development are crop emergence at GS10, stem extension at GS31, flowering at GS61 and the end of grain filling at GS87 (AHDB, 2015b). Growth of the crop is also dependent on increasing temperatures and day length. Knowing what stage the majority of a field crop is at is important in crop management and fertilisation regimes to maximise the crop quality and yield.

The grain filling section of the plants development is critical to the yield, most of the nutrients in carbohydrates that are incorporated into the grain are produced from the flag leaf and sheath. A crop canopy in a field of wheat is measured by its green area index (GAI). Most of the protein found in the grains at harvest is from redistributed nitrogen, with 84% of grain nitrogen coming from this source, and only 16% of grain N produced from uptake of nitrogen after flowering (AHDB, 2015b). By the stage of a typical harvest at GS93 the nitrogen distribution in the plant has mostly transferred into the grain with 68% of plant nitrogen present there. The remaining 32% of plant nitrogen is found within the straw and chaff (AHDB, 2015b).

During development it is common for most wheat varieties to produce tillers, which are secondary stems forming off the main stem. This increases the yield of the crop and a typical high yielding wheat variety will produce one main stem and around 3 tillers (Perkins, 1997). The optimum yield of wheat is a balancing act between the protein content and crop yield as a variety that produces grain with a high protein content usually shows a reduction in the yield of the crop (Cornell and Hoveling, 1998).

3.3 Aim and objective

The aim of this study is to replicate the methodology of the large scale field trials and add more treatment combinations to compare the effects of different organic treatments. This is in comparison to the mineral fertilisers with and without the inclusion of a biochar amendment.

Objective: To study the effects of different organic fertiliser treatments against a mineral fertiliser used as a control both with and without the addition of a biochar application.

3.4 Methodology

The small scale arable trials were completed at the Leeds University research farm in Tadcaster, West Yorkshire (lat. 53^{o} 86'; long. -1^{o} 33'; alt.55m). These trials took place in a polytunnel at the university farm as a pot trial. The OMW products used in these trials were produced using chicken manure and pig manure as their respective base materials.

To enable comparisons with the large scale trials completed in 2013 and 2014 the treatments with the OMW product were compared against the fertilisation products used in the large scale arable trials. Spring planted wheat was used as the trial crop to allow for comparisons with the large scale trials. In addition to the OMW products used in the trial a biochar application was used on half of the treatments to give a comparison with the biochar used in the soil in 2013 and 2014. The plots were arranged in a RCBD within the polytunnel and there were eight different treatments being trialled, these are listed in Table 3.1. The treatments were designed to test the objective of how the source of nitrogen input in the form of inorganic fertiliser, chicken manure, a chicken manure based OMW compost, a higher nutrient pig manure based OMW compost with and without biochar present compare in terms of plant growth. The biochar used in these trials is the same as for Chapter 2, a commercially produced product from Carbon Gold. The application rate of the treatments was based on the total N available in the amendments.

Table 3.1 Treatment descriptions for the small scale arable trials in 2015

Treatment	*Description*
1	Standard ammonium nitrate fertiliser treatment
2	Standard ammonium nitrate fertiliser treatment + biochar
3	Replacement fertiliser in the form of pig manure based OMW compost
4	Replacement fertiliser in the form of pig manure based OMW compost + biochar
5	Replacement fertiliser in the form of chicken manure based OMW compost
6	Replacement fertiliser in the form of chicken manure based OMW compost + biochar
7	Replacement organic fertiliser in the form of pelletised organic chicken manure
8	Replacement organic fertiliser in the form of pelletised organic chicken manure + biochar

For each of the eight treatments five replicates were used, giving a total of 40 pots. Each pot had a 2 l capacity and a depth of 11cm which replicates the depth of soil utilised by plants in the field of 15cm reasonably well. The RCBD gave an arrangement of pots as shown in Figure 3.1 arranged on five benches within the polytunnel.

3	4	7	8	3	6	8	2	4	5	6	1	4	3	5	1	6	3	2	8
1	2	6	5	7	4	1	5	8	3	7	2	2	8	7	6	1	4	7	5
Block 1				Block 2				Block 3				Block 4				Block 5			

Figure 3.1 Plot layout of the treatments for the small scale arable trials. The bold line denotes benches and the numbers refer to the treatments

The fertiliser treatments and biochar where applicable were applied to the top of the soil in each pot and gently incorporated into the soil by hand. Eight spring wheat seeds were then planted in each pot in two rows across the middle. These were planted on the 23 June 2015, with the first seedling emergence in the week following on the 30 June 2015. Pots were watered by hand in a methodical pattern several times a week, with additional watering during particularly hot periods to prevent desiccation of the seedlings.

The treatments were applied at a rate of 150kgN/ha in two stages. The initial fertilisation occurred at planting of the seeds and the second fertilisation of the plants took place once the plants had reached GS31, listed as when the plants have their first node at the extension of the stem. This is as per the recommendations for successful fertilisation of wheat as originally cited by the Home Grown Cereals Authority (HGCA) now the Agriculture and Horticulture Development Board (AHDB) (AHDB, 2015b). The biochar for treatments 2, 4, 6, and 8 was applied at a rate of 10t/ha.

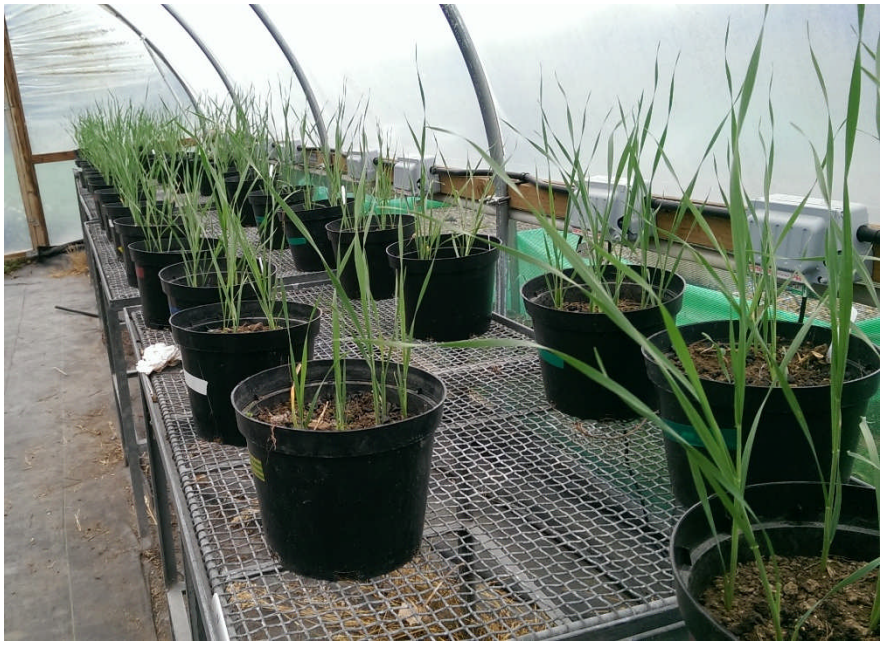


Plate 3.1 Pot set up for spring wheat in the polytunnel

3.4.1 Characterisation of the fertiliser products

A laboratory analysis of the composts and manure applied to the soil in the wheat trial are shown in Table 3.2. This shows that the chicken manure based

OMW (COMW) had the highest pH of any of the treatments at 10.1, this compost also had the highest EC at 14000µS/cm and the highest concentrations of calcium at 154505mg/kg and magnesium at 1.26%.

The highest total nitrogen and ammonium nitrogen concentrations were found in the chicken manure pellets with levels of 4.48% and 861mg/kg respectively. The chicken manure pellets also have the highest amounts of phosphorus and zinc at 1.53% and 461mg/kg respectively. The pig manure based OMW has the highest levels of potassium at 8.6% and the highest levels of sulphur at 0.7%.

Table 3.2 Characterisation of the fertiliser products and manure

	Units	***Chicken manure pellets***	***Pig OMW***	***Chicken OMW***
pH water (1:2.5)		8.74	9.69	10.1
EC	µS/cm	5830	11610	14000
Total Phosphorus	% w/w	1.53	1.055	1.38
Total Potassium	% w/w	2.38	8.645	5.48
Total Magnesium	% w/w	0.697	0.6545	1.26
Nitrate Nitrogen (fresh)	mg/kg	13.3	<10	<10
Ammonium Nitrogen (fresh)	mg/kg	861	138	129
Total nitrogen	% w/w	4.48	3.41	2.27
Total Sulphur	% w/w	0.51	0.7145	0.578
Total Copper	mg/kg	73	74.35	57.9
Total Zinc	mg/kg	461	209.5	434
Total Sodium	% w/w	0.447	0.4105	0.616
Total Calcium	mg/kg	80340	52823	154505
Total Phenols (index)	mg/kg	<1	<1	<1
Dry Matter (fresh)	%		75.95	92.9

The biochar used in the trials was commercially produced by Carbon Gold, and is described in further detail in Chapter 2.

3.4.2 Limitations of the trials

These trials started later than was planned due to seed availability, with the spring wheat seeds being planted on the 23 June which is too late in the year to achieve a successful grain yield, despite the protection afforded by being grown undercover. The trial was stopped before the start of autumn as the crops were unprotected and the risk of a cold spell and destruction of the trial by vermin was a real risk that has affected trials in the past at the University farm. This meant that during the trial period the crops did not grow to their final development stage.

3.4.3 Sampling for soil and plants

Once the plants had established and the second application of fertiliser had been applied the leaf blades were measured for chlorophyll content. The chlorophyll measurements were carried out on the same plant in each pot on the leaf below the flag leaf, in order to ensure consistency of the measurements throughout the monitoring period. The chlorophyll measurements were taken on four occasions before the plants were harvested

On reaching growth stage 55 - 59 after the ear had completely emerged the plants were harvested with fresh and dry weight of the roots and shoots being recorded. The plants had at this stage had 4 - 5 internodes (AHDB, 2015b) and the developing grains were still soft. This was completed for five plants from each pot meaning that 25 plants were measured for height, spike length and number of grain for each treatment. Height and spike length are a marker of plant growth and development, the length of the spike also gives an indication of the number of grains that might be present. At the end of the trial period the soil was removed by removing the bulk by hand and then rinsing with water and the plants shaken to remove excess water before weighing. The plants were then measured for total height (including spike), spike length and the number of grains on each was counted. The plants were then placed in a drying oven for 24 hours until a consistent weight was reached.

After the plants had been removed from the pots a soil sample was obtained from four of the blocks for each treatment and sealed in a ziplock bag in preparation for laboratory analysis.

3.4.4 Analysis methods

The methods used for soil analysis in this chapter follow those previously described in Chapter 2. The further work analysis in this chapter was carried out on the dried plants

3.4.4.1 Protein analysis

The protein in the plant was analysed using a Leco Truspec/FP628 N analyser. This analysis is completed using a dried, crushed sample which is weighed prior to being placed in the analyser. The analyser works through a process of dual combustion with temperatures up to 1050^{o} in order to allow for complete combustion of organic matter to obtain an accurate result. The analyser automatically produces a result of the protein concentration in solid samples.

3.5 Results

3.5.1.1 Chlorophyll levels

The mean concentrations of chlorophyll in the leaves recorded from the treatments can be seen in Figure 3.2. The figure shows that generally, with the exception of treatments 2 and 3 the concentration of chlorophyll shows a slight increase from the start of the measurements on the 6 August up to the 13 August. After this date most of the treatments showed a decrease in chlorophyll concentrations. The mean highest concentration of chlorophyll recorded was for treatment 3 (pig manure based OMW compost with no biochar) on the 20 August with a SPAD measurement of 52.6. The lowest recorded mean chlorophyll concentrations were also in treatment 3 on the 13 August with a value of 35.6. The data for the chlorophyll was normally distributed so it was analysed using an ANOVA. This showed that there were no significant differences between any of the treatments on any of the dates that chlorophyll was measured throughout the monitoring period. As mentioned earlier, this is

illustrated in Figure 3.2 by the fact that the data for all the treatments show a similar pattern and have similar values for each measuring date. The graph appears to show a large difference between the chlorophyll concentrations for treatments 3 and 6 on the 13 August, which would suggest that there may be a significant difference between these two treatments. However the data analysis showed that the data for treatment 3 had a large standard deviation for that date of 21.4, whereas treatment 6 has a small standard deviation of 2.6. This shows there was much greater variability in the data for treatment 3 and that overall, despite the apparent difference shown on the graph, the data were in fact not significantly different.

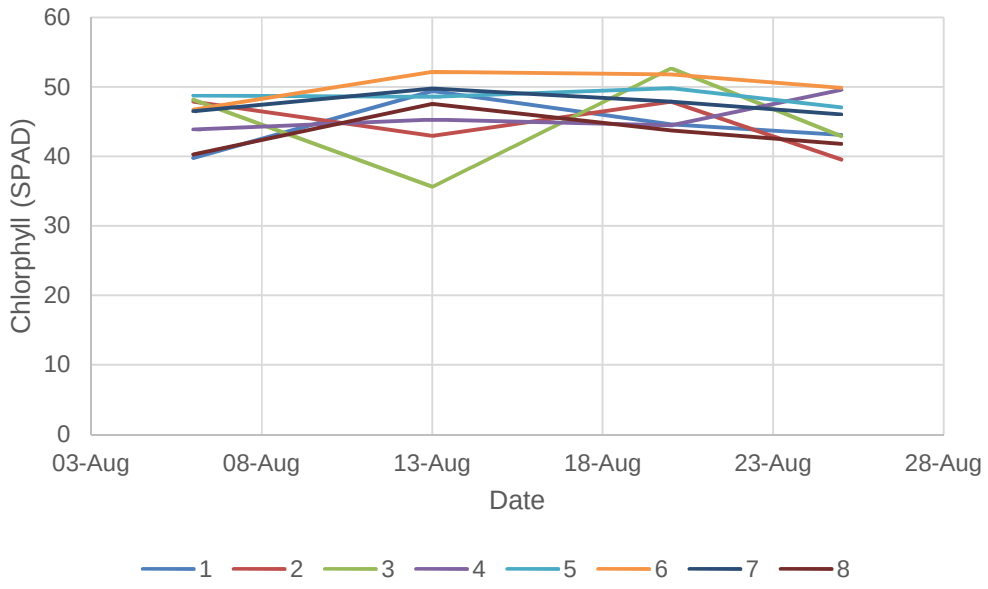


Figure 3.2 Mean chlorophyll levels in the leaves of all 8 treatments

3.5.1.2 Fresh and dry weight

The fresh and dry weight was determined by taking 5 plants from each pot in the trial and grouping them together. The results of this are shown as a mean fresh and dry weight with the standard deviation shown on the graph as error bars in Figure 3.3. It is apparent from the data that there was very little difference in the fresh and dry weights of the plants from each treatment and that only small differences were observed for plant dry matter.

The treatment with the highest mean fresh weight for the 5 plants was treatment 5 at 15.4g and the treatment with the lowest mean fresh weight for the 5 plants was treatment 3 with a value of 12.8. This data shows that the variation in the fresh weight over all the treatments 2.6g which does not sound like much but represents a 20% increase from treatment 3 (lowest) to treatment 5 (highest).

For the dry weight of the plants treatment 6 had the highest mean dry weight of 4.9g and treatment 7 had the lowest mean dry weight at 3.6g. This is a difference across all the treatments of 1.3g which represents a 36% increase from treatment 7 (lowest) to treatment 6 (highest).

The data for the dry matter content of the plants (Figure 1.3) shows that there was some variation across the different treatments. The data for the dry matter content shows that treatment 6 had the highest DM content of 32.6% with treatment 7 having the lowest of 25.8% DM content. This is a difference across all the treatments of 6.8% from treatment 7 (lowest) to treatment 6 (highest).

This data was normally distributed so it was analysed using an ANOVA. The results of the ANOVA showed that none of the treatments had significantly different fresh or dry weights or dry matter content when $p<0.05$. The error bars on the graph in Figure 3.3 show the overlap between the data for the different treatments.

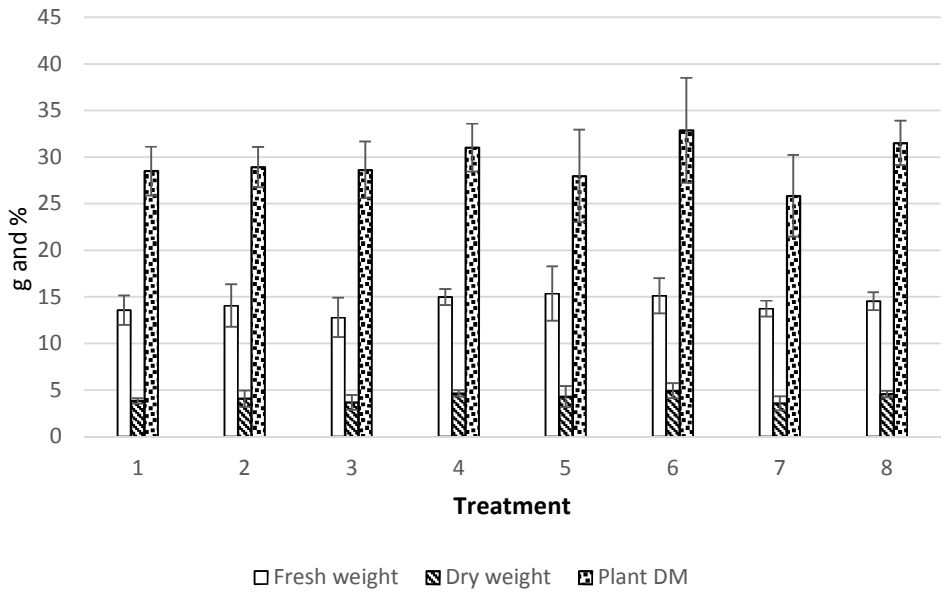


Figure 3.3 Mean fresh and dry weight of the wheat plants with standard deviation indicated by the error bars. (Fresh weight and dry weight values are in grammes and the dry matter is given as a % of the fresh weight)

3.5.1.3 Protein analysis

The protein measurement is an indication of the nitrogen uptake by the plant as nitrogen is assimilated into the proteins contained within the stem and leaves. The protein analysis was carried out by NRM laboratories. The results of the total plant protein analysis are shown in Figure 3.4. This shows the treatment with the highest concentration of protein in the harvested plant was treatment 7 with 14.4% protein as a percentage of dry matter. The treatments with the lowest mean plant protein content were 1 and 4 with the same amount of protein in the plant of 12.9%. The data for the protein content was normally distributed when tested using a Shapiro-Wilk test so it was analysed using an ANOVA. A Tukey's test in an ANOVA showed no significant differences between treatments when $p < 0.05$. The error bars on the graph in Figure 3.4 showing standard deviation show some overlap in protein concentration between treatments.

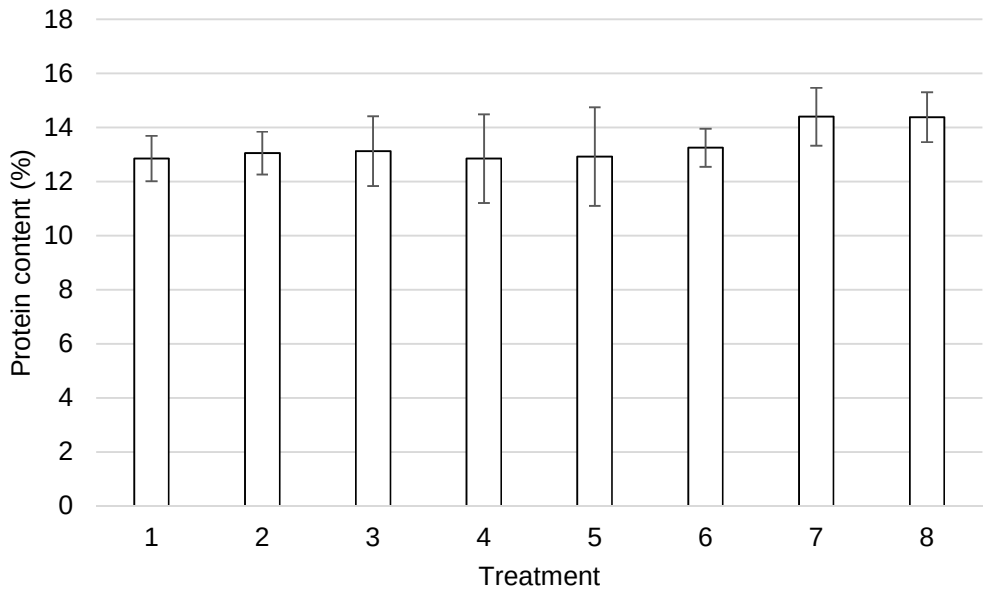


Figure 3.4 Mean protein percentage of dry matter in the harvested wheat plants with error bars showing standard deviation

3.5.1.4 Plant height and spike length

The results for the plant height and spike length are shown in Figure 3.5. This shows that the treatment 5 had the tallest plants after the growing period with a mean height of 509.3mm. The treatment with the shortest plants was treatment 3 with a mean height of 476.2mm. This data shows that the variation in the plant height over all the treatments was 33.1mm which represents only a 7% increase from treatment 3 (smallest) to treatment 5 (largest).

The data for the spike length shows that treatment 4 had the largest mean spike length at 79.4mm and treatment 7 had the smallest mean spike length of 72.7mm. This data shows that the variation in the spike length over all the treatments was 6.7mm which represents only a 9% increase from treatment 7 (shortest) to treatment 4 (longest).

The data for the plant height and spike length was not normally distributed according to a Shapiro-Wilk test so a cube transformation was performed on the data which normalised it. The results of an ANOVA using a Tukey's test

showed no significant differences between the treatments in terms of both plant height and spike length when $p < 0.05$.

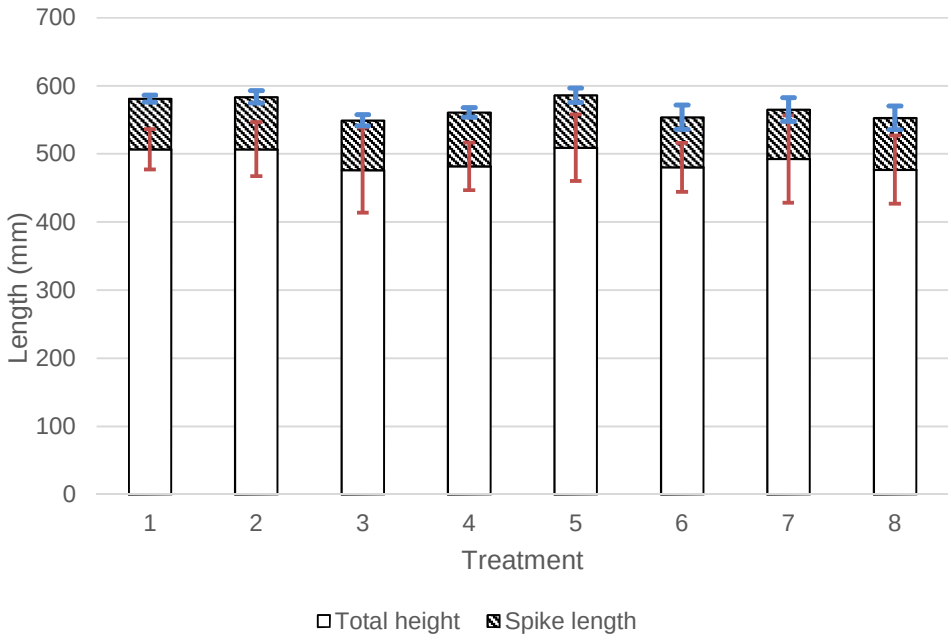


Figure 3.5 Mean values for plant total height and spike length with error bars showing standard deviation

3.5.1.4.1 Visual assessment of the plant growth

The photographs in Plate 3.2 to Plate 3.6 show the growth of the wheat plants by block at the end of the experiement. The grouped stems in the picture are 5 stems from each of the treatments within the block. The photographs support the data presented in 3.5.1.2 and 3.5.1.4 as they do not show any patterns in terms of growth or production of the plants between treatments when lined up side by side.

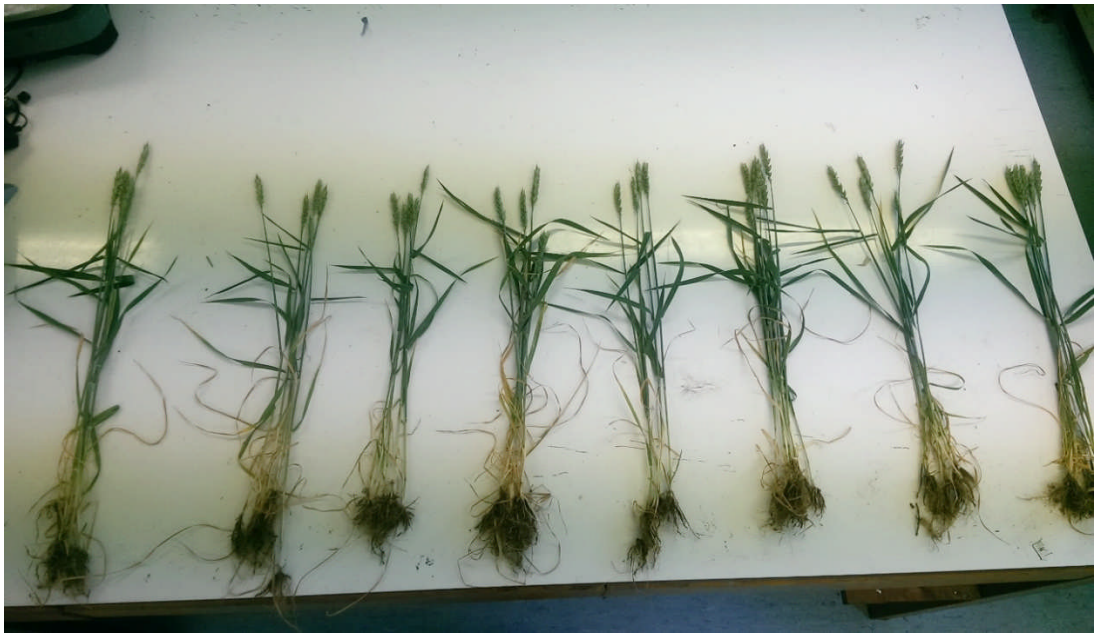


Plate 3.2 Growth of the plants in block 1, treatments 1-8 from left to right.

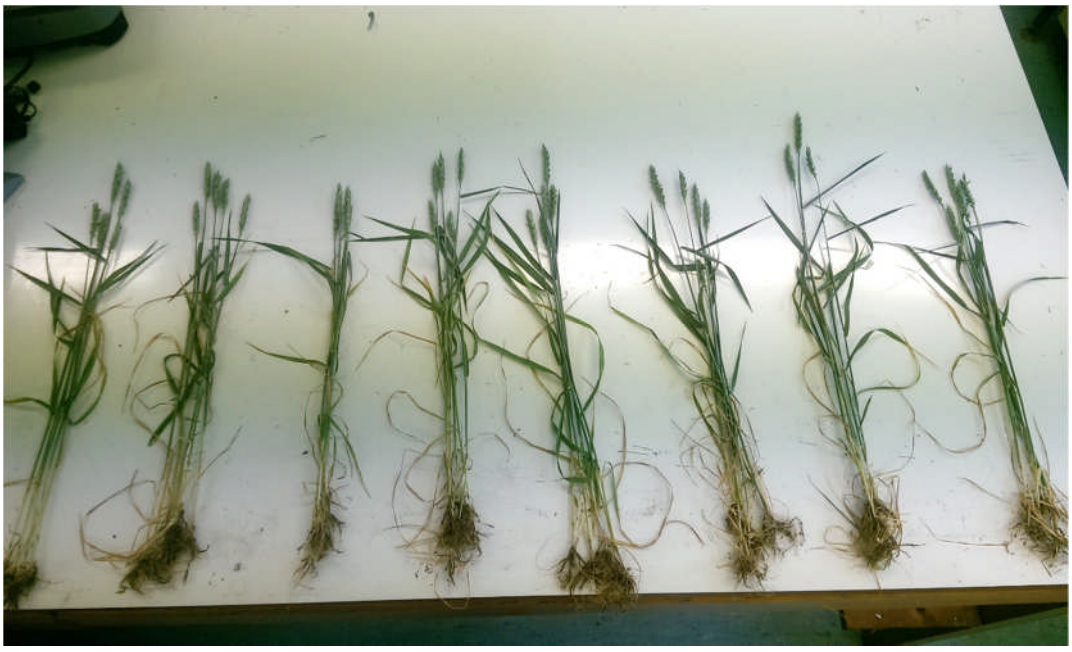


Plate 3.3 Growth of the plants in block 2, treatments 1-8 from left to right.

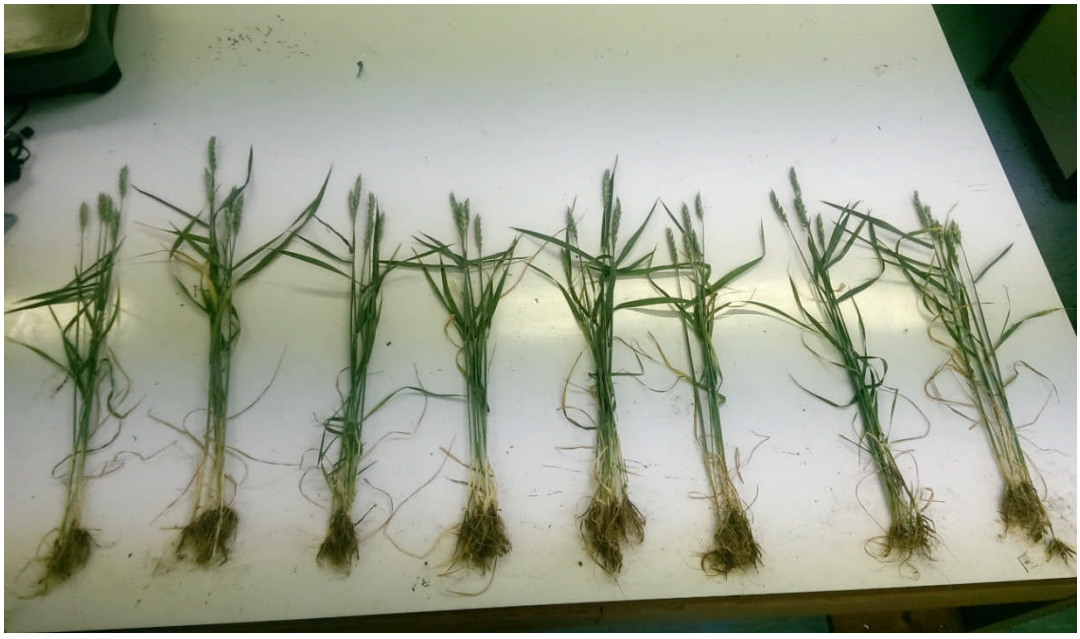


Plate 3.4 Growth of the plants in block 3, treatments 1-8 from left to right.

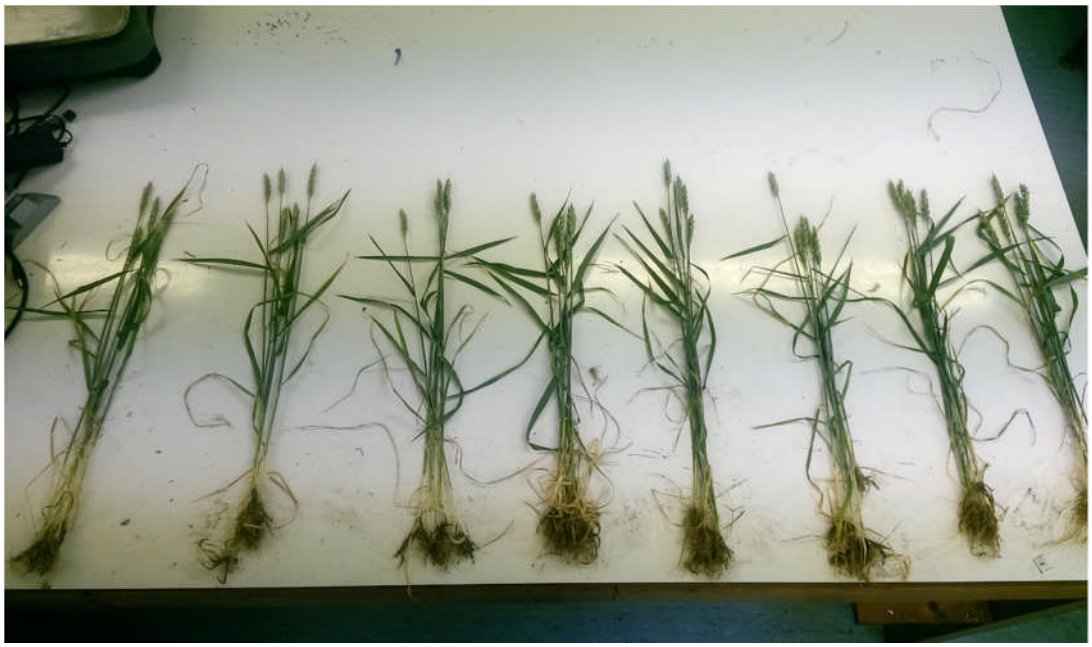


Plate 3.5 Growth of the plants in block 4, treatments 1-8 from left to right.

Plate 3.6 Growth of the plants in block 5, treatments 1-8 from left to right.

3.5.1.5 Number of grains

The number of grains produced per plant was determined on each of the 5 plants from each replicate and the results are given in Figure 3.6. The data is presented as a median value which gives the best representation when looking at count data which is non-parametric. The greatest number of grains per plant was observed for treatment 8 with 30 grains per plant. The treatment with the fewest number of grains was treatment 6 with 25 grains per plant. The data was not normally distributed which is expected as the data is non-parametric. A Kruskal-Wallis test using a Chi-Square was applied and did not show any significant differences in the number of grains between the treatments. As the yield was not measured on this trial that number of grains can be used as an indicator of the fertiliser effect on yield for the crop. From the results the fertiliser effect on yield can be said to be the same between all the treatments.

