

**Biochar as a Soil and Seed Compost Amendment**

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**Project Thesis in partial fulfilment of the degree of Masters in  
Organic Horticulture**

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**September 2014**

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## **List of Abbreviations**

ADE	Amazonian Dark Earth
AWCD	Average Well Colour Development
CEC	Cation Exchange Capacity
CLPP	Community Level Physiological Profiling
DM	Dry Matter
EC	Electrical Conductivity
GHG	Greenhouse Gas
HTT	Highest Treatment Temperature
MVSP	Multivariate Statistics Package
PCA	Principal Component Analysis
RRB	Replicated Randomised Block
SOM	Soil Organic Matter
WHC	Water Holding Capacity

## **1 – Abstract**

Biochar is pyrolised biomass that is used as a soil amendment. It has potential to sequester carbon, reduce agricultural pollution, and boost crop yields. Its effects in the tropics are well understood but far less is known about its effects on crops in fertile temperate soils. The present study investigated the effects of biochar on the growth of white turnip (*Brassica rapa* subsp. *rapa*). The first part of the study assessed the effects of biochar on plant growth when used as a seed compost amendment, with germination, seedling growth and growth after transplanting to the field recorded. The second part examined the effects on crop growth when used as a soil amendment in field trials. Various soil parameters were recorded: pH, EC, WHC, infiltration time and microbial communities. Overall, there was no effect on plant growth, but there was weak evidence that dry matter is affected when biochar is used as a seed compost amendment. Soil microbial communities underwent significant changes and WHC and infiltration time increased. Biochar is recommended as a method of sequestering carbon with no detrimental effects on plant growth, but on this evidence cannot be recommended as a viable soil or seed compost amendment.

## 2 – Introduction

### 2.1 *The Development of Biochar*

Current practice in the production and distribution of food and materials has caused wide spread degradation and destruction of soils while also creating problems related to the inappropriate disposal of organic waste (Diacono and Montemurro, 2010). These issues are essentially two sides of the same coin – both can be solved by returning organic wastes to the soil but this will take large scale organization as well as investigation into the safest and most efficient methods. The application of biochar to agricultural and degraded soils has been promoted as one such method and has gained popularity for several other reasons, summarised by Lehmann and Joseph, (2009). As a stable form of carbon (Glaser *et al.*, 2001, Lehmann, 2007b), biochar possesses great potential for reversing the greenhouse effect by acting as a long-term carbon sink. Most of the carbon in organic matter, including organic waste, is ultimately released back to the atmosphere if composted or left to decay naturally (Lehmann, 2007b). Biochar production and application could lock this carbon in the ground, potentially for centuries or even millennia, and reduce net emissions of greenhouse gases. Emissions of greenhouse gases (GHGs) may also be reduced in soils to which biochar is added (Augustenborg *et al.*, 2012). The biochar production process degrades a large amount of the carbon-based material of the biomass to oily and tarry vapours that can be harnessed to produce bio-oil (Amonette and Joseph, 2009). This can be done at high and low temperatures (Lehmann, 2007a). Lastly, biochar can be used in agriculture and land reclamation where, through its effects on soil properties, crop yields can be increased and pollution levels reduced.

The ‘*Terra Preta*’, also known as *Terra Preta de Indio*, or the Amazonian Dark Earths (ADE) are carbon rich, fertile anthropogenic soils, covering an estimated 50,000 ha in the Amazon rainforest. Terra Preta soils have higher concentrations of nutrients and more stable organic matter than surrounding unmodified soils and this has been linked with charred substances or black carbon, including charcoal (Glaser *et al.*, 2001). Between 7000 and 500 years ago (Neves *et al.*, 2004), these charred substances were added to the soils during human inhabitation as a result of fires used for cooking and other purposes, although it is not known whether the soil modification was intentional or inadvertent (Glaser and Birk, 2012). The hot, rainy

climate of the humid tropics means that soil organic matter (SOM) decomposes rapidly (Tiessen *et al.*, 1994) and subsequently nutrients are leached easily but the *Terra Preta soils* do not present such a problem. The beneficial effects of black carbon are thought to be mainly due to their stability in soil compared to other forms of organic matter and their prevention of leaching (Glaser *et al.*, 2001).

Other less well-known examples of incorporating charred organic matter exist in several regions. Across mainland Europe, charcoal deposits of both natural and anthropogenic origins have been found (Vaccari *et al.*, 2011), and Downie *et al.* (2011) described soils (Terra Preta Australis) with similar properties to the Terra Preta at sites where Aboriginal oven mounds once existed. In 18<sup>th</sup> and 19<sup>th</sup> century Ireland the practice of paring and burning was often practiced on marginal or tough land. This involved removing the top layer of soil along with the vegetation and burning it in large piles after allowing it to dry (Bell and Watson, 2008). The resulting ashes and charred material were returned to the land and were said to improve the fertility, although this point was much debated.

Biochar is a contemporary label for charcoal when it is used as a soil amendment. It is produced by pyrolysis, a process distinguished from burning by the absence of char remaining after the latter (Lehmann and Joseph, 2009). Pyrolysis is carried out under conditions of relatively low temperature and oxygen and the material does not burn completely. Biochar is now quite well established as an effective amendment for poor, infertile soils in the tropics, with a meta-analysis by Jeffery *et al.* (2011) showing variable but overall positive effects. Soils in temperate climates are more stable and hence may not benefit as much from biochar amendment. Interest in this topic has heightened recently – most studies on this topic are from within the last decade. Biochar has several qualities that mediate its effects on soil properties. Its physical and chemical characteristics affect soil structure and nutrient dynamics, with resultant effects on soil biota.

## **2.2 Physical Characteristics**

The characteristics of different biochars are inconsistent, mainly due to the variety in feedstocks and the highest treatment temperature (HTT) during the production

process. The porosity of the biochar is largely determined by the original structure of the biomass used. Additionally, the chemical composition of the organic matter determines the degree of alteration that occurs – a material with a higher ratio of less heat resistant chemicals such as hemicelluloses will lose more mass during pyrolysis. Conversely, a material with a higher proportion of more resistant chemicals such as lignin will retain more mass as charcoal. Thus more charcoal can be produced from wood than a similar volume of grass. A higher pyrolysis temperature also burns off more material and hence the final yield of biochar depends on both the feedstock and the HTT (Amonette and Joseph, 2009).

Biochar develops a greater surface area with increasing HTT (Downie *et al.*, 2009) with surface areas ranging in one experiment (Brown *et al.*, 2006) from  $10\text{m}^2\text{ g}^{-1}$  at a HTT of  $450^\circ\text{C}$  to  $400\text{m}^2\text{ g}^{-1}$  at  $600\text{--}750^\circ\text{C}$ . At higher temperatures, melting and deformation of the chemical structure can occur, resulting in a reduction of surface area (Downie *et al.*, 2009). Other variable characteristics that influence biochar effects on soil include micro- and macroporosity (macropores can provide a habitat for microorganisms), solid density and bulk density (Downie *et al.*, 2009).

### 2.3 Chemical Characteristics

One quite consistent characteristic of biochars that are used as a soil amendment is alkalinity (Chan and Xu, 2009), although even this can be altered with various treatments, and a lower HTT results in a lower pH (Lehmann, 2007a). The C/N ratio varies, but its effects on N availability are not always as expected. Many terra preta soils have C/N ratios above that at which N immobilisation normally occurs but have agriculturally suitable levels of available N (Lehmann *et al.*, 2003). Cation exchange capacity (CEC) also tends to increase with increasing HTT, but this can change when biochar is added to the soil. In the Amazonian dark earths, where ambient temperatures range from  $30^\circ\text{C}$  to  $70^\circ\text{C}$ , CEC increases over time but it has not been established whether this would happen in colder climates (Lehmann, 2007a). In temperate soils, one study (Prommer *et al.*, 2014) found that biochar addition to soil affected multiple microbially mediated stages of the N cycle. Several transformation processes of organic N decreased in rate but overall N mineralization rates were unchanged, while nitrification increased substantially.



Based on investigations into the chemical composition of various biochars, higher pyrolysis temperatures cause a greater loss of nutrients and reduction of nutrient availability, although more study is needed regarding the latter point (Chan and Xu, 2009). Several plant growth studies (e.g. Chan *et al.*, 2007) show improved growth when nitrogen fertiliser is added in combination with biochar, suggesting that biochar is relatively lacking in nutrients. Nutrient content also varies with the type of feedstock used. Thus biochar is more likely to affect plant growth through altered nutrient dynamics, i.e. cation retention or CEC, rather than by adding nutrients.

#### 2.4 Biological Effects

According to Lehmann *et al.* (2011), biochar amendment generally increases the microbial biomass. They suggest that two particular qualities are likely to contribute to this: the existence of macropores (as stated above) and relatedly its large surface area; and its high capacity to adsorb inorganic nutrients, soluble organic matter and gases. These qualities serve as, respectively, habitat/refuge and food source.

Kolb *et al.* (2009) studied the effect of biochar addition on microbial biomass and activity in 4 different temperate soils in the US. All of the soils varied in texture but had similar histories, being originally wooded land (pine or deciduous) and then actively cropped for at least several years prior to the study. Both activity and biomass increased in each soil, and N availability also increased. The magnitude of the response did vary between soil types however, and the authors suggested this was due to differences in nutrient availability. The increases correlated with biochar over 5 different concentrations, ranging from 0-0.1kg kg<sup>-1</sup>. Increased microbial biomass and heightened levels of activity affect nutrient dynamics by increasing the rate of mineralisation. As a result of mineralisation more nutrients are made available, supporting further microbial and/or plant growth.

Watzinger *et al.* (2014) provide another study on the microbial effects of biochar in temperate soils. Minor increases in the microbial biomass were observed, and they were mainly attributed to pH changes. They also found greater increases in the biomass of gram negative bacteria and actinomycetes compared to gram positive

bacteria and fungi. The authors speculated that this was a result of the availability of extra nutrients and the sudden change in environmental conditions facilitating the fast growth habits of the former. The slower growth habits of gram positive bacteria and fungi make them more competitive in more stable, low nutrient environments. Ameloot *et al.* (2014) assessed the microbial communities at four sites in the UK and Italy several years after biochar field trials took place there. Microbial activity and abundance were both lower or equal in biochar plots compared to control plots and the authors concluded that biochar had ceased to function as a substrate for microorganisms after several years in the ground. However, lower activity is also seen as a sign of increased metabolic efficiency (Thies and Rillig, 2009). Liang (2008, in Thies and Rillig, 2009) found increased metabolic efficiency in the Amazonian Dark Earths, while Jin *et al.* (2008) reported the same effect occurring in the US. Increasing nutrient availability using microbially mediated pathways is advocated by Drinkwater and Snapp (2007) for boosting the productivity of sustainable agroecosystems, including organic holdings. The benefits include decreased use of fertilisers, reduced leaching and slow but more consistent release of nutrients to crops over time.

According to Thies and Rillig (2009), there is a lack of direct data regarding biochar effects on macrofauna populations. Interestingly, a study in an Irish soil (Augustenborg *et al.*, 2012) examined the combined effects of biochar and earthworms on soil emissions of the greenhouse gases CO<sub>2</sub> and N<sub>2</sub>O, finding that biochar reduced the emissions that earthworms cause.

### **2.5 Biochar in Temperate Soils**

Overall, reviews and meta-analyses have found that the effects of biochar are not consistent across the various studies. While an increase in crop production, rather than a decrease or no change, is the most common outcome, Spokas *et al.* (2012) suggest that publication bias may have occurred. This is the tendency for negative or neutral scientific results to remain unpublished, with the result being that a review of the literature finds a more positive overall effect that is not representative of the true overall effect. Spokas *et al.* (2012) also reported a greater likelihood of finding positive effects in weathered or low fertility soils.

