



School of  
**Biological, Earth and  
Environmental Sciences**

# **Biochar as a Plant Growth Substrate Amendment**

**Thesis presented by:**

Eric Hynes, MRes

116728981

for the degree of

**Master of Research**

School of Biological, Earth and Environmental Sciences,  
University College Cork, 2021.

**Head of School/Department:**

Prof. Astrid Wingler

**Supervisors:**

Dr. Eoin Lettice and Dr. Barbara Doyle Prestwich

<b>2. <u>ABSTRACT</u></b>	<b>5</b>
<b>3. <u>AN INTRODUCTION AND BACKGROUND TO BIOCHAR</u></b>	<b>6</b>
3.1 Global Pressures for Alternative Growth Media	6
3.2 Definition, Production and Interest of Biochar	6
3.3 Biochar as a Substrate Amendment: an Ancient Agricultural Tool	8
3.4 The Microscopic Structure and Functionality of Biochar	10
3.5 The Environmental Need To Replace Peat in Horticulture	11
3.6 Abiotic and Biotic Stress Suppression	12
<b>4. <u>MATERIALS AND METHODS WITH RESULTS</u></b>	<b>14</b>
4.1 Overall Experimental Aims	14
4.1.1 Data Observations and Analyses	14
4.2 Biochar Source, Pyrolysis and PF Compost	14
4.3 Treatments	15
4.4 Glasshouse Experiment Watering and Harvesting	16
4.4.1 Watering	16
4.4.2 Plant Fresh Weights and Dry Weights	16
4.5 Treatment Preparation	16
4.6 Glasshouse Experiment #1: Testing Plant Growth Response of PRG and OSR using Treatment 1	17
4.6.1 Hypotheses	17
4.6.2 Experimental Design	18
4.6.3 Glasshouse Experiment #1 Results	18
4.6.3.1 OSR Plant Heights	19
4.6.3.2 OSR Fresh Weights and Dry Weights	19
4.6.3.3 PRG Plant Heights	20
4.6.3.4 PRG Fresh Weights and Dry Weights	20
4.7 Glasshouse Experiment #2: Testing Growth Response of PRG and OSR to Treatment 2	21
4.7.1 Hypotheses	21
4.7.2 Experimental Design	21
4.7.3 Glasshouse Experiment #2 Results	21
4.7.3.1 OSR Plant Heights	21

4.7.3.2 OSR Fresh Weights and Dry Weights	
22	
4.7.3.3 PRG Plant Heights	23
4.7.3.4 PRG Fresh Weights and Dry Weights	
24	
4.8 Glasshouse Experiment #3: Testing Plant ‘Growth Response’ of PRG to Treatment 3 and 4 with Abiotic Stress Exposure	24
4.8.1 Preparing Charged BC	25
4.8.2 Hypotheses	26
4.8.3 Experimental Design	26
4.8.4 Inducing Plant Stress via Abiotic Stress Exposure	27
4.8.4.1 Salt Stress	27
4.8.4.2 Drought Stress	27
4.8.4.3 No Stress	27
4.8.5 Glasshouse Experiment #3 Results	27
4.8.5.1 PGR Plant Heights	27
4.8.5.2 PGR Fresh Weights	28
4.8.5.3 PGR Dry Weights	29
4.9 Glasshouse Experiment #4: Testing Plant Growth Response of OSR to Treatment 3 and 4 with Abiotic Stressor Exposure	30
4.9.1 Hypotheses	30
4.9.2 Glasshouse Experiment #4 Results	30
4.9.2.1 OSR Plant Heights	30
4.9.2.2 OSR Fresh Weights	31
4.9.2.3 OSR Dry Weights	32
4.10 Glasshouse Experiment #5: Testing Plant Health/Stress Response of OSR to Treatment 3 and 4 with Abiotic Stressor Exposure	33
4.10.1 Methodology of Acquiring Data on Plant Stress	33
4.10.2 Hypotheses	34
4.10.3 Glasshouse Experiment #5 Results	34
4.11 SEM Microscopy Experiment #1: The Embedding of Rush BC and Oak BC Samples in Resin for Structural and Mapping Analyse	36
4.11.1 Methodology of Preparation of BC Samples for Analysis	36
4.11.2 Preparing BC Samples and ‘O-Rings’	36
4.11.3 Methodology of the Preparation of Epoxy Resin and O-Rings	37

4.11.4 Hypotheses	38
4.11.5 Microscopy Experiment #1.1 Results: Determining the Mean Pore Surface Area of Each Biochar	38
4.11.6 Microscopy Experiment #1.2 Results: Determining the Mean Pore Frequency per Biochar Surface Area	39
4.11.7 SEM Images of Oak BC	39
4.11.8 SEM Images of Rush BC	41
4.12 SEM Microscopy Experiment #2: The Preparing of Oak BC and Rush BC Samples for Qualitative EDS Elemental Analyses	43
4.12.1 Hypothesis	43
4.12.2 Methodology of Preparing BC Samples on SEM Stubs	43
4.12.3 Microscopy Experiment #3 Results: Qualitative EDS Elemental Analyses of Oak BC	44
4.12.4 Microscopy Experiment #4 Results: Qualitative EDS Elemental Analyses of Rush BC	47
<b>5. <u>DISCUSSION</u></b>	<b>51</b>
5.1 OSR Plant Growth Response to Treatments 1 and 2	51
5.1.1 Response to Treatment 1	51
5.1.2 Response to Treatment 2	51
5.2 PRG Plant Growth Response to Treatments 1 and 2	52
5.2.1 Response to Treatment 1	52
5.2.2 Response to Treatment 2	52
5.3 OSR Plant Growth Response to Treatments 3 and 4 with Abiotic Stress Exposure	53
5.3.1 OSR Response to Treatments 3 and 4 with Abiotic Stress Exposure	53
5.4 PRG Plant Growth Response to Treatments 3 and 4 with Abiotic Stress Exposure	55
5.4.1 PRG Response to Treatment 3 and 4 with Abiotic Stress Exposure	55
5.5 OSR Stress Response to Treatments 3 and 4 with Abiotic Stress Exposure	57
5.6 Quantitative Analyses of Both BCs Using Scanning Electron Microscope	58
5.7 Qualitative Analyses of Both BCs Using Scanning Electron Microscope	59
<b>6. <u>CONCLUSIONS AND RECOMMENDATIONS</u></b>	<b>60</b>
<b>7. <u>ACKNOWLEDGEMENTS</u></b>	<b>61</b>
<b>8. <u>REFERENCES</u></b>	<b>61</b>

## **2. ABSTRACT**

Biochar (BC) is an alternative growth media, a potential soil health ameliorant and climate change mitigation strategy. The effects of 2 BC and peat-free compost treatments (treatments 1 and 2) were tested on plant growth of perennial ryegrass (*Lolium perenne L.*) and oilseed rape (*Brassica napus*) in potted glasshouse trials. Overall, plant height and yield of *L. perenne* and *B. napus* were not affected by treatment 1. Treatment 2 had no effect on plant height and yield of *B. napus*, but did have significant positive height and yield effects on *L. perenne*. Treatment 1 and 2 were ‘charged’ over 6-months of outdoor incubation and re-tested (as treatments 3 & 4) on the same plants concurrently in the presence of abiotic stressors. The effects of both charged treatments and stressor combinations on both plants were shown to be individually statistically different with varying results between group interactions. The control treatment had the most positive effect on plant height and yield for both plant species. SPAD measurements on *B. napus* plant health showed that treatment 3 was most effective at alleviating abiotic stress. The structural and elemental components of 2 biochars were analysed using SEM. The vastly porous nature of a hardwood BC and its elemental constituents compared to a grass BC was confirmed, strengthening other findings in the scientific literature.

### **3. AN INTRODUCTION AND BACKGROUND TO BIOCHAR**

#### **3.1 Global Pressures for Alternative Growth Media**

Accelerated human population growth in the 20th and 21st centuries has put relentless pressure on the most diverse and complex ecosystem in the world - the soil (DeLong et al., 2015). Monocropping and other unsustainable, intensive agricultural practices such as excessive fertilizer and pesticide applications are some of the main anthropogenic causes of soil nutrient depletion and degradation (Nearing et al., 2017). Global soil degradation endangers soil as an immeasurably important ecosystem services, from carbon sequestration and storage (CSS) to global food security (DeLong et al., 2015). With estimates for the global population set to reach over 9 billion people by 2050, there has never been a greater need for research into and implementation of sustainable agricultural practices to improve and maintain soil health (Tahat et al., 2020).

#### **3.2 Definition, Production and Interest of Biochar**

Biochar (BC) is a carbon rich and vastly porous substance, generated as a co-product of pyrolysis (see *fig.1*), along with syngas and bio-oils (Basu, 2018). The substance is principally used as an environmental and agronomic tool for soil amendment (Huber et al., 2006) as well as for a wide range of other potentially sustainable industry uses. Uses like wastewater treatment (Xiang et al., 2020), immobilization of soil contaminants (Jeffrey et al., 2015), thermal and mechanical insulation applications (Lazzari et al., 2019), adsorption of toxic chemicals in functional clothing (Hanoğlu et al., 2019), energy storage via BC electrodes in supercapacitors (Caguiat et al., 2018), biodegradable food-packaging (Diaz et al., 2020), media in air pollution control systems (Gwenzi et al., 2020) or as an animal health food supplement which subsequently produces an enriched BC manure after excretion (Schmidt et al., 2019) to name a few. The pyrolytic production of biochar from biomass and its various industrial uses can be observed in Figure 1.

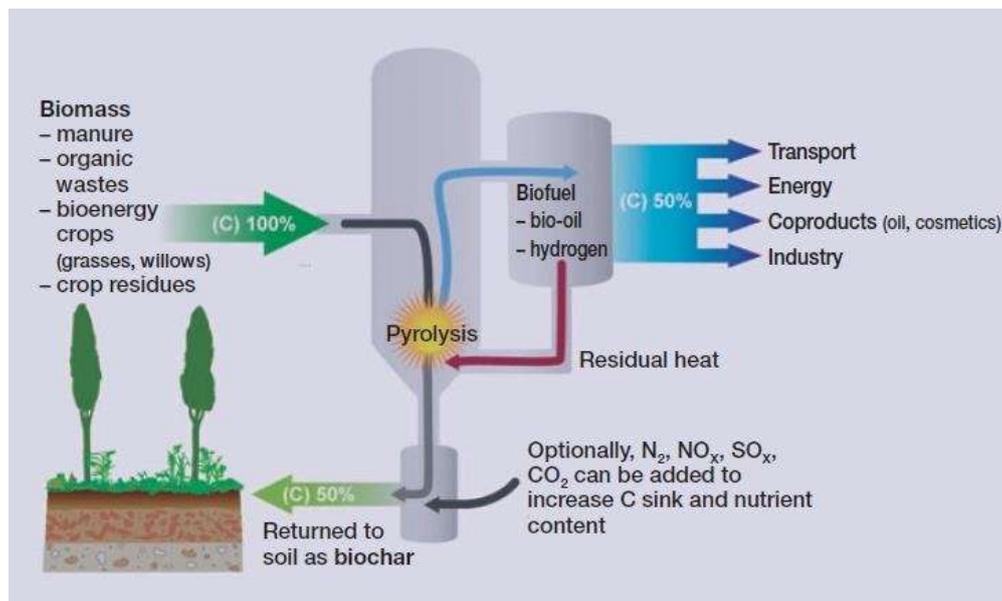


Figure 1: A diagram showing the production of BC (and other by-products) via pyrolysis and their potential uses. Source: Lehmann, 2007.

Global interest in BC has increased due to its carbon capture and sequestration (CCS) capabilities (Lee et al., 2017). Climate change is recognised as a global problem (Hanna et al., 2021) bringing with it increased instances of extreme weather with increasing intensity (Droste et al., 2020). BC becomes carbon negative when applied to soils and thus represents a potential climate change mitigation strategy for greenhouse gas (GHG) emission reduction (Sagrilo et al., 2015).

By 2050, the global population is estimated to be about 9 billion, thus increasing global pressure on energy and food production (Strange, 2015). Of these, 300 million will be living on floodplains due to global sea level rise (Kulp and Strauss, 2019). BC can help mitigate greenhouse gas (GHG) emissions by reducing GHGs emitted from agricultural soils (Case et al., 2014). BC made from sustainable feedstocks could reduce the net emissions of CO<sub>2</sub>, N<sub>2</sub>O and CH<sub>4</sub> by 1.8 Gt CO<sub>2</sub> eq at maximum, equalling 12% of total CO<sub>2</sub> emissions annually (Woolf et al., 2010). Gu and Bergmann (2015) estimated that syngas, used for energy production had a much lower global warming potential of 0.142 kg CO<sub>2</sub>-eq /kWh, as opposed to natural gas (0.72 kg CO<sub>2</sub>-eq /kWh) and coal (1.08 kg CO<sub>2</sub>-eq /kWh).

BC's capacity to sequester carbon is a process involving the deceleration of the carbon cycle (Harvey et al., 2012). Once atmospheric carbon is fixed as plant biomass through photosynthesis and later pyrolysed into BC, it produces a more chemically and structurally robust material with a greater capacity to resist microbial degradation (Thomazini et al., 2015). Information in the literature regarding carbon emissions during pyrolysis is scarce and what data can be found is very limited. This highlights the need for more research into BC production for sustainability purposes.

Upon soil application, BC's physical occlusion from saprophytic soil microbes allow these microbes to interact with soil clay mineral deposits (Lehmann et al., 2015). This has been hypothesized to also promote the recalcitrant nature of BC in soils (Lehmann et al., 2015). Thus, the persistent BC can last from hundreds to thousands of years in soil (Kuzyakov et al., 2014). However, the timescale of BC humification and degradation within a soil depends on the heterogeneous nature of the BC (Ippolito et al., 2020; Hilscher and Knicker, 2011). This is determined by the influence of the types of feedstock, temperature and duration of pyrolysis of a BCs characteristic (Ippolito et al., 2020; Hilscher and Knicker, 2011). Ippolito et al (2020) extensive meta-analysis showed that pyrolysis temperatures greater than 500 °C together with hard-wood as the feedstock, led to BC with half-lives greater than 1000 years, thus improving C sequestration and storage.

### **3.3 Biochar as a Substrate Amendment: an Ancient Agricultural Tool**

BC was first described in 1966 in the literature as “terra preta” (Sombroek, 1966) or “Amazonian dark earth” within ancient central Amazonian soils, with evidence of its use as far back as 6000 B.C. (Glaser et al., 2001). Terra preta appears to have been formed by Amerindian cultures who practiced agricultural methods of burning crop residues and other biomasses which lead to a charring of the soils (Mann, 2002). Since then, there have been many studies (Gwenzi et al., 2020; Lee et al., 2017; Stavi and Lal., 2013; Huber et al., 2006) into the potential benefits and uses for such an archaic agricultural method with much greater amounts of research yet to be done.

BC also suppresses other GHGs including N<sub>2</sub>O (Laird, 2008). A potential mechanism for this is the absorption of nitrate and ammonia to or by BC within the soil, thus suppressing nitrate leaching and N<sub>2</sub>O emissions (Thomazini et al., 2015). Using a 15 N gas flux method Cayuela et al (2013) observed the influence of BC on denitrification, which decreased N<sub>2</sub>O emissions from 10 to 90% between 14 individual agricultural soils. Other research has also shown an increase in genes associated with denitrification and N-fixation in the rhizosphere by soil microbial communities (Ducey et al., 2013).

In a study by Thomazini et al (2015), soil CO<sub>2</sub> and N<sub>2</sub>O levels emitted to the atmosphere were found to be impacted significantly (P = 0.04 & P = .03, respectively) by BC application. Furthermore, the study found that BC addition suppressed N<sub>2</sub>O emissions by an average of 63 % across all soils.

Estimates show that BC, as a negative emissions technology (NET), applied at a rate of 50 t ha yr<sup>-1</sup> over 14 Mha could potentially remove 0.7 GtC eq. yr<sup>-1</sup> (Smith, 2016; Genesio et al., 2012). Applied at the same rate over 26 Mha, BC could remove 1.3 GtC eq. yr<sup>-1</sup> (Smith, 2016; Genesio et al., 2012) with projected figures for C sequestration between 0.5-2 GtC eq. yr<sup>-1</sup> by 2050 (Fuss et al., 2018; Smith, 2016). Furthermore, Smith (2016) demonstrated BC's added advantages of lowering impacts on water use, land, nutrients, energy needs and costs

as well as surface albedo compared to other NETs afforestation/reforestation or bioenergy production with carbon capture and storage (BECCS).

In comparison to BC the average individual mature tree removes 22 kgC eq. yr<sup>-1</sup> (Schröder et al., 2013). Also contrasted is the projections for allowing the regrowth of global forests potentially sequestering 8.9 GtC eq. yr<sup>-1</sup> up to 2050, while sustaining global food production and grassland ecosystems (Cook-Patton et al., 2020). This, together with C sequestration delivered by present global forests would remove 53% of atmospheric carbon per annum.

The application of BC as a soil amendment tool was shown to have a beneficial effect on soil fertility and crop productivity (Stavi and Lal., 2013). However, the ability of BC to ameliorate soil health and plant productivity rests on the performance of such variables, together with the climate of a region. Areas with degraded soils with low relative pH, nutrient, soil organic matter (SOM), cation exchange capacity (CEC), water retention etc. levels tend to experience the best of yield responses (Dokoochaki et al., 2019). In 2017, Jeffery et al. conducted a meta-analysis of 109 papers which demonstrated significant yield response variations depending on geographical positioning.

Ruyschaert et al (2016) facilitated a North Sea ring trial between 7 different countries (UK, The Netherlands, Belgium, Germany, Denmark, Sweden and Norway) all with varying soil types and soil parameters. It was found that BC applications increased the soil organic content (SOC) at all field sites which had low initial SOC (<3%). BC applications were found to increase pH across all field sites but only the Norway site, which had an initially low pH was found to be significant. There were significant grain yield increases in the Sweden and UK sites, while the Norway site also experienced a significant straw yield increase.

A quantitative review by Biederman and Harpole (2013) performed a different meta-analysis from 114 sources containing 371 studies, providing the first review of BC effects on multiple ecosystem functions and responses. On average, the incorporation of BC to soils transpired to increase crop yields (above and belowground), productivity above ground and plant potassium tissue concentration (Biederman and Harpole, 2013). Within this meta-analysis was a study from Asai et al. (2009) where both an improved rice cultivar (*Oryza sativa* cv. Apo) and a traditional rice variety (*Oryza sativa* var. Vieng) on average experienced increased leaf chlorophyll content (SPAD) and grain yields compared to the controls (SPAD 38.9 to 38.7 and yield 4.7 to 4.5, respectively).

Another study by Hossain et al. (2010) demonstrated that cherry tomato (*Lycopersicon esculentum*) yields increased by 64% when BC was applied to chromosol soil compared to the control. Overall, soil productivity increased, compared to the controls in rhizobia nodulation, soil microbial biomass, and soil nutrients (K, P, N & C) as well as elevations in soil pH levels, making soils less acidic (Biederman and Harpole, 2013). In Colombia, an unproductive savanna oxisol experienced increasing yields (from 28 to 140%) of maize (*Zea*

*mays*) in the second, third and fourth years after the addition of a solitary application of 20t ha<sup>-1</sup> of BC (Major et al., 2010).

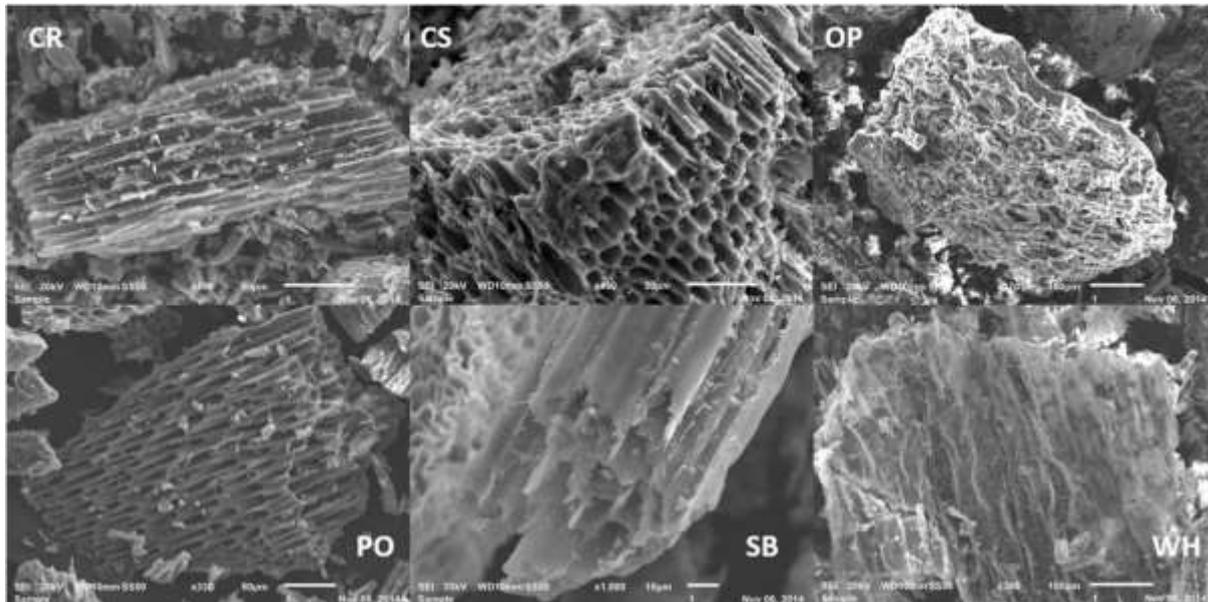
However, not all crop responses to BC applications are positive. In a study by Jay et al (2015), short term BC applications failed to increase various growth parameters and yields of strawberry (*Fragaria ananassa*) fruit, spring barley (*Hordeum vulgare*) grain/ear counts and potato (*Solanum tuberosum*) tubers, even with the presence of fertilizers. In 2020, Melaku et al. showed that similar short-term BC applications required increased fertilizer inputs to achieve the same crop yield potential as the control.

As a soil amendment tool, BC can directly benefit arable soils most in need of amelioration through improving soil density, texture, structure, porosity and particle size distribution (Fox et al., 2014). These improvements can lead to an increased cation exchange capacity (CEC) and together can affect a soil's ability to retain essential plant nutrients for assimilation and water retention within the soil thus influencing various plant growth effects and health (Hagner et al., 2016; Hazelton and Murphy., 2016; McKenzie et al., 2004). In Germany, Haider et al. (2020) demonstrated improved maize crop (*Zea mays*) growth in unproductive sandy soils through BC application, leading to better water holding capacity and nutrient retention as a result.

As outlined earlier, plant growth improvements through BC application are obtained via the amelioration of soil health, as well as soil productivity and function. These advantages consist of increased crop yield, productivity, priming of plant defences against biotic and abiotic stresses and more (Wang et al., 2019; Elad et al., 2011). In 2015, Jeffrey et al. demonstrated how BC applications increased crops yields of soybean (*Glycine max L. Merr.*), maize (*Zea mays L.*), wheat (*Triticum aestivum L.*), and rice (*Oryza sativa L.*) by 22, 19, 17 and 16 % respectively.

### **3.4 The Microscopic Structure and Functionality of BC**

The microscopic structure of a BC and its characteristic, beneficial carbonous pores and their frequency are influenced by the types of feedstock used, as well duration and temperature at which they undergo pyrolysis (Żukiewicz-Sobczak et al., 2020; Narthey and Zhao, 2014 ;Hilscher and Knicker, 2011). It is the surface area and charge of these pores that are the physical and chemical influencers in a BC's adsorption functionality (Narthey and Zhao, 2014). Adsorption functionality affects the water/nutrient retention abilities of a BC, while creating a prosperous niche for soil microbial communities (SMCs) to inhabit (Azeem et al., 2020). The links between various BC structures, characteristics and functionality have been examined and compared using physicochemical analyses (structural and elemental) by scanning electron microscope (SEM) to gain greater insights into the mechanics of a BC's functionality (Yang et al., 2021; Batista et al., 2018; Dong et al., 2017).



*Figure 2: A collection of scanning electron micrographs of various BCs made from 6 different feedstocks. Exhibiting their variations in structural differences. CR = charcoal fines, CS = coconut shell, OP = orange peel, PO = Palm oil bunch, SB = sugarcane bagasse, WH = water hyacinth. Source: Batista et al., 2018.*

### **3.5 The Environmental Need To Replace Peat in Horticulture**

Ireland's raised bogs are some of the oldest ecosystems on the planet (Renou-Wilson, 2018). However, habitat destruction under unsustainable peat mining for horticultural co-composting and electricity generation in the 20th century (Mackin et al., 2017) has removed 84% of these active raised bogs (ARBs) (Renou-Wilson et al., 2019). These habitat losses prompted the EU to safeguard active raised bogs under the EU Habitats Directive, requiring Ireland as an EU member state to implement conservation measures (Mackin et al., 2017). This was achieved through ARBs being designated and regularly monitored as special areas of conservation (SACs) (Schouten, 2002).

The need to protect environmental regulatory capabilities of ARBs (Evans et al., 2014), their benefits towards biodiversity and carbon sequestration (Wilson et al., 2013) have contributed to the phasing out of peat in the EU by 2024 (Renou-Wilson et al., 2019). Peat harvesting in Ireland has ceased since 2019, following a high court ruling. However, with a horticulture industry worth €477m and no economically viable peat alternative yet available, the peat-reliant sectors of mushroom, vegetable and ornamental cultivation and some 17,000 related jobs are at risk unless alternatives can be found (Hilliard, 2021; McCormack, 2021).

BC has been investigated as a potential alternative for peat within peat-based compost used in horticulture (Margenot et al., 2018). Like peat, BC is the carbonized biomass of plants (Steiner and Harttung., 2014) however, the main difference between them is the pyrolysis of biomass. Research shows the benefit of adding BC with peated composts, yet studies are

lacking into using BC as the replacement for peat in horticultural soil free mixes (Wang et al., 2019; Margenot et al., 2018; Steiner and Harttung., 2014).

### **3.6 Abiotic and biotic stress suppression**

BC applications suppress biotic stresses i.e. disease-causing plant pathogens in a number of ways. Firstly, by (i) promoting beneficial organisms which antagonise pathogens through nutrient competition or antibiotic production (Bonanomi et al., 2015). Beneficial organisms are attracted to the nutrients provided by the pore volume, surface area and negative surface charge of a BC through the mechanism of sorption (Chen et al., 2012).