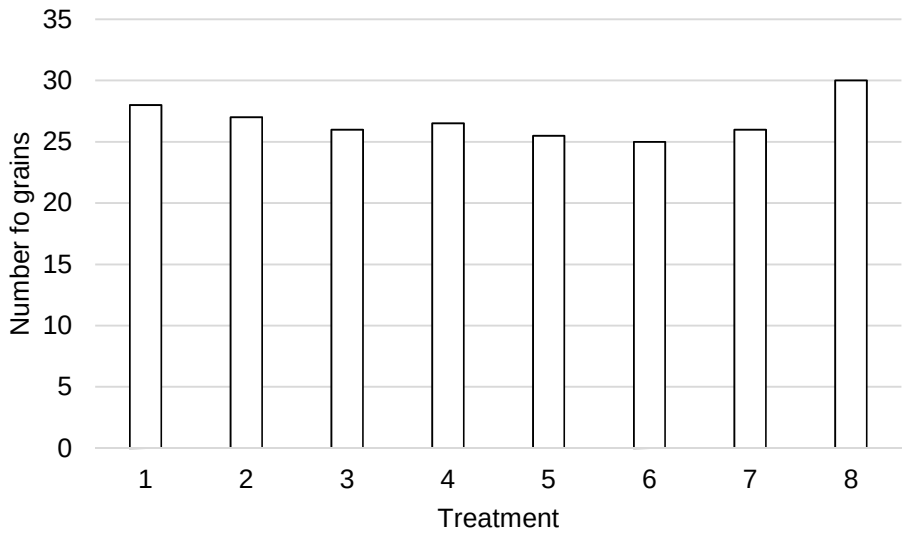


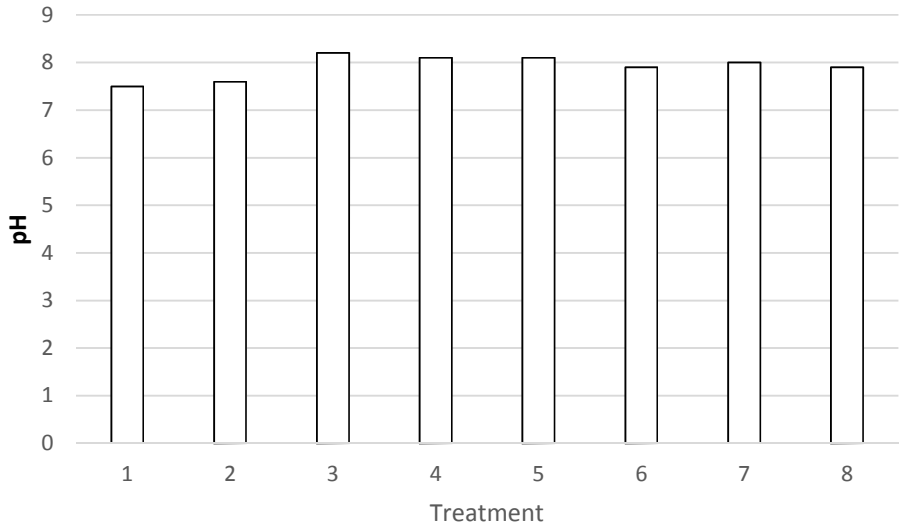
Figure 3.6 Median number of grains produced by the plants for each treatment

3.5.1.6 Soil analysis

The data for the soil analysis was normally distributed when analysed using a Shapiro-Wilk test, except for the data for the pH values. Applying three different transformations to the pH data still did not normalise it, so this is treated as non-parametric data. The results of the soil analysis are shown in Figure 3.7, Figure 3.8, Figure 3.9, Figure 3.10 and Figure 3.11.

The pH values are shown in Figure 3.7, these are shown as median values as the data was non-parametric. From the graph it can be seen that treatment 3 has the highest pH of 8.2 and that treatment 1 has the lowest median value of 7.5. A Kruskal-Wallis test showed significant differences between the data, and to analyse these further a post hoc, Man-Whitney U test was performed to compare treatments directly against each other. The Mann-Whitney test showed that treatments 1 and 2 significantly different from all the other treatments for pH, this is shown in the table included as part of Figure 3.7 in the superscript notation. Treatments 1 and 2 are the ones that received the standard ammonium nitrate fertiliser as a source of nitrogen, whereas all other treatments received nitrogen in an organic form. In Figure 3.7 the pH values show that the treatments that received biochar had a lower pH than the

equivalent treatment without biochar except for the control treatment, shown in the decreases shown from 3-4, 5-6 and 7-8.



Treatment differences in pH							
1[a]	2[a]	3[b]	4[b]	5[b]	6[b]	7[b]	8[b]

Figure 3.7 The pH values for the soil at the end of the trial period

The results for the conductivity in the soil samples are given in the graph in Figure 3.8. It is apparent from the graph that there is a degree of variation in the conductivity levels for the different treatments. The treatment with the highest conductivity was treatment 2 (mineral fertiliser with biochar) with a value of 160.6 µS/cm. The lowest conductivity was found in treatment 3 (pig manure based OMW with no biochar) with value of 96.6 µS/cm. All the treatments show an increased conductivity with the biochar addition. The data was normally distributed so a test for significance between the treatments could be carried out using an ANOVA. This showed that despite the apparent variability observed from the mean data in the graph there were no significant differences between treatments for conductivity.

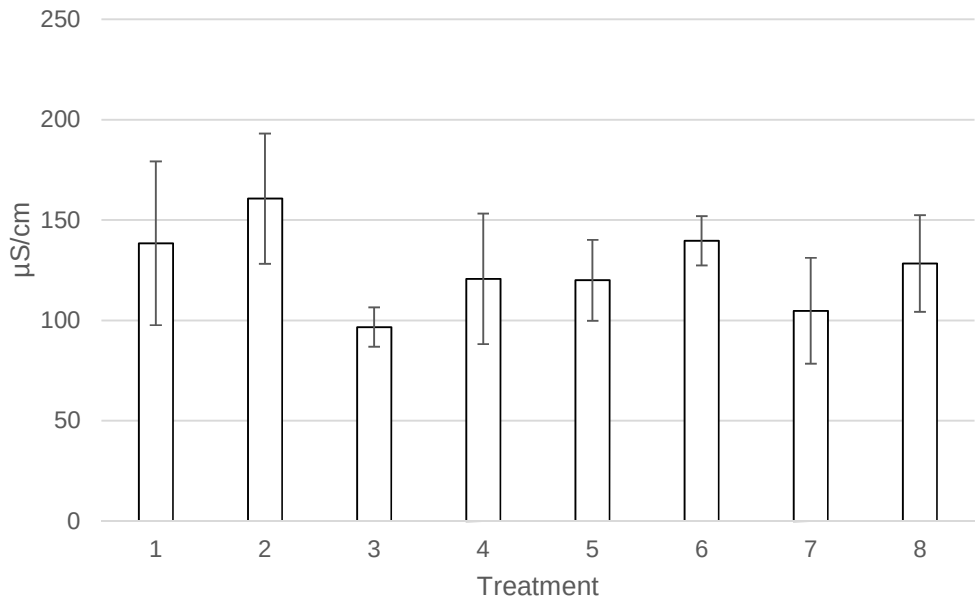


Figure 3.8 Mean conductivity of the treatments with the standard deviation shown by the error bars

The data for the ammonium results is shown in Figure 3.9, with error bars to indicate the standard deviation. The graph shows that the highest concentrations of ammonium were recorded in treatment 8 with a mean value of 4.3 mg/kg and that the data for this treatment varied between replicate measurements as illustrated by the large standard deviation of 2.1. The treatment with the lowest concentrations of ammonium was treatment 5 with 1.4 mg/kg. The graph shows a trend in the treatments, with an increase in the concentrations of ammonium from treatment 5 through to 8. The increase between treatments 5 – 6 and 7 – 8 suggests that treatments with a biochar amendment (6 and 8) retained more ammonium than those without a biochar amendment, although this pattern is not repeated between treatments 1 – 2 and 3 – 4.The only significant differences in the data are between treatments 5 and 8, with 5 being significantly lower.

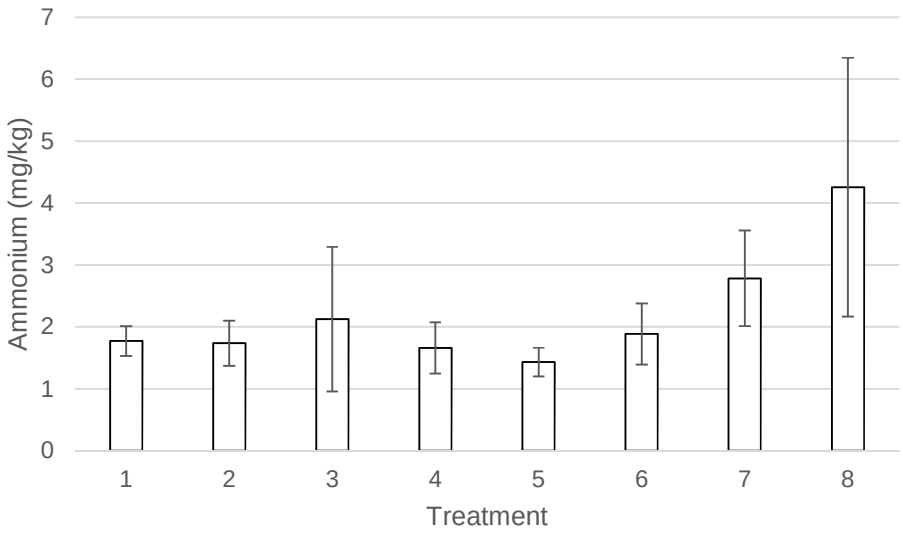
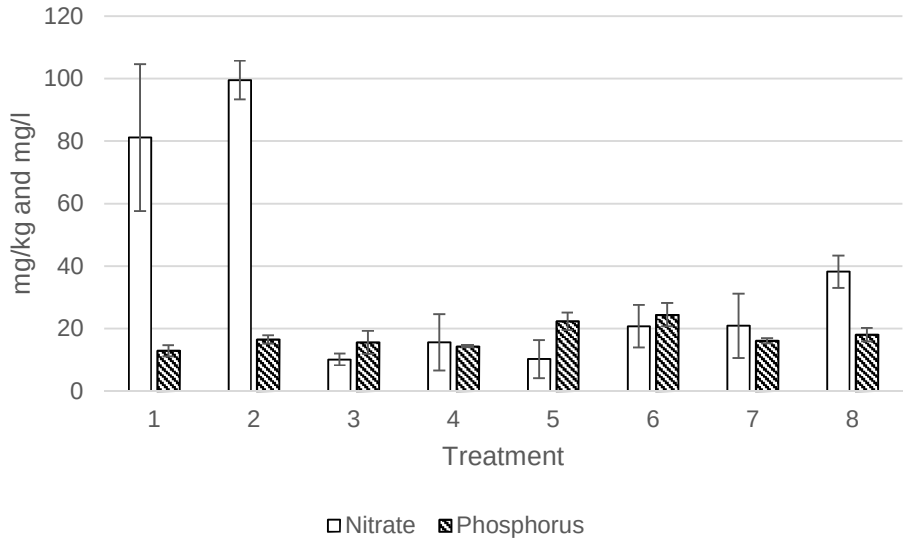


Figure 3.9 Mean values for ammonium within the soil samples with error bars demonstrating the standard deviation.

The results for the concentrations of nitrate and phosphorus in the soil samples are shown in Figure 3.10. This shows the concentrations of nitrate were much higher in treatments 1 and 2 than all other treatments, with the highest being in treatment 2 with 99.5 mg/kg nitrate. Treatment 1 had the largest standard deviation of all treatments for nitrate indicating a large amount of variation in the replicate analysis. The lowest levels of nitrate were recorded in treatment 3 with 10.1 mg/kg of nitrate in the sample. The results of the Tukey's test show that treatments 1 and 2 have significantly higher levels of nitrate than all other treatments. This is to be expected as the nitrogen applied in the mineral fertiliser is applied as nitrate, the nitrogen in the organic products has to be oxidised into nitrates to show up on an analysis. The same trend as the ammonium is seen in the nitrate too, with treatments with biochar applied having higher concentrations of nitrate than those without biochar.

The results for the phosphorus in the samples show that the highest mean concentration was from treatment 6 with 24.5 mg/l and the lowest in treatment 1 with 12.9 mg/l. The treatments that are significantly different from one another are indicated using the notation in the table as part of Figure 3.10. The main treatment with significant differences was treatment 6 that was significantly

higher than treatments 1 – 4 and treatment 7. The same pattern as measured in the nitrate and ammonium is also seen here with the phosphorus showing higher concentrations in those treatments that received biochar than those that did not.

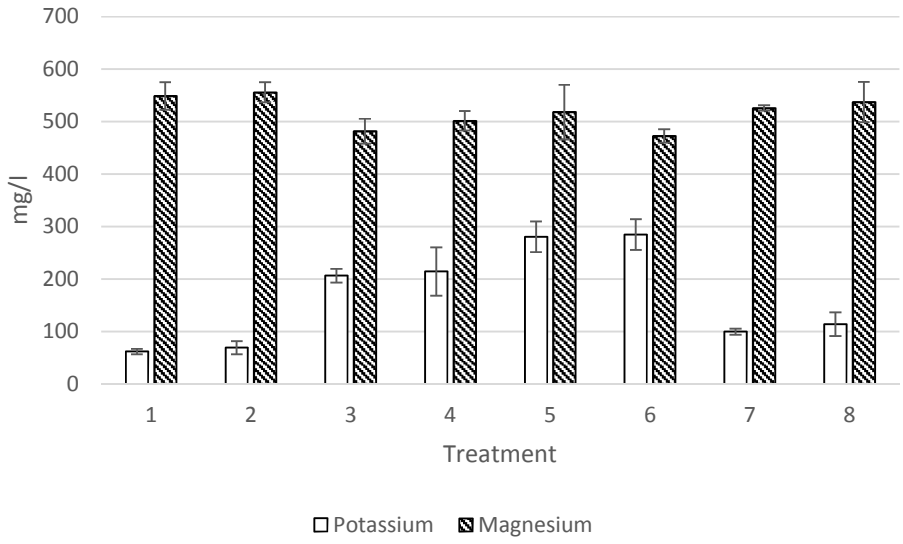


Treatment – differences in phosphorus concentrations							
1^{c}	2^{bc}	3^{bc}	4^{c}	5^{b}	6^{ab}	7^{bc}	8^{abc}

Figure 3.10 Mean levels of nitrate (mg/kg) and phosphorus (mg/l) in the soil samples with standard deviation shown on the error bars.

The results for the potassium and magnesium found in the soil samples are shown in Figure 3.11. The concentrations of potassium were highest in treatment 6 with a value of 284.3mg/l and the lowest in treatment 1 at 66.6mg/l. The trend for this data showed increasing levels of potassium from treatments 1 – 6 then lower again for treatments 7 and 8. This shows that the treatments receiving the OMW compost had higher levels of potassium than treatments receiving the mineral fertiliser, and those receiving a chicken manure fertiliser. There were significant differences in the concentrations of potassium present between the treatments. Notation describing these significances is given in the table as part of Figure 3.11.

The results for magnesium show that treatment 2 had the highest value of 555.6mg/l. The lowest concentrations of magnesium were recorded from treatment 6 with 472.3mg/l found in the samples. These are the only treatments that showed significant differences between each other for magnesium, with treatment 2 having significantly more magnesium in the samples than treatment 6.



Treatment differences in potassium							
1^{a}	2^{a}	3^{bc}	4^{bcd}	5^{cd}	6^{d}	7^{a}	8^{a}

Figure 3.11 Mean levels of potassium and magnesium in the samples, with the standard deviation shown on the error bars.

3.6 Discussion

The objective of these trials was to study the effects of different organic treatment combinations against a mineral fertiliser with and without the addition of a biochar amendment. These are split into two sections similar to the results section of plant characteristics and soil analysis.

3.6.1 Plant characteristics

The results from the data for all the plant characteristics showed no significant differences between treatments. This would suggest that all of the treatments produced plants with a similar number of grains, a similar height, and similar fresh and dry weight. Looking at the values for chlorophyll (leaves) and protein content at harvest (whole plant) they also showed no significant differences across all the different treatments. The assumption from this data is that the nutrient uptake and storage in the plants was similar regardless of the treatment applied. The treatments all had nitrogen applied at the same rate, which has led to the plant's production and development being similar to one another. This would suggest that the nitrogen uptake of the plants is consistent across all treatments regardless of the form the nitrogen is applied in. This is a similar observation to the work completed by Quillam in 2012, and also to the large scale arable trials carried out in 2013 and 2014 discussed in Chapter 2 Quilliam (2012) found that there was no significant differences in the chlorophyll concentrations in the leaves comparing plots that received 0MKg/ha, 25MKg/ha, or 50MKg/ha of biochar over three years, with half of the treated plots receiving a double dose in year 3 (25MKg/ha + 25MKg/ha and 50MKg/ha + 50MKg/ha) when trialled on dwarf beans in Wales (Quilliam et al., 2012). No additional fertiliser was applied in the trials by Quillam.

The field trials in Chapter 2 on winter wheat showed no significant differences in chlorophyll concentrations between treatments that received no biochar and those that received 10MKg/ha of biochar. It also shows that in this instance the addition of a biochar amendment had no impact on the nitrogen assimilation by the wheat plants. By the similar success of each treatment in this case it is shown that none of them are phytotoxic when applied at these levels. They also don't have a detrimental effect on germination or early developmental stages of the plant. This is perhaps in part due to the short length of the trials as the addition of a biochar amendment has more of an accumulative long term effect on nutrients in the soil. This is thought in part to be the result of reducing leaching and therefore making more nutrients plant available. Cumulative effects of biochar additions at higher rates than 10mkg/ha were shown in the work by Jones (2012). In these trials biochar was applied at the same rates as

the work by Quilliam (2012) (0MKG/ha, 25MKg/ha and 50MKg/ha only) and the grass grown in year 2 and 3 of the trial on plots treated with biochar showed greater foliar N uptake than those that received no biochar (Jones et al., 2012)

As the measurements taken from the trials for the large scale arable work were slightly different, and no yield measurements were taken from the pot trials they are difficult to compare directly. The results for the large scale arable trials for WW in 2013 and 2014 showed no difference in yield between treatments that received biochar and those that did not. This compares to trials completed by Quillam in 2012 (Quilliam et al., 2012) in the trials described above. The pot trials also showed differences between the different fertiliser treatments with biochar and those without when it comes to measuring plant development by size and protein content, and by the number of grains. The biochar in these trials was applied at the same rate as the large scale arable trials.

The different characteristics of the materials applied, apart from their nitrogen content, had no significant impact on plant growth. The short length of the trial could be a factor in this, and had the plants been able to finish their growth cycle, some differences may have been apparent due to the nutrients available in the amendments.

Much of the research in this area compares the growth with OMW amendments (treated and untreated) with a control plot that receives no nutrient amendment. In studies such as those of Rinaldi in 2003 where plots had OMW applied or did not, an OMW amendment produced a similar crop yield to a water only application (Rinaldi et al., 2003). Santonoceto in 2010 and Mekki in 2006 also completed similar experiments with the OMW amendment only compared to a control receiving water and no nutrients (Santonoceto et al., 2010, Mekki et al., 2006a). This trial aimed to replicate a realistic situation of crop production and to achieve this a typical mineral fertiliser was used as a control as it was considered the best situation to realistically reflect the most likely scenario for the majority of UK famers.

3.6.2 Soil analysis

The results of the soil analysis between the treatments did show some significant differences. The pH of the soil in the analysis showed that treatments 1 and 2 had a significantly lower pH than all other treatments. The pH of the organic amendments were all alkaline, ranging from 8.7 – 10.1 and the pH of ammonium nitrate was acidic, typically a pH around 5.4. This would account for the lower pH in the soil following harvest. Lowering the pH of the soil makes it easier for the plants to absorb ammonium, whereas a higher soil pH makes it easier for the plant to absorb nitrates (Masclaux-Daubresse et al., 2010). The pattern in the pH results showed that the treatments that received biochar had lower pH than the equivalent treatment without biochar. The lower pH of the biochar treated pots could be a result of the biochar adsorbing more nutrients onto its surface, shown in the pattern of increased levels of nitrate and ammonia in the treatments with biochar, which in turn lowered the pH of the soil following harvest.

There were no differences in the conductivity between the treatments, which is interesting as both the OMW composts had much higher EC than the chicken manure compost, the value for chicken manure was 5830µS/cm with the OMW compost value ranging from 11610 - 14000 µS/cm. The high conductivity of the OMW had a negative impact on plant growth for the strawberry plants in Chapter 5, but in the wheat trials the high conductivity did not affect growth or have any effects on plant mortality. This was probably due to the small amount of OMW compost used in the wheat pot trial, being applied based on nitrogen loading rates instead of the typical higher rates used for compost.

The levels of ammonium were significantly lower in treatment 5 with the chicken based OMW than in treatment 8 with the chicken manure pellets. This was to be expected as the chicken manure pellets in Table 3.2 had over four times the amount of ammonium present compared to the chicken manure based OMW. The results showed that the treatments using chicken manure (7 and 8) and the treatments using chicken manure based OMW (5 and 6) both had an increasing amount of ammonium remaining in the soil when biochar was added. The percentage of ammonium present at the end in comparison to

the concentrations in the materials that were applied was similar for the OMW products with the percentage remaining in the sample post-harvest ranging from 1.1 – 1.5%. The amount remaining from the chicken manure was lower with values at 0.3 and 0.5% for the treatments without and with biochar respectively. The long term effects of biochar addition could be to improve the retention of ammonium in the soil to allow its application to be reduced in future years.

The levels of nitrate were significantly higher in the treatments that received the ammonium nitrate fertiliser than all other organic fertilisers that were applied. This could suggest that as the nitrogen in the mineral fertiliser is presented in a more assimilable form that there is a greater amount left in the soil as the plant uses only what it needs. In all cases for nitrate the treatments with the biochar addition showed increased levels of nitrate in the soil at the end of the trial would which suggest that the biochar was assisting in nutrient adsorption and retention and as a result reducing nutrient leaching and loss to the atmosphere (Stavi and Lal, 2013). This is a similar result to the meta-analysis of studies on biochar completed by Biederman and Harpole in 2013 (Biederman and Harpole, 2013). Biederman (2013) analysed the results of 371 studies taken from 114 published manuscripts. It was found that disregarding different soils and climate, on average a biochar amendment improved the crop yield and total soil nitrogen when compared to control conditions. In relation to soil chemistry the aim of applying biochar is to improve nutrient retention and reduce fertiliser use in subsequent years.

Treatment 6 with the chicken based OMW with biochar addition had higher levels of phosphorus present than all other treatments except for treatment 5 and 8. This is at odds with the chemical analysis of the amendments as the chicken manure pellets amendment has higher levels of phosphorus than the chicken manure based OMW.

The results for magnesium are perhaps an anomaly, as treatment 2 with the mineral fertiliser and biochar addition had significantly more magnesium than treatment 6. Treatment 6 was the chicken based OMW with biochar, with the

chicken based OMW product having the highest magnesium content of all of the nutrient amendments.

The levels of potassium in the soils following harvest was highest in all treatments receiving an OMW compost amendment. This corresponds to the potassium content of the OMW application which is higher than the chicken manure amendment as given in Table 3.2, and the mineral fertiliser contains no potassium. The levels of potassium in the treatments that received biochar as an amendment were higher than in the treatment with the same fertiliser. This would suggest that the biochar was assisting in retaining the potassium within the soil and preventing it from being leached out, and also that biochar was acting potentially as a source of potassium. Biederman in 2013 recorded similar results for most studies that used biochar as an amendment to soil (Biederman and Harpole, 2013).In the meta-analysis by Biederman (2013) the application of a biochar amendment in 371 studies was found on average to increase the levels of potassium in the soil.

3.7 Conclusions

The trials in this chapter show that for the plant monitoring aspects of the results there are no significant differences in any of the treatments. The results also show that when applied at a nitrogen loading rate the chicken manure, chicken manure based OMW, pig manure based OMW all compare similarly with each other. This suggests the same as the results from Chapter 2 with no differences in plant development as a result of different fertiliser treatments. The biochar addition to the treatments also made no difference to an of the plant development characterisations.

Chapter 4 Horticultural trials 2014

4.1 Introduction

This chapter will discuss in detail the pot trials carried out in 2014 using strawberries as a crop to test the effect of different substrates on the development and yield of strawberry plants and fruit characteristics. This will consist of background information to support the data and a review of the previous work carried out in this area in addition to a detailed account of the methodology used in this study, and a full description and discussion of the results from trials carried out in 2014. The work described in this chapter leads into the research described in Chapter 5 Horticultural trials in 2015.

The trials in this chapter used two forms of composted OMW as compost replacers, these were both produced with chicken manure as a base material. In addition to this a pelletised chicken manure was utilised as a fertiliser and also a biochar amendment was applied as an organic amendment. It is expected that the chicken manure fertiliser will provide too much nitrogen for the plants and that the biochar addition may reduce fungal pathogens on the fruit.

4.2 Literature review

4.2.1 Strawberry plants and their fruit

4.2.1.1 Development and popularity

As a result of its popularity as a tasty and nutritious food source the strawberry *Fragaria x ananassa* has been subject to extensive selective breeding and genetic selection to produce different varieties able to withstand specific conditions. The development of these varieties began as early as Roman times with the first mention being made around AD23, and the first discussion of

cultivation appearing in France in the 1300s (Hancock, 1999). The first studies on the genetics of strawberries began in the 1700s. The two most prominent 60 day varieties that are grown in the UK are Elsanta and Sonata which are prized for their large, well-shaped fruit with a good colour. Hancock (1999) lists 112 strawberry varieties that are grown globally. Having this number of varieties means that there are strawberry plants to suit most climates and as a result strawberries are successfully grown on every habited continent with the largest producer of fruit being the United States. The strawberry fruit is the most widely cultivated and most in demand berry species on a global basis (Garriga et al., 2015). Throughout the period 1990 to 2011 the US was the largest producer of strawberries (ERS, 2012, Hancock, 1999) and the UK was the 13th largest producer producing 92 000 tonnes in 2011 (ERS, 2012).

The success of the annual crop is important to the sustainability of the crop being produced and its value. The strawberry crop in the UK in 2014 was valued at £244 million for that year, with total production of strawberries at 104 thousand tonnes of fruit which was a new high for this crop (DEFRA, 2015b). It is due to this large consumption of strawberries and the widespread commercial and economic benefits that the fruit creates that it is perhaps the most studied berry in terms of nutrition, its genome and the agronomics surrounding its production (Giampieri et al., 2014).

4.2.1.2 Plant physiology

The growth of a strawberry plant is split into vegetative and fruiting stages. All fruits produced in any one year from a plant will have been laid down in the crown in the previous autumn, as soon as daylight hours begin to shorten the plants prepare for the next season's growth. The fruit grow on trusses from the crown of the plant and the plants produce fruit on the truss in order and produce primary fruit, followed by secondary, tertiary and in some cases quaternary fruit. Due to their physiology this leads to the plants flowering in several successive stages throughout a season, with the flowers decreasing in size each time (Klatt et al., 2014). Strawberry fruits are not a true berry but are composed of numerous ovaries making up many fruiting cells that structure one

fruit, referred to as an ‘aggregate accessory fruit’ (Agarwal, 2013, Hancock, 1999).

A diagram showing the main parts of a growing strawberry plant are shown in Figure 4.1. Stolons, or runners, are produced from healthy strawberry plants to propagate daughter plants (Hughes, 1970), with the mother plants capable of transferring water, nutrients and assimilates for over a year (Hancock, 1999). Stolons are horizontal above ground connections between plants, this is in contrast to rhizomes which perform a similar function to stolons but grow horizontally underneath the soil surface (Rose et al., 2006). Healthy plants of the *F x ananassa* can produce up to 10-15 stolons per plant. Stolon production is optimised when day length is greater than ten hours, and within a temperature range of 21-30^{o}C (Hancock, 1999). Strawberry leaves are usually trifoliate, being composed of three leaflets, a new leaf is grown every 8 to 12 days when conditions are favourable (Strand, 2008)

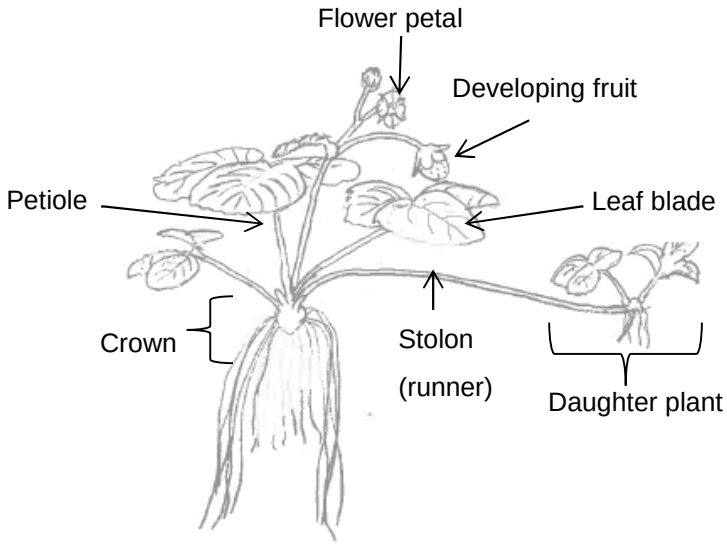


Figure 4.1 Diagram showing the parts of a growing strawberry plant with fruit growing. Adapted from (Strand, 2008)

4.2.1.3 Health benefits of strawberries

The popularity of strawberry fruit as a commodity and as an aid to a healthy lifestyle is rising due to their abundance of nutrients, sweet flavour and the fact

that they are priced at a reasonable level (Pokhrel et al., 2015). Strawberries contain nutrients that are thought to improve health benefits beyond our basic nutritional needs, for example they are a major source of dietary antioxidants (Wang and Lin, 2003, Paredes-López et al., 2010). Some studies have shown strawberries to have had beneficial effects in combatting cancer due to these antioxidant properties and the ability of the ascorbic acid within the fruit to reduce the proliferation of cancer cells (Olsson et al., 2006, Fan et al., 2012). This benefit is thought to be due to the strawberries ability to reduce and reverse the damage brought about through oxidative stress (Seeram, 2008). As well as the potential health benefits for cancer, they are also referred to as being heart healthy and having positive impacts in combatting cardiovascular disease. Their cardiovascular beneficial components are bound in the chemical composition with polyphenols, anthocyanins, vitamin C, and folate all important for health (Agarwal, 2013, Vandendriessche et al., 2013).

4.2.2 Use of peat and other growing media for strawberry production

The total area covered by peat soils in the UK is approximately 1.65 million hectares, of which around 70 000 hectares are lowland raised bogs, which is where the peat used for horticultural use is harvested (Alexander et al., 2008). The harvesting of peat involves the draining and cutting of peatlands and in 1993 the total area of peat extraction for horticulture was reduced to 5400ha (Robertson, 1993), this has later changed to only 4000ha (Alexander et al., 2008). Peat plays an important function in sequestering carbon and therefore plays a role in the alleviation of climate change. When peat is forming from *Sphagnum* spp. mosses it takes up carbon from the atmosphere and stores it (House et al., 2010). In addition to this important role, peat also provides ecosystem services in flood alleviation, water filtration and provision of habitat to maintain ecological biodiversity and a refuge for protected species such as the golden plover *Pluvialis apricaria* (Holden et al., 2007) nightjar *Caprimulgus europaeus* , reed bunting *Emberiza schoeniclus* and adder *Vipera berus* (Wildlife, 2010). The destruction of peat resources is problematic as they are

habitats that are difficult to recreate due to their lifecycle and ecosystem function (Ledoux et al., 2000).

The use of peat as a growing media for food is prolific in the UK both in commercial and domestic production, this is due to its qualities of being very stable both physically and chemically and being slow to degrade (Farrell and Jones, 2010). On a global scale peat is the predominant source of a substrate material for potted plants (Altieri et al., 2014). The increasing costs of using peat as a soilless growth media (Herrera et al., 2008) as well as pressure from environmental groups such as Natural England, Scottish Natural Heritage and the RSPB to reduce the use of this finite resource have meant that alternatives to solely using peat for production have been examined. This need for a peat replacement is recognised within the UK horticulture sector, but it is not a switch that can be made overnight. There are positive trends however in both the retail/domestic and commercial sectors regarding the use of peat. Total use of peat within domestic and commercial settings fell between 2011 and 2014 from 58.2% (1.83Mm^3) to 51.1% (1.39Mm^3) and 72% (0.93Mm^3) to 65.2% (0.72Mm^3) of total growing media used respectively (AHDB, 2015a).

One of the most important attributes of growing media is to provide a structure within the pot containing air-filled pores to allow the substrate to be free draining and to provide space for gas exchange (Evans, 2004, Evans and Gachukia, 2007, Olle et al., 2012). Food producers in the UK have other sources to exploit as growing media. The use of coir from coconut husks as well as perlite and wood fibre are substrates which negate the need for peat. Coconut fibre or coir dust have similar physical properties to peat having high physical stability (Cresswell, 2002) in most cases, however their properties are also largely dependent on their origin (Abad et al., 2005). Perlite is inorganic and is produced from mining aluminosilicate which is then heated to produce a suitable product (Evans, 2004, Evans and Gachukia, 2007). Experiments with tomato *Lycopersicon esculentum* showed no difference in fruit yields when grown in various peat, perlite and coir combinations (Arenas et al., 2002).

There have been many studies using composted waste as a peat substitute. Successful substitutions, those that performed better, or similarly to peat as a

control were using combinations of peat with composted water hyacinth to grow:

- Chinese cabbage at 50% substitution (Fan et al., 2015),
- composted food and green waste on sunflowers at 75% substitution (Farrell and Jones, 2010),
- municipal solid waste on tomatoes at 30% substitution (Herrera et al., 2008); and
- spent tea grinds to grow Lantanas up to 100% substitution when co-composted (Wells et al., 2012).

4.2.3 Effects of using alternative media for growing plants: control of pathogens

Controlling soil and substrate borne pathogens in crop production has changed since the implementation of the Montreal Protocol on Substances which Deplete the Ozone Layer (the Montreal Protocol) in 1989 which banned the use of methyl bromide, a common soil fumigant and a prominent ozone depleting substance (ODS) (UNEP, 2015, Gareau, 2010). The ban came into force for developed countries from 2005 and worldwide by 2015 (Millner et al., 2004). Methyl bromide was an effective and economically viable fumigant and worked well in systems of high density cropping with the same plants produced year on year leading to a build of damaging biological factors (Ajwa et al., 2003). There are chemical alternatives to methyl bromide in; chloropicrin, metam sodium, 1,3-dichloropropene, methyl iodide and propargyl bromide which are legal for use (Leandro et al., 2007b, Ajwa et al., 2003, Millner et al., 2004), however with an increase in the production of organic food, it is important to look at biological alternatives in controlling pathogens.