The three meta-analyses found in this literature search reported positive effects. None of them investigated temperate soils exclusively. Jeffery *et al.* (2010), in a meta-analysis of 16 studies, reported an average increase in crop productivity of 10%, but with a range of -28% to 39%. The effects were most apparent in acid to neutral soils with a coarse to medium texture, suggesting that increased pH and water holding capacity (WHC) were the main mechanisms through which charcoal affected plant growth. Biederman and Harpole (2013), investigating ecosystem responses rather than crop productivity, analysed 114 studies in total. Several parameters were found to be positively influenced by biochar: above ground productivity, soil microbial biomass, nodulation of rhizobia, and soil N, P, K and C. However, this analysis was criticized by Jeffery *et al.* (2013) for overstating the positive effects of biochar and making claims not substantiated by their own analysis. Verheijen *et al.* (2009) also found a small positive effect overall, again most commonly in acidic soils suggesting that a liming effect is responsible. Nelissen *et al.* (2014) point out the importance of the liming effect of biochar in temperate soils and propose that this is the main reason for crop improvement. Their reasoning is that many studies that find a positive effect on crop growth use soils with low pH, and when a neutral or higher pH is used no effect is seen.

According to Nelissen *et al.* (2014), the analysis by Jeffery *et al.* (2010) included just one study in a temperate region (New Zealand), while Biederman and Harpole's (2013) more extensive analysis included several. One noteworthy point that emerged from the latter study is that the biochar effect correlated significantly with latitude – greater positive effects were more likely near the tropics while smaller and negative effects increased away from the equator.

Experiment	Location	Feed-stock	HTT	Application rate(s), kg m <sup>-2</sup>	Crop	Crop Effect	Soil effects	Suggested reason	Reference
Measurement of N-use efficiency and N leaching	USA	Maize stover	600°C	0, 0.1, 0.3, 1.2, 3	Maize	Crop growth and N-uptake unchanged	Increased retention of N fertiliser in the soil	Increase in microbial biomass, more fertiliser N converted to organic forms	Güereña <i>et al.</i> (2013)
Greenhouse trial	Germany	Beech wood, composted with organic matter	450°C	0, 5, 10, 20	Oats	Biochar increased biomass, with a TOC content stronger effect in sandy soil than in loam soil	Increased TOC content significantly	Possibly due to TOC and/or increased availability of nutrients	Schultz <i>et al.</i> (2013)
Greenhouse trial	USA	8 different feedstocks compared	4 temp.s compared - 300°C, 400°C, 500°C, 600°C	0, 0.26, 0.65, 2.6, 9.1	Corn	Plant biomass increased, but effect stopped or reversed at highest application rate	Not investigated	Possibly increased available N and/or increased pH	Rajkovich <i>et al.</i> (2011)

**Table 1.** Summary of studies investigating the effects of biochar on fertile soils in temperate climates using pot trials

Experiment	Location	Feed-stock	HTT	Applica-tion rate(s), kg/m <sup>2</sup>	Crop	Crop effect	Soil effects	Suggested reason	Reference
Lab based soil tests	USA	Oak	550°C	0.1g biochar per gram of soil	None		Increased WHC, reduced extractable NO <sub>3</sub> <sup>-</sup> , little change in NH <sub>4</sub> <sup>+</sup>	immobilization following increased microbial growth, NH <sub>4</sub> <sup>+</sup> due to lack of	Zheng <i>et al.</i> (2011) (CITE)
Greenhouse trial	Austria	Vineyard pruning; wheat straw; mixed woodchips	525°C	0, 3, 9	Mustard, barley, red clover (successively)	Significant decrease in mustard and barley yield; no effect on clover	pH increased in acid soil	Not clear, N immobilization and micronutrient deficiencies unlikely	Kloss <i>et al.</i> (2014)
Pot trial, comparing fresh biochar with steam activated biochar	Germany	Beech (90%), oak (10%)	475°C	0, 2, 7.5, 15 g kg <sup>-1</sup> of soil	Ryegrass	Plant N uptake decreased with fresh biochar but increased with activated biochar	Total N increased, leaching reduced; activated biochar effects twice as strong as fresh biochar	Activated biochar had higher C/N ratio, hence reduced immobilisation	Borchard <i>et al.</i> (2012)

**Table 1.** continued

A number of studies have been conducted in recent years in regions with a temperate climate and fertile soil. These include both pot trials using local soil as well as field trials on agricultural land and are summarised in tables 1 and 2. Jones *et al.* (2012)

and Borchard *et al.* (2014) speculated that root development facilitated by biochar amendment could account for plant growth – in both studies maize, which is deep rooting, did not benefit while the grass in the study by Jones *et al.* (2012) showed an increase in biomass, height and nutrition. Another possible reason for this is the age of the biochar in the soil. The grass was planted in the second and third years after biochar addition, after which certain positive effects on nutrient availability and soil microorganisms may have materialised. CEC is often low in fresh biochar but can increase in the first few months (Lehmann, 2007a) so in the first year there may be no change to nutrient retention or availability in the soil. Similarly, any changes to the soil biota may occur gradually.

Karer *et al.* (2013) also found a reduction in plant growth in the first two years after biochar addition, this time due to nutrient shortages. Biochar, being high in carbon but often low in other nutrients and having a high CEC, often reduces nutrient availability in soil when added without other fertiliser (Chan and Xu, 2009). However, some pot trials have shown increased N availability or N uptake efficiency after biochar addition, in one case causing increased plant growth (Rajkovich *et al.*, 2011, Güereña *et al.*, 2013, Schultz *et al.*, 2013). Chan *et al.* (2007) found a synergistic effect of nitrogen fertiliser and biochar on plant growth in a hardsetting Australian farmland soil – biochar amendment alone did not positively affect yields of radish (dry weight) but biochar in combination with a nitrogen fertiliser had a greater effect than a control with just fertiliser. This suggests that biochar increased the plant nitrogen-use efficiency.

Hammond *et al.* (2013, Table 2) suggested that CEC may have played a large role in their results. Although a meta-analysis of seven field trials showed a positive effect of biochar application on crop yield, there was one without which the overall effect would have been nil. In this trial on spring barley there were two major differences from the others: the biochar had been aged for several years prior to application and it was applied to the surface as a top dressing rather than being mixed in immediately. This biochar, probably as a result of the aging process, had exchangeable nutrient and CEC values an order of magnitude greater than the biochars in the other trials. The top dressing method may have had effects on soil temperature and moisture that also contributed to the final yield.

Experiment Location	Feed-stock	HTT	Application Rate(s), kg/m <sup>2</sup>	Crop	Crop effect	Soil effects	Suggested reason	Reference
3-year field trial	Wales Ash, beech and oak wood	450°C	0, 2.5, 5	Maize (1st year), grass (2nd and 3rd years)	No effect on maize growth, grass showed increased crop height, dry biomass and nutritional quality	Increased respiration and growth of fungi and bacteria; short term pH increase.	Possibly due to increased rooting facilitated by biochar	Jones <i>et al.</i> (2012)
As above	As above	As above	As above	Dwarf bean	Freshly added biochar caused increased growth compared to control soil and soil with 3-year old biochar	Fresh biochar caused increase in NH <sub>4</sub> <sup>+</sup> availability	Inconclusive	Quilliam <i>et al.</i> (2012)
3-year field trial	Finland Pine and spruce chips	600°C	0, 0.5, 1	Wheat, turnip rape, faba bean	No effect on plant yield	Increased soil WHC during a low rainfall year; possibly increased immobilization of N	Inconclusive	Tammeorg <i>et al.</i> (2014)

**Table 2.** Summary of studies investigating the effects of biochar on fertile soils in temperate climates using field trials

Experiment	Location	Feed-stock	HTT	Applicat ion Rate(s), kg/m <sup>2</sup>	Crop	Crop effect	Soil effects	Suggested reason	Reference
2-year field trial	Austria	Beech wood	550°C	0, 2.4, 7.2	Maize, barley, wheat and sunflower	Yield decrease in plots without fertiliser; no effect in plots with fertiliser; yield increase in barley in first year	Bulk density decreased; WHC increased; pH and EC temporarily increased	Yield decreases due to nutrient shortage caused by biochar; greater barley yield due to increased WHC in a low rainfall year	Karet <i>et al.</i> (2013)
3 year pot trial in field conditions	Germany	Beech and spruce wood with three different pyrolysis processes	450°C - 1100°C	0, 4.5, 30	Maize	No effect at lower application rate, decrease in yield at higher rate.	WHC increased	Deep rooting characteristics of maize may have made biochar redundant	Borchard <i>et al.</i> (2014)
2-year field trial	Italy	Beech, hazel, oak and birch wood (coppice)	500°C	0, 3, 6	Durum wheat	Increased biomass and grain yield at both application rates in both years	Bulk density decreased, pH and temperature increased	Several reasons suggested as possibilities, speculative	Vaccari <i>et al.</i> (2011)

**Table 2.** continued



Experiment	Location	Feed-stock	HTT	Applicat ion Rate(s), kg/m <sup>2</sup>	Crop	Crop effect	Soil effects	Suggested reason	Reference
1-year field trial	UK	Not given	Not given	0, 0.5, 1, 2, 5	Spring barley	No effect from biochar alone; 30% increase in crop yield when combined with N fertiliser	Not given	Increased nitrogen use efficiency	Gathorne-Hardy <i>et al.</i> (2009)
Seven field trials on multiple working farms	Scotland, England	Sycamore, oak, Scots pine, beech amongst others	500°C	Several rates ranging from 0-8	Spring barley, oil seed rape, winter wheat, carrots, beetroot, spinach, various legumes	Overall, a significant positive effect (meta-analysis). Average increase in crop yield of 0.4t ha <sup>-1</sup> . Application rates of ≤2kg m <sup>-2</sup> were most successful.	Not given	Various, see main text	Hammond <i>et al.</i> (2013)

**Table 2.** continued

The nutrient dynamics of biochar in soil are highly variable and the dearth of long-term studies means that it is not known whether positive effects develop over time. Currently, knowledge of biochar effects is limited to the first few years, when the soil system has just undergone a major change. Results can therefore be expected to be erratic, and this can be seen in the literature. The study by Quilliam *et al.* (2012) is significant in this regard. Their experiment investigated plant growth and several other parameters in soils to which biochar had been added three years previously. Fresh biochar was added to half of these so that three treatments were being compared – a control soil, a soil with 3-year old biochar and a soil with 3-year old biochar plus fresh biochar. Plant growth, microbial activity and colonisation of plant roots by mycorrhizal fungi all increased in the fresh biochar treatment, whereas the old biochar treatment did not differ from the control. These results are in stark contrast to studies of the Amazonian dark earths, which appear to retain their characteristics over centuries.

Soil WHC can also be increased with biochar addition – Karer *et al.*, (2013) reported a greater yield of barley grown during a drought in biochar amended soil. This quality might become valuable as climate becomes increasingly erratic and weather less predictable. Farmers and growers may wish to increase the ability of their soil to buffer against unfavourable growing conditions. Biochar can reduce leaching in temperate soils. Laird *et al.* (2010) reported lower levels of N, P, Mg and Si leaching from a biochar amended soil in Midwestern USA even though, interestingly, the biochar itself contained substantial quantities of these nutrients. Reduction in leaching does not always lead to an immediate positive effect on crop growth (Borchard *et al.*, 2012). Several field studies have reported positive effects of biochar when used in combination with fertiliser. Gathorne-Hardy *et al.* (2009) found no effect from biochar when added to the soil alone, but achieved a 30% increase in crop yield when combined with N fertiliser. Interestingly, nutrients apart from N were added in amounts far above what was needed by the crop so that the focus of the trial was on N alone.

## 2.6 Potential in Irish Organic Horticulture

Positive effects on plant growth are somewhat rare, as described above, and this is a weak point in the commercial potential of biochar. More consistent effects, such as reduction of leaching, are less obvious and therefore may not be as highly valued amongst growers in general, except among the environmentally conscious and economically prudent. Quilliam *et al.* (2012) reported a reduction in emergence of weed seeds in soil with biochar added, with no difference in the growth of the main crop. The cause of this was unclear but increased biomass of AMF and/or altered nutrient dynamics were suggested as possibilities by the authors.

Kasten Dumroese *et al.* (2011) suggested using biochar as an amendment for seeding compost, since it has several suitable qualities including high CEC and WHC, as well as low nutrient levels. The researchers created pellets of biochar to minimise the release of dust and to facilitate more homogenous mixing with compost. Their tests showed that biochar can contribute to seed compost qualities when added to peat based compost. It should be noted that peat based composts are not permitted under organic certification guidelines.