By (ii) inhibiting soilborne plant pathogens via the presence of volatile organic compounds (VOCs) e.g. furan (C<sub>4</sub>H<sub>4</sub>O) in a BC which incite direct toxicity to the pathogens (Graber et al., 2010). Persistent free radicals (PFRs) e.g. semiquinones, phenoxylys, cyclopentadienyls and more, are also inhibitory to microbial pathogens by inducing oxidative stress (Zhu et al., 2017).

By (iii) inducing plant pathogenic resistance against such foliar disease-causing fungal pathogens as *Leveillula taurica* and *Botrytis cinerea* (Elad et al., 2010). This defence mechanism is regulated by the interactions in the rhizosphere between plant growth promoting rhizobacteria/fungi (PGPR/PGPF) and plant roots (Harel et al., 2012). Arbuscular mycorrhizal fungi (AMF) interactions in the rhizosphere also induce systemic resistance against pathogens like *Fusarium oxysporum* by upregulating plant antioxidant production of L-Ascorbic acid, reducing cellular glutathione through increased redox reactions (Begum et al., 2020; Akhter et al., 2015; Winkler et al., 1994).

By (iv) enhancing disease resistance due to improved nutrient assimilation e.g. increased defence against root rot (*Phytophthora sojae*) and others (Jaiswal et al., 2020). Increased nitrogen adsorption and assimilation increases plant nitrogen utilization efficiency, helping to promote root morphology, thus increasing plant resistance to soilborne pathogens (Feng et al., 2021). Nitrogen utilization is increased through upregulation of genes related to glutamate dehydrogenase expression (Zhang et al., 2020), improving root growth and strengthening plant resistance against pathogens in the rhizosphere such as *P. sojae* (Jaiswal et al., 2020).

By (v) disruption of intra and inter-specific communication molecules within microbial communities (Zhu et al., 2017). BC can disrupt molecules essential for quorum sensing within microbial communities (Gao et al., 2016) e.g. a signalling molecule of the acyl-homoserine lactone (AHL) group, N-3-oxo-dodecanoyl-L-homoserine lactone crucial for the regulation of gene expression in gram negative soil borne microbes, like *Pseudomonas aeruginosa* (Masiello et al., 2013). BC induced signal inhibition occurs via the sorption capacity of BC (Gao et al., 2016). However, a BC's feedstock and pyrolysis conditions greatly affect its sorption capacity - BC made from honey mesquite (*Prosopis glandulosa*) pyrolyzed at 700 °C exhibited ten times the AHL cell to cell signal inhibition than that of the same BC but pyrolyzed at 300 °C (Masiello et al., 2013).

By (vi) disrupting pathogenic mobility within the soil (Bonanomi et al., 2015; Lehmann et al., 2011). Through adsorption, BC can impede and deactivate the mobilization of toxic metabolites i.e. mycotoxins and cell wall degrading enzymes i.e. polygalacturonase from pathogens such as *Fusarium oxysporum* (Jaiswal et al., 2018). All of these mechanisms linked to BC induced pathogen resistance contribute to greater plant growth promotion (Wang et al., 2019; Lehmann et al., 2011; Elad et al., 2011).

Abiotic stresses are also suppressed by BC (Bonanomi et al., 2015). In a study by Meng et al (2019) BC was found to decrease fomesafen toxicity and fomesafen assimilation by wheat plants while concurrently increasing wheat plant performance, soil mycobiome and microbiome diversity and the promotion of beneficial plant-microbe interactions in the rhizosphere.

Zhang et al (2020) demonstrated that soybean crops experiencing the negative effects of salinity and drought stress could be alleviated by the application of BC. In a high temperature stress experiment using BC and P applications on two separate rice (*Oryza sativa*) cultivars, both were found to be more resistant to the heat stress.

Contamination of crops by heavy metals present in agricultural soils can be detrimental to plant and consumer health, due to the production of harmful reactive oxygen species like hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and hydroxyl radical (OH<sup>-</sup>) (Engwa et al., 2019; Mani and Sankaranarayanan., 2018). The sorption capacity of BC can bioremediate soils of various heavy metals and rates of pollution (Senthilkumar and Prasad, 2020). In an experiment by Shakya and Agarwal (2019), BC made from discarded pineapple peel and pyrolyzed at 350 °C yielded the greatest adsorptive result of a group of BCs, by bioremediating hexavalent chromium (Cr (VI)) from an aqueous solution at 41.7 mg/g. The polarity of the BC's surface area was found to be the influencing factor on the adsorption of Cr (VI).

Heavy metal contamination of agricultural land causes abiotic stress to the crop plant as well as has a drastic effect on humans. Increased metal concentration in plants leads to the production of reactive oxygen species which results in cell death and thus affects the crop production in plants.

In this present study, two BCs, one from oak (*Quercus sp.*) and other from rush (*Juncus effusus*) were mixed separately with a peat-free (PF) compost. These treatments were then individually tested on perennial ryegrass (*Lolium perenne*) and oilseed rape (*Brassica napus*) in various growth experiments on plant height and yield.

## **4. MATERIALS AND METHODS WITH RESULTS**

### **4.1 Overall Experimental Aims**

The aims of this project were to:

- Evaluate the effects of 2 biochar (BC) and peat-free (PF) compost treatments on the plant growth of perennial ryegrass (PRG) (*Lolium perenne*) and oilseed rape (OSR) (*Brassica napus*).
- To ‘charge’ and improve treatments via 6-month outdoor incubation while also abiotically stressing both plants so as to observe plant performance under such conditions.
- Observe and gain quantitative physical structure results and qualitative elemental results of the microscopic structures for both biochars via scanning electron microscope (SEM).
- Gain further insight into the relationship between structure and elemental analyses of both biochars.
- Map the porous surface area of the biochars.
- Embed biochar in epoxy resins so as to obtain greater structural mapping analyses under SEM.
- Obtain soil plant analysis development (SPAD) measurements from stressed OSR plants at the end of growth trials in glasshouse experiments.

#### **4.1.1 Data Observations and Analyses**

Data observations (histograms and line graphs) were generated by Google Sheets. Images and spectra were generated by a JEOL JSM-IT200 InTouchScope™ SEM and subsequently mapped and analysed using ImageJ. Statistical analyses (Welch two-sided t-tests, 2-way ANOVA tests and tukey post hoc tests) were generated by Rstudio. SPAD measurements were obtained using the Photosynq Multispeq™ phytometer.

#### **4.2 Biochar Source, Pyrolysis and PF Compost**

The two BCs used for these experiments were produced via pyrolysis of feedstocks of (a) oak (*Quercus spp.*) and (b) rush (*Juncus effusus*) in a mobile pyrolysis unit (fig.3). They were kindly provided to the School of BEES by Bernard Carey of Biomass to Biochar Ltd (BTBL) for research purposes.

The oak BC pyrolysis regime had a temperature of between 450-600 °C and a duration of roughly 3 hours. The regime for the rush BC had a temperature of between 400-500 °C and a duration of around 2 hours.

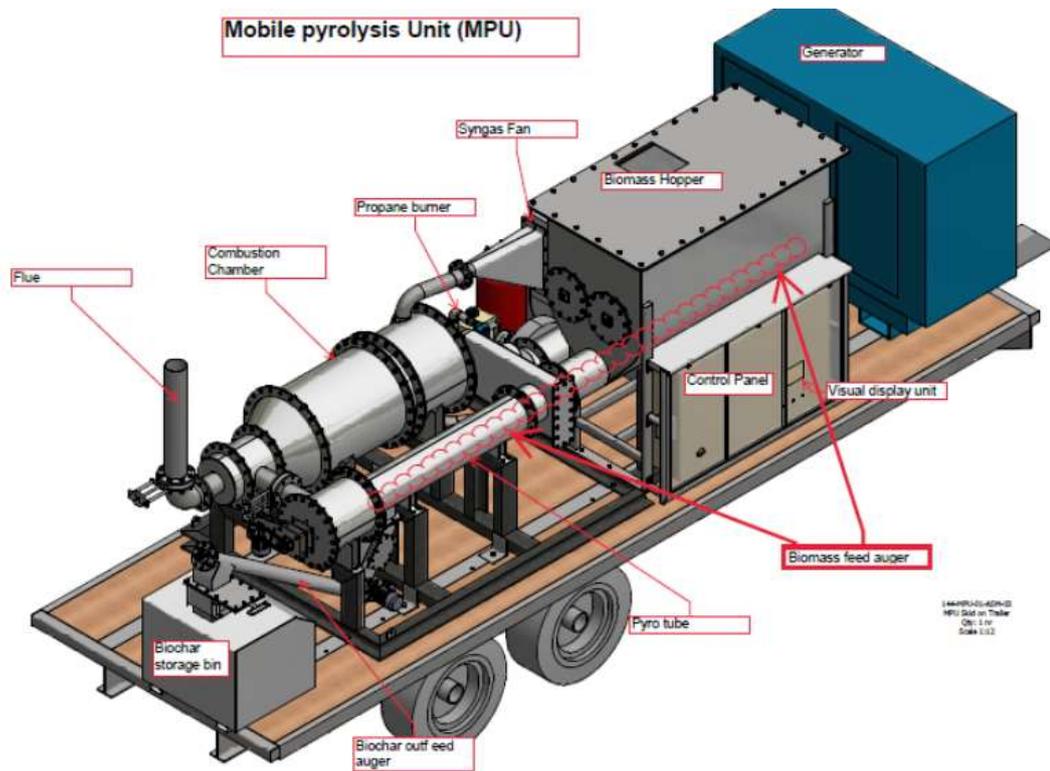


Figure 3: A diagram showing an example of a MPU and its various components used in the production of biochar, syngas and bio-oils. Source: <https://www.biomasstobiochar.ie/mpu.html>

The PF compost used for these experiments was purchased from Fruit Hill Farm, Colomane, Bantry, Co. Cork, Ireland. The PF compost had the following feedstock ratios:

- 55% coconut pith/coir, 20% green waste compost and 25% wood fibre compost.
- Predative mites (*Stratiolaelaps scimitus*) were added as a specific biological control agent of sciarid fly (*Sciaridae spp*).

### 4.3 Treatments

- Treatment 1 = PF compost and oak BC
- Treatment 2 = PF compost and rush BC
- Treatment 3 = PF compost and oak BC (6-month incubation)
- Treatment 3 = PF compost and rush BC (6-month incubation)

### 4.4 Glasshouse Experiment Watering and Harvesting

#### 4.4.1 Watering

All treatments throughout the glasshouse experiments in this thesis were kept sufficiently hydrated by having all replicate plant pots placed in self containing trays. These trays were then filled with enough water to keep treatments moist to the touch. Trays were inspected weekly and water poured into trays accordingly, if needed.

#### **4.4.2 Plant Fresh Weights and Dry Weights**

At the end of the 6-week growth trials, plants were removed from their pots and had any excess soil removed. Using a precision balance, the plant fresh weights were taken and put into brown paper bags. Each replicate number and treatment type were then inscribed onto each bag and placed in a tray. Once all fresh weights were taken the tray was then placed into a drying oven (60°C) for a period of at least 48 hours (fig.4). Afterwards the trays were removed, brown bags discarded, and all plant dry weights were recorded using a precision balance.



*Figure 4: The plant drying oven in the UCC glasshouse with trays filled with fresh weight plant material, segregated into PRG and OSR groups*

*Photos: Eric Hynes, 2021.*

#### **4.5 Treatment Preparation**

Amounts of either oak or rush biochar pieces were pulverized using a pestle and mortar (fig.5). 1.6 g aliquots (Aherne, 2017; Murphy, 2017; O'Sullivan, 2015) of pulverized biochar were then weighed out with weighing scales (fig.6). 426 cm<sup>3</sup> (aka 9 cm) plant pots were filled with PF compost. Both biochar and PF compost were subsequently mixed heterogeneously in an open container using a laboratory spoon (fig.7). The BC and PF compost treatment was then transferred back into 426 cm<sup>2</sup> plant pots. This sequence was completed 20 times so as to have 20 pots of test material (10 pots for PRG and 10 pots for OSR). Following on, 20 pots of PF compost were also used as control to the experiment. All test and control pots were put into 2 trays (10 test pots and 10 control pots for each tray) and transferred to the UCC glasshouse for use in subsequent experiments.



*Figure 5-7: Pulverising of biochar using a pestle and mortar, followed by weighing out of 1.6g of pulverized biochar and subsequent mixing with PF compost before potting.*

*Photos: Eric Hynes, 2021.*

#### **4.6 Glasshouse Experiment #1: Testing Plant Growth Response of PRG and OSR using Treatment 1**

Once the trays were transferred to the glasshouse, one tray was labelled for PRG testing and the other for OSR testing. In the PRG tray, 10 PRG seeds were carefully and evenly sown into each test plant pot. In the OSR tray, 5 OSR seeds were carefully and evenly sown into each test plant pot. Once all the seeds were sown for the experiment, plant pots were arranged as shown in table 1 of the next section.

##### **4.6.1 Hypotheses**

- **Does treatment 1 affect PRG/OSR growth?**
- **Does treatment 1 affect plant fresh weight?**
- **Does treatment 1 affect plant dry weight?**

##### **4.6.2 Experimental Design**

The experimental design for glasshouse experiment #1 can be found below in Table 1. This approach was used to ensure randomisation within the experiment's design.

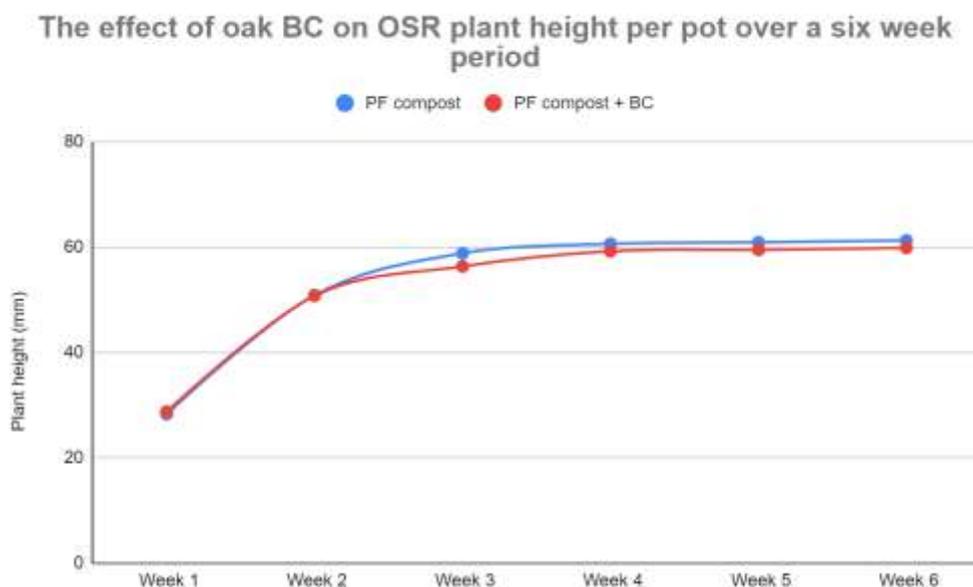
*Table 1: Shows the complete randomized design (CRD) of plant pots within each tray.  
A = test plant pot, B = control plant pot.*

A	B	A	B	A
B	A	B	A	B
A	B	A	B	A
B	A	B	A	B

**4.6.3 Glasshouse Experiment #1 Results**

**4.6.3.1 OSR Plant Heights**

When a two-sample t-test was performed on the week 6 data of the below figure (fig.8) it was found that plant heights were not affected by treatment 1. A p-value of 0.7683 was generated, with mean plant heights of 61.3 mm & 59.9 mm for PF compost and treatment 1, respectively.



*Figure 8: Shows the difference in plant heights taken weekly over a 6-week period between OSR plants grown in PF compost and OSR plants grown in the PF compost + BC.*

**4.6.3.2 OSR Fresh Weights and Dry Weights**

When a two-sample t-test was performed on the data of the below figure (fig.9) it was found that plant fresh weights were not affected by treatment 1. A p-value of 0.5465 was generated, with mean plant fresh weights of 8.998 g & 8.647 g for PF compost and PF compost + BC, respectively.

The same test was carried out on plant dry weights and were found to not be affected by treatment 1. A p-value of 0.519 was generated. Mean plant dry weights were 0.94 g for PF compost and 0.88 g for PF compost + BC.

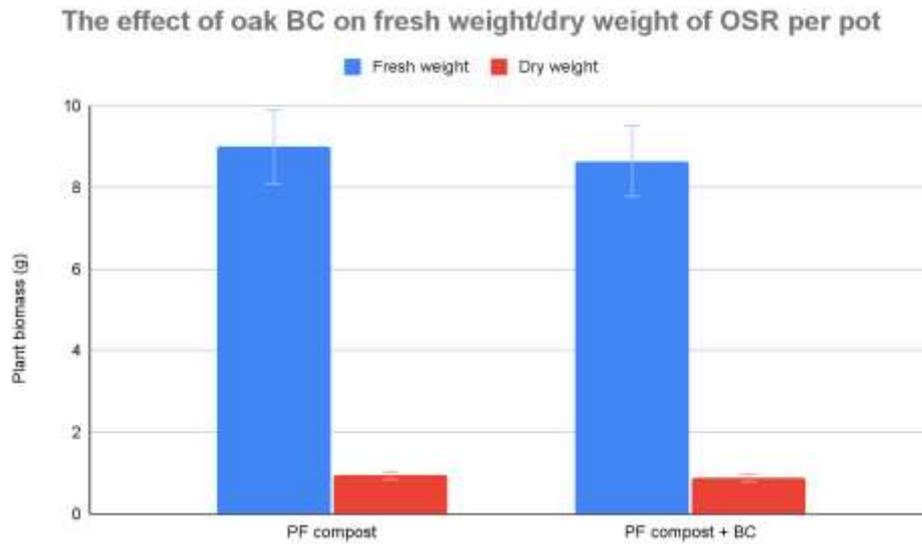


Figure 9: Shows the difference in plant fresh weights and dry weights taken after 6 weeks of growth between OSR plants grown in PF compost and OSR plants grown in the PF compost + BC.

#### 4.6.3.3 PRG Plant Heights

When a two-sample t-test was performed on the week 6 data from the below figure (fig.10) it was found that plant heights were not affected by treatment 1. A p-value of 0.6404 was generated, with mean plant heights of 309.52 mm & 317.04 mm for PF compost and PF compost + BC, respectively.

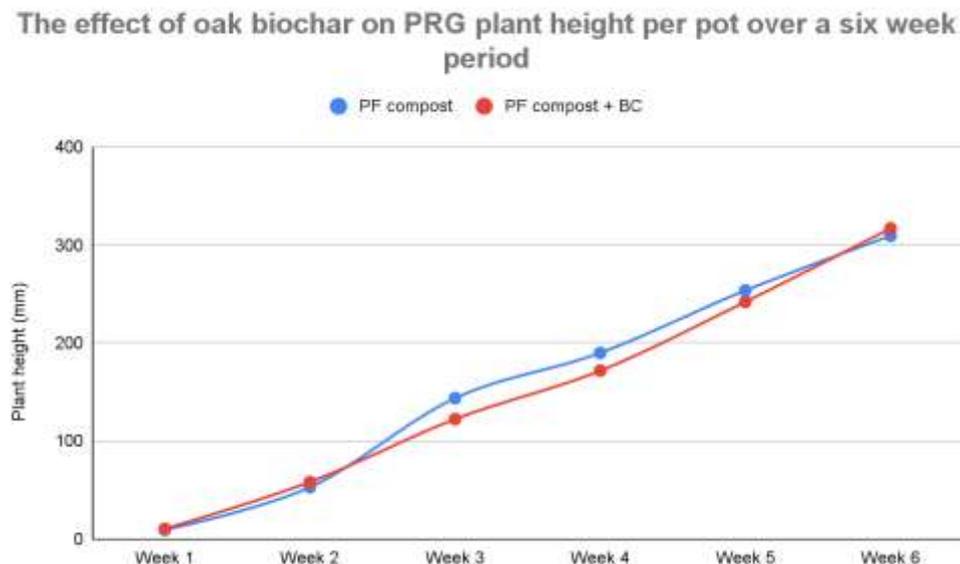
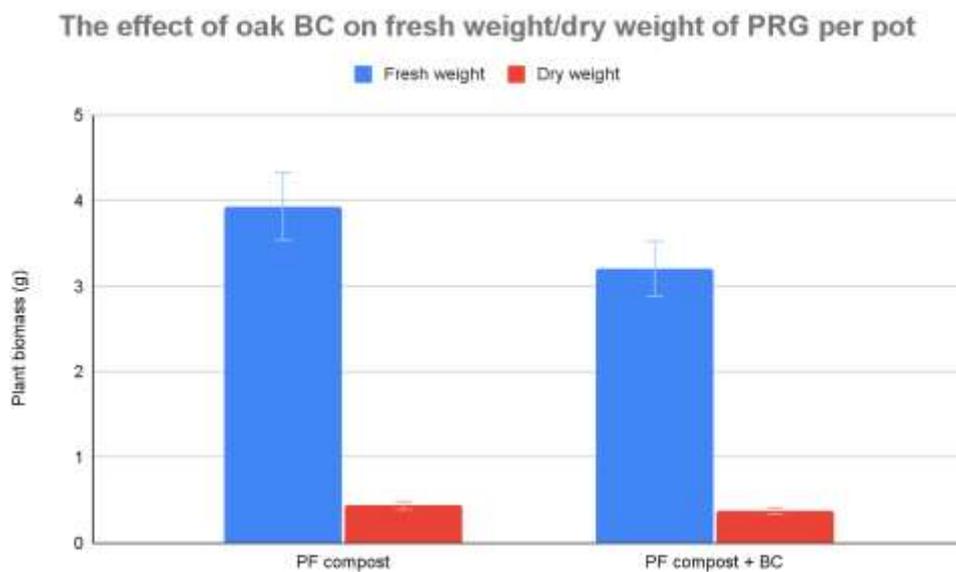


Figure 10: Shows the difference in plant heights taken weekly over a 6-week period between PRG plants grown in PF compost and PRG plants grown in the PF compost + BC.

#### **4.6.3.4 PRG Fresh Weights and Dry Weights**

When a two-sample t-test was performed on the data of the below figure (fig.11) it was found that plant fresh weights were not affected by treatment 1. A p-value of 0.2201 was generated, with mean plant fresh weights of 3.207 g & 3.937 g for PF compost and PF compost + BC, respectively.

The same test was carried out on plant dry weights, which were found to not be affected by treatment 1. A p-value of 0.333 was generated. Mean plant dry weights were 0.374 for PF compost and 0.437 for PF compost + BC.



*Figure 11: Shows the difference in plant fresh weights and dry weights taken after 6 weeks of growth between PRG plants grown in PF compost and PRG plants grown in the PF compost + BC.*

#### **4.7 Glasshouse Experiment #2: Testing Growth Response of PRG and OSR to Treatment 2**

The same methods were used here as in sections 4.4 & 4.5 but with a rush BC instead of an oak BC.

##### **4.7.1 Hypotheses:**

- Does treatment 2 affect plant growth?
- Does treatment 2 affect plant fresh weight?

- Does treatment 2 affect plant dry weight?

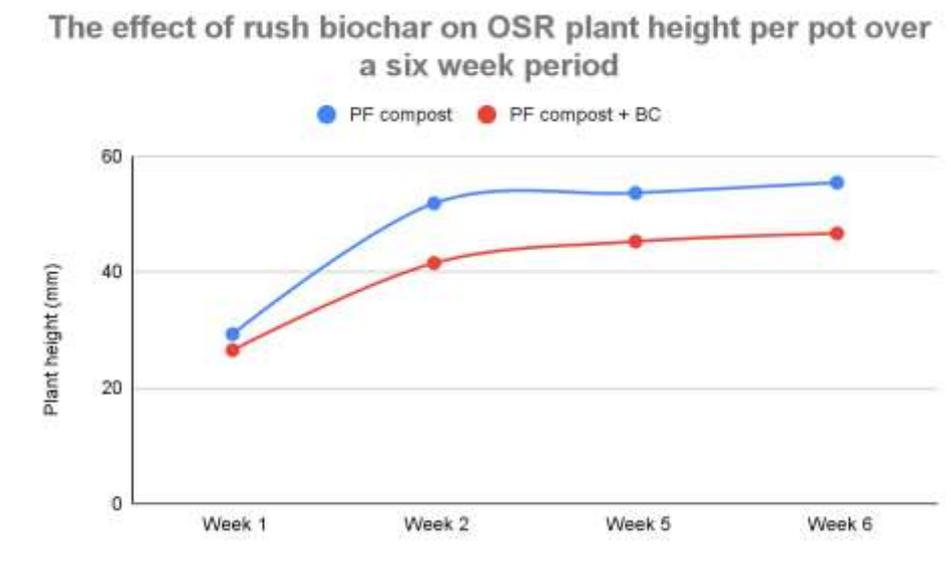
#### 4.7.2 Experimental Design

The experimental design here remained the same as in section 4.5.2.

#### 4.7.3 Glasshouse Experiment #2 Results

##### 4.7.3.1 OSR Plant Heights

When a two-sample t-test was performed on the week 6 data of the below figure (fig.12) it was found that plant heights were not affected by treatment 2. A p-value of 0.4803 was generated, with mean plant heights of 55.43 mm & 46.65 mm for PF compost and PF compost + BC, respectively. Week 3 & 4 were omitted from the data due to UCC and the glasshouses being inaccessible during the holiday season.

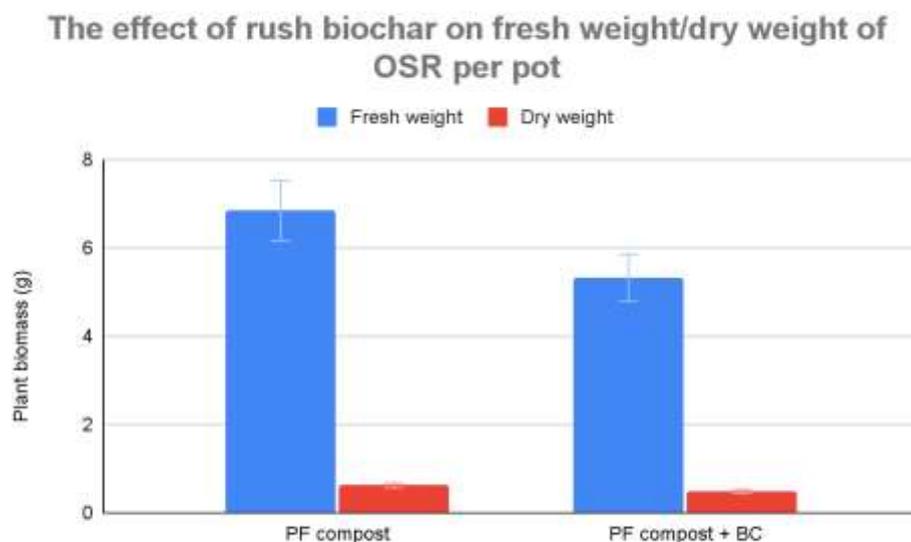


*Figure 12: Shows the difference in plant heights taken weekly over a 6-week period between OSR plants grown in PF compost and OSR plants grown in the PF compost + BC.*

##### 4.7.3.2 OSR Fresh Weights and Dry Weights

When a two-sample t-test was performed on the belonged to the below figure (fig.13) it was found that plant fresh weights were not affected by treatment 2. A p-value of 0.3115 was generated, with mean plant fresh weights of 6.84 g & 5.32 g for PF compost and PF compost + BC, respectively.