There are some biological fungal controls in the form of *Trichoderma* spp strains that are present in soil and show aggressive responses to soil pathogens (Leandro et al., 2007a, Leandro et al., 2007b). A reduction in plant pathogens from the use of compost is known to occur and be beneficial to different plant species which in turn can lead to improvements in plant growth. This has been demonstrated in some instances when growing strawberries

(Wang and Lin, 2003, Leandro et al., 2007b) although not when looking at black root rot (Millner et al., 2004) Millner found that both poultry and cow manure composts at 5%,10% and 20% rates had significant control over the *Phythopthora fragariae* disease versus unamended soils. In the same experiment the occurrence of black root rot was low across all treatments, and no treatments had roots that were symptomatic of the disease (Millner et al., 2004).

Grey mould *Botrytis cinerea* is a fungus which affects strawberry fruit by causing rotting of the green and ripening fruits and can cause the tell-tale appearance of grey, dusty spores on the fruit's surface (Hughes, 1970, Hancock, 1999) this lifecycle strategy is referred to as being necrotrophic (Meller Harel et al., 2012). This disease is widespread and the most likely cause of fruit rot in strawberries (Hancock, 1999). Dead plant matter provides an excellent habitat for the *Botrytis* to thrive and fruit that are in contact with the soil are most at risk. The incidence of grey mould in strawberry fruit can be exacerbated by environmental conditions, if there is not enough air flow or the conditions are kept too humid (Hughes, 1970).

Biochar can have beneficial effects on soil borne pathogens and in particular in some cases on the reduction of grey mould *B. cinerea* infection in strawberry fruit. The first record of a charcoal product reducing the occurrence of pathogens in nursery stock was in 1915. In more recent years biochar has been trialled on asparagus and cucumber and has been successful in reducing the occurrence of soil borne pathogens from the *Fusarium* and *Rhizochtonia* genus for these crops (Mehari et al., 2015). The application of biochar to fruit crops has been shown to have benefits on the quality of the fruit produced through a reduction in the number of fungal pathogens attacking the produce (Meller Harel et al., 2012).

4.2.4 Substrates produced from waste products used on strawberries

The use of composts produced from olive mill wastes in crop trials with strawberry were carried out by Altieri et al in 2010. This research showed a

positive effect of using a compost produced with solid olive mill waste, using wool waste, wheat straw and sawdust as bulking agents, and stored for three months under aerobic conditions before being used as a peat substitute for strawberries. The trials combined the OMW compost with peat at 25%, 50% and 75% v/v ratios. The addition of a liquid fertiliser to the trials meant that the substituted compost performed adequately alongside the peat and therefore could be a viable alternative to growers (Altieri et al., 2010). Municipal solid waste compost (MSWC) and a compost tea made from MSWC were compared in a trial by Hargreaves, Adl and Warman in 2008. The strawberry fruit produced in these trials using two treatments with a foliar compost tea application and three different N rates of MSWC application, added to the soil at 75 kg N/ha, 150 kg N/ha, and 300 kg N/ha. The highest rate of MSWC had a negative impact on fruit production, attributed to salt content of the compost, but all the composts had lower than expected yields, although all had a similar quality of fruit produced (Hargreaves et al., 2009).

Vermicompost produced from vegetable waste and cow dung provided the alternative fertiliser base for Singh *et al* 2008. Their experiment showed that an application rate of 7.5Mkg/ha of vermicompost onto the soil along with an inorganic fertiliser produced the best results. The results included a reduction in albinism, fruit malformation and grey mould occurrence indicating that the addition of vermicompost had an effect by reducing these disorders of the fruit, and as a result, increased the yield over the control of inorganic fertiliser (Singh et al., 2008). In more recent work by Singh et al in 2010 it was found that the yield and quality of strawberry fruit was improved, and disease occurrence reduced with a foliar vermicompost leachate application in comparison to a water only application (Singh et al., 2010b).

4.3 Aims and Objectives

The aim of these trials was to assess and analyse the effects of using alternative treatments to peat on the health and production of strawberry plants and fruit. These trials used a compost produced using olive mill wastewater

which is a by-product of olive oil production. This was composted with manures and green wastes to create a substitute product.

There were three main objectives of this research:

Objective 1: To compare the effects of liquid fertiliser with chicken manure as a replacement fertiliser on strawberry growth with and without the addition of biochar.

Objective 2: To compare ratios of OMW compost varieties and their effects on strawberry growth with and without the interaction with biochar.

Objective 3: To compare the addition of a significant amount of biochar at a 50% rate and its effect on strawberry growth.

4.4 Methodology

4.4.1 Trial set up

The horticultural trials in 2014 were conducted at the Leeds University farm located in Tadcaster, West Yorkshire (lat. 53º 86' long. -1º 33' alt.55m). The farm has facilities for growing crops outside in the field and also undercover, in these trials the plants were grown undercover in a polytunnel in a bench set up.

Two varieties of OMW compost were trialled, the first was twice composted chicken manure with OMW (OMWa) and the second was three times composted chicken manure with OMW which was then sun dried (OMWb).

The OMW compost product used in these trials was developed using repeated composting and was carried out at the Technological Educational Institute of Crete (TEIC). The two compost products were composted two (OMWa) and three times (OMWb) respectively with chicken manure as the base component. The initial composting stage for both products was carried out in windrows using chicken manure mixed with green waste as the bulking agent. OMW was sprayed onto the windrows in order to control the moisture content of the material and as an alternative to using fresh water or leachate. During this initial composting period over 132 days the windrows were turned a total of 14

times. The windrows were 50m long, 2m wide and 1.1m high. Photographs showing this stage of composting are shown in Plate 4.1.

Plate 4.1 Photographs showing the initial composting period

At the beginning of the second composting period, again for both products, fresh chicken manure was added with the compost from the first stage being used as the bulking agent. The windrows were turned 8 times with 2.8l of OMW added at each turning. This second composting period was 70 days long with windrows 30m long, 2m wide and 1.1m high, photographs showing this period are shown in Plate 4.2.

Plate 4.2 Photographs showing the 2nd composting period

For the OMWb product an additional composting period was carried out in which a portion of the final product from the second composting stage was used as a bulking agent for the third round of composting, with fresh chicken manure being added. The bulking agent was screened in order to separate it into fine and coarse particles to allow for two variations of the final compost, using either fine or coarse material as the bulking agent. The compost used in

these trials was the variant with the coarse bulking agent. This final composting stage took place in ring composters with 8 turnings over 96 days with a total of 70l of OMW added in that time. Photographs showing the method for this composting are shown in Plate 4.3.

Plate 4.3 Photographs showing the 3rd composting

The OMWb compost was then sun dried by spreading in a thin (15-20cm) layer over the floor in a glasshouse and this was turned daily over a period of 55 days with a total of 84l of OMW added in that time. This process is shown in Plate 4.4.

Plate 4.4 Photograph showing the sun drying process

After 55 days the material was then transferred into plastic tanks for the final drying for 115 days being turned daily with 260l of OMW added. This part of the process is shown in Plate 4.5.

Plate 4.5 Photograph showing the tanks used for the final stage of the process

The analyses of the composts used in these trials are shown in Table 4.1. The analysis of the biochar used in these trials was produced by Carbon Gold and the analysis for this is given in Chapter 2. The analysis shows that OMWb has a lower pH and higher conductivity than OMWa and also has higher phenols content. A raised conductivity can have benefits in nutrient transfer up to a point, levels higher than 3mS/cm have shown to have phytotoxic effects on plants, and strawberry plants are known to be salt sensitive (Parida and Das, 2005, D'Anna et al., 2003). (See Chapter 5 Section 5.2.1 for more information). The effects of high levels of phenols in the growth medium can have phytotoxic effects on plants, see Chapter 2 for more information. The levels of potassium were higher in OMWb being nearly double that of OMWa, also having higher levels of S, Cu, Zn and Na in OMWb. The chicken manure had a much greater ammonium nitrogen content than either of the OMW composts but with a

comparative amount of K and P to OMWa. The level of nitrogen in the form of nitrates was highest in OMWa with the chicken manure and OMWb having comparatively similar values. The chicken manure had much lower Ca and Mg levels than either OMW compost.

Table 4.1 Analysis of the OMW compost used in 2014

	Units	*Chicken manure*	*OMWa*	*OMWb*
pH water (1:2.5)		8.74	8.96	7.89
EC	µS/cm	5830	5320	7970
Total Phosphorus	% w/w	1.53	1.39	1.31
Total Potassium	% w/w	2.38	2.73	5.49
Total Magnesium	% w/w	0.697	1.29	1.12
Nitrate Nitrogen (fresh)	mg/kg	13.3	564	<10
Ammonium Nitrogen (fresh)	mg/kg	861	395	161
Total nitrogen	% w/w	4.48	2.41	2.6
Total Sulphur	% w/w	0.51	0.447	0.571
Total Copper	mg/kg	73	56.1	66.8
Total Zinc	mg/kg	461	344	564
Total Sodium	% w/w	0.447	0.374	0.51
Total Calcium	mg/kg	80340	163555	135914
Total Phenols (index)	mg/kg	<1	<1	4.9
Dry Matter (fresh)	%		83.8	89

The OMW compost (a and b) was trialled within a polytunnel using strawberries as the chosen crop since these are readily grown locally and over an extended season when grown undercover. They are a high value traditional English grown crop and also grown across Europe in many Mediterranean countries who also produce OMW. Spain, Italy and Turkey were among the world's top ten strawberry producers from 2001 – 2010 (FAOSTAT, 2014).

The strawberries in this trial were subject to treatment with chicken manure, OMW and biochar. The biochar used in these trials is produced commercially by Carbon Gold and has been described in more details in Chapter 2.

The strawberry variety chosen for this trial was a 60 day variety that will produce a crop on a 60 day cycle within the growing season; the variety used was Elsanta *Fragaria x ananassa*. Each of the different treatments had 5 replicates (pots) with each of these pots containing two strawberry plants, giving 10 plants for each treatment. This was following a similar methodology to Altieri (Altieri et al., 2010, Altieri et al., 2014). The different ratios of OMW product were mixed with a commercially produced peat free compost (PFC) in order to give the plants a suitable medium for growing in and taking root. The PFC and the OMW product were combined together and mixed by hand in each of the different ratios, with biochar added to those treatments which included it. The OMW composts were both dry to touch with a hard texture with varying sizes of particle within the mixture. An example of the OMW compost size, shape and texture can be seen in Plate 4.6.

Plate 4.6 OMWa compost as received for use in the strawberry trials

As discussed in section 4.3 there were three objectives of these trials, further information on these with details of the experimental units are described in the following sections.

Objective 1: To compare the effects of liquid fertiliser with chicken manure on strawberry growth with and without the addition of biochar.

The treatments used to achieve objective 1 are shown in Table 4.2.

Table 4.2 Treatments in objective 1 in the trials in 2014

Treatment number	Combination	Fertiliser type
1	Control: 100% peat free compost (PFC)	Liquid
2	PFC and biochar (BC) addition at 10t/ha	Liquid
3	PFC	Pelletised chicken manure
4	PFC + BC	Pelletised chicken manure

Objective 2: To compare ratios of OMW compost varieties and their effects on strawberry growth with and without the interaction with biochar. Details given in Table 4.3.

To achieve the objective 3 ratios of OMW compost incorporated with the base substrate were used. The biochar was added to the compost at a rate of 10Mkg/ha, which was calculated based on the surface area of the compost in the 2 l pots.

Table 4.3 Treatments used to achieve objective 2 in the trials in 2014

Treatment number	Combination	Fertiliser type
1	Control: 100% peat free compost (PFC)	Liquid
2	PFC and biochar (BC) addition at 10t/ha	Liquid
5	10% OMWb + 90% PFC	Liquid
6	25% OMWb + 75% PFC	Liquid
7	50% OMWb + 50% PFC	Liquid
8	10% OMWb + 90% PFC + BC	Liquid
9	25% OMWb + 75% PFC + BC	Liquid
11	10% OMWa + 90% PFC	Liquid
12	25% OMWa + 75% PFC	Liquid
13	50% OMWa + 50% PFC	Liquid
14	10% OMWa + 90% PFC + BC	Liquid
15	25% OMWa + 75% PFC + BC	Liquid

Objective 3: To determine whether biochar with PFC affected strawberry growth.

The treatments used to achieve this objective are given in Table 4.4. No OMW compost was added during this trial as this was a trial to determine the effects of having a high percentage of biochar. A high rate of biochar was chosen to enable any differences afforded by this addition to be magnified.

Table 4.4 Treatments for objective 3 in the trials in 2014

Treatment number	Combination	Fertiliser type
1	Control: 100% peat free compost (PFC)	Liquid
10	50%PFC + 50% biochar	Liquid

The ratios of OMW were chosen to show any changes over different levels of compost in the soil. These ratios were decided upon using similar experiments to those in the literature (Millner et al., 2004, Altieri et al., 2010) and to obtain the best results given the amount of raw materials available. The plants were purchased in a frozen state, allowed to defrost for 24 hours and then transplanted into pots, this took place on the 9 July 2014. Each of the pots was labelled twice, once on the pot using tape, and also with a plant marker labels. A drip irrigation system was set up to allow each pot to be watered separately; this allowed the watering to be relatively consistent across all of the treatments. The watering was automatic and programmed to take into account forecasted weather for the day. At the start of the trial the strawberries were watered three times a day for half an hour, after three weeks this was reduced to twice a day to allow for the change in ambient daily temperatures. This change was based on the substrate moisture content as conditions that are too damp can lead to increased risk of pathogens on the fruit.

The pots were arranged in a completely randomised layout on tables within the polytunnel to account for any variables such as heat and edge effects on the plants due to positioning. This was in a bench set up with three rows of plants in pots as shown in Figure 4.3. An ibutton® temperature logger was used to record daily temperatures every hour throughout the trial period. The average daily maximum and minimum temperatures within the polytunnel were calculated and are displayed in Figure 4.2. This shows the temperature gradually dropping throughout the trial period with the peaks and troughs showing the different temperatures between day and night. The lowest minimum temperature of 3.5^{o}C was recorded on 12 October, 3 days before the

last harvest date of fruit. The highest maximum temperature was 41.5^{o}C on the 17 July, a little over a week after transplanting from their frozen state. This temperature high is greater than the optimum temperature for strawberry fruit production, however given that the temperatures otherwise maintained day and night temperatures within a suitable range it is not expected that this adversely affected the fruit growth (Wang and Camp, 2000).

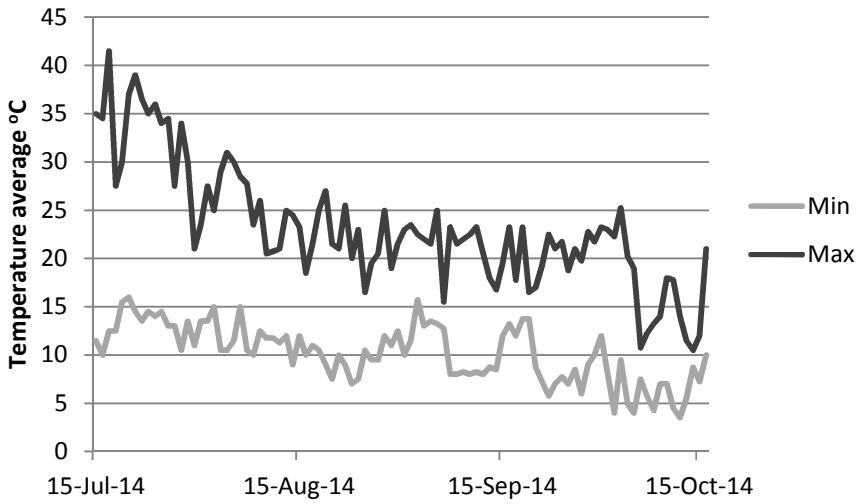


Figure 4.2 The average daily maximum and minimum temperatures throughout the growing and harvest season in the polytunnel where the strawberries were grown

The strawberries were covered during the fruit ripening period to prevent birds or rodents from harvesting the crop, the mesh allowed passage of insects to allow for pollination of the fruits. The setup is shown in Plate 4.77. Strawberries that do not receive adequate pollination will not develop into a marketable fruit, more on this can be read in Chapter 5 section 5.2.3.

Side	4	13	10	9	2	9	12	6	10	2	12	7	5	11	13	14	2	11	15	13	14	15	5	8	12
Middle	7	14	15	3	4	7	4	8	13	9	5	6	14	1	12	8	1	8	8	11	9	4	12	13	6
Aisle	1	1	10	7	1	3	3	14	2	10	2	5	9	15	11	4	3	3	5	6	10	6	7	11	15

Figure 4.3 The layout of the completely randomised pots within the polytunnel at the Leeds University farm (numbers refer to treatments shown in Table 4.2, Table 4.3 and Table 4.4)

Plate 4.7 The bench layout of the strawberry plants showing the net cover to prevent loss of fruit to pests.

The first application of fertiliser followed the appearance of the first flowers on the strawberry plants. The fertiliser used for this was Levington® Tomorite with a nitrogen:potassium:phosphorus ratio of 4:4.5:8. The strawberries were then fertilised twice a week with the liquid fertiliser designed for use on fruits at the recommended rate, the chicken manure fertiliser was applied once a week. The pelletised chicken manure was ground then spread on the compost and incorporated into the soil by hand. The chicken manure was applied less often as being a solid product the risk of nutrient leaching from the substrate was lower. The chicken manure was applied at the same nitrogen loading rate as the liquid fertiliser. This programme of fertilisation followed practices for strawberry fertilisation by hand as closely as possible.

4.4.2 Sampling fruits and substrate

Weekly monitoring of the strawberry plants was undertaken for a variety of different parameters. Chlorophyll levels in the leaves from each replicate plant

were measured using a Minolta soil plant analysis development (SPAD) meter. This was done on a weekly basis and the leaf chosen for measurement was selected at random each time. The number of flowers per replicate were counted every day for a week at the start of the flowering period and then twice weekly for the two following weeks. The number of fruits per replicate were counted from the end of July after the appearance of the first green fruit and then on 5 occasions on a weekly basis until the first harvest of ripe fruit. This was done to determine if any of the treatments produced more flowers or fruit, and whether it occurred earlier or later within the growing season.

The strawberries were harvested when ripe and then assessed for quality. The yield of strawberries in number per replicate and the weight of the marketable and discarded fruit was recorded. Assessing the marketability of fruits was completed in accordance with the Class I standard as detailed in Council Regulation (EC) No 1234/2007 (Council, 2000) . This is the standard used for high quality strawberries in the UK.

Flesh firmness, sugar content and width were measured on the first 10 marketable fruits from each replicate using a digital penetrometer and a refractometer. A Novanna FT02 penetrometer was used, which is ideal for strawberry flesh as it has a capacity of 1kg and a small gradation. Fruit firmness is a good indicator of shelf life for fruit produced and is generally an inherited quality (Hancock, 1999). For this test the penetrometer is pushed into the side of the fruit. For measuring sugars a Novanna MR200ATC Refractometer was used as it had a suitable range, resolution and accuracy for soft fruits and gives a measurement in Brix. Brix, or degrees Brix is a standard measure for sugar content with 1 degree equal to 1g of sucrose in 100g of solution (Boulton et al., 2013). For this test a few drops of juice from the fruit are squeezed onto the glass of the refractometer and then held up to the light. To maintain the consistency of all the measurements taken from the fruit, data was collected by the author only. The selection of the first ten fruits from each replicate means that it is likely that all of these were primary or secondary fruits which are the largest the plant generally produces throughout the season. Total yield from each plant from all harvests was calculated with intact fruits with a diameter greater than 18mm and a weight more than 4g representing

marketable yield. Smaller fruits, malformed and rotten fruits were counted, weighed and discarded. Strawberries were classified into one marketable category and five discarded or unmarketable categories rated on their condition as outlined below:

- P – perfect marketable strawberries – weighed and measured
- I – infected with grey mould *B. cinerea,* discarded
- M – misshapen, discarded
- S – too small, discarded
- D – damaged, discarded
- O – overripe, discarded

An example of a *B.cinerea* infected strawberry is shown in Plate 4.8, this is shown next to healthy marketable fruit. The incidence of grey mould infection increases with the fruit becoming overripe and in damp conditions with reduced airflow.

Plate 4.8 Infection of a strawberry fruit with *B.cinerea* alongside healthy fruit of marketable quality

The fruits were harvested on ten dates throughout the growing season with the first fruits harvested on day 55, the 2 September 2014. The harvest dates following were after 56, 61, 64, 68, 69, 76, 83, 92 and 98 days growth with the final harvest date on 15 October 2014.

Sampling of the substrate took place on the day following the final fruit harvest and was carried out by initially removing the plants from the pots. Any compost in the roots of the plants was removed as effectively as possible and the compost was stored in a labelled ziplock bag and kept refrigerated to await laboratory analysis for its chemical components such as pH, EC, nitrogen, ammonia, ionic analysis including potassium and its CEC.

4.4.3 Analysis methods

The analysis methods for the substrate in this chapter are the same as for Chapter 2.

4.5 Results

The analysis of the fruit produced is split into two sections. Analysis of the total fruit produced from plants from each treatment and whether they were marketable or not, and a comparison of the quality of the fruits produced from the 10 fruits from each replicate that was subject to additional measurements of their width, firmness and sugar content. A repeat of the tables given in 4.3 Aims and Objectives is shown below in in Table 4.5.

Table 4.5 A summary of the treatments applied to the plants

Treatment number	Combination	Fertiliser type
1	Control: 100% peat free compost (PFC)	Liquid
2	PFC and biochar (BC) addition at 10t/ha	Liquid
3	PFC	Pelletised chicken manure
4	PFC + BC	Pelletised chicken manure
5	10% OMWb + 90% PFC	Liquid
6	25% OMWb + 75% PFC	Liquid
7	50% OMWb + 50% PFC	Liquid
8	10% OMWb + 90% PFC + BC	Liquid
9	25% OMWb + 75% PFC + BC	Liquid
10	50%PFC + 50% biochar	Liquid
11	10% OMWa + 90% PFC	Liquid
12	25% OMWa + 75% PFC	Liquid
13	50% OMWa + 50% PFC	Liquid
14	10% OMWa + 90% PFC + BC	Liquid
15	25% OMWa + 75% PFC + BC	Liquid

When comparing the results against one another, it must be kept in mind that unequal sample sizes are being compared due to plant mortality in some of the treatments. When the data are normally distributed these have been compared using an ANOVA, and when the data is assessed as being not normally distributed then a non-parametric test such as Kruskal-Wallis, and a Mann-Whitney U test have been performed.

4.5.1 Plant growth and development

4.5.1.1 Chlorophyll

The chlorophyll levels in the leaves of the plant give an indication of the nitrogen uptake in the plants because the majority of leaf nitrogen is stored in the chloroplasts. The levels in the leaves were measured over the course of 6 weeks with the chlorophyll measured 5 times during this period.

It can be seen in Figure 4.4 that there was very little difference between all the treatments in terms of the chlorophyll measurements and that the trend over time appears to be the same with the exception of treatment 13 which has a spike on the 14 August that is not observed in any of the other treatments. At the start of the monitoring period the SPAD values ranged from a low of approximately 28 (treatments 11 and 14) up to a high value of approximately 45 for treatment 9.

The general trend in chlorophyll is a falling one throughout the measuring period with the levels on the final day of a high of 33.7 for treatment 1 and a low of 12 for treatment 11. On the 14 august the chlorophyll measurements between treatments 8 and 14 were significantly different with treatment 8 having a significantly lower value than treatment 14, with a p value of 0.05. On the same occasion treatment 9 was significantly lower than treatment 13 with a p value of 0.05. Other than these differences the levels of chlorophyll indicative of nitrogen uptake into the leaves was consistent through all of the treatments. A table has been incorporated into Figure 4.4 to show the standard deviations for all of the treatments throughout the monitoring period. This shows up the anomalous result for treatment 13 on the 14 August as having a very high standard deviation which counters the difference shown on the graph that might suggest that this result is significantly different from the other treatments. This value is highlighted in red in the table in Figure 4.4. There is another higher standard deviation for treatment 9 on the 22 July, however this did not translate into any significant differences or a deviation from the trend shown on the graph in Figure 4.4.

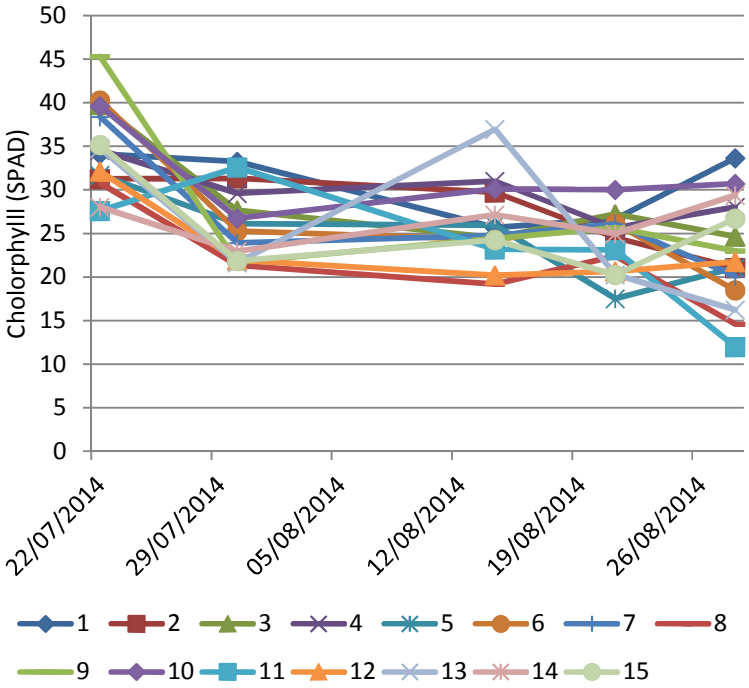


Figure 4.4 The chlorophyll levels in the leaves of the plants for each treatment over a 6 week period shown on the line graph with figures for the standard deviation given below.

4.5.1.2 Flower and fruit emergence

The flowers and fruits on the plants were counted after their first appearances on the plant during the early and middle stages of the trial. The data for these was predominantly normally distributed except for the first few occasions when there were many replicates that had not yet produced any flowers or fruit. For the purposes of comparisons and common sense for the fruit and flower analysis, the mean values are rounded up and down in the discussion of results as appropriate.

4.5.1.2.1 Flowers

The dates for the flowers recording are from the 22 – 26, 28 and 29 July and the 5 and 14 of August. A line graph showing the number of flowers throughout the recording period is shown in Figure 4.5. Figure 4.5 shows that there was

very little variation in the number of flowers on the plants for the different treatments and also that the overall trend was similar regardless of the treatment applied. The general trend shows an initial rapid increase in the number of flowers over the first 6 days followed by a drop on day 8 that was observed across all treatments. This was then followed by a slight increase on day 15 and another drop on the final day, day 24.

The treatment with the greatest number of flowers at any one point was treatment 15 on the 28 July with an average of 8 flowers on each replicate. The data is non-parametric so was analysed using a Kruskal-Wallis test with a Chi-Square analysis. This showed only one of the dates on the 25 July had any significant differences between treatments. Further analysis using a Mann-Whitney U test was completed to compare treatments against one another. This showed that treatment 2 had significantly more flowers than treatment 1, 4, 6 14, 15 and treatment 9 had significantly more flowers than treatment 1 and 4.

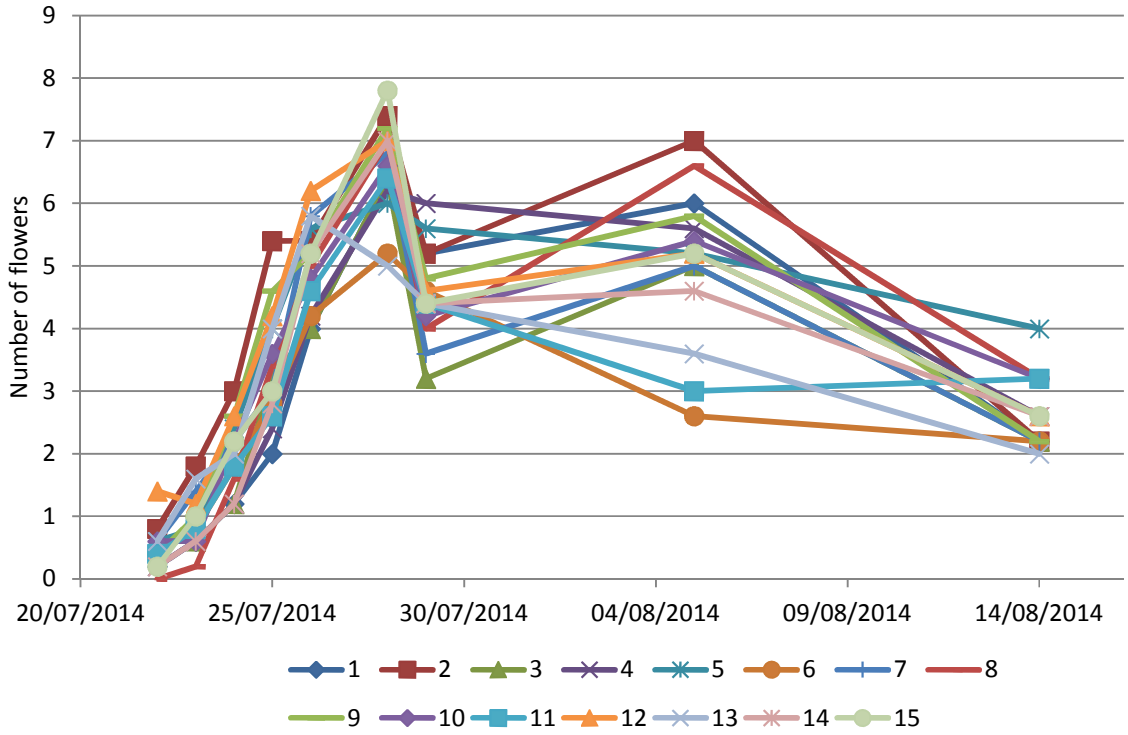


Figure 4.5 Line graph showing the mean numbers of flowers on each treatment throughout the measurement period with figures for the standard deviation given below.

4.5.1.2.2 Fruits

The dates for recording the fruit were the 29 July, and the 5, 14, 21 and 28 August. It can be seen in Figure 4.6 that once again that the number of fruits on the plants for the different treatments was very similar and that the overall trend for all treatments showed a consistent increase in fruits throughout the monitoring period. The standard deviation for the number of fruits is shown in the table included as part of Figure 4.6. The greatest number of unripe fruit recorded on the plants was for treatment 2 on the 21 of August with an average number of approximately 50 strawberries per replicate. As the data for the fruits was count data it was best analysed using a Kruskal-Wallis with Chi-Square test. Of the five dates that fruit counts were completed, only two of them produced data that was significantly different from each other on the 21 and 28 August. These data were then further scrutinised using a Mann-Whitney U test to examine which treatments were different from one another. On the 21

August treatment 2 had significantly more fruits than treatments 1, 3, 4, 6, 8, 13, 14, and 15. Treatment 9 had significantly more fruits than treatments 1, 6, 14, 15 and treatment 5 had significantly more fruit than treatments 6, 14, 15. On the 28 August treatment 2 had significantly more fruit than treatments 1, 3, 4, 5, 6, 7, 8, 10, 12, 13, 14, 15

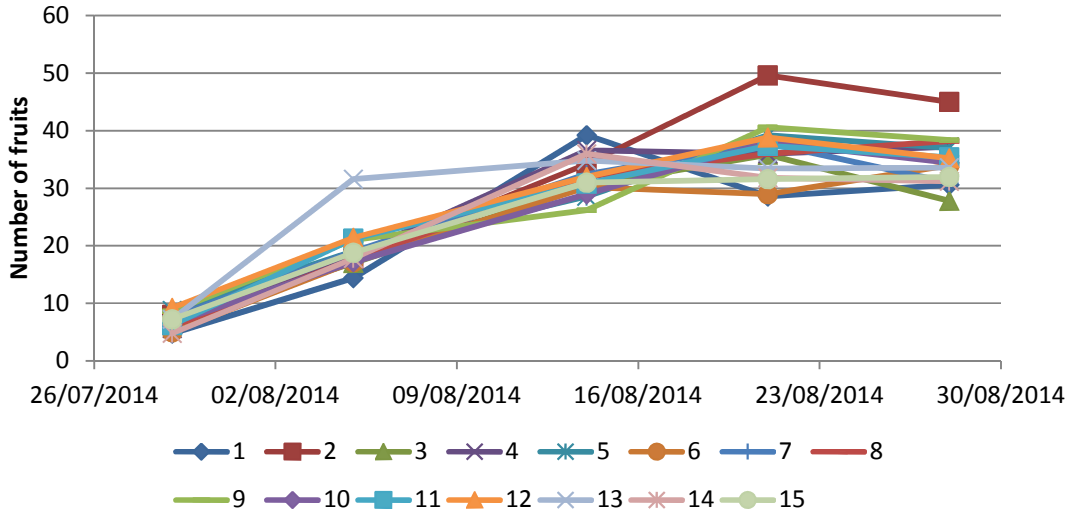


Figure 4.6 Line graph showing the mean numbers of fruiting bodies on each treatment during the recording period

4.5.2 Total fruit harvest analysis

The treatment that produced the most fruit was treatment 2 with 189 strawberries produced from all ten plants; 79% or 150 of these were marketable. The treatment with the least fruit produced was treatment 3 with 120 strawberries, with 79 of those or 66% of them being of marketable quality. The mean number of fruits for all treatments was 146 with 71% of them on average being of marketable quality. To compare production with commercial production using mineral fertilisers, it would be expected that each plant would yield around 1kg of fruit during its first season, and 3kg of fruit during its second season (Peter Overoode, pers comm). This can be considered as optimal conditions for strawberry production. The treatment that produced the highest weight of total fruit was treatment 2 with 1.8kg of fruit produced from 10 plants. This is around 10 times lower than expected production in a commercial set up.

To give a direct comparison between the weights of the average fruit produced throughout the season and the average weight of the first ten fruits harvested from each experimental unit these are displayed together in Figure 4.7. Treatment 4 produced the heaviest fruit over all other treatments for both weight criteria with weights of 10.6 and 13.8 g for all fruits and the first ten fruits respectively. This is in comparison for the lowest weight fruit from treatment 12 of 8.5g from the all fruits category and for treatment 14 with a value of 10.6 for the first ten fruits category.