Several studies have investigated the effects of biochar on germination. A recent UCC based study showed promising effects of biochar on oat seed germination (Rice, 2014). Solaiman *et al.* (2012) found that it caused increased rates of germination in wheat, reduced germination of subterranean clover and mixed results for mung bean. Root/shoot ratios were also affected but not correlated with germination success. In a somewhat less relevant study, Robertson *et al.* (2012), biochar enhanced the biomass of pine and alder seedlings in a sub-boreal forest soil. Free *et al.* (2010) found no effect on maize germination in a New Zealand based study and Bargmann *et al.* (2013) reported similar results with spring barley. Mulcahy *et al.* (2013) showed reduced wilting in tomato seedlings after biochar application to soil and stated that biochar may be more feasible at a smaller scale considering the cost of production.

### 3 – Aims

One of the aims of this study was to investigate whether plants germinated in biochar-amended compost showed increased yield when transplanted to outdoor field conditions. The second aim was to study the effect on crop yield of biochar application to soil, the intention being to add to the growing body of work being done on biochar-amended soil in temperate regions. Effects so far have been variable and the causes as yet unclear, with several mechanisms potentially responsible. An investigation into several soil physical, chemical and biological characteristics was needed to provide insight into the effects on crop growth. An overall aim of the project was to establish a long-term outdoor trial area to test the effects of biochar over time, considering the scarcity of studies spanning more than 1-3 years.

### 4 – Materials and Methods

#### 4.1 *Biochar*

In keeping with suggestions for biochar research to describe in as much detail as possible the source material and production method (Lehmann and Joseph, 2009) specifics of the biochar used are provided here. The biochar was sourced from Biochar Ireland, located in Donegal, and was produced from thinnings from sustainably managed hardwood forest. Temperatures exceeded 650°C during production. The charcoal was ground to a fine powder and rainwater was added at a rate of  $\sim 0.251 \text{ kg}^{-1}$  prior to delivery to the site in order to minimise the release of dust. Before application to the soil, the charcoal was sieved through a 1cm x 1cm mesh sieve.

#### 4.2 *Study Site*

The study took place at the School of Biological, Earth and Environmental Sciences, University College Cork, Ireland. Outdoor plots were established during the summer of 2014 by the Centre for Organic Horticultural Research (COHR). The latitude is 51°53'57" north and the grid reference is W664 719.

### 4.3 Seed Compost Amendment Trial

For the germination trial, biochar was mixed with Klassmans peat based compost at rates of 0 kg m<sup>-2</sup> (control), 0.5 kg m<sup>-2</sup>, 2.5 kg m<sup>-2</sup>, 5 kg m<sup>-2</sup> and 20 kg m<sup>-2</sup>. Seeding trays containing 150 cells were used, with an individual cell volume of ~40ml. A replicated randomized block (RRB) design was employed, with 6 replicates of each treatment and each replicate containing 25 cells (blocks of 5 x 5 cells). Two experiments using this design were carried out, each with a different cultivar of white turnip (*Brassica rapa* subsp. *rapa*). The cultivars used were 'Tokyo Cross' and 'Market Express'. Seeds were sown, one per cell, in a greenhouse on 18/7/2014 and watered daily before noon for the duration of the study. Initially, both cultivars were to be planted outdoors after a few weeks growth but it was later decided to plant out only the 'Market Express' seedlings and measure the fresh and dry biomass of the 'Tokyo Cross' seedlings. This was partly due to a lack of space in the outdoor plots but also allowed a more detailed picture of early plant growth to emerge. 'Market Express' seedlings were planted outdoors on the 15/8/2014. The design was again RRB with 6 replicates of each treatment, but plants from different replicates were used in each of the new replicates. The planting was done in rows spaced 0.25m apart, with 0.1m spacing within the rows and 12 plants per row. Each of the 30 replicates was represented by one row of plants.

The 'Tokyo Cross' seedlings were weighed on 15/8/2014, and dried in an oven at 50°C. They were weighed two days later, returned to the oven and weighed again the next day to ensure that they were completely dry. 'Market Express' plants were harvested on 26/8/2014, the roots and leaves separated, weighed immediately to measure fresh biomass and a sub-sample of 3 plants per replicate dried and weighed as above.

### 4.4 Soil Amendment Trial

Seeds were planted in Klassmans peat based seeding compost on 18/7/2014. Cultivars, seed trays, location, watering regime and plant spacing were as above. Biochar was applied to the outdoor plots at the end of July 2014. The design was RRB with 4 treatments and 6 replicates of each treatment. The application rates were 0 kg m<sup>-2</sup> (control), 0.2 kg m<sup>-2</sup>, 0.8 kg m<sup>-2</sup> and 2.4 kg m<sup>-2</sup>. The plot size was 2m x 0.5m and

a buffer strip of 0.3m was left between plots. 2 rows, one for each cultivar and each containing 18 plants, were planted in each plot on 5/8/2014.

Plants were harvested on 16/8/2014 and 17/8/2014, the roots and leaves separated and weighed immediately to measure fresh biomass. The roots of a sub-sample of 3 plants per replicate were dried and weighed as above.

#### **4.5 Chlorophyll Readings**

A SPAD-502 chlorophyll meter was used to test the leaf chlorophyll levels in all outdoor study plots. The biochar amended plots were tested on 15/8/2014. Three readings were taken from one of the youngest fully formed leaves of each 'Tokyo Cross' plant, and the average of these readings recorded. Chlorophyll readings from the seeding amendment trial were taken prior to harvest on 26/8/2014, but a sub-sample of 4 plants per row was used.

#### **4.6 Statistics**

The RRB design allowed Friedman's test to be carried out on all results above.

#### **4.7 Soil Tests**

Soil was collected from each replicate and pooled for each treatment. Soil tests were carried out using pooled samples of soil from each treatment that were dried for 3 days at 50°C. EC and pH were tested using deionised water filtered through a sample of the soil. EC and pH meters provided the results. Infiltration time and WHC were calculated by placing 25g soil in a large syringe and pouring 35ml water into the syringe. The time between addition of water and when the first drop emerged from the needle of the syringe was recorded for the infiltration time. The WHC was calculated by measuring the amount of water that emerged from the syringe and subtracting this value from the original amount poured in.

#### **4.8** *Community Level Physiological Profile*

Soil was collected as above. For each treatment, soil was sieved and 10g weighed out, then suspended in 10ml of half-strength Ringer's solution. This was diluted to a ratio of 1:5,000 and pipetted in 150 $\mu$ l amounts to each well on a Biolog plate. The plates were stored in darkness for seven days and read using the BIORAD plate reader (model 680). Results were analysed using the statistical software package MVSP. AWCD, species richness (R), and the Shannon index were calculated, and PCA and cluster analysis performed. The 3 diversity indices were tested for differences using the Kruskal-Wallis test.

## 5 – Results

### 5.1 Soil tests

Biochar amendment caused an overall increase in the infiltration time and water holding capacity of the soil (Table 3). Both measurements dropped slightly with the low treatment but were higher in the medium and high treatments. The infiltration time was almost double in the high treatment compared to the control, from 22.7 seconds to 38 seconds. WHC increased in the medium and high treatments, the latter by 11%. The low treatment decreased compared to the control.

**Table 3.** Soil test results. Low = 0.2 kg m<sup>-2</sup>; Medium = 0.8 kg m<sup>-2</sup>; High = 2.4 kg m<sup>-2</sup>.

<b>Biochar Treatment</b>	<b>Infiltration Time (sec)</b>	<b>WHC (ml g<sup>-1</sup>)</b>	<b>pH</b>	<b>Conductivity (μS cm<sup>-1</sup>)</b>
Control	22.7	34.9	5.98	295
Low	18.5	32.9	5.76	238
Medium	33.0	37.1	5.96	268
High	38.0	38.9	6.1	249

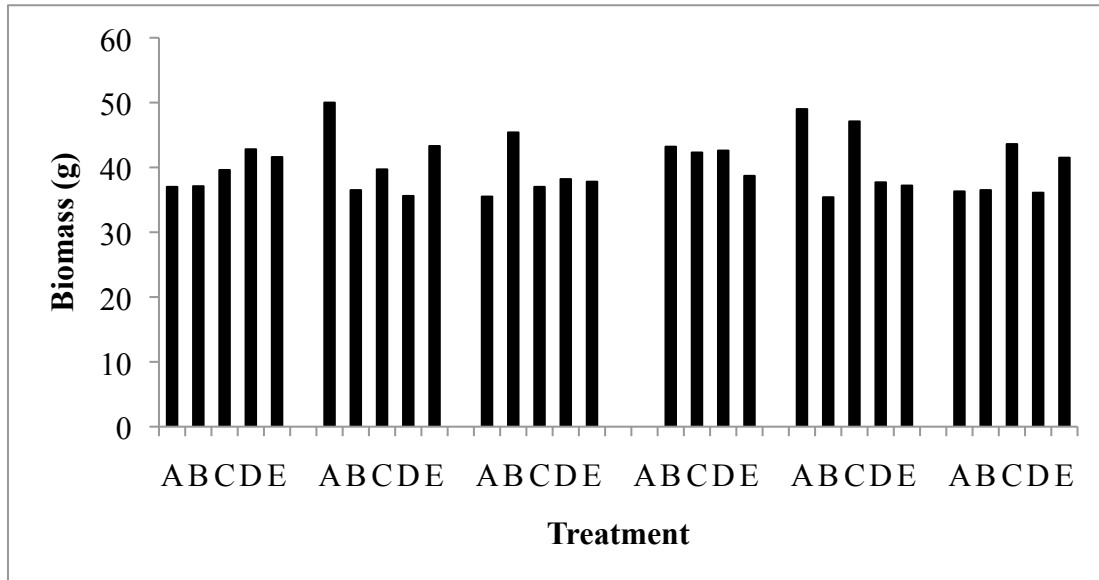
Soil pH showed minor changes (Table 3) – the highest rate of biochar application had a very slight reduction in acidity compared to the control, from 5.98 to 6.1. The low treatment had the largest deviation from the control at 5.76 while the medium treatment remained relatively unchanged. The control treatment had the highest conductivity reading at 295μS cm<sup>-1</sup>. The low treatment again had the lowest value at 238μS cm<sup>-1</sup>.

### 5.2 Seed Compost Amendment Trial

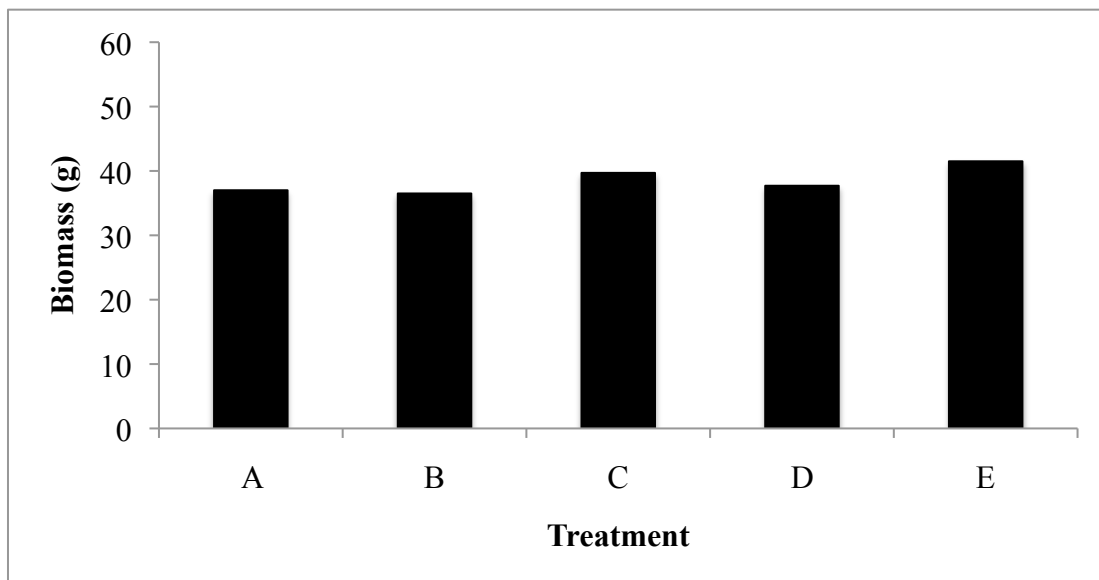
Plant growth was variable overall – plants at tray edges were quite small compared to those in the middle. The seeding compost amendment trial showed no significant differences between treatments (Friedman’s test), but differences at the P<0.1 level were detected in ratios of plant dry weight to fresh weight (described below, figs. 6 and 10). The results for total seedling biomass within each treatment across all replicates are shown in fig. 1 and the mean biomass values for each treatment are



shown in fig. 2. The biomass varied within replicates with no clear trend emerging overall. The lowest and highest biomass values, 35.4g and 45.4g, were found in B treatments from different blocks. Median values showed much less variation and, again, no clear trend (fig. 2).