The same test was carried out on plant dry weights and showed that OSR plants were not affected by treatment 2. A p-value of 0.424 was generated. Mean plant dry weights were 0.62 for PF compost and 0.484 for PF compost + BC.



*Figure 13: Shows the difference in plant fresh weights and dry weights taken after 6 weeks of growth between OSR plants grown in PF compost and OSR plants grown in the PF compost + BC.*

#### **4.7.3.3 PRG Plant Heights**

When a two-sample t-test was performed on the week 6 data from the below figure (fig.14) it was found that plant heights were affected by treatment 2. A p-value of 0.0268 was generated, showing there was a significant difference between the two treatments. The mean plant heights of 223.4 mm & 281.59 mm for PF compost and PF compost + BC, respectively.

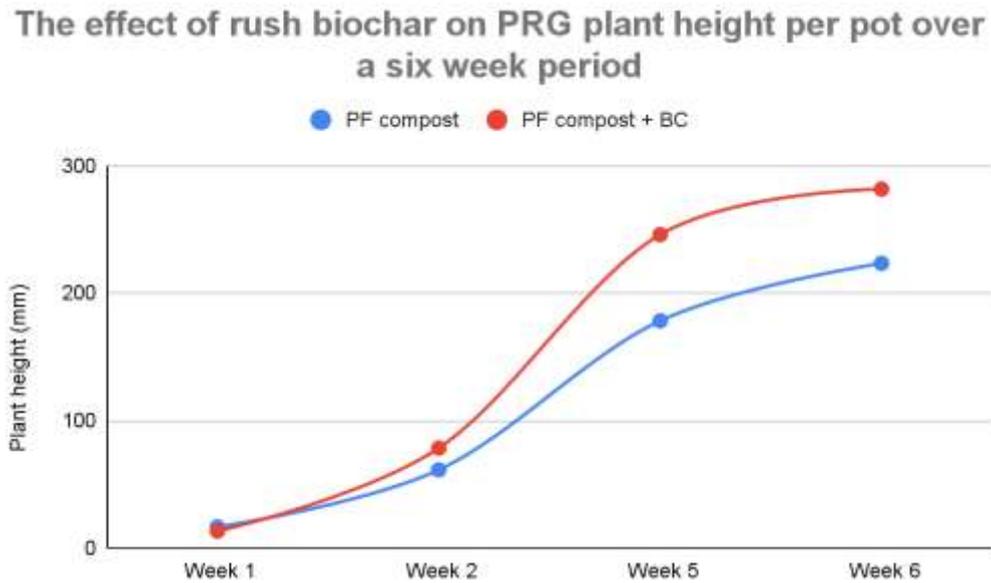
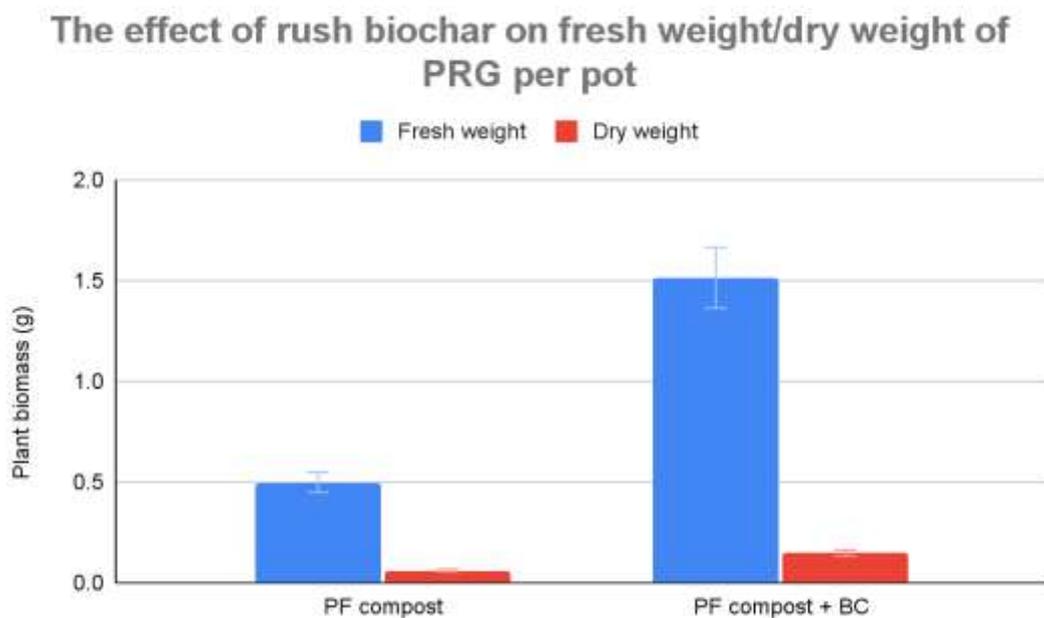


Figure 14: Shows the difference in plant heights taken weekly over a 6-week period between PRG plants grown in PF compost and PRG plants grown in the PF compost + BC.

**4.7.3.4 PRG Fresh Weights and Dry Weights**

When a two-sample t-test was performed on the data from below figure (fig.15) it was found that plant fresh weights were affected by treatment 2. A p-value of <0.001 was generated, so there was a highly significant difference between the two treatments. The mean plant fresh weights were 0.498 g & 1.513 g for PF compost and PF compost + BC, respectively.

The same test was carried out on plant dry data, showing plant dry weights had been significantly increased by treatment 2. A p-value of 0.012 was generated. Mean plant dry weights were 0.062 for PF compost and 0.148 for PF compost + BC.



*Figure 15: Shows the difference in plant fresh weights and dry weights taken after 6 weeks of growth between PRG plants grown in PF compost and PRG plants grown in the PF compost + BC.*

#### **4.8 Glasshouse Experiment #3: Testing Plant ‘Growth Response’ of PRG to Treatment 3 and 4 with Abiotic Stress Exposure**

Following on from the two previous experiments which had mostly neutral to negative results, newer experiments were designed in a bid to induce positive plant growth and yield responses in the potted glasshouse experiments.

##### **4.8.1 Preparing Charged BC**

Large amounts of treatment 3 and 4 (same ratio as treatments 1 and 2 but x10) were left in their own individual large containers outside of the UCC glasshouses. They remained there for 6 months, exposed to the atmosphere, in order to charge and induce positive plant growth responses. They would then be subsequently used in later plant growth experiments.

The 4 large containers (2 x treatment 3 and 2 x treatment 4) used for the long-term experiment had 5 drainage holes drilled into each container. These holes were evenly distributed around the circumference of the base of the large containers. After this, small rocks were placed above each hole, in an effort to improve drainage and for the more efficient flow of rain through the treatments (fig.16). This was followed by the loading of 4.26 L of PF compost into the container and the successive adding of 16 g of pulverized BC (fig.17). This was then carefully and thoroughly mixed together using a garden trowel.



*Figure 16 & 17: An empty, large container with rocks added above modified drainage hole areas, followed by the large amounts of BC and PF compost treatment, ready to be mixed.*

*Photos: Eric Hynes, 2021.*

At the end of the 6-month period, these treatments were transferred into the same size pots used in experiments 1 & 2. The next phase of the experiment was to test PRG plant growth in the presence of treatments 3 and 4 as well as abiotic stressors (salt stress and drought stress) and to observe the interactions that may occur.

#### **4.8.2 Hypotheses**

- **Does treatment 3 have an effect on PRG growth and on PRG plants under abiotic stress?**
- **Does treatment 4 have an effect on PRG growth and on PRG plants under abiotic stress?**

#### **4.8.3 Experimental Design**

The experiment has 90 x 426 cm<sup>3</sup> pots and 10 PRG seeds were then carefully sown into them. Experiments using treatment 3 were designated 30 replicates. Pots of 10 were separated in a tray and used for the salt stress test. Another 10 were separated in another tray and used for the drought stress test and the remaining 10 were isolated as a control. This same method was repeated for the treatment 4 experiments (30 replicates) and control experiments (30 replicates).

*Table 2: Shows the experimental design of Glasshouse Experiment #3 demonstrating the positioning of each 10 replicates within each self-contained tray. A = treatment 3, B = treatment 4, C = control, D = drought stress, S = salt stress, NS = no stress.*

	<b>D</b>	<b>S</b>	<b>NS</b>
<b>A</b>	<b>10</b>	<b>10</b>	<b>10</b>
<b>B</b>	<b>10</b>	<b>10</b>	<b>10</b>
<b>C</b>	<b>10</b>	<b>10</b>	<b>10</b>

#### **4.8.4 Inducing Plant Stress via Abiotic Stress Exposure**

All seeds were allowed a 2-week period to germinate and grow before any abiotic stressors were applied to the respective replicate plant pots.

##### **4.8.4.1 Salt Stress**

Using a technique designed by He et al. (2018) plants undergoing salt stress testing were given 20 ml of 17.532 g/L NaCl solution every second day into the plant pots via syringe for greater accuracy. Firstly, a high precision analytical weighing scale, a weighing boat and a small laboratory spoon were used to weigh out 17.532 g of NaCl. Measures of tap water were taken from the UCC glasshouse for consistency with the same water used for watering of plants in previous experiments. Tap water (1 L) was measured using a large graduated cylinder. The NaCl and tap water were subsequently mixed as a solution in a glass media bottle and transported back to the glasshouse for the experiments. These steps were repeated to keep a ready supply of NaCl solution for plant salt stress testing throughout the experiment.

##### **4.8.4.2 Drought Stress**

Plants to be drought stress tested were put aside in drought stressing trays. Each plant pot replicate was given 20ml of tap water per week and monitored regularly so plants did not die of dehydration. If plants appeared observably near death as such, then a booster hydration shot of 10ml tap water was given to any replicate pots in need of the boost.

##### **4.8.4.3 No Stress**

The same number of replicates underwent a no-stress regime, acting as the control in the presence of either treatment and no BC control. The replicates had water applied the same way as in section 4.3.

#### 4.8.5 Glasshouse Experiment #3 Results

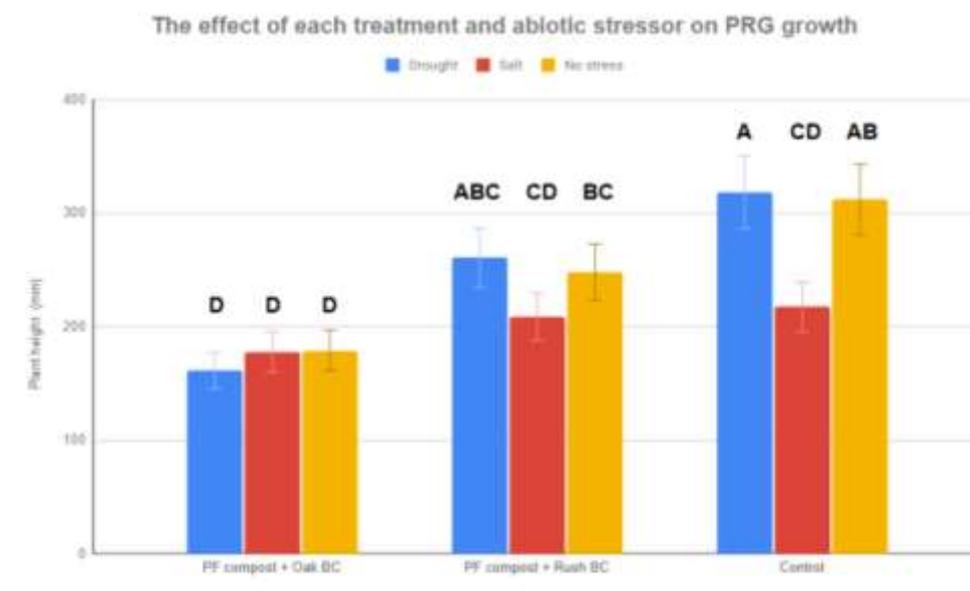
##### 4.8.5.1 PGR Plant Heights

A two-way ANOVA was carried out on the plant height data of the below figure (fig.18). A p-value of <0.001 was generated for the 3 categories of the effect of treatment, stressor and treatment:stressor interaction on plant height. This shows the data analyses to be highly significant and that the presence of both charged treatments did influence the growth of PRG.

A Tukey post hoc test was subsequently carried out on plant height data of the below figure (fig.18). A p-value of <0.001 was generated for all 3 treatments showing they were statistically different from each other. Between the samples, the treatment 3 was the most dissimilar to the other groups and no statistical difference was found between the drought, salt and non-stressors of this group.

Within the treatment 4 group, the salt stressor was shown to be most statistically similar to all 3 stressors in the treatment 3 group. The same can be said for the no BC and salt stressor effect.

The treatment 4 and drought stressor shared statistically significant similarities with all other treatment:stressor effects (except for the treatment 3 group) on plant heights. The control and drought stressor combination had the most statistically unique effect to all other combinations, followed by the control and no stressor.



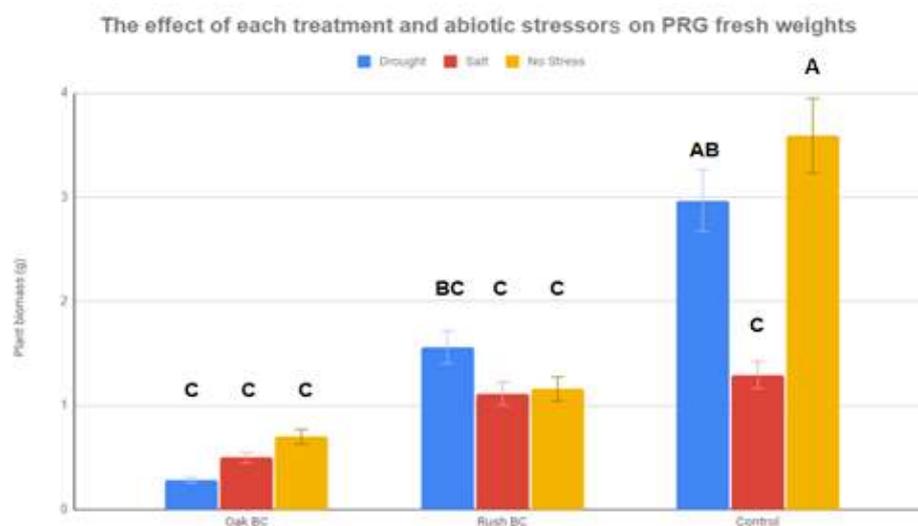
*Figure 18: Shows the effect of each treatment:stressor on the growth of PRG plants and the interaction between them. Any samples sharing a common letter are not significantly different.*

#### 4.8.5.2 PGR Fresh Weights

Using a two-way ANOVA, statistical analysis was performed on the plant fresh weight data belonging to the below figure (fig.19). A p-value of <0.001 was generated for the 2 categories of treatment and treatment:stressor interaction and was therefore highly significant. A p-value of 0.01 was generated for treatment:stressor interactions showing the data to be significant. This confirms that treatment 3 and treatment 4 did have an effect on the fresh weights of PRG as well PRG exposed to abiotic stressors.

A Tukey post hoc test was then carried out on fresh weight data of the below figure (fig.19). A p-value of <0.001 was generated for the treatment 3 to control treatments and the treatment 4 to control treatments, showing the relationship of treatments 3 and 4 to be highly significantly different from the control treatment. A p-value of 0.01 was generated for the relationship between treatments 3 and 4, showing their effects to be significantly different.

Treatment 3 and stressor group were all statistically similar and shared common effects on plant fresh weights with the treatment 4 group, as well as the control and salt stressor combination. The treatment 4 and drought stressor shared some similarities to the control and drought stressor combination. The most significantly different effect on fresh weights was experienced with the control and no stress combination, which also shared statistical similarities to the control and drought stressor combination.



*Figure 19: Shows the effect of each treatment:stressor on the growth of PRG plants and the interaction between them. Any samples sharing a common letter are not significantly different.*

#### 4.8.5.3 PGR Dry Weights

A two-way ANOVA was performed on the plant dry weight data from the below figure (fig.20). The p-value of <0.001 was generated for the treatment category and was therefore highly significant. The p-values for stressor and treatment:stressor categories were found to be 0.06 and 0.01, respectively showing the differences to be significant. This confirms that treatment 3 and treatment 4 did have an effect on the dry weights of PRG as well PRG exposed to abiotic stressors.

A Tukey post hoc test was then carried out on the same plant dry weight data. A p-value of <0.001 was generated for all 3 treatments showing they were statistically different from each other.

Treatment 3 and stressor group were all statistically similar and shared common effects on plant fresh weights with treatment 4 group, as well as the control and salt stressor combination which was highly similar to the treatment 4 effect and drought stressor combination. The control and drought stressor combination had the most overall statistically different effect, which was shared with the control and no stress combination.

Treatment 4 and drought stressor, control and salt stressor and control and no stressor combinations all shared statistically significant similarities.

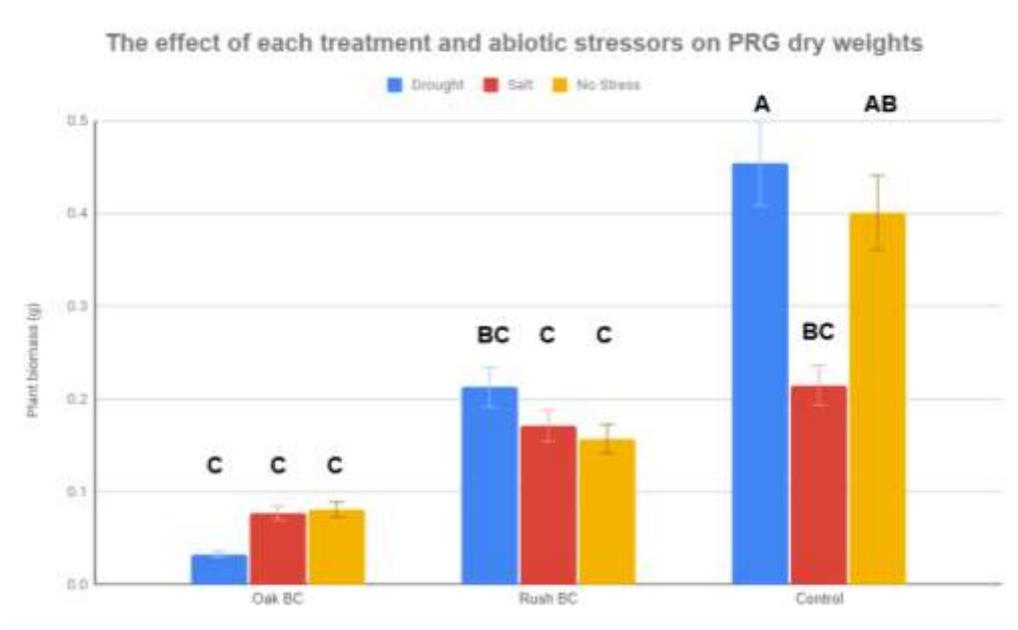


Figure 20: Shows the effect of each treatment:stressor on the growth of PRG plants and the interaction between them. Any samples sharing a common letter are not significantly different.

#### **4.9 Glasshouse Experiment #4: Testing Plant Growth Response of OSR to Treatment 3 and 4 with Abiotic Stressor Exposure**

The methods of preparation for replicates, trays and experimental design remained the same here as in section 4.8. Except for the sowing of 5xOSR seeds per replicate pot instead of PRG seeds.

##### **4.9.1 Hypotheses**

- **Does treatment 3 have an effect on OSR growth and the growth of OSR plants under abiotic stress?**
- **Does treatment 4 have an effect on OSR growth and the growth of OSR plants under abiotic stress?**

##### **4.9.2 Glasshouse Experiment #4 Results**

###### **4.9.2.1 OSR Plant Heights**

A two-way ANOVA was carried out on the plant height data from the figure below (fig.21). The p-value of <0.001 was generated for the 3 categories of the effect of treatment, stressor and treatment:stressor interaction on plant height. This shows the data analyses to be highly significant. It demonstrates that the presence of both charged treatments in conjunction with abiotic stressors did have an effect on the growth of OSR.

A Tukey post hoc test was then carried out on the same data (fig.21). A p-value of <0.001 was generated for the treatment 3 to control treatments and the treatment 4 to control treatments, showing the relationship of treatments 3 and 4 to be highly significantly different from the control treatment. A p-value of 0.01 was generated for the relationship between treatments 3 and 4, showing their effects to be significantly different.

Both treatment:stressors (3 and 4) had the most similar effects (D, E and DE), distinct to that of the control treatment:stressors, which were all dissimilar to one another (A,B and C). The combinations that had the most positive effects on OSR plant growth were firstly the control:no stress combination, followed by the control:salt stress and the control:drought stress.

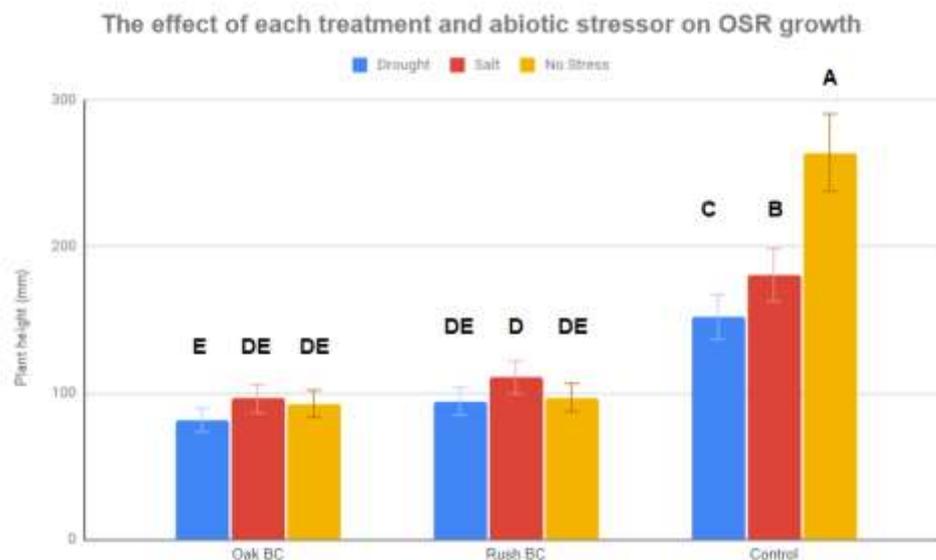


Figure 21: Shows the effect of each treatment:stressor on the growth of OSR plants and the interaction between them. Any samples sharing a common letter are not significantly different.

#### 4.9.2.2 OSR Fresh Weights

Using a two-way ANOVA, statistical analysis was performed on the plant fresh weight data (fig.22). p-values of <0.001 were generated for the 3 categories of the effect of treatment, stressor and treatment:stressor interaction on plant height. This shows the data analyses to be highly significant. It demonstrates that the presence of both charged treatments in conjunction with abiotic stressors did have an effect on the growth of OSR.

A Tukey post hoc test was then carried out on the same data (fig.22). A p-value of <0.001 was generated for treatment 3 to control treatments and the treatment 4 to control treatments, showing the relationship of both charged treatments to be highly significantly different from the control treatment.

Both the charged BC treatments exerted highly similar effects across all combinations of treatment:stressor. The control:salt stress (B) and control:no stress (A) combinations were the only groups found to be different to all other combinations.

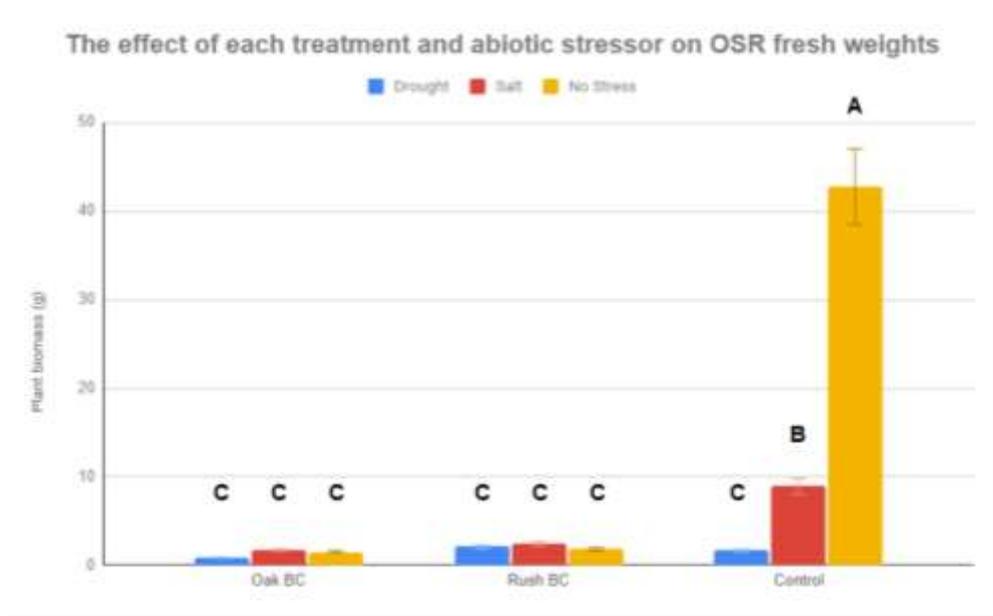


Figure 22: Shows the effect of each treatment:stressor on the fresh weights of OSR plants and the interaction between them. Any samples sharing a common letter are not significantly different.

#### 4.9.2.3 OSR Dry Weights

A two-way ANOVA was carried out on the plant dry weight data from the below figure (fig.23). The p-value of <0.001 was generated for the 3 categories of the effect of treatment, stressor and treatment:stressor interaction on plant height. This shows the data analyses to be highly significant. It demonstrates that the presence of both charged treatments in conjunction with abiotic stressors did have an effect on the growth of OSR.

A Tukey post hoc test was then carried out on the same data (fig.23). A p-value of <0.001 was generated for the treatment 3 to control treatments and the treatment 4 to control treatments, showing the relationship of both treatment 3 and 4 to be highly significantly different from the control treatment.

Both treatment 3 and 4 exerted highly similar effects across all combinations of treatment:stressor. The control:salt stress and control:drought stress combinations (B) were statistically similar. The effect of the control:no stress (A) combination massively outperformed all other combinations.