The data for total fruits when examined using a Shapiro-Wilk test for normality identified that the weight data for the fruits was not normally distributed so it was subject to a square root transformation which had the effect of normalising the data to enable it to be analysed using an ANOVA. There was a significant difference between treatment 4 and treatments 9 and 12 for the average weight of total fruit produced with p values of 0.025 and 0.009 respectively with 4 having a higher mean weight. This suggests that the combination of chicken manure as a fertiliser and the biochar addition has acted as a better fertiliser for the strawberry fruits in this instance. For the first ten fruits harvested there was also a significant difference between treatment 4 and treatments 8 and 14 with values of 0.036 and 0.001 respectively again with treatment 4 having a higher mean weight. There were no other significant differences between any treatments for weight of fruit. From the graph in Figure 4.7 the first ten fruits harvested from each replicate have a higher average fruit weight for every treatment when compared with the average fruit weight of all fruits harvested. The standard deviation for the first ten fruits is also greater than that for total fruits as shown by the error bars in Figure 4.7.

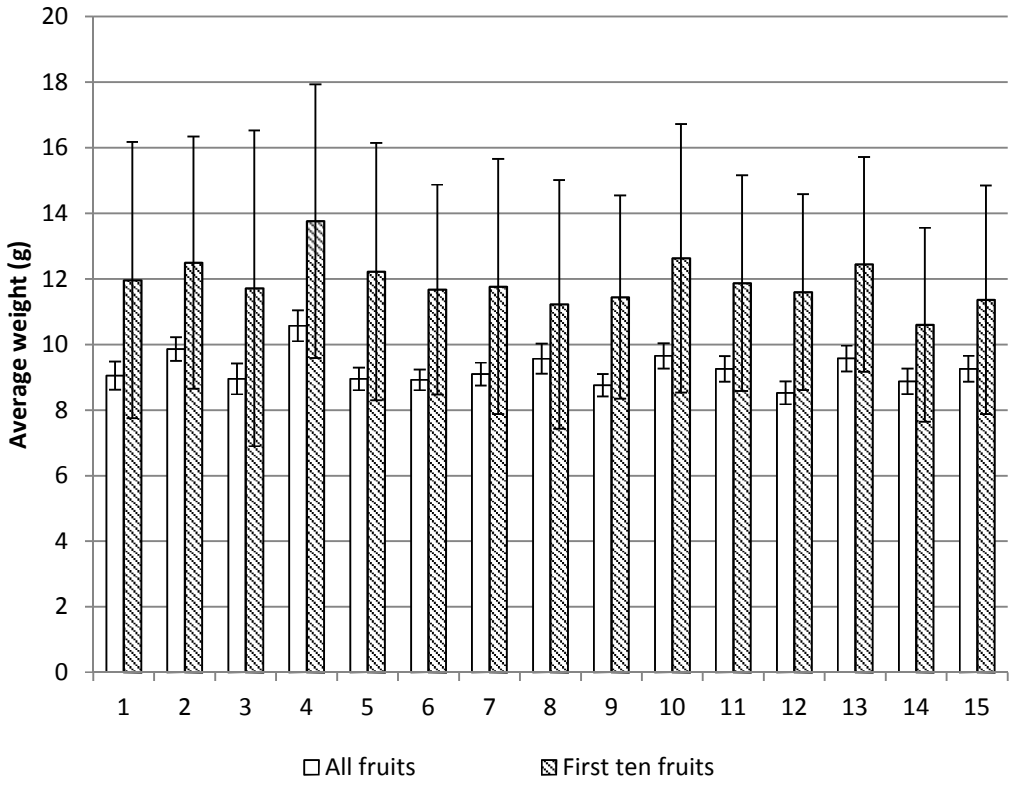


Figure 4.7 Bar graph showing the average weights of the marketable fruit from each treatment of total fruits and the first ten fruits

4.5.3 Impact of treatment on marketability

4.5.3.1 Number of marketable and non-marketable fruits

The results of this analysis are presented as averages per replicate plant with standard deviation indicated by the error bars, displayed in Figure 4.8. Limitations of this analysis are that treatments 3 and 12 both had one replicate that suffered the loss of one plant in one of their five replicates leaving 9 plants instead of 10 for the analysis.

From Figure 1.8 it can be seen that treatment 2 stands out as the highest performing treatment with an average of 30 strawberries produced per replicate, the lowest producing treatment was 3 with an average of only 15 fruits per replicate. Taking into account the limitations for treatment 3, the next lowest producing treatment was 15 with an average of 18 fruits per replicate.

Data for fruit quality for all fruits harvested (whether marketable or not, and reason for non-marketable) was not normally distributed and therefore it was subjected to a square root transformation to normalise the data prior to analysis through an ANOVA. There were no significant differences between any of the treatments 1-15 and their effect on whether the fruit was marketable or not, and on the condition status of the marketable or discarded fruits.

Treatment 2 performed significantly better than 6 other treatments when it came to number of marketable fruits produced per replicate (two plants). Treatment 2 was significantly different from 1 (0.024), 3 (0.004), 8 (0.049), 12 (0.035), 14 (0.035) and 15 (0.020) with p values shown in brackets. Taking into account the limitations of this analysis it may be possible to discard the significant results in comparison to treatment 2 for treatments 12 and 3 which had the loss of one plant each, although given the very low p value for both of these when compared to treatment 2 it is possible that the loss of these plants had affected the outcome of the statistical test.

There were no significant differences in the number of discarded strawberries produced per replicate between treatments. Treatment 10 had the greatest number of discarded or non-marketable fruits.

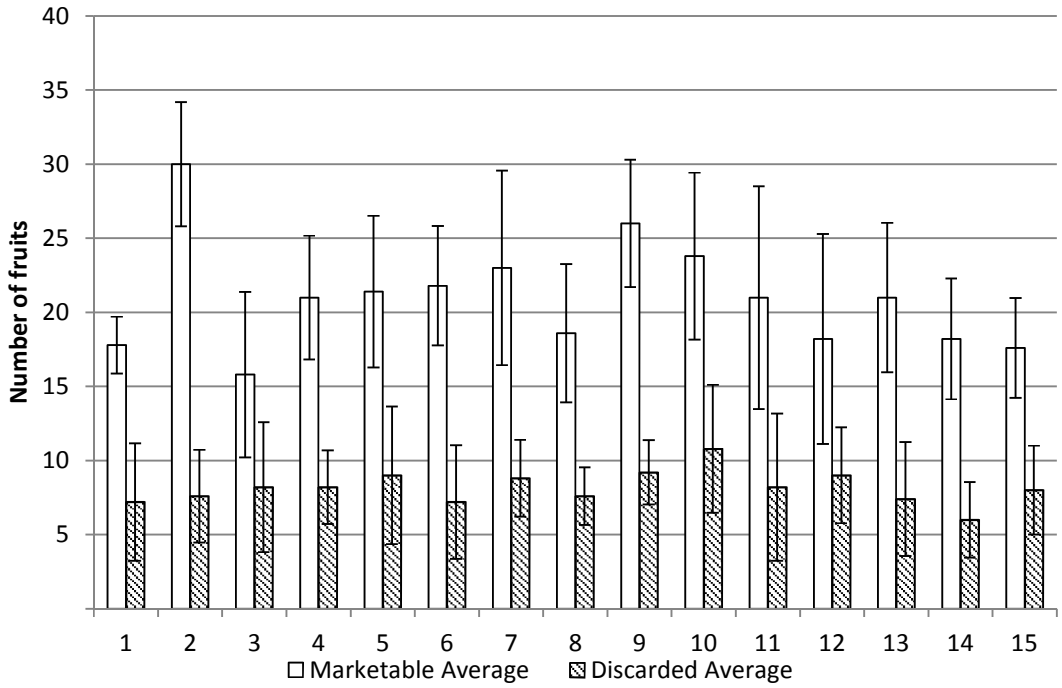


Figure 4.8 Graph showing the average number of marketable and discarded strawberries produced by each replicate for every treatment, with standard deviation showing on the error bars.

4.5.3.2 Fruit infected with grey mould

The number of strawberries infected with *B. cinerea* as part of the discarded cohort were counted and assessed for each treatment (Table 4.6). The greatest number of infected strawberries occurred in treatment 10 with a 50% biochar ratio which had 22 infected fruits. The lowest infection rates occurred in treatment 4 with a 10Mkg/ha application rate of biochar with a total of 5 infected fruits. There are no significant differences between the numbers of *B.cinerea* infected strawberries between any of the treatments. The details of the fruit numbers and their quality produced by each treatment are given in Table 4.6.

Table 4.6 Details of the fruit produced by each treatment

Treatment	Number of fruit	Marketable	Non-marketable	*B.cinerea* infected	Average weight (marketable fruit)
1	125	89	36	12	9.05
2	189	150	39	12	9.86
3	120	79	41	10	8.95
4	146	107	39	5	10.57
5	152	107	45	9	8.95
6	145	109	36	13	8.92
7	159	115	44	16	9.1
8	131	93	38	12	9.57
9	176	130	46	16	8.76
10	173	119	54	22	9.65
11	146	105	41	6	9.26
12	136	91	45	7	8.52
13	142	105	37	18	9.57
14	121	91	30	6	8.88
15	128	88	40	11	9.26

4.5.4 Quality of ten fruits

The data for treatments when compared using ten fruits for width, firmness and sugar content was not normally distributed using the Shapiro-Wilk test for normality. A Log10 transformation provided the best solution and normalised the data for analysis using ANOVA.

4.5.4.1 Width of fruit produced

There was little variation between treatments for the width of the fruit produced when measuring the first ten fruits from each experimental unit. The treatment which produced the fruits with the greatest width was treatment 4 with a mean

width of 32mm. The smallest fruits by width were produced by treatment 14 with a mean width of 28.8mm. The error bars showing standard deviation for width do not vary greatly between treatments with the largest deviation in treatment 6 with a value of 4.7mm and the lowest deviation in treatments 9 and 14 with deviation of 3.2mm. The only treatment that showed any significant difference was treatment 4 which was significantly wider than treatments 6 ($p = 0.039$) and 14 ($p = 0.012$). The information for the width of fruit produced is shown in Figure 4.9.

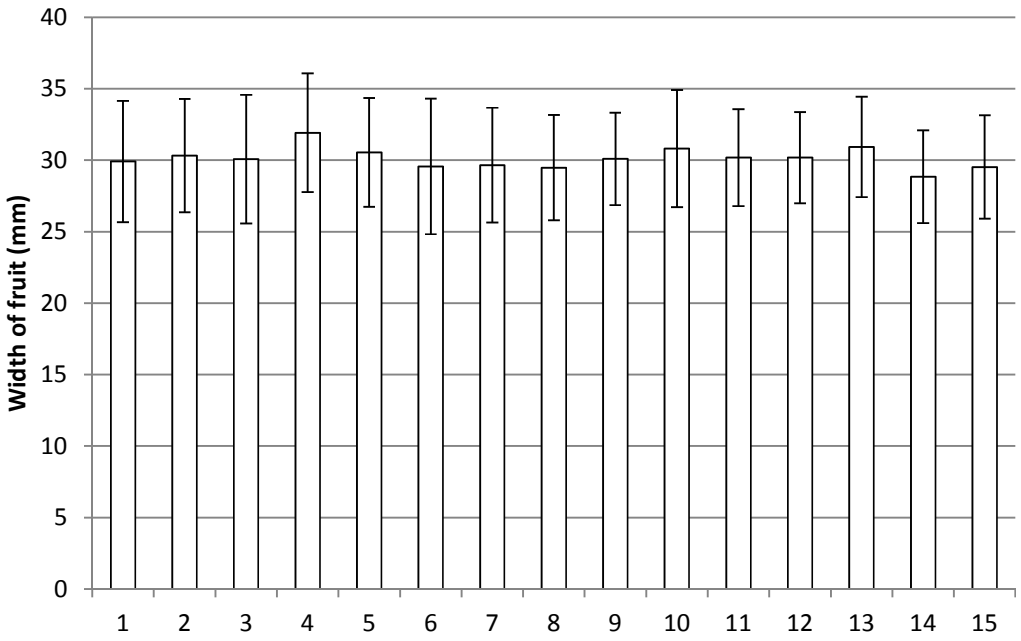


Figure 4.9 Bar graph showing the width of fruit for all treatments from the ten fruits measured per each replicate, error bars show standard deviation

4.5.4.2 Firmness of fruit

There was considerable variation in the results for the firmness of the first ten fruits as measured by the handheld penetrometer. This data is shown and described in Figure 4.10 and Table 4.7.

Treatment 4 produced the firmest fruits with a mean penetrometer recording of 736N. The least firm fruits were produced by treatment 13 with a mean recorded firmness of 575N. The treatment with the most significant differences

from other treatments was treatment 4 being significantly higher than twelve treatments as indicated in the boxplot in Figure 4.10 and Table 4.7. Treatment 3 was significantly higher than seven other treatments, and treatment 13 had values that make the penetrometer results significantly lower than seven other treatments.

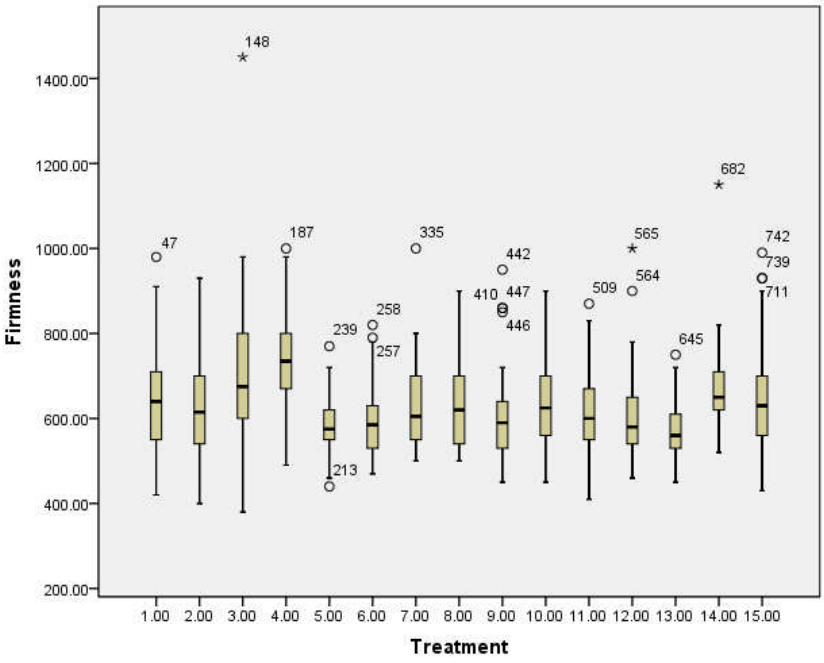


Figure 4.10 Boxplot showing the results of the penetrometer test for all 15 treatments with the interquartile ranges and minimum and maximum values shown.

The information displayed in Table 4.7 can be read and understood by reading down and across from each number to understand which treatments are significantly different to one another. This table shows that treatment 4 had the most significantly different data.

Table 4.7 Table showing the treatments that are significantly different from each other as indicated in the cell with a ✓

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*
1	-			✓									✓		
2		-	✓	✓											
3			-		✓	✓			✓		✓	✓	✓		
4				-	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓
5					-									✓	
6						-								✓	
7							-						✓		
8								-							
9									-					✓	
10										-			✓		
11											-				
12												-		✓	
13													-	✓	✓
14														-	
15															-

4.5.4.3 Sugar content of the fruit

The results for the sugar content of the fruit are shown in Figure 4.11 with the mean values for Brix with standard deviation shown on the error bars. This data shows that treatment 10 had the highest concentration of sugar in the fruit with a mean of 7.3 and a standard deviation of 1.3. The treatment with the lowest sugar concentration in the fruit was treatment 8 with a Brix mean of 6.1 and a

standard deviation of 1.3. The difference in the sugar levels between the treatments was the greatest between treatment 10 which had a significantly higher level of Brix than treatments 5 (0.022), 8 (0), 11 (0.003), 12 (0.005), 13 (0.040) and 14 (0.004); p values for each shown in brackets. This suggests that treatment 10 had significantly sweeter fruit than any of these other treatments. Treatment 8 has the least sweet fruits of any of the treatments, and treatment 6 was significantly different from treatment 8 with a p value of 0.015. The mean values for the sugar content with standard deviation shown on the error bars is in Figure 4.11.

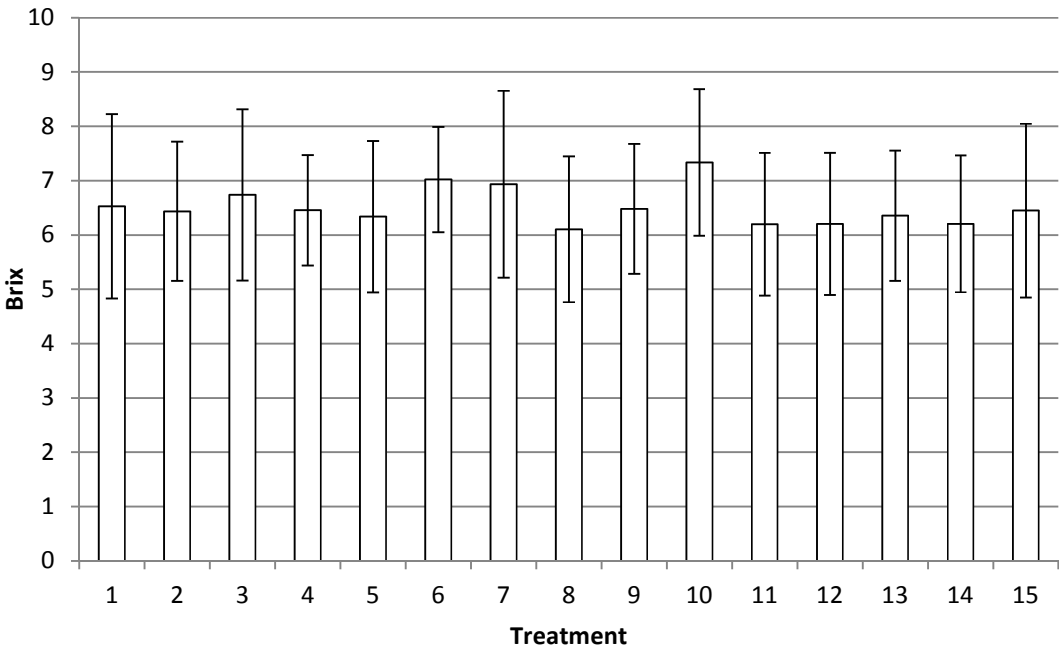


Figure 4.11 Bar graph showing the sugar levels in the fruit as measured in Brix

4.5.5 Effects of time on fruit harvested

Another effect that can be seen from the harvested fruits is the impact of time on fruit. There were ten harvest dates in total, however not all treatments were harvested on the same days. The information in Table 4.8 gives the details of the harvest dates and how these are grouped to give comparisons between the

data for each treatment. The graph in Figure 4.12 shows the mean weight of the fruit for each treatment on the days when they were harvested.

Table 4.8 Information on the harvest dates and days after planting and how these are grouped together to give 7 harvest dates.

Harvest	*Time after planting (days)*	*Dates*
1	55, 56	02/09/14, 03/09/14
2	61, 64	08/09/14, 11/09/14
3	68, 69	15/09/14,16/09/2014
4	76	23/09/14
5	83	30/09/14
6	92	09/10/14
7	98	15/10/14

It can be seen in Figure 1.12 that regardless of the treatment applied the mean weight of fruit for each treatment falls throughout the harvest season as would be expected given the way the fruit is produced on the trusses. There was no significant difference in the weight of fruit on each harvest day, and not all treatments produced fruit for harvest days 6 and 7 at 92 and 98 days after planting. Comparable to the results in 4.5.2 treatment 4 has the heaviest fruit on the first harvest date with a mean weight of 15.6g. The lowest weight fruit on the first harvest date were those from treatment 3 with a mean weight of 10.3g. As some treatments did not produced fruit past harvest date 5 this is taken as the last date on which fruit can be reliably compared. On this date towards the end of the harvest at day 83 treatment 11 produced the heaviest fruit with a mean weight of 9.2g and treatment 10 had the lightest fruit with a mean of 5.9g.

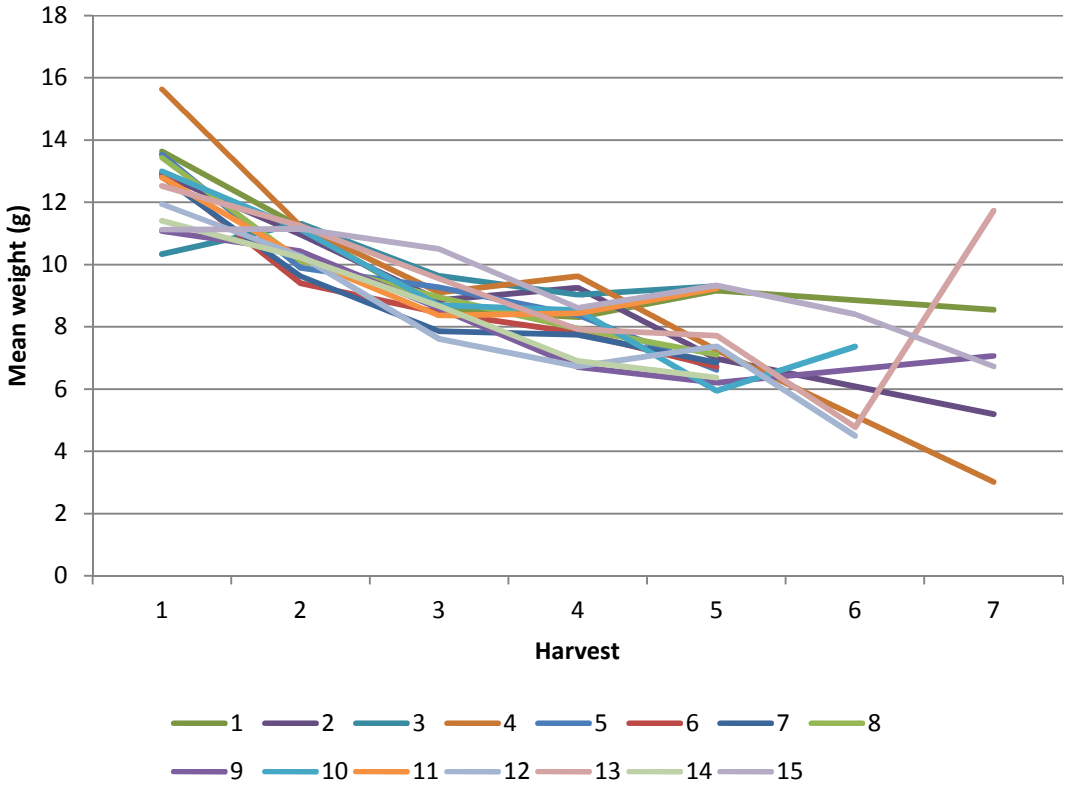


Figure 4.12 Line graph showing the mean weight of fruit for each of the 15 treatments throughout the harvest season over 7 harvest days.

4.5.6 Post-harvest substrate analysis

4.5.6.1 Analysis of pH and EC

The results of the pH and EC analysis are given in Table 4.9. The data collected for the pH and EC of the samples was normally distributed which made it possible for it to be directly analysed using an ANOVA. The stand out results from the pH was for treatment 7 which was significantly higher than all other treatments apart from treatment 13 with a pH of 8.14 (p values range from 0.0 to 0.0018). The lowest pH of all the treatments was for treatment 1 with a value of 7.4. The value for the EC for treatment 7 at 474.8µS/cm was also significantly higher than many of the other treatments, all of them barring treatments 5, 9 and 13. The treatment with the lowest EC was treatment 8 with a value of 196.94 µS/cm.

Table 4.9 Results of the pH and EC analysis of the substrate samples

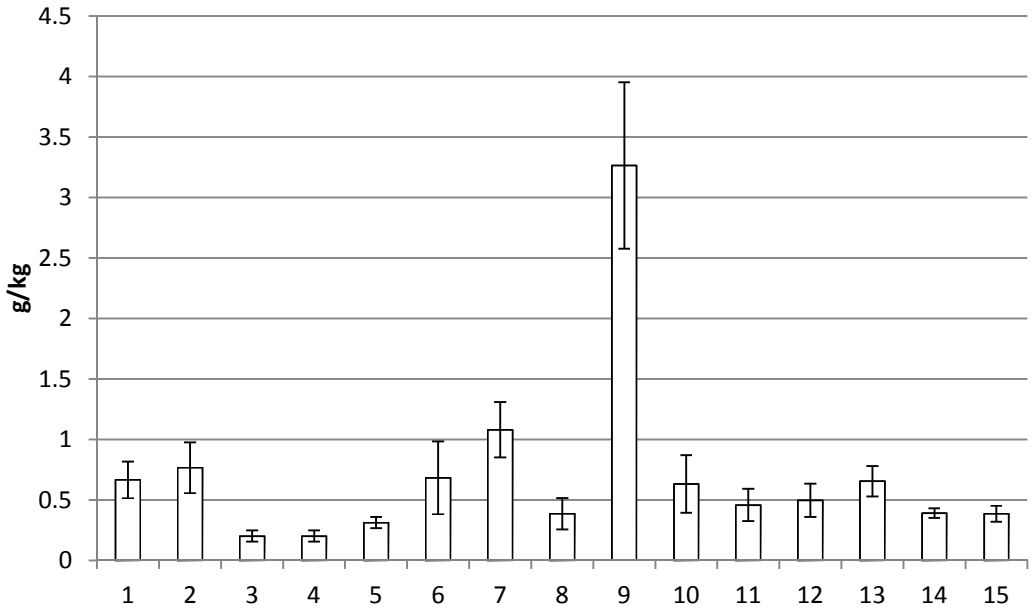
Treatment	Details	pH	EC (µS/cm)
1	Control: 100% peat free compost (PFC)	7.40	222.74
2	PFC and biochar (BC) addition at 10t/ha	7.45	208.18
3	PFC	7.65	227.76
4	PFC + BC	7.54	250.22
5	10% OMWb + 90% PFC	7.60	301.08
6	25% OMWb + 75% PFC	7.75	268.26
7	50% OMWb + 50% PFC	8.14	474.80
8	10% OMWb + 90% PFC + BC	7.68	196.94
9	25% OMWb + 75% PFC + BC	7.56	302.20
10	50%PFC + 50% BC	7.81	209.98
11	10% OMWa + 90% PFC	7.64	221.92
12	25% OMWa + 75% PFC	7.75	241.60
13	50% OMWa + 50% PFC	7.82	386.20
14	10% OMWa + 90% PFC + BC	7.62	201.12
15	25% OMWa + 75% PFC + BC	7.73	201.20

4.5.6.2 Nitrogen and ammonia

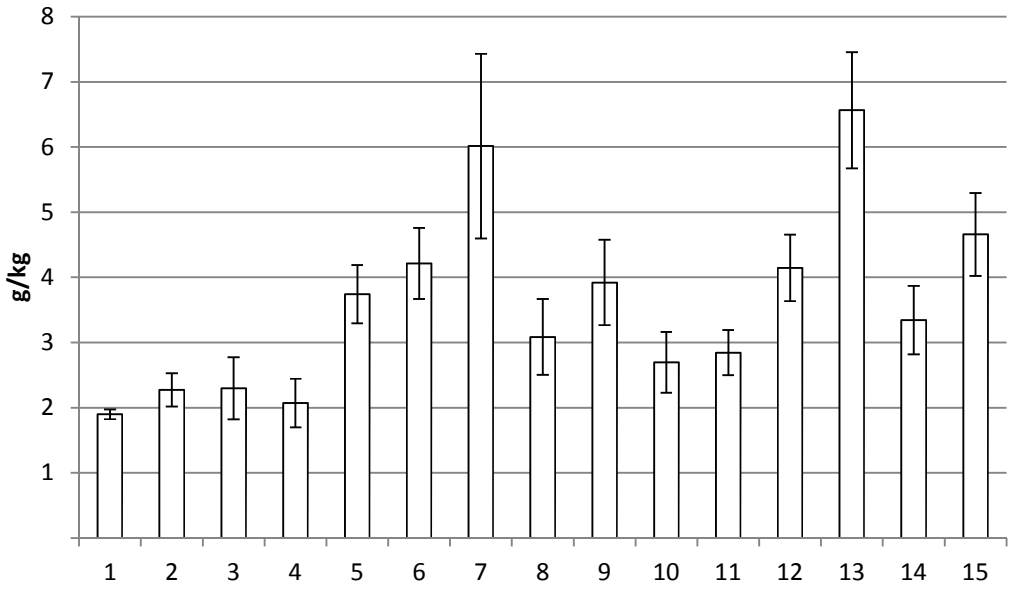
Nitrogen in the substrate samples was measured using the total Kjeldhahl nitrogen (TKN) method and ammonia NH_3 analysis. The results for this analysis are shown in Figure 4.13. The levels of ammonia shown in the treatments using both OMWa and OMWb in Figure 4.13a show an increasing amount of ammonia in the substrate to correlate with the increasing percentage v/v of OMW. This is shown from 5-7 with values of 0.3/kg, 0.6/kg and 1.0g/kg; treatments 8-9 at 0.3g/kg and 3.3g/kg and treatments 11-13 with values of 0.4g/kg, 0.5g/kg and 0.6g/kg respectively. The treatments with the lowest mean value for ammonia were treatments 3 and 4 both having values of 0.2g/kg. The

treatment with the highest mean value was treatment 9 with a mean of 3.3g/kg and a standard deviation of 0.68g/kg. The inclusion of biochar into the treatments has the effect of reducing the levels of ammonia in the analysis when comparing treatment 11 and 14, however the biochar increases the levels of ammonia in treatments 9 and 8 when compared to treatments 6 and 5 respectively.

The levels of TKN in Figure 4.13b show a mirror of this pattern seen for the treatments in the ammonia results, but it also extends to treatments 14-15. This is shown from 5-7 with values of 3.7g/kg, 4.2g/kg and 6.0g/kg; treatments 8-9 at 3.1g/kg and 3.9g/kg; treatments 11-13 with values of 2.8g/kg, 4.1g/kg and 6.6g/kg and treatments 14 and 15 with values of 3.3g/kg and 4.7g/kg respectively. The treatment with the lowest mean value for ammonia was treatment 2 with a value of 1.9g/kg and a standard deviation of 0.08g/kg. The treatment with the highest mean value was treatment 13 with a mean of 6.6g/kg and a standard deviation of 0.89g/kg. There is a trend of a reduction in TKN with the addition of biochar for OMWb and a trend of an increase in TKN when biochar is combined with OMWa.



a)



b)

Figure 4.13 Bar graphs showing the amount in g/kg (fresh weight) of ammonia in the samples in part a) and of TKN in part b)

The treatments that are significantly different from one another are shown in Table 4.10. This table shows their difference using a coding when comparing any two treatments. Treatment 9 was significantly different from all other

treatments for the ammonia levels in the sample and treatment. Treatment 13 was significantly different from all others for the TKN concentration, except treatment 7 when comparing the results for the TKN analysis. Treatment 7 was also significantly different from all other treatments for TKN concentration, except for treatment 13.

Table 4.10 The treatments and how they compare with one another when compared in SPSS, treatments with a significant difference from one another are shown using the N for the TKN analysis and Am for the ammonia analysis

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*
1	-				N	N	N		N Am			N	N	N	N
2		-	Am	Am	N	N	N		N Am			N	N		N
3			-		N	N	N Am		N Am			N	N		N
4				-	N	N	N Am		N Am			N	N		N
5					-		N Am		Am				N		
6						-	N		Am	N			N		
7							-	N Am	N Am	N	N Am	N Am		N Am	Am
8								-	Am				N		N
9									-	Am	Am	Am	N Am	Am	Am
10										-		N	N		N
11											-		N		N
12												-	N		
13													-	N	N

14	-
15	-

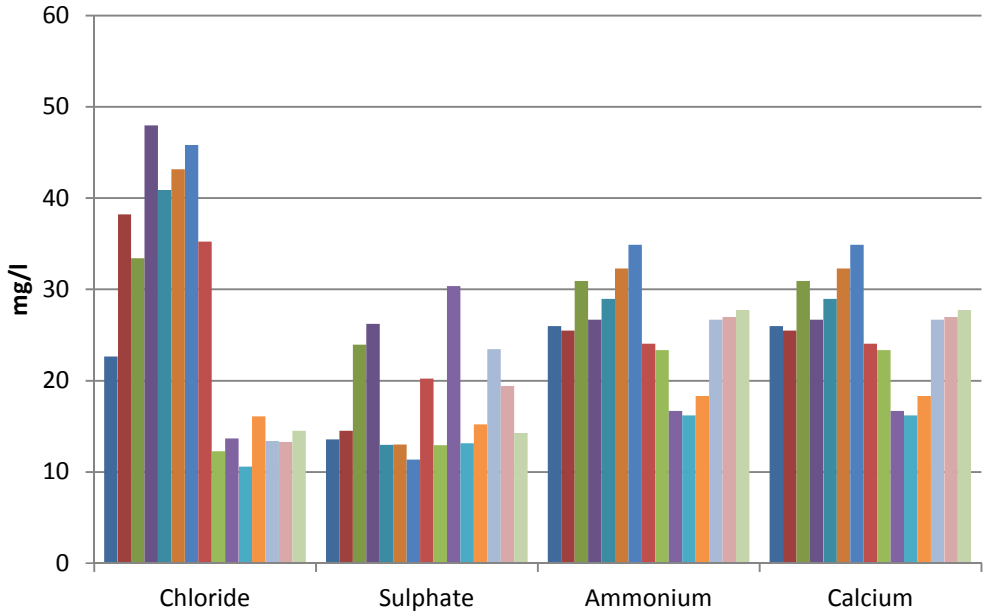
4.5.6.3 Ionic analyses

The substrate following harvest was sampled and analysed for: ammonium (NH_4^+), K, P, Mg, Na, Ca, and Cl. The analysis was carried out using an ion chromatograph. The methods for these are further described in Section 2.4.6 with a glossary of reagents in Appendix A. The data collected from these analyses was normally distributed and was analysed using an ANOVA.