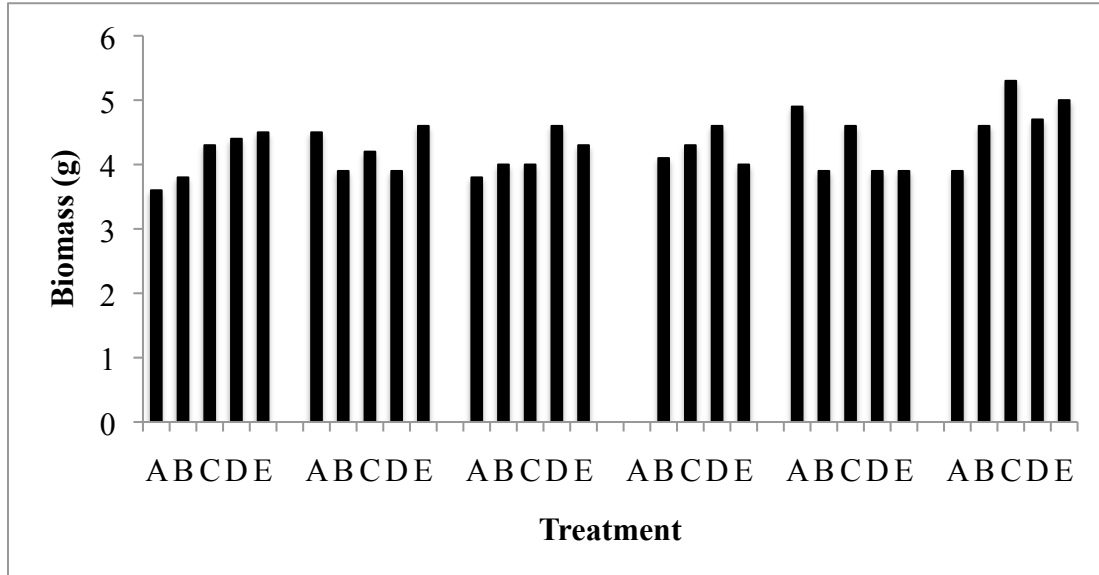


**Figure 1.** Total fresh biomass of white turnip (cv. ‘Tokyo Cross’) seedlings over five biochar treatments and six replicated blocks. A = Control; B = 0.5 kg m<sup>-2</sup>; C = 2.5 kg m<sup>-2</sup>; D = 5.0 kg m<sup>-2</sup>; E = 20 kg m<sup>-2</sup>.

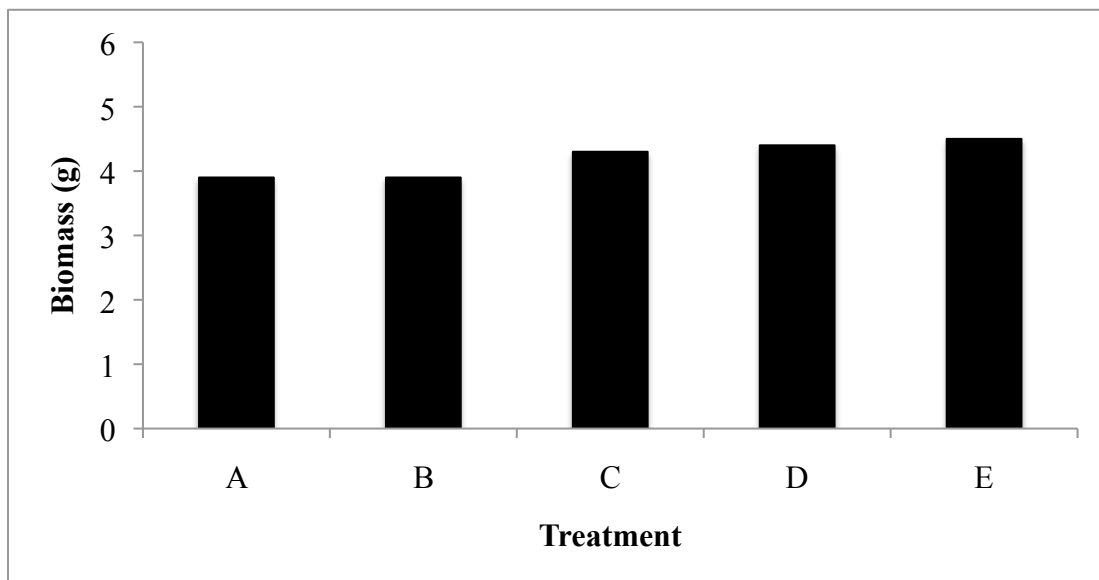


**Figure 2.** Median white turnip (cv. ‘Tokyo Cross’) fresh biomass over five biochar treatments. A = Control; B = 0.5 kg m<sup>-2</sup>; C = 2.5 kg m<sup>-2</sup>; D = 5.0 kg m<sup>-2</sup>; E = 20 kg m<sup>-2</sup>.

Dry weight values were similarly variable within blocks and showed no particular trend across blocks (fig. 3). Median dry weight values for each treatment (fig. 4) showed, less variability but there was also no significant difference between treatments.



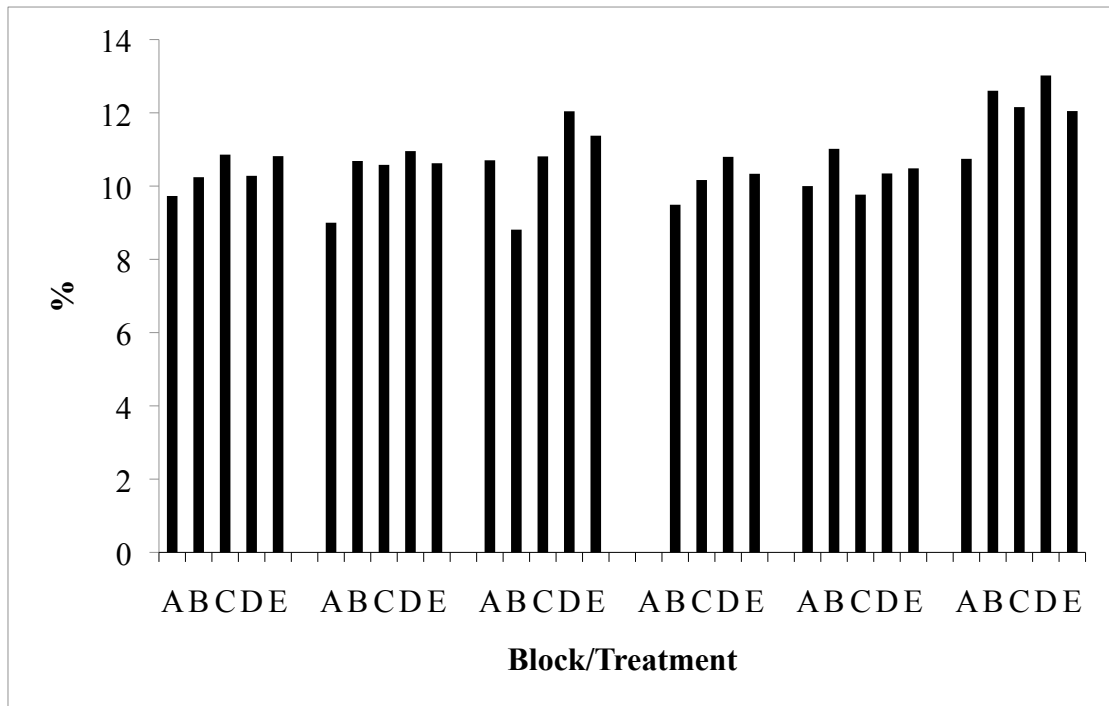
**Figure 3.** Total dry weight of white turnip (cv. Tokyo Cross) seedlings over 5 biochar treatments and six replicated blocks. A = Control; B = 0.5 kg m<sup>-2</sup>; C = 2.5 kg m<sup>-2</sup>; D = 5.0 kg m<sup>-2</sup>; E = 20 kg m<sup>-2</sup>.



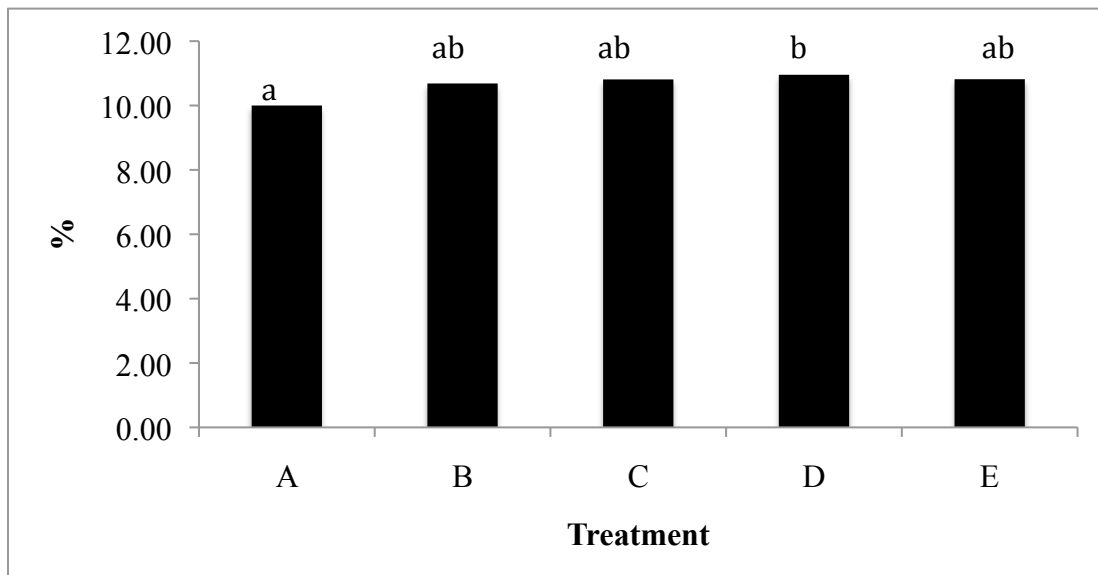
**Figure 4.** Median dry weight of white turnip (cv. Tokyo Cross) seedlings for each treatment. A = Control; B = 0.5 kg m<sup>-2</sup>; C = 2.5 kg m<sup>-2</sup>; D = 5.0 kg m<sup>-2</sup>; E = 20 kg m<sup>-2</sup>.

The ratios of dry biomass to fresh biomass in the seedling trial are shown as percentages in fig. 5. There was a trend toward higher percentages with increasing

biochar rates, and this is quite clear in the median values for each treatment (fig. 6). The dry: fresh ratio of plants grown at the 5 kg m<sup>-2</sup> biochar rate was greater than those grown in the control treatment at the P<0.1 level.



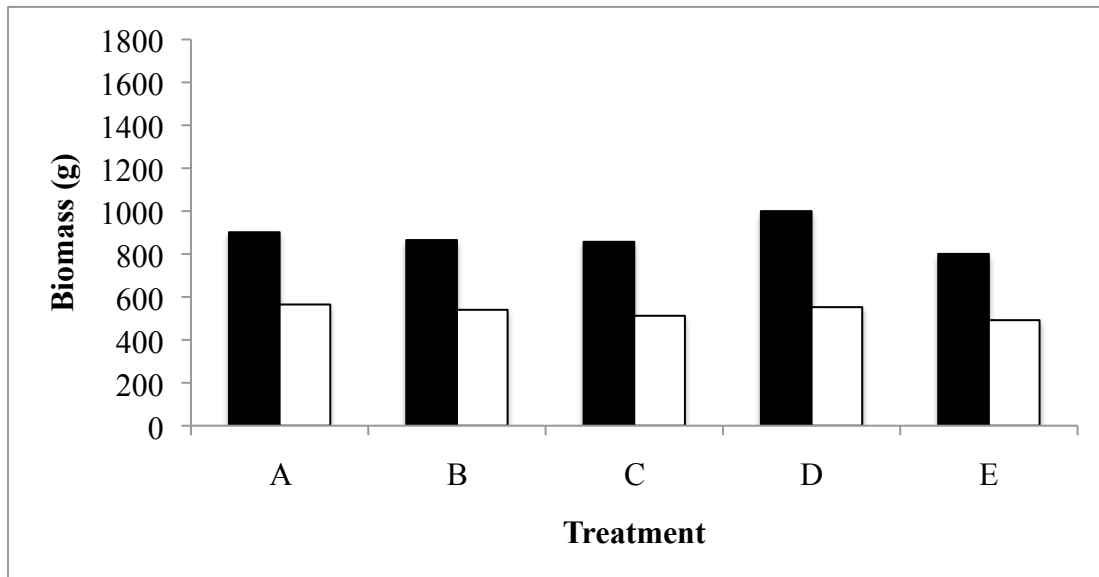
**Figure 5.** Dry Weight: fresh weight ratio of white turnip (cv. ‘Tokyo Cross’) seedlings over five treatments and six replicated blocks A = Control; B = 0.5 kg m<sup>-2</sup>; C = 2.5 kg m<sup>-2</sup>; D = 5.0 kg m<sup>-2</sup>; E = 20 kg m<sup>-2</sup>.