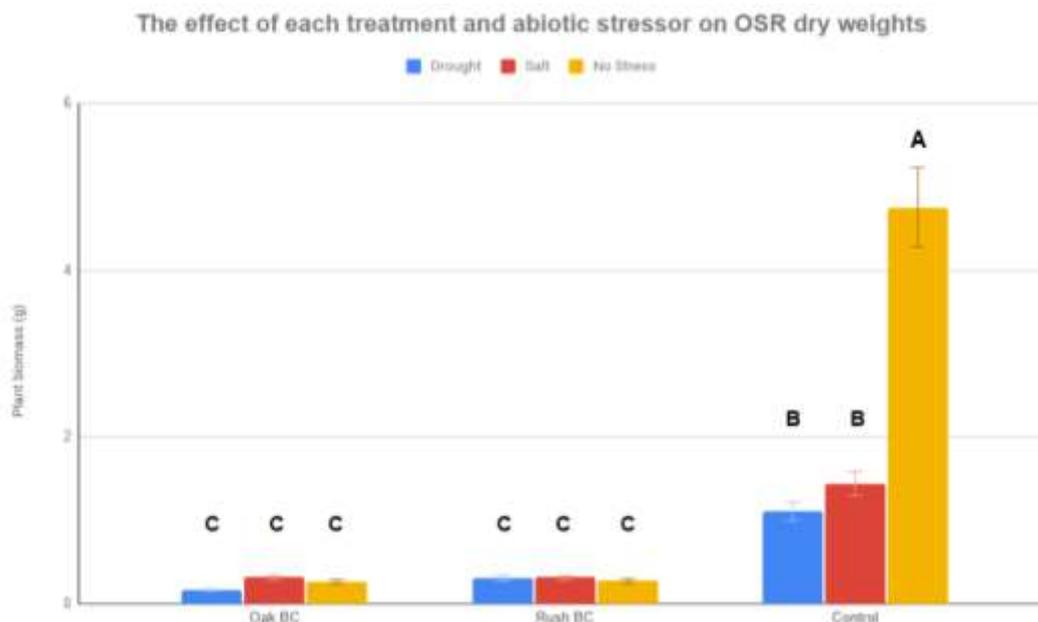


Figure 23: Shows the effect of each treatment:stressor on the growth of OSR plants and the interaction between them. Any samples sharing a common letter are not significantly different.

#### **4.10 Glasshouse Experiment #5: Testing Plant Health/Stress Response of OSR to Treatment 3 and 4 with Abiotic Stressor Exposure**

##### **4.10.1 Methodology of Acquiring Data on Plant Stress**

At the end of the glasshouse experiment #4, SPAD measurements were taken from each OSR plant within each replicate. The Photosynq Multispeq™ and its partner software Multispeq v2.40 was downloaded from Google Play and installed onto a smartphone to allow the pairing of smartphone and the handheld photosynq device. After pressing the button at the back of the device, a leaf from an OSR plant was carefully placed where the LED sensor for spectroscopy is located on the underside of the device.

Using a trigger in the smartphone software, SPAD measurements were taken from the leaf and subsequently transmitted to the smartphone and noted (fig.24). Pressing the button on the back of the device once more to carefully release the leaf, further measurements of the other leaves of all other OSR plants were taken and noted. SPAD demonstrates leaf chlorophyll content and fluorescence and is an indicator of plant health.



*Figure 24: A demonstration of the Photosynq Multispeq™ taking SPAD measurements from a sample leaf, via the paired device and smartphone. Managed through the v2.40 software.*

*Photo: Eric Hynes, 2021.*

#### **4.10.2 Hypotheses**

- **Does treatment 3 have an effect on OSR health and the health of OSR plants under abiotic stress?**
- **Does treatment 4 have an effect on OSR health and the health of OSR plants under abiotic stress?**

#### **4.10.3 Glasshouse Experiment #5 Results**

A two-way ANOVA was carried out on the SPAD data acquired for the below (fig.25). The p-value of  $<0.001$  was generated for the 3 categories on the effect of treatment, stressor and treatment:stressor interaction on plant height. This shows the differences to be highly significant. It demonstrates that the presence of both charged treatments in conjunction with abiotic stressors did have an effect on the plant health of OSR.

A Tukey post hoc test was then carried out on the same data (fig.25). Treatment 3 and drought stressor, treatment 3 and salt stressor, treatment 4 and drought stressor, treatment 4 and no stressor and the control substrate and no stressor combinations all shared the most statistically similar effects. Followed by all of the charged BC combinations and control and salt stressor and control and no stressor as being next most significantly similar in effect. The control and drought stress effect of SPAD measurement was the most significantly different combination.

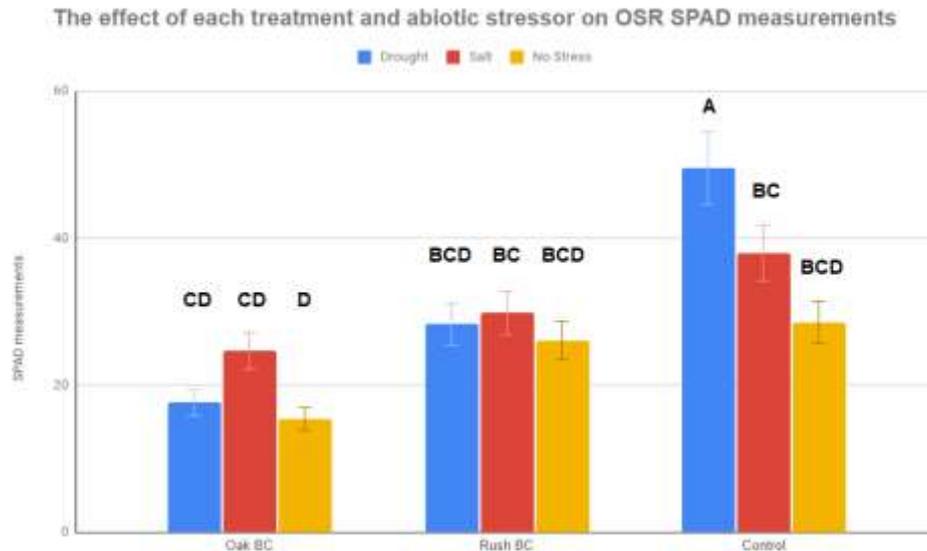


Figure 25: Shows the effect of each treatment:stressor on the plant health/stress of OSR plants and the interaction between them. Any samples sharing a common letter are not significantly different.

#### **4.11 SEM Microscopy Experiment #1: The Embedding of Rush BC and Oak BC Samples in Resin for Structural and Mapping Analyse**

To gain insights into the microstructures of both biochars, it was decided that SEM analyses would provide the best data of such aspects of the material. The structural parameters of pore frequencies, biochar pore surface area and biochar sample surface area were mapped so as the data acquired from both biochars could be compared. Thus, helping to contrast the differences between the two types.

For the structural analyses of porous microstructures such as biochar the fixing of samples for SEM analyses in an epoxy resin is the preferred embedding media option (Besserer et al., 2016). Good adhesion, minimal sample shrinkage, vapor pressure and low viscosity are some of reasons for its choice (Besserer et al., 2016).

#### **4.11.1 Methodology of Preparation of BC Samples for Analysis**

10 pieces of each BC type were separated from larger pieces of each BC type. They were carefully cut on top of aluminium foil using a scalpel (fig.26).



*Figure 26: Shows pieces of BC being cut into smaller pieces using a scalpel, on top of a small piece of tinfoil. Photo: Eric Hynes, 2021.*

#### **4.11.2 Preparing BC Samples and ‘O-Rings’**

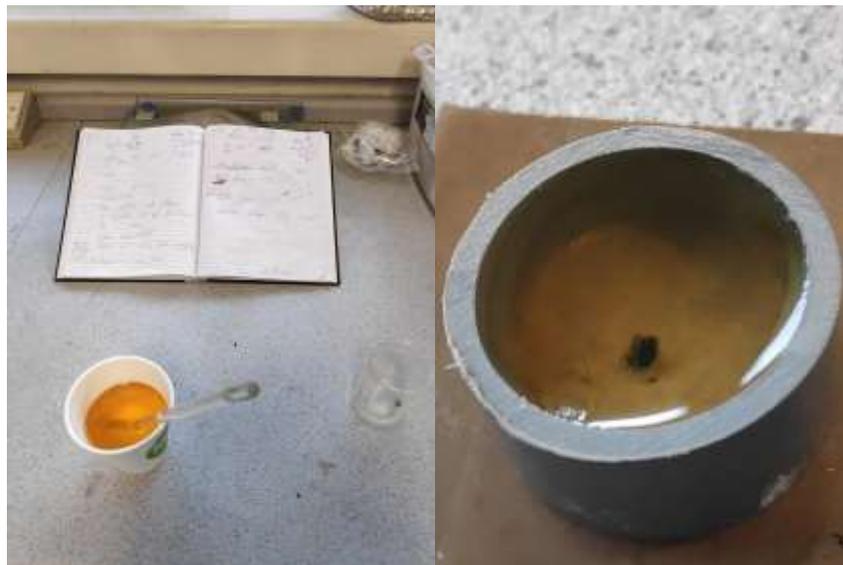
20 pieces of 27 mm diameter grey pipe were cut roughly 15 mm in length by a UCC technical officer. These ‘o-rings’ had one side sanded down using 2 different grades of sandpaper (240 grit, 600 grit) on sanding plates. Pieces of brown sticky tape of similar square shape and size were cut from the sticky tape roll, and placed on the workbench, sticky side up. Each of the o-rings were then placed, sanded side down onto the sticky side of each piece of tape. Using a forceps, a BC sample was then placed into the centre of each o-ring, firmly sticking the sample to the sticky tape (fig.27).



*Figure 27: Shows brown sticky tape left on the workbench, sticky side up. O-rings are stuck to the sticky tape with samples of biochar on the inside, securely stuck to the tape. Photo: Eric Hynes, 2021.*

#### **4.11.3 Methodology of the Preparation of Epoxy Resin and O-Rings**

Before starting to use the resin materials, laboratory goggles and facemask were ensured to be worn before using the highly toxic and carcinogenic resins. Using a ratio 2:1 of EpoThin™ 2 Epoxy Resin to EpoThin™ 2 Epoxy Hardener, 80ml resin and 40ml hardener measured in a beaker and poured into a disposable coffee mug for mixing. A glass rod was used to slowly and carefully stir the mixture for 2 minutes taking extra care not to allow bubble formation (fig.28). Once the mixture was ready, it was subsequently poured carefully into the o-rings, on top of the biochar samples attached to sticky tape. Then, the resin and samples were left to harden overnight for a 24-hour period (fig.29).



*Figure 28 & 29: Shows the preparation of epoxy resin and careful following of the noted protocol from a lab-book. Figure... shows an o-ring with the resin set after 24 hours and a piece of rush BC visibly fixed at the opposite side of the resin/o-ring. Photo: Eric Hynes, 2021.*

Once hardened, each resin filled o-ring was sanded down using a ‘figure of 8’ sanding technique, to mitigate any lob-siding of the o-ring samples. Resin samples for SEM analyses are expected to have a ‘mirror finish’ appearance at the end of the sanding down process. To achieve this, each o-ring had around 20 minutes of sanding on each grade of grit sandpaper (coarse 240 grit to ultra-fine 2500 grit - fig.30) and finally on to the Kemet Polishing Pad with a 6  $\mu\text{m}$  cloth to be used in conjunction with a Kemet Diamond Suspension 3 $\mu\text{m}$  solution (fig.31) to put the final mirror-finish glaze on to the sample. After this, samples were ready to be inputted into the SEM chamber for analysis and photography (post SEM image analysis via ImageJ).



*Figure 30 & 31: Shows the sanding plates of varying grades of sanding grit. As well as the Kemet polishing pad, cloth and diamond solution. Photo: Eric Hynes, 2021.*

#### **4.11.4 Hypotheses**

- **Does a hardwood BC like oak BC display distinct structural differences to that of a grass BC such as rush BC?**

#### **4.11.5 Microscopy Experiment #1.1 Results: Determining the Mean Pore Surface Area of Each Biochar**

A two-sample t-test was performed on the above data and it was found that the mean pore surface area was not significant. A p-value of 0.078 was generated. The oak BC mean pore surface area and the rush BC mean pore surface area were 259.45  $\mu\text{m}$  and 133.24  $\mu\text{m}$ , respectively.

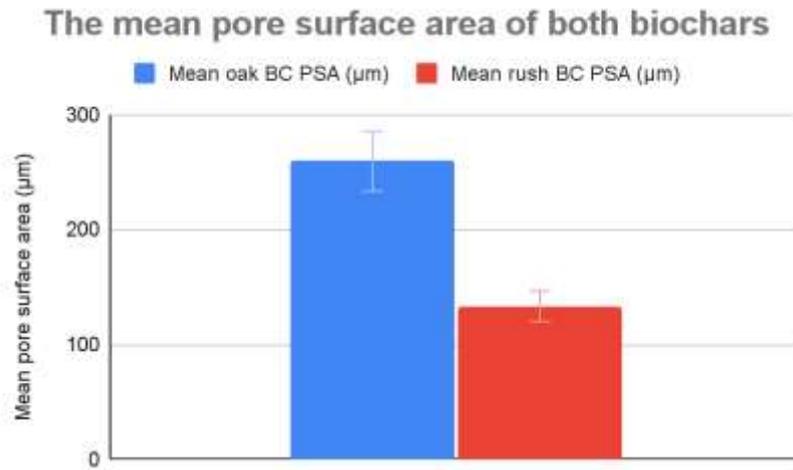


Figure 32: Shows the mean pore surface area of each BC type.

#### **4.11.6 Microscopy Experiment #1.2 Results: Determining the Mean Pore Frequency per Biochar Surface Area**

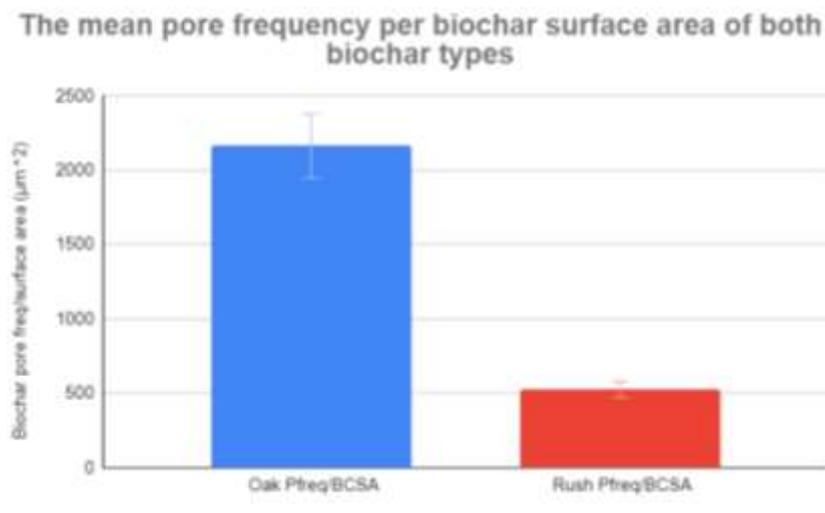


Figure 33: Shows the mean pore frequency per biochar surface area of both BC types.

When a two-sample t-test was performed on the above data, it was found that the mean pore surface area was not significant. A p-value of 0.1083 was generated. The oak BC mean pore frequency per BC surface area was 2164.79  $\mu\text{m}^{-2}$  and the rush BC mean pore frequency per BC surface area was 526.01  $\mu\text{m}^{-2}$ .

#### **4.11.7 SEM Images of Oak BC**

The SEM images below (fig.34-37) exhibit the vastly porous nature of the structure of oak BC. The BC pieces were photographed by high def SEM at various magnifications and subsequently mapped using ImageJ.

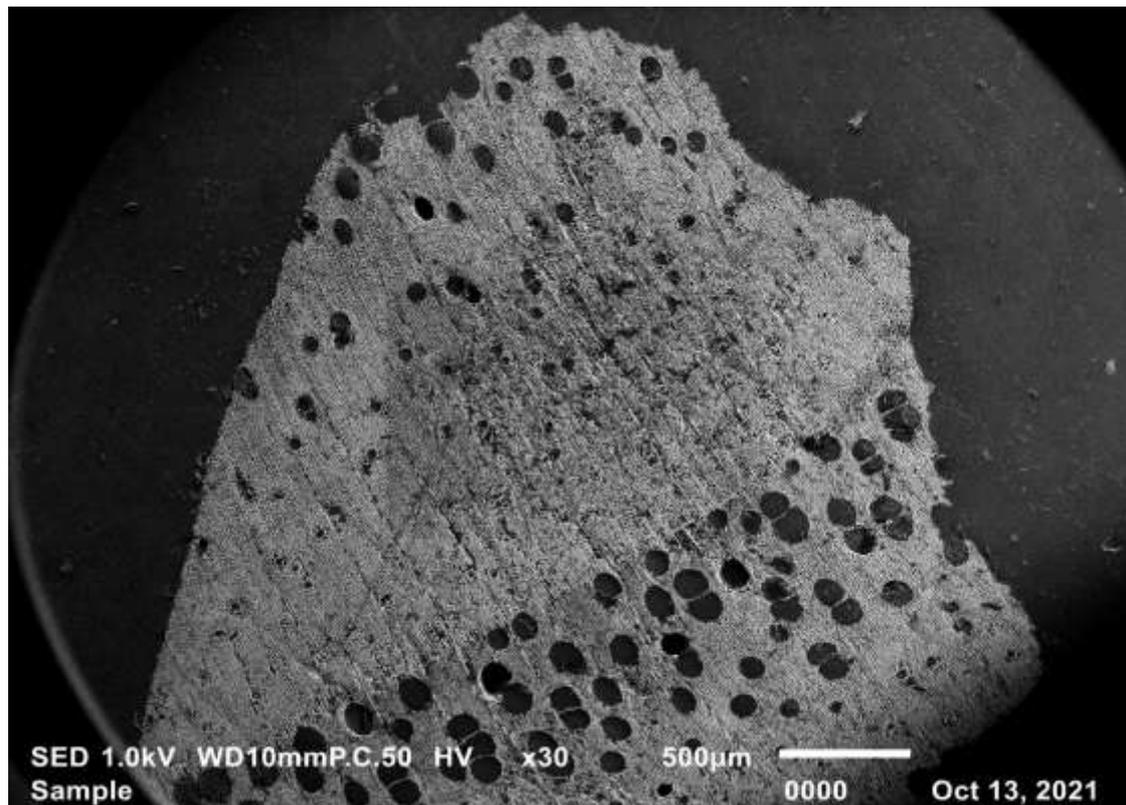


Figure 34: Shows a sample of oak BC embedded in epoxy resin at minimum magnification (x30). Photo: Eric Hynes, 2021.

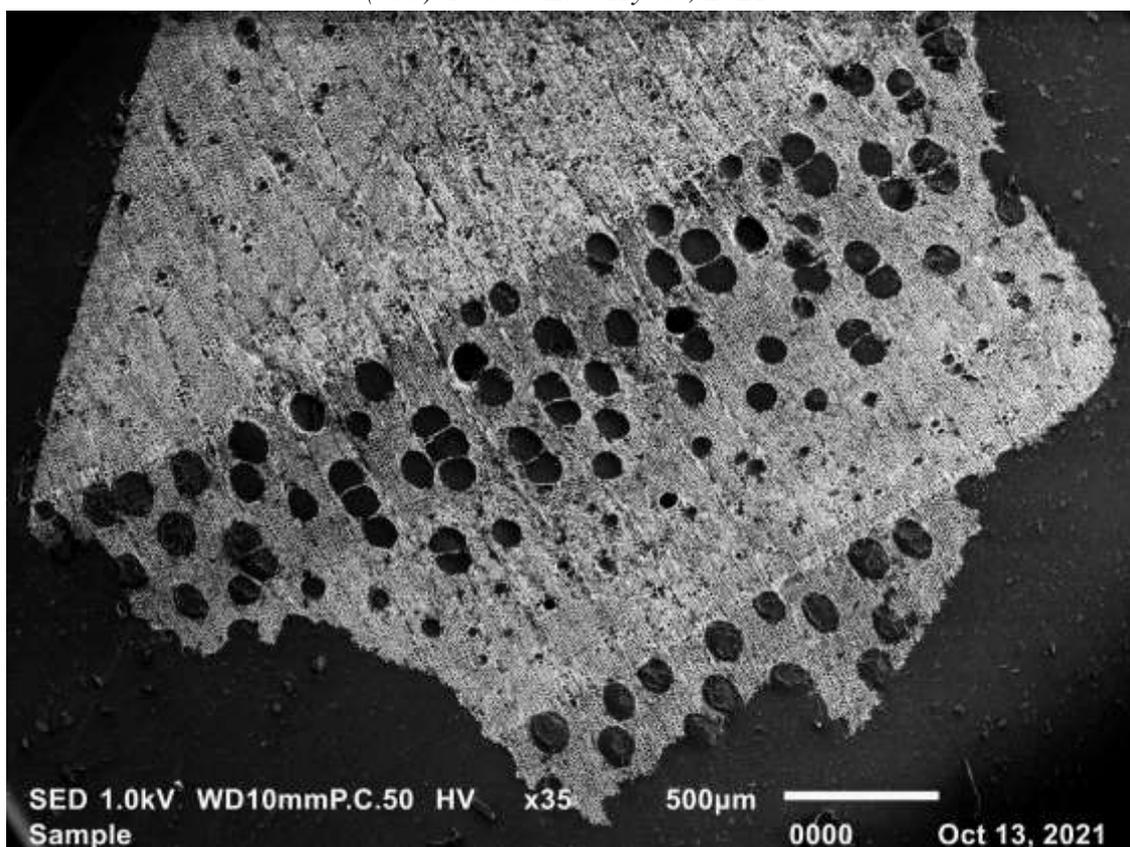
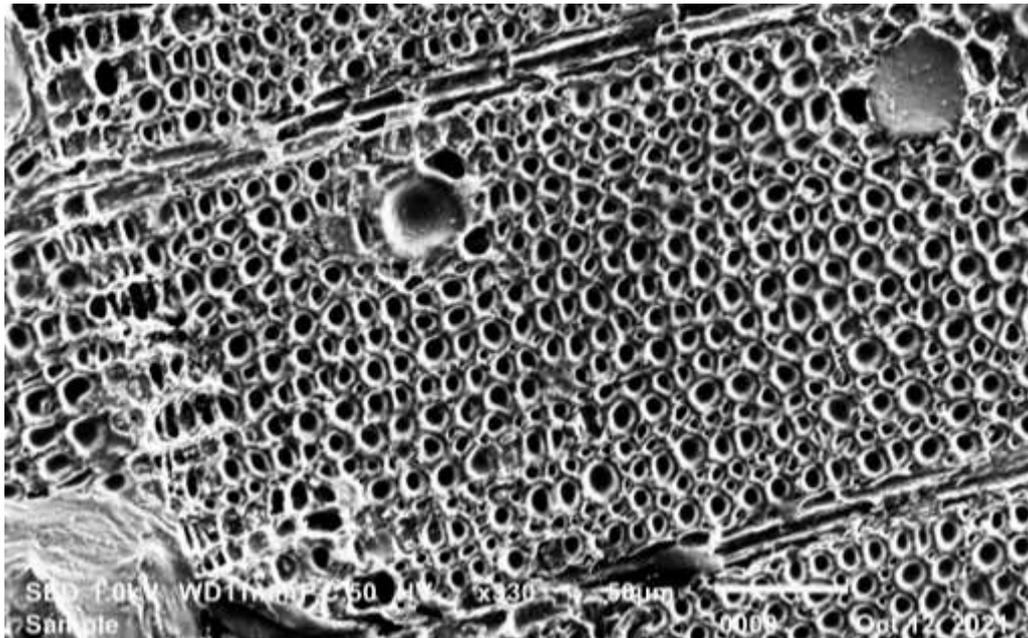
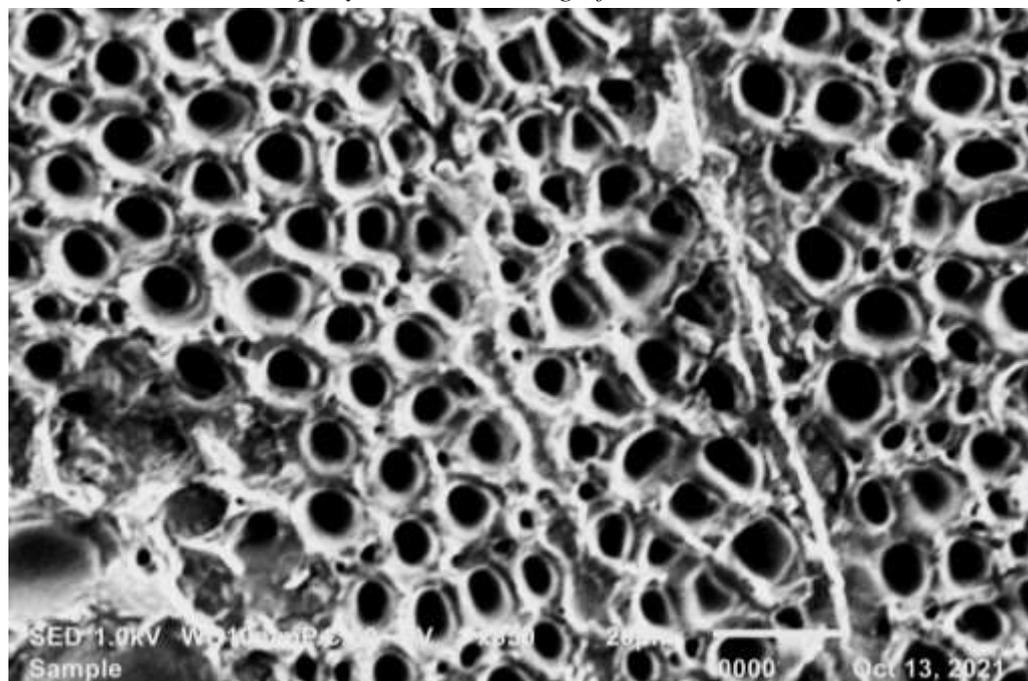


Figure 35: Shows the omitted southern section of the sample from figure 32. Embedded in epoxy resin, x35 magnification. Photo: Eric Hynes, 2021.