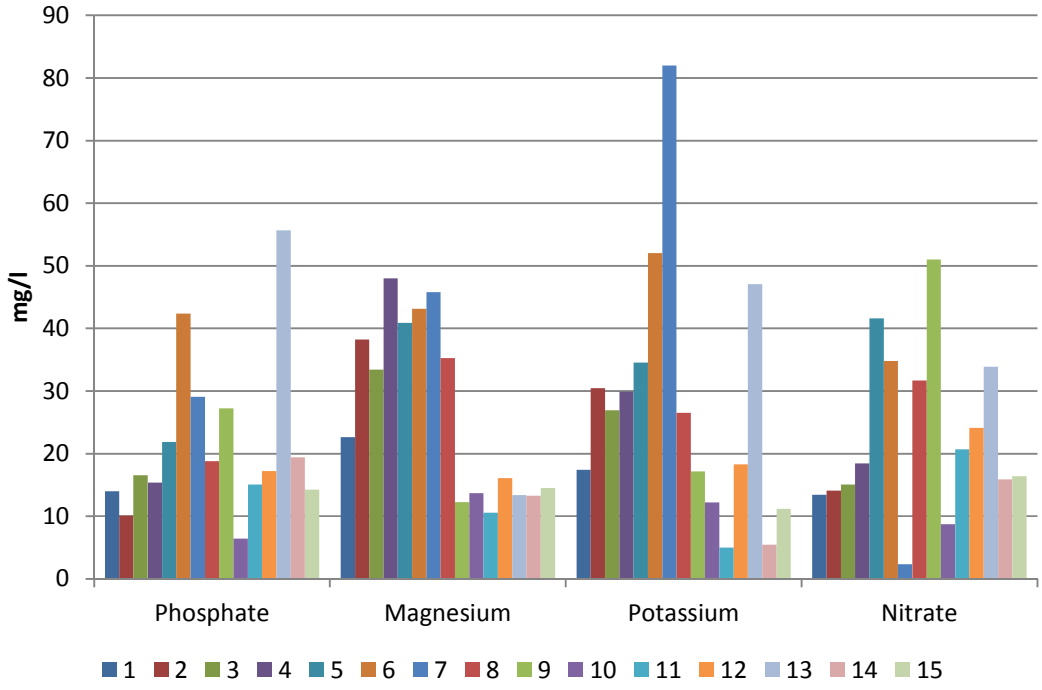
The results of the chemical analysis of the compost samples are shown graphically in Figure 4.14a) and b) and Table 4.11 shows those that are significantly different from one another. There are no error bars shown on the graphs as due to the spread of data some of the error bars were greater than the mean value. The trends expected in this data were a pattern relating to increasing amounts of compost, so there should be a pattern with treatments 5 to 7, 8 and -9, 11 to 13 and 14 and 15.

This increase in nutrients can be seen for OMWb in treatments 5 to 7 for Cl, Ammonium, Ca, Mg, and K. However when biochar is added to the treatments in 8 and 9, there is a falling trend in nutrients for Cl, S, Ammonium, Ca, Mg and K. The nutrients in treatments using OMWa are in general lower than those where OMWb was used. There is still an increasing pattern with the OMWa treatments in increasing amount of nutrients from 11-13 for S, Ammonium, Ca, Phosphate, K, and Nitrate.

Figure 4.14 shows those treatments which have significantly different levels from one another, the one that stands out most is the value for potassium in graph b) for treatment 7 where it is significantly different from 11 other treatments. This treatment was the 50% rate of OMWb. The levels of phosphate in treatment 13 are significantly different from all the other treatments.



a)



b)

Figure 4.14 Bar graphs a) and b) showing the levels of nutrients in the compost after harvesting the fruit.

The information shown in Table 4.11 can be understood by reading down and across from each number for the treatments to see any treatments from which it is significantly different and for which ion.

Table 4.11 The treatments and how they compare with one another when compared in SPSS, treatments with a significant difference from one another are shown using the chemical symbol for that element in the specific cell. N3 is used to show Nitrate (NO_3-).

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*
1	-			Cl	N3	Mg N3 P	Ca K Mg		N3	Ca	Ca		Mg N3 P		
2		-			N3	Am Mg N3, P	Ca, K Mg P	Am	Am Cl N3	Am	Ca Cl	Am	Mg N3, P		Am
3			-		N3		K		N3	Ca Mg	Ca Mg	Ca N3	Mg, P		
4				-	N3	P	K		Cl N3	Ca Mg Cl	Ca Mg Cl	Cl	Mg Cl P	Cl	Cl
5					-		N3		Cl	Ca Mg Cl N3	Ca Mg Cl N3	Ca	Mg Cl, P	Cl N3	Cl N3
6						-	N3	Ca Mg	Ca Mg	Ca Mg N3, P	Ca Mg N3, P	Ca Mg	P	Mg, P	

7	-	Ca K Mg N3	Ca K Cl N3	Ca K Mg Cl P	Ca K Mg Cl	Ca K Mg Cl N3	Cl N3, P	K Mg Cl	K Cl
8		-	N3				Mg, P		
9			-	Mg N3, P	N3	N3	Mg P	N3	N3
10				-			Ca Mg N3, P	Ca	Ca Mg
11					-		Ca Mg, P	Ca	Ca Mg
12						-	Mg, P		Ca
13							-	Mg, P	Mg, P
14								-	
15									-

4.5.6.4 CEC of the compost

The cation exchange capacity of a substrate indicates its ability to hold onto simple cations for release when needed (Russell, 1973). The CEC is responsible for nutrient mobility through a substrate and making these available for plants to take up (Schiefer et al., 2015). Increasing the CEC of a growth medium can improve its fertility, leading to enhancements in the growth and yield of the crop.

The methodology for the CEC was adapted from that used by Gaskin (2008) and is explained in Chapter 2 (Gaskin et al., 2008). The CEC analysis was carried out only on the treatments using the compost produced from OMW and in ratios that could be compared with the use of OMW compost in combination with biochar. The analysis for the CEC was completed using 3 replicates and not 5 as in other analyses. This shows that the control treatment with no OMW compost added but with added biochar had the lowest value for CEC with a value of along with the largest standard deviation of all of the treatments. This treatment (treatment 2) was significantly lower from treatments 8 (0.023), 9 (0.007), 11 (0.027), 12 (0.027), 14 (0.002), and 15 (0.002) with p values shown in brackets. In addition to this, treatment 10 was significantly lower than 14 (0.045) and 15 (0.034) and treatment 1 was also significantly lower than treatment 15 with a p value of 0.045.

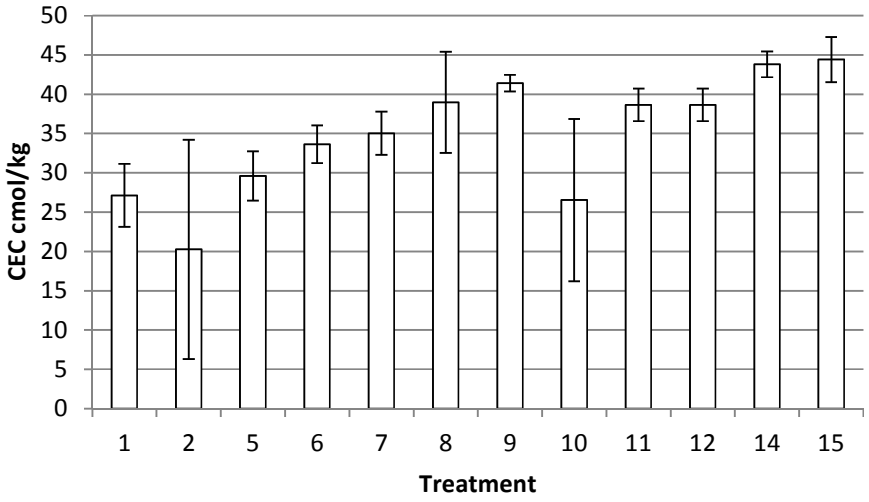


Figure 4.15 Bar graph showing the mean value for the CEC of the samples with error bars displaying standard deviation

4.6 Discussion

4.6.1 Objective 1

To compare the effects of liquid fertiliser with chicken manure as a replacement fertiliser on strawberry growth with and without the addition of biochar.

The chlorophyll levels in all treatments did not vary significantly from one another, indicating that the nitrogen uptake rate was similar across all treatments regardless of organic amendment. This shows that the plants were able to absorb nitrogen as efficiently from the chicken manure fertiliser as they were from the inorganic fertiliser and that the biochar had no effect on this in this experiment. In a trial by Asai in 2009 it was found that a biochar amendment on two rice cultivars reduced chlorophyll levels at 8Mkg/ha and 16Mkg/ha biochar application rates (Asai et al., 2009). This suggests that the biochar amendment may be supressing the nitrogen uptake from the chicken manure fertiliser, which is rich in nitrogen.

Treatment 4 was the most successful treatment when comparing the fruit for weight, width and firmness against all other treatments. This would suggest that

using chicken manure as a fertiliser in the presence of biochar can have a positive impact on the production of quality fruit from the plant. This contrasts with the accepted wisdom of not using chicken manure as a fertiliser for strawberry production due to its high nitrogen content. This is due to nitrogen not being required once the plants start fruiting. Applications of nitrogen during the vegetative stage for root and shoot development are beneficial however once the first flowers have appeared too much nitrogen can cause unnecessary vegetative growth and reduce fruit quality and increase the risk of powdery mildew (Pritts, 2015, Hancock, 1999). Chicken manure is also variable in its content of micro nutrients such as copper and manganese essential for strawberry plant growth (Pokhrel et al., 2015). It is possible that the biochar amendment had some effect on this as biochar has been recorded to reduce nitrogen uptake in some instances when nitrogen is in short supply (Asai et al., 2009, Lehmann et al., 2003). In this instance the reduction of nitrogen uptake from the chicken manure amended pots may have allowed the strawberry plants in treatment 4 to grow and develop successfully.

When it comes to the number of strawberries produced, treatment 2, the control treatment with the addition of biochar performed the best, being significantly more productive than the control without biochar added (treatment 1). The differences in the numbers of fruit and flowers produced on the plant are indicative of the yield result from the plants. Treatment 2 had more flowers than treatments 1 and 4 on one occasion and more fruits on the plants pre-harvest than treatments 1, 3 and 4 on two occasions during the monitoring period. The results for objective 1 would suggest that treatments that received biochar performed better than those that didn't when all other parameters are kept the same.

The mechanism for biochar to improve the yield as in treatment 2 or fruit quality as in treatment 4 could be due to the biochar adsorbing necessary nutrients onto its surface to release when the plants require them. The biochar addition could have increased the soil water permeability, as Asai found in rice crops in 2009 (Asai et al., 2009). However, in other cases it has been known to improve water retention in the soil. Glaser in 2002 found that increased amounts of charcoal increased the water retention capacity of the soil and the specific

surface area of the soil was three times higher than soils without the charcoal amendment, this increased the water holding capacity by 18%. Improving the water holding capacity of a soil makes water more plant available and can help reduce erosion (Glaser et al., 2002). The addition of biochar could also have reduced the bulk density of the substrate as Rogovska found in a soil column study in 2011. In this study it was found that increasing amounts of biochar application reduced the bulk density of the soil in the column when compared to a control with no biochar added (Rogovska et al., 2011). The rate of water movement through the pots was not measured so this could be a measurement for future work using biochar on strawberries to enhance growth. The bulk density of the substrates was also not measured as a part of these trials which is a potential parameter for measurement when considering future trials with strawberries and biochar.

The chemical analysis of the substrate after harvest showed that the treatments (3 and 4), fertilised with chicken manure, both had significantly lower levels of ammonia than the control treatment with biochar added (treatment 2). The analysis also showed that treatment 1 had significantly lower concentrations of chloride than treatment 4. It is possible that the nitrogen present in the chicken manure more readily volatilises as ammonia than the nitrogen source present in treatment 2 (Bitzer and Sims, 1988). Biochar is known for ammonia sorption and storing the ammonia so that it can be made available to the plant when needed (Spokas et al., 2012b). However given the differences between treatments 2 and 4, both with biochar added it is more likely the difference in the levels of ammonia can be attributed to the chicken manure fertiliser than the biochar.

4.6.2 Objective 2

Compare ratios of OMW compost varieties and their effects on strawberry growth with and without the interaction with biochar.

The chlorophyll measurements show that treatment 8 and 14 had one incidence when they were significantly different from one another. These both had the incorporation of 10% of OMWb or OMWa respectively and had a

biochar addition. On one occasion the levels of chlorophyll found in leaves on plants in treatment 9 were significantly lower than those found in treatment 13. These are not readily comparable as treatment 9 had a 25% level of OMWb with a biochar addition and treatment 13 had a 50% OMWa addition with no biochar. It is not possible to infer anything for this result of any significance regarding nitrogen uptake in relation to the type of compost used as it only occurred once throughout the monitoring period. If there was a significant effect of the compost affecting nitrogen uptake by the leaves it could be expected to occur more than once. In the growth of tomato seedlings in olive waste compost Ceglie found the incorporation rates of 20% and 45% had no significantly different impacts from one another when measuring the chlorophyll, although they were significantly lower than the control of 100% peat based substrate. The olive waste compost when used at 70% and 90% incorporation in a peat base had lower chlorophyll values (Ceglie et al., 2011). A greater difference in chlorophyll levels in these trials could have been shown if a higher percentage OMW rate had been trialled.

During the flower monitoring, treatment 9 had more flowers than the control (treatment 1) on one occasion. This was followed up by treatment 9 having more fruit per-harvest than treatments 1, 6, 14, and 15 on one occasion. As a comparison between the OMW compost with biochar this shows that during the fruit monitoring OMWb at 25% with a biochar addition performed better than OMWa at 25% with a biochar addition. This also shows that the OMWb performed better with biochar than without. This was only recorded on one occasion throughout the monitoring period so it is hard to draw anything conclusive from this. In experiments with orchids Gijbels found that a fertilisation regime containing NPK against a control receiving only water had no effect on the number of flowers on each inflorescence (Gijbels et al., 2015). Arancon conducted trials where food and paper waste vermicomposts were amended with mineral fertilisers to make their nutrient levels of NPK the same as the inorganic fertiliser used as a control. These were applied to strawberry crops, and those grown in the vermicompost treated plots had significantly more flowers than those grown in the inorganic fertiliser, along with a significantly higher marketable yields. This is attributed to the increased

microbial activity in the vermicompost amendment, and not to any differences in the micronutrients in the composts (Arancon et al., 2004).

There were some patterns in the chemical analysis of the compost that fall in line with the increasing amounts of OMW compost used within the treatments, this is shown in the increasing amounts in the nitrogen and ammonia that follow the increasing amount of OMW compost used for all treatments. This would suggest the idea that using a rich compost as a substitute substrate adds additional nutrients into the media that can potentially be made available to the plants. The values for pH and EC were significantly higher for treatment 7 than for most of the other treatments. The levels for EC can be understood by looking at the analysis of the OMWb used in treatment 7 which had a higher EC than OMWa, and treatment 7 received a 50% rate of OMWb. The two stand out results from the ionic analysis are for treatment 7 for the levels of potassium and for treatment 13 for the levels of phosphate. The levels of potassium in treatment 7 are explained by OMWb having double the concentration of potassium than the other two treatments, and that treatment 7 was incorporated at a 50% rate. The levels of phosphate in treatment 13 are not as well explained with both the OMW compost having similar initial concentrations of phosphorus.

The levels of CEC in the treatments follow the increasing amounts of product used, with the treatments with biochar added having higher CEC values than the same treatment without biochar. Increasing amounts of soil organic matter (SOM) are associated with a higher CEC of the soil (Liang et al., 2006) so the addition of the OMW as an organic component could have caused this increase in CEC. The results for the treatments with biochar that showed higher levels of CEC are supported by the literature that a biochar amendment can improve the CEC of the soil. This is due to the oxidation of the biochar particles and also the adsorption of particles of organic matter on the surface of the biochar (Liang et al., 2006). A combined effect of added organic matter in the OMW compost along with the biochar could have an accumulative effect on the CEC on the substrate, with both amendments contributing.

4.6.3 Objective 3

Does biochar with PFC affect strawberry growth?

Treatment 10 when compared with treatments 1 and 2 performed better for weight of the first ten fruits produced and better than treatment 1 for weight of all fruits produced. The aspect where the fruit produced in treatment 10 performed the best was for sugar content, being significantly higher in sugar than all of the other treatments. Treatment 10 with a 50% rate of biochar had the greatest number of discarded fruits from any of the treatments, and also the highest number of *B. cinerea* infected fruits. This opposes the results cited by Meller Harel et al 2012 who found that the addition of biochar reduced the incidence of *B. cinerea* on the plants due to the biochar inducing the plant to express defence-related genes to improve plant resistance to pathogens (Meller Harel et al., 2012). This difference in reaction could be due to the quality of the feedstock for the biochar, however Meller Harel used two biochars, one from a citrus wood char and another from a greenhouse waste char at rates of 1% and 3%. Both biochars showed improvement in the plant defence against pathogens. Mehari used biochar on tomatoes to reduce the occurrence of *B.cinerea* and found that in the plants without a jasmonic acid mutation the biochar reduced the effects of grey mould on the fruits (Mehari et al., 2015).

The CEC for treatment 10 with the 50% biochar addition is lower than for the control which is unexpected given that biochar has in the literature been known to enhance the CEC of a substrate (Liang et al., 2006). This reduction in the CEC could be due to an over application of biochar, with a 50% rate being outside the levels of application used in commercial situations. The increase in the proportion of biochar meant that there would have been less of the peat free compost base, which provided much needed organic matter for the fruits.

4.7 Conclusions

These trials showed that there are no consistent patterns in plant development and fruit quality and quantity when different OMW compost were used as a substrate base. The addition of biochar as an organic amendment made no significant differences in the results that showed a trend.

In these trials only 3 dosages of OMW compost were applied to the substrate base, in further trials it would be beneficial to be able to complete more ratios in a gradual increase so that any effects of adding the compost can be more clearly seen. In addition to this, it would be ideal to have greater replication of each treatment as in these trials there were only 5 experimental units to test each treatment. This need for further work has been addressed in Chapter 5 Horticultural Trials 2015.

Chapter 5 Horticultural trials 2015

5.1 Introduction

Building on the work completed with the strawberry compost trial in 2014, this trial was set up to obtain more robust data to gain a better understanding of the effects of an OMW based compost and allow for improved data analysis. This trial was designed to look more in depth at the effects of the OMW compost on the growth of the strawberry plant by using a more incremental inclusion of the OMW compost. This study also looked into the effects of the compost on the successful pollination of the fruit alongside quality parameters measured in Chapter 4.

The organic amendment used in this chapter is an OMW based compost using pig manure as a base material. It has been used as a substrate replacement. It is expected that this may have effects on strawberry growth due to its nutritional value.

5.2 Literature review

5.2.1 Substrate and fertiliser effects on plant growth

Using waste derived composts, or compost teas, can be a way of promoting a circular waste economy and utilising useful nutrients for plant growth. However, a weakness in using waste derived compost as a substrate base for food production is the possible presence of high levels of undesirable constituents that can have a negative impact on plant development and production. The composts designed for such use either need to have these components mediated in some way to negate their effects or these components need to be at tolerable levels for the plants to cope with and maintain normal growth. Two potentially phytotoxic components present in the waste from olive oil production

are the relatively high concentration of phenols and also extremes of conductivity. Phenolics can have a dramatic phytotoxic effect on plants, more information on this effect was given in Chapter 1.

Salt stress on plants is common for many food producing regions including the Mediterranean (Cardeñosa et al., 2015) and it is estimated that between a quarter and a third of productive land worldwide is affected by salinity (Karlidag et al., 2009, Keutgen and Pawelzik, 2009). Strawberries are considered to be a salt sensitive species (Saied et al., 2005) and their response to salt stress can vary depending on the length of exposure time, the type of system they are grown in and the developmental stage at which the stress occurs (Cardeñosa et al., 2015). The recommendations set out by DEFRA suggest that strawberries are not subjected to salinity levels greater than 2.0mS/cm, and that it should be maintained at around 1.4mS/cm (DEFRA, 2010). The negative effects of toxic levels of salinity can be seen at the whole plant level, having an effect on photosynthesis and the plants' metabolism (Parida and Das, 2005). High levels of salinity are known to reduce leave number, leaf area, and shoot dry weight. Pirlak in 2004 found that high salinity of 5mS/cm also reduced the number of and the length of the runners produced on the plants and the number of crowns (Pirlak and Eşitken, 2004).

It has been reported that in some cases increasing the levels of salt can increase the yield of fruit up to levels of 2.6mS/cm conductivity (D'Anna et al., 2003) however it was found that above these levels more unmarketable fruit was produced. This is in agreement with Giuffrida (2001) who also found that salt levels between 2.6 – 4.6mS/cm reduced the number and fresh weight of strawberry fruit produced (Giuffrida et al., 2001). It has been suggested that the quality of fruit measured by sugar content can be improved by increased salinity. Cardeňosa in 2015 found that strawberry plants grown under salt conditions had better tasting fruit and fruit of better nutritional quality with higher antioxidant levels than fruit in lower salt conditions (Cardeñosa et al., 2015). The higher salt concentrations compared during that study were levels of 2mmol/L NaCl and 7mmol/L NaCl equivalent to approximately 0.2mS/cm and 0.7mS/cm respectively. In comparison to other trials under salt conditions the

experiment carried out by Cardeňosa et al cannot be described as putting the strawberry plants under salt stress.

The effects of salt stress can vary within plant species, and trials on different strawberry varieties showed that the effects of increased salinity were different on two commercial varieties of fruit. Between 2004 and 2009 Saied (2005) and Keutgen (2009) completed trials on two varieties of strawberry: Korona and Elsanta. Saied in 2005 found that the Korona variety were more able to cope with salt stress with no effect on the taste of the fruit however, the Elsanta variety had a decrease in taste quality by 24% when measured by 7 untrained panellists, when comparing salt levels of 2.6mS/cm and 5.1mS/cm. They found that the levels of Brix in the fruit were lower in the Elsanta than in the Korona variety, however there were no differences in fruit number or fruit yield (Saied et al., 2005). Keutgen (2009) completed trials with the same varieties with different salinity exposure at 3.4mS/cm and 6.9mS/cm and found that the salt stress reduced plant fresh and dry weight, especially in the Elsanta variety (Keutgen and Pawelzik, 2009).

5.2.2 Substrate and fertiliser effects on nectar production

Nectar is a high source of sugar in the form of glucose, sucrose and fructose (Nepi, 2014, Vaudo et al., 2015) for pollinators. Cardoza reported that flowers on cucumber *Cucumbus sativus* plants grown with a vermicompost amendment showed higher nectar sugar content than the control which was a commercial potting mix, although this result did not prove to be significantly different from the control (Cardoza et al., 2012). The use of this different compost in the experiments by Cardoza has also been shown to have an effect on the foraging behaviour of bumble bees, with an increase in visit length on plants grown in experimental vermicompost (Cardoza et al., 2012). The nectar from flowers is also an important source of amino acids for protein production as these are the second highest component of nectar (Gardener and Gillman, 2002). The presence of nitrogenous compounds in the form of amino acids is ubiquitous in nectar, however different types of amino acid produced by plants will influence the taste of the nectar and its attractiveness to pollinators. Gijbels et at (2015)

reported that in orchids *Gymnadenia conopsea* it was possible to change the amino acid composition of the nectar by making changes to fertilisation (Gijbels et al., 2015). This was conducted in the field with fertilisation added via mineral liquid NPK addition. The work concluded that additional fertilisation can affect the patterns of reproduction and pollination success (Gijbels et al., 2015).

There are also non-protein amino acids (NPAA) incorporated into the nectar available from a flower. There are around 250 amino acids that have specific roles in the plant when involved in interactions with bacteria, herbivores, fungi and other plants. These NPAAs have antibacterial or antifungal properties or can promote the allelochemical or antiherbivory attributes of the plant (Nepi, 2014). The volume of nectar present may also be as important as the concentrations of the sugars and amino acids (Guerra-Sanz, 2008).

5.2.3 Pollination methods and mechanism

Intensification of fruit production due to an increased demand from a rising population has led to a global demand for the services of pollinators, in particular bumble bees. The development of new methods, monoculture systems and improved and more controlled management has increased global production and is going towards reducing world food poverty. This has had the corresponding effect of reducing biodiversity and ecosystem services, particularly pollinator services (Deguines et al., 2014).

Pollination with animals as vectors involves using them to transfer the pollen present from the anther of one flower to the stigma of another flower (Nepi, 2014). An example of a strawberry flower in a cross sectional view is given in Figure 5.1. This shows the main features of the flower including the stamen where the pollen is produced and the pistils where the ovules are produced (Hancock, 1999).

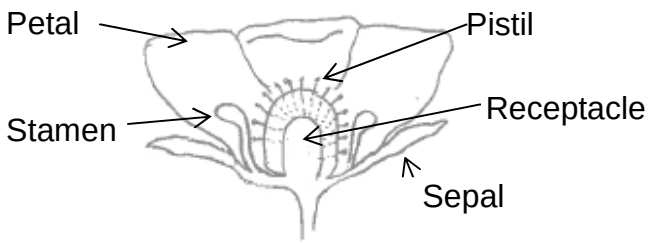


Figure 5.1 Diagram showing the cross section of a strawberry flower. Adapted from (Strand, 2008)

Of the flowering plants used for food production estimates suggest that pollinator input ranges from 75% of the 115 world leading crop species requiring pollination (Klatt et al., 2014) to nearly 90% of crops using animals as vectors for pollination for seed and fruit production (Cardoza et al., 2012). Whichever estimate is used, it is clear that pollination is crucial for the maintenance of current global food production. It is possible for plants to self-pollinate through windblown, or mechanically driven pollen spreading, however pollination using vectors, typically insect vectors is most common in commercially produced food. Pollination using soundwaves is also a viable method of pollination in the absence of insect pollinators and is a valued method working alongside cutting edge food production in seawater greenhouses and has been used on tomatoes (Hitchin, 2014, DeTar, 1968). Vibration pollination has also been used on strawberries grown under artificial light conditions in the absence of ultraviolet light that is necessary for bee pollination (Shimizu et al., 2015).

Bees as pollinators select flowers based on their attributes and will visit flowers that are rich in nectar. Bees need to derive all their energy requirements from floral resources and as such will choose those with the richest provisions. Bees can be subject to nutrient deficiencies when floral resources are poor and pollination of plants can suffer as a result (Vaudo et al., 2015). The provision of an attractive flower, a refined odour and a tasty nectar source will attract bees to pollinate the flower (Gijbels et al., 2015) and hence promote the production of a marketable fruit. The effects of using an alternative compost as a substrate

base and source of nutrients could have an impact on the attractiveness of flowers (in size and shape) and also on the quality of the nectar. Successful bee pollination has been shown to produce more fruit of better quality in strawberry plants and with a longer shelf life than either wind or self-pollination (Klatt et al., 2014). Experiments with blueberries also showed that pollination can be a limiting factor on the overall production of fruit (Benjamin and Winfree, 2014).

As described in Chapter 4 strawberries are an aggregate fruit (Agarwal, 2013) and are composed of cells, each with their own achene which is the true fruit of the strawberry plant. Strawberry fruits may have over 200 achenes or seeds on their surface and each of these needs to be pollinated to achieve an evenly formed marketable fruit. In the absence of pollination, strawberry fruits will not form an even shaped berry and as a result will not be commercially viable. A cross section of a strawberry fruit is shown is Figure 5.2 where the achenes and their accessory bodies can be clearly seen making up a strawberry 'fruit' as it is known. The pollination of each achene causes them to produce auxin, a hormone responsible for the development of the strawberry fruit through the initialisation of the build-up of gibberellic acid, another hormone that works with the auxin to increase the size of the cell. This in turn induces fruit growth and increases fruit size and weight (Klatt et al., 2014).

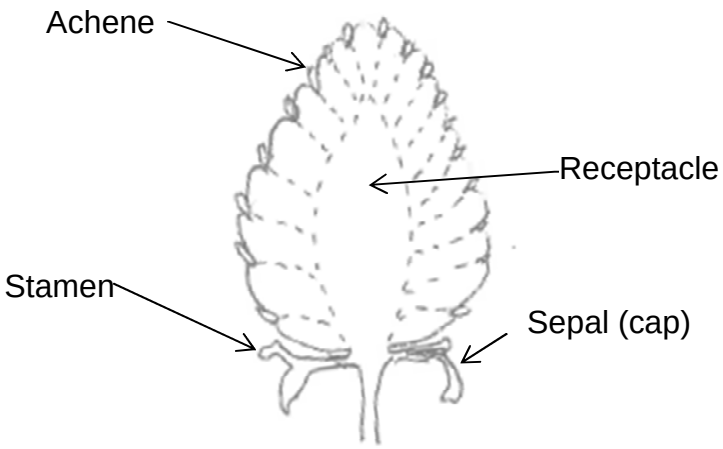


Figure 5.2 Cross section of a strawberry. Adapted from (Strand, 2008)

5.3 Aims and objectives

The aim of these trials was to assess the effects of increasing concentrations of a compost produced using olive mill wastewater in smaller increments than the trials in 2014. These trials are also designed to give a more robust data analysis due to the improved design of the trials and the increase in the number of experimental units. Increasing the OMW compost in smaller increments should give a better idea of any effects this may have on plant productivity and fruit production.

Objective 1: Determine the impacts of increasing the fraction (v/v) of OMW compost present in the growing media for strawberry plants and alongside this compare the effects of two common base substrates, coir and peat against each other. The different treatments set up in order to achieve this objective are given in Table 5.1

Table 5.1 Treatments set up to fulfil objective 1

Treatment number	*Combination*	*Type of irrigation*
1	Control – 100% coir base	Fertigation
2	100% Peat compost (PC)	Fertigation
3	5% OMW & 95% PC	Fertigation
4	10% OMW & 90% PC	Fertigation
5	15% OMW & 85% PC	Fertigation
6	20% OMW & 80% PC	Fertigation
7	25% OMW & 75% PC	Fertigation

Objective 2: Compare the effects of a substrate with OMW compost added in the presence and absence of liquid fertilisation

Table 5.2 Treatments set up to fulfil objective 2

Treatment number	*Combination*	*Type of irrigation*
F	15% OMW & 85% PC	Fertigation
NF	15% OMW & 85% PC	Hand watered – no fertiliser in the water

5.4 Methodology

5.4.1 Trial set up

The trials in 2015 were carried out at the Stockbridge Technology Centre (STC) located at Cawood, North Yorkshire (lat 53.82^{o}, long -1.15) and used an OMW compost produced using pig manure as the base manure. The compost used in these trials was produced differently to the chicken manure based compost used in 2014. The compost for the 2015 trials was subjected to the solar drying process with the OMW applied repeatedly, before being composted. It is suggested that using this method will help to reduce the amount of phenols in the product as they will be denatured during the composting process. The analysis of the compost used in these trials in combination with the peat product is given in Table 5.3. The data that potentially was a cause for concern was the high EC of over 11000 µS/cm.

Table 5.3 Analysis of the OMW compost produced for the trials in 2015

Parameter	*Units*	*Value*
pH		9.69
EC	µS/cm	11610
Total Phosphorus	% w/w	1.055
Total Potassium	% w/w	8.645
Total Magnesium	% w/w	0.6545
Nitrate Nitrogen (fresh)	mg/kg	<10
Ammonium Nitrogen (fresh)	mg/kg	138
Total Nitrogen	% w/w	3.41
Total Sulphur	% w/w	0.7145
Total Copper	mg/kg	74.35
Total Zinc	mg/kg	209.5
Total Sodium	% w/w	0.4105
Total Calcium	mg/kg	52823
Total Phenols (index)	mg/kg	<1
Dry matter	%	75.95

The characterisation of the raw pig manure and OMW are given in Table 5.4. The OMW used to produce the OMW compost for these trials had an EC of 2310 µS/cm, and the dried pig manure had a starting value EC before the OMW addition of 14270 µS/cm. The addition of the OMW in this case reduced the EC of the pig manure. The foliar nutrient ranges for strawberry for copper (6 – 20mg/kg), zinc (20 – 50mg/kg), calcium (7000 – 17000mg/kg) are also exceeded by the OMW compost (Hancock, 1999). It can be seen from the data in Table 5.4 the OMW is rich in nutrients with a large concentration of potassium in the waste product.

Table 5.4 Characteristics of the raw products

	Pig manure	*OMW*
pH	7	4.9
EC	14270 µS/cm	2310 µS/cm
Total Phosphorus	1.2% w/w	152.36mg/l
Total Potassium	1.64% w/w	2345.07mg/l
Total Magnesium	5.31g/kg	143.24mg/l
Total Nitrogen	2.14% w/w	331.95mg/l
Total Copper	63.71 mg/kg	229.36µg/l
Total Zinc	210.78 mg/kg	5441.06 µg/l
Total Phenols (index)	1.95g gallic acid/l	3.4375g gallic acid/l

The plants were grown in a controlled glasshouse with pollinators provided in bee hives. Pollination for the plants was provided by a commercially available bee hive provided by a BCP® BeeSure hive providing bumble bee worker bees.

The trials described here were designed to build on the work completed in 2014 and included more defined treatments. There were a total of 9 treatments carried out in 2015, given in Table 5.1 and Table 5.2. The control used for 2015 was based on the standard way in which many of the commercially grown strawberries in the UK are produced. These are grown in a coir base to give the strawberry plants a structure to root in, but all the nutrition comes from being fertigated. Fertigation is a type of management where irrigation is combined with fertilisers to provide nutrients to plants in a liquid form.

The strawberry plants were purchased at 60 days growth in a frozen state, allowed to defrost and then transplanted into pots. Each of the pots was labelled twice, once on the pot using tape, and also with a plant marker cane. The strawberry variety chosen for the trials in 2015 was the Sonata variety

(*Fragaria x ananassa)*. The seven treatments for objective 1 had fifteen replicates each, with each replicate being one strawberry plant. The pots were arranged in the glasshouse in a randomised complete block design (RCBD) as shown in Figure 5.3. The layout of the block was lying adjacent to each other so that all of the blocks were next to the edge of the bench. The two treatments in objective 2 had five replicates each and were not subject to a RCBD due to the small number of replicates and the necessary management for the plants. For this objective a full 15 replicates was not possible due to supply constraints, so 5 replicates per treatment was selected as this amount had worked effectively in the trials in 2014.