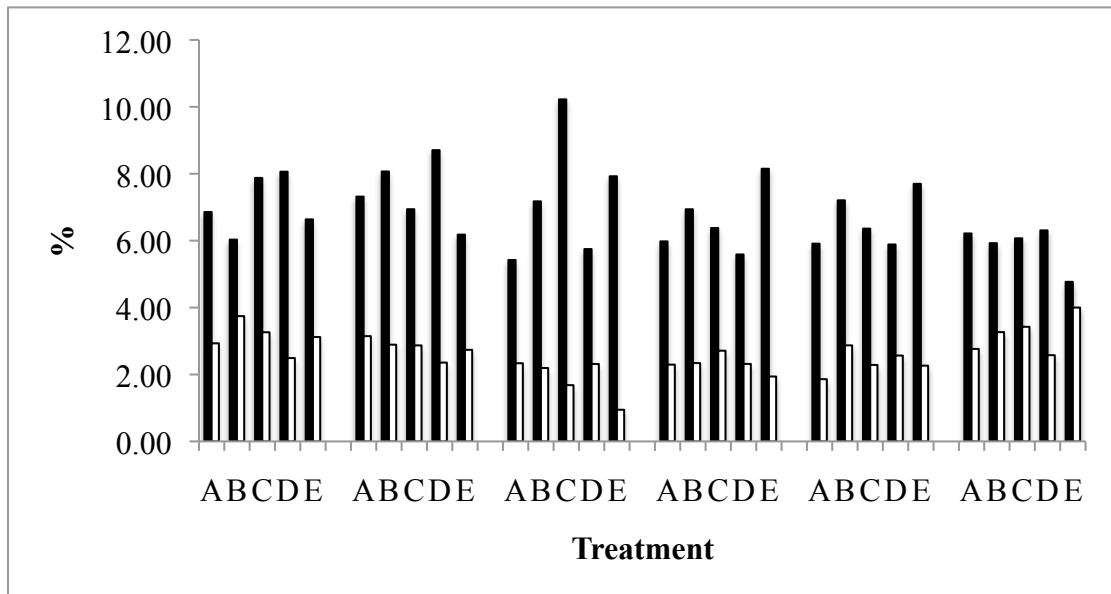
**Figure 6.** Median dry weight: fresh weight ratio of white turnip (cv. Tokyo Cross) seedlings for each treatment. A = Control; B = 0.5 kg m<sup>-2</sup>; C = 2.5 kg m<sup>-2</sup>; D = 5.0 kg m<sup>-2</sup>; E = 20 kg m<sup>-2</sup>. Columns which do not share a common letter are significantly different (Friedman’s test, P < 0.1)

Germination success did not differ significantly between treatments. The lowest germination rate was 21/25, or 84%, and the most common (or modal) rate was 100%. The high germination rate meant that the values for the average biomass per plant showed broadly similar variation between treatments as the total biomass values. No significant differences were found using the average values for fresh biomass, dry biomass and dry biomass as a percentage of fresh biomass.

There were no significant differences in the total fresh biomass, fresh root biomass, fresh leaf biomass, or leaf/root fresh biomass ratio of the 'Market Express' seedlings planted outdoors. However, certain trends are visible from the median values. Root biomass appears to be greatest at the second highest biochar amount, but the control treatment appears to have the next highest root biomass (fig. 7).

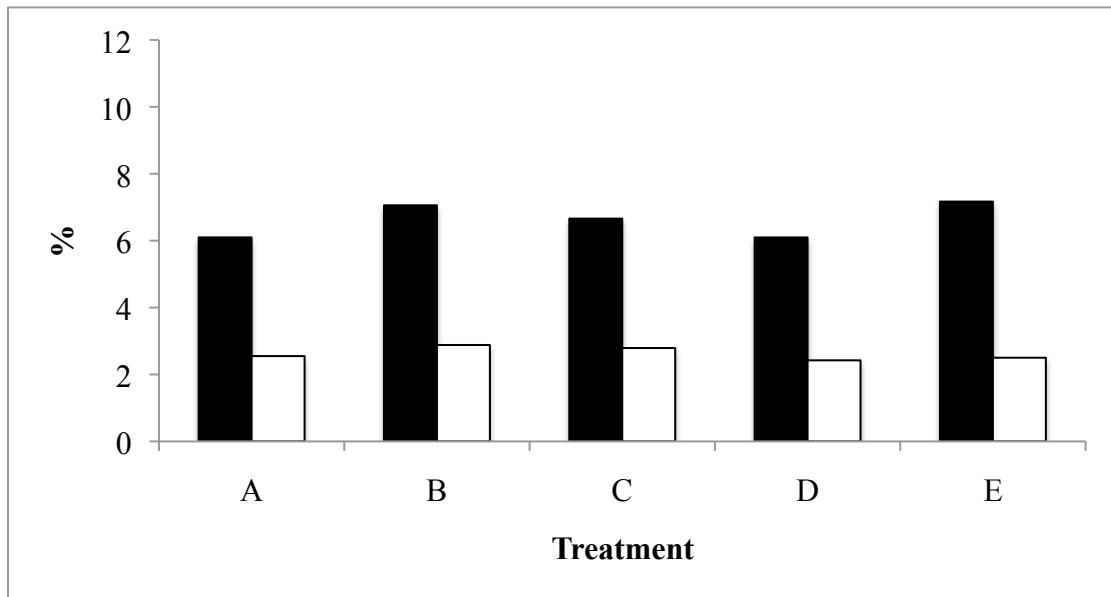


**Figure 7.** Median fresh biomass of roots (■) and leaves (□) of white turnip (cv. 'Market Express') from the outdoor trial. A = Control; B = 0.5 kg m<sup>-2</sup>; C = 2.5 kg m<sup>-2</sup>; D = 5.0 kg m<sup>-2</sup>; E = 20 kg m<sup>-2</sup>.

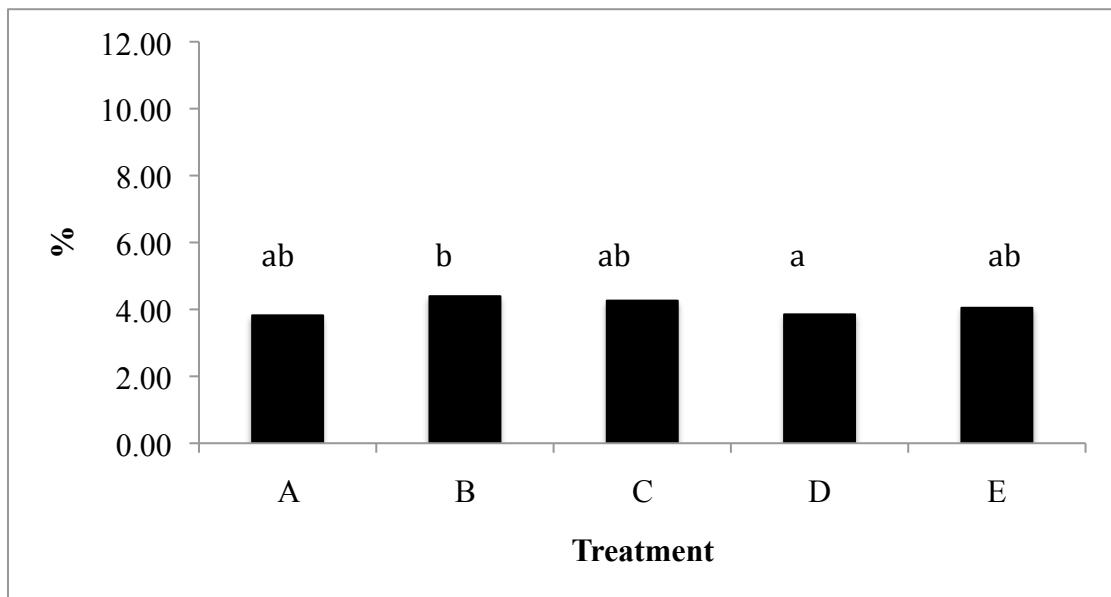


**Figure 8.** Dry weight:fresh weight ratios of roots (■) and leaves (□) of white turnip (cv. ‘Market Express’) over five treatments and six replicated blocks. A = Control; B = 0.5 kg m<sup>-2</sup>; C = 2.5 kg m<sup>-2</sup>; D = 5.0 kg m<sup>-2</sup>; E = 20 kg m<sup>-2</sup>.

Dry:fresh weight ratios were calculated for leaves and roots separately (fig. 9) and combined (fig. 10). There were no significant differences between the treatments but differences at the P<0.1 level were found when whole plants were compared (fig. 10). In this case the lowest biochar rate (0.5 kg m<sup>-2</sup>) was found to have a greater dry:fresh weight ratio than the second highest rate (5.0 kg m<sup>-2</sup>).



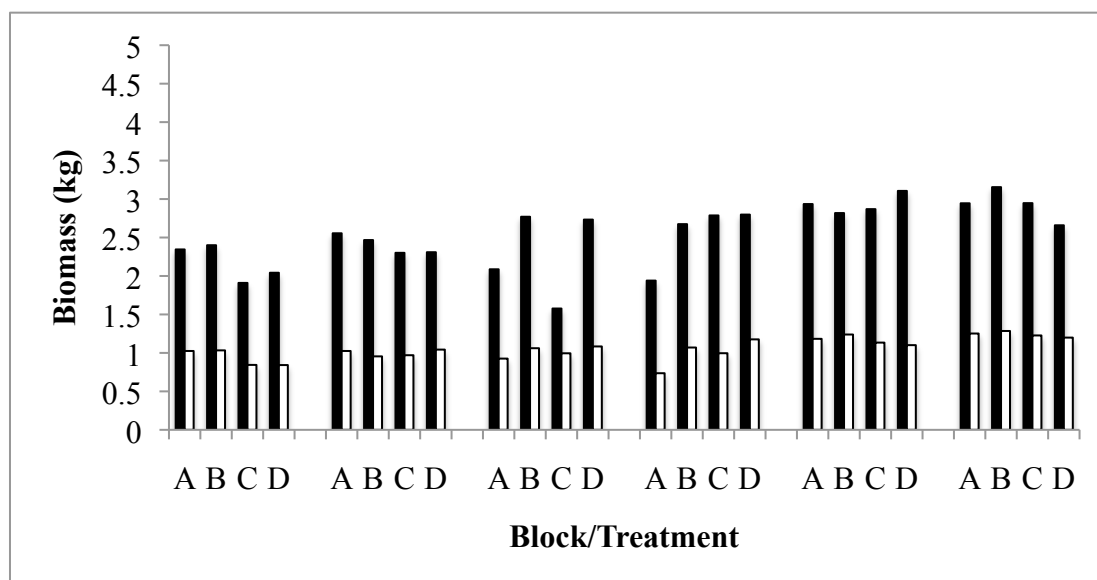
**Figure 9.** Median dry weight:fresh weight ratios of roots (■) and leaves (□) of white turnip (cv. ‘Market Express’) over five treatments. A = Control; B = 0.5 kg m<sup>-2</sup>; C = 2.5 kg m<sup>-2</sup>; D = 5.0 kg m<sup>-2</sup>; E = 20 kg m<sup>-2</sup>.