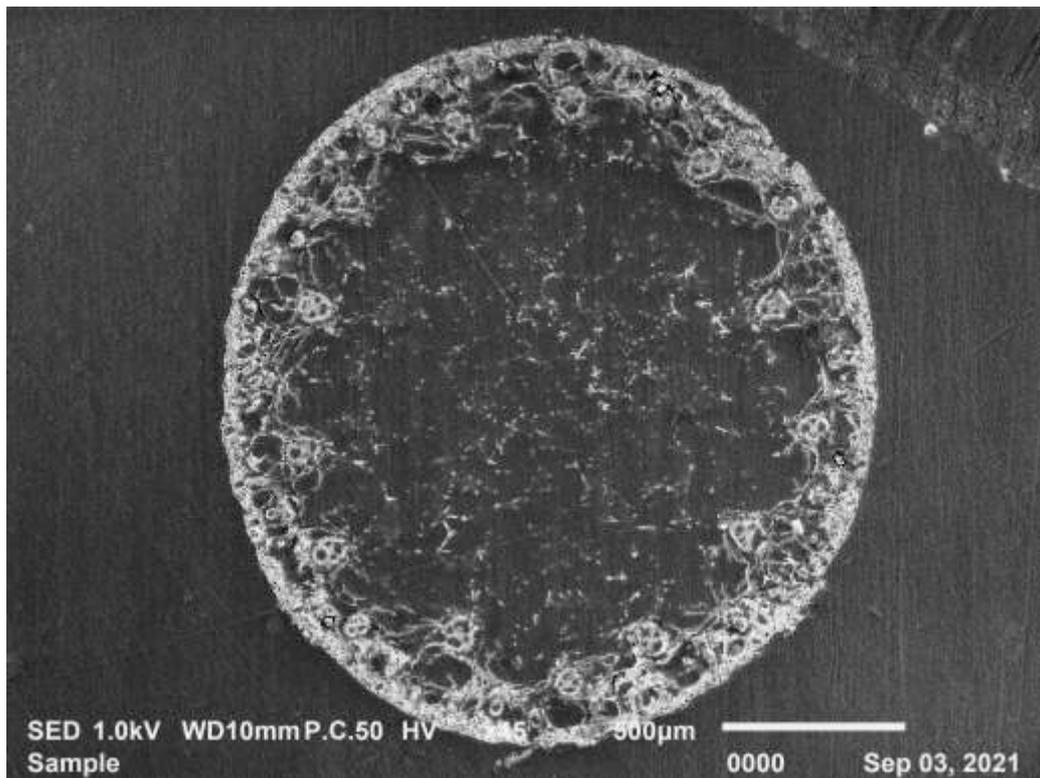
*Figure 36: An up-close perspective of the strong and numerous presences of deep pores of oak BC. Embedded in epoxy resin, x330 magnification. Photo: Eric Hynes, 2021.*



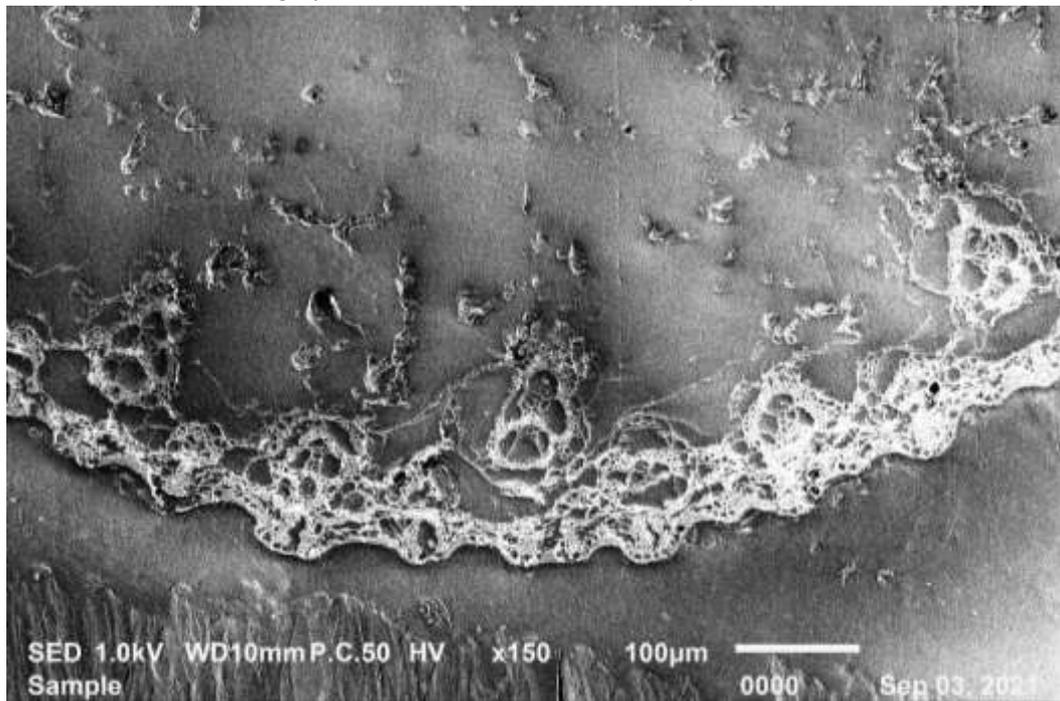
*Figure 37: An up-close perspective of the strong and numerous presences of deep pores of oak BC. Embedded in epoxy resin, x850 magnification. Photo: Eric Hynes, 2021.*

#### **4.11.8 SEM Images of Rush BC**

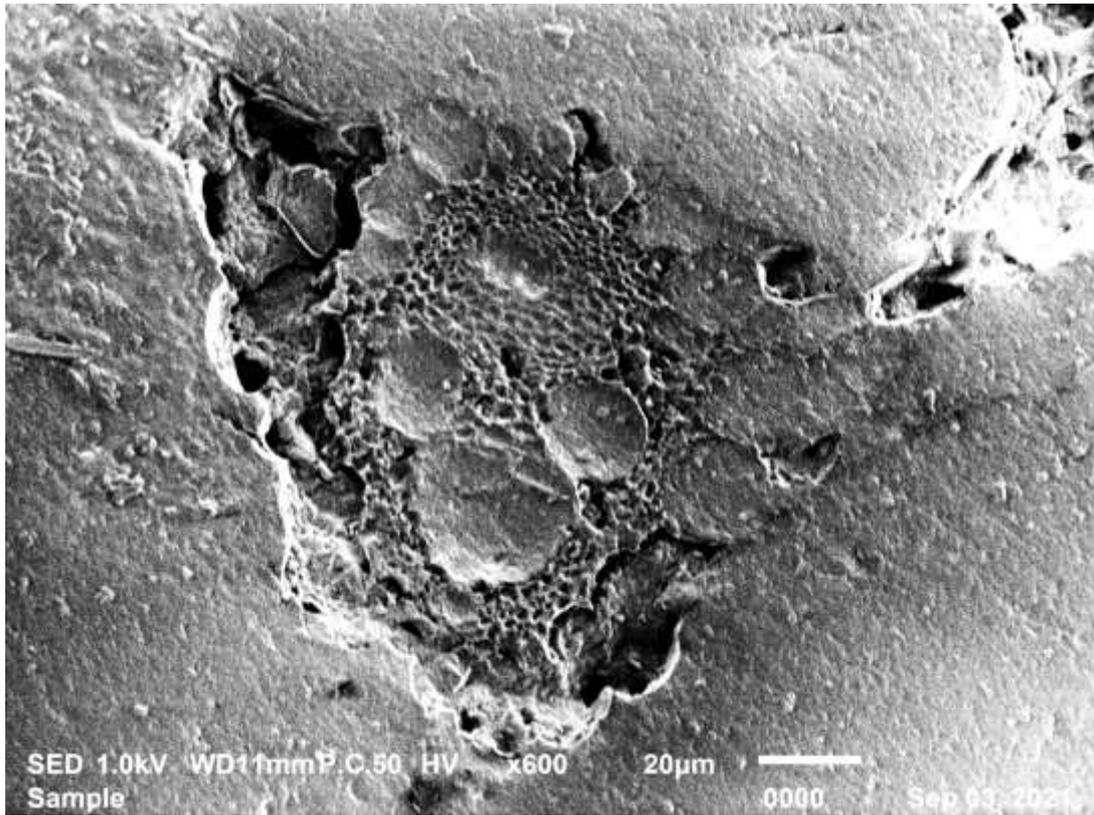
The SEM images below (fig.38-40) exhibit the sparse nature of rush BC pores. The epidermal tissues of the plant (vascular bundle etc) is where these pores reside after pyrolysis occurs. While the larger central area of the culm pith see no formation of pore structures. The BC pieces were photographed by high def SEM at various magnifications and subsequently mapped using ImageJ.



*Figure 38: Shows a whole cross section of a rush BC embedded in epoxy resin at magnification (x45). Photo: Eric Hynes, 2021.*



*Figure 39: Shows a section of rush BC embedded in epoxy resin (x150 magnification). Photo: Eric Hynes, 2021.*



*Figure 40: Shows a close up of a xylem and phloem plant anatomy of a rush BC embedded in epoxy resin at magnification (x600). Photo: Eric Hynes, 2021.*

#### **4.12 SEM Microscopy Experiment #2: The Preparing of Oak BC and Rush BC Samples for Qualitative EDS Elemental Analyses**

Furthering on from section 4.10, an experiment on the elemental analyses of BC was the next step in utilising the efficiency of SEM to compare and contrast one biochar against the other. Energy dispersive X-Ray spectroscopy (EDS) was performed on 10 samples of each BC to gain further insight into the elemental constituents on the materials.

##### **4.12.1 Hypothesis**

- **Does a hardwood BC like oak BC display the presence of different elemental constituents to that of a grass BC such as rush BC?**

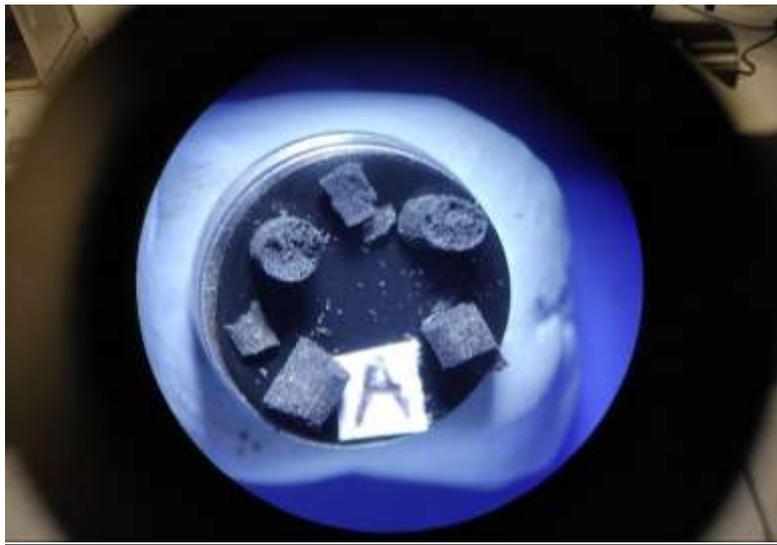
##### **4.12.2 Methodology of Preparing BC Samples on SEM Stubs**

The same methods used in section 4.10.1 were repeated here.

Once the 10 small pieces of each BC were ready, 4 x 12.55 mm diameter, high purity aluminium SEM pin stubs were taken from a pack of 100 (2 x stubs per biochar type). 4 x 12 mm double sided, electrically conductive, carbon adhesive discs (aka Leit tabs) were taken

from a pack of 100 and stuck carefully to the top of the stubs. Both the stubs and Leit tabs were manufactured and ordered from Agar Scientific Ltd.

Taking a piece of bluetack, a SEM stub was carefully pushed into the adhesive bluetack and placed in the centre of a light microscope stage. Here, using the light microscope to carefully observe and guide, small fragments of BC samples were lifted by forceps and placed onto the Leit tab and the orientation fixed slightly, if needed (fig.41). After all specimens were satisfactorily placed onto the stubs, the bluetack and SEM stubs with samples stuck to the Leit tab were carefully lifted and placed into the SEM stub specimen mount storage box. These were then transported to the SEM and subsequently loaded into the SEM for EDS analyses.



*Figure 41: Shows pieces of fragmented rush BC, carefully placed on top of a cohesive Leit tab resting on top of the SEM pin stub, through the lens of a light microscope.*

*Photo: Eric Hynes, 2021.*

#### **4.12.3 Microscopy Experiment #3 Results: Qualitative EDS Elemental Analyses of Oak BC**

Observations of the qualitative data produced by the 10 EDS spectra below confirm the presence of primarily carbon, and secondarily oxygen in all 10 samples of oak BC tested by EDS in the SEM. The spectra for replicates #1-8 (fig. 42-49) confirmed the presence of just carbon while the remaining 2 replicates (fig.50-51) confirmed the presence of both carbon and oxygen, where spikes of carbon were up to 8 times greater than the spikes of oxygen.

It should be noted that even though there are observable spikes of data in the region of the x-axis where oxygen's presence should be confirmed, it can be seen the 'O' has not been assigned on the EDS spectra (except fig.50-51). If the duration of EDS performed on the samples was perhaps extended or shortened the presence of carbon may well have been detected.

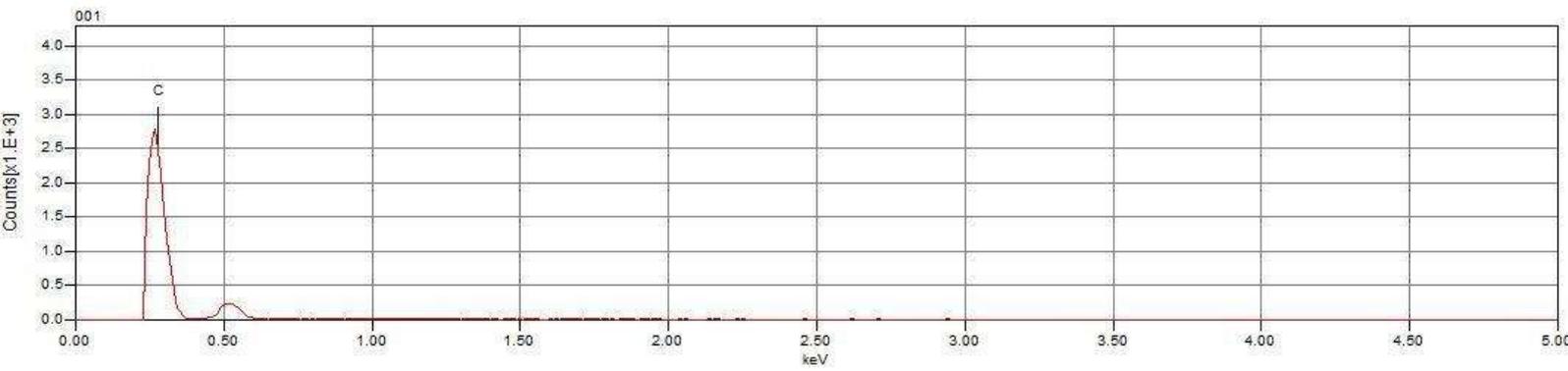


Figure 42: An EDS spectrum of oak BC (replicate #1) showing the presence of carbon.

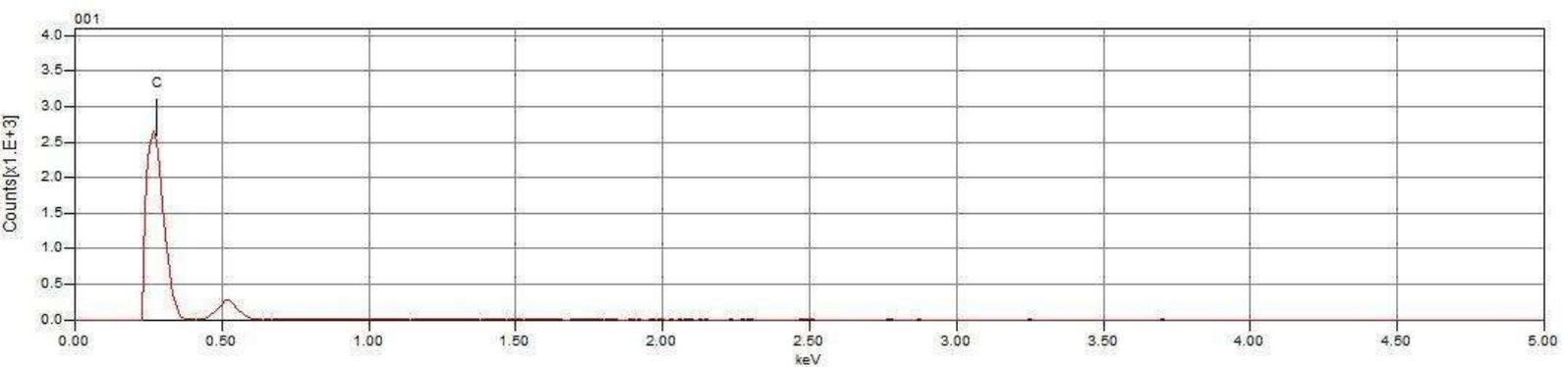


Figure 43: An EDS spectrum of oak BC (replicate #2) showing the presence of carbon.

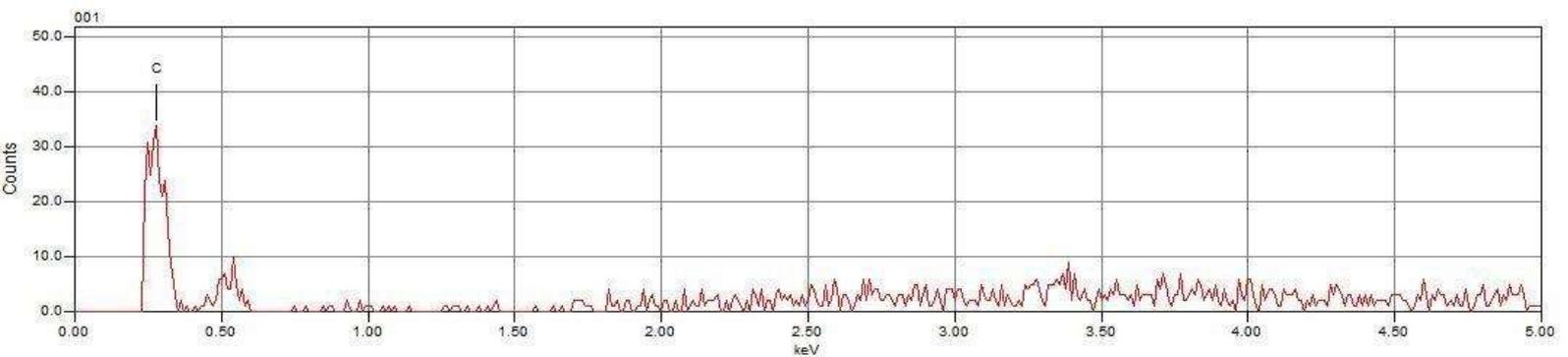


Figure 44: An EDS spectrum of oak BC (replicate #3) showing the presence of carbon.

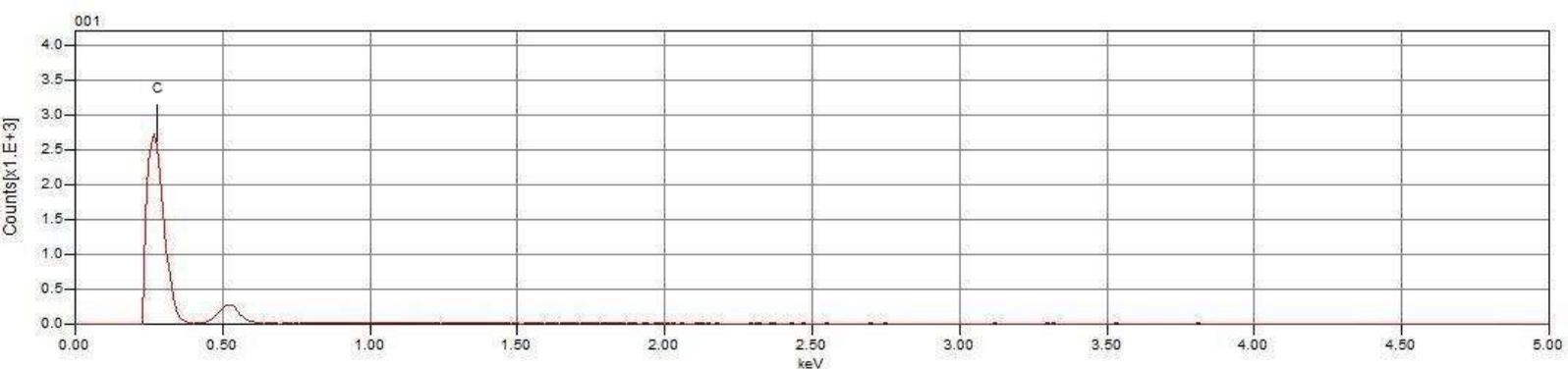


Figure 45: An EDS spectrum of oak BC (replicate #4) showing the presence of carbon.

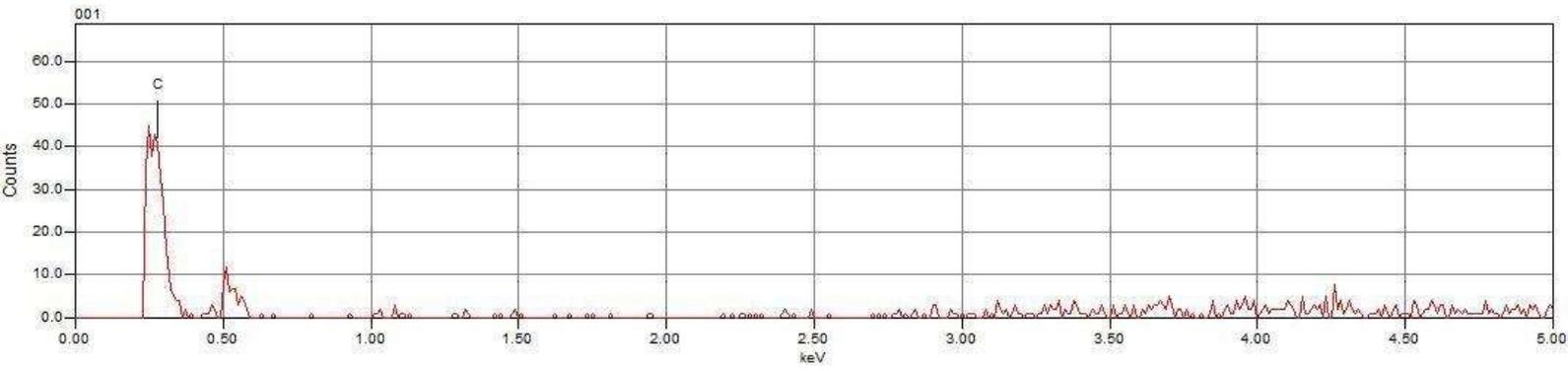


Figure 46: An EDS spectrum of oak BC (replicate #5) showing the presence of carbon.

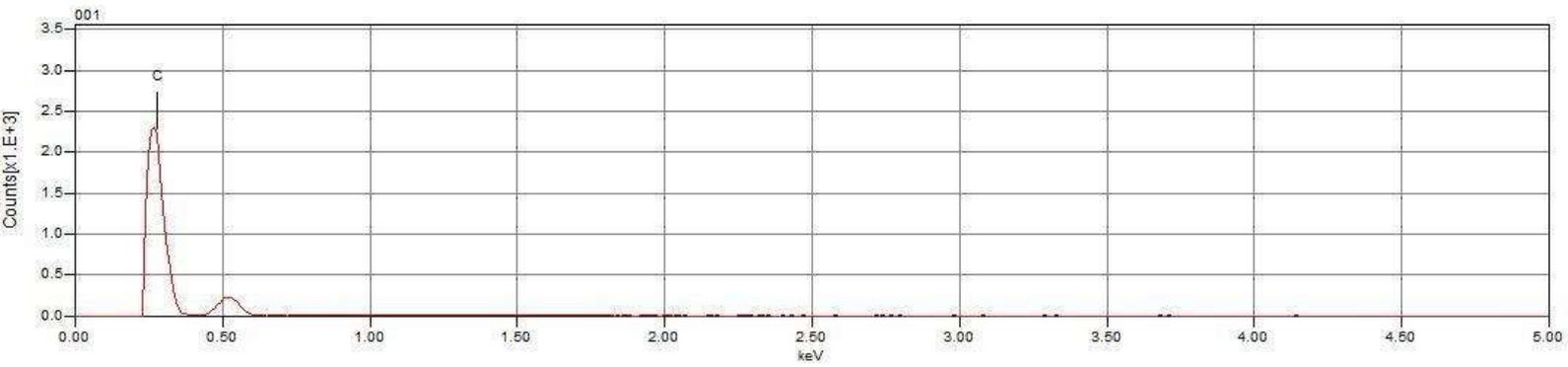


Figure 47: An EDS spectrum of oak BC (replicate #6) showing the presence of carbon.

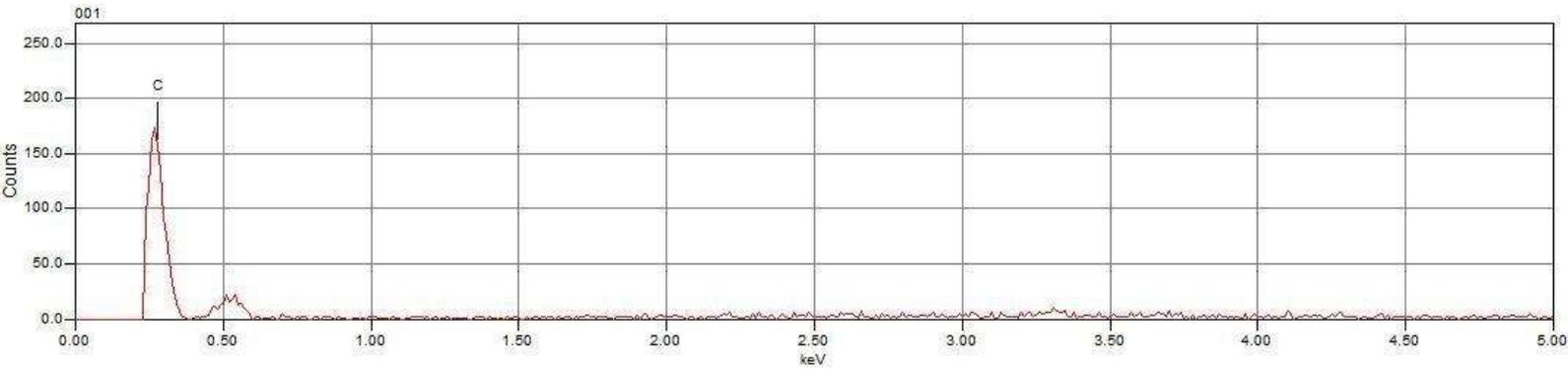


Figure 48: An EDS spectrum of oak BC (replicate #7) showing the presence of carbon.