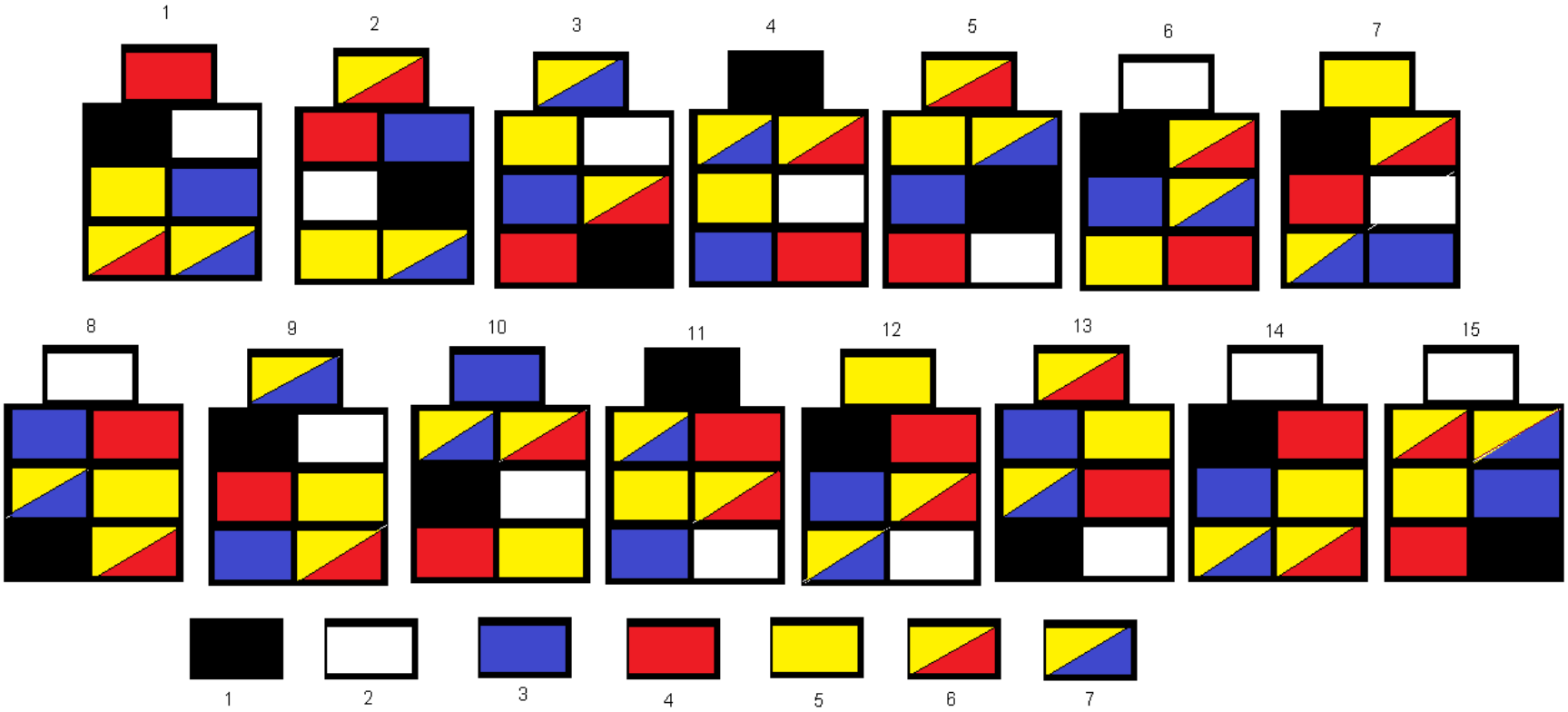


Figure 5.3 Layout of RCBD within the glasshouse at Stockbridge. Treatment numbers are given underneath the single cells at the bottom and the block number 1-15 is given above the groups of 7 cells.

The plants were watered using an automatic drip feed system, with the fertiliser added alongside the water using a Dosatron® dispenser from the D3 range. The fertiliser was added into the water as a mixture of calcium nitrate, Iron EDTA, Nitric acid and Sangral® 1.1.3. Sangral is a brand of NPK fertiliser that also contains iron, zinc, manganese and copper. These nutrients were used as they are recommended for strawberry growth by DEFRA (DEFRA, 2010). The temperature in the glasshouse were measured at half hourly intervals by a Priva® climate control system that is permanently mounted in the glasshouse. A line graph showing the daily minimum and maximum temperatures is shown in Figure 5.4. The peak in the temperatures was at 42ºC and the low was at 4ºC. The temperatures shown on the graph in Figure 5.4 rise above the optimal temperatures for strawberry growth as discussed in Chapter 4 however this will affect all plants and treatments equally due to the block design so no differential limitations as a result of temperature are anticipated.

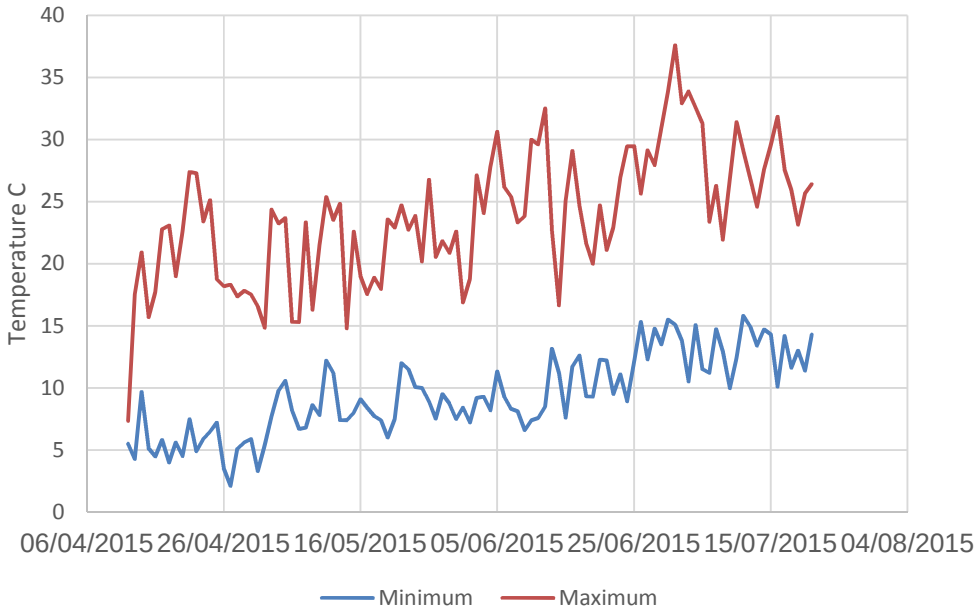


Figure 5.4 Daily minimum and maximum temperatures as recorded in the glasshouse throughout the monitoring period.

A photograph of the set up within the glasshouse is shown in Plate 5.1. This shows the layout of the bench and the proximity of the bee hive to the experimental units.

Plate 5.1 The set up within the glasshouse including markers, the bee hive (cardboard box with yellow top) and the fertigation drip-feed system.

5.4.2 Sampling of fruits and substrate

The sampling carried out in 2015 used the trials in 2014 as a basis to inform the new trials and to improve the methodology. The cold stored plants were defrosted and transplanted on the 14 April 2015, the first flower appeared on the 14 May and the first harvest of marketable fruit was on the 18 June. This means that the plants took 65 days to produce fruit after transplanting.

In these trials the chlorophyll measurements were taken on a weekly basis from each replicate throughout the trial period totalling 9 measuring occasions. In contrast to the trials in 2014, a leaf from each replicate was tagged around the petiole – the petiole is the stalk that attaches each leaf to the stem (Rose et al., 2006) - to ensure that the same leaf was measured on each visit. This was

done in order to obtain a clearer pattern of nitrogen uptake into the leaves during the growing season. A leaf count per replicate plant was also completed along with a count of the number of runners per plant. The number of leaves and the number of runners on each plant were counted three times during the monitoring period, at the start, in the middle of the trial and on the final day.

The fruits were harvested twice a week from the appearance of the first ripe fruit for a total of 8 harvest dates. It was the aim to measure five marketable fruits from each plant for all treatments for weight, width, firmness and sugar content. This gave approximately 75 fruits analysed for each treatment given 15 replicates x 5 fruits from each. A fruit for this analysis was selected on each plant on each visit to give a representation of fruit throughout the harvest period, in contrast to the method used in 2014 which sampled predominantly primary fruit. All other fruits were classified into being marketable or nonmarketable and were weighed.

The second fruit sampled from each plant for the extended measurements was stored in a ziplock bag and frozen in preparation for an assessment of pollination success on the fruit. Pollination success is a measure of how well the fruit was pollinated, and as a result how well the fruit has formed. This was determined by blending the fruit with water using a handheld Braun® MQ100 stick blender, then the pollinated and unpollinated achenes (seeds) were collected. The amount of water the fruit was blended in varied from 100-200ml depending on the size of the fruit to increase the effectiveness of the fruit blend. A photograph to show the process of blending and filtering in preparation for counting is shown in Plate 5.2. Pollinated achenes are denser than unpollinated ones so that they sink to the bottom when suspended in water, leaving the unpollinated ones floating on the surface. The unpollinated achenes can then be collected from the surface of the water using a sieve and counted, and the pollinated achenes filtered over a fine mesh before being counted. This method is adapted from Klatt (Klatt et al., 2014).

Plate 5.2 Photograph to show the process of separating pollinated and unpollinated achenes

In the middle of the harvest period fruits from a sufficient number of replicates to provide a large enough sample size from all treatments were pooled together and submitted for a vitamin C analysis. These were sealed in a ziplock bag and delivered immediately to the laboratory for analysis.

On completion of the harvesting the plants were removed from the pots and all substrate removed by hand and by washing with water. A substrate sample was taken from each replicate from four of the blocks with a full suite of treatments and stored in a ziplock bag to be sent for laboratory analysis. The plants from each block were then photographed next to one another, and then stored in paper bags for weighing and drying. Prior to weighing plants and roots were measured for total length and an image of his process is shown in Plate 5.3. They were measured from the base of the plant to the end of the tallest stem or longest root, the length of the biggest runner on each plant was also measured in this way. The plants from the four blocks with each treatment still remaining were sent for plant protein analysis following drying.

Plate 5.3 Photograph showing the way in which the plants were measured for height prior to the weighing and drying process.

5.4.3 Analysis methods

The analysis for substrate in this chapter has mostly been covered by chapters 2 and 3, with only one analysis of the strawberry fruit being novel in this chapter in the metals analysis discussed below.

5.4.3.1 Vitamin analysis

The vitamin analysis was completed on pooled fruit collected from each replicate. This was done using a high pressure liquid chromatography (HPLC) following sample preparation using extraction using a centrifuge and an acetate buffer with a pH of 4.8. Before injection into the HPLC samples were subject to ultrasonic waves to ensure dissolution. The vitamin C in the sample is then calculated by measuring the size of the peak produced in the HPLC.

5.4.3.2 Metals analysis

The metals analysis was completed on a filtered sample from a water suspension by using Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP – OED). This uses argon gas to create a plasma and then the sample is misted onto a plasma flame where the sample is broken down into its constituent elements. This then emits the individual wavelength for each

element present. The apparatus measures the strength of the wavelength for each element and can give a result based on a calibration curve.

5.5 Results

This section presents the results obtained from the data collected from the plants and fruits produced as part of this trial. Although each treatment set up for objective 1 and 2 started with 15 and 5 replicates respectively, the addition of the OMW compost resulted in the complete loss of some plants. Therefore it will be noted that the number of replicates in the field and harvest data may be less as the dead plants were then removed from the trial and not replaced. Treatments 1-4 all have 15 replicates for all the data analysis, treatment 5 has 11, treatment 6 has 10 and treatment 7 has 4 replicates to represent that treatment.

5.5.1 Plant growth and development

5.5.1.1 Chlorophyll content of the leaves

The data collected for the chlorophyll levels in the leaves was normally distributed according to a Shapiro-Wilk test and therefore could be analysed using an ANOVA. The results for the chlorophyll measurements for those treatments set up for objective 1 are shown in Figure 5.5 and are the mean values obtained from the surviving replicate plants. The table below the figure shows the standard deviation for the mean values throughout the monitoring period.

It can be seen in Figure 5.5 that the overall trend for the levels of chlorophyll in the leaves increases throughout the monitoring period for all treatments. In addition to this the general observation is that the higher the proportion of OMW compost used the lower the leaf chlorophyll content. The lowest chlorophyll contents were found for treatment 7 in which 25% OMW compost was combined with the peat compost which is the highest proportion of OMW compost used. The chlorophyll concentrations for treatment 7 started the

monitoring period at 6.56 and ended on the 20 July on 26.87. For treatment 7 the chlorophyll content in the leaves remained below the other treatments throughout the monitoring period.

The highest chlorophyll concentration during the entire monitoring period was observed for treatment 1 on the 15 June with a value of 50.84. Treatment 1 was the 'control' treatment in which the substrate was 100% coir with no OMW compost or peat added. This high chlorophyll concentration is an unusual spike in the data and the values for the standard deviation on this date show no great difference between the standard deviation for treatment 1 against all other treatments. There appeared to be no anomalies in the data for this high mean value. Treatment 1 also ended the monitoring period on the highest value of 43.53.

Figure 5.5 shows that treatments 5, 6 and 7 have consistently lower SPAD values for chlorophyll than treatments 1-4. On every occasion throughout the monitoring period, statistical analysis shows that treatment 7 had significantly lower values for the SPAD chlorophyll than treatments 1, 2, 3, and 4. The statistical analysis also shows that treatment 6 was also significantly different from treatments 1, 2, 3, and 4 on most occasions except on 15 June when it was not significantly different from any other treatments and on the 20 July when it was significantly different from treatments 1, 2, and 3. Treatment 5 is located in between the high and low SPAD values and as a result has occasions when it is significantly higher than treatment 7 (26 May, 1, 8, 22 and 28 June) and other occasions when it is significantly lower than treatment 1 (29 June), treatment 2 (26 May, 8, 15, 22 and 29 June) and treatment 3 (26 May, 1, 8, 22 and 28 June). The data from the chlorophyll measurements indicates that nitrogen uptake from the substrate into the leaves is significantly lower in those treatments receiving 15%, 20% and 25% OMW compost, i.e. treatments 5, 6, and 7. There were no significant differences between the controls and those treatments that received a 5% or 10% addition of OMW compost. The standard deviations are much greater for treatments 6 and 7 throughout the monitoring period. This would suggest that the impact on the plants in these treatments is a lot more variable that for the other treatments.

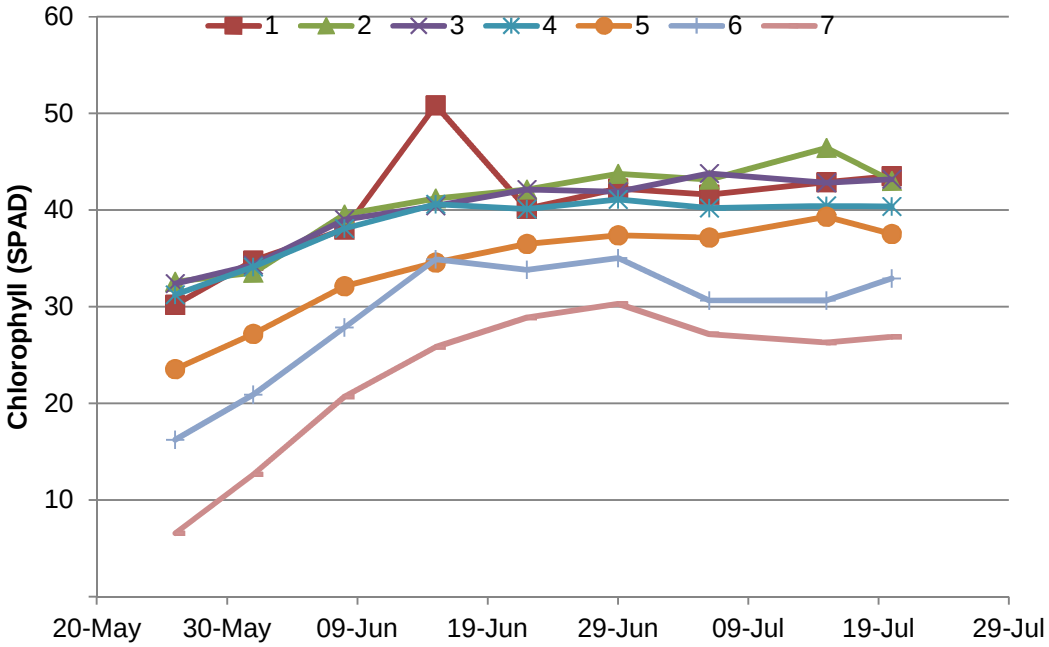


Figure 5.5 Mean leaf chlorophyll content for each of the treatments in objective 1 throughout the monitoring period.

The mean chlorophyll concentrations in the leaves for the plants set up for objective 2 are shown in Figure 5.6. Due to their only being two categories (fertigation - F and hand watered - NF) this data was compared using an independent sample T-test. Figure 5.6 shows that the initial chlorophyll content of the leaves in both treatments was similar and that the chlorophyll levels in treatment NF, the unfertilised treatment, showed a rapid decrease throughout the monitoring period from an initial value of 36.04 with the average on the final date of the 20 July being only 10.52. In contrast the fertilised treatment showed a consistent if small increase over the monitoring period from an initial value of 32.84 and ended the monitoring period on a high of 43.81. Data analysis showed that the values for the fertilised treatment (F) are significantly higher than the unfertilised treatment (NF) when compared using a T-test for the second half of the monitoring period from the 23 June onwards.

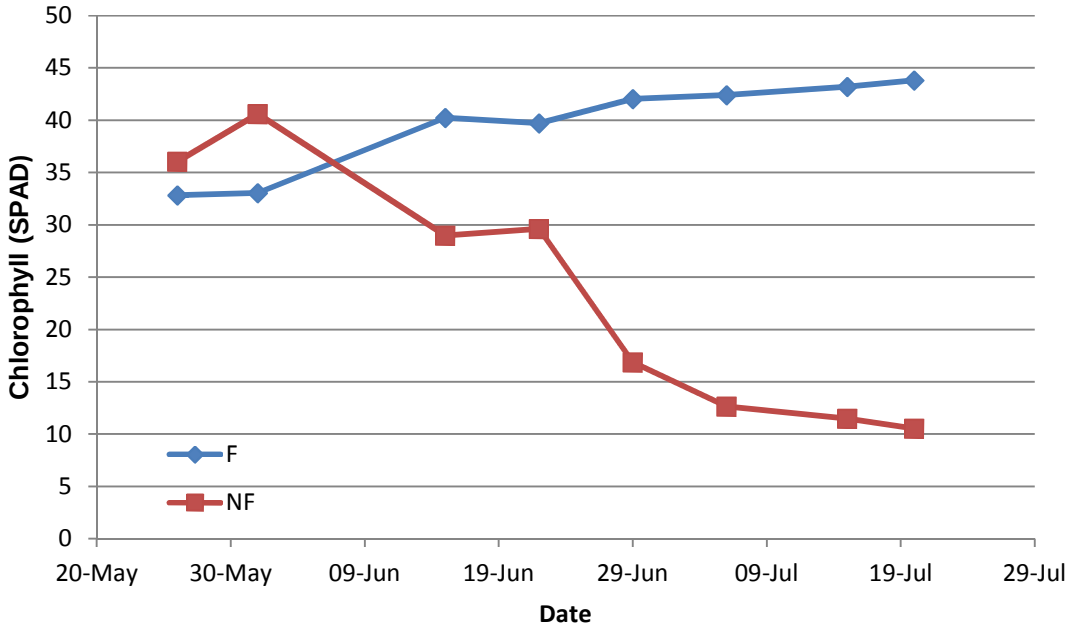
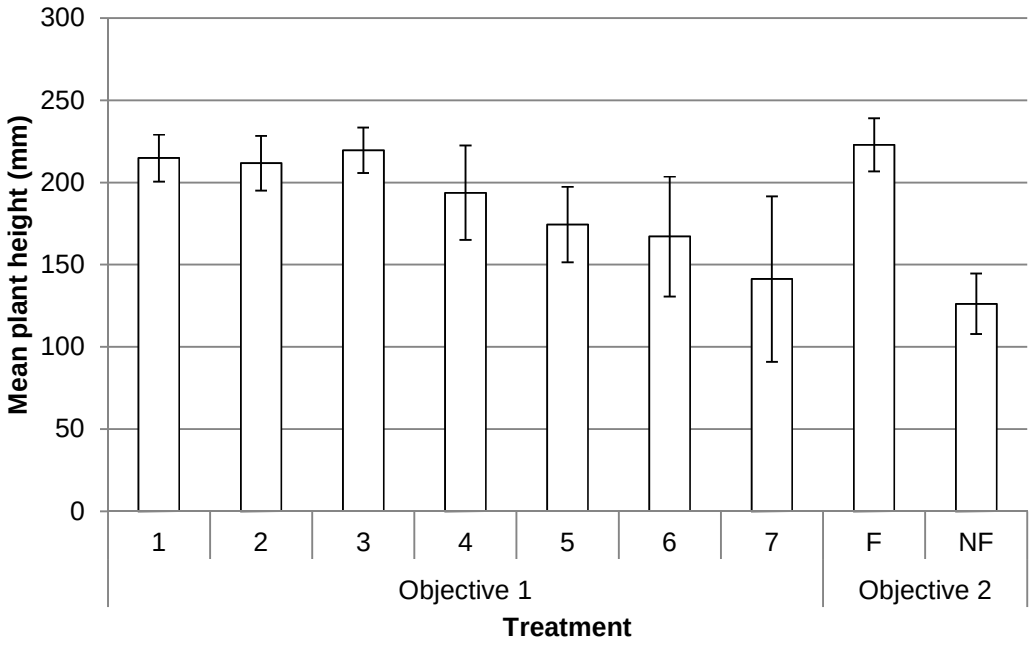


Figure 5.6 Mean leaf chlorophyll content for each of the treatments in objective 2 throughout the monitoring period.

5.5.1.2 Plant height

The plant height data was obtained from all remaining viable plants at the end of the trial and was measured prior to drying, the results are shown in Figure 5.7. The tallest plants were those for treatment 3 in the objective 1 trial which was the one in which 5% OMW compost was added to peat compost (the lowest fraction of OMW compost used) with a mean height of 219.6mm, this also had the lowest standard deviation of 13.8mm. The treatment with the smallest plants was treatment 7 in which 25% OMW compost was combined with the peat compost (which is the highest proportion of OMW compost used) with a value of 141.3mm. The overall trend observed in Figure 5.7 is that treatments 1, 2 and 3 appear to be similar and then there is a steady decrease in mean plant height for treatments 4, 5, 6 and 7. This would suggest that as the fraction of OMW compost used increases the mean height of the plants decreases. The results for the objective 2 trial are very different from each other with the fertilised treatment (F) having a value of 223mm for mean plant height and NF having smaller plants with a mean plant height of 126.2mm.

The data for plant height were normally distributed according to the Shapiro-Wilk test and can be analysed using an ANOVA. This test for significance shows that treatments 1, 2, 3, 4 and F are similar to each other and can be grouped together. Treatments 5, 6, 7 and NF can also be grouped together as they are similar to each other but are significantly different from treatments 1,2,3,4 and F. These differences are shown with notations (a and b) in the table shown below the graph in Figure 5.7. All those treatments which are not significantly different from each other have the same notation.



Treatment								
1^a	2^a	3^a	4^a	5^b	6^b	7^b	F^a	NF^b

Figure 5.7 Mean plant height for each treatment with error bars indicating standard deviation with a table below to show the significance between each treatment – different superscripts indicate a significant difference in the results.

5.5.1.3 Number of leaves and runners

As the data for the number of leaves is non-parametric it is best displayed as a median value on a graph and has be to analysed for significance using a Kruskal Wallis test with a Chi Squared (χ^2) analysis, followed up by pairwise Mann-Whitney U tests for all occasions with significant data. The data for the median number of leaves for all treatments is shown in Figure 5.8.

In all treatments except the non-fertilised treatment (NF) the number of leaves increases throughout the growth season. For treatment NF the number of leaves remained constant until the middle of the monitoring period. The general observation is that the number of leaves for treatments 1, 2, 3 and 4 were consistently higher than those for treatments 5, 6 and 7. Overall it would appear that there is a downward trend in the number of leaves with increasing amounts of OMW compost. The greatest median value for number of leaves was from treatments 1 and 3 with 27 leaves on the third and final count. The fewest number of leaves was for NF which had 0 leaves by the time count 3 was undertaken. This shows a significant difference in the data between treatments for all counts.

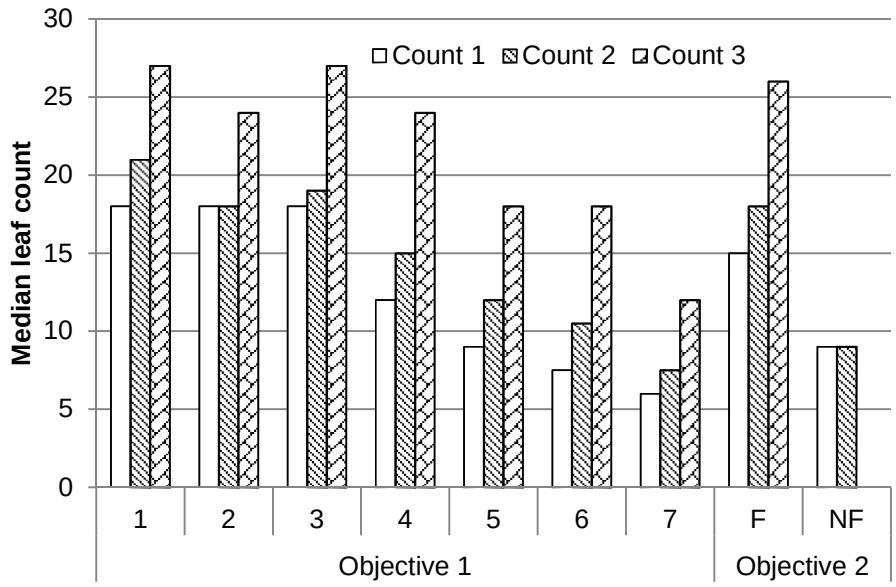


Figure 5.8 Median leaf counts for each treatment measured on three occasions (start – count 1, middle – count 2 and final day – count 3) throughout the monitoring period

The data for the number of runners on the strawberry plants was also no-parametric so it is also displayed using a median value and analysed using a Kruskal-Wallis test. Figure 5.9 shows that for the first two counts in the growing season only treatments 1, 2 and 3 produced runners. By the end of the growing season (count 3) all treatments had produced runners, however unlike the leaf count there did not appear to be a relationship (either positive or negative) between the fraction of OMW used and the number of runners. It was observed that all treatments with any runners present had the greatest number for the third count, and the overall trend was that plants treated with OMW above a 5% rate had fewer runners. The greatest median number of runners recorded in any treatment was in treatment 2 on the final count with a median of 5 runners. Comparing the fertilised and non-fertilised treatments for objective 2 shows that the fertilised treatment produced an increasing number of runners over the growing season with a final median count of 4 runners. In stark comparison the non-fertilised treatment failed to produce any runners during the whole growing season.

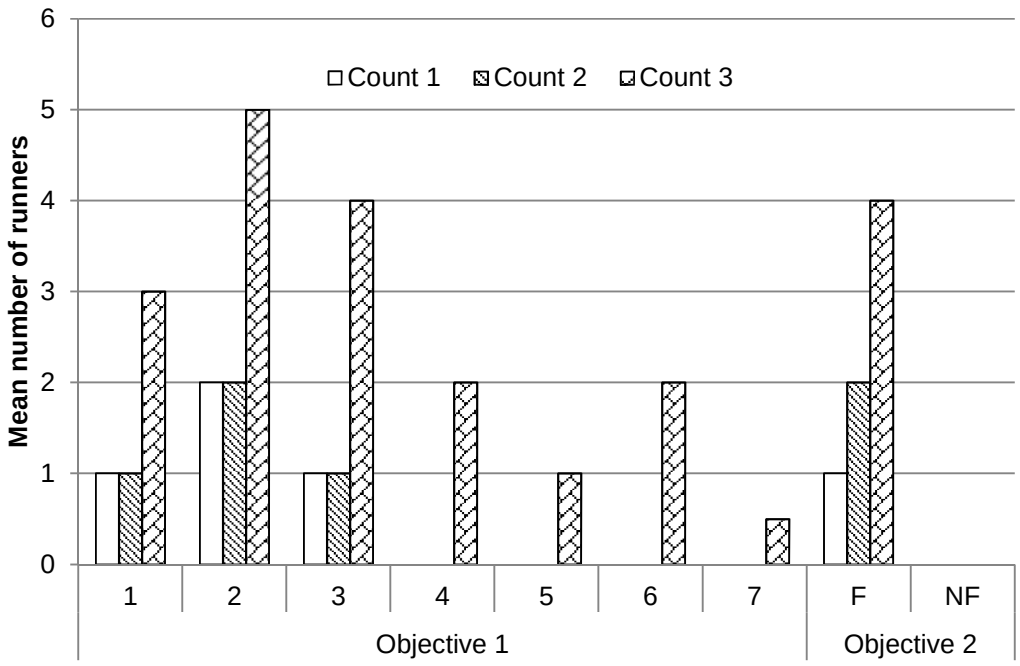


Figure 5.9 Median number of runners counted for each treatment measured on three occasions (start – count 1, middle – count 2 and final day – count 3) throughout the monitoring period

The information to describe the statistically significant differences between the leaves and runners on each treatment is given in Table 5.5. This is a result of the Mann-Whitney U tests carried out after the Kruskal Wallis test for significance. The notation describing the data is different when data is significantly different from one another.

Table 5.5 The count data for the leaves and runner, with superscript text denoting significant differences between the treatments.

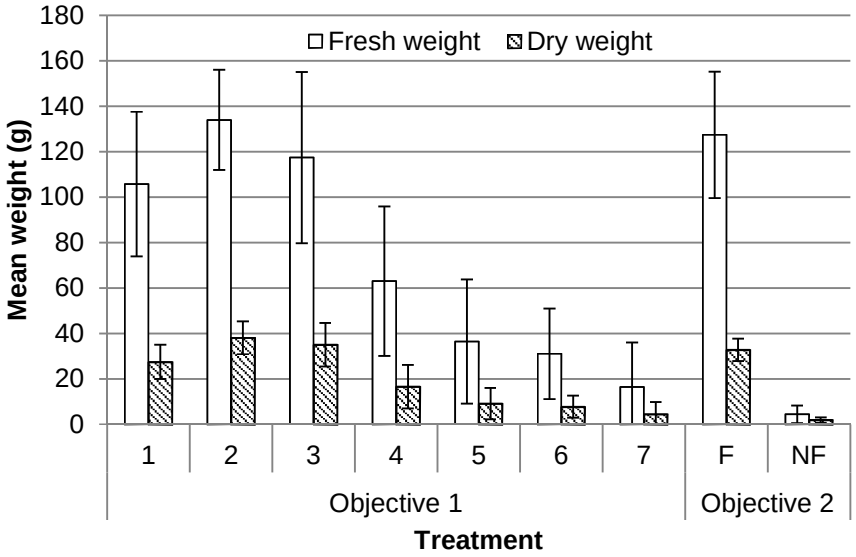
Leaves 1	*Leaves 2*	*Leaves 3*	*Runners 1*	*Runners 2*	*Runners 3*
1^{a}	1^{a}	1^{ad}	1^{ab}	1^{ab}	1^{a}
2^{a}	2^{ae}	2^{bd}	2^{a}	2^{a}	2^{a}
3^{a}	3^{ae}	3^{abd}	3^{b}	3^{b}	3^{a}
4^{bc}	4^{bc}	4^{cd}	4^{c}	4^{c}	4^{c}
5^{bd}	5^{bd}	5^{e}	5^{c}	5^{c}	5^{c}
6^{bd}	6^{bd}	6^{e}	6^{c}	6^{c}	6^{c}
7^{bd}	7^{bd}	7^{ef}	7^{c}	7^{c}	7^{c}
F^{ac}	F^{ce}	F^{ab}	F^{ac}	F^{c}	F^{ab}
NF^{bd}	NF^{bde}	NF^{f}	NF^{c}	NF^{c}	NF^{d}

5.5.1.4 Fresh and dry weight

The results for the fresh and dry weight of all treatments are shown in Figure 5.10. The graph shows the mean fresh and dry weight for the different treatments and the table shows the calculated dry matter and the standard deviation of the data for each treatment.

The fresh and dry mean weight of the plants was highest in treatment 2 with values of 133.9g and 38.1g respectively. The treatment with the lowest value in objective 1 was treatment 7 with wet and dry values of 16.5g and 4.5g. With the exception of treatment 1 the data for the treatments in the objective 1 trial suggests that there was a negative relationship between the fresh and dry weights and the fraction of OMW used. The difference between treatments in objective 2 is large with the fresh weight of F being 127.4g and the fresh weight of NF at 4.5g. The data was normally distributed so could be analysed using a

Tukey's test in an ANOVA. Notation indicating significance between treatments for fresh weight is shown in the table in Figure 5.10. The notation shows that the treatments fall into two groups with treatments 1, 2, 3 and F being similar to each other but dissimilar to treatments 4, 5, 6, 7 and NF.



Treatment	Mean plant dry matter (%)	Standard deviation
1[a]	26.34	2.99
2[a]	28.42	2.71
3[a]	27.59	3.02
4[b]	29.01	12.31
5[b]	25.21	3.05
6[b]	25.35	2.99
7[b]	33.32	10.99
F[a]	26.02	1.77
NF[b]	55.21	29.01

Figure 5.10 Bar graph and table with the bar graph showing the mean fresh and dry weights of the plants from each treatment and the table showing the mean dry matter content and standard deviation.

5.5.1.5 Visual assessment of plant growth

The inclusion of the OMW compost at the higher ratios did not take long to have plant damaging effects on the strawberry plants. Treatments 1-4 kept a full suite of replicates, with none of the plants in the 15 blocks dying. Treatment 5 had a loss of four of the replicates, treatment 6 lost five of the replicates and treatment 7 lost eleven replicates to plant mortality. This information would suggest that the larger the fraction of OMW used the greater the detrimental impact on plant survival.

During the monitoring and harvest period the plants were also checked for signs of pests such as aphids, and deficiency symptoms. From treatment 3, seven of the replicates exhibited signs of calcium deficiency, and two of the replicates in treatment four showed the same signs. Calcium deficiency is shown through a tip-burn in the runner tips, and fully developed leaves becoming crinkled with a band of necrosis across the centre (Hancock, 1999).