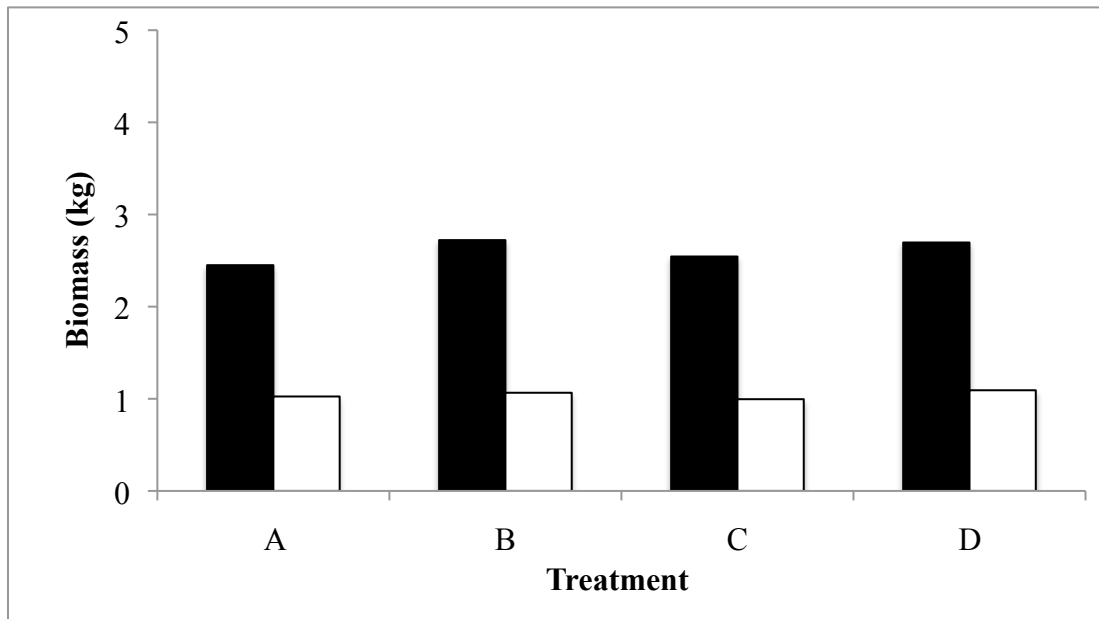
**Figure 10.** Median whole plant (roots and leaves combined) dry weight:fresh weight ratio over five treatments. A = Control; B = 0.5 kg m<sup>-2</sup>; C = 2.5 kg m<sup>-2</sup>; D = 5.0 kg m<sup>-2</sup>; E = 20 kg m<sup>-2</sup>. Columns which do not share a common letter are significantly different (Friedman’s test, P < 0.1)

### 5.3 Soil Amendment Trial

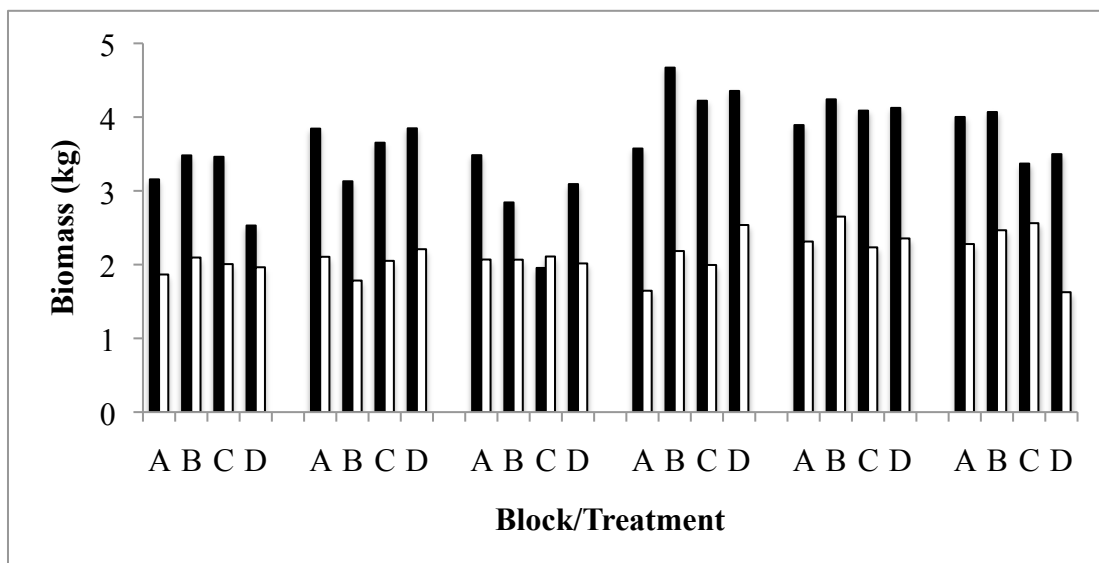
Overall, white turnip biomass showed no significant differences between treatments, including the control, in both cultivars. Due to the variation in plant numbers between replicates at harvest, ranging from 13 to 18, values for total biomass within replicates were compared (figs. 11 and 13) rather than mean biomass per plant. The biomass values for each replicate were variable and showed no clear trend in either cultivar.



**Figure 11.** Total fresh biomass of white turnip (cv. ‘Market Express’) roots (■) and leaves (□) over four biochar treatments and six replicated blocks. A = Control; B =  $0.2 \text{ kg m}^{-2}$ ; C =  $0.8 \text{ kg m}^{-2}$ ; D =  $2.4 \text{ kg m}^{-2}$ .



**Figure 12.** Median fresh biomass (kg) of white turnip (cv. ‘Market Express’) roots (■) and leaves (□) over four biochar treatments. A = Control; B = 0.2 kg m<sup>-2</sup>; C = 0.8 kg m<sup>-2</sup>; D = 2.4 kg m<sup>-2</sup>.

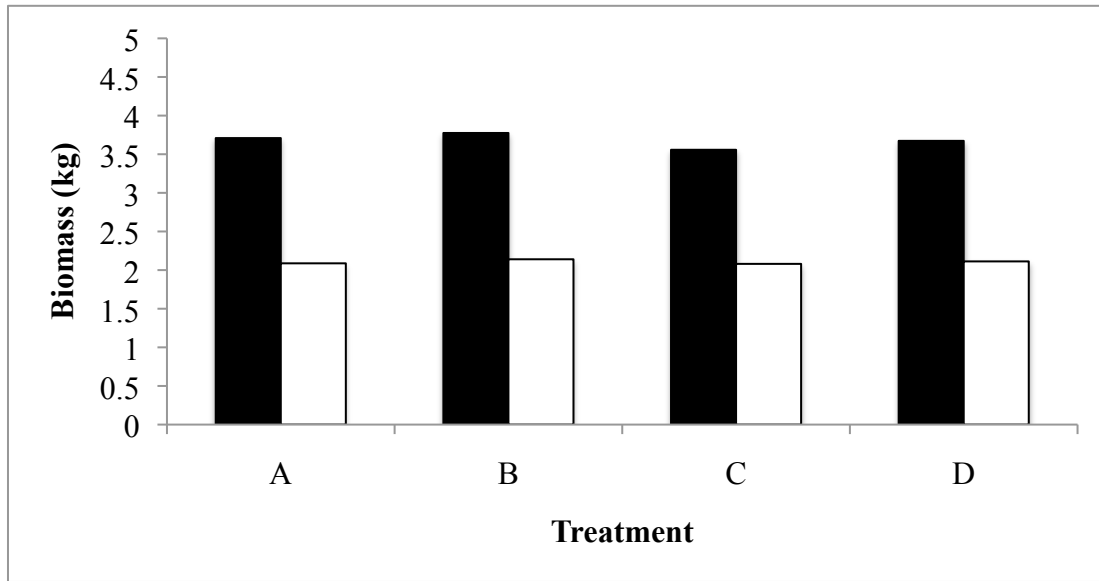


**Figure 13.** Total fresh biomass of white turnip (cv. ‘Tokyo Cross’) roots (■) and leaves (□) over four biochar treatments and six replicated blocks. A = Control; B = 0.2 kg m<sup>-2</sup>; C = 0.8 kg m<sup>-2</sup>; D = 2.4 kg m<sup>-2</sup>.

The median biomass for each treatment showed slight differences, with treatments B and D greater than A and C in the ‘Market Express’ cultivar (fig. 12), and treatment B greater than the others in ‘Tokyo Cross’ (fig. 14). No significant differences were

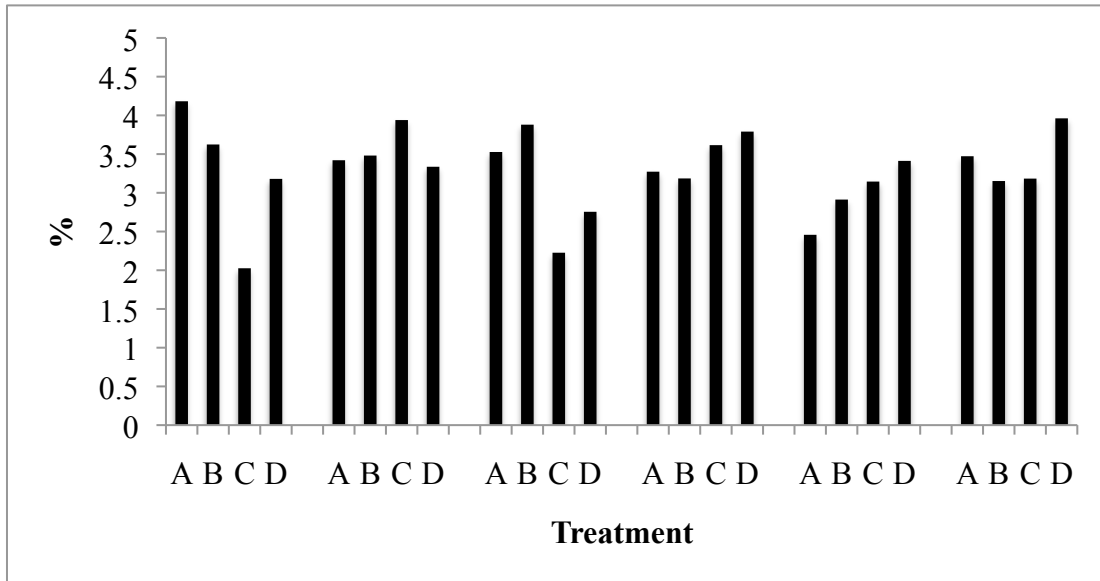


detected (Friedman's test). The ratios of root biomass to leaf biomass were also compared (Friedman's test) but these did not vary significantly among treatments.

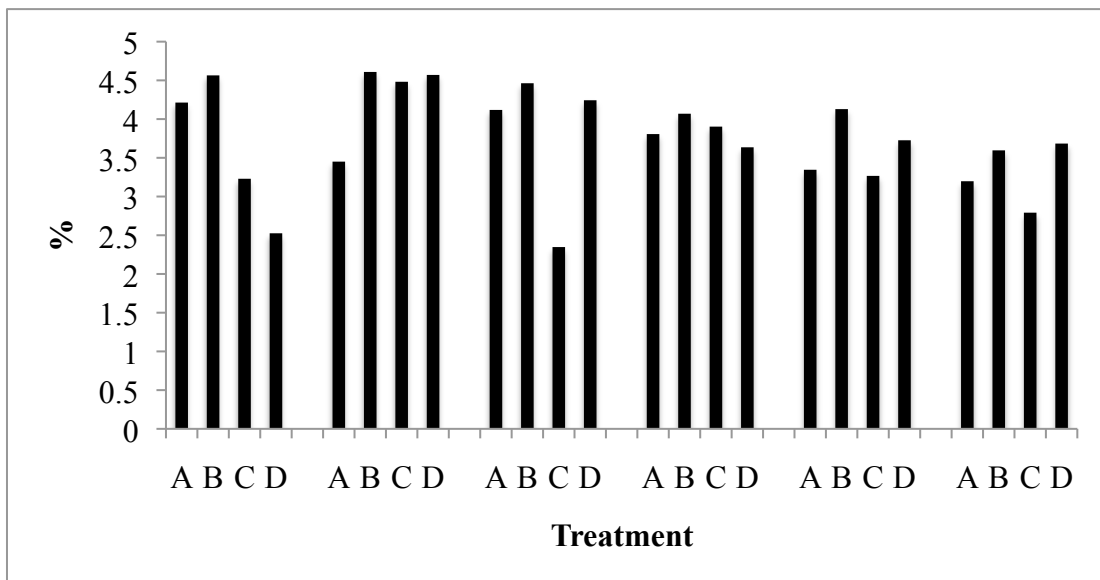


**Figure 14.** Median fresh biomass (kg) of white turnip (cv. 'Tokyo Cross') roots (■) and leaves (□) over four biochar treatments. Black columns = root biomass; white columns = leaf biomass. A = Control; B = 0.2 kg m<sup>-2</sup>; C = 0.8 kg m<sup>-2</sup>; D = 2.4 kg m<sup>-2</sup>.