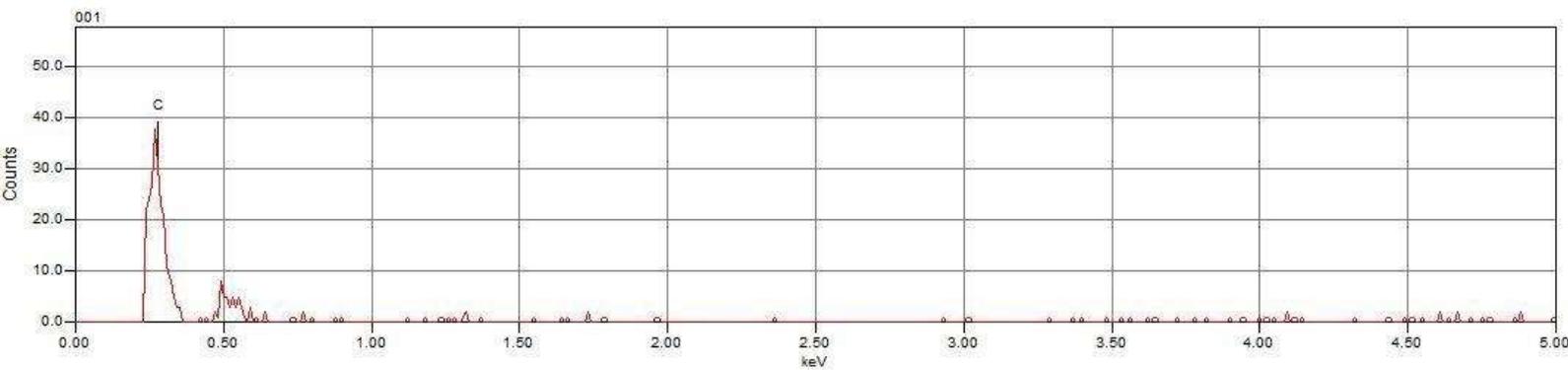


Figure 49: An EDS spectrum of oak BC (replicate #8) showing the presence of carbon.

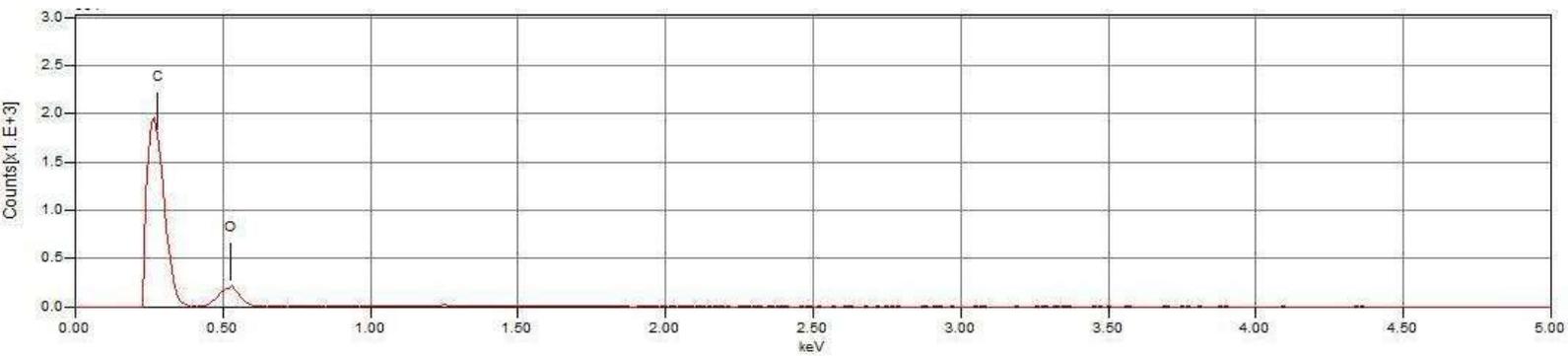


Figure 50: An EDS spectrum of oak BC (replicate #9) showing the presence of carbon and oxygen.

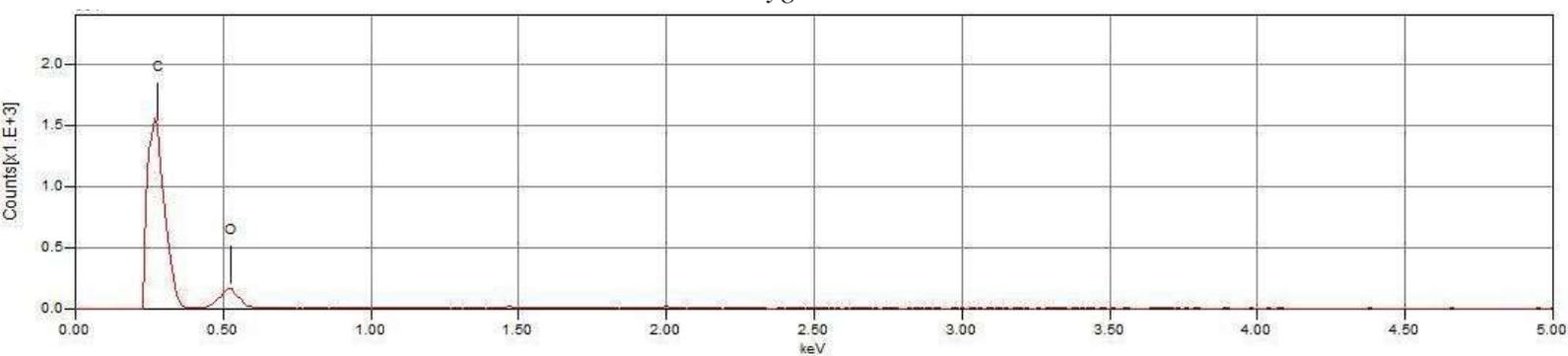


Figure 51: An EDS spectrum of oak BC (replicate #10) showing the presence of carbon and oxygen.

#### **4.12.4 Microscopy Experiment #4 Results: Qualitative EDS Elemental Analyses of Rush BC**

Observations of the qualitative data produced by the 10 EDS spectra above confirm the presence of carbon, oxygen, chlorine, potassium, sodium, magnesium, calcium and silicon in all 10 samples of rush BC tested by EDS in the SEM. The elements confirmed for each replicate (fig. 53-62) was as follows:

- Replicate #1: oxygen, sodium, phosphorous, chlorine, potassium and calcium
- Replicate #2: oxygen, sodium, magnesium, silicon, chlorine and potassium
- Replicate #3: chlorine and potassium
- Replicate #4: phosphorous chlorine potassium
- Replicate #5: carbon, sodium, phosphorous, chlorine and potassium
- Replicate #6: sodium, chlorine and potassium
- Replicate #7: chlorine and potassium
- Replicate #8: sodium phosphorous chlorine potassium
- Replicate #9: chlorine and potassium
- Replicate #10: phosphorous chlorine potassium

It should be noted that even though there are observable spikes of data in the region where carbon's presence should be confirmed, it can be seen that 'C' has not been assigned on the

EDS spectra (except fig.57). If the duration of EDS performed on the samples was perhaps extended or shortened the presence of carbon may well have been detected

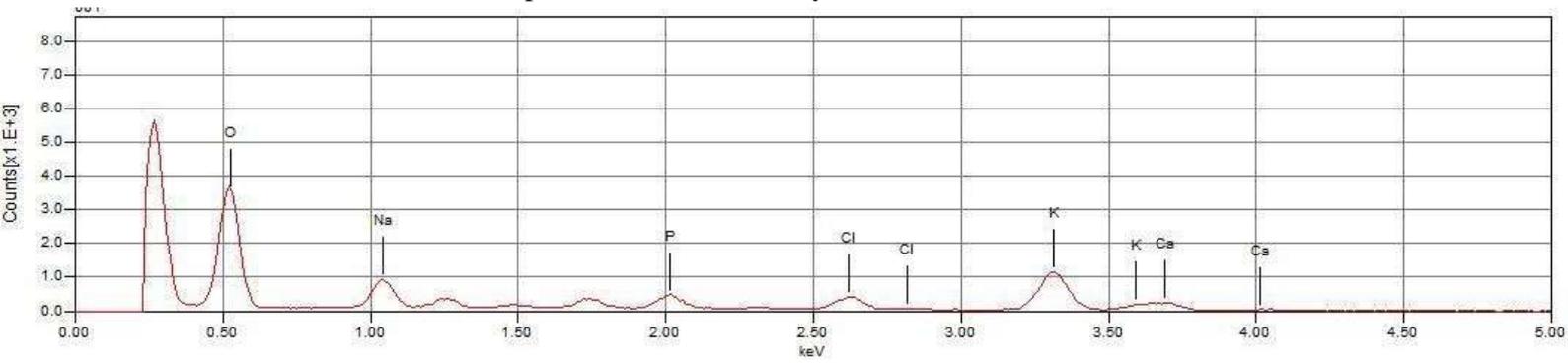


Figure 53: An EDS spectrum of oak BC (replicate #1) showing the presence of elements.

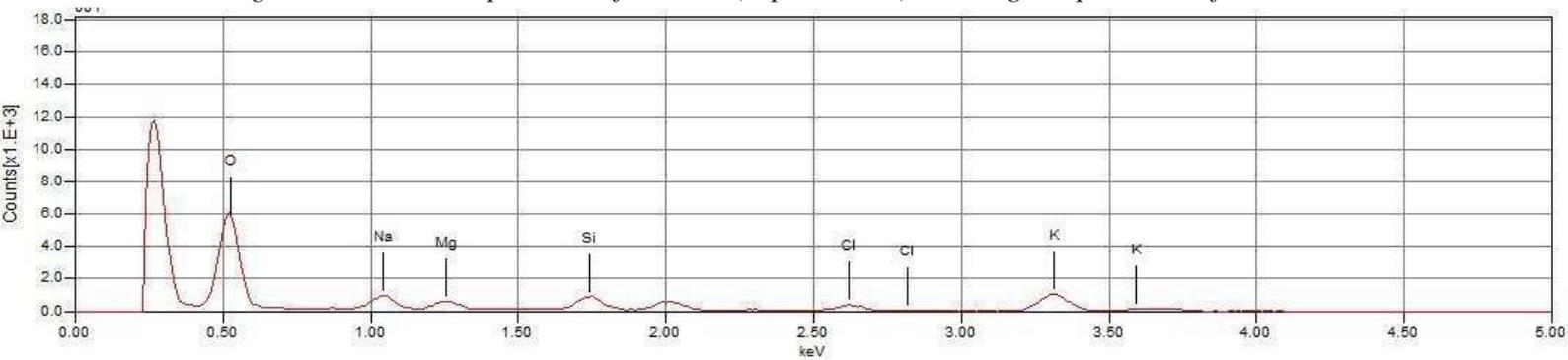


Figure 54: An EDS spectrum of rush BC (replicate #2) showing the presence of elements.

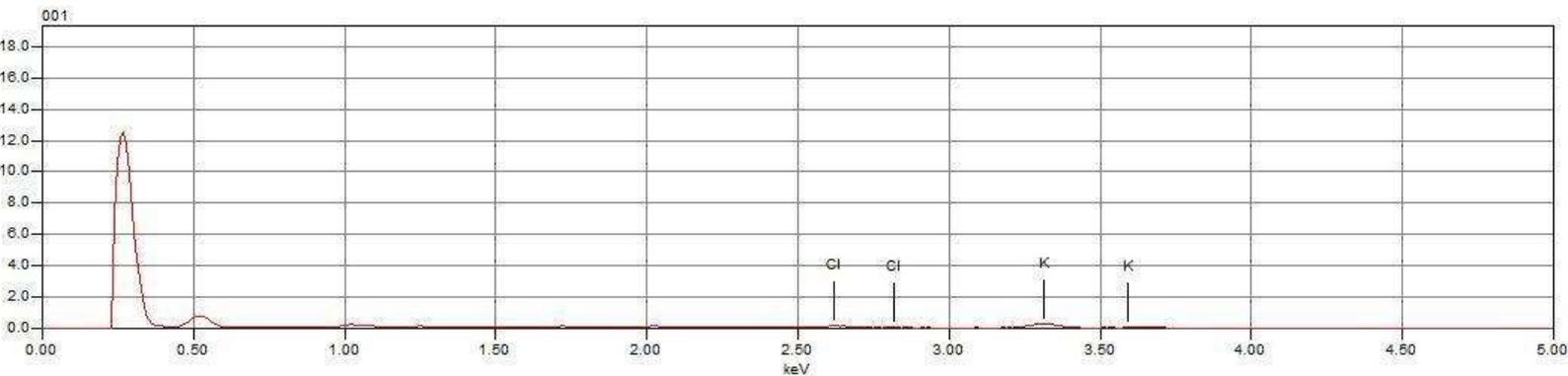


Figure 55: An EDS spectrum of rush BC (replicate #3) showing the presence of elements.

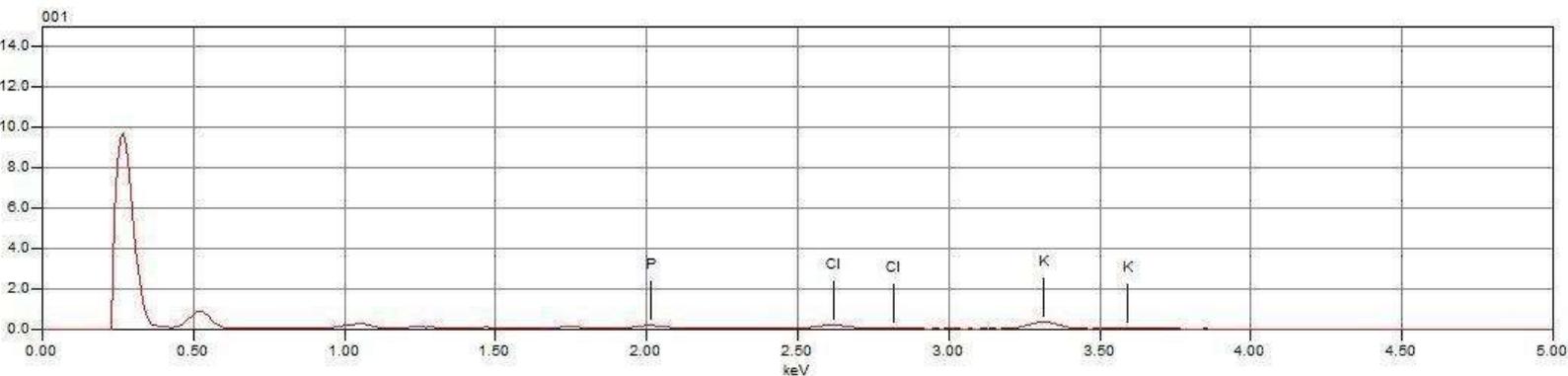


Figure 56: An EDS spectrum of rush BC (replicate #4) showing the presence of elements.

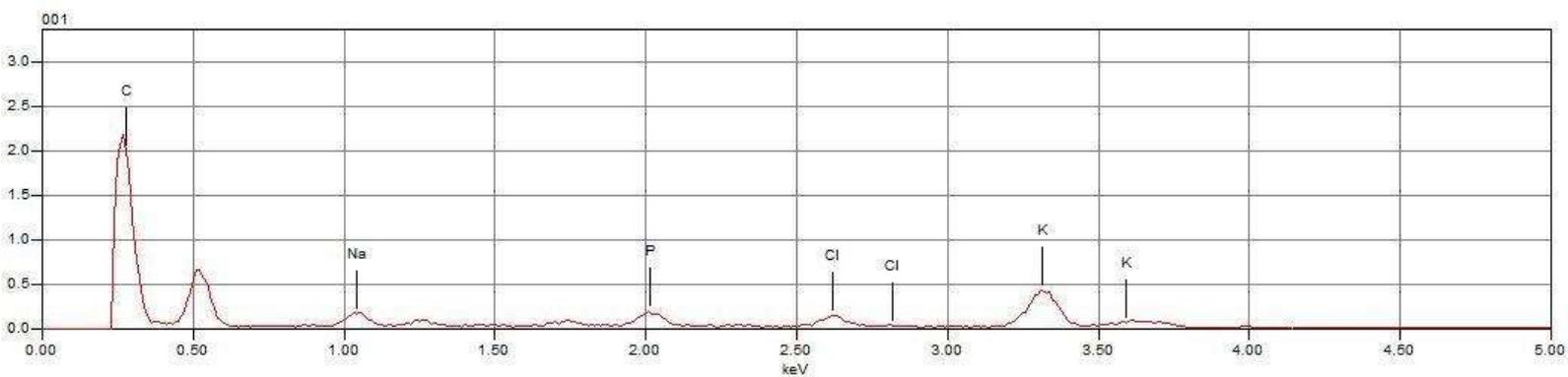


Figure 57: An EDS spectrum of rush BC (replicate #5) showing the presence of elements.

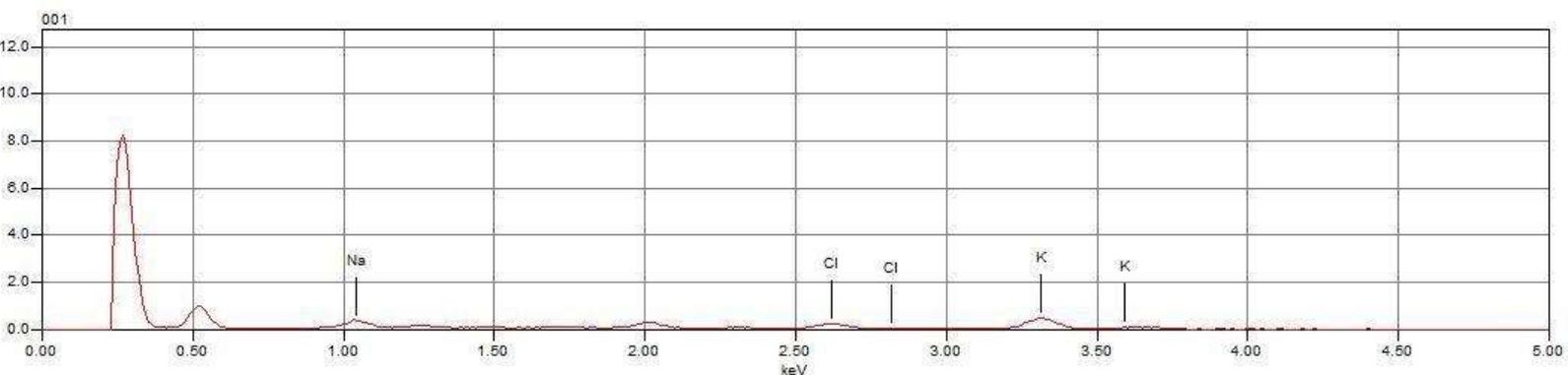


Figure 58: An EDS spectrum of rush BC (replicate #6) showing the presence of elements.

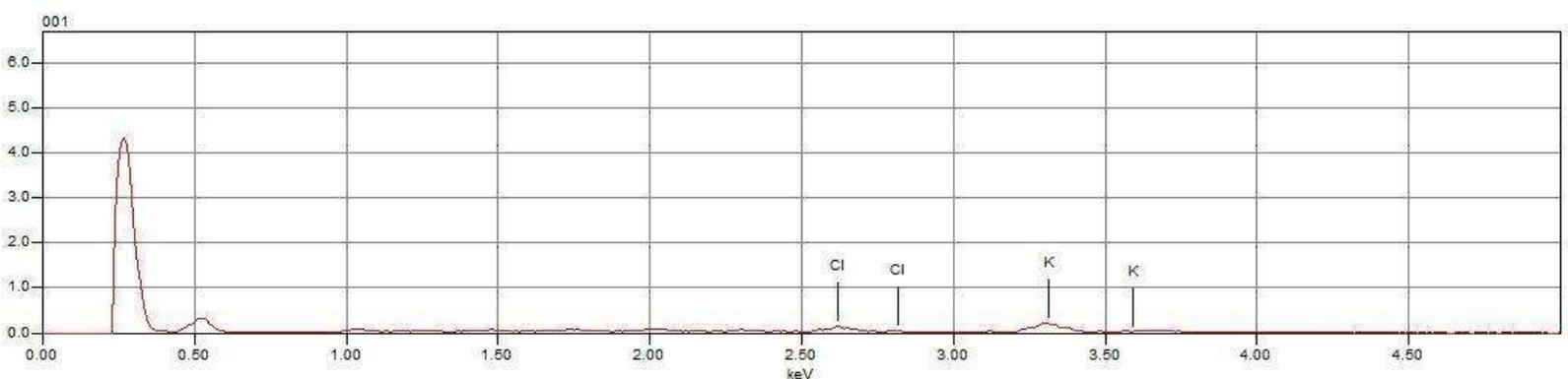


Figure 59: An EDS spectrum of rush BC (replicate #7) showing the presence of elements.

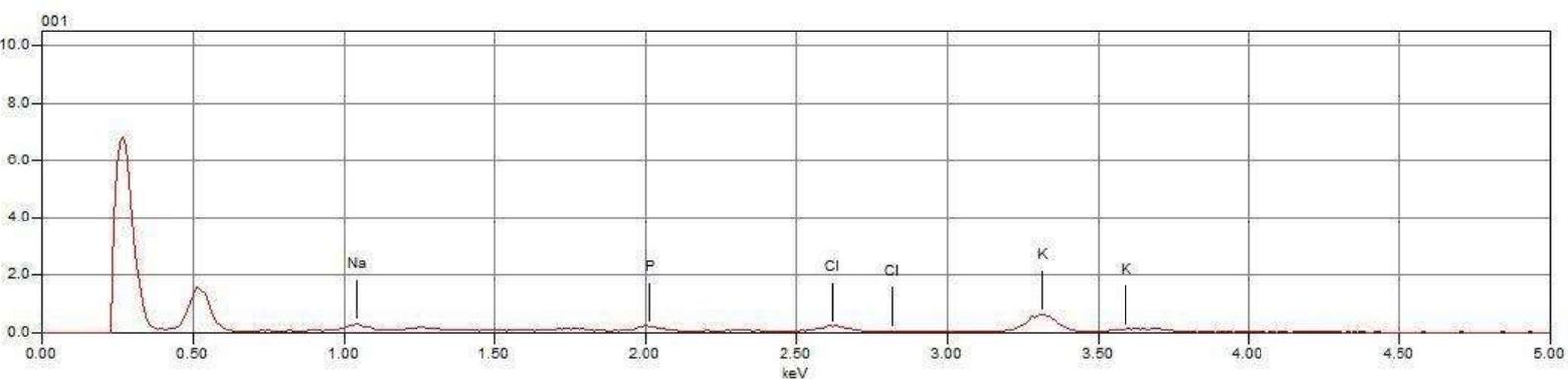


Figure 60: An EDS spectrum of rush BC (replicate #8) showing the presence of elements.

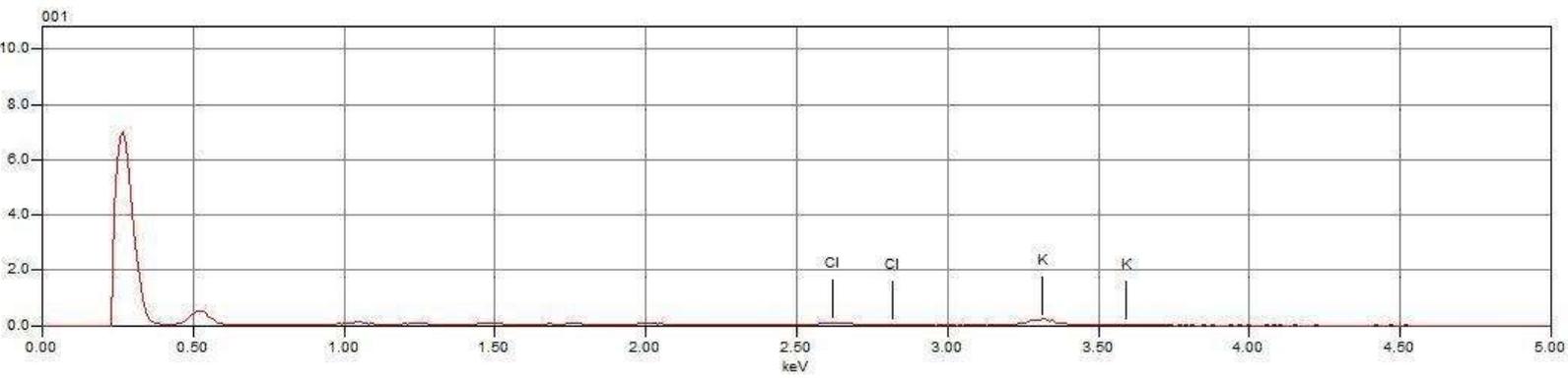


Figure 61: An EDS spectrum of rush BC (replicate #9) showing the presence of elements.

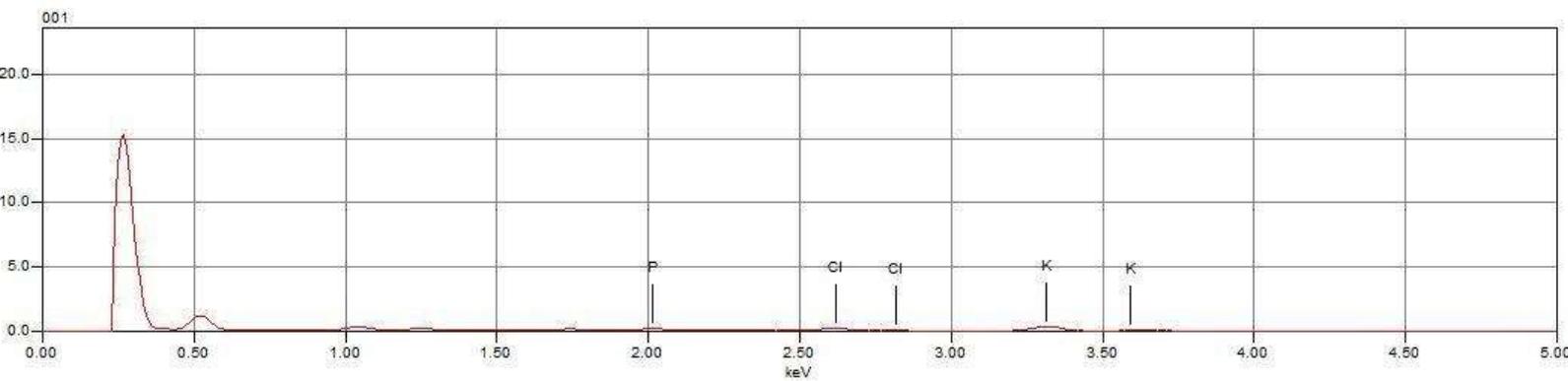


Figure 62: An EDS spectrum of rush BC (replicate #10) showing the presence of elements.