Following the final harvest the plants were removed from the substrate and the roots washed. The plants could then be photographed to show any differences in growth between the treatments. The photographs in Plate 5.4 show the plants in blocks 4, 9 and 11 as these were the only blocks that maintained the full number of treatments throughout the trials. The treatment numbers can be read left to right 1-7 and the pictures show that there was a decrease in the size of the plant as the treatment number and therefore the amount of OMW compost in the substrate increases. There was a decrease in the amount of plant material as well as the visible height of the plants, this ties in with the data discussed in 5.5.1.2, 5.5.1.3 and 5.5.1.4.

a) Block 4

b) Block 9

c) Block 11

Plate 5.4 The photographs shown in a), b) and c) show the growth of the plants from blocks 4, 9 and 11 respectively.

5.5.1.6 Leaf protein analysis

Four samples from each treatment for both objectives were sent to the Sciantec laboratories for leaf protein analysis following the final harvest date of strawberries and the removal of the plants from the substrate; the results of this are shown in Figure 5.11. This shows the mean for each treatment along with an error bar depicting standard deviation. The data from each objective were possible to compare on a similar graph in this case as the same number of replicates for each treatment were submitted for analysis. The data was normally distributed so could be analysed using a Tukey's test in an ANOVA.

The data shows that the values for leaf protein for treatment 5 were higher than others at 15.45%, treatment 5 also had a small standard deviation of 0.65. The lowest leaf protein was in the non-fertilised treatment with 9.97% protein in the leaves, but with a large standard deviation of 4.14. There were no significant differences between the seven treatments for objective 1 or between the two treatments for objective 2. There was however a significant difference between

the leaf protein content of treatment 5 and the non-fertilised treatment which were comparable as they had the same amount of OMW compost in the substrate, but had had different management. This does show up a discrepancy in the data between treatment 5 and the F treatment which should have had similar values, and whilst they weren't significantly different from one another, there was some variation in their values. This could be due to the different number of replicates between treatment 5 and the F treatment.

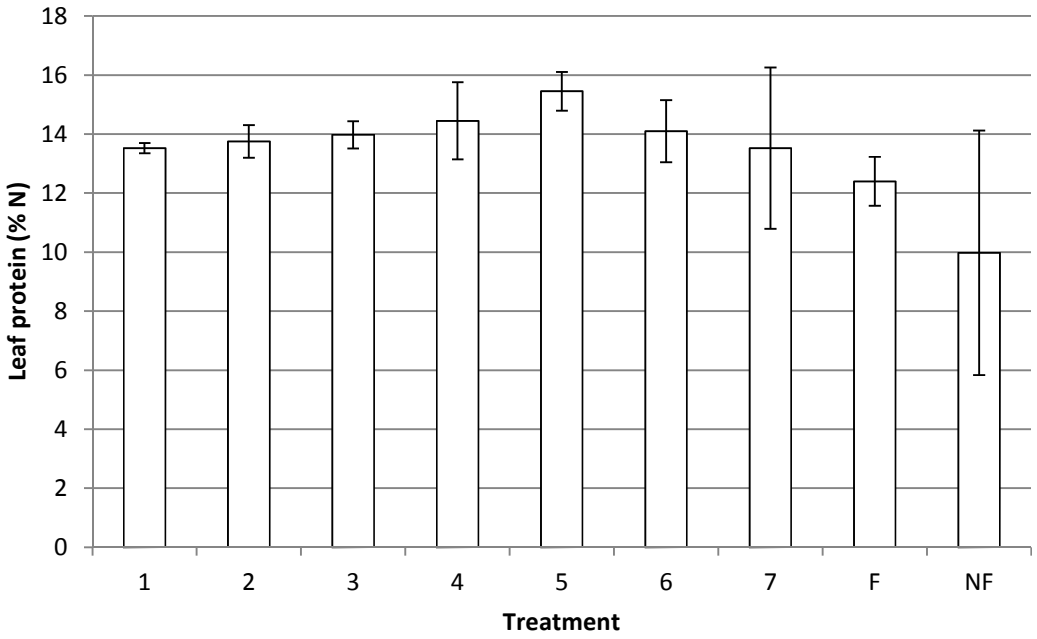


Figure 5.11 Leaf protein analysis from all treatments

5.5.2 Total fruit harvest analysis

The total amount of fruit produced from each experimental unit was weighed and counted. This gives a large cohort of data for strawberry weight and the results of this are shown in Figure 5.12. Aside from treatment 1 having slightly lighter fruit than treatment 2 the graph in Figure 5.12 shows an overall downward trend in mean weight with increasing treatment number and hence percentage of OMW compost used. The treatment which produced the heaviest fruits from all fruit produced was treatment 2 with a mean weight of 16.6g. The treatment that produced the lowest weight fruit in the objective 1 trial was

treatment 7 with a mean weight of 8.0g, the treatment producing the lowest weight fruits was NF with a mean value of 6.6g. All these values represent the marketable fruit. The data were not normally distributed and transformations of the data (cubing, square root, Lg10, LN) were not able to convert it into normally distributed data so a Kruskal-Wallis test was performed. This showed a χ^2 value of 78.3 and a significance of 0 which shows that there were significant differences within the data. A Mann-Whitney U test identified that treatments 1-3 were grouped together and significantly higher than treatments 4-7 and NF. Treatment F was significantly higher than treatments 6, 7 and NF.

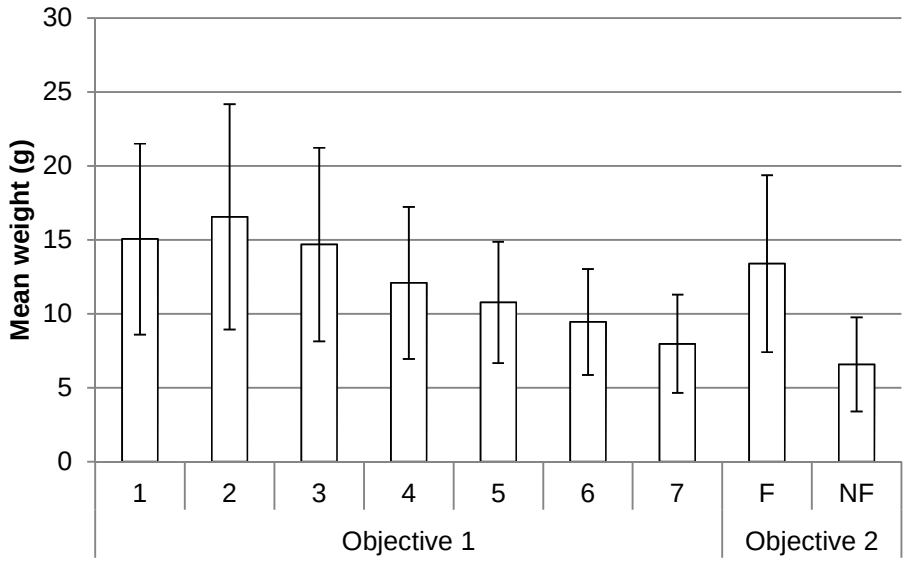


Figure 5.12 The mean weight of the strawberries produced from each treatment with error bars indicating standard deviation.

5.5.3 Fruit marketability

The data to show the number of marketable fruits as a percentage per treatment are shown in Figure 5.13. This shows that the overall trend appears to be that as fraction of OMW compost used increases the number of marketable fruit decreases. Treatment 1 had the greatest number of marketable fruits with a median number of 12 fruits per replicate. The treatment with the fewest number of marketable fruits is treatment 7 with 1.5 fruits per replicate. As the data is count data is it best analysed using a non-parametric

test, in this case a Kruskal-Wallis H test was performed. The treatment with the highest number of discarded strawberries was treatment 5 with a median number of 2 discarded fruits. The treatments with the fewest discarded strawberries were treatments 1 and F with a median number of 0 discarded fruits per replicate.

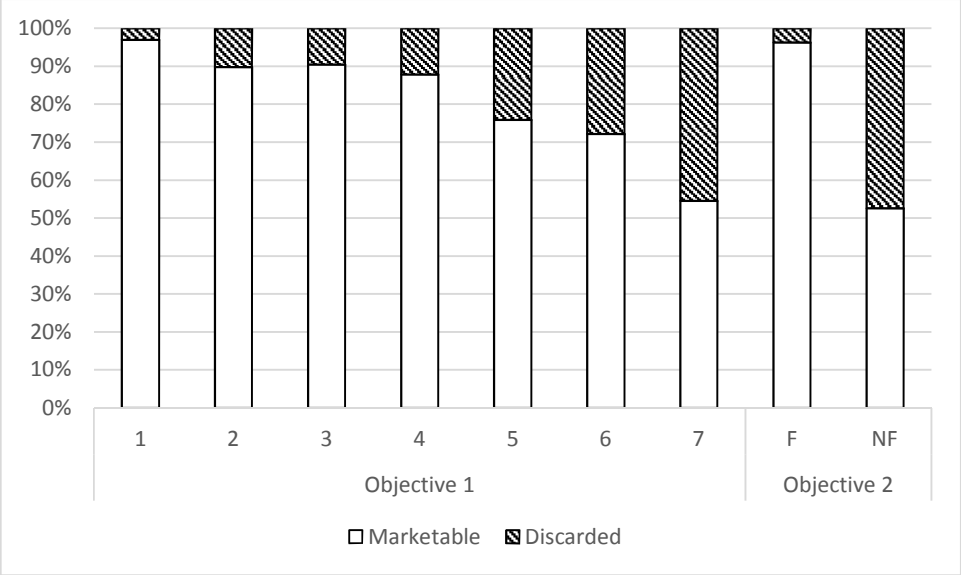


Figure 5.13 The percentage of marketable and discarded fruits from each treatment

The results for the Kruskal-Wallis test showed there to be some significant differences within the data so Mann-Whitney U tests were performed to test all treatments. The results of the tests for significance are shown by the notation in Table 5.6 next to the median values for the number of marketable and discarded strawberries.

Table 5.6 The results of the Mann-Whitney U test for significance with differences shown using notation.

Treatment	*Marketable*	*Discarded*
1	12^{a}	0^{acd}
2	10^{abd}	1^{abcd}
3	10^{ad}	1^{d}
4	7^{bd}	1^{bcd}
5	5^{c}	2^{c}
6	2^{ce}	1^{cd}
7	1.5^{ce}	1.5^{bcd}
F	5^{fcd}	5^{bd}
NF	2^{e}	2^{bcd}

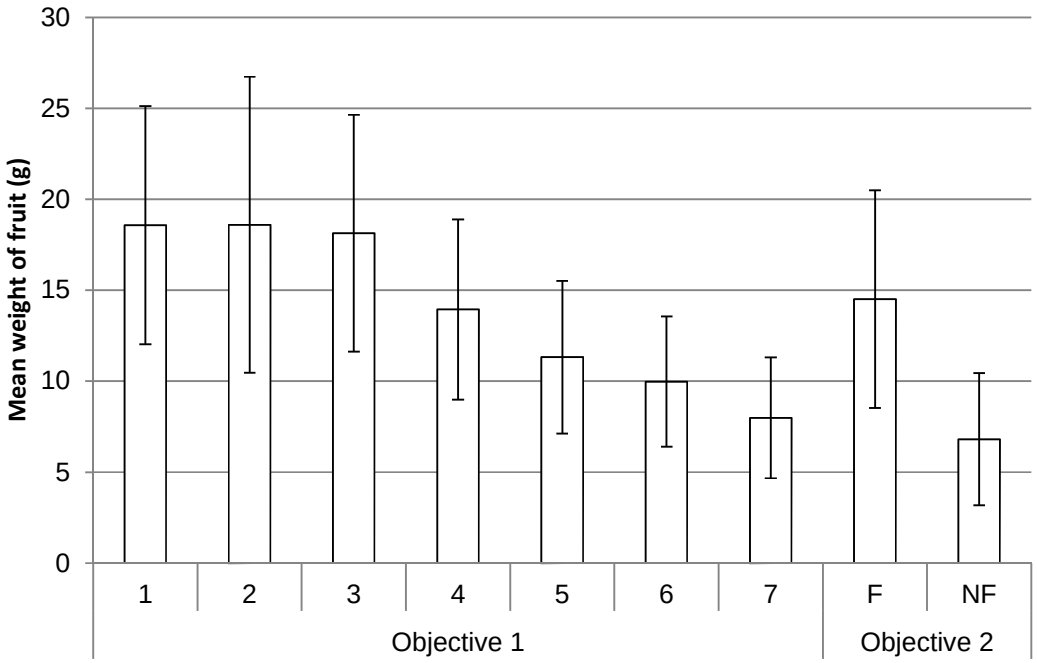
5.5.4 Quality of the fruits

The aim of measuring five fruits from each experiment unit for the detailed characteristics was met in most cases, however, some of the replicates did not produce enough fruit throughout the harvest period for five to be assessed. In some cases fruit were substituted from other plants from the same treatment but from a different block, but in some cases this was also not possible. The data for the quality measurements was all normally distributed when analysed using a Shapiro-Wilk test so it was possible to carry out an ANOVA on all the data using a Tukey's test.

5.5.4.1 Weight of fruit produced

The mean weight calculated from analysis of five fruits together with the standard deviation of the 5 fruits measured from the plants is shown in Figure 5.14. The mean weights for treatments 1-3 are very similar, with treatment 2 having the highest mean weight of fruit with a value of 18.6g. The trend for the

data for treatments 4-7 was a decrease in the fruit mean weight with increasing treatment number and hence increasing percentage of OMW compost. The standard deviation across all measurement ranges from 3.3g for treatment 7 to 8.1g for treatment 2. The test of statistical significance using the Tukey's test showed that the data could be split into two groups. Treatment 1-3 and F are similar to one another, and are all significantly different from treatments 4-7 and treatment NF. This data is shown in the table below the bar graph in Figure 5.14 with the notation indicating treatments that are significantly different from one another.



Treatment								
1[a]	2[a]	3[a]	4[b]	5[b]	6[b]	7[b]	F[a]	NF[b]

Figure 5.14 The mean weight of the five fruits from each plant measured along with an error bar indicating standard deviation with a table below to show the significance between each treatment.

5.5.4.2 Width of fruit produced

The mean width of the measured fruit is shown in Figure 5.15. A photograph showing the digital callipers used to measure the width of the fruit is shown in Plate 5.5. The data shows that the width of the fruit for treatments 1-3 was similar to one another, with all values within a range of 34.83mm to 34.97mm. Treatments 4-7 then show a progressive decrease in the width of fruit with treatment 7 having the smallest mean value for objective 1 of 25.5mm. The lowest mean width observed was for the fruits taken from NF with a mean width of only of 23.8mm. The standard deviation shown by the error bars in Figure 5.15 show that there is a similar amount of variability within each of the treatments. The tests for significance show that treatments 1-3 were similar to each other but were significantly different from treatments 4 -7 and NF. Notation to describe the relationships are shown in the table in Figure 5.15.

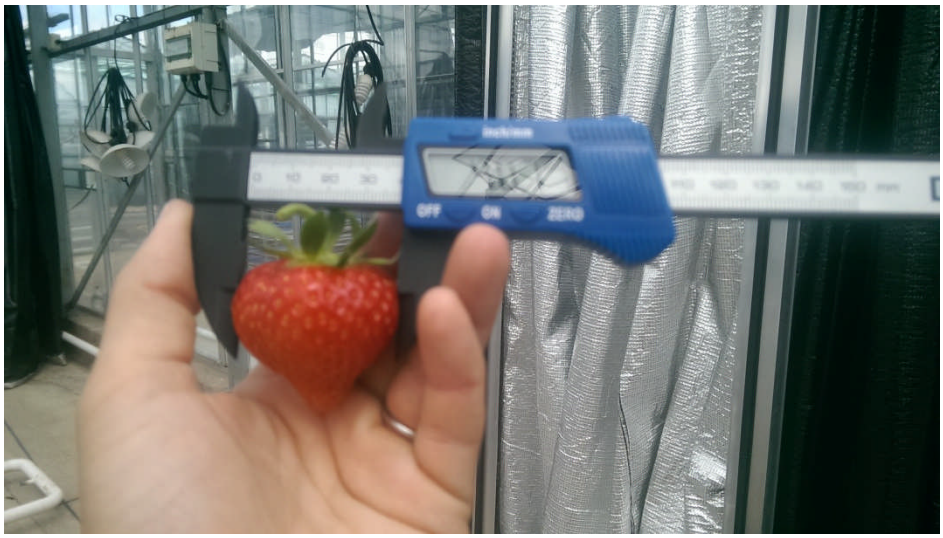
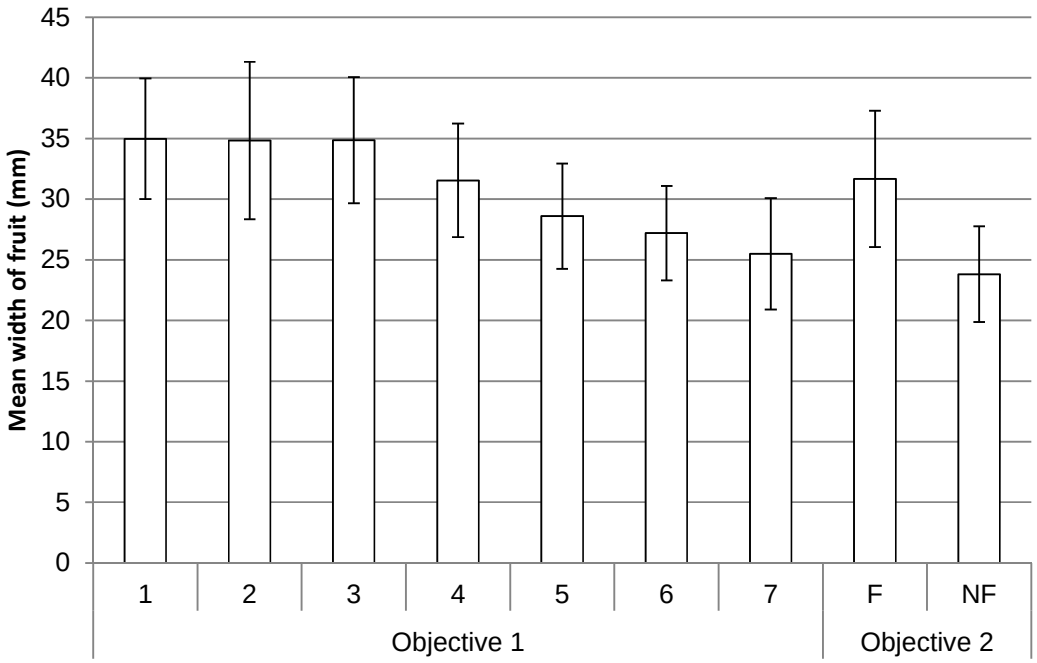


Plate 5.5 The method for fruit width measurement



Treatment								
1[a]	2[a]	3[a]	4[ab]	5[bc]	6[bc]	7[bc]	F[ba]	NF[c]

Figure 5.15 The mean width of the measured fruit from each treatment, with an error bar to show standard deviation with a table below to show the significance between each treatment.

5.5.4.3 Firmness of fruit

The firmness of the measured fruit is shown as a mean value in Figure 5.16. The firmness of fruit across the treatments was variable and there was no discernible pattern with the amounts of OMW compost present in the substrate. The firmest fruits were those from treatment 2 with a value of 613N, this treatment also had the largest standard deviation of all treatments of 236N. Along with the variation in the mean values the error bars showing standard deviation show a lot of overlap between treatments. The Tukey's test showed that there were no significant differences between any of the treatments.

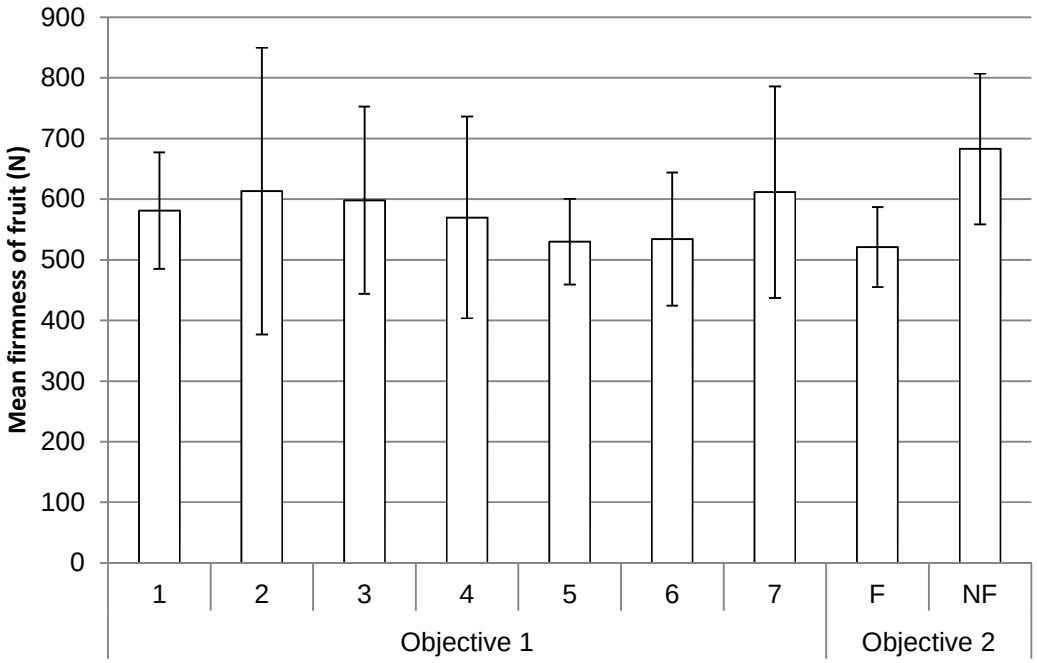


Figure 5.16 The mean firmness of the fruits from each treatment, measured in Newton's with error bars to show standard deviation.

5.5.4.4 Sugar content of the fruit

The mean sugar content of the fruit is shown in Figure 5.17. In contrast to the values for weight and width of fruit there was no obvious pattern in the sugar content of the fruit sampled although there was a minor trend in decreasing sugar content with increasing treatment number. The highest sugar content was observed in treatment NF with a mean value of 8.4. The lowest value for sugar content was in treatment 5 with a mean value of 6.8. The error bars show some overlap of recorded data in the standard deviation. The Tukey's test showed a significant difference between treatment 5 and treatments 1 and NF.

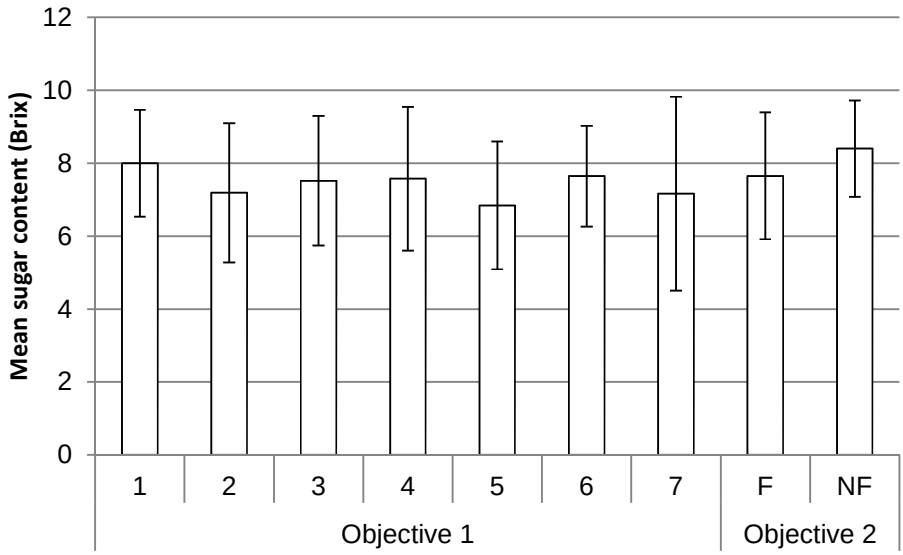


Figure 5.17 The mean sugar content in the fruit measured in Brix with error bars to show standard deviation

5.5.5 Vitamin C analysis

The vitamin C analysis was carried out on a pooled sample of strawberries from each treatment. The analysis was completed by Sciantec and due to the high cost of the analysis only one sample per treatment was submitted for analysis. No samples for objective 2 trial were submitted for this analysis. Figure 5.18 shows that there was an incremental increase in the levels of vitamin C from treatments 2-4 with treatment 4 having the highest levels of all the treatments with a value of 790.5 mg/kg. Treatments 5, 6, and 7 have the lowest vitamin C levels of all the treatments, with treatment 5 having the lowest at 572.8 mg/kg vitamin C.

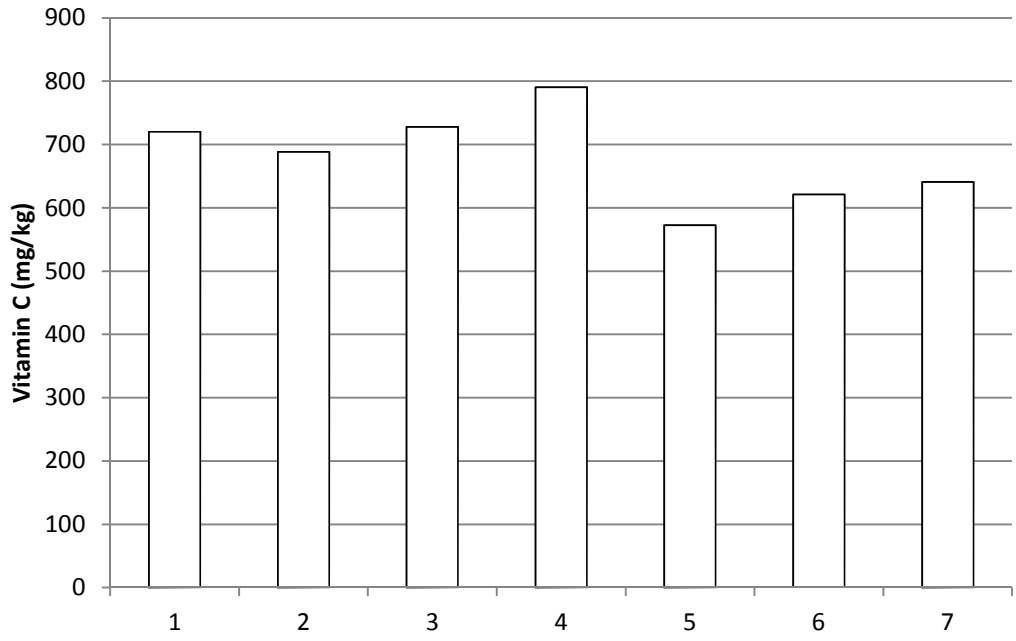


Figure 5.18 Vitamin C content of the strawberry fruit

5.5.6 Pollination success

The pollination success for fruits from all treatments is shown in Figure 5.19. This shows the median number of pollinated and unpollinated achenes counted from the fruit collected. The data in Table 5.7 shows the percentage of pollinated and unpollinated achenes for each treatment. The data shows that with the exception of treatment 1 there was a drop in the number of pollinated achenes as the treatment number and therefore the fraction of OMW compost used increased. Treatment 2 had the highest number of pollinated achenes with a mean number of 231. Treatment 1 had fewer than this and treatments 3-6 show a trend for a decreasing number of pollinated achenes on the fruit with 6 having the lowest mean number at 48 achenes. Treatment F shows a higher number of pollinated achenes than the NF treatment.

Comparing the number of unpollinated achenes shows that it was lowest on treatment 2 with a mean value of 33. Treatment 6 has the highest number of unpollinated achenes with a mean number of 52. Treatment 6 is the only treatment to have more unpollinated than pollinated achenes. There were no

significant differences in the number of unpollinated achenes between any of the treatments. There were however some significant differences between the numbers of pollinated achenes and notation to show this is given in Table 5.7. The pattern again for the data was that the first 3 treatments, up to 5% inclusion of the OMW product, have similar values with the rest similarly grouped.

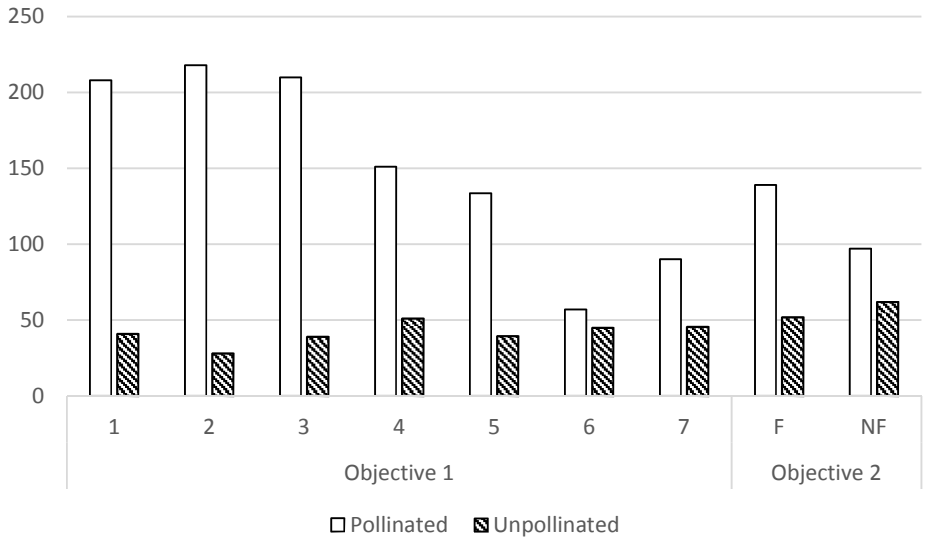


Figure 5.19 The median number of pollinated and unpollinated achenes on the fruit from each treatment

Table 5.7 The percentage of pollinated and unpollinated achenes, along with notation to describe the significant relationships between the treatments

Treatment	*% Pollinated*	*% Unpollinated*
1	84.4 [ab]	15.6
2	87.3 [b]	12.7
3	82.8 [a]	17.2
4	77.5[c]	22.5
5	75.7[c]	24.3
6	48.0 [d]	52.0
7	66.6 [d]	33.4
F	74.5[c]	25.5
NF	57.7[d]	42.3

The photograph shown in Plate 5.6 gives an example of a poorly pollinated fruit. The fruits are misshapen due to the fact that not all of the achenes have been pollinated and as a result the cells of the fruit have not developed uniformly.

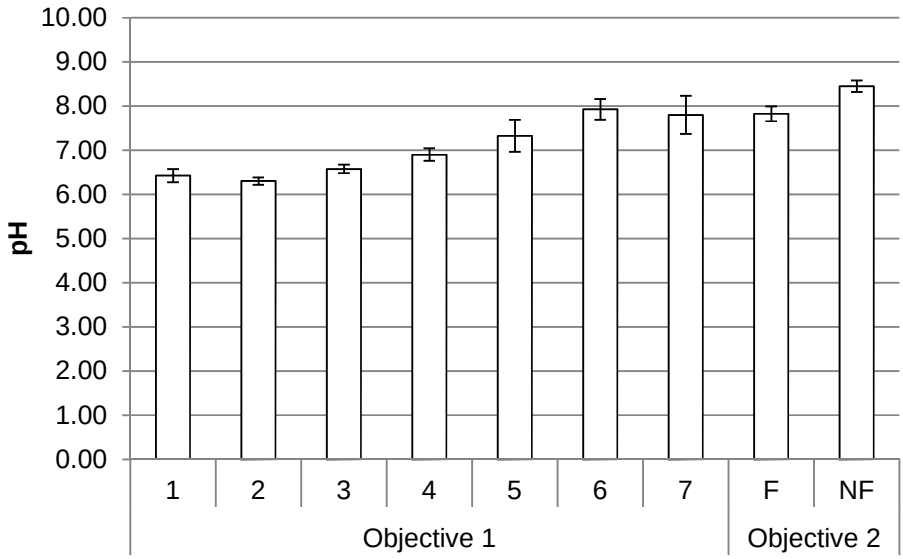
Plate 5.6 Poorly pollinated fruits

5.5.7 Substrate analysis

The substrate samples were collected following the final harvest on the 20 July, and were sent to the laboratories at Natural Resource Management (NRM) for analysis. The results of this are shown in Figure 5.20, Figure 5.21, Figure 5.22, Figure 5.23 and Figure 5.24. The data for this analysis were predominantly normally distributed so were analysed using an ANOVA. The concentration of the phenols in the OMW compost in 2015 showed values of less than 1 and for this reason the phenols in the strawberry substrate following harvest were not analysed as it was thought that since the concentration was so low in the initial test the chance of having a phytotoxic effect from this was negligible.

The mean values for the pH of the treatments are shown in Figure 5.20. This shows a tendency of increasing pH with increasing treatment number and therefore the amount of OMW compost used, all of the mean values have similarly small standard deviations. The highest pH was observed for treatment NF with a mean pH of 8.45; the lowest pH is from treatment 2 with a mean value of 6.30. The notations to describe the relationships between the

treatments and any significant differences are given in the table below the graph in Figure 5.20.



Treatment								
1^{abc}	2^{b}	3^{abc}	4^{acd}	5^{cd}	6^{ef}	7^{de}	F^{de}	NF^{f}

Figure 5.20 The pH of all the treatments as a mean value with error bars showing standard deviation

The results of the conductivity are shown in Figure 5.21, where a large difference can be seen between treatment NF and all other treatments, with treatment NF having the greatest mean EC of 1090.8µS/cm in the substrate. The treatment with the lowest EC was treatment 1 with a value of 278.5µS/cm. As would be expected from looking at the data graphically, data analysis showed that treatment NF was significantly greater than all other treatments, with no other differences in the data. The plants in treatment NF were much less developed than the other plants, and perhaps this is shown in the lack of uptake of any salts from the substrate.

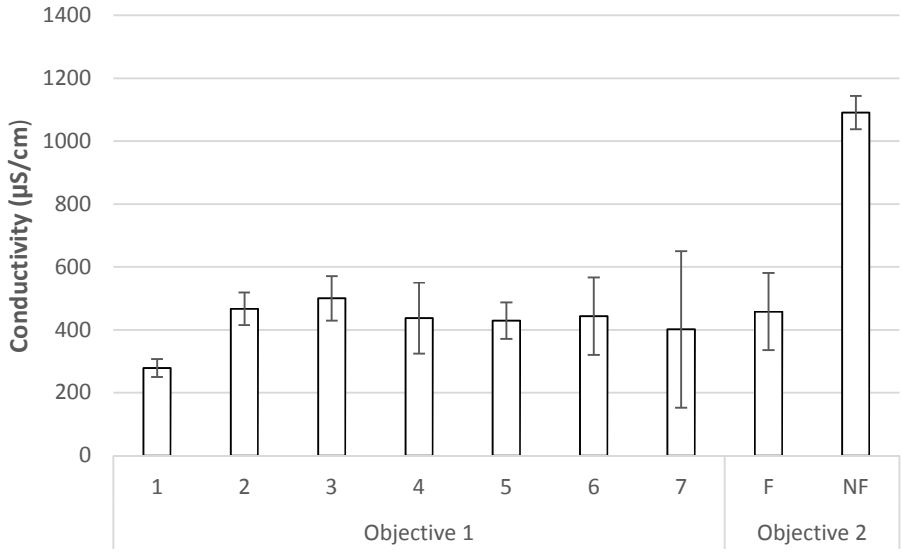


Figure 5.21 The levels of conductivity mean values within the substrate samples with standard deviation shown on the error bars.