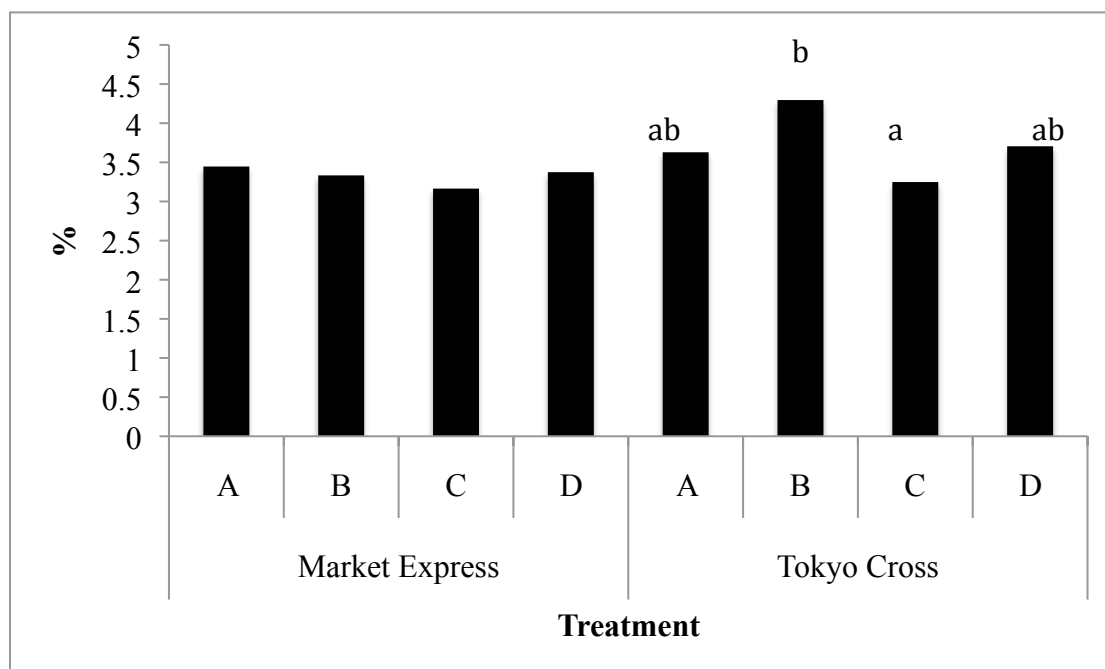
The dry:fresh weight ratios of the roots were calculated using the fresh weights and dry weights of a sub-sample from each replicate. Figs. 15 and 16 show the values for individual replicates. Results were quite variable within and between blocks. Median values (fig. 17) show that, overall, the 'Market Express' cultivar did not appear to respond to biochar. In the 'Tokyo Cross' cultivar, dry:fresh weight ratios were greater in the low treatment than in the medium treatment.



**Figure 15.** Dry weight:fresh weight ratio of white turnip (cv. 'Market Express') root over four biochar treatments and six replicates. A = Control; B = 0.2 kg m<sup>-2</sup>; C = 0.8 kg m<sup>-2</sup>; D = 2.4 kg m<sup>-2</sup>.



**Figure 16.** Dry weight:fresh weight ratio of white turnip (cv. 'Tokyo Cross') root over four biochar treatments and six replicated blocks. A = Control; B = 0.2 kg m<sup>-2</sup>; C = 0.8 kg m<sup>-2</sup>; D = 2.4 kg m<sup>-2</sup>.



**Figure 17.** Median root dry weight: fresh weight ratio of both white turnip cultivars over four biochar treatments. A = Control; B = 0.2 kg m<sup>-2</sup>; C = 0.8 kg m<sup>-2</sup>; D = 2.4 kg m<sup>-2</sup>. Columns which do not share a common letter are significantly different (Friedman's test, P < 0.05)

#### 5.4 Chlorophyll Test

Mean chlorophyll meter readings using the SPAD Chlorophyll Meter are given in table 6. They range from 37.65 (treatments A and D) to 38.87 (treatment B), a variation of 3.2% from the control, but there were no significant differences (Friedman's test).

**Table 4.** Mean SPAD Chlorophyll Meter readings on 15/09/14. A = Control; B = 0.2 kg m<sup>-2</sup>; C = 0.8 kg m<sup>-2</sup>; D = 2.4 kg m<sup>-2</sup>.

Treatment	A	B	C	D
Mean Reading	37.65	38.87	38.77	37.65

#### 5.5 Community Level Physiological Profile

Table 5 summarises the diversity indices derived from the community level physiological profiling test using Biolog plates. All treatments showed a significant

increase compared to the control (Kruskal Wallis,  $P < 0.05$ ) in all three indices. AWCD was highest in the low treatment at 1.30 but there were no further significant differences between the soils with biochar added.

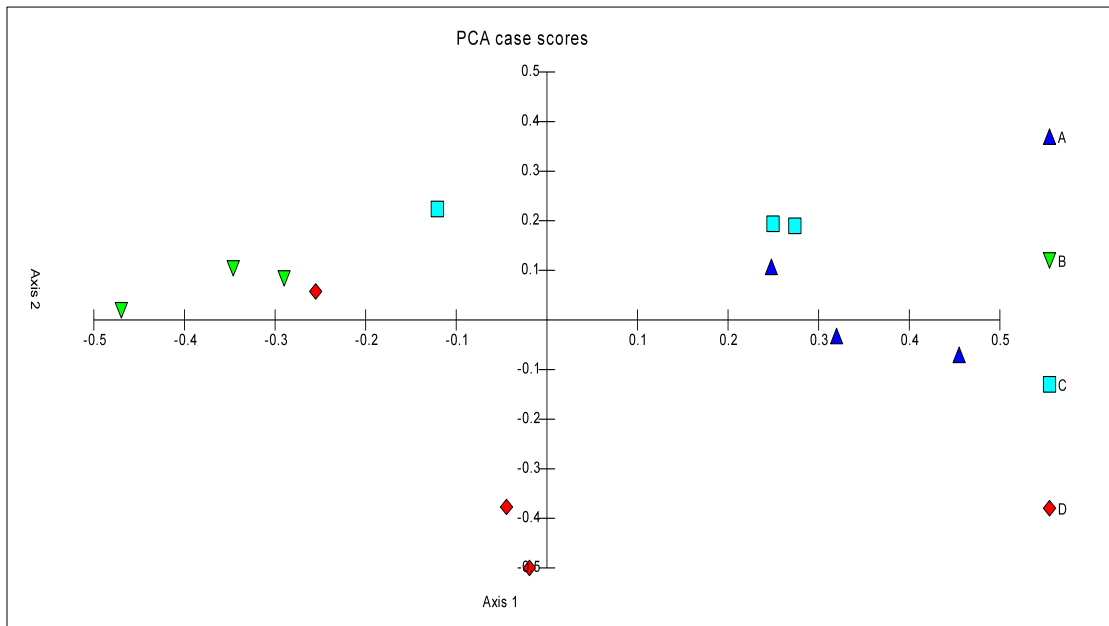
**Table 5.** Mean Microbial Diversity Indices from Biolog analysis. Low = 0.2 kg m<sup>-2</sup>; Medium = 0.8 kg m<sup>-2</sup>; High = 2.4 kg m<sup>-2</sup>.

<b>Biochar Treatment</b>	<b>AWCD</b>	<b>R (Species Richness)</b>	<b>H (Shannon Index)</b>
Control	0.80 a	25.67 a	3.17 a
Low	1.30 b	30.33 c	3.27 c
Medium	1.00 b	29.00 b	3.22 b
High	1.05 b	28.33 b	3.26 c

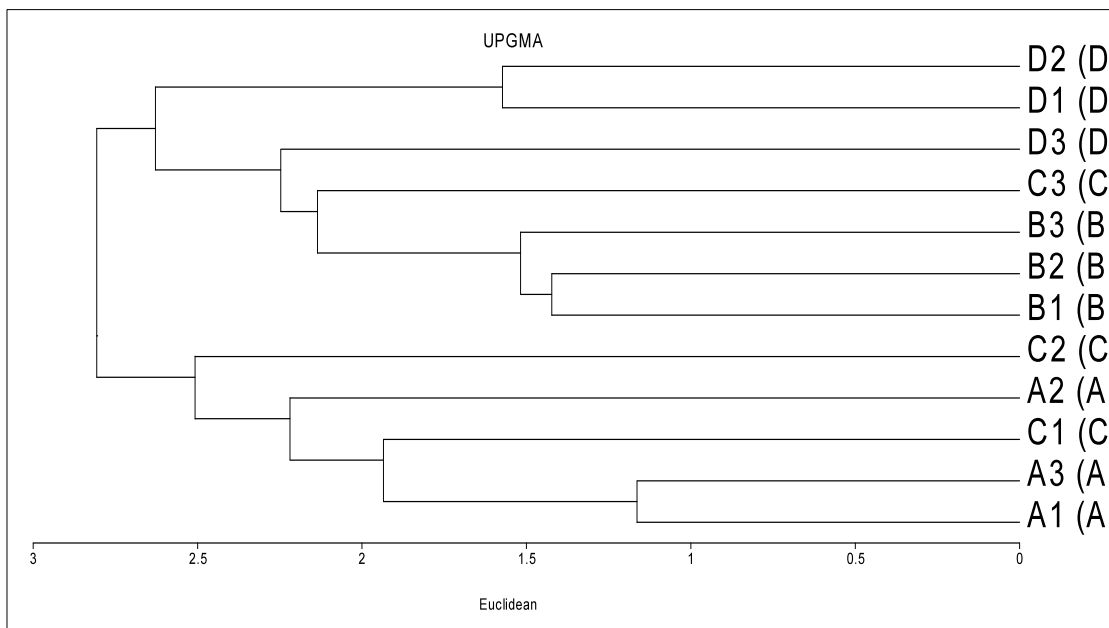
Values within a column which do not share a common letter are significantly different (Kruskal Wallis,  $P < 0.05$ )

Species richness (R) showed a similar trend – the low treatment had the highest well development, but in this case was significantly different from the other two biochar treatments, as well as the control. In two of the low treatment replicates, 30 wells developed a colour registering above the minimum (0.25) in the light meter, while all 31 did this in the third one. All the soil samples produced the minimum well colour development in most wells, the lowest number being 25 in two of the control replicates. The low and high treatments had significantly higher Shannon index values than the medium treatment, and all were significantly higher than the control.

Principal component analysis (fig. 18) showed clear variation between treatments. The first two principal components accounted for 52.2% of variability of the data. The cluster analysis, represented by a dendrogram (fig. 19), showed a similar pattern. Two groups, one consisting of treatments B and D and the other of A and C, emerged in both visual representations. The first node in the dendrogram (fig. 19) separates the B and D treatments from all but one of the C treatments and all of the control (A) treatments. Similarly, one of the C treatments in the PCA diagram (fig. 18) is on the same side of the y-axis as all the B and D treatments, and on the opposite side to the rest of the A and C treatments.



**Figure 18.** Scatter plot of the four treatments according to the first and second principal components. A = Control; B = 0.2 kg m<sup>-2</sup>; C = 0.8 kg m<sup>-2</sup>; D = 2.4 kg m<sup>-2</sup>.



**Figure 19.** Dendrogram following cluster analysis of biolog plates. A = Control; B = 0.2 kg m<sup>-2</sup>; C = 0.8 kg m<sup>-2</sup>; D = 2.4 kg m<sup>-2</sup>.