## 5. DISCUSSION

## **5.1 OSR Plant Growth Response to Treatments 1 and 2**

### **5.1.1 Response to Treatment 1**

A hypothesis was to test that treatment 1 could have an effect on OSR growth and yield. Looking at the data presented in section 4.5.4.1 (fig. 5), this mixture does not appear to affect the growth of OSR. Growth of OSR over the 6-week growth period was uniform across both treatments until after week 2 where growth remained similar until the end of the trial. Therefore, results were found to be statistically insignificant. The plant fresh weights and dry weight data were also found to not be significant.

Statistical significance aside, an observation of a recurring theme was noted. OSR plants grown without treatment 1 had higher yields than those grown with the treatment.. All OSR plant measurements with treatment 1 were negatively affected compared to the control. There have been many studies on short term applications of BC or COMBI (co-composted biochar) such as in this thesis, which have shown positive initial effects on soil N<sub>2</sub>O emissions but have failed to have a positive effect on plant growth and yield (Melaku et al., 2020; Bonanomi et al., 2017; Darby et al., 2016; Jay et al., 2015).

In 2015, Jay et al demonstrated that short term applications of hardwood BC from *Castanea sativa* into a sandy loam soil in the UK saw no statistically significant increases in plant growth. Specifically, they found no increases in potato (*Solanum tuberosum L.*) shoot growth, tuber quality or yield; strawberry (*Fragaria x ananassa*) leaf growth, yield or fruit quality; and spring wheat (*Hordeum vulgare L.*) plant growth or yield and grain quality. As mentioned before, those soils in greatest need of amelioration tend to have the most positive responses to BC treatments.

Naveed et al (2021) demonstrated that COMBI used in chromium contaminated soils, growing OSR had positive results. They found that the plant growth of OSR (height, shoot dry weight, root dry weight, root length) and phytostabilization were significantly increased while also significantly reducing enzymatic antioxidant activities against chromium related stress.

A putative reason for the absence of an increase in crop growth of OSR with a short-term oak biochar treatment is the lack of additional P being made available to the plant (Jay et al., 2015). Initial biochar applications without the application of extra P can lead to direct decline in available P (Jay et al., 2015; Nelson et al., 2011).

### **5.1.2 Response to Treatment 2**

To test whether treatment 2 had an effect on OSR growth and yield was another hypothesis of this thesis. In glasshouse experiment #2, the control and test replicates demonstrated an

observable but insignificant difference ( $p = 0.4803$ ) in plant height over the 6-week growth period. Plants treated with treatment 2 appeared to have reduced growth compared to that of the control (fig.12). Again, there was an observable but insignificant difference between the means for OSR plant fresh and dry weights (fig.13) with a  $p$ -value of 0.424, results were not significant.

In section 5.1.1. OSR plants were negatively affected by treatment 2 compared to the control. A study by Thers et al (2020) showed that after an initial growing season of OSR in a sandy loam soil with a wheat (*Triticum aestivum L.*) straw BC (a grass BC more similar to rush BC), there was no significant increase in plant growth or yield. Wang et al (2019) showed in a meta-analysis that grain yield was only significantly increased (up to 39.7%) in cereal crops ( $p < 0.001$ ) like maize (*Zea mays*), barley (*Hordeum vulgare*), quinoa (*Chenopodium quinoa*), wheat (*Triticum aestivum*) and oat (*Avena sativa*) compared to other plants like bok choy (*Brassica rapa*) and OSR.

Another study demonstrated how OSR grown in a sandy loam soil was not affected after each growing season by the presence of a wheat (*T. aestivum L.*) BC, even after 3 consecutive years of application (Hansen et al., 2017). A meta-analysis by Wang et al (2019) of crops grown with a COMBI treatment showed that only a statistically significant increase in yield was experienced by cereal crops. That other plants such as OSR did not show a significant positive difference in yield, however the control (compost) did show a significant increase.

However, another study showed that wheat (*T. aestivum L.*) straw biochar inoculated with inorganic phosphate solubilizing bacteria (iPSB), saw positive OSR growth due to the biochar sorption abilities, making available phosphate made possible by the iPSB, available to the plant (Zheng et al 2019).

## **5.2 PRG Plant Growth Response to Treatment 1 and Treatment 2**

### **5.2.1 Response to Treatment 1**

A hypothesis was to test that treatment 1 could have an effect on PRG growth and yield. Looking at the data from the tables presented in section 4.5.3 there appeared to be little difference between plant height ( $p = 0.7683$ ), fresh weight ( $p = 0.5465$ ), dry weight ( $p = 0.519$ ) and dry weight values. All 3 parameters were found to be insignificant. Mean plant heights varied at the end of week 6, OSR plants were found to be positively affected by the test treatment than the control, but only slightly.

Plant yields have been known to be neutrally or negatively affected by the over-liming effect of a BC (Vijay et al., 2021). Woody BC (such as oak) can have an inherently high pH and when in the presence of soil which is acidic can have a negative or neutral impact on plant growth and yield (Shetty and Prakash., 2020). On average composts are between 6 - 8 pH (Taylor et al., 2016), however some composts can be as low as 4.5 (Jamal et al., 2018).

### **5.2.2 Response to Treatment 2**

The effect of treatment 2 (fig.11) on plant height appears to have had a positive effect on PGR growth here compared to the control treatment and all previous test treatment trials (sections 5.2.1, 5.1.2 and 5.1.1). Looking at the plant fresh and dry weights the same can be observed, both weights for the test treatment have been more positively and significantly affected than the control treatment. This would suggest that treatment 2 had a greater effect on PRG as a target crop.

Głąb et al (2020) demonstrated in a sandy loam soil potted, glasshouse experiment that 2 grass biochars *Miscanthus* (*Miscanthus x giganteus*) and wheat (*Triticum aestivum L.*) both had positive effects on PRG growth and productivity. Their results saw highly significant effects of almost double the biomass yields and root dry matter density than that of the control. Głąb et al also found that root length density, root volume density and root surface area were all significantly greater than that of the control.

In 2016, Vandecasteele et al saw a significant increase in PRG of 34.7% when using a holm oak (*Quercus ilex*) COMBI treatment in field trials.

### **5.3 OSR Plant Growth Response to Treatments 3 and 4 with Abiotic Stress Exposure**

To obtain positive results for OSR growth and yield in the presence of treatment 1 and treatment 2, large amounts of the treatments (the same ratios and components used in glasshouse experiments #1 and #2, uncharged) were left outside to ‘charge’. This was an attempt to incubate the BC and PF compost and determine its plant growth promoting effect. Allowing the BC more exposure for nutrient absorption in the presence of the PF compost and atmosphere.

After subsequently repotting for the following revised experiments, the same plant species were tested against both charged treatments concurrently with an abiotic stressor.

#### **5.3.1 OSR Response to Treatments 3 and 4 with Abiotic Stress Exposure**

Treatment 3 and 4 did have an effect on OSR plants. However, these were both negative effects compared to the control.

In this experiment an attempt was made to establish if a BC based, growth promoting treatment media could potentially fill the gap left by the phasing out of peat used for peat-based composts. Since many before have used BC in combination with composts as COMBI to induce positive plant growth (Wang et al., 2019; Bonanomi et al., 2017; Darby et al., 2016; Jay et al., 2015) either with or without added fertilisers, it was hypothesised that leaving mixtures outside for long periods could significantly charge the treatments for subsequent repotting. Schmidt (2008) had said that a minimum of two weeks was required to charge a BC without any extra chemical/fertiliser components needing to be added.

However, when comparing the observational and statistically significant differences between experiments 1 and 2 to these charged treatment experiments, the latter appears to have had a much greater (albeit negative) effect on plant growth.

It is likely that this decreased growth effect on OSR is a matter of nitrogen immobilisation caused by the overly high ratio of nitrogen to carbon within the charged treatment (Vijay et al., 2021). When microbes typical of the soil or in the mixture are experiencing growth and proliferation, they require a balanced N:C ratio of 1 mol of N for every 10 mol of C as food (Bonanomi et al 2017). If there is not a sufficient amount of nitrogen in the mix when an extra carbon source is added, this leads to the scavenging by microbes of available N in the soil/mixture. Thus, leaving too little N for the plant to assimilate and greatly affecting the growth of the plant (Bonanomi et al 2017). Another cause of nitrogen immobilisation is the insufficient pyrolysis of a feedstock into biochar, which can leave a lot of unnecessary carbon available to soil microbes (Brewer and Brown., 2012). Thus, negatively affecting plant or crop growth.

Despite all the above, many COMBI experiments have improved crop yields when applied either in the first year of application or in the subsequent years following an initial COMBI application.

Willow wood co composted with bagasse, green waste, poultry fertiliser increased grain yields of maize by 10% to 13% compared to the control (Agegnehu et al., 2016). The same COMBI experiment saw increases of peanut pod yields by 17 % to 24% compared to the control.

A COMBI made with pyrolyzed wood chippings, compost and horse, poultry and cow manure was left to charge for 60 days (Kammann et al., 2015). This COMBI mixture was grown with quinoa and in the first growing season obtained a very large yield of 305% whilst the mixture of uncharged biochar showed a 60% decrease in yield compared to the control. The composting process used was proven by scanning electron microscopy to have a direct effect on the BCs organic content (Kammann et al., 2015).

#### **5.4 PRG Plant Growth Response to Treatments 3 and 4 with Abiotic Stress Exposure**

Both treatments were demonstrated to have a statistically significant effect on the growth on the PRG plants. However, these effects were shown to be negative compared to the control, rather than the desired effect of positive growth promotion.

##### **5.4.1 PRG Response to Treatment 3 and 4 with Abiotic Stress Exposure**

Much like the response of OSR in section 5.3.1, the PRG response to the control:abiotic stressor combination out-performed both charged treatments. However, the charged

rush:abiotic stressor treatments was the most statistically significant and similar to the control:abiotic stressor treatments. Echoing the performance of treatment 2 with PRG plants (sections 4.6.3.3 and 4.6.3.4) the treatment 4 was statistically the better treatment out of the two charged treatments. Between the 3 combination treatments the salt stressed plants remained statistically similar.

As with section 5.3.1, nitrogen immobilisation is a likely mechanism within the charged treatments leading to decreased yields of the tested plants. The over-liming effect could also be a potential factor playing a role.

Another mechanism that may have influenced further decreases in plant growth and yields would be the exudation of allelopathic compounds by weeds which have colonised the large tubs of treatments 3 and 4 as a substrate over the 6-months. As much as was possible (every few weeks) every effort was made to remove the haulm-based portion of the juvenile weeds present (fig.63&64). Common Irish weeds such as Dandelion (*Taraxacum officinale*) Cat's Ear – (*Hypochaeris radicata*) Nipplewort – (*Lapsana communis*) Ragwort – (*Senecio jacobaea*) have been known to exudate allopathic compounds aqs biotic stressors to crops currently or subsequently growing in the substrate they are present (Możdżeń et al., 2021; Jankowska et al., 2014; Jacobs and Sing., 2009; Kim et al., 2005).



Figure 63 & 64: Firstly shows juvenile weeds growing on the charged biochar treatment substrate and the latter shows most of the weeds removed. Photos: Eric Hynes, 2021.

Remaining plant litter such as extensive roots systems (fig.65) left behind by germinated weeds (once again, every effort was made to remove as much as possible) can directly affect crop root growth and thus overall crop growth and yields or indirectly by mediating microbial activity within the pots (Bonanomi et al 2017). All of which could be working synergistically

with the N-immobilization and liming mechanisms, thus affecting subsequent crop growth further.



*Figure 65: Extensive roots left behind by the growth of weeds in the large tubs of biochar treatments being charged outside of the glasshouses. Photos: Eric Hynes, 2021.*

Regularly overturning a compost (every 3 to 7 days) or composting feedstock is needed to provide oxygen for microbial respiration, aerobic digestion, kills weed seeds and antagonises germinated weeds and provides greater moisture distribution (Dahlquist et al., 2007).

Lashari et al (2013) demonstrated that a COMBI mixture including wheat biochar, pyroligneous solution (aka wood vinegar - a pyrolysis by-product) and poultry fertiliser gave a 36.5% increase to wheat yields which were salt stressed in an antisol soil, the second year of crop growth. This result was in comparison to the control without the pyroligneous solution and also after neutral results obtained in the first growing season.

Schmidt et al (2017) demonstrated that a COMBI co composted with NPK fertilizer and resulted in yields around 20% with a standard deviation of 5.1% over 4 experiments testing different cash crops. However, another COMBI, this time co composted with cow urine produced yield of up to 123% with a standard deviation of 76.7% over 13 experiments testing different cash crops. However, these sorts of powerful N fertilising approaches could have potential detrimental effects to the environment via soil nitrate leaching (Haider et al., 2016).

### **5.5 OSR Stress Response to Treatments 3 and 4 with Abiotic Stress Exposure**

The SPAD measurements taken by the Photosynq Multispeq™ showed that the 3 categories of treatment, stressor and treatment:stressor interaction all significantly affected the plant health/stress response of OSR. Unfortunately, the surface area of PRG was too small for the

Photosynq Multispeq™ to be able to take a measurement. Therefore, it was omitted from this experiment.

SPAD measurements demonstrate leaf chlorophyll content and fluorescence. The higher the SPAD value means the more a plant is unhealthy and stressed from reactive oxygen species caused by oxidative stress caused by abiotic or biotic stressors (Chunjiang et al., 2007).

In section 4.10.3 it can be seen (fig.25) that the charged oak BC treatment:stressor treatment combinations had the greatest effect on positive plant health. Plants treated with oak BC but not stressed performed the best in this experiment as would be expected. Followed by plants treated with a charged rush BC treatment that were stressed, which were uniquely quite similar across the stressors and no stressor categories. Leaving the control:abiotic stressor treatment group to last, with drought stress being the worst (fig.25) affected, followed by salt stress and then no stress.



*Figure 66: The poor appearance of OSR plants growing in the control:drought stress plant pots (bottom right) with contrasting treatment 4:drought stress plant pots (top left). Photo: Eric Hynes, 2021.*

Haider et al (2020) demonstrated that the application of BC mitigated the negative effects of drought stress on wheat (*Triticum aestivum*). They showed that plants being drought stressed in the presence of a BC produced from the pyrolysis of wheat stems significantly increased crop yield (13.92%).

In a study by Kumar et al (2020) a hardwood BC was tested on a *Z. mays* crop exposed to salt stress. The greatest SPAD measurement was obtained in control plants with no BC (42.9) demonstrating BCs ability to alleviate the effects of stress caused by salt stress.

## **5.6 Quantitative Analyses of Both BCs Using Scanning Electron Microscope**

The results from section 4.10.5 show that the mean pore surface area of an oak BC is almost double (259.45  $\mu\text{m}$ ) that of a rush BC (133.24  $\mu\text{m}$ ) (fig.32). However, the two sample t-test which was run for these findings was not significant and this is most likely due to the very high variance sizes of the pore surface areas of both BC types.

The results from section 4.10.6 demonstrated that the mean pore frequency per BC surface area for oak BC was over 4 times (2164.79 $\mu\text{m}^2$ ) that of the rush BC (526.01 $\mu\text{m}^2$ ) (fig.33). However, as above, the two-sample t-test which was run for these findings was not significant and this is most likely due to very high variance found between the pore frequencies of the BCs (especially oak BC) and varying surface area sizes of each biochar sample.

The sheer magnitude of structural differences exerted between the two types of biochars can be explained by the percentage of lignin, cellulose and hemicellulose content constituting the biomass of a given species of feedstock (Rangabhashiyam and Balasubramanian, 2019).

Trees (such as oak) and woody plants are known to have high levels of lignin to cellulose and hemicellulose content (Novaes et al., 2010). Pore composition (size and frequency) of a biochar is influenced by the percentage of lignin content of a feedstock and the pyrolysis temperature (Břendová et al., 2017). The temperature needed for polymer decomposition to form a BC from a high lignin feedstock is between 250-500°C (Kong et al., 2014). The frequency of micropores can be increased by elevating the pyrolysis temperature and the amount of lignin available to the pyrolysis regime (Břendová et al., 2017).

Grasses (such as rush) and herbaceous plants have inherently low amounts of lignin compared to a high cellulose and hemicellulose content and are said to have a lignocellulosic biomass (Waliszewska et al., 2021). The temperature needed for polymer decomposition to form a BC from a lignocellulosic feedstock is between 200-350°C (hemicellulose) and 305-375°C (Kong et al., 2014). Thus the lack of lignin content and lower temperature needed for polymer decomposition results in far fewer pores. This explains the large differences in pore frequencies between the 2 types of BC.

### **5.7 Qualitative Analyses of Both BCs Using Scanning Electron Microscope**

The qualitative results generated by energy dispersive X-Ray spectroscopy in section 4.11.2 show us that C and O were the elements present in both BC types. However, with the oak BC it can be seen that these elements were the only one's present. Whereas with the rush BC there was also Cl, K, P, Na, Mg, Ca and Si present in the samples.

The presence of C and O in biochar samples are due to a couple of reasons. The condensed aromatic structures of the feedstock biomass have C as the principal element, controlling the organic phase of the BC (Uchimiya et al., 2011). Furthermore, O is a central elemental to the

organic, polar functional groups (Uchimiya et al., 2011). These both influence the reactivity of a BC within a soil ecosystem (Bakshi et al., 2020).

The presence of the Cl, K, P, Na, Mg, Ca and Si elements found in the rush BC are based on whether a low or high heating rate was used and the overall pyrolytic temperature. For lignocellulosic biomasses a low rate of 2 - 5°C/min should be utilized (Mohanty et al., 2013). The agricultural and geographical location of where a plant has grown can also influence what microelements which are present in a lignocellulosic biomass (Bakshi et al., 2020).

## **6. CONCLUSIONS AND RECOMMENDATIONS**

Overall, the addition of an oak BC and rush BC to a peat-free compost as separate growth media treatments had mostly neutral to negative effects on plant-growth promotion. However, the positive effect of rush BC on PRG in treatment 2 and 4 would suggest that PRG may be an ideal target plant for rush BC or as part of a BC and peated/PF compost treatment. Furthermore, PRG has known green manure capabilities which directly affect soil health (Vystavna et al., 2020) e.g. the plants extensive root system mitigates nitrogen loss via leaching and soil physical structure via amelioration of soil compaction or drought affected soils (Mauro et al., 2015). If PRG was used synergistically with a rush BC or COMBI treatment could further benefit a particular soil experiencing low soil health/productivity.

The effort to develop a potentially new COMBI method/alternative growth media to induce positive plant growth was largely unsuccessful. As detailed in this project, the COMBI approach to BC application has seen many benefits in crop production/soil health amelioration. However, these COMBI studies share a common theme - BC was added at the start of composting (a COMBI standard), therefore aerobic digestion of plant material and reactions with BC is key to charging a BC. Antonangeloe et al (2021) showed that mixing a BC with a mature compost yielded neutral to negative results in plant growth, compared to the standard COMBI method.

The Irish horticultural industry is in desperate need of a replacement to peat which is still being imported into the country in large quantities. They are calling on the Irish government to allow peat harvesting from Irish bogs while increasing research funding for suitable alternatives i.e. BC (O'Sullivan., 2021). The banning of peat for environmental reasons makes little sense when such quantities are being imported for horticultural and domestic uses.

Given the lack of positive plant growth increases in temperate soils, further BC potted trials should also include the target soil type with its COMBI mixture i.e. podzol with a standard COMBI mixture. Directing further BC research to find the right target crops/soil types and pyrolysis regime of a feedstock to optimize COMBI production is essential for creating stable alternative growth media. Furthermore, increasing our understanding of the pH and electrical

conductivity of a BC and target soil type will allow us to optimise amendment capabilities for greater sustainable agriculture.

## **7. ACKNOWLEDGEMENTS**

The author would like to thank Dr. Eoin Lettice and Dr. Barbara Doyle Preswitch for their helpful guidance and feedback and Prof. Maria McNamara for providing SEM training and support. Also to the lab technicians at UCC BEES campus for their technical support as well all authors and researchers cited in the reference list.

## **8. REFERENCES**

Agegnehu, G., Bass, A.M., Nelson, P.N., Bird, M.I., 2016a. Benefits of biochar, compost and biochar–compost for soil quality, maize yield and greenhouse gas emissions in a tropical agricultural soil. *Sci. Total Environ.* 543, pp.295–306.

Aherne, R., 2017. The effect of biochar incorporation on soil biodiversity. BSc Applied Plant Biology. University College Cork. Cork, Ireland.

Akhter, A., Hage-Ahmed, K., Soja, G. and Steinkellner, S., 2015. Compost and biochar alter mycorrhization, tomato root exudation, and development of *Fusarium oxysporum* f. sp. *lycopersici*. *Frontiers in Plant Science*, 6, p.529.

Antonangelo, J.A., Sun, X. and Zhang, H., 2021. The roles of co-composted biochar (COMBI) in improving soil quality, crop productivity, and toxic metal amelioration. *Journal of Environmental Management*, 277, p.111443.

Asai, H., Samson, B.K., Stephan, H.M., Songyikhangsuthor, K., Homma, K., Kiyono, Y., Inoue, Y., Shiraiwa, T. and Horie, T., 2009. Biochar amendment techniques for upland rice production in Northern Laos: 1. Soil physical properties, leaf SPAD and grain yield. *Field crops research*, 111(1-2), pp.81-84.

Bakshi, S., Banik, C. and Laird, D.A., 2020. Estimating the organic oxygen content of biochar. *Scientific reports*, 10(1), pp.1-12.

Basu, P., 2018. Biomass gasification, pyrolysis and torrefaction: practical design and theory. *Academic press*, 5(2), pp. 160-161.

Batista, E.M., Shultz, J., Matos, T.T., Fornari, M.R., Ferreira, T.M., Szpoganicz, B., de Freitas, R.A. and Mangrich, A.S., 2018. Effect of surface and porosity of biochar on water holding capacity aiming indirectly at preservation of the Amazon biome. *Scientific Reports*, 8(1), pp.1-9.

- Besserer, H.B., Boiarkin, V., Rodman, D. and Nürnberger, F., 2016. Qualifying electrically conductive cold embedding-media for scanning electron microscopy. *Metallography, Microstructure, and Analysis*, 5(4), pp.332-341.
- Begum, N., Qin, C., Ahanger, M.A., Raza, S., Khan, M.I., Ashraf, M., Ahmed, N. and Zhang, L., 2019. Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in abiotic stress tolerance. *Frontiers in plant science*, 10, p.1068.
- Biederman, L.A. and Harpole, W.S., 2013. Biochar and its effects on plant productivity and nutrient cycling: a meta- analysis. *GCB bioenergy*, 5(2), pp.202-214.
- Břendová, K., Száková, J., Lhotka, M., Krulíková, T., Punčochář, M. and Tlustoš, P., 2017. Biochar physicochemical parameters as a result of feedstock material and pyrolysis temperature: predictable for the fate of biochar in soil?. *Environmental geochemistry and health*, 39(6), pp.1381-1395.
- Brewer, C.E. and Brown, R.C. 2012. Biomass and Biofuel Production, *Comprehensive Renewable Energy*. Elsevier, 5.18. Pp.357-384.
- Bonanomi, G., Ippolito, F., Cesarano, G., Nanni, B., Lombardi, N., Rita, A., Saracino, A. and Scala, F., 2017. Biochar as plant growth promoter: better off alone or mixed with organic amendments?. *Frontiers in plant science*, 8, p.1570.
- Cayuela, M.L., Sánchez-Monedero, M.A., Roig, A., Hanley, K., Enders, A. and Lehmann, J., 2013. Biochar and denitrification in soils: when, how much and why does biochar reduce N<sub>2</sub>O emissions?. *Scientific reports*, 3(1), pp.1-7.
- Caguiat, J.N., Arpino, G., Krigstin, S.G., Kirk, D.W. and Jia, C.Q., 2018. Dependence of supercapacitor performance on macro-structure of monolithic biochar electrodes. *Biomass and bioenergy*, 118, pp.126-132.
- Case, S.D., McNamara, N.P., Reay, D.S. and Whitaker, J., 2014. Can biochar reduce soil greenhouse gas emissions from a *Miscanthus* bioenergy crop?. *Gcb Bioenergy*, 6(1), pp.76-89.
- Chen, Z., Chen, B., Zhou, D. and Chen, W., 2012. Bisolute sorption and thermodynamic behavior of organic pollutants to biomass-derived biochars at two pyrolytic temperatures. *Environmental science & technology*, 46(22), pp.12476-12483.
- Cook-Patton, S.C., Leavitt, S.M., Gibbs, D., Harris, N.L., Lister, K., Anderson-Teixeira, K.J., Briggs, R.D., Chazdon, R.L., Crowther, T.W., Ellis, P.W. and Griscom, H.P., 2020. Mapping carbon accumulation potential from global natural forest regrowth. *Nature*, 585(7826), pp.545-550.
- Chunjiang, Z., Aning, J., Wenjiang, H., Keli, L., Liangyun, L. and Jihua, W., 2007. Evaluation of variable- rate nitrogen recommendation of winter wheat based on SPAD chlorophyll meter measurement. *New Zealand Journal of Agricultural Research*, 50(5), pp.735-741.

Dahlquist, R.M., Prather, T.S. and Stapleton, J.J., 2007. Time and temperature requirements for weed seed thermal death. *Weed Science*, 55(6), pp.619-625.

Darby, I., Xu, C.Y., Wallace, H.M., Joseph, S., Pace, B. and Bai, S.H., 2016. Short-term dynamics of carbon and nitrogen using compost, compost-biochar mixture and organo-mineral biochar. *Environmental Science and Pollution Research*, 23(11), pp.11267-11278.

Diaz, C.A., Shah, R.K., Evans, T., Trabold, T.A. and Draper, K., 2020. Thermoformed Containers Based on Starch and Starch/Coffee Waste biochar Composites. *Energies*, 13(22), p.6034.

DeLong, C., Cruse, R. and Wiener, J., 2015. The soil degradation paradox: Compromising our resources when we need them the most. *Sustainability*, 7(1), pp.866-879.

Dokoohaki, H., Miguez, F.E., Laird, D. and Dumortier, J., 2019. Where should we apply biochar?. *Environmental Research Letters*, 14(4), p.044005.

Dong, X., Li, G., Lin, Q. and Zhao, X., 2017. Quantity and quality changes of biochar aged for 5 years in soil under field conditions. *Catena*, 159, pp.136-143.

Droste, N., May, W., Clough, Y., Börjesson, G., Brady, M. and Hedlund, K., 2020. Soil carbon insures arable crop production against increasing adverse weather due to climate change. *Environmental Research Letters*, 15(12), p.124034.

Ducey, T.F., Ippolito, J.A., Cantrell, K.B., Novak, J.M. and Lentz, R.D., 2013. Addition of activated switchgrass biochar to an aridic subsoil increases microbial nitrogen cycling gene abundances. *Applied soil ecology*, 65, pp.65-72.