Figure 5.22 shows the mean values for the potassium, sulphate, phosphorus and chloride within the samples, error bars have been omitted from this graph as they made the data less clear to understand. The data for potassium, shows that with the exception of treatment 7 there appears to be a progressive increase in the concentration of potassium as the treatment number and therefore the fraction of OMW compost used increases. The lowest potassium concentration was observed for treatment 1 with 174.9 mg/l and the highest was for treatment NF with an extreme value of 1787.5mg/l. Data analysis showed that concentration of potassium in the substrate for treatment NF was significantly higher than all other treatments and that there were no other significant differences between the treatments in terms of the concentration of potassium. The reason for the high amount of potassium in the substrate is likely due to the poor fruiting of the plants and as a result, little potassium being taken up by them.

The data for sulphate concentration shows a pattern of increasingly concentration of sulphates through treatments 1 to 3 with a subsequent decrease from treatments 4 to 7. It was also found that the sulphate concentration in treatment F was similar to treatment NF. The lowest

concentration of sulphate was in treatment 7 with 155.4mg/l and the highest concentration was in treatment 3 with 556.4mg/l. Data analysis showed that treatment 3 has a significantly higher concentration of sulphate in the substrate than treatments 5 – 7 and treatment 2 also has a significantly higher concentration than treatment 7.

The data in Figure 5.22 shows that the concentrations of phosphorus progressively increase from treatment 1 through to treatment 7 with the lowest value for treatment 1 of 27.5mg/l and the highest value for treatment 7 of 165.8mg/l. The concentration of phosphorus in treatment NF was higher than in treatment F. Data analysis shows that the phosphorus concentration for treatment 7 was significantly higher than treatments 1 – 4 and treatment F. Treatment NF had a significantly higher concentration of phosphorus than treatments 1 – 3.

The final component presented in Figure 5.22 is chloride which shows a pattern of variability between the treatments and no apparent relationship to the amount of OMW compost used. The lowest value for chloride was in treatment 5 with 58.3mg/l and the highest value was for treatment NF with 472.5mg/l. Treatment NF was found to be significantly higher than all other treatments, which can be interpreted from the graph in Figure 5.22. There were no other significant differences between the data for chloride.

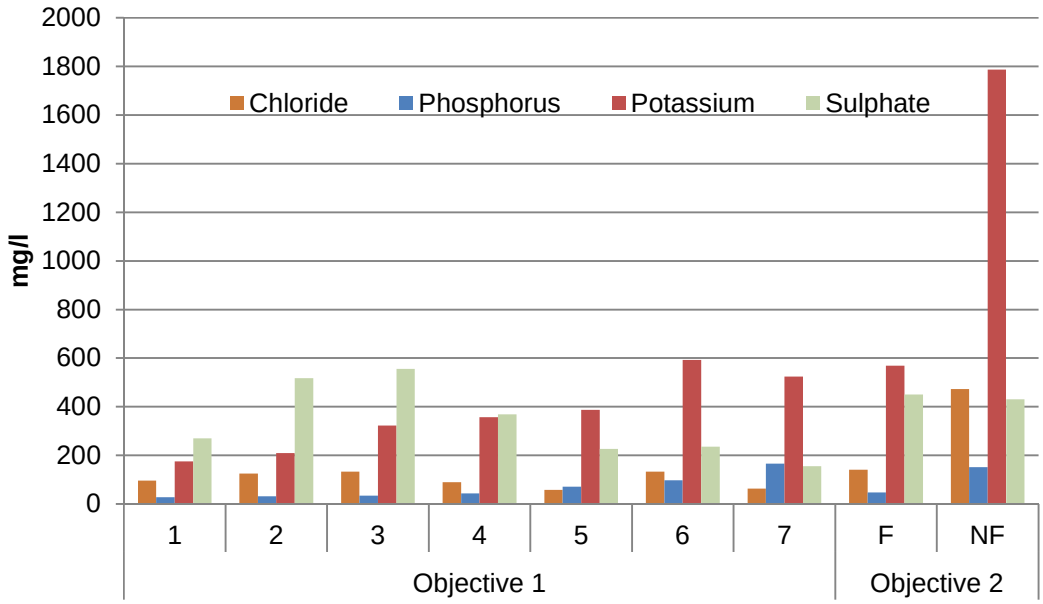


Figure 5.22 The chemical analysis of the substrate for Chloride, Phosphorus, Potassium and Sulphate.

Figure 5.23 shows the results of the substrate analysis for magnesium, calcium, sodium, ammonium, nitrate and soluble-N. The variability in the concentration of magnesium was somewhat mixed with the concentration increasing from treatment 1 to 2, decreasing from 3 to 6 and then increasing again for treatment 7. Treatment NF has a higher concentration of magnesium than treatment F. The lowest and highest concentrations of magnesium were found in treatments F (22.5mg/l) and 2 (64.5mg/l) respectively. Data analysis shows that there was no real pattern in the treatments when compared using an ANOVA. The results of the ANOVA showing significance are shown in Table 5.8.

The concentrations of calcium in the substrate follow a similar trend to that observed for the magnesium, with the highest in treatment 2 (164.7mg/l) and the lowest in treatment F (51.46mg/l). The results showing the significant differences between treatments for calcium are shown in Table 5.8.

Sodium also had a variable pattern in the terms of the concentrations in the treatments with the lowest and highest values in treatment 5 (47.9mg/l) and treatment NF (139.1mg/l) respectively. Data analysis shows that treatment NF

had the greatest significant difference from other treatments being significantly higher than treatments 1, 4, 5, 6 and 7.

The variation in the concentrations of ammonium in the substrate was low between treatments, except for treatment 7 which had a high of 100.8mg/l. The treatment with the lowest concentration of ammonium was treatment F with a value of 12.2mg/l ammonium. There were no significant differences between any of the treatments for ammonium.

The concentrations of both nitrate and soluble-N in the substrate followed a similar trend for all treatments, at higher concentrations up until treatment 4, before dropping off. Both are present at the lowest concentrations in treatment NF with nitrate at 2.7mg/l and soluble-N at 30.5mg/l. The treatment with the highest concentrations of nitrate was treatment 5 with 111.4mg/l and the highest concentration of soluble-N was present in treatment 2 with 134.4mg/l. The results for the significant differences between these treatments are shown in Table 5.8.

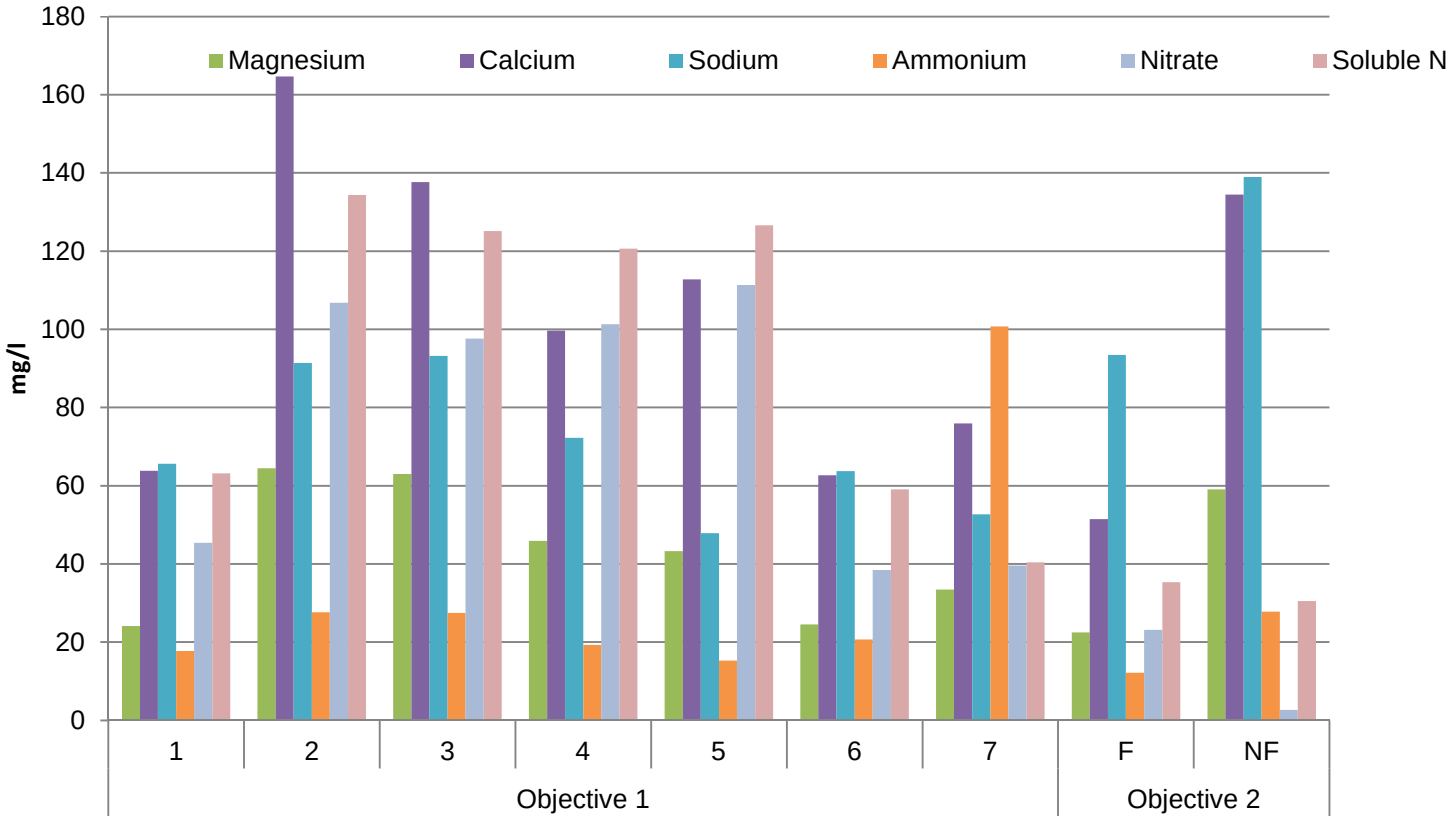


Figure 5.23 Chemical analysis of the substrate for magnesium, calcium, sodium, ammonium, nitrate and soluble N.

Figure 5.24 shows the results for the metals in the substrate samples on a two panel chart to show the variability of the data on one graph, metals include boron, copper, zinc, iron and manganese.

There was an increase in the concentrations of boron shown in Figure 5.24 from left to right, but the changes in the concentration were small with the lowest value at 0.13mg/l (treatments 1 and 2) and treatments F and NF having the highest values of 0.18mg/l. Treatment 2 had significantly lower levels of boron than both F and NF, although this isn't obvious from looking at the graph or by looking at the mean values rounded up/down.

There was more variability in the concentrations of copper in the substrate samples with treatment NF having the highest concentration of 0.96mg/l. Treatment 1 had the lowest concentration of copper in the substrate with only 0.10mg/l recorded as a mean. Treatment NF had significantly higher concentrations of copper than all other treatments, as would be expected by examining the bar graph.

The concentrations of manganese increase from left to right on Figure 5.24 with treatment NF having the highest concentration with a value of 0.58mg/l. The lowest mean concentration of manganese in the substrate samples was in treatment 1 with 0.12mg/l. There were no significant differences between any of the treatments for the concentrations of manganese.

The concentrations of zinc follow a similar pattern to that of manganese with treatment 1 having the lowest concentration (0.22mg/l) and treatment F having the highest concentration (1.94mg/l). Treatment F is had significantly more zinc than treatments 1 – 5 and treatment 7. Treatment NF had significantly higher concentrations of zinc than treatment 1.

The concentration of iron in the substrate show variability between the treatments, with no obvious trend and treatment 4 having the lowest concentration at 1.19mg/l. Treatment NF had by far the highest concentration of iron in the substrate samples with a mean value of 18.54mg/l, leading to it being significantly different from all other treatments. Similar to the high potassium levels in the substrate the high iron levels could be due to the poor plant development and fruiting leading to none of the iron present in the

substrate being taken up by the plant. There were no other significant differences between the treatments for the concentration of iron.

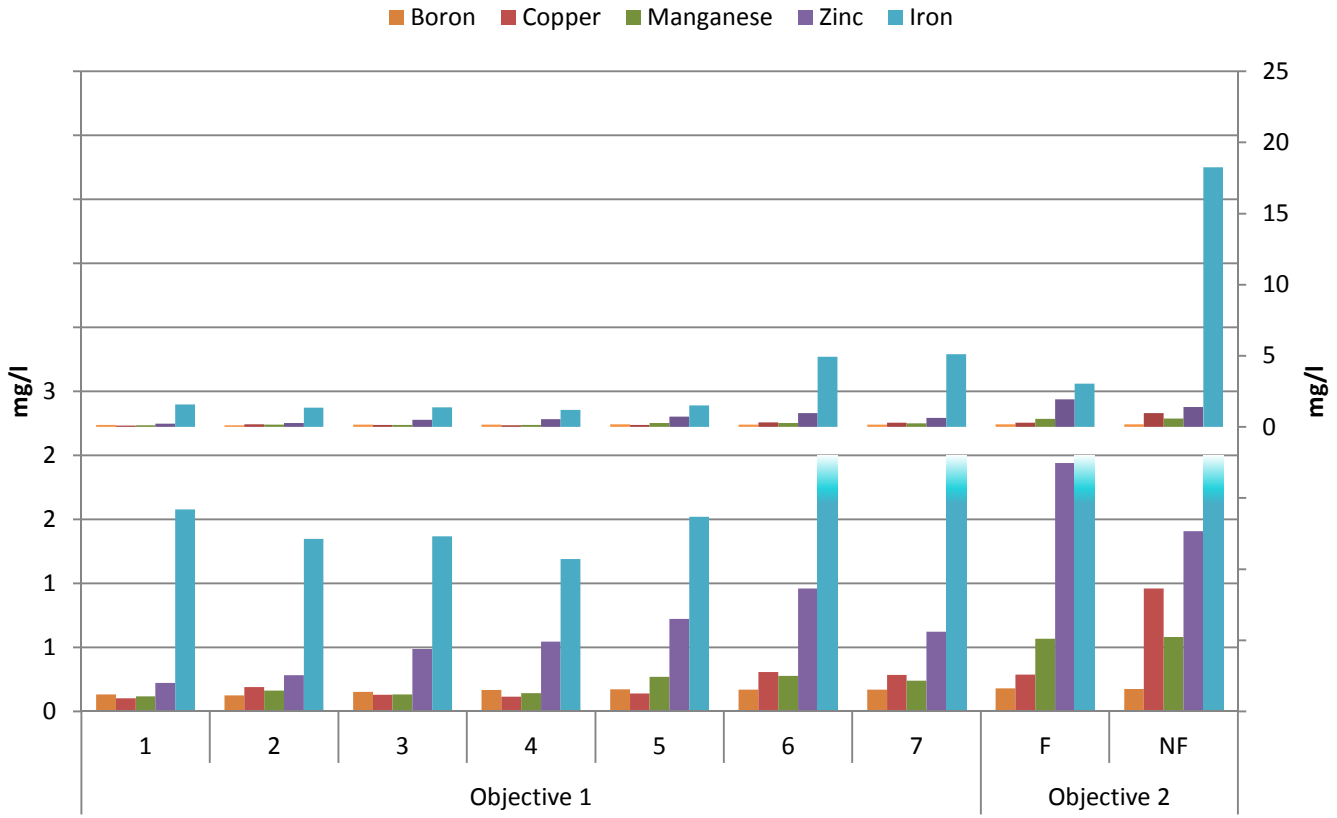


Figure 5.24 Chemical analysis of the substrate for boron, copper, manganese, zinc and iron. The graph is a split panel chart with two axes to allow for a better visual understanding of the data.

The significant differences between the treatments are described in Table 5.8, this shows clearly which treatments have significantly different concentrations of nutrients in the substrate.

Table 5.8 The treatments that have significant differences from one another for the chemical analysis using chemical notations for ID, with N3 standing for nitrate, and N denoting soluble-N.

	1	2	3	4	5	6	7	F	NF
1	-	Mg, Ca, N	Mg, N	N	N3, N				Mg, Na
2		-				Mg, Ca, N3, N	Ca, N3, N	Mg, Ca, N3, N	N3, N
3			-			Mg, N	N	Mg, Ca, N3, N	N3, N
4				-		N	N	N3, N	Na, N3, N
5					-	N3, N	N3, N	N3, N	Na, N3, N
6						-			Mg, Na
7							-		Na
F								-	Mg, Ca
NF									-

5.6 Discussion

5.6.1 Phenols

The concentrations of phenols in the raw OMW compost were low which suggested that these were not an important factor when considering the plant mortality at the start of the trial period. The low levels of phenols in the product could be attributed to the composting process as removal of phenols using

composting has previously been reported. Altieri and Esposito in 2008 conducted trials with destoned olive mill waste combined in a mixture with bulking agents including wool waste, wheat straw and sawdust. They found that a 3 month storage period in net sacks protected from rain was sufficient to reduce the phenols in the OMW by a factor of 10 (Altieri and Esposito, 2008).

5.6.2 Conductivity

Conductivity is the ability of a soil or substrate to carry an electric current, it is a good indicator of the amount of nutrients available for crops to absorb. The high initial levels of conductivity in the OMW product were predominantly down to the levels in the pig manure which were very high. The addition of the OMW and repeated drying increased the levels of EC in the pig manure to over 20mS/cm. This level was reduced to 11.6mS/cm by the time the product was analysed prior to the trials in the UK. These levels are higher than those reported in the literature with pig manure considered to have a high EC with some values at 2.9mS/cm (Ye et al., 1999, Huang et al., 2004, Huang et al., 2006). Raw olive mill wastewater also can have high levels of EC with levels ranging from 5.3mS/cm, 8.3mS/cm to 8.7mS/cm (Paredes et al., 2005, Komilis et al., 2005, Mekki et al., 2006b). Raised conductivity in soil has been shown as an effect of using raw OMW as an amendment (Saadi et al., 2007).

Levels of conductivity in the final post-harvest substrate are not that different given the plant mortality rates for treatments 5, 6, and 7. They are not outside of the tolerable levels for strawberry plants as discussed in 5.2.1. This could be a result of the watering and fertigation programme throughout the trial period, with the conductivity in the substrate gradually being reduced over time. Problems with substrates under saline conditions can be ameliorated through several mechanisms, one of which is leaching (Qadir et al., 2000). The same effect was found in trials with strawberries and OMW by Altieri in 2010 when the EC of the substrate dropped throughout the trial. The high EC at the start was marked by necrosis of the plant leaves, however this recovered through successive watering and leaching of the plants which reduced the EC progressively through the trial (Altieri et al., 2010, Karlidag et al., 2009) However it would appear that

this process was not rapid enough for the plants with the high percentage of OMW inclusion to recover from the high and phytotoxic levels of conductivity in the initial stages of the trial. The mortality of plants in the treatments with higher levels of conductivity tie in with the experiments conducted by D'Anna and Giuffrida (D'Anna et al., 2003, Giuffrida et al., 2001)

D'Anna found that the number of marketable fruits decreased as the EC of the substrate increased when using a nutrient solution with increasing salt content on strawberries grown in a coir base (D'Anna et al., 2003). Giuffrida found that increasing salt concentrations in two soilless media (clay and coir) systems found a reduction in fruits produced per plant, a decrease in fruit weight and the progression of plant mortality (Giuffrida et al., 2001).

In other trials using a compost produced from MSW, the high sodium levels present were shown to have a detrimental effect on fruit production when used on strawberries (Hargreaves et al., 2009). In the work completed by Pirlak in 2004 adding a NaCl solution to plants, it was found that strawberries receiving a treatment of 7.5mS/cm suffered complete mortality of all replicates (Pirlak and Eşitken, 2004). The OMW compost mixes were not analysed prior to these trials and irrigation starting so the starting EC of the combinations is not accurately known. However given the high EC of the OMW compost (11.6mS/cm in Table 5.3) and the high mortality of the plants at the 25% incorporation level it is possible that the EC of the starting mix was similar to that of Pirlak's 7.5mS/cm.

The treatment for NF with hand watering suggests that irrigation in the absence of additional fertiliser was not sufficient to counter the high levels of salt in the OMW product. Treatment NF had significantly higher sodium than treatments 1 and 4-7 which could explain some of the mortality. NF had significantly more potassium and iron than all other treatments.

5.6.3 Chlorophyll

Chlorophyll levels in the leaves have been shown in previous studies with strawberries to have been reduced when exposed to higher levels of salinity (Kaya et al., 2002). In studies with tomatoes using a solid olive waste compost with a conductivity of 2.4mS/cm Ceglie (2011) showed that the levels of

chlorophyll in the leaves decreased with increasing amounts of the olive mill waste compost incorporated into the substrate. However, plant production was optimal when OMW compost was included at a 20% rate in a peat substrate base (Ceglie et al., 2011). Chlorophyll levels have also been shown to be lower in strawberry trials in plants receiving a solid organic fertiliser and a liquid organic fertiliser when compared to treatments solely receiving inorganic solid and liquid fertiliser (Pokhrel et al., 2015).

In these trials there were no significant differences in the chlorophyll levels between the controls and the treatments that received 5% and 10% OMW compost addition. This shows that the nitrogen uptake from these ratios is similar to the control and that the OMW compost can be used safely as an addition to the base substrate at these ratios with no adverse effects of increased nitrogen uptake by the plants. In the treatments receiving more OMW compost the nitrogen uptake by the plants was reduced as reflected by the significantly lower levels of chlorophyll within the leaves that were measured.

Treatments F and NF had notable differences in the levels of chlorophyll in the leaves, which can be attributed to the absence of any liquid fertilisers being applied, with low nitrogen uptake a result of this and the higher conductivity present in treatment NF. The high conductivity in the NF treatment would have impacted on the plants ability to uptake nutrients.

5.6.4 Plant characteristics

Plant fresh and dry weight were highest in the two control treatments with no OMW product added and in the 5% OMW treatment and treatment F, with all other treatments having significantly lower weights. If the reduction in plant growth can be attributed to the salt content of the OMW the pattern in the growth of these plants corresponds to the data collected by Keutgen in 2009. In this trial treatments with increasing levels of salinity applied showed a reduction in the plant fresh and dry weight (Keutgen and Pawelzik, 2009).

Runner and leaf development showing plant growth and production increased throughout the monitoring period for all treatments except for NF. There were differences in the number of leaves and runners between the treatments as

shown by a Mann-Whitney U test. Arancon showed that a vermicompost amendment applied at 5Mkg/ha and 10MKg/ha produced significantly more runners on strawberry plants than a control which received an inorganic fertiliser (Arancon et al., 2004). This is a much lower application rate than the ratios used in this chapter which show the opposite of the Arancon's results.

5.6.5 Fruit production

The effects on both the amount and the quality of the fruit produced between treatments was significant. The treatments with the higher concentrations of OMW compost were unable to produce fruit at the same number and quality as the control, or the low concentration OMW treatments. Altieri in 2010 posted similar results with the control treatment having the highest yield of all treatments and the yield falling with greater additions of an OMW compost (Altieri et al., 2010). Altieri used combinations of 25%, 50% and 75% OMW incorporated into the peat substrate with the raw OMW having an EC of 2.74mS/cm. In trials using organic versus inorganic fertilisers strawberry plants again have shown reduced yields. Pokhrel in 2015 found that organic fertiliser strategies produced the firmer and sweeter fruits than the inorganic fertiliser, however the inorganic fertiliser produced a higher yield of heavier fruits (Pokhrel et al., 2015). In the trials completed as part of this research the strawberry plants managed to cope with up to 10% inclusion of the OMW compost, but higher ratios than this resulted in high plant mortality and a significantly poorer crop.

The taste of the fruits as measured by the sugar content in this trial for objective 1 responded to the OMW addition in a similar way to the fruits tested at different levels of salinity in the work completed by Saied (Saied et al., 2005). The trend through the OMW treatments was of falling sugar concentrations with higher OMW amounts. Saied used two varieties of strawberry (Korona and Elsanta) and in both the sugar levels in the fruits were significantly lower in the plants treated with NaCl solution containing 2.6mS/cm and 5.1mS/cm than the control receiving tap water with an EC of 0.3mS/cm.

The results for objective 2 showed that the treatment NF with a significantly higher conductivity than treatment F also had fruits with a higher sugar content. Studies by Cardeňosa in 2015 show a similar result to this with taste improving with increasing salinity (Cardeñosa et al., 2015) as discussed in 5.2.1. This increase in sweetness is due to the salts present reducing the amount of water in the fresh fruit, leading to an increase in the concentration of reducing sugars and acids giving a sweeter taste (Awang et al., 1993).

5.6.6 Pollination

Effects of compost on the visits by pollinators was also important due to the increased numbers of unmarketable fruit in the treatments that received higher concentrations of the OMW compost.

The effects of the compost with respect to pollination were shown by the significant differences between the number of pollinated achenes and the treatments. The OMW product not only affected the plant development and strawberry production, but has also affected the quality and attractiveness of the strawberry flower to pollinating bees. The greater the addition of the OMW compost, the less attractive the flowers of the plant became. The attractiveness of floral nectar is known to affect pollinator attraction to a flower and therefore influence the reproductive success of a plant (Gijbels et al., 2015). A mutualistic relationship between pollinators and flowering plants is one that is integral to the success of horticultural and agricultural systems, as well as natural ecosystems (Cardoza et al., 2012). The use of a vermicompost as a soil improver to promote plant development in cucumbers was trialled by Cardoza in 2012. It was found that plants treated with vermicompost had significantly longer visits by pollinating bumble bees. In return the bumblebees that fed on the plants in the vermicompost amendment had significantly larger and more active ovaries, which is a measure of good nutrition. The sugar content of the plants in the vermicompost amended plots tended to be higher than the control with no vermicompost however this was not significantly different (Cardoza et al., 2012). Fertilisation of flowers to improve the nectar amino acid content has also shown

to improve reproductive rates in orchids when compared to unfertilised flowers (Gijbels et al., 2015).

5.7 Conclusions

The conclusions of these trials are that the effects of using the OMW with a pig manure base were definitive. The inclusion of the OMW in the pots had a noticeable effect on all aspects of plant development and production. There was a clear trend shown in decreasing productivity of the plants with increasing amounts of OMW compost combined in the peat mixture. The OMW used at higher rates above 20% had an effect on the mortality of the plants, with more dying off with increasing amounts of OMW used

The effects of this OMW product in this way suggest that it should not be used as a peat substitute for strawberries, and potentially other pot grown plants that are salt sensitive. This OMW product given it does have some nutrient qualities in it may be better placed being applied as a fertiliser.

Chapter 6 Conclusions

The results discussed in the preceding chapters highlight that the effects of organic amendments on plant growth and development can be variable depending on their method of application and the plants they are applied to.

The work on the large scale arable trials demonstrated that the different types of nitrogen application had no significant effect on the crop yield. A similar result was shown in the arable pot trials in 2015 with no differences shown in plant growth and development. The one difference in yield that occurred due to the biochar amendment can be attributed to an effect of temperature and insulation of the crop during a cold season during the first year of large scale arable trials.

The use of the OMW on strawberries showed a difference in the plant mortality between the two trials which was dependent on the variety of OMW used and on the conductivity of the compost. The OMW used in 2014 was able to be included at higher rates in the compost without having a negative effect on plant growth. The compost used in Chapter 4 could be a suitable peat replacement as it did not show any particular effects on plant mortality. The OMW used in 2015 was included at more incremental rates which showed up the critical point where the OMW became toxic to the plants.

Given the intensity of the production method for this compost product, and the relatively small amounts that can be produced, it is likely to make a better amendment on higher value pot grown crops such as fruit, rather than field crops that require a high volume of fertilisation. The ability to utilise a compost such as this is dependent on the product being able to meet criteria that deem it suitable to be used as a substrate for food production. Compost produced in the UK has to meet regulatory criteria and have consistent physical, chemical and biological characteristics. In the UK the regulatory criteria is set out by the Compost Quality Protocol and British Standard BSI PAS 100. Compost products must meet this specification set out by the British Standards Institute in order to be sold as a compost. The parameters for testing the compost quality

have upper limits set for *Escherichia coli*, *Salmonella spp*, potentially toxic elements (PTEs) such as copper, cadmium, nickel and mercury, microbial respiration rate, weed seeds and physical contaminants such as stones.

The EU fertiliser regulation includes biochar under its scope, and one of the main concerns for biochar under these regulations is the potential presence of heavy metals.

The economic benefits of reusing the waste from olive oil production this way could reduce the costs of the waste management required to process this waste. However as the waste is essentially dealt with using free solar radiation through drying lagoons for the wastewater it is unlikely that any other process will cost less than this. It is therefore important that any product created from this waste is worth the additional cost and benefit over the current lagoon drying method.

The environmental benefits of using a compost produced from waste will reduce the impact of the OMW as a phytotoxic raw waste product into something that can benefit crop growth when applied in the most appropriate way. The use of this material in composting may also reduce the emissions from the OMW that will be produced during solar drying in lagoons. The further reaching environmental benefits of producing a reliable compost material from waste could provide an organic peat alternative for use in horticulture. This would lead to less peat being extracted for food production meaning that peat environments were left to provide ecosystem services such as flood alleviation, carbon sequestration and providing habitat for protected species.

Chapter 7 Further work for consideration

The large arable trials indicated that biochar can have a positive impact on crop yield in some seasons, further work would be to investigate this over a longer time period with detailed weather observations and different methods of application running concurrently on the same crops. Soil temperature monitoring to compare the differences between plots that received surface applied biochar and those that did not would allow any differences in yield due to temperature to be quantified. This longer piece of work would also record data from downstream non target species and habitats to assess any impact of organic amendments in the form of biochar on sensitive riparian and terrestrial habitats.

In the small arable work more could be done to complete the same pot trials over consecutive growing seasons to see if there were any cumulative effects of the OMW product treatments and the biochar on crop growth and development in a small scale set up. It would also be interesting to compare the growth of winter wheat that was used in the large scale arable trials in 2013 and 2014 and spring wheat that was used in 2015 in relation to the amendments applied to the soil.

The addition of biochar to horticultural substrates to improve pathogen resistance is an interesting concept, but as yet there is no definitive method that has been developed. Further trials using different crops and biochar produced from known feed stocks would be an area for further research.

Using the OMW product used in the 2015 horticultural trials it would seem that it would only be possible to grow very salt tolerant plants in the OMW used without any phytotoxic effects on the plant growth and development. Modifications in the production of this compost need to be made to ameliorate the salt content and reduce the conductivity. In future trials, measurements of the leachate from each pot could be made, along with periodic sampling and

analysis of the substrate to monitor the nutrient levels within the soilless media base.

In addition to this work, further work into making the most of the chemical attributes of the OMW compost could be worked on. Making up a compost tea in order to provide fertilisation in a soilless cultivation, either in a growth media such as coir, or in a hydroponic system could be the next steps for compost that is unsuitable to be used as a substrate base.

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Abbreviations

ALC	Al perujo compost
BC	Biochar
BOD	Biochemical oxygen demand
CEC	Cation exchange capacity
DEFRA	Department of Environment, Food and Rural Affairs
ENSO	El Niño Southern Oscillation
FAO	Food and Agriculture Organisation of the United Nations
GI	Germination index
LED	Light emitting diode
MSW	Municipal solid waste
NIAB	National Institute of Agricultural Botany
OMW	Olive mill wastewater
OSR	Oilseed rape
PFC	Peat free compost
RCBD	Randomised complete block design
SPAD	Soil plant analyses development
WFD	Water Framework Directive
WW	Winter wheat

Appendix A Glossary of laboratory reagents

Ammonium molybdate solution

- Dissolve 10g ammonium molybdate into 100ml distilled water in a 250ml volumetric flask
- Make up to 250ml and transfer to a Duran bottle for storage

Ascorbic acid solution – 0.1M

- Weigh out 1.76g ascorbic acid and dissolve in a 100ml volumetric flask
- Make the solution up to 100ml and store in a duran bottle.
- This solution is stable for a week at 4^{o}C

Combined reagent

- Using an acid washed duran bottle make up 100ml combined reagent using
- 50ml 2.5M sulphuric acid
- 5ml potassium antimonyl tartrate solution
- A5ml ammonium molybdate solution
- 30ml 0.1M ascorbic acid solution
- Mix after the addition of each reagent
- The reagent is stable for 4 hours

Hydrochloric acid solution – 1M

- Fill 1l volumetric flask with 700ml distilled water
- Add 86ml concentrated hydrochloric acid
- Leave to cool then make up to 1l with distilled water and transfer into a duran bottle for storage

Indicating boric acid

- Weigh out 20g boric acid into a 1l volumetric flask in approximately 500ml distilled water
- Add 10ml mixed indicator solution
- Make up to 1l with distilled water and transfer to a duran bottle

Potassium antimonyl tartrate solution

- Dissolve 1.372g potassium antimonyl tartrate solution in 100ml of distilled water in a 500ml volumetric flask
- Make up to 500ml and transfer to a duran bottle

Sodium hydroxide – 2M

- Weigh out 80g sodium hydroxide and dissolve in distilled water in a 1l volumetric flask
- Make up to 1l using distilled water

Standard phosphate solution

- Dissolve 0.22g anhydrous potassium dihydrogen orthophosphate in a 1l volumetric flask in 100ml water
- Make up to 1l with distilled water and transfer into a duran bottle

Sulphuric acid – 2.5M

- Wearing nitrile gloves and safety goggles add 68ml concentrated sulphuric acid to 300ml distilled water in a 1l volumetric flask
- Allow to cool and then make up to 1l with distilled water
- Store in a duran bottle

Sulphuric acid – 10mM

- Half fill a v1l volumetric flask with distilled water
- Wearing nitrile gloves and safety goggles Add 0.55ml concentrated sulphuric acid
- Mix thoroughly
- Make up to 1l with distilled water and transfer into duran bottle