## 6 – Discussion

### 6.1 Soil Characteristics

The rate at which water moves through soil is governed by several factors, at least one of which must have been affected by biochar application since it took twice as long for water to move through soil with the highest application rate compared to soil with no biochar added. Root growth can increase the infiltration rate, and if this contributed to the variation between treatments it could be hypothesised that biochar had a negative effect on root development. The soil samples were taken from the spaces between plants however, and considering the narrow rooting structure of white turnip it is unlikely that this was a cause. Therefore a direct effect of biochar on water infiltration is more likely to be responsible. Change to soil structure is an unlikely cause since any structure was lost during preparation for the soil tests.

The change to infiltration time may be related to the water holding capacity, which increased in the biochar treated plots. This is in line with several studies from temperate regions. Basso *et al.* (2013) reported a 23% increase in water holding capacity in a sandy loam in Iowa, USA while Karhu *et al.* (2011) found an 11% increase in an agricultural soil in Finland. In the present study, the highest biochar application rate also caused an 11% increase. This change may have been caused directly by the porosity of biochar. It follows that since the water had more pore spaces to infiltrate, it took longer for the first drop of water to appear in the infiltration test. If the enhanced WHC results in more plant available water, these results suggest a potential application of biochar as a buffer against drought.

The lack of change in pH was an unexpected result considering the wide occurrence of increases in alkalinity following biochar application. The difference between the pH value for the highest treatment and the control was just 0.03, meaning the pH was essentially unaffected. The decrease of 0.2 in the lowest treatment is also unremarkable. Kloss *et al.* (2014, see table 1), investigating biochar effects on three different soils, found that pH increased in the acidic soil from 5.4 to 6.1. The more alkaline soils (with pH values of 6.6 and 7.2) did not undergo changes to pH. The biochar used in this study had a HTT of 650°C and hence would be expected to have a higher pH than that used by Kloss *et al.* (2014). Streubel *et al.* (2011) observed

increases in pH in 5 different soils with biochars from 4 different feedstocks. Such results suggest that the liming effect of biochar is very robust, which is in contrast to the unclear results from this study. However, it is not unprecedented for soil pH to remain unchanged after biochar application – similar results were reported by Güereña *et al.* (2013).

Conductivity, which can be used as a surrogate measure for CEC, appeared to decrease compared to the control. This is a noteworthy finding, because the effect of biochar on CEC in temperate soils is not yet clear (Lehmann, 2007a). Biochar is often credited with the ability to reduce leaching and this strongly related to CEC (Rajkovich, 2012). At the beginning of this study, one of the expected effects of biochar was to reduce leaching but these results, though tentative, suggest that this biochar did not increase the soils ability to retain nutrients and therefore would not reduce leaching.

## **6.2 Seed Compost Amendment Trial**

The method used to test germination success may have benefitted from more in depth recording of data. Daily records in the week following sowing might have revealed variation in germination time and early seedling growth. Rice (2014) reported faster germination and a higher success of germination in biochar amended seeding compost. The high quality of the seed compost used in the present study may be difficult to improve upon and hence any benefit from biochar could be redundant in this particular medium. This would explain the failure to replicate the findings by Rice (2014). As stated in the introduction, other studies have observed no effect of biochar on germination and early seedling growth (Bargmann *et al.*, 2013, Free *et al.*, 2010), similarly to this study.

The variation in the fresh biomass of the ‘Tokyo Cross’ seedlings may have been responsible for the lack of any observed effect of biochar. Perhaps if more uniformity had been achieved in the growing process, a clear effect would have been observed. Despite the lack of a biologically significant effect, the trend of increasing dry matter (DM) with higher application rates of biochar (fig. 6) is noteworthy. Chan *et al.* (2007) found that DM of radish increased with biochar application in a poor soil when

fertiliser was also added, but not when fertiliser was absent. The authors attributed this effect to better plant root development due to biochar effects on the soil quality – specifically reducing the tensile strength and increasing the field capacity water content (similar to the WHC). This root development was credited with facilitating increased N use, and hence causing a higher DM content. In this study however, seedling growth took place in mixtures of seeding compost and biochar, which were low in nutrients compared to the soil in the field trial.

The trend noted in the seedlings was not repeated in the outdoor trial. The difference noted in the outdoor trial was between two of the biochar treatments rather than between a biochar treatment and the control. This finding may suggest a negative effect of biochar on plant growth since the lowest treatment, which contained a very small amount of biochar ( $0.5 \text{ kg m}^{-2}$ ) had a greater dry: fresh weight ratio than a treatment with 10 times as much biochar. However, since a similar contrast was not seen between this low treatment and the highest treatment, a negative effect of biochar is unlikely.

The main aim of this part of the study was to examine growth of seedlings outdoors after germination with biochar, specifically whether enhanced seedling growth translated to better growth after planting outdoors. In that regard, the present study can be considered at best a partial success, since there were no biologically significant effects on seedlings. While differences at the  $P < 0.1$  level are noteworthy, they cannot be used as a basis for making any strong claims.

Plant dry matter levels are an indicator of nutrient density – plants with higher dry matter concentrations can be expected to contain more nutrients and this is important for human nutrition. Organically grown food appears to contain more dry matter and hence may have more health benefits than conventionally grown food (Lairon, 2009, Brandt *et al.*, 2011). It is not clear whether increased dry matter leads to an actual increased intake of nutrients and vitamins in humans (Brandt *et al.*, 2011).



### 6.3 Soil Amendment Trial

The overall lack of variation in plant growth between treatments across several parameters was not particularly unexpected. The effects of biochar application to fertile soils in temperate regions are as yet unclear and variable between studies. The one significant difference observed (fig. 17) was between the low and medium treatments, with the high treatment and the control falling roughly halfway between them. This pattern is very difficult to explain, since it appears to be quite independent of biochar application.

The variation in biomass within the replicated blocks may be responsible for the lack of significant effects or trends. Due to logistical constraints the seedlings were planted later than originally planned, by which stage they had begun to show substantial variation in height, possibly due to competition for light. Therefore the seedlings were already of variable size when they were planted. This could have caused a considerable obscuring of any differences that may have existed between the treatments.

Several field trials conducted in temperate regions and on fertile soils have found no effect of biochar on crop growth (Table 1). Jones *et al.* (2012) found no effect on maize growth in a Welsh study while Borchard *et al.* (2014) found the same in Germany, but suggested that this may have been due to the rooting structure of the plant. Out of seven trials in Scotland and England examined by Hammond *et al.* (2013), three showed no significant effect. Tammeorg *et al.* (2014) found no effect on wheat, turnip rape and faba bean crops in Finland. Karer *et al.* (2013) observed negative effects without fertiliser and no effect with fertiliser in crops of maize wheat and sunflower. Gathorne-Hardy *et al.* (2009) found no effect on spring barley when no fertiliser was added to the soil, but a 30% increase when fertiliser and biochar were added in combination. Based on the latter trial, it is possible that addition of fertiliser in this study could have brought about a yield increase in the biochar plots. Most of the studies in Table 1 used N fertiliser of various types, either in all plots or as a second treatment in order to study the combined effects of biochar and fertiliser. Due to the richness of the soil in this study, it was decided against using fertiliser. The finding by Schultz *et al.* (2013) that biochar had a stronger positive effect on oat crop growth in poor sandy soil than in a rich loam soil also points to a possible reason for

the lack of an effect in this study. The soil may be of such high fertility that it is difficult to make any substantial improvements.

Summer rainfall was below average (Met Éireann, 2014) in the study area and after the first week of planting out, the trial plots were not watered. However, based on regular visual inspection of the plots, the plants did not suffer from drought stress to any large degree. Hence the potential effect of biochar on WHC was not tested. Karer *et al.* (2013) found that biochar caused an increase in spring barley growth in a drought year, and this may be a potential use of biochar.

#### **6.4 Community Level Physiological Profile**

AWCD is seen as a representation of the inoculum density in the test soil and hence an indicator of microbial biomass. The Biolog test results from this study imply that biochar increased the soil microbial biomass. However, bearing in mind that there are far more reliable tests for microbial biomass, these results are tentative at best. Garland (1997) notes that a higher density of microbes can cause differences in the other two indices, even if the same species are present. This can be seen from the calculation used to determine species richness (R), which counts the number of wells scoring above 0.25. Two samples with the same species richness but different inoculum densities could cause different numbers of wells to develop a colour score above 0.25. These two indices must therefore be treated with a certain amount of caution. Despite these reservations, the indication from the species richness (R) and Shannon indices is that the number of species increased in the biochar plots. Importantly, the Shannon Index suggests that the relative abundances of species are fairly equal. Increased microbial diversity in the soil has the potential to enhance nutrient transformations and hence improve plant nutrition (Drinkwater and Snapp, 2007, Brady and Weil, 2010)

CLPP is reliable in showing whether there has been a change to a microbial community but it is limited in describing what changes have occurred (Garland, 1997). The PCA and cluster analysis results are quite clear in showing differences between the treatments and consistent in showing which treatments differ the most – they both agree the treatments are divided into two groups, A and C in one and B and

D in the other. The Shannon Index (table 5) has a similar division, with B and D significantly different from A and C. However, the grouping together of the control soil with the medium biochar treatment does not allow a simple interpretation such as a linear relationship between biochar application rate and microbial community structure. The biodiversity indices do not appear to correlate with the PCA and cluster analysis but, interestingly, the grouping together of treatment A with C and B with D in the latter matches the very slight trend seen in the plant biomass (figs. 12 and 14) and the one significant finding of differences in dry matter concentration between treatments (fig. 17).

Though microbial activity is linked with nutrient availability and hence plant growth (Drinkwater and Snapp, 2007), biochar application is unlikely to be responsible for this grouping. In the studies from the literature review (see table 2), significant biochar effects had simple patterns unlike the one found here – the studied parameter either increased, decreased or increased and then decreased in response to increasing rates of biochar application (Jones *et al.*, 2012, Quilliam *et al.*, 2012, Karer *et al.*, 2013, Vaccari *et al.*, 2011, Gathorne-Hardy *et al.*, 2009). Attempts to link similar patterns within the study must be treated with caution since there is a risk of reporting trends that do not exist. The grouping discussed here ignores the majority of plant growth results obtained in the present study, which did not show a pattern similar to the Biolog results.

Anders *et al.* (2013) noted changes to microbial communities in three agricultural soils in Austria. Altered nutrient availability and pH were thought to be largely responsible. Nutrient enrichment was linked strongly with the changes in the two less fertile soils. As stated in the introduction, several researchers have found changes to microbial communities with biochar application, including in temperate soils, with the causes varying among studies. Kolb *et al.* (2009) attributed increases in microbial biomass to increased mineralization, and Watzinger *et al.* (2014) made a similar assertion, noting that pH may also have contributed. In the present study, the soil tests showed at least slight changes in all parameters and therefore none can be linked directly to the changes seen in the CLPP.

## 7 – Conclusions

The lack of any clear effect, either positive or negative, is an important point. As discussed in the introduction, several aspects of the interaction between biochar and soil have to be carefully examined before it can be considered a viable technology. One of these is the effect on crop growth in fertile temperate soils. From this study, soil amendment with biochar can be recommended as a method of carbon sequestration with no negative consequences on plant yield or soil qualities. While these results do not add to the case for biochar as a commercial proposition, it must be recognized that this is one amongst many similar studies, several of which have found positive effects.

As well as causing changes to soil hydrology and the soil microbial community, there is weak evidence for an increase in the dry matter concentration of seedlings and mature plants. These all suggest that there is potential for biochar to become a useful horticultural material. Based on this, a number of recommendations can be made for future studies. Tests on germination and early seedling growth could involve different seed composts and more detailed measurement, specifically keeping a daily record of growth in the first few weeks after sowing. The lack of clarity in these results may have been largely due to heterogeneity in plant size when transplanting took place. This could be avoided with better planning of planting dates.

It is hoped that the biochar plots will remain available for future studies. This is important because, as discussed above, there are very few long-term trials in temperate climates. The short space of time between application to the soil and planting that occurred in this study will not be an issue. An examination of the soil nutrients would be extremely pertinent as the prevention of leaching is a widely touted quality of biochar. Future studies could also investigate combinations of biochar with organic fertiliser since other studies have found no effect of biochar amendment on its own but positive effects when combined with fertiliser.

## Acknowledgements

The author wishes to thank Prof. Peter Jones, Mr. Eoin Lettice, Mr. Klaus Laitenberger and Ms. Mairead Kiely for advice and assistance with all aspects of the project.

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