Elad, Y., David, D.R., Harel, Y.M., Borenshtein, M., Kalifa, H.B., Silber, A. and Graber, E.R., 2010. Induction of systemic resistance in plants by biochar, a soil-applied carbon sequestering agent. *Phytopathology*, 100(9), pp.913-921.

Elad, Y., Cytryn, E., Harel, Y.M., Lew, B. and Graber, E.R., 2011. The biochar effect: plant resistance to biotic stresses. *Phytopathologia Mediterranea*, 50(3), pp.335-349.

Engwa, G.A., Ferdinand, P.U., Nwalo, F.N. and Unachukwu, M.N., 2019. Mechanism and health effects of heavy metal toxicity in humans. *Poisoning in the modern world-new tricks for an old dog*, 1(10). pp.1-23.

Evans, C.D., Bonn, A., Holden, J., Reed, M.S., Evans, M.G., Worrall, F., Couwenberg, J. and Parnell, M., 2014. Relationships between anthropogenic pressures and ecosystem functions in UK blanket bogs: Linking process understanding to ecosystem service valuation. *Ecosystem Services*, 9, pp.5-19.

Feng, L., Xu, W., Tang, G., Gu, M. and Geng, Z., 2021. Biochar induced improvement in root system architecture enhances nutrient assimilation by cotton plant seedlings. *BMC plant biology*, 21(1), pp.1-14.

Fischer, D. and Glaser, B., 2012. Synergisms between compost and biochar for sustainable soil amelioration. *Management of organic waste*, 1. p.168.

Fox, A., Kwapinski, W., Griffiths, B.S. and Schmalenberger, A., 2014. The role of sulfur-and phosphorus-mobilizing bacteria in biochar-induced growth promotion of *Lolium perenne*. *FEMS Microbiology Ecology*, 90(1), pp.78-91.

Fuss, S., Lamb, W.F., Callaghan, M.W., Hilaire, J., Creutzig, F., Amann, T., Beringer, T., de Oliveira Garcia, W., Hartmann, J., Khanna, T. and Luderer, G., 2018. Negative emissions—Part 2: Costs, potentials and side effects. *Environmental Research Letters*, 13(6), p.063002.

Gao, X., Cheng, H.Y., Del Valle, I., Liu, S., Masiello, C.A. and Silberg, J.J., 2016. Charcoal disrupts soil microbial communication through a combination of signal sorption and hydrolysis. *Acs Omega*, 1(2), pp.226-233.

Genesio L, Miglietta F, Lugato E, Baronti S, Pieri M, Vaccari F.P ,2012. Surface albedo following biochar application in durum wheat. *Environmental Research Letters*, 7, p. 014025.

Głąb, T., Gondek, K. and Mierzwa-Hersztek, M., 2020. Pyrolysis improves the effect of straw amendment on the productivity of perennial ryegrass (*Lolium perenne* L.). *Agronomy*, 10(10), p.1455.

Glaser, B., Haumaier, L., Guggenberger, G. and Zech, W., 2001. The Terra Preta phenomenon: a model for sustainable agriculture in the humid tropics. *Naturwissenschaften*, 88(1), pp.37-41.

Graber, E.R., Harel, Y.M., Kolton, M., Cytryn, E., Silber, A., David, D.R., Tsechansky, L., Borenshtein, M. and Elad, Y., 2010. Biochar impact on development and productivity of pepper and tomato grown in fertigated soilless media. *Plant and soil*, 337(1), pp.481-496.

Gu, H. and Bergman, R., 2015. Life-cycle GHG emissions of electricity from syngas produced by pyrolyzing woody biomass. In *Proceedings of the 58th International Convention of Society of Wood Science and Technology June 7-12, 2015 Jackson Lake Lodge, Grand Teton National Park, Wyoming, USA*, pp. 376-389. (pp. 376-389).

Gwenzi, W., Chaukura, N., Wenga, T. and Mtisi, M., 2020. Biochars as media for air pollution control systems: Contaminant removal, applications and future research directions. *Science of the Total Environment*, p.142249.

Hagner, M., Kempainen, R., Jauhiainen, L., Tiilikkala, K. and Setälä, H., 2016. The effects of birch (*Betula* spp.) Biochar and pyrolysis temperature on soil properties and plant growth. *Soil and tillage Research*, 163, pp.224-234.

Haider, G., Joseph, S., Steffens, D., Müller, C., Taherymoosavi, S., Mitchell, D. and Kammann, C.I., 2020. Mineral nitrogen captured in field-aged biochar is plant-available. *Scientific reports*, 10(1), pp.1-12.

Haider G, Steffens D, Müller C, Kammann CI. 2016. Standard extraction methods may underestimate nitrate stocks captured by field-aged biochar. *Journal of Environmental Quality* 45: 1196–1204.

Haider, I., Raza, M.A.S., Iqbal, R., Aslam, M.U., Habib-ur-Rahman, M., Raja, S., Khan, M.T., Aslam, M.M., Waqas, M. and Ahmad, S., 2020. Potential effects of biochar application on mitigating the drought stress implications on wheat (*Triticum aestivum* L.) under various growth stages. *Journal of Saudi Chemical Society*, 24(12), pp.974-981.

Hanna, R., Abdulla, A., Xu, Y. and Victor, D.G., 2021. Emergency deployment of direct air capture as a response to the climate crisis. *Nature communications*, 12(1), pp.1-13.

Hanoğlu, A., Çay, A. and Yanık, J., 2019. Production of biochars from textile fibres through torrefaction and their characterisation. *Energy*, 166, pp.664-673.

Hansen, V., Müller-Stöver, D., Imperato, V., Krogh, P.H., Jensen, L.S., Dolmer, A. and Hauggaard-Nielsen, H., 2017. The effects of straw or straw-derived gasification biochar applications on soil quality and crop productivity: a farm case study. *Journal of environmental management*, 186, pp.88-95.

Harel, Y.M., Elad, Y., Rav-David, D., Borenstein, M., Shulchani, R., Lew, B. and Graber, E.R., 2012. Biochar mediates systemic response of strawberry to foliar fungal pathogens. *Plant and Soil*, 357(1), pp.245-257.

Harvey, O.R., Kuo, L.J., Zimmerman, A.R., Louchouart, P., Amonette, J.E. and Herbert, B.E., 2012. An index-based approach to assessing recalcitrance and soil carbon sequestration potential of engineered black carbons (biochars). *Environmental science & technology*, 46(3), pp.1415-1421.

Hazelton, P. and Murphy, B., 2016. *Interpreting soil test results: What do all the numbers mean?* CSIRO publishing, p.1.

He, F., Xu, C., Fu, X., Shen, Y., Guo, L., Leng, M., et al. (2018). The microRNA390/trans-acting short interfering RNA3 module mediates lateral root growth under salt stress via the auxin pathway. *Plant Physiol.* 177, 775–791.

Hilliard, M., 2021. Ireland starts importing peat following wind-up of domestic production. *The Irish Times*, 21 September. URL: <https://www.irishtimes.com/news/environment/ireland-starts-importing-peat-following-wind-up-of-domestic-production-1.4679135>

Hilscher, A. and Knicker, H., 2011. Carbon and nitrogen degradation on molecular scale of grass-derived pyrogenic organic material during 28 months of incubation in soil. *Soil Biology and Biochemistry*, 43(2), pp.261-270.

Huber, G.W., Iborra, S. and Corma, A., 2006. Synthesis of transportation fuels from biomass: chemistry, catalysts, and engineering. *Chemical reviews*, 106(9), pp.4044-4098.

Hossain, M.K., Strezov, V., Chan, K.Y. and Nelson, P.F., 2010. Agronomic properties of wastewater sludge Biochar and bioavailability of metals in production of cherry tomato (*Lycopersicon esculentum*). *Chemosphere*, 78(9), pp.1167-1171.

Ippolito, J.A., Cui, L., Kammann, C., Wrage-Mönnig, N., Estavillo, J.M., Fuertes-Mendizabal, T., Cayuela, M.L., Sigua, G., Novak, J., Spokas, K. and Borchard, N., 2020.

Feedstock choice, pyrolysis temperature and type influence biochar characteristics: a comprehensive meta-data analysis review. *biochar*, pp.1-18.

Jacobs, J. and Sing, S., 2009. Ecology and management of tansy ragwort (*Senecio jacobaea* L.). Invasive Species Technical Note MT-24. Bozeman, MT: US Department of Agriculture, Natural Resources Conservation Service. 13, p. 5.

Jaiswal, A.K., Frenkel, O., Tsechansky, L., Elad, Y. and Graber, E.R., 2018. Immobilization and deactivation of pathogenic enzymes and toxic metabolites by biochar: a possible mechanism involved in soilborne disease suppression. *Soil Biology and Biochemistry*, 121, pp.59-66.

Jaiswal, A.K., Alkan, N., Elad, Y., Sela, N., Philosoph, A.M., Graber, E.R. and Frenkel, O., 2020. Molecular insights into biochar-mediated plant growth promotion and systemic resistance in tomato against *Fusarium* crown and root rot disease. *Scientific reports*, 10(1), pp.1-15.

Jamal, Q., Lee, Y.S., Munir, S., Malik, M.S. and Kim, K.Y., 2018. The combined use of five bacteria including *Bacillus amyloliquefaciens* Y1 as biofertilizer in compost improved low bush blueberry growth, rhizosphere bacteria and enzyme at various pH. *Pakistan Journal of Agricultural Sciences*, 55(2).

Jankowska, J., Ciepiela, G.A., Jankowski, K., Kolczarek, R., Sosnowski, J. and Wiśniewska-Kadzajan, B., 2014. The allelopathic influence of *Taraxacum officinale* on the initial growth and development of *Festuca rubra* (L.). *Journal of Ecological Engineering*, 15(1).

Jay, C.N., Fitzgerald, J.D., Higgs, N.A. & Atkinson, C.J. 2015, "Why short-term biochar application has no yield benefits: evidence from three field-grown crops", *Soil use and management*, vol. 31, no. 2, pp. 241-250.

Jeffery, S., Abalos, D., Spokas, K.A. and Verheijen, F.G., 2015. Biochar effects on crop yield. *Biochar for environmental management: science, technology and implementation*, 2, pp.301-325.

Jeffery, S., Bezemer, T.M., Cornelissen, G., Kuyper, T.W., Lehmann, J., Mommer, L., Sohi, S.P., van de Voorde, T.F., Wardle, D.A. and van Groenigen, J.W., 2015. The way forward in biochar research: targeting trade-offs between the potential wins. *Gcb Bioenergy*, 7(1), pp.1-13.

Jeffery, S., Abalos, D., Prodana, M., Bastos, A.C., Van Groenigen, J.W., Hungate, B.A. and Verheijen, F., 2017. Biochar boosts tropical but not temperate crop yields. *Environmental Research Letters*, 12(5), p.053001.

Kammann, C.I., Schmidt, H.P., Messerschmidt, N., Linsel, S., Steffens, D., Müller, C., Koyro, H.W., Conte, P. and Joseph, S., 2015. Plant growth improvement mediated by nitrate capture in co-composted biochar. *Scientific reports*, 5(1), pp.1-13.

Kim, O.Y., Park, S.I., Jung, I.M. and Ha, S.Y., 2005. The allelopathic effects of aqueous extracts of *Hypochaeris radicata* L. on forage crops. *Journal of Life Science*, 15(6), pp.871-878.

- Kong, S.H., Loh, S.K., Bachmann, R.T., Rahim, S.A. and Salimon, J., 2014. Biochar from oil palm biomass: A review of its potential and challenges. *Renewable and sustainable energy reviews*, 39, pp.729-739.
- Kulp, S.A. and Strauss, B.H., 2019. New elevation data triple estimates of global vulnerability to sea-level rise and coastal flooding. *Nature communications*, 10(1), pp.1-12.
- Kumar S, M., Mounkaila Hamani, A.K., Sootahar, M.K., Sun, J., Yang, G., Bhatti, S.M. and Traore, A., 2021. Assessment of Acidic Biochar on the Growth, Physiology and Nutrients Uptake of Maize (*Zea mays* L.) Seedlings under Salinity Stress. *Sustainability*, 13(6), p.3150.
- Kuzyakov, Y., Bogomolova, I. and Glaser, B., 2014. Biochar stability in soil: decomposition during eight years and transformation as assessed by compound-specific <sup>14</sup>C analysis. *Soil Biology and Biochemistry*, 70, pp.229-236.
- Lashari, M.S., Liu, Y., Li, L., Pan, W., Fu, J., Pan, G., Zheng, J.J.J.J., Zheng, J.J.J.J., Zhang, X., Yu, X., Hai, F.L., Muhammad, S.L., Xiao, Y.L., Lashari, M.S., Liu, Y., Li, L., Pan, W., Fu, J., Pan, G., Zheng, J.J.J.J., Zheng, J.J.J.J., Zhang, X., Yu, X., 2013. Effects of amendment of biocharmanure compost in conjunction with pyroligneous solution on soil quality and wheat yield of a salt-stressed cropland from Central China great plain. *F. Crop. Res.* 144, pp.113–118
- Lazzari, L.K., Perondi, D., Zampieri, V.B., Zattera, A.J. and Santana, R.M., 2019. Cellulose/biochar aerogels with excellent mechanical and thermal insulation properties. *Cellulose*, 26(17), pp.9071-9083.
- Lee, J., Kim, K.H. and Kwon, E.E., 2017. Biochar as a catalyst. *Renewable and Sustainable Energy Reviews*, 77, pp.70-79.
- Lehmann, J., 2007. Bio-energy in the black. *frontiers in Ecology and the Environment*, v. 5, n. 7, p. 381-387.
- Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C. and Crowley, D., 2011. Biochar effects on soil biota—a review. *Soil biology and biochemistry*, 43(9), pp.1812-1836.
- Lehmann, J., Abiven, S., Kleber, M., Pan, G., Singh, B.P., Sohi, S., Zimmerman, A., 2015. Persistence of biochar in soil, In: Lehmann, J., Joseph, S. (Eds.), *Biochar for Environmental Management: Science, Technology and Implementation* (second edition). Rouge, New York, pp. 235-282.
- Mackin, F., Flynn, R., Barr, A. and Fernandez-Valverde, F., 2017. Use of geographical information system-based hydrological modelling for development of a raised bog conservation and restoration programme. *Ecological Engineering*, 106, pp.242-252.
- Major, J., Rondon, M., Molina, D., Riha, S.J. and Lehmann, J., 2010. Maize yield and nutrition during 4 years after biochar application to a Colombian savanna oxisol. *Plant and soil*, 333(1-2), pp.117-128.

Mani, A. and Sankaranarayanan, K., 2018. Heavy metal and mineral element-induced abiotic stress in rice plant. *Rice Crop: Current Developments*, 149.

Mann, C.C., 2002. The Real Dirt on Rainforest Fertility. *Science*, 297, pp. 920–923.

Margenot, A.J., Griffin, D.E., Alves, B.S., Rippner, D.A., Li, C. and Parikh, S.J., 2018. Substitution of peat moss with softwood biochar for soil-free marigold growth. *Industrial Crops and Products*, 112, pp.160-169.

Masiello, C.A., Chen, Y., Gao, X., Liu, S., Cheng, H.Y., Bennett, M.R., Rudgers, J.A., Wagner, D.S., Zygourakis, K. and Silberg, J.J., 2013. Biochar and microbial signaling: production conditions determine effects on microbial communication. *Environmental science & technology*, 47(20), pp.11496-11503.

McCormack, C., 2021. Vegetable growers protest against peat laws as sector ‘wipe out’ looms. *The Irish Independent*, 14 July. URL: <https://www.independent.ie/business/farming/tillage/vegetable-growers-protest-against-peat-laws-as-sector-wipe-out-looms-40652376.html>

McKenzie, N., Jacquier, D., Isbell, R. and Brown, K., 2004. Australian soils and landscapes: an illustrated compendium. CSIRO publishing, (15), p.96.

Melaku, T., Ambaw, G., Nigussie, A., Woldekirstos, A.N., Bekele, E. and Ahmed, M., 2020. Short-term application of biochar increases the amount of fertilizer required to obtain potential yield and reduces marginal agronomic efficiency in high phosphorus-fixing soils. *biochar*, 2(4), pp.503-511.

Meng, L., Sun, T., Li, M., Saleem, M., Zhang, Q. and Wang, C., 2019. Soil-applied biochar increases microbial diversity and wheat plant performance under herbicide fomesafen stress. *Ecotoxicology and environmental safety*, 171, pp.75-83.

Mohanty, P., Nanda, S., Pant, K.K., Naik, S., Kozinski, J.A. and Dalai, A.K., 2013. Evaluation of the physiochemical development of biochars obtained from pyrolysis of wheat straw, timothy grass and pinewood: effects of heating rate. *Journal of analytical and applied pyrolysis*, 104, pp.485-493.

Możdżeń, K., Barabasz-Krasny, B., Stachurska-Swakoń, A., Turisová, I. and Zandi, P., 2021. Germination and growth of radish under influence of nipplewort aqueous extracts. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 49(1), pp.12195-12195.

Murphy, S.R., 2017. Effect of biochar on weed emergence, seed germination. BSc Applied Plant Biology. University College Cork. Cork, Ireland.

O’Sullivan, A., 2015. The effect of biochar incorporation on soil biodiversity. MSc Organic Horticulture. University College Cork. Cork, Ireland.

O’Sullivan, K., 2021. Peat imports into Ireland ‘make no environmental, economic or ethical sense’. *The Irish Times*, 14 November. URL:

<https://www.irishtimes.com/news/environment/peat-imports-into-ireland-make-no-environmental-economic-or-ethical-sense-1.4728349>

Naveed, M., Tanvir, B., Wang, X., Brtnicky, M., Ditta, A., Kucerik, J., Subhani, Z., Nazir, M.Z., Radziemska, M., Saeed, Q. and Mustafa, A., 2021. Co-composted biochar enhances growth, physiological and phytostabilization efficiency of *Brassica napus* and reduces associated health risks under Cr stress. *Frontiers in Plant Science*, p.2568.

Nelson, N.O., Agudelo, S.C., Yuan, W. & Gan, J. 2011. Nitrogen and phosphorus availability in biochar-amended soils. *Soil Science*, 17, pp.218–226.

Novaes, E., Kirst, M., Chiang, V., Winter-Sederoff, H. and Sederoff, R., 2010. Lignin and biomass: a negative correlation for wood formation and lignin content in trees. *Plant Physiology*, 154(2), pp.555-561.

Nartey, O.D. and Zhao, B., 2014. Biochar preparation, characterization, and adsorptive capacity and its effect on bioavailability of contaminants: an overview. *Advances in Materials Science and Engineering*, 2014.

Rangabhashiyam, S. and Balasubramanian, P., 2019. The potential of lignocellulosic biomass precursors for biochar production: performance, mechanism and wastewater application-a review. *Industrial Crops and Products*, 128, pp.405-423.

Renou-Wilson, F., 2018. Peatlands. In *The Soils of Ireland* (pp. 141-152).

Renou-Wilson, F., Moser, G., Fallon, D., Farrell, C.A., Müller, C. and Wilson, D., 2019. Rewetting degraded peatlands for climate and biodiversity benefits: Results from two raised bogs. *Ecological Engineering*, 127, pp.547-560.

Ruysschaert, G., Nelissen, V., Postma, R., Bruun, E., O'Toole, A., Hammond, J., Rödger, J.M., Hylander, L., Kihlberg, T., Zwart, K. and Hauggaard-Nielsen, H., 2016. Field applications of pure biochar in the North Sea region and across Europe. In *biochar in European Soils and Agriculture*, pp. 121-157.

Sagrilo, E., Jeffery, S., Hoffland, E. and Kuyper, T.W., 2015. Emission of CO<sub>2</sub> from biochar- amended soils and implications for soil organic carbon. *Gcb Bioenergy*, 7(6), pp.1294-1304.

Schröder, C., Mancosu, E. and Roerink, G.J., 2013. Methodology proposal for estimation of carbon storage in urban green areas. *European Environment Agency*, 3, p. 7-9.

Schmidt, H.P., 2008. Ways of making Terra Preta: biochar activation. *Journal for terrier-wine and biodiversity*, ISSN, pp.1663-0521.

Schmidt, H.P., Hagemann, N., Draper, K. and Kammann, C., 2019. The use of biochar in animal feeding. *PeerJ*, 7, p.e7373.

Schmidt, H.P., Pandit, B.H., Cornelissen, G. and Kammann, C.I., 2017. Biochar- based fertilization with liquid nutrient enrichment: 21 field trials covering 13 crop species in Nepal. *Land Degradation & Development*, 28(8), pp.2324-2342.

- Schouten, M. G. C. (Ed.) (2002). Conservation and restoration of raised bogs: geological, hydrological and ecological studies. Department of Environment and Local Government, Dublin.
- Senthilkumar, R. and Prasad, D.M.R., 2020. Sorption of Heavy Metals onto biochar. In Applications of biochar for Environmental Safety (p. 207). IntechOpen.
- Shakya, A. and Agarwal, T., 2019. Removal of Cr (VI) from water using pineapple peel derived biochars: Adsorption potential and re-usability assessment. Journal of molecular liquids, 293, p.111497.
- Shetty, R. and Prakash, N.B., 2020. Effect of different biochars on acid soil and growth parameters of rice plants under aluminium toxicity. Scientific Reports, 10(1), pp.1-10.
- Smith, P., 2016. Soil carbon sequestration and biochar as negative emission technologies. Global change biology, 22(3), pp.1315-1324.
- Sombroek, W.G., 1966. Amazon soils: A reconnaissance of the soils of the Brazilian Amazon region (Doctoral dissertation, Pudoc), p. 125.
- Stavi, I. and Lal, R., 2013. Agroforestry and biochar to offset climate change: a review. Agronomy for Sustainable Development, 33(1), pp.81-96.
- Steiner, C. and Harttung, T., 2014. Biochar as a growing media additive and peat substitute. Solid Earth, 5(2), pp.995-999.
- Strange, R., 2015. Feeding more than 9 billion by 2050: challenges and opportunities. Food Sec, 7 (2), pp.177–178.f
- Tahat, M.M, Alananbeh, K., Othman, Y. and Leskovar, D., 2020. Soil health and sustainable agriculture. Sustainability, 12(12), p.4859.
- Taylor, M.D., Kreis, R. and Rejtö, L., 2016. Establishing growing substrate pH with compost and limestone and the impact on pH buffering capacity. HortScience, 51(9), pp.1153-1158.
- Thers, H., Abalos, D., Dörsch, P. and Elsgaard, L., 2020. Nitrous oxide emissions from oilseed rape cultivation were unaffected by flash pyrolysis biochar of different type, rate and field ageing. Science of the Total Environment, 724, p.138140.
- Thomazini, A., Spokas, K., Hall, K., Ippolito, J., Lentz, R. and Novak, J., 2015. GHG impacts of biochar: Predictability for the same biochar. Agriculture, Ecosystems & Environment, 207, pp.183-191.
- Uchimiya, M., Wartelle, L.H., Klasson, K.T., Fortier, C.A. and Lima, I.M., 2011. Influence of pyrolysis temperature on biochar property and function as a heavy metal sorbent in soil. Journal of agricultural and food chemistry, 59(6), pp.2501-2510.

Vaccari FP, Baronti S, Lugato E, Genesio L, Castaldi S, Fornasier F, Miglietta F (2011) biochar as a strategy to sequester carbon and increase yield in durum wheat. *Eur J Agron* 34:231–238.

Vandecasteele, B., Sinicco, T., D'Hose, T., Nest, T.V. and Mondini, C., 2016. Biochar amendment before or after composting affects compost quality and N losses, but not P plant uptake. *Journal of Environmental Management*, 168, pp.200-209.

Waliszewska, B., Grzelak, M., Gawel, E., Spek-Dźwigala, A., Sieradzka, A. and Czekala, W., 2021. Chemical Characteristics of Selected Grass Species from Polish Meadows and Their Potential Utilization for Energy Generation Purposes. *Energies*, 14(6), p.1669.

Wang, Y., Villamil, M.B., Davidson, P.C. and Akdeniz, N., 2019. A quantitative understanding of the role of co-composted biochar in plant growth using meta-analysis. *Science of the Total Environment*, 685, pp.741-752.

Wilson, D., Farrell, C., Mueller, C., Hepp, S. and Renou-Wilson, F., 2013. Rewetted industrial cutaway peatlands in western Ireland: a prime location for climate change mitigation?. *Mires & Peat*, 11, pp.3-5.

Winkler, B.S., Orselli, S.M. and Rex, T.S., 1994. The redox couple between glutathione and ascorbic acid: a chemical and physiological perspective. *Free Radical Biology and Medicine*, 17(4), pp.333-349.

Woolf, D., Amonette, J.E., Street-Perrott, F.A., Lehmann, J. and Joseph, S., 2010. Sustainable biochar to mitigate global climate change. *Nature communications*, 1(1), pp.1-9.

Xiang, W., Zhang, X., Chen, J., Zou, W., He, F., Hu, X., Tsang, D.C., Ok, Y.S. and Gao, B., 2020. Biochar technology in wastewater treatment: A critical review. *Chemosphere*, 252, p.126539.

Yang, C., Liu, J. and Lu, S., 2021. Pyrolysis temperature affects pore characteristics of rice straw and canola stalk biochars and biochar-amended soils. *Geoderma*, 397, p.115097.

Zhang, Y., Ding, J., Wang, H., Su, L. and Zhao, C., 2020. Biochar addition alleviate the negative effects of drought and salinity stress on soybean productivity and water use efficiency. *BMC plant biology*, 20(1), pp.1-11.

Zhang, Z., Dong, X., Wang, S. and Pu, X., 2020. Benefits of organic manure combined with biochar amendments to cotton root growth and yield under continuous cropping systems in Xinjiang, China. *Scientific reports*, 10(1), pp.1-10.

Zheng, B.X., Ding, K., Yang, X.R., Wadaan, M.A., Hozzein, W.N., Peñuelas, J. and Zhu, Y.G., 2019. Straw biochar increases the abundance of inorganic phosphate solubilizing bacterial community for better rape (*Brassica napus*) growth and phosphate uptake. *Science of the total environment*, 647, pp.1113-1120.

Zhu, X., Chen, B., Zhu, L. and Xing, B., 2017. Effects and mechanisms of biochar-microbe interactions in soil improvement and pollution remediation: a review. *Environmental Pollution*, 227, pp.98-115.

Żukiewicz-Sobczak, W., Latawiec, A., Sobczak, P., Strassburg, B., Plewik, D. and Tokarska-Rodak, M., 2020. Biochars Originating from Different Biomass and Pyrolysis Process Reveal to Have Different Microbial Characterization: Implications for Practice. *Sustainability*, 12(4), p.1